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PROCEEDINGS

3RD INTERNATIONAL CONFERENCE ON BIOSCIENCES AND BIOTECHNOLOGY

MAINTAINING WORLD PROSPERITY THROUGH BIOSCIENCES, BIOTECHNOLOGY AND REVEGETATION

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BALI, SEPTEMBER 21ST-22ND, 2011

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UNIVERSITAS UDAYANA

YAMAGUCHI UNIVERSITY

HIMPUNAN MAHASISWA PEMINATAN BIOTEKNOLOGI DAN BIOSAINS UNIVERSITAS UDAYANA

INHEALTH

KONSORSIUM BIOTEKNOLOGI INDONESIA

AIPKES

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PREFACE

This proceedings compiles all papers presented in the 3rdInternational Conference on Biosciences and Biotechnology (ICBB) held at the Udayana University, Bali on 21st - 22nd September 2011.

Sixteen plenary presentations were delivered by keynote and invited speakers with international reputations from Japan, Australia, Vietnam and Indonesia and a total of 123 papers (oral and poster presentation) compiled in this proceedings, which were presented in the conference by 279 contributors from 52 institutions in eight countries (Indonesia, Japan, Australia, Vietnam, Korea, USA, Spain, The Netherlands).

We thank those who involve in the organizing committee for their hardworking. While it was a huge task, it was a privilege for us in editing this proceedings and work together with the referees who reviewed papers.

We hope that the papers contained in this proceeding will prove useful in developing further study in the area of Bioscience and Biotechnology.

Editors

FOREWORDS-HEAD OF ORGANIZING COMMITTEE

I would like to sincerely thank all of the authors who contribute their papers in this proceedings. I would therefore give my high appreciation on all of those effort and dedication.

The conference was held in relation to the 49th Udayana University Anniversary and in collaboration with Yamaguchi University, Japan. This conference was aimed to gather scientists, academics, engineers and industries in biological related areas to discuss and share their expertise and ideas in the field of Biosciences and Biotechnology. The conference theme "**Maintaining World Prosperity through Biosciences, Biotechnology and Revegetation**" has appealed participants presenting their studies on four major fields of **Health, Agriculture, Agricultural Technology and Food Sciences, and Environment and Biodiversity**. This 3rd ICBB also focused on revegetation as one way to prevent global warming and conserve biodiversity. The conference was financially supported by Rector of Udayana University and several sponsors.

I hope this International Conference has created an international networking and collaboration and open up new ideas in maintaining world prosperity in all aspects in Biotechnology and Biosciences.

I will use this opportunity to invite you again to join us in The 4th International Conference on Biosciencesand Biotechnology which will be held on 21st - 22nd September 2012 in conjunction with the golden anniversary of Udayana University.

Last but not least, I will highly appreciate all of the members of the Organizing Committee for the good teamwork to make the 3rd International Conference on Biosciences and Biotechnology (ICBB-Bali 2011) possible and the team of editors for the hard work compiling and editing 123 papers presented in this book.

See you again in Bali at ICBB 2012

Dr. dr. I Dewa Made Sukrama, M.Si.,SpMK(K)

FOREWORDS
RECTOR OF UDAYANA UNIVERSITY

I would like to express my great appreciation to the organizing committee who worked so hard to make the 3rdInternational Conference on Biosciences and Biotechnology (ICBB-Bali 2011) to happen smoothly. This conference was held in conjunction to the 49th Anniversary of Udayana University and being our annual agenda. The main aim of this conference was to respond the problems related to the world prosperities, including deforestation, illegal logging, food and live stock shortage, as well as climate change which lead to the world disasters.

I was so happy to have you all in Bali which is well known in the world as a favorite tourist destination as well as recently a favorite site for holding International events, such as International Conference. As this conference was designed to gather scientists, engineers, practitioners, and industries in Biological related disciplines,I expected intense discussion has happened among them so that some brilliant ideas to be used to improve the quality of human life have been formulated and published in this proceeding.

Here, I would also like to acknowledge the National and International invited speakers for their willingness to come miles away to Bali and present their high standard papers. I understand that you all spent much time for this conference, and therefore I must give high appreciation on all of those effort and dedication.

I hope this International Conference was an ideal forum for communication and sharing ideas as well as experience in Biosciences and Biotechnology-related disciplines in the future. I also hope that this forum served as a forum for promoting advanced Biosciences and Biotechnology with regard to economic growth and social welfare.

Finally, I wished you most successful conference and hope that it provided new ideas and strategies for the application of Biosciences and Biotechnology in all aspect of our life.

See you again in Bali in 2012

Prof. Dr. dr. I Made Bakta, SpPD.(KHOM)
Rector of Udayana University

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KEYNOTE PRESENTATION

GENETICS, GENOMICS AND EVOLUTIONARY THEORY FOR SUSTAINABLE CROP GENETIC IMPROVEMENT

Wallace A. Cowling
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The University of Western Australia

ABSTRACT

Plant breeders have achieved significant progress during the first 100 years of "modern" crop breeding, but genetic improvement has been bought at the expense of genetic diversity within crop breeding programs. This is not sustainable. Small effective population size has resulted in inbreeding and genetic drift, and in some cases has stalled genetic improvement. Evolutionary theory indicates that this does not have to be the case – it is possible to achieve high rates of genetic improvement in the long term, and maintain genetic diversity, with more sustainable outcomes.

Relatively simple changes to crossing, selection and migration strategies can increase genetic diversity in plant breeding programs, with faster genetic progress and long-term sustainable genetic improvement. It is now possible to select on the whole population (or at least a significant portion of it), to measure economic traits (such as yield) over many years and sites, and to measure genotype by environment interaction (GxE) in multi-environment trials (MET). One of the critical differences between animal and plant breeding is GxE – every plant breeding trial gives a unique estimate of the genetic value of a line, which changes over environments. I refer to a recent analysis of canola breeding yield and oil data in Australia to demonstrate these points.

In genetic selection, a matrix of genetic relationship based on pedigrees is integrated into analysis of MET data, and this improves estimates of additive and non-additive genetic value of breeding lines at each site. In theory, genomic selection, which integrates a matrix of genetic relationship based on whole genome markers such as SNPs, can provide even better estimates of genetic values and more rapid cycles of selection. There are many challenges in the application of genomic selection to plant and animal breeding. The jury is still out on how successful genomic selection will be in animal breeding. Genomic selection is most likely to succeed in plant breeding programs based on large effective population size (more crossing parents), moderate selection pressure and migration. Genetic and genomic selection will therefore motivate the adoption of "evolutionary best practice" in breeding programs, which will improve long-term sustainability in crop genetic improvement.

ENVIRONMENTAL RESTORATION BY THE DEVELOPMENT OF NEW TECHNOLOGY ON PREVENTION OF SOIL EROSION AND REVEGETATION IN BARREN AREAS.

Takuya Marumoto
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ABSTRACT

Environmental destruction has occurredfor past 30 years by natural disaster and human activities. Forest was been destructed due to the development of industry, town and road. And then, content of carbon dioxide in atmospherehas increased gradually. Furthermore, much pollution as air, water, soil and food has increased.The environmental restoration such as prevention of soil erosion and reforestation is emergent necessity for sustainable human life.The new biological technology developed using symbiotic microorganisms with plant root (mycorrhizal fungi) for environmental restoration is introduced in this report.

PLANT IMMUNITY AS HOST RESISTANCE ACTIONS AGAINST PATHOGEN ATTACK –ARABIDOPSIS NSL2 AND THE INTERACTING-PROTEINS-

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ABSTRACT

Plants respond to pathogen infection by activating a defense mechanism known as plant immunity. One of the most efficient and immediate resistance reactions against pathogen attack in plants is the hypersensitive response (HR), which leads to rapid local cell death at the site of pathogen entry that is characterized by restricted growth and spread of the pathogen. Concomitant with the appearance of the HR, a secondary response known as systemic acquired resistance (SAR) is induced in uninfected tissues.

Arabidopsis *nsl2* (*necrotic spotted lesion 2*) mutant, which has been originally reported as the *cad1* (*constitutively activated cell death 1*), shows a phenotype that mimics HR-like cell death 1). The NSL2 protein is suggested to negatively control the salicylic acid (SA)-mediated pathway of HR-like cell death in plant immunity. Induction of SAR results in induction of pathogenesis-related (*PR*) genes in systemic organs triggered by local HR. In this study, we establish a *NSL2* knockdown system in transgenic Arabidopsis based on constitutive or dexamethasone (DEX)-induced RNAi. The constitutive knockdown plants showed *nsl2*-like phenotype. In DEX-induced RNAi, localized knockdown resulted in induction of *PR1* gene expression and restriction of bacterial growth in both DEX-treated and systemic leaves 2). These results indicate that the NSL2 negatively controls SAR via HR.

To clarify functions of NSL2 protein, we performed yeast two-hybrid screening to identify proteins interacted to the NSL2. Consequently, we succeeded in identification of Arabidopsis F-box protein NIFC1 (NSL2-interacting F-box group C1) as the interactors. The F-box protein consists of ubiquitin ligase which is involved in ubiquitin/26S proteasome pathway functions to degrade short-lived regulatory proteins that are involved in cell cycle regulation, signal transduction, apoptosis and metabolic regulation as well as misfolded proteins 3-4). Further characterizations of the NIFC1 which controls plant immunity will be reported.

INFLUENCE OF ADDITIONAL CALCIUM IN POULTRY DIETS ON GROWTH, BLOOD LIPIDS PROFILE, SPERM MOTILITY, AND SPERM CRYOSURVIVABILITY

F. Kanyinji and T. Maeda
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ABSTRACT

This study investigated the changes in the lipoprotein profiles and sperm quality of male birds fed with additional Ca. Thirty 21-wk-old Barred Plymouth Rock roosters were fed diets containing 0 (Control) or 2% additional Ca in the form of fossil shell (Aragonite) flour (Treatment) from 18 to 53 wk of age. Blood samples were collected from individual males at 21, 25, 29, 34, 37, 42, 46, 51 and 53 wk of age to assess and compare levels of blood Ca concentration ($[Ca^{2+}]$), total cholesterol (Ch), low-density lipoprotein cholesterol (LDL-Ch), high-density lipoprotein cholesterol (HDL-Ch) and triglycerols (TG). Semen samples were collected from individual males at 34, 35, 36, 37 and 38 wk of age to assess sperm quality and cryosurvivability measured as motility. Males in the treatment group exhibited significantly high blood $[Ca^{2+}]$ and HDL-Ch, but reduced total Ch, LDL-Ch and TG, compared to those in the control group. Sperm motility of fresh and frozen-thawed semen from treated males was significantly higher than that of control males. In conclusion, this study showed that feeding additional Ca (2%) in the form of Aragonite flour has beneficial modifications of not only blood plasma lipid profiles, but also of sperm quality.

Keywords: additional dietary calcium, total cholesterol, low-density lipoprotein, high-density lipoprotein, triglycerols, cryosurvivability

INTRODUCTION

In male reproduction, composition pattern of individual lipoproteins, i.e., low-density lipoprotein Ch (LDL-Ch), high-density lipoprotein Ch (HDL-Ch) and triglycerols (TG) in blood, is of great importance because of its potential effects on sperm functions (Samir Bashandy, 2007). For instance, reactive oxygen species release can lead to activation of sperm oxidative stress (Araujo *et al*., 1995), mediating sperm membrane damage and thus sperm malfunction (Iwasaki and Gagnon, 1992). It is positively correlated with LDL-Ch levels in hyperlipidemic patients, and negatively correlated with HDL-Ch concentrations (Samir Bashandy, 2007). Ca-related changes in total Ch do not always yield reciprocal alterations in individual lipoproteins such as LDL-Ch, HDL-Ch and TG, which may increase sperm quality and cryosurvivability. This study investigated the nutritional effects of feeding additional Ca to male birds as reflected in their lipoprotein profiles and sperm quality such as fresh sperm motility and cryosurvivability.

MATERIALS AND METHODS

Thirty 21-wk-old Barred Plymouth Rock roosters were fed diets containing 0 (Control) or 2% additional Ca in the form of fossil shell (Aragonite) flour (Treatment) from 18 to 53 wk of age.

Blood samples were collected from individual males at 21, 25, 29, 34, 37, 42, 46, 51 and 53 wk of age to assess and compare levels of blood Ca concentration ($[Ca^{2+}]$), total Ch, LDL-Ch, HDL-Ch and TG. Blood $[Ca^{2+}]$ was determined colorimetrically using a locally available commercial diagnostic kit (Wako Ca 272-21801, Wako Pure Chemical Industries Ltd., Tokyo, Japan). Total Ch, LDL-Ch, HDL-Ch and TG were determined from a 20 µmL plasma sample using an automated Beckman Coulter AU480 instrument (Beckman Coulter Inc., Fullerton, CA, USA).

Semen samples were collected from individual males at 34, 35, 36, 37 and 38 wk of age to assess sperm quality and cryosurvivability measured as motility. Collected semen from control and treatment groups was diluted eighty times (1:79, v/v) with their own seminal plasma, and analyzed in triplicates for sperm motility just after collection. Also, a 0.5 mL aliquot of fresh semen from both groups was diluted four times (1:3, v/v) with the diluent based on a 11.2% (w/v) trehalose solution supplemented with 0.3% (w/v) bovine serum albumin, gentamicin (50 µg/mL), and 10% (v/v) N-methylacetamide (cryoprotectant), and then mixed well. The cryoprotectant-diluted semen was frozen by liquid nitrogen vapor, and then stored in liquid nitrogen at -196°C. An hour later, the semen was thawed in a water bath set at 41°C. Analysis of sperm motility to assess quality at ejaculation and cryosurvivability was done in triplicates

by computer-assisted sperm analysis (CASA) method using HTM IVOS Motility Analyzer 8.1 (Hamilton Thorne Research, Beverly, MA, USA).

The data collected, i.e., blood [Ca^{2+}], total Ch, LDL-Ch, HDL-Ch, TG and sperm motility (fresh, cryoprotectant-diluted and frozen-thawed), were analyzed by ANOVA, followed by Student's t-test. Differences among means with $P < 0.05$ were accepted as representing statistically significant differences.

RESULTS

From 22 wk of age onwards, there was a significant difference in blood [Ca^{2+}] between control and treatment. Similarly, treated males exhibited significantly lower blood total Ch levels than control birds between 25 and 53 wk of age. LDL-Ch levels declined while HDL-Ch increased between 25 and 53 wk of age. LDL-Ch and HDL-Ch values for treated birds were significantly different from those of the control group. Additional Ca in the diet significantly reduced TG in treated birds, when compared to the control.

When fresh semen was diluted with cryoprotectant prior to freezing, sperm motility (fraction of spermatozoa population that displays movement with a path velocity>10 μm/sec) declined from 76 and 80% to 65 and 77% for the control and treated males, respectively. Likewise, the pattern in progressive motility, defined as the fraction of spermatozoa swimming forward with path velocity>25 μm/sec and straightness>80%, was similar to that of motility. The trend in sperm motility after thawing was also similar to that of cryoprotectant-diluted semen; the value of the treatment (28%) was significantly higher than the control (19%). Statistical analysis of the interaction effect of the age at which semen was sampled on sperm motility did not show any significant effect.

DISCUSSION

The results of this study agree with the general assumption that dietary induced changes in total Ch levels yield essentially reciprocal alterations in individual lipoprotein profiles (Grundy *et al*., 1990). An important observation in this study is that the hypocholesterolemic characteristics exhibited by additional Ca in the form of Aragonite flour incorporated in diets for Barred Plymouth Rock male birds, reduced both total Ch and LDL-Ch, but caused an increase of HDL-Ch. These results conform to observations of Denke *et al*. (1993) and Ditscheid *et al*. (2005) who found additional Ca-blood total Ch reductions in rats, and of Reid *et al*. (2002) who documented such dietary Ca supplementation-serum LDL-Ch and HDL-Ch changes in humans. These results suggest a beneficial influence of additional Ca in the form of Aragonite flour on the lipid and lipoprotein profiles of the birds.

Yamamoto *et al*. (1999) observed low sperm motility in rabbits fed high Ch diets, and attributed this observation to a decline in Sertoli and Leydig cell functions caused by hypercholesterolemia. Thus, increase in sperm motility in males fed 2% supplemented Ca may also be attributed to a reduction in blood Ch that optimized the functions of the Sertoli and Leydig cells, leading to production of spermatozoa with high membrane integrity.

Cryopreservation damages spermatozoa and systematically decreases the quality of spermatozoa (Wishart, 1985; Holt, 2000). The decrease in semen quality due to dilution or freezing also magnifies any damage that was initially present in fresh semen. According to Thurston and Harris (1970), the freezing process disrupts the outer sperm membrane protein layer resulting in the loss of protein to the seminal plasma, which in turn exposes the inner phospholipids and their accompanying negative charges. Ca^{2+} from seminal plasma migrates to these sites and forms insoluble complexes that deprive seminal plasma of Ca^{2+}. In the present study, spermatozoa from males fed 2% supplemented Ca also maintained superiority in post thaw motility compared to the control, concurring with the proposition of Blesbois (2007) that the viability of frozen-thawed spermatozoa depends on the quality of fresh semen. Because the spermatozoa from this group had higher sperm membrane integrity, the freezing-thawing process probably had minimal damage to the spermatozoa, compared to control spermatozoa.

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NEUROBIOLOGICAL STUDY ON ALTERED STATES OF CONSCIOUSNESS

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INTRODUCTION

Based on a survey of 488 human societies worldwide, Bourguignon reported that 90% had institutionalized some form of altered states of consciousness and 57% associated these states with a possession trance1). Although various procedures in different cultures are undertaken to bring people to trance states, it is likely that such altered states of consciousness involve common altered physiological states. Nevertheless, its underlying neurobiological mechanism remains unknown, in part, due to a lack of physiological investigation of these phenomena. Hereafter we refer to altered states of consciousness as trance states or trances.

One reason for this paucity of scientific data is that the induction of an authentic trance state is difficult to achieve in a laboratory setting, because a trance state is always induced by a specifically controlled procedure according to the specific culture. Therefore, *in vivo* field studies of naturally induced trances are necessary to provide meaningful data concerning the neurobiological mechanism underlying trance states.

At the same time, there are at least two major difficulties in undertaking *in vivo* field studies of trances. First, it is extremely difficult to obtain volunteer subjects because of the sacred context of trances. Second, it is not easy to establish controlled experimental conditions in the field, not only at a technical level, but also since the conditions of any such research must exist within the institutional and religious circumstances of the ritual ceremonies that induce the trance.

To overcome these ongoing challenges, we have been endeavoring, for nearly two decades, to establish mutual trust with members of several communities of local Balinese and administrative officers in Bali, Indonesia, so as to be allowed access to subjects participating in their sacred ritual dramas. In the present study, we report on the results of our investigations there, having for the first time been able to make use of electroencephalograms (EEG) and measure plasma concentrations of several psychoactive substances: catecholamines and their metabolites, and neuropeptides, obtained from Balinese subjects involved in ritual dramas under natural conditions.

MATERIALS

In Bali, Indonesia, the participants and/or spectators of ritual ceremonies and dramas are known to go into trance states without the use of any psychoactive drugs2,3). This phenomenon is called Kerauhan. One of the prominent characteristics of Kerauhan is the fact that it normally occurs, not in a professional shaman, but rather in ordinary, healthy individuals en masse during ritual ceremonies. In this study, we focused on a Kerauhan that frequently occurs during a dedicatory ritual drama called ''Calonarang'' throughout many areas on Bali Island. In this drama, several participants usually exhibit a similar pattern of trance state. Therefore, the homogeneity of this drama is potentially suitable for neurobiological investigation.

METHODS

3-1 Electroencephalogram (EEG)

Subjects

Twelve healthy male volunteers (aged 33.5 ± 6.8 years) were investigated. Nine subjects were Calonarang drama actors portraying a warrior fighting against a witch, while two subjects played musical instruments in the dramas, and one subject was a spectator.

According to the appearance and behavior of the subjects in this drama (*see Results*), the subjects were divided into two groups. Seven subjects exhibited specific trance behaviors (e.g., fixed, unfocused

eyes; mask-like expression; stiffening of the limbs; tremor) during their acting (trance group), whereas five subjects exhibited no possession trance behavior but simply performed actions similar to those of the trance group (control group).

EEG Equipment and Procedure

We developed a custom-modified, portable EEG telemetry system for field use. It was designed so as not to restrict the vigorous movement of the subjects[4]. EEGs were recorded in parallel from two subjects using two electrocaps and two telemetry systems (Synact &5 14x modified, NEC Corporation, Tokyo, Japan). The EEGs were recorded from 11 scalp sites (linked Fpl-Fp2, F7, Fz, F8, C3, C4, T5, Pz, T6, O1, and O2 according to the lnternationa1 10-20 System), using linked earlobe electrodes as reference electrodes. Data were filtered at 60 Hz (-3dB), time constants of 0.3 sec, sampled at 100 or 256 Hz, digitized, and stored on a portable personal computer (VAIO PCG-868, SONY lnc., Tokyo, Japan). The subjects provided written informed consent prior to the study. In an interview prior to the experiment, basic personal data on each subject were obtained, including family background, clinical history, right- or lefthandedness, and history of possession trance experience. Special care was taken to ensure that the subjects were in good health and did not have any medical indication of neurological disease or mental disorder, either personally or among family members.

Approximately 30 minutes prior to the performance of the drama, we placed the electrodes on the subjects and confirmed that the equipment did not make them feel uncomfortable or restricted. Just before the drama began, we recorded each subject.'s eyesclosed resting-state EEG for 3 minutes. After that, their EEGs were continuously recorded throughout the entire performance. Within 3-to- 8 minutes after the drama and each subject.'s concomitant recovery from their trance state, we again recorded their eyes-closed resting-state EEG for 3 minutes. Blood pressure and heart rate were also measured just before recording the eyes-closed resting-state EEG both before and after the drama. Subjects were then interviewed once again after the drama as to their memory of the drama, any subjective symptoms, and etc.

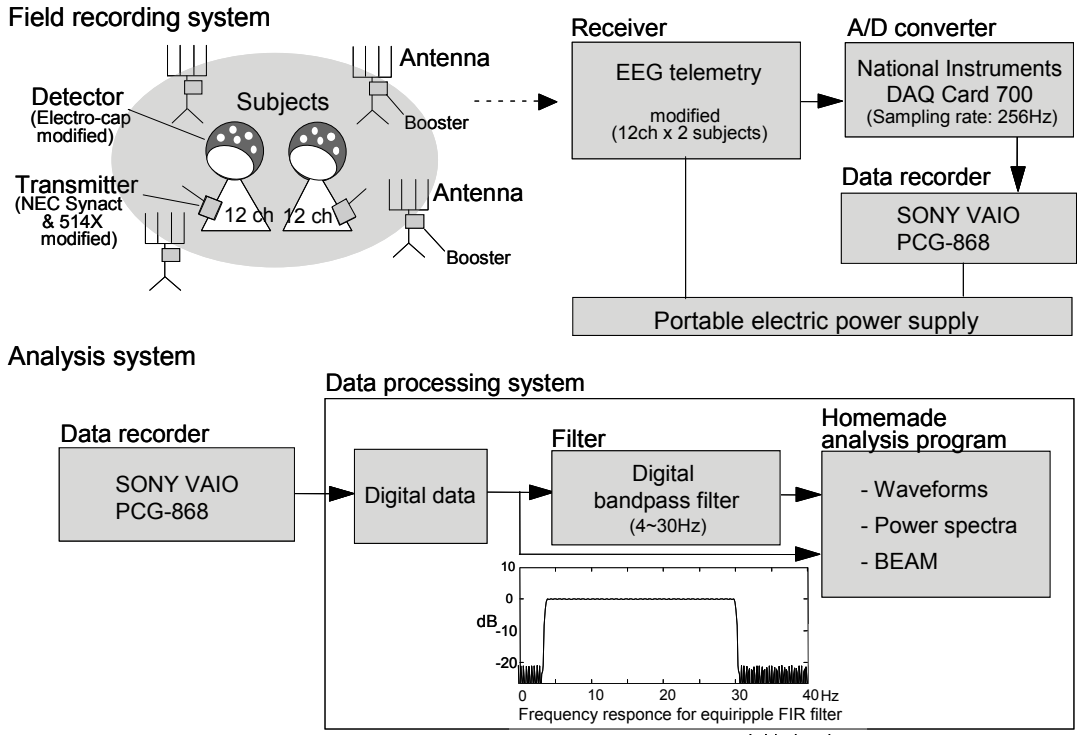

Fig.1 The multi-channel portable EEG recording s ystem and analysis system was specifically developed for field use.

EEG Data Analysis

Filtering data using a digital band pass filter

A range of frequencies that included the 4 bands of interest, namely, theta (4-7.5 Hz), alpha l (8-9.5Hz), alpha 2 (10-12.5Hz), and beta (13-30 Hz), was precisely extracted from the original data using a high-performance digital bandpass filter with equiripple FIR under the following conditions: (1) the passband cut-off frequencies were 4 Hz and 30 Hz; (2) the stopband cut-off frequencies were 3.5 Hz and 30.5 Hz; (3) the stopband attenuation was 20 dB/0.5 Hz; (4) the passband ripple was 0.1 dB; and, (5) the filter order was 768. Under these filtering conditions, any high-frequency artifacts caused by myogram or low-frequency artifacts including baseline wobble caused by body or cable movements were greatly decreased. The extracted EEG was then analyzed to examine the 4 frequency bands described above.

Visual inspection of the EEG waveforms

The EEG waveforms were visually inspected by comparing the raw waveforms, the filtered waveforms, and the frequency spectrum of each 2-second block. Any 2-second block during which the telemetry data was not received or which contained artifacts was then excluded from further analysis.

Categorization of EEG data

The data were categorized based on the behavioral transition of the states of the subjects and the macroscopic structural changes of the EEG waveform indicated by the power spectra. Based on behavioral alterations, the EEG data were divided into two states of consciousness: the normal state and the trance state. The normal state was divided into three phases: the resting period prior to the drama, the standby period after the start of the performance, and the resting period after the end of the drama. The trance state consisted of one phase: the period from the appearance of trance behavior to the disappearance of trance behavior. Thus, the four phases were as follows:

1. PRE: eyes-closed resting phase in a normal state before the start of the drama
2. WAIT: eyes-open waiting phase in a normal state before acting
3. MOVE: eyes-open moving phase in a trance state during acting
4. POST: eyes-closed resting phase in a normal state after the end of the drama

EEG topography

The EEG topography was mapped for the theta, alpha 1, alpha 2, and beta frequency bands for each phase, as follows: Frequency spectra of the filtered EEG data, with artifacts excluded as described above, were calculated for each 2-second block with an overlap of 1 second (frequency resolution 0.5 Hz, sampling 256 Hz). The root of the power was computed for each bandwidth (theta: 4-7.5 Hz; alpha 1: 8-9.5 Hz; alpha 2: 10-12.5 Hz; beta: 13-30 Hz) for each scalp site, which together then comprised the equivalent EEG potential for each bandwidth. These values were averaged over the period of each phase. The average equivalent EEG potential of 11 scalp sites was extended to 25 scalp sites. Then a Brain Electrical Activity Map (BEAM) was calculated, illustrating the distribution of the equivalent EEG potential on the scalp in a 15-grade color map, using direct interpolation[4,5,6].

Statistical analysis of EEG

An average EEG potential value for the four frequency bands examined was calculated across channels. To remove inter-subject variability, the averages for each phase were normalized with respect to those averaged across all the phases. The data of each group were separately subjected to the analysis of variance (ANOVA) with a within-group factor of four phases. Then, a post-hoc test of Fisher's PLSD was used to test for differences in the normalized EEG potential among phases. To explore a characteristic change during possession trances, the DELTA was calculated by subtracting the data during the WAIT phase from those during the MOVE phase for each of the four frequency bands. An unpaired Student's /-test was used to test for differences in the DELTA between the trance group and the control group.

3-2 Plasma Concentration of Psychoactive Substances

Subjects

In total, 24 healthy Balinese males (aged 35.4 ± 8.3 years) participated. All of them took the role of the same character in the drama, namely, warriors fighting with a witch, in the ritual drama "Calonarang". Data from these 24 subjects were collected on 7 separate occasions; 3 or 4 subjects on each occasion. As for EEG measurement, according to their appearance and observable behavior, these subjects were divided into 2 groups. Fifteen subjects exhibited specific possession trance behaviors (e.g., fixed, unfocused eyes; mask-like expression; stiffening of the limbs; tremor) during their performance (trance group), whereas 9 subjects exhibited no possession trance behavior and simply performed actions similar to those of the trance group (control group). As noted above, the subjects were fully informed by a Balinese medical doctor beforehand about the nature of the experiment and had provided their written consent prior to the experiment itself.

Procedure

Blood sampling

All the subjects fasted for at least 4 hours and refrained from smoking or drinking tea or coffee for at least 2 hours before the first blood sample was collected. Approximately 15 minutes before the ritual drama began, the subjects sat in a relaxed position and their blood pressure and heart rate were measured. Then 24 ml of blood was collected from an antecubital vein of each subject. Within 6 minutes after the drama ended, the same procedure was repeated for each subject. Baseline levels were established in 19 of 24 subjects using the same sampling procedure on a day that the ritual was not performed, matching the pre-ritual time, considering altered daily rhythms. Basic personal data including medical history, trance history, and family history were obtained by interview before the drama. Via a standard clinical interview, episodic recall and subjective impressions before, during, and after the trance were obtained from each subject after the ritual drama ended.

Quantitative analysis of psyclioactive substances

Blood was collected into 3 vacuum tubes containing ethylene-diamine-tetraacetic acid 2 sodium (EDTA-2Na) and 1 tube containing EDTA-2Na and trasylol. The tubes were centrifuged at 800g for 10 minutes to separate the plasma using a custom-built, battery-powered portable centrifuge developed for field use. The plasma samples were rapidly frozen in dry ice and stored at -70°C until assayed for 3 catecholamines: adrenaline (AD), noradrenaline (NA), and dopamine (DA); 3 catecholamine metabolites: vanillylmandelic acid (VMA), 3-methoxy-4-hydroxy-phenylglycol (MHPG), and homovanillic acid (HVA); and 2 neuropeptides: adrenocorticotropic hormone (ACTH) and (3-endorphin. All substances were examined by SRL Inc. (Tokyo, Japan) according to the manufacturer's instruction. The catecholamines and their metabolites were assayed using high-performance liquid chromatography, and the neuropeptides using radioimmunoassay.

Statistical analysis ofpsychoactive substances

Statistical evaluation was performed using SPSS software (SPSS Inc., Chicago, IL, USA). First, the heart rate, systolic blood pressure, and plasma concentration of each substance examined were subjected to repeated measures analysis of variance (RM-ANOVA) with a within-subject factor of condition (pre-ritual vs. post-ritual measures) and a between-subject factor of group (trance group vs. control group). For the substances exhibiting non-negligible condition by group interaction, delta was defined as the difference in the plasma concentration of each substance between pre-ritual and post-ritual measures. Because the equality of variance of delta between the two groups was rejected for all the above substances with a significance level of $P < .05$ using Levene's test and because there was no evidence to reject normal distribution of the data, we used unpaired Student's /-tests with separate-variance estimates for the two groups to examine the difference in delta between the two groups. Additionally, in comparing the plasma concentrations of the pre-ritual condition with baseline levels that were measured on a different day, separate RM-ANOVAs were performed on the data from 19 subjects.

RESULTS

4-1 Appearance of the subjects

During the climax scene, the subjects later included in the trance group attacked the person playing a witch with a sword. They threw themselves at the witch, glowered at her, staggered around for a while, and then threw themselves at her once again. They performed these automatism-like behaviors repeatedly. They exhibited a mask-like face with their eyes fixed but unfocused. They vigorously poked their sword against their chest, abdomen, head, and face. Furthermore, some of the subjects devoured live chicks as sacrifices. Finally, they fell to the ground with stiffened limbs. A few of them exhibited tremors. After a priest sprinkled a few drops of holy water on their faces and bodies and assistants patted them, the subjects were able to stand (with assistance) and gradually returned to a normal state within a few 4 minutes. The mean duration of the trance state was around 10 minutes. Without exception, all subjects in the trance group exhibited anterograde amnesia of the episode.

In contrast, the subjects later included in the control group also performed as warriors fighting against the witch. None of these subjects ate live chicks, but otherwise they performed the same actions as those of the trance group. Nevertheless, their facial expressions (including their eyes) appeared normal, which markedly differed from those of the trance group. They also fell down in the final scene, but shortly afterwards, when receiving holy water, they suddenly and easily rose up without assistance and behaved normally. No muscle rigidity or tremor was observed. Without exception, none in the control group exhibited any memory disturbance regarding the episode.

Although heart rate increased significantly from pre- to post-ritual (condition: $P < .001$), this change was the same for both groups (group: $P = .16$, interaction: $P=.70$). Systolic blood pressure did not differ (condition: $P = .38$, group: $P = .59$, interaction: $P = .33$).

4-2 EEG

EEC topography

Transition of the BEAM of the trance subjects

The averaged BEAMs of the 7 trance subjects for each of the 4 phases are shown in Fig. 2.

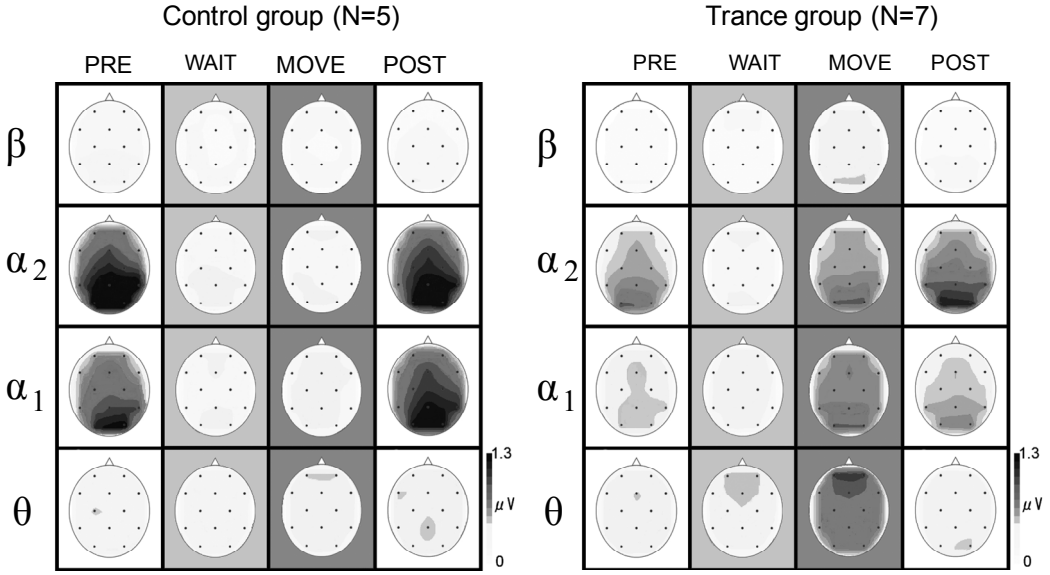

Fig.2 The averaged BEAMs in control group and trance group

PRE : Although some subjects exhibited a low-voltage brain wave pattern, all of the subjects exhibited an occipital dominant alpha power quite similar to the usual pattern of healthy subjects.

WAIT : A small theta power was observed in 3 subjects; otherwise, all of the trance subjects exhibited low power in the 4 frequency bands, with no unusual changes observed.

MOVE : The alpha 1 and 2 powers increased markedly for all trance subjects. Some theta and beta powers were also apparent. Every trance subject exhibited slightly different distribution patterns of EEG power during the MOVE phase. In some, the theta power was highest. In others, the alpha 1 and 2 powers were highest. In others, the theta, alpha 1, and alpha 2 powers were all high.

POST : The frequency powers that had increased during the MOVE phase decreased in all of the trance subjects. However, in some trance subjects, the alpha 1 and 2 powers did not return to PRE phase levels but remained higher.

Transition of the BEAM of the control subjects

The averaged BEAMs of the 5 control subjects are illustrated for the 4 phases in Figure 2. A similar transition patterns were observed in the BEAMs of control subjects. During the PRE phase, alpha 1 and 2 powers with an occipital dominant pattern appeared as they generally do in normal, healthy subjects. During the WAIT phase, the alpha 1 and 2 powers decreased and were not apparent again until the POST phase, in which phase they were similar to that during the PRE phase.

Statistical evaluation of the change in spontaneous EEGs

There was a highly significant main effect of the phase for all 4 frequencies for trance subjects as well as for control subjects. The post-hoc test showed a highly significant difference for all 4 frequencies between the WAIT phase and the MOVE phase for the trance subjects (theta, alpha 1, beta: *P<,001;* alpha 2: P<01; Fig. 3A). In contrast, there were no significant differences between the WAIT phase and the MOVE phase for the control subjects (Fig. 3B).

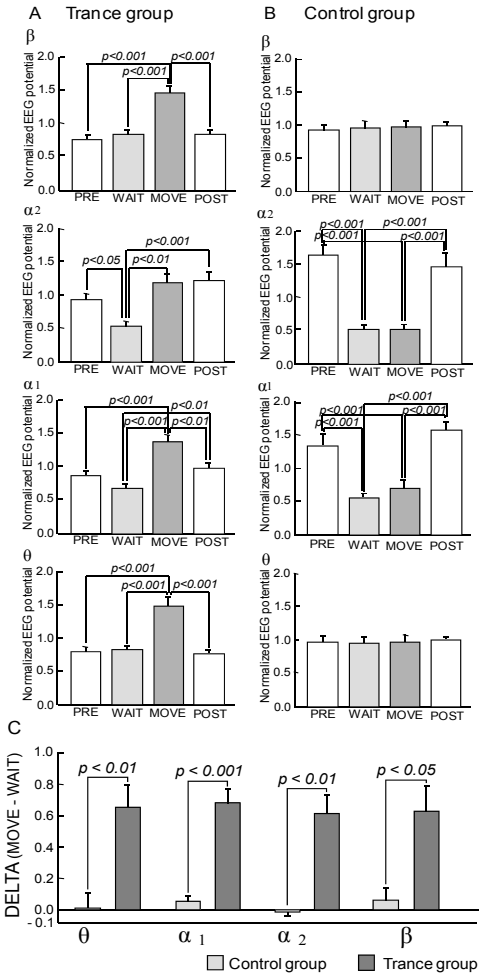

Fig. 3 Statistical evaluation of normalized EEG potentials

The differences between the MOVE phase and the WAIT phase (i.e., DELTA) were significantly higher in the trance group than in the control group (alpha 1: *P<.00l;* theta and alpha 2: 7'<01; beta: *P<.05;* Fig. 3C).

4-3 Psychoactive Substances

All of the substances studied during this experiment significantly increased post-ritual compared with pre-ritual measures. Moreover, the conditions for group interaction were significant for NA and DA *(P<* .05) and approached significance for p-endorphin (P = .052). Further analysis using unpaired Student's /-tests with separate-variance estimates for the two groups revealed that the trance group exhibited a significantly greater increase in NA, DA, and p-endorphin post-ritual compared with pre-ritual measures (i.e., delta) than did the control group (Fig. 4). We also confirmed that the conclusions of this study would not change even with non-parametric statistics (Mann-Whitney U-test: NA, *P< .05;*

DA, P< .01; p-endorphin, *P< .05).* There was no significant main effect or interaction between baseline and pre-ritual plasma concentrations for all substances examined, except that a main effect in condition was observed for AD (pre-ritual > baseline, *P< .05)* and a main effect in group was observed for DA (trance group > control group, *P<* .05). The baseline and pre-ritual values for all subjects for all substances were within the normal range of values for healthy people.

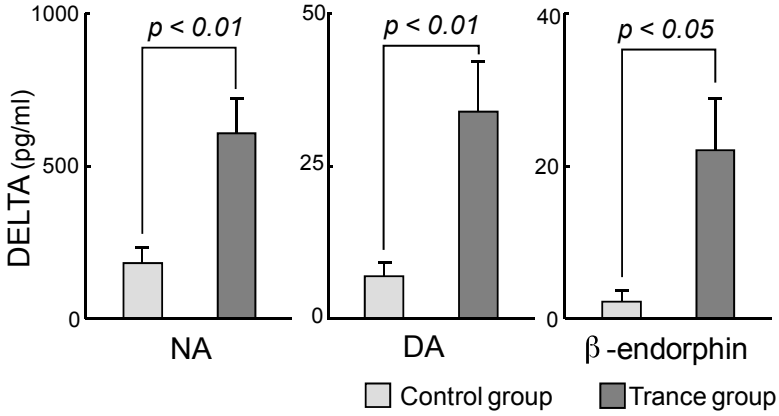

Fig. 4 Difference in plasma concentrations between pre- and post-ritual measures (DELTA)

DISCUSSION

EEC measurement

Through this study, we have been able, for the first time, to successfully record EEGs during trances under natural conditions. There is a possibility that a physiological difference between the normal state and a trance state can be detected in a spontaneous EEG as a difference in the stationary aspect of the frequency structure described by our analysis. On the other hand, transitional characteristics in microtime domains and relationships across different EEG bands cannot be assessed by the present method. Other techniques of spectral analysis, such as the maximum entropy method, may be useful for this purpose[8].

EEG characteristics of the trance state

The results of the present study demonstrate that several significant changes in the EEG recordings of subjects occurred during possession trances induced under natural conditions, as compared to EEGs recorded before and after trance state and compared with control subjects under similar circumstances who had not entered trance state. Moreover, certain trance-related changes were sustained into the post-trance period.

The common features for all trance subjects, summarized in comparison with those for the control subjects, are as follows:

1. Within the trance group, but not the control group, there was a highly significant difference in the frequency power between the WAIT phase (normal-state phase) and the MOVE phase (trance-state phase). The increase in the alpha power during the MOVE phase in trance subjects was the most significant change. This increase remained into the POST phase.
2. Between the two groups, there was a highly significant difference in the alpha 1 and 2 powers. The difference in the beta and theta powers between the groups was also significant.
3. The trance subjects tended to exhibit lower frequency waves during the MOVE phase (eyes open) than in the PRE phase (eyes closed).

There were also various individual differences between each subject; however, the EEG characteristics of the trance group, as described above, were very consistent and statistically quite different from those of the control group. This suggests that such characteristics represent general electro-physiological hallmarks of a trance state of consciousness. The additional individual variations in EEG power during the MOVE phase may be due to standard biological variation or to differences in the depth of the trance.

Enhancemant of the EEG in the alpha rhythm

Although the mechanisms underlying the generation of the alpha rhythm have yet to be fully clarified, an animal model suggests the involvement of at least the thalamocortical and intracortical networks^. A focal decrease of the background EEG in the alpha band occurs in association with corresponding cortical activation. It is known that an occipital alpha-EEG closely reflects the activation of the visual cortex; a significant negative correlation between the occipital alpha rhythm and activity in the visual cortex has been reported^. Importantly, the enhancement of the alpha-EEG in eye-open phase is evident in the trance state. Although there is marked variability in alpha power in normal individuals from one period of time to another, depending on their level of mental activity, this finding may suggest some linkage between the depth of trance and alpha-EEG. A positive correlation has been shown between the occipital alpha-EEG and the regional cerebral blood flow in the deep brain structure[9]^ including the thalamus'[0]. Therefore, we need to consider the possibility that a trance state may be associated with a change of activity in deep-lying structures, including the thalamus.

NA, DA, and fS-endorphin characteristics of the trance state

The significant increase in NA, DA, and p-endorphin in the trance group raises the possibility that activation of these neuronal circuits in the CNS plays a role in the altered states of consciousness, memory deficits, and unusual behavior observed during trances.

The plasma concentration of NA correlates with that in the cerebrospinal fluid, which, in turn, correlates with its activity in the CNS [10]. In the medulla, NA-producing neurons are located in the ventral (Al) and dorsal (A2) columns, which primarily project to the hypothalamus and control cardiovascular and endocrine functions[11]' 12,13) -^^ neurons also exist in the ventral (A5 and A7) and dorsal (A6) columns of the pons"' [12'13]\ The dorsal A6 group projects from the locus ceruleus to the mesencephallic periaqueductal gray, medial forebrain bundle, amygdala, hippocampus, thalamus, and cerebral cortex"' [12''3)]. These areas of the human brain are involved in vigilance and responsiveness to environmental stimuli. The ventral A5 and A7 neurons primarily project to the spinal cord and modulate autonomic reflexes and pain sensation[11'12'1^]. We suggest that an increase in 7 the plasma concentration ofNA may reflect, in part, the activation of the central NA system, which may represent the neurobiological mechanism underlying the hyperarousal state, tensity, stenosis of consciousness, and decreased pain sensation during trances.

DA-producing neurons are located primarily in the substantia nigra (A9), adjacent retrorubral field (A8), ventral tegmental area (A 10), and hypothalamus (All, A12, A13, A14) [13•14•15)]. There are major ascending DA projections from the A8-A10 neurons to the striatum, limbic system, and frontal and temporal cortices[13'14'15^] These pathways have been implicated in motor initiation, emotion, and memory formation and storage. The mesostriatal DA system lacks DA autorecepters, and, therefore, these neurons

readily exhibit bursts of action potentials'[5]"^. Furthermore, it has been reported that DA transport proteins, which are reversed by cocaine resulting in increased extracellular DA[16'17]-"[0], are located primarily in the projections from substantia nigra to corpus striatum (motor control) and from the ventral tegmental area to the limbic system (memory formation)[19]. The alterations in behavior, such as psychomotor excitement, hyperlocomotion, automatism-like actions, cataleptoid posture, and memory disturbance, observed in the trance group are consistent with a greatly increased activation of the DA system.

P-endorphin is one of the major endogenous opioid peptides. p-endorphin-producing neurons exist in a limited area of the arcuate nucleus in the hypothalamus and the commissural nucleus in the medulla oblongata[20'20]. The opioid peptidergic system in the spinal cord primarily has an analgesic function, whereas that in the brain affects the emotional state and induces a sense of well-being and euphoria[2]^. In addition, intraventricular injections of a small amount of morphine or p-endorphin induce hyperactivity in rats, while a large amount induces exophthalmos, myoclonus, and decreased deep tendon reflex[23]^. The cataleptoid behavior with decreased pain sensation observed in the trance group is consistent with the response to opioid peptides observed in an animal model as well as in humans.

Opioid peptides are released with monoamines during periods of high monoamine neuron activation, and act synergistically with monoamines in their effect on postsynaptic cells[24]^. These substances are known to have a role in generating pleasure sensation[22]^ Indeed, some subjects in the trance group reported euphoria or eudemonia at the post-ritual interview, suggesting that the reward generation system is involved in trances.

CONCLUSION

We consider that we have been able to verify, through this experiment, the functionality of the portable EEG recording system we developed and the utility of the EEG analysis method for subjects with movement in the field. Indeed, this methodology shows itself quite useful for EEG recording under severe conditions and for investigating the features of spontaneous EEGs. We also succeeded in our goal of measuring, for the first time, the EEG of a trance-state subject under natural settings.

Furthermore, we were able to investigate, also for the first time, the plasma concentrations of psychoactive substances from healthy subjects exhibiting behaviors typical of trance states in the field[25]^. Plasma concentrations of NA, DA, and p-endorphin increased to a significantly greater degree in the trance group compared to the control group. The results suggest that catecholamines and opioid peptides in the CNS are involved in trance states "Kerauhan," including markedly altered states of consciousness, memory, pain sensation, and behavior. The present study represents a solid foundation for future attempts to scientifically clarify the neurobiological mechanism underlying altered states of consciousness.

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THE SEDATIVE EFFECT AND THE PHYSIOLOGICAL MECHANISM OF CEDROL

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ABSTRACT

This study showed that cedrol, which is a sesquiterpene alcohol contained in cedarwood oil, significantly prolonged the R-R interval of the ECG, decreased systolic and diastolic blood pressures. These results demonstrated that inhaled cedrol eased sympathetic excitation, shifted the autonomic activities to parasympathetic dominance, and reduced mental tension. Based on these effects on the parasympathetic nerve system, cedrol was expected to affect the sleep. Therefore we performed polysomnograph analysis to investigate the effect of cedrol. The findings showed that cedrol also sedated the body and mind, improved the onset of sleep, and maintained sleep and symptoms as complementary therapy in menopouse. The effects of cedrol on the autonomic nervous system, perception of its odor, and liking or disliking of it were evaluated in females living in the capital cities of 4 countries(Japan, Norway, Thailand and USA). Cedrol was shown to induce parasympathetic dominance in the subjects of all 4 countries despite marked differences in their physical and social environments. In modern Asia, stress is increased by increasing social complexity, life is changing to an urban style, and various sleep-interfering factors affect our lifestyle. The results of this study suggest that cedrol is useful for the preparation of a more relaxing living environment.

Keywords:cedrol, parasympathetic dominance, sedative effect, sleep

FUNCTION OF A MULCHING SHEET FOR THE PREVENTION OF SOIL EROSION AND REVEGETATION

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ABSTRACT

From the experiments by model and actual slopes, several effective functions of a mulching sheet (MS) were indicated on the prevention ofsoil erosion and revegetation as follows: 1) drainage of flow water, 2) reduction of muddy water, 3) prevention of soil erosion, 4) decrease of drought damage to soil, 5) decrease of the fluctuation of temperature and moisture in surface soil, 6) reduction of wintry withering, 7) promotion of plant growth, 8)environmental conservation of root zone, 9)environmental improvement of soil microorganisms.

DETERMINATION AND EFFECT OF ARBUSCULAR MYCORRHIZAL FUNGI, *GIGASPORA MARGARITA* CK INOCULATED IN REVEGETATION AT NUKUI DAM SITE

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ABSTRACT

The establishment of survival arbuscular mycorrhizal (AM) fungi, *Gigaspora margarita* Becker & Hall inoculated in revegetation at Nukui dam was studied. There were different soil amendments, in experimental design, in every plot. The population of AM fungi and AM colonization of 3 species, Japanese hill cherry (*Prunus jamasakura* Sieb.ex Koidz, Yamazakura-inoculated plant), Eulalia (*Miscanthus sinensis* Anderss*,* Susuki) and Mugwort (*Artemisia vulgaris* Pampan*,* Yomogi) were investigated to compare among inoculated and non-inoculated treatments. The AM colonization was enhanced in inoculated plots although natural or accidental AM fungi involved into un-inoculated plots and successfully colonized. Difference in the combination of soil amendments did not affect AM colonization apparently. Under given conditions, inoculation simply resulting in the higher colonization level after 4 years of execution. The colonization intensity and spore number in rhizosphere and zone root soil was positive correlated.

In order to conduct detection of *Gigaspora margarita* CK, inoculated strain after revegetation at Nukui dam for long time, the gene analysis was carried out by a specific molecular sequence marker method. All of the inoculated treatments gave positive specific band at 235-bp on some templates from DNA preparations of AM spores collected from Susuki and Yamazakura root zone soil. Moreover, from trap culture, spores were differed among host plant and soil inoculum and several spores also gave image of *G. margarita,* at the same time the density of AM structures colonized in host roots was high, especially auxiliary cell - a typical structure in Gigasporaceae was presented. The results seemed to denote the inoculation of AM fungi improving the AM establishment of symbiosis between AM and plant and increasing the diversity of AM associations. Inoculated AM, *G. margarita* CK could survive for long times in the field condition at Nukui dam.

Keywords: Detection, inoculation, molecular sequence marker, soil amendment, survival Arbuscular mycorrhizae (AM), revegetation.

INTRODUCTION

Arbuscular mycorrhizae (AM), the most widespread symbioses on the earth (Hayley and Smith, 1983) are receiving attention because of the increasing range of their application in practical fields as diverse as sustainable agriculture, reforestation programs and ecosystem management (Bethlenfalvay and Schuepp, 1994).

Some plant species are almost totally depends on mycorrhizal association for nutrient uptake (Warcup and McGee, 1983). Improved growth and survival of such species will help plants to establish themselves in a diverse ecosystem, an important criteria for revegetation success. Plants, which are normally mycorrhizal will be at a competitive disadvantage in revegetation if phosphorus is limiting and if there are few effective mycorrhizal fungi in the soil. Therefore, the presence of effective mycorrhizal fungi is likely to be re-established. In general, plants from mature ecosystems require the presence of mycorrhizae for their development (Janos, 1980).

Revegetation of disturbed soil should aim to establish a stable ecosystem with fully functioning nutrient cycling processes. Mycorrhizal fungi are likely to be important in helping achieve that aim by increasing nutrient uptake by plant and contributing to the restoration of soil structure (Miller and Jastrow, 1991). Optimizing the survival of mycorrhizal fungi could provide a useful focus for revegetation strategies, giving wider benefits in enhancing the overall microbial activity of the soil (Jasper, 1994).

It is well known that AM fungi species of the genus *Gigaspora* appear to favour fluxes of C compounds from plant to soil biota, resulting ultimately in enhanced soil aggregation, while *Glomus spp.* tend to favour root colonization, plant growth and productivity through improved mineral nutrition (Bethlenfavay and Linderman, 1992). The management of AM on revegetation and reforestation are well documented (Jasper, 1994; Marumoto *et al*., 1997). But little still is known about the establishment and effect of survival AM after revegetation for long time. The overall objective of the work presented was closely linked to the project "Utilization and management of symbiosis associations - Mycorrhiza (AM and

EM (Ectomycorrhizae) in revegetation program at Nukui dam".

The effect of AM fungi inoculation as a surplus on the various effects of the soil amendments after revegetation at Nukui dam was analysed. In this study, the attempt was made to answer the question, whether an inoculated strain of AM fungi, *Gigaspora margarita* Becker and Hall contained in the amendment used in revegetation at Nuikui dam could be survived for several years (from 1998). For this purpose, a molecular method was applied (Yokoyama *et al*., 2002). The performance of PCR and hybridization were combined. In the actual course of detection, a marker DNA sequence that is diagnostic for the *G. margarita* CK strain was introduced.

MATERIALS AND METHODS

Description of the study site

The Nukui Dam is the second highest arch dam in Japan, located across a narrow section of the Takiyama River with hillside slopes and both banks ranging from 45° to 60° and a river bed width ranging from approximately 40 to 60m in Hiroshima Prefecture with a catchment-area of 1,700 km^2. The annual precipitation is approximately 2000 mm and average annual temperature is about 13°C.

The study site was the berm number (No) 10 - a part of planting area at Nukui dam, which was executed revegetation process on May 1998. Total area of this berm was 40.5 m^2 and set into 8 plots (each 3 x 1.5 m). Each plot was divided by plywood with thickness of 2 cm soil planting.

The field sampling was carried out in November 2002 – in the autumn season because the AM fungi spore populations are, in general, greatest in the autumn in areas where there are marked warm and cold seasons (Douds and Chaney, 1982; Klironomos *et al*., 1993).

Revegetation program

Construction of the Nukui dam began in July 1992, the dam concrete placing was commenced in May 1994, and the work completed in December 1998. Regarding the preservation of the natural environment around the dam site, the research project of revegetation at Nukui dam was started from 1997 by the corporation betweens the dam construction office, Yamaguchi and Hiroshima Universities. The test on the No 10 berm started at 1998. Each plot (3m x 1.5m) was filled with Masa soil (Granite) and added with some amendments (sand, zeolite, vermiculite, bark or wheat straw compost). Each five seedlings of three tree species: Yamazakura (*Prunus jamasakura* Sieb.ex Koidz), Arakahi (*Quercus glauca* Thunb.) and Konara (*Quercus serrata* Thunb. Ex Muuray) were transplanted to every plot.

Inoculation of AM fungi was carried out by mixing 15 g of an AM fungi spore-containing material, Cerakinkong product (a commercial inoculum, Central Glass Co. Ltd, Tokyo) with soil around Yamazakura roots at transplanting in order to promote early rooting and initial growth of planted vegetation. The material contains at least spores of *Gigaspora margarita* Becker and Hall and *Glomus sp*. (ca. 2000 spores 100 g^{1}).

Soil and plant sampling

Soil was sampled along 8 plots of the No 10 berm, each with 5 cores (5 x 5 x 10 cm, each about 250 g in fresh weight) and composite giving pooled top soil samples per plot. The soil samples were passed through a 2 mm sieve and then mixed cautiously before sub-sampling ca.500 g of soil for physical, chemical and biological analyses.

Additionally, randomly selected plants (containing rhizosphere and root zone soil) were gathered from 3 cores of each plot (3 plants/ plot) in order to estimate AM association and detect survival of AM inoculated to host plants (with 3 replications). Three host plants were chosen for sampling: Japanese hill cherry (*P. jamasakura* Sieb.ex Koidz, Yamazakura-inoculated plant), Eulalia (*M. sinensis* Ander, Susuki) and Mugwort (*A. princes* Pampan, Yomogi). The hair roots were taken for Yamazakura. After getting the root zone soil, the roots were washed gently under the tap water to remove the adhering rhizosphere soil. To confirm whether *G. margarita* CK in the experimental field was affected from native vegetation by flow runoff or other, the AM association in natural forest was investigated. Five (5) plant species were sampled from natural forest in the upper of experimental field at Nukui with rhizosphere and root zone soil (3 replications) as above.

Quantification of the spore and AM colonization

The whole amount of rhizosphere and root zone soil from 3 plants of each species was processed to extract the resting spores using the modified method of wet-sieving and decanting suggested by Gerdeman and Nicolson (1963).

Spores were counted under a dissecting microscope and collected individually using a pipette with a finely extruded tip and fine forceps and separated into different groups based on their morphology, color, and type of hyphal attachment. After collecting, spores were sterilized by 2% Chloramin T with 1200 mg/l streptomycin and stored at 4°C for further study.

The roots were cleared and stained by the modified method of Kormanik and McGraw (1982). The stained roots were estimated by a magnifel-intersect method (McGonigle *et al*., 1990), where roots are observed at 200 x magnification. Each arbuscular, vesicular or hyphae were quantified separately. For quantification and observation of AM structures inside the root by glass slide method, root samples were mounted in lacto-glycerol solution on microscope slides and then observed under a compound microscope at 100-400 x magnifications. The frequency on arbuscular, vesicular, spore, auxiliary cell, external and internal hyphae connected to arbuscular at each observed across section were estimated. The AM infection was calculated as below:

Percentage of AM colonization (%) = (AM root length / root length) x 100

AM structure (%) = (AM structure / AM root length) x 100

DNA extraction

After extracting from soil and sterilizing, spores were transferred into a micro-centrifuge tube containing 0.1% SDS and heated at 60°C for 10 min by followed sonication 2 min. Spores and roots were extracted DNA and performed PCR as described by Yokoyama *et al*. (2002) using HS *Tag* polymerase (Takara Bio, Kyoto). The roots DNA was extracted with the aid of plant DNA mini-kit (Quiagen K.K, Tokyo).

Detection of a DNA marker sequence

The DNA sequences of PCR primers and an oligonucleotide probe, PCR and hybridization condition were identical as those described by Yokoyama *et al*. (2002) with primers pair of ITS 1 and ITS 4 and primers set VANS1 and AM1-2. The preparations giving a 547-bp band were subjected to PCR with M13 mini-satellite and 639R primers. PCR products were electrophoresis with 1.5 % agarose gels. Gels were stained in ethidium bromide solution and photographed under UV illumination.

Southern hybridization was carried out as described by Yokoyama *et al.* (2002). The oligonucleotide was labeled by ECL 3'-oligo labeling kit (Amersham Pharmacia Biotech city). The DIG oligonucleotide tailing kit and DIG luminescent detection kit (Roche Diagnostics) were used in place of the probing and detecting kits in the experiments on DNA preparations, respectively.

Trap culture

Soils using as inoculum for trap culture were taken from root zone of Yamazakura and Susuki at each inoculated treatment. The Masa soil (Granite), a common soil type in Japan, was autoclaved and used as pot growing medium with Peter's No-Phosphorous fertilizers 15-0-15 (150 $g.kg^{-1}$ N, none P_2O_5, 150 $g.kg^{-1}$ K_2O). A hundred gram of sieved soil was layered at the 5 cm depth in disinfected substrate (1.8 kg Masa soil) in a plastic pot (2 l volume) with 7.83 g of fertilizer. The fertilizer was added because the available nitrogen in the potting medium was too low to support initial growth of the host plants. The seed of Alfalfa (*Medicago sativa* L.) and Bahia grass (*Paspalum notatum* Fluegge) were sowed and pots were placed in a growth chamber at 27°C (15 hrs light and 9 hrs dark time). After one month, the pots were placed in a green house until harvest. Sampling and gene analysis were estimated after 3 months.

Soil analysis

Soil chemical, physical and microbiological properties were determined according to general methods (Method of soil analysis).

Statistical analyses

Results were tested with STATISTICA (1998) by the analysis of variance (Microsoft office 97). Means and standard deviations (StDev) were produced in Microsoft Excel (Windows 97). Correlation within and between the soil parameters and the AM fungi data were initially determined by performing linear regressions in Microsoft Excel (Windows 97). A single ANOVA analysis was simultaneously carried out to prove the validity of the regression (Zar, 1984). In one indicated case, the least significant difference (LSD) test was applied to reveal tendencies.

RESULTS AND DISCUSSIONS

Soil properties and Microflora

The soil properties of each plot at sampling were according to inoculation and non-inoculation treatment. pH_{H2O}, MWHC and bulk density were similar for both inoculated and non-inoculated treatments. However, there were 2 plots giving the EC value strongly higher than other as 10-3 and 10-7 plots (Table 1). Those plots had same soil components (data not shown). The total carbon and nitrogen content and exchange cations in inoculated plots were slightly higher than those in non-inoculated plots, but the differences were statistically insignificant.

Table 1. The physical, chemical properties and microflora of soil at Nukui dam (sampled in November, 2002)

Parameter	pH		EC	MWHC	Bulk density		C
	H_2O	KCl	(µS/cm)	(g/100g)	(g/100ml)		(%)
Treatment					Crude	Fine	
Non-inoculation	5.58±0.43	4.34±0.27	109.63±52.37	32.81±4.08	101.83±2.86	160.37±3.181	1.01±0.35
Inoculation	5.99±0.65	4.62±0.15	70.19±6.68	32.78±1.06	96.29±13.32	151.64±12.7	1.15±0.32

Parameter	N	Available P	Ca	K	Mg	Na	Fungi ($x10^4$)	Bacteria ($x10^6$)
Treatment	(%)	(mg/100g)					CFU/g dry soil	
Non-inoculation	0.05±0.035	0.33±0.08	4.31±0.41	0.69±0.22	0.88±1.05	0.72±0.77	4.29±2.79	5.58±5.38
Inoculation	0.06±0.02	0.33±0.11	4.45±1.28	0.83±0.20	1.44±1.73	0.56±0.42	3.86±2.94	5.20±5.13

There was a little higher of fungal and bacteria number in inoculated than in non-inoculated treatments (Table.1) but did not significantly differ among the experimental treatments (StDev, ANOVA). The fungi quantity was ranged from 2.2 to 6.8 $x10^4$ CFU/g dry soil. The highest bacteria number was obtained at 10-1 plot-inoculated treatment and the lowest was in non-inoculated treatment.

The fact that the total nitrogen was highest up now compared to formerly data and that the phosphate and exchangeable cations improved with time confirmed the raising tendency of nutrients content after carrying out revegetation at Nukui. In both undisturbed and cultivable systems, potential productivity is directly related to soil organic matter concentration and turn over (Reeves and Redente, 1991).

Spore number in rhizosphere soil of plant roots

Spores picked up from the Yamazakura and Susuki rhizosphere soil in the inoculated plots were mainly got from the sieved size range of 106-210 µm or 210-500 µm. There was significant increasing

number of spore in inoculated treatments at 5% level (ANOVA, $p<0.05$) in Susuki and Yamazakura (Fig.1a).

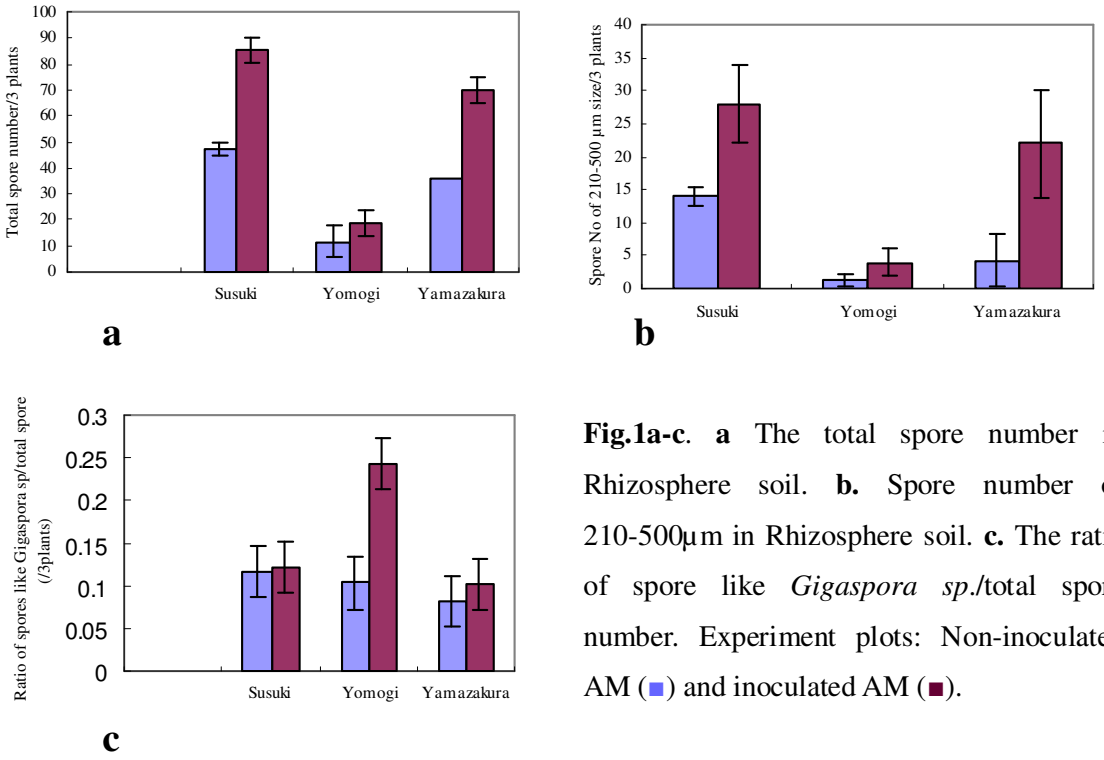

Fig.1a-c. **a** The total spore number in Rhizosphere soil. **b.** Spore number of 210-500µm in Rhizosphere soil. **c.** The ratio of spore like *Gigaspora sp.*/total spore number. Experiment plots: Non-inoculated AM (■) and inoculated AM (■).

The difference between inoculated and non-inoculated treatments of spore number of big size (> 210µm) in Yamazakura was 5.2 times (Fig.1b). Clearly, plant hosts vary markedly in their ability to affect spore production by *G. margarita.* By observation of morphology and size of spores, the spores looked like *Gigaspora.sp* in inoculated plots were more than in non-inoculated ones in all of investigated plants (Fig.1c).

The AM colonization in plant roots

In Yamazakura, high potential plots showing a very high percentage of root infection were inoculated treatments with range of infection was 72.16 – 84.0%. The minimum infection was found in non-inoculated treatment as 38.33%. Moreover, the coiling hyphae of infection roots was noticeable increasing in inoculated treatments, showed twice times higher comparing to in non-inoculated ones. At the same time, there was significant increasing of arbuscular as well as vesicular and auxiliary cell in inoculated plots (Fig. 2a-e).

The similar results were obtained from Susuki roots on AM colonization. The AM colonization was high in both of inoculated and non-inoculated treatments, ranged from 47.06 to 88.18 %. In inoculated plots, coil hyphae formed the main components of the AM colonization. In non-inoculated treatments, a small proportion of infected root length (< 10%) contained arbuscular, auxiliary cell and vesicular. The AM colonization include infected root rate and AM structures in inoculated treatments given at higher than in non-inoculated ones (Figure 2a-e).

A considerable proportion of root length (28.25 – 61.62%) was arbuscular mycorrhizae in infection rates of Yomogi roots with AM fungi. Colonization by AM fungi was highest in 10-6 plot, inoculated treatment. The level of colonization in non-inoculated treatments was significantly lower than that of inoculated ones (ANOVA, $p<0.05$). There was a significant increase in typical AM structures in the inoculated treatments (Figure 2a-e). On the other hand, arbuscular, auxiliary cell and vesicular were absent in some non-inoculated plots and very low in other ones.

The density of AM structures was increasing not only in arbuscular, coil hyphae, auxiliary cell but also in vesicles, while Gigasporaceae not form vesicles on its life. This due to inoculum product, Cerakinkong includes both of *Gigaspora margarita* and Glomus *sp*. Those AM strains might have an effect

on plant root together.

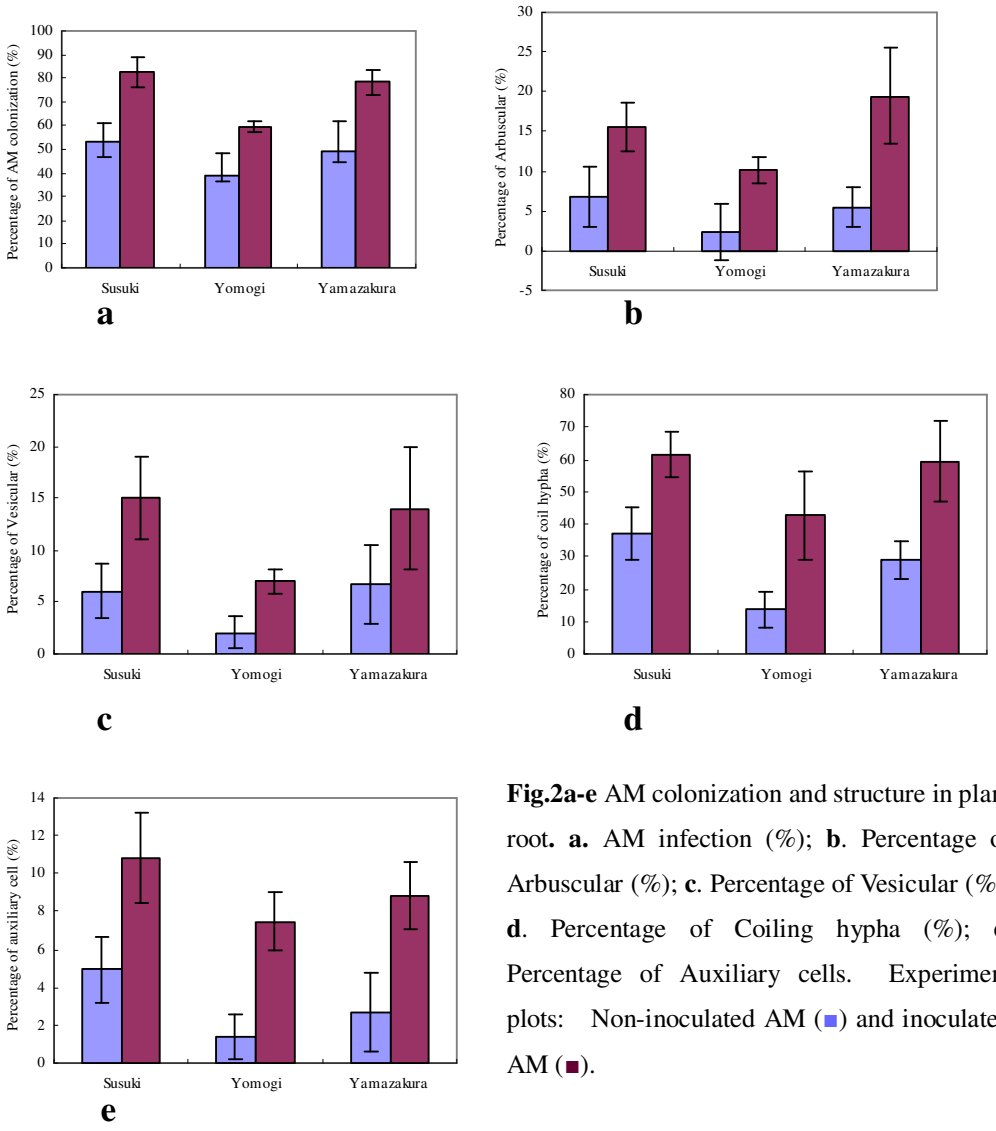

Fig.2a-e AM colonization and structure in plant root**. a.** AM infection (%); **b**. Percentage of Arbuscular (%); **c**. Percentage of Vesicular (%); **d**. Percentage of Coiling hypha (%); **e**. Percentage of Auxiliary cells. Experiment plots: Non-inoculated AM (■) and inoculated AM (■).

Inoculation of AM fungi promoted occurrence of spore and colonization to plant roots. The obtained results showed that the AM colonization rate in inoculated plots was higher than in non-inoculated plots. Moreover, the density of typical structures (arbuscular, vesicular, coil hyphae, auxiliary cell) of AM was also much more in inoculated treatments compare with non-inoculated treatments. This is a well know response which has been widely reported in the literature (Draft and Niscolson, 1969).

Through observation of AM structure under compound microscope, it was found that density of typical AM structures in inoculated treatments was higher than in non-inoculated ones. There was much more arbuscules and auxiliary cell (Fig 2b,e), which are typical structure of Gigasporaceae family in roots of Yamazakura and Susuki plants in inoculated plots than in non-inoculated plots. Meanwhile, arbuscules and auxiliary cell were very low in inoculated plots and absent in non-inoculated plots in Yomogi roots. These suggest that there have been different kind of host-AM fungi interaction and that Yamazakura-*G.margarita* containing material could be a better combination for the start of vegetation.

There was a linear correlation between spore numbers in rhizosphere soil and AM formation for each inoculated and non-inoculated plots (Fig.3). The efficiency of AM fungi propagules to form symbiosis seemed to be higher in inoculated and non-inoculated plots. This, in turn, suggests the vegetation developed in inoculated plots depend on AM more strongly than those in non-inoculated plots.

In investigated plants, Yamazakura would receive the strongest benefits for their initial growth just after transplanting among three species. The primary effect of AM inoculation might hold AM-propagule

level at higher by comparing to non-inoculated plots (Fig.3). This might help the establishment of invading weeds such as Susuki and Yomogi rather than Konara and Arakashi seedlings. The higher propagule level might support AM formation with Susuki (Fig.1a) and Susuki could make abundant AM propagule in the next generation. This could result AM active in inoculated plots for long time. The above advantages, however, do not show statistical differences among plant flora and biomass because the effect of soil amendment remains active.

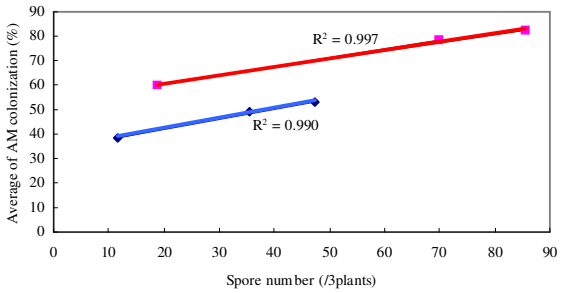

Fig.3. The correlation between spore number and AM colonization.
(--■--) Non-inoculated AM;
(--▲--) Inoculated AM.

Although Yamazakura was inoculated plant but the AM colonization was highest in Susuki. The reason was might be *Gigaspora margarita* gives more effect to Susuki than other host plants, confirming the results of other workers in our laboratory (Takahiro Tateishi, unpublished data). As far as tested, *G. margarita* CK seemed to be not effective for Yomogi. In turn, Susuki has been an alternative good host for *G. margarita* CK rather than Yamazakura, the later should be the first host for *G. margarita* CK at the beginning of the experiment. Susuki may support the survival of *G. margarita* CK in addition to Yamazakura.

A part from AM efficiency on vegetation development, the rate of inoculated AM fungi at start should be discussed. There were some spores looked like *Gigaspora sp.* Much more spores looked like *Gigaspora sp.* occurred in rhizophere soil of Yamazakura and Susuki plant in inoculated plots than in non-inculated plots (2.33 and 3.73 times, respectively). Further study is necessary to detect the inoculated strain, *Gigaspora margarita* after revegetation process.

The spores looked like *Gigaspora sp.* in rhizosphere soil and arbuscular and auxiliary cell in root formed much more in inoculated plots than in non-inoculated plots at Nukui dam site. This indicated the possibility of survival and propagation of inoculated AM fungi, *Gigaspora margarita*. Regarding why AM inoculation enhance AM establishment even after several years, a hypothesis suggested that there was interaction and competition among host plants. When pairs of plant species are growth together with or without the addition of AM inoculums, the outcome of the interaction between them is known to be very different (Francis and Read, 1994). Clearly, AM inoculum lead to improved the symbiosis between AM associations and plant communities. It seems to be a key factor for revegetation success.

Analysis of sporal DNA from rhizosphere soils

In evaluation of all those circumstances, DNA analysis is very important for isolated AM species of *G. margarita*. The method of specific molecular sequence marker was strongly recommended for the detection of AM species, such as *G. margarita* CK and MAFF 520054.

For Yamazakura, the positive reaction of DNA templates was 8, higher in inoculated treatments than in non- inoculated ones. All of inoculated treatment also gave 235 bp band at some templates (Fig.4). There were 3 DNA preparations from plot No 1; 2 from plot No 2 and No 6 and ones only from plot No 5 given band at 235 bp. On the other hand, no band appeared in DNA preparations from non-inoculated treatments (data not shown).

In sporal templates from Susuki rhizosphere soil, the positive band was highest in inoculated treatments. In inoculated treatments, 6 of DNA preparations in plot No 1 and 2 appeared band at 235 bp. There were 2 of 5 DNA preparations from plot No 6 gave 235 bp band and 2 templates from plot No 5 has 235 bp band in 6 DNA preparations. Those results demonstrated that some AM spores were isolated as *G. margarita* CK. However, there were 3 templates in non-inoculated treatments (2 from plot No 7 and other from plot No 8) also appeared the band at 235 bp. This maybe due to *G. margarita* CK came from upper

site of No 10 berm, where all of slopes were inoculated Cerakinkong during revegetation process.

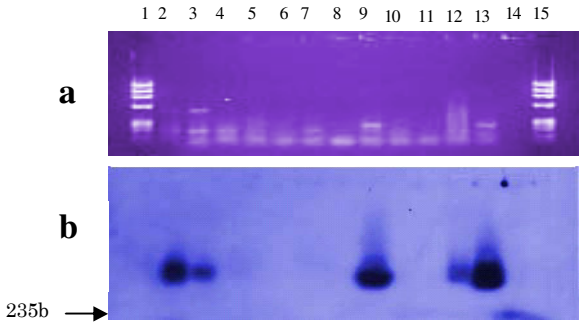

Fig.4. The isolated-specific *G. margarita* (in 5 and 6 plot of Yamazakura). **a**: PCR with M13 mini-satellite and 639R; **b**: Southern hybridiration with 230PC
Lanes 1, 15: Ø X 174 *Hae* III fragments.
Lane 2: *G.. margarita* MAFF520054
Lanes 3-9: sporal DNA preparations at plot 5.
Lanes 10-14: sporal DNA at plot 6

There was no 235 bp band in all of DNA preparations from both of inoculated and non-inoculated treatments in Yomogi rhizosphere soil. The results showed that Yomogi might be unsuitable host for *G. margarita* CK symbiosis.

Table 2. Detection of spore of *Gigaspora margarita* CK inoculated at Nukui dam site

Plot No	No marker positive / No spore tested		
	Susuki	Yomogi	Yamazakura
1*	3/6	0/6	3/6
2*	3/6	-	2/6
3	-	-	0/3
4	-	0/1	0/1
5*	2/6	0/2	1/6
6*	2/5	0/5	2/5
7	2/6	0/2	0/5
8	1/5	0/2	0/3

Spores appearing as *G .margarita* sp. were subjected
(*): Inoculated treatment; (-): no spore appeared as *G .margarita sp.*

In general, 10 spores from Susuki and 8 spores from Yamazakura rhizosphere soil in inoculated plots were identified as *Gigaspora margarita* CK. At the same time, 3 spores from Susuki rhizosphere soil in non-inoculated plots were also identified as *G. margarita* CK (Table 2). Those results confirmed that *G. margarita* CK still survived after inoculation for long time (4 years) in the field condition at Nukui dam.

Revegetation with inoculation of AM promoted the increase in AM populations and diversity of spore in soil. There were AM spore similar in size with *G. margarita* CK from rhizosphere soil of Yamazakura, Susuki and Yomogi in every plot. Regarding to detect inoculated AM, *G. margarita* CK, the marker sequence was detected for these spores collected from rhizosphere of Susuki and Yamazakura in inoculated plots. It was the fact that the positive reaction of DNA templates was much more in inoculated treatments than in non- inoculated ones. All of inoculated treatment also gave 235 bp bands at some templates from DNA preparations of Susuki and Yamazakura spores. The DNA preparations gave highest positive reaction in Susuki. However, there was contamination of *G. margarita* CK in non-inoculated plot. This maybe due to *G. margarita* came from upper site of No 10 berm, where all of slopes were inoculated with Cerakinkong during revegetation process. Although there were some *G. margarita*- like spores existed in non-inoculate treatments, it was evident that inoculated AM, *G. margarita* CK still survived in the field condition after revegetation process at Nukui dam even 4 years later.

The sporulation and detection of root DNA from native plants

The spore number (> 210µm size) from rhizosphere and root zone soil of native plants was investigated. The number of spore was different among plant species. In general, the spore density was low, highest of spore number got from rhizosphere of Yomogi (Table 3). The density of spores in soil and their morphology diversity were very variable, i.e. white to dark color, spherical to ellipse. It was low comparing to samples from No 10 berm, where the spore number of big size (> 210µm) ranged from 2 to 35 in inoculated plots and from 1 to 15 in non-inoculated plots. On the other hand, the AM infection rate, in correlative, was also low less than 45% in all of investigated plants (Table 3). In some habitats, spore was not found in all seasons but much higher numbers have sometimes been found in samples from No 10 berm.

Table 3. AM infection in native plants

Species	Spore number (> 210µm size)	AM Colonization (%)
Yomogi (*Artemisia vulgaris* Pampan)	16	43.58
Chisimazasa (*Sasa kurilensis*)	0	7.02
Kumazasa (*Sasa veitchii*)	2	12.47
Suge (*Carex sp.*)	4	25.29
Susuki (*Miscanthus sinensis* Andrerss)	5	32.37

Chisimazasa, Kumazasa: Dwarf bamboo grass

Based on morphology, the results expressed more than 80% of the spore sampled belonged to only one or two species, in contrast with the experimental field where all species collected were evenly distributed. Almost spore colored in black or brown and only some ones had yellow color. By observation of morphology of those spores, at the same time reference from INVAM homepage, we realized that they did not belong to *G. margarita*. In order to check this result, the analysis of root DNA was carried out.

Although there was positive reaction with primer set VANS1/AM 1-2, no any specific band at 235 bp appeared in all of DNA templates of native roots with primer set M13/639R (data not shown). Those result proved that there was no AM fungi possessing the marker DNA sequence and *G. margarita* strains and that *G. margarita* existed in experimental field not be affected from natural vegetation.

Detection of spore from trap culture

One set of pot experiment was prepared according to soil trap culture method. In regard to elucidate the strong effects of an inoculation bioassay in plant root, at the same time confirm inoculated AM, alternative appropriate trap culture was done in the green house by pot experiment. Two plant species, Alfalfa (*M. sativa* L.) and Bahia grass (*P. notatum* Fluegge), were chosen because of well document on AM establishment (Hetrick and Bloom, 1986). The root zone soil from Yamazakura and Susuki root in inoculated plots was collected and used as a low AM inoculum potential for setting up the trap culture.

By observing morphology under dissecting microscope, spores were differs among host plant and soil inoculum (Table.4) and several spores gave image of *G. margarita*. Moreover, the density of AM structures colonized in host roots was high, especially auxiliary cell – a typical structure in Gigasporaceae was presented (Table. 4). In order to confirm this, further work of specific isolated of sporal DNA from trap culture was done by using a specific molecular sequence marker method as above.

There were 5 templates from sporal DNA preparations of Alfalfa (each one from Susuki and Yamazakura inoculum of plot 1, two from Susuki inoculum of plot 2 and other from Yamazakura inoculum of plot 5) and 3 of 14 DNA preparations of Bahia grass (each one from Susuki inoculum of plot 1 and 2

and other from Yamazakura inoculum of plot 5) appeared specific band at 235 bp (Table.4). Those results suggested that some AM spores from trap culture were also isolated as *G. margarita* CK.

Moreover, by trap culture, the result showed that the propagules of *G. margarita* CK in soil were active and infective. There were some sporal DNA preparations in both of Alfalfa and Bahia grass host gave the positive band at 235 bp. These suggested that *G. margarita* has positive effect on establishment symbiosis in host plant and could survive with time in field condition.

In conclusion, inoculated AM, *G. margarita* CK could survive long times in the field condition at Nukui dam suggested that AM fungi are likely to be important in successful revegetation by the interaction and co-development of introduced plants and inoculated AM, resulting in recovery of vegetation in devastated and disturbed areas that may contribute to land and environmental reclamation.

able 4. Detection of *G .margarita* CK by trap cultures

Table 4. Detection of *G .margarita* CK by trap cultures

Trapping host	Plot No							
	1		2		5		6	
	Susuki	Yamazakura	Susuki	Yamazakura	Susuki	Yamazakura	Susuki	Yamazakura
Alfalfa*1								
Infection rate*2	+++	++	+++	+++	++	+++	++	++
Auxiliary cell*3	+	±	++	+	±	++	+	±
Spore like *G. margarita*4	+	+	+++	+	+	++	+	+
Marker positive*5	3(1)	1(0)	7(2)	3(1)	1(0)	4(1)	2(0)	1(0)
Bahia grass*1								
Infection rate*2	++	++	+++	+	+	++	+	+
Auxiliary cell*3	±	±	+	± ±	±	±	±	
Spore like *G. .margarita*4	+	+	++	+	+	+	+	+
Marker positive*5	3(1)	2(0)	4(1)	1(0)	1(0)	2(1)	1(0)	0(0)

*1: Triplicate pots were prepared
*2: Intensity level are shown as: below 30% (±), 31-60% (+), 61-80% (++) and more than 80% (+++)
*3: Frequently of auxiliary cells ere ranked as rare (±), low (+), middle (++) and high (+++)
*4: The ratio of *G. margarita*-like spores number to total spore number of sum of 3 replications such as: 0 to 20% (+), 21 to 50 % (++) and more than 50% (+++).
*5: The number in parenthesis mean marker-positive to tested spore numbers.

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EFFECTS OF PULSED ELECTRIC TREATMENT ON CULTIVATION SYSTEM OF CATERPILLARFUNGUS, *CORDYCEPSSINENSIS* (BERK.)SACC., A MEANS OF PROMOTING MANNITOL PRODUCTION

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ABSTRACT

Effect of pulsed power was investigated on fruit body formation of caterpillar fungus,*Cordycepssinensis* (Berk.) Sacc.Pulsed power of 30-120 kV was directly charged to the substrate just before fructification. The effect of the pulsed power resulted to promote for *C. sinensis*fructification. The treatment especially stimulated the fructification on *C. sinensis*, and also promoted the production of mannitol.

INTRODUCTION

Cultivation of edible mushrooms on sawdust-based substrate is steadily improving to effective methods. Especially, efficient techniques are useful for this cultivation method. Biological efficiency has been improved by optimizing various factors, such as substrate formula, strain type, culture maturity, water condition and other environmental conditions of the cultivation room.

Electric pulsed power utility is now applied to various agricultural crops, especially in horticultural fields. Cultivation system has been improved with electricity utilization in the field of systematic house crops such as tomato, lettuce, strawberry and various kinds of flower. Effect of air ions on plants have experimented. Fruit body production was promoted by the electric pulsed power treatment on the logwoodor sawdust substrate of *L. edodes*. We also investigated the effect of the electric pulsed power on the fruit body formation in the sawdust-based substrate of 10 edible mushrooms.

Medicinal mushroom is useful for human life, especially caterpillar fungi has been evaluated for most important folk medicine. We tested the electric pulsed power for the cultivation of *Cordycepssinensis* (Berk.) Sacc.

MATERIALS AND METHODS

Strain of *C. sinensis*was used stocked in the laboratory of Kyushu University. Strains were maintained on a potato dextrose agar medium (Difco) at 4°C.Fruit body production was tested on a plastic bag. The plastic bag contained 1.0 kg substrate. The medium was sterilized by autoclaving at 120°C for 30 min and then allowed to cool to room temperature. The liquid spawn was inoculated at the surface of the plastic bag substrates.

All cultures were cultured in the dark during the early phase of fungal growth until 14 days from inoculation, and then were exposed to 500 lux intensity of 12 hr intervals of cool-white fluorescent illumination. The plastic bag was continuing incubated longer to continue vegetative mycelial growth. Chilling treatment (shift down of temperature) was given to all species cultures after various incubation days. Flush occurred spontaneously, and all fruit bodies were weighed.

Pulsed power treatments were done just before the fruiting stages. Pulsed power of 30-120 kV was directly shot from the instrument to the mature sawdust-based substrates.

Two hundred mg of lyophilized fruit body (grounded powder) was mixed with 25 ml distilled water and boiled at 80°C for 3-4 hr at 100 rpm in a rotary shaker (multiple extractions). The slurry was filtered with a 0.45 µm membrane filter (Millipore, Ireland) and concentrated in a vacuum and cooled, while four volumes of 95% ethanol were added, than allow to precipitated overnight at 4°C. The precipitated crude inner polysaccharide (IPS) was collected by centrifuging at 10,000 *g* for 20 min, re-washed with ethanol and then lyophilized then the weight of crude IPS was determined.

Two g of lyophilized fruit body (grounded powder) was homogenized with a glass homogenizer at 1500 rpm with 10 ml of 80% ethanol for 3 min. The resulting suspension was heated in a hot-water bath

for 30 min, and ethanol was removed in an evaporator. The residue was treated with ion-exchange resin (Amberlite IR-120 and IR-45), then condensed, freeze-dried, and treated with trimethylsilyl (TMS). The samples were analyzed with a high pressure liquid chromatography (HPLC).

RESULTS AND DISCUSSION

Treatment of pulsed power promoted the fruit body formation of *C. sinensis*. Electric pulsed power resulted to more production of fruit-body in all tested voltage. Difference between the electric pulsed power and control was already recognized in the primodia formation stage. Number of primodia on the treated substrate was more than that of control. Shapes of the fruit body were normal in the treated substrates. The fruiting capacity was actually promoted by the pulsed power.

Caterpillar fungus, *C. sinensis* was stimulated by the pulsed power treatment. The applied voltage was tested 30-120 kV for fruit body formation. The electrical stimulation was effective for fruiting about 2 times increase. The results indicate that more radical stimulation is not so effective for fruiting.Polysaccaride (IPS) and mannitol content also clearly increase with the electric pulsed treatment.

OVERVIEW OF GUNUNG MERAPI NATIONAL PARK IN THE POST ERUPTION 2010

DhaniSuryawan
GunungMerapi National Park

INTRODUCTION

GunungMerapi National Park (TNGM)is an area of nature that has a genuine ecosystem of tropical high terrain rain forest which surrounds the Volcano of Merapi. Merapi itself is an active volcano that erupts in a quite uncertain pattern of time and scale. Geologically, Merapi is predicted approximately 10.000 years old with a long track record of several tremendouse volcanic erruptions that ever occured in history, and the 2010 eruption was the latest until today. Due to this natural phenomenon, it is obvious that managing the Gunung Merapi National Park (TNGM) has to be based on this aspect among the others.

The Gunung Merapi National Park (TNGM) were formed pursuant to the Forestry Minister Regulations No.: 134/Menhut-II/2004 dated on 4th May 2004 concerning the shifting functions of State Forest Area in Daerah Istimewa Yogyakarta Province, which consists the Nature Sanctuaries of*Plawangan Turgo,* Natural Parks of *Plawangan Turgo*, Forest Protected Area in Forest Merapi Groups, and State Forest Area in Central Java Province which consists the Forest Protected Area in Forest Merapi Groups. The total area of TNGM region is 6.410 ha.

Administratively, GunungMerapi National Park (TNGM) region located in two provinces: the Daerah Istimewa of Yogyakarta (DIY) Province (consist 1.728 ha) and the Jawa Tengah Province (consist 4.682 ha). Part of the TNGM region located in Jawa Tengah Province consists of three districts: Klaten, Boyolali, and Magelang. While Sleman is the only district in DIY Province that part of it included in the TNGM region. Institutionaly, the TNGM region can be divided into two sections: Section-1 consists of Sleman and Magelang District while Section-2 consists of Klaten and Boyolali District. Each Section has one or several resorts. TNGM region has a total of seven Resorts.

In the post eruption 2010 the GunungMerapi National Park institution (henceforth abbreviated as TNGM) there are several enormous 'homeworks' to be done in order to restore the all biophysics conditions that has changedby the eruption 2010.

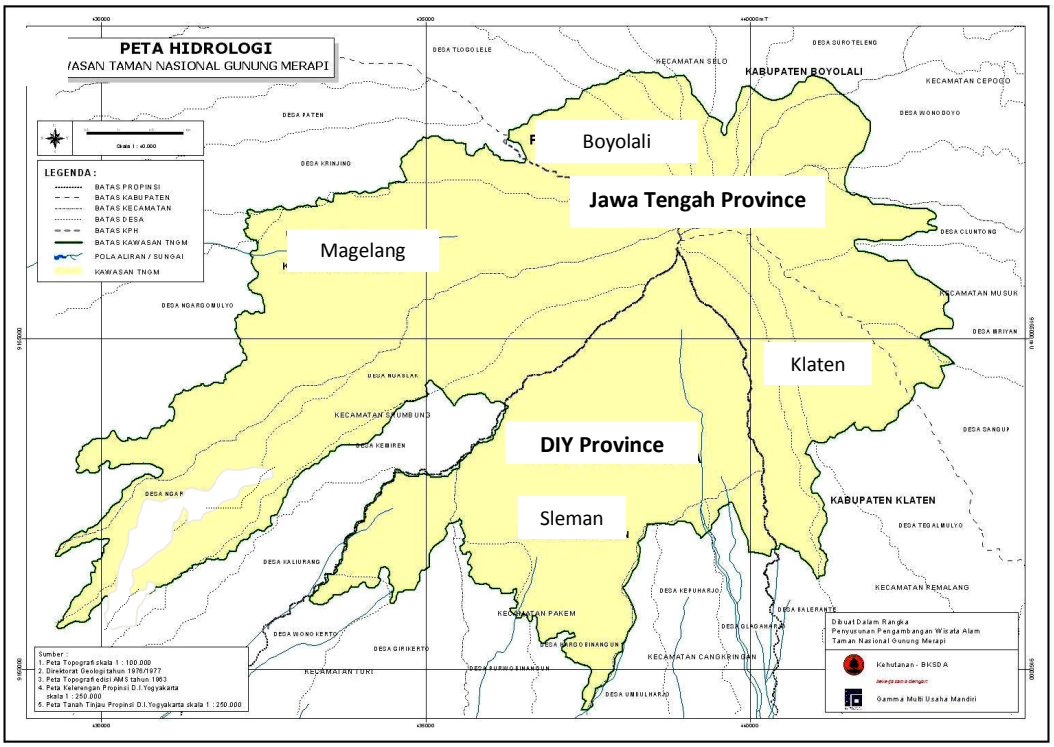


Figure 1. Administrative map of TNGM region

Biophysics Conditions After The 2010 Eruption

The eruption of Merapi on November 2010 has released approximately 150 million meter cubic of volcanic materials into the atmosphere and adjacent environment causing a large scale of changes to TNGM severalbiophysics' features such as vegetation, water resource, soil, wildlife animals, etc. But only few aspects will be discussed on this writing.

The latest recent plant and animal species identification (joint survey by TNGM and Forestry UGM, 2011) reported that in TNGM region as much as 108 plant species, 97 bird species, and 15 mammal species has beenidentified. All of the plant species can be grouped into 52 plant families, while the birds can be grouped into 32 families, and the mammals into 10 families.

Focusing on birds, before the eruption it was recorded as much as 147 bird species can be found in the region. After the eruption, only 94 bird species remain (temporary) as the other 53 bird species assumed hasn't return to the habitat (probably waiting until the biophysics conditions improved). This roughly suggests that the lost of significant forest area caused by the eruption has significant impact to the birds species diversity in the region.

Below is shown a list of 8 tree species found in TNGM-Sleman District and 10 most widespread species of birds and mammals in TNGM after the eruption.

Table 1.List of tree species found in TNGM-Sleman

No	Local Name	Latin Name
1	Rasamala	*Altingia excels*
2	Puspa	*Schimawallichii*
3	Kina	*Cinchona pubescens*
4	Bendo	*Artocarpus elastic*
5	Pasang	*Quercussundaica*
6	Jirek	*Symplocoscochinchinensis*
7	Dadap	*Erythrinalithosperma*
8	Kayumanis	*Cinnamomumburmannii*

Source :Susanto, JM 2011

The 10 most widespread species of birds and mammals in TNGM are shown below:

Table 2.List of the 10 most widespread species of birds and mammals in TNGM

No	Local name	Latin name
1	Waletlinci	*Collocalialinchi*
2	Cucakkutilang	*Pycnonotusaurigaster*
3	Cicakkorengjawa	*Megaluruspalustris*
4	Kacamatabiasa	*Zosteropspalpebrosus*
5	Wiwikuncuing	*Cacomantissepulcralis*
6	Tekukur	*Streptopeliachinensis*
7	Bentetkelabu	*Laniusschach*
8	Monyet/bedes/motak	*Macacafascicularis*
9	Kucinghutan	*Felisbengalensis*
10	Musang	*Paradoxussp*

Source : TNGM – Forestry UGM, 2011

Ecologically, forest and its soil in TNGM region has a vital role in keeping water balance for several rivers that originate from Merapi such as Gendol, Kali Kuning, Boyong, Krasak, Putih, Senowo, Apu River, etc. Based on the latest survey after the eruption, there are 18 water sources (natural spring water) identified.

Although the eruption has caused an increase thickness of soil layers in several parts of TNGM, and fertilized it, but it couldn't be judged that every part of TNGM region are favorable for planting or replanting. Only parts of Merapi which has rocky surface and an insignificant thickness of soil that couldn't be planted. The total are that couldn't be planted approximately 30-40% of the total of 6.410 ha of the TNGM region.

Based on the latest survey, damages to the TNGM region causd by the eruption can be classified

into 3 classes of damages : light damage covers up to 2.543,94 ha (41,40%), moderate damage 1.207,91 ha (19,66%) and heavily impacted/damaged 1.242,16 ha (20,21%). While the lava flow area covers 1.151,46 ha (18,74%).

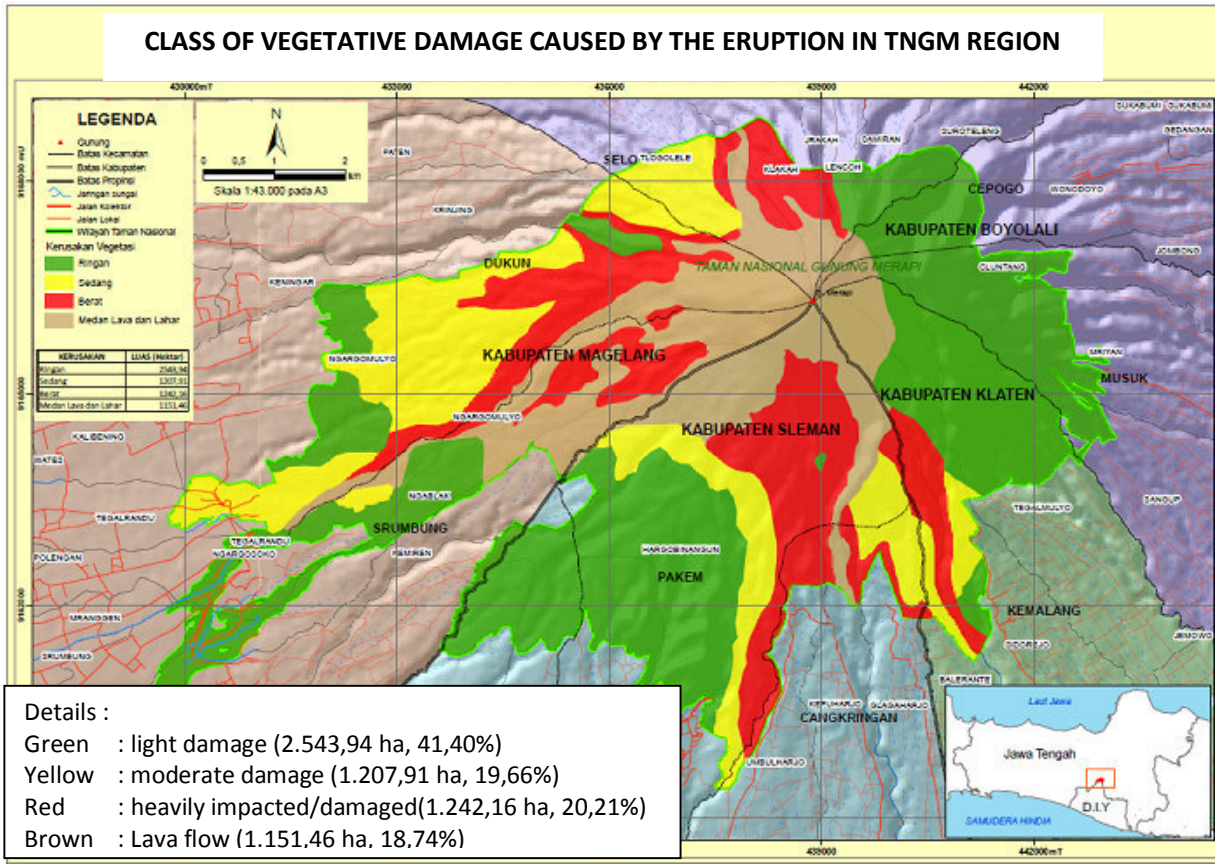


Figure 2. Class of vegetative damage caused by the eruption in TNGM region. Source : Satellite Interpretation (ASTER) and ground check (2011)

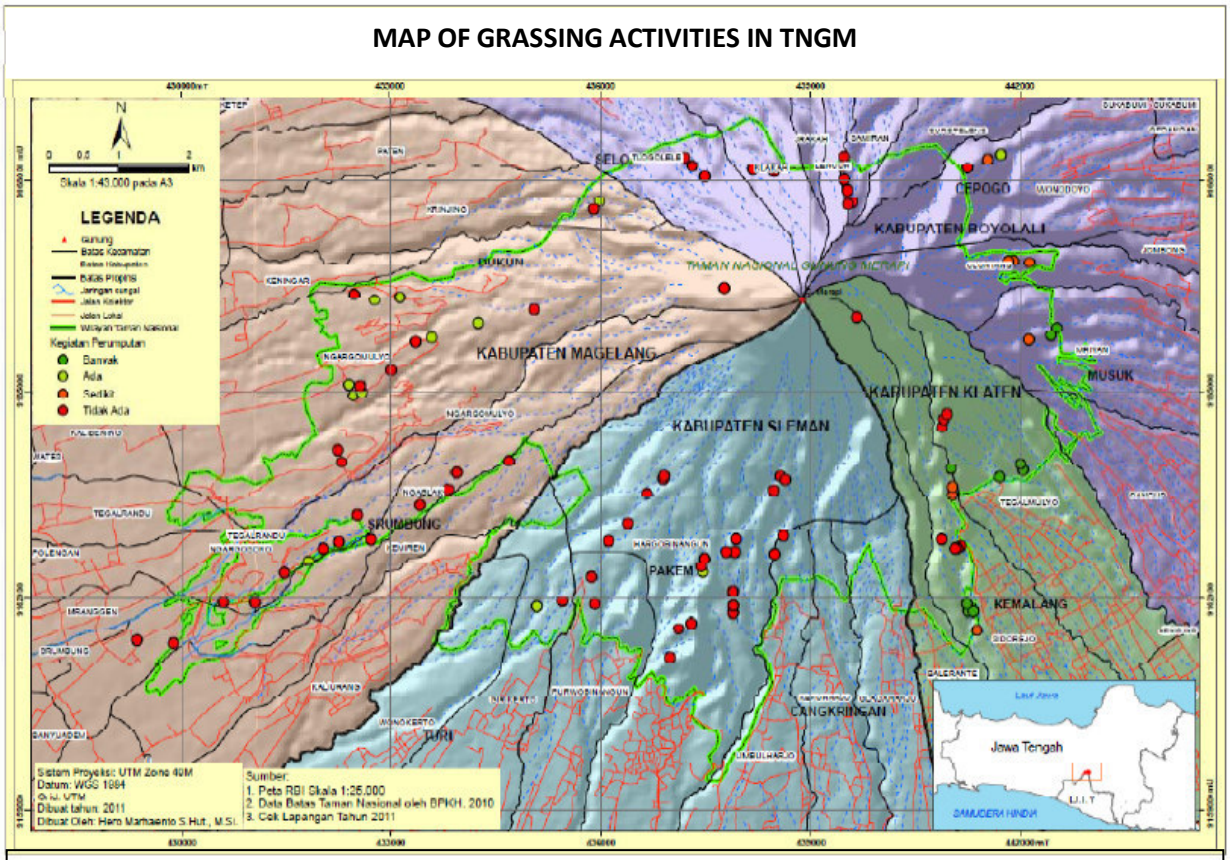
Volcanic eruption-materials which covered the TNGM region consist of sand and volcanic ashes eventually turned into layers which vary in thickness. According to the Yogyakarta Volcanic Technology Development and Research Center (BPPTK), a large amount of these materials still remain in upper slope of Merapi. BPPTK predicted it could require as much as 3 rainy seasons to uncover the whole materials from the slope. So, this implies that cold lava flood potentially may occur again.

Figure 3. As seen from this picture (left), it is obvious that some part of the Merapi are not favorable for planting.

Impacts to Social and Management Aspects

The eruption has burned and blown down large amount of forest, especially pine species. The wild animals have fled to outside the region. The eruption also caused a significant impacts to the economic and social life of the local community, one of the hit sector is farming. The deformation/changes of ecological features, landscapes, and other mattersmay imply to rezoning the TNGM region and to reformulate TNGM management based on the ground conditions of multi dimensional aspects.

Due to the farming impact, grassing activities by some people also affected. Below is shown a map of grassing activities in the TNGM region after the eruption.



Red dots	: no grassing recorded activities / identified
Orange ots	: few grassing recorded activities / identified
Yellow	: Moderate grassing activities recorded/ identified
Green	: Lots of grassing activities recorded/ identified

Figure 4. Map of grassing activities by some people

Rezoning the region maybe time consuming considering comprehensive aspects that may have to be accommodated in order to accomplish well-steady conservation. While reformulating the management depends on the accuracy and comprehensive data and information gathering.

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AGRICULTURE AND BIOTECHNOLOGY

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ABSTRACT

The invention of agriculture 10,000 years ago heralded the dawn of civilisation. Humans have progressed from hunters and gatherers to urban dwellers; more than 50% of today's 6.8 billion people live in cities! The "green revolution" of the 1960s and 1970s helped triple the world's food supply and, for a while, kept pace with the increasing world population. Yet, the distribution remained uneven and the problem of chronic under-nutrition for millions of people around the world remained. In the face of predicted increases in the world population to around 10 billion by 2050 and the challenges faced by agriculture as a result of climate change, providing adequate food and fibre for humanity is a pressing issuerequiring urgent attention. Since more and more of the arable land is used to house the growing world population, provide feed for stock to supply animal protein and to grow crops for bio-energy, how could agriculture keep pace and remain productive without further degradation of the soil or damage to the environment? Could biotechnology be the key to solvingworld hunger given the challenges of climate change and immense population growth? This is the focus of this presentation.

THE ROLE OFMYCORRHIZAL FUNGI ASSOCIATED WITH PLANT ROOT FOR THEIR GROWTH

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ABSTRACT

Mycorrhizal symbiosis is in the spotlight positioned as the key function of revegetaion in wasteland for zero emission challenge. Focusing on this, the role of mycorrhizal (chiefly, arbuscularmycorrhizal (AM) and ectomycorrhizal (ECM)) fungi has been discussed. Mycorrhiza is just the pivot of metabolism between plant and fungi as shown in the structure and mechanism. The enormous biomass of mycorrhizae and hyphae in soil is grounds that we have been paying attention to the ecological functions. Noteworthy mycorrhizal functions are as follows; early high growth of host plant, development into multipartite symbiosis, contribution to the soil conservation, a physical, chemical and biological defense, influence to vegetation succession, alleviation of chemical fertilizers, and response to host, edaphic, and ecological specificity.

BIOINDUCTION TECHNOLOGY INCREASED AGARWOOD (GAHARU) PRODUCTION OF *AQUILARIA* SPP. DAN *GYRINOPS* SPP.

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ABSTRACT

Agarwood signifies as one of the non timber forest products (NTFPs) commodities which affords high economy value in enhancing the state earnings. Agarwood presents a resin product that evolves fragrant smell occurring to the secondary metabolism reaction between the agarwood-yielding and particular fungi. In nature, the agarwood-yielding trees can be induced by fungi through the nature injury, but only with limited extent. Until this occasion, there are no fewer than 26 tree species that yield agarwood, two of which, namely *Aquilaria* spp. dan *Gyrinops* spp. still belong to the category regarded as scarce. The Forest Microbiology Laboratory, under the R&D Centre for Forest Conservation and Rehabilitation so far has collected particular fungi able to develop agarwood, called *Fusarium* spp. from Aceh until Papua, which in reached 54 fungi species. Meanwhile, 8 out of those 54 fungi species have been trial tested, comprising FORDA CC-00499 (from West Kalimantan), FORDA CC-00500 (Jambi), FORDA CC-00509 (FORDA CC-00509), FORDA CC-00501 (West Sumatera), FORDA CC-00512 (Papua), FORDA CC-00495 (South Kalimantan), FORDA CC-00497 (Central Kalimantan), and FORDA CC-00511. Further 3 out of those 8 species afforded their virulence consecutively FORDA CC-00509, Papua, and Kalimantan Tengah (as the highest virulence), followed in decreasing order by those from Jambi, West Nusa Tenggarta and Kalimantan Barat (as the medium virulence), and ultimately those from West Sumatera dan South Kalimantan Selatan (as the lowest). For the bio-inducement on agarwood development, it needs standardization and effectiveness toward such bio-inducement in order to develop agarwood with favorable qualities.

Keywords: Agarwood, *Fusarium* spp., bio-induction, standardization.

INTRODUCTION

The agarwood products originated from the nature, their sources tend to become alarmingly limited. Meanwhile, agarwood products in shape can form like cut sizes, chips, lumps, or flour. The commercial value of agarwood is determined by the fragrance smell, and wood aroma that evolves when being burnt. The community recognizes (identifies) the class and qualities of agarwood through the names of consecutively sapwood, kemedangan, and flour. Besides, in raw material (unprocessed) form as wood chips, at present through the distillation can be obtained agarwood-essential oil with high value. The uses of agarwood in Indonesia by the community particularly the inland Sumatera and Kalimantan has procceded for quitea long time. Traditionally, agarwood is used as among others incense for ritual and religious ceremony, body fragrance, room scent, cosmetics, and simple drugs. Recently, the market demand of agarwood by the Middle East and several European, American countries, and East Asian countries (Korea, Japan, and China) tends to increase, whereby such agarwood is used as rawm material for herbal drugs (Siran and Turjaman, 2010).

As of this occasion, the agarwood distributed in the market either domestic or abroad is still originated from mostly the nature with its varying qualities. The increase in agarwood traded since the last tree decades has brought about the scarcity in the production of agarwood sapwood from the nature. For these reasons, the particular species of agarwood-yielding trees, that comprise *Aquilaria* dan *Gyrinops* have been included in the Appendix II of the CITES, as the protected species. Besides being due to the high intensity of agarwood hunting, the decrease in agarwood production is also brought about by the declining supportive ability of natural production-forest that goes concomitantly with the uncontrolled illegal logging and converstion of forest area for other purposes (e.g. plantation establishment, community resettlement, etc).

In an attempt to accelerate agarwood production, the R&D Centre for Forest Conservation and Rehabilitation has invented the technology for agarwood inducement with the help of agarwood-developing fungi. Santoso *et al*. (2006) reported that results of purification on those agarwood-developing fungi were indicatively dominated by the particular fungi species of *Fusarium* spp . For this technology, the agarwood development as such afforded to reach 90-100%. The research on agarwood by the group of Forest Microbiology researchers started in 1984, who conducted the bio-inducement using soild isolates, whereby the agarwood-developing fungi was grown on wood sawdust, and then those isolates were inoculated into

the stem of agarwood-yielding trees. Prior to the inoculation, the boring (drilling) was performed on the stem surface, using the drill bit with 10-15 mm in diameter, while the direction of holes inclined at 45° angle to the stem surface. Afterwards, the solid isolates were induced into the stem through a pipe that was pushed inward using a wood stick, thereby causing such isolates entering the induction holes that were further closed with a paraffin. With such treatment, it turned out that the success of agarwood development reached 40-60%. Usually, the rotting (decay) occurred to the resulting agarwood, when the raindrops entered into the induction holes.

In 2000, the R&D Centre for Forest Conservation and Rehabilitation researchers improved the induction technology, and used the liquid-inoculant media. Meanwhile, the diameter of drill (boring) bit was reduced to 3 mm. The induction treatment in direction was almost perpendicular to the stem surface, and as such the resulting holes reached as a third (1/3) in depth inward as the steam diameter. The drilling (boring) work should be such that it avoid reaching or hitting physically the pith. The liquid inoculant as induced into the holes in amount reached about 1 cc, and afterwards the holes left unclosed (unplugged).

The group of Forest Microbiology researchers has collected 54 fungi isolates from Aceh until Papua. Out of those 54 fungi species, as many as 8 species was already induced, comprising FORDA CC-00499, FORDA CC-00500, FORDA CC-00509, FORDA CC-00501, FORDA CC-00512, FORDA CC-00495, FORDA CC-00497, and FORDA CC-00511. Further, from those 54 species of agarwood-developing fungi, only 36 fungi species was already identified (Sitepu *et al*., 2010).

Objective of these researchs were to acquire the data and information about the distance and amount of liquid inoculant as induced into the stem of the agarwood-yielding trees, therefore the reliable standard can be determined for the induction and regarding the appropriate amount of liquid inoculant as induced into the trees that develop agarwood, and to acquire the data/information about the direction and the depth of induction holes, which afterwards remain unclosed.

MATERIALS AND METHODS

Object Description

The process of inoculant preparation was done at the Forest-Microbiology, R&D Centre for Forest Conservation and Rehabilitation (Bogor) The location as selected for the inducement process took place at consecutively Sukabumi, Carita (Banten), Bodok (West Kalimantan), and West Nusa Tenggara, that each served as the demonstration plot. In this research, there was also a demplot situated at Sukabumi, where thein inducement was done on the agarwood-yielding trees, growing (plots).

Materials

The materials consisted of consecutively:

- Agarwood–yielding trees, comprising *Aquilaria malaccensis* (plot Sukabumi), *Aquilaria microcarpa* (West Kalimanta), *Gyrinops* sp. (West Nusa Tenggara).
- Fungi species, comprising (FORDA CC-00509/FORDA CC-00509), FORDA CC-00500/Jambi), (FORDA CC-00501/West Sumatera), (FORDA CC-00499/Wst Kalimantan), (FORDA CC-00497/ Central Kalimantan), (FORDA CC-00495/South Kalimantan), (FORDA CC-00511/West Nusa Tenggara), and FORDA CC-00512/Papua).
- Agarwood-yielding trees which were already induced, which in number reached 15 trees

  Meanwhile, the equipment as used consisted of portable electric generator set, electric drill (borer), drill bits with 3-mm diameter, inducement device, measuring (gauge) tool, writing sets, camera, and labels.

Methods

The method as implemented presented the induction using liquid-inoculant as done in the following:

1. The induction using four species of agarwood-developing fungi, comprising (FORDA CC-00509/ FORDA CC-00509), (FORDA CC-00500/Jambi), (FORDA CC-00511/West Nusa Tenggara), (FORDA CC-00501/West Sumatera), dan (FORDA CC-00499/West Kalimntan). Such induction

was done in Sukabumi, on *Aquilaria malaccensis* tree species.

2. Other induction using 4 species of agarwood-developing fungi, comprising (FORDA CC-00497/ Central Kalimantan), (FORDA CC-00495/South Kalimantan), (FORDA CC-00511/West Nusa Tenggara), and (FORDA CC-00512/Papua). Such induction took place was performed on two species of agarwood-yielding trees (*Aquilaria microcarpa* dan *Gyrinops* sp.) originated from West Kalimantan and West Nusa Tenggara.
3. For induction no. 1, the stem of on *Aquilaria malaccensis* tree was drilled (bored) using a drill bit with 3-mm diameter, through its surface, until reaching the depth inward one third (1/3) of the stem diameter. The distance between the resulting holes and the next holes was 10 cm. After drilling, into the holes was injected 2 cc of liquid inoculant. The drilling in direction was perpendicular in direction to stem surface.
4. For induction no. 1, the drilling manner was similar to no. 1, but distance between the inoculatio holes varied in 4 levels, namely 5 cm, 10 cm, 15 cm, and 25 cm. This was indended, because the varying distances would determine the induction standard regarded as effective in agarwood development.
5. All the resulting inoculation should not be closed (remain unplugged).
6. The agarwood-yielding trees as induced with four fungi species still not yet recognized (identified) regarding their role on those trees comprised the fungi from consecutively Papua, South Kalimantan, Central Kalimantan Tengah, and West Nusa Tenggara. Each of those 4 fungi specues was inoculated to *Aquilaria microcarpa* trees. For the fungi originated from West Nusa Tenggra location, the distances between injection/inoculationi holes varied at 5 cm, 10 cm, 15 cm, and 20 cm, each with 3 replications, or 4 *Fusarium* sp. fungi species x 4 injection treatements x 3 replicates; and therefore as many as 48 trees were needed. Likewise, for the fungi originated from West Kalimantan location, the distances between injection/inoculation holes varied at 10 cm, 15 cm, 20 cm, and 25 cm, each with 3 replications, or 4 *Fusarium* sp. fungi species x 4 injection treatements x 3 replicates; and therefore as many as 48 trees were needed as well.
7. For further induction test in West Kalimantan and West Nusa Tenggara, these activities revelaed a part of a series regarding the trial test on agarwood induction at various species of agarwood-yielding trees in various locations with their varying micro-climate conditions. The induction activities took place in locations of West Kalimantan and West Nusa Tenggara. The four fungi isolates which were already recognized/identified afforded their favorable qualities 3 years after their induction, comprising FORDA CC-00509, FORDA CC-00500, FORDA CC-00499, dan FORDA CC-00501. Such induction was done on each of their particular trees, in each of their locations, thereby reaching the total of 80 trees, with the details involving 4 isolates x 10 trees x 2 species of agarwood-yielding trees (i.e. *Aquilaria microcarpa* dan *Gyrinops* sp.) with the induction distance as far as 10 cm.

Parameters

The parameters as performed were as follows:

1. Observing the infection symptom, and effect of the agarwood-developing fungi
2. Measuring the induction symptom in length and in width, when the induction results reached 3-month age.
3. Observing the effect of environment which revealed the role in the infection caused (induced) by the agarwood-developing fungi.
4. For the trees which were induced by the fungi species of consecutively FORDA CC-00509/FORDA CC-00509, FORDA CC-00500/Jambi, FORDA CC-00505/West Sumatera, and FORDA CC-00499/ West Kalimantan, the sample-taking was done when the induction results reached the age of 1 year, 2 years, and 3 years, respectively.

RESULTS AND DISCUSSION

Results of induction using the particular fungi (i.e. FORDA CC-00509, FORDA CC-00500, FORDA CC-00501, dan FORDA CC-00499) on *Aquilaria malaccensis* trees, under the condition facing the infection

by those fungi, the trees would repond to defend and restore themselves. The tree resistance would determine who was the winner between the trees themselves and the disease caused (induced) by those microorganisms (fungi). In the agarwood development, certainly the disease was expected to win, thereby developing the agarwood as desired. The chemical compounds owned by in this regard the *Aquilaria malaccensis* trees signified as an attempt of tree resistance against the disease-inducing microorganisms (fungi). The agarwood itself was already identified as containing among others sesquiterpenoid, a defending compund of phytoalexin type. The vulnerability of trees in facing the fungi infection was related to development of agarwood, whereby the agarwood qualities either qualitatively or quantitatively could be each reflected by the extent of infection and the content of other compounds.

In Figure 1 can be seen that the length of infection that occurred to the stem of *Aquilaria malaccensis* trees, when the inoculation results reached 2-month and 6-month age. At two-month age, the isolat FORDA CC-00509 isolate exhibited the highest infection value (4.13 cm in length), followed in decreasing order by the mixed isolates, Padang, West Kalimantan, until the lowest as shown by the infection induced by the isolates from Jambi. From the analysis of variances, it revealed that the isolate origin significantly affected the infection length as occurred to *Aquilaria malaccensis* (Tabel 4). Further test using the Duncan's mulitiple range test convinced that 2 months after inoculation the FORDA CC-00509 isolates brought about the largest infection on the stem of this agarwood-yielding tree species, followed in decreasing order by the mixed isolates (Table 1).

Different from the condition at 2-month inoculation age, at 6-month inoculation age the fungi (isolates) exhibited their typical infection symptom. At this 6-month age, statistically the isolate did not inflict significant effect on the infection that occurred at *Aquilaria malaccensis* stem. This was shown by the analysis of variance (Table 2). Nevertheless, similar to the condition at 2-month inoculatiuon age, from Figure 1 could be seen that the highest infection was caused (induced) by the FORDA CC-00509 isolates and its mixture.

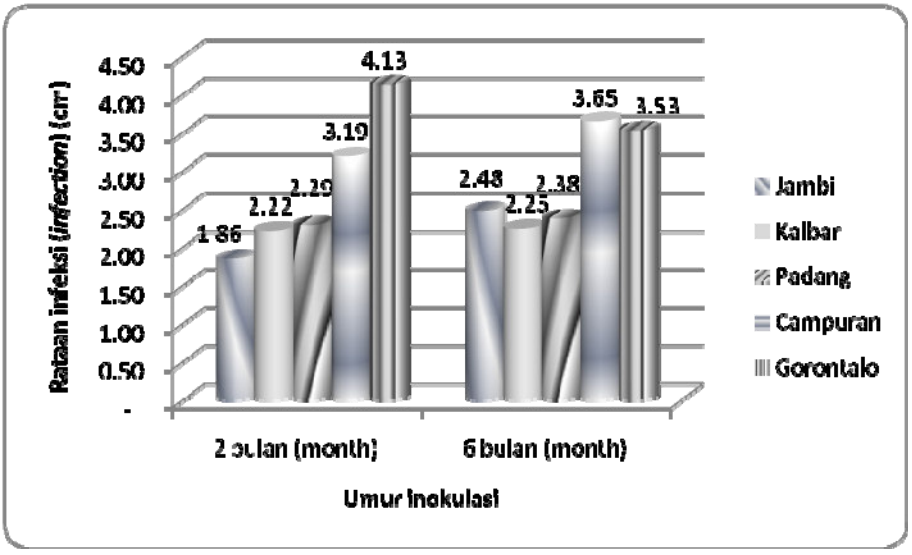

Figure 1. Length of infection that occurred to the stem of *Aquilaria microcarpa* trees
Remarks: Umur inokulasi = Inoculation age; Asal isolat = Isolate origin; panjang infeksi = infection rate; campuran = mixed isolates; Kalbar = West Kalimantan

Figure 2 shows that the changes in infection length that occurred beginning 2-month inoculation age until 6-month age. Although the FORDA CC-00509 isolates still inflicted the largest infection, the infection at 6-month inoculation age did not undergo significant changes. Meanwhile, the infection by another four isolates (with their different origins) revealed the varying increase. Nevertheles, statistically at 6-month inoculation age the different isolate origins did not bring about significant effect on the infection rate (the significant level reaching 0.186 at $\alpha = 5\%$)

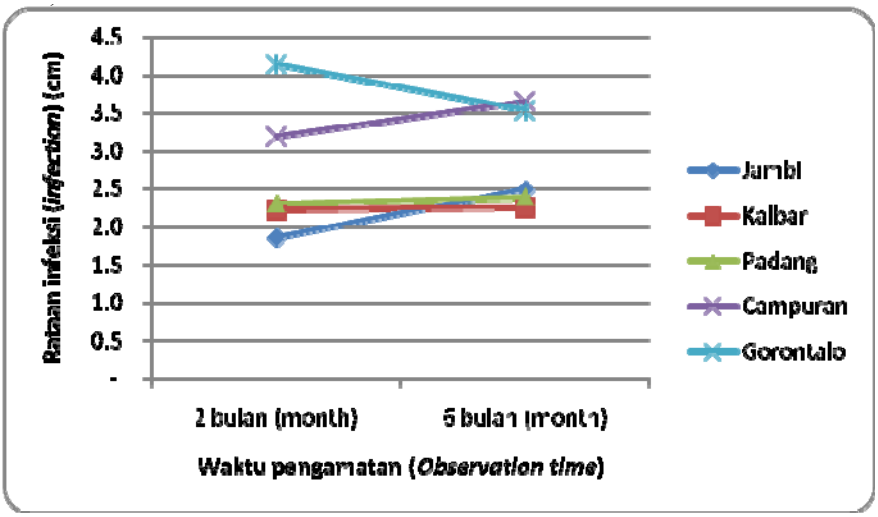

Figure 2. Infection rate at the stem of *Aquilaria mcrocarpa* trees
Remarks: Umur inokulasi = Inoculation age; Asal isolat = Isolate origin; panjang infeksi = infection rate; campuran = mixed isolates; Kalbar -= West Kalimantan

Tabel 1. Analysis of variance regarding the effect of treatment (region origin of the *Fusarium* sp. isolates) on the length of infection at the stem of *Aquilaria microcarpa* trees (α=0,05)

Pengamatan (*Observation time*)	Sumber keragaman (*Source*)	Db (df)	Jumlah kuadrat (*Sum of square*)	Kuadrat Tengah (*Mean of square*)	Fhit (*F-calc*)	Sig.
2 bulan (2 *months*)	Asal isolat (*Isolate origin*)	4	10,172	2,543	16,760	0,000 **
	Galat (*error*)	10	1,517	0,152		
	Total	14	11,689			
6 bulan (6 *months*)	Asal isolat (*Isolate origin*)	4	3,809	0,952	2,894	0,079 **
	Galat (*error*)	10	3,290	0,329		
	Total	14	7,099			
Laju infeksi (*Infection rate*)	Asal isolat (*Isolate origin*)	4	0,153	0,038	1,907	0,186 ns
	Galat (*error*)	10	0,201	0,020		
	Total	14	0,354			

Remarks: ** = significant at 1% level, ns = not significant

Table 2. Further test using the Duncan's multiple range tests on the infection length on the stem of *Aquilaria microcarpa* trees, at 2-month inoculation age

Asal isolat (Isolate origin)	Rataan (Mean value of the infection length)
Jambi	1,857a
Kalimantan	2,223a
Padang	2,297a
Campuran	3,193b
FORDA CC-00509	4,133c

Note: *values followed by the same letters are insignificantly different*); α=0,05

The development of infection as occurred 6 months after inoculation revealed that region origin did not bring about significant effect any longer on the infection length (Table 1), although the largest infection was still caused (induced) by the mixed isolates, and the FORDA CC-00509 isolate caused the largest infection, the consistency in the infection development still deserves firther research by viewing the development of infection rate by the isolat FORDA CC-00509 isolates until reaching the particular period.

Scrutinizing the infection development on the stem of *Aquilaria malaccensis* trees, it can be inferred that the FORDA CC-00509 isolates brought about the largest infection (in length), which implied that this isolate afforded the development of agarwood the most favorable qualities. Although the mixed isolates exhibited the longest infection length 6 months after inoculation, there was a possibility that this was merely caused (induced) by the FORDA CC-00509 isolates themselves. Meanwhile, the longer duration as allowed for those 4 species of agarwood-developing fungi as described as above, then the better the qualities of the resulting agarwood.

For the induction using other fungi species, that comprised FORDA CC-00497, FORDA CC-00495, FORDA CC-00511, and FORDA CC-00512, as induced on the stems of *Gyrinops* sp. that existed in West Nusa Tenggara, 3 months after inoculation could be presented in Table 3.

Tabel 3. Inoculation some isolates of *Fusarium* spp. to *Gyrinops* sp. after 3 months di West Nusa Tenggara.

No	Number of replication	Tree Code No.	Inoculant origin	Distance between injection holes (cm)	Infection development (in average), cm	
					Vertical direction	Horizontal direction
1	3	1	FORDA CC-00512	5	X	x
2	3	1	FORDA CC-00512	10	0,50	2,00
3	3	1	FORDA CC-00512	15	1,00	6,00
4	3	1	FORDA CC-00512	20	1,33	8,57
5	3	2	FORDA CC-00495	5	0,63	3,67
6	3	2	FORDA CC-00495	10	0,70	2,57
7	3	2	FORDA CC-00495	15	0,67	1,47
8	3	2	FORDA CC-00495	20	0,60	2,43
9	3	3	FORDA CC-00497	5	0,50	2,50
10	3	3	FORDA CC-00497	10	0,53	3,83
11	3	3	FORDA CC-00497	15	0,77	2,17
12	3	3	FORDA CC-00497	20	0,47	2,17
13	3	4	FORDA CC-00511	5	0,40	3,40
14	3	4	FORDA CC-00511	10	0,23	2,43
15	3	4	FORDA CC-00511	15	0,40	3,33
16	3	4	FORDA CC-00511	20	0,37	2,77

Remarks: distance (between injection holes) 5 cm, 10 cm, 15 cm, and 20 cm

From Table 3, it can be indicated that the injection using the FORDA CC-00512 isolates at 5 cm distance (between the injection holes), all the injected trees become dead, while at the distance of 10 cm and 15 the tree death portion reached 66.67%. Likewise, the tree induction using the FORDA CC-00511 isolates at 5 cm distance, the tree death portion reached 66.67 as well (Table 4), and this was brough by among others the distance effect, the ferocity (severity) of *Fusarium* fungi, and the resistance of the trees themselves.

Table 4. The portion (percentage) of tree death at 3-month age (duration) after inoculation by the fungi isolates.

Inoculant origin	Number of dead trees	Number of replications	Distance of injection	Portion the dead trees
FORDA CC-00512	3	3	5	100
FORDA CC-00512	2	3	10	66.67
FORDA CC-00512	2	3	15	66.67
FORDA CC-00512	0	3	20	0
FORDA CC-00495	0	3	5	0
FORDA CC-00495	0	3	10	0
FORDA CC-00495	0	3	15	0
FORDA CC-00495	0	3	20	0
FORDA CC-00497	0	3	5	0
FORDA CC-00497	0	3	10	0
FORDA CC-00497	0	3	15	0
FORDA CC-00497	0	3	20	0
FORDA CC-00511	2	3	5	66.67
FORDA CC-00511	0	3	10	0
FORDA CC-00511	0	3	15	0
FORDA CC-00511	0	3	20	0

Remarks: injection distance (between injection holes)

The induction using those fungi isolates, that comprised FORDA CC-00497, FORDA CC-00495, FORDA CC-00511, FORDA CC-00512, as done on the stem of pada *Aquilaria microcarpa* trees growing in Bodok (West Kalimantan), when the inoculation results reached 3-month age is presented in Table 5.

Table 5. Induction results using *Fusarium* spp. isolate conducted on the stem of *Aquilaria* spp. (in West Kalimantan) at 3-month inoculation age

No	Number of replication	Tree code No.	Inoculant origin	Injection distance	Infection development (in average), cm	
					Vertical direction	Horizontal direction
1	3	1	FORDA CC-00512	10	0.80	3.80
2	3	1	FORDA CC-00512	15	0.77	4.47
3	3	1	FORDA CC-00512	20	0.80	4.60
4	3	1	FORDA CC-00512	25	0.83	4.73
5	3	2	FORDA CC-00495	10	0.67	1.70
6	3	2	FORDA CC-00495	15	0.70	1.50
7	3	2	FORDA CC-00495	20	0.70	1.73
8	3	2	FORDA CC-00495	25	0.70	1.70
9	3	3	FORDA CC-00497	10	1.00	3.47
10	3	3	FORDA CC-00497	15	1.00	4.30
11	3	3	FORDA CC-00497	20	1.00	3.73
12	3	3	FORDA CC-00497	25	1.00	3.57
13	3	4	FORDA CC-00511	10	0.80	2.63
14	3	4	FORDA CC-00511	15	0.87	2.80
15	3	4	FORDA CC-00511	20	0.77	2.53
16	3	4	FORDA CC-00511	25	0.87	2.73

Remarks: injection distance (between injection holes)

Viewing Table 5, it turns out that with the injection using the isolates of consecutively FORDA CC-00512, FORDA CC-00495, FORDA CC-00497, and FORDA CC-00511 at 10-cm, 15-m, dan 25-m injection distance, all the injected trees survived, or no tree death occurred. This could be so, due to the suitability (compatibility) between the induction (injection) distance, the *Fusarium* isolates, and the resistance of the injected trees themselves. To examine the average reaction regarding the agarwood development in vertical and horizontal direction, it is presented in Figures 3, 4, 5, and 6.

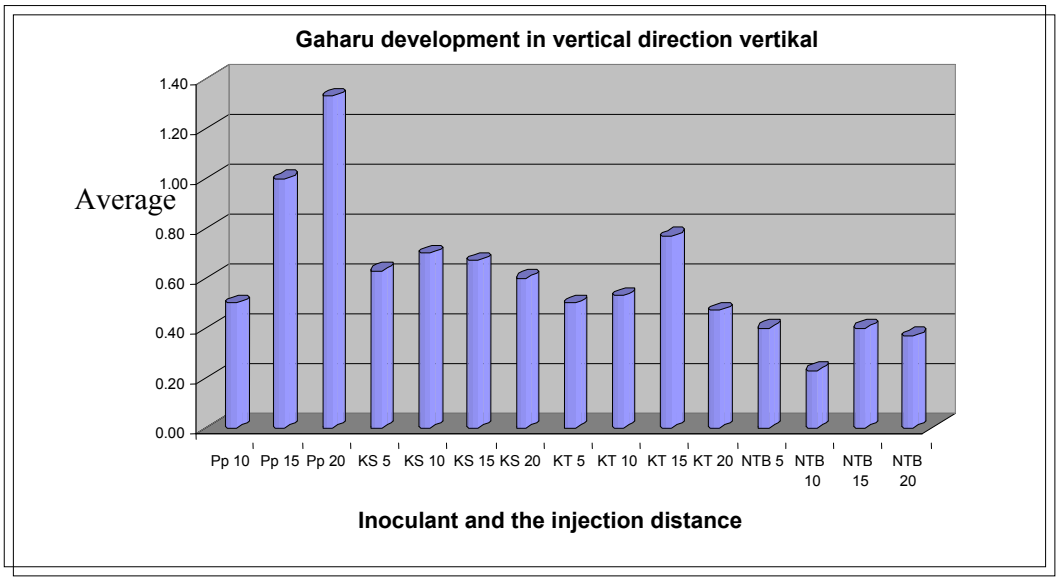

Figure 3. The reaction of agarwood development in vertical direction, as observed 3 months after the inoculation treatment (the experiment took place in West Nusa Tennggara

Remarks:
Pp : Isolates originated from Papua (FORDA CC-00512)
Ks : Isolates originated from South Kalimantan (FORDA CC-00495)
Kt : Isolates originated from Central Kalimantan (FORDA CC-00497)
NTB : Isolates originated from West Nusa Tenggara (FORDA CC-00511)

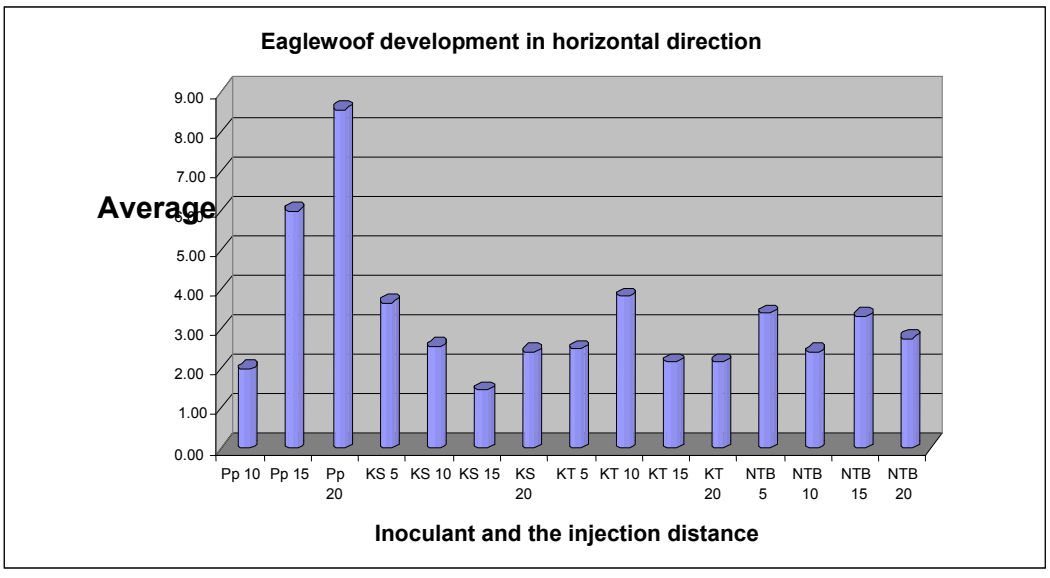

Figure 4. The reaction of agarwood development in horizontal direction, as observed 3 months after the inoculation treatment (the experiment took place in West Nusa Tennggara

Remarks:
Pp : Isolates originated from Papua (FORDA CC-00512)
Ks : Isolates originated from South Kalimantan (FORDA CC-00495)
Kt : Isolates originated from Central Kalimantan (FORDA CC-00497)
NTB : Isolates originated from West Nusa Tenggara (FORDA CC-00511)

Figures 3 and 4 reveal that all the isolates afforded significant effect/role on the agarwood development on the stem of *Aquilaria* spp. trees. Meanwhile, with 20-cm injection distance, the isolates originated from Papua inflicted the most favorable responses/role (average agarwood development in vertical and horizontal directions reaching consecutively 1.33 cm and 2.87 cm) compared to other isolates from South Kalimantan, Central Kalimantan, and West Nusa Tenggara.

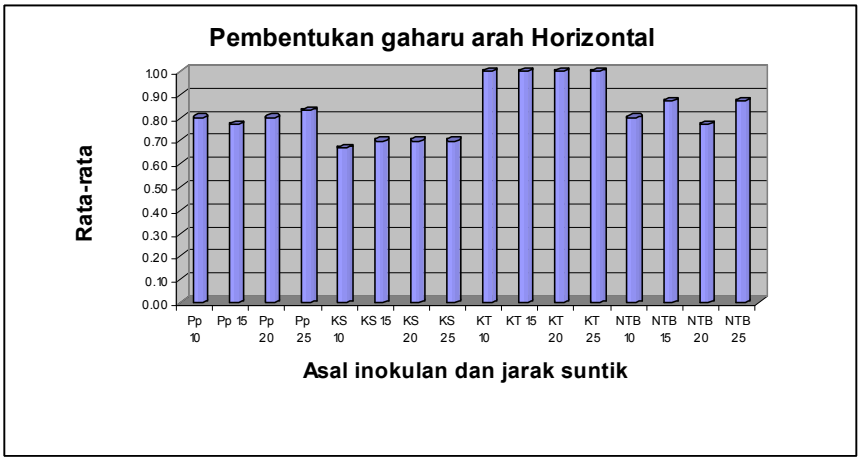

Figure 5. The reaction of agarwood development in horizontal direction, as observed 3 months after the inoculation treatment (the experiment took place in West Kalimantan)

Remarks:
Pp : Isolates originated from Papua (FORDA CC-00512)
Ks : Isolates originated from South Kalimantan (FORDA CC-00495)
Kt : Isolates originated from Central Kalimantan (FORDA CC-00497)
NTB : Isolates originated from West Nusa Tenggara (FORDA CC-00511)

Rata-rata = Average; Pembentukan agarwood arah vertikal = Eglewood development in vertical direction; asal inoculum dan jarak suntik = inoculant origin and the injection distance (between the inoculation holes)

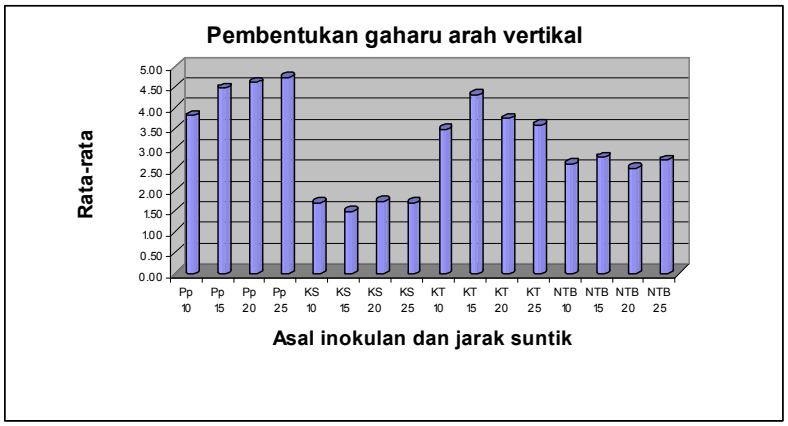

Figure 6. The reaction of agarwood development in vertical direction, as observed 3 months after the inoculation treatment (the experiment took place in West Kalimantan)

Remarks:
Pp : Isolates originated from Papua (FORDA CC-00512)
Ks : Isolates originated from South Kalimantan (FORDA CC-00495)
Kt : Isolates originated from Central Kalimantan (FORDA CC-00497)
NTB : Isolates originated from West Nusa Tenggara (FORDA CC-00511)

Rata-rata = Average; Pembentukan agarwood arah vertikal = Eglewood development in vertical direction; asal inoculum dan jarak suntik = inoculant origin and the injection distance (between the inoculation holes)

Examining Figures 5 and 6, it reveals that all the isolates as inoculated to the tree stem using the injection (inoculation) distance that rached consecutively 10 cm, 15 cm, 20 cm, and 25 cm inflicted the favorable responses on agarwood development. Meanwhile, the inoculation isolates originated from Papua inflicted the remarkable responses in that the agarwood development reached 4.73 cm (in vertical direction) and 0.83 cm (in horizontal direction).

In activities regarding the standardization and effectiveness of isolates which were already recognized such as FORDA CC-00509, FORDA CC-00500, FORDA CC-00501, and FORDA CC-00499, the induction was done using those 4 isolates, on the stem of *Aquilaria microcarpa* dan *Gyrinops* sp. trees.

Further tests on activities of agarwood induction were done in two locations, comprising West Kalimantan and West Nusa Tenggara by observing the measurement of agarwood development as induced in vertical and horizontal directions (presented in the Appendix). To look into the measurement results on the agarwood-development symptom in vertical and horizontal directions, it is presented in Tables 6 and 7.

Table 6. Analysis results on agarwood-development sympton in vertical and horizontal directions (the research taking place in West Kalimantan location)

No	Repetion	Tree code	isolates code	average vertical (cm)	Duncan test	average Horizontal (cm)	Duncan test
1	10	1	FORDA CC-00509	3,11	A	0,97	A
2	10	2	FORDA CC-00501	2,29	C	0,58	C
3	10	3	FORDA CC-00500	2,81	B	0,81	B
4	10	4	FORDA CC-00499	2,75	B	0,77	B

Table 7. Analysis results on agarwood-development sympton in vertical and horizontal directions (the research taking place in West Nusa Tenggarta)

No	Ulangan	Kode pohon	Asal inokulan	Rata-rata Vertikal (cm)	Uji Duncan	Rata-rata Horizontal (cm)	Uji Duncan
1	4	1	FORDA CC-00509	5.00	A	0.85	A
2	10	2	FORDA CC-00501	3.73	B	0.67	AB
3	8	3	FORDA CC-00500	2.60	C	0.55	BC
4	10	4	FORDA CC-00499	1.67	C	0.41	C

Remarks for Tables 6 and 7: Ulangan = Number of replications; Asal inokulan = Inoculant origin; Rata-rata vertikal = Average of the symptom in vertical direction; Rata-rata horizontal = Average of the symptom in horizontal direction; Uji Dunan = Duncan's multiple range test; The figures followed horizontally by the same letters (A, B, C) are not significantly different

Data in Tables 6 and 7 reveals that the isolates in West Kalimatan that exhibited high virulence were FORDA CC-00509 (with agarwood development reaching 3.11 cm in vertical direction and 0.97 cm in horizontal direction, respectively). Likewise, in West Nusa Tenggara, the FORDA CC-00509 isolates were also still very virulent, but unfortunately caused the death to the agarwood trees. This occurred due to the aspects of relation between stitability (compatibility), *Fusarium* spp. severity/ferocity, and the resisteance of the trees themselves. The percentage of tree death is presented in Table 8.

Table 8. The percentage level of tree death, as observed 3-year after the inoculation treatment (the research took place in West Nusa Tenggara)

No	Tree code no	Inoculant origin	Number of dead trees	Number of replications (trees)	Percentage of tree death (%)
1	1	FORDA CC-00509	6	10	60
2	2	FORDA CC-00501	0	10	0
3	3	FORDA CC-00500	2	10	20
4	4	FORDA CC-00499	0	10	0

To examine the induction reaction that induced agarwood development, it is presented in Figures 7, 8, 9, and 10.

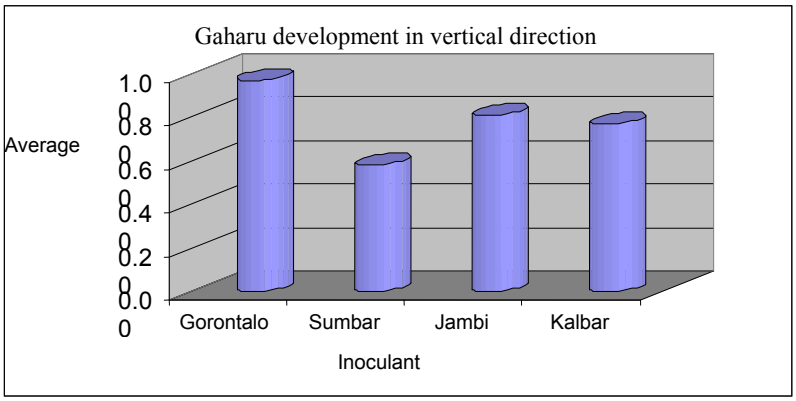

Figure 7. The induction that occurred at agarwood trees development in vertical direction (the experiment took place in West Kalimantan)

Remarks : Isolate origin
Gorontalo : (FORDA CC-00509)
West Sumatera : (FORDA CC-00501)
Jambi : (FORDA CC-00500)
West Kalimantan : (FORDA CC-00499)

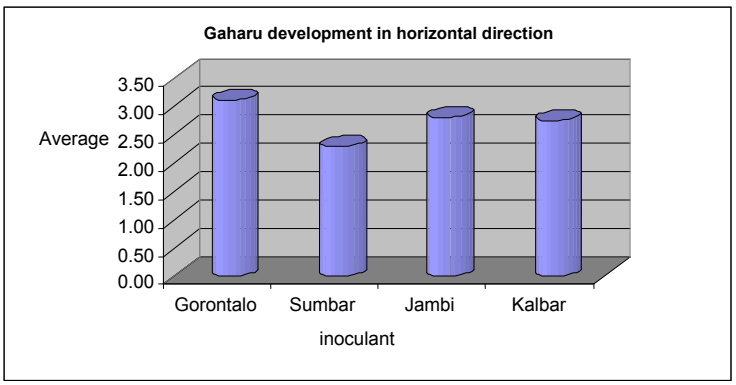

Figure 8. The induction that occurred at agarwood trees development in horizontal direction (the experiment took place in West Kalimantan)

Remarks : Isolate origin
Gorontalo : (FORDA CC-00509)
West Sumatera : (FORDA CC-00501)
Jambi : (FORDA CC-00500)
West Kalimantan : (FORDA CC-00499)

Examining Figures 7 and 8, it reveals that the FORDA CC-00509 isolates inflicted the virulence, which were higher than those of FORDA CC-00500 dan FORDA CC-00499 isolates, followed in decreasing order the FORDA CC-0050.

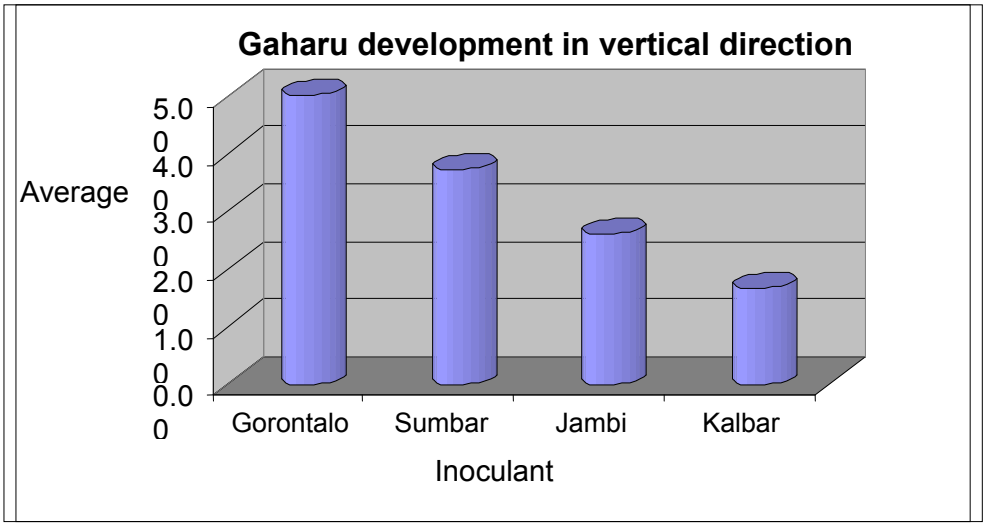

Figure 9. The induction that occurred at agarwood trees development in vertical direction (the experiment took place in West Nusa Tenggara)

Remarks : Isolate origin
Gorontalo : (FORDA CC-00509)
West Sumatera : (FORDA CC-00501)
Jambi : (FORDA CC-00500)
West Kalimantan : (FORDA CC-00499)

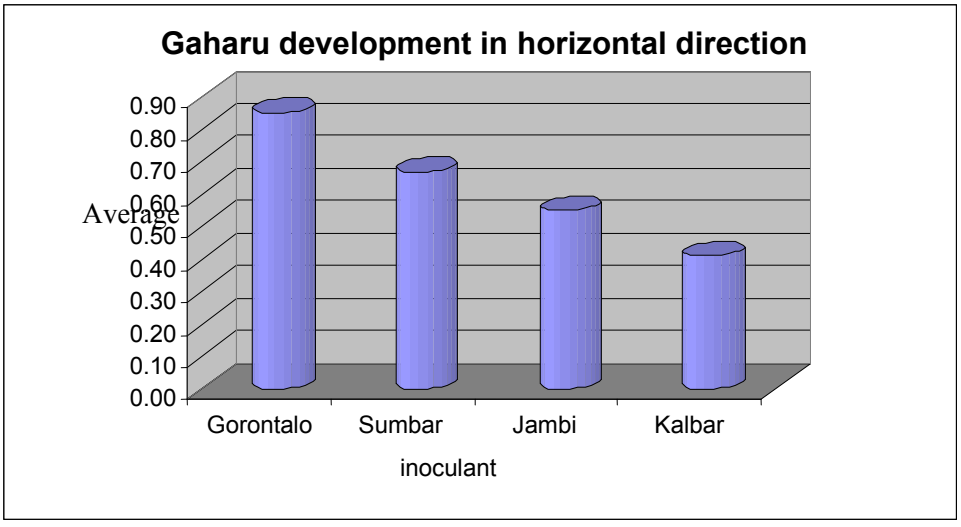

Figure 10. The induction that occurred at agarwood trees development in horizontal direction (the experiment took place in West Nusa Tenggara)

Remarks: Isolate origin
Gorontalo : (FORDA CC-00509)
West Sumatera : (FORDA CC-00501)
Jambi : (FORDA CC-00500)
West Kalimantan : (FORDA CC-00499)

Examining Figures 9 and 10, it reveals that the FORDA CC-00509 isolates inflicted the highest virulence, followed in decreasing order by the FORDA CC-00501 dan FORDA CC-00500 isolates, until the FORDA CC-00499 isolates as the lowest virulence.

From those data, it can be inferred that the induction distance of those isolates as induced to the stem of agarwood-yielding trees could be figured out. The induction distance for each of those isolates as induced to the stem of agarwood-yielding *Aquilaria* spp. trees reached about 10-15 cm, and this should recognize the environment conditions such as humidity, temperature, and light intensity. The isolates such as CC-00509, FORDA CC-00512, and FORDA CC-00497 afforded high virulence, thereby being very effective to all species of agarwood-yielding trees that grow in almost any locations or regions.

Meanwhile for *Gyrinops* sp., it turns out that the induction distance between should be 20 cm. When examining the results of induction tests on *Gyrinops* sp., it states hat the induction distance ranged about 5 cm - 15 cm. Most of the *Gyrinops* sp. trees as induced by the FORDA CC-00509 dan FORDA CC-00512 isolates suffered from their death. As such, at 5-cm injuction distance the tree death reached 100%, while at consecutively 10-cm and 15-cm induction distances, the portion of the dead trees were equal (66.67%, respectively). Meanwhile, for the FORDA CC-00511isolates at 5-cm induction distance, the tree death reached 66.67% as well. This implied the particular agarwood-yielding trees (in this regard *Gyrinops* sp. species) exhibited different resistance against the different induction isolates.

CONCLUSION AND RECOMMENDATION

Conclusions

Isolates of FORDA CC-00509 dan FORDA CC-00512 inflicted the highest virulence on the agarwood-yielding trees, followed in decreasing order by the FORDA CC-00497, FORDA CC-00500, FORDA CC-00511, FORDA CC-00499 dan FORDA CC-00501. The standard of distances between inoculation-hole distance for the agarwood-yielding *Aquilaria* spp tree species was 10 cm, and for *Gyrinops* sp. species, it was 20 cm. The FORDA CC-00509 dan FORDA CC-00512 isolates turned out very effective in agarwood development. Each of the species of agarwood-yielding trees exhibited different resistance, such as *Aquilaria malaccensis*, *Aquilaria microcarpa*, and *Gyrinop*s sp. species which were more sensitive (vulnerable) to the FORDA CC-00509 dan FORDA CC-00512 isolates. The induction using FORDA CC-00500 on *Aquilaria malaccensis* with the induction duration for 3 years afforded the agarwood development with favorable qualities.

Recomendations

The Forest Microbiology Laboratory (under the R & D Centre for Forest Conservation and Rehabilitation) currently own 54 isolates, and so far 8 isolate species have been trial tested, and therefore the remaining isolates (46 species) still deserve further trial tests.

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ANNEX

Table 9. Results of measurement on gaharu development at *Aquilaria* spp. in vertical and horizontal direction (the experiment took place in West Nusa Tenggara), as observed 3 months after inoculation treatment

No.	Number of replications	Tree code no.	Inoculant origin	Injection (inoculation) distance	Gaharu development	
					Vertical direction	Horizontal direction
1	1	1.5.1	FORDA CC-00512	5	X	X
2	2	1.5.2	FORDA CC-00512	5	X	X
3	3	1.5.3	FORDA CC-00512	5	X	X
4	1	1.10.1	FORDA CC-00512	10	2	0,5
5	2	1.10.2	FORDA CC-00512	10	X	X
6	3	1.10.3	FORDA CC-00512	10	X	X
7	1	1.15.1	FORDA CC-00512	15	X	X
8	2	1.15.2	FORDA CC-00512	15	X	X
9	3	1.15.3	FORDA CC-00512	15	6	1
10	1	1.20.1	FORDA CC-00512	20	10	1
11	2	1.20.2	FORDA CC-00512	20	10	1
12	3	1.20.3	FORDA CC-00512	20	5,7	2
13	1	2.5.1	FORDA CC-00495	5	2	0,5
14	2	2.5.2	FORDA CC-00495	5	3	0,6
15	3	2.5.3	FORDA CC-00495	5	6	0,8
16	1	2.10.1	FORDA CC-00495	10	3,5	0,7
17	2	2.10.2	FORDA CC-00495	10	2,2	0,6
18	3	2.10.3	FORDA CC-00495	10	2	0,8
19	1	1.15.1	FORDA CC-00495	15	1,6	0,7
20	2	1.15.2	FORDA CC-00495	15	1,3	0,7
21	3	2.15.3	FORDA CC-00495	15	1,5	0,6
22	1	2.20.1	FORDA CC-00495	20	2,8	0,6
23	2	2.20.2	FORDA CC-00495	20	2,8	0,8
24	3	2.20.3	FORDA CC-00495	20	1,7	0,4
25	1	3.5.1	FORDA CC-00497	5	2,3	0,6
26	2	3.5.2	FORDA CC-00497	5	2,5	0,4
27	3	3.5.3	FORDA CC-00497	5	2,7	0,5
28	1	3.10.1	FORDA CC-00497	10	7	1
29	2	3.10.2	FORDA CC-00497	10	2	0,3
30	3	3.10.3	FORDA CC-00497	10	2,5	0,3
31	1	3.15.1	FORDA CC-00497	15	3,1	1
32	2	3.15.2	FORDA CC-00497	15	2,2	1
33	3	3.15.3	FORDA CC-00497	15	1,2	0,3
34	1	3.20.1	FORDA CC-00497	20	2,5	0,4
35	2	3.20.2	FORDA CC-00497	20	1,8	0,4
36	3	3.20.3	FORDA CC-00497	20	2,2	0,6
37	1	4.5.1	FORDA CC-00511	5	X	X
38	2	4.5.2	FORDA CC-00511	5	3,4	0,4
39	3	4.5.3	FORDA CC-00511	5	X	X
40	1	4.10.1	FORDA CC-00511	10	2,5	0,4
41	2	4.10.2	FORDA CC-00511	10	2,5	0,2
42	3	4.10.3	FORDA CC-00511	10	2,3	0,1
43	1	4.15.1	FORDA CC-00511	15	3,5	0,4
44	2	4.15.2	FORDA CC-00511	15	1,5	0,5
45	3	4.15.3	FORDA CC-00511	15	5	0,3
46	1	4.20.1	FORDA CC-00511	20	3	0,5
47	2	4.20.2	FORDA CC-00511	20	2,5	0,2
48	3	4.20.3	FORDA CC-00511	20	2,8	0,4

Table 10. Results of measurement on gaharu development at *Aquillaria* spp. in vertical and horizontal direction (the experiment took place in West Kalimantan), as observed 3 months after inoculation treatment

No.	Number of replications	Tree code no.	Inoculant origin	Injection (inoculation) distance	Gaharu development	
					Vertical direction	Horizontal
1	1	1.10.1	FORDA CC-00512	10	3.3	0.9
2	2	1.10.2	FORDA CC-00512	10	4.2	0.7
3	3	1.10.3	FORDA CC-00512	10	3.9	0.8
4	1	1.15.1	FORDA CC-00512	15	4.5	0.8
5	2	1.15.2	FORDA CC-00512	15	4.4	0.7
6	3	1.15.3	FORDA CC-00512	15	4.5	0.8
7	1	1.20.1	FORDA CC-00512	20	4.6	0.9
8	2	1.20.2	FORDA CC-00512	20	4.7	0.8
9	3	1.20.3	FORDA CC-00512	20	4.5	0.7
10	1	1.25.1	FORDA CC-00512	25	4.8	0.9
11	2	1.25.2	FORDA CC-00512	25	4.7	0.8
12	3	1.25.3	FORDA CC-00512	25	4.7	0.8
13	1	2.10.1.	FORDA CC-00495	10	1.6	0.7
14	2	2.10.2	FORDA CC-00495	10	1.8	0.6
15	3	2.10.3	FORDA CC-00495	10	1.7	0.7
16	1	2.15.1	FORDA CC-00495	15	1.6	0.6
17	2	2.15.2	FORDA CC-00495	15	1.4	0.8
18	3	2.15.3	FORDA CC-00495	15	1.5	0.7
19	1	2.20.1	FORDA CC-00495	20	1.9	0.8
20	2	2.20.2	FORDA CC-00495	20	1.6	0.7
21	3	2.20.3	FORDA CC-00495	20	1.7	0.6
22	1	2.25.1	FORDA CC-00495	25	1.7	0.6
23	2	2.25.2	FORDA CC-00495	25	1.8	0.7
24	3	2.25.3	FORDA CC-00495	25	1.6	0.8
25	1	3.10.1	FORDA CC-00497	10	3.5	1
26	2	3.10.2	FORDA CC-00497	10	3.3	1
27	3	3.10.3	FORDA CC-00497	10	3.6	1
28	1	3.15.1	FORDA CC-00497	15	4.5	1
29	2	3.15.2	FORDA CC-00497	15	4.1	1
30	3	3.15.3	FORDA CC-00497	15	4.3	1
31	1	3.20.1	FORDA CC-00497	20	3.7	1
32	2	3.20.2	FORDA CC-00497	20	3.6	1
33	3	3.20.3	FORDA CC-00497	20	3.9	1
34	1	3.25.1	FORDA CC-00497	25	3.4	1
35	2	3.25.2	FORDA CC-00497	25	3.7	1
36	3	3.25.3	FORDA CC-00497	25	3.6	1
37	1	4.10.1	FORDA CC-00511	10	2.6	0.8
38	2	4.10.2	FORDA CC-00511	10	2.5	0.7
39	3	4.10.3	FORDA CC-00511	10	2.8	0.9
40	1	4.15.1	FORDA CC-00511	15	2.8	0.9
41	2	4.15.2	FORDA CC-00511	15	2.9	0.9
42	3	4.15.3	FORDA CC-00511	15	2.7	0.8
43	1	4.20.1	FORDA CC-00511	20	2.7	0.8
44	2	4.20.2	FORDA CC-00511	20	2.5	0.7
45	3	4.20.3	FORDA CC-00511	20	2.4	0.8
46	1	4.25.1	FORDA CC-00511	25	2.9	0.8
47	2	4.25.2	FORDA CC-00511	25	2.6	0.9
48	3	4.25.3	FORDA CC-00511	25	2.7	0.9

ORAL PRESENTATION: AGRICULTURE

THE POTENTIAL IMPLEMENTATION OF COCOA BASED AGROFORESTRY IN CRITICAL LAND AT NAGARI NAN TUJUAH, PALUPUH DISTRICT, WEST SUMATERA WITHIN THE VOLUNTARY CARBON MARKET (VCM)

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ABSTRACT

Difficulties of forestry activities in entering CDM have stimulated emerging of Voluntary Carbon Market. Agroforestry has been practised in many part of Indonesia including West Sumatera with cocoa as main commodity, particularly in Palupuh District. This type of agroforestry is potential form of land use that can be applied in critical land under VCM scheme. Therefore, research is needed to build a typology of existing cocoa agroforestry, analyze the trade off between the potential of carbon accumulation and economic benefit of agroforestry versus monoculture and analyze the potential implementation of cocoa agroforestry in VCM. Data collection was performed in two stages, i.e. measurements of Diameter Breast Heigh and species of trees on cocoa based agroforestry and monoculture stands in Nagari Gadut; and social-economic survey. There were five types of cocoa based agroforestry that are developed in Palupuh, i.e. cocoa – durian, coconut (model I); cocoa – rubber, coconut (model II); cocoa – cinnamon (model III); cocoa – cinnamon, surian, durian, jengkol, rambai (model IV); and cocoa – cinnamon, duku, durian, areca nut (model V). Biomass carbon of cocoa based agroforestry ranged from 12,54–49,3 ton/ha, while monoculture ranged from 2,77–26,25 ton/ha. For newly planted land, monoculture practice is more profitable than agroforestry practice that composition has been compromised by the community of Rp.11 million/ha. For land that was planted earlier, model V has highest feasibility and model III has the lowest. By following the VCM scheme, farmers of agroforestry model I-V can increase their income by 9-21% per ha at the price of 6 USD/ton CO_2, 12-27% at the price of 8 USD/ton CO_2 and 14-35% at the price of 10 USD/ton CO_2. Although the VCM scheme can increase the income of agroforestry farmers up to 30%, but the value is still below the income from monoculture eventhough at the price of 10 USD/ton CO_2. For afforestation project that will run by farmers with compromised composition, VCM scheme can increase the income so that equivalent to the revenue gained from the practice of monoculture on the price of USD 8/ton CO_2.

Keywords: Cocoa based agroforestry, VCM, carbon accumulation potential, economic potential

INTRODUCTION

Background

Climate change caused by rising emissions of greenhouse gases (GHG) is a serious problem of world today. In 1997, the Kyoto Protocol was agreed with three flexibility mechanisms that can be chosen by the developed countries to reduce GHG emissions. One such mechanism is Clean Development Mechanism (CDM), i.e. cooperation between developed countries with developing countries to reduce GHG emissions. Carbon market through CDM is known as the regulated market (Panjiwibowo et al., 2003). In addition to regulated carbon market, another emerging carbon market is Voluntary Carbon Market (VCM). Difficulties for forestry activities in entering CDM market have stimulated emerging of non regulatory market that is known as Voluntary Carbon Market (VCM). Most of the VCM project is a small-scale project that can reach out to the participation of small communities (Taiyab, 2006).

Indonesia potentially participates in the activities of VCM, especially in the forestry sector. Form of forestry that is potentially developed under VCM project is agroforestry because this sector is generally developed in Indonesia, one in West Sumatra Province. Agroforestry commodity that is known and widely grown in West Sumatra Province is cocoa. West Sumatra province has a lot of land that have the potential to be developed into cocoa based agroforestry. Yet the problem often faced by cocoa based agroforestry farmers is that short-term productivity of cacao based agroforestry products are lower than the production of monoculture practice, whereas cocoa based agroforestry has environmental benefits far greater than monoculture practices including carbon sequestration services. Therefore research is needed to determine how well the potential of agroforestry in absorbing carbon dioxide and the economic potential of this small-scale cocoa agroforestry when compared with the monoculture that can be implemented in VCM

project by considering the advantages of co-benefits that accompany it and how well the activity of the VCM is able to increase revenue of agroforestry cocoa farmers.

Problem

Palupuh local communities are developing small-scale cocoa agroforestry. This type of agroforestry is potential form of land use that can be applied in critical land under VCM scheme. Afforestation by agroforestry techniques could potentially be included in the VCM project through intermediaries World Agroforestry Centre (ICRAF). In Palupuh, especially at Jorong Paninggiran Ateh, Nagari Nan Tujuah, there are many unproductive critical land. ICRAF as an intermediary corporation in the VCM so far has done the mapping of critical land to be used potential agroforestry. By few times of Focal Group Discussion (FGD) which were facilitated by ICRAF with the community, it was known that the Palupuh community chose cocoa as the major commodities with five other types of plants as the shade, among others, surian, mahogany, durian, clove and rubber.

In addition to producing agricultural products for dayly life, agroforestry is one form of climate change mitigation activities recognized in the VCM. In this project, conducted orientation agroforestry is seen from two points of interest. Community run the agroforestry project by the orientation of agricultural products with optimum results. While ICRAF here as intermediaries as well as project coordinator of VCM looked agroforestry as carbon sequester as well as to improve farmers' welfare.

Both of these perspectives will be incorporated into a scheme of cooperation agreement between ICRAF and the community, so that optimization of both objectives can be achieved. The existence of opportunities for cooperation between ICRAF with local communities to raise revenue is expected to stimulate farmers to keep agroforestry culture. It is not yet known how the shape of the existing agroforestry development in Palupuh and how potential of environmental and economic services of their agroforestry. Therefore, research is needed to build a typology of existing cocoa agroforestry in Palupuh District, analyze the trade off between the potential of carbon accumulation and economic benefit of cocoa agroforestry versus monoculture and analyze the potential implementation of small scale cocoa agroforestry in VCM.

Objectives

1. To build a typology of existing cocoa agroforestry in Palupuh District
2. To analyze the trade off between the potential of carbon accumulation and economic benefit of cocoa agroforestry versus monoculture
3. To analyze the potential implementation of small scale cocoa agroforestry in VCM.

METHODS

The study was conducted from December 2010 until March 2011. Broadly speaking, this study is divided into several activities ranging from observation by the researchers, Focal Group Discussion (FGD) which was facilitated by ICRAF and data retrieval by researchers. Retrieval of data includes field measurements of Diameter Breast Heigh (DBH) and species of individual trees and social-economic survey.

Description of Location

The study was conducted in Jorong Paninggiran Ateh, Nagari Nan Tujuah, Palupuh District, Agam Regency, West Sumatra. *Jorong* equivalent to the *dusun* (bahasa) and is the smallest unit of governance in the West Sumatra Province forming a *Kanagarian*. Palupuh borders with Pasaman District in the north, Lima Puluh Kota District in the east, Tilatang Kamang Subdistrict in the south, and Palembayan Subdistrict in the west. Approximately 6.32 % of land in this subdistrict is agroforestry. More than 80% of land is still forested and shrub (BPS Agam, 2009).

Procedures

Preliminary Observations

At the stage of preliminary observations, we identified patterns of agroforestry that were run by the community in general. Interviews were conducted to some figures, especially Panorama Indah farmer groups builder that will develop cocoa agroforestry in Nagari Nan Tujuah, government and local farmers. By this stage, we could obtaine some plot candidates which can be measured in the data collection phase of the carbon potential and the subsequent data of cocoa based agroforestry farmers who could be interviewed further.

Focal Group Discussion (FGD)

FGD was facilitated by ICRAF for the early introduction of carbon trading to the public. Activities undertaken in the FGD were the introduction of carbon markets, map-making and discussion of VCM contract design as type and cropping pattern, contract period, system verification, sanctions, liability and the parties involved. From the results of the FGD facilitated by ICRAF, the researchers got the data about plant type preference by farmers for planting on their land which will potentially be included in the VCM. In the subsequent discussion, the development of agroforestry with plants as agreed in the FGD is called with 'Model Agreed'.

Data Retrieval

Measurements of biomass carbon were performed on cocoa stands of 3, 5, 7 and 9 years old. For each stand age, measurements were performed three times, two measurements on cocoa based agroforestry stands in Nagari Nan Tujuah, Palupuh District and a single measurement on monoculture stand in Nagari Gadut, Tilatang Kamang District. Interviews for exploring the economic potential of agroforestry activities and socio-economic condition of the society was conducted to 40 agroforestry farmers in Nagari Nan Tujuah and 10 monoculture farmers in Nagari Gadut.

Data Analysis

Carbon Analysis

Plot size varied according to the condition of the garden area of agroforestry in the field ranged from 1000 - 2000 m^2. Parameters calculated were diameter of tree at breast height (DBH), jorquette diameter (for cocoa) and tree height. Biomass of trees of each model were determined using equation allometrik agroforestry as in table II.1. Total levels of biomass carbon in tree biomass was determined using the equation:

$$\textbf{Carbon Biomass (ton/ha) = Tree Biomass (ton/ha) x 0,45}$$

Asumption:

* The content of C in tree biomass was 0.45

Data of carbon biomass was converted in to form of carbon dioxide biomass (CO_2 Equivalent) with the formula:

$$\textbf{CO}_2\textbf{ Equivalent} = \frac{\textbf{Mr CO}_2}{\textbf{Ar C}}$$

Mr CO_2 : Relative molecular weight of the compound of CO_2 (44)

Ar C : Relative molecular weight of the atom C (12)

Furthermore, the calculation of MAI (Mean Annual Increment) (tonnes/ha/year) and carbon accumulation rate (tons/ha/year). MAI is the average growth of trees per year with the calculation: MAI = X (t) / t where X (t) = biomass of trees at age t. Accretion rate of carbon accumulation is the average amount of carbon in tree biomass per year with the calculation: MAI = Y (t) / t where Y (t) = biomass carbon at age t

Economic Analysis

To see the extent to which an undertaking agroforestry is profitable, then the most appropriate analysis to be used is project-based financial analysis. The size of the feasibility of farming used are as follows:

a. Net Present Value (NPV)

Net Present Value (NPV) is the current value that reflects the value of profits earned during the period of exploitation taking into account the time value of money or time value of money (Suharjito et al., 2003). NPV value obtained by the equation:

$$NPV = \sum_{t=0}^{t=n} \frac{Bt - Ct}{(1+i)^t}$$

NPV : Net Present Value
Bt : Benefit
Ct : Cost
i : Interest
t : Time period

Determinatin:
NPV > 0 (nol) → project is feasible to run
NPV < 0 (nol) → project is not feasible to run
NPV = 0 (nol) → project is in *Break Event Point* (BEP)

b. Benefit Cost Ratio (BCR)

Benefit Cost Ratio (BCR) is the ratio between revenues and expenses during the period of exploitation (taking into account the time value of money or (time value of money) with the model formulation as follows (Suharjito et al., 2003):

$$BCR = \frac{\sum_{t=0}^{t=n} \frac{Bt - Ct}{(1+i)^t}}{\sum_{t=0}^{t=n} \frac{Bt - Ct}{(1+i)^t}} \quad \begin{array}{l} \longrightarrow \quad Bt - Ct > 0 \\ \longrightarrow \quad Bt - Ct < 0 \end{array}$$

BCR : Benefit Cost Ratio
Bt : Benefit (income cash flow at t-period)
Ct : Cost at t period
I : Interest
T : Time Period

Determination:
BCR > 1 → project is feasible to run
BCR < 1 → project is not feasible to run

c. Internal Rate of Return (IRR)

Internal Rate of Returns (IRR) showed the maximum interest rate that can be paid by a project/ business or in other words the ability to earn income from money invested. Agroforestry efforts will be said feasible if IRR is greater than the interest rate prevailing in the market at that time by the following formula (Suharjito et al., 2003):

$$IRR = i_1 + \frac{NPV_1}{(NPV_1 - NPV_2)}(i_2 - i_1)$$

IRR : Internal Rate of Return
NPV1 : NPV at i1
NPV2 : NPV at i2
i1 : *Discount Factor* 1
i2 : *Discount Factor* 2

Determination:
If the IRR is greater than the interest rate prevailing in the bank then the project is feasible to run

RESULTS AND DISCUSSION

Agroforestry Models in Palupuh District

In the 1980s, Palupuh communities developed mixed farms, which were dominated by cinnamon (*Cinnamomum burmanii*) and rubber (*Hevea brasiliensis*). But lately these two commodities have decreased of profitability and productivity. Cinnamon and rubber farmers began to switch their gardens, planted with cocoa trees (*Theobroma cacao*). Farmer chose cocoa because these plants can be harvested within three to four years, they produce fruit throughout the year and the price of cocoa in the market tend to be stable. In addition, as plants that require shade, cacao considered suitable to be planted in the garden of the former mixture of cinnamon, because people no longer need to replant crops as shade. Therefore, it is very prevalent in Palupuh farmers who still maintain the cinnamon plant in their gardens as a protector of cocoa. On the basis of the above conditions, cocoa is used as a core consideration to determine which form of agroforestry was observed in this study. From the results of field review, interviews and local Kenagarian data then fixed at five model agroforestry as a sample in this study and monoculture plantations as a comparison as in table III.1. Comparison of the density of components on each model can be seen in figure III.1.

Comparison of Carbon and Economic Aspects of Agroforestry Practices vs Monoculture of Cocoa

Carbon Aspect

Parameters to see the dynamics of the binding of carbon dioxide in a land are tree biomass, carbon biomass (tons/ha), Mean Annual Increment (MAI) and the rate of carbon accumulation on average per year (t/ha/yr). MAI and rate of carbon accumulation on average per year for each of the agroforestry model above is a cumulative of the basic components of the model with the proportion of components as can be seen in figure III.1. Estimates of carbon potential of each agroforestry in the table III.1 shown in table III.2. From the table III.2, it can be seen that the model V (cocoa age 9 years) is a model of agroforestry with highest tree biomass. Besides having high individual density per hectare (see picture III.1), it can can be seen in figure III.2 that most components as main contributor of tree biomass are durian and cacao that are characterized by their large diameter. However, MAI on the model V is not the highest since the components of durian that has a high biomass has little value of MAI because the age of durian was more than 50 years old. When compared with the practice of monoculture cocoa with the same age, model V has biomass carbon per hectare of two and a half times greater (figure III.2). Similarly, model V has the rate of carbon accumulation of the annual average of one and a half times larger than nine years old cocoa monoculture.

Model III (cocoa stands of 5 years) is a model of agroforestry in Palupuh which has the lowest value of MAI and carbon accumulation rate among the five models (table III.2 and image III.3). Cocoa and cinnamon had the same character of low biomass. Generally, the practice of planting that is classified in this model III is that farmers do not cut down the young cinnamon plant was left on their land and then replant cocoa randomly at open land locations.

From the table III.2, it can be concluded that the average model of agroforestry has a higher carbon biomass compared with monoculture practices. Cacao based agroforestry has carbon biomass ranged from 12.54 to 49.3 tonnes/ha, while the practice of monoculture ranged from 2.77 to 26.25 tons/ha. Model I has carbon biomass 8.6 times greater than the practice of monoculture; 3.5 times by model II; 1.3 times by model IV and 1.9 times by model V. Biomass carbon value of model III is similar to the practice of monoculture of the same age. In addition to considering carbon biomass, it is also important to see how much the rate of carbon accumulation due to the age of each component of different models. Models I, II and V have the carbon accumulation rate greater than the practice of monoculture of 3.8; 1.9, and 1.1 times. Model III has the rate of carbon accumulation that is similar to the practice of monoculture of the same age. While model IV has a value of carbon accumulation rate that is lower than the practice of monoculture of the same age because of the density of individuals per hectare that is low.

Value of the rate of accumulation of carbon per year on cocoa agroforestry practices in Palupuh ranged from 2.17 to 4.27 tonnes/ha/year. This value is more variable compared with cacao agroforestry research in Lima Puluh Kota District, West Sumatra conducted by Sorel (2007) ranged between 3.24 to 3.88 tonnes/ha/year. Presumably this is due to lack of clarity in cacao agroforestry management options in Palupuh Subdistrict related to the effectiveness of using the land area. In addition, the form of cocoa agroforestry in Lima Puluh Kota District as the center of cocoa in West Sumatra is more structured, where cocoa farming as the main crop has been there since twenty years ago with the pattern of proportional spacing and better treatments such as fertilization and pruning on a regular basis (Sorel, 2007)

Economic Aspect

This discussion will be assessed on the comparison of the economic benefits obtained by cocoa based agroforestry with monoculture farmers through farm business analysis for each practice. Discussion of this aspect of the economic comparisons based on several premises and assumptions as follows:

1. Model I, II, and III are agroforestry practices with limited initial capital and belonging to the semi-subsistence farming. Labor also comes from his own family who are not paid professionally. Models IV and V are examples of agroforestry that are already financed by the farmers in the form of fertilizer costs. For comparison, samples of monoculture in Nagari Gadut can already be classified as a market-oriented farming and classified as commercial farming.
2. The five models that are being developed by cacao based agroforestry farmers were planted on land that already contains trees that have been productive. In the calculation of financial analysis, all models (except model III with cinnamon components) already has a beneficial since year-1 that will affect the eligibility standards of farming when compared with monoculture farming planted on vacant land not yet in production. For comparison, shown also projected the feasibility of agroforestry farming on land that is not yet in production which will be run by the community Palupuh with VCM scheme. From the results of FGDs conducted, farmers in Paninggiran Ateh chose 6 major crops to be planted in their fields of cocoa, durian, clove, rubber, mahogany and surian. For further discussion, the term projection of farming that will run by this society will be called Model Agreed.
3. Data on the proportion of the components of land per hectare for each practice was needed to obtain a comparison of the proportion of the economic benefits of cocoa in monoculture farming practices and agroforestry:
    a. Monoculture. The average cocoa monoculture plantations in Nagari Gadut use a spacing of 3 x 3, with an average age of first fruit of four years. Age of monoculture farming is estimated to 20 years (Obiri et al., 2007). Monoculture cocoa in Nagari Gadut preceded by planting bananas for a period of three years and then harvested when the canopy is covered the cocoa land.

b. Model Agreed. Proportion and spacing proposed by farmers shown in Table III.3. Comparative analysis of the financial period follows one-time rotation of the cocoa production as main crop that is 20 years.
c. Agroforestry. The proportion of each of the five components of agroforestry model shown in Figure III.1. Although both belong to the practice of agroforestry, Model Agreed and five models of agroforestry in Palupuh differ on the value of production in year-0 that affects the subsequent financial analysis.

Based on these assumptions, it can be done comparisons among the advantages to be gained from cocoa monoculture practices in Gadut, five cacao agroforestry models which were run by farmers and Model Agreed. In general, factors that are needed in conducting the financial analysis are model of cost and production.

Cost model

Comparison of the cost model of cocoa farming in general can be divided into two types, namely cost of infrastructure/production facilities and labor costs. In monoculture farming and Model Agreed later, the cost of infrastructure/facilities include the purchase of seeds, fertilizers, herbicides and equipment. Labor costs include the cost of land preparation, planting, fertilizing, maintenance and harvesting. The main difference of these two types of farming practices is that in the practice of monoculture in Gadut, labor are rewarded according to local daily wage work, whereas most of the practice of agroforestry in Palupuh use labor of his own family members. But in this financial analysis, the labor itself remains incorporated in the model of financing to pay the opportunity cost of doing business in another.

Model Production

Based on field observation, cocoa can generally be harvested at the age of four years. Specifically to estimate the harvest of cocoa and other co-benefits, conducted interviews with farmers agroforestry and monoculture of various ages cocoa to get an overview of growth projected crop production per year. For reference, the projected harvest of 20 years is also based on research conducted by Obiri et al (2007). In addition, fertilization is also a factor to be considered in estimating harvest cocoa. Harvesting cocoa without fertilization produces only 2/3 of the total harvest of cocoa by fertilizing at the same age (Siregar, 2010).

Financial analysis

Calculation of financial analysis is based on pricing data taken in December 2010 to February 2011, with mortgage interest rates averaged 14%. Table III.4 shows comparison of NPV, B/C ratio, and IRR for each type of cocoa farming. There are two comparisons that can be analyzed in table III.4, namely: (1) comparison of the feasibility of the newly planted area (output value = 0 at year 0) that is between farming monoculture and Model Agreed (2) comparison of the feasibility of farming on land that has been previously planted (production value > 0 at year 0) that is among the farming model I - V.

Based on financial analysis that has been done, monoculture farming is more profitable than Model Agreed. Magnitude of the differences obtained over a span of twenty years amounted to 11 million/ha. From the table III.4, it can be seen that the model V has highest value of the feasibility of farming the and model III is the lowest. Based on the discussion above we can see the trade off between the practice of agroforestry and monoculture. In general, agroforestry practices are capable of binding carbon better than monocultures, but in terms of revenue, the economic potential value of monoculture better than Model Agreed activities that are equally planted on empty land. Therefore, VCM is an appropriate strategy for agroforestry farmers to increase their income rather than having to change the system into monoculture farming. How big is this VCM can increase farmers' income will be discussed further in section III.3.

Potential Implementation of Small Scale Cocoa Agroforestry within VCM in Paninggiran Ateh

Socio-cultural background of society in Paninggiran Ateh

To get a background of the potential implementation of carbon projects, in addition to physical potential of ability of land to absorb carbon and the potential economic benefits, we need to know in advance about the profile of Paninggiran Ateh community that will carry out these agroforestry activities such as social conditions, cultural and economic of the community.

a. Age Range

Half of the population in Paninggiran Ateh are in productive age range 18-56 years (Figure III.4)

b. Livelihood

Productive labor in Palupuh numbered more than 400 people. From Figure III.5 it can be seen that most of their living as farmers (39.51%) consisting of agroforestry farmers, rice growers and farmers fields. Furthermore, as much as 24.63% of the population living as a self-employed business or trade in the capital district in the town of Bukittinggi.

c. Family Size

Population data of Jorong Paninggiran Ateh showed that the average population is a small-medium family, where nearly a quarter of the total families in Paninggiran Ateh have four family members (figure III.6)

d. Education

Highest level of education in Jorong Paninggiran Ateh is just graduated from elementary school or equivalent of 29.31% and 20.42% of junior high graduates. Only about 2% of the population Paninggiran Ateh who continue their education to higher education (figure III.7).

e. Tenure

Most respondents (45%) work more than one hectare of land (figure III.8). Generally they cultivate land as agroforestry and *sawah* (paddy field) in groups. Most farmers own and cultivate the private land or customary land that is intended for them. The land that they cultivate in the form of agroforestry, farm and *sawah*. Besides working on his own land, 25% of respondents also worked on land owned by someone else with a sharing system. In addition to working on their own land and land owned by local residents, 17.5% of respondents also worked on village-owned land that is free to be used by the community without any tax.

f. Houses Quality

Houses quality of farmers respondents interviewed in Palupuh are quite simple. More than 70% of respondents have a cement floor, nearly 80% of respondents have a wall of brick and all the respondents are already using iron sheeting as roof (figure III.9). More than half of respondents have a home with a simple type with sizes ranging from 30-60 m^2.

Of socioeconomic backgrounds, it can be concluded that the population of Paninggiran Ateh have the potential to develop coco agroforestry-based VCM. Judging from the level of the age range and availability of labor, community of Paninggiran Ateh have a high productive workforce. Nearly 40% of the population of Paninggiran Ateh already experienced in living as agroforestry farmers. Most of the farmers were also able to cultivate the land more than 1 ha/person. Low economic circumstances affect the low level of education of the population of Paninggiran Ateh. Most people only complete primary school, so they only have skills as a laborer and farmer. Only 2% of the population that is able to continue their education into college. Judging from the quality standards of buildings, the average resident has a simple house that shows the low state of the economy of local community. Therefore, VCM is one of the potential for community of Paninggiran Ateh to increase income from agroforestry enterprises they run.

Comparative Financial Analysis Without and With VCM

Carbon biomass values of each model can be seen in table III.2. The biomass carbon data was converted to carbon dioxide biomass (CO_2 Equivalent). From the result of conversion, it can be calculated the value of the content of carbon dioxide uptake for each model of 88.37 tons CO_2/ha; 174.21 tons CO_2/ha; 46.02 tons CO_2/ha; 122.32 tons CO_2/ha ; and 180.93 tons CO_2/ha. By using the data of MAI of various

components of plant at five model, it can be also estimated the potential value of carbon dioxide uptake of Model Agreed which is about 269.52 tons CO_2/ha.

Financial analysis in this study take into account transaction costs. Based on research conducted by Antoko (2011) and some experience of Plan Vivo project (Plan Vivo, 2008), the overall transaction costs to be incurred in the carbon project no more than 40% of the total project value of carbon. Unit sales that are used in this scheme is VER (Voluntary Emission Reduction) that is equivalent to 1 ton of CO_2. VER price used is USD 6/ton CO_2 (Plan Vivo, 2010). At the time of this study, the price of USD 1 is considered equivalent to Rp 8700,-. In a sensitivity analysis, it is also used carbon price of USD 8/ton CO_2 and USD 10/ton of CO_2 as a comparison. Interest rates by 14%. The results of the calculation of financial analysis monocultures, Model Agreed and five models without/with VCM schemes can be seen in Table III.5.

Unlike the financial analysis in section III.2.2, the value of farm income of agroforestry model I-V without VCM scheme in table IV.6 was assumed = 0 at year 0. Based on calculations in Table III.5, it is known that the value of agroforestry model I-V without following the carbon scheme is financially viable although its economic potential is still under the practice of monoculture. Following the VCM scheme on the price of 6 USD/ton of CO_2, the the agroforestry activity in model I-V could increase total revenues up 8.8% to 20.76% per ha. Value of the increased revenues would be higher to a range of 11.8% to 27.34% on the price VER 8 USD/ton CO_2 and 14.11% - 34.79% on the price VER 10 USD/ton. Although the VCM scheme can increase revenue by more than 30%, but the income is still below the value of monoculture farming income, although at a price of 10 USD/ton CO_2 though.

Trade off between the monoculture and the agroforestry model I-V is quite large. But it should also be noted that the basic calculation of financial analysis that compares the two types of businesses are using the projected time for 20 years following the time the productivity of cocoa. Agroforestry production other than cocoa as durian, coconut and other fruit trees will still provide income for farmers after a period of 20 years. In the economic calculations that were done in this study, the practice of monoculture is more favorable for small-scale. But for the development of widespread, the practice of monoculture is not necessarily better and more profitable. The practice of monoculture would be more susceptible to disease risk and commodity price dynamics that can not be predicted precisely.

From the table III.5 it can also be compared Model Agreed to be run by people of Paninggiran Ateh without and with the VCM scheme. Model Agreed has NPV of Rp 54 million/ha with a projected twenty years, smaller Rp 11 million/ha compared to the practice of monoculture. For carbon project agroforestry that will run by farmers of Paninggiran Ateh with Model Agreed composition, VCM scheme can increase the income of farmer so that equivalent to the revenue gained from the practice of monoculture on the price of USD 8/ton CO_2 and can increase revenue up to 19%.

Implementation Possibility of VCM Scheme in Paninggiran Ateh

Based on calculations, by using carbon schemes, models II and V has the highest NPV (Table III.5). High NPV on models II and V indicate that the model II and V are the best option for farmers in Paninggiran Ateh if they want to use carbon as an alternative financing scheme in addition to using Model Agreed composition that was agreed in advance. However both the calculation of the carbon potential for model I-V or Model Agrred limited to the estimated biomass of trees and not taking into bellows-ground biomass. In addition, during the twenty years of agroforestry activities, of course, farmers will do the thinning of trees and harvesting fruit that will affect the value of net absorption of greenhouse gases by sinks so that the estimated magnitude absorption in this study was too high.

Associated with the implementation of environmental services payoff, there are indications that can be used as a reference strategies and policies required that the transaction mechanism and driving forces (Nugroho and Kartodihardjo, 2009 in Antoko, 2011). Mechanism of transactions that could be conducted through the Plan Vivo scheme is in the form of carbon trading called VERs from community-based projects of land use. Carbon service providers in this regard is agroforestry farmers in Jorong Paninggiran Ateh incorporated in Panorama Indah Farmers Group, while the buyer of services come from companies or individuals who want to buy the services of carbon sequestration. Services buyers are from companies or individuals interested in buying carbon. ICRAF's role as project coordinator, intermediaries (facilitators) and at the same time as the initial investors for initiating carbon projects.

VCM project that will run in the near future is the greening of former oil palm plantation land and grass lands that was unproductive in an area approximately 15 hectares. This land is customary land (lahan kaum) and owned by the majority in Paninggiran Ateh namely Kaum Koto. In the customary land management arrangements in West Sumatra, customary land can be used freely by the members concerned with the approval of the customary chief (Datuak) but only limited in 'use rights'. In this activity, members of the Koto agreed to give 'use rights' of the development of cocoa-based agroforestry carbon projects to Farmers Group Panorama Indah. Members of farmer groups are largely composed of members of the Koto and several other members of the Jorong around.

Based on FGD results, they agreed that the outcome of the VCM agroforestry activities will be divided by the proportion of 60% for the Farmers Group Panorama Indah, 30% for members of the Koto as the owner of the land, 5 % for tax and the remaining 5% to charity (zakat). This agreement should be sharpened again by the farmer groups with the parties as landowners. From interviews conducted on sharing system in the local area, most farmers in Paninggiran Ateh that the land belongs to other people, used the system for farm products with a proportion of 2 : 1 for each of the agricultural commudity. The proportion of 2 : 1 was also reflected in the FGD outside of tax and zakat. But the division of the harvest cocoa agroforestry in the form of farm products and their accompanying co-benefits should be calculated beyond the additional incentives from the VCM. Farmer groups as actors and producers in the VCM are entitled to a full incentive from the sale of carbon services that benefits can be felt by the members of farmer groups in full.

Farmers that are represented by the Farmers Group are expected to access information about the pathways of carbon sales activities, ranging from registration, validation, monitoring, verification through sales at the company. Transparency of the financial chain path between the producer (farmer), initial investors and facilitators (ICRAF), and the buyer must be guaranteed so that each party can benefit equally. Carbon projects in Paninggiran Ateh still fairly early in the initiation stage. Until now it is still not been agreed what percentage of the division of the proceeds of carbon between the farmers' groups with the facilitators (ICRAF). But from some carbon activities project experience undertaken, the facilitator get 15-20% proportion of total sales of carbon (MEETB, 2009; ECL, 2010).

Farmers as providers of services will be accompanied by a project coordinator, in this case ICRAF, an independent environment institution. Project Coordinator will be responsible for overall management of projects including the construction of farmer groups, monitoring and verification. Local governments (Pemda) in this case Kenagarian parties is not directly involved in project activities. However, the local government acts as the driving factor in encouraging the development of the local cocoa farmers. Participation of local government is required to make regulatory instruments in the form of regulations and policies at regional level that support the practice of voluntary carbon trading; reactivate farmers' cooperative that has been underrepresented on Paninggiran Ateh, supervise and provide information on market price of cocoa at the level of local producers. With the growing standards of transparency in cocoa prices, the expected income of farmers in cocoa agroforestry in Paninggiran Ateh can be improved, and the trade off between the practice of agroforestry-monoculture can get smaller so as to prevent the agroforestry farmers to switch to monoculture.

In most VCM projects using the Plan Vivo scheme, period project is designed at least for 10 years (MEETB, 2009). For VCM projects in Nagari Nan Tujuah, it is assumed that the project period lasted for 20 years, following the rotation cycle of productive cocoa. This means, for a period of 20 years, agroforestry farmer as a produser is responsible for the existence of quantity and quality of agroforestry in accordance with the rules of how to care for the cultivation of agroforestry and timber tree cut down during the period of the project. Besides the consequences for not cutting down trees during the project period, farmers must also meet the requirements of the project mainly to avoid carbon leakage.

In practice, like most reward environment project, there is likely to occur leakage. Leakage is the possibility of rising greenhouse gas emissions in areas outside the project boundary due to afforestation/ reforestation activities (AR) (Kollmuss, 2008). Most cases of leakage that occurs is when AR activity is done, the farmers can no longer cultivate their land with seasonal crops that provide short-term productivity that provides daily income for them. There is a tendency for farmers to open new land with cutting down the trees on forests outside the project area for arable crops so there was leakage.

To minimize leakage, it must be assured that farmers that are incorporated in the Farmers Group Panorama Indah still have enough land to grow other crops. From Figure III.8 we can see that 45% of the population have enough land for farming, more than 1 ha per person. Most of the land is the land belongs to the line of mothers that have clear management system in accordance with the prevailing system of Minangkabau custom. In its management, most farmers allocate land they own into two categories, namely sawah and agroforestry. Generally, farmers in Nagari Nan Tujuah focus the allocation of labor to till the sawah belonging to the time of planting or harvest and cultivate agroforestry on the sidelines of the time period of growing rice. With this system, communities are able to meet the needs of food (rice) for subsistence, and earn additional income from agroforestry activities they do on the sidelines of sawah activities.

In addition to the VCM scheme, the practice agroforestry such as those in Paninggiran Ateh can earn an additional incentive to explore more about the opportunities in obtaining additional incentives such as service water storage and conservation of biodiversity through the improvement and provision of habitat for many species of flora and fauna . Therefore it is necessary to do further research about the prospects and other reward mechanisms that are available with the existence of cacao agroforestry in Paninggiran Ateh.

CONCLUSION AND SUGGESTION

1. There were five types of cocoa based agroforestry that are developed in Nagari Nan Tujuah, i.e. cocoa – durian, coconut (model I); cocoa – rubber, coconut (model II); cocoa – cinnamon (model III); cocoa – cinnamon, surian, durian, jengkol, rambai (model IV); and cocoa – cinnamon, duku, durian, areca nut (model V)
2. Agroforestry practices capable of binding carbon better than monocultures. Carbon biomass of cocoa agroforestry were ranged from 12,54 to 49,3 tonnes/ha, while cocoa monoculture ranged from 2,77 to 26,25 tons/ha. Model V has the highest carbon biomass (49,3 tonnes/ha). Model II has the highest carbon accumulation rate (4,27 tonnes/ha/year).
3. Monoculture farming is more profitable than Model Agreed amounted to 11 million/ha. Model V has the highest feasibility of farming & model III has the lowest.
4. By following VCM, farmers could increase their income 9-21% per ha in the price of 6 USD/ton of CO_2; 12-27% on the price VER 8 USD/ton of CO_2, and 14-35% on the price VER 10 USD/ton CO_2
5. By following VCM, farmers who will run the project with the Model Agreed compotition are able to match the revenue generated from the practice of monoculture on the carbon price USD 8/ton CO2 and increase revenue up to 19%.

Further research is needed to explore the prospects of other environmental services reward mechanisms that can be provided by cacao agroforestry in raising an additional incentive for farmers in Nagari Nan Tujuah

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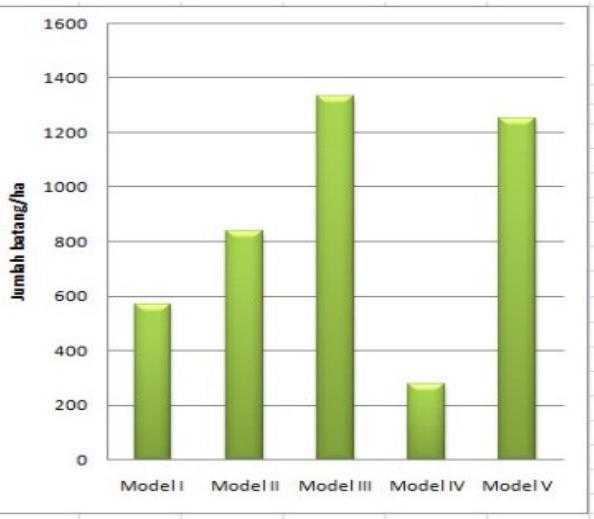
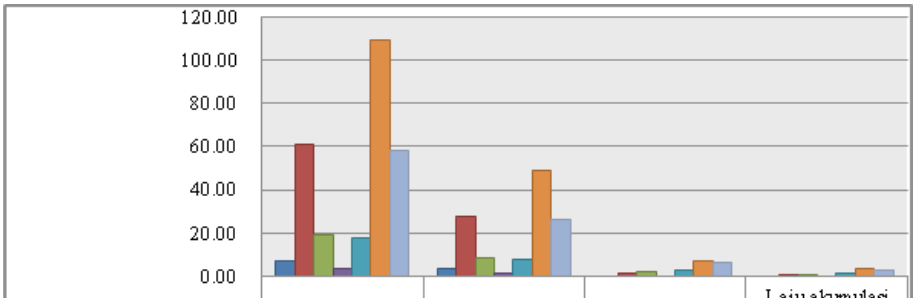
Images and tables that are referenced in the text:

1. Figure III.1 Comparison of the density of components on each model

Model Agroforestri	Jenis komponen	Jumlah batang/ha	Jumlah total/ha
Model I	Kakao	340	568
	Durian	100	
	Kelapa	128	
Model II	Kakao	580	840
	Karet	140	
	Kelapa	120	
Model III	Kakao	782	1334
	Kulit manis	552	
Model IV	Kakao	143	279
	Durian	15	
	Jengkol	14	
	Kulit manis	57	
	Rambai	15	
	Surian	35	
Model V	Kakao	689	1254
	Durian	62	
	Duku	78	
	Kulit manis	158	
	Pinang	267	

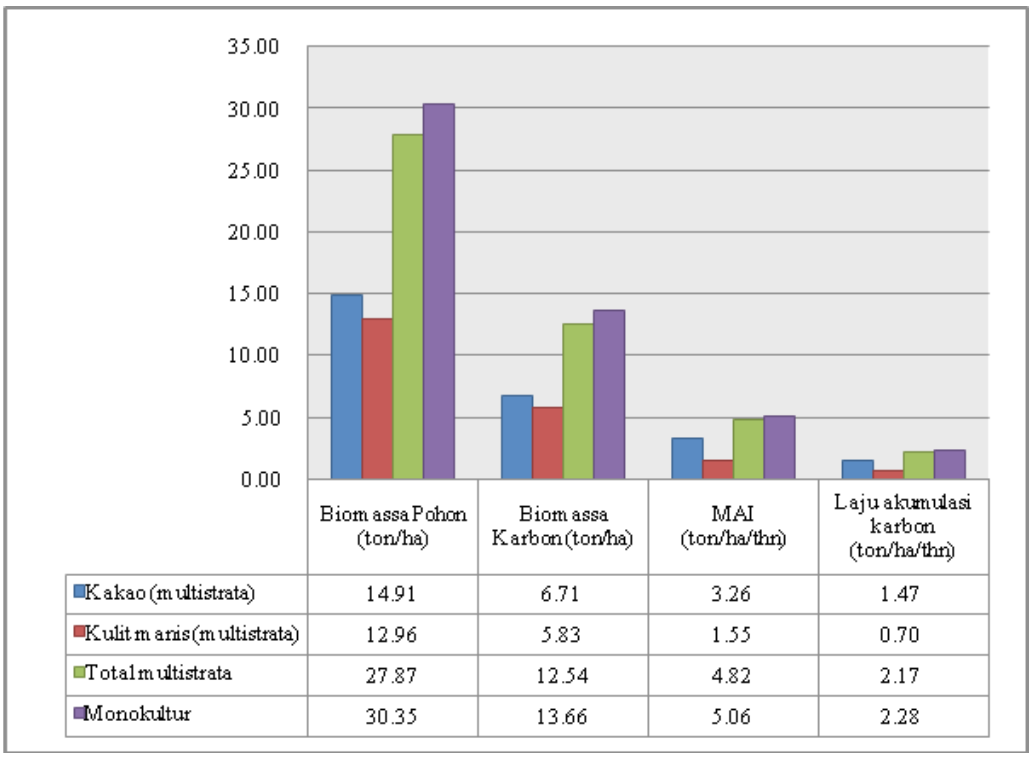


2. Figure III.2 Comparison of accumulated carbon model V (cocoa based agroforestry age 9 years)



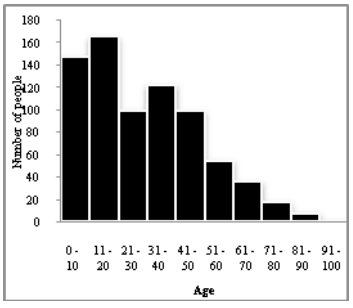
	Biomassa Pohon (ton/ha)	Biomassa Karbon (ton/ha)	MAI (ton/ha/thn)	Laju akumulasi karbon (ton/ha/thn)
Duku (multistrata)	7.57	3.41	0.33	0.15
Durian (multistrata)	61.22	27.55	1.28	0.57
Kakao (multistrata)	19.10	8.60	2.12	0.96
Kulit manis (multistrata)	4.06	1.83	0.38	0.17
Pinang (multistrata)	17.59	7.91	3.07	1.38
Total multistrata	109.55	49.30	7.18	3.23
Monokultur	58.34	26.25	6.48	2.92

3. Figure III.2 Comparison of accumulated carbon model V (cocoa based agroforestry age 5 years)

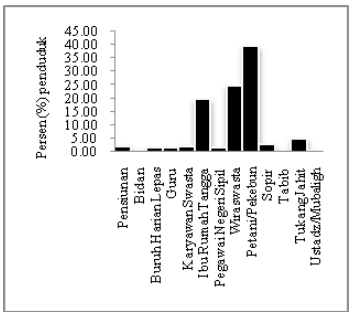


	Biomassa Pohon (ton/ha)	Biomassa Karbon (ton/ha)	MAI (ton/ha/thn)	Laju akumulasi karbon (ton/ha/thn)
Kakao (multistrata)	14.91	6.71	3.26	1.47
Kulit manis (multistrata)	12.96	5.83	1.55	0.70
Total multistrata	27.87	12.54	4.82	2.17
Monokultur	30.35	13.66	5.06	2.28

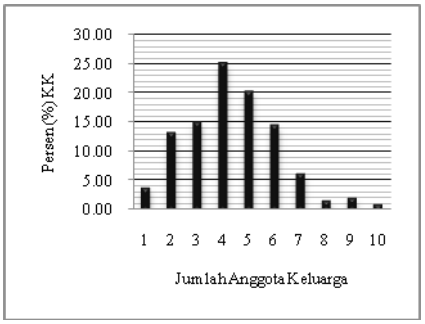
4. Figure III.4 Age range



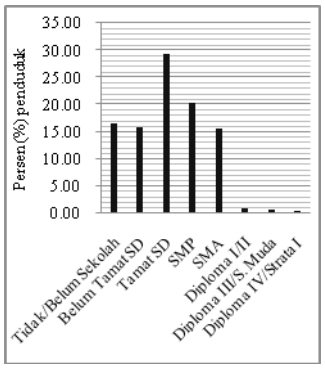
5. Figure III.5 Profil mata pencarian penduduk



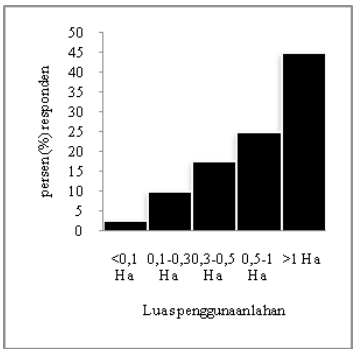
6. Figure III.6 Family size



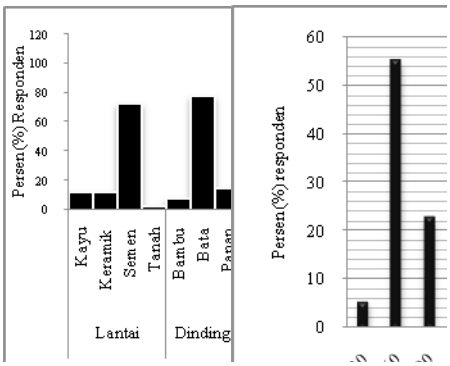
7. Figure III.7 Education



8. Figure III.8 Land Tenure



9. Figure III.9 House Quality



10. Table II.1 Equation allometrik some tree species

Type of Tree	Allometric equation
Branching trees	$B = 0{,}11\rho D^{2{,}62}$
Slash crop (coffe, cocoa)	$B = 0{,}281\ D^{2{,}06}$
Palm (coconut, areca nut)	$B = BA*H*\rho$

B : Tree biomass(ton/ha)
P : Wood density (gram/cm^3)
List of wood density is obtained from the link:
(http://www.worldagroforestry.org/sea Products/AFModels/treenwood/treenwood.htm)
D : DBH (cm)
H : Tree Height (cm)
BA : *Basal area* (cm^2)

11. Tabel III.1 Agroforestry model in Nagari Nan Tujuah

Option	Group	Plant component	Scientific name	Location
Model I (Kakao 3 yr)	-Tanaman pohon pelindung -Tanaman pohon utama -Tanaman semusim	Durian Kelapa Kakao Gardamungu	*Durio zibethinus* *Cocos nufifera* *Theobroma cacao* *Amomum compactum*	Palupuh
Model II (Kakao 5 yr)	-Tanaman pohon pelindung -Tanaman pohon utama -Tanaman semusim	Karet Kelapa Kakao Gardamungu	*Hevea brasiliensis* *Cocos nucifera* *Theobroma cacao* *Amomum compactum*	Palupuh
Model III (Kakao 5 yr)	-Tanaman pohon pelindung -Tanaman pohon utama	Kulit manis Kakao	*Cinnamomum burmanii* *Theobroma cacao*	Palupuh
Model IV (Kakao 7 yr)	-Tanaman pohon pelindung -Tanaman pohon utama -Tanaman semusim	Kulit manis Surian Durian Jengkol Rambai Kakao Gardamunggu	*Cinnamomum burmanii* *Toona sureni* *Durio zibethinus* *Archidendron pauciflorum* *Baccaurea motleyana* *Theobroma cacao* *Amomum compactum*	Palupuh
Model V (Kakao 9 yr)	-Tanaman pohon pelindung -Tanaman pohon utama -Tanaman semusim	Kulit manis Duku Durian Pinang Kakao Gardamungu	*Cinnamomum burmanii* *Lansium Domesticum* *Durio zibethinus* *Areca catechu* *Theobroma cacao* *Amomum compactum*	Palupuh
Monokultur	-Tanaman pohon budidaya -Tanaman semusim	Kakao Pisang	*Theobroma cacao* *Musa paradisiaca*	Nagari Gadut

12. Table III.2 Carbon biomass profile

	Model I	Model II	Model III	Model IV	Model V	Mono-3	Mono-5	Mono-7	Mono-9
Tree Biomass (ton/ha)	52,61	105,49	27,87	73,99	109,55	6,17	30,35	54,85	58,34
Carbon Biomass (ton/ha)	24,08	47,47	12,54	33,30	49,30	2,77	13,66	24,68	26,25
MAI (ton/ha/thn)	7,76	9,49	4,82	5,30	7,18	2,05	5,06	7,84	6,48
Carbon Accumulation Rate (ton/ha/thn)	3,52	4,27	2,17	2,38	3,23	0,93	2,28	3,53	2,92

* MAI: *Mean Annual Increment*

13. Tabel III.3 Model Agreed Proportion

Name of plant	**Number of trees**	**Space of planting (m)**
Cocoa	512	3 x 6
Suren	16	25 x 25
Mahogany	25	18 x 18
Durian	16	25 x 25
Clove	25	18 x 18
Rubber	150	9 x 6

14. Tabel III.4 Comparative financial analysis of cocoa farm per hectare

Type of cocoa panting	**NPV**	**B/C ratio**	**IRR**
Monoculture	70.429.333	6,38	26,13%
Model Agreed	59.424.772	4,18	19,75%
Model I	88.510.620	14,62	26,79%
Model II	94.325.645	15,18	26,93%
Model III	16.296.620	2,60	19,13%
Model IV	64.723.961	20,66	25,49%
Model V	151.736.638	21,47	27,04%

15 Table III.5 Sensitivity analysis feasibility of Agroforestry without / with VCM scheme

Model	NPV, B/C ratio & IRR	With/without VER	VER Price (USD/ton CO2; kurs Rp 8700/USD)		
			6 USD	8USD	10 USD
Monoculture	NPV (Rp/ Ha)(million)	70,43			
	BCR	6,38			
	IRR	26,13%			
Model I	NPV (Rp/ Ha)(million)	Without VER	**18,73**		
		With VER	21,50	22,42	23,35
		Difference	14,78%	19,70%	24,66%
	BCR	Without VER	3,1		
		With VER	4,48	5,27	6,39
	IRR	Without VER	19,03%		
		With VER	19,77%	20,02%	20,27%
Model II	NPV (Rp/ Ha)(million)	Without VER	**47,29**		
		With VER	52,75	54,57	56,39
		Difference	11,55%	15,39%	19,24%
	BCR	Without VER	6,3		
		With VER	16,25	25,1	25,9
	IRR	Without VER	20,84%		
		With VER	21,63%	21,89%	22,15%
Model III	NPV (Rp/ Ha)(million)	Without VER	**16,30**		
		With VER	17,74	18,22	18,60
		Difference	8,80%	11,78%	14,11%
	BCR	Without VER	2,6		
		With VER	3,03	3,21	3,36
	IRR	Without VER	19,13%		
		With VER	19,59%	19,74%	19,86%
Model IV	NPV (Rp/ Ha)(million)	Without VER	**18,25**		
		With VER	22,04	23,24	24,60
		Difference	20,76%	27,34%	34,79%
	BCR	Without VER	4,09		
		With VER	9,48	9,94	10,46
	IRR	Without VER	19,23%		
		With VER	20,33%	20,68%	21,07%
Model V	NPV (Rp/ Ha)(million)	Without VER	**46,85**		
		With VER	52,52	54,40	56,29
		Difference	12,10%	16,11%	20,15%
	BCR	Without VER	5,85		
		With VER	14,14	25,14	25,98
	IRR	Without VER	20,95%		
		With VER	21,78%	22,06%	22,34%
Model Agreed	NPV (Rp/ Ha)(million)	Without VER	**59,42**		
		With VER	67,87	70,68	73,49
		Difference	14,22%	18.94%	23,67%
	BCR	Without VER	4,18		
		With VER	7,62	10,5	11,28
	IRR	Without VER	19,75%		
		Dengan VER	20,57%	20,85%	21,11%

APPLICATION OF MICROSPORE CULTURE TO PRODUCE HOMOZYGOUS LINES OF A NEW HEXAPLOIDBRASSICA SPECIES

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ABSTRACT

*Brassica*species include many important leafy and tuber vegetables, fodder and oilseed crops around the world. *B. napus*,in particular, is an important crop species for edible oil and biofuel. However, *B. napus*has a relatively narrow genetic based due to extensive breeding and a relatively shortdomestication period and cultivation history. All three of the allotetraploid *Brassica* species (*B. napus*, *B. juncea* and *B. carinata*) suffer from this problem, and narrow regions of adaptation. Therefore, we investigated a new hexaploid *Brassica* species which would have a broad genetic base, and combine genetic diversity from all domesticated *Brassica* species to generate new varieties of vegetables, edible oil, fodder and biodiesel, potentially with broader adaptation and tolerance of stress and disease. In this study we used the microspore culture technique, also called doubled haploid technique,to generate diverse populations of pure homozygous lines of putative hexaploid *Brassica*in one generation. Seventeen hexaploid*Brassica* genotypes, resulting from wide crosses, were employed as donor plants. Steps of microspore culture technique, started from bud collection, microspore isolation and embryo development in *Brassica*hexaploid followed Cousin and Nelson (2009) method.

Keywords: *Brassica*, microspore, embryo, hexaploid, doubled haploid

INTRODUCTION

*Brassica*species include many important leafy and tuber vegetables, fodder and oilseed crops around the world. *B. napus*,in particular, is an important crop species for edible oil and biofuel. All three of the allotetraploid *Brassica* species (*B. napus*, *B. juncea* and *B. carinata*) has a relatively narrow genetic based due to extensive breeding and a relatively short domestication period and cultivation history and also narrow regions of adaptation. Therefore, we investigated a new hexaploid *Brassica* species which would have a broad genetic base, and combine genetic diversity from all domesticated *Brassica* species to generate new varieties of vegetables, edible oil, fodder and biodiesel, potentially with broader adaptation and tolerance of stress and disease.

The production of hybrid cultivars requires the use ofhomozygous parental (Yuan *et al*., 2010). Doubled haploid technique has become an important tool in assisting breeding program. Doubled haploid plants reach homozygosity (pure breeding) in one generation and they can be used as parents for hybrids immediately. It is an effective way to shorten the breeding cycle. This technique has proved useful in canola (*Brassica napus)* (Zhou *et al*., 2002).

In this study we used the microspore culture technique, also called doubled haploid technique,to generate diverse populations of pure homozygous lines of putative hexaploid *Brassica*in one generation.

MATERIALS AND METHODS

Seventeen hexaploid *Brassica* genotypes, resulting from wide crosses, were employed as donor plants (Table 1). Hexaploid *Brassica* seedlings were grown and maintained in controlled environment growth room (CER) with 16 hour photoperiod with a light intensity of around 200 $\mu molm^{-2}s^{-1}$. Four replicates, i.e. 4 seedlings of each genotype were grown from seed to first flowering at temperature of 15°C/5°C (day/night). The first flower shoot was then trimmed to induce more flower branches.

About 25 – 90 flower buds were collected from each genotype depend on the number of buds available. Collected flower buds were transferred from growth room to laboratory on ice and were freshly used. It can also be stored at 4ºC within 2-3 days, if necessary.

Steps of microspore culture technique, starting from bud collection, microspore isolation, embryo development, plantlet development and acclimatization in *Brassica*hexaploid followed Cousin and Nelson (2009) method. Isolated microspores were heat treated at 32.5°C for 3 days to induce embryo production.

Colchicine 0.1% was incorporated on culture media to induce chromosome doubling. Ploidy level of donor plants and the progenies were examined using flow cytometry technique.

Table 1.Hexaploid*Brassica* genotypes used for microspore culture

No	Line no	Category	Combination (♀ x ♂)
1	H20-1	Li x Yan	C23-4 (54)/Y54 (54)
2	H04-6	Meng x Li	7H183-3-3 (54) / C23-1 (54)
3	H01-6	Li x Li	C23-4 (54) / C26-1 (54)
4	H21-3	Li x Yan	C23-4 (54)/Y54-2 (54)
5	H02-1	Li x Meng	C23-1 (54) / 7H183-3-2 (54)
6	H03-5	Li x Meng	C26-1 (54) / 7H183-3-3 (54)
7	H16-1	Meng x Yan	7H170-5-1 (54)/Y54-2 (54)
8	H24-1	Yan x Li	Y54-2 (54)/C26-3 (54)
9	H22-1	Li x Yan	C26-1 (54) / Y54-2 (54)
10	H12-1	Li x Cowling	C26-1 (54) / 5xC3 (?)
11	H23-1	Li x Yan	C26-4 (54)/Y54 (54)
12	H18-1	Meng x Yan	7H183-3-4 (54)/Y54-2 (54)
13	H15-2	Cowling x Meng	5xC3 (?) /C23-1 (54)
14	H10-1	Li x Cowling	C23-1 (54) / OP02 (?)
15	H06-4	Cowling x Li	5xC3 (?) / C28-3 (?)
16	H09-3	Li x Cowling	C21-4 (54) / SP50 (?)
17	H11-2	Li x Cowling	C23-3 (54) / SP28 (?)

RESULTS AND DISCUSSION

Number of buds collected was varied among genotypes, from 27 buds on line H06-4 to 90 buds on genotype H20-1 (Figure 1a). This variation occurs due to flower availability on each genotype. Some genotypes grow vigorously and produce more flower buds, while other genotypes grow weaker and produce less flower bud.

Number of microspore plated varied among genotypes, between 1 – 7 plates (Figure 1a). There was a good correlation (correlation coefficient = 0.70) between number of flower buds used for microspore culture and number of microspore plates produced, showing that the more flower bud collected, the more microspore culture/plate produce. Collecting flower buds below 40 buds should be avoided because it produced less than 3 plates. Cousin and Nelson (2009) suggested that about 80 flower buds should be collected for optimum microspore culture.

Number of embryo produced varied among genotypes (Figure 1c; 1d) and it was strongly correlated with number of microspore plates (correlation coefficient = 1), except for H04-6. Small number of embryo produce in this genotype may due to crossing incompatibility of the parent, resulted in weak regenerating capability.

Heat shock treatment was effective to induce embryogenesis in almost all genotypes. Similar results has been reported, in which microspore embryogenesis in broccoli was increasedby a standard heat shock stress treatment of 32.5^{o}C for one day (Dias, 2001). Heat shock has been shown to be an effective trigger in switching the gametophytic development of microspore to the sporophytic pathway, especially in *Brassica* microspore culture (Yuan *et al*., 2010).

Genotype with few numbers of embryos produced such as H04-6 and H18-1 may need a different treatment to induce embryogenesis, such as using alternate heat and cold treatment or incorporation of PEG on the media (Cousin and Nelson, 2009).

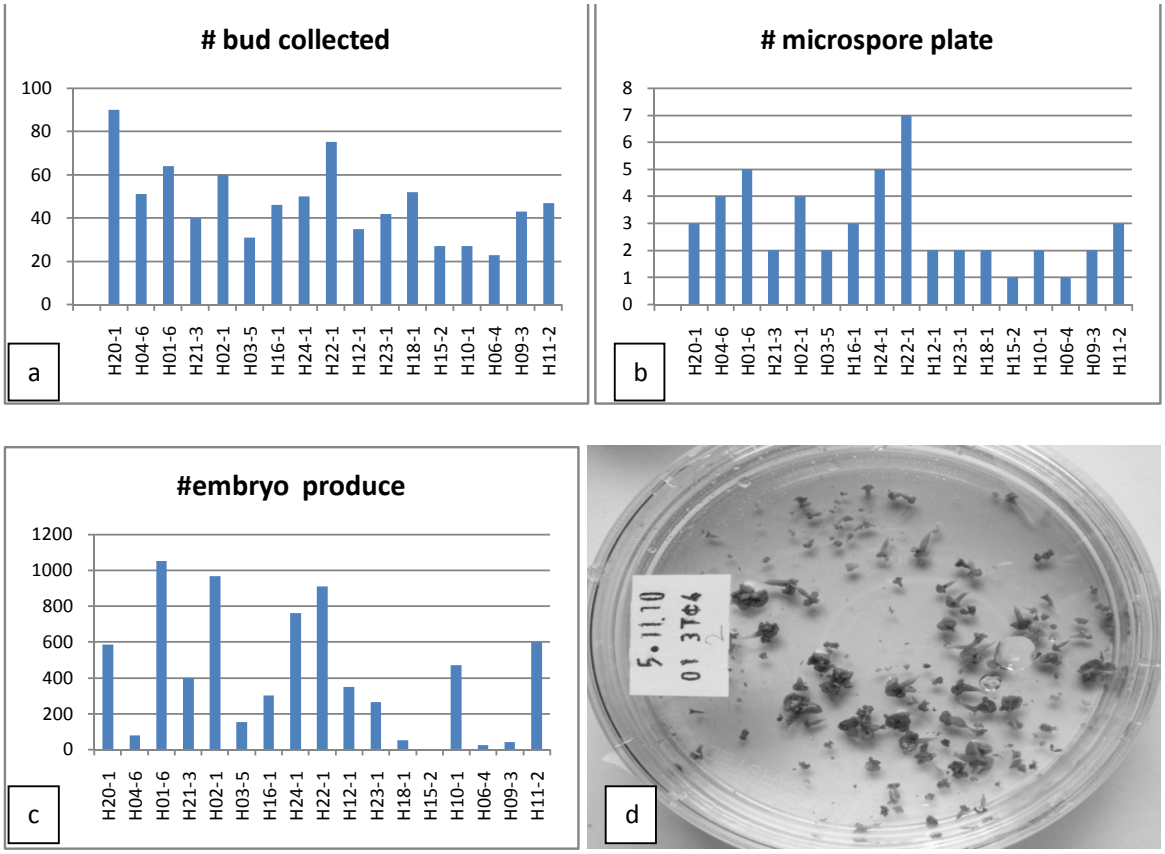

Figure 1. Number of bud collected (a), number microspore plates (b) number of embryo produce from each genotypes (c), picture of embryo produce (d).

CONCLUSION

In conclusion, microspore culture technique was successfully employed to produce emmbryos of putative hexaploid*Brassica* species examined. Further study need to be done to obtained plantlet, and confirmation of chromosome number using flow cytometry technique.

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AGROBACTERIUM-MEDIATED TRANSFORMATION OF AN INDONESIAN WILD ORCHID *VANDA TRICOLOR* LINDL. VAR. *SUAVIS*

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ABSTRACTS

Vanda tricolor Lindl. var. *suavis* is an Indonesian wild orchid which is now extremely rare in nature due to its habitat destruction. Development of an appropriate method for improving *Vanda* orchid through genetic modification could be valuable for horticulture and, indirectly, also for conservation.

The aim of the research was to investigate the efficient method of *Agrobacterium*-mediated transformation for *V. tricolor* orchid. This research investigated that *Agrobacterium*-mediated transformation of protocorms of *Vanda tricolor* orchid can be established with the apllication of acetosyringone and ascorbic acid. The transgene can be integrated in to the plant genome with the transformacy frequency of 4.35%, which was achieved by application of 25 ppm of acetosyringone in inoculation and co-cultivation and then treated the targets with 50 ppm of ascorbic acid in the step of *Agrobacterium* elimination, soon after 3 days of co-cultivation.

Keywords: *KNAT1*, micropropagation, *V. tricolor*, ascorbic acid, acetosyringone

INTRODUCTION

Vanda tricolor Lindl. var. *suavis* is an Indonesian wild orchid which spreads in some places in Indonesia, i.e. West Java, D.I. Yogyakarta (specifically in slope of Mount Merapi), East Java, Bali and Sulawesi (Gardiner, 2007). This species has white - color flower with red / purple / brown spots, and red / purple labellum and also fragrant.

V. tricolor is now to be extremely rare in its habitat due to forest destruction, either caused by natural disaster (volcano eruption) or by over gathering. Genetic transformation of this species could be valuable for improving horticultural traits as well as for conservation.

In this research, genetic transformation of *V. tricolor* Lindl has been established for conservation purposes. This research was encouraged by research done by Semiarti *et al*. (2007). The research by Semiarti *et al.* (2007) found *35S::KNAT1* (*KNOTTED1-LIKE Arabidopsis thaliana*) transformant of *Phalaenopsis amabilis* orchid which has phenotype of multiple shoots. Other previous researches done by Lincoln *et al.* (1994) Chuck *et al*. (1996) and Frugis, *et al.* (1999) also found that overexpression of *KNAT1* gene in several species induced meristematic structures and phenotype of multiple shoots. Based on the results of those previous researches, therefore in the recent research, *KNAT1* gene was transferred in to the plant genome of *V. tricolor* orchid to create transformant plants which has high totipotency. The transformant then can be used as sources of explants for micropropagation, an in vitro method of plant propagation. With insertion of *KNAT1* gene, micropropagation in order to establish ex situ conservation will be more efficient. However, method for transfer gen in to the genome of *V. tricolor* orchid has not yet been established.

Agrobacterium-mediated transformation offers several advantages such as the possibility to transfer only one of few copies of DNA fragments carrying the gene of interest at higher efficiencies with lower cost and the possibility of producing transgenic plants wich are free of marker genes (Kuta and Tripathi, 2005). The aim of the recent research was to investigate the efficient method of *Agrobacterium*-mediated transformation for *V. tricolor* orchid.

MATERIALS AND METHOD

The research was conducted in The Laboratory of Plant Tissue Culture of Faculty of Biology and The Laboratory of Genetics and Plant breeding of Faculty of Agriculture, Gadjah Mada University in 2010

(during a year)..

Transfer of the transgene was done using *Agrobacterium*-mediated transformation and used 8 week-old protocorms as target of transformation. Protocorms were collected from seed culture of *V. tricolor* Lindl. var *suavis* from Bali. Protocorms were then precultured on *New Phalaenopsis* / NP (Islam *et al*., 1998) medium added with 1.5 ppm 2,4D, 3 days before inoculating with suspension of *Agrobacterium*.

The T-DNA harbored the *KNAT1* gene under the control promoter of 35S from Cauliflower Mosaic Virus (*35 S CaMV* promoter) and the *NPTII* gene, a kanamycin resistant gene as selectable marker for transformant selection. This T-DNA was constructed in the binary vector PGreen and transfer to the plant genome mediated by *A. tumefaciens* strain LBA4404 as mentioned in Semiarti *et al*. (2007)(Figure 1).

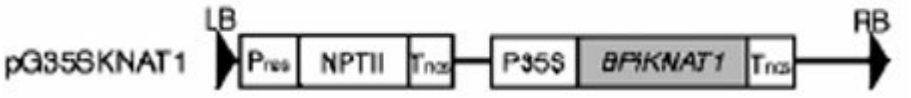

Figure 1. T-DNA structure of *pG35S :: KNAT1*. *KNAT1* gene (1,2kb) was under controlled of 35 S promoter from *cauli flower mosaic virus*(CaMV). RB = *Right Border*; LB = *Left Border*; Pnos = promoter of nopaline synthase gene; Tnos = *polyadenylation site* from nopaline synthase gene; *NPTII* = Neomycin Phosphotransferase gene; P35S = promoter from 35S *CaMV* . *KNAT1* F1 and *KNAT1* R1 are the oligonucleotida primer for amplifying *KNAT1* gene of 1,2 kb (Semiarti *et al*., 2007).

Application of acetosyringone (AS) and ascorbic acid (C6H8O6) was treated during the transformation process. Concentration of 0 (none) and 25 ppm AS were added in the inoculation (30 minutes) and / or co-cultivation (3 days). Ascorbic acid with concentration of 0 (none) and 50 ppm were applied in the step of bacterial elimination (post co-cultivation). Selection for transformants was done using 300 ppm kanamycin. Protocorms that were still green and survive after 5 week on the selection medium were collected and counted. These protocorms then were maintained and grown for 4 weeks in the NP medium added with 5 µM 2-*isopentenyladenine* (2iP), and 0.15 µM *Napthalene acetic acid* (NAA) for shoot induction. Protocorms that were still green after this regeneration step, were judged as transformant candidates. Transformed candidates were confirmed by PCR (polymerase chain reaction) that amplified 1200bp *KNAT1* gene fragment using a pair of *KNAT1* gene specific primer (F1*KNAT1* and R1*KNAT1*, as mentioned in Semiarti *et al.*, 2007).

RESULTS AND DISCUSSION

Table 1 shows the percentage of green protocorms after selection process and after plant regeneration. The percentage of green protocorms after selection and the percentage of candidates transformant increased with application of acetosyringone, and the greatest was found when 25 ppm of AS was applied in both inoculation and co-cultivation. Acetosyringone is phenolic compounds, naturally it is exuded from wounding of tissue of dicots and induces activation of *Vir* genes and subsequently mediates T-DNA transfer in to the plant genome. However, orchid is monocots, while Kuta and Tripathi (2005) stated that monocots do not show the wound response characteristic of the dicot species. Escudero and Hohn (1997) demonstrated that competence of plant cells for *Agrobacterium*-mediated transformation DNA transfer is not necessarily linked to wounding. In this case, exogenous acetosyringone, added in inoculation and co-cultivation medium, replaced the need for wounding. In this research, the greatest percentage of transformant candidates was obtained when 25 AS added in both the inoculation and co-cultivation, compared to the treatment of AS in co-cultivation or in the inoculation only. Theese data indicated that the longer period of contact between *Agrobaterium* and AS achieved greater percentage of transformant candidates, i.e. contact periods of 30 minutes + 3 days (in both inoculation and co-cultivation) > 3 days (in co-cultivation only) and > 30 minutes (in inoculation only). Beside of the role of AS in the induction of *Vir* genes, Kuta and Tripathi (2005) proposed that it is possible that AS also perturb the *Agrobacterium*-induced defense signal transduction events in plant cells, leading to reprogramming of *Agrobacterium*-incompetent cell to a competent one. The effectiveness of AS in the *Agrobacterium*-mediated transformation of orchid

was reported previously for *Dendrobium* (Men *et al.*, 2003), *Phalaenopsis* hybrid (Mishiba *et al.*, 2005), and *Cymbidium* (Chin *et al*., 2007).

The percentage of transformant candidates also increased with application of 50 ppm of ascorbic acid. It increased to be 3 fold (for the treatment of "AS 0 + 0" / without AS) , 10 fold (for "AS 0 + 25" / application of AS in co-cultivation), 4 fold (for "AS 25 + 0" / application of AS in inoculation), and 10 fold (for "AS 25 + 25" / application of AS in inoculation and co-cultivation) compared to those of without ascorbic acid (Table 1).

Ascorbic acid is an antioxidant that can reduce browning in tissue culture (Wu and Toit, 2004; Park *et al*., 2006) and reduce necrosis in *Agrobacterium*-mediated transformation (Enriquez-Obregon *et al*., 1998; 1999). In tissue culture use, Dan (2008) has been classified ascorbic acid as an antioxidant that can reduce tissue browning, and promote organogenesis, somatic embryogenesis, and shoot growth from buds during micropropagation across different plant species. While in the use of ascorbic acid in *Agrobacterium*-mediated transformation, Dan (2008) also concluded that ascorbic acid is a suitable antioxidant to minimize browning/necrosis for both dicotyledonary and monocotyledonary plant species.

Table 1. Effect of Acetosyringone (AS) and ascorbic acid (AA) on the percentage of green protocorms after selection and after regeneration in the *Agrobacterium*-mediated transformation of *V. tricolor*

Treatments				The number of protocorms used	The percentage of green protocorms after 5 weeks selection on 300 ppm kanamycin	The percentage of green protocorms after regeneration (transformant candidates)
Application of AS in the inoculation (ppm)	Application AS in the co-cultivation (ppm)	Application of ascorbic acid in the bacterial elimination step (ppm)	Name of treatments			
0	0	0	AS 0+ 0,AA 0	393	4 (1, 02 %)	1 (0,25 %)
0	25	0	AS 0+25,AA 0	932	28 (3,00 %)	5 (0.54 %)
25	0	0	AS 25+0,AA 0	692	11(1, 59 %)	3 (0,43 %)
25	25	0	AS 25+25,AA 0	561	30 (5,35%)	5 (0,89%)
0	0	50	AS 0+ 0, AA 50	932	31 (3,33 %)	8 (0,86 %)
0	25	50	AS 0+25, AA 50	450	52 (11,56 %)	25 (5,56 %)
25	0	50	AS 25+0,AA 50	498	43 (8,63 %)	9 (1,81 %)
25	25	50	AS 25+25, AA 50	449	83 (18,49 %)	39 (8,69 %)

Dan (2008) proposed that transformation of plant genome using *Agrobacterium tumefaciens* is the exploitation of process of pathogen infection. The initial response of plants to pathogen attack is an oxidative burst with production of reactive oxygen species (ROS) (Wojtaszek, 1997), followed by hypersensitive response (HR) to pathogens leading to necrosis (Greenberg et al., 1994). Production of ROS is a defense mechanism of plants as ROS can kill pathogens (Dan, 2008). Kuta and Tripathi (2005) proposed that *Agrobacterium*-induced ROS production can be quenched with antioxidant, such as ascorbic acid. Dan (2008) stated that ascorbic acid is ROS scavenger.

However, the non-susceptibibility of plant cells to colonization by pathogen (non competent cell) is known to be due to successful recognition of the invading pathogen by the plant cell, which generates an internal signal that triggers early defense responses in the plant cells (Somssich and Hahlbrock, 1998). The earliest defense reaction observed in non-susceptible plant cells following pathogen attack is oxidative burst (Mehdy, 1994). It can be concluded that the greater the defense mechanism of the plant cell, it will be more non susceptible (non-compatible) to be colonized by *Agrobacterium*. In the case of *Agrobacterium*-mediated transformation of *V. tricolor*, the defense mechanism of the plant cells could be relatively high following *Agrobacterium* infection, leading to necrosis and reduction of green protocorms achieved after selection. Therefore, the role of ascorbic acid in preventing browning/necrosis in the recent research is very

significant. However, this is still in question and needs further research to unravel the phenomenon.

Although it is still unclear that why plant cells of *V. tricolor* did have high defense mechanism following *Agrobacterium* infection, however, high total phenolic compound was observed in protocorms of *Vanda* tricolor (data not shown), this may induce greater defense mechanism as a response to *Agrobacterium* infection, as Arditti (1991) suggested that phenolic compound in orchid protocorms may function as a protector against predators and pathogens.

Regardless of those above theory and suggestion, in the recent research, the transgene had been successfully integrated in to the plant genome of *V. tricolor* orchid. When DNA of putative transformant was PCR amplified using specific primer of *KNAT1*, the 1200bp of *KNAT1* fragment was detected in the electrophoregram (Figure 2), indicated that *KNAT1* gene was inserted in to the plant genome.

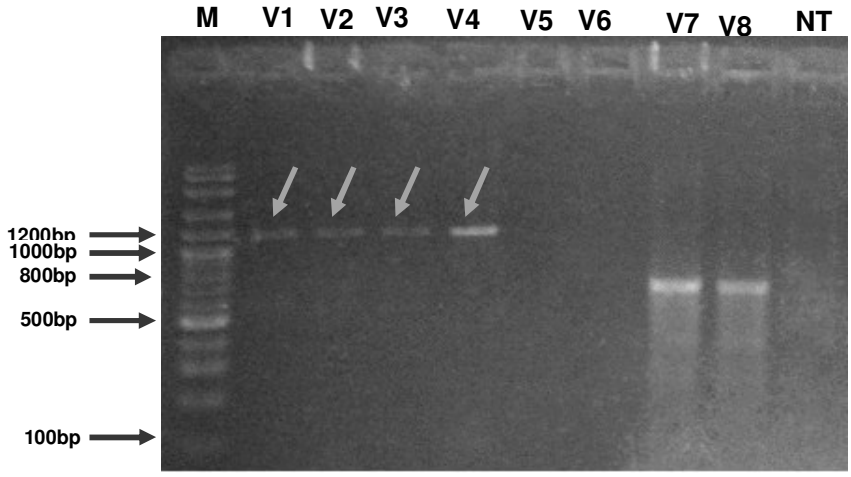

Figure 2. The electrophoregram of PCR amplified DNA of *35S:KNAT1* transformants using specific primer of *KNAT1* (*KNAT1*F1 - *KNAT1*R1). V1-V8 are transformant candidates of *V. tricolor*. Yellow arrows shows 1200bp fragment that amplified from putative transformants (V1-V4). V5-V8 are non-transformants. A non-specific band of 800bp was detected in V7 and V8. M = DNA marker of "100bp Plus DNA Ladder" from Vivantis. NT = non-transformant.

Theese samples of transformant candidates were randomly taken from treatment of "AS 25 + 25, AA 50", that gave the highest percentage of transformant candidates. The frequency of transformation can be calculated to be 4.35 % (4/8 x 8.69%).

CONCLUSION

Agrobacterium-mediated transformation of protocorms of *Vanda tricolor* orchid can be established with the apllication of acetosyringone and ascorbic acid. The transgene can be integrated in to the plant genome with the transformacy frequency of 4.35%, which was achieved by application of 25 ppm of acetosyringone in inoculation and co-cultivation and then treated the target with 50 ppm of ascorbic acid in the step of *Agrobacterium* elimination, soon after 3 days of co-cultivation.

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POSSIBLE ROLE OF CELL WALL DEGRADATION ENZYMES FOR BACTERIOPHAGES INFECTION AND PRODUCTION

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ABSTRACT

The utilization of chemical pesticide have many negative effects, such as the insecticide resistance, resurgence, outbreak of secondary pests and diseases, disappearance of parasitoid and predator, residual effect of food and environmental. Over the past 60 years both the number of agricultural toxicants in the environment and rates of toxin-related diseases have increased dramatically. The one of smart way to break that problem is by utilization the natural enemies. To control diseases caused by bacteria the one advance approach is by bacteriophages. Bacteriophage has been reported infect the bacteria with specific hosts. The mechanism of infection is very interesting study. To break the cell wall of bacteria the bacteriopages produced the many kind of cell wall lytic enzymes. In this review, the cell wall lytic enzymes with specific cleavage site and its role in the bacteriophages infection will be discussed.

Keyword: Cell wall lytic enzymes, biological control, bacteriophages

INTRODUCTION

The number of population and the food consumption is still increasing however the productive land of agriculture is decreasing. In solving this problem, in 1992 UNCED (United Nations Conference on Environmental and Development) recommended a concept of sustainable development (Djuniadi, 2003). Its recommendation is the sustainable development must has the attention to social, economic, and ecological aspect in the local, regional, and global level. On the other hand, the food production during the past decades 1940s has been conducted to use the chemical intensively, such as chemical pesticides and fertilizers (Erickson 2009). The utilization of chemical pesticide releasing the negative effect, such as the insecticide resistance, resurgence, outbreak of secondary pests and diseases, disappearance of parasitoid and predator, residual effect of food and environmental. Over the past 60 years both the number of agricultural toxicants in the environment and rates of toxin-related diseases have increased dramatically. These 'toxicants' are widely distributed in nature and many lack an established NOAEL (no observed adverse effect level) or consensus about long term health effects (Oates and Cohen, 2009).

The one possible way to reduce the utilization of chemical pesticides in agriculture area is by the utilization of natural enemies. Naturally in ecosystem the pests and diseases have enemies, competitors and/or antagonists, such as parasitoids, predators, pathogens, viruses (bacteriophages) (Lacey *et al*., 2001; Loessner 2005; Susila *et al*, 2005; Supartha *et al*, 2005; Sumiartha *et al*, 2006;). In organic farming system to utilize of them is possible to make the ecosystem balance and sustainable.

One advance way to control diseases causing by bacteria is utilize the bacteriophages. The some study about the mechanism of infection of bacteriophages has been reported. Bacteriophage T7 was reported infect the *Escherichia coli* cells, and the role of gp16 cell wall lytic enzyme is beneficial during that infection (Moak and Molineux, 2000). Loessner (2005) reviewed about the application of bacteriophage endolysins (cell wall lytic enzymes). Endolysins in food and in biotechnology has been used, with the specific action. Therefore they offer a unique possibility for the biological control of unwanted bacteria without having any effect on other organisms, such as the natural flora. In medical application the purified preparations of cell wall lytic enzymes could also be used astherapeutic agents, either alone or in combination with classical antibiotics, particularly in external applications (Loeffler *et al*, 2001; Loessner, 2005). However in my knowledge in agriculture area to control bacteria the information and application of bacteriophage cell wall lytic enzymes are limited. For more clear, in this review the possible role of cell wall lytic enzymes in the mechanism of infection of bacteriophahe will be summarized and discussed.

RESULTS AND DISCUSSION

Cell wall lytic enzymes

Cell wall lytic enzymes were reported play many important roles in the life cycle of bacteria, as well as vegetative growth, sporulation and germination. The roles of individual enzymes in each process have been studied by constructing mutant. The one of the best study about cell wall lytic enzymes has been conducted in *Bacillus subtilis. B. subtilis* is the best characterized member of the Gram-positive bacteria, has been studied for more than 50 years as a model of microorganism, in its biochemistry, physiology and genetics. Its genome of 4,215,810 base pairs comprises about 4,100 protein-coding genes, with the origin of replication coinciding with the base numbering start point, and terminus about 2,017 kilo bases. The genome contains at least ten prophages or remnants of prophages. *B. subtilis* is an aerobic, non-pathogenic bacterium, endospore-forming, rod-shaped bacterium, commonly found in soil, water, and in association with plants. (Kunst *et al*., 1997).

The involvement of cell wall degradation enzymes includes peptidoglycan maturation, cell separation, motility and competence, cell expansion, cell wall turnover, protein secretion, differentiation, mother-cell lysis and pathogenicity (Fig. 1) (Foster 1992; Blackman *et al.,* 1998; Smith *et al.,* 2000).

B. subtilis has more than 30 cell wall lytic enzymes. The cell wall lytic enzymes in *B. subtilis* are classified according to their hydrolytic bond specificity as muramidases, lytic transglycosylases, glucosaminidases, amidases, and endopeptidases (Fig. 2) (Shida and Sekiguchi 2005, Sudiarta *et al.*, 2010a, Sudiarta *et al*., 2010b). In applied science, cell wall lytic enzymes, as well as muramidase (lysozyme) have been utilized in wide area (biochemistry, agriculture, textile as well as food industry). In addition some of cell wall lytic enzymes were utilized in medical field, such as for controlling *Staphylococcus aureus.* The advent of *S. aureus* strains that are resistant to virtually all antibiotics has increased the need for new antistaphylococcal agents. An example of such a potential therapeutic is lysostaphin, an enzyme that specifically cleaves the *S. aureus* peptidoglycan, thereby lysing the bacteria (Kumar, 2008; Francius *et al.,* 2008).

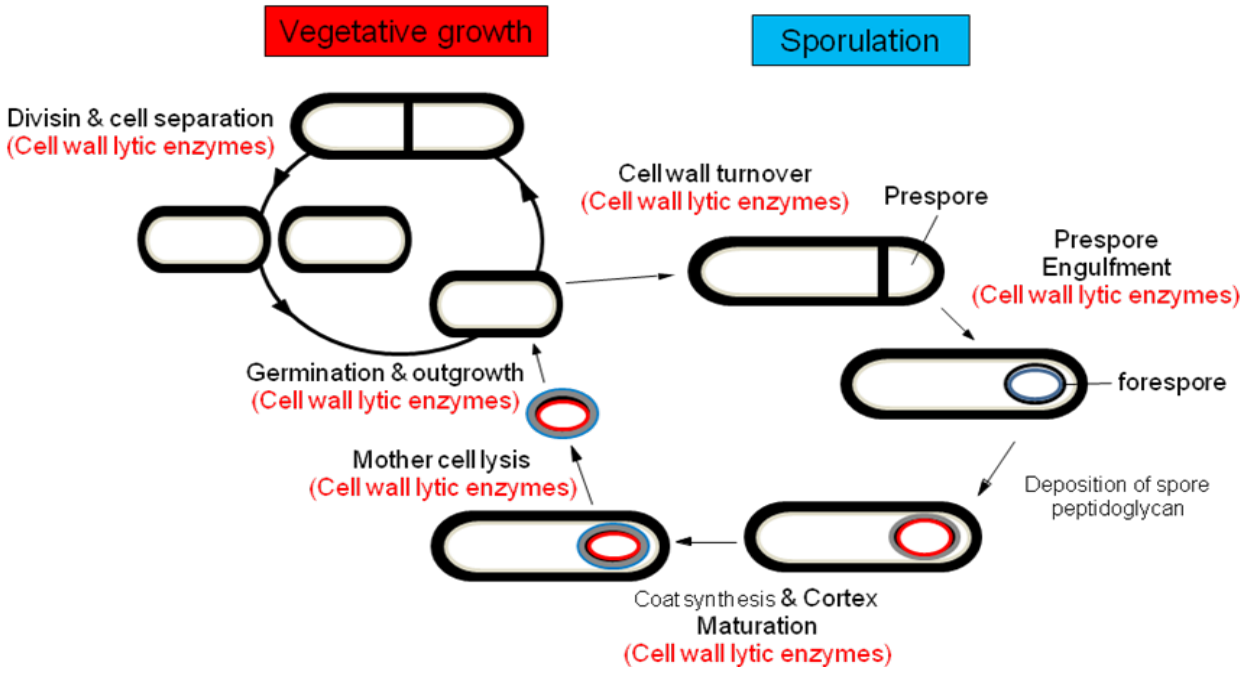

Figure 1. Life cycle of *Bacillus subtilis***.** Important roles of cell wall lytic enzymes in life cycle of *B. subtilis*.

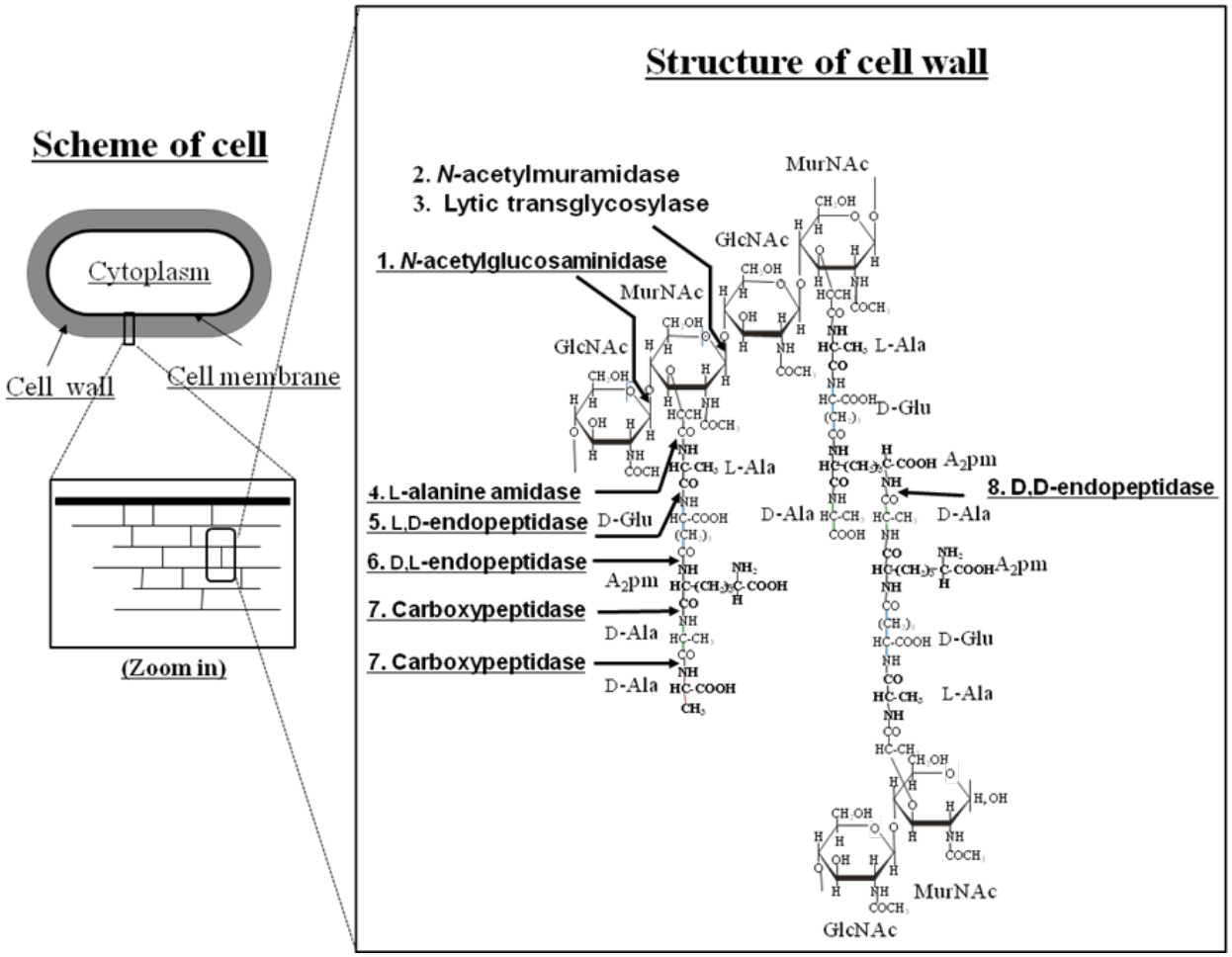

Figure 2. Structure of typical *B. subtilis* peptidoglycan of vegetative cells. The *arrows* indicate hydrolytic bonds attacked by cell wall hydrolases.

Propose the role of cell wall lytic enzymes in bacteriophages infection

On the other hand, the cell wall lytic enzymes are also reported to play important role in phage infection (Fig. 3). Cell wall lytic enzymes (endolysins) are phage-encoded enzyme that degraded the peptidoglycan of host at the terminal stage of the phage production (Loessner, 2005). The gp 13 of bacteriophage Φ29 has cell wall lytic activity and has very importance role for bacterial virus entry (Cohen *et al*., 2009).

The cycle of bacteriophages to infect the microorganisms seems to be simple; adsorption, insertion of nucleic acids, production of nucleic acids and proteins of bacteriophages, and host cell lysis, sequentially. However, the infection of suitable target microorganisms is very specific. Cell wall hydrolases encoded in bacteriophage genomes are involved in host cell lysis (final infection cycle) and in adsorption (facilitation of infection) (first infection cycle) Fig. 3 (Piuri and Hatfull, 2006).

Recently, we reported the possible role of cell wall lytic enzymes (CwlP) related with phage infection in *B. subtilis* (Sudiarta *et al.*, 2010b). The target protein, CwlP, is a phage-related protein whose gene is located in the SP-beta prophage (Regamey and Karamata, 1998). CwlP is the largest protein, comprising 2,285 a.a. (252 kDa), in the prophage region. Since CwlP has a phage-related minor tail domain, it is possible that the protein acts as a tail protein. Recently, Piuri and Hatfull (2006) described that gp17 of tape measure protein (*Tmp*) is the mycobacteriophage TM4 tail protein (1,229 a.a.). The protein contains a cell wall lytic enzyme, and that hydrolysis by the hydrolase facilitates efficient infection of stationary phase cells. Interestingly, Kenny *et al.* (2004) showed that Orf50 of bacteriophage Tuc2009 (906 a.a.) encodes tail-associated cell wall-degrading activity and is involved in infection through cell wall hydrolysis. To deliver the DNA and to break the cell wall of host completely in the end of life cycle, the bacteriophages were reported produced the cell wall lytict enzymes (Fig. 3).

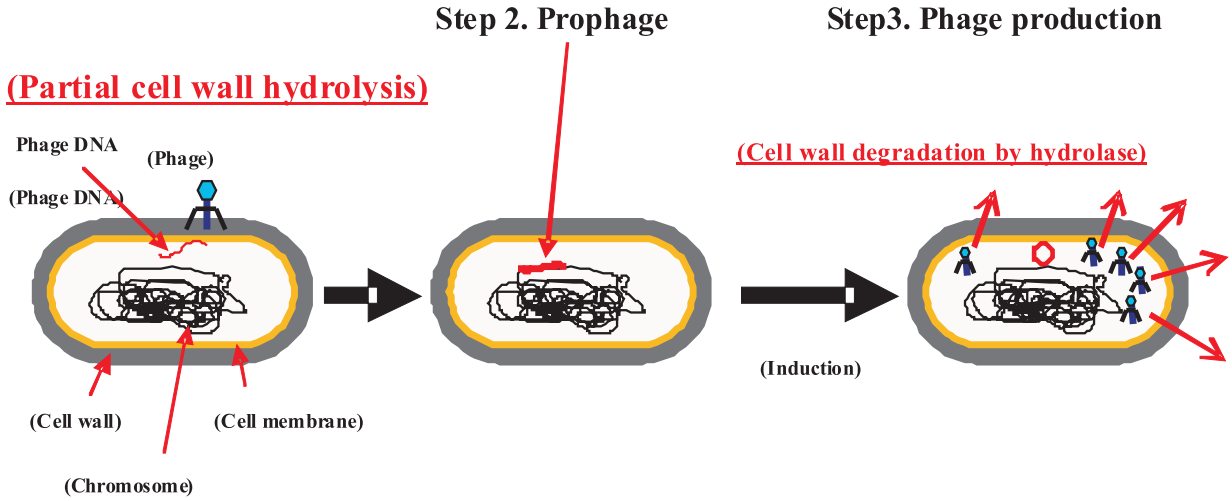

Figure 3. Life cycle of lysogenic phage. Cell wall lytic enzymes play important roles in life cycle of lysogenic phage, at least in the periods of phage DNA insertion into the host cell (step 1) and host cell lysis after phage production (Step 2).

CONCLUDING REMARK

To support the organic farming system and sustainable agriculture the biological control for pests and diseases is essential approach. The biological control by utilization of natural enemies is one of importance step to reduce the utilization of chemical. The advance approach to control diseases causing by bacteria is by bacteriophages. The high technology and depth of knowledge is needed for this way, however is to be simple if understand the specific step of infection mechanism of bacteriophage, as well as the role of cell wall lytic enzymes. That information will be necessary for basic and applied study, particularly to utilization of bacteriophages in agriculture area.

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OPTIMIZATION OF VITAMIN-MINERAL SUPPLEMENTATION IN THE KING GRASS-BASED RATIONS TO MINIMIZE METHANE EMISSIONS IN BALI CATTLE FEEDLOT

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ABSTRACT

Several scientists estimate that livestock contributes up to thirty-seven percent (37%) of the global total methane (CH_4). The majority of sources of methane emissions from cattle is through burping. It can be significantly reduced through the modification of livestock rations. Reduction efforts typically use feed supplements or other food while other efforts have concentrated on changes in the genetic composition of livestock. This study aims to obtain optimal vitamin-mineral supplementation in the king grass-based rations to minimize methane emissions in feedlot of bali cattle. Randomized block design was used in this study consisting of 4 treatments and 5 groups based on differences in steer weight. The treatments tested consisted of: R0 = as much as 5 kg of concentrate + the king grass was given ad libitum; R1, R2 and R3 = R0 + 0.1%; 0.2%, and 0.3% vitamin-mineral (pignox) in concentrate respectively. Variables observed include dry matter intake, methane emissions, energy retention, live weight gain per day and utilization efficiency ration (feed conversion ratio, FCR). The data obtained were analyzed by analysis of variance, if the treatment has a significant effect, then the analysis was continued with an orthogonal contrast test at the level of 5%. Optimal supplementation levels were predicted by the regression analysis. Results showed that vitamin-mineral supplementation significant effect ($P < 0.05$) for all variables observed in bali cattle fed king grass-based rations with vitamin-mineral supplementation. Supplementation level of 0.2 to 0.3% can reduce the dry matter intake. However, vitamin-mineral supplementation from 0.1 to 0.2% can increase the retention of energy by 12%, efficient utilization of the ration increased to 16%, pressing the methane emissions of up to 18%, and live weight gain can increase up to 14% (0.58 vs 0.66 kg/day) than steer without supplementation. Based on regression analysis, obtained the level of vitamin-mineral supplementation optimal 0.16% to produce methane emissions at a minimum, maximum energy retention and live weight gain was maximal in Bali cattle feedlot fed king grass-based rations. The results can be concluded that vitamin-mineral supplementation 0.1 - 0.2% in the king grass-based rations can reduce methane emissions by 18%, improve the efficiency of utilization of the ration, increasing the live weight gain of cattle, and obtained the optimum supplementation of 0.11% which produces the minimum methane emissions 33.72 mM.

Keywords: *supplementation, vitamins, minerals, methane, king grass, Bali cattle.*

INTRODUCTION

Methane is a significant greenhouse gas. The emission of greenhouse gases (GHG) including methane from bovines and other ruminants is believed to be a significant contributor to global warming. Some scientists estimate that livestock contributes up to thirty-seven percent (37%) of the total global methane (CH_4) budget. One ton of methane is equivalent to approximately 21 to 22 tons of carbon dioxide with respect to its global warming potential. As a result, the reduction of one ton of methane emissions can be considered as achieving a reduction of 21 or more tons of carbon dioxide and can generate about 21 tons of carbon credits (as carbon dioxide) in the evolving Greenhouse Gas (GHG) market. Moreover, methane has about a 12 year half life in the atmosphere and is increasing worldwide at an annual rate of around one half of one percent (0.5%) per year. Accordingly, reduction of presently excessive methane emissions into the atmosphere is most desirable.

Ruminants emit methane (CH_4) as a natural part of their digestive processes. A dairy cow can produce as much as 500 L per day. Methane is a potent greenhouse gas, 21 times as effective as CO_2 in its 'radiative forcing'. If the calculated methane production in Bali originating from bali cattle with a population currently at 637,473 head, then the methane gas production by 318 736 500 L per day, a high figure to contribute to global warming. Methane production does not include other ruminants such as goats and buffaloes.

Methane emission from bovine sources, of which the majority is through belching, can be significantly reduced through modification of cattle diet. Attempts at reduction typically involve using nutrient blocks or other feed supplements while other efforts have concentrated on modification of the genetic composition of the animal herd.

Greenhouse and Agriculture Australian Greenhouse Office (2006) has recommended that the GHG emissions reduction strategy called Best Practice Development, including through improved nutritional quality, the optimization of feed intake and feed digestibility and rumen manipulation. Meanwhile, scientists at the Rowett Institute of Nutrition and Health (RINH) have led two major EC initiatives to discover plants and plant extracts that, when added to the feed, could lower the methane emissions of cattle and sheep. Plants such as *Rheum nobile*, *Allium sativum* (garlic), and *Salix caprea* (goat willow) can suppress methane emissions 25% respectively.

Another strategy adopted by scientists is using the 'hydrogen sink' compounds that can divert H_2 away from the forming microbial methane. This compound is fumaric acid, a natural metabolite of the Krebs cycle that is found in all animals. Rumen bacteria convert fumaric acid into succinic acid using the H_2. So, less H_2 is available for the production of methane by the forming microbial methane. Succinic acid is converted to propionic acid, which is a normal product formed by rumen bacteria and are absorbed into the tissues of animals to form glucose and amino acids. However, using fumarate as a feed additive may pose a problem because cattle are too acidic stomach. To overcome these problems are designed slow-release fumaric acid mechanism. The results of experiments on sheep showed that the formation of methane can be reduced 75% and feed conversion efficiency increased 10%, but the results of this study did not succeed in cattle.

Loss of energy in the form of methane is significantly decreased utilization of energy for cattle. Therefore the technology of feeding in cattle that are easy to apply to suppress methane emissions is needed. Technology supplementation in the ration has been shown to increase the efficiency of feed energy utilization in Bali cattle (Partama *et al*., 2010b), but very few studies are available on methane production in bali cattle. This study aims to obtain vitamin-mineral supplementation is optimal in the King grass-based rations to minimize methane emissions in feedlot of bali cattle.

MATERIALS AND METHODS

This study consisted of a series of field and laboratory experiments. Field trials conducted in the Village Serongga, district of Gianyar, Gianyar regency. Laboratory tests were conducted at the Lab. Nutrition and Food Livestock, Faculty of Animal Husbandry, Udayana University, and Lab. Analytical Chemistry, Udayana University. Experiments were conducted during six months, starting from the preparation up to the observation or measurement activities in the field.

The experiment was conducted in individual cages. Cage was designed to meet the maintenance requirements of fattening Bali cattle. Required 20 individual cages to accommodate 20 steers of Bali cattle with an average live weight of 319 kg or with the range of 279-367 kg. Cattle were randomly placed in individual cages with a capacity of one steer per cage and given ration treatment according to the experimental design used.

Ration materials consist of, King grass, concentrates and pignox (commercial product as a source of vitamins and minerals). The materials used to formulate diets with cedar multi vitamin and mineral supplementation differently.

Ration treatments consisted of King grass and concentrates supplemented with pignox. There are four treatments in concentrate rations for four treatments ie S0, S1, S2, and S3. Concentrate on S0 is without adding pignox, while concentrate on the S1, S2, and S3 supplemented with as many consecutive pignox 0.1%, 0.2% and 0.3% (Table 1).

Provision of rations conducted two times a day in the morning at 9:00 and lunch at 14.00 pm. Given 5 kg of concentrate per cattle per day, divided into the two times of the morning and afternoon, as mentioned above. King grass given unlimited (ad libitum). The concentrate is given first and followed the King grass on every provision of both morning and afternoon. Similarly, water was supplied ad libitum provided separately with the feeding.

Table 1. Nutrient content of diets material experiment.

Nutrient	Concentrates S0	S1	S2	S3	King grass
Dry matter (%)	87,64	87,64	87,64	87,64	24,80
Organic matter(%)	64,13	64,13	64,13	64,13	71,84
Crude protein (%)	12,13	12,13	12,13	12,13	5,01
Crude fiber (%)	7,76	7,76	7,76	7,76	27,20
Energy (GE, Mcal/kg)	3,22	3,22	3,22	3,22	3,39
Sulfur (S, ppm)	685,50	694,09	702,68	711,28	-
Zinc (Zn, ppm)	45,09	65,09	85,09	105,09	26,12

Description: S0, S1, S2 and S3 = vitamin-mineral supplementation in concentrate respectively 0%, 0.1%, 0.2% and 0.3%.

Experimental Design

This research used randomized block design with four treatments ration S0, S1, S2, and S3 and five groups based on the weight of livestock. Thus there were 20 male bali cattles in this experiment. One unit is the first experiment male Bali cattle were randomly placed in individual cages in accordance with the experimental design. Dietary treatments were: S0 = 5 kg concentrate + King grass was given ad libitum; S1 = S0 + 0.1% pignox in concentrate; S2 = S0 + 0.2% pignox in concentrate; and S3 = S0 + 0.3% pignox in concentrate.

Observed Variables

Variables observed are dry matter intake and energy intake, methane gas emissions, retention of energy, live weight gain of steers, and feed efficiencies (FCR = feed conversion ratio).

Variables Measurement Procedure

Live Weight Gain. Live weight gain of cattle is known by calculating the difference in initial weight final weight. Furthermore average daily live gain weight can be detected by dividing the weight difference with the time trial. Livestock weight was measured every two weeks once in the morning before the animals were given food and water. Weighing is done by using electronic scales with a capacity of 1000 kg with accuracy 100 g.

Dry Matter and Energy Intake. Dry matter intake was measured by calculating the difference between the amount (weight) given the remaining rations are not consumed. Steaming feed intake is done every day until the experiment ended. Ration energy content be determined by adiabatic bomb calorimeter. Furthermore, the amount of energy intake can be detected by using the equation:

Energy intake = total feed intake X % of dry matter X energy content of rations.

Methane Gas Emissions. Methane gas emissions (M) can be calculated using the formula: M = 0.5 A - 0.25 P + 0.5 B with the understanding M = Methane gas, A = Acetic acid, P = propionate acid, and B = butyric acid. Methane energy value (Mcal) = 0.2108 X mol Methane (Orskov and Ryle, 1990). Analysis of individual VFA levels were measured by gas chromatography technique. Rumen fluid immediately disentrifius with speed 10000 rpm for 15 minutes at a temperature of 4 º C to obtain supernatants. A total of 2 ml supernatant was taken with a pipette to be inserted into a small plastic tube is closed. Into these tubes 30 mg 5 sulphosalicylic acid (C_6H_3 (OH) $SO_3 2H_2O$) was added, then whipped. Then disentrifugation was done at 3000 rpm for 10 minutes at a temperature of 4 º C, then filtered with melipori order to obtain clear liquid. A total of 1 ml of fluid was injected into the gas chromatography, the previously injected standard solution VFA. Concentrations of individual VFA (cM) rumen fluid samples can be calculated using the following formula:

(cM) = (High Sample / High Standard) X Standard Concentration

Energy Retention. Energy retention be calculated by deposition of nutrients (fat, protein, and minerals) and deposition of nutrients can be calculated by converting live weight gain with body composition. Based on this nutrient deposition can be calculated the energy retention with the provisions of 1g of fat deposition is equivalent to 9.32 kcal, while the deposition of 1 g protein, equivalent to 5.5 kcal (Ørskov and Ryle, 1990). So the retention of energy per day per cattle can be calculated by summing the energy content of the deposition of body fat and protein per cattle per day.

Data Analysis

The data obtained were analyzed by variance. When there was a significant effect on treatment response variables, it was then followed by orthogonal contrast test at level 5%. Regression analysis was used to determine the optimal mineral-vitamin supplementation to obtain methane emission the minimum and live weight gain the maximum of bali cattle in accordance with their genetic potential (Steel and Torrie, 1986).

RESULTS AND DISCUSSION

RESULTS

Effect of Vitamin-Mineral Supplementation on Feed Intake

Vitamin-mineral supplementation significantly ($P<0.05$) effect on dry matter intake, Supplementation of 0.1% showed no significant difference compared with no supplements, but the dry matter consumption decreased significantly at 360-470 g in the vitamin-mineral supplementation from 0.2 to 0.3% (Table 2).

Energy intake is also affected by vitamin-mineral supplementation in cattle fed basic ration king grass. Ration energy intake in cattle fed the highest vitamin-mineral supplementation of 0.1% in concentrate, but not significantly different from cattle fed a control diet (Table 2). The range of energy intake is 247.33 to 271.52 Kcal/kg$W^{0.75}$/d, equivalent to 17.8 to 19.6 Mcal per cattle per day, when the average weight of cattle is 300 kg.

Table 2. Dry matter intake, emission methane, retention of energy and live weight gain of bali cattle fed King grass-based rations with vitamin-mineral supplementation.

Variables	Supplementation Treatment			
	S0	S1	S2	S3
Dry matter intake (kg)	6,65[b]	6,58[b]	6,29[a]	6,18[a]
Energy intake (Kcal/kg$W^{0,75}$/d)	271,52[b]	267,47[b]	254,54[a]	247,33[a]
Methane (mM)	42,56[b]	34,99[a]	39,41[a]	64,10[c]
Methane energy (Kcal/kg$W^{0,75}$/d)	15,45[b]	12,46[a]	14,07[a]	22,71[c]
Retention of Energy (NEp, Kcal/kg$W^{0,75}$/d)	31,14[a]	34,92[b]	34,64[b]	32,06[a]
Feed Conversion Ratio (FCR)	11,48[b]	10,06[a]	9,68[a]	10,18[a]
Live weight gain (kg/d)	0,58[a]	0,66[b]	0,65[b]	0,61[a]

Description: The variable is similar to the number with superscript not the same, significantly different at $P<0.05$ when compared with orthogonal contrast test; NEp = Net energy for production (live weight gain of cattle); FCR = total dry matter intake divided by live weight gain per day of Bali cattle ; S0, S1, S2 and S3 = vitamin-mineral supplementation in concentrate respectively 0%, 0.1%, 0.2% and 0.3%, $W^{0,75}$ = metabolic weight of cattle; d = day.

Effect of Vitamin-Mineral Supplementation on Methane Emission and Energy Utilization

The results of this study showed that vitamin-mineral supplementation of 0.1 to 0.2% in concentrate to reduce methane emissions at the Bali cattle fed King grass-based rations (Table 2). Vitamin-mineral supplementation at 0.1% in concentrate to reduce methane emissions up to 18% compared with cattle without supplementation (34.99 mM vs. 42.56 mM). However, increasing levels of supplementation up to 0.3% of methane emissions increased by up to 51% of cattle without supplementation (64.10 mM vs. 42.56 mM). There is a clear relationship between vitamin-mineral supplementation with methane emissions of bali cattle as following regression quadratic equation: $Y = 42.976 – 172.88\ X + 806.4\ X^2$ with a coefficient of determination (R^2) = 0.816* with the understanding of X = supplementation of vitamin-mineral (%) , Y = methane concentration (mM) as seen in Figure 1. From this regression equation can be predicted optimal vitamin-mineral supplementation was 0.11% which produces the minimum methane emissions was 33.72 mM.

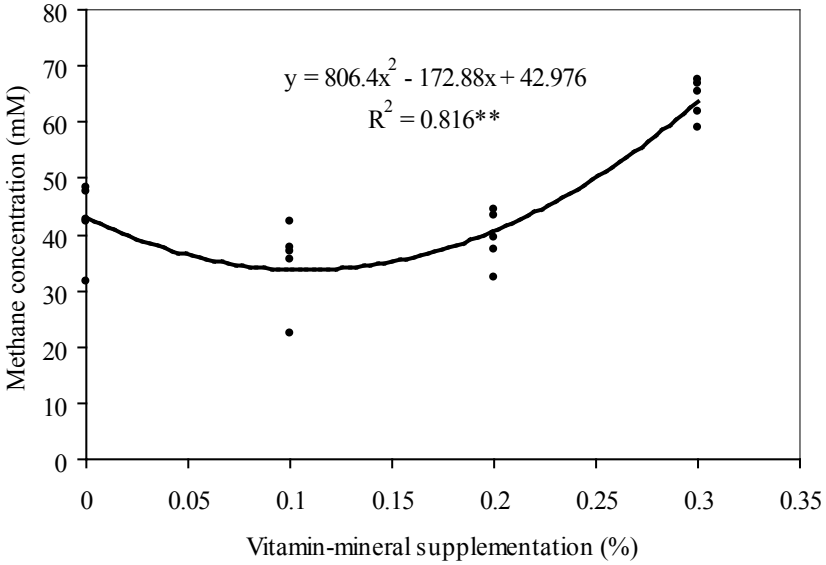

Figure 1. The relationship between vitamin-mineral supplementation with methane emissions of bali cattle fed King grass-based rations.

Vitamin-mineral supplementation in concentrate significantly affect on the energy lost in the form of methane gas. Increased levels of supplementation up to 0.2% can reduce the energy of methane, but supplementation of 0.3% can increase the energy of methane (Table 2). The energy lost in the form of methane ranged from 12.46 to 22.71 Kcal/kg$W^{0.75}$/day, equivalent to 0.9 - 1.64 Mcal/head/day when the average weight of 300 kg of Bali cattle.

Energy retention to live weight gain of cattle is also influenced significantly by vitamin-mineral supplementation. Vitamin-mineral supplementation up to 0.2% in concentrate level can increase the energy retention was significantly ($P < 0.05$) in Bali cattle fed King grass-based rations (Table 2). Supplementation at 0.3% level in concentrate can also increase the retention of energy, but the increase was not statistically significant. Energy retention to live weight gain of Bali cattle in this study ranged from 31.14 to 34.92 Kcal/kg$W^{0.75}$/day equivalent to 2.42 to 2.52 Mcal /head/day, when the average weight of 300 kg of Bali cattle. The highest energy retention was in cattle with supplementation of 0.1% in concentrate and the lowest was in cattle without supplementation.

Ration utilization efficiency is also affected by vitamin-mineral supplementation. Vitamin-mineral supplementation can improve the efficiency of feed utilization in Bali cattle characterized by increasingly low value of FCR (feed conversion ratio). In Table 2 shows the lowest FCR values in cattle with supplementation of 0.2% and the highest in cattle without supplementation.

Vitamin-mineral supplementation in concentrate may increase the live weight gain of Bali cattle fed King grass-based rations, but supplementation of 0.2 to 0.3% levels did not differ significantly with live weight gain of Bali cattle fed a control diet (Table 2). The highest live weight gain of Bali cattle was reached at 0.1% supplementation and the lowest on the control diet. There is a clear relationship between vitamin-mineral supplementation with live weight gain of Bali cattle as following regression quadratic equation: $Y = 0.583 + 0.959\ X - 2.95\ X^2$ with a coefficient of determination (R^2) = 0.414* with the understanding of X = supplementation of vitamin-mineral (%) , Y = live weight gain of Bali cattle (kg/day) as seen in Figure 2. From this regression equation can be predicted optimal vitamin-mineral supplementation was 0.16% which produces the maximum live weight gain of Bali cattle was 0.66 kg / day.

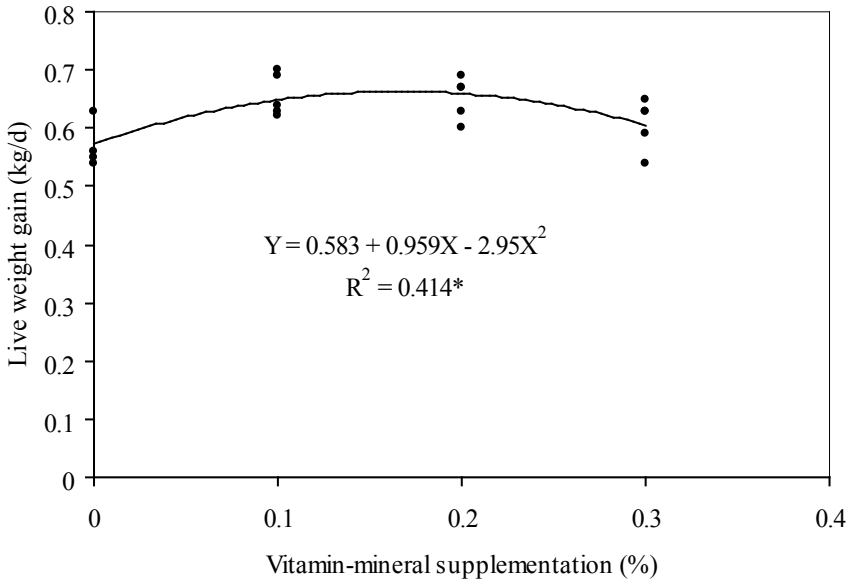

Figure 2. The relationship between vitamin-mineral supplementation with live weight gain of Bali cattle fed King grass-based rations.

DISCUSSION

In principle, feed intake in animals is to meet the nutrient and energy needs. Livestock will stop eating when energy needs are met or stomach has been filled by the feed nutrient needs exceed the capacity, although not yet fulfilled. Level of feed intake is influenced by physiological status of livestock, feed quality and palatability. To obtain the optimal level of consumption required ration formulations that match the needs of livestock rations containing adequate and balanced nutrient.

Ration is attempted in this study meets the nutrient needs of ration ingredients consisting of 5 kg of concentrate, grass king was given ad libitum or given an average of 15 kg per cattle per day, so that these rations containing dried material (BK) 40.51%, crude protein (CP) 10.31%, and energy (GE) 3.27 Mcal / kg, equivalent to 56.29% levels of TDN (Total Digestible Nutrients). However, the results of this experiment showed that feed intake and nutrient levels such as dry matter and energy decreased when given vitamin-mineral supplementation in concentrate, especially the level of supplementation of 0.2 to 0.3% caused a decrease in consumption statistically significant (Table 2). This decline in consumption was caused by an imbalance of nutrients in diet, especially minerals. This causes the excess mineral supplementation, especially Zn in cattle's digestive tract that can cause metabolic disturbances are loss of appetite, reduce the accumulation of Fe and Cu in the liver and increase spending on S in the feces. Thus, this will suppress metabolism disorders feed intake and nutrients. Decrease in consumption level has not yet led to nutrient-deficient cattle and this can be proved by the accretion of a cattle live weight during the experiment, although feed intake decreased with increasing levels of vitamin-mineral supplementation (Table 2).

Vitamin-mineral supplementation from 0.1 to 0.2% in concentrate to give a positive influence on energy utilization and productivity of Bali cattle fed King grass-based ration are characterized by higher energy retention and live weight gain (Table 2). This is supported by the levels of ammonia ($N\text{-}NH_3$) and propionate acid is higher, lower methane emission and rumen microbial protein synthesis is higher at the level of supplementation from 0.1 to 0.2%. Rumen microbial protein as a major source of amino acids for the host animal, so the higher rumen microbial protein production of higher protein deposition in the body of cattle. Similarly, the higher the acid production propionat higher nutrient deposition in the form of body fat. Meanwhile, the lower the methane gas production means less energy is wasted so that more energy is stored in the form of animal protein and fat body (Partama *et al*., 2010a).

The high methane production in cattle without supplementation and cattle with vitamin-mineral

supplementation of 0.3% in line with crude fiber digestibility coefficients (Partama *et al*,. 2010a). The higher the crude fiber digestibility coefficients were in line with the higher production of methane gas. These data indicate that the pattern of fermentation leads to the portion of acetic acid of a larger line with the increased production of methane gas because crude fiber is structural carbohydrate which the result of fermentation more acetic acid (Arora (1995). Methane production in cattle with high levels of supplementation of 0.3% probably due to the existence of advanced fermentation process so many of liberate H^2 utilized by methane-forming microbes such as *Methanobacterium ruminantium*, and *Methanobacterium mobilis* (Arora, 1995).

Retention of energy for livestock production is associated with deposition of nutrients (Partama *et al*., 2010). The results of this study show that the increase of retention of energy for live weight gain of bali cattle is supported by a higher nutrient deposition as well (Partama *et al*., 2010). This is understandable because the fat and protein the body can be converted into energy which the energy content of one gram of fat and one gram of protein, respectively 9.32 and 5.5 kcal/g (Orskov and Ryle, 1990).

The high energy retention and live weight gain in cattle with vitamin-mineral supplementation of 0.1% due to the sufficient and balanced nutrients in the ration. Concentrate with a vitamin-mineral supplementation 0.1% containing ratio of N:S are balanced, and contains enough minerals Zn and S (Table 1). S is an essential mineral in amino acids synthesis contains sulfur, and is needed in large numbers to microbial protein synthesis. Meanwhile, Zn minerals were involved in metalo enzyme synthesis such as DNA and RNA polymerase, alkaline phosphatase, amylase and neutral protease (Jouany, 1991). Thus, this strongly supports rumen microbial protein synthesis and its activity so that the feed material entering the rumen degraded to produce energy efficiently utilized with a low indicator of wasted energy in the form of methane gas.

The research was supported by $N\text{-}NH_3$ concentration sufficient to offset the relatively high concentration of VFA (from 166.33 to 198.06 mM) would strongly support the efficiency of rumen microbial protein synthesis. $N\text{-}NH_3$ is the result of protein degradation, while the VFA degradation products from carbohydrates in the rumen (Partama *et al*., 2010a). The balance between these two components is an ideal prerequisite for the optimization of microbial protein synthesis. Stern *et al* (2006) states that the rumen bacteria can use protein and carbohydrates as energy sources. Carbohydrate is the main energy source for bacteria, and can also be used as a carbon skeleton that combines with ammonia (NH_3) to rumen microbial protein synthesis. The high rumen microbial protein synthesis will be followed by high energy retention and low emission of methane (Partama *et al*., 2010a)

Vitamin-mineral supplementation of 0.1% in concentrate to give the best effect in the process of fermentation and microbial protein synthesis in Bali cattle fed King grass-based rations (Partama *et al*., 2010). . The highest concentration of $N\text{-}NH_3$ followed by the highest propionate acid and the lowest concentration of methane gas caused the highest rumen microbial protein synthesis compared to other treatments. This shows that supplementation at the level of 0.1% produce rations contain enough nutrients and balanced. Mineral content of Zn to 65.09 ppm (Table 1) in concentrate, slightly higher than the recommendation Georgievskii (1982), ie 40-60 ppm is based on the needs of the cattle outside Indonesia. This means that it is true especially of mineral nutrient requirements of Zn in Bali cattle is higher than the cattle in foreign countries. Zn minerals needed by cattle small amounts (micro minerals), but it has a very important function in the various activities of enzymes in the body of cattle.

CONCLUSIONS

The results can be concluded that vitamin-mineral supplementation 0.1 - 0.2% in the king grass-based rations can reduce methane emissions by 18%, improve the efficiency of utilization of the ration, increasing the live weight gain of cattle, and obtained the optimum supplementation of 0.11% which produces the minimum methane emissions 33.72 mM.

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YIELD OF THREE CASTOR (*Ricinus communis* L.) HYBRID VARIETIES TREATED WITH DIFFERENT RATES OF FERTILIZER

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ABSTRACT

The biggest challenge in growing energy crop of castor (*Ricinus communis* L.) as an industrial crop is to improve its daily productivity. Hybrid varieties have many been suggested as one of the ways to overcome the problem. This study aimed at testing yielding ability of three Chinese hybrid varieties of castor grown on dryland and treated with different rates of NPK (15-15-15) fertilizer. The varieties were Zhibo-5, Zhibo-7 and Zhibo-8 and the fertilizer rates were 0 g plant^{-1}, 125 g plant^{-1} and 250 g plant^{-1}. The experiment was conducted during the rainy season of 2010/2011, from February to May, 2011. There were 300 plants in each treatment and 10 plants from each treatment were taken as sample plants. The results show that most of the plants in zero fertilizer treatment failed to grow and those able to grow failed to produce panicle. Zhibo-5 treated with 250 g plant^{-1} of NPK fertilizer produced moderate number of panicles per plant but high in panicle length, panicle weight and number of fruit per plant. The highest seeds dry weight per plant (543.6 ± 92.8 g plant^{-1}) was also produced by Zhibo-5 treated with 250 g of fertilizer while the lowest (205.2 ± 92.1 g plant^{-1}) was also produced by the same variety treated with 125 g of fertilizer. The highest 1000 seeds weight (349.9 ± 29.6 g) was produced by Zhibo-7 treated with 250 g fertilizer and the lowest (252.7 ± 30.7 g) was in Zhibo-8 treated with 125 g fertilizer. In all, the three hybrid varieties require high input of fertilizer and may not as a good option to improve castor daily productivity.

Keywords: *energy crop, annual, productivity*

INTRODUCTION

Climate change has become a hot issue and government of all over the world has spent so much money to study this issue. Fossil fuels and coal burning for industries and transportation as well as high deforestation rate, that lead into high CO_2 concentration in the air, are considered as the main cause of the climate change. The use of renewable energies to replace fossil fuels uses in order to reduce CO_2 emission and to sustain the fossil fuels availability, has been intensified. One of the renewable energies is biofuel. Unfortunately, recent data shows that current world biofuels production is still low, less than 2% of the total demand (Zah and Rudy, 2009). In Indonesia, palm oil (*Elaeis guineensis*) and physic nut (*Jatropha curcas* L.) have long been utilized as feedstocks for biofuels. However, palm oil plantation in Indonesia has a bad record because of deforestation activities (Zhou and Thompson, 2009), while physic nut is considered as a high water foot print plant (Garbens-Leenes *et al*., 2009).

One of the non-food crops that is catagorized as an energy crop as well as an industrial crop is castor (*Ricinus communis* L.). Castor oil was reported as a good source of biodiesel (Baldwin and Cossar, 2009; Barbosa *et al*., 2010). Other uses of castor oil are as lubricans, plant-based paint, natural coating in chemical industries and cosmetics (Derksen *et al*., 1995; Johnson Jr., 2007). Castor plant stem was also reported as a good source of particle board, even though the quality is not the best (Grigoriou and Ntalos, 2001). Based on that many uses, castor has been extensively grown as an industrial crop in countries like China, India and Brazil. In Indonesia, castor is mainly grown in East Java and West Nusa Tenggara. Considering the high demand of biofuels and the many uses of the castor oil, castor has a good future market.

Most cator plants grown in Indonesia are perennial. There are three national variety available in Indonesia, namely Asembagus-22 (ASB-22), Asembagus-60 (ASB-60) and Asembagus-81 (ASB-81). The rests are mainly local variety, such as Beaq Amor, Klikit Kayangan and Gundul Bayan, that are mainly found on drylands Lombok. There are at least two basic problems in growing perennial castor varieties on drylands. The first problem is short period of rainy season. With this condition, the best plant productivity is limited only during the rainy season only and the rest of the year the castor plants tend to shed their leaves, and hence no yield is expected. The second problem is that this plant requires many harvests. Time lag between the first panicle harvest and the second and the third can be two weeks. This harvest practice costs a lot of labours that make perennial castor daily productivity is low. Castor varieties with shorter growing period (annual like) with high productivity is suggested to be grown on drylands to improve the

plant daily productivity (Anjani, 2010).

Some annual castor hybrid varieties have been developed in China, such as Zhibo-5, Zhibo-7, Zhibo-8. The adaptability of these hybrid varieties on dryland of Pringgabaya, Lombok has been reported by Jaya and Hadi (2010). In that study, all the hybrid varieties were given 240 g of NPK (15-15-15) fertilizer per plant. That dosage is considered as high, and since the castor population was 4.444 plants Ha^{-1}, in 1 Ha was applied more than 1 ton of fertilizer. Considering the price of NPK fertilizer is IDR 115.000 per 50 kg, the cost of fertilizer only will be around IDR 2.500.000 per hectare, which is way too expensive. The purpose of this study is to test and to explore yielding ability of three Chinese castor hybrid varieties that treated with different rates of fertilizer. The results of this study are expected to be used as guidelines in choosing annual hybrid castor variety that has high daily productivity.

MATERIALS AND METHODS

An experiment was conducted during the rainy season of 2010/2011, from February to May, 2011 in Akar-akar, North Lombok, at elevation of around 12 m above sea level. Soil texture was sandy loam with 0.09% N, 815 ppm of P, 0.74 me/100 g of K and soil pH 6.5. Three Chinese castor hybrid varieties were grown and fertilized with NPK (15-15-15) at three different rates, 0 g, 125 g and 250 g per plant. Spacing was 1.5 m x 1.5 m and planting holes of 30 cm x 30 cm x 30 cm of length, width and depth were made for each plant. Each planting holes received 1 kg of chiken manure and 4 g of Furadan 3G that had been mixed with the soil. Sowing was done on February 14, 2011 and no other treatment was given to the growing plants. Each treatment plot had 300 plants and 10 plants from each treatment were selected as sample plants for measurements. Parameters measured were panicles number, panicles length, panicles weight, number of fruits, seeds dry weight and weight of 1000 seeds. All the collected data were analyzed using One-Way Analysis of Variance in Minitab.

RESULTS AND DISCUSSION

Total rainfall during the experimental period was 900 mm with 40 days of rain. This ammount of rain is considered as high because usually the total rain at the experimental site was about 700 mm. Since there was sufficient rain that created enough moisture in the soil until the end of the experimental period, all the plants in the fertilized treatments grew and yielded well. Those plants in the unfertilized plots, for all the three hybrid varieties, failed to grow and to yield. More than 90% of the plants died at about flowering stage and the rest, produced one panicle only with a very small number of fruits and the seeds inside the fruits were empty. This fact shows that hybrid varieties, especially castor hybrid varieties from China, need high input of fertilizer to grow and to yield.

Zhibo-8 was harvested the earliest at 85 days after sowing (DAS), followed by Zhibo-7 at 90 DAS and the last was Zhibo-5. All the three varieties expressed their genetics as annual varieties on dryland tropics as what they performed in their country of origin, Guandong, China, which is catagorized as sub-tropical region. These results can be used as a guideline in choosing castor hybrid varieties to be grown in drylands areas in Indonesia that have short period of rainy season.

All the varieties were affected significantly by fertilizer application rates. NPK fertilizer with higher rate (250 g $plant^{-1}$), gave better result in all three varieties for all parameters, followed by the lower rate (125 g $plant^{-1}$). In term of number of panicles per plant, Zhibo-8 produced the highest and the lowest was in Zhibo-5 (Table 1). Earlier, Jaya and Hadi (2010) reported that when these three varieties of castor were grown in a less rainfall condition, such as in Pringgabaya, East Lombok, Zhibo-7 produced the highest number of panicles (14 panicles), followed by Zhibo-8 (10 panicles) and the lowest was Zhibo-5 (9.4 panicles). In the experiment that was reported earlier, the plants were given the same NPK fertilizer but the dosage was 240 g $plant^{-1}$. This data shows that panicles number produced by both Zhibo-5 and Zhibo-8 are unstable and varied with environment conditions. Panicles number produced by Zhibo-7, on the other hand, were relatively constant in both locations showing that this variety is the most adapted on drylands Lombok conditions.

Table 1. Average number of panicles per plant, average length of panicles per plant and average weight of dry panicles per plant of the three hybrid varieties at two different levels of fertilizer rate

Treatments	Panicles number/plant (± SE)	Panicles length (cm)/plant (± SE)	Panicles weight (g) /plant (± SE)
Zhibo-5 + 125 g NPK	6.4 ± 1.96	42.0 ± 10.34	485.5 ± 286.7
Zhibo-5 + 250 g NPK	13.9 ± 3.78	45.5 ± 5.22	1601.4 ± 563.8
Zhibo-7 + 125 g NPK	9.6 ± 3.56	34.6 ± 5.56	811.8 ± 207.7
Zhibo-7 + 250 g NPK	15.0 ± 4.71	36.7 ± 8.14	1211.3 ± 391.4
Zhibo-8 + 125 g NPK	12.6 ± 5.82	38.3 ± 10.25	1161.6 ± 526.8
Zhibo-8 + 250 g NPK	16.3 ± 8.28	39.3 ± 6.21	1383,0 ± 673.0
P (5%)	0.000	0.048	0.000

The highest panicles number in Zhibo-8 was not followed by the highest in panicles length and panicles weight. The highest panicles length and panicles weight were produced by Zhibo-5 (Table 1). Looking at the panicles length data, the statistical analysis results show that there was a significant effect of fertilizer rate in all varieties. However, the average values of panicles length in both fertilizer rate in each of the variety treated, show not much different. This indicates that fertilizer has little effect on panicles length as compared to the effect of fertilizer on panicles number. Great effect of fertilizer was also shown in panicles weight data, especially in Zhibo-5 and Zhibo-7. Doubling fertilizer rate in Zhibo-5 resulted in more than triple of panicle weight, but not in other varieties. This data shows that among the three varieties, Zhibo-5 is the most responsive to fertilizer treatment.

Table 2. Average number of fruits per plant, average seeds dry weight per plant and weight of 1000 seeds of the three castor hybrid varieties treated with different rates of NPK fertilizer

Treatments	Fruits number/plant (± SE)	Seeds dry weight (g) /plant (± SE)	Weight of 1000 seeds (± SE)
Zhibo-5 + 125 g NPK	293.8 ± 98.00	205.2 ± 92.1	280.2 ± 18.07
Zhibo-5 + 250 g NPK	757.3 ± 170.60	543.6 ± 92.8	307.1 ± 5.35
Zhibo-7 + 125 g NPK	362.1 ± 82.9	266.7 ± 71.2	296.0 ± 14.06
Zhibo-7 + 250 g NPK	393.8 ± 105.3	315.0 ± 84.0	349.9 ± 29.63
Zhibo-8 + 125 g NPK	542.0 ± 199.0	358.3 ± 168.1	252.7 ± 30.68
Zhibo-8 + 250 g NPK	549.7 ± 264.4	360.1 ± 179.7	289.9 ± 11.58
P (5%)	0.000	0.000	0.000

The highest panicles weight in Zhibo-5, that was treated with 250 g plant^{-1} NPK fertilizer, resulted in the highest number of fruits per plant and the highest dry seeds weight per plant (Table 2). Fruits number data also show that NPK fertilizer rate has a great effect only in Zhibo-5, while in the other two varieties, fertilizer rate seems to have a very little influence. This data show that for Zhibo-7 and Zhibo-8, a fertilizer rate of 125 g plant^{-1} of NPK is the most economical in producing number of fruits, but not in Zhibo-5. Data in Table 2 also show that Zhibo-5 is the most responsive to fertilizer application, where doubling the dosage of NPK fertilizer resulted more than double in seeds dry weight. In Zhibo-7, doubling the rate of NPK fertilizer resulted only 18% increased in seeds dry weight while in Zhibo-8, no increase was recorded. With this kind of yield, Zhibo-5 will produce approximately 2.4 ton Ha^{-1} of dry seeds. Current price of 1 kg of castor dry seeds in Lombok is IDR 4.000, which means that from 1 Ha, a farmer can receive IDR 9.600.000 bruto.

In term of weight of 1000 seeds, that shows the quality of seeds, Zhibo-7 treated with 250 g NPK fertilizer per tree produced the best result, followed by Zhibo-5 and Zhibo-8. Zhibo-7 was also the most responsive to the additional fertilizer rate applied, where doubling the fertilizer rate resulted in 17% increased in 1000 seeds weight, while in Zhibo-8 and Zhibo-5 were only 14% and 9%, respectively. The 17% increase in 1000 seeds weight in Zhibo-7 as caused by doubling the fertilizer rate should have

resulted in a very high yield. Unfortunately, the number of fruits per tree in Zhibo-7 variety was the lowest among the three varieties tested. More works are needed by breeders to improve the number of fruits per tree in Zhibo-7 since this variety is the most stable in producing number of panicles in different dryland environments in the island of Lombok.

CONCLUSIONS

The three castor hybrid varieties relay heavily on inorganic fertilizer of NPK in order to be able to grow and to yield well on dryland Lombok. The effect of fertilizer rates on some parameters were varies amongst the three varieties. In general, Zhibo-5 was the most responsive to the additional application of NPK fertilizer, especially in panicles weight, fruits weight and dry seeds weight parameters. In contrast to the other parameters, panicle length in each variety with 125 g and 250 g NPK per tree treatments showed not much different, even though statistically the fertilizer rate showed a significant effect. Overall, application of 250 g plant^{-1} of NPK only needed by Zhibo-5 to improve its yield while the other two varieties, 125 g plant^{-1} of NPK is considered as an optimum dosage.

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THE INCREASE OF PROTEIN DIGESTIBILITY AND METABOLIZABLE ENERGY OF RICE BRAN BY *SACCHAROMYCES CEREVISIAE* FERMENTATION

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ABSTRACT

Yeast culture product, which have some fermentation ability consist of yeast (*S. cereviseae*) and the media which the yeast grew on. This research was carried out to study the increase of protein digestibility and metabolizable energy of rice bran by *S. cereviseae* fermentation. The research used a completely randomized design (CRD) with four treatments in six replicates. There were four birds in each replicate with relative homogenuous body weight. The experimental basal diets for the experiment period were formulated to 15% unfermented rice bran as a control diet (A), diets with 15% fermented rice bran by 0,10% *S. cereviseae* (B), diets with 15% fermented rice bran by 0,20% *S. cereviseae* (C), and diets with 15% fermented rice bran 0,30% *S. cereviseae* (D), repectively. Experimental diets and drinking water were provided *ad libitum* during the entire experimental period. The results of this experiment showed that the used of 15% fermented rice bran by *S. cereviseae* (B, C, and D treatments) in diets, increased body weight gains and feed efficiencies significantly (P<0,05) on Bali drake than control groups (A). The used of 15% fermented rice bran by *S. cereviseae* before offered for the bird, increased significantly different (P<0,05)t on protein digestibility and metabolizable energy of rice bran than without fermented treatments or control group (A). It is concluded that used of *S. cereviseae* for fermenting rice bran were increased protein digestibility, metabolizable energy, and performance of Bali drake aged 0-8 weeks.

Keywords*: S. cereviseae*, rice bran, metabolizable energy, protein digestibility, Bali drake

INTRODUCTION

Alternative feed sources include milling and distillery by-products as well as forages, and are acceptable energy sources for poultry. The potential of forages as energy sources for poultry depends considerably on such factors as cell wall content, degree of microbial fermentation in the large intestine, and extent of absorption and utilization of the volatile acids produced (Kahlique *et al*., 2003). Agro-industry by-product as well as rice bran, is one such product abundantly and cheaply available during the season. These toxic factors are trypsin inhibitor, lectin (hemaglutinin), phytic acid as phytate, and crude fiber. These anti-nutritive factors have been reported to reduce feed intake and depress performance of poultry (Bidura *et al*., 2010).

Bach Knudsen (2001) reported that dietary fiber (DF) has been defined as the complex macromoleculer substances in food plants that are not degraded by mammalian digestive enzymes. With the exception of lignin, all of the materials called DF are carbohydrates in nature. DF is thought to mediate protective effects on the colonic epithelium through their fermentation products and fecal bulking capacity (Wang *et al*., 2003).

Feeding high fiber resulted in a lowered rate of lipogenesis and tendency of an increased capacity to utilize acetyl-CoA in pigs (Zhu *et al*., 2003). Non starch polysaccharide (NSP) are the carbohydrate components of DF and are the predominant substrates for anaerobic fermentation. NSP can, to a certain degree, be broken down by microflora permanently colonizing the gastrointestinal tract and their breakdown in all nonruminant including human and pig mainly occurs in the hindgut by microbial fermentation (Weng *et al*., 2003).

Most of the recent studies focus on the effect of the bacterial and fungal enzymes used in rice bran based diets. More than 50% of phosphorus in rice bran is in the form of phytate, which is poorly available in the digestive tract of monogastric animals (Ilyas *et al*., l995). Phytic acid found in vegetable feed sources affect the protein and amino acid digestibilities negatively by preventing the activities of the proteolytic enzymes such as pepsin/trypsin. Futhermore, phytic acid has a higher P content and chelating ability and so phytate form of phytic acid diminishes the availability of Ca and P (Pointillart, 1991). Monogastric animals can not make use of phytin phosphorus due to lacking of phytase enzyme in their digestive systems and consequently phytin posphorus is mostly excreted in the faeces.

Therefore, it is suggested that fermented of feeding by yeast (*S. cerevisiae*) can be used in order to alleviate the negative effect of phytic acid. The use of *S. cerevisiae* as a probiotics sources in poultry production as become an area of great interest, because continued use of probiotics in animal feeds may result in the presence of antibiotics residues in animal products. Gut microfloral enzymes are beneficial to the nutrition of the host because they increase the digestion of nutrients, especially in the lower intestine (Sissons, 1989). Previous experiments showed that the inclusion of microorganisms in the diets improved feed efficiency and digestibility (Suciani *et al.,* 2011 and Warmadewi *et al*., 2008).

Aspergillus oryseae (AO) and *yeasts*, particularly *Saccharomyces cerevisiae*, have been used as probiotics by many workers. Both *Aspergillus spp*. and *Saccharomyces* belong to the *Ascomycoyina* subdivision and have many industrial applications in the brewing, destilling, and baking industries (Ahmad, 2005).

Park *et al.* (l994) showed that yeast added to a diet could reduce animal wastes. But, Piao *et al*. (l999) reported no significant improvement in weight gain, feed intake and feed efficiency with 0,10% yeast culture. Feeding live yeast to broiler breeder reduced colonization of salmonela in their ceca and improved phosphorus utilization in growing chickens.

The objective of this study was to determine the increase of protein digestibility and metabolizable energy of rice bran by *S. cereviseae* fermentation and performance of Bali drake.

METERIAL AND METHOD

Management of experimental Birds

One hundred twenty of Bali drake day-old-duck (DOD) were randomly allotted to colony wire-floored cages, 5 birds per cages. A 500 ml plastic bottle equipped was placed of each cage. Experimental diets and drinking water were provided *ad libitum* during the entire experimental period (for a 8-week period). Body weight and feed intake were recorded weekly.

Diet and Drinking Water

The four experimental diets (Table 1) based on corn-rice bran were formulated to 15% unfermented rice bran as a control diet (A), diets with 15% fermented rice bran by 0,10% *S. cereviseae* (B), diets with 15% fermented rice bran by 0,20% *S. cereviseae* (C), and diets with 15% fermented rice bran 0,30% *S. cereviseae* (D), repectively. The basal diets (Table 1) were formulated to meet or exceed nutrient requirement (NRC, 1994). All of diets in mash form and compiled by iso-energy (2900 kcal ME/kg) and iso-protein (CP: 18%). Through all the experimental period, birds were allowed *ad libitum* acces to feed and water. The composition of ration compiler substances and nutrient which is used in diets can be seen in Table 1.

Saccharomyces cereviseae

Saccharomyces cerevisiae used in this experiment were isolated from "Ragi Tape". It's a culture produced locally by fermenting the rice bran with *S. cereviseae*. *Saccharomyces cerevisieae* from ragi tape which used is the common yeast used in "tape" making title "Na Kok Liong", ensiled in number 26895.

Fermented of Rice Bran

Fermented of rice bran were prepared from the same batch approximately 0.0-0.3% *Saccharomyces cerevisiae* were added to each feeding. Following the fermentation, water was added to bring the product to 50% content and fermented for 3 days. After fermentation, fermented of rice bran was dried at 45^0C for 6 h.

Tabel 1. Formula and chemical composition of diets of growing Bali drake aged 0-8 weeks (as-fed basis)[1)]

Ingredient (%)		Level of *S.cerevisiae* in Rice Bran Fermented[2)]				
		0% (A)	0.1% (B)	0.2% (C)	0.3% (D)	
Yellow corn		53,95	53,95	53,95	53,95	
Rice bran		10,00	**10,00**	**10,00**	**10,00**	
Fish meal		13,05	13,05	13,05	13,05	
Coconut meal		13,60	13,60	13,60	13,60	
Soybean		8,00	8,00	8,00	8,00	
Palm oil		0,50	0,50	0,50	0,50	
NaCl		0,40	0,40	0,40	0,40	
Mineral mix		0,50	0,50	0,50	0,50	
Total		100	100	100	100	
Metabolizable Energy	(kkal/kg)	2902	2902	2902	2902	2900[3)]
Crude Protein	(%)	18,0	18.0	18.0	18.0	18.00[3)]
Crude Fibre	(%)	4.85	4.85	4.85	4.85	5-7[3)]
Eter Extract	(%)	6.76	6.76	6.76	6.76	5-10[3)]
Ca	(%)	1.08	1.08	1.08	1.08	0.9-1.2[3)]
P-available	(%)	0.63	0.63	0.63	0,63	0.40[3)]
Argynin	(%)	1.52	1.52	1,52	1.52	1.00[3)]
Lysine	(%)	1.31	1.31	1.31	1.31	0.82[3)]
Metyonine+systeine	(%)	0.79	0.79	0.79	0.79	0.60[3)]

Note:
1. Calculation based ingredient by Scott *et al*. (1982)
2. Level of *S.cerevisiae* on Rice Bran Fermented in rations
3. Standard of Farrell (1995)

Retention and excretion of nutrients

After of feeding trial, 6 birds from each treatment were randomly assigned to individual metabolic cages to determine the retention and excretion of dietary nutrients. Excreta were collected for 6 h. Foreign substances (feathers, scurf, etc) mixed in the collected excreta were removed before drying at 60^0C for 48 h and subsequent grinding. Feed and feces were analyzed by AOAC (l994) procedures for proximate components. The retention of nutrients was calculated by dividing the amount of retained nutrient (ingested nutrient minus excreted nutrient) by the amount of ingested.

Dry matter (DM), organic matter (OM), CP and CF determinations were done according to the Assocciation of Official Analytical Chemists (l994). The CP content of the diets was determined using the Kjeldahl procedure (AOAC, 1994). Crude fibre in the feeds were determined using the procedure of Van Soest *et al.* (l991) on oven-dried samples.

Statistical Analysis

All data were subjected to a one-way analysis of variance test (Steel and Torrie, 1980). Statistical significances among treatment means were determined by method of New Multiple Range Test of Duncan when the F value was significant at 5 % level.

RESULTS AND DISCUSSION

Table 2 shows the chemical composition of unfermented rice bran (UF) and fermented rice bran (F) ingredient. The digestibility both of crude protein and and metabolizable energy (ME) were slightly increased significantly (P<0,05) by *S.cerevisiae* fermentation.

Table 2. The effect of *S. cerevisiae* fermented on nutrient digestibilities (% dry matter) and metabolizable energy of rice bran

Digestibility of Rice Bran	Level of *S. cerevisiae* in Rice Bran Fermented[1)]			
	0% (A)	0.1% (B)	0.2% (C)	0.3% (D)
Crude protein (%)	66.71b[2)]	72.95a	73.62a	73.26a
Crude fiber (%)	46.79a	48.51a	47.98a	48.06a
Metabolizable Energy (kcal/kg)	1723b	1849a	1901a	1870a

Note:
1. Level of *S.cerevisiae* on rice bran fermented in rations: 0.0% (A); 0.1% (B); 0.2% (C), and 0.3% (D), repectively.
2. The different superscript at the same row is significantly different (P<0,05)

S. cerevisiae can not affect crude fiber digestibility of rice bran. Becouse, among the cell wall polysaccharides of rice bran known as nonstarch polysaccharides (NSP) are celluloses, pectins, and oligosaccharides. NSPs can not be degraded enzymitically in the digestive systems of the birds due to the lacking of enzymes degrading the NSPs in their digestive systems (Choct, 2002).

The metabolizable energy on birds were offered fermented rice bran was increased and it significantly (P<0,05) different than metabolizable energy on birds were offered unfermented rice bran (Table 2).

Final Body Weight and Live Weight Gains

Weight gain, feed intake, and feed efficiency are given in Table 3. At the end of the experiment (at 56 days of age) the body weight gain was significantly (P<0.05) increased in the group fed with fermented rice bran by *S. cerevisiae* diet compared to groups that received control (unfermented rice bran). In general the body weight gain tended to increase with increasing levels of *S. cerevisiae* which is probably due to increase of crude protein digestibility and metabolizable energy of the fermented rice bran used in experiment (Bidura *et al*., 2009).

Table 3. Addition effect of fermented rice bran by *S. cerevisiae* in diets on performance of Bali drake eged 0-8 weeks

Variabel	Level of *S. cerevisiae* in Rice Bran Fermented[1)]				SEM[2]
	0% (A)	0.1% (B)	0.2% (C)	0.3% (D)	
Final body weight (g/birds)	974.36b[3)]	1067.09a	1105.72a	1098.43a	25.902
Body weight gains (g/birds/8 weeks)	974.62b	1004.41a	1041.60a	1035,71a	20.937
Feed consumption (g/birds/8 weeks)	5633.30a	5283.20a	5301.74a	5344.26a	215.08
Feed Conversion Ratio (feed/gains)	5.78a	5.26b	5.09b	5.16b	0.148

Note :
1. Level of *S.cerevisiae* on rice bran fermented in rations: 0.0% (A); 0.1% (B); 0.2% (C), and 0.3% (D), repectively.
2. Standard Error of The Treatment Means
3. The different superscript at the same row is significantly different (P<0,05)

For the body weight gain there was no significantly (P>0.05) difference between the 0.1% and 0.3% *S. cerevisiae* levels, but they were significantly lower in the group control (A). This finding was consistent with previous results reported by Cao *at al*. (l998).

The average of final body weight during eight weeks observation at birds which having the ration control (A) is 974.36 g/birds (Table 3). The average of final body weight of the birds having ration with fermented rice bran by *S. cerevisiae* levels were increased significantly different (P<0,05) than the birds were offered unfermented rice bran.

Feed Consumption and Feed Conversion

Feed consumption was not affected by fermented rice bran in diets. The averages of feed consumption in all of treatment were not significantly different (P>0,05) than control groups. Feed conversion ratio were significantly different (P<0,05) between unfermented rice bran and fermented rice bran. Feed consumption was not affected by fermented feed (FF) in diets. The averages of feed consumption between in treatments B, C, and D were not significantly different (P>0,05) than control groups.

Feed conversion ratio and live weight gains in control groups are lower significantly (P<0.05) difference than other groups. This may be coused by the fact that poultry can not utilize the fibrous parts of the rice bran ingredients.

The average of feed conversion ratio (feed : gains) during eight weeks observation at birds which having the ration control (A) is 5.78/birds (Table 3). The average of feed conversion ratio (feed : gains) of the birds having ration FF were decreased significantly (P<0,05) than control groups.

Table 3 shows the chemical composition of unfermented rice bran and fermented rice bran ingredient. The digestibility of crude protein, crude fiber, and ME were slightly increased by the fermentation. These results indicated that carbohydrates other than fibres were used for microbial growth (*Saccharomyces cerevisiae*) and the reduction of nitrogen free extract resulted in increased concentration of the other components. Yi *et al.* (l996) reported that supplementation of microbial in diets improved N retention in broiler chickens and *in vitro* digestibility of vegetable protein. Also, Chen *et al.* (2005) reported that addition of 0.20% complex probiotic (*L. acidophilus* and *S. cerivisae*) in basal diets were inceased digestiblities of dry matter.

Fermented of rice bran ingredient by ragi (*Saccharomyces cereviseae*) and it's addition to the diet had better digestibilities, because *Saccharomyces cereviseae* in the gastrointestinal tract can part of an probiotics sourches. *Saccharomyces cereviseae* as part of an probiotics were increased retention of mineral Calsium, Posphor, and Mangane (Nahashon *et al.,* l994 dan Piao *et al.,* l999). Also, Piao *et al.* (l999); Sibbald and Wolynetz (l986), suggested that probiotics in the gastro intestinal tract can improve protein and energy retention on the body of birds. These fungal are effective in degrading of the complex compounds such as b-glucans and arabinoxylans (Bedford and Classen, 1992).

According to Ilyas *et al.* (l995), when the fermented feeding was suplemented in formula feeds, phytase in the fermented product might partly degrade the phytate in order ingredient in the digestive tract. The fermented product is possibly used as a phytate-free protein source of feed, which contains high available phosphorus. It's was reported that fermentation of soybean meal by *Aspergillus usami* reduced phytate phosphorus levels.

Fermentation process by ragi which contains *Saccharomyces cerevisiae*, according to Wallace dan Newbold (1993), *Saccharomyces cerevisiae* can improve crude fibre digestibility on the ceca of birds to become volatile fatty acid (acetate, provionate, and butirate acid).

Ilyas *et al.* (l995) reported if the enzyme effectively degrades phytase in the digestive tract, phytase in fermented rice bran can be degraded phytate from other ingredients of ration. The reasons for the reduction both of excreta protein and energy by the feeding fermentations may be related to the fact that fermentation process may improve dietary protein and energy digestibility (Chiang dan Hsieh, 1995). Jaelani *et al*. (2008), reported that fermented of palm kernel meal by *Trichoderma reesei* was increased both of metabolizable energy and crude protein contents of palm kernel meal.

Chen *et al.* (2005) reported that dietary supplementation of complex probiotic increased the body weight gain and decreased fecal NH_3-N concentration, and slightly improved digestibility of nutrients. Fermented feed product to the rations coused numerical increases in the body weight gain. This study is consistent with some studies which indicated that fermented diets effect performance positively (Bidura *et al*. 2008b). This case can be attributed to the positive effects of fermented feed product on phytates and protein. Wu *et a*l. (2005) reported that supplementation of *Aspergillus xlanase* can improve the performance of the broilers fed the wheat-based diet.

Piao *et al*. (l999) reported that used of 0.10% *yeast* (*Saccharomyces cerevisae*) in diets were increased body weight gains, feed efficiency, and absorption of nutrient in broiler, and were decreased N and P excretion in manure. Park *et al*. (l994) suggested that body weight gain and feed efficiencies were significantly improve by the addition of 0.10% *yeast culture* in diets of broiler. Bidura (2008) reported that

birds were offered fermented diets coaused body weight dan carcass weight of drake were increased.

It is concluded that used of *S. cereviseae* for fermenting rice bran were increased protein digestibility, metabolizable energy, and performance of Bali drake aged 0-8 weeks.

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THE PERFORMANCE OF BALI CATTLE FED ON UREA-CONTAINING DIETS

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ABSTRACT

A feeding experiment was conducted in Bali cattle which were given a basal diet of urea ammoniated rice straw supplemented either with urea molasses block, rice bran or urea molasses block plus rice bran. The experiment was designed in completely randomized design (CRD) with 5 treatments and 4 animals in every treatment.

The objective of the experiment was to examine the effects of supplementation on bodyweight gain and the efficiency of feed conversion in Bali cattle, feed on urea ammoniated rice straw basal diets.

Cattle on the supplemented diets produced an average bodyweight gain of 230-326 g/h/d. The highest live weight (326 g/h/d) was observed on cattle given basal diet supplemented with 400 g urea molasses block plus 600 g/h/d of rice bran. The use of urea molasses block as a supplement was superior to rice bran supplement. Although the straw intake of all groups of animals was not significantly different (P>0.05), the supplemented groups used diets more efficiently. The lowest FCR in this experiment was 15.0 observed on cattle given a basal diet of urea ammoniated rice straw, supplemented with 400 g urea molasses block plus 600 g/h/d of rice bran.

Keywords: Bali cattle, urea ammoniated rice straw, urea molasses block, rice bran supplement

INTRODUCTION

Bali cattle are the only pure-breed cattle in Indonesia. The origin of this breed is *Bos banteng*. The Indonesian government is trying to develop this breed intensively at the village level. This reason for this is that this breed is the original one, adapted well to the environment and culture in Indonesia, especially in Bali. Slow growth rates of the Bali cattle have reduced the effectiveness of the government program.

In Bali, the cattle are raised for domestic use as meat producing drought animals and as an investment by the farmers. The feeding system is based cut-and-carry. The feed mainly consisted of field grass, tree leaves, banana stem and rice straw. Concentrate in the diet are not common. The diet is apparently inadequate, so that the weight gain of the animals is usually low, less than 200g/a/d (Rika *et al*., 1981).

It would be very valuable if the conventional cattle diet in Bali could be improved by a simple treatment or supplementation that is not expensive and easy to manage by the farmer at the village level. The available and cheap feed (rice straw) is highly indigestible and low in N content. The use of *urea ammoniated rice straw* (Mudita,2008) and urea molasses block or rice bran or the combination of both as a supplement is a good approach (Sudana, 1992;Wanapat and Khampa,2007). Therefore, studies were designed on the use of urea ammoniated rice straw ("*jerea"*) UMB, and rice bran in increasing the utilization of rice straw in the Bali cattle diet and increasing the performance of the Bali cattle itself.

MATERIAL AND METHODS

After 2 weeks of adjustment period, 20 uncastrated Bali cattles (147 – 156kg bodyweight) were randomly allocated to 5 experimental diets show in table 1. "*Jerea*" were made by ammoniated the fresh rice straw with urea (4% urea/straw DM) and fermented in un-aerobic condition for 6 weeks. The urea molasses block (UMB), (which consisted of: 16% urea, 37% molasses, 32% rice bran, 6.5% copra meal, 8% lime and 2% minerals mix) was given to the animals twice a day in a total quantity of 400g/h/d. Rice bran were fed to the animals one a day (600g/h/d). Daily feed intake was recorded and the animals were weight every 2 weeks prior to feeding. The treatment covered 12 weeks. Data were analyzed using an analysis of variance (Snedecor, 1968).

Table 1. Composition of the diets

Diet component	Treatment Groups				
	A	B	C	D	E
Field grass	*ad lib*	0	0	0	0
Ammoniated rice straw ("*jerea*")	0	*ad lib*	*ad lib*	*ad lib*	*ad lib*
Urea molasses block (UMB) (g DM)*	0	0	400	0	400
Rice bran (g DM)	0	0	0	600	600
Water	*ad lib*	*ad lib*	*ad lib*	*ad lib*	*ad lib*

**Manufactured by sub. BPTP, Grati, East Java- Indonesia*

RESULTS AND DISCUSSION

Mean total DM intake, straw intake, rice bran intake, live-weight change and FCR for each group of cattle are given in Table 2.

According to the literatures (See Leng *et al*, 1973, Leng 1984; Loosli and McDonald, 1968 and Block, 2006) the rumen fluid ammonia concentration should be kept higher than 50 mg Nl^{-1} to sufficiently support microbial production in the rumen. The actual level of ammonia in the rumen required for growth and activity of rumen microorganism depend on variety of factors including diet and feeding regime (Leng and Nolan, 1984; Bach *et al.,* 2005). Rice straw basal diet usually produced low level of ammonia rumen (Sudana, 1984; Astawa, 2007) and can be increase by ammoniated the straw with urea or by supplemented the diet with urea molasses block (Sudana and Leng, 1986). In all studies the first limiting nutrient for microbial growth has been assumed to be ammonia.

Table 2. Feed intake and live-weight gain

Parameter	Treatment groups				
	A	B	C	D	E
Live-weight gain (g/h/d)	120^{a}	89^{b}	257^{c}	230^{c}	326^{d}
Field grass intake (g DM/h/d)	3262	0	0	0	0
Jerea intake (g DM/h/d)	0	3070^{a}	3846^{b}	3542^{c}	3890^{d}
UMB intake (g DM/h/d)	0	0	400	0	400
Rice bran (g DM/h/d)	0	0	0	600	600
Total feed intake(g DM/h/d)	3262^{a}	3070^{a}	4246^{b}	4142^{b}	4890^{c}
FCR (feed intake/live-weight gain)	27.18^{a}	34.50^{b}	16.52^{c}	18.01^{c}	15.0^{c}

Value on different alphabet on column showed significant ($P<0.05$)

The data presented here tend to suggest that the urea molasses block provided some deficient nutrient (s) despite supposedly complete supplementation with minerals including Na, Cu, P, S and trace elements, since the urea molasses block increased straw and total intake as well as live-weight change above that for the basal diet and the diet supplemented with rice bran only. Molasses itself is a concentrated plant juice and as such, contains a wide range of trace minerals, vitamins, sugars (sucrose, glucose and fructose usually about 2:1:1) and is particularly rich in potassium and sulphur. The intake of molasses was about 180 g/h/d only and therefore the additional nutrients must have acted mainly to increase the efficiency of utilization of the feed. Due to the improved rumen conditions, presumably small intestinal protein supply increases, resultantly increasing the proportion of protein deposition relative to fat.

An important practical aspect here is that these studies emphasize the obvious advantage of using molasses block as a "coverall" in the variable and largely unknown feeding system that pertain to animals fed on agro-industrial by products under village conditions in developing countries (Leng, 1984). The urea molasses bock, which has been much for grazing animals (Loosli and Mc.Donald, 1986, Leng et al., 1973, and Bal and Ozturk,2006) is, however, a most effective way to providing urea and of overcoming a number of potential mineral deficiencies and acid or peptides. The approach is now finding application and appears

to be a method of choice of providing these nutrients to ruminants in the tethered husbandry system of some developing countries (Sudana and Leng, 1986).

CONCLUSION

Rice straw and other agro-industrial by products are low quality for livestock because they are low in digestibility but their support to growth is lower than their potential since they are deficient in particular N/Protein, Minerals and vitamins.

Even after urea treatment, the treated straw alone is still roughage which provides only sufficient nutrients for maintenance on low production and only converts this diet from a sub maintenance to a little better than a maintenance diet.

Urea-ammonia treatment seems to be a reliable choice for farmers in developing countries since it is probably more economical and practical than other methods.

To obtain high animal productivity from treated or untreated rice straw (if production is the objectives), rice straw should be supplemented with urea-molasses block, rice bran or the combination of booth, and the use of urea-molasses-block for this purpose appears to be agood approach.

As a source of a supplement to rice straw basal diet, urea molasses block is superior to rice bran because it is contain, mineral, salt and vitamin completely as well as additional energy provided.

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THE EFFECT OF LACTIC ACID BACTERIA ADMINISTRATION ON THE PERFORMANCES, TOTAL BACTERIA IN THE DIGESTIVE TRACT, AND THE BLOOD AND MEAT CHOLESTEROL CONTENT OF *KAMPONG* CHICKENS

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ABSTRACT

The main objective of this study was to find out the effect of lactic acid bacterial (LAB) levels in the water (drink) on the performance, blood and meat cholesterol contents, and total bacteria in the digestive tract of village (*kampong*) chickens. A complete randomized design was applied in the study. Four variants or levels of LAB in the water (0, 5, 10, and 15 % w/v) were administered to the treated chickens. Six chickens, weighed between 270.20 - 315.02g, were used in each treatment and five replicates were set per treatment. The amount of energy and protein contents in the diet during the period of the study were 2.900 kcal ME/kg and 18%, respectively. Diet in mash form and drinking water were provided *ad libitum* for 6 weeks of the study (from the age of 6 - 12 weeks old).

The result of the study showed that inclusion of lactic acid bacteria in the drinking water significantly increased the daily weight gain ($P<0.05$), *rate of passage,* and total lactic acid bacteria in the digestive tract of the village or *kampong* chickens. This treatment was also found to reduce the serum and meat cholesterol level, total *coliform* and *E. coli* in the digestive tract of the chickens, especially when applied at the concentrations of 10 - 15% (w/v).

Keywords: lactic acid bacteria, performances, cholesterol, village chickens

INTRODUCTION

Village chickens, one of the local Germplasm, have a good potential to be developed as a meat source. This is due to their relative compact and inexpensive meat when compared to other livestock. Besides that their maintenance are also very simple. However, growth and development of this type of chickens in the rural areas are extremely slow, probably due to the threat of a global crisis, narrow availability of land, and reduced feed sources. Therefore, the poultries of village chickens either in semi-intensive or intensive methods require the availability of quality sustainable diets. The use of local sources that is cheap and sustainably available is expected to solve these problems. The use of yoghurt, supplemented in the drinking water of the chickens, as a source of lactic acid bacteria (probiotic) is an interesting case that needs to be investigated. Yogurt is a fermented milk produced by incubation of LAB-inoculated milk at 30 - 45^{o}C for at least 2.5 hours (Tamime and Robinson, 1999).

Plain yoghurt mainly contains lactic acid bacteria (*Lactobacillus, Bifidobacterium, Pediococcus* etc.). These types of bacteria are reported to play important role in maintaining human health (Fardiaz, 1992). Tsuchiya *et al*. (1982) reported that *Lactobacillus* and *Bifidobacterium* could reduce problems related to human digestive tract. *Lactobasillus* culture has also been used as food supplement to reduce the content of C*oliform* in the intestine of chicks. Jin *et al*., in Jin *et al*., 1997, for example, supplemented single or mixed culture of *Lactobacillus* in the diet of broiler and found that these cultures significantly reduced the C*oliform* content of this broiler. The use of *Lactobacillus* at the rate of 75 mg/kg feed was also found to lower *Coliform* level in the intestinal and caecum of turkey (Francis *et al*., in Jin *et al*., 1997). However, only a few information on the use of lactic acid bacteria (contained in plain yoghurt) in the drinking water of chickens are available. Therefore, this study was conducted on village (*kampong*) chickens in order to find out the influences of lactic acid bacteria levels in water on the village chicken performance, blood and meat cholesterol content, as well as the total number bacteria in digestive tract of this type of chickens.

MATERIALS AND METHODS

The study was conducted at Badung regency-Bali for 6 weeks. The calculation of feed consumption, daily weight gain and *rate of passage* were conducted weekly. The number of lactic acid bacteria in the digestive tract was calculated on microscopic methods Breed (Lay and Hastowo, 1992) at the Laboratory

Bio Science Udayana University, and the cholesterol analysis of serum and meat was conducted at the Laboratory of Sanglah General Hospital, Denpasar.

Feed in mash form and drinking water added with 3% sugar and plain yogurt as a source of lactic acid bacteria was given *ad lib*. The purpose of sugar addition was to stimulate the growth of the LAB. The detail composition of the diet applied in this experiment is shown in Table 1.

A complete randomized design with four treatments (0, 5, 10 and 15% w/v of lactic acid bacteria from plain yoghurt) and five replications per treatment was applied in the project. Six chickens, weighed from 270.20 – 315.02g, per treatment were set per cage in the experiment.

The serum and meat cholesterol contents were analyzed using the method of Enzymatic Cholesterol High Performance CHOD-PAP KIT (Boehringer, 1993). The results were then analyzed using the analysis of variance (Anova) followed by Duncan test (Steel and Torrie, 1993) when any significant different at $p < 0.05$ was indicated.

Table 1: Nutrients composition of the village chicken diet during 6 weeks experiment

Nutrients of diet	Lactic acid bacteria in the drinking water (% w/v)				
	0	5	10	15	Standard[1]
Metabolizable energy (kcal ME/kg)	2,900.11	2,899.73	2,899.67	2,900.24	2,900.00
Crude protein (%)	18.00	17.99	17.99	17.99	18.00
Fats (%)	6.33	5.91	5.32	4.80	6 - 9[2])
Crude fibres (%)	4.82	5.94	7.07	8.30	3 - 8[2])
Calcium (%)	1.03	1.22	1.21	1.24	0.60
Phosphorous (%)	0.56	0.57	0.57	0.58	0.40
Lysine (%)	1.18	1.20	1.22	1.24	0.70
Methionine (%)	0.41	0.38	0.38	0.36	0.36
Tryptophan (%)	0.19	0.17	0.17	0.16	0.16

Note: I) Standar Scott *et al.* (1982). 2) Standard NRC (1994).

RESULTS AND DISCUSSION

Administration of LAB via the drinking water of *kampong* chickens has significant increment on the total LAB in the intestine of the chickens ($p<0.05$). The level of total LAB increment was proportional to the level of LAB administration. The presence of the LAB in the intestinal tract of the chickens was found to significantly ($p<0.05$) suppress the growth of pathogenic bacteria (C*oliform* and *E. coli*) (Table 2). This was probably due to: (1) the reduction in pH level (from 6 or 7 to 3 or 4.3) within the intestinal tract of the chickens as a result of the formation of lactic acid by the metabolic pathways of the LAB in the intestine of the chickens; (2) the formation of small amount peroxide (sufficient to inhibit the growth of gram-negative bacteria) in addition to the lactic acid within the intestinal tract of the chickens by LAB that worsen the environmental condition for the pathogenic bacteria (Tamime and Robinson, 1999); (3) production of volatile acids with a certain level of antimicrobial effects gram-negative bacteria (Fardiaz, 1992). A contradictory growth patterns shown by the LAB and the pathogenic bacteria within the intestinal tract of the chickens following administration of LAB via drinking water is expected to increase the efficiency of feed used for growth, although feed intake decreased ($P> 0.05$) (Table 2). An increase in the level of LAB in the diets of the chickens (Table 2) increased the availability of carbohydrate and protein in feed solution through which the chickens obtained the nitrogen source for growth. Besides that these LAB helped to digest the protein so that the availability and absorption of amino acids for the chicken's growth increased (Fardiaz, 1992). Surono (2004) reported that LAB also produced bacteriocin called bio-preservat ion amino acids and other compounds, such as various vitamins, folic acid, flavor compounds (such as 2-acetyl) and peroxide. These compounds were suspected to have contribution to improve significantly the growth rate or daily weight gain of the chickens (Table 2). This is in line with the statement of Scott *et al*. (1982) who stated that the availability of amino acids or the amino acid retention determined the opportunity of the chicken to grow faster than normal growth rate. As shown in Table 2, the growth rate or the daily weight gain of the chickens treated with LAB in yoghurt was significantly higher ($p<0.05$) when compared to the control. Other positive effect of the administration of LAB to k*ampong* chickens was a

reduction in the cholesterol level of the serum and the meat of the chickens. When compared to the control, the cholesterol level of the serum and the meat of the LAB-treated chicken was significantly lower (P<0.05) (Table 2). This phenomenon was presumably due to: (1) the production of lactic acid bacteria that suppress the decomposition process of feed in the digestive tract and increase the rate of passage of feed (Table 2), so that the absorption of nutrients, especially fat as an energy source to produce acetic acid (Acetyl-Co-A) decreased (Scott *et al*., 1982); (2) the process of fermentation by lactic acid bacteria in the digestive tract produces a factor that can inhibit cholesterol synthesis from acetate (Mann and Spoerry. in Fardiaz, 1992). This inhibited the formation of acetyl-CoA, mevalonate, scualen, lanosterol and cholesterol, which in turn caused a decrease of TG, LDL, VLDL (Sutarpa, 1998; North and Bell, 1990).

Table 2: Performance, lactic acid bacteria, *Coliform*, *E. coli*, serum and meat cholesterol, rate of passage village chickens 12 week old.

No	Response	Lactic acid bacteria in the drinking water (% w/v)**			
		0	5	10	15
1.	Feed Consumption (g)	500.35 a	500. 45 a	495.78 a	492.85 a
2.	Daily weight Gain (g)	439.25 a	434.60 a	545.20 b	500.02 b
3.	Lactic Acid Bacteria in Digestive Tract (cfu/g)	$1.9x10^4$ b	$2.5x10^4$ b	$1 .3x10^6$ a	$1.1x10^6$ a
4.	Coliform (cfu/g)	$1.1x10^6$ a	$2.6x10^6$ a	$3.8x10^4$ b	$2.7x10^4$ b
5.	E. coli (cfu/g)	$3.1x10^4$ a	$1.9x10^4$ a	$5.3x10^3$ b	$4.2x10^3$ b
6.	Serum Cholesterol (mg/dL)	159.40 a	152.08 b	153. 60 b	154.10 b
7.	Meat cholesterol (mg/dg)	58.49 a	57.03 a	40.75 b	43.10 b
8.	Rate of passage (minutes)	98.50 a	91.02 b	87.98 b	89.90 b

****Values in the same raw followed by the same letter are not significant statistically at p<0.05**

Based on the results above, it can be concluded that administration of lactic acid bacteria at the levels of 10% and 15% (b/v) via drinking water significantly improved the daily weight gain and the total count of the LAB in the digestive tract of kampong chickens, but it reduced the cholesterol level of the serum and meat of the chickens.

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ORAL PRESENTATION: AGRITECH AND FOOD

AN ENDO-B-D-1,4-GALACTANASE SECRETED BY *ASPERGILLUS NIGER* DURING SOLID STATE FERMENTATION ON SOYBEAN PULP

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ABSTRACT

Production of protein soybean curd tofu is one of the important food industries in Indonesia. However, huge materials as soybean pulp (SP) wastes were produced without any economic value. We examined, this material consist of around 60% total sugars, 30% of crude proteins and 10% lipids. Through GC analysis, SP mainly composted by galactose (40.3%), arabinose (21.1%), xylose (11.7%) and mannose (10.9%). Solid state fermentation of soybean pulp by introducing *Aspergillus niger* revealed that this fungus could grew well, indicated by the bulky black spores emerged after four days solid state fermentation. An extracellular galactanase was harvested by water extraction containing 1% NaCl and 0.1% toluene (v/v). For the purification, the crude enzyme was precipitated using 70% saturated and further chromatographed on a DEAE Butyl Toyopearl 650M, Q-Sepharose and Mono-Q column chromatography. This purification steps, resulted in 3.1% yield and 3,330 fold purification of enzyme. Estimated by SDS-PAGE, galactanase has molecular weight 44 KDa approximately. The enzyme exhibited maximum activity at pH 3.6 and 55°C, and retained nearly 100% activity in a pH range of 3-6 and below 55°C after 30 minutes exposure to respective temperatures. By TLC analysis, it was detected to be capable of releasing galactose and galactosyl oligomers (dimer, trimer, tetramer, pentamer, etc) from soybean pulp alkali extract substrate, in similar manner as from citrus pectin galactan which is known to have backbone chain of β-D-(1,4) linked. The results, confirmed that this galactanase is an Endo-β-D-1,4-Galactanase (E-βGAL) which specifically hydrolyzed internal β-D-(1,4)-galactopyranose linkage at random.

Keywords: galactanase, *Aspergillus niger,* soybean pulp

INTRODUCTION

Cellulose which occurs as microfibrils embedded in pectic and hemicellulolytic materials in cell wall are most important component of plant biomass, while the hemicellulose (xylans, arabinans, galactans, glucans and mannans) rank next to cellulose as the second most abundant group of the renewable polysaccharides in agriculture biomass (3, 4,8,10). They also found in food industry where generally some of the hemicelluloses cannot be processed, and a significant amount remains as organic wastes. For example, the protein soybean curd *tofu* which was known as one of the important traditional food industries in Indonesia also discarded plenty of secondary organic products as soybean pulp (SP) wastes. The usage of this waste is very limited with no economical value.

Microbial utilization of SP may possible to increase the value of this waste. In this research we evaluated the solid state fermentation of SP by introducing *Aspergillus niger* and its possibility to produce an endo-β-D-(1,4)-galactanase (E-βGAL). Isolation and characterization of this enzyme was reported.

MATERIALS AND METHODS

Materials

Fresh soybean pulps were collected from local industry at Jember. The chromatography media DEAE Toyopearl 650 M and Butyl Toyopearl were supplied by TOSOH Corp. (Tokyo, Japan). Q-Sepharose High Performance and Mono-Q HR 5/5 were produced of Pharmacia, (Uppsala, Sweden). The molecular weight marker was obtained from Daiichi Pure Chemicals Co., Ltd. Citrus pectin polygalacturonic was obtained from Sigma (St. Louis, Mo., USA).

Cultivation and optimization of crude enzyme production

Aspergillus niger was used for producing crude enzyme by inoculating to 500 g of sterilized SP in a 5 liter Erlenmeyer flask and incubating at 30°C. After 4-5 days, the culture was stopped by adding 500 ml water containing 1% NaCl and 0.1% toluene (v/v), followed by shaking at room temperature for 9 hours. The suspension was centrifuged to recover the supernatant as a crude enzyme. Then the crude enzyme was concentrated to about one-tenth of the initial volume by ammonium sulfate precipitation at 70% saturation.

The precipitate was dissolved and dialyzed against water for 3 days. This solution was stored at 4°C till used for SP hydrolysis. For optimization of cultivation period, 10 g of SP in 100 ml Erlenmeyer flasks were inoculated with *A. niger* and the cultures were stopped by adding 10 ml containing 1% NaCl and 0.1% toluene (v/v) daily. Then, the crude enzyme was obtained with the same procedure above.

Degree of hydrolysis and total sugar content analysis

The degree of hydrolysis was examined by incubating the reaction mixture of concentrated crude enzyme and substrate at 37°C. The hydrolyzate was obtained by centrifugation and the release of reducing sugars measured by the method of Nelson (7) as modified by Somogyi (9) using glucose as a standard sugar for calibration. The degree of hydrolysis was calculated as follows:

$$Degree\ of\ hydrolysis(\%) = \frac{Total\ reducing\ sugar\ of\ hydrolyzate\ (w/v)}{Total\ substrate\ (w/v)} * 100\%$$

The total sugar content of hydrolyzate was also measured by the phenol-sulfuric acid method (1).

Analysis of SP components

Twenty mg pulverized-dried SP was hydrolyzed with 4 ml of 1 N sulfuric acid at 100°C for 2 hours and total carbohydrate was measured by the phenol-sulfuric acid method. Total lipid was measured by ether refluxing of 9 g freeze-dried SP for 16 hours at 50°C. Lipid content was calculated as percentage of SP. The crude protein content was analyzed by gas chromatograph (GC 8A, Shimadzu, Tokyo, Japan) equipped with combustion chamber (NC 80, Sumigraph, Tokyo, Japan). Fifteen milligrams of freeze-dried samples were ashen at 830°C and the released nitrogen was measured to estimate crude protein content, using acetanilide as for calibration. All measurements were done in duplicates.

Analysis of sugar composition was performed by using gas chromatograph (G-3000, Hitachi, Tokyo, Japan) as alditol acetates. A sample of 20 mg of SP was fully hydrolyzed with 2 ml of 2 N HCl for 6 hours at 100°C. The hydrolyzate was filtered, evaporated to dryness, mixed with 1 mg of 2-deoxy-D-glucose as an internal standard, and reduced with 2 ml of 0.2 M $NaBH_4$ at room temperature overnight. Five to six drops slurry of dowex resin H type 100-200 mesh (Bio-Rad Laboratories, CA) were then added to the mixture and incubated at room temperature for 1 h, followed by filtration. The filtrate was evaporated to dryness and any residual boric acid removed by repeated evaporation with methanol. The sugar alcohols obtained were acetylated in 2 ml of acetic anhydride:pyridine (1:1) at 100°C for 10 min. The mixture was then diluted with chloroform:water (1:4), shaken well and the upper layer removed by centrifugation at 2000 rpm for 10 minutes. Remaining pyridine was removed from the chloroform extract by washing with water, followed by centrifugation. The resulting alditol acetates were dried and dissolved using chloroform. Gas chromatograph (GC) analysis was performed on a stainless steel column, 2 mm I.D. x 1.83 m, packed with 3% (w/w) ECNSS-M on Gas Chrom Q 100-120 Mesh (GL Sciences, Tokyo, Japan). Nitrogen gas flowing at 30 ml min^{-1} was used as carrier gas with the initial column temperature of 190°C for 5 minutes and then increased to 210°C at a rate of 1°C/min. The sugar component of SPAE, residual SP, soybean arabinogalactan, coffee bean arabinogalactan and citrus pectin galactan were analyzed also with the same procedure above.

Analysis of enzymatic hydrolysis product

The enzymatic hydrolysis product was analyzed by TLC on a silica gel plate (silica gel 60 F254, Merck, Darmstadt, Germany). Butanol:ethanol:chloroform:25% ammonia (4:5:2:8) was employed as a solvent system. Sulfuric acid with 1% vanillin was used for detection.

Preparation of substrates

Soybean Pulp Alkali Extract (SPAE) was prepared as follows: 500 g of SP were suspended in 10% NaOH and shaken overnight. The mixture was filtered and the filtrate adjusted to pH 5 with acetic acid. Filtrate was made to 50% in ethanol and the precipitate was obtained by centrifugation at 12000 rpm, for 20 minutes. The pellet was dried under reduced pressure at 40°C. The quantity of the dry powder was about 3% of the starting material.

The citrus pectin galactan (1,4)βgalactan was prepared as described by Labavitch et al. (5). Forty grams of citrus pectin were dissolved in 1.5 liters of 5 N NaOH containing 100 mM NaBH4. The solution, in 5 liter round bottom flask, was stirred while being heated at 90oC. The solution was refluxed for 20 hr, cooled, and adjusted to pH 5.0 by the addition of glacial acetic acid. Solid residues were removed by centrifugation. The resulting supernatant was dialyzed for 3 days against running tap water and for 1 day against distilled water. The dialyzed preparation was made 10 mM in potassium phosphate, pH 7.0. The buffered preparation was passed through a column (5 x 25 cm) DEAE-cellulose that had been equilibrated in 10 mM potassium phosphate, pH 7.0. The solution which passed directly through the column and 10 mM phosphate wash were combined, concentrated at 40oC under reduced pressure. The concentrated preparation was dialyzed against deionized water at 4oC and then made 50% in ethanol by the addition of absolute ethanol. The precipitate that formed was collected by centrifugation, dissolved in deionized water, and lyophilized. The final recovery of citrus pectin galactan was about 2% of the total citrus pectin polygalacturonic. The arabinogalactan of coffee bean was prepared by the method of Emi et al. (2). Coffee beans were pulverized and successively extracted with 2 portions of a mixture of ethanol and benzene (1:2), 10 portions of water (25°C) and 20 portions of 1.0% sodium hypochlorite. The residue obtained was boiled with 20 portions of water for 8 hr. After concentration to an adequate volume under reduced pressure, the supernatant was mixed with 3 volumes of cold ethanol. The precipitate obtained was dried by washing it successively with ethanol and ether. Final yield was 2% of the starting material.

Enzyme assays

Enzyme activities were determined by incubating the reaction mixture at 37°C and measuring the released of reducing sugars by the method of Nelson (7) as modified by Somogyi (9). One unit of enzyme activity was defined as the amount of enzyme that produced reducing sugar at rate of 1 µmol per minute. For pH range 1-6 and 6-8, acetate and phosphate buffer were used respectively. All assays were performed in 1 ml total volume.

Purification E-βGAL

All purification steps were carried out at room temperature, using 20 mM acetate buffer, pH 5. Details of the purification are mentioned under 'Result and Discussion'.

Enzyme Properties

The effect of pH and temperature on activity and stability of these enzymes were determined at the pH range of 1 to 8 and at a temperature range of 20 to 70°C. The molecular weight of enzymes was estimated by sodium deodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Molecular weights of the denatured enzymes were estimated by comparison of their migration rates with those of protein standards. Proteins standard (in Dalton) used, were phosphorylase (97,400), albumin (66,300), α-L-Arabinofuranosidase (64,400), aldolase (42,400), carbonic anhydrase (30,000), trypsin inhibitor (20,100) and lysozyme (14.400).

RESULT AND DISCUSSION

The successful of growth of *A. niger* proved SP could be used as carbon and nitrogen sources for this organism during solid state fermentation. We investigated that fresh SP contained around 75-85% water content, while dried SP consist of around 60% total sugar, 30% of crude protein and 10% lipid. The gas chromatograph analysis of sugar components showed that fresh SP mainly consists of galactose (40.3%), arabinose (21.1%), and also the minor components were xylose, mannose, rhamnose, fucose, and glucose (Table 1*)*. It is also reported by Morita (6) that the major component soybean arabinogalactan contains galactose and arabinose in the approximate proportion of 2:1. Furthermore, the SPAE and residual SP also contain galactose and arabinose in a ratio nearly 2:1.

Table 1. Gas Chromatograph Analysis Sugar Components of SP and Various Substrates

Substrates	Sugar compositions (%)						
	Rha	Fuc	Ara	Xyl	Man	Gal	Glc
Fresh SP	3.5	2.7	21	12	11	40	9.8
SP alkali extract (SPAE)	2.7	2.3	13	20	19	24	20
Residual SP	6.3	2.5	27	10	4	46	4.5
Soybean arabinogalactan	6.9	1	4.1	17	0.2	68	3.2
Coffee bean arabinogalactan	5.7	-	25	-	10	59	-
Citrus pectin galactan	0.3	-	2.7	-	-	91	6

During fermentation, the appearance of liquefied forms as hydrolyzed product during cultivation started between fourth or fifth day. As the cultivation time increased, the liquefied forms also increased. It is believed that these fungi secrete some potential liquefying enzymes on SP solid media. The optimum cultivation period was 4 days, optimized by daily harvesting (Figure 1). The crude enzyme had maximum digestion (50% as monosaccharides) of SP after 35 hours at 37°C.

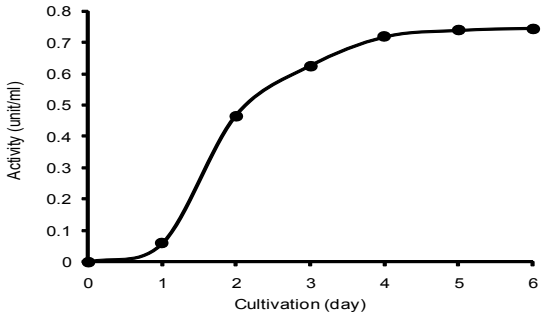

Figure 1. Optimum Cultivation of *Aspergillus niger* on SP Medium. The optimum cultivation period was determined by daily monitoring activity of crude enzyme at 37°C in 1 ml total volume of 0.1 M acetate buffer pH 5 containing 1% SPAE for 10 minutes.

Crude enzyme properties

The effect of pH and temperature on the enzyme activity was measured after 10 minutes incubation at 37°C of each enzyme in 1 ml 1% substrate at various pH and temperatures. Optimal pH and temperature ranges for assay of crude enzyme activity were pH 4 and 60°C, respectively (Figure A and B). The stability pH and temperature of crude enzyme were 2.5 – 7.5 and below 55°C after incubated 30 min to the corresponding pH and temperatures.

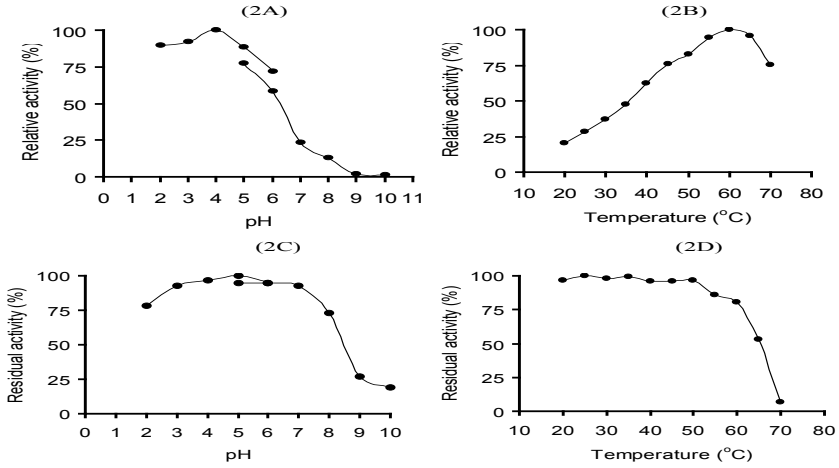

Figure 2. Effect of pH and Temperature on activity and stability of crude enzyme. The crude enzyme was obtained from 4 days cultivation at 30°C and the optimum pH (2A), optimum temperature (2B), pH stability (2C), and temperature stability (2D) of crude enzyme were assayed for 10 min at 37°C in 1 ml total volume of 0.1 M acetate buffer pH 5 containing 1% SPAE.

Purification

The crude enzyme was concentrated with 70% saturated ammonium sulfate and centrifuged at 12000 rpm for 20 min. The precipitates were dissolved in buffer. Remaining ammonium sulfate was removed by overnight dialysis against the same buffer at 4°C. This was followed by DEAE Toyopearl 650M ion exchange column pre-equilibrated with buffer. The column was eluted by 0–0.5M NaCl linear gradient. The fractions which actively hydrolyzed SPAE were pooled separately, dialyzed against buffer to remove NaCl. This dialyzed active fractions was then reloaded on a DEAE Butyl Toyopearl 650M column, followed by using Q-Sepharose column chromatography. Finally, Mono-Q (anion exchange) was used. All of the column were pre-equilibrated with buffer and eluted with linear gradient 0-0.5 M NaCl. This purification scheme resulted in 3.1% yield and 3,330 fold purification of E-βGAL.

Tabel 2. Purification of E-βGAL

Purification Step	Total ABS-280	Total Activity (unit)	Spec. act. (unit) / ABS280	Yield (%)	Fold
Crude enzyme	54,340	1,700	0.03	100	1
Ammonium sulfate Precipitation	10,560	910	0.09	54	3
DEAE Toyopearl 650M	73	185	2.53	10.8	84
DEAE Butyl Toyopearl 650M	15	85	5.7	5	190
Q-Sepharose-HP	1	71.4	74.3	4.2	2,480
Mono-Q	0.52	51.9	99.8	3.1	3,330

Enzymes properties

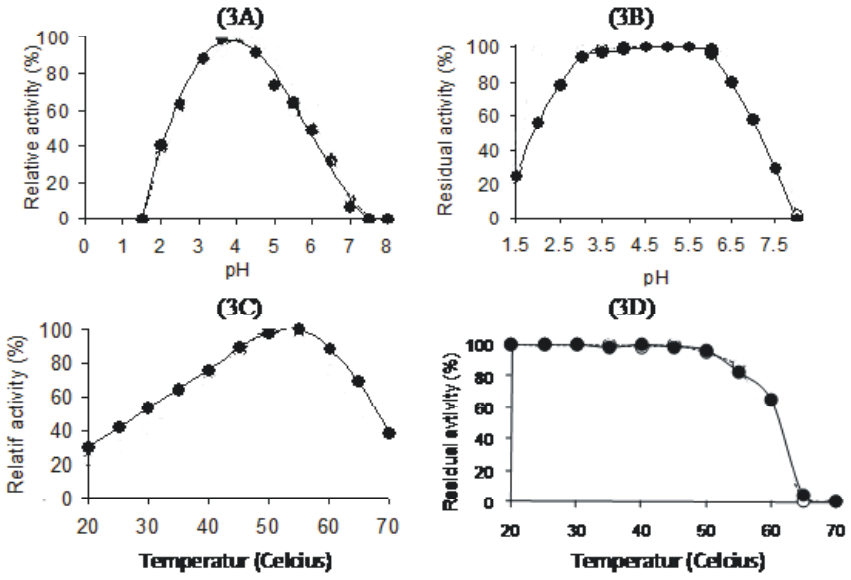

Figure 4. Effect of pH and Temperature on Activity and Stability. The optimum pH (3A), pH stability (3B), optimum temperature (3C) and temperature stability (3D) of E-βGAL (•) were assayed in 1 ml total volume containing 1% SPAE at 37°C.

The effect of pH on the enzyme activity was measured after 10 minutes incubation at 37°C of each enzyme in 1 ml 1% substrate at various pH values. E-βGAL exhibited maximum 3.6 (Figure 3A) and retained nearly 100% activity in a pH range of 3-6 respectively after 30 minutes exposure to corresponding pH values (Figure 3B). E-βGAL showed optimum activity at 55°C respectively (Figure 3C), and is nearly 100% stable below 55°C after 30 minutes exposure to respective temperatures (Figure 3D). As shown in Figure 5 the molecular weight of E-βGAL was 44 KDa approximately.

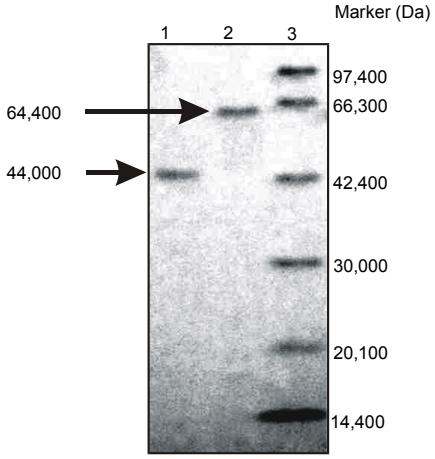

Figure 5. SDS-PAGE of Purified Enzymes. Lane 1 is purified E-βGAL, lane 2 and 3 are markers

Substrate specificity and analysis of hydrolysis product.

The substrate specificity had been identified toward various substrates for purified enzymes E-βGAL. SPAE was hydrolyzed by and E-βGAL to 7.4% (Table 3). TLC analysis revealed E-βGAL released galactose as well as some oligomers (Figure 5).

Tabel 3. The Degree of Hydrolysis (%) towards Various Substrates

Substrates	Degree of Hydrolysis (%)
Soybean pulp alkali extract (SPAE)	7.4
Soybean-arabinogalactan	20.3
Coffebean-arabinogalactan	2.1
Citrus pectin galactan	37.4

E-βGAL besides active on SPAE also effectively hydrolyzed soybean arabinogalactan or citrus pectin galactan with degree of hydrolysis 20.3 % and 37.4% respectively. However, it hydrolyzed coffee bean arabinogalactan with low efficiencies (Table 3). By TLC analysis,it was detected to be capable of releasing galactose and galactosyl oligomers (dimer, trimer, tetramer, pentamer, etc) from SPAE (Figure 6), in similar manner as from citrus pectin galactan or soybean arabinogalactan (data not shown) which they were known (3, 9) to have similar backbone chain of β-D-(1,4) linked. The results, confirmed that the E-βGAL is an endohydrolase which specifically hydrolysis internal β-D-(1,4)-galactopyranose linkage at random.

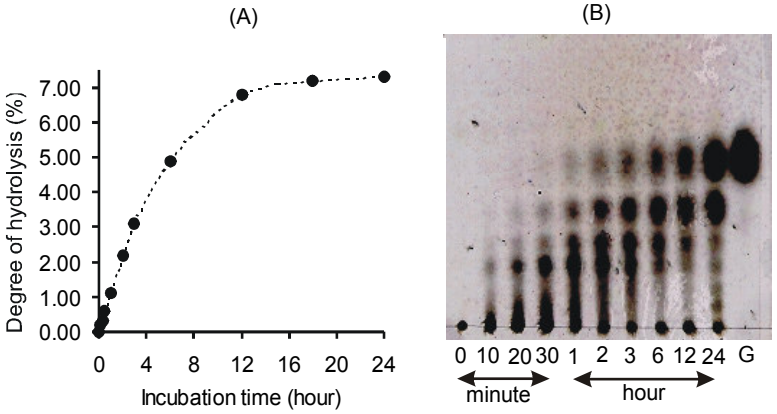

Figure 6. Hydrolysis of SPAE by E-βGAL. 1% SPAE was used as substrate in 50 mM acetate buffer, pH 4. The mixtures were incubated at 37°C for different duration as mentioned. The degree of hydrolysis measured as percentage of substrate hydrolyzed (A) and the products were analyzed by TLC, using galactose (G) as standard (B).

CONCLUSION

Microbial utilization of SP was investigated. During SP fermentation a liquefying enzymes were produced and identified as E-βGAL with molecular weight 44 KDa approximately. The enzyme exhibited maximum activity at pH 3.6 and 55°C, and retained nearly 100% activity in a pH range of 3-6 and below 55°C after 30 minutes exposure to respective temperatures. By TLC analysis, it was detected to be capable of releasing galactose and galactosyl oligomers (dimer, trimer, tetramer, pentamer, etc) from SPAE substrate. Suggested, this galactanase is an Endo-β-D-1,4-Galactanase which specifically attacked internal β-D-(1,4)-galactopyranose linkage at random.

KNOWLEDGEMENT

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EFFECT OF AMYLOSE CONTENT AND HEATING TEMPERATURE ON CHARACTERISTICS OF FRESH RICE FLOUR-BASED SPRING ROLL WRAPPERS

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ABSTRACT

The effects of amylose content and heating temperature on characteristics of fresh rice flour-based spring roll wrappers were investigated by using added free amylose of cassava to rice flour. The used rice flour in this research was from variety Mentik (an Indonesian local rice variety). Amylose content of blended rice flour ranged from 25% up to 40%. The fresh rice flour-based spring roll wrappers were made without frying oil on Teflon frying pan at 72°C and 82°C during 4 minutes. After heating, the product was tempered for 30 minutes at 25°C. The product was evaluated for rice starch granules size, moisture content, water activity and elongation at break. Each experiment was conducted by three replications. All of the data were analyzed by analysis of variance (α 5%). Duncan multiple range test (α 5%) was used to determine the significant difference among the treatments. The result showed that free amylose adding to rice flour blends homogenized the swelling of rice starch granules. The increasing of amylose content more than 34% increased water activity. The amylose content from 31 % up to 40% increased the moisture content. Increasing amylose content decreased elongation at break. Calculated amylose content in the range of 25% to 31% at 72 ° C for 4 minutes produced products with relatively high elongation at break of 14.45% -16.84%. Heating temperature at 82 ° C for 4 minutes produced lower elongation at break characteristics which ranged between 10, 16% to 12.02%.

Keywords: amylose content, heating temperature, fresh spring roll, rice flour, characteristics.

INTRODUCTION

Characteristics of fresh rice flour-based spring roll wrapper are affected by several factors, such as natural properties of rice, heating temperature and duration of heating. Gertz (2000) in Jack (2006) described the changes during heating, it is involved mass transfer, heat transfer, removal of heat, internal cooking and several physicochemical reactions. The rate of evaporation and water migration within the product will determine the final characteristics of the food product. If the rate of evaporation and water migration were not optimal, the gummy texture will be produced. There are some parts that bind more hydration water, so that the impression is not mature (under-cooked). Evaporation of water in the layers of material (subsurface water) will also cause heat loss from the surface toward the center of the material. Lanner *et al*. (2001) stated that the heating temperature must be regulated in order to optimize the evolution of water vapor from the constant rate period of moisture loss during heating or frying. Certain degree of gelatinization should be reached in order to get sufficient tensile strength and to prevent cracking due to uneven distribution of water (Wanous, 2004).

Low heating temperature resulted insufficient gelatinization process. The gummy texture due to excessive hydration during inefficient heating process (Lanner et al., 2001). Mechanical properties of formed gel are strongly influenced by time and temperature in addition to tempering conditions. It was also affected by protein and polysaccharide content, pH and the addition of salt (Nunes *et al*., 2003). Heating temperature has a role in the decomposition of the biopolymer structure into smaller parts which results in increased molecular interactions. Heating temperature is an important factor in the formation of a stable film, in related to the implementation of thermal gelation, denaturation, and precipitation (Li, *et al*., 2005). Heating temperature has to provide enough energy to denature various proteins of rice varieties, at 73.3 ° C for albumin and 82.2 ° C for glutelin, whereas the temperature is required for protein-starch interaction of rice around 80.5 ° C (Ju *et al*., 2001). Egg white albumin began to coagulate at 62 ° C and becomes very solid at temperatures higher than 70 ° C (Endre and Monegle, 1987 in Mukprasirt *et al*., 2000). Increasing heating temperature 70°C to 80°C induced protein denaturation that produce an increase in the number and localization of a stronger bond between the chains of the protein. It will decreased ability of detention stretches that can be observed with elongation decreasing of edible films made from green bean protein (Bourtoom, 2008). Thus the heating temperature will determine the characteristics of food product, which is included spring roll wrapper.Too long heating will result in fracture of the matrix due to shrinkage as

a result of evaporation of water and damage the binding material ability of starch (Anderson and Hodson, 2006). Proper heating temperature will produce a cohesive mass that can be caused by an increasing amount of amylose in a continuous phase of the system and the occurrence of strong interactions between starch granules and the continuous matrix system (Rodriguez-Sandoval *et al*., 2008). Thus, the temperature and duration of heating determine the characteristics of the product.

Starches with higher amylose content will form stronger gel and will be more difficult to damage. Increasing of amylose content will inhibit the swelling of the granules thus maintained the integrity of the swollen starch granules. The purpose of this study is to investigate the influence of amylose content and heating temperature on the characteristics of fresh rice flour-based spring roll wrappers.

MATERIALS AND METHODS

Materials

Mentik rice from Candi, Nglames, Madiun, obtained from the UD. Eka Jaya rice mill at Surabaya. Rice flour obtained by grinding the rice in dry process (without soaking) and be sifted by a 80 mesh sieve size. Amylose extraction from tapioca used modified method of Takeda et al. (1986) and Patindol et al. (2003). Leghorn chicken eggs obtained from a local shop in Surabaya.

Methods

The research design was factorial experiment with randomized completely block design. Various factors is the amylose content consists of six levels: 25%; 28%; 31%; 34% ; 37% and 40% (w / w); while heating temperature on 72ºC and 82 ºC. The observed dependent variables are starch granule size, $a_{w,}$ moisture content and elongation at break. The data were processed by analysis of variance, the difference of among treatments were tested by Duncan Multiple Range Test with $\alpha = 5\%$. Starch granules size was measured by using Olympus DP 20 Digital Camera Microscope. Water activity was measured with a Rotronic hygrometer AW1 Hygro Palm at 85% RH + / - 1% at temperature of 25 º C + /-2 º C. Moisture content was measured by gravimetric method (AOAC, 2000). Elongation at break was measured by Shimadzu Autograph. The batter has been mixed to be homogeneous by placing the mixture on a Labinco magnetic stirrer hot plate model L-81with speed of 100 rpm for 2 minutes, then placed on a Teflon material frying pan (diameter 10 cm). Heating was held for 4 minutes. The formulation are listed in Table 1.

Table 1. Formula of Fresh Rice flour-based Spring Roll Wrapper

Ingredients (g)	Amylose Content (%)					
	25	28	31	34	37	40
Rice Flour	3.00	2.85	2.70	2.55	2.40	225
Crude amylose of 85% purity*	0.00	0.15	0.30	0.45	0.60	075
White Egg	3.50	3.50	3.50	3.50	3.50	3.50
Water	6.00	6.00	6.00	6.00	6.00	6.00
Tapioca	0.50	0.50	0.50	0.50	0.50	0.50
Total (g)	13.00	13.00	13.00	13.00	13.00	13.00

RESULTS AND DISCUSSION

Rice Starch granules size

Data in Table 2. showed a significant difference in the effects of amylose content and there was interaction between two factors to the size of rice starch granules.

Table 2. Rice Starch Granule Size of Fresh Rice Flour-based Spring Roll Wrappers on Different Levels of Amylose and Heating Temperature

Heating Temperature (º C)	Rice Starch Granule Size (μm2)*					
	Amylose Content (%)					
	25	28	31	34	37	40
72	1673.14 **h**	1531.39 **f**	1476.12 **d**	1408.92 **c**	1323.34**b**	1271.57 **a**
82	1700.85 **i**	1609.67 **g**	1506.48 **e**	1484.40 **de**	1415.69 **c**	1344.85 **b**

*Values in same column with different letter are significantly different based on DMRT test with α =5%

Starch granules size decreased significantly with increasing amylose content on both heating temperatures. This is caused by increasing levels of amylose starch, the more hydrophilic side of the dough system, resulting in competition between the granules in water absorption and produced restriction of swelling. Swelling restrictions would affect the cohesiveness and elongation (Shih, 1996). It also affected distance between the granules with one another, so at the optimal distance will result cohesive and flexible product with sufficient high elongation characteristics. The data also showed an increase in the size of rice starch granules in the respective levels of amylose starch at 82°C compared to products of heating temperature at 72 ° C. Increased temperature lead to breaking of some bonds between and intra molecular which makes the structure of starch granules are more open and to facilitate the entry of water in the structure of starch granules (Hongsprabhas, 2007).

Water activity (a_w)

Table 3 showed a trend of increasing in a_w as increased levels of amylose. This phenomena could be influenced by amylose alignment molecules which produced free water.

Table 3. A_w of Fresh Rice Flour-based Spring Roll Wrappers on Different Levels of Amylose and Heating Temperature

Heating Temperature (º C)	Amylose Content (%)*					
	25	28	31	34	37	40
72	0.509 **a**	0.512 **ab**	0.514 **b**	0.521 **c**	0.527 **c**	0.535 **d**
82	0.653 **h**	0.659 **i**	0.665 **j**	0.609 **g**	0.603 **f**	0.598 **e**

*Values with different letter are significantly different based on DMRT test with α =5%

This phenomenon is supported by the strong correlation between size of rice starch granules and A_w with a coefficient of determination (R2) of 0.92 (Y = 7506.6 X -11688.05) as shown on Fig. 1.

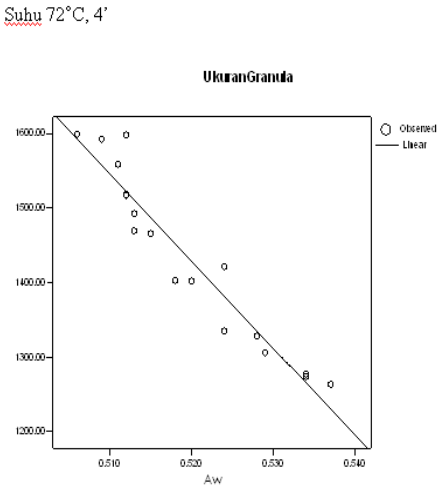

Figure.1 Regression Curve between Starch Granule Size and A_w on 72 º C Heating **Moisture Content**

The average moisture content showed a trend of increasing water content as increasing levels of amylose.

Table 4. Water Content of Fresh Rice Flour-based Spring Roll Wrappers on Different Levels of Amylose Content

Amylose content (%)	Water content (%)*
25 %	34.76 **a**
28 %	36.49 **ab**
31 %	38.15 **bc**
34 %	38.85 **bc**
37 %	40.35 **c**
40 %	40.74 **c**

*Values with different letter are significantly different based on DMRT test with α =5%

This phenomenon is caused by the amount of water trapped in the gel system will increase along with greater levels of amylose in the material system. The data in Table 5 showed an average decline in water levels with an increase in heating temperature from 72 ° C to 82 ° C. This is due to the increase in heating temperature means an increase in the rate of evaporation of free water that produce lower water content.

Table 5. Water Content of Fresh Rice Flour-based Spring Roll Wrappers on Different Heating Temperature

Heating Temperature (º C)	Water content (%)*
72	39.67 **b**
82	36.74 **a**

*Values with different letter are significantly different based on DMRT test with α =5%

Elongation

An increasing of amylose content decreased elongation due to longer distance of molecular components as the consequence of more amount of water trapped in gel system (Table 6). The increasing levels of amylose starch provided the more hydrophilic side of the dough system. In this condition,water acted as a plasticizer materials (Chang et al., 2006).

Table 6. Elongation at Break of of Fresh Rice Flour-based Spring Roll Wrappers on Different Levels of Amylose Content and Heating Temperature

Heating Temperature	Amylose Content (%)*					
(º C)	25	28	31	34	37	40
72	16.84**f**	14.62 **e**	14.45**e**	13.68**d**	11.85 **c**	10.02**a**
82	12.02**c**	11.83**c**	11.11**b**	10.97**b**	10.85**b**	10.16**a**

*Values with different letter are significantly different based on DMRT test with α =5%

CONCLUSION

Based on the study of all the response of depended variables, it can be concluded that the treatment of amylose content of rice flour and heating temperature influenced the characteristics of fresh rice flour-based spring roll wrappers. Amylose content in the range of 25% to 40% tend to increase the moisture content of product. Heating temperature at 72°C for 4 minutes with 34% of calculated amylose content

produced best characteristics of fresh rice-based spring rolls wrappers, based on elongation. Heating temperature at 82°C for 4 minutes decreased elongation significantly compared with product at 72°C for 4 minutes. Higher heating temperature could strengthen the chemical bonds among the polymers and affected elongation of product.

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ISOLATION AND IDENTIFICATION OF YEASTS FROM *Broussonetia papyrifera* VENT. FROM TROWULAN, INDONESIA

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ABSTRACT

Isolation and identification of yeasts from *Broussonetia papyrifera* Vent (Moraceae) from Trowulan, Indonesia has been carried out. A total of 1.027 yeast isolates were obtained by using direct, washing, and filter membrane methods. Based on similarity of colony morphology, 15 representative yeast isolates were selected. All representative isolates were identified based on the sequence analyses of internal transcribed spacer (ITS) regions of rDNA. Seven representative yeast isolates belong to the phylum *Ascomycota,* and consist of two classes, i.e. *Hemiascomyetes* (3 isolates of *Candida*), and *Euascomycetes* (1 isolate of *Dothideaceae*, and 3 isolates of *Aureobasidium*).Eight isolates belong to the phylum *Basidiomycota* and consist of three classes, i.e. *Ustilaginomycetes* (1 isolate of *Pseudozyma*), *Urediniomycetes* (2 isolates of *Rhodotorula*), and *Hymenomycetes* (5 isolates of C*ryptococcus*).

Keywords: *Ascomycota*, *Basidiomycota*, *Broussonetia papyrifera*, identification, yeasts.

INTRODUCTION

Broussonetia papyrifera Vent. is a small tree which grows naturally in Asia (Thailand, China, Myanmar, Laos, Japan, Korea) and Pacific countries. The plant grows to 1,500 m high (Whistler & Elevitch, 2006). The plant leaves provide nutrient resources to yeast colonists. Isolation of yeasts from plants have been reported by Stohr et. al (2008) and they were identified as *Cryptococcus* Vuillemin dan *Sporobolomyces* Kluyver & van Niel. De Errasti (2009) reported that *Coprinellus micaceus*, *Lecythophora hoffmannii*, and *Rhizopus microsporus* were isolated from *Broussonetia papyrifera* plant. This paper reported the yeast species found on leaves of *Broussonetia papyrifera* from Indonesia.

OBJECTIVE

The objectives of the present study were (i) to obtain yeast isolates from *Broussonetia papyrifera* plant of Trowulan, Indonesia (ii) to identify their species using the ITS rDNA sequence information.

MATERIAL AND METHOD

Three methods were used for isolation of yeasts, i.e. *direct-washing method*, *spread method*, and *Millipore membrane filtration method*. Isolation medium was *Yeast Malt Agar (*YMA) with tetracycline. Total DNA was extracted from yeasts by boiling method according to Sjamsuridzal and Oetari (2003). The DNA extract was amplified by PCR technique using ITS4 and ITS5 primers according to White (1990). The amplification consisted of 40 cycles of denaturation at 95°C for 15 sec, primer annealing at 58°C for 30 sec, and primer extension at 68°C for 1 min; followed by post extension at 68°C for 10 min. PCR products were analyzed on 2% (w/v) agarose gel, stained with ethidium bromide and visualized under UV light using the Gel doc. The sequencing results were compared with DNA sequences from GenBank database at National Center of Biotechnology Information (NCBI) using the Basic Local Alignment Search Tool (BLAST) program for screening sequence similarity.

RESULTS AND CONCLUSION

Gene-sequence determinations have also provided a rapid, accurate means for identification of individual strains to the species level. Sequencing of species-diagnostic genes represents the most accurate means for isolate identification,and several rapid identification methods using molecular probes based on these sequences have been developed andcare becoming available to identification yeast isolates expecially from plant.

The results of isolation and identification of yeast isolates from saeh plant obtained this study are reported in Tabel 1. Isolation and identification of yeasts from a plant (*Broussonetia papyrifera* Vent., family Moraceae) from Trowulan, Indonesia has been carried out. A total of 1.027 yeast isolates were obtained by using direct, washing, and filter membrane methods. Based on similarity of colony morphology

The identification results indicated that 15 representative yeast isolates consist of two classes from phylum *Ascomycota* and three classes from phylum *Basidiomycota.* Seven representative yeast isolates belong to the phylum *Ascomycota,* and consist of two classes, i.e. *Hemiascomyetes* (3 isolates of *Candida*), and *Euascomycetes* (1 isolate of *Dothideaceae*, and 3 isolates of *Aureobasidium*). Eight isolates belong to the phylum *Basidiomycota* and consist of three classes, i.e. *Ustilaginomycetes* (1 isolate of *Pseudozyma*), *Urediniomycetes* (2 isolates of *Rhodotorula*), and *Hymenomycetes* (5 isolates of C*ryptococcus*).

Table 1. Yeast isolates from *B.papyrifera* Vent, Trowulan Indonesia

NO	CODE	LOCATION	IDENTIFICATION
1	TR1S1 WM 2.3	Trowulan, Jawa Timur	*Cryptococcus flavescens*
2	TR1S1 WM 2.6	Trowulan, Jawa Timur	*Dothioraceae* sp.
3	TR1S2 WM 1.3	Trowulan, Jawa Timur	*Candida* sp.
4	TR1S2 WM 1.5	Trowulan, Jawa Timur	*Candida* sp.
5	TR1S2 MF3.5	Trowulan, Jawa Timur	*Aureobasidium* sp.
6	TR2S2. WM1.1	Trowulan, Jawa Timur	*Cryptococcus flavescens*
7	TR2S1.WM1.11	Trowulan, Jawa Timur	*Cryptococcus flavescens*
8	TR2S2. WM 1	Trowulan, Jawa Timur	*Cryptococcus flavescens*
9	TR2S1. WM 1.3	Trowulan, Jawa Timur	*Cryptococcus sp.*
10	TR2.5	Trowulan, Jawa Timur	*Aureobasidium pullulans*
11	TR2S2 WM 2.9	Trowulan, Jawa Timur	*Aureobasidium* sp.
12	TR2S1 WM 1.5	Trowulan, Jawa Timur	*Candida* sp.
13	TR2S1. WM 1.51	Trowulan, Jawa Timur	*Pseudozyma thailandica*
14	TR2S1.DW 1.1	Trowulan, Jawa Timur	*Rhodotorula mucilaginosa*
15	TR2S1.DW 1.2	Trowulan, Jawa Timur	*Rhodotorula mucilaginosa*

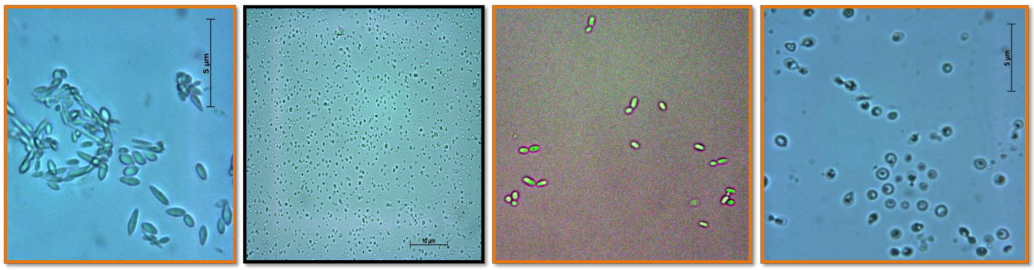

Figure 1. Yeast isolates from *Broussonetia papyrifera*: a. *Pseudozyma thailandica*; b. *Candida* sp., c. *Rhodotorula mucilaginosa,* d. *Cryptococcus* sp.

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PHYSIOLOGICAL AND GENETIC CHARACTERIZATION OF *LACTOBACILLUS RHAMNOSUS* FROM SUMBAWA MARE MILK

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ABSTRACT

Sumbawa mare milk is an Indonesian indigenous fermented milk product, which recently gained interest due to its claimed health promoting effects. Previous studies on microbiology of Sumbawa mere milk revealed that the *Lactobacillus rhamnosus* was one of dominant bacterial species in the milk. The objective of this study was to verify the physiological and genetic characters of *L. rhamnosus* isolated from Sumbawa mare milk and their potential application as probiotic for human health.Thirty *L. rhamnosus* strains were used in this study. The physiological features were analyzed using API CHL for lactobacilli, while their genetic diversities were determined using PCR-RAPD. The probiotic properties of the strains were analyzed based upon their resistant to low pH, resistant to deoxycholic acid, primary bile acid metabolism, and inhibitory activities against pathogenic bacteria. The selected strains then was further analysed by sequencing of their 16S rDNA.Sugar metabolisms of *L. rhamnosus* from Sumbawa mare milk were varies and likely depent on the individual properties of strain. Most of *L. rhamnosus* from Sumbawa mare milk could not metabolize maltose, mellibiose, turranose and lyxzose, but they metabolized fucose. The latter is rarely found in *L. rhamnosus*. Most *L. rhamnosus* resistant to pH 2-4. It was no strain resistant to 0.4mM DCA, but most strains resistant to 0.2mM DCA. Most strain did not transform cholic acid into deoxycholic acid. Genetic variation verified by PCR RAPD showed that the 30 srains could be placed into 7 genetic groups. Sequences of the variable area I (VI) of the 16S rDNA showed 97-99% homologies to the *L. rhamnosus*. The siginificant of *L. rhamnous* from Sumbawa mare milk for human health is under investigation. This studies demonstrated the diversities of *L. rhamnosus* in Sumbawa mare milk, which may potential link to their specific functions for human health.

Keywords: *Lactobacillus rhamnosus*, Sumbawa mare milk, probiotic.

ORAL PRESENTATION: BIODIVERSITY AND ENVIRONMENT

BIODESULPHURIZATION OF DIBENZOTHIOPHENE BY GROWING AND IMMOBILIZED CELLS OF KWN5 STRAIN

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ABSTRACT

A new strain, KWN5 was tested for the ability to use dibenzothiophene in n-tetradecane as the sole sulphur source with two phase oil-water system. The desulphurization ability of KWN5 strain was evaluated by growing and immobilized cells with dibenzothiophene (DBT) as substrates. Growing cells of KWN5 could degrade 200 ppm DBT around 72.13% within 96 h at 37°C, pH 7. Cells immobilized by entrapping with sodium alginate had high DBT desulphurization activity and could degrade DBT of 46.76-100% (depend on concentrations of sodium alginate and cells) within 24 h at 37°C, pH 7 with shaking (90 rpm).

Keywords: *Biodesulphurization; dibenzothiophene; growing cells; immobilized cells; KWN5*

Fossil fuels such as light gas oil and diesel oil contain various heterocyclic organosulphur compounds, including alkylated form of dibenzothiophene (DBT), which cannot be completely desulphurized by hydrodesulphurization process using metal catalysts. However, biodesulphurization process using microbial biocatalysts capable of desulphurizing aromatic sulphur compounds, such as alkylated DBTs is suitable for this purpose, and several studies on DBT desulphurizing bacteria and their enzymes involved in DBT desulphurization have been reported (Gray et al., 1996; Ohshiro et al., 1996).

Konishi and co-workers (1997) reported that biotransformation by growing cells are usually conducted in complex growth media which can interfere with analysis of product owing to the presence of a complicated mixture of other metabolites produced by the biocatalysts and the medium component. To avoid these problems, we are able to used immobilized cells for quantitative analysis of their specific desulphurizing activities.

In this work, the desulphurization activity by growing and immobilized cells of KWN5 strain in two phase system of saline and *n*-tetradecane containing DBT have been evaluated.

MATERIALS AND METHODS

Chemicals

DBT was purchased from Tokyo Kasei Kogyo Co. Ltd. (Tokyo, Japan). Tetradecane and sodium alginate were purchased from Wako Pure Chemical Co., Osaka, Japan. All other reagents were analytical grade available.

A concentrated fraction of aromatic compounds (CA) was prepared by fractionation of commercial light gas oil (Gunam et al., 2006).

Bacterial strains and cultivation

KWN5 strain (Gunam et al. 2009) was used in this study because of its ability to desulphurize DBT. KWN5 strain was grown in a mineral salt sulphur-free (MSSF) medium (pH 7) with CA as the sole sulphur source as reported in our previous report (Gunam et al., 2009). The cultivation of seed culture was carried out at 37°C for 4 days in test tube containing 5 ml of MSSF medium and 10 µl of CA with shaking at 190 rpm. For the production of large quantity of cells, KWN5 strain was cultivated in 500 ml Erlenmeyer flasks containing 100 ml of MSSF medium with 50 µl CA as the sole source of sulphur at 37°C for 4 days.

Entrapment in a calcium alginate gel

Sodium alginate with various concentrations [1-4% (w/v)] was dissolved in deionized water and sterilized at 121°C for 15 min. After cooling, 6 ml of sodium alginate solution was mixed with 3 ml of cell suspension (31.7 mg dry cells/ml). Cell immobilization was performed by extruding the mixture through

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an injector into a gelling solution of 4% (w/v) calcium chloride ($CaCl_2$) at room temperature and solidified for 6 h at 4°C. The immobilized beads formed were washed with saline, and then stored at 4°C until use.

Biodesulphurization by immobilized cells in a model oil system

Biodesulphurization (BDS) was carried out using immobilized cells in 100 ml flask containing 17.5 ml saline and 2.5 ml *n*-tetradecane containing 100 mg DBT/l at 37°C on rotary shaker at 90 rpm. The immobilized cells were activated for 48 h in culture medium (MSSF-CA medium) before used.

The immobilized cells were separated from the medium by transferring of the model oil and saline into another tube and the beads were washed twice with saline. The beads were regenerated by 20 h incubation in saline solution containing 1% glucose at 37°C with shaking at 90 rpm. Then, the beads were ready to use for the next cycle to desulphurize fresh oil after being washed 2 times with saline.

Analytical methods

The turbidity of water layer of the model oil after reaction was measured with a spectrophotometer at 660 nm. The cell concentration was determined from the linear relationship between the optical density at 660 nm (OD_{660}) and dry cells (drying at 105°C for 36 h). The concentration of DBT was measured by GC equipped with a flame ionization detector. A portion of the oil layer was centrifuged, and the supernatant was analyzed by GC (GC-17A, Shimadzu) equipped with a DB-17 column (0.25 mm i.d. x 30 m length; J&W Scientific, Folsom, CA) and a flame ionized detector. The GC analysis was carried out under the following conditions: the initial temperature of the column oven was 220°C, and heated to 270°C at a rate of 10°C/min. The carrier gas was helium, and both the injector and detector temperatures were set at 250°C.

RESULTS AND DISCUSSION

Effect of carbon sources on desulphurization activity

In order to know the effect of carbon sources on desulphurization activity, KWN5 strain was grown on MSSF-medium containing various carbon sources. KWN5 strain was able to grow in MSSF-medium with different carbon sources, but the highest growth occurred in MSSF-medium containing CA as the sole carbon source (Fig. 1).

The cells grown on MSSF-medium without carbon source the bacterium could not grow well. These results indicate that this strain utilized dibenzothiophene as a sulphur source but not as a carbon source. In addition, the strain could not utilize the solvent *n*-tetradecane as a carbon source.

Another important thing that this strain is the lack of the utilization of hydrocarbons in the organic layer, this characteristics is preferable and ideal for desulphurization because it keeps the remaining hydrocarbon molecules fully active as energy sources without any loss of their thermal units (Konishi et al. 1997).

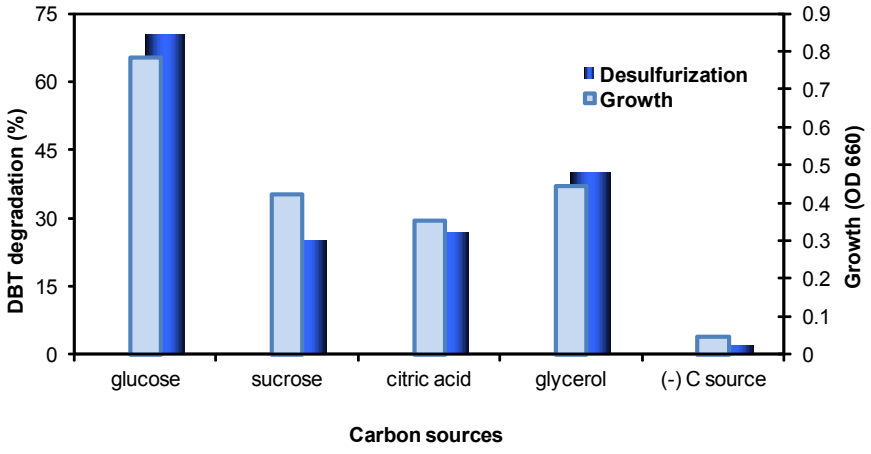

Fig. 1. Effects of various carbon sources on the growth and desulphurization activity of KWN5 strain

Immobilized Cells Activity

Many results have been reported on BDS, using cell suspensions in oil/water/cell was triphasic systems. Yu (1998) tried to apply a cyclone to isolate cells and water from emulsified oil. Chang et al (2000) used celite beads as an immobilization material. Both trials were based on triphasic systems to maintain the desulphurization rate, but did not result in perfect separation. Therefore, we tried to apply the entrapped-cell method.

The biodesulphurization activity of cells immobilized in Na-alginate with the entrapped-cell method, was conducted and the results were shown in Fig. 2. Cells immobilized in 1-4% (w/v) sodium alginate and cells OD_{660} of 20-40, exhibited high activities in the model oil and showed a slightly higher activity than the others.

Cells of KWN5 was immobilized by entrapping with sodium alginate had high DBT desulphurization activity and could degrade DBT of 46.76-100% (depend with concentrations of sodium alginate and cells) within 24 h at 37°C, pH 7 with shaking (90 rpm). These results indicated that the support material was appropriate for cells immobilization and demonstrated that cells immobilization was a very essential technique for the degradation of sulphur in oil. This technique has many advantages such as easy to separate the beads with desulphurized oil, more stable, and so on. Naito et al., (2001), reported that BDS using ENT-4000-immobilized cells in a n-tetradecane/immobilized cell biphasic system made it easy to recover desulphurized oil and to use the biocatalyst repeatedly for long periods with reactivation.

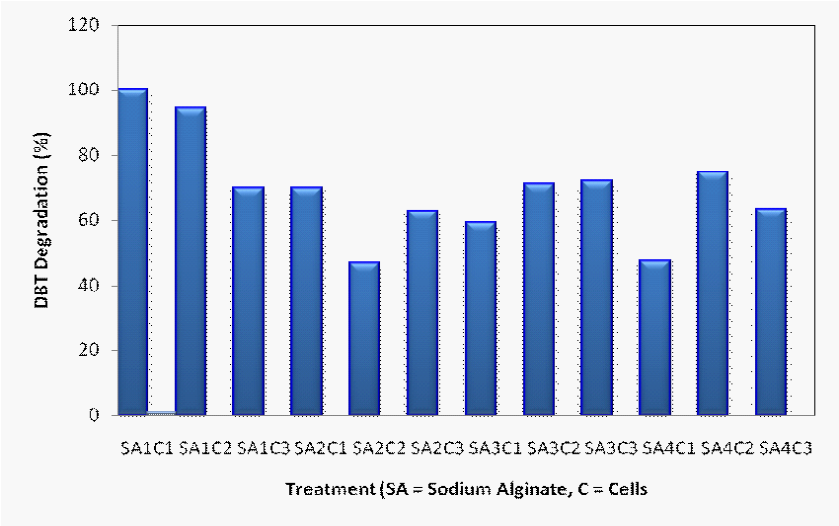

Figure 2. Effects of sodium alginate and cells concentrations on desulphurization activity of immobilized cells KWN5

CONCLUSION

KWN5 strain could grow on mineral salt sulphur-free (MSSF) medium with the *n*-tetradecane oil phase containing 200 ppm dibenzothiophene (DBT) as the sole sulphur source and desulphurize this compound. Growing cells of KWN5 could degrade 200 ppm DBT around 72.13% within 96 h at 37°C, pH 7. Cells immobilized by entrapping with sodium alginate had high DBT desulphurization activity and could degrade DBT of 46.76-100% (depend with concentrations of sodium alginate and cells) within 24 h at 37°C, pH 7 with shaking (90 rpm).

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SOME ISSUES RELATED TO WILDLIFE (FLORA AND FAUNA) THAT NEED TO BE MANAGED BY HOTELS SEEKING SUSTAINABLE TOURISM CERTIFICATION IN BALI

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ABSTRACT

Some hotels in Bali (Indonesia) recently have been seeking for certification on sustainable tourism. Some certification that are chosen including EarthCheck, Green Globe or Tri Hita Karana Tourism Awards and Accreditations. Criteria on those certifications, among others, are related to wildlife or flora and fauna. This paper describes and discusses some issues related to biodiversity (flora and fauna) that needs to be managed by hotels which seek sustainable tourism certification in that province. The data were collected by the author when undertaking onsite observation on the trainings / consultations or audits / pre audits that were undertaken on those hotels in the past ten years, between the year of 2000 and 2010. These data were supported by other information available on literatures related to flora and fauna aspects. Some issues recorded including: (1) souvenirs sold made of protected species, (2) lack of knowledge on protected species, (3) lack of information on protected species passed to guests, employees, suppliers and other stakeholders, (4) the use of protected species as materials/accessories on the hotel, (5) lack of concern in buying eco-building materials, (6) lack of care for the danger of keeping invasive species, (7) lack of systems in preventing misuse of protected species, (8) problems related to animal feeding, (9) problems of hotels attacked by monkeys, (10) difficulties faced by hotels in handling feces (of birds and bats), (11) the danger of snake attacks, (12) no space kept for "conservation zone", (13) relase of "feral animals". Some thought on strategies to manage these issues are also provided in this paper.

Keywords: flora, fauna, hotel, conservation, sustainable tourism, certification, Bali

INTRODUCTION

Some hotels in Bali (Indonesia) recently have been seeking for certification on sustainable tourism (Dalem, 2009; Dalem, 2011). Some certifications that are chosen including EarthCheck, Green Globe or Tri Hita Karana Tourism Awards and Accreditations. Criteria on those certifications, among others, are related to wildlife or flora and fauna. This paper describes and discusses some issues related to biodiversity (flora and fauna) that need to be managed by hotels which seek sustainable tourism certification in that province. This paper is expected to provide a better understanding on issues related to flora and fauna in the hotel environments in Bali, the information of which will be useful for improvement of environmental management related issues in those hotels.

MATERIAL AND METHODS

The data were collected by the author when undertaking onsite observation on the trainings / consultations or audits / pre-audits that were undertaken on hotels in Bali in the past ten years, between the year of 2000 and 2010. These data were supported by other information available on literatures related to flora and fauna aspects.

RESULTS AND DISCUSSIONS

Some issues recorded including: (1) souvenirs sold made of protected species, (2) lack of knowledge on protected species, (3) lack of information on protected species passed to guests, employees, suppliers and other stakeholders, (4) the use of protected species as materials/asesories on the hotel, (5) lack of concern in buying eco-building materials, (6) lack of care for the danger of keeping invasive species, (7) lack of systems in preventing misuse of protected species, (8) problems related to animal feeding, (9) problems of hotels attacked by monkeys, (10) difficulties faced by hotels in handling feces (of birds and bats), (11) the danger of snake attacks, (12) no space kept for "conservation zone", and (13) relase of "feral animals".

(1) Souvenirs Sold Made of Protected Species

Some hotels do not aware they are selling souvenirs made of protected species. For example, a

hotel was observed to sell a souvenir made of the turtle shells. They were sold in the form of comb. But after audited the souvenir had not been sold in this hotel anymore, following recomendation provided by the audithor on sustainable tourism.

(2) Lack of Knowledge on Protected Species
Lack of knowledge on protected species make the hotel people doing illegal actions. Some of them think that they still can keep shells of protected species, because they have been dead already, even though not allowed to keep living animals. In fact, keeping "dead animals", part of the shells, and products made of part of protected species actually also against the law.

(3) Lack of Information on Protected Species Passed to Guests, Employees, Suppliers and other Stakeholders
In order to achieve succcessful actions in handling protected species, the hotel needs to get data on protected species and work together with stakeholders in protecting those species. If just one of the stakeholders only protect the species while others do not then there is a possibility that one of them take actions that against the law. The result of conservation then will be still not satisfying. When the data have been available, then they need to be communicated to other parties/stakeholders. Information provided can be in the form of "warning signs" and a little bit words on them, part or clause of the agreement or contract for the tenants or contractors/suppliers, or in the form of information letters for guests.

(4) The Use of Protected Species as Materials/Accessories on the Hotel
Not only souvenirs, accessories or decorations of the hotel may be made of protected species. They may be in the form of stuffed turtles, mollusc shells, chunck of black corals, etc. They actually should not be used, because against the law. For example, the souvenir made of the shell of *Nautilus pompilius*. The species were 15-25 cm in size, inhabit deep sea, and generally sold as a whole, not cut in pieces. The species is protected based on Indonesian Forestery Ministry Decission "SK Menetri Kehutanan No. 12/Kpts II/1987" dated January 12, 1987 (Suartini *et al*., 2010).

(5) Lack of Concern in Buying Eco-building Materials.
Many hotels do not take attention wether their wood for building materials taken from a legal logging or not. An environmental conscious operation needs to find out about this. In addition the hotel needs to find out wether their building materials of protected species or not. If they were protected, they should not be used.

(6) Lack of Care for the Danger of Keeping Invasive Species
In few hotels, at least on two hotels I was observed, a species of fish, locally kown as "ikan sapu-sapu" is considered important tools for cleaning the aquarium and ponds. The ability of the fish in cleaning the aquarium or ponds has been recognised, but the risk of the incidental release of the species into the closeby waterbody has not been considered. In any waterbodies, this fish may decreases the fisheries production because it may clear fish eggs (I Wayan Restu, fisheries expert, pers. comm.). One species of freshwater turtle which might not be native/indigenous to Bali has been sighted, and frequently considered to be invasive by the turtle expert (Prof. Shelley Burgin, pers. comm.). Dispite the benefits of attraction for the guest especially the kids, this turtle may cause environmental risk because has dispersed incidently to Bali's waterbody without enough attention from the hotel managements.

(7) Lack of Systems in Preventing Misuse of Protected Species.
For example, one hotel which has been found to sell souvenirs of protected species, and then in few years latter this hotel again found to do so. To overcome this case, the tenant needs to be ask to sign a contract within which given clause prohibition of selling souvenirs made of protrected species with the risk faced if doing so. This make them aware, so the risk for selling souvenirs of protected species become lower. In addition, monitoring needs to be undertaken by environmental committee of the hotel to find out whether protected species, or part of them, used in souvenir materials and warning them not to sell them any more if that happened.

(8) Problems Related to Animal Feeding
Some hotels have made animals feeding as part of their tourist attractions. For example feeding themed squirels may be a good fun for many guests. Feeding fish may be a great fun for guests

including the children.

Some guests may thing that by feeding wildlife that will good for the life of those creatures. Actually feeding them may change their behaviour so become more dependent on foods provided by humans and decrease their survival capability. In additions, feeding animals may cause the risk of beeing bitten, the risk of stolen foods by animals/fauna, etc. Feeding too much may cause the monkeys population increased too fast so they need more habitats, the situation of which might be making a difiifcult situation for their management, because providing more habitats might not be possible.

Feeding animals may cause animals to be sick. So, healthy food for them need to be considered. When animals understand that stealing things will be paid by getting 'reward of food' from the victims, animals may steal guest's belongings and kept them in custody and only willing to release them when 'rewards' have been given. This part of learning process.

(9) Problems of Hotels Attacked by Monkeys

Even though in some places in Bali monkeys have been providing benefit for the local people, because being used as tourist attactions (Suryawan Wiranatha and Dalem, 2010; Suputra, 2003), few hotels in Bali have been facing problems because of being attacked by monkeys**.** They came to the hotel in individual or in groups steal food in the hotels, especially during the dry season, when food stocks in their natural habitats are limited. Severe dought may cause them less likely to get food from their natural habitats and then seek for food in human settling, including in hotels closeby to their habitats. The "monkey attacks" may cause problems related to cleanliness, spread out of diseases, and may also cause scared to the guests staying in the hotel.

(10) Difficulties Faced by Hotels in Handling Faeces (of Birds and Bats)

When many birds and bats available within the site of hotels, either in the long term or just in a short visit, in a short term, they may cause a problem related to faeces dropped on the walls or on the pavements or other part of those hotels. These related to the bad smell caused and aesthetics values decreased because of the existence of the faeces in inappropriate locations. For the bat, some hotels have tried to kill them by shooting them, some have tried to get rid of them by using electronical tools that providing high thone sounds which make bats go away! Unfortunately, this does not always successful.

(11) The Danger of Snake Attacks

Some hotels especially those close to the forests or vegetations usually got a higher risk of visited by snakes than others. The snake may be venomous or not. Even though they are not venumous, they still make guests scared. Venomous snakes, such as named locally as "ular hijau / green snake" may cause death tolls to the beatten guests. Snakes actually become important part of the ecosystems, because, for example they become predator of mice, "the farmer's enemy". More snakes killed may cause the growth of mice population growing faster, because less predator available, and this may cause more preblems to humans, so it is important to keep them alife as part of the ecosystem.

(12) No Space Kept for "Conservation Zone".

Based on "sanga mandala" on Balinese life phylosophy of the "Tri Hita Karana", at least 3/9 (or 33%) of the hotel sites should be kept as open green area/natural vegetation or "conservation zone". Dalem *et al*. (2010) reported that the average of site of hotels in Bali that kept as natural open green area/ natural vegetation was 29% only, which is below the standard minimum considered enough, 33%. In addition 4 out of 7 hotels were kept their natural green area for below 33%. Meanwhile based on the author personal observations, many of the hotels actually have got no third zone anymore, or 0%. This has decreased one site on earth which considered to be a source of genetic, for the conservation purposes.

(13) Relase of "Feral Animals".

One hotel's staff was interviewed and he said that has a program to release wildlife especially birds for simbolizing 'loving the environment'. Among bird species released was some *Cacatua goffini* (Tanimbar Cockatoo) which is feral for Bali. The species actually naturally available in Tanimbar Island, eastern part of Indonesia (Strange, 2001). Some of these species have been probably naturalized as frequently seen around Nusa Dua lagoon, southern part of Bali (see Dalem *et al*., 2003). Impacts of feral animals might be disastrous to ecosystem, related to possible disease

spreaded, killing other animals (prey) or eating vegetations, etc. In developed countries, such as Australia, this case usually taken seriously, and the feral might be killed or forced to get out of their 'new habitat' (Prof. Shelley Burgin, pers. comm.).

CONCLUSION

Some issues related to wildlife (flora and fauna) that need to be managed by hotels seeking sustainable tourism certification in Bali, including: (1) souvenirs sold made of protected species, (2) lack of knowledge on protected species, (3) lack of information on protected species passed to guests, employees, suppliers and other stakeholders, (4) the use of protected species as materials/accessories on the hotel, (5) lack of concern in buying eco-building materials, (6) lack of care for the danger of keeping invasive species, (7) lack of systems in preventing misuse of protected species, (8) problems related to animal feeding, (9) problems of hotels attacked by monkeys, (10) difficulties faced by hotels in handling faeces (of birds and bats), (11) the danger of snake attacks, (12) no space kept for "conservation zone", (13) relase of "feral animals".

Some thought on strategies to manage these issues including no animal feeding, getting enough infomation on protected species, providing appropriate information to guests and other stakeholders, include the protected species aspect on the contract with suppliers or contractors, and carry out monitoring of products/souvenirs/facilities related to protected species regularly.

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IDENTIFICATION OF MARINE TURBO SPECIES (MOLLUSCA: GASTROPODA) FROM PEMUTERAN AND CANGGU BEACH BALI USING DNA BARCODES

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ABSTRACT

Molecular sequence data are a powerful tool for species identification, particularly in cases where morphological differences are obscure. Distinguishing species in the *Turbo species* of tropical marine gastropods has long been difficult, because descriptions and identification has relied exclusively on shell and operculum characters. Here we use molecular sequence data from mitochondrial gene cytochrome oxidase subunit I region as taxon barcodes to characterize the genetic and as the core of a global bioidentification system for animals. This study used 33 turbo marine snail samples from Pemuteran and Canggu beach. Mitochondrial DNA (mtDNA) was isolated by Chelex 10% followed by Polymerase Chain Reaction (PCR) technic. DNA sequence analyses were done using the MEGA5 program. The result from this research indicate that 20 sample from Pemuteran were identified as *T. chrysostomus* While from 13 sample of Canggu beach 5 sample were identified as *T. bruneus*, 4 sample as *T. sparverius* and 4 samples has similarities sequence with *T. kenwilliamsi* and *T. sparverius* but genetic distances showed that the four samples were distinct species. We conclude that sequence variation in the COI barcode region will be very effective for discriminating species of marine turbo snail.

Keywords: *Turbo species,* mitochondrial DNA, barcoding, molecular identification

INTRODUCTION

Mitochondrial deoxyribonucleic acid (mtDNA) sequence data have been both heralded and scrutinized for their ability to discriminate among species for identification (DNA barcoding) or description (DNA taxonomy) (Hebert et al 2003). For animals, mitochondrial genes are attractive targets because they are shared across diverse taxa and do not contain introns that can complicate amplification using the polymerase chain reaction (PCR). The availability of broad-range primers for amplification of mitochondrial COI from diverse invertebrate phyla establishes this gene as a particularly promising target for species identification in animals (Folmer et al. 1994).

DNA barcoding is designed to provide accurate, and automated species identifications through the use of molecular species tags based on short, standardized gene regions (Hebert et al 2003). The availability of broad-range primers for amplification of a 645 bp fragment of cytochrome c oxidase subunit I (COI) from diverse invertebrate and vertebrate phyla establishes this gene sequence as a particularly promising target for species identification in animals (Folmer et al. 1994). DNA barcoding was proposed as a means for identifying unknown specimens by collecting sequence data from a specific gene region and comparing these data to an established reference database (Hebert and Gregory, 2005).

Turbo is a species of sea snail, marine gastropod mollusk in the family *Turbinidae,* occur in intertidal and shallow sub tidal rocky shore of ocean water, and has an important role both in terms of ecology and economy. Distinguishing species in the *Turbo species* of tropical marine gastropods has long been difficult, because descriptions and identification has relied exclusively on shell and operculum characters. To identify the species need both morphological characteristics and genetic techniques. Limited information about diversity and species in Indonesia encourage research on the molecular identification of a turbo species . Here we assess the efficacy of using cytochrome c oxidase subunit I data as a guide to help confirm and describe a species of turbo snail.

MATERIALS AND METHODS

A 33 turbo snails samples collected from northern and southern coastal areas of Bali. From the north coast region is represented by Tangkad Jaran (n = 19) and Sumberkima (n = 1) Pemuteran. From the southern region represented by the Echo beach Canggu (n = 13). The morphological characteristic

identification suggests a whole sample has similarities with *Turbo Chrysostomus*.

Genomic DNA was isolated from muscle foot tissues using 10% Chelex (Walsh et al. 1991). Polymerase chain reaction (PCR) was carried out on the DNA extracts, using standard reagents and the universal COI primers LCO1490 (5′-GGTCAACAAATCATAAAGATATTGG-3′) and HCO2198 (5′-TAAACTTCAGGGTGACCAAAAAATCA-3′) (Folmer *et al*. 1994), amplify a 658 l bp region of COI. A hot start PCR was performed by a thermal cycler, iCycler (Bio-Rad), in a 25-µl reaction volume containing 1 µl of template total DNA and 24 µl of premix made with 14,5µl deionized water, 2.5 µl PCR Buffer (PE-II), 2.5µl dNTPs (8µM), 2.0 µl $MgCl_2$ (25mM), 1.25µl each primer (each 10 mM), and 0.125µl PE Amplitaq (5 unit/ µl). Thermal cycling condition comprised an initial denaturation at 94°C for 15 sec; 38 cycles of denaturation at 94°C for 30 sec, annealing at 50°C for 30 sec, and elongation at 72°C for 45°C and a final elongation at 72°C for 5 min. The PCR product was purified using a digestion of 5U shrimp alkaline phosphatase and 5U exonuclease for 30 min at 37 °C, followed by 15 min at 80 °C. Both strands were sequenced with a BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems) following the manufacturer's protocol, using the same primer set as the initial PCR amplification. Sequencing was performed with ABI Prism 3730 DNA Analyzer (Applied Biosystems) at the Life Sciences Laboratory Core Center at Cornell University. Forward and reverse sequences were proofread using Sequencher 4.7 (GeneCodes Corp), visually checked for accuracy, then aligned in MUSCLE, and analyzed using MEGA5 software (Tamura *et al.* 2011). We identified the samples using sequence similarity and K2P distance approaches. For sequence similarity we used NCBI's nucleotide BLAST server against NCBI GenBank (http://blast.ncbi.nlm.nih.gov), to identify each sample sequence. The sample was identified as the species with which it shares the highest percent pairwise identity for BLAST.

RESULTS AND DISCUSSION

A total of 602- 625 bp of COI sequence was determined from 33 turbo snail samples. A BLAST search at the National Center for Biotechnology Information resulted candidate species with the maximum percentage identity value more than 93%. The sequence in GenBank that showed the highetst homology with all samples from the Tangkad Jaran and Sumberkima Pemuteran beach sequence was AM403904.1, a sequence from a gastropod identified as *Turbo chrystotomus*, with the maximum percentage identity value being 99%. while samples from Echo beach Canggu 5 were identified as *Turbo bruneus* the sequence in GenBank that showed the highetst homology sequence was AM049381.1 with the maximum percentage identity value being 93%. The sequence in GenBank that showed the highetst homology with 4 samples from Echo beach Canggu sequence was AM403911.1 and identified as *Turbo sparverius*, with the maximum percentage identity value being 96%. The last 4 samples sequence from Echo beach were showed the highetst homology with sequence AM403911.1, a sequence from a gastropod identified as *Turbo sparverius*, with the maximum percentage identity value being 93% and the next highest was the following sequences with the maximum percentage identity value being 93%: AM049384.1, identified as *Turbo kenwilliamsi* (figure 1).

The sample TU71002, TU71005, TU71012, and TU71013 from Echobeach were showed the highetst homology with sequence *Turbo sparverius* as well as *Turbo kenwilliamsi* in GenBank. Genetic distance between these 4 samples with *T.kenwilliamsi* ranged between 7,9% to 8,3% whereas with *T.sparverius* ranged between 7,2% to 7,9%. This genetic distance indicated that the fourth samples is a different species with *T.sparverius* and *T.kenwilliamsi*. The fourth sample of marine turbo snails from Echo beach Canggu is probably were a species that have not been listed in GenBank yet.

The present study evidenced that turbo species can be efficiently identified with DNA barcodes using highest BLAST sequence similarity, and that the present COI library can be used for subsequent applications in ecology and systematic.

In the future, we need to collect a larger samples size from Bali for a more comparison with the other turbo species, need more research for other species of *Turbo* to gain a better knowledge of the genetic diversity and relationship among species in the phylogenetic tree.

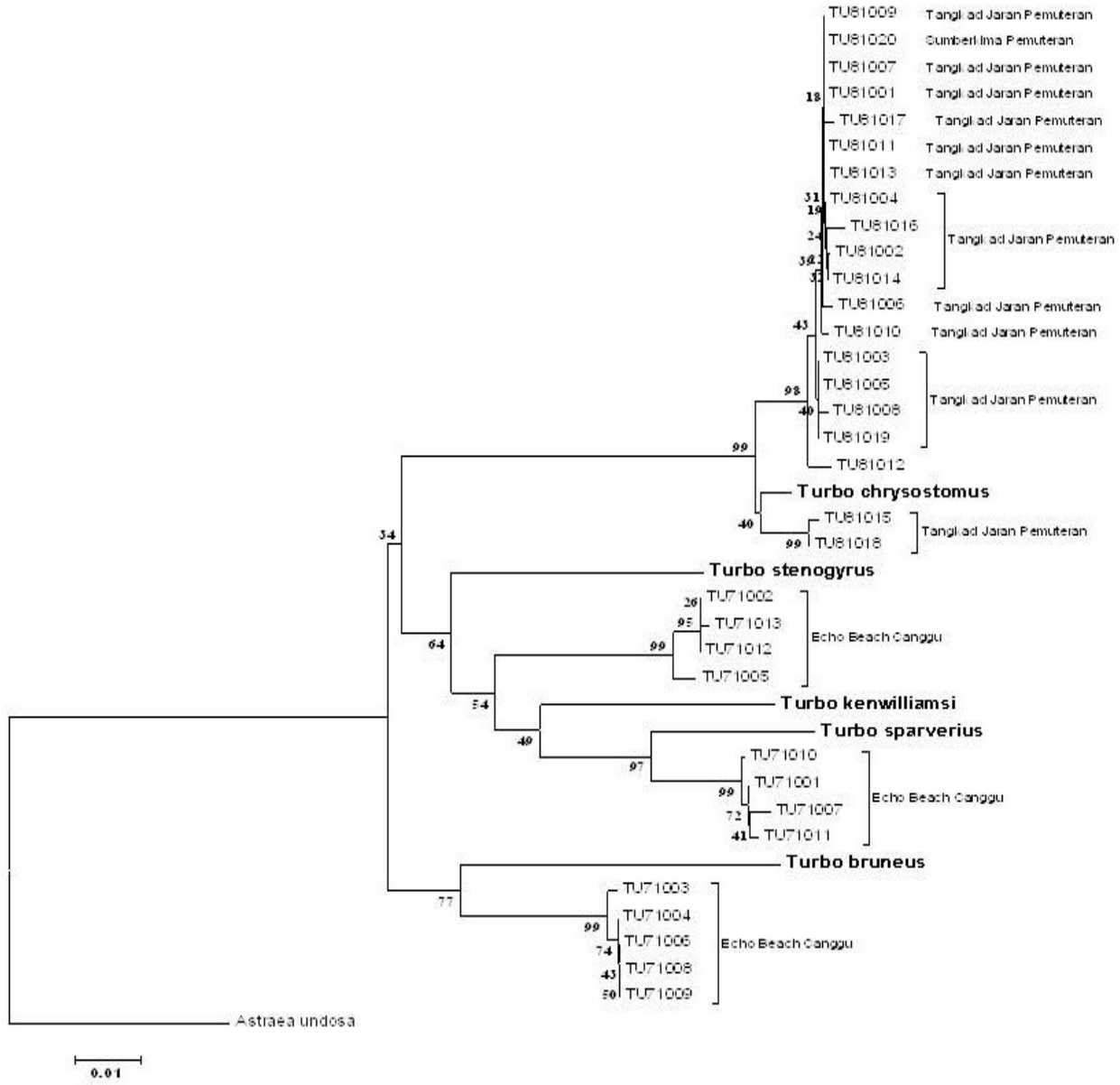

Figure 1. Neighbour joining tree from the analysis of the COI gene sequences of 33 samples with bootstrap value were computed over 1000 replication.

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RHIZODEGRADATION OF OIL WASTE

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ABSTRACT

Oil waste entering the aquatic environments is one of the water pollutions which is difficult to deal with. In this paper the biodegradation of liquid waste containing mineral oil (engine fuel and oil) by Bermuda grass (*Cynodon dactylon L*) is reported. The waste was treated in a bath consisting of the grass supported by layers of sands and gravels for a varied of periods (residence times). The total oil contents of the effluents were determined against the various residence times and the correlation between the two parameters was used to study the kinetics of the oil waste degradation. The degradation of the oil waste was found to follow a first order reaction with a rate constant of 0.09 hour^{-1}. The rate of the degradation was determined to follow the equation: r = - 0.09 c, where 'r' is the degradation rate and 'c' is the total oil contents. The optimum residence time for the degradation in this experiment is 30 hours, during which 81.5% of the waste has been reduced.

Keywords: rhizodegradation, oil waste, kinetics, reaction rate.

INTRODUCTION

The high consumption of energy has been driven humankind to use all available resources in every possible ways and means. Fossil fuel is one major energy source that has been harnessed in large amount and the number seems to keep increasing, perhaps until it actually runs out. This in turn produces huge amount of oil waste loaded to the environment. More than 570 tonnes oil waste are dumped into the marine environments each year (USNRC, 2003). In Indonesia alone, about 400 million barrels oil are consumed each year (BPS, 2010). The oil waste, or the liquid waste containing oil, has definitely to be treated before loaded to the water environments. Common waste treatments use floatation or oil trap mechanisms to isolate or separate oil from liquid waste. However, such methods require further treatment to degrade the separated oil. Amongst others, bioremediation is one sound method to overcome this problem. Bioremediation conducted through degradation of contaminants by the association of soil microorganisms and root exudates is now known as rhizodegradation (Dzantor, 2007). This method has been used world wide to degrade various types of contaminants. Apart from degrading fossil fuels and their derivatives (Etsuko, 2007; Unterbruner, et.al., 2007), rhizodegradation has been successfully treated other chemicals polluting the soil such as PAH (Lippencott, 2005) and perchlorates (Nzengung, et.al., 2004; Yifru and Nzengung, 2008).

The kinetics of the degradation including the reaction order and the rate of the degradation is desirable to investigate in order to be able to design a robust treatment process using this method. The reaction order can be determined by analyzing the concentration changes with time. A zeroth order degradation would proceed independent of the concentrations of the contaminant studied, so the concentrations decrease in a constant rate. This is shown by a linear curve between concentrations as Y axis and times as X axis. A first order degradation means one proceeding with a rate linearly depends on the concentrations, thus the concentrations decrease continuously over time, shown by an exponential curve between the concentrations (Y axis) and times (X axis). The rate is mathematically expressed as follows (Dogra and Dogra, 1984):

$$\frac{\partial C}{\partial t} = -kC^n$$

where '*C'* represents concentration, '*k*' represents the rate constant, *'t'* is time, and *'n'* is the order of reaction. For a zeroth order reaction the equation is rewritten as:

$$\frac{\partial C}{\partial t} = -k \qquad or \qquad \partial C = -k\,\partial t$$

which, upon integration becomes:

$$C_0 - C_t = k\,t \quad or \quad C = C_0 - kt$$

thus the concentrations *'C'* are linearly correlated to times *'t'*, with *'-k'* is the slope of the curve. Similarly, a first order degradation is represented by the equation:

$$\frac{\partial C}{\partial t} = -\,kC^1 \quad or \quad \frac{\partial C}{C} = -\,k\,\partial t$$

which is integrated to give:

$$\ln C = \ln C_0 - kt$$

thus the correlation between *lnC* and *t* is linear with *–k* as the slope.
Furthermore, a second order degradation is expressed by the equation:

$$\frac{\partial C}{\partial t} = -\,kC^2 \quad or\ C^{-2}\partial C = -\,k\,\partial t$$

or, as an integrated expression:

$$C = \frac{1}{\frac{1}{C_0 + kt}}$$

and thus the correlation between *1/C* and *t* is linear with *k* as the slope. The degradation or reaction order can therefore be determined by determining the most linear correlation between *C* and *t*, *lnC* and *t*, or *1/C* and *t*

In this paper the reduction of total oil contents of waste by rhizodegradation using Bermuda grass (*Cynodon dactylon L*), along with the order and the degradation rate, are reported.

METHODS

Thirty litres of waste containing oil (flotation effluents) was collected from the Indonesia Power Electricity Generating Plant in Denpasar. Portions of 250 mL samples were analysed for their total oil contents (Clesceri, et.al.,1998). Ten litres was treated in the 0.6 m x 0.6 m x 0.3m biofilter constructed by grass grown on sandy soil supported by layers of gravels. Three 250 mL samples were drawn from the biofilter after 30, 36, 42, 48, 60, and 72 days, and their total oil contents were determined by the same method as before. The correlation between the total oil contents and the residence times were used to determine the order and the rate of the rhizodegradation.

RESULTS AND DISCUSSIONS

As shown in Table 1, the total oil concentration decreases from 455 to about. 86 mgL^{-1} (approximately 81.5 % reduction) in 30 hours, and then degrades slowly with 34 mgL^{-1} remaining after 42 hours (total reduction of approximately 93 %). After 60 hours the concentration drops to approximately 3 % of the initial concentration and then stays fairly constant for another 12 hours when it further declines to about 0.2 % of the initial concentration.

-

Table 1. The total oil during degradation

Residence time (hrs)	Total oil content (mgL^{-1})
0	455 ± 51.1
30	86 ± 24.9
36	64 ± 22.2
42	34 ± 15.1
48	17 ± 4.6
60	1.7 ± 0.6
72	1.33 ± 0.5

The decrease in concentration of total oil with time is shown in Figure 1. The graph shows that the concentration decline can be considered to comprise of two different speed decays : A rapid decay over the initial thirty hours (with average decay rate of about 12.3 $mgL^{-1}hour^{-1}$, followed by a slower decay with average decay rate of approximately 2 $mgL^{-1}hour^{-1}$).

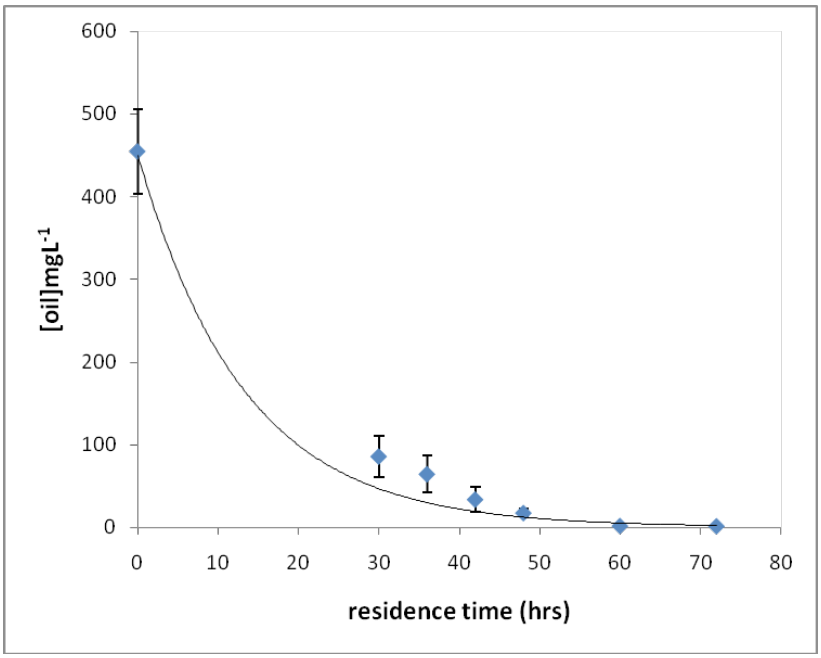

Figure 1. The decline of total oil concentrations with residence times

The continuous degradation suggests that the rhizodegradation may be first or second order reaction, meaning that the decay rate is concentration dependent. Comparing the linearity of the curves of *lnC* versus *t* ($R^2 = 0.921$, Figure 2) and *1/C* versus *t* ($R^2 = 0.603$, Figure 3), it is clear that the degradation follows a first order kinetics.

The constant of degradation rate is therefore determined by the slope of the curve shown in Figure 2, which is 0.09. This means that by this biofilter the total oil contents of the waste degrade with a rate of 0.09 times the initial content ppm per hour. Almost 100% oil waste has been removed during the experiment which means that it is worth wile to apply this treatment technique on larger scale. Further research would also include the determination of the fate of the oil compounds fractions, as well as the microbial population taking part in the rhizodegradation.

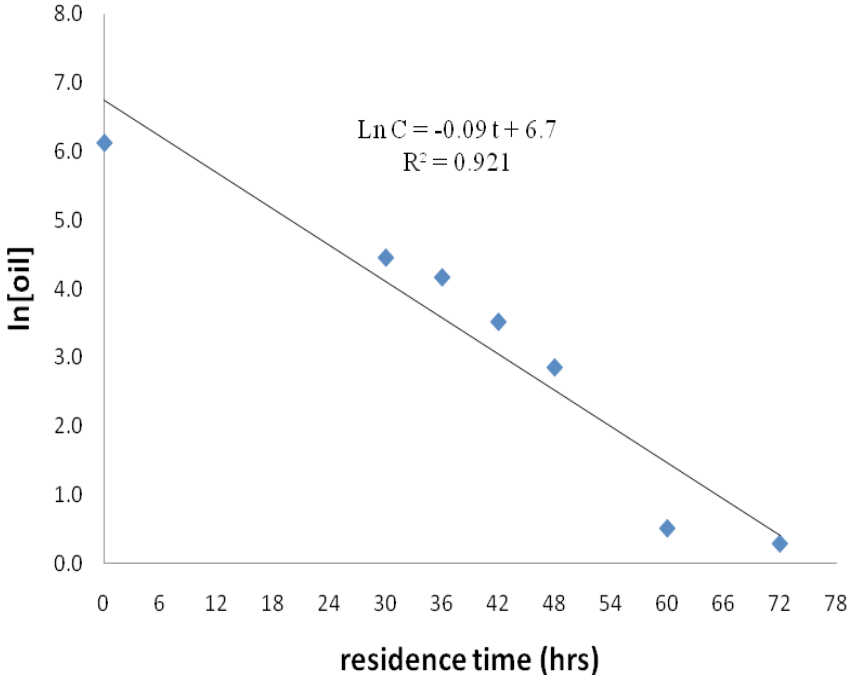

Figure 2. Linear regression of natural logarithm of total oil contents with residence times

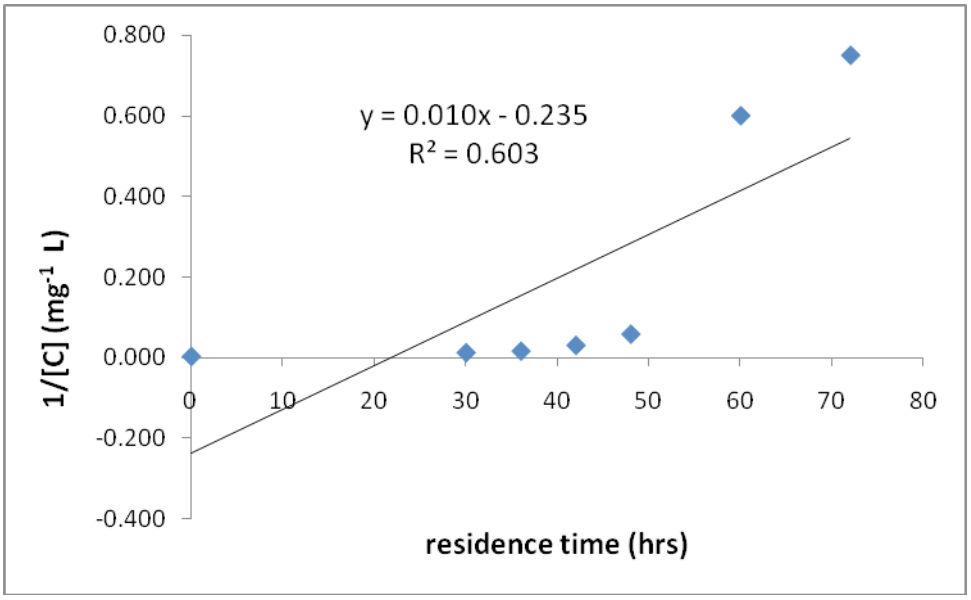

Figure 3. Linear regression of (total oil contents)$^{-1}$ with residence times

CONCLUSION

It is evident that the 0.108 m^3 biofilter constructed of Bermuda grass grown on sandy soil successfully degrades the oil waste through a first order kinetics with a rate expressed by 'rate = - 0.09 [oil] ppm hour^{-1}'. The rhizodegradation has removed 99.8% of the oil from the waste in 72 hours, although the decomposition products are yet to be determined. Investigation is underway to determine the microorganisms responsible for the rhizodegradation.

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POTENTIAL OF SEAWEEDS COLLECTED AT SANUR BEACH AS BIOFUELS

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ABSTRACT

Some 30 species of seaweads (mainly green and brown seaweeds) were collected from Sanur Beach area and were investigated for alternative raw materials in the biofuel production. The potency of the seaweeds as an alternative raw materials for biofuel production was assessed by analysing the fat, polysaccharide, protein and crude fiber content of the seaweeds. The identity of the species was determined by comparing the collected specimens with those specified in the literatures. It was found in this project that the major component of the seaweeds was crude fiber, ranging from 6.5 to 74.3% g/g (dw). The highest fat content was found in Sargassum sp., amounting at 3.7% w/w. Protein and polysaccharide were only present in minute amount in all samples. In the long run, suitable candidate of seaweeds will be developed into bioethanol or biodiesel.

Keywords: biofuel, seaweed

INTRODUCTION

An increase in fuel consumption in Indonesia has made this country to become a net importer of fossil fuel. The unrenewable feature of the fossil fuel has forced the world to search for other renewable sources of energy, e.g. biofuel, some of which are biodiesel from soybean oil (Manuel, 2007), olive oil (Sanchez and Vasudevan, 2006), palm oil in Malaysia (Tamunaidu dan Bhatia, 2007) and *minyak jarak* in Indonesia, canola oil in Germany and France (Schmidt, 2007). Bioethanol can be produced from sugarcane, Brazil is the pioneer in this case (), or from corn in the USA (Schmidt, 2007, Torney et al., 2007), or from water hyacinth (Aswathy et al., 2010). Bioethanol and biodiesel can be used in pure form in conventional transportation maschine without any modification of the maschinery. However, there are some disadvantages of the use of these raw materials for biofuels, because high energy is needed in its production (Worldwatch Institute, 2006) and also cause environmental problem (Fargione et al., 2008, Searchinger et al., 2008, Groom et al., 2008). Besides, it can be counterproductive to the food security issue since it competes with food raw material (Manuel, 2007) as reported by Schmidt (2007) that in Germany and France, the producer of margarine has complained the scarcity of raw material. When analyzed from harvest yield, the price of the commodity and the price of the biofuel, energy and chemicals needed in its cultivation and production, the efficiency of the production, side products, greenhouse effect, and other environmental problems, corn ethanol has 25% ENB (*net energy balance*) and soya oil 93% which means that corn ethanol produced 25% more energy than the amount its consumed and soya oil 93% (Schmidt, 2007). There is no report yet on biofuel from seaweed.

Bali with a coastline of 436,5 km and is located in the tropical country, it has a big potency of seaweeds, which can grow or be cultivated throughout the year and used as the raw materials for biofuel. Seaweeds biofuels is environmentally sound and renewable. The aim of this research is to collect and determine the fat, sugar, crude fiber, and protein content of the seaweeds to investigate the potency of its use for biofuels. Seaweeds with high fermented sugar and fat can be used as raw materials for the production of bioethanol whereas seaweeds with high fat content can be used as raw materials for biodiesel.

MATERIALS AND METHODS

Seaweeds were collected at the Sanur Beach, Bali, sorted and washed using tapwater, then wind- or sun-dried and the water content was determined gravimetrically. Each seaweeds were ground to obtain fine powder. Primary identification of the seaweeds was carried out manually by comparing sample seaweeds with the one in the literature.

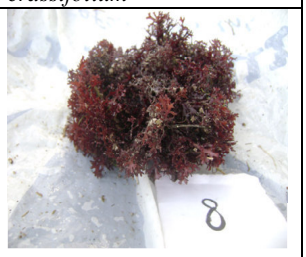
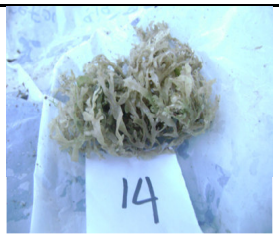
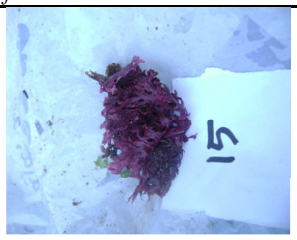
Starch concentration was determined using Nelson-Somogyi method (spectrophotometric at 520 nm, Robyt and White, 1990). Fat content was measured by extracting dried powder of samples using 2: 1 chloroform-methanol using soxlet. The extract was then dried using rotary vacuum drier followed

by heating at 50^{0}C (Sudarmadji et al., 1997). Protein content was measured using Biuret method which were measured using spectrophotometer at 520 nm (Robyt and White, 1990). Crude fiber was measured according to method of Sudarmadji et al. (1997); the residu of fat extraction was reextracted using 0.255 N sulphuric acid, boiled, filtered, and washed using 0.313 N NaOH, K_2SO_4 and boiled aquadest respectively. The content of crude fiber is measured as dry weight of the residu of this procedure.

RESULTS AND DISCUSSION

Sampling was carried out at fullmoon at the time that the lowtide was at its lowest to collect seaweeds far into the sea.

Thirty nine types of seaweeds were sampled at Mertasari Beach in Januari 2010 (differentiate visually). The results are presented in Figure 1. Samples were dominated by green seaweeds, however red and brown seaweeds were also found in less numbers. Some were identified manually using some seaweed literatures (Druehl, 2000, Hillson, 1977, Gakken, 1975).

			
Sampel 1*Ulva fasciata*	Sampel 2 *Ulva reticulata*	Sampel 3 *Gelidiella acerosa*	Sampel 4 *Sargassum crassifolium*
			
Sampel 5 *Sargasum piluliferum*	Sampel 6 *Padina australis*	Sampel 7 *Vidalia obtusiloba*	Sampel 8 *Stenogramme interupta*
			
Sampel 9 *Galaxaura subverticillata*	Sampel 10 *Glacillaria arcuata*	Sampel 11 *Ganonema farinosum*	Sampel 12 *Prionitis patens*
			

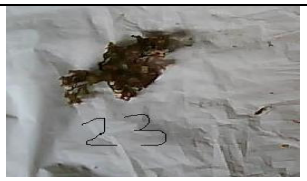
Sampel 13 *Gloiopeltis complanata*	Sampel 14 *Dictyopteris polypodioides*	Sampel 15 *Stenogramme interupta*	Sampel 16 *Eucheuma* sp.
			
Sampel 17 *Ulva* sp.	Sampel 18 *Eucheuma cottoni*	Sampel 19 *Halimeda* sp.	Sampel 20 *Sargassum* Sp.
			
Sampel 21 *Glacillaria* sp.	Sampel 22 *Sargassum* sp.	Sampel 23 *Sargassum* sp.	Sampel 25 *Gelidium* sp.
			
Sampel 26 *Hypnea* sp.	Sampel 27 *Hypnea* sp.	Sampel 28 *Gracillaria* sp.	Sampel 29 *Euchema spinosum*
			
Sampel 30 *Acanthophora sp.*	Sampel 31 *Ulva* sp.	Sampel 32 *Gigartina* sp.	Sampel 35 *Sargassum* sp.
			
Sampel 36 *Glacillaria* sp.	Sampel 37 *Codium* sp.	Sampel 38 *Gelidium* sp.	Sampel 39 *Glacillaria* sp.

Figure 1. Samples of seaweeds collected at Mertasari Beach, Sanur, Bali

Determination of Fat, Protein, sugar and crude fiber content of seaweeds samples

The content of sugar, fat, protein and crude fiber was presented in Table 1. It shows that the content of the seaweeds was dominated by crude fiber, which range from 6.5 % in *Ulva fasciata* to 74.3% in *Glacillaria arcuata*. The highest concentration of fat was 3.7% w/w found in *Sargassum crassifolium*. As a comparison, the fat content of seaweeds in the west coast of Malaysia Peninsula ranged between 17,6% in *Dictyota dichotoma* (Phaeophyta) and 1,1% in *Glacillaria changii* (Rodhophyta) (Chu et al., 2003). Whereas the seaweeds found at the northeastern part of Mediterranean Sea has fat content between 1.10% in *S.filamentosa* and 11.53% dry weight in *S.schimperii* (Polat and Ozogul, 2007).

Starch and crude fiber can be hydrolized into sugar monomers which then can be fermented into ethanol by employing *Saccharomyces cereviceae* or *Zymomonas mobilis* or other ethanol producing microorganism. Whereas fat can be further processed by esterification to produce biodiesel. The result showed that some seaweeds found at Mertasari Beach, Sanur, Bali can be used as the raw materials for biofuels especially *Glacillaria arcuata*, *Gelidium sp*. (sample 25), and *Ulva sp.* (sample 17) which will be developed further into bioethanol.

Table 1. Sugar, fat, protein, and crude fiber content of seaweeds of Mertasari Beach, Sanur, Bali

No.	Sample code	Sample name	% fat	Starch content (g/L)	% Protein	% Crude Fibre
1	1	*Ulva fasciata*	3.2%	0.0194	0.0143	6.5%
2	2	*Ulva reticulata*	3.1%	0.0403	0.0104	20.1%
3	3	*Gelidiella acerosa*	0.6%	0.0493	0.0338	15.3%
4	4	*Sargasum crassifolium*	3.7%	0.0093	0.0260	31%
5	5	*Sargasum piluliferum*	1.3%	0.0195	0.0818	41%
6	6	*Padina australis*	1.9%	0.0046	0.0182	6.6%
7	8	*Stenogramme interupta*	0.2%	0.0613	0.0091	33.2%
8	9	*Galaxaura subverticillata*	0.4%	0.0037	0.0519	30.3%
9	10	*Glacillaria arcuata*	0.0%	0.4233	0.0091	74.3%
10	12	*Prionitis patens*	0.5%	0.0423	0.0675	34.2%
11	13	*Gloiopeltis complanata*	1.2%	0.0060	0.0377	10.9%
12	14	*Dictyopteris polypodio Ides*	0.7%	0.0464	0.0883	19.9%
13	15	*Stenogramme interupta*	1.9%	0.0037	0.0468	29.2%
14	16	*Eucheuma sp.*	1.4%	0.0074	0.0234	29.3%
15	17	*Ulva sp.*	1.4%	0.0148	0.0584	66.2%
16	18	*Eucheuma cottoni*	1.1%	0.0566	0.0169	19.7%
17	19.	*Halimeda sp.*	0.7%	0.0097	0.0623	9.7%
18	20	*Sargassum sp.*	0.4%	0.0434	0.0078	23.7%
19	22	*Sargassum sp.*	0.03%	0.0409	0.0961	38.2%
20	25	*Gelidium sp.*	1.2%	0.4286	0.0000	38.4%
21	27	*Hypnea sp.*	0.1%	0.5132	0.0221	54.7%
22	28	*Gracillaria sp.*	0.9%	0.0445	0.0130	44.8%
23	29	*Euchema spinosum*	0.5%	0.6667	0.0519	45%
24	30	*Acanthophora sp.*	2.2%	0.0295	0.1558	40%
25	32	*Gigartina sp.*	2.3%	0.2733	0.0545	33.7%
26	35	*Sargassum sp.*	2.6%	0.0127	0.0442	26.5%
27	36	*Glacillaria sp.*	0.4%	0.0214	0.0519	37.5%
28	37	*Codium sp.*	0.0%	0.0087	0.0844	54.2%
29	38	*Gelidium sp.*	0.1%	0.7725	0.0260	66.4%

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CARBON STORAGE BY URBAN TREES IN SRENGSENG AND PT. JIEP URBAN FOREST OF JAKARTA

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ABSTRACT

Urban forests can have important role in climate change mitigation in city by reducing carbon dioxide levels through sequestration mechanism. This study aimed to estimate carbon storage potential of standing trees in two urban forests in Jakarta, namely Srengseng and PT. Jakarta Industrial Estate Pulogadung (JIEP) urban forest. Carbon data were taken from ten 25 m × 25 m plots in which tree biomass were measured using allometric equation. Carbon organic content in tree biomass was converted using 0.5 conversion factor. Results showed that Srengseng urban forest store higher carbon, with average carbon storage density of 104.85 tonC/ha, compared with 51.56 tonC/ha in PT. JIEP urban forest. With a vegetated area of 12 ha and 8.9 ha, total carbon storage potential of trees in Srengseng urban forest and PT. JIEP urban forest were 1,258.16 tonC and 458.89 ton C, respectively. These data can be used to help assess the actual and potential role of urban forests in reducing atmospheric carbon dioxide in Jakarta.

Keywords: carbon storage, urban forestry, tree biomass, Srengseng, PT. JIEP

INTRODUCTION

Increasing levels of atmospheric greenhouse gases are thought to contribute to an increase in atmospheric temperatures. Globally, the averaged surface air temperature increased 0.5°C in the 20th century. In Jakarta, there was an increasing of 0.6 °C in the averaged daytime temperature in 2000, which higher than those in 1970s (Tokairin *et al*., 2009). A current estimate of the expected rise in average surface air temperature globally is between 1 and 3.5 °C by the year 2100 (Hamburg *et al*., 1997). A more recent study suggests that the rate of warming for Indonesia will be slightly greater from 0.2 to 0.3 °C per decade (Boer and Faqih, 2004).

Among other greenhouse gases, carbon dioxide (CO_2) is the most dominant one, accounting for three quarters of the total emissions (Olivier *et al.,* 2005). Increased atmospheric CO_2 is attributable mostly to fossil fuel combustion (about 80–85%) and change in land use (Hamburg *et al*., 1997). Impacts of climate change may be worse in urban areas (Nowak and Crane 2002). This is because high levels of fossil fuel combustion increase surface air temperature in urban areas, compares to their surrounding rural environments (Lo *et al*., 1997).

Trees are important sinks for atmospheric carbon. Trees fix carbon during photosynthesis and storing excess carbon as biomass, in which 50% of their standing biomass is carbon itself (Ravindranath *et al*., 1997). Urban forests are expected to perform this function. In Good Practice Guidance for Land Use, Land-Use Change and Forestry (IPCC, 2003), urban forests are regarded as being one of the carbon sinks. Therefore, urban forests can play a critical role in helping to combat increasing levels of atmospheric CO_2 through carbon sequestration mechanism. Importance of forested areas in carbon sequestration is already accepted, and well documented (Tiwari dan Singh, 1987; Ramachandran *et al*., 2007; Terakunpisut *et al.,* 2007). However, scarcely attempts have been made to study the potential of trees in carbon sequestration from urban area, especially in Jakarta.

In this study, we make an attempt to explore ecological conservation values of such areas in urban ecosystem. The study constitutes an assessment of standing biomass and carbon storage potential of trees in two urban forests in Jakarta, namely Srengseng urban forest and PT. JIEP urban forest. The role of such areas in urban ecosystem needs to be addressed. These data can be used to help assess the actual and potential role of urban forests in reducing atmospheric CO_2. In addition, they provide insights for decision-makers and the public to better understand the role of urban forests in reducing atmospheric CO_2, and make better management plans for urban forests in Jakarta.

METHODS

Study sites

The research was conducted in DKI Jakarta province. Jakarta is the capital city of Indonesia and it has five municipalities with a total population of 8,524,190 in 2011 (Suku Dinas Kependudukan dan Pencatatan Sipil Kota Administrasi Jakarta, 2011). Jakarta is located in the coastal lowland ecosystem with average elevation of 7 m a.s.l. Based on Koppen climate classification, Jakarta has a hot and humid tropical climate (Koppen, 2011). It has an average monthly rainfall of 2.000 mm with daily temperature range of 23,4-37,1 °C and humidity of 77,97% (Pemerintah Provinsi DKI Jakarta, 2009).

Our study area included two urban forests in two municipalities, namely Srengseng urban forests in West Jakarta (6°12' S, 106°45' E) and PT. Jakarta Industrial Estate Pulogadung (JIEP) urban forest in East Jakarta (6°12' S, 106°55' E) (Figure 1). Srengseng urban forest is located in residential area in Kembangan District, West Jakarta. Srengseng urban forest is a conservation forest which mainly serves as river catchment area. It has a total area of 15 ha, with a vegetated area of 12 ha (Pemerintah Provinsi DKI Jakarta, 2009). PT. JIEP urban forest is located in industrial area of Pulogadung, Cakung District, East Jakarta. PT. JIEP urban forest serves as buffer zone for industrial area. It has a total area of 8.9 ha (Pemerintah Provinsi DKI Jakarta, 2009).

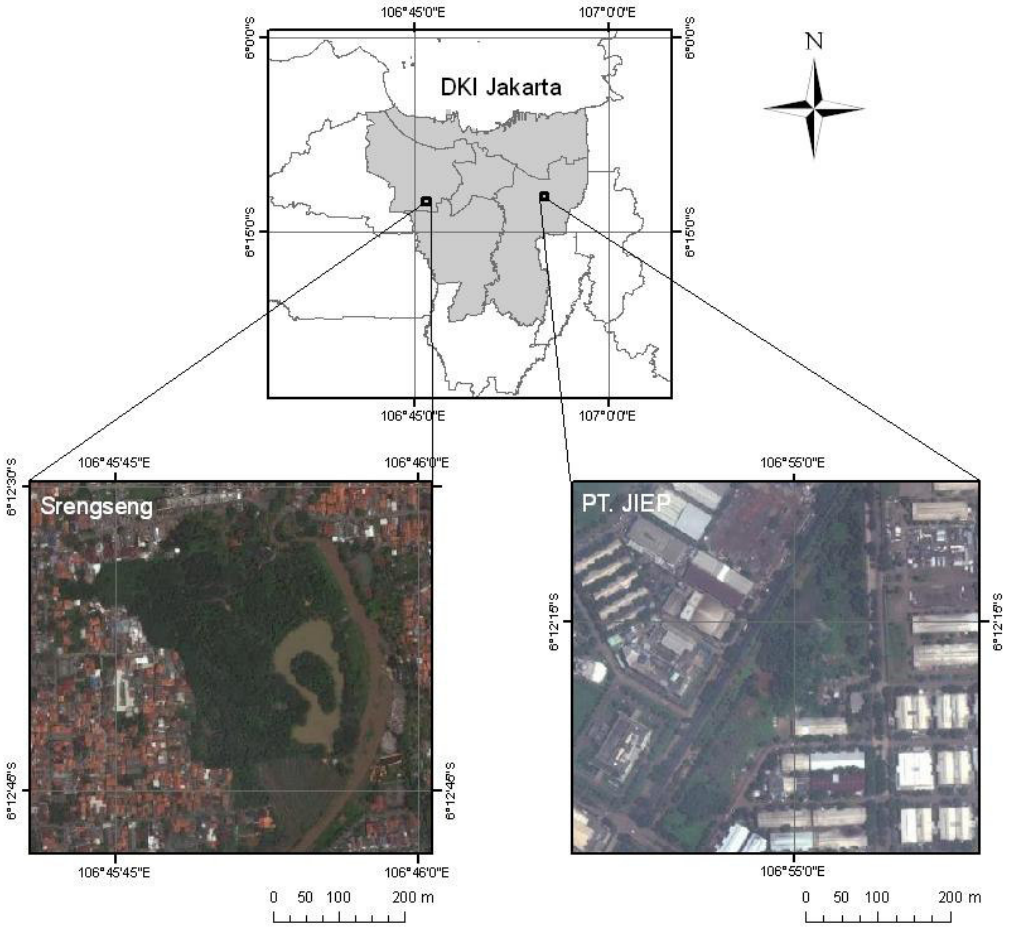

Figure 1. The study area: Srengseng urban forest and PT. JIEP urban forest of Jakarta as seen with Google Earth.

Field survey

From June to July 2011, we investigated ten plots in our study sites, five plots in Srengseng forest and five plots in PT. JIEP forest. Plots with size of 25 m × 25 m were allocated randomly in each study area. Within each plot, we recorded the species name and stem diameter at 1.37 m above the ground (DBH) for each tree.

Data analysis

Biomass for each measured tree was calculated using allometric equations for general forest species groups in tropical ecosystem with moist climate classification: ABD = exp. (-2.289 + (2.649 × ln dbh) - (0.021 × ln dbh^2)) (Brown, 1997). The biomass of an individual tree was calculated based on the DBH alone. Open-grown, maintained trees tend to have less above-ground biomass than predicted by forest-derived biomass equations for trees of the same diameter at breast height (Nowak, 1994). To adjust for this difference, biomass results for urban trees were multiplied by a factor 0.8 (Nowak, 1994). The dry biomass was then converted to carbon by multiplying 0.5 (Nowak, 1994; Nowak and Crane, 2002). After that, carbon density (tonC/ha) was calculated for each plot by extrapolating carbon stocks from a per plot basis to a per hectare basis using a conversion factor (Pearson et al., 2005). The expansion factor is calculated as the area of a hectare in square meters divided by the area of the sample in square meters.

RESULT AND DISCUSSION

We identified 44 tree species in Srengseng forest and 18 tree species in PT. JIEP forest. Based on importance value index calculation (Table 1), *Bauhinia acuminata*, *Swietenia macrophylla* and *Aleurites moluccana* were the most common species in Srengseng urban forest, while *Lagerstroemia speciosa*, *Swietenia macrophylla* and *Pithecellobium dulce* were commonly found in PT. JIEP forest. PT. JIEP forest had higher tree density, with a density of 829 trees per ha compared to 614 trees per ha in Srengseng. However, the majority of the trees in PT. JIEP forest were recently planted, with small DBH. Nearly half of the trees had DBH smaller than 7 cm.

Table 1. Ten tree species with highest Importance Value Index (IVI) in Srengseng and PT. JIEP urban forest.

Species	Relative Density	Relative Dominance	Relative Frequency	IVI (%)
Srengseng urban forest				
Acacia auriculiformis	9.90	6.24	4.76	20.90
Aleurites moluccana	7.29	9.42	6.35	23.06
Bauhinia acuminata	16.15	9.56	7.94	33.64
Ceiba pentandra	1.56	8.36	1.59	11.51
Delonix regia	5.21	10.14	3.17	18.52
Filicium decipiens	7.29	7.55	1.59	16.43
Leucaena leucocephala	8.33	7.78	4.76	20.87
Pithecellobium dulce	1.56	4.21	3.17	8.95
Swietenia macrophylla	13.02	10.63	4.76	28.41
Tectona grandis	4.69	5.78	3.17	13.64
PT. JIEP urban forest				
Acacia mangium	1.54	4.73	3.57	9.85
Adenanthera pavonia	1.93	4.37	3.57	9.88
Canarium commune	0.39	3.28	3.57	7.23
Erythrina lithosperma	1.93	3.63	7.14	12.70
Ficus lyrata	6.95	0.06	3.57	10.58
Lagerstroemia speciosa	26.64	23.93	14.29	64.86
Leucaena leucocephala	3.09	10.93	10.71	24.74
Mimusops elengi	7.72	0.02	10.71	18.45
Pithecellobium dulce	7.72	29.72	3.57	41.01
Swietenia macrophylla	37.07	11.28	10.71	59.06

With a vegetated area of 12 ha and 8.9 ha, carbon stored by urban trees were 1,258.16 tonC in Srengseng forest and 458.89 tonC in PT. JIEP forest, respectively. The average carbon density was 104.85 tonC/ha in Srengseng forest and 51.56 tonC/ha in PT. JIEP forest (Table 2). The difference in carbon density between Srengseng and PT. JIEP forest may be due to the different structure of the urban forests. According to Nowak (1994), the ages of the trees or the size of the trees (DBH) affect carbon storage and sequestration. Moreover, tree density (trees/ha) is also one dominant factor that affects carbon storage density in urban forest (Nowak and Crane, 2002). Carbon densities will tend to increase with tree density and/or incresed proportion of large diameter trees. Large trees greater than 77 cm in diameter sequester approximately 90 times more carbon than small trees less than 8 cm in diameter (Nowak, 1994).

Table 2. Tree density, average DBH, aboveground biomass density and carbon density of trees in Srengseng and PT. JIEP urban forest.

Plot	Tree density	Average DBH (cm)	Aboveground biomass density (ton/ha)	Carbon storage (ton/ha)
Srengseng urban forest				
1	592	24.52	184.66	92.33
2	608	22.59	178.34	89.17
3	432	25.47	221.30	110.65
4	608	22.21	190.14	95.07
5	832	20.17	274.02	137.01
Average	614.14	22.99	209.70	104.85
Total				1,258.16
PT. JIEP urban forest				
1	1056	3.69	42.02	21.01
2	896	14.35	87.65	43.83
3	1072	7.24	69.03	34.51
4	688	10.04	71.65	35.82
5	432	24.92	245.27	122.63
Average	828.8	12.05	103.12	51.56
Total				458.89

PT. JIEP urban forest, which had higher tree density (614 trees/ha) had lower carbon density estimate (51.56 tonC/ha) relative to Srengseng forest (104.85 tonC/ha). One reason for the lower carbon density in PT. JIEP forest was smaller diameter structure and younger age of trees compared to trees in Srengseng, with more than 40% of the trees were recently planted and still in sapling category. Srengseng forest had higher big trees, and around 2% of its tree population had DBH greater than 50 cm.

Urban trees in PT. JIEP were dominated by fast-growth species, such as *Lagerstroemia speciosa*, *Leucaena leucocephala*, *Erythrina lithosperma*, *Ficus lyrata*, *Adenanthera pavonia* and *Acacia mangium* (Vozzo, 2002). This fast-growth species has relatively high rates of carbon sequestration, which in turn leading to a higher carbon sequestration rate (Zhao et al., 2010). Therefore, eventhough PT. JIEP forest has lower carbon storage, it has high potential to sequester more carbon.

The average carbon density in both urban forests was generally lower than those stored in forest stands. Due to their relatively low tree cover, urban forests typically store less carbon per hectare in trees than forest stands (Nowak and Crane, 2002). Forest stands typically have about twice the tree density as urban areas, and about half the average carbon density per unit of tree cover. However, on a per unit tree cover basis, individual urban trees, on average, contain approximately four times more carbon than individual trees in forest stands, due to a larger proportion of large trees in urban environments (Nowak and Crane, 2002).

Polunin (1997) noted that tropical forests have carbon density of 225 tonC/ha in average, ranging from 30 to 400 tonC/ha, depend on type of vegetation and soil of the forest. Furthermore, Tomich et al. (1998) noted that natural forests can store carbon up to 497 tonC/ha. Karo (2011) measured that carbon density of trees in Bukit Barisan forest, North Sumatera was 485.01 tonC/ha. Carbon storage in Srengseng and PT. JIEP urban forest were higher compared with Taman Eden natural park (95.82 tonC/ha) (Bakri, 2009), and urban forests in other cities such as Shenyang, China (33.22 tonC/ha) (Liu and Li, 2011), Hangzhou, China (30.25 tonC/ha) (Zhao et al., 2010), Atlanta, US (35.74 tonC/ha), Baltimore, US (25.28 tonC/ha), and Jersey City, US (5.02 tonC/ha) (Nowak and Crane, 2002).

Quantifying the benefits of carbon storage by urban forests is important in understanding the role of urban forests in reducing atmospheric CO_2 and make better management plans (Jim and Chen, 2008). Our results showed that the Srengseng and PT. JIEP urban forest had relatively high carbon density, however they were much lower than that of the natural forests. These results suggest that urban forests in Jakarta have high potential to store and sequester carbon, and there are still great potential to increase the capacity of carbon storage and sequestration by urban forests in Jakarta. In 2009, green open spaces in DKI Jakarta province was only 6,825 ha or 10.5% of the total land area, with a target of 13.94% in 2010 (Pemerintah Provinsi DKI Jakarta, 2009). We suggest to increase the total area of urban forests and planting more trees, in particular trees of native species with fast growth rates. These will further improve the capacity of carbon storage and sequestration of urban forests in Jakarta.

CONCLUSION

Srengseng and PT. JIEP urban forest can play a significant role in mitigating the impacts of climate change by reducing atmospheric CO_2 levels in urban areas of Jakarta. This research provides a quantification of carbon storage by Srengseng and PT. JIEP urban forests in Jakarta, a city with the highest fossil fuel combustion in Indonesia. Carbon storage was different in both urban forests due to difference in species composition and age structure. These results can be used to help assess the actual and potential role of urban forests in reducing atmospheric CO_2 in Jakarta.

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PREPARATION OF REGULAR MICRO-PATTERNEDTHIN POLYMER FILMS FOR POTENTIAL CELL CULTURE SUBSTRATES

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ABSTRACT

Regular micro-patterned films were prepared based on star polystyrenes using self assembly method. The method was relatively simpler and cheaper than using lithography techniques which commonly used to fabricate such regular micro-patterned films. The casting conditions were varied to alter the pore size of the films. The morphology of the film surface was studied using Field Emission Scanning Electron Microscopy. The films were found to be very potential for being used for cell culture substrates.

Keywords: *micro-pattern, thin film, morphology, polystyrene, cell culture.*

INTRODUCTION

Polymers have been widely used as materials for tissue engineering, especially as substrates for cell culture [1,2]. Therefore, direct patterning on polymer films is required. However, most of micro-patterning of such films has been carried out using lithography methods which is relatively complicated and expensive [3,4].

In recent years, self-assembled polymer films with narrow pore size and high degrees of ordering have been created by using water droplets or bubbles to template the surfaces of polymer and inorganic films. Francois and co-workers found that honeycomb structure films could be prepared without using any lithography techniques by simply spreading star-polystyrenes (PS-PPP) solution onto a glass slide under humid condition [5-7]. Star-shape polystyrenes were found to create honeycomb structure films easily under a wide range of conditions [8-10].

Not all types of polymers appear to produce honeycomb structured films. The materials that have been found to support facile self-assembly honeycomb structure formation are star polymers, rod-coil block copolymers, amphiphilic block copolymers, amphiphilic copolymers, polyion complexes, comb polymers and organic/inorganic hybrid materials, were described by Stenzel [9]. Researchers are still not certain why these types of materials can be more readily to produce honeycomb structure films than the others. Rapid interfacial precipitation and low viscosity of these solutions may provide time for the droplets to organize into hexagonal arrays during the evaporation process. However, recent papers have shown that the formation of homogenous surface pores in polymer films is not limited to star polymers as coated nanoparticles, linear polymers have also shown to form these surface structures as well as wide range of other polymers, nanoparticles and inorganic materials [11-15].

The mechanisms of the film formation have been studied experimentally by number of researchers but are still not well understood. Despite early observations of 'breath figures' from the days of Lord Rayleigh, the fact that condensation droplets on the film surfaces can promote self-assembly of honeycomb structureshas attracted much attention recently due to the interest in micro and nanoscaled structures [13, 16]. Karthaus et al. and others believed that self assembly of water droplets in the solution surface play an important role in the formation of the cellular structures [6, 17, 18]. They found that water droplets could arrange themselves into hexagonal arrays on the surface of hydrophobic polymer-solvent systems (such star polymers in carbon disulfide). These arrays can be observed even on the pure solvent surfaces before they coalesce each other then evaporate. The presence of polymers in the solution appears to prevent the droplets from coalescing by creating a solid film about the droplet and thus films with very uniform pores were obtained [8, 19].

Moreover, it is clear that minor changes in casting parameters could change the film morphology in both conventional phase inversion and the vapor induced templating process. However, there has been little systematical study about the influence of sucrose core star-polymer properties and casting parameters on the film morphology. Therefore, in this study, the effect of the casting parameters on film morphology

was investigated. The casting parameters include: concentration of polymer solution, relative humidity, and temperature.

The interest in studying the formation has been motivated by the potential application of honeycomb structure films as cell substrates, patterned templates, membranes, and optical application [20, 21]. In this work, the influence of the casting parameters on the morphology of honeycomb structure film using sucrose-core star-polystyrene for potential cell culture substrates is the focus.

MATERIALS AND METHODS

Materials

Sucrose-core star-polystyrenes were used as the polymer materials. Carbon disulfide (CS_2) supplied by Aldrich applied as solvents.

Methods

Film Casting

Film casting was carried out in a casting chamber with the relative humidity more than 50%. Approximately, 120 µl of star-polymer solution was drop-wised using a pipette onto a glass support in the casting chamber. A constant moist airflow was applied horizontally over the solution surface. The drop of polymer solution was kept in the casting chamber for 10 minutes and then the resultant membrane was collected. The casting conditions were varied and the influence of those conditions on the membrane morphology was studied.

Film Characterization

The resultant films were characterized using Hitachi S-900 Scanning Electron Microscope (SEM). The film pore size and pore size distribution were then analyzed using image analysis software, i.e. Soft Imaging System (SIS) Analysis software, for determining the pore size and pore size distribution of the membranes.

RESULTS AND DISCUSSION

Regular micro-patterned films were prepared in this study using self-assembly formation that was simpler and need relatively low cost. A key issue of the formation is the evolution of the droplets as their growth and coalescence under appropriate casting conditions seem to be constrained to produce very even droplet size despite their relatively large size compared to thermodynamically stable microemulsions and micelles. Capillary forces such as those described by Nagayama and co-workers are attributed to the aggregation of such droplets but thermal and surface tension gradients have been also suggested as contributory forces in the formation mechanisms of the ordered arrays [6, 24, 25]. Considerable theoretical work on 'breath figures' or condensation droplet formation and evolution has been undertaken, but the complexity of their formation and stabilization on a labile surface with evaporative cooling, interfacial precipitation, and mass transfer simultaneously occurring poses a difficult problem [26-28]. The 'breath figures' templating and vapor induced phase inversion processes reported in other literature have essentially similar features, and some preliminary modeling of the phenomena have been carried out recently[29-33].

Altering the casting conditions had found to be significantly changed the film pore size. The results showed that using higher concentration of polymer solution created smaller pore size and shorter distance between pores, and therefore produced higher pore-density of the films (Figure 3.1). At higher concentration of the polymer solution the precipitation rate is more rapid, or in other words the phase inversion occurred more quickly as such the water droplets had less time to grow further and therefore creates smaller pore sizes.

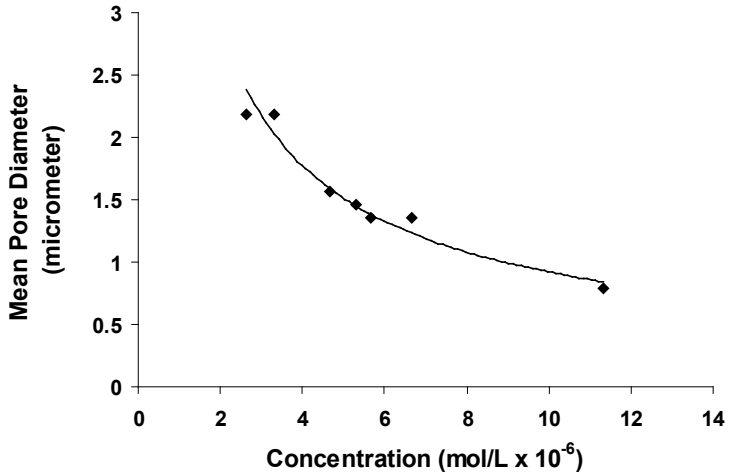


Figure 3.1 Relationship between concentration of polymer solution and membrane pore size. The casting was carried out using 10 mg/mL – 20 mg/mL of star polystyrenes with the mol. wt. of 17,670-37,722 in CS_2, R= 85% at room temperature (26˚C) using 2.4 L/min airflow rate.

At low concentrations of polymer, there may be insufficient polymer to precipitate and stabilize the droplets before coalescence. At higher concentrations, local viscosity may become high during the evaporative process, hindering droplet organization into ordered arrays.

Moreover,increasing the relative humidity in the chamber could change the film pore size by convecting more water droplets to the surface. Therefore, the higher the relative humidity in the chamber, the larger the pore size of the films was obtained (Figure 3.2). Moreover, we found that at higher humidity, the precipitation of the solution occurred more slowly than at lower humidity. As such, at higher humidity condition, the water droplets on the polymer solution surface had more time to grow further in size, and thus created films with larger pore size than at lower humidity condition. The results were consistent with the recent works by Peng et al [11]. Furthermore, we also found that if the humidity was too high, the pore size of the membranes was mostly irregular because the coalescence of water droplets might have occurred in some parts of the surface.Similarly, if the humidity was too low (dry condition), we could not obtain regular structure either, because less amount of water vapor to condense on the solution surface.

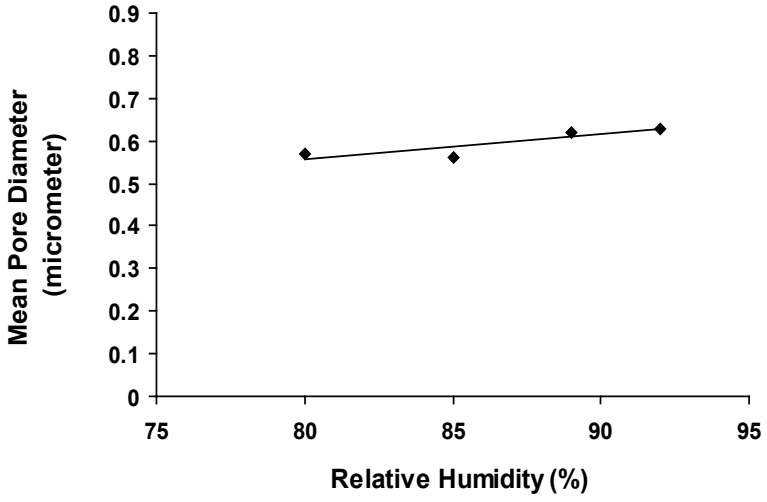
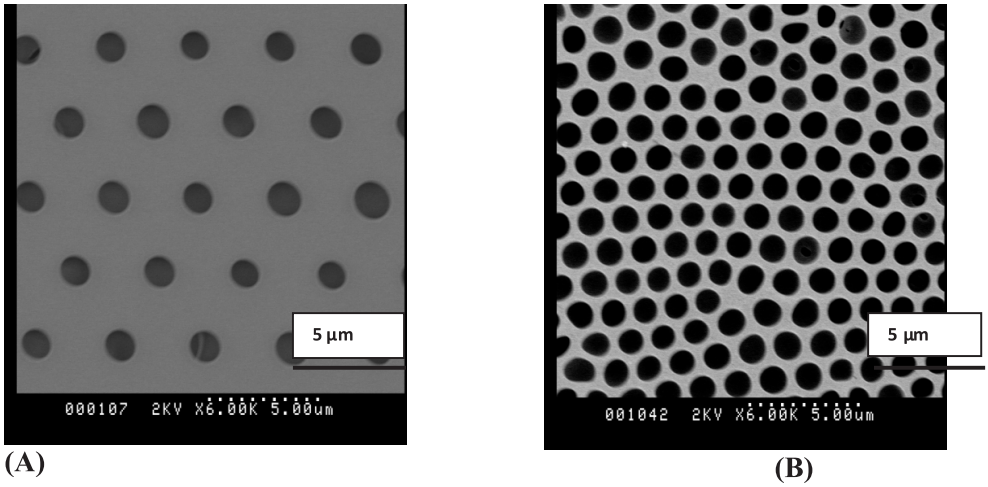


Figure 3.2 The plots of relative humidity against membrane pore size which obtained using star polystyrenes of Mw 37,722 in DCM as solvent with concentration of 20 mg/mL and airflow rate of 2.6 L/min, under room temperature (22˚C).

The casting stage temperature is also an important parameter that influences the membrane morphology. Angus et al. (2002) have believed that the use cold stage (< 10°C) for casting of uniform films is more favorable, as the temperature of the casting stage influences the quality of the films [19].

We found that the use of cold stage (low temperature) tended to produce higher pore density of the films than at room temperature. The colder the casting stage, the faster the condensation occurs allowing more water vapor could condense as water droplets on the solution surface. Therefore, using low temperature created more number of pores (on the same surface area) compared to using room temperature.

The images of the films which cast with different casting stage temperature are shown in Figure 3.3. SEM images of the membranes in larger scale can be seen in Figure 3.3(A) and 3.3(B) showing that using the cold stage tends to create less regular films.



(A) (B)

Figure 3.3 The influence of casting stage temperature on the film pore size. (A) at room temperature (23 ºC); (B) using cold-stage (5 ºC). The membranes cast using star polystyrenes with Mw of 17,670, concentration of 10 mg/mL in CS_2 with airflow rate of 2.4 L/min and R=90%.

SUMMARY

In summary, desirable film pore size and morphology can be achieved by optimizing the star-polymer structures and the casting conditions. Interestingly, the pore size was found to correlate strongly to the molar concentration. Higher concentration of polymers solution created smaller pore size of the films. On the other hand, increasing the relative humidity of the casting chamber produced films with larger pore size. Casting on the cold stage tended to form films with higher number of pores compared to casting at the room temperature by promoting condensation and reducing solvent evaporation. The range of pore size of the film was approximately between 0.5 and 2.0 micrometers. Films with this range of pores are very potential to be applied for cell cultured substrates.

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PHYTOPLANKTON AS WATER QUALITY INDICATOR AT SASAK LAKE CIPUTAT

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ABSTRACT

Phytoplancton has a very important role in water, both as primary producer of foodchain and as an indicator for trophic state in lake. The objective of this study was to analyze the water quality of Sasak lake using phytoplankton, sampled at three different location. The result showed that water quality of Sasak lake was noted with highly pollution level which indicated by diversity index of phytoplankton based on Shanon-Wiener index (H'<1). It also showed by the mostly found phytoplankton as indicator for polluted water categorie (82,56%), that were *Diatoma* (Bacillariophyceae, 15,42%), *Euglena, Phacus and Trachelimonas* (Flagellata, 67,14%). Thus, local government should manage well this lake especially waste disposal from human activities surrounding the lake and gives high attention to restore water quality toward the good condition, followed by consistantly law enforcement for polluters.

Keywords: Water quality, phytoplankton, Sasak Lake, diversity

INTRODUCTION

One of small lakes in Java is Sasak lake located in Ciputat, South Tangerang district. Water of Sasak lake has been used for drinking water source for many species of both large and small animals living there. Water of lake are often used to irrigate agricultural lands belonging to the people around and can also be used for bathing and washing purposes.

Most of people living or doing activities surrounding lake has been disposing of waste into the water body of lake which will cause water pollution problems in further. In order to assess level of pollution regarding to water quality, biological monitoring is a valuable choice. Bioindicators of pollutants are useful in predicting the level and degree of pollutants before the efffect of the pollutants starts, which caused large proportions (Verma, 2002). Study of these organisms is generally linked to the use of mathematical distribution of these organisms in the communities to which the bioindicator species belong (Singh and Singh, 2003).

Qualitative and quantitative analysis of different groups of organisms have led to establishment of bioindicators, indices and systems which can be used to assess the pollution status of water body, such as phytoplankton (Mahadev *et al*, 2005; Kumari *et al*., 2008).

Phytoplankton as an indicator of water quality has a cosmopolitan nature, which can live in various types of waters and has the pattern of spreading widely. The presence of phytoplankton varies from one place to another because of different water quality, so that phytoplankton can be used as an indicator of water quality (Nontji, 2006). This study was aimed to assess water quality using phytoplankton as indicator of pollution status at Sasak lake, Ciputat, South Tangerang.

MATERIALS AND METHOD

The research was conducted from March to August 2008 in Sasak Lake, Ciputat, South Tangerang. Further laboratory analysis was conducted at the Centre of Integrated Laboratory, State Islamic University Syarif Hidayatullah (UIN) Jakarta. Sampling sites of three locations were taken randomly (Figure 1), the water entrance (inlet), water bodies and water out (outlet). At the first sampling site was adjacent with the settlement/residential area where there were more human activities than other sampling sites. Location 2 was located at the center of lake (water bodies) and surrounded by Floating Fish Net (FFN). Location 3 was an outlet area adjacent to residential areas and roads. Each location was conducted by five repetitions with intervals of three days to produce more accurate data and to have a higher level of accuracy (Arisandi, 2003).

Figure 1. Sampling sites of study

Phytoplankton sampling methods are adopted from Ikasari (2005). For water sample was taken at the surface layer of water vertically with a depth of 30 cm using a Kemmerer water sampler, amounted 1 liter of water. One liter of water then concentrated to 10 ml by pouring the water sample into the plankton net. Type of phytoplankton was identified using the identification key of Fukuyo (2000) and Sachlan (1982).This procedure was replied to each sampling sites. Environmental physics and chemistry data were also collected in situ including brightness, turbidity, conductivity, water temperature, pH and dissolved oxygen (DO). It is informed that Sasak lake is shallow lake which has depth of about 0.5 to 2.0 m (Krisanti, 2006).

Data was analyzed descriptive quantitatively based on community structure including index of diversity, uniformity, dominance and abundance of phytoplankton. Those were calculated using Shannon-Wiener index, as follows:

Shannon-Wiener diversity index

$$H' = - \Sigma \, Pi \log_2 Pi$$

Where:
H' : Shannon diversity index
Pi : the relative abundance
ni : number of individuals of all species
N : total number of all types in community

criteria:
H '<1 : The community is not stable or the quality of water is poor
1 <H '<3: The stability of the community is moderate or the water quality is moderate.
H '> 3: stability in the community is stable or the quality of water is good

Uniformity Index

$$E = H'/ H \max$$

Where:
E : uniformity index
H' max : maximum diversity (Ln S)
H' : Diversity indices

Dominance index

$D = \sum (ni/N)^2$

Where:
Ni : number of individuals of all kinds
N : total number of all types in the community dominance index between 0-1
D = 0, there is no species dominating others or community structure is stable.
D = 1, there is species dominate other species or community structure is unstable, due to ecological pressures

.

Plankton Abundance

Plankton abundance was calculated based on the formula developed by Effendi (1997), as follow:
$N= n(Vr/V_0)(1/Vs)$

Where:
N : planton abundance (individu/L)
n : number of plankton
Vr : vol of filtered water sample (ml)
Vo : vol of water sample in the sample botle (ml)
Vs : vol of water sample (1000 ml)

Table 1. Criteria for assessment of environmental quality of Plankton

No	Divesity Index	Community Structure	Category
1	> 2.0	Very unstable	Unpolluted
2	2.0-1.0	More stable	Polluted
3	1.5-1.0	Stable	More polluted
4	< 1.0	Not stable	High polluted

(Fachrul, 2006).

RESULTS AND DISCUSSION

This research found 17 genera of phytoplankton classified into four families (Figure 2), namely Cyanophyceae (1 genus), Flagellata (3 genera), Bacillariophyceae (2 genera) and Chlorophyceae (11 genera). They were *Oscillatoria* (Cyanophyceae, 4,22%) *Euglena, Phacus, Trachelimonas* (Flagellata, 67,14%); *Diatoma, Melosira* (Bacillariophyceae, 15,42%); *Actinastrum, Cosmarium, Pediastrum, Scenedesmus, Tetraedron, Chlorella, Coelastrum, Oocystis, Anacystis, Pandorina and Chlorogonium* (Chlorophyceae, 13,23%).

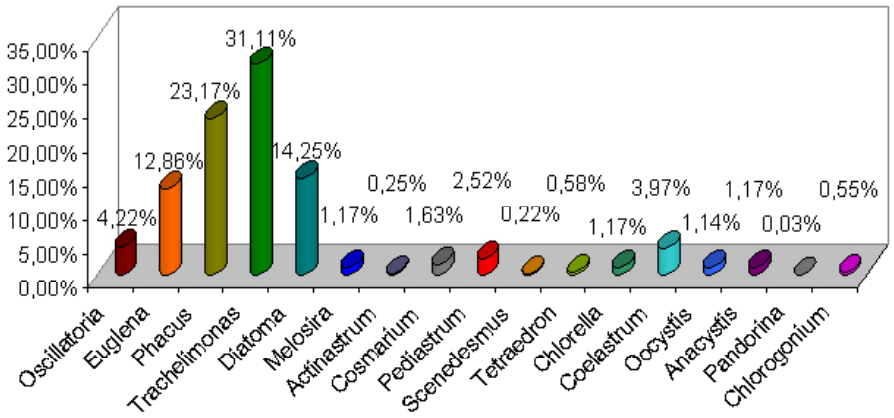

Figure 2. Type of phytoplankton found in Sasak Lake on 3 locations sampling sites

Euglena, Phacus and Trachelimonas were the principal type of phytoplankton throughout the study site which were found the highest amount among other phytoplankton found in water of Sasak lake (67,14%). The abundance of them was due to adaptive ability to the aquatic environment. According Sachlan (1982), those type of phytoplankton are able to protect themselves from toxic substances in waters with a protective cyste, therefore they are able to live in polluted water and used as bioindicator for pllution status in water body. Besides that, they also have flagellum to make widen movement in the waters, while the other genera have not.

Diatoma was other type of phytoplankton grouped in indicator for polluted water. This organism is one of the principal type of phytoplankton found in Bacillariophyceae group which have a very important role for fisheries. Diatoma abundance in water lake of Sasak has caused enormous tolerance and the cosmopolitan nature of life. This is as pollution indicator, as reported by Kumari *et al* (2008) at the lakes of Nagpur city. As one of higher percentage of phytoplankton genus found in water of Sasak lake, the presence of Diatoma indicated the pollution occured.

Phytoplankton in family of Chlorophyceae were found in lower percentage in water of Sasak lake. The low abundance of the genus was due to physical and chemical factors of water (Table 2) in Sasak lake which did not supported the living of organism. According Sachlan (1982), *Actinastrum, Coelastrum, Pediastrum and Scenedesmus* can be used as an indicator of fresh water is slightly acidic in nature with a pH between 5.5 or 6.5. Besides the low brightness on these waters may cause some difficulties in phytoplankton photosynthesis and finally caused anaerobic process which in further polluted the water body. This indicated decreasing dissolved oxygen concentration as other environmental parameter in assessing pollution status in water.

Table 2. Water quality in Sasak Lake

Parameter	unit	Location		
		1	2	3
Brightness	cm	58,8	62,0	66,8
Turbidity	FTU	10,374	10,434	14,346
Conductivity	S/cm	0,163	0,152	0,150
Temperature	^{o}C	29,02	29,24	29,2
pH		6,89	6,81	6,83
Dissolved oxygen	mg/L	0,69	0,60	0,60

Other assessment of pollution status of water in Sasak lake was the community structure of phytoplankton. The result showed the average value of the diversity of phytoplankton ranged from 0721-0775, the diversity of each sites was relatively similar (Table 3). The lowest phytoplankton diversity occured at the location 1 (0.721) which was due to the abundance of pollutants appearing in this sampling site, lower brightness value than the other locations.

Table 2. Community structure of phytoplankton

Parameter	Location of Sampling		
	1	2	3
Diversity	0,721	0,725	0,761
Abundance	2000	2980	1518
Dominance	1,076	1,084	1,002

This caused to limitation of light come into the water and was used by phytoplankton. The highest diversity was at location 2 (0.775) which was due to the location adjacent with FFN as the sources of nutrient for phytoplankton.

Based on the Shannon-Wiener index, it was found that the level of diversity in Situ Sasak H '<1:00 which was in a low category, and this indicates the level of heavy pollution. Dispersal and species richness of phytoplankton in water of Sasak lake were relatively low which was meant the phytoplankton community was not stable and only a few phytoplankton were able to live in water Sasak lake. During the study was found the number of species were not too many but some species had a high abundance. This showed that

some types of phytoplankton can adapt and survive in polluted water but others can not.

Average abundance of phytoplankton in the water was ranging from 1518-2980 Ind/L. The lowest abundance was occurred in locations 3 (1518 Ind/L) because the location was in outlet sites where was assumed fewer nutrients found. The highest abundance was occurred at the location 2 with the average value of 2980 Ind./L where was adjacent to the FFN supplied more nutrients for phytoplanton. In addition, water physical and chemistry parameters at this location were also good for the life of phytoplankton. However, if the nutrient content on this site are not controlled well then in long term phytoplankton will be blooming and can cause eutrophication phenomenon in the waters. It was found that aquaculture activities with FFN techniques that took place in water body will directly affect to water quality of Sasak lake.

Average uniformity index of phytoplankton in the water was ranging at 0333-0344 which showed very low categorie. This was due to the abundance of species that are uneven and there was a tendency of a genus dominated the population in the water. Uniformity index describes the level of equilibrium species composition of phytoplankton. Not equally abundant genus causes a low uniformity.

Dominance index in the water was ranging from 1.002 to 1.084 which indicated highly criteria for all sampling locations. According Fachrul (2006), an index of dominance in a water with a value of D>1 indicates domination of one or more types of phytoplankton and further the community structure becomes unstable due to ecological pressures. There were four genera of phytoplankton dominating in the waters of the Sasak Situ, namely *Diatoma, Euglena, Phacus and Trachelimonas*. Unfortunately, those types of phytoplankton are as bioindicator for polluted water categorie. Thus, it can be said that the water body of Sasak lake had already polluted indicated by phytoplankton structure.

CONCLUSION

Water quality of Sasak lake was very bad and indicated as a highly level of pollution status based on total percentage of phytoplankton indicators for polluted water (82,56%) which were grouped in family of Flagellata (67,14%) and Bacillariophyceae (15,42%). Other types of phytoplanton found in Sasak lake's water were Cyanophyceae and Chlorophyceae. Lower percentage of Chlorophyceae (13,23%) and Cyanophyceae (4,22%) indicates lower productivity level of Sasak Lake related to lower nutrient content.

To prevent further pollution, it is expected the participation of communities in maintaining and keeping clean Sasak lake through empowering the young local people. Continously monitoring water quality of Sasak lake is also useful to anticipate water pollution derived from upland wastes. For upland area, government has to facilitate it for useful activities such as sport, playing ground and park in order to attract people to maintain the lake indirectly. The most important is law enforcement for people who dispose waste into the water or destruct the ecosystem of lake.

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EFFECTIVENESS OF *TRICHODERMA* SP. ISOLATE TD_{22}-GROWN COMPOST IN PROTECTING PYRETHRUM PLANTS FROM*SCLEROTINIAMINOR* INFECTION

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ABSTRACT

The main objective of this study was to investigate the effectiveness of 4.5 months old of *Trichoderma* sp. isolate Td_{22} grown in a mix of 20% millet seed and 80% WFW compost in protecting pyrethrum plants from *Sclerotiniaminor* infection.The trial was conducted in 0.5 L capacity pots containing field soil amended with 5% v/v compositions of a suppressive compost previously inoculated with 4.5 months old Td_{22}. *S. minor* grown on millet seed was then evenly inoculated approximately 20 mm below the soil surface at rate of 2.0 g inoculum per pot. Soil without compost amendment, amended with pathogen only, or without pathogen inoculation served as controls. Four, three-week old pyrethrum seedlings were sown per pot following pre-incubation of the soil mixture in a shade house for four days, with eight replicates per treatment. The experiment was maintained for seven weeks in the shade house, with the number of healthy seedlings/plants being recorded at weekly intervals, starting one week after sowing. The trials were destructively sampled at week eight. All surviving plant tops were harvested, dried at 65°C and subjected to dry weight determinations. The sclerotia of the pathogen (*S. minor*) in pots inoculated with either *S. minor* alone (A0B1) or with *S. minor* and Td_{22}-grown compost (A1B1) were retrieved using a 0.5 mm sieve.

The results showed that the suppressive mix consistently protected the pyrethrum plant from *S. minor* attack. Protection of 100% was recorded when soil was amended with Td_{22}-compost at the rate of 5% (v/v) seven weeks after sowing. Mortality of controls at this time was 78%. It was indicated in the trial that the Td_{22} was not pathogenic to pyrethrum (plants grown with compost-grow Td_{22} in the absence of the pathogen showed 100% survival). The average dry weight per survive plant in the *S. minor* control treatment (A0B1) was significantly lower ($p<0.05$) than that in Td_{22} amended treatments A1B0 (amended with Td_{22} only) and A1B1 (amended with both Td22 and the pathogen *S. minor*). However, the dry weight of the plant in this control treatment (A1B0) was not statistically significant ($p>0.05$) from that recorded in the nil-pathogen, nil-Td_{22} control (A0B0). The density of *S. minor* sclerotia in the control treatment (A0B1) was significantly higher (almost double) than that in pots where *S. minor* and compost grown Td_{22} were co-inoculated (A1B1), suggesting inhibition of growth or sclerotial development of the pathogen, or parasitism and death of the sclerotia.

Keywords: Compost, *Sclerotinia minor*, pyrethrum,*Trichoderma* sp.

ORAL PRESENTATION: HEALTH

THE EFFECT OF DARK RED ROSELLE (*Hibiscus Sabdariffa* var Sabdariffa rubber) TO THE LEVEL OF MALONDIALDEHYDE (MDA) IN RATS

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ABSTRACT

Hyperlipidemia is one of some factors that cause cardiovascular disease. This condition will increase lipid peroxidation caused by free radicals in the body. Lipid peroxidation produces toxic compound malondialdehyde (MDA). One effort to prevent lipid peroxidation is antioxidant intake. Dark Red Rosella contains active compound as antioxidant such as Vitamin C, anthocyanin and phenols. This study was performed to investigate the effect of Dark Red Rosella (Hibiscus sabdariffa var sabdariffa rubber) to the level of plasma malondialdehyde (MDA) of mice's Sprague dawley plasma given high lipid diet. Randomnized control trial was used as a design in this study. Twenty eight males *Sprague dawley* aged 2 months divided into 4 groups. Control group (K) received standard diet without rosella. Group A, B, and C as intervention groups received standard diet and different rosella doses 0.0225 gram/1 ml/ 250 gram BW, 0.045 gram/1 ml/ 250 gram BW and 0.09 gram/1 ml/ 250 gram BW for each group. Every group was adapted for 7 days and then every group received high lipid diet for 7 days. Their blood plasma was taken for measure MDA on the 7th day adaptation; 7 day after high lipid diet; 14th and 28th day after intake rosella. The result show that MDA plasma significantly different in all groups ($p<0.05$). Group C has the lowest MDA plasma than group K, A and C. Group C were decreased 67,83% MDA plasma during in this study. Dark Red Rosella (Hibiscus sabdariffa var sabdariffa ruber) can reduce the lecel of MDA of of mice's Sprague dawley plasma given high lipid diet

Keywords: Hyperlipidemia, Dark Red Rosella, Antioxidant, MDA

INTRODUCTION

Cardiovascular disease (PCV) is one example of some of the prevalence of many degenerative diseases happened lately increased. One cause of cardiovascular disease is hyperlipidemia. Hyperlipidemia is a condition which marked the high concentration of lipids with increasing concentrations of triglycerides, LDL (low density lipoprotein), and blood cholesterol beyond normal limits (in humans> 200 mg / dl) (10). Hyperlipidemia may increase lipid peroxidation caused by free radicals in the body, such as the liver (20). Lipid peroxidation is a reaction that occurs between free radicals with polyunsaturated fatty acids (polyunsaturated fatty acids, PUFA) found in cell membranes and LDL. Polyunsaturated fatty acids that have formed peroxidation products those are toxic to the body of malondialdehyde (MDA) (12). Malondialdehyde reflect peroxidation associated with serum cholesterol, arterial injury and severity of atherosclerosis. Increased plasma MDA can also lead to myocardial infarction caused by platelet aggregation and cause endothelial damage. (11.15.18.19). Increased MDA also obtained in patients with metabolic syndrome compared with normal individuals (8). One of the plants that contain high antioxidant is Dark Red Roselle (Hisbiscus sabdariffa var sabdariffa rubber). Flowers Rosela has active components that act as antioxidants are vitamin E, beta-carotene and anthocyanins (25.30.31). Rosella flower extracts also contain polyphenolic acids, flavonoids, and PCA (protocatechuic acid) (4).

METHOD

This research is experimental research with the study design used a complete randomized design (CRD). Dark Red Roselle Flower taken from Calix and harvested after 4-5 months old. Dark Red Roselle Flower petals are dried in the sun for 3-5 days. Dark Red Roselle Flower petals have dried brewed with hot water with a temperature of 75-90 C for 15-20 minutes. High-fat feed was made from standard feed Japfa Comfeed Br II of lard mixed with liquid 10% (20 grams of standard feed mixed with 2 grams of fat pork).

Animal used in this research is *Sprague Dawley* male rats with the age of 28 ± 60 days at the tail of rats weighing 200-250 grams. Rats adapted for 7 days in individual cages with standard feeding of Japfa Comfeed BR II and drinks in an ad libithum. Experimental animals have blood drawn for analysis

via orbital sinus malondialdehyde (MDA) initial plasma. Feeding high-fat treatment given after 7 days adaptation period. High-fat feed given for 7 days with a high-fat food made from standard feed mixed with liquid lard 10%. Animals try to have blood drawn for use as an analysis of plasma MDA levels after treatment of high-fat feed. Mice were then divided randomly into 4 treatment groups with each group of seven rats that were fed the standard diet without the infusion of feed Dark Red Roselle Flower petals. (Group K), standard feed group with flower petals infusion doses of 0.0225 gr Dark Red Roselle / 1 ml / 250 g BW (group A), standard feed group with flower petals Rosela infusion dose of 0.045 g / 1 ml/250 g BW (Group B), and the standard feed group with Dark Red Roselle Flower petals infusion dose of 0.09 g / 1 ml / 250 g BW (Group C). Analysis of plasma MDA levels was conducted by Thiobarbituric acid-reactive substance (TBARS)(14). Data was analyzed by multivariate analysis using ANOVA to determine differences between groups. If there is a difference then followed by post hoc tests using Tukey HSD.

RESULT

Sprague Dawley rats body weight during the study. *Sprague Dawley* rats weight known to have increased during the study. *Sprague Dawley* rats body weight during the study are presented in Table 1. The results of analysis using a statistical test known that found a significant difference to the growth of body weight during the study ($p < 0.05$). However, based on statistical analysis was found that there is no significant difference in body weight of rats between the treatments groups ($p > 0.05$).

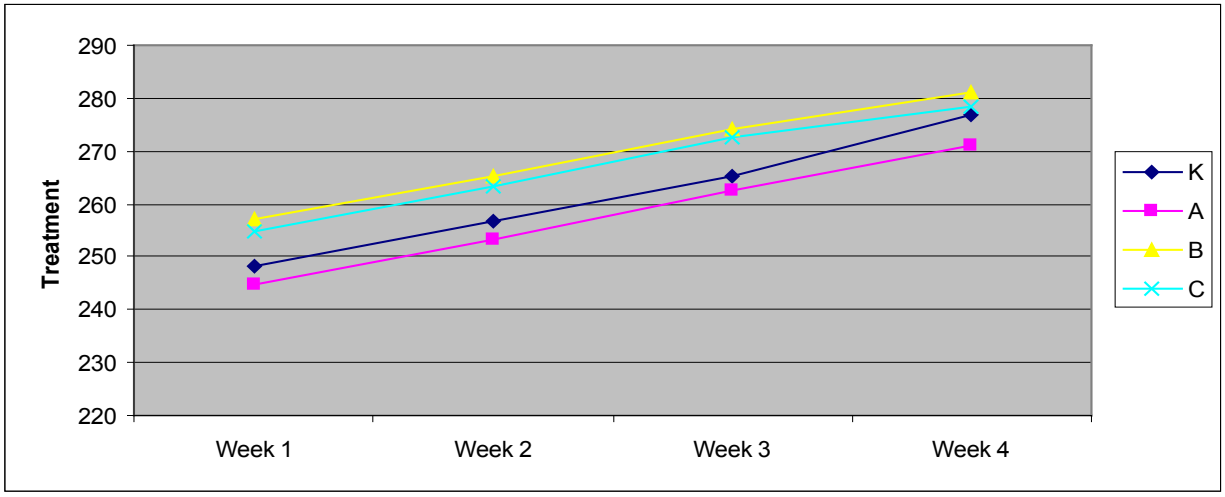

Figure 1 *Sprague Dawley* rats body weight during the study (C: control, A: Dosage 1 (g/ml/250 0.0225 g), B: Dosage 2 (0.045 g / ml / 250 g), C: Dosage 3 (0.09 g / ml / 250 gr)

Sprague Dawley rats feed intake during the treatment

The average feed intake of Sprague Dawley rats during the treatment of infusion Dark Red Roselle Flower petals are known in the group K of 16.90 ± 0.22 grams, group A 16.79 ± 0.16 g, group B 17.12 ± 0.31 ± 16.95 grams and 0.38 grams of the group. The results of statistical tests on the average feed intake during the 4 weeks of treatment known to feed intake of rats during the treatment is not obtained significant differences ($p = 0.188$) between treatment groups.

MDA plasma levels during treatment in Sprague Dawley rats

The results of analysis using One Way ANOVA test on the 14th and the 28th day of treatment in mind that there are significant differences between groups of fate ($p = 0.000$). MDA plasma levels of Sprague Dawley rats can be seen in Table 1.

Table 1 MDA Level in Sprague Dawley Plasma

Group	Adaptation	Hyperlipidemia	Treatment	Treatment
Control	1.97±0.23	7.60±0.24	7.40±0.12	7.97±0.14
Dosage 0.0225 g/ml/250 g w	1.75±0.22	7.98±0.23	6.48±0.12	6.11±0.20
Dosage 0.045 g/ml/250 g w	1.85±0.16	8.19±0.33	4.72±0.10	4.11±0.15
Dosage 0.09 g/ml/250 g w	1.95±0.19	8.02±0.36	4.00±0.12	2.58±0.17

DISCUSSION

The results showed that Dark Red Roselle Flower petals give effect to the decrease of plasma MDA levels of *Sprague Dawley* rats fed a diet high in fat. Percentage decrease in plasma MDA levels in the group who received Purple Rosella steeping group A of 23.43%, 49.82% for group B and group C of 67.83%. While, controls groups who did not get a Purple Rosella steeping increased plasma MDA in the amount of 4.87%. MDA plasma levels inverse proportional to the dose of steeping Dark Red Roselle given to groups of experimental animals. The results of statistical tests show that there are differences in plasma MDA levels between groups of rats ($p < 0.05$). Group C with a dose of 0.09 g / 1 ml / 250 g BW were found to have the strongest activity against the decrease in plasma MDA levels when compared with group A at a dose of 0.0225 g / 1 ml / 250 g BW and group B with a dose of 0.045 g / 1 ml / 250 g BW.

Rosella is a plant that is widely consumed as a beverage cold and heat as well as having antioxidant effect of activity (5,23,29). Rat plasma levels of MDA decreased by steeping the petals on this research Dark Red Roselle allegedly caused by steeping Dark Red Roselle petals contain antioxidant compounds that anthocyanins, phenolic compounds and vitamin C. Antioxidants work to stabilize free radicals with a complete lack of electrons that are owned by free radicals and inhibiting the formation of a chain reaction of free radicals that can cause oxidative stress (21). Antioxidant that has been giving its electrons become free radicals are not dangerous because they have the ability to accommodate changes in the electron without becoming reactive. The combination of several antioxidants Provides better protection (synergism) against oxidation compared with only one type of antioxidant (17). Compounds delphinidin-3-sambubioside and cyanidin-3-sambubioside is an anthocyanin compound in rosella who played a major role in antioxidant activity (6).

Rosella anthocyanins act as antioxidants that are beneficial to bind free radicals in the body (26). Anthocyanins inhibit lipid peroxidation that can form the MDA by acting as a powerful scavenger of O2 .- as well as O2 quenchers and also react with peroxyl radicals that are responsible for the radical chain reaction during lipid peroxidation (3.28). Anthocyanin in rosella shown counteracts oxidative stress (2).

Phenol compounds are antioxidant compounds contained in Dark Red Roselle. Antioxidants can prevent the oxidation of phenolic compounds that produce MDA with lipid peroxyl radical trapping (7). Phenolic compounds can inhibit the oxidation reaction and is able to act as a reducing hydroxyl radical (OH. -), superoxide anions (O2. -), and peroxyl (16). Phenol compounds other than as an exogenous antioxidants also increase the synthesis of endogenous antioxidants (22). Phenol proved to have an influence on the transcription process is the synthesis of endogenous antioxidants reduced glutathione (13,16,27). Reduced Glutathione (GSH) is an enzyme that plays an active role in protecting cellular in removing hydrogen peroxide radicals in the body (33). Purple rosella contains ascorbic acid which are antioxidants. Vitamin C is water soluble vitamin that has the ability to suppress free radicals that will attack the lipids. As a free radical scavenger, vitamin C can directly react with super oxide (O2. .-) and hydroxyl anion (OH. -), hydrogen peroxide (H2O2) and various lipid hydroperoxide (LOOH) that play a role in the prevention of lipid peroxide (1.8).

Ascorbic acid can react with oxygen terkativasi like superoxide anions. Ascorbic reaction with superoxide is physiologically similar to the work Superokside dismutase (SOD). Superoxide radicals

react with vitamin C to hydrogen peroxide and dehydroascorbic. Hydrogen peroxide formed vitamin C with a catalyst modified by the enzyme peroxidase into water and monohidroaskorbat (33). Ekawanto results (9) showed that steeping rosella flower petals can enhance enzyme activity Superokside dismutase (SOD) in mice induced by alcohol. Superokside dismutase is a major antioxidant produced by the body's mitochondria, lysosomes and nucleus. Superokside dismutase serves as a scavenger against superoxide radicals and prevents the formation of new radicals because it can alter the existing free radicals to be reduced molekui negative impact (17).

SOD is an antioxidant mechanism as the reductant reaction dismutase and superoxide anions into hydrogen peroxide and oxygen (32). Prevention of superoxide radicals by SOD enzyme inhibits lipid peroxidation chain reactions that can reduce the formation of MDA. Prevention means of free radicals with antioxidants to prevent lipid peroxidation process and the formation of MDA, because MDA is the end result of lipid peroxidation process. This study proves that the compounds that produce lipid peroxidation MDA can be prevented by administering infusion Purple Flower petals. The content of active compounds Dark Red Roselle Flower petals infusion of anthocyanin, a compound suspected of phenol and Vitamin C can prevent lipid peroxidation is characterized by low levels of plasma MDA in the group who received infusion Dark Red Roselle Flower petals.

CONCLUSION

The conclusion of this research is Dark Red Roselle Flower petals infusion (Hibiscus sabdariffa var sabdariffa rubber) lowered levels of malondialdehyde (MDA) plasma Sprague Dawley rats fed a diet high in fat. Based on the conclusions will need further research to determine the effect of infusion Dark Red Roselle Flower petals on levels of body aktioksidan unit (SAT) and the body's endogenous antioxidant levels and need further research to determine the effect of infusion Dark Red Roselle Flower petals with human subjects.

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INTAKE OF SARDINELLA LONGICEPS OIL AS ANTI DISLIPIDEMIA THROUGH DECREASE OF LDL-C ON RAT WISTAR

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ABSTRACT

Nowadays,fish consumption behaviour needs to be improved since the chandes of consumption paten ti instant food. Fish, especially from deep see, is good for health, due to its rich content of polyunsaturated fatty acid (omega-3) which level is investigated in this research using true experimental study with randomized pre and posttest control group design. This research employed 50 Wistar rats which were divided into 5groups, i.e.control group ()%SLO), treatment group 1 (10% SLO), treatment group 2 (15% SLO),treatment group 3 (20%SLO), and treatment group 4 (25%SLO). To obtain dislipidemia, the rats were fed with food rich in factor for 8 weeks, followed by determination of HDL level (pretest). After that,all rats were fed with sardinella longiceps oil for 6 weeks for dislipidemia and followed by determination of HDL.

Intake of 20% SLO resulted in an increase of blood LDL levels of dislipidemia Wistar rat about 22,78% from 55,97 ± 5.1,25 to 68,73 ± 1,25 MG/dL. Futher research regarding of whether the intake of SLO inhuman results in a similar effect to anti dislipidemia need to be carried out.

Keywords: Fish consumption,antidislipidemia,sardinella longiceps

INTRODUCTION

Nowdays, fish consumption behaviour needs to be improve since the changes of consumption patern to instan food. Fish, especially from deep sea is good for health, due to the fish rich of polyunsaturated fatty acid (omega-3). Such fish including *Thunnus Scombridae*, *Euthymrus sp*, *Scamberamorus*, *Decapterus*, *Rastrellinger*, *Sardinella Longiceps*, *Carangidae*, *Clupeidae*, and *Psettodeserumei*. *Sardinella Longiceps* are a highly abundance fish found in Indonesian sea. Outbreak during peak season occures and as a consequence no economic value. Therefore, to increase their economic value, the fish can be managed to produce oil (Abdullah, 200; Ihsan, 2009).

Research on fish oil advantages to decrease dislipidemia have already widely carried out. This was intiated by the evidence that Japanese life longer compare to Mongolianese. This is as a results of Japanese have much more fish on their diet compare to meat consumption by Mongolianese. Fish consumption leads to lower of total cholesterol, LDL-cholesterol, and trigliceride and higher HDL cholesterol compare to meat consumption. This is because of fish contain much more polyunsaturated fatty acids omega-3 (Komatsu, *et al*., 2006; Fadilah, 2007).

Many researchs reported that there is a correlation between high lipid serum levels to incidence of atheroschlerosis, a triger of coronary heart disease. Coronary heart disease occures as a consequence of one of general mechanism, i.e. blood circulation disturbance and abnormality of chardiac charge or any other form of aritmias. These will lead to the present of unorganize myocardiac contraction, blood circulation obstruction, and blood circulation regugirtation. All of these conditions result in shunting, abnormal circulation, and end up with congestive heart failure (Vinay, *et al*. 2004). Ateroschlerosis is a slow progressive disease present in large to small muscular and elastic arteris. Main sites of atheroschlerosis are abdominal aorta, coronaria arteri, poplitea arteri, desendens toracali aorta, intern carotic arteri, and willisi circulation. Other risk factors, such as hypertension, chronic hypercholesterolemia, immune system disturbance, toxin and virus have also affect destruction of arteri endothelial wall cells. This destruction induces endotel cell permeability changes and lead to plasma constituent, i.e. lipoprotein easily flow to the inner of arterial wall. Endotel cell destruction will also change thrombocystein lumen arteri which is lead to adhesion of thrombocyte to damaged and inflamated blood. Finally, results in contact of lower connected tissue to other elements in blood circulation. If, this damage goes chronically and ages, atherosclerosis process continually occurs and leads to thick tunica intima and disturbance of blood circulation on that site (Szmitko, *et al*., 2003).

Dyslipidemia is an abnormal lipoprotein metabolism, generally correlated to decrease or overproduction of lipoprotein. Dyslipidemia is frequently also known as hyperlipidemia associated with

increase of lipid serum as a risk factor of cardiovascular disease. This is because of there is a cholesterol behavior which is play an important role on atherosclerosis in dyslipidemia. Therefore, differentiation between hypercholesterolemia with dyslipidemia is hypercholesterolemia is define as an increase of serum cholesterol more than 200 mg/D1 after 9-12 h starving. On the other hand, dyslipidemia define as the present of increase LDL-cholesterol serum more than 160 mg/D1, increase of triglyceride serum more than 150 mg/D1 or HDL-cholesterol below 40mg/D1 for man and below 50 mg/D1 for women, besides hypercholesterolemia criteria. High cholesterol symptom in dyslipidemiacannot be felt by dyslipidemia patients, however, this can only be known through routine check of blood cholesterol. High cholesterol diet and genetic induce dyslipidemia (Anonym, 2009).

Based on background explained above, the author want to investigate the effect of anti dislipidemia, through an decrease of LDL.

RESEARCH METHOD

Research Design

This is a true experimental study with randomized pre and posttest control group design. This research employing 100 Wistar rats divided into two research, i.e. dislipidemia and atheroschlerosis. Each researchs applying 50 Wistar rat classified into 5 groups, i.e. control group (0% SLO), treatment group 1 (10% SLO), treatment group 2 (15% SLO), treatment group 3 (20% SLO), and treatment group 4 (25% SLO).

Initial step is to prepaired all rats in similar condition, therefore, all rat were feeded with ITB diet for 4 weeks. Then to obtain dislipidemiarat (Research I), rats were fed with food rich of fat (UNAIR diet) for 8 weeks, followed by determination LDL-C levels (pretest data).

Location And Time Schedule

Rats preparation were carried out at Center Study of Animal Diseases(CSAD)Veterinary Faculty UdayanaUniversity.LDL-C determination was carried out at UPT. Laboratorium Analitik Universitas Udayana. Research was carried out for 12 months including data analysis and writing the results.

Data Analysis

All data were analyzed descriptively. Mean difference decrease of decrease of LDL-C were analyzed using anova one way at 5% significant levels.

RESULTS

Decrease of LDL-C levels of dislipidemia Wistar rat

Mean decrease data of LDL-C of dislipidemia Wistar rats for pre and posttest were listed on Table 1. The data on Table 1 were normally distributed and their variance were also homogeneous insicates by $p > 0.05$. Mean different profile of LDL-C levels after treatment of various SLO intake was presented on Figure 1

Decrease of LDL-C levels of dislipidemia Wistar rat

Mean LDL-C levels of Wistar rat pretest and postest data were listed on Table 1.

Table 1 Decrease of LDL-C Levels

Treatment	LDL-C (mg/dL)	
	Pretest	Posttest
SLO 0% (control)	144.05 ± 0.02	141.06 ± 1.54 a
SLO 10%	144.09 ± 0.03	140.99 ± 1.38 a
SLO 15%	144.05 ± 0.08	134.52 ± 1.89 b
SLO 20%	144.08 ± 0.02	126.12 ± 1.61 c
SLO 25%	144.08 ± 0.03	134.52 ± 3.51 b
	LSD 5% = 0.95	LSD 1% = 1.29

Notes : Mean followed by same letter indicates insignificant different (p>0.05). LSD for posttet.

Data on Table 1 are normaly distributed and their variance also homogeneous with $p > 0.05$. Mean profile different of LDL-C difference of various SLO intake was presented in Figure 1

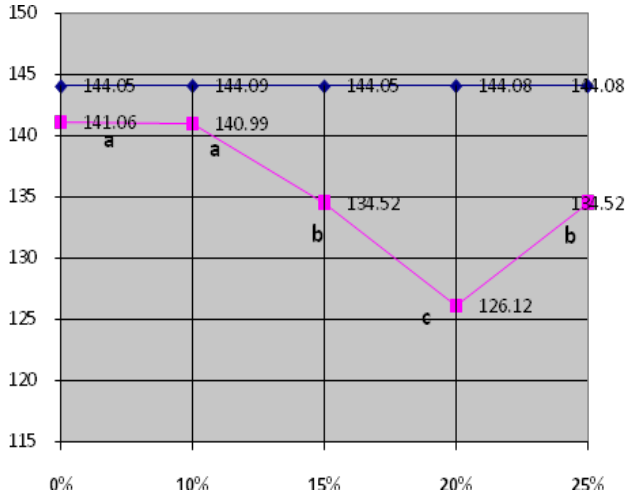

Figure 1
Mean Different Profile of LDL-C Difference for Posttest

Anova test indicates that there is a significant different of SLO various intake with $p < 0.05$. Resume of the test was presented on Table 2.

Table2 Resume of Post Hoc LDL-C Levels

Treatment		Mean Difference LDL-C (mg/dL)	*p**
SLO 0% (control)	- SLO 10%	0.14	0.880
	- SLO 15%	3.35	0.001
	- SLO 20%	14.99	0.001
	- SLO 25%	6.75	0.001
SLO 10%	- SLO 15%	- 3.21	0.001
	- SLO 20%	- 14.85	0.001
	- SLO 25%	- 6.61	0.001
SLO 15%	- SLO 20%	- 11.64	0.001
	- SLO 25%	- 3.41	0.001
SLO 20%	- SLO 25%	8.23	0.001

*Significant $p < 0.05$

DISCUSSION

Dyslipidemia

A number of 50 Wistar rat for dislipidemia have already investigated. Dislipidemia research was applied by feeding of high fat diet (UNAIR diet) for 8 weeks, compare to 13 weeks for atheroschlerotic research. Mean weight of initial Wistar rat is 49.78 ± 0.77) g. After the present of dislipidemia, the mean weight is about 201.75 ± 0.51 g (pretest data). Mean weight of Wistar rat after intake of various SLO for 6 weeks is 203.78 ± 1.03 g (posttest data).

Decrease of LDL-C blood levels of dyslipidemia Wistar rat

In this research, it was obtained that intake of various SLO concentration decrease blood LDL-C levels of dyslipidemia Wistar rat. Mean LDL-C blood levels of dyslipidemia Wistar rat were presented on Table 1. Mean HDL-C blood levels of dyslipidemia Wistar rat for pretest with intake of 0%, 10%,

15%, 20%, and 25% SLO were kolest-LDL *pre* dan*posttest* (144,05±0,02) and (141,06 ±1,54) mg/dL, respectively. The mixture of MIL 10% results in the levels of kolest-LDL *pre* and*posttest* (144,09±0,08) and (140,99±1,38) mg/dL. The table also showd the level of kolest-LDL for MIL 15%, 20% dan 25% *pre* and*posttest* (144,05±0,08) and (134,52±1,89) mg/dL; (144,08±0,02) and (126,12±1,61) mg/dL; (144,08±0,03) and (134,52± 3,51) mg/dL respectively. This research shows that lowering of the levels of kolest-LDL took place at all treatments (Figure 5.3).From the same table, it can also be seen that mean HDL-C blood levels of dyslipidemia Wistar rat for posttestwith intake of 0%, 10%, 15%, 20%, and 25% SLO were (56.84 ± 1.25); (58.14 ± 1.38); (59.26 ± 0.84); and (68.72 ± 0.86) mg/dL, respectively. The increase different of HDL-C blood levels of dislipidemia Wistar rat caused by intake of 0%, 10%, 15%, 20%, and 25% SLO can be seen on Table 4.10. This table reveals that the highest increase of 11.88 mg/dL HDL-C blood levels of dislipidemia Wistar rat was observed for intake of 20% SLO, compare to 1.30 mg/dL for intake of 10% SLO, and 2.72 mg/dL for intake of 15% SLO. These were compared towards HDL-C blood levels of dislipidemia Wistar rat with intake of 0% SLO. An interesting trend observed in this research was increase of 2.54 mg/dL HDL-C blood levels of dilipidemia rat due to intake of 25% SLO, as indicated by Figure 4.5. However, that increase is not as big as for intake of 20% SLO about 11.88 mg/dL. These trends occure in all treatments of SLO intake, including decrease of LDL-C and MDA blood leves.

In dyslipidemia case, cell membrane was saturated of cholesterol due to exessive of LDL acceptance and endogenebiosintesys. To overcome this situation, naturaly cholesterol balancing occures through pick cholesterol up and distributed to extracelular flood and return it to liver. This event known as reverse cholesterol transport and was carried out by HDL as an antithrombin (Devlin, 1986; Murray, 2004). Possible mechanism is due to anti oxidant inactivation, that function to break down unsaturated fatty acids. Long and continue exposure induce leucocyte adhesion to vascular endotel which leads to endotel cells promote cholesterol effux that have as role to control vascular (Tatong, 2003). This is conform to hypothesis 5, i.e intake of SLO increase HDL-C blood levels of dislipidemia Wistar rat.

CONCLUSION

It can be concluded that:

1. Intake of 20% SLO results in increase of LDL-C blood levels of dislipidemia Wistar rat about 22.78%, from 55.97±1.25 to 68.73 ±1.25 mg/dL; and
2. further research regarding of whether intake of SLO to human results in a similar effect for anti dislipidemia.

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HUMAN IMMUNODEFICIENCY VIRUS DETECTED IN THE GIGOLO COMMUNITY IN SURAKARTA

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ABSTRACT

To build an adequate molecular epidemiology database of human blood borne virus in the high risk communities in Indonesia, a molecular epidemiology study has been performing since 2009 started from Central Java. In 2011, the gigolo community in Surakarta started to be enrolled in the study. For the best of our knowledge, the molecular epidemiology data of blood borne virus including that of Human Immunodeficiency Virus in the gigolo community is quite rare in Indonesia. In May-June 2011, 30 gigolos were recruited by respondent driven sampling method. The study participants completed an interviewer administered questionnaire that assessed general socio-demographic and epidemiological characteristics associated with blood borne virus infections including that of their sexual behaviors. Blood sample was drawn for Human Immunodeficiency Virus serologic test using Determine HIV-1/2 confirmed by molecular amplification of part of the Human Immunodeficiency Virus gag gene by nested RT-PCR. In the ongoing study, 53.33% (16/30) of gigolos have tattoos and or piercings. With respect to sexual behavior, 90% (27/30) of gigolos have bisexual orientation, with 86.67% (26/30), 83.33 % (25/30), and 96.67% (29/30) of anal sex, coitus, and oral sex activities, respectively. Unprotected sex was found in 80% (24/30) of gigolos. Importantly, Human Immunodeficiency Virus infection was detected in three out of thirty gigolos involved in the study. The gigolo community in Surakarta found at high risk for Human Immunodeficiency Virus infection and transmission due of their high risk sexual activities. Since Human Immunodeficiency Virus was detected in the community, prevention programs are needed to reduce the virus dissemination.

Keywords: Surakarta, gigolo, HIV

INTRODUCTION

Indonesia, the Asia's most rapidly growing epidemic of HIV/AIDS, recorded 21,770 AIDS cases with 1,206 additional new cases in 2010 (UNAIDS, 2010). In Central Java, numbers of people with HIV/AIDS is still increasing, reported from April thru' June 2011 as many as 306 additional cases of AIDS (Ministry of Health, 2011). There were no data showing cases among bisexuals especially male sex workers until now.

Male sex workers, as well as gigolos are not yet listed as high risk population in HIV/AIDS condition data and not included as a target of surveillance in National AIDS Commission (KPA, 2007). Epidemiological research, especially molecular epidemiology of HIV in gigolo community in Indonesia is also extremely rare. So that, the epidemiology of HIV-related research on gigolo community is indispensable as efforts to control the spread of HIV/AIDS.

To build an adequate molecular epidemiology database of HIV in the high risk communities Indonesia, a molecular epidemiology study has been performing since 2009 started from Central Java (Prasetyo *et al*., 2009). In 2011, study in the gigolo community in Surakarta started to be enrolled. This paper reports on quantitative and qualitative surveys of HIV molecular detection and risk behavior in gigolo community in Surakarta.

METHODS

Sampling and data collection procedures

In May-June 2011, cross sectional surveys among community recruited and self recognized gigolos were undertaken. Strategy of respondent driven sampling was selected because this population is very hard to reach.

First of all, trained field staff mapped the locations where these populations gathered. After that, they were invited and driven into a designated place to be fully explained about the objective of the research

activities by our supervisor. For those who want to participate had to sign letter of consents; the limits of thirty gigolos were included in analysis. And the last, respondents were pleased to go into private rooms provided for interviewing process.

The study was anonymous; biological specimens and questionnaires were linked by unique numbers, which researchers could use to access test results. Blood was drawn and trained interviewers administered a structured questionnaire with sections on socio-demographics, sexual behavior, and epidemiological characteristics associated with blood borne virus infections.

Laboratory procedures

Samples collecting and serologic test

Immediately after collection, blood samples were transferred to an EDTA tube and putted in a cooler. Blood samples were gathered and processed at Biomedical Laboratory of Sebelas Maret University in Surakarta. In the laboratory, blood samples were transferred to polypropylene tube 15 ml and centrifuged 2000 rpm for 15 minutes to get blood plasma (Haryanto, *et al*., 2008). Blood plasma were taken and divided into aliquots, labeled, then stored in -80 ^{o}C. Fifty micro liters blood plasma were pipetted and tested for antibodies to HIV using Determine HIV-1/2 (Inverness, Princeton, New Jersey) as shown in its manual.

Nucleic acid extraction

Viral RNA was extracted from blood plasma using PureLink™ Viral RNA/DNA Mini Kit (Invitrogen, Carlsbad, California) and performed using procedures that recomended by Invitrogen. Then the viral RNA from extraction product was used for reverse transcriptase PCR.

Synthesis of cDNA

Viral RNA from extraction product was synthesized into cDNA using SuperScript III First-Strand Synthesis SuperMix (Invitrogen, Carlsbad, California) and performed using protocols in the manual. The product was used as a template for first round of nested PCR.

Nested PCR

Oligonucleotides primers sets for amplification of first round using outer primer set H1G777 (5′-TCACCTAGAACTTTGAATGCATGGG-3′) as forward primer and H1P202 (5′-CTAATACTGTATCATCTGCTCCTGT-3′) for backward primer with amplification product 830 bp in length. For second round amplification was used inner primer set H1Gag1584 (5′-AAAGATGGATAATCCTGGG-3′) as forward primer and g17 (5′-TCCACATTTCCAACAGCCCTTTTT-3′) for backward primer with amplification product 460 bp in length (Miura *et al*., 1990).

First round and second round amplification nested PCR was performed by *GoTaq® Green Master Mix* (Promega, Madison, Wisconsin) with condition initial denaturation 95 ºC for 2 minutes, followed by 35 cycles of amplification including denaturation for 1 minute at 95 ºC, annealing for 1 minute at 45 ºC, and extension for 1 minute at 72 ºC. At the end of the amplification was completed with final extension at 72 ºC for 5 minutes. For second round amplification, PCR product from first round amplification was used as template. Amplification cycle consist of initial denaturation at 95 ºC for 2 minutes, then denaturation for 1 minute at 95 ºC, annealing for 1 minute at 55 ºC, and extension for 1 minute at 72 ºC. At the end of amplification was completed with final extension at 72 ºC for 5 minutes. Second round amplification was performed in 35 cycles (Miura *et al.,* 1990).

Ethical and other issues

Test results for HIV were available to respondents who want to know. The respondents only need to leave their contact numbers. Presenting individuals who tested HIV positive were referred to VCT facility at RSUD Dr. Moewardi in Surakarta for care and support.

The study received ethical clearance from Ethical Committee Faculty of Medicine University of Sebelas Maret Surakarta/RSUD Dr. Moewardi Surakarta.

RESULTS

Demographics and blood contact-related

In Mei-June 2011, thirty respondents who admitted themselves as gigolo were obtained. Respondents who participated in this study had an age range between 19 to 49 years with an average age of 30.5. All of gigolos are citizens from Java tribe and could serve and sell sex to men, women, or transgender clients.

From the interview results, all of the respondents claimed to have been circumcised, did not have job that come into contact with human blood, and never received blood transfusion, dialysis, and organ transplantation. There were 53.33% (16/30) of gigolos had tattoos and or piercings; with 31.25% (5/16) of them had done it with needle that was used interchangeably. As much as 86.67% (26/30) of all respondents had to be gigolo to meet their economic needs, only 13.33% (4/30) stated that they just want to have fun.

There was only one respondent who had lived in a correctional institution. The respondent lived inside a room together with seven prisoners for two months and a week. In the correctional institution, the respondent had been injected for the first time with a needle was used interchangeably and had homosexual activity without condom.

The results of anti-HIV serology, HIV-related history, sexual behavior and condom use

As much as 90% (27/30) of all respondents had bisexual orientation, 10% (3/30) had homosexual orientation. Oral sex was a sexual activity that mostly done by gigolos, 96.67% (29/30) of gigolos had reported oral sex, with 86.67% (26/30) and 83.33% (25/30) of anal sex and vaginal sex activities, respectively. Condom use varied by each individual, none of the respondent reported 86.67% (26/30) of gigolos admitted rarely use condom and 13.33% (4/30) never used condom in anal and or vaginal sex.

Anti-HIV serologic test was performed on thirty respondents and only three of them (10%) were HIV-seropositive. Based on the interviews, the three of them were never punctured by any blood-contaminated objects, never received blood transfusions, had no history of sharing needles together, and never lived in a correctional institution.

One of the seropositive respondents had sexual intercourse with foreigners by more than one hundred people who came from France, Canada, Australia, and Malaysia without ever using a condom. He carried out the sexual intercourse with foreigners about 4-5 times a week. French kiss, oral sex, anal sex, and petting were usual sexual activities done with those foreigners. Beside the foreigners, the respondent also had hundreds of male clients from local citizen. In Bali, this respondent had plastic surgery and body piercings with needles that were used interchangeably. This respondent had never done an HIV tests before and have never used antiretroviral drugs (ARVs). According to this 45-year-old man's history, in October 2010, he was hospitalized for 6 months due to pulmonary tuberculosis.

The second HIV-seropositive respondent claimed to have conducted sex parties and had sexual intercourse with more than 500 men. He had sexual intercourse with those men routinely and almost every day. He honestly said that he rarely used condom because his clients complained every time he tried to use condom. Sexual activities which usually done by him were French kiss, anal sex, and oral sex. According to this 32-year-old men's history, he had used shabu-shabu and marijuana for ten years from age 10. At the same age, the respondent also had tattoos and body piercings with shared used needles. The respondent also been hospitalized due to illness of the lungs. Unexpectedly, the respondent had HIV testing twice with the last test performed in 2004 but he never took or received the results. Unfortunately, he never knew the test result so that he never used ARVs.

The third respondent was 19 years old but had sexual intercourse with more than 10 men and more than 5 women. Sexual activities which usually done by him were vaginal sex, anal sex, petting, and oral sex. He had no history of HIV testing before and never taking antiretroviral drugs.

HIV molecular detection results

Molecular detection of HIV *gag* gene by nested PCR gave negative results in all three HIV-seropositive respondents. Negative results also appeared in the molecular detection of the HIV *pol* gene.

DISCUSSION

The main perpetrators of sexual transmission of HIV are sex workers (UNAIDS, 2010). One kind of the sex workers is male sex worker or are better known as the gigolo. According to the result of this study, 10% of all gigolos infected with HIV. The greatest possibility of HIV infection among the community is derived from risky sexual behavior because all the seropositive respondents had no history of a direct contact with blood. Those findings are appropriate with the data of HIV/AIDS transmission risk cases, from National AIDS Commission in Indonesia (KPA, 2007).

The gigolos were very often had anal sex and oral sex because most of their clients were men. The gigolos were also recorded very often had vaginal sex because some of them already have a wife and the others were frequently went to female sex workers. All of the gigolos were not consistent to use condom in every sexual activity. Most of their reasons for not using condom were sexual satisfaction and requests from clients who didn't want to use condom.

Included in risky sexual behavior is sexual intercourse with foreigners from endemic countries. In this study, HIV-seropositive respondents also had history of sexual intercourse with foreigners. This risky behavior is a possible cause of HIV infection among respondents, especially when the foreigners were from the area continues to experience an increase in HIV cases such as Eastern Europe and Central Asia (UNAIDS, 2010). Moreover, the respondents did not know about the risk of contracting HIV when dealing with foreigners. So that, educating the sex workers about the dangers of having sex with foreigners needs to be done by social activist and the government to tackle the spread of HIV/AIDS in Indonesia.

The risk of HIV transmission through sexual intercourse is influenced by the condom use and circumcision (Siegfried *et al*., 2009). From the results in this study, many respondents were already circumcised and they were all HIV-uninfected. Condoms have been approved and widely accepted as the choice of prevention of HIV transmission through sexual contact (Weller dan Davis, 2002; de vincenzi, 1994). Consistent condom use can reduces HIV transmission through sexual contact up to 85% (Weller dan Davis, 2002). From the result in this study, as much as 86.67% (26/30) of respondents had been accustomed to use condom (although rarely) during sexual intercourse. The respondents who always use condom during intercourse were HIV negative in serological tests. It shows the success of the government and other stakeholders in promotion of condom use among sex workers.

History of tattoos and piercings with shared needles was initially suspected as the cause of HIV-positive respondents. Even though there were no cases of HIV/AIDS reported due to tattoos and body piercings, the users will susceptible to hepatitis b and or hepatitis c virus infection (Nishioka *et al*., 2002; Nurutdinova *et al*., 2011).

The results of molecular detection for all three seropositive respondents were all negative. Negative results were obtained not from technical errors because the molecular detection procedures had been repeated several times to ensure the results. Moreover, external amplification control were applied and used in the molecular detection process. Besides, molecular detection of HIV *pol* gene had been done using the same kit and the same samples and the results were also negative. So that, there were no errors in techniques, kits, and primers used in the study.

The possible causes of negative results may be due to the amount of molecular detection of viral RNA below our limit of detection (10^4copies/ml). The low level of HIV RNA may have three possible explanations. First, the respondents may be individuals with long-term non-progressive HIV infection because of mutation in co-receptor CCR5 and CCR2 as had been well described in previous reports (Simon *et al*., 2006; Pantaleo *et al*., 1995). In Indonesia, as far as researchers know, the data of mutation in CCR5 and CCR2 have not been investigated.

An alternate possibility is that the respondents have relatively strong immune system and the virus less pathogenic due to deletions in the HIV *nef* gene (Kirchhoff *et al*., 1995; Cao *et al.,* 1995; Pantaleo *et al*., 1995). The immune system that plays a role is CD8+ which can suppress viral replication, thereby

reducing the amount (Simon *et al*., 2006). Moreover, increasing the amount of CD8+ can inhibit virus replication to 90% (Cao *et al*., 1995). Meanwhile, monitor the amount of CD4+ in a sustainable manner can assign these individuals as non-progressive HIV cases (Pantaleo *et al*., 1995). Unfortunately, this study didn't check the amount of CD4 and CD8. The last possibility is that the respondent may be infected by other subtypes that cannot be detected by our PCR assay (Kovacs and Scott, 2001; McCutchan, 2000).

In this study, there were two limitations. First, it was not possible to draw truly random sample of the community because the high mobility of the population. Second, because this population is very hard to reach, so the study did not find the exact amount of gigolo in Surakarta.

CONCLUSIONS

Results of HIV serological test conducted by Determine HIV-1/2 (Inverness) gave positive result in three respondents. All three respondents had a history of HIV-related risk through unsafe sex and needles were used interchangeably. Nested PCR with molecular detection of HIV *gag* gave negative results in all three HIV-seropositive respondents.

CD4 and CD8 examinations need to be done as well as monitoring for all three HIV-seropositive respondents. Further research on the pathophysiology and pathogenesis of HIV with undetected RNA and further surveillance measures to complement the data of molecular epidemiology of HIV in gigolo community in Indonesia need to be done too, so that it can be used in prevention and eradication of HIV in Indonesia.

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MOLECULAR VARIATIONS OF KUNITZ DOMAIN-LIKE SEQUENCE HEPATITIS B VIRUS X PROTEIN

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ABSTRACT

The hepatitis B virus X protein (HBx) is a multifunctional protein that can regulate cellular transcription, signal transduction pathways, proteasome activity, cell cycle progretion, apoptosis, and HBV replication. HBx is also known to a major causative of hepato cellular carcinoma (HCC). To predict and understand more on the molecular pattern of HBV X gene, a bioinformatics study had been performed. All of HBV X gene complete coding sequences in GenBank were downloaded and retrieved. All of the sequences than were molecular analyzed with ClustalW and MEGA4 software, especially the regions between aa 52 to 65 and 88-154 that already known important for the augmentation of HBx in viral replication. Several aa variations were found in the molecular analysis. In N-terminal part of the Kunitz domain-like sequence (residues 56-72), the motif was revealed as follows: R/P56, G/E/S 57, L/F/I58, P/A59, V/A/C/T/I/G/E60, C/S61, A/S/L/T/G62, F/Y/S63, S/T/P64, S/A/C/P65, A/S/T/G/N66, G/A/D/E67, P/S/L68, A/T/P/G70, L71, and R/C/G72. In C-terminal part of the serine protease inhibitor Kunitz domain-like sequence, the motif was revealed as follows: V/T/I/E/A/N/S/A/L131, F/Y/C132, V/E/G133, L/S/P134, G/R/E/V135, G/D/R/S136, C/Y/S137, R/S/M/T138, H/Y/D/Q/N 139, K/T140, L/W141, and V/Y/H/G/D142. The alignment data also showed that 99 sequences from total 2589 isolate sequences had aa lacking within the aa 134 to 154; therefore, could modulate the HBx functions and have differential effects on expression of P21 and P53. Variations were found in a few motifs which are well known to be fully conserved, including that of in the Kunitz domain. The implication of variations in Kunitz domain in clinical practice is still undiscovered, further study is needed.

Keywords: hepatitis B virus X gene, Kunitz domain

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INTRODUCTION

Hepatitis B virus (HBV) infection is a major global public health problem. It is also a major cause of acute and chronic hepatitis worldwide. Nowadays, approximately 2 billion people who have been infected worldwide, more than 350 million are chronic carriers of HBV. Some of the infected patients (15-40%) will develop cirrhosis, liver failure, or hepatocellular carcinoma (HCC). HBV infection accounts for 500 000 to 1.2 million deaths each year and is the 10th leading cause of death worldwide. The incidence of Hepatocellular carcinoma has increased worldwide, and the disease is now the 5th the most frequent cancer, killing 300 000–500 000 people each year (Lavanchy, 2004).

HBV belongs to the family of *Hepadnaviridae* and has several unique properties. It has a very compact circular genome, with overlapping reading frames, and unlike any other animal DNA virus, it replicates through an intermediate reverse transcription (Erik et al. 2000). HBV has a compact genome 3.2 kb with four partially overlapping open reading frames (ORFs), encoding three structural proteins such as the polymerase (P), the core antigen (preC-C), and the surface antigen (preS-S) and one nonstructural protein, X protein (HBx) (El Monem, et al. 2010). The average amount of the nucleotide of the X ORF is 465 bases, which encodes the 154 amino acid X protein. (Kidd-Ljunggren, et al. 1995). Different members of hepadnavirus family, separated from woodchuck (WHV) and ground squirrel (GSHV) also own an ORF encoding a polypeptide with similar size and function as the HBV X protein. The avian Hepadnaviruses, however, does not have homologous ORF(Kidd- Ljunggren, et al. 1995).

HBV X protein plays a role in viral replication. Furthermore, it is known that HBV X protein has an oncogenic function (Jin, et al. 2006). In point of virus pathogenesis, HBV X gene and X protein are

interesting objects for study. To predict and to understand more the molecular pattern of HBV X gene, we performed bioinformatics study for all HBV X gene deposited in GenBank. Here, we present our current analysis results of the HBV X gene.

MATERIALS AND METHODS

Complete X gene of HBV Sequence

First, we downloaded all HBV genes X sequence deposited in GenBank. We then manually selected and grouped them depending on their genotype and subtype, followed by manual screening for their complete coding sequence. For this report, we analyzed the complete coding sequence of HBV gene X.

Alignment and Molecular Analysis of HBV X gene Sequence

In total, 2589 sequences with complete coding sequence were retrieved from GenBank. The sequences then were aligned by ClustalW, for both of nucleotide and amino acid sequences, using MEGA4 (Tamura, et al. 2007). From this alignment, we revealed the nucleotide and amino acid consensus sequences. We also performed motif analysis based on their nucleotide and amino acid sequences.

RESULTS AND DISCUSSION

HBx has 154-amino-acid (154-aa) that contains N-terminal negative regulatory domain and a C-terminal transactivation or coactivation domain. The N-terminal is one-third (aa 1 to 50) of HBV X region and not required for HBx function. The C-terminal is positioned at two-thirds of HBV X region (amino acids [aa] 51 to 154). HBx is a multifunctional protein that exhibits effects on gene transcription, signaling pathways, genotoxic stress responses, protein degradation, cell cycle control, cell proliferation, and apoptosis. These activities may affect viral replication and viral proliferation directly or indirectly and may also be relevant to HBV-associated pathogenesis, especially hepatocarcinogenesis. Its effects on transcription are mediated through protein-protein interaction with endogenous cellular proteins, transcription factors and cofactors, or basal transcription machinery (Liu et al. 2008; Murakami, 1994; Murakami, 2001). HBx has been shown to interact with transcriptional factors such as RPB5 of RNA polymerase (Cheong et al. 1995), basic region/leucine zipper (bZIP) proteins (Perini et al. 1999), the tumor suppressor p53 (Wang et al. 1994), and TATA-binding protein (Qadri et al. 1996). HBx can be also associate with serine protease TL2 (Takada et al. 1994) and cellular DNA repair protein (Lee et al. 1995). The interaction of HBx with these proteins leads to activation of signal transduction pathways.

A. N-terminal Domain of HBx

N-terminal negative regulatory domain of HBx is not required for its stimulation function on HBV RNA synthesis and HBV DNA replication, as the truncated HBx protein (HBxD1) lacking the N-terminal one-third (aa 1 to 50) activated HBV replication to an extent similar to that of the full-length HBx (Tang et al. 2005). The N-terminal region of HBV X gene, spanning amino acids is necessary for the negative regulations. The negative effect of the regulatory domain is specifically interfered with the HBx transactivation. The N-terminal region has a repressive effect on X-protein-mediated transactivation and is reported to be involved in protein-protein interactions leading to HBx oligomerization. N-terminal HBx had no effect on expression or stability of the full or truncated HBx proteins (Murakami et al. 1994). The N-terminal region spanning amino acids 1 to 78 of HBx is known well as the site of direct protein-protein interactions between X molecules (Li et al. 2006).

B. C-Terminal Domain of HBx

Amino acids 50 to 140 are essential for activation of the mitogenic pathways (Li et al. 2006) and X-protein-dependent transactivation (Arii et al. 1992; Renner et al. 1995). HBx activates transcription directly via protein-protein interactions with specific components of the basal transcriptional apparatus and via direct interactions with the basic Leucine Zipper (bZIP) family of CREB/ATF proteins. CREB/ATF proteins, located between amino acids 50 to 115 (Barnabas and Adrisani, 2000), are direct interaction targets

of HBx (Li et al. 2006). The HBx-dependent activation of mitogenic pathways and the direct transcriptional effects of HBx contribute to HBx-mediated hepatocyte transformation. X_{50-140} also contains transcription factor IIB (TFIIB) at amino acids 102 to 136 (Lin et al. 1997), and TFIIH (amino acids 110 to 143) (Qadri et al. 1996). Amino acids 58 to 119 of HBx are sufficient to activate the MAPK pathways (Nijhara et al. 2001). Lin et al. (1997) reported that the HBx-binding sites are located within the oligomerization and specific DNA-binding domains of p53, and that the p53-binding site is confined in a small region (102–136 aa) of the HBx C-terminal. Amino acids 116 to 140 of HBx are necessary for mitogenic pathway activation and endogenous gene expression and for mediating cell growth outcomes, such as apoptosis. HBx variants encoded by integrated HBV genomes contain C-terminal deletions generally lacking aa 134 to 154 might alter the potential of HBx in activating the mitogenic pathways. The C-terminal region spanning aa 141 to 154 decreases X-protein stability. HBx variants lacking aa 134 to 154 may play a role in HCC development since its stability is increased by aa 134 to 154 lacking. A larger C-terminal deletion of amino acids 116 to 154 in HBx1-115 abolishes activation of all the mitogenic pathways (Li et al., 2006). Our alignment data shows that 99 sequences from total 2589 isolate sequences have aa lacking within the aa 134 to 154. Total 0.03 percents of all isolates collected from GenBank have a potential to develop to HCC.

There may be three regions of the X gene essential for the transactivation function of the X protein (at codons 46-52, 61-69 and 132-139) (Arii et al., 1992). Low HBx protein expression and a high rate of HBx mutation, particularly deletions of amino acids at the C-terminal end, were found in the tumor tissues (Liu et al., 2008a). C-terminal region of HBx is crucial to its transcriptional function and that, in tumor tissues, most HBx natural mutants have lost their capacity for controlling cell proliferation, viability and transformation (Tu et al., 2001) thus abrogate both the transactivation and anti-proliferative effects of wild-type HBx. Different deletions at the C-terminal end may have different biological consequences in regulating cell proliferation and invasion. HBx with 40 amino acid deletion at C-terminal mutant promotes cellular proliferation, focus formation, tumorigenicity, invasive growth and metastasis by promoting the cell cycle progression from G0/G1 to S phase; in contrast, HBx with 30 amino acid deletion at C-terminal mutant represses cell proliferation by arresting cells in G1 phase (Liu et al., 2008a; Liu et al., 2008b).

HBx can interact with mitochondria. Potential pathways through which HBx may act in order to modulate mitochondrial physiology, thereby altering many cellular activities and ultimately contributing to the development of HBV-associated liver cancer. One study shows that a putative mitochondrial localization signal for HBx was located in C-terminal region between amino acids 54 and 70; however, another study shows that a mitochondrial localization signal is also located between amino acids 68 and 117 of HBx. HBx activities in mitochondrial physiology involve stimulation of signal transduction pathways by a HBx induced elevation of cytosolic calcium levels, which involves modulation of the mitochondrial permeability transition pore (MPTP), a channel that spans through the outer mitochondrial membrane into the mitochondrial matrix and controls the migration of molecules into and out of mitochondria (Clippinger et al., 2008). HBx is localized to both the cytoplasm and the nuclei of cells (Henkler et al., 2001). HBx can interact with voltage-dependent anion channel 3 (VDAC3), the channels that span the outer mitochondrial membrane and can function as a component of the MPTP (Orrenius et al., 2001). HBx regulates mitochondrial membrane potential in hepatocytes, and this function varies depending on the status of NF-$_k$B activity. Status of NF-$_k$B may change in the course of an HBV infection or in the process of hepatocyte transformation. If the activity of NF-$_k$B is blocked in hepatocytes, HBx induced depolarization of mitochondria could enhance activation of apoptotic pathways and eventually participate in the selection of hepatocytes that are resistant to proapoptotic signals. Therefore, both HBx activation of NF-$_k$B and HBx modulation of the mitochondrial membrane potential could contribute to the development of HBV-associated HCC (Clippinger et al., 2008).

HCC is shown to be more prevalent in men than in women. Estrogen, which exerts its biological function through estrogen receptor (ER), can inhibit HBV replication. Estrogen exerts its function through its two nuclear receptors, estrogen receptor α and β (ER α and ERβ). ER α has been well characterized in human liver. ERΔ5, an Erα variant lacking exon 5, is found to be preferentially expressed in patients with HCC compared with patients with normal livers. ERΔ5 interacts with Erα in vitro and in vivo and functions as a dominant negative receptor. Both Erα and ERΔ5 associate with HBx. HBx decreases Erα-dependent transcriptional activity, and HBx and ERΔ5 have additive effect on suppression of Erα transactivation.

The HBx deletion mutant that lacks the Erα-binding site abolishes the HBx repression of Erα. Han et al., (2006) constructed HBx mutant in which the ERα binding site region from amino acids 73 to 120 of HBx C-terminal was deleted. The mutation abrogated the repression of ERα transactivation function by HBx when co-transferred to HepG2 cells. HBx (Δ73–120) lose the ability to interact with ERα in HepG2 cells (Han et al., 2006). These finding suggest that interaction of HBx ERα binding site at amino acids 73 to 120 with ERα is required for repression of ERα transactivation function (Han et al., 2006).

C. Kunitz Domain of HBx.

According to Takada et al., (1994) one of the transactivating functions of the X protein is mediated trough inhibition of hepatic serine protease activity, thereby protecting cellular transcription factors from degradation or processing. The X protein carries sequences corresponding to the Kunitz domain, which is a characteristic property of these inhibitors. This region is known to be well conserved and has the consensus sequence ^{131}VFVLGGCRHKLV^{142}. The region spanning amino acid residues 131 to140 represents the C-terminal part of the serine protease inhibitor Kunitz domain-like sequence. The N-terminal part of the Kunitz domain-like sequence (residues 56-72) which has the consensus sequence ^{56}RGLPVCAFSSAGPCALR^{72} is distant from C-terminal part by some 50 amino acids, but both of these relatively hydrophobic regions appear to be essential for transactivation (Arii et al., 1992; Takada and Koike, 1994).

Compared to our alignment data, variations were found in the molecular analysis. Variations especially were found in N-terminal part of the Kunitz domain-like sequence (residues 56-72) were R56P, G57(E/S), L58(F/I), P59A, V60(A/C/T/I/G/E), C61S, A62(S/L/T/G), F63(Y/S), S64(T/P), S65(A/C/P), A66(S/T/G/N), G67(A/D/E), 68P(S/L), A70(T/P/G), and R72(C/G). Only Leucine^{71} (L^{71}) is conserved. In C-terminal part of the serine protease inhibitor Kunitz domain-like sequence, there was variation on V131(T/I/E/A/N/S/A/L), F132(Y/C), V133(E/G), L134(S/P), G135(R/E/V), G136(D/R/S), C137(Y/S), R138(S/M/T), H139(Y/D/Q/N), K140T, L141W, and V142(Y/H/G/D). However, the implication of the clinical practice is still unknown, further study is needed.

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SEROLOGICAL AND MOLECULAR AMPLIFICATION OF HUMAN IMMUNODEFICIENCY VIRUS INTEGRASE GENE IN THE MALE SEX WORKERS IN SURAKARTA

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ABSTRACT

Human Immunodeficiency Virus/Acquired Immunodeficiency Syndrome (HIV/AIDS) is one of the global problems in health. As the HIV/AIDS epidemic is becoming larger and more complex, effective and efficient efforts must become more sophisticated for the purpose of public health action. One of the main factors of HIV transmission is free sex among sex worker, including that of the male sex workers. However, for the best of our knowledge, there is limited data of molecular epidemiology of Human Immunodeficiency Virus in male sex workers in Indonesia, especially in Surakarta. In total, 30 male sex workers in Surakarta were enrolled in the study by respondent driven sampling method in May-June 2011. After signed the informed consent the respondents were interviewed and their blood were retrieved. The blood samples were then fractionated, aliquoted, and kept frozen until analyzed; however, the fresh plasma aliquot was used for detection of anti-HIV-1/2 by Determine-HIV-1/2. The serological positive samples were then performed for nucleic acid extraction, followed by cDNA synthesized, and the amplification of part of Human Immunodeficiency Virus integrase gene by nested RT-PCR. All the male sex workers involved in the present study were on productive age (19-49 years old). Seventeen out of thirty respondents were having low level education and poor knowledge of Human Immunodeficiency Virus infection and transmission. Alcohol and narcotics abuse history was found in 86.7% (26/30) and 26.7% (8/30) of respondents, respectively. All of respondents had high-risk sexual activities in point of the Human Immunodeficiency Virus. Human Immunodeficiency Virus infection was detected in three out of thirty respondents. The present results indicate that the male sex workers in Surakarta are vulnerable to acquire and spread HIV infection due to high-risk behaviors and other related factors; therefore, interventions to diagnose, treat and prevent HIV infections among this population is needed.

Keywords: HIV, male sex workers, Surakarta

INTRODUCTION

Human Immunodeficiency Virus/Acquired Immunodeficiency Syndrome (HIV/AIDS) is one of the major challenges in health. The number of people infected with HIV worldwide is estimated at 33.3 million and 1.8 million of whom died of AIDS (WHO, 2009). In Indonesia, the number of reported AIDS cases increased rapidly every year and recorded 21.770 cases by the year 2010. Of these, the comparisons between men and women is about 3:1. The distribution pattern showed a high percentage of AIDS in young adults, account for 48.1% (Ministry of Health, 2010). Free sex has influenced the lifestyle of young people so that impact on increased risk factors for HIV infection (Sigarlaki, 2008).

National AIDS Commission reported that commercial sex worker is the main factor contributing the spread of HIV infection (National AIDS Commission, 2007). Among some communities are at high risk of HIV transmission, male sex worker is the most vulnerable communities as possible have a wide sexual network of communities, either with male or female sex workers (IBBS, 2007). Therefore, male sex workers tend to be hidden and difficult to reach by the HIV prevention efforts by the government (National AIDS Commission, 2007). However, for the best of our knowledge, there is limited data of epidemiology of HIV in male sex workers in Indonesia, especially in Surakarta.

As the HIV/AIDS epidemic is becoming larger and more complex, effective and efficient efforts must become more sophisticated for public health action. HIV detection is a key to determine the pattern and epidemiological characteristics of HIV infection that developed in Indonesia. The present study was conducted to know the status of male sex workers in Surakarta based on serological and molecular detection. The products of this research will be analyzed in a molecular epidemiological study.

METHODS

In total, 30 male sex workers in Surakarta were enrolled in the study by respondent driven sampling method in May-June 2011. After signed the informed consent the respondents were interviewed and their blood samples were retrieved. The blood samples were then fractionated, aliquoted, and kept frozen until analyzed; however, the fresh plasma aliquot was used for detection of HIV-1/2 antibodies by Determine-HIV-1/2 (Inverness Medical, Tokyo, Japan). The serological positive samples were then performed for nucleic acid extraction using PureLink™ Viral RNA/DNA (Invitrogen, Carlsbad, CA), followed by cDNA synthesized using SuperScript® III First-Strand Synthesis SuperMix (Invitrogen, Carlsbad, CA). A part of the pol sequences covering the HIV integrase gene (approximately 288 bp) was amplified by nested polymerase chain reaction (PCR) using the primers unipol 5 (5'-TGG GTA CCA GCA CAC AAA GGA ATA GGA GGA AA-3') / unipol 6 (5'-CCA CAG CTG ATC TCT GGC CTT CTC TGT AAT AGA CC-3') in the first round; and unipol 1 (5'-AGT GGA TTC ATA GAA AGC AGA AGT-3') / unipol 2 (5'-CCC CTA TTC CTT CCC CTT CTT TTA AAA-3') in the second round (Ndembi et al., 2003). The nested PCR were performed by *Platinum® PCR SuperMix* (Invitrogen). Amplification of PCR I was done with 1 cycle of 94°C for 2 min; and 45 cycles of 94°C for 30 sec, 45°C for 30 sec, and 72°C for 1 min; with a final extension of 72°C for 10 min. Amplification of PCR II was done with 1 cycle of 94°C for 2 min; and 45 cycles of 94°C for 30 sec, 55°C for 30 sec, and 72°C for 1 min; with a final extension of 72°C for 10 min. The final amplified PCR products were detected after electrophoresis on a 1.5% agarose gel with ethidium bromide staining of the gel.

RESULTS

In the present study, a number of 30 male sex workers in Surakarta were participated. The sociodemographic data of respondents can be seen in table 1. All the male sex workers were on productive age (19-49 years old). A total of 93.3% (28/30) of respondents were Javanese. Seventeen out of thirty respondents were having low level education and poor knowledge of HIV infection and transmission. Based on marriage status, most of the respondents were not married 70% (21/30). Twenty one out of thirty respondents were employed, but the income per month was mostly less than Rp 1.000.000,00.

Table 1. Sociodemographic data survey respondents

History	Number (%)
Age (years)	
< 20	2/30 (6.7%)
20-34	19/30 (63.3%)
35-49	17/30 (56.7%)
Ethnicity	
Java	28/30 (93.3%)
Outside of Java	2/30 (6.7%)
Education	
Not completed high school	17/30 (56.7%)
Completed high school	12/30 (40%)
College	1/30 (3.3%)
Marital status	
Married	9/30 (30%)
Not married	21/30 (70%)
Employment status	
Employed	21/30 (70%)
Not employed	9/30 (30%)
Income per month (Rp)	
< 1.000.000	20/30 (66.7%)
1.000.000-3.000.000	9/30 (30%)
> 3.000.000	1/30 (3.3%)

The HIV was detected in three out of thirty respondents by Determine HIV-1/2 serological test. Further investigation by nested RT-PCR amplifying a part of integrase gene showed the negative results.

We obtained the preliminary data of HIV status in male sex workers in Surakarta. Overall, respondents were active in commercial sex with some reasons, such as the economic needs 86.7% (26/30), and biological needs 13.3% (4/30). Based on a history of sexual behavior, HIV-seropositive respondents had sexual orientation of transgender 3.3% (1/30), bisexual 3.3% (1/30), and homosexuals 6.7% (2/30). Age at first intercourse performed by all HIV-infected respondents were at a very young age, specifically less than 20 years. Respondents had a history of commercial sex activity with men 10% (3/30), noncommercial sex with men 3.3% (1/30), commercial sex with women 3.3% (1/30), and group sex 3.3% (1/30). One out of thirty respondents had a history of sex with foreigners, and injecting drug users (IDU). Overall, the respondents had ever done unprotected sex. In addition, alcohol and narcotics abuse history was found in 86.7% (26/30) and 26.7% (8/30) of respondents, respectively. Data on history of sexual behavior, drug use, and tattoos/piercings can be seen in Table 2.

Table 2. A history of sexual behavior, drug use, and tattoos/piercings

History	HIV seropositive
Sexual	
Transgender	1/30 (3.3%)
Bisexual	1/30 (3.3%)
Homosexual	2/30 (6.7%)
Age at first sex (years old)	
< 10	1/30 (3.3%)
10-19	2/30 (6.7%)
≥ 20	0/30 (0%)
Commercial sex activities with men	3/30 (10%)
Number of partners	
<10	0/30 (0%)
10-50	1/30 (3.3%)
50-100	0/30 (0%)
>100	2/30 (6.7%)
Condom use	
Always	1/30 (3.3%)
Sometimes	2/30 (6.7%)
Never	0/30 (0%)
Noncommercial sexual activities with men	1/30 (3.3%)
Number of partners	
<10	1/30 (3.3%)
≥10	0/30 (0%)
Condom use	
always	0/30 (0%)
Sometimes	0/30 (0%)
never	1/30 (3.3%)
Commercial sexual activities with women	1/30 (3.3%)
Number of partners	
<10	1/30 (3.3%)
≥10	0/30 (0%)
Condom use	
always	1/30 (3.3%)
Sometimes	0/30 (0%)
never	0/30 (0%)
History of group sex	1/30 (3.3%)
History of sex with foreigners	1/30 (3.3%)
History of sex with IDU	1/30 (3.3%)
History of anal sex without protection	
always	0/30 (0%)
Sometimes	2/30 (6.7%)
never	1/30 (3.3%)
History of drug users	1/30 (3.3%)
Tattoos/piercings	3/30 (10%)
Sterilization of tattoo/piercing	1/30 (3.3%)
Shared needles of tattoos/piercings	2/30 (6.7%)

DISCUSSION

The positive rate of HIV infection in the male sex workers in Surakarta was 10%. The results of serological detection were positive in all three samples, but the molecular detection by nested-RT PCR of integrase gene was negative. These conditions might be the possibility of false-negative results. Investigation of the negative reactions showed that a low viral burden in some infected subjects was the primary cause for the false-negative PCR results (Schechter et al., 1991; Zazzi *et al*., 1995). The results might occur because the specific PCR only detected the integrated cDNA. Integration is an advanced stage in the HIV life cycle and characterized by the entry of proviral DNA into the host chromosome. If the HIV cDNA is not integrating to the nucleus, the viral cDNA will form 2-LTR circles. Therefore, the unintegrated HIV cDNA is not amplified by PCR (Friedrich *et al*., 2010). However, the serological evidence in this study suggests the early data of HIV infection describing the status of HIV in the male sex workers in Surakarta.

The male sex worker is a small community. Unlike female prostitutes, the male sex workers often hide their commercial sexual relationships so that they can have sex freely (Shinde *et al*., 2009). However, this community has a relevant role in the transmission of HIV in their own community as well as their network to the other communities. The data of this study indicates the sexual transmission of HIV in the male sex workers. HIV transmission in this community is known by the history of high-risk sexual behaviors. Almost of all respondents (97%) had engaged in sexual activity since the age of less than 20 years old. All of respondents were "money boys" that enabled sexual intercourse with their partners of various ages, including the older partners. It had been proven that young men who have sex with older partners had a higher risk of acquiring HIV (Rassjo et al*.,* 2006). This study also showed that the rate of condom use was inconsistent, that was "always" (3.3%) and "sometimes" (6.7%) in commercial sexual activities with men, "never" (3.3 %) on non-commercial sexual activity with men, and "always" (3.3%) in commercial sexual activity with women. However, the condom use could be influenced by some reasons, such as the awareness of safety wear (33%), availability (30%), and partner demand (13%). Most of the respondents stated to use the condoms in reason of preventing HIV infection (60%) and had used condoms in sexual intercourse (87%). Condom use could be associated with a low level of consciousness before sexual intercourse, such as the consumption of alcohol and narcotics, so they did not protect themselves. In the present study, we found a history of alcohol intoxication (86.7%) and narcotics abuse (26.7%) among male sex workers. The effect of alcohol and narcotics use in sex worker had been associated with an increased risk of HIV infection (Urassa *et al*., 2008).

The type of sexual activity also affected the use of condoms. In respondents with HIV seropositivity, the risk of sexual activity might be associated with their behaviors of anal intercourse (10%), orogenital (6.7%), coitus (3.3%), and French kiss (3.3%). It had been reported that the condom use in sex orogenital was relatively low due to the transmission of sexually transmitted diseases, especially through anal sex (de Graaf et al., 1994).

The high-risk sexual activity was also due to the relationship with foreigners. In the history of HIV positive male sex workers, we found the data of their sexual intercourse with hundreds of foreigners from various countries, including France, Canada, Australia, and Malaysia. Sexual activities was performed 4-5 times per week by not using any security.

The community of male sex workers also served to spread HIV infection to other communities. It was known that this community had sexual network among their community and heterosexual network to the other communities, either by commercial or noncommercial sex (Pisani et al., 2004; IBBS, 2007). Moreover, we found 36.7% of respondents have history of group sex and 3.3% of them was HIV positive.

In addition to sexual networking to the other communities, the HIV positive respondents in this research also reported a history of sexual intercourse with IDUs (3.3%), so it impacted the transmission of HIV infection to the IDU community. Although relatively small of an amount, we found that the infected respondent was also drug user (3.3%). This possibly increased the risk of HIV infection. Unsafe injecting behavior, in the other studies, had been shown to be associated with the spread of HIV (Sarkar et al., 2010; Tran et al., 2006). The high prevalence of HIV infection might cause using shared needles. However, the drug users had been studied in relation to HIV transmission through a shared use of drug ampoules, water

for washing syringe before injection, and cotton to stop bleeding (Sarkar et al., 2010).

HIV transmission also reported because of unsafe tattoo/body piercing (UNAIDS, 2008). The HIV infection could spread through tattooing/body piercing if the infected blood contaminated the equipment used by the user's tattoo. In this study, all of HIV positive respondents had a history of tattoos/body piercing. The use of nonsterile equipment was found in 6.7% of respondents.

The present study shows the characteristics of male sex workers in Surakarta, who are vulnerable to HIV transmission. One of the limitations of the study was the number of samples, and hence may not be representative of the sex work in the population. In spite of the limitation, this is an important study which provides information on male sex workers in Surakarta. Therefore, the further research is needed to determine the factors associated risk behaviors in the male sex workers with a wider scale.

CONCLUSION

The present results indicate that the male sex workers in Surakarta are vulnerable to acquire and spread HIV infection due to high-risk behaviors and other related factors. Surveillance from the other communities of high-risk HIV transmission in Surakarta is also needed, so it can be used in programs of prevention, diagnosis, and eradication of infectious diseases due to HIV.

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SEROLOGICAL AND MOLECULAR BASED DETECTION OF HEPATITIS C VIRUS INFECTION AMONG MALE COMMERCIAL SEX WORKERS IN SURAKARTA, INDONESIA

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ABSTRACT

Male commercial sex workers community is been associated with high risk of human blood borne virus infection including that of the hepatitis C virus (HCV). However, at present there is neither data nor publication about hepatitis C virus infection in the male commercial sex workers community in Indonesia yet, for the best of our knowledge. In May 2011 until June 2011, 30 male commercial sex workers were enrolled in the study by respondent driven sampling method. Socio-epidemiological data were retrieved and blood sample were collected from all respondents. The blood samples were screened for antibody of hepatitis C virus using particle agglutination assay. The nucleic acid was extracted from the anti-HCV positive samples. The RT-PCR nested was performed to detect part of E1-E2 region of the HCV genome. The report presents preliminary data results from on going molecular epidemiology study of human blood borne viruses in male commercial sex workers in Surakarta. High risk sexual activities were found in 73.3% (22/30) of respondents with 33.3% (10/30) of respondents had sexually transmitted infections history. Drug abuse history was found in 23.3% (7/30) of respondents; however, only one of them had injecting drug use history. In total, HCV infection was detected in 23.3% (7/30) of respondents.Hepatitis C virus infection had been found in the male commercial sex workers community in Surakarta, therefore, indicate the need for interventions to diagnose, treat and prevent HCV infections among this population.

Keywords: Surakarta, hepatitis C virus, male commercial sex workers.

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INTRODUCTION

Hepatitis C virus (HCV) infection is major cause of chronic hepatitis, liver fibrosis, liver cirrhosis, and hepatocellular carcinoma (HCC). There are at least 170 million people worldwide are infected with HCV (Drexler et al., 2009). About 45.000 cases of liver cirrhosis and 18.000 cases of liver cell carcinoma are detected in Indonesia each year (WHO, 2011).

Hepatitis C virus is transmitted through various channels. Percutaneous exposures, such as blood transfusions and injecting drug use, are well-established risk factors for HCV infection (Terrault, 2002). HCV transmission also occurs through other percutaneous channel such astattooing, piercing, acupuncture, and accidental syringes use (Ishietal., 2001; Shepardetal., 2005; Terrault, 2002). Sexual contact can also transmit HCV (Halfon et al., 2001; Lai et al., 2004; Nakayama et al., 2005; Terrault, 2002). Individuals who areengaged in commercial sexual practice, including male commercial sex workers, have a higher frequency in contact with many sexual partners and more likely to have high-risk sexual partners and engaged in bisexual behavior compared to common people (Baseman et al., 1999; Weber et al., 2001; Weber et al., 2002).

The HCV antibody detection is the current method used to detect HCV infection. However, there are many seronegative HCV infections. Reverse transcriptase polymerase chain reaction (RT-PCR) for HCV RNA provides a better diagnostic tool to detect the infection (Colin et al., 2001; Re et al., 2005; Yi-Jung, 2006).

MATERIALS AND METHODS

Subjects and Samples

In May until June 2011, 30 male commercial sex workers were enrolled in this study by respondent driven sampling method. After ethical approval and informed consent were obtained, a structured questionnaire was administered. The questionnaire waseliciting information on socio-epidemiological characteristics, medical history, including sexually transmitted infections (STIs), sexual practices and behaviors, history of injecting drug use, and incarceration. Blood samples were collected from all respondents. Plasma were separated from whole blood by centrifugation and subjected to the following tests.

Serological Test

Detection of anti-HCVin blood plasma was carried by particle agglutination assay method using kits of Ortho HCV Ab PA II ® (Fujirebio, Tokyo, Japan). Assay was performed according to the manufacturer's instructions.

RNA Extraction, Reverse Transcription, and Polymerase Chain Reaction (PCR)

The HCV RNA was extracted from 200 µL anti-HCV positive plasma by using PureLink™ Viral RNA/DNA (Invitrogen, Carlsbad, CA), and reverse-transcribed according to First-Strand cDNA Synthesis protocol (Invitrogen). A part of E1-E2 region of the HCV genome includinghypervariableregion1 (HVR1) was amplified by nested PCR with Lqz188senseprimer(5'-CAY CGB ATG GCH TGG GAY ATG ATGATG AA) and Lqz187 antisenseprimer(5'-CCY ACB ACM ACD GGG CTN GGD GTG AAR CAR TA) in the first round, and Lqz189senseprimer(5'-TGG GAY ATG ATGATG AAY TGG TC) andLqz187 in the second round (Zhang et al., 2004). PCRreaction was performed usingPlatinum®PCRSuperMix(Invitrogen).First-round PCR was done with one cycle of 94°C for 10 min, and 45 cycles of 94°C for 30 sec, 50°C for 30 sec and 72°C for 1 min with a final extension of 72°C for 10 min. Second-round PCR was done under the same conditions. PCR products were separated on a 1.5% agarose gel, stained with ethidium bromide, and visualized under UV light.

Data Analysis

Analysis was performed descriptively, focusing on serological and molecular detection result, high risk sexual practice, injecting drug use, sexually transmitted infections (STIs), and incarceration.

RESULTS

Thirty male commercial sex workers in Surakarta, Indonesia were included in this study. Of all respondents, 50% (15/30) were born in and residentsof Surakarta, while 36.7% (11/30) came from cities outside Surakarta. There were 13.3% (4/30) of respondents resides outside Surakarta. The sociodemographic characteristics of respondents are shown in Table 1. Approximately three-fourths were less than 36 years old and 30% (9/30) were recognized being engaged in a marital relationship. Most of respondents had income account for Rp500.000,00 - Rp3.000.000,00 per month. There were 33.3% (10/30) of respondents whose income lower than Rp 500.000,00 per month. Almost all of respondents had no knowledge of HCV.

Of all respondents, 86.7% (26/30) were found have bisexual orientation. Respondents were reported various sexual activities with 100% (30/30) practiced petting and 83.3% (25/30) practiced *french* kiss. Coitus, anal intercourse, and oral sex were practiced in more than two-thirds of respondents. We found 36.7% (11/30) of respondents practiced group sex. Sexual activities of respondents are shown in Table 2.

Table 1 Sociodemographic characteristics of respondents (n=30)

Characteristic	Distribution
Age group (years)	
18-25	30% (9/30)
26-30	26.7% (8/30)
31-35	20% (6/30)
36-40	10% (3/30)
>40	13.3% (4/30)
Marital status	
Married	30% (9/30)
Unmarried	70% (21/30)
Education	
Unfinished Elementary School	10% (3/30)
Elementary School	10% (3/30)
Junior High School	36.7% (11/30)
High School	40% (12/30)
Bachelor	3.3%(1/30)
Income as a male commercial sex worker	
<Rp 500.000,00	33.3% (10/30)
Rp 500.000,00-Rp 1.000.000,00	30% (9/30)
Rp 1.000.000,00-Rp 3.000.000,00	33.3% (10/30)
Rp 3.000.000,00-Rp5.000.000,00	3.4% (1/30)
Knowledge about HCV	
Known	3.3% (1/30)
Not known	96.7% (29/30)

Table 2 Sexual activities of respondents

Sexual activity	Distribution
Petting	100% (30/30)
French kiss	83.3% (25/30)
Coitus	86.7% (26/30)
Oral sex	96.7% (29/30)
Anal intercourse	86.7% (26/30)
Rimming	20% (6/30)
Hand sex	43.3% (13/30)
Group sex	36.7% (11/30)

In sexual intercourse, 73.3% (22/30) of respondents used condoms inconsistently and 26.7% (8/30) of respondents used condoms consistently. Of all participants, 33.3% (10/30) of respondents had experienced sexually transmitted infections (STIs). We found no respondent in this study with history of sexual contact with partners who suffer hepatitis C.

There were 23.3% (7/30) of respondents with history of narcotic drug use and14.3%(1/7) of themhad a history of injecting drug use. From the study we also found 53.3%(16/30) of respondents had tattoos and or piercings.There were norespondent who had a history of acupuncture. A total of37.5%(6/16) of the respondents obtained tattoo and or piercingin commercial site, while62.5%(10/16) obtained it in a non-commercial site, such as home or correctional institution. Respondents were then differentiated based on the use of tattoos and or piercings equipments. A total of 16.7% (1/6) of respondents who obtained tattoos and or piercings in commercial site always used sterilized and non-interchangeably used equipments. There were 83.3% (5/6) of respondents who were inconsistent in the use of sterilized and non-interchangeably equipments. Among respondents who obtained tattoo and or piercing in non-commercial site, we found respectively 20% (2/10) and 80% (8/10) of respondents were consistent and inconsistent in using sterilized and non-interchangeably used equipments. One out of thirty (3.3%) respondents was found had history of incarceration. This respondent practiced sexual intercourse, injecting drug use, and obtained tattoo inside correctional facility.

Overall, anti-HCV positivity was found in 23.3% (7/30) of respondents. However, HCV RNA was undetected in all anti-HCV positive respondents.

DISCUSSION

Hepatitis C virus can be transmitted through sexual intercourse. Correct and consistent use of latex condoms can significantly reduce the risk of HCV transmission through sexual intercourse since latex condom is not permeable to infectious agents contained in genital secretions (Holmes, 2004; United Nations Population Fund, 2004). In the present study we found 26.7% (8/30) of respondents with consistent use of condoms. However, a total 73.3% (22/30) of respondents used condoms inconsistently.

Unprotected sexual intercoursemay not necessarily cause a personinfected with HCV since HCV is rarely found in semen or vaginal fluids(Villena, 2006). HCV RNA titer in semen or vaginal fluidis low (equal to 10^2 copies/mL). Therefore, HCV is transmitted less efficiently than other blood born viruses such as hepatitis B virus (HBV) and human immunodeficiency virus (HIV). Additionally, there may be an absence of suitable target cells in genital tract to allow infection to occur or infection may require the presence of abnormal mucosa (Terrault, 2002).

Respondents in this study generally practiced both vaginal and anal sexual intercourse. HCV is transmitted easier in anal intercourse compared to vaginal intercourse as anorectal mucosal layer is thinner compared to vaginal mucosa. Anal intercourse may traumatize the mucosa so that blood contact can occur. Blood, compared with semen, is more influential medium of HCV transmission. Anorectal mucosal lesions of anal intercourse in a prolonged sexual relationship can be an entry portal and source of HCV infection (Schmidt, 2011). Anal intercourse generally practiced by respondents for male to male sexual intercourse. In total, 86.7% (26/30) of respondents were bisexual. Moreover, in group sex, blood contaminated penile insertion can act as vectors for the receptive partner in a group sex activity, when the condom is not used or are not replaced for every partner in the group sex (Schmidt, 2011). We found 36.7% (11/30) of respondents practiced group sex.

In addition, sexually transmitted diseases (STDs) that manifests on the genitals caused by bacteria or viruses can also be a pathway of HCV transmission. An individual with STD is more susceptible to transmission of HCV because the disease is causing injury to the genital area so that the possibility of blood contact during sexual activity is higher (Terrault, 2002).

Percutaneous exposures history to HCV infection were also studied. HCV is efficiently transmitted through injecting drug use (Murphy et al., 2000; Sherman, 2001). In this study we found 23.3% (7/30) of respondents who had used narcotics and 14.3% (1/7) among them had injecting drug use history. This respondent practiced injecting drug use for the first time while living in a correctional institution.

Correctional institutions (jails and prisons) are important site for blood-borne viruses transmission. The prisoners tend to use a syringe alternately instead of using new syringes. This behavior is principal risk factor for HCV transmission among injecting drug users (IDUs) (Hahn, 2001). IDUs showed positive results for anti-HCV examination quickly after initiation of interchangeably drug injection (Garfein, 1996). In addition to injecting drug use, tattooing, piercing, and sexual coercion are also risks for HCV spreading in correctional institution (Hellard, 2000). Respondent with a history of incarceration found in this study, as well as having a history of injecting drug use, also had a history of tattooing and sexual contact with fellow prisoners while living in the correctional institution.

Safe tattooing practice includes proper sterilization techniques of reusable equipment and using a disposable syringe (World Health Organization, 1999). Although sterilization is not expensive, it can increase the cost for each tattooing for about 15% (Behrens, 2008). This procedure may not allow a safe tattooing practice in commercial site where respondents obtained a tattoo and or piercing.

Detection of HCV infection in this study was conducted by using particle agglutination assay and nested RT-PCR method. RT-PCR was used to detect circulating HCV RNA in blood. The presence of HCV RNA indicates HCV is actively replicating (reproducing and infecting new host cells) (Carithers, 2000; Pawlotsky, 2002). If HCV RNA was undetected by RT-PCR, viral load of HCV in blood may not reach the minimal amount to be detected by RT-PCR. An individual with very low viral load may still have HCV circulating in his blood so that transmission to others may still able to occur. In total, anti-HCV

was detected in 23.3% (7/30) of respondents in this study. Of all anti-HCV positive samples, no RT-PCR positive result was found.

After an acute exposure to HCV, HCV RNA is generally detectable in blood preceding anti-HCV seroconversion (Ghany, 2009). HCV RNA can be identified within two weeks after exposure, whereas anti-HCV was not detected before 8-12 weeks. However, false negative results may occur when an individual with active HCV infection is in the period of intermittent low level viremia. Intermittent low levelviremia imply negative results on HCV RNA detection, followed by positive results for following detection of HCV RNA (Bruden et al., 2004; Scott et al., 2006). Intermittent low level viremia may occur around two months before HCV infection enter the period of exponential increase in viral load and high titers plateau phase preceding seroconversion (Glynn, 2005). Such cases require repeat RT-PCR test 4-6 months after the first RT-PCR test (Ghany, 2009).

Negative RT-PCR result in anti-HCV positive individuals can also show that HCV infection has resolved. These individuals may have HCV infection in the past (Gretch, 1997; Sakugawa, 1995). Individuals with self-limiting hepatitis C may be susceptible to clearance of HCV RNA or HCV core antigen within 12-16 weeks after clinical onset (Mondelli, 2005). Strong immune response from cytopathic T helper cells (Th) 1, CD8 T cell, CD4 T cells, type 1 cytokineare likely to be involved in the clearance of circulating HCV in blood (Mondelli, 2005; Thimme, 2001). In addition to immune response, Scott et al. (2006) also reported that young-onset when a person infected with HCV is associated with spontaneous HCV resolution.

An individual with chronic hepatitis C may also show a negative result for HCV RNA detection. It is related to low titer of HCV RNA (Scott et al., 2006). Small proportion of patients with chronic HCV infection may be non-viremic, especially if they experienced disease progression to hepatocellular carcinoma (Pawlotsky, 1997; Yokosuka, 1999; Zaaijer, 1993).

CONCLUSION

Hepatitis C virus infection had been found in male commercial sex workers community in Surakarta. In consideration of sex as a common behavior, sexual contact likely contributes to the total burden of HCV infection in Indonesia. This indicates the need for interventions to diagnose, treat and prevent HCV infections among this population, and to prevent further HCV spreading to society.

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GROWTHHORMONE REDUCESLOW DENSITY LIPOPROTEIN OF DYSLIPIDEMIC WISTAR RAT

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ABSTRACT

The use of growth hormone (GH) as anti aging treatment is still controversial. The benefit of this treatment to prevent age associated disease, especially cardiovascular disease, is still in question due to the lack of study. In this study, the effect of growth hormone therapy to low density lipoprotein is observed in dyslipidemia, a risk factor of cardiovascular disease. A randomized, pre and post control group experimental study was done using 20 male Wistar rats, age 11 – 12 months. The subjects were divided into 4 groups, aquadest (P0), GH 0,02 IU/day (P1), GH 0,04 IU/day (P2), and GH 0,08 IU/day (P3). All subjects were given high cholesterol diet for 3 weeks to achieve dyslipidemic state (total cholesterol level > 200 mg/dL) and the diet was continued until the end of the study. After 3 weeks, aquadest and GH were injected once daily for 2 weeks. Plasma low density lipoprotein level was measured before and after treatment by colorimetric enzymatic CHOP test.The mean of low density lipoprotein level before treatment were the same (p > 0,05) between P0 (130,82 mg/dL) and P3 (133,19 mg/dL), and also between P1 (127,52 mg/dL) and P2 (125,33 mg/dL). Growth hormone therapy significantly reduced low density lipoprotein level of P1 by 40,71% (75,61 mg/dL, p< 0,05), P2 by 64,5% (44,49 mg/dL, p < 0,05), and P3 by 90,68% (12,41 mg/dL, p , 0,05). The difference of post test and pre test low density lipoprotein level between all groups were also significant (p < 0,05).This study concluded that growth hormone reduced low density lipoprotein in dyslipidemic male wistar rat.

Keywords: growth hormone, low density lipoprotein, dyslipidemia

INTRODUCTION

Aging process leads to decrease of mental and physical capacity. The process is responsible for deterioration of various physiological function of human body that will result in age related diseases. Age related disease, such as cardiovascular, is a major cause of death around the world (WHO, 2008). According to the neuroendocrine theory of aging, aging is caused by the change of hormone level, a systemic regulator of physiological function. One of the important hormones decreased in aging is growth hormone (GH). This hormone has now been used as anti aging treatment(Djuanda, 2007; Pangkahila, 2007).

Growth hormone is a polypeptide hormone; consist of 191 amino acids with 22kDa molecular weight. It is synthesized by somatotroph in the anterior pituitary (Tien, 2000). The secretion of GH is regulated centrally by hypothalamic hormones, growth hormone releasing hormone (GHRH) and somatostatin. Growth hormone releasing hormone induces GH secretion, whereas somatostatin prevents it. Growth hormone secretion is also influenced by neurohormonal response. Conditions such as physical activity, starvation, anorexia, stress, and sleep duration can increase GH secretion (Fanciulli, 2009; Jørgensen, 2010). Growth hormone exerts its effect and prevents its own production by a feedback mechanism through GH itself or indirectly through insulin like growth factor-1 (IGF-1). The effect of GH to physiological process is complex. Growth hormone is the main key in controling growth and metabolism of carbohydrate, protein and lipid. The level of GH is low in the old age and children (Gardner danShoback, 2007; Jørgensen, 2010).

Low density lipoprotein (LDL) is a component of lipoprotein produced by the liver. LDL level is increase in dyslipidemia. Dyslipidemia is a major risk factor of cardiovascular disease. Increase oxidized LDL can lead to deposition of fat in the intima wall, which initiates the progression of atherosclerosis (Shao danHainecke, 2009).Growth hormone may modulate the level of this lipoprotein, because its effects on lipid metabolism.

The use of GH as anti agingtherapy is still in debate. Although it has been clinically proved to increase quality of life, but controversial evidences have been found regarding the benefit of this treatment to actually prevent age associated disease, such as cardiovascular disease (Colao*et al*., 2008; Olivier*aet al*., 2007). Meanwhile, considerable indirect evidence and theorythical assumption of the side effect of this treatment toward cancer incidence can not be fully eliminated (Jenkins *et al.*, 2006; Renehandan Brennan, 2008). Further research is needed to elaborate the effect of GH treatment to cardiovascular disease. In this

study, the effect of GH to low density lipoprotein level, a predictor of cardiovascular disease, is observed in dyslipidemia subject.

MATERIALS AND METHODS

Growth hormone used in this study was recombinant GH (Eutropin; Novell). High cholesterol diet was composed of 1% cholesterol, 5% yolk, 10 % lard, 1% coconut oil, prophyiltiouracyl 0,01%, and added with standard chow up to 100%(Farmacology Department, Udayana University). The subjects of the study were male Wistar rats and the weights were 200 – 225 mg. Eleven to twelve month-old rat was used in this study, which was equal to the third decade of human year where GH was started to decline (Hanson, 2010). All of the samples were dyslipidemic (total cholesterol > 200 mg/dL) (Hardini *et al*., 2007). According to previous study the sample needed in this study was 5 rats for each group, includingthe 20% additional sample (Parini*et al*., 1999). The samples were obtained from Animal Laboratory Unit, Farmacology Department, Udayana University.

All animals were adapted for 1 week and then given high cholesterol diet for 3 weeks to achieve dyslipidemic state. After 3 weeks of high cholesterol diet, blood was taken from the animal to measure plasma total cholesterol and pre test LDL level. The diet was continued until the end of the study. Twenty eligible subjects were then randomized and divided into 4 groups; (1) aquadest 0,01 mL, (2) GH 0,02 IU/0,01 mL, (3) GH 0,04 IU/0,01 mL, and (4) GH 0,08 IU/0,01 mL.Subcutaneous injection of aquadest and GH were given once daily at 7 am for 2 weeks. Plasma LDL level was then examined for post test.

Cholesterol and LDL level were measured using CHOP – PAP method (Bochringer-MennheimGmBp) in the Interuniversity Central Laboratory, GadjahMada University. Blood were collected from periorbital sinus, after 15 minutes, the blood were centrifuged 3000 rpm for 20 minutes. Plasma (0,01 mL) were mixed with cholesterol reagent (1 mL). The mixture was placed in room temperature for 20 minute before cholesterol measurement in 500nm absorbancy. Plasma (0,02 mL) were mixed with precipitative solutionand placed for 10 minutes in room temperature. The mixture was then centrifuged 4500 rpm for 20 minutes. Supernatant (0,01 mL) were then mixed with cholesterol reagent (1 mL). The mixture was placed again for 20 minutes in room temperature before LDL measurement in 500 nm absorbancy (Dachriyanus*et al*., 2007). Data were analized with Anovaand paired t test to detect the effect of GH to plasma LDL.

RESULTS

One way Anova analysis showed significant difference in pre test plasma LDL level between groups ($p < 0,05$) (table 1). Post hoc test showed LDL similarityof group P0 – P3 and P1 – P2 (data not shown). Post test plasma LDL level were different between groups ($p < 0,05$) both in Anova or Post hoc test.

Table 1.Pre test andpost testplasma LDL level in control group (P0) and treatment groups (P1, P2, P3)

Group	N	LDL *Pre Test*			LDL *Post Test*		
		$\bar{x}$	F	p	$\bar{x}$	F	p
Aquadest (P0)	5	130,82 ± 2,79	4,13	0,024	157,79 ± 6,31	1473, 73	0,000
GH 0,02 IU (P1)	5	127,52 ± 4,65			75,61 ± 2,01		
GH 0,04 IU (P2)	5	125,33 ± 2,78			44,49 ± 2,17		
GH 0,08 IU (P3)	5	133,19 ± 4,60			12,41 ± 2,06		

Difference of post test and pre test were also analized because pre test plasma LDL levels were not equal between groups. Anova analysis showed that the difference of post test and pre test plasma LDL level were significant between groups (table 2). Post hoc test also showed the differences were observed between each group (data not shown). Decrease of plasma LDL level in P3 were greater than P1 and P2 ($p<0,05$), eventhoughpre testLDL level in P3 were higher than P1 and P2.

Table 2.The difference of post test and pre test plasma LDL level in control group (P0) and treatment groups (P1, P2, P3)

Group	N	LDL difference	F	p
Aquadest (P0)	5	26,97 ± 5,05	1365,55	0,000
GH 0,02 IU (P1)	5	-51,91 ± 3,57		
GH 0,04 IU (P2)	5	-80,84 ± 1,50		
GH 0,08 IU (P3)	5	-120,76 ± 4,10		

GH decreased plasma LDL level by 41% in P1, 65% in P2, and 91% in P3, whereas in aquadest injected group the LDL level increased. Paired t test analysis on group P0, P1, P2, and P3 showed those changes were significant ($p < 0,05$) (Figure 1).

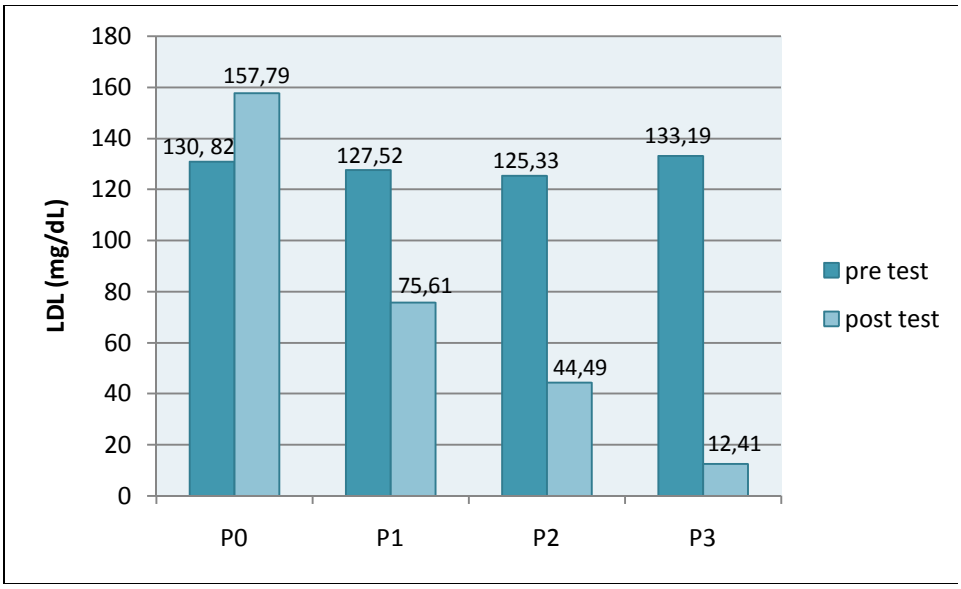

Figure 3.Pre test and post test plasma LDL level in control group (P0= aquadest 0,01 mL)and GHtreatment group (P1= 0,02 IU, P2=0,04 IU, P3=0,08 IU)

DISCUSSION

Growth hormone therapy could effectively decrease plasma LDL level in male wistar rat with dyslipidemia, despite the continous high cholesterol diet in all groups. Plasma LDL leveldecreasedby up to 41% in P1, 65% in P2, dan 91% in P3 after 2 weeks of GH treatment. This result is supported by other studies using mice and rats (Frick *et al*., 2002; Lopez-Olivia *et al*., 2009) and also observed in human (Lind *et al*. 2004; Maison*et al*., 2004; Colao*et al*., 2005; Abs *et al*., 2006; Olivier*aet al*., 2007). The decrease of LDL level is associated with the increase of GH dose. Dose of GH 0,08 IU/day caused the highest decrease of LDL, then followed by 0,04 IU/day, and the smallest dose, 0,02 IU/day, produced the lowest LDL decrease. It should be noted that the effect could be obtained by relatively low dose of GH. Appropriate dose of GH should be considered in the GH therapy, because fewer doses may not give effect on LDL (Pfeifer *et al*., 1999), and an overdose could lead to higher incidence of side effects (Pangkahila, 2007). Eventhough the dose in Pfeifer *et al*. (1999) was higher than in this study and another study by Lind *et al*. (2004). The subjects in the study were having growth hormone deficiency, whereas in the later studies using subjects with noor slight decrease of GH. Long term exposure to excessive GH could also cause an increase of LDL level (Frick *et al*., 2001). This could be happened due to a dominant very low density lipoprotein (VLDL) secretion and decrease of hepatic lipase. Hepatic lipase is an enzyme that functions in the uptake and breakdown of LDL (Frick *et al*., 2001;*Verhelst*dan Abs, 2009).

The mechanism through which GH increases LDL is by increasing the expression of LDL receptor. Growth hormone also influences editing of mRNA ApoB100 and increases production of ApoE by the liver.

The modification in the apolipoprotein composition of LDL can induce the uptake of LDL through LDL receptor by the liver, therefore GH can still lower LDL eventhough the secretion of VLDL is increased (Frick *et al*., 2002; Lind *et al*., 2004; Verhelstdan Abs, 2009).

In conclusion GH by its internal regulation on the uptake of lipid effectively decreases plasma LDL level in diet-induced dyslipidemiaWistar rat.

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THE INFLUENCES OF ETHANOL EXTRACTS OF SAMBILOTO AGAINTS THE DIFFERENCES OF INTERFERON GAMMA CONCENTRATION IN LUNG GRANULOMAS CULTURE OF MICE INFECTED WITH *Mycobacterium tuberculosis*

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ABSTRACT

The spesific mark of pulmonary tuberculosis is the granuloma, consisting of lymphocytes and macrophages that show the interaction of immune cells with Mycobacterium tuberculosis. The good structure of granuloma is important to limit the spread of germs, but when the immune system declines, granulomas can no longer afford to limit the spread of bacteria and become pathologic with liquifaction necrosis. The failure of treatment with anti-tuberculosis drugs is high enough to cause an increase in the number of tuberculosis patients. The use of additional immunotherapy gives attention to treat tuberculosis beside the use of anti tuberculosis drug, particularly to the patients who are resistant to antituberculosis drugs. Sambiloto (Andrographis panniculata) is a plant that has been proven to have immunostimulatory effects. Natural materials are attracted to research because of high levels of availability in Indonesia. The aim of this study is to know the effectiveness of the ethanol extract of sambiloto against the concentration difference of interferon gamma on the formation of pulmonary granuloma in mice infected with *Mycobacterium tuberculosis*. The methods of study is experimental with post test only control group design. Forty mice infected with *Mycobacterium tuberculosis* were divided into 4 groups. Group 1 and 3 are given anti-tuberculosis drugs (isoniasid with doses of 25 mg/kg), group 2 and 4 are given anti-tuberculosis drugs and ethanol extracts of sambiloto (with dose of 75 mg/20 g bb). The measurement of interferon gamma concentration on cultured lung granulomas of mice for groups 1 and 2 performed at the fifth week, while groups 3 and 4 on the seventh week. Measurement of cytokines was done by using interferon gamma kit with enzyme-linked immunosorbent assay technique. Analysis of data was using one-way ANOVA statistical test with $p < 0.05$. Results showed an average concentration of interferon gamma from groups 1, 2, 3, and 4 are 188; 256; 290; 350 pg / ml. Analysis of results showed significant differences between the four groups, with highest concentrations in group 4. Ethanol extract of sambiloto increasing interferon gamma concentration on cultured lung granulomas of mice infected with *Mycobacterium tuberculosis.*

Key word: The ethanol extract of sambiloto, granuloma of pulmonary tuberculosis, interferon gamma.

INTRODUCTION

Pulmonary Tuberculosis is a chronic disease that attacks the lung parenchyma. WHO data from 2003 showed, Indonesia has contributed to the tuberculosis cases in the world, after India and China. Information from Health Department 2008 in the last year of tuberculosis in Bali reached 1200 cases. Tuberculosis problem was increased by the emerging multidrug resistance (MDR), and also tuberculosis co-infection with HIV.[1,2,3] The characteristic of pulmonary tuberculosis is granulomas, which is predominantly composed of lymphocytes and macrophages that show the interaction of immune cells with *M. tuberculosis*. Granuloma formation is a process organization that depends on the recruitment of lymphocytes is facilitated by the adhesion molecule and chemokine.[4] Granuloma is the site of the infection process, may play a role persistency, pathological, and protection against *M. tuberculosis*.

T cells and macrophages cells participate in controlling tuberculosis. IFN-γ and TNF-α produced by T cells is important as a macrophage activator. TNF-α, and lymphotoxin (LT)-α3 plays a role in the formation and maintenance of structural integrity of the granuloma.[5] Granuloma is a collection of mononuclear phagocytes (macrophages and or epitelioid cells) that can continue or not with necrosis or leukocyte infiltration of inflammatory cells. Granulomas are generally functioning in limitation or control the spread of pathogens that enter the network so that the damage can be limited.[6]

The use of additional immunotherapy draws attention to deal with tuberculosis, particularly because of the increased percentage of patients who are resistant to antituberculosis drugs. Immunomodulatory expected to be used to repair or rebuild (imunorestorasi) immune system is deficient or dysfunctional. The weakness of the immunomodulator is a repeated exposure necessary to produce cytokines capable of activating macrophages in the absence of repeated exposure for a period of time resulted in a body will not be found that specific T lymphocytes secrete cytokines that can activate macrophages.[7] Therefore, it

would require a level of immunomodulatory high availability so that it can be repeated in the long term as immunomodulator derived from nature. Sambiloto is the natural ingredients are easily found in Indonesia, which proved to have properties as an immunomodulator.

Sambiloto (*Andrographis panniculata*) proved to enhance the immune response (immunostimulant and has a bactericidal effect).[8] Natural material above is very interesting because it is as immunomodulator capable of enhancing the activity of immune cells.

MATERIAL AND METHODS

This study is an experimental study with a post test only with control group design. This experiment tested of the effectiveness of ethanol extract of sambiloto to the differences of IFN-γ concentration in lung granulomas in mice infected with *Mycobacterium tuberculosis*. There are 40 mice were divided into four study groups P1, P2, P3 P4. In first week carried out the intervention of *M.tb* infection to all groups (P1-P4) with 60 µl dose (bact. conc 105/ml). In the week III: P1-P4 are given OAT (isoniasid and) with dose: 25 mg / kg; P2 and P4 are also given the extract of sambiloto (S) with dose: 75 mg/20 grbb. At week V: P1-P2 were sacrificed for granuloma culture to examined for concentrations of IFN-γ whereas P3 and P4 groups were sacrificed at week VII for granuloma cultured and examined for IFN-γ concentration. Prepared pure cultures of *M. tuberculosis* strain H37Rv references 3-4 weeks old (strain virulens).

Preparation of sambiloto extract was done as follow: sambiloto was dried with aerated, and make powder. Five kg of sambiloto powder was extracted by using 50 lt ethanol maceration, at 60 º C, then filtered and the extract obtained was concentrated and dried. Experiments: all group were infected with 10^5 perml bacteria intranasally, initially created as many as 10^7 bacteria suspension is equivalent to a Mc Farland, then diluted until a concentration of 10^5 bacteria, as many as 60 micro liters of suspension was inoculated into the nostrils of mice with a micropipette . Then the mice were placed in cabinet, fed and watered ad libitum, observed daily for 3 weeks.[9] Then after 3 weeks, some mice were sacrificed for histopathological lung examined by haematoxylin eosin to prove the presence of granulomas and proven BTA (Basil smear) with ZN staining (Ziehn Nelsen). If it is proven granulomas then followed by OAT administration (group P1 & P2): OAT (isoniasid) dg dose: 25 mg / kg; group P3 & P4: OAT + 75 mg / grbb extract sambiloto then observed daily until week V and VII termination for review the profiles of the cytokines. The examination of IFN-γ cytokines by ELISA method : a termination carried out in each group at weeks V and VII. Then the macroscopic of lung granuloma, (granuloma tissue) ; in perfusion with cold isotonic buffer (40^0C cold sterile PBS), then the lungs were incubated in medium RPMI 1640 (Sigma) containing collagenase (Sigma), for 60 minutes at 37^0C. Granuloma tissue was taken and cultured in RPMI 1640 medium 10% FCS (CSL Bioscience, Melbourne, Australia), 2 mM L-glutamine (Flow Laboratories, Sydney, Australia), 10 mM HEPES (Sigma), and 10 mM sodium bicarbonate (BDH, Melbourne, Australia), after incubation of 48 hours, the supernatant was taken and checked the concentration of cytokines IFN-γ by ELISA method (R & D Systems, Minneapolis, MN and Pharmingen, San Diego, CA).[8]

RESULT AND DISCUSSION

Figure 1. showed concentrations of IFN-γ in mice lung granulomas cultured in RPMI medium after incubation of 48 hours in a CO2 incubator. The average concentration of IFN- γ in group P1 to P4 respectively (pg / ml) is 188; 256; 290; 350. The concentration of IFN- γ lowest in the group that only received OAT which are terminated at 5 week while the highest concentration of IFN- γ in the group who get sambiloto and OAT which is terminated at 7 week, when analyzed by one way ANOVA, there are significant differences between groups with the same termination time (P1-P2 at weeks 5 and P3-P4 at week 7) ($p < 0.05$).

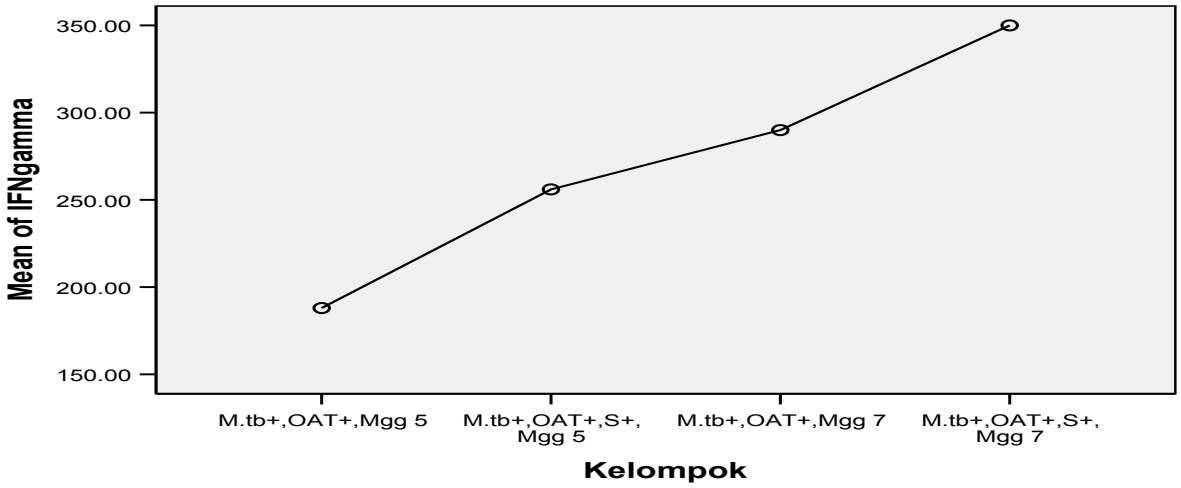

Figure 1. Result of IFN-γ concentration from mice lung culture which is terminated in V and VII week.

The above result might be due to the effect of sambiloto, in which the research results has a good effect in the secretion of cytokines IFN-γ. Granuloma is a typical sign of the mycobacterial disease, granulomas are indispensable in limiting the spread of mycobacterial, the intact of granuloma structure will keep bacterial load in the lung tissue that can agravate lung damage. In the structure of granuloma there are interaction between the mycobacterial, T cells, infected macrophages that already contains the bacilli in preventing the later spread of mycobacterial. Various chemical and cellular factors controlling the formation of granulomas, including cytokines.

Granuloma is microenvironment important in limiting the growth of mycobacteria in macrophages, and granulomas at the same time have the potential to cause tissue damage through necrosis and fibrosis.[9] One signal is needed in the formation of a granuloma is IFN-γ as a signal for the activation of macrophages and lymphocytes. In one study in mice which the concentration of IFN-γ was lacking, when they was given aerosol mycobacterial, the granuloma formation was fail. IFN- γ stimulates macrophages to kill M.tb through various channels including activating enzymes and inos phagocyte oxidase, to produce reactive oxygen and nitrogen metabolites, which increase the GTPase-stimulating fusion of fagolysosomes.[6]

Granulomas in the lungs of mice also did not reveal any multinucleated giant cells and central necrosis with caseous because it occurs co-location of CD4 T cells with macrophages and some of which turned into epithelioid cells. Sambiloto (Andrographolide panniculata = A.panniculata) as a medicinal plant has done various research on its role in controlling tuberculosis. From the results above it is associated with granuloma structure that results from *m.tb* infection, in the group given sambiloto than OAT seems lung damage that occurs lighter and levels of cytokines IFN- γ also higher than the group that only received OAT alone. This could happen probably because the effect of antimicrobial of sambiloto extract can work synergistically with OAT in mycobacterial killing so that the process of lung damage does not occur further. According to a research conducted by Wahyunitisari, *et al*. (2006) sambiloto show antimicrobacterial effects on macrophages cultured infected *M.tb*. apart from that sambiloto is also mentioned as an immunostimulant that has an effect on this study appears elevated concentrations of cytokines IFN- γ in cultured lung granulomas of mice infected *M.tb* than the group that only received OAT alone, where IFN- γ as mentioned earlier is very important in the formation and maintaining granuloma structure to remain intact so that the spread of germs does not occur and more severe damage can be prevented.

The research was also supported by in vitro studies conducted by Batkhuu (2002), which mentions the role of sambiloto in lymphocyte proliferation and production of cytokines secreted by Th1 (IFN- γ). Sambiloto used in this study came from the region of Badung, Bali which grows wild in the farm population, unknown active compounds dominant in these plants may make a somewhat different effect if it grows in other areas.[10,11]

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THE RELATIONSHIP BETWEEN KNOWLEDGE AND ATTITUDE OF WORKER'S IN USING PERSONAL PROTECTIVE EQUIPMENT IN DEPARTMENT OF FORGING, DIVISION FORGING AND CASTING OF METAL COMPANY IN BANDUNG WEST JAVA

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ABSTRACT

Personal Protective Equipments (PPE) is not used by workers properly to protect their body from job hazards and dangers. Accident which often occurs in the workplace mostly caused by human factors. Its caused due to their behavior than neither mechanical failure nor administrative failures.This research aim is to find out the correlation between knowledge and attitude of workers in using their Personal Protective Equipment in the Department of Forging, Division Forging and Casting of a metal company in Bandung. Analytical survey was used in this research, and cross-sectional approach to 62 respondents who work directly on department of producing. By means of questionnaire and check-list for the observation method were used to collect the data needed in this research. Statistical Analyzing is used Product Moment Correlation to determine the relationship between variables.The research results that 90.32% respondents have a good level of knowledge and 53.23% have unfavorable attitude in using Personal Protective Equipment, while the using of the safety equipment was only 61.29% of which properly. The statistical analysis resulted in correlation between knowledge and the using of PPE of workers at 95 Confidence Level (p value: 0,000) and No significant correlation between attitude and the using of PPE of workers. In short, there is no correlation between knowledge and attitude of workers' in using PPE. The research recommendation is the enhancement of supervision in using PPE, because on its case, PPE is very important to wear, to protect worker from danger and hazard and prevent from any cases that could be happened worse.

Keywords: *Knowledge, Attitude, Personal Protective Equipment (PPE),Metal.*

INTRODUCTION

Occupational disease and occupational accidents in many levels will cause a negative impact directly to the industrialization, in case of the decrease of workers' productivities. International Labor Organization (ILO) stated that occupational ill-health and occupational accidents in work environment have killed many more victims compared with World War. Research conducted by International Labor Organization (2003) had concluded that every day, more than 6.000 people die, equivalent with one died people every 15 seconds, or 2.2 million people/workers die every year because of the accident and illness related to work in the work places. Men who work died two times more than women workers because men workers usually do the more dangerous jobs and activities than women workers. Over all, occupational accidents had killed approximately 350.000 workers.

The recent data from Worker and Transmigration Department in Indonesia, the occupational accident in the work places are still high. There is a regular increased from year 2000 to 2007. Furthermore some researches revealed that Indonesian's workers accident in the workplace is very tragic.[a] Those accidents mostly occur because of the negligence and human factors (80-85)%.[b]

Every company should integrate the execution of occupational safety and health management system and company management system.[c]Thismetal Company where this research had been conducted is one of the companies in Bandung, which carries out the occupational and safety health management system in every process of the job activity. This metal companyis a company which runs service sector and mental industry, especially not only in the field of Military Product Corporation (Arm and Ammunition Division) but also in the field of commercial production (Industrial and Service Division), Industrial Engineering Division, especial Function Vehicle Unit, and Forging and Casting Division.

This metal company, especially in the Forging Department engaged with high risk in working and environmental places that can endanger the safety and health of the workers. Forging department is a unit of company which has many difference machinesin its production activities. Forging activity is a metal process in increasing the usefulness of metal by forming and quality improvement of plastic deforming in hot and cold condition and also by blowing and pressing regularly. The higher the engine is used the more variety of the safety and health risks. Moreover, the material was used in production activities have many characteristics that can become a negative effect to the workers, so that they will complaint because of the

unwanted impact to their health.

The result of preliminary study revealed that there are someaccident cases during 2006 to 2008. Although the amount of the accident during the recent year is only a little, however this situation has not already reached the company target in reaching the "Zero Accident". The unwanted situation not only happens in the small company but also in the big company that needed a big problem solution and gave a negative impact for their financial expenditure.[d]

This metal company has 87 workers, composed of 62 workers who work directly in production unit and 25 workers who work in administration unit. Based on the interview to the supervisor of the Occupational safety and health and work environment, the using of Personal Protective Equipment (PPE) is not effective. There are so many workers who work in high risk work environment such as a high temperature in forging division they do not use the PPE properly. So many reasons they gave that using PPE limited their activity, too hot, heavy and make them uncomfortable. These reasons make workers not use their PPE when they worked.

Many accidents related to the job occur in the work environment caused by human factors, such as the accident happened because of the worker behavior than the mechanic failure or the work system limitation. Behavior is a response or one's reaction to the stimulus.[e] The behavior that based on the good knowledge will last longer than without good knowledge of the workers.[f] The attitude of workers will be influenced by their level of knowledge of the stimulus so that attitude and knowledge have an effect to the behavior.[g] In the execution of using the Personal Protective Equipment, knowledge and attitude will also give influence to the usage of the PPE.

To explore the using of Personal Protective Equipment of the worker in the metal company, a quantitative research was conducted. In particular, this research aimed to identify the relationship between knowledge, attitude of occupational safety and health and the behavior of workers in using Personal Protective Equipment.

METHODS

This research population involved 87 workers in the forging and casting division of metal company in Bandung, however only 62 workers who are eligible to this study because they work directly to the production unit. A cross-sectional study was held to find the relationship of the variables of this research, dependent variable and independent variable. The using of PPE is dependent variable, while knowledge and attitude of Occupational Safety and Health are two of the independent variables. By means of questionnaire the data of this research was collected. A self reported questionnaire should be fulfilled by workers to know their knowledge and attitude of safety and health in the work place and also their behavior in using PPE.

A cross-sectional study was conducted in order to collect the information from respondents. Analytical quantitative method was used to explain every variable in this research and Pearson's Product Moment Correlation was used to analyze the relationship between dependent variable and independents variables. The using of Personal Protective Equipment is the dependent variable and the independents variables are knowledge and attitude of workers about Occupational Health and Safety at their work.

Knowledge about Occupational Health and Safety is an ability of workers to understand what occupational health and safety at work including the using of personal protective equipment. Workers' attitude is their opinion about the using of personal protective equipment in workplace and the behavior in using personal protective equipment is their habit to use the device in order to keep their safety and health at work.

RESULTS

Interviewing to respondents results data and than being analyzed using computer program. Coding, tabulating and presenting in table or figure had been done to make the explanation of this research clear. The descriptive statistics of each variable as shown in Table 1.

Table 1. Descriptive Statistics of Variables

Var	N	Range	Min	Max	Mean	Std. Dev
Knowledge	62	7	11	18	16.37	1.61
Attitude	62	21	55	76	64.97	5.27
Practice/Behavior	62	7	11	18	16.37	1.621

Pearson's Product Moment Correlation coefficients established that there is significant correlation between knowledge and the using of personal protective equipment of workers in metal company in Bandung, (p value=0,001; coefficient correlation=0.994), while there is no significant correlation between attitude and the using of personal protective equipment of workers in metal company in Bandung, (p value=0,49; coefficient correlation=0.89).

This research makes a description for knowledge, attitude and the using of personal protective equipment to present the data in tables. The knowledge is categorized using presentation of the scores, as: Bad (< 60% answers are true; enough if 61-75% answers are true and Good grade if the true answers are more than 75% (76-100%). To measure and distribute the attitude categories using T score. Favorable attitude is defined if their scores of attitude are more than mean T, and Unfavorable attitude is defined if the scores of attitude lower than mean T. For the dependent variable, using of personal protective equipment is being carried out if their score is more than mean score, and not being carried out if their score is lower than the mean score of its variable.

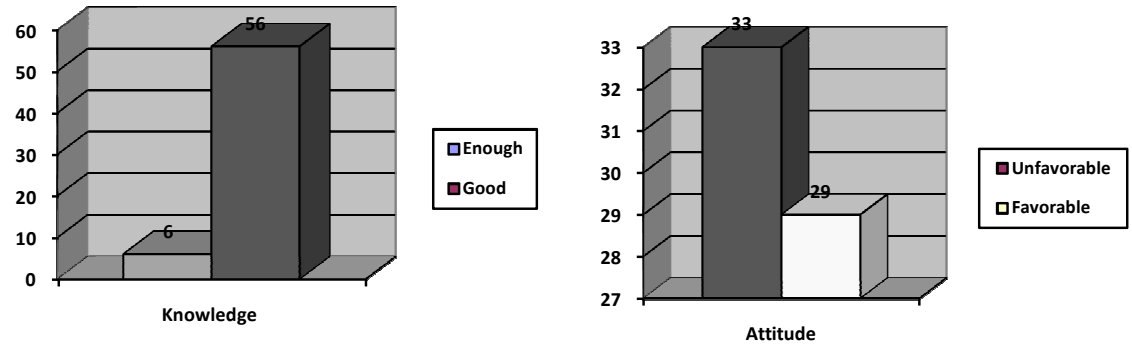

Diagram 1Knowledgeof Workers about Occupational Health and Safety and Attitude of Using of Personal Protective Equipment

The first table shows that workers who have good knowledge of occupational safety and health are higher than the other. More than 50% the workers in a good knowledge and education.Second table indicates that workers' attitude about the using of personal protective equipment. More than a half of workers' attitude are in unfavorable state. They do not agree to use the device when they do their job at workplace.

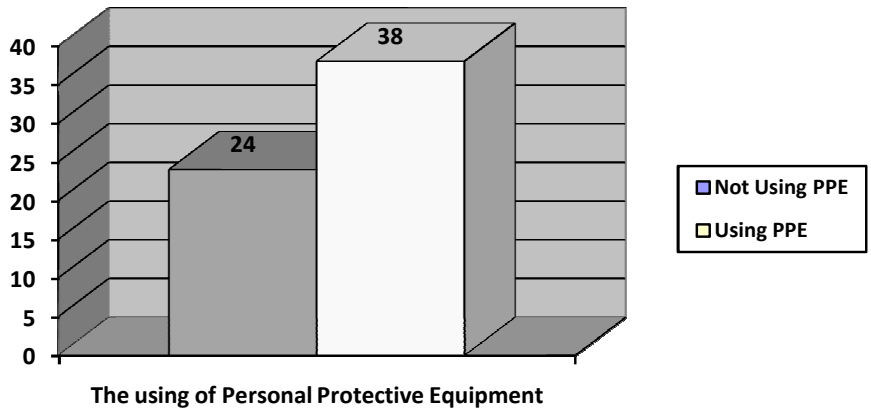

Diagram 2The Using of Personal Protective Equipment by workers in Metal Factory

Although the using of personal protective equipment is very important for their safety and health, workers often do not care about it. Only 38 workers who do use personal protective equipment, while there are still a few workers who do not use those devices.

To know the relationship between knowledge and behavior, attitude and behavior are using cross-tabulation. Pearson's Product Moment is used to test the Correlation analysis. The result of analysis between independent and dependent variable are shown by Table 2, the correlation between knowledge, attitude and the using of personal protective equipment.

Table 2. Correlation between Independent Variables and Dependent Variable

Variables	Categories	Behavior						*p value*
		Not Using PPE		Using PPE		Total		
		f	%	f	%	f	%	
Knowledge	Enough	1	4.2	5	13.2	6	9.7	0.001
	Good	23	95.8	33	86.8	56	90.3	
	Total	24	100	38	100	62	100	
Attitude	Unfavorable	13	54.2	20	52.6	33	53.2	0.089
	Favorable	11	45.8	18	47.4	29	46.8	
	Total	24	100	38	100	62	100	

The table indicates that there is significant correlation between knowledge about occupational safety and health and the using of personal equipment (p value=0,001). From 24 workers who do not use personal equipment, 95.5% in state of Good knowledge, while knowledge in enough condition is only 4.2%. Workers who have use personal equipment every time in their workplace (38 workers), more than 85% are in Good state of knowledge.

Pearson's Product Moment Correlation Coefficient of this test is 0.994. It means the correlation between the two variables is very close. The higher their knowledge, the better their behavior in using personal protective equipment. There is no correlation between attitude of workers and the behavior in using personal protective equipment. The coefficient of the Pearson's correlation is 0.493. Based on the coefficient correlation, the relationship between attitude and behavior in using personal protective equipment is significant at level alpha = 10%.

DISCUSSION

This quantitative study revealed that knowledge about occupational health and safety has a significant correlation with the employee's behavior in using personal protective equipment. Knowledge is one of the predisposing factors which influenced the behavior. Knowledge is the result of what people feel, hear, watchingfrom their senses. Mostly they received the knowledge from their sight and hearing.

A few workers have their knowledge in good level because they often get the right information about the benefit and the opportunity if they use personal protective equipment. Accident in the workplace and occupational disease can be reduced or to be eliminated from work environment so that it will not happen in the future.Good knowledge will support their behavior in using PPE (overt behavior).

Attitude is one of enabling factors in forming peoples' behavior. Attitude is a hiding response from someone to the stimulus or objects. Manifestation of attitude couldnot be seen directly, however it will be a covert behavior. According to Newcomb[h], attitude is a readiness or willingness to act or do something although He or She has no specific motivation. Furthermore, attitude is overt behavior that represents the combination of cognitive, affective and psychomotor to the object or stimulus.[7] Attitude is an evaluative response. The response will appear when a person facing the problem that needs a solution.[7]

Every company has an occupational safety and health program. It will last longer and more effective whenever employee and employer collaborate and cooperative in executing the program.[i] This research showed that there is no correlation between attitude and workers' behavior in using personal protective equipment (p value > 0,05) while knowledge of workers has a significant correlation to workers' behavior

in using personal protective equipment whenever they are in the workplace doing their daily job that full of hazards.

Observation was also done to make sure that workers use their personal protective equipment properly in the workplace. More than a half of workers (61.29%) in forging department use their personal protective equipment in the right way, but 39.7% did not use their safety devices properly. The equipment that usually used by workers in forging and casting division are shoes, helmet, gloves and masker. The equipments should be used in every activity in workplace to prevent the accident or the bad effect of the work environment that can release any dangerous materials for their health.

In every activity that involved human being, the effectiveness of its activity depends on the point of view of the workers to their jobs.[3] To prevent the unwanted incidence especially accident in the workplace and diseases related to work can also by using personal protective equipment for every worker. Personal protective equipment is a set of devices which is used by workers to protect their part and whole body from potential dangerous of the work environment hazards.[1]

CONCLUSION

Based on the discussion of this research, we can conclude that workers' knowledge has significant correlation with the workers' behavior in using personal protective equipmenthowever attitude has no correlation to the workers' behavior in using personal protective equipment.

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NEUROCYSTICERCOSIS WITH PARTIAL SECONDARY GENERALIZED EPILEPSY IN AN 11 YEAR OLD GIRL

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ABSTRACT

Neurocysticercosis, defined as infection of the brain and covered by the larval stage of the *Taenia solium* tapeworm. *Taenia solium* is the most common helmintic infection of the central nervous system and leading cause of acquired epilepsy worldwide. The aims of this study were to report a case of neurocystisercosis with epilepsy in 11 years old children and succesfull of seizure controll only with carbamazepine after treatment with albendazole and methyl-prednisolon. We reported a case, 11 years old girl, who presented with a new onset seizure, and then became two episodes of status epilepticus and the seizure were uncontrolled with 3 kind of anti epileptic drugs. The type of seizure was partial secondary generalized, initially with motor clonic of both upper and lower extremities and sometime on the trunk, followed with unconscious and drooling. The leading cause of intractable seizure was confirmed by neuroimaging head contras computed tomography (CT) scan and magnetic resonance imaging. The type of seizure was confirm by electroencephalography (EEG). The MRI revealed multiple cysts with scolex, which absolutely support the diagnosis of neurocysticercosis. The EEG showed multifocal epileptiform spike wave, sometime become to generalized. Other differential diagnosis such as toxoplasmosis and tuberculoma were excluded by clinical examination and negative serologic test. After the diagnosis confirmed, the patient were treated with albendazole 28 days, methyl-prednisolone for 7 days to prevent inflammation response after antiparacitic treatment and carbamazepine were used to control the seizure. Other 3 kind antiepileptic drug were tappering off. However the seizure could be controlled well only with single antiepileptic drug. Every child who presents with new onset seizures or other unexplained neurologic features and whose CT or MRI show cystic lesion or contrast enhancing rounded lesion should raise a suspicion of neurocysticercosis. So that we can treat the patient correctly after the diagnosis confirmed.

Keyword : neurocysticercosis, epilepsy, children

INTRODUCTION

Human neurocysticercosis (NC) refer to infection of the nervous system by the larvae stage of *Taenia solium*, is a major cause of epileptic seizures and other neurologic morbidity worldwide. The disease is widely prevalent, especially in several low- and middle income countries of the world, including India, China, sub-Saharan Africa, and Central and South America.[1-3]

Human cysticercosis occurs when a human host ingests infective eggs by fecal contamination and replaces the pig as intermediate host. Humans are the only host for the adult tapeworm and thus the only source of cysticercosis for pigs or other humans. Vegetarians and other people who do not eat pork can also acquire cysticercosis. Infection occurs when humans eat raw vegetables contaminated with *Taenia solium* eggs or food prepared by carriers of tapeworm.[4]

Human cysticercosis occurs anywhere in the human body, but becomes symptomatic almost exclusively in the nervous system or the eye. NC is generally classified into parenchyma and extra-parenchyma which include ventricular, cisternal, ophthalmic or spinal. The clinical manifestations of NC are pleomorphic. Several varieties of NC have been recognized depending upon the number, location, and evolutionary stage of the cysticerci in the human brain. The common clinical manifestation due to cyst location in the parenchymal, such as seizures, signs of increased intracranial pressure, focal neurodeficits, meningitis, and psychiatric disturbances. Seizure are the most common symptom in 70-90% of patients. Most children present with partial seizure (84-87%) particularly complex partial seizure; about a quarter have simple partial seizure. Most seizure are short duration, generally lasting for less than 5 minute. Status epilepticus has been reported in 1.7% to 32% cases.[4-6]

CASE REPORT

An 11 year old girl who was born and lived in Jimbaran, in a rural area of Bali. Shewas admitted to Sanglah Hospital because of recurrence seizure. The first seizure happened on August 2010, without fever, lasting approximately for 30 minute and type of seizure was initialy with motor clonic of both extemities,

and then she become unconscious and drolling. After seizure stopped, she was conscious.

Previously, she had no experience of seizure with or without fever. She also had headache since one year before the first seizure, she felt like pounding pain lasting 15-30 minutes without nausea or vomiting. Headache was become worse if she done more activities and relieved by rest. She had no history of chronic coughing, decreased of body weight and sweating in the night. She did not have history of closed contact to family with active tuberculosis. In her house there were no pet such as pig, chicken and cat, except a dog. When she was second grade at elementary school, her parents saw proglottids, the size about 1-2 cm but her parents reported never seen other worm in her feces previously. Her teacher also complaint that she had learning disability recently.

When she had first seizure, she was presented to the emergency department of private hospitals in Denpasar on August 2010, the type of seizure were tonic clonic, unconscious, and without fever. She had been hospitalized for four days. At the time, the diagnosis was epilepsy and she had medication with clobazam and valproic acid. Soon after hospital discharge, she had recurence the same type of seizures with duration approximately until 30 minute. She had been hospitalized and she was treated with the same medication from neurologist.

Three weeks after hospital discharge at October 2010, she had seizure without fever lasting approximately for two hours and hospitalized at intensive care unit (ICU) in private hospital. During in ICU, seizure recurring about 4-5 times a day. The seizures did not respond to treatment with diazepam, intravenous phenytoin and propofol. Because of seizure still uncontrolled, she was directly admitted to the Pediatric Intensive Care Unit (PICU) at Sanglah hospital. The seizure was successfully controled by phenobarbital (5 mg/kg iv) and Phenytoin (20 mg/kg iv). At the time, the working diagnosis was refractory status epilepticus. Computed tomography (CT) was done and the results was normal. After hospitalized for seven days, she refused continuing treated at Sanglah Hospital because her parents want to found alternative therapy.

In November 2010, she had seizure lasting for 15 minute and hospitalized at private hospital. Types of seizure were the same like first seizure. CT with contras was done and the result showed small multiple cysts at right and left sub-cortical temporal and cysts calcification at right and left parietal according to intraserebral cysticercosis or cerebral toxoplasmosis (figure 2). She had been treated by neurologist with anticonvulsant such as clobazam, phenytoin and topiramate.

On April 2011, The seizure were relapse again, the type of seizure were general, motor clonic that affected on both her upper and lower extremities symmetrically, sometime her trunk up and down look like dyspnea. The seizures were occured 3 times a day, duration 5-10 minutes each time, without fever. After seizure finished, she woke up normally. She was hospitalized and treated by phenobarbital intramuscular and continued with oral phenobarbital and other AED who used previously. Magnetic resonance imaging (MRI) and serologic for toxoplasmosis was done. she received albendazole 15 mg /kg/ day orally, prednisone, and antiepileptic drug.

She had no history of specific disease and no recent history of traumatic or infective episodes, neither ingestion of drugs. Daily activities prior to illness were normal. Her development was normal according to her age. History of immunization was completed according to the recommendation of Indonesian Pediatric Society. In family habits sometimes they were ate pork and Balinese food called "*Lawar*" (vegetables mixed with uncooked pork and fresh blood). Her parent did not realize if pork contained egg of *T. solium.* They used to eat pork 1-2 times a week.

Upon physical examination, she looks healthy with normal vital sign. Her body weight 33 kg and height 144 cm, appropriate with height for age z score -1,34 and BMI for age z score -1,15 (WHO Anthro-Plus). The general condition was normal (heart, lung and abdomen were normal, no sign of anemia, lympadenopathy nor icteric)

The examination of upper and lower extremities showed no deformities, no sign of joint or skin inflammation and the muscular examination showed normal strength. Patellar and Achilles tendon reflex were increased. There were no Babinski's sign, no ankle or knee clonus, muscle tone and sensibility were normal. Cervical spine, lumbar spine and neurological examination were unremarkable.

Based on history and physical examination, the clinical diagnosis was intractable epilepsy, caused by intracerebral cysts suspected neurocysticercosis, dd/ tuberculosis and toxoplasmosis.

Laboratory data included a complete blood count of WBC 9,0 k/µL (neutrophil 43,3%; lymphocyte 43,7%; monocyte 8,6%; eosinophil 3,5%; basophil 0,9%); Hb 13,0 g/dL; Hct 39,6% and Plt 448 k/µL. The result of serum electrolyte were sodium 140 mmol/L; potassium 3,6 mmol/L; chloride 102 mmol/L; calcium 8,8 mmol/L. Urine analysis was normal. Stool analysis revealed normal and no worm such as ancylostoma/necator, ascariasis, taeniasis and enterobius vermicularis. The result of serologic test for toxoplasmosis, both IgG and IgM were negative.

MRI of the brain showed small multiple cystic round lesion located at cortical parietal and frontal right and left side (figure 3).

The EEG was done to confirm the type of seizure. EEG showed background wave 6-7 wave mixed with 3-4 Hz mild voltage, without asymmetric. There were focal epileptiform wave become general at left temporal, parietal and frontal. So the conclusion of EEG was abnormal with focal to general epileptiform wave.

Based on the clinical manifestation, laboratory result and neuroimaging finding, our assesment was neurocysticercosis **(B69.0)** with partial secondary generalized epilepsy **(G40.2).** After the diagnosis was confirm in April 2011, appropriated therapy was started, she received albendazole orally for 28 days, prednisone, carbamazepine, and calcium. Other antiepileptic drug such as phenobarbital, clobazam, topiramate were tappering off immediately but one by one for about 1 month. In 2-4 weeks after those treatment completed, the seizure less relapsed with less duration. Two months later, there were no seizure anymore. So the seizure already controlled well until this report for 6 months. She has planning for MRI evaluation after 6 months.

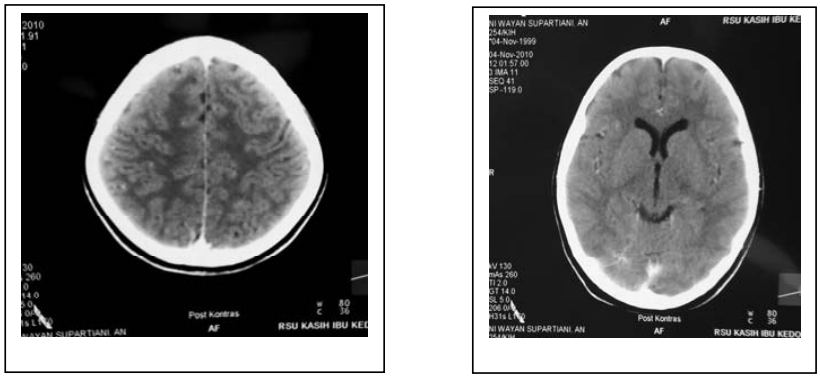

Figure 2. CT with contras showed small multiple cyst at right and left sub cortical temporal and cysts calcification at right and left parietal.

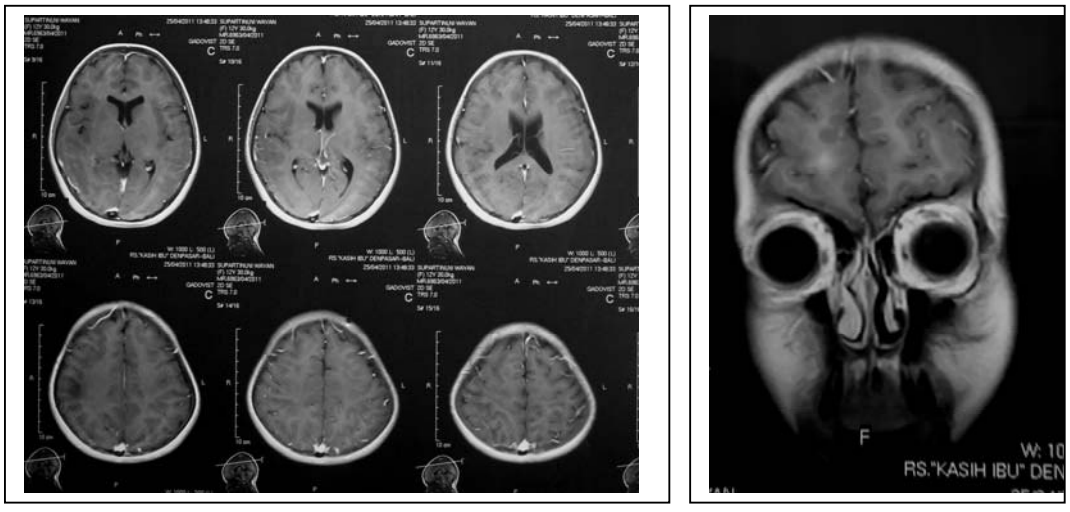

Figure 3. Brain MRI with contrast show calcified multiple lesions in cortex area of occipital, parietal and frontal of the brain.

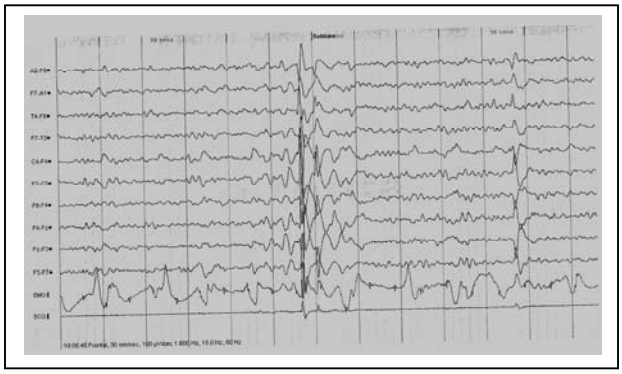

Figure 4. EEG record showing focal to general epileptiform wave.

DISCUSSION

We report, a girl at age 11 years old with NC and partial secondary generalized epilepsy. Over seven months she treated with multiple AED for epilepsy, but her seizure still uncontrolled. After MRI investigation and NC has already proven as a caused of epilepsy, prompt treatment can be given.

Taenia solium, responsible of the disease, can cause, intestinal infection and cysticercosis in human. In the classic life cycle, humans develop taeniasis after eating undercooked pork, contained cysticerci. Larvae develop into adult tapeworm inside human's small intestine. The tapeworm releases daily eggs disseminated to the environment through feces. In developing countries, where sanitary regulations are often neglected, roaming pigs may ingest food polluted by eggs, become an intermediated host.[4,5]

In our case, the parasite perceives human as an intermediate host and disseminates to a variety of organs, including the central nervous system, where cysticerci developed. She might got NC, because of eat undercooked pork related to Balinese habit.

The clinical manifestations of NC are variable, depends on the number, stage, location as well as the host response. A large part of individuals holding the parasite in the central nervous system are asymptomatic. In some patients symptoms may develop many years after brain infection, as it has happened to our patient. The main clinical manifestations of NC are seizures, headache, and focal neurological deficits, and it can have such sequelae as epilepsy, hydrocephalus, and dementia.[3,4,6]

In our case, she had recurrence seizure and type of seizure is partial secondary generalized. EEG was abnormal and showed epileptiform wave at left temporal become general.

Neurocysticercosis predominantly affects adults in their third or fourth decade of life; it is uncommon in children and elderly people because the incubation period of *T. solium* is long. The disease is recognized mainly in children older than 7 years, owing to this incubation period.[3]

In our case, NC had been diagnosed when she had recurrent seizure at 11 years old.

Histological demonstration of parasites from a brain biopsy should be as a "gold standard" for fixed diagnosis of NC. Clearly this procedure is limited because of very invasive. Therefore neuroimaging remains the main instrument to confirm the diagnosis. In the identification of extraparenchymal cysts MRI is more sensitive than CT scan. The only reliable tool for diagnosis of NC is imaging by CT or MRI. The presence of viable cysts with a mural nodule, associated with degenerative cysts and calcifications, is typical.[3,6]

In our case, using **C**T and MRI, the multiple cystic lesion found at cortex of occipital, parietal and frontal of the brain.

Immunological tests have low sensitivity and specificity, especially for single lesion. The ELISA has 65% specificity and 50% sensitivity and it can give false negative results in case of parenchymal NC, inactive lesion or helminthes infections. The enzyme-linked immuno-electrotransfer blot assay, using purified glycoprotein antigens from *Taenia solium* cysticerci, has been reported to be highly specific 100% and nearly 98% sensitive for patients with either multiple active parenchymal cysts or extraparenchymal NC.[2,3,7]

In our case, she had no examined by Enzyme linked immuno-absorbent assay (ELISA) nor Enzyme linked immuno-electrotransfer blot assay (EITB) because limitation of laboratory facility in Bali to do this examination. The only serologic test for toxoplasmosis was done in this patient, to exclude other etiology of cystic lesion and the result of serologic test for toxoplasmosis was negative.

Other differential diagnoses is tuberculoma, microabcesses, low grade astrocytoma, cystic cerebral metastasis, toxoplasmosis and fungal lesion.[4,5]. **In our case,** MRI revealed multiple cystic lesion appropriate with NC, but other diagnosis for exclude differential diagnosis showed single small enhancing computed tomographic lesion (SSECTL).

Treatment should be individually fitted for each patient, with antiepileptic drugs, analgesics, corticosteroids, or a combination of these. Anthelmintic drugs (praziquantel and albendazole) are used routinely, but so far no controlled clinical trial has established specific indications or definitive doses of treatment. Antiparasitic drugs are the mainstay of treatment; in particular, albendazole may be favourable for the treatment of parenchymal cysts because of its power to pass into cerebral spinal fluid. Previous studies recommended the administration of albendazole at a dosage of 15 mg/Kg/day for 8-28 days, but later studies prove that a 1 week course is equally effective. Corticosteroids are useful for reducing local edema and inflammation around dying parenchymal cysts; so they are often administered together with anti parasitic drugs. Corticosteroids are commonly administered in NC on the premise that they reduce inflammation and edema around dying parenchymal cysts and are also recommended for treatment of large subarachnoid cysts, arachnoiditis, and cysticercotic encephalitis.[6] However, the dose, duration, form, mode and most importantly, timing of administration of corticosteroids are not clear.[3,7,8]

In our case, patient treated with albendazole 200 mg twice daily for 28 days and prednisone 15 mg twice daily for seven days, carbamazepin 15 mg/kg, and supplement of calcium because of the patient also had hypocalcaemia. We stopped other AED one by one. The seizure were controlled well 2-4 weeks after those treatment completed.

Neurosurgical intervention should be considered for hydrocephalus requiring ventriculoperitoneal shunt, accessible racemose cysts in the basal cisterns, or a cyst in an intraventricular location. Transitional or degenerative cysts, of any size or location, should not be sampled or removed because the parasite was dead and will disappear or be calcified spontaneously.[7-9] **In our case**, no indication for neurosurgical intervention.

Parenchymal forms of neurocysticercosis have a good prognosis in terms of clinical remission. **In our case** the prognosis is good because the seizure already controlled well until now only with single AED (carbamazepine), although repeted MRI and EEG still needed to make sure.

CONCLUSION

WS, 11 years old girl, Balinese, had chief complaint recurrence seizure and unresponsive with AED's drug. Seizure was occured since August 2010 and became worse until April 2011. The type of seizure was partial secondary generalized. Other symptoms were headache and decreased of kognitif achievement. The physical examination only found increased of patella reflexes of both lower extremities. Based on CT, MRI and EEG diagnosis of NC with partial secondary generalized were confirmed. Patient was administered with albendazole as antiparasitic days, prednisone as anti inflamation, and carbamazepine as AED. The seizure was controlled well until now only with single AED (carbamazepine). So a good clinically prognosis was aimed after thetreatment.

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EFFECT OF DIETARY LIPIDS RATIO AND CHOLINE DEFICIENCY ON HEPATIC ANTIOXIDANT ENZYME ACTIVITIES

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ABSTRACT

The composition of fat rather than the amount in the diet was the major factor influencing the amount of liver lipid in choline deficiency rats. Therefore this study was undertaken to learn the effect of lard/soybean oil ratio on antioxidant status in liver of rats fed the choline deficiency diet. The choline-deficient diet was prepared similarly as the basal diet except choline was omitted. The ratio of dietary lipid was prepared as CF1 which contains a mixture of lard : soybean oil (19 : 1 w/w) and CF2 contains a mixture of lard : soybean oil (1 : 19 w/w).

The activities of total SOD, Cu,Zn-SOD, and glutathione peroxidase were significantly lower in the liver of CF1 rats than the basal and CF2 rats. Catalase and Mn-SOD activities in liver were not significantly different among three groups. The levels of α-tocopherol were significantly depressed in liver of CF1 rats, while of α-tocopherol values were not different between the basal and CF2 rats. Lipid peroxidation (TBARS) levels were significantly higher in the liver of CF2 rats than the basal and CF1 rats.

Choline deficiency induced significantly lowered antioxidant enzymes activities in response to increasing dietary lard ratio in the diet. In addition, the increased in liver lipid contens and serum ornithine carbamoyltransferase activity indicated the liver was damaged in rats fed the choline deficient which contains a mixture of lard : soybean oil (19 : 1) in the diet. Thus, dietary lipid plays an important role in determining cellular susceptibility to oxidative stress and liver damage induced by choline deficiency.

Keywords: choline deficiency, antioxidant enzymes

INTRODUCTION

Choline deficiency induces lipid accumulation in the liver (Lombardi et al., 1966; Aoyama et al., 1971) because choline is required for hepatic secretion of lipoproteins. Rats fed choline deficient diet showed a very reproducible pattern of lipid peroxidation in the liver nucleous followed by a similar effect in liver mitochondria (Blusztajn, 1989). Free radical-mediated lipid peroxidation has been implicated in a variety of pathological processed. The susceptibility of given organism to oxidative damage is influence by the overall balance between the degree of oxidative stress and antioxidative capabilities.

Dietary lipid plays an important role in determining cellular susceptibility to oxidative stress. Buckingham (1985) has reported that during marginal vitamin E elevated the ratio of polyunsaturated/ saturated fatty acids may directionally enhance the rate of in vivo lipid peroxidation. The composition of the fat, rather than amount in the diet, was the major factor influencing the amount of liver lipid in choline deficiency rats. Therefore this study was undertaken to learn the effect of lard/soybean oil ratio on antioxidant status in liver of rats fed the choline deficiency diet.

MATERIALS AND METHODS

Animal

This study complied with the animal experimental guides according to the committee of experimental animal care at Hokkaido University, Japan. Male rats of Wistar strain of specific pathogen free-type (Japan SLC, Inc., Hamamatsu, Shizuoka Japan), weighting approximately 65 g at the beginning of experiment were housed individually in stainless steel wire-bottom cages in an air-conditioned room at approximately 23°C. Lighting was regulated to provide equal periods of light (08:00- 20:00 h) and dark (20:00-08:00 h). Rats were divided into three groups each contained 10 rats, and allowed free access to the appropriate experimental diet and water for 21 d *ad libitum*

Diets

The compositions of diets are shown in Table 1. The choline-deficient diet was prepared similarly as the basal diet except choline was omitted. The ratio of dietary lipid was prepared as CF1 wich contains a mixture of lard : soybean oil (19 : 1 w/w) and CF2 contains a mixture of lard : soybean oil (1 : 19 w/w). Body weight and food intakes were measured daily throughout the entire experimental period.

Table 1. The composition of Diet

	Basal	CF1	CF2
Casein (vitamin-free)	140	140	140
Vitamin Mixture (AIN-93G)	10	10	10
Choline Bitartrate	2,5	-	-
Mineral mixture (AIN-93G)	35	35	35
Soybean oil	10	10	190
Lard	190	190	10
Sucrose	612,5	615	615

CF1 = choline deficient diet which contain lard : soybean oil = 190 :10 (g/kg) diet.
CF2= choline deficient diet which contain lard : soybean oil = (10 : 190 g/kg) diet

Serum and tissue collection

After feeding for 21 d, the rats were decapitated and blood samples were collected. The liver samples were immediately removed, weighed, and frozen by dropping into liquid nitrogen. The serum samples were obtained from the blood samples by centrifugation at 1000 x g for 10 min. The liver and serum samples were stored in -80^{o}C until need for assay.

Analytical procedures

Hepatic total lipids were extracted and purified according to the method of Floch et al., (1957) the liver lipids being gravimetrically estimated after removing the solvent. Triacylglycerol (Nagele et.al., 1985) and cholesterol (Siedel et.al., 1983) in the liver were estimated by enzymatic methods, respectively. Phospholipids in the liver were calculated by the method of difference [total lipids – (triacylglycerol and cholesterol]. Serum triacylglycerol (Nagele et.al., 1985), cholesterol (Siedel et.al., 1983) and phospholipids (phospholipids containing choline) (Takayama et.al., 1977) were measured by enzymatic methods, respectively.

Serum ornithine carbamoyltransferase activity was estimated according to the method of Oshita et. al. (1976). Superoxide dismutase (EC 1.15.1.1) was measured according to the method of McCord et. al., 1969. Catalase (EC 1.11.1.6) activity was determined by following reduction of hydrogen peroksidase as describe by Aeibi et. al. (1974). Gluthathione peroxidase (EC 1.11.1.9) activity was measured by the method of Paglia (1967). The level of lipid peroxidation was quantified according to the method of Ohkawa et al (1979). α-Tocoperol was measured by HPLC according to the method of Ueda et al (1987).

Significant of the differences among mean values were determined by Duncan's multiple range test. Values of $P<0.05$ were considered to be significant.

RESULTS AND DISCUSSION

Body weight gain of rat fed choline deficient diet both of CF1 and CF2 were significantly decrease as compared to basal diet, while food intake were not different among three groups. The liver weight were significantly different among three groups, and the highest value was noted in CF1 groups (Table 2)

Choline deficiency caused an accumulation of lipids in the liver (Table 3). The total lipids in the liver of rats fed the choline-deficient diet both of CF1 and CF2 were significantly higher than those of rats fed the basal diet. Choline is needed for hepatic secretion of lipoproteins. Hepatic secretion of very low density lipoprotein (VLDL) from liver into blood requires active phosphatidylcholine biosynthesis (Yao et al., 1989) and in choline deficiency, the diminished capacity of liver cells to synthesize new phosphatidylcholine molecules results in accumulation of triacylglycerol in the liver. In fact, serum triacylglycerol content

was significantly different among three groups (Table 3). Benton et al.,1956, Have reported that different dietary fat have marked differences in ability to increase the liver lipids in rat fed hypolipotropic diets. In this study was found that the ratio of lard : soybean oil (19 : 1) in the choline deficient diet produces higher liver lipids than that of the ratio of lard : soybean (1 : 19) in the diet.

Table 2. Food intake, body weight gain, and liver weight of rat fed either the basal or the choline deficient diets which contains different ratio of fat.

Diets	Food intake (g/21 d)	Body wt. gain (g)	Liver wt. (g/100 g of body wt.)
Basal	243 ± 7 a	92,6 ± 2,6 a	4,91 ± 0,05 c
CF1	230 ± 5 a	75,8 ± 2,2 b	8,34 ± 0,12 a
CF2	230 ± 5 a	74,9 ± 2,4 b	6,40 ± 0,10 b

Data represent means ± SEM for 10 rats
a,b,c Means within the seam vertical column that do not share a common letter were significantly different (P<0,05)

Table 3. Lipids profil in the liver and serum of rat fed either the basal or the choline deficient diets which contains different ratio of fat.

Diet	Lipids profil in the liver				Lipids profil in the serum		
	total lipid (mg/g)	triacylglycerol (mg/g)	cholesterol (mg/g)	phospholipid (mg/g)	triacylglycerol (mg/dl)	cholesterol (mg/dl)	phospholipid (mg/dl)
Basal	61,3 ± 2,0 c	18,4 ± 0,9 c	3,79 ± 0,19 a	39,1 ± 1,3 c	287 ± 29 a	78,9 ± 2,9 a	219 ± 9,0 a
CF1	358 ± 8,0 a	86,4 ± 5,0 b	3,64 ± 0,13 a	228 ± 17 a	110 ± 8,0 b	55,6 ± 2,1 b	138 ± 4,0 b
CF2	249 ± 9,0 b	114 ± 4,0 a	3,06 ± 0,11 b	131 ± 7,0 b	125 ± 20 b	46,8 ± 2,1 c	132 ± 8,0 b

Data represent means ± SEM for 10 rats
a,b,c Means within the seam vertical column that do not share a common letter were significantly different (P<0,05)

Serum ornithine carbamoyltransferase (OCT) activity in basal diet was 3,91 ± 0,60 b, CF1 was 12,1 ± 2,3 a, and in CF2 was 5,05 ± 0,42 b. This enzyme is specially located in the liver with low values in other tissues. The increased in circulating OCT are considered to be highly specific for hepatotoxicity. In this study was found that rats fed CF2 induced accumulation of lipids in the liver, but did not induced liver damage.

The antioxidant defense system is composed mainly of three enzymes such as superoxide dismutase, catalase, and glutathione peroxidase. A significant decline in the level of liver total superoxide dismutase, Cu, Zn- superoxide dismutase, glutathione peroxidase, and α-tocopherol (Table 4) of rat fed the choline deficient diet which contain lar : soybean oil ratio (19 ; 1) CF1 was observed in this study. The decrease in enzymatic and non enzymatic antioxidant protection might induce elevation in lipid peroxidation level (Yu, 1994). Lipid peroxidation contentof CF2 groups are significantly increased as compared to the basal and CF1 groups. Huang et al (1992) have reported that the elevation of the dietary soybean oil level in the protein diet significantly increases tissue lipid peroxidation and reduced the activities of antioxidant enzymes. However, in this study, was observed that the antioxidant enzymes activities of CF2 group were not significantly different to that of the basal group. The mechanism of choline and dietary soybean oil on the activities of antioxidant enzymes is unknown. Although an effect of choline in decomposition of lipid hydroperoxide has been reported (Miyazawa et al., 1984)

In conclusion, Choline deficiency induced significantly lowered antioxidant enzymes activities in response to increasing dietary lard ratio in the diet. In addition, the increased in liver lipid contens and serum ornithine carbamoyltransferase activity indicated the liver was damaged in rats fed the choline deficient which contains a mixture of lard : soybean oil (19 : 1) in the diet. Thus, dietary lipid plays an important role in determining cellular susceptibility to oxidative stress and liver damage induced by choline deficiency.

Table 4. Antioxidan status in the liver of rat fed either the basal or the choline deficient diets which contains different ratio of fat.

Diet	Superoxide Dismutase (SOD) U/mg Protein			Catalase	GPx	TBARS	α-tocopherol
	Total SOD	Cu, Zn - SOD	Mn - SOD	U/mg Protein	(U/mg Protein)	nmol/g liver	nmol/g lipid
Basal	58,1 ± 2,3 a	49,4 ± 2,2 a	8,75 ± 0,57 a	1,42 ± 0,04 a	0,493 ± 0,012 a	187 ± 7 b	181 ± 6 a
CF1	44,5 ± 2,2 b	34,7 ± 3,0 b	9,83 ± 0,93 a	1,60 ± 0,07 a	0,373 ± 0,025 b	197 ± 3 b	100 ± 3 b
CF2	57,4 ± 5,3 a	48,2 ± 4,8 a	9,18 ± 0,14 a	1,38 ± 0,05 a	0,445 ± 0,020 a	257 ± 8 a	157 ± 16 a

Data represent means ± SEM for 10 rats
a,b,c Means within the seam vertical column that do not share a common letter were significantly different (P<0,05)

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INHIBITION OF IODIZED TABLE SALT ON GROWTH OF STREPTOCOCCUS MUTANS IN-VITRO

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ABSTRACT

Caries is a disease similar to other disease, because this disease can disturb daily activities. This research aims to determine the use of table salt for inhibiting the growth of *Streptococcus mutans*. This is a true experimental with *post test only control group design*, applying non and mark table salt. In this study, it was obtained that unmark table salt with 4%, 6%, and 8% concentration are not effective to inhibit the growth of *Streptococcus mutans*. In addition, mark table salt with 4% and 6% were also not effective against *Streptococcus mutans*. However, mark table salt with 8% concentration was found effective towards *Streptococcus mutans*. In this study, inhibition of *Streptococcus mutans* was also treated by applying iodine solution. With concentration of iodine similar to mark table salt concentration of 4%, 6%, and 8%. It was obtained that the iodine solution concentration similar to 4%, 6%, and 8% inhibit the growth of *Streptococcus mutans*, indicated by inhibition zone of 22.17 ± 2.02 mm; 26.33 ± 3.18 mm; and 24.33 ± 1.52 mm, respectively. From this study, it can be concluded that mark table salt with concentration of 8% inhibit the growth of *Streptococcus mutans*. Future study should be carried out in order to determine the growth of *Streptococcus mutans* using the combination of iodine and sodium chloride to understand the combination effect of these two compounds.

Keywords: caries, *Streptococcus mutans*, unmark table salt, mark table salt, iodine solution.

INTRODUCTION

Caries is a disease similar to other disease, because this disease can disturb daily activities. There are many factors stimulate of caries, i.e. host (dental structure and saliva), diet, microorganism and time (Willet, *et al*., 1991; Kidd, *et al*., 1992; Samaranayake, 2002). Miller theorem reveals that caries occurs as results of decalcification of solid substance of the teeth due to an acid product. As an acid source are bacteria activities that fermented carbohydrate. On mouth cavity, there are many microorganisms as a normal flora and these microorganisms live in balance with the host (Kidd, *et al*., 1992; Samaranayake, 2002).

Streptococcus, Staphylococcus, Lactobacillus, and filament bacteria are common microorganisms found in the deep of lyses caries. Amongst these bacteria, *streptococcus* is the most found, therefore it was said that these bacteria play an important role on teeth pulpa disease. The most common *Streptococcus* observed is *Streptococcus mutans* (Willett, *et al*., 1991; Sidarningsih, 2000; Samaranayake, 2002; Nomura, *et al*., 2004).

There are many ways to prevent dental caries before undergoing dental medical care, such as providing aseptic environment of mouth cavity. This can be achieved by using antiseptic material that can decrease population of bacteria in mouth cavity (Ford, 1993; Forrest, 1995; Laksminingsih, 2000; Soeherwin, *et al*., 2000). Mouthwash in the market are too many and each of them have advantages and disadvantages, such as have side effect of discoloring teeth and tongue, disturbance of taste after gargling. Besides that, not all of society can easily obtain mouthwash as well as not affordable. Therefore, a cheap and easily to gain traditional material also no adverse effect is the interesting thing that can be applied as an alternative to replace the mouthwash (Soeherwin, *et al*., 2000).

Washing mouth by applying 0.9% sterile physiologic sodium chloride (NaCl) decrease 35% of population bacteria (Muthalib and Mangundjaya, 1975). Sodium chloride inhibition test towards 211 *Enterococcus* strain and 68 D-*Streptococcus* strain isolated in clinical laboratory to determine different between *Enterococcus* and D-*Streptococcus* on human gastro intestinal tract, indicates that D-*Streptococcus* are not growing in *Brain Heart Infusion* (BHI) agar media mixed with dextrose and 6.5% NaCl. Meanwhile, *Enterococcus* is surviving (Soeparmin, 1991).

MATERIALS AND METHOD

This is a true experimental study with post test only control group design to determine whether *Streptococcus mutans* ATCC 35668 persist towards varies percentages of table salts (marked an unmarked). There were 4 groups in this study, i.e. control group with sterile aquadest, the second group treatment with unmarked table salts (4%, 6%, and 8%), the third group treated with marked table salts (4%, 6%, and 7%), and the last group treated with iodine solution with concentration similar to 4%, 6%, and 8% marked table salts.

RESULTS AND DISCUSSION

Iodine concentration obtains from marked and unmarked table salts and iodine solution for treatment in this study were presented in Table 1.

Table 1 Iodine Concentration of Each Table Salts and Iodine Solution

No	Table Salts Type	Iodine (ppm)
I	Unmarked Table Salts	
	1. 4%	1.51
	2. 6%	2.26
	3. 8%	3.01
II	Marked Table Salts	
	1. 4%	2.19
	2. 6%	3.29
	3. 8%	4.38
III	Iodine Solution	
	1. Equal to 4%	2.19
	2. Equal to 6%	3.29
	3. Equal to 8%	4.38

Table salts solution effectively towards *Streptococcus mutans* was carried out following inhibition zone method using paper disc. This paper was immersed in table salts solution for 60 second and for control negative was immersed on aquadest. The disc was then placed on MH media agar and incubated overnight at temperature of 37^0C as indicated on Figure 1.

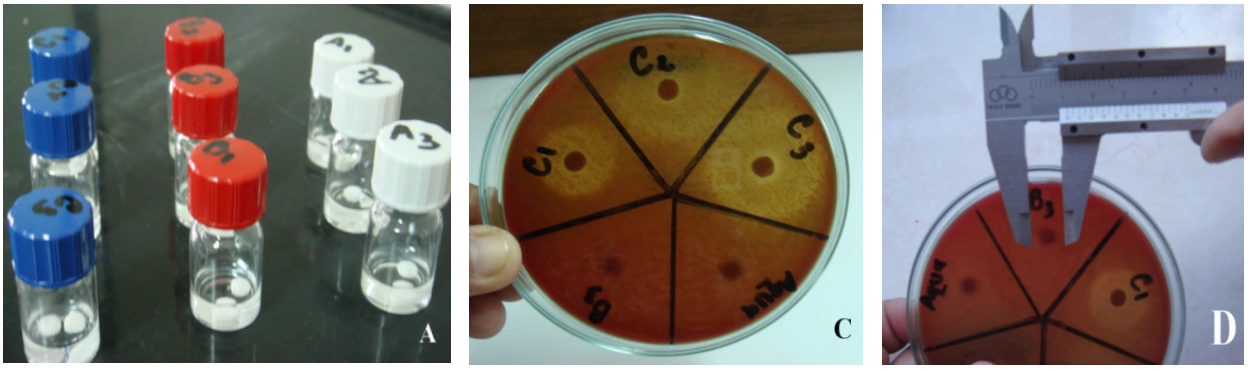

Figure 1
Affectivity Test of Table Salts Solution towards *Streptococcus mutans*

Inhibition zone for all experiments were presented on Table 2.

Table 2 Inhibition Zone of Table Salts and Iodine Solution towards *Streptococcus mutans*

No	Treatment	Inhibition Zone (mm)		
		4%	6%	8%
1	Control	0	0	0
2	Unmarked Salt 1	0	0	0
	Unmarked Salt 2	0	0	0
	Unmarked Salt 3	0	0	0
3	Marked Salt 1	0	0	8.0
	Marked Salt 2	0	0	8.5
	Marked Salt 3	0	0	8.5
4	Iodine Solution 1	20.0	24.0	22.5
	Iodine Solution 2	24.5	24.5	30.0
	Iodine Solution 3	23.0	24.0	26.0

Data on Table 2 were not normally distributed and their variances were also not homogenous ($p < 0.05$). Therefore, to determine the different between treatments, non parametric Kruskal-Wallis test was performed. From the test it was observed that there were a significant different among marked table salt, unmarked table salt, and iodine solution ($p < 0.05$). The difference was then performed by applying *Post Hoc Test* Tamhane and the resume of the results were presented on Table 3.

Table 3 Different among varies treatment towards Inhibition Zone of *Streptococcus mutans*

Multiple Comparisons Tamhane

Dependent Variable	(I) treatment	(J) treatment	*Mean Difference* (I-J)	*Std. Error*	*Sig.* (*p*)	*95% Confidence Interval*	
						Lower Bound	*Upper Bound*
Inhibition Zone 4%	control	Unmarked table salts	0.000	0.000	.	0.000	0.000
		Marks table salts	0.000	0.000	.	0.000	0.000
		Iodine	-22.17*	1.167	0.016	-34.731	-9.603
Inhibition Zone 6%	control	Unmarked table salts	0.000	0.000	.	0.000	0.000
		Marked table salts	0.000	0.000	.	0.000	0.000
		Iodine	-26.33*	1.833	0.029	-46.077	-6,589
Inhibition Zone 8%	control	Unmarked table salts	0.000	0.000	.	0.000	0.000
		Marked table salts	-8.333*	0.167	0.002	-10.1282	-6.5385
		Iodine	-24.33*	0.882	0.008	-33.8309	-14.8357

**. The mean difference is significant at the 0.05 level.*

In this research, it was obtained that treatment of 4% table salts in either marked or unmarked did not inhibit the growth of *Streptococcus mutans* compare to control. However, for 4% iodine solution has already significantly inhibited the bacteria growth indicated by the present of inhibition zone of 22.16 mm with $p < 0.05$.

This research indicates that even though marked and unmarked table salts have already contained of iodine, did not inhibit the growth of *Streptococcus mutans* as shown by iodine solution. The inhibition was performed by 8% marked table salts.

CONCLUSION AND FUTURE WORK

Among varies concentration of 4%, 6%, and 8% marked table salts, only marked table salts with concentration of 8% inhibit the growth of *Streptococcus mutans* invitro. Further research regardless of invivo test need to be carried out.

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ANTIBIOTIC SENSITIVITY PATTERN AMONG *SERRATIA MARCESCENS*I SOLATES FROM BLOOD SPECIMENS IN SANGLAH HOSPITAL

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ABSTRACT

Serratia marcescens is a member of the family Enterobacteriaceae. It is one of the causative agent of hospital acquired pneumonia, corneal ulcer among contact lenses wearer, bone and joint infection, bacteremia, and any other infections in patients treated in Intensive Care Unit. This bacterium is also found to contaminate infusion solution. *Serratiamarcescens* is often found to be resistant to commonly used beta lactam antibiotics.This study was aimed to demonstrate antibiotic sensitivity pattern among *Serratiamarcescens* isolates from blood specimens in Sanglah Hospital from January to June 2011 using Disc Diffusion Test method standardized by Clinical and Laboratory Standards Institute (CLSI) 2011.During 6 months of this descriptive study, there were 29 *Serratia marcescens* isolates found. All those isolates showed resistance to Ampicillin/Sulbactam, Amoxicillin/Clavulanic acid, Cephalohtin, Cefuroxime, Ceftazidime, Aztreonam, and Gentamicin. Sensitivity to Chloramphenicol, Ciprofloxacin, Trimethoprim/Sulfamethoxazole, Amikacin, Meropenem, and Imipenem were shown in 89,7%, 96,3%, 96,6%, 77,3%, 81,2%, and 65,2% isolates, respectively. Some problems in the treatment management of infection caused by *Serratiamarcescens* in Sanglah Hospital persist since a few antibiotic choices sensitive to this bacterium are available. Moreover, multi resistant *Serratia marcescens* strains have begun to arise.

Keywords: *Serratia marcescens*, bacteremia, antibiotic sensitivity

INTRODUCTION

Bacteremia have serious impacts, especially in the pediatric patients.In Sanglah Hospital, most blood samples suitable for culture come from pediatric ward and *Serratiam arcescens*was the second most bacteria isolated from blood specimens.The most common bacteria isolated was still *Pseudomonas sp.*

Serratia marcescens is a Gram negative bacilli classifiedi n the family of Enterobacteriaceae[1]. This bacteria normally resides human gastrointestinal tract, but they are also found in the environment and can even survive on the human skin for extended period of time[2]. Morever, soap and detergents have been reported as potential sources for *Serratia marcescens*outbreak[2]. This bacteria is also found to contaminate infusion solution[3]. In clinical setting, this bacteria was confirmed to be one of the causative agent of hospital acquired pneumonia, corneal ulcer among contact lenses wearer, bone and joint infection, bacteremia, and any other infections in patients treated in Intensive Care Unit[3]. This bacteriais a opportunistic agent that can rapidly spread in the nosocomial setting[2]. About 50% nosocomial infection are caused by aerobic Gram negative bacilli, including *Serratia marcescens*[3]. There are several outbreaks of *Serratia marcescens* in Neonatal Intensive Care Unit (NICU) all around the world in recent years that has caused potentially fatal sepsis, meningitis, or pneumonitis in the very premature and low birth weight infants, with mortality rates about 44%[2]. Based on Clinical and Laboratory Standards Institute (CLSI) 2011, the first antibiotic of choices for infection caused by this bacteria are Ampicillin, followed by Cefazolin, Gentamycin, Tobramycin, and then Amikacinin relative order of choice[4].

MATERIALS AND METHOD

Blood specimens were first incubated into Bactec until positivity observed. The blood specimens identified positive by Bactec were then cultured to sheep blood agar and MacConkey agar and incubated at 37°C. After 18-24 hours of incubation period, the growth of *Serratia marcescens* was first suspected by its colony morphology. The colony of suspected *Serratia marcescens*appears brick red in MacConkey agar plate after 37°C incubation for about 24 hours and prolonged incubation at room temperature.After characterization of the colony growth, Gram staining and further identification using Microgen were done. On Gram staining, it was shown to be Gram negative bacilli. Identification was done using commercial kit (Microgen) to confirm the presence of *Serratia marcescens* in the colony.

On the same day of identification, colony of *Serratia marcescens* were then cultured to the Mueller

Hinton agar and some antibiotic discs were placed. This agar was then reincubated for another 24 hours before antibiotic sensitivity pattern examined.

RESULTS

During six months period (January – June 2011) of this observational study, there were 29 *Serratia marcescens*isolates identified. For the blood specimens, *Serratia marcescens* was the second most common bacteria isolated. All those isolates showed resistance to Ampicillin/Sulbactam, Amoxicillin/Clavulanic acid, Cephalothin, Cefuroxime, Ceftazidime, Aztreonam, and Gentamicin. Sensitivity to Chloramphenicol, Ciprofloxacin, Trimethoprim/Sulfamethoxazole, Amikacin, Meropenem, and Imipenem were shown in 89,7%, 96,3%, 96,6%, 77,3%, 81,2%, and 65,2% isolates, respectively. There was also found panresistant isolate. Furthermore, *Serratia marcescens*was not isolated from any other specimens type.

DISCUSSION

This observational study clearly show that *Serratiamarcescens*still become a problem in the clinical setting in Sanglah Hospital. Antibiotic sensitivity pattern among *Serratia marcescens* isolated from blood specimens in Sanglah hospital show all isolates resistant to nearly all β-lactam antibiotics and some degree of sensitivity persists to any other group of antibiotics. However, problems in the management of bacteremia caused by this bacteria persist since antibiotic choices is quite restricted, especially for Pediatric patients. Continuing reports in hospitals all around the world regarding *Serratia marcescens* outbreaks should alert all of us about the significance of this bacteria and the importance of preventing any infection by any measures such as proper hand washing, proper antiseptic use before specimens collection, and practicing general precautions.

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IDENTIFICATION MITOCHONDRIAL NUCLEOTIDE 3130-3404 MUTATION IN 30 INDONESIAN SUBJECTS WITH TYPE 2 DIABETES MELLITUS*

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ABCTRACT

The objective of this study was to investigate the roles of various mtDNA mutations or variants of mitochondrial nucleotide 3130-3404 in Type 2 Diabetes Mellitus in Indonesian subject. The method used was described as follow: a total of 30 Indonesian subjects with type 2 diabetes mellitus were recruited for this study. Mitochondrial nucleotide 3130-3404 including 16s rRNA gene, tRNA leu gene and NADH Dehydrogenase 1 gene were detecting using PCR amplification and Direct DNA sequencing. The result showed that four homoplasmic nucleotides were observed: T3200C, C3206T, C3210T and T3398C. Mutation T3200C, C3206T and T3398C has been detected in indonesian subject with type 2 diabetes mellitus. C3210T mutation always found in patient as a linkage with T3398C mutation.

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INTRODUCTION

Numerous mitochondrial DNA mutations are significantly correlated with development of some diseases. One kind of genetic disease is type 2 diabetes mellitus. Number of people with type 2 diabetes mellitus increase rapidly also for Indonesian population. Mutations in mitochondrial DNA (mtDNA) are associated with type 2 diabetes mellitus because ATP plays a critical role in the production and the release of insulin. In 1992, Ouweland *et al* first described a large pedigree with maternally transmitted type 2 diabetes mellitus and deafness caused by A to G transition at 3243 in the mitochondrial tRNA leu gene. Since then, the association of mtDNA and diabetes mellitus has been further examined by many other researchers (Tang *et al* 2006).

Previous efforts were focused on several site, the most common mutant site was A3243G in tRNA Leu gene, followed by G3316A, and T3394C in ND1 gene. T3200C mutation in 16S rRNA gene also related with type 2 diabetes Mellitus (Tao *et al,* 2002). The most common mitochondrial point mutation, A3243G tRNA leu gene reported associated with any disorder like MELAS and type 2 Diabetes Mellitus. The mutation resides in the mitochodrial transcription terminator binding site, which adjoin 3' end of the 16S rRNA gene and therefore, could affect not only the amino sequences of the structural genes but also their termination (Tao *et al*, 2002). To investigate the roles of various mtDNA mutations or variants in Type 2 Diabetes Mellitus in indonesian subject, we developed a detection system using PCR and Sequencing analysis for defining mtDNA mutation in the tRNA leusin gene region.

MATERIALS AND METHODS

In this study, we only used 30 subjects with type 2 diabetes mellitus and informed concern was obtained. The selection was based on clicinal test completely. Total DNA was prepared from peripheral blood samples using wizard kit DNA extraction (Promega Inc). The primer used mt 3243 forward 5'-AGG ACA AGA GAA ATA AGG CCT-3' and mt3243 reverse 5'-AAC GTT GGG GCC TTT GCG T-3' (Zhong *et al,* 2000) to amplified 294 bp tRNA leu gene. PCR reaction was performed in a final volume 20 µl as

follows: DNA sample was added to reaction mixture 10X taq Buffer, 10 mM dNTPs, 5 U taq takara, 0,2 µl for each Primer Mt3243 forward and reverse (1st Base). The fragment followed electhrophoresis on 2% agarosa gel, EtBr staining. PCR product wer purified using DNA Purification kit (Qiagen) and used as template for sequencing. DNA template were analized by a DNA Sequencer (ABI Prism 3130), mtSNP were identified by comparison with human mitochondrial genom sequence from Genebank NCBI.

RESULTS

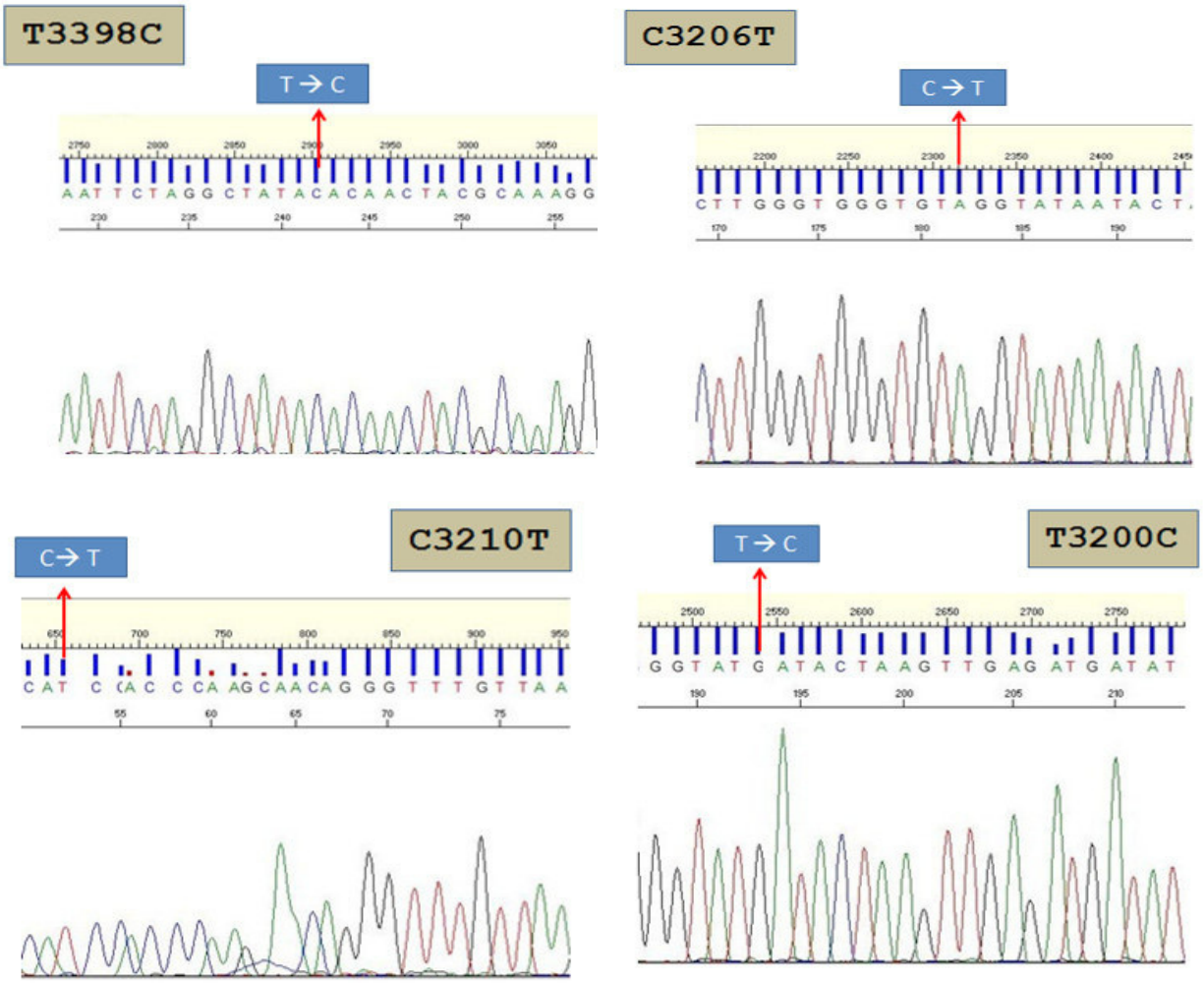

Figure 1. Sequences of mtDNA point mutation. Arrows for mutant base; A: mtDNA T3200C mutation; B: mtDNA C3206T mutation; C: mtDNA C3210T mutation; D: mtDNA T3398C mutation.

DISCUSSION

Thirty Type 2 Diabetes Mellitus patients have four different point mutations. The mutations were detected by sequencing analysis, namely T3200C, C3206T, C3210T, T3398C. No A3243G mutation found in all subjects in this research. A3243G is heteroplasmy mutation. A pathogenic mtDNA usually exists in heteroplasmic form, with the existence of both mutant and wild-type mtDNA in affected cells. The degree of heteroplasmy also varies considerably in different tissues and among different individuals. Leucocytes, which are currently used by most workers as the source of mtDNA, generally contain a lower proportion of mutant mtDNA than other cells (Zhong *et al*, 2000).

T to C transition at 3200 in the 16S rRNA gene has been reported in chinese population (Tao *et al*, 2002). T3200C mutation is sufficient to disrupt normal ribosomal functioning and could contributeto susceptibility to adult onset type 2 diabetes mellitus. It is located in the 3' end of the 16S RNA and had a

most stable secondary structure greatly different from wild type, indicating a likelihood of causing a disease (Tao *et al*, 2002). C to T transition at 3206 in 16S rRNA gene also has been reported and associated with type 2 diabetes mellitus. Nucleotide transition in this site may also caused an abnormality for secondary structure rRNA. C3206T mutation minimal free energy modeling showed similiar secondary structurewith the wild type. So it its probably a non functional polymorphism without causative significance to tyip2 2 diabetes mellitus (Tao *et al,* 2002).

In this research we found that T3398C and C3210T mutation is a linkage. Because from the three samples has mutation C3210T , also has a T3398C mutation. Mutation C3210T have been reported before. But T3398C has been reported. This mutation converts a methionine to a threonine at amino acid position 31. The 3398 mutation appears to fulfill the suggested criteria for definition of disease-related nucleotide variants (Jacksh, 1996). However in this research, we have not checked a normal samples. In order to complete data, examination normal samples needed to determine the mutation C3210T correlated or not with type 2 diabetes mellitus. Increase samples number and spread in kind of Indonesian tribes also need to determine another point mutations caused type 2 diabetes mellitus in indonesian subjects.

CONCLUSION

Mutation of T3200C, C3206T and T3398C has been detected in 30 indonesian subject with type 2 diabetes mellitus. Mutation of C3210T found in patient and this mutation always found as a linkage with T3398C mutation.

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EFFECTS OF SEAWEED EXTRACT ON PLASMA LEVELS OF HDL (HIGH DENSITY LIPOPROTEIN) AND LDL (LOW DENSITY LIPOPROTEIN) OF WISTAR RATS

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ABSTRACT

Hypercholesterolemia or hyperlipidemia has been established as an important risk factor of cardiovascular disease. Patients with hypercholesterolemia usually require a prolonged treatment; and the newer and more potent generation of antilipid agents are costly. In Bali there are several types of seaweed that are generally consumed by the local people and known by the local names of *Bulung Boni* (Caulerpa spp.) and *Bulung Sangu* (Gracilaria spp.). Studies on the effect of *Bulung Boni* and *Bulung Sangu* extracts on plasma levels of HDL and LDL still very limited, therefore further investigations were considered relevant and needed. This experimental study used completely randomized design, using a total of 24 Wistar rats divided into six sample groups of equal size, all fed with a diet high in cholesterol content. The six sample groups were respectively designated as negative control group, positive control group, and four treated sample groups, respectively fed orally with a dose of 20 mg and 60 mg extracts of *Bulung Boni* and *Bulung Sangu* per 100g of body weight per day. Our study showed that rats fed with high-cholesterol diet with *Bulung Boni* or *Bulung Sangu* extract at a dose of 20 mg and 60 mg/100 g bw/ day were associated with statistically significantly increased plasma HDL levels ($p < 0.05$), and decreased plasma LDL levels ($p< 0.05$) as compared with those of rats fed with high cholesterol diet without *Bulung Boni* or *Bulung Sangu* extracts. From our data it could be implied that *Bulung Boni* and *Bulung Sangu* extracts significantly increasing plasma HDL level, and lowering LDL level.

Keywords: Seaweed extracts, Caulerpa spp, Gracilaria spp, HDL cholesterol, LDL cholesterol, and anti-lipid agent

INTRODUCTION

The development of people live style that consume more fatty foods, especially of saturated fatty acid intake tend to cholesterol to be higher than the level of need. Intake of foods with high cholesterol content can increase cholesterol levels in the blood. This condition called hypercholesterolemia. One of the major atherosclerosis risk factors are dyslipidemia, and the prevalence of dyslipidemia in Indonesia has increased (Anwar, 2006).

Anticipating the effect of hypercholesterolemia in cardiovascular disease, has developed several hypolipidemic drugs such as niacin, gemfibrozil, and the class of statins (Hangbao et al., 2008). The treatment of patients with hypercholesterolemia require a long period, and high costs, therefore the study should be developed to obtain more effective drugs with a cheaper price, and less side effects. Natural ingredients from the sea, such as seaweeds need to be explored because of its carotenoids content that has function as an antioxidant and lowering blood cholesterol levels.

In Bali there are several types of seaweed that have long been used as a source of food by people. Types of seaweeds local name are *Bulung Boni* (Caulerpa spp.) and *Bulung Sangu* (Gracilaria spp). However studies on the effect of *Bulung Boni* and *Bulung Sangu* extract on plasma levels of HDL and LDL of Wistar rats are very limited, therefore it is still very relevant for further study.The results of study can provide information to the public about *Bulung Boni* and *Bulung Sangu* benefits in plasma HDL and LDL levels in cases of dyslipidemia.

MATERIAL AND METHODS

Material in this study consists of *Bulung Boni* (Caulerpa spp.), *Bulung Sangu* (Gracilaria spp.), white male rats of Wistar strain, 2.5 up to 3 month of age, 200 g to 225 g of weight, profile thio uracil (PTU), standard diet, foods high in cholesterol is made in the Laboratory of Pharmacology Faculty of Medicine Udayana University with a mixture of 1% dried pig brain, 5% egg yolk cooked, 10% lard,

1% coconut oil, and 83% standard diet with drinking water containing 0.01% PTU (Pengembangan dan Pemanfaatan Obat Bahan Alam, 1991), and cholesterol kit.

This experimental study used completely blind randomized design, using a total of 24 Wistar rats divided into six sample groups of equal size, all fed with a diet high in cholesterol content. The six sample groups were respectively designated as negative control group (KN), positive control group (KP), and four treated sample groups, respectively fed orally with a dose of 20 mg and 60 mg extracts of *Bulung Boni* per 100g of body weight per day (BB20 and BB60), and 20 mg and 60 mg extracts of *Bulung Sangu* per 100g body weight per day (BS20 and BS60).

Preparation of seaweed extracts

Dried seaweed were grinded until smooth and become powder. Seaweed powder is mixtured with 96% ethanol solution with a ratio of 3:1, then stirred with a magnetic stirrer for one hour at room temperature. Furthermore, filtered with Whatman paper No. 42 in order to obtain a filtrate, then concentrated by rotary evaporator.

Seaweed extracts treated in rats

In one cage was placed as many as four rats that had previously adapted for one week in the laboratory. Standard diet, cholesterol diet, and beverages rats administered daily ad libitum. Seaweed extract was administered orally by zonde with dose of 20 mg and 60 mg/100 g body weight rat/day according to treatment.

Blood sample

After 30 days treatment, rats was fasted for 18 hours. Bloods sample was taken through the sinus orbitalis as much as 2 cc. Measurement of cholesterol levels using enzymatic CHOD PAP Test colorimeter method. Blood samples of rats as much as 1 cc put into test tube, and plasma from blood was separated with centrifuge for 20 minutes at 1500 rpm. 10 µl of plasma was mixed with 1000 µl of reagent. Sample tubes incubated for 10 minutes at temperature of 20-250 ^{0}C. Samples inserted into the spectrophotometer with a wavelength of 500 nm (Rahayu, 2005).

Data Analysis

Analysis of cholesterol HDL and LDL performed with test of homogeneity of variance using Levene's Test at 5% significance level. If the variance is not homogeneous, the data must be transformation. To determine the effect of treatment, the data were analyzed with analysis of variance at 5% significance level. If the F test showed a significant difference , the treatment was tested furthermore by BNJ at 5% significance level.

RESULTS AND DISCUSSIONS

Levels of high density lipoprotein (HDL)

Levene's test showed that the treatment with *Bulung Boni* and *Bulung Sangu* extracts on observations of HDL and LDL cholesterol have a homogeneous variance ($p > 0.05$) (Table 1).

Table 1. Levene's Test for Homogeneity of Variances

Variable	MS effect	MS error	F	P
HDL	2,0870	1,0409	2,0049	0,1266
LDL	149,0894	77,1862	1,9315	0,1387

The highest plasma HDL level found in negative control was 78.25 ± 1.72 mg / dl, then BB60 was 62.34 ± 1.19 mg / dl, BS60 61.53 ± 3.84 mg / dl, 58.44 ± BS20 1.19 mg / dl, BB20 55.68 ± 1.44 mg / dl, and the lowest in the positive control with plasma HDL level 45.29 ± 1.11 mg / dl (Figure 1).

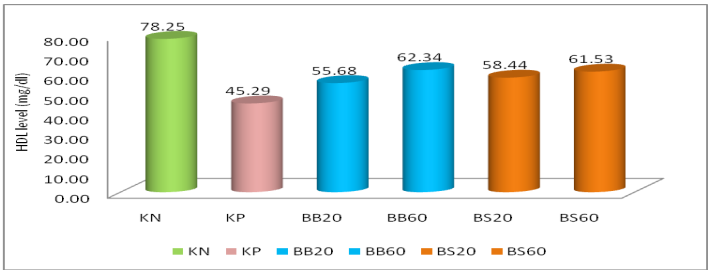

Figure 1. Plasma HDL Level of Wistar Rats in Negative Control (KN), Positive Control (KP), BB20, BB60, BS20, and BS60

Analysis of variance of plasma HDL level of Wistar rats fed high-cholesterol diet with treated *Bulung Boni* and *Bulung Sangu* extracts showed significantly differences ($p < 0.05$) in various treatments. To determine the effect of each treatment on plasma HDL level performed with multiple comparation test. Plasma HDL level in positive control (KP) was significantly lower compared with KN, BB20, BS20, BB60, and BS60. Plasma HDL level in BB20 was significantly higher compared with positive controls, but did not differ significantly with BS20. Plasma HDL level in BB60 was significantly higher compared with the KP, BB20, and BS20, but non significantly different with BS60. Plasma HDL level in KN was significantly higher compared with KP, BS20, BB20, BS60, and BB60.

Bulung Boni and *Bulung Sangu* extracts with a dose of 60 mg/100 g rat bw/day were increased plasma HDL level significantly higher compared with dose of 20 mg/100 g bw rat/day. *Bulung Boni* and *Bulung Sangu* extracts dose of 60 mg/100 g bw rat/day can increase plasma HDL level above 60 mg/dl, approximately 62.34 ± 1.19 mg/dl in BB60, whereas in BS60 was 61.53 ± 3.84 mg /dl. This plasma HDL level were high category. According to Stapleton et al.(2010), plasma HDL level of less than 40 mg /dl are low, whereas above 60 mg/dl is high category. HDL cholesterol level above 60 mg/dl can be considered as a protective effect against heart disease (Colpo, 2005).

Bulung Boni extract increased plasma HDL level in rats were significantly higher than fed only high cholesterol diet, but did not differ significantly with *Bulung Sangu* extract. This is due to in *Bulung Sangu* and *Bulung Boni* content several types of the same carotenoid such as beta carotene, chlorophyll b, antheraxanthin, astaxanthin free, and neoxanthin, therefore the ability to increase plasma HDL level did not differ significantly. Any increase in plasma HDL cholesterol level at 1 mg/dl can reduce the risk of coronary heart disease in men by 2% and 3% in women (Adam, 2005). High level of HDL in the blood will accelerate the process of transporting cholesterol to the liver, therefore reducing the possibility of accumulation of cholesterol in the blood vessels (Fuhrman et al., 2002). Kagami et al. (2008), reported that the carotenoids from the type of lycopene, and beta-carotene plus LDL receptor activity of macrophages, have effects similar with effect fluvasfatin.

Levels of low density lipoprotein (LDL)

The lowest plasma LDL level showed in negative control was 21.26 ± 3.29 mg/dl, BB60 48.00 ± 10.97 mg/dl, BS60 53.86 ± 20.89 mg/dl, 113.18 ± BB20 18.91 mg/dl, BS20 117.33 ± 26.41 mg/dl, and positive controls with the highest average level of plasma LDL 233.38 ± 19.86 mg/dl (Figure 2)

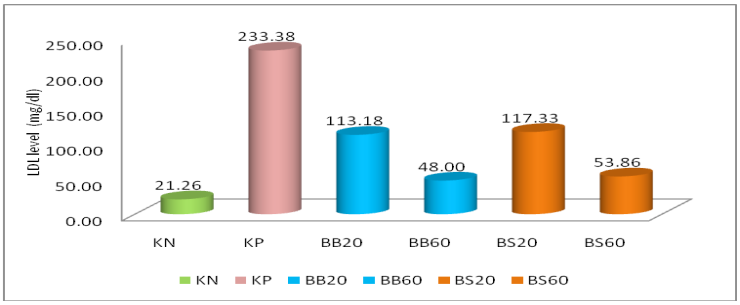

Figure 2. Plasma LDL Level of Wistar Rats in Negative Control (KN), Positive Control (KP), BB20, BB60, BS20, and BS60

Analysis of variance of plasma LDL level of Wistar rats fed high-cholesterol diet with treated *Bulung Boni* and *Bulung Sangu* extracts showed significantly differences ($p < 0.05$) in various treatments. To determine the effect of each treatment on plasma LDL level performed with multiple comparation test. Plasma LDL level in positive control (KP) was significantly higher compared with negative control (KN), BB60, BS60, BB20, and BS20. Plasma LDL level in BB20, significantly higher compared with BB60, BS60, and KN, but did not differ significantly with BS20. Plasma LDL level in BB60 significantly lower compared with the KP, BS20, BB20 but non significantly different with BS60. Plasma LDL level in negative control were significantly lower compared with BB60, BS60, BB20, BS20, and KP.

In rats fed high-cholesterol diet with *Bulung Boni* and *Bulung Sangu* extracst with a dose of 20 mg/100 g bw rat/day had plasma LDL level were significantly lower than rats only fed high cholesterol diet without *Bulung Boni* or *Bulung Sangu* extracts. In BS20 was 117, 33 ± 26.41 mg/dl, and BB20 was 113.18 ± 18.91 mg/dl. *Bulung Boni* and *Bulung Sangu* extracts with dose of 60 mg/100 g bw rat /day can reduce plasma LDL level were significantly lower compared with dose of extract 20 mg/ 100 g bw rat /day. Plasma LDL level in BB60 was 48.00 ± 10.97 mg/dl, while in BS60 plasma LDL level higher at 53.86 ± 20.89 mg /dl, but did not differ significantly with BB60. This shows that *Bulung Boni* and *Bulung Sangu* extracts with a dose of 60 mg/100 g bw rat /day can reduce plasma LDL level achieve to optimal LDL level.

LDL level less than 100 mg /dl is optimal LDL level category, 100-129 mg/dl is near optimal level of LDL, 130-159 mg/dl is the highest normal range, 160-189 mg/dl is high LDL category, more than 190 mg/dl, very high category (Jae, 2008). *Bulung Boni* and *Bulung Sangu* extracts can lowering cholesterol level, this is due to carotenoids content in *Bulung Boni* and *Bulung Sangu* function as hypocholesterolemic. Myers (2005), reported that carotenoids has a very important biological functions as an antioxidant, immune system, prevent degenerative diseases, anti-inflammatory, anti-stress, inhibiting lipid peroxidation, and lower blood cholesterol level.

CONCLUSION

1. Plama HDL level of Wistar Rats fed high cholesterol diet treated *Bulung Boni* and *Bulung Sangu* extracts significantly higher compared with Wistar rats fed high cholesterol diet without *Bulung Boni* and *Bulung Sangu* extracts
2. Plama LDL level of Wistar Rats fed high cholesterol diet treated *Bulung Boni* and *Bulung Sangu* extracts significantly lower compared with Wistar rats fed high cholesterol diet without *Bulung Boni* and *Bulung Sangu* extracts

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WATER EXTRACT OF PURPLE SWEET POTATO TUBERS REDUCES BLOOD PRESSURE 0F HYPERTENSIVE RATS INDUCED BY NaCl

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ABSTRACT

Compliance of hypertensive patients to take medication is one of many determinant factors to achieve successful treatment. Side effects and the expensive price of drugs are the causes of the incompliance of patients taking the medication. Utilization of herbal medicine is a new hope to resolve the issue. Purple sweet potato tuber is a plant part that expected has beneficial effect in lowering blood pressure because it contains anthocyanins which are antioxidants and can preserve endothelial function. To prove these hypothesis, a study was conducted with randomized control group pre and post-test design. The study was done on 20 adult male Wistar rats that were divided into two groups of 10 rats. Both groups of rats were made hypertensive by administering high doses of NaCl. Control group of rats given only NaCl alone for 14 days. Treatment group were given NaCl and water extract of purple sweet potato tuber with a dose of 4 cc per day for 14 days. Before treatment and during treatment, blood pressure were taken everyday with special sphygmomanometer. The results indicate a significant difference in blood pressure between the control group with treatment (P = 0.0001). In the treatment group, it was observed that there was a significant decrease in blood pressure compared to the control group (P=0,0001). From the results of this study, it can be concluded that administration of purple sweet potato tuber water extract may lower high blood pressure of rats induced by NaCl.

Key words: Purple sweet potato tuber, blood pressure, hypertensive rats.

INTRODUCTION

Successful treatment of hypertensive patient requires adherence to taking medication regularly, because hypertension usually require long life treatment. Side effects caused by antihypertensive drugs, and expensive of antihypertensive drugs often a cause of decreased compliance of hypertensive patients to take medication. Irregular treatment will cause the occurrence of various complications of hypertension. The use of herbal medicine is inexpensive and easy, because medicinal plants could be planted around the yard. Purple sweet potato is the one which has the potential as herbal medicine. It has been observed that purple potato to have high content of anthocyanins which are flavonoids that have antioxidant and anti-inflammatory effects. Water extract of purple sweet potato tubers can lower blood cholesterol in rabbits and rats given high-cholesterol diet, and increasing blood total antioxidant level (Jawi, 2008; Jawi and Budiasa, 2011). Flavonoids can improve vascular endothelial function (Engler, 2004), through regulation of eNOS(endothelial nitric oxide synthase) expression and increased production of NO (nitric oxide), that is a strong vasodilator (Erdman, 2007; Han, 2007; Morris, 2007).

In the presence of oxidative stress, the bioavailability of NO will decrease. Giving antioxidants can reduce free radical, so the oxidative stress does not happen and will ultimately increase the bioavailability of NO. Flavonoids can improve the bioavailability of NO because it can be as an antioxidant. Flavonoids are found in vegetables and fruits. If they are consumed regularly, they can protect the body from cardiovascular disease and some other chronic diseases, through the antioxidant effect (Knekt, 2002). Anthocyanin pigment is one important type of flavonoid that has been widely studied and has a beneficial effect on cells in mammals such as having antioxidant effects, antimutagenic, and hepatoprotective antihipertensive (Middleton, 2000; Lila, 2004). Provision of anthocyanin-rich foods such as corn, purple, and red radish for 15 weeks, can lower blood pressure and pulse rate (Shindo, 2007). Provision of anthocyanins from *Aronia melanocarpa* on the patient's metabolic syndrome can lower blood pressure, reduce endothelin-1 and may improve lipid profiles in the blood (Broncel, 2007). Giving red wine poliphenol extrac red wine, an extract which contains flavonoids, can increase the expression of endothelial eNOS, and increased the release of endothelial NO as astrong vasodilator that (Leikert, 2004). Purple sweet potato tubers in Bali proved to have high anthocyanin content (Suprapta, 2004), and antioxidant effects on blood and various organs of mice with oxidative stress (Jawi, 2008).

Water extract of purple sweet potato tuber can also improve lipid profiles and increases total antioxidant level in blood of rabbit(Jawi and Budiasa, 2011). These findings generate a question whether

the water extract of purple sweet potato tubers can lower blood pressure through the effects of these antioxidants. The purpose of this study is to prove that the water extract of purple sweet potato tubers can be used as easily available food substance and can be developed in community as healthy food for hypertensive people.

MATERIALS AND METHODS

The design of this study was an experimental laboratoric with randomized control group pre and post-test design. The study was conducted at the Center of Food Study at Gajah Mada University, Yogyakarta. The population in this study were white wistar male rats aged 3-4 months are obtained from Food Study Center of Gadjah Mada University in Yogyakarta. The sample size is determined by the Fiderer formula, each group of 10, with an average weight of 200 grams. The independent variable in this study is water extract of purple sweet potato tuber. Dependent variable is systolic blood pressure is measured with a special rat sygmomanometer. Before the research began, two groups of that rats adapted for 2 weeks. After two weeks the blood pressure were examine as a pre-test data. After that the control group given NaCl at a dose 2% of rat body weight every day for two weeks. In addition to the treatment group given NaCl and purple sweet potato tuber water extract at a dose of 4 cc per day with oral rote.

Water extract of purple sweet potato tuber was made by the following procedure: purple sweet potato tubers obtained from farmers in the area of Tabanan Bali, washed with water and then peeled. Once peeled sweet potatoes were cut into pieces crosswise with a thickness of 2 to 2.5 cm. Sweet potato chunks were mixed with clean water at a ratio of 1 kg sweet potato plus 1 liter of water and then blended and filtered with three layers of gauze. Fluid obtained from filtration was boiled, for 30 minutes. The content of anthocyanin of this material was 146 mg / ml. During the study blood pressure in both groups of rats were evaluated every two days until day four, then performed the evaluation of blood pressure every day. The test statistic used is the T-test.

RESULTS

Body Weight

The results showed that there was no effect of water extract of purple sweet potato tuber on body weight. The average weight of white rats before and after the study can be seen in the Figure 1. On the graph, it can be seen the changes in body weight of rats for 2 weeks. Initial weight and final weight did not differ between the control group with treatment group. The average weight gain early control group was 228.60 ± 16.92 grams, while the treatment group was 225.10 ± 13.55 grams. The average final weight of control group 306.00 ± 42.45 grams, while the treatment group was 301 ± 39.30 grams.

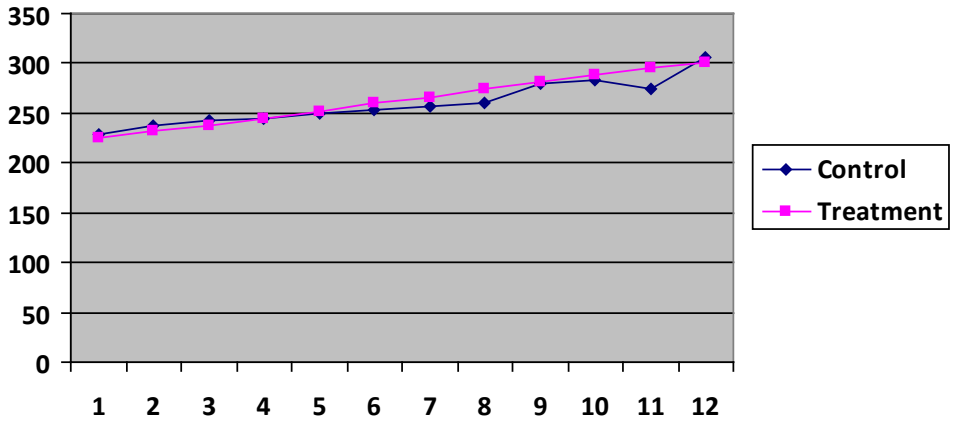

Figure 1: Comparison of average increase in body weight between control group and the treatment group, during 12 days of observation.

Blood Pressure.

The results of this study indicates that the effect of NaCl at a dose of 2% body weight of rats caused increases in systolic blood pressure in both groups of rats. The increase in systolic blood pressure is much higher in the control group compared to the treatment group (Figure 2).

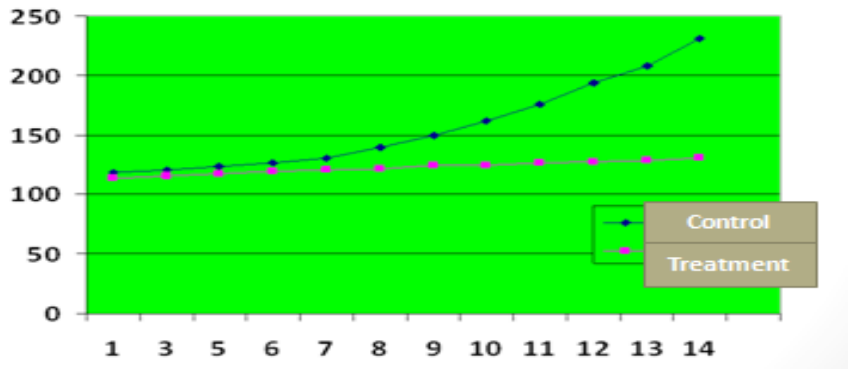

Figure 2: Comparison of average increase in blood pressure between control group and the treatment group, during 14 days of observation.

Figure 2 above shows the real blood pressure differences between the control group with treatment group. Average blood pressure of control group at pre-test was 118.60 ± 6.05 mmHg. After administering a high dose of NaCl daily for 14 days an increase in average blood pressure of 231 ± 6.92 mmHg. Graph of the average change in blood pressure during the study, can be seen in the figure No. 2. On the graph are visible results of the evaluation of blood pressure of white rats for 2 weeks of treatment. The increase in blood pressure in the control group was much higher than the treatment group (group given NaCl and water extract of purple sweet potato tuber for 2 weeks (P = 0.0001).

DISCUSSION

The results of weight measurements in this study shows that the increases in body weight did not differ between the control group with treatment group. Therefore, there is no effect of water extract of the purple sweet potato tuber on weight of white mice. The results of the evaluation of systolic blood pressure for 2 weeks showed the role of water extract of purple sweet potato tubers in maintaining blood pressure remained normal in mice induced by NaCl. Water extract of purple sweet potato tubers that contain high anthocyanins (Suprapta, 2004) will increase the anthocyanin levels in the blood of rats. Anthocyanin will accumulate in endothelial cells and protect endothelial cells from the effects of free radicals, so it can maintain NO as a strong vasodilator (Han, 2007). The role of NO in decreasing blood pressure has been widely studied. Oksidative stress can reduce the bioavailability of NO. The relaxation response of blood vessels is impaired due to decreased NO. Giving antioxidants (especially antioxidants derived from plants such as anthocyanin, which is one member flavonoids) in this condition is very useful (Mann, 2007). Purple sweet potato tubers contain high level of anthocyanin (Suprapta, 2004), so it can lower blood pressure in rats given high doses of NaCl, possibly through the mechanism of increased antioxidant which improves the bioavailability of NO, so the endothelial function will be stabilized (Wallace 2011).

The results are consistent with several studies that prove the polyphenols / flavonoids from a variety of foods may protect endothelial function of blood vessels, so blood vessels can maintain a stable blood pressure. Giving RWPCs (red wine polyphenolics compounds) capable of inhibiting the proliferation and migration of vascular cells and blood vessels were able to induce relaxation by increasing NO, as a result of increased eNOS activity by various mechanisms. RWPCs also can increase the release of prostacyclin and inhibit the synthesis and the effects of endothelin-1 resulting in relaxation of blood vessel (Han, 2007). Research in vitro and in vivo by providing an isoflavone that as one of flavonoids may lower systolic and diastolic blood pressure by increasing NO, causing dilatation of the blood vessels (Mann, 2007). Flavonoids from purple grape extract increases the production of NO by platelet (Fredman 2001). Cyanidin-

3-glucoside, which is a typical anthocyanin pigment may increase NO by increasing eNOS expression in endothelial cells (Xu, 2004). Provision of anthocyanin-rich foods such as purple corn and red radish for 15 weeks with anthocyanin content of approximately 1% of the total diet, can lower blood pressure and pulse rate (Shindo, 2007). Giving Anthocyanins from chokeberry, bilberry and eldeberry can maintain endothelial function properly after being exposed to ROS in invitro (Bell, 2005).

Water extracts of purple sweet potato tuber in this study, supporting the theory and studies with extracts that contain anthocyanins from various sources may lower blood pressure through maintaining endothelial function by increased NO, because anthocyanin as antioxidants which can cope free radical.

CONCLUSION

From this results of study, it can be concluded that water extract of purple sweet potato tuber can lower systolic blood pressure in white rats with NaCl-induced hypertension.This study has several weaknesses, among others, does not measure the amount of food eaten by each individual so that the influence of food is less controllable. It is therefore advisable to conduct research with respect to individual food intake. Further research needs to be done to measure levels of NO and eNOS in endothelium, so that it can explain the action mechanism of anthocyanin in purple sweet potato tubers in lowering blood pressure.

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COMPARTMENTALIZED NOTCH SIGNALING SUSTAINS EPITHELIAL MIRROR SYMMETRY

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ABSTRACT

Bilateral symmetric tissues must interpret axial references to maintain their global architecture during growth or repair. The regeneration of hair cells in the zebrafish lateral line, for example, forms a vertical midline that bisects the neuromast epithelium into perfect mirror-symmetric plane-polarized halves. Each half contains hair cells of identical planar orientation but opposite to that of the confronting half. The establishment of bilateral symmetry in this organ is poorly understood. Here, we show that hair-cell regeneration is strongly directional along an axis perpendicular to that of epithelial planar polarity. We demonstrate compartmentalized Notch signaling in neuromasts, and show that directional regeneration depends on the development of hair-cell progenitors in polar compartments that have low Notch activity. High-resolution live cell tracking reveals a novel process of planar cell inversions whereby sibling hair cells invert positions immediately after progenitor cytokinesis, demonstrating that oriented progenitor divisions are dispensable for bilateral symmetry. Notwithstanding the invariably directional regeneration, the planar polarization of the epithelium eventually propagates symmetrically because mature hair cells move away from the midline towards the periphery of the neuromast. We conclude that a strongly anisotropic regeneration process that relies on the dynamic stabilization of progenitor identity in permissive polar compartments sustains bilateral symmetry in the lateral line.

Keywords: bilateral symmetry, Notch signaling, hair cell regeneration, planar cell inversion. Has been published in *Development 2011 (138), 1143-1152*

ANTIMICROBIAL EFFECTS OF INDONESIAN MEDICINAL PLANTS EXTRACTS ON PLANKTONIC AND BIOFILM GROWTH OF *PSEUDOMONAS AERUGINOSA AND STAPHYLOCOCCUS AUREUS*

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ABSTRACT

In nature, most microorganisms live together in large numbers, attached to a surface and forming structured layers, known as a biofilm. Bacterial biofilms are different from planktonic grown cells, most importantly being their resistance to detergents and antimicrobial agents. Some 54 ethanol extracts were obtained from a variety of known selected Indonesian medicinal plants. Most of the obtained extracts were previously reported as anti-bacterial. Anti-bacterial assays were performed against *Pseudomonas aeruginosa* PAO1 and *Staphylococcus aureus* Cowan I, two known pathogens. The above derived extracts show an inhibitory effect on planktonic grown bacteria and more interesting on biofilms. Up to a concentration as low as 125 µg/ml, biofilm of *P. aeruginosa* PAO1 and *S. aureus* Cowan I is inhibited for 50% (IC 50) by 7 plants ethanol extract: *Kaempferia rotunda*, *Zingiber aromaticum, Caesalpinnia sappan, Cinnamomum burmanii, C. sintoc, Sesbania grandiflora*, and *Nelumbium nelumbo*. Limited bacteriostatic activity was evident. These data makes the obtained extracts interesting sources as putative anti-biofilm agents. This research can contribute to the development of new strategies to prevent and treat biofilm infections.

Keywords: medicinal plants, biofilms, *Pseudomonas aeruginosa* PAO1, *Staphylococcus aureus Cowan I*

POSTER PRESENTATION: AGRICULTURE

THE EFFECT OF INCLUSION OF CHICKEN FEATHER MEAL POWDER AT VARIOUS LEVELS IN THE RATION ON PRODUCTION OF JAPANESE QUAIL (*Coturnix-coturnix japonica*)

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ABSTRACT

This study was carried out to determine the effect of level chicken feather meal powder (CFMP) in the ration on production of Japanese quail (*Coturnic-coturnic japonica*). A total of 60 birds (2 week old) female Japanese quails were used in the present experiment which was arranged in a Completely Randomized Design with 5 treatments and 4 replications (3 birds each). Dietary inclusions of different levels of feather meal powder were examined, namely R0 which was designed for ration without feather meal powder, and R1, R2, R3 and R4 for rations containing feather meal powder at 2.5%, 5%, 7.5% and 10%, respectively. Diets were formulated to be iso-caloric and iso-protein (2785kcal/kg and 22% CP). The variables noted were body weight, body weight gain, feed consumption, feed conversion, age at 5% lay, egg weight at 12 week, shell thickness, haugh unit (HU) and egg yolk color. Four eggs from each treatment were analysis every week and the whole experiment used 100 eggs. Data were analyzed using the analysis of variance and when significant different was noted, the analysis was continued with Duncan's Multiple Range Test. The results concluded that the use of 10% of chicken feather meal powder on the ration significantly affected to the body weight, body weight gain, feed consumption, feed conversion ratio, but not significantly affected the age at 5% lay, egg weight, shell thickness, haugh unit and egg yolk color.

Keywords: *Japanese Quail, performance, feather meal powder, shell thickness, Haugh unit.*

INTRODUCTION

Common quail and Japanese quail belong to Aves, Coturnix. Their scientific names are *Coturnix coturnix* and *Coturnic Japonica,* respectively (Chang *et al*., 2001 and 2005). Quails are divided into wild quail and domestic quail. All wild quails belong to one of two species, Common Quail (*Coturnix coturnix*) and Japanese Quail (*Coturnix japonica*). For commercial purposes, domestic quails are available in both laying and meat strains (Wang *et al.,* 2003). Poultry raisers are not the only ones who get benefit from raising quail, hence the continuing survival and growth industry is recognized and will largely depend on its ability to compete poultry product (Suprijatna, 2010). Agreement of ASEAN-China free trade (ACFTA) has been put in effect since January 2010. In regard to trading, Indonesia with its population of 125 million is a remarkable market share because food consumptions for Indonesian people are quite large. On the other hand, up to now the consumption of animal protein for Indonesian people still very low (5.861 g/capita/day), though recommendation by "Widya Karya Pangan dan Gizi Nasional" in 1998 is 6 g/capita/day. The low intake of animal protein at household level may risk the occurrence of malnutrition and impaired brain growth in infants. The poultry products have become food sources with very high nutritive values, particularly quail meat and eggs. These can be the major protein source for consumers.

Rearing breeds of quail is relatively easier than raising other breeds of poultry. Moreover, quail also need little space and need ration only 14 g/quail/day (Wu and Self, 2005). Quail can grow faster than other breed of bird and also there is a good opportunity in supplying the protein need. Quail at 1.4 years of age can produce 200 - 300 eggs (Abidin, 2002). The average egg weight is about 9 g (Chang, 2005). Body weight of male and female quail is 143 g and 117 g, respectively (Rama Rao, 2006). Quail have ability to produce 3 to 4 generations per year in a particular breeding program; female start to lay eggs at 35 day of age, or at an average of 40 day (Dillak *et al*., 2010). Eggs of quail would be benefit the nutritional quality of protein depends on amino acid content and physiological utilization of specific amino acids after digestion and absorption (Muguruma *et al*., 2010).

Quail mostly kept in extensive systems, where they need to get high quality of feeding. . Feed is a vital component in quail rearing; feed cost is approximately 75% of total capital cost. One reason is the dependence on imports of poultry sector which is very high, in term of poultry feed ingredients (corn, soybean meal and fish meal). To reduce the cost of the fish meal on the ration it may be worth to change

the resource of protein with substitution using feather meal powder (Laualata, 2004).

According to Bidura (2008), the non conventional meal of feather meal powder (FMP) rich in mineral and contain 61% protein, 3080 Kcal/kg energy and 1 % fiber. This form can provide protein sources for amino acid requirement apart from difficulties in providing diet of the birds.

This experiment was carried out to determine the best level of chicken feather meal powder (FMP) used in ration for production Japanese of quail (*Coturnix-coturnix japonica)*.

MATERIALS AND METHOD

Birds and Treatments

Sixty (60) female Japanese quails were used as materials in an experiment using a Completely Randomized Design (CRD). The quails were divided into 5 treatments with 4 replicates of 3 quails each. The treatments were as follows. R0: diet without feather meal powder (FMP); R1: diet with 2.5% FMP; R2: diet with 5% FMP; R3 : diet with 7.5% FMP; and R4: diet with 10% FMP.

Diet was formulated to be iso-caloric and iso-protein (2.785 kcal/kg and 22% CP), using the following ingredient: yellow corn, rice bran, soybean meal, fish meal, and coconut meal.

Diet and drinking water were provided *ad libitum*.

Traits Measured

Body weight and feed intake were recorded weekly by an electronic weighing balance. The variable recorded were: body weight, body weight gain, feed consumption, feed conversion, age at egg lay 5% production, egg weight, shell thickness and haugh unit (HU) which were studied for 10 weeks. The age at the attainment of sexual maturity and sexual weight were determined when the quails laid their first eggs in each pen, this situation forms 5% productivity. Egg shell thickness was an average measure taken at two different locations of eggshell using a stainless steel digital caliper (Mitutoyo, Model 500-196-20, Japan). Height of thick albumen was measured with albumen height apparatus (Teclock Corporation, Japan) approximately at 1 cm away from yolk. The formula of Williams (1997) was used to calculate haugh unit (HU) as follow: HU value =100 Log (H +7.7 –1.7 $W^{0.37}$); where H is albumen height (mm) and W is egg weight (g). Egg yolk color was scored using a 15-point scale of yolk color fan of DSM (DSM Nutritional Product Ltd., Basel, CH-4002, Switzerland).

Average egg weights were determined, cell thickness and haugh unit (HU). Four eggs from each treatment were analyzed every week and the whole experiment used 100 eggs.

The data were analyzed by analysis of variance and mean comparison was tested by Multiple Range Test (Steel and Torrie, 1990).

RESULTS AND DISCUSSION

The effect of chicken feather meal powder on performance of quail

The feed consumption of birds following the treatment is given in Table 1. There were variation in feed consumption among treatment and it was found to be significant ($P<0.05$). Quails received diets consisting of 10 % of feather meal powder (R4) have the lowest value compared to the other treatments. But, there were no significant differences found between R0, R1, R2 and R3. The highest feed consumption was found in quail received treatment level of 2.5% feather meal powder (R1). The gradually decreasing in feed intake with the increase in level feather meal powder is shown in Table 2. This might be due to the fact that FMP had physiological effect on the digestive system and increased metabolism on the quail tract. According to Waught *et al.* (2006), feed palatability was important in stimulating feed ingestion by the birds. These diets may have other problems associated with them such as anti-nutritional factors, low digestibility and not be dissolved properly (Bidura *et al.,* 2008).

The recorded body weight and weight gain of the birds following treatments are given in Table 1. The effect of treatment on body weight and weight gain were found to be significant ($P < 0.05$). The body weight and weight gain for the R4 group were noted to be the lowest compared to R0, R1, R2 and R3. Moreover, the highest body weight and weight gain was demonstrated in birds receiving the R1 treatment.

The result showed that the treatments can't give negative affect for the quail growth, because the chickens feather meal powder at 10% level contain high fiber. But chicken feather meal powder may have other problems associated with anti-nutritional factors, low digestibility and it can not be dissolved properly. This, in turn, may have an effect on protein content of the given ration. According Wu and Self (2005), amino acid must be provided from the diet in order to meet the requirements for maintenance, growth and reproduction.

The worst feed conversion ratio was noted for the quail receiving 10% level of feather meal powder (R4). However, feed conversion ratio for quails receiving FMP at levels of 0% (R0), 2.4% (R1), 5% (R2), 7.5% (R3) were not significantly decreased. The inclusion up to 10% feather meal powder in ration affected non palatability for quail. Tini (2010) reported that 16 - 20% protein may increase the consumption of the rations**.** Protein in poultry feed consumed expressed just enough to their energy requirements for the maintenance for body weight (Dewi, 2009).

Table 1. The Effect of Chicken Feather Meal Powder for Performance of Quail

VARIABLE	TREATMENT				
	0 % CFMP (R0)	2.5% CFMP (R1)	5% CFMP (R2)	10% CFMP (R3)	15% CFMP (R4)
Feed Consumption (g/bird)	355.09[a]	356.49[a]	354.08[a]	354.88 [a]	351.82 [b]
Body weight(g/ bird)	152.09[a]	153.55[a]	153.05[a]	152.95[a]	149.05[b]
Weight gain (g)	97.07[a]	98.53 [a]	98.03[a]	152.95[a]	149.05[b]
Feed Conversion Ratio (FCR)	3.66 [b]	3.60 [b]	3.62[b]	3.63 [b]	3.73 [a]

Mean with different superscripts in the same rows differ significantly (P<0.05), CFMP: chicken feather meal powder

The effect of inclusion of chicken feather meal powder on production of quail

The effect of chicken feather meal powder on production of quail is given in Table 2. The quails received 0–10% feather meal powder with respect to egg weights were found to be significant (P<0.05). The egg weight at 12 week was noted to be lowest than the other evaluations. Ipek *et al*. (2007) determined that the mean egg weight at 12 weeks of age is 10.80 g/egg. The Mean egg weight is related to protein consumption and feather meal powder is source of amino acid (methionine and cysteine). Consumption all of the FMPP treatment in the current experiment gave similar results as that reported by Suhermiayati dan Irianti (2010).

The age at 5% lay, which is a determinant of egg production in quails, was evaluated as the age when the first egg was laid. Age at first egg is of importance since it indicates the sexual maturity age. The first laying egg of the quail in this experiment varied, ranging from 42.04 – 42.33 days. Quails receiving treatment of 5 % chicken feather meal powder (R2) has the highest value but among the treatment there is no significantly different (P> 0.05). Nazligul *et al*. (2001) found that age when the first egg was laid in combine type quails was the 43rd day which was higher than the present research results. According to Sartika *et al*. (2008), there are several factors that of importance in sexual maturity attainment, that are particular strain, genetics, and feed management. Chicken feather meal powder as source of protein or amino acid has potential benefit for improving egg protein (Martin *et al*., 2003).

The average shell thickness, haugh unit (HU) and egg yolk color of the treatment are presented in Table 2. Egg weight was influenced by several factors among other things, which were sexual maturity, genetic, quantity and quality of diet especially the requirement of protein and amino acid (Resnawati and Sartika, 2010). The quality of ration with the increasing level of the feather meal powder inclusion can gave important point, protein for requirement on daily basis as well as growth rate and protein content of the bird on body weight (Bidura, 2008). Moreover, according to (Hartono, 2004) and Abidin (2002), protein added to the ration gave egg weight about 8 -11 g/egg and 9.39 g, respectively.

Table 2. The Effect of Chicken Feather Meal Powder (CFMP) Inclusion on Production of Quail

VARIABLE	TREATMENT				
	0 %CFMP (R0)	2.5% CFMP (R1)	5% CFMP (R2)	10% CFMP (R3)	15% CFMP (R4)
Egg weight (g/ 12 week)	9,93[a]	10.14[a]	10.20[a]	10.06 [a]	9.98 [b]
Age at 5% lay (day)	42.30	42.32	42.33	42.04	42.30
Shell thickness (mm)	0.29	0.30	0.30	0.29	0.29
Haugh Unit	87.90	88.10	88.08	88.15	87.85
Egg yolk colour	6.12	6.22	6.25	6.15	6.12

Mean with different superskrips in the same rows is differ significantly (P<0.05)

The observation and measurement of various kinds of egg shells is presented in Table 3. The treatment of R1 and R2 (2.5 and 5% chicken feather meal powder) gave the highest value of the shell thickness (P > 0.05) among the treatment. Egg shell thickness varies, but the range of value is not so large (Table 3). This is caused by the differences in the synthesis and secretion of egg shell membrane (Whittow, 2000). According to Sofwah (2007), the quality of shell thickness is influenced by calcium and phosphor balance in the ration, genetic trait and management of the light (Tri-Yuanta and Nys, 1990). Calcium and phosphor in the current ration experiment was 0.5 - 0.69% Ca and 1.30 - 1.55% and this results was similar to what it has been reported by Rama Rao (2003) and Rama Rao *et al*. (2006).

The effect of treatments on Haugh unit (HU) was found no significantly different (P > 0.05). According to Caner (2005), Haugh unit is an indication of quality of albumen weighed by high albumen and egg weight; this also can indicate the egg quality. The factor to influence of Haugh Unit is value of resources new egg. The requirement of the consumer, the targets that have to be pigmented are the yolk, the skin, and the subcutaneous fat. For laying hens, the position in egg yolks was 30 to 45% for canthaxanthin, 14% for astaxanthin, and 25% for zeaxanthin. The effect of nutrition egg yolk color with varying level on chicken feather meal powder gave markedly different results but statistically there was no significant different (P > 0.05). Several studies have indicated similarity with Naszligul *et al*. (2001) that when the fist egg was laid in combine type quail was lower than other works. A study with quail indicated a satisfactory effect of cassava leaves meal (CLM) at 5% for yolk yellowness than control (Nguyen *et al*., 2003). But according to Ismoyowati (2010), Mojosari duck egg yolk was higher at color scores than Tegal duck (6.3 ± 1.10), it caused by resource the xanthophyl feeds.

CONCLUSION

The results concluded that the used of 10% of chicken feather meal powder on the ration was significantly affected to the body weight , body weight gain, feed consumption, feed consumption ratio, but not significantly affected the age at 5% lay, egg weight, shell thickness, Haugh unit and yolk color of Japanese quail (*Coturnix-coturnix japonica*).

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ESTRADIOL AND PROGESTERON CONCENTRATION OF SIX-MONTH AND ONE-YEARS OLD FEMALE RATS THAT SUPPLEMENTED BY SOMATOTROPIN

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ABSTRACT

The aims of this research is to investigate the effects of somatotropin supplementation on estradiol and progesteron concentration of six-month and one-year old female rats. Forty eight female rats were assigned into a randomized block design with 4 factors. The first factor was age with 2 levels (6 months and 12 months). The second factor was somatotropin dosage with 2 levels (0 and 9 mg/kg body weight). The third factor was duration of injection with 2 levels (3 weeks and 6 weeks), and the fourth factor was sampling period with 2 levels (0 and 14 days after somatotropin termination). Plasma estradiol and progesterone concentration was assayed by RIA (Radioimunoassay) method. The results showed that somatotropin supplementation had significant effects on estradiol concentration (P=0.013) and low concentration on progesterone .

Keywords: estradiol, progesteron, female rat, somatotropin

INTRODUCTION

Somatotropin is a scientific name of *growth hormone*, a hormone secreted by anterior pituitary gland that plays a significant role in cell proliferation. This hormone stimulates the growth of all tissues that can grow by increasing cell size and increase cell mitosis (Guyton. 1995).

In its infancy, when growth is rapid, very high levels of somatotropin. With age, levels of somatotropin in the body decreases. Since the age of 21 years, somatotropin production declined 3% each year, so that after the age of 60, somatotropin production declined by about 80% (Bengtsson et al. 2000).

This resulted in decreased secretion of somatotropin emergence of signs of skin aging such as wrinkles, eyelids dropped, changes in body composition, osteoporosis and menopause in women. This happens because many cells die, decrease in cell mitosis (cell division) to replace damaged cells. Accumulation of dead cells that are disturbing transport between cells so that disrupt the function of tissues, organs and functions of the body's overall physiological functions (Ganong. 2001).

Hertoghe (1996) reported that the decline in somatotropin secretion in patients with pituitary difesiens followed by a decrease in the secretion of other hormones, including reproductive hormones such as estradiol and progesterone. Estradiol and progesterone are steroid hormones that play a role in the reproductive cycle. Estradiol is produced by cells of the ovary growing internal and in very small amounts by the adrenal glands, whereas progesterone is produced by the corpus luteum, a yellow body on the ovaries. Estradiol and progesterone stimulate the growth and development of the female genital tract and the occurrence of estrous cycles in females. This hormone works in the uterus, causing structural changes in the glandular epithelium of the uterus by stimulating hypertrophy of epithelial cells and organelles uterine protein synthesis (Huang et al. 1997).

In addition to working in the reproductive cycle, estradiol and progesterone also have an important influence in maintaining the body's physiological functions such as increasing protein synthesis, collagen synthesis and protect the bone from porous (osteoporosis). This function is stimulated by somatotropin, so these hormones work together (synergy) in maintaining the body's physiological functions. However, with increasing age, levels of somatotropin in the body continues to decline and so do levels of estradiol and progesterone decreased in their secretions. Decrease in estradiol levels cause the cessation of reproductive cycles in female (menopause). Decreasing estradiol levels also increase the risk of osteoporosis in women, loss of skin elasticity and moisture due to decreased protein synthesis and collagen, accumulation of fat in the abdomen and pelvis due liposisis decreased fat and increased risk of hypertension and cardiovascular disease (Veldhuis et al. 2005)

MATERIALS AND METHOD

Forty eight female rats were assigned into a randomized block design with 4 factors. The first factor was age with 2 levels (6 months and 12 months). The second factor was somatotropin dosage with 2 levels (0 and 9 mg/kg body weight). The third factor was duration of injection with 2 levels (3 weeks and 6 weeks), and the fourth factor was sampling period with 2 levels (0 and 14 days after somatotropin termination)

Somatotropin was injected intramuscularly following an research design. To homogenize the estrous cycle in rats, injection of 1000 µg PGF2αy (Prostaglandin F2α)/ kg. body weight dosage was conducted. It was conducted twice Injection of PGF2αy (Prostaglandin F2α) ie; 3 days before the treatment ended and 3 days after the first injection of PGF2αy (Prostaglandin F2α) After homogenization the estrous cycle, rats dissected as much as 12 tails. Taking blood samples for testing levels of estradiol and progesterone do when surgery is taken directly from the heart, red blood allowed to clot and then separated from the serum. Serum was taken and stored in a refrigerator at a temperature of-20oC until ready to be tested.

Levels of estradiol and progesterone were measured by radioimmunoassay method (RIA) technique using a solid phase coat-acount estradiol kit (Diagnostic products cooporati on, Los Angeles, CA),-acount coat progesterone kit (Diagnostic products cooporati on, Los Angeles, CA). Enumeration is done by gamma counter at Balitna Ciawi.

The data obtained were analyzed using GLM ANOVA statistics with the program Minitab 11 for Windows and when there is a real difference or a very real test followed by Duncan.

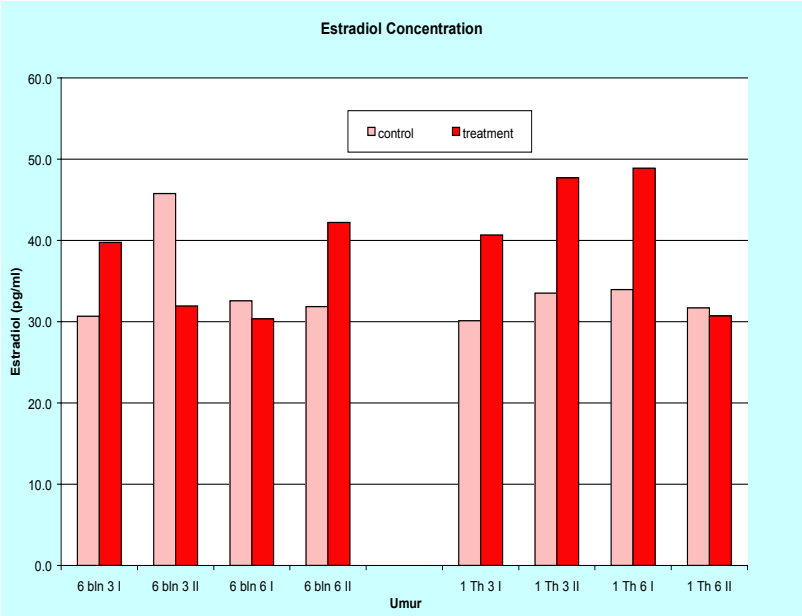
RESULTS AND DISCUSSION

Somatotropin supplementation in female rats aged six-months and one-year increased the concentration of estradiol ($P = 0.013$). Mice aged one year had higher estradiol levels compared with rats aged six- months ($P = 0.007$). There was interaction between age, dosage, injection and long sampling time after injection in increasing levels of estradiol ($P = 0.000$).

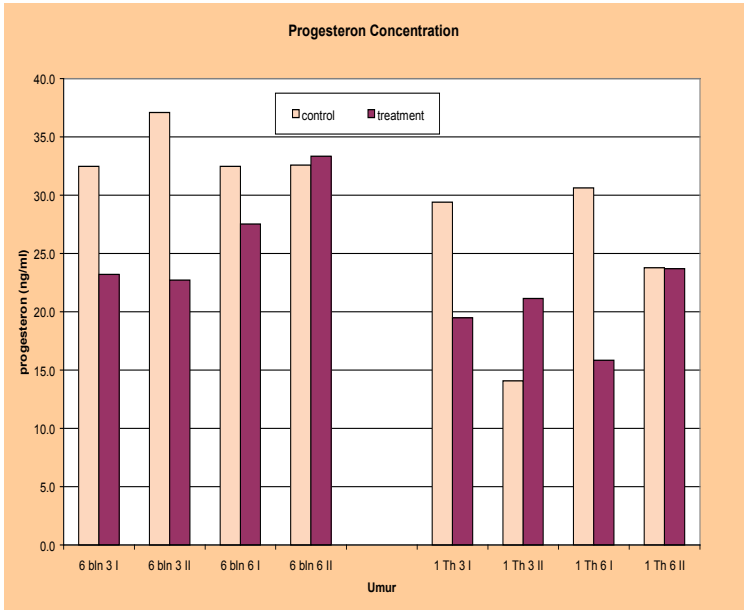
At age six-months female rats somatotropin injection for 3 weeks of estradiol concentrations decreased by 6.23% compared with controls. While the groups of mice aged six-months were injected for 6 weeks there was an increase of 12.61% of the estradiol compared with control. Rats aged one year has 35.48% higher estradiol compared with control rats aged one year

In rats aged 1 year, somatropin injections can stimulate increased levels of estradiol ($P <0.01$). Giving injections of somatropin and duration also increase levels of estradiol. There was interaction between age, dosage, injection time, and time of sampling in affecting levels of estradiol ($P = 0.000$).

The cells of ovarian follicles produce estradiol and estradiol concentrations peaked during the estrous phase. Jump in elevated levels of estradiol at estrus phase gives positive feedback to the hypothalamus for the secretion of LH to ovulation. Increased levels of estradiol produced by cells of ovarian follicle cells compared with controls stimulated by somatotropin treatment, where somatotropin stimulates growth of cells that grew to include cells of ovarian follicles. With the number of follicle cells that mitotic, more estradiol also produced. Estradiol is known to be mitotic and stimulate proliferation of ovarian granulosa cells by increasing the sensitivity of granulosa cells to the presence of substances that are mitogenic, such as insulin and growth factors (growth factor) produced by T cells (Adelien 2001).



Somatotropin injection dose, and age is very real rats interact in influencing the levels of progesterone (P = 0.000). Progesterone levels obtained in this study is quite low in both treatment groups and control groups. This is because the measurement is made at the estrus phase when progesterone concentrations are low. Some of the combination treatment showed results not significantly different (P> 0.05) but there are some combination treatment responded significantly different. In female rats aged six-months and 1 year, somatotropin injections decrease the concentration of progesterone, respectively 26.63% and 19%.



CONCLUSION

From the analysis and discussion it can be concluded that supplementation of somatotropin in female rats aged six months and one-year, age and time of sampling rats caused an increase in estradiol levels in plasma and somatotropin supplementation and age of rats interact very real impact on levels of progesterone female rats aged six months and one year. Further research needs to be done by using female rats older than one year (have gone through menopause), to see the effect of somatotropin injection on levels of estradiol.

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CADMIUM TOXICITY IN GROWTH OF RED LETTUCE (*LACTUCA SATIVA* L. CV RED SUN)

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ABSTRACT

An experiment using a mini hydroponic set up was carried out to study the effect of cadmium (Cd) on growth of red lettuce (*Lactuca sativa* L. cv Red Sun). The culture was maintained in 1/10 MS medium at 25°C with 12 h light. Five levels of Cd were evaluated (0ppm, 10ppm, 20ppm, 30ppm and 40ppm) in the form of $CdCl_2$. Treatment was given to three week seedlings for two weeks. Solution was refreshed every week. Several growth characteristic were monitored including number of leave, length and width of leave, shoot fresh weight and root fresh weight. There was a significant decrease in the number of leave when seedlings were treated by 20ppm, 30ppm and 40ppm. The length and width of leaf were reduced in each treatment of Cd levels. However there was no significant different between 10ppm, 20ppm, 30ppm and 40ppm. Fresh weight of shoot decreased around 50% when 10ppm Cd was applied. The reductions of hoot fresh weight were bigger at 20ppm, 30ppm and 40 ppm, but there is significant different between these three Cd concentration. Same pattern was observed for fresh weight of root. The reduction of growth in plant due to exposure to Cd is caused by alteration of mineral uptake, stomatal opening and photosynthesis.

Keywords: cadmium, growth, *Lactuca sativa* L.

INTRODUCTION

Cadmium is one of heavy metals that contaminate soil. Cadmium contamination comes from fossil fuel, industrial activities, sewage sludge applications, agricultural practices such as the use of pesticides and fertilizers (Clemens, 2001). Cadmium is a mobile heavy metal and its accumulation is affected by soil pH. In acidic soil, Cd accumulation increases up to 20 times (Clemens, 2001).

In high concentration, cadmium is dangerous for most living organisms. Horticultural products can be a place for accumulation of cadmium and then enter the food chain (John *et al*., 2009). Lettuce (*Lactuca sativa* L.) has become a common leafy vegetable in Indonesia. It is used as fresh vegetable in salad. Although it is not original plant of Indonesia, lettuce can be cultivated easily in Indonesia. The objective of this study was to evaluate the effect of Cd treatment on growth of *L. sativa* L. cv Red Sun. in hydroponic system.

MATERIALS AND METHODS

Seeds of red lettuce (*L. sativa* cv Red Sun) were germinated in petri dishes with wet filter papers. After five days, seedlings were transferred to hydroponic system with 1/10 MS medium. The cultures were maintained for three weeks and the solution was refreshed every week.

The three weeks old plants then were treated with different concentration of Cd (0ppm, 10ppm, 20 ppm, 30ppm and 40 ppm) in the form of $CdCl_2$. Treatments were given for two weeks and the media were refreshed every week to prevent depletion of heavy metal and nutrient. The plants were grown at 25°C with 12 h light. The treatments were repeated three times.

Several growth parameters were studied including leaf number, length and width of leaf, fresh weight of shoot and root. The inhibitory rate (IR, %) of shoot and root fresh weight was calculated by the following formula :

$$IR = \left(1 - \frac{x}{y}\right) \times 100$$

where x and y are the average values detected in the treated sample and control.

RESULTS AND DISCUSSION

Growth of *L. sativa* L. cv Red Sun was strongly inhibited by cadmium (Figure 1). Generally when higher concentrations of cadmium were applied, the reduction of growth was stronger. The number of leaves decreased in response to high concentration of cadmium (Figure 2a). Concentration of 20 ppm, 30 ppm and 40 ppm significantly reduced leaf number compared to control plants and 10 ppm treatment. However, 30 ppm cadmium has the same effect as 40 ppm cadmium.

The length of leaves (without length of petioles) and the width of leaves were greatly affected by cadmium (Figure 2b and 2c). Compare to control plants, treatment of 10 ppm, 20 pmm, 30 ppm and 40 ppm cadmium significantly decreased leaf length and width. For leaf length, there were no significant different effects between 10 ppm and 20 ppm. While for leaf width, there were no significant different effects between cadmium concentrations used.

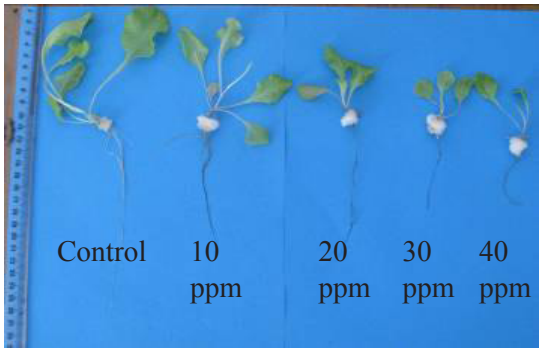

Figure 1. The growth of *L. sativa* L. in different concentrations of cadmium (from left to right : control, 10 ppm, 20 ppm, 30 ppm and 40 ppm)

The biomass was also reduced in the presence of heavy metal. Table 1 demonstrates the decreased of shoot fresh weight and root fresh weight as well as the inhibitory rate of cadmium for fresh biomass in *L. sativa* L. The higher concentration of Cd used, the more the inhibition rate of fresh weight shoot and root.

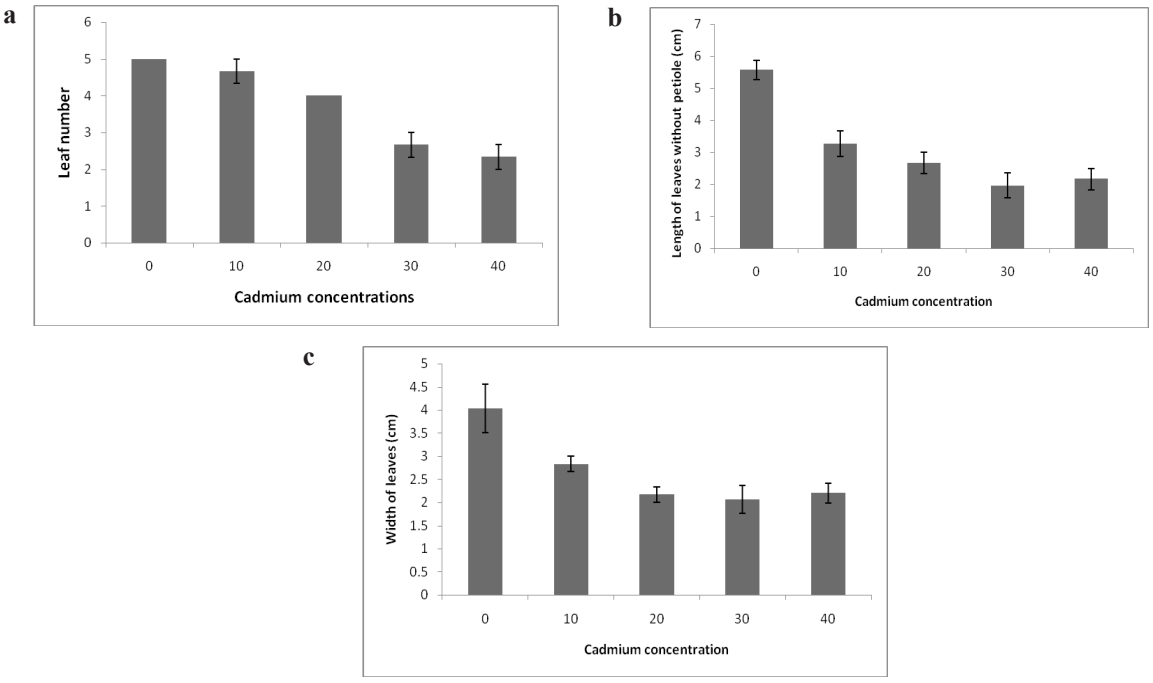

Figure 2. The effects of cadmium treatment on leaf number (2a), length of leaves (2b) and width of leaves (2c)

Table 1. Shoot and Root Fresh Weight of Lettuce Treated with Different Concentrations of Cadmium and the Inhibitory Rate of Cadmium

Cadmium concentration (ppm)	Shoot fresh weight (g)	Inhibitory rate %	Root fresh weight (g)	Inhibitory rate %
0	0.5454 ± 0.101165a	0	0.0325 ± 0.006897	0
10	0.2171 ± 0.048219b	60%	0.0159 ± 0.004632	52%
20	0.0981 ± 0.016001b	81%	0.011133 ± 0.00372	63%
30	0.0646 ± 0.014268c	88%	0.0036 ± 0.000351	90%
40	0.0577 ± 0.014855c	89%	0.0025 ± 0.000153	92.5%

Surprisingly, at lower concentrations, the growth of shoot in cadmium-contained media was more affected that the root. In heavy metal contaminated soil, it is well known that the growth of root is heavily affected (Hakmaoui *et al*., 2006). However, in this study, stronger inhibition of root growth compared to shoot growth only occurred at higher concentration of cadmium. This fact may due to that the root cells of *L. sativa* cv Red Sun has a well-functioning defense system for lower concentration of cadmium.

The inhibition of plant growth during stress is due to low water potential, which restricted nutrient uptake (John *et al*., 2009). It was shown that Cd treatment caused inhibition of chlorophyll synthesis (Padmaja *et al*., 1990) and the decrease of photosynthetic rate (Dong *et al*., 2005). Furthermore, it was also suggested that the growth inhibition is driven by direct effects on both cell division and elongation (Vassilev *et al*., 2005). This is supported by Kiran and Sahin (2006), who suggessted that there was a decrease in mitotic index because of cadmium treatment.

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POST HARVEST MANAGEMENT OF ROSE (*Rosa hybrida)* AS CUT FLOWER

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ABSTRACT

Rose is one of the well known cut flower that requires proper maintenance after plant were grafted.

The study was explored by surveying some farmers at Cipanas, West Java. The rose were grown using root stock (wild rose)(*Rosa indica*) and scion from shoot of hybrid rose plant. Flowers can be harvested in 2-2.5 months after the scion grafted to mother plant. Flowers were harvested when one or two petals had opened. Flower were cut using sharp knife to avoid stalk injury. Farmers usually harvest the flower in the morning, collect and grade them into class A and B depend on the size of the flower. Every 20-25 flowers were tied up and wrapped using banana leaves to prevent loss of water due to transpiration. Farmers do not use chemical to prolong the self life of the flower

INTRODUCTION

Rose (*Rosa hybrida)* is belonged to cut flower in ornamental plants. Its flower is very attractive due to its colour and shape. Rose colour of flower depends on cultivar, such as red, pink, black, purple, yellow and white. The flowers give fragrance with their special aroma. At Sindanglaya, Cipanas, West Java, Indonesia, some cultivars were cultivated to produce cut flowers. The cultivars were introduced from overseas and adapted to Cipanas area.The others were domestic cultivars that cultivated by some farmers.

Production of cut flower required proper cultivation in the field and post harvest handling before marketed to consumers or suppliers. This paper is pointed to discuss how to manage the post harvest of rose as cut flower.

MATERIALS AND METHODS

Data were collected by surveying some farmers and some data were presented from Balithor Cipanas (Horticultural Research Centre) at Cipanas, West Java.

RESULTS AND DISCUSSION

Cultivar

Farmers grew some cultivars, such as Sekul, Ema, Amerika, Merah Bata, Merah Tua, Camelot, and Pink Negeri. Farmers prefer to grow Merah Bata, Sekul, and Pink Negeri because the flower can be harvested faster and has lots of flower. Cultivars with white and yellow flower blossoms longer than the other cultivars thus only a few farmers grow them in special occasion for christmas day.

Balithor Cipanas (Horticultural Research Centre) at Cipanas, West Java grew other cultivars such as Lady X, Camelot, Crymson Glory, and Mr. Lincoln

Planting materials

There were two types of planting materials, rootstock as mother plant and scion for bud grafted. Root stocks were planted in soil for 5-10 cm in depth. The root stocks were used to combine with scion from rose hybrid to produce new type of flower. Root stock plant usually has vigorous root for mother plant to stand in the soil and absorb water and also mineral from soil. Three months after bud was grafted (the scion grafted to the mother plants), the plant were moved to collection area for maintenance until the new plant flowering.

Plants maintenance

The rose plants were fertilized using Urea, TSP and KCl during planting time. Pesticide were applied

once a month to keep plant healthy. Mehran *et al*. (2007) reported that using pre-harvest fertilization of Ca is a conventional treatment to improve storage life of apple and also rose. Ca fertilization with concentration of 10 mM $CaCl_2$ at 40, 30 and 25 days before harvest, could improved vase life compared to control. Ten days after harvesting the cut flowers treated with $CaCl_2$ had good quality while the quality of control flowers decreased on fifth day after harvesting.

Others maintenance such as watering/irrigating, application of pesticide, staking and earthing is required for the plants in the field. Anonim (2008) reported that earthing was done after 6 to 8 weeks of planting. Staking was required after the emergence of flower spikes, so the spikes may not fall down when there is a strong wind. Care should be taken not to injure the leaves at any stage as the injury is harmful to the plants (Anonim, 2008).

Harvesting

Harvesting the rose flower can be done 2-2.5 months after bud was grafted. New rose flower will bloom from the shoot of grafted branch. The flower can be harvested if 1-2 petals have opened.

Farmers usually harvest the flower every morning. Flowers were cut using sharp knife from the flower stem at 10-15 cm from the soil. The root stock was left in the field.

After harvesting, the flowers were collected, graded based on colour of flower and held together by rubber band.

Post harvest handling by local farmer is very traditional. Nunes reported that in order to minimize these losses, studies were conducted to determine the effects of some chemicals wich promote a later senescense, through the following treatments: control sample destillated water, potassium nitrate 40 ppm, gibberellic acid 60 ppm, magnesium sulfate 40 ppm. After harvesting, the flower branches were immersed in the solutions, where they stayed till the senescence began.

PACKAGING

Bundles of 20-25 flowers were first wrapped in banana leaves for protection from sudden temperature fluctuations, bruising and moisture loss. Then they were fasted with rubber bands, packed in bamboo basket, then it ready for distribution to consumers or supplier at around Cipanas and Jakarta, Indonesia. Farooq *et al.* (2004) showed that storage and vase life of rose cut flower can be improved by harvesting flower at tight bud, packing in aluminum foil then stored at 3°C. By proper post harvest handling, the benefit of cut flower can be extended.

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MOLLUSC SPECIES AT RICE FIELD AREAS OF KESIMAN KERTALANGU CULTURAL VILLAGE, DENPASAR

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ABSTRACT

Indonesia has vast areas of freshwater habitats, including rice fields. These areas sometimes have been drain and inundated by water. These water-inundated areas have been utilised as habitat by molluscs. Research has been undertaken at rice field areas of Kesiman Kertalangu Cultural Village, Denpasar. Some of the sites were explored to sample molluscs occured in that areas. The mollusc samples were preserved in 70% alcohol prior to identification in the Laboratory. Identification was based on morphology of the mollusc, referring to Dharma (1988), Djajasasmita (1999), Burch (1980) and Jutting (1956). Seven species of molluscs were observed occured on the site, namely *Lymnaea rubiginosa*, *Bellamya javanica*, *Pomacea canaliculata*, *Pomacea* sp., *Melanoides tuberculata*, *Melanoides* sp. and *Corbicula javanica*. *Lymnaea rubiginosa* was observed to have the highest species density compared to others, while *Corbicula javanica* has only been sighted to inhabit irrigation channels that composed of sandy muddy substrates.

Keywords: *species, mollusc, Kesiman Kertalangu Cultural Village*

INTRODUCTION

Indonesia has high biodiversity including biodiversity on freshwater habitat. Lake, swamp, rivers and reservoir with the irrigation channels including in freshwater habitat. Rice fields can also be classified as freshwater habitat. Rice field is an agricultural land sometimes have been drain and inundated by water. Although only watery in a relatively short time but the variety of fauna can live there. Mollusc is one of the fauna used the rice field as habitat. Recorded about 24 species of freshwater mollusc commonly found in the rice field (Djajasasmita, 1993; Djajasasmita, 1999).

Molluscs have some benefit for humans for example can be used as a protein source. The shell of mollusc can be mixed with livestock feed, especially fowl feed, as source of calcium (Dharma, 1988). Recently, many sea-shells have been used for materials of handycrafts and assessories (Leimena, 2002). The use of shells of mollusc as souvenir or handycraft, has providing economic benefits for the sellers, as source of income, including when they were exported.

On the other hand, mollusc also caused a problem in agriculture, which attack the plant (Djajasasmita,1993). One of the mollusc species which caused the problem for agriculture is *Pomacea canaliculata.* This species is very potential to become a major pest because it reproduce very fast and attack young plants (Budiyono, 2006). Freshwater mollusc also known serves as intermediate host of parasite trematodes and nematode. *Lymnaea rubiginosa* is one of the mollusc species infected by rediae or cercariae of *Fasciola hepatica*. Darmono *et al* (1983) in Djajasasmita (1985) examined specimens of *Lymnaea rubiginosa* collected from six villages in Bali, 9,52% of which were infected by rediae and cercariae of *Fasciola hepatica*.

The study was conducted because it is believed that the mollusc play an important role in the ecosystem. Meanwhile, the data available related to them in rice fields are still limited. Thus, a preliminary study of them was carried out in rice fields of Kertalangu Cultural Village of East Denpasar. Putra *et al*. (2010) stated that the site is considered to be a productive agricultural land.

MATERIALS AND METHOD

Samples of mollusc were taken at rice field of Kesiman Kertalangu Cultural Villages, Tohpati-East Denpasar. Samples were taken in a flooded rice field (newly planted with rice), unflooded rice field (rice plant is already tall) and dry rice field. Sampling was carried out by the exploration some of the rice terraces in the village and mollusc found in rice field was sampled, including from irrigation channels that irrigate the rice fields. The mollusc samples were preserved in 70% alcohol prior to identification in

the Animal Taxonomy Laboratory-Udayana University. Identification was based on morphology of the mollusc, refering to Dharma (1988), Djajasasmita (1999), Burch (1980) and Jutting (1956).

RESULTS AND DISCUSSION

Seven species of mollusc has been identified at rice field of Kesiman Kertalangu Cultural Villages as listed in Table 1.

Table 1. Mollusc Species at Rice Field of Kesiman Kertalangu Cultural Villages

No	Species	Family	Class
1.	*Lymnaea rubiginosa*	Lymnaeidae	Gastropoda
2.	*Bellamya javanica*	Viviparidae	Gastropoda
3.	*Pomacea canaliculata*	Ampullariidae	Gastropoda
4.	*Pomacea* sp.	Ampullariidae	Gastropoda
5.	*Melanoides tuberculata*	Thiaridae	Gastropoda
6.	*Melanoides* sp.	Thiaridae	Gastropoda
7.	*Corbicula javanica*	Corbiculidae	Bivalvia

Lymnaea rubiginosa was observed to have the highest species density compared to others. The density of the species was 35 to 100 individuals in the area of 50 cm x 50 cm. The species are also observed almost cover the entire surface of each flooded rice fields (newly planted with rice). According Djajasasmita (1985), *Lymnaea rubiginosa* is very common freshwater species, inhabit almost all types of freshwater habitats.

In the dry rice field (soil cracks and rice plants are dry), many species of *Lymnaea rubiginosa* were found dead. This is likely related to the fact that the species does not have shell covers (operculum), so there is nothing to protect its body from high temperatures. Marwoto (2011) stated that in general the species that do not have shell covers (operculum) is more susceptible to changes in temperature, substrate and flow.

Bellamya javanica has only been sighted to inhabit rice field and also found in low density. The density of the species was 1 to 5 individuals in the area of 50 cm x 50 cm. *B. javanica* was observed together with *Lymnaea rubiginosa* on each flooded rice fields (newly planted). Genus of Bellamya was included on Operculata group because the genus has shell covers (operculum). The genus prefers freshwater habitat that composed of muddy substrate, overgrown with aquatic plants and slow water flow, such as rice field, swamps, the edge of lakes and small rivers. This species prefers clear and clean water.

Melanoides tuberculata and *Melanoides* sp. was found in rice field and irrigation channels that irrigate the rice field. Both places have sandy mud and muddy substrate. Supian and Ikhwanuddin (2002) stated that *Melanoides tuberculata* is the most common and most wide-ranging member of the family Thiaridae, and is found in almost any kind of freshwater.

Pomacea canaliculata, known in Indonesia as *keong mas* or *siput murbei*, has been found with eggs attached to the rice plants. The species can be found either in flooded rice field (newly planted), non-flooded rice field (rice plant is already tall) and also in the irrigation channels. These species has good adaptation and also able to eat everything in the environment or called as polifagus (Ghesquiere, 2003). According to information from local farmers, the species is quite damaging although not as much *Lymnaea rubiginosa* because *P. canaliculata* eat young rice plants. Budiyono (2006) stated that *P. canaliculata* has been devastating to young and soft stems plants such as rice seedlings. The habitat for this species is usually ponds, swamps, rice fields, water drainages, and other inundated habitats. The species needs special attention because they can reproduce very fast and attack plants on the early stages of their growth. Uncontrolled growth of the population of *P. canaluculata* may cause severe damage to rice especially on the area which is continuously planted by rice.

Corbucila javanica has only been seen to inhabit at irrigation channels that composed of sandy mud substrates. This is due to *C. javanica* prefers sandy mud substrates while rice fields is generally muddy

areas. This is the same with what has been reported by Djajasasmita (1985) who observed the species on the irrigation channels.

CONCLUSION

Seven species of molluscs were observed existed in the rice field. *Lymnaea rubiginosa* was observed to have the highest species density compared to others, while *Corbicula javanica* has only been seen to inhabit irrigation channels that composed of sandy muddy substrates.

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ANTIBABESIAL ACTIVITY FROM *EUCHRESTAHORSFIELDII* AGAINST *BABESIAGIBSONI* IN CULTURE

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ABSTRACT

Antibabesial activity of *Euchrestahorsfieldii* seeds extract against *Babesiagibsoni* in culture were evaluated. The air dried seeds of *E.horsfieldii*were boiled. The boiling water was filtered and extracted with EtOAc to give aqueous andEtOAc extracts. Evaporation of the EtOac extracts afforded a residue which was subjected to chromatographic SiO_2 column and eluted with each 500 ml of $CHCL_3$, 3% MeOH/$CHCL_3$, 20% MeOH/$CHCL_3$, 70%MeOH/$CHCL_3$,and MeOH successively. To evaluate the antibabesial activity, *B.gibsoni* used in this study was maintained in culture medium consist of 60% RPMI 1640, 40 % normal dog Serum, 6 % normal dog RBC_s (red blood cells).. The test was done using 96-well plates. Concentration of each extract were 1000, 100, 10, and 1µg. The plate was incubated at 37^0 C, 5% CO_2, 90% N_2 for 72 hours. A Giemsa stained thin smeared specimen was prepared and the percentage of parasitemia was counted. Effects of the extract on *B. gibsoni* growth showed that the crude extract and fraction 3 % MeOH/$CHCL_3$ produced 93 % and 89% inhibition of parasite growth, respectively when tested at a concentration of 10 µg/ml.

Keywords: Antibabesial activity, *Euchrestahorsfieldii,*Parasitemia,Inhibition

INTRODUCTION

Babesiosis is a tick-borne hemolytic disease of domestic and wild mammals caused by intraerythrocytic parasites of Babesia. *Babesiagibsoni* and *B. canis* are two species of parasites commonly known to infect dogs and cause canine babesiosis.In general, *B gibsoni,* as well as malaria parasites of *plasmodium falciparum,* invade red blood cells of host and induce severe anemia. The disease is characterized by remittent fever, progressive anemia, hemoglobinurea, marked splenomegaly, hepatomegaly and sometimes causes death (Adachi *et al*., 1993; Farwell *et al*., 1982). Many kinds of commercial medicines able to treat this diseases. It sometimes induces severe side effects such as weakness, irritability, paralysis, and fatal central nervous system haemorrhage. Therefore an alternative chemotherapeutic agent with less side effects is urgently needed for the treatment of *B. gibsoni* from natural sources such as medicinal plants.

MATERIALS AND METHOD

Preparation of *E. horsfieldii* seed extracts

E. horsfieldii seeds were ground into small pieces and air dried in the shade for several days. The air dried seeds were boiled two time with each 1 L water for 30 min. The boiling water was filtered and extracted with EtOAc to give aqueous and EtOAc extracts. Evaporation of the EtOac extracts afforded a residue which was subjected to chromatographic SiO_2 column and eluted with each 500 ml of $CHCL_3$, 3% MeOH/$CHCL_3$, 20% MeOH/$CHCL_3$, 70%MeOH/$CHCL_3$,and MeOH successively. The aqueous extracts was also evaporated (Subeki, 2005)

Antibabesial activity assays in vitro

Babesiagibsoni parasites used in this study were maintained culture medium as previously reported by Yamasaki *et al.* (2000) Heparinized venous blood from a normal dog was washed three times with Vega y Martinez solution (Vega, 1985).) and then washed twice with RPMI 1640 supplemented with sodium pyruvate (0.1 mg/ml), glutamine (0.3 mg/ml), sodium bicarbonate (2 mg/ml), penicillin (100 units/ml) and streptomycin(100 µg/ml). After being washed, erythrocytes were resuspended to a final packed

cell volume of 5% in culture medium consisting of 60% RPMI 1640 medium and 40 % normal dog serum. The culture medium was mixed with Babesia gibsoni infected erythrocytes and incubated at 37^0 C under a humidified atmosphere 0f 5% CO_2,5% O_2, 90% N_2. Every three days, 60 % volume of the culture supernatant was aspirated and an equal volume of uninfected fresh erythrocytes was mixed with infected cultured erythrocytes every 7 days.

To evaluate the antibabesial activity, each of the crude and fractions extract were dissolved in a small quantity of dimethyl sulfoxide (DMSO) and further diluted in RPMI 1640 medium. The cultured *B.gibsoni* parasites were mixed with fresh medium get to 25 % parasitemia at the start incubation. The test was performed in a 96-well culture plate, and each well contained 30 µl of parasitized erythrocytes suspension and 30 µl of extract solution containing the respective concentration of each extract. The concentration were 1000, 100, 10, and 1 µg/ml. The parasite in the culture plate was further incubated for 72 hour.and a Giemsa stained thin smeared specimen was prepared.

***B. gibsoni* on bloodsmear slide with cytospin**

After keeping the plate in the incubator, was prepared a Giemsa stained thin smeared specimen with cytospin. Culture that contain *B. gibsoni* and plant extract from the plate (60 µl) added 80 µl cytospin solution. The culture was centrifuged in cytospin with 450 rpm for 5 minute remains residues on glass slides. The glass slides soaked in MeOH for 2 min and dried at room temperature. Soaked in Giemsa stain. After 60 min. washed with water and were dried.

Calculated % of Parasitemiaand Inhibition

Parasitemia level was determined by counting the number of parasitized cells in 100 erythrocytes. Inhibition of *B. gibsoni* parasite growth was counted by :

Inhibition = { (% PE - % PC) : %PC}x 100

Inhibition = Inhibition of B gibsoni parasite growth

PE = Percentage parasitemia of erythrocytes on the culture that contain *E. horsfieldii*extracts

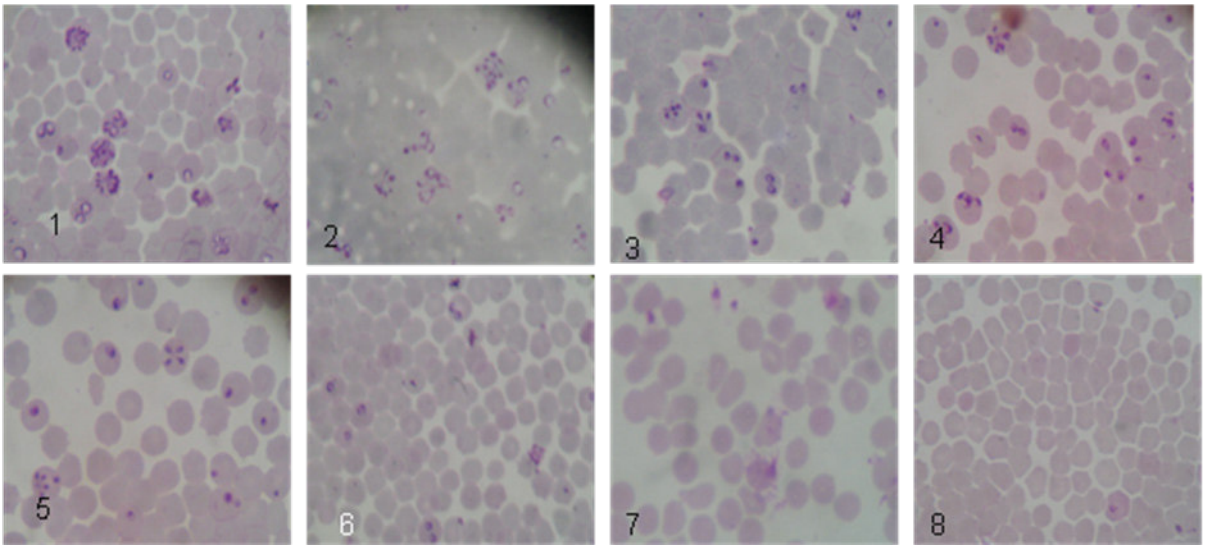
PC = Percentage Parasitemia of erythrocytes on the control

Resultsand Discussion

Antibabesial activities of the *E. horsfieldii* extracts on B.*gibsoni* in culture are shown in Table 1. Result showed that EtOAc extract and 3 % MeOH/$CHCl_3$ fraction have appreciable activity with 0.6 % and 1 % parasitemia at concentration 10µg/µl. The inhibitions of *B gibsoni*parasite growth are 89 % for EtOAc and 85% for 3 % MeOH/$CHCl_3$.

Table 1.Antibabesial activity of *E. horsfieldii*extracts against *B.gibsoni* in Culture

	Parasitemia (%)	Inhibition (%)
Crude extract	4.3%	52 %
H_2O	1.3%	82 %
EtOAc	0.6 %	89%
$CHCl_3$	6 %	35 %
3 % MeOH/$CHCl_3$	1%	85 %
20 % MeOH/$CHCl_3$	1.8 %	77 %
70 % MeOH/$CHCl_3$	4.92%	46 %
MeOH	4.2%	53 %



Changes of *Babesia gibsoni* on Giemsa stained blood smear with *Euchresta horsfieldii* seed extract treated.(1) control. (2) $CHCl_3$. (3)70 % MeOH/$CHCl_3$ (4) Crude extract (5) 20 % MeOH/$CHCl_3$ (6) H_2O (7) 3 % MeOH/$CHCl_3$ (8) EtOAc

E.horsfieldii is an important component of folk medicine for the treatment of malaria, an antidote of snake poisoning, as aphrodisiac and antidiabetes. The phytochemical investigation of *E. horsfieldii* has previously shown the presence of alkaloid, cytosine, flavonoid, saponin, and polyfenol. Mizuo Mizuno *et al* (1990) reported new compounds ;euchrenones, glabrol (flavanone), pterocarpan, and ferulic acid ester.

CONCLUSION

In general , results from this study showed that difference of inhibition *B. gibsoni* parasite growth was observed among the different fractions of *E. horsfieldii,* and this may be attributed to the presence of the active compounds in more than one fraction. It would be of interest to further investigate, isolate and identify the possible active principle component of the extracts towards development of antibabesial drugs.

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OPTIMISING RUMEN FUNCTION OF BALI CATTLE FED RATION BASED ON AGRICULTURE BY-PRODUCTS WITH SUPPLEMENTATION OF MULTIVITAMINS-MINERALS

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ABSTRACT

A research has been carried out to optimize the rumen function and the rumen microbial protein synthesis in Bali Cattle (Steer). Sixteen Bali cattle were used in this experiment. This experimen used a Completely Randomized Block Designed with four treatments and four blocks. The first treatment was ration based on agriculture by products without supplementation (R_0), while the other three treatments were supplemented with 0.075% (R_1), 0.150% (R_2) and 0.225% (R_3) multivitamins-minerals. The variables of rumen microbial protein synthesis including urinary exretion of allantoin, purin derivatives absorbsion, rumen microbial protein synthesis and efficiency of rumen microbial protein synthesis were observed. Feed and nutrients degradation on the rumen, pH, concentration of NH_3-N, VFA and amount of protozoa in the rumen fluid also were measured in the experiment. The result showed that rumen microbial protein synthesis and amount of microbial purines absorbed in suplementation of 0.150% and 0.225% multivitamins-minerals were significant difference (P<0,05) compared with without supplementation (222.83 and 222.24 gram/day Vs 197.83 gram/day) and (49.45 and 49.35 Vs 44.76 mmol/day), but they were not significantly different with without supplementation on rumen microbial protein synthesis (213.57 gram/day) and on amount of microbial purines absorbed (48.67 mmol/day). Degradable organic matter and Sulfur absorbed in the rumen, amount protozoa, and ruminal pH, concentration of NH_3-N, totally VFA and propionate acids of rumen fluid significantly (P<0,05) increased as well. Increasing supplementation until 0.15% multivitamins-minerals were decreased concentration of acetate acid, butirate acid and methane gas production compare with without supplementation, but increasing until 0.225% supplementation of multivitamins-minerals increased concentration of butirate acid and methane gas production. Moreover, regression analyzed showed that suplementation of multi vitamins-minerals of 0.188% coused maximum rumen microbial protein synthesis of 223,39 gram/day. It was concluded that rumen microbial protein synthesis of Bali cattle given ration based on ammoniated rice straw could be increased as maximum 12.92.%. The level optimal multivitamins-minerals supplementation used in this experiment was 0,188%.

Keyword: *Agriculture by-products, Bali cattle, Multivitamins-minerals, Microbial Protein Synthesis, Rumen Function.*

INTRODUCTION

Optimise the rumen function on degrading fibrous feed such as non-conventional feedstuffs is one of Bali cattle productivity improvement strategy. The products of rumen function such as degraded nutrients in the rumen, metabolic rumen product (VFA, and NH_3-N) and microbial biomass are essential nutrients to meet the nutrient demands of the anaerobic microbes and body tissues of ruminant animals (Mohamed and Chaudhry, 2008). Microbial protein synthesis in the rumen supllies 40 to 80% of the amino acids absorbed by ruminants (Karsli, 1999) and the amino acid composition of microbial protein is similar to that of protein in the main animal product such as milk, lamb or beef (Orskov, 1992 cited by Verbic, 2002). Microbial protein synthesized in the reticulo-rumen constitutes almost the only source for protein digestion in the small intestine on the animals given feeds with such low quality fibrous diets like as agricultural by product (Oosting *et al*., 1993). That ways better strategies minded national program to support feed security especially ruminants feed focused on optimise use agriculture waste and agro-industry by products as feed (Ilham, 2006).

The maximum potential of rumen microbes to produce microbial protein and degraded nutrients in the rumen can be explored only by the provision of high quality diet (Verbic. 2002), so increasing rumen function in cattle fed ration based on agriculture by product like as rice straw should be focused on strategies for through many problems especially deficiency nutrient and low degistibility at that feed materials. Applied urea ammoniation technologies verified can increased digestible rice straw (Chenost and Kayouli, 1977; Partama, 2005), in spite of inadequacy micro-nutrients such as minerals Ca, P, Mg, Cu, Zn, Mn, Co, Fe, S, and vitamins A, D_3 and E can not increased (drake *et al*., 2002). Multivitamin-minerals supplementation of cattle given ration base on waste is one of the best strategy a farmer can make. That statements founded on vitamins and minerals are important factor to affected the effectivity rumen fermentation and efficiency of microbial protein syntheis (Karsli dan Russell, 2001).

Minerals and vitamins supplement to enhance fermentative digestion and microbial growth efficiency in the rumen of cattle on poor quality feed. The macro-minerals (Ca, P and Mg), micro-minerals (Cu, Co,

Zn, Mn, Fe and S) and multi-vitamin (especially Vitamin A and E) most important to rumen fermentation activity, feeds degradation, and microbial protein synthesis (Leng, 1997). The levels of sulfur and ammonia in rumen fluid which maximize digestibility of fibrous carbohydrates appear to be 1–2 mg S/l and 50–80 mg ammonia N/l respectively, whereas maximum microbial growth efficiency seems to require 4–10 mg S/l and 150–200 mg ammonia N/l respectively (Leng, 1997). Even though, Bal and Ozturk (2006) showed optimise rumen microbial protein synthesis needed 1.6 – 1.9 g Sulfur/kg organic matter digested with N/S ratio 18.5:1. Limited intake of sulfur may restrict rumen activity and microbial protein synthesis when large amounts of non-protein nitrogen are fed to ruminant animals, such as urea or effect given urea ammoniated feeds (Karsli and Russell, 2001). In spite of, given fed with very high sulfur can decreased fed consumsion, respiration stress even death (Kandylis, 1994 disitasi Parakkasi, 1999). Phosphorus is another mineral required for the synthesis of ATP and protein by rumen microbes. Microbial protein synthesis can be limited by an insufficient supply of P for microbial growth. Mills *et al*., 1969 (cited by Parakkasi 1999) describe the mineral zinc (Zn) as components and activator of any enzyme such as *deyidrogenase, peptydase* and *fosfatase* with functions at nucleat acid metabolisms, carbohydrate metabolisms and protein synthesis. Rumen microbes needed 130-220 mg Zn/kg for optimise microbes growth and yields. Other minerals such as Ca, Mn, Fe, Co and Cu also important for microbial protein synthesis, synthesis of ATP and vitamins B, microbes activities, nutrient degradable (Karsli dan russel, 2001; Parakkasi, 1999). Supply of vitamins A and E important on given ration based on urea ammoniated rice straw because that materials deficience (Chenost dan Kayouli, 2008). Vitamins A and E function for microbes growth especially microbial protein synthesis and energy supply for microbes activities (Parakkasi, 1999). Supply of vitamins B-complex also important couse low of supply mineral precursor finding that vitamin such as minerals S or Co on feed material.

This experiment was conducted to determine the best level of multivitamins-minerals supplementation in the fermentation process on ration based on agriculture by-product that could increase of rumen function to degrade nutrients, produce high quality of rumen metabolic product, and increase supply of nutrients for host animal. The research results are expected to give early illustration of science and technology development especially in optimizing the utilization of local resources based on agriculture by-product feedstuffs to support feed security of ruminant production.

MATERIALS AND METHODS

Location, Animals, Diet and Experimental Design

A research has been carried out at farm of "Nandi Abian" farmers group association, Abianbase of Gianyar Regency used sixteen Bali cattle mean body weight 244.19 + 33.78 kg. They were kept in feedlot pens (individual concrete pens) on site for duration of the study. This experiment was run for 12 weeks at the farm and continously laboratory experimental for data analysis at Animal Nutrition Laboratory, Faculty of Animal Husbandry and Analytic Laboratory, Udayana University.

This experiment used a Completely Randomized Block Designed with four treatments and four blocks base on body weight of cattle. The treatment were as follows:

R_0 = Basal Ration without suplemented multivitamins-minerals
R_1 = R_0 were suplemented with 0.075% multivitamins-minerals
R_2 = R_0 were suplemented with 0.150% multivitamins-minerals
R_3 = R_0 were suplemented with 0.225% multivitamins-minerals

Basal ration composed by urea ammoniated rice straw and agroindustry by product. Ration and water provided *ad libitum*. Materials Feedstuffs and Nutrient composition can see at Table 1 and 2.

Table 1. Material Composition of Ration Experimental

Feedstuffs Composition	Composition Ration (%)			
	R_0	R_1	R_2	R_3
Urea Ammoniated Rice Straw	25.000	25.000	25.000	25.000
Pollard	34.000	33.966	33.932	33.898
Coconut Meal	25.000	24.975	24.950	24.925
Sugarcane	6.000	5.994	5.988	5.982
Coconut Oil	5.000	4.995	4.990	4.985
Limestone ($CaCO_3$)	2.000	1.998	1.996	1.994
Urea	1.000	0.999	0.998	0.997
Salt	2.000	1.998	1.996	1.994
Pignox	0.000	0.075	0.150	0.225
T o t a l	100.000	100.000	100.000	100.000

Table 2. Nutrient Compositions of Ration Experimental

Nutrient[1]	Ration			
	R0	R1	R2	R3
Dry Matter (%)	89.420	89.420	89.420	89.420
Organic Matter (%)	83.728	83.663	83.598	83.533
Crude Protein (%)	14.596	14.584	14.572	14.559
Crude Fibre (%)	12.216	12.211	12.207	12.202
Gross Energy (Mkal)	3.888	3.885	3.882	3.878
Ca (%)[2]	1.348	1.347	1.346	1.345
P (%)[2]	0.450	0.449	0.449	0.449
S (%)[2]	0.300	0.303	0.305	0.308
Zn (%)[2]	0.015	0.016	0.018	0.019
N/S ratio	7.785	7.709	7.634	7.561

Reference: [1)]Analysis by Animal Nutrition Laboratory, Faculty of Animal Husbandry, Udayana University, and [2)]Analysis by Analitic Laboratory, Udayana University

Data Collection, Sampling Procedure and Analysis

Parameters observed on this research are the variable of rumen microbial protein synthesis (MPS), including urinary exretion of allantoin, purin derivatives absorbsion, rumen microbial protein synthesis and efficiency of rumen microbial protein synthesis (E_{MPS}), feed and nutrients (DM, OM, CF, CP, Ca, P, S and Zn) degradation/absorbsion on the rumen, pH, concentration of NH_3-N, Totally VFA, Partial VFA (Acetate Acids, Propioniate Acids and Butirate Acids) and amount of protozoa in the rumen fluid

Feed were randomly collected and fecal samples were taken from total collection of individual cattle during the last 7 day of the study. They were analyzed for chemical composition such as DM, ash, CP and CF contents with proxymate analyzed (AOAC, 1980), and concentrations of minerals Ca (with EDTA method), S (Iodometry method), P and Zn (with Atomic absorption Spectrophotometre/AAS).

At the end total collection period, rumen fluid samples were collected at 4 hours post feeding. Approximately 200 ml of rumen fluid was taken from the middle part of the rumen by using a hand vacum pump. Rumen fluid was immediated measure for pH using portable pH meter (HANNA instrument HI 9025). Concentration of NH3-N rumen fluid calculated with using micro difution Conway technic (AOAC, 1990), Totally Volatile Fatty Acid calculated from concentration of Acetate Acids + Propionate Acids + Butirate Acids. Partial VFA (Acetate, Propionate and butirate) analyses using a high performance liquid chromatography HPLC.

The total direct count of protozoa on rumen fluid were counting with using MFS solution at counting chamber/hemocytometer (Ogimoto and Imai, 1981).

Urine samples were analyzed for allantoin in urine was determined by HPLC as described by

Matsumono et al., 1985 (cited by Partama, 2005). The amount of microbial purines absorbed (X mmol/day) corresponding to the purine derivates ecreted (Y mmol/day) was calculated based on the relationship derivated by Bowen (2003).

$$Y = 0{,}85\ X + 0{,}190\ W^{0,75}$$

Where Y is the excretion of purine derivates (mmol/day); X is the microbial purines absorbed (mmol/day), 0.190 $W^{0.75}$ is contribution of endogenous purine each kg metabolic body weight from Bos indicus

The supply of microbial protein in gram per day was estimated as follows:

$$\text{Microbial Protein (gram/day)} = \frac{70X}{0.116 \times 0.83 \times 1000} \times 6.25$$

With X being the absorption of purine derivates in mmol/day, digestibility of microbial purine is 0.83, the N content of purines is 70 mg N/mmol and the ratio of purine-N : total N in mixed rumen microbes is 11.6:100, the convertion factor N to Protein is 6.25

The E_{MPS} which denote the microbial protein supplied to the animal per unit of DOMR was calculated using the following formula:

$$E_{MPS}\ (g/1000\ DOMR) = \frac{MP\ (g/day)}{DOMR\ (g)} \times 1000$$

Where DOMR = DOMI x 0.65 (ARC, 1990 cited by Khampa and Wanapat, 2006), DOMR = digestible organic matter apparently fermented in the rumen, DOMI = digestible organic matter intake, E_{MPS} = efficiency microbial protein synthesis

Statistical Analysis

Data Collecting were analyzed by Analysis Variance/ANOVA and continued with Honestly Significant Difference test (HSD-test) if necessary (Sastrosupadi. 2000). Contras polinomial analysis were using for optimum supplemented multivitamins-minerals (Winarsunu, 2004).

RESULTS AND DISCUSSION

Effect on Degradable Nutrients on Rumen

The degradable nutrients and absorbed micro-nutrients (minerals) on rumen are presented in Table 3. Degradable organic matter, Sulfur absorbed and N:S ratio on rumen were affected ($P<0.05$) by multivitamins-mineral supplementation, while degradable DM, CP, CF, energy and absorbed Ca, P and Zn on rumen were similar in all treatments. Degradable organic matter and S absorbed on rumen were significantly higher ($P<0.05$) in Bali cattle fed R_3 (2078.10 g/d and 8.66 g/d) than in R_0 (1846.32 g/d and 6.88 g/d) while N : S ratio on rumen was significantly lower ($P<0.05$) in Bali cattle fed R_3 (6.81) than in R_0, R_1 or R_2 (7.78; 7.83; 8.26).

Similar degradable nutrients on rumen (except organic matter and S absorbed) were affected by quality of ration given. In general, rate of digestion nutrien on rumen is the major factor controlling the energy available for growth of rumen microbes (Fellner, 2004). Furthermore, nutrient composition of all ration were similar (Table 2).

Significantly degradable organic matter and S absorbed on rumen by supplementation of 0.225% multivitamins-mineral show increasing efectivity of rumen microbes esspecially rumen bacteria where showed by high microbial protein yield were similar with R_2 but higher ($P<0.05$) than R_0 (Table 5) and with lower (quantitative) amount protozoa than R_2 ($5.80x10^5$ Vs $6.20x10^5$ cells/ml) (Table 4). The low populated protozoa will increasing populated of bacteria and than increasing rumen degradable nutrients (Polan, 1987).

Table 3. Influence of Multivitamins-Minerals Supplementation on Nutrient Degradable and Absorbed on Rumen in Bali Cattle

Item	Treatment[1]				SEM[3]
	R_0	R_1	R_2	R_3	
- Degradable DM (g/d)	2170.02[a2]	2325.66[a]	2417.05[a]	2422.55[a]	67.18
- Degradable OM (g/d)	1846.32[a]	1989.86[ab]	2070.04[ab]	2078.10[b]	52.96
- Degradable CP (g/d)	334.60[a]	370.70[a]	379.28[a]	367.62[a]	11.73
- Degradable CF (g/d)	219.75[a]	212.89[a]	242.05[a]	219.15[a]	13.28
- Degradable Energy (Mkal/d)	8.34[a]	8.85[a]	9.08[a]	9.32[a]	0.27
- Ca Absorbed on Rumen (g/d)	24.64[a]	25.46[a]	26.38[a]	26.63[a]	2.04
- P Absorbed on Rumen (g/d)	6.34[a]	5.15[a]	5.74[a]	4.61[a]	0.77
- S Absorbed on Rumen (g/d)	6.88[a]	7.57[ab]	7.35[ab]	8.66[b]	0.31
- Zn Absorbed on Rumen (g/d)	0.08[a]	0.13[a]	0.20[a]	0.07[a]	0.04
- N:S ratio on rumen	7.78[b]	7.83[b]	8.26[b]	6.81[a]	0.13

Note:
[1)] R_0 = Ration without multivitamins-minerals supplementation, R_1 = Ration with 0.075% multivitamins-minerals supplementation, R_2 = Ration with 0.150% multivitamins-minerals supplementation, and R3 = Ration with 0.225% multivitamins-minerals supplementation, [2)] The same letter in same row is not significantly difference (P>0.05), [3)] SEM = Standard Error of the Treatment Means

Effect on Amount Protozoa and Rumen Fermentation

Table 4 present amount protozoa and rumen fermentation characteristic. As for amount protozoa, supplementation of 0.150% multivitamins-minerals (R_2) increasing amount populations and 60% higher ($P<0.05$) than R_0 but not significantly than R_1 dan R_3. Ruminal pH values were found in a range of 6.94 – 7.34 which were significantly different among treatment. The supplementation of R_3 resulted in highest pH, while the supplementation at R_0, R_1 and R_2 were similar among treatment. The supplementation at R_1 and R_2 also similarly with at R_3. Ruminat pH rumen fluid in this study there are in the normally range pH like as reported by Owen and Goetsch (1988) (except at R_3) are 5.5-7.2 and supplementation of multivitamins-mineran can as buffer for to prevent reduce pH as effect from increase bacteria population. Furthermore, normally ruminal animals depend on cellulolytic bacteriato digest cellulose, but these bacteria cannot resist the low ruminal pH and an increase in pH gradient leads to anion toxicity (Russell and Wilson, 1996). In addition, most ruminal bacteria prefer pH near neutrality for growth although some species (e.g., *Streptococcus bovis* and *Prevotella ruminicola*) can growth in pH 5 to 6 ranges.

The concentration of ruminal ammonia-N (NH_3-N) were significantly higher ($P<0.05$) in the R_3 than in R_0, but not significantly compared with R_1 and R_2. The supplementation of 0.075% (R_1) and 0.150% (R_2) multivitamins-minerals also result high concentration NH_3-N but not significantly ($P>0.05$) compare with R_0 (Table 4). The higher NH_3-N concentration associated with high supplementation of multivitamins-minerals may have been due to increasing effectivity used N component from feeds by rumen microbes. Zinc on multivitamins-minerals have been increasing microbes enzyme activities so that degrable protein process to peptide, olygopeptide, amino acids and ending to ammonia can produce with well (Arora, 1995). Furthermore, higher NH_3-N concentration associated with high organic matter degradable on rumen may have been due to the continuous supply of new substrates from soluble fractions especially N-component (Chen and Hsu, 1998).

Total and partial VFA concentration were significantly ($P<0.05$) by supplementation of multivitamins-minerals. Concentration of totally VFA and propionate acids in Bali cattle fed R_2 were significantly higher ($P<0.05$) than those fed without supplementation of multivitamins-minerals (R_0). The higher VFA concentration associated with high organic matter degradable on rumen (Table 3), protozoa populated (Table 4) and estimation bacteria populated (shown by high microbial protein synthesis, at Table 5). The increasing rumen microbes populated (bacteria and protozoa) as effect the supplementation of multivitamins-minerals have been increasing carbohydrate fermentation to produce VFA. Furthermore, the higher propionate acids concentration on R_2 as effect of high organic matter content with low crude fiber content on ration given (Table 2). The higher total VFA and propionate acids concentration in this study suggested by Hermawan

(2006) were reported supplementation 0.05% ammonium sulfate and 0.03% *pignox* have been increasing totally VFA and propionate acids proportion on rumen.

Table 4. Influence of Multivitamins-Minerals Supplementation on amount protozoa and Rumen Fermentation Characteristic in Bali Cattle

Item	Treatment[1]				SEM[3]
	R_0	R_1	R_2	R_3	
Amount Protozoa (x 10^5 cells/ml)	3.88^{a2}	5.20^{ab}	6.20^{b}	5.80^{ab}	0.51
Ruminal pH	6.94^{a}	6.95^{a}	7.23^{ab}	7.34^{b}	0.09
NH3-N (mM)	5.33^{a}	7.13^{ab}	7.89^{ab}	8.98^{b}	0.66
Total VFA (mM)	114.80^{a}	117.09^{ab}	120.08^{b}	117.24^{ab}	0.89
Partial VFA					
Acetate Acid (mM)	73.10^{b}	72.90^{ab}	72.24^{a}	73.05^{ab}	0.19
Propionate Acid (mM)	22.00^{a}	26.70^{abc}	29.40^{c}	22.60^{ab}	1.33
Butirate Acid (mM)	19.69^{a}	17.49^{a}	18.44^{a}	21.58^{a}	1.14
Concentration of Methane (mM)	40.90^{ab}	38.52^{ab}	37.99^{a}	41.67^{b}	0.83

Note:[1)] R_0 = Ration without multivitamins-minerals supplementation, R_1 = Ration with 0.075% multivitamins-minerals supplementation, R_2 = Ration with 0.150% multivitamins-minerals supplementation, and R3 = Ration with 0.225% multivitamins-minerals supplementation, [2)] The same letter in same row is not significantly difference (P>0.05), [3)] SEM = Standard Error of the Treatment Means.

The methane gas concentration on rumen fluid were significantly (P<0.05) by supplementation of multivitamins-minerals on ration based on urea ammoniated rice straw. Concentration of methane gas production in Bali cattle fed R_3 were significantly highest than the others treatment, while given fed R_2 result lowest methane gas production (Table 4). This case may be happened as given fed R_3 in Bali cattle to result minerals concentration on rumen highest so that negative respond for growth and rumen microbes activities. Furthermore, given fed R_3 may be also to result run of secondary fermentation process so more reduction H^2 molecule which used by *Methanobacterium ruminantium* and *Methanobacterium mobilis* to produce methane (Hungate, 1966; Johnson and Johnson, 1995). Moreover, the lower methane gas concentration on rumen fluid at R_1 and R_2 are surprising, while show the optimizing bio process on rumen and reduced acidosis risk in Bali cattle. Furthermore, low methane production is surprising because reduced global warming risk and can positive for animal, farmer and environment (Hegarty, 2001).

Effect on Microbial Protein Supply

As shown in Table 5, the allantoin excretion in urine and efficiency microbial protein synthesis (E_{MPS}) in the rumen were not significantly (P>0.05) in all treatment with range 51.23 – 55.76 mmol/d and 107.17 – 107.74 g/1000g DOMR, while the allantoin absorbed and microbial protein synthesis have been increased by suplementation of 0.150% - 0.225% multivitamins-minerals on ration.

Table 5. Influence of Multivitamins-Minerals Supplementation on Microbial Protein Supply in Bali Cattle

Items	Treatments[1]				SEM[3]
	R_0	R_1	R_2	R_3	
Allantoin excretion (mmol/d)	51.23^{a2}	55.13^{a}	55.52^{a}	55.76^{a}	1.34
Allantoin Absorbed (mmol/d)	44.76^{a}	48.67^{ab}	49.45^{b}	49.35^{b}	1.19
Microbial Protein Synthesis (g/d)	197.83^{a}	213.57^{ab}	222.83^{b}	222.24^{b}	5.43
E_{MPS} (g/1000 g DOMR)	107.17^{a}	107.36^{a}	107.74^{a}	107.42^{a}	4.96

Note:[1)] R_0 = Ration without multivitamins-minerals supplementation, R_1 = Ration with 0.075% multivitamins-minerals supplementation, R_2 = Ration with 0.150% multivitamins-minerals supplementation, and R3 = Ration with 0.225% multivitamins-minerals supplementation, [2)] The same letter in same row is not significantly difference (P>0.05), [3)] SEM = Standard Error of the Treatment Means.

The higher allantoin absorbed and microbial protein supply in Bali cattle fed R_2 and R_3 may be due to synchronization of available fermentable energy and degradable nitrogen in rumen and also supply and availability trace mineral and vitamins as effect of supplementation multivitamin-minerals. Karsli and Russell (2001) reported in addition to N and carbohydrate supply, microbial yield is affected by concentration of trace minerals and vitamin. In addition, dietary sulfur concentration has been found to effect microbial growth. The amount of sulfur required by rumen microbes for synthesis of methionine and cysteine range from 0.11 to 0.20% of the total diet, depending on the status of the cattle. Limited intake of sulfur may restrict microbial protein synthesis when large amounts of NPN are fed to ruminal animals, such as urea or urea were fixation in product urea ammoniated rice straw.

Similar of the Allantoin excretion and efficiency protein microbial synthesis (E_{MPS}) of Bali cattle fed ratio based on urea ammoniated rice straw were 51.23 – 55.76 mmol/day and 107.17 – 107.74 g/1000g DOMR shown Bali cattle can be adaptation of environment as well. Moreover, that are may be as effect of increasing populated of protozoa in Bali cattle fed ration with supplementation of multivitamins-mineral especially R_2 (Table 4). High populated protozoa on rumen can take advantages and disadvantages (Kamra, 2005). Protozoa on rumen can increase protein degradable, supply ammonia nitrogen (NH_3-N) on rumen and reduce acidosis risk, in spite of high populated of protozoa also can decrease the number of bacteria and fungi in the rumen liquor as effect protozoa is a predator of bacteria. Bacteria is a sources of nitrogen/ protein of protozoa (Coleman, 1988). So protein microbial synthesis can be decrease of high populated protozoa and efficiency microbial protein synthesis on digestible organic matter apparently fermented in the rumen can be reduced.

Generally, efficiency microbial protein synthesis (E_{MPS}) in the rumen of Bali cattle given ration based on urea ammoniated rice straw with supplementation of multivitamins-minerals ere enough (107.36-107.42 g PMS/1000g DOMR). This is surprising because Bali cattle were supplemented of multivitamins-minerals on ration based on urea ammoniated rice straw have a much better Allantoin excretion from dairy cattle fed cottonseed meal at 0.5 kg/head/day and urea-treated rice straw was offered *ad lib.* with supplementation of corn meal and cassava chip as energy sources and with vary levels were result 28.1 – 45.8 mmol/d (Khampa and Wanapat, 2006), in spite of lower than Allantoin excretion of crossbreed (Brahman Vs Local) given urea treated rice straw with supplemented 1-3% per metabolic body weight ($BW^{0.75}$) concentrate which result 130.7 – 244.6 mmol/d (Wanapat and Khampa, 2007).

The E_{MPS} of Bali cattle given ration based on urea ammoniated rice straw with supplementation of multivitamins-minerals was 107.17 – 107.74 g MP/1000g DOMR equal with 69.51 – 69.97 g MP/1000g DOM or 17.27 – 17.78 g N/kg DOMR were there in range values E_{MPS} fattening cattle reported Karsli and Russell (2001 and 2002) were 70–237g MPS/1000g DOMR. This is surprising because ration based on urea ammoniated rice straw have a much better E_{MPS} from crossbreed cattle (Brahman Vs Local) were given fed various of energy source and level supplementation which calculate 4.3 – 10.7 g N/kg DOMR (Khampa and Wanapat, 2006).

Generally, relation supplementation of multivitamins-minerals with rumen microbial protein synthesis in Bali cattle given ration based on urea ammoniated rice straw following regression formula: $Y = 197.67 + 273.29\ X - 725.78\ X^2$, where $R^2 = 0.4697$ (Figure 1), where Y = rumen microbial synthesis (g/d), and X = supplementation of multivitamins-minerals on ration. Based on that regression analyses known that suplementation of multi vitamins-minerals of 0.188% coused maximum rumen microbial protein synthesis of 223.39 gram/day or maximum increased 12.92%.

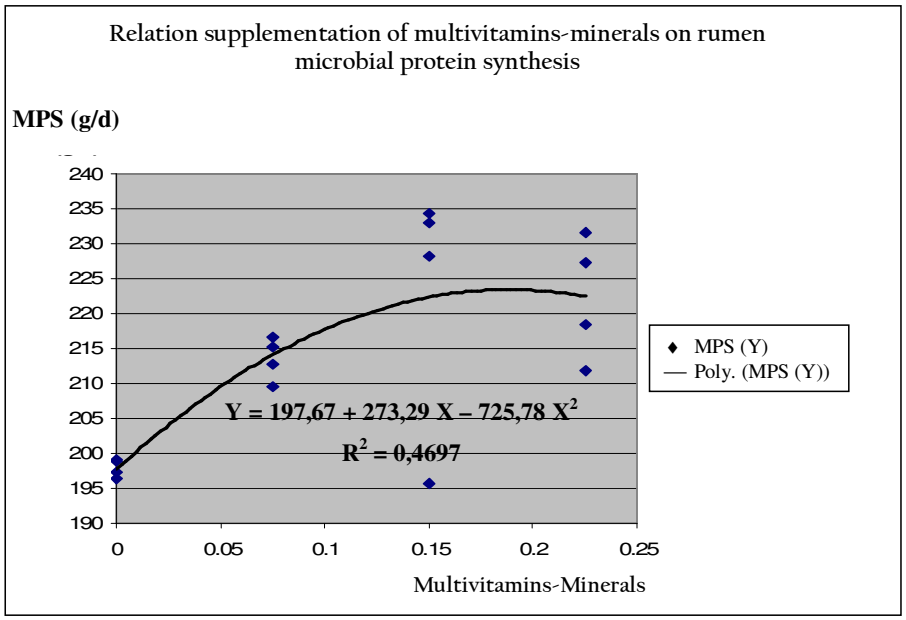

Figure 1.Relation supplementation of multivitamins-minerals on the ration based on urea ammoniated rice straw with rumen microbial protein synthesis

CONCLUSION

Supplementation of multivitamins-minerals on ration based on ammoniated rice straw can increasing maximum microbial protein supply as 223.39 g PMS/day (increase 12.92.%) with optimum supplementation are 0.188%.

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ANTIOXIDANTS COMPOUNDS IN STEM BARK OF *INOCARPUS FAGIFERUS* FOSB

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ABSTRACT

This paper describes the screening and isolation of active compound of antioxidants on *gayam* (*Inocarpus fagiferus* Fosb) stem bark. An amount of 21.05 g of concentrated extract was resulted from 300 g dry powder of *gayam* stem bark macerated in ethanol. This extract was dissolved in the mixture of ethanol-water (7:3) and then was partitioned with chloroform. It was evident that only chloroform extracts was which most active as an antioxidant. Separation of chloroform extract as an antioxidant using column chromatography (stationary phase silica gel 60, mobile phase chloroform) resulted in five fractions (F_1, F_2, F_3, F_4, and F_5) and the fraction of F_3 had the highest reduction percentage against the free radicals after 60 minute observation. The results of the identification F_3 fraction using gas chromatography mass spectrometry analysis showed that the fraction contains five components consisted of one steroid compound (cholesta-4,6-dien-3-ol) and four organic acids (tetradecanoic acid, hexadecanoic acid , 9,12-octadecadienoic acid, and octadecanoic acid 9,10-dibromo -methyl ester).

Keywords: [*Inocarpus fagiferus* Fosb, antioxidants, steroids, organic acids]

INTRODUCTION

Screening of bioactive compounds from plant can be conducted with the phytopharmacologic approaches and phytochemical screening approaches. One of the phytopharmacologic approaches relies on etnobotany, screening bioactive compound of plant based on its use as traditional medicine by certain society (Farnsworth, 1996). Traditional medicine represents one of Indonesia's cultural assets and has been empirically proven from generation to generation (Kardinan and Taryono, 2003).

A plant which can be exploited as traditional medicine is *gayam* (*Inocarpus fagiferus* Fosb). The stem bark of this plant is applicable to heal the dysentery disease and potential as antioxidant agent (Segatri, 1995). This is possibly because of the chemical compounds content of the stem bark, especially those of secondary metabolic compounds such as steroid, saponin, flavonoid, alkaloid, triterpenoid, and anthraquinone (Santi, 2000). Nevertheless there is no research explaining the correlation between the compounds in the stem bark with the traditional healing, although many people have proven the effect and benefit from *gayam* stem bark. Only the statement of Agestia and Sugrani (2009) which explained that there was correlation between structure of steroid or flavonoid and its possibility as antioxidant activity.

Moreover, results of antioxidant activity tests indicate that ethanol extract of *gayam* can reduce free radicals of diphenylpikril hydrazil (DPPH) equal to 98.21% at fifth minute. Therefore it is crucial to investigate the chemical contents of the *gayam* stem bark and their bio-activity as an antioxidant agent. In this paper the isolation process of antioxidant compounds from the stem bark and the identification of its components using GCMS are described.

MATERIALS AND METHODS

Materials

Materials used in this research are: *gayam* (*Inocarpus fagiferus* Fosb) stem bark, obtained from Padang Sambian Klod West Denpasar Bali and taxonomycally identified by LIPI's Kebun Raya " Eka Karya" Bali. Chemicals used are ethanol (p.a and technical), ether p.a, chloroform p.a, hydrochloric acid, concentrated sulphuric acid, anhydrous acetic acid, ethanol 96%, aquadest, silica gel 60, silica gel GF_{254}, DPPH (diphenylpikril hydrazil), and phytochemical reagent.

Equipments

Equipments used include a set of glass wares, analytical balance, blender, knife, rotary vacuum evaporator, UV lamp (254 and 366 nm), thin layer and column chromatographs, desiccators, test tubes, testing dishes, centrifuge, volumetric pipettes, gas chromatographs mass spectrometers.

Extraction and isolation of antioxidant compounds from *gayam* stem bark (*Inocarpus fagiferus* Fosb)

About 300g dried powder of *gayam* (*Inocarpus fagiferus* Fosb) stem bark was macerated using 3 L ethanol 96% (EtOH). Maceration process was conducted 4 times at 24 hours each. The ethanol extract was evaporated using rotary vacuum evaporator to obtain a concentrated EtOH extract. The EtOH extract was dissolved in a mixture of EtOH-H_2O (7:3). Furthermore EtOH-H_2O extract was evaporated until only water (H_2O) extract remained. Then H_2O extract was partitioned with chloroform. Each extracts was then evaporated to obtain concentrated water extract and chloroform extract. Each concentrated extract was tested for antioxidant activity with the DPPH test. Most active antioxidant extract was fractionated by column chromatograph using 60 g of silica gel 60 and chloroform as the mobile phase. Each fraction was tested by DPPH and the active antioxidant fractions were pooled and purified to obtain a relatively pure isolate. It was then analyzed using Gas Chromatographs Mass Spectrometers (GCMS) to determined its components.

RESULTS AND DISCUSSION

Extraction and isolation of antioxidant compounds from *gayam* stem bark (*Inocarpus fagiferus* Fosb).

The result of maceration of about 300 g dried powder of *gayam* (*Inocarpus fagiferus* Fosb) stem bark using 3 L ethanol was about 21.05 g red concentrated ethanol extract. After fractionation, 3.02 g of concentrated chloroform extract and 15.18 g of concentrated water extract were resulted.

Antioxidant activity tests using DPPH showed that chloroform extract was more active than the water extract (see Table 1). Separation of 1.5 g of the chloroform extract using column chromatograph yielded 5 fractions (Faction F_1-F_5). Antioxidant activity test to these five fractions showed that fraction F_3 was the most active as antioxidant toward DPPH and it consisted of one component (Table 2).

Table 1. The results of antioxidant activity test using DPPH

Sample	Time (minute)	Test	Absorbance (A) at λ			(A) Calculate 517	Reduction
			497	517	537		
Chloroform extract	5	DPPH	0,776	0,797	0,779	0,0195	71,80 %
		Sample	0,812	0,824	0,825	0,0055	
	60	DPPH	0,765	0,785	0,768	0,0185	91,89 %
		Sample	0,810	0,815	0,817	0,0015	
Water extract	5	DPPH	0,776	0,797	0,779	0,0195	67,18 %
		Sample	0,675	0,681	0,675	0,0064	
	60	DPPH	0,765	0,785	0,768	0,0185	67,58 %
		Sample	0,674	0,681	0,675	0,0060	

Table 2. The results of chloroform extract fractionation using column chromatograph and antioxidants activity test

Sample (amount of/ Rf) weight (g)	Time (minute)	Test	Absorbance (A) at λ			(A) Calculate 517	Reduction
			497	517	537		
F_1 (2/0.80; 0.90) 0.28	5	DPPH	0,764	0,848	0,725	0,1035	54,11 %
		Sample	0,432	0,463	0,399	0,0475	
	60	DPPH	0,788	0,872	0,734	0,111	69,81 %
		Sample	0,421	0,415	0,342	0,0335	
F_2 (1/0.65) 0.23	5	DPPH	0,764	0,848	0,725	0,1035	55,56 %
		Sample	0,520	0,541	0,470	0,046	
	60	DPPH	0,788	0,872	0,734	0,111	71.17 %
		Sample	0,490	0,503	0,452	0,032	

F_3 (1/0.43) 0.29	5	DPPH	0,764	0,848	0,725	0,1035	95,65 %
		Sample	0,460	0,412	0,355	0,0045	
	60	DPPH	0,788	0,872	0,734	0,111	103,15 %
		Sample	0,452	0,295	0,245	-0,0035	
F_4 (3/0.11; 0.21; 0.34) 0.24	5	DPPH	0,764	0,848	0,725	0,1035	33,33 %
		Sample	0,480	0,515	0,412	0,0690	
	60	DPPH	0,788	0,872	0,734	0,111	60,81 %
		Sample	0,395	0,403	0,324	0,0435	
F_5 (1/0.08) 0.30	5	DPPH	0,764	0,848	0,725	0,1035	31,88 %
		Sample	0,490	0,527	0,423	0,0705	
	60	DPPH	0,788	0,872	0,734	0,111	53,60 %
		Sample	0,391	0,408	0,322	0,0515	

Purity test to F_3 fraction was carried out by TLC with various mobile phases of chloroform, chloroform-n-hexane (3:2), chloroform-ethanol (1:1), n-hexane-ethanol (3:1), and n-hexane-ethyl acetate-ethanol (3:1:2) mixtures that was shown pure relatively.

Identification of Isolate (F_3 Fraction)

The result of phytochemical test indicated that the isolate contained the steroid compound which was shown by the color change of isolate from yellow to blue green. Chromatograph of GCMS analysis indicated that the isolate contained five components. The WILEY229.LIB data base approach suggested that the isolate contains five components consisted of one steroid compound (cholesta-4,6-dien-3-ol (**5**)) and four organic acids namely tetradecanoic acid (**1**), hexadecanoic acid (**2**), 9,12-octadecadienoic acid (**3**), and octadecanoic acid 9,10-dibromo-methyl ester (**4**) (see Figure 1 and Figure 2).

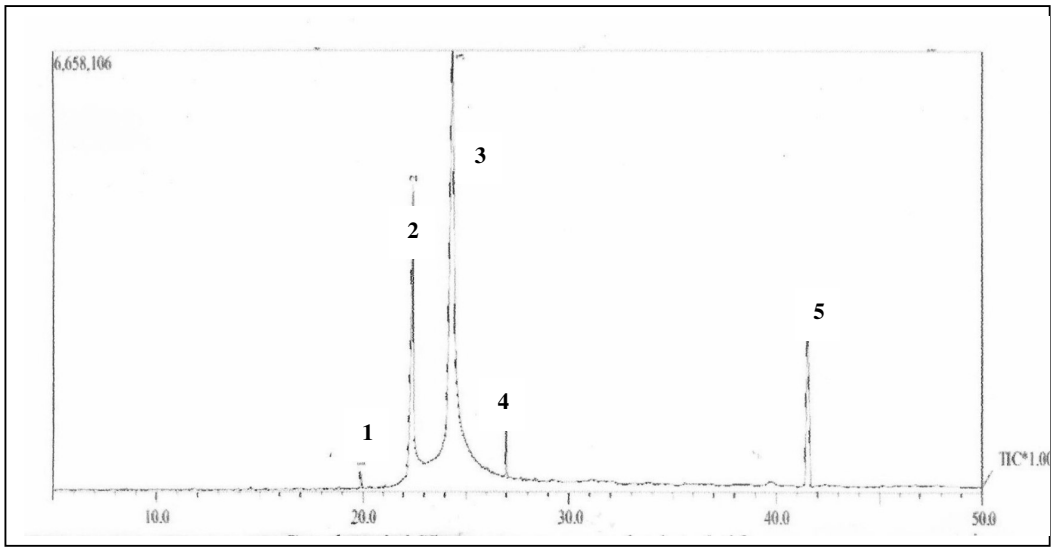

Figure 1. Chromatograph of GCMS analysis of isolate

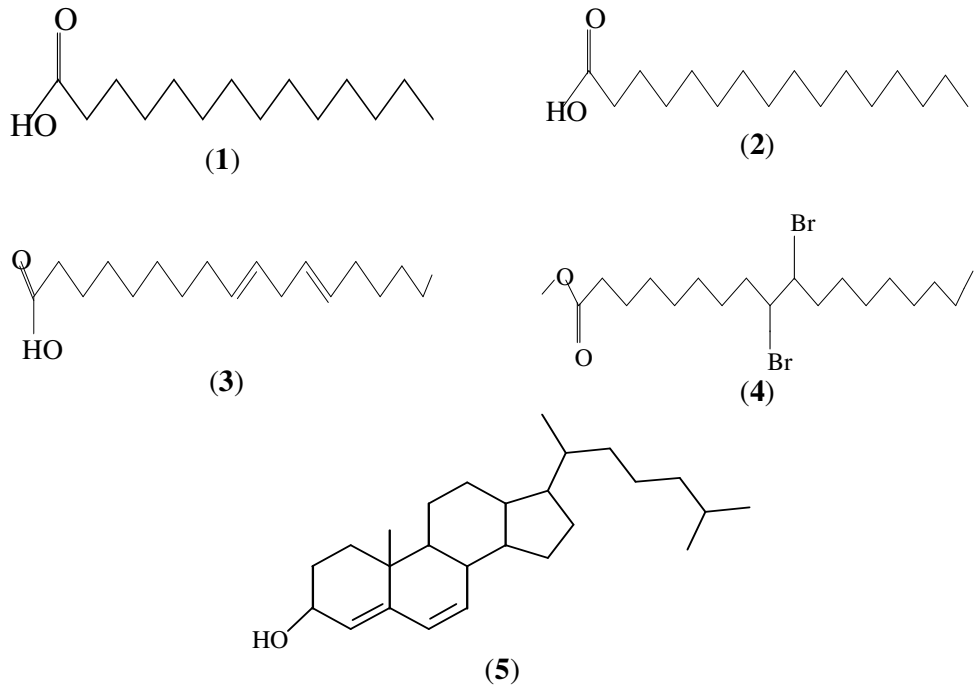

Figure 2. The structures of the compounds in the isolate

CONCLUSION

Conclusions of these researches are:

1. The isolate (F_3 Fraction) obtained from chloroform extract is identified as antioxidant compounds with reduction percentage to free radicals equal to 95.65% and 103.15% at fifth and sixty respectively.
2. The isolate contains five components consisted of one steroid compound namely cholesta-4,6-dien-3-ol and four organic acids namely tetradecanoic acid, hexadecanoic acid, 9,12-octadecadienoic acid, and octadecanoic acid 9,10-dibromo -methyl ester.

Further research

Separation of the isolate to obtain single compound its analysis using NMR to determine the chemical structure.

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THE POTENTIAL OF EXPOSURE TO NATURAL COLOR OF MIXED ARECA SEED, BETEL LEAF, GAMBIER WITH KAlSO4 MORDANT AND IT'S UTILIZATION TOWARDS COLORATION OF ALBASIA WOOD

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ABSTRACT

The research about natural dye potency from the mixtures of areca seed, betel leaf, gambier with $KAlSO_4$ mordant and it's utilisation towards coloration of albasia wood (*Paraserianthes falcataria*) was carried out. The preparation of the dye material in form of powder for each sample that has been determined for its water. The Betel leaf approximately has 15,83% water content, areca seed has 14,47%, and Gambier has 20,89%. The extraction of mixture of natural dye was conducted by heating in water for two hours. 10,00 gram of each samples of betel leaf, areca nut, and gambier were refluxed and resulted in 28,60%, 40,10%, and 30,20% respectively. According to phytochemical test, the betel leaf extract contain flavonoid, tannin, and carotenoid, whereas the areca nut and gambier extracts contain flavonoid and tannin.

The mixture of three of them with ratio (5g : 5g : 5g) was extracted by 375 mL water and the resulted extract was brown. The optimum mass of the adsorbed natural dye by 0,90 gram addition ans 60 minutes soaking time was 0,1111 g with, betel leaf, areca nut and gambier ratio (5g : 5g : 5g) It's showed that $KAlSO_4$ mordant and various seaking time strengthen the bond between dye and wood fiber and clarify the resulting color.

After the color endurance test by using 1% detergent water, and soaked for 15 minutes, the dye without $KAlSO_4$ easily washed out. The resulting color after staining process which varies from light brown, reddish brown, to dark brown.

Keywords: areca seed , betel leaf, gambier, water content, $KAlSO_4$

INTRODUCTION

Indonesian is a country rich in biological resources, both animal and vegetable sources. This is supported by the geographical situation of Indonesian tropical climate with average rainfall is high throughout the year. Indonesian is famous for its rich diversity-grown vegetation, especially forest that is wood. Unlimited use of wood for household appliances (interior), but also used for the exterior, for example, to create a bridge. Whereas with a decorative colors and patterns, some types of wood used to make objects of high artistic value such as carvings and statues (Herawati, 2005).

Wood has a high decorative value caused by the color, type of fibers and patterns in the wood. Furniture made of wood are usually given a transparent color to show its natural beauty, while furniture or other equipment made of wood that has no color and pattern of interest will be assigned a specific color so as to produce a more attractive color display (Herawati, 2005).

Staining associated with the creation of a surface area of interior (wood), parts of the plant has a relatively large potential to be used as natural dyes. Each plant can be a source of natural dyes because they contain the natural pigment. Potential sources of natural dyes is determined by the intensity of color produced and depending on the type of coloring matter that exists. Coloring matter is the substance that determines the color of natural dyes, an organic compound contained in the source of these natural dyes. These natural dyes found in plant parts like leaves, stems, bark, flowers, fruits, roots, sap, and so on, with the levels and types of coloring matter is varied. All parts of the plants are basically a dye that can be used as a dye in the fabric for interior or be used as natural dyes in the interior furniture and other interior elements are directly or used in conjunction with other products made from basic materials chemistry (Setiawan,2003)

Natural dyes have been recommended as a friendly dyes good for the environment and health since it contains natural component has a value of relatively low pollution load, easily degraded biologically and non-toxic. Plants used as a dye can be obtained around the environment so that cost-effective, using natural dyes indirectly help preserve the plant species. Behind the excess is stored several disadvantages which are generally not all natural dyes can directly color the wood fibers, therefore, necessary auxiliary substance called mordant. Mordant derived from the Latin, meaning modere bite. Mordant also referred to as a special substance that can improve the adhesion on a variety of fabric dyes. Functioning as a substance that can help form a bridge between the dye chemical natural fiber structure thus increasing dye affinity(Fitrihana,2007).

The mixture of natural dyes to be used in dyeing albasia wood surfaces in this study is gambier, betel leaves, and areca seed . In the daily life of the parents chew the three materials, it is believed to strengthen the tooth root and the most interesting is the color of the result are discarded chewing is reddish brown dye that can be used as timber.

According to research Bogoriani (2009, 2010 and 2011) mixtures of natural dyes from gambier, betel leaves and areca seed was produced russet-brown to reddish wood fiber acacia and albasia, where the auxiliary substances used is whiting ($CaCO_3$), $KMnO_4$ and citric acid.

Based on the above description of the mixture of natural dyes, and mordant influence on the resulting color, then the replacement of this research was conducted mordant $KMnO_4$ with alum ($KAlSO_4$). Alum in solution can produce Al_3+ ions and hydrated ions to form $[Al(H_2O)_6]_3$+. Then the ion hydrate deposits have hydrolysis of Al $(OH)_3$ (p) onto the wood fiber and dye is then absorbed by the Al $(OH)_3$. The presence of Al can bridge bond active groups between dye with wood fiber, so it produced a different hue.

Besides the treatment of natural dyes by the addition of various auxiliary substances may increase the possibility of adsorption capacity, because the active groups on the reactive dye more so that it can improve adhesion of natural dyes on the surface of the wood fibers become larger and produce more brilliant colors. For that conducted research on the effect of the addition of auxiliary substances ($KAlSO_4$) on natural dyes to wood fiber surface albasia (*Paraserianthes falcataria*).

MATERIALS AND METHOD

The chemicals used in this study is $KAlSO_4$ powder, concentrated sulfuric acid (H_2SO_4), concentrated hydrochloric acid (HCl), magnesium powder, 1% $FeCl_3$, ethanol (pa), 1% detergent water and aquades, areca seed powder, betel leaf powder, powdered gambier, and albasia wood.

Equipment

The tools used in this study were analytical balance, filter paper, knives, blenders, sieves, funnels, stirring rods, spatulas, pipettes drops, glass beaker, place the sample, a spray bottle, electric bath, a set of tools reflux, stopwatch.

Preparation of natural dye materials

Areca seeds cut into small pieces, dried in the sun, blended to a powder, then sifted. Betel leaf is cleaned, cut into small pieces, dried to a powder blend, then sifted, whereas in Gambier just blended to a powder and then sifted. Because the gambier used already in the form of biscuits. Sieve results from each sample of dye is determined water content and stored for subsequent procedures.

Compound Content Determination of Dyes Areca Seed, Betel Leaf, Gambier

A total of 10.00 g of areca seed aquades added as much as 100 mL. ± then refluxed for 2 hours. The extract obtained was concentrated using a rotary vacuum evaporator so that the concentrated extract obtained is then weighed and calculated compound content. Concentrated extract obtained and tested tannins, flavonoids and carotenoids with a color reaction. In the same way as above performed on betel leaves and gambier.

Extraction of natural dyes

Natural dyes (Gambier, betel leaves and areca seed) were made by comparison (5g: 5g; 5g) was then inserted in a glass beaker in the extraction with aquades ratio (1 g of natural dyes: 25 mL aquades). The mixture is heated for 30 minutes.After the cold extract was filtered and stored for subsequent procedures.

Staining of The Albasia Wood Surface without The Influence of Auxiliary Substances KAlSO4

The filtrate water is added natural dyes without alum as much as 100 ml is used to color the wood as a control. Albasa wood that has been sanded soaked by the time variation of: 15m: 30m: 60m, observed changes. Furthermore, wood is dried in the sun, the wood is dry weighed until a constant mass to determine how the adsorbed dye, the color of the wood surface is observed and tested for durability of color on the wood.

Effect of Addition of Auxiliary Substances in a Mixture of Substances KAlSO4 Natural Color of Wood Surface Albasia

The filtrate was added mordant natural dyes ($KAlSO_4$) with varying masses are: 0.5, 0.7; 0.9 to 100 ml volume was heated for 30 minutes, stirring until thoroughly homogenized and then cooled and albasa wood that has been sanded, soaked by the time variation of: 15m: 30m: 60m on a mixture of dyes containing $KAlSO_4$. Furthermore, wood is dried in the sun. Dry wood that has been weighed up to constant, the color of the wood surface is recorded and observed masses that produce optimum $KAlSO_4$ dye absorption maximum at albasia wood surface and tested durability color on wood

Endurance test the dye on the surface of albasia wood

Albasia colored wood that is dry of the treatment carried out as follows: to test durability, each such treatment, wood A011, A012, and A013, not soaked with water detergent 1% (control), A014 wood soaked with water 1% detergent for 15 minutes. Further observation on the color and weighed mass of each timber to determine the amount of wood mass are reduced.

RESULTS AND DISCUSSION

Yield of Betel Leaf, Areca seed and Gambier

The sample powder of betel leaf, areca seed and gambier, each of which is used as much as 10.00 g was extracted by refluxing for 2 hours using 100 ml of solvent water, where the process of reflux at 3 replications. The extract obtained is filtered and evaporated, resulting in dry extract. The yield of dry extract each count, to know how much dye is contained in the sample. The yield of the three samples can be seen in Table 4.1

Table 4.1 The results of calculation yield

Observation Results	Betel Leaf	**Areca nut**	**Gambier**
Initial weight (g)	10,00	10,00	10,00
Weight extract (g)	2,86	4,01	3,02
Yield (%)	28,60	40,10	30,20
The color of extracts	brown	red brick	red brick

Based on the results of the calculation of the yield data of each extract in Table 4.1 shows that the betel leaf extract has a yield of 28.60%, 40.10% areca seed, gambier and 30.20%. This means that all three samples have a dye which is pretty much in 10.00 g of the sample. To determine the content of dye in each sample extract then tested the phytochemicals.

Phytochemical Test Areca Seed Extract, Betel Leaf and Gambier

To determine the dye content of each sample then tested the phytochemicals in each sample extract. Based on test results of phytochemical data indicate that betel leaf extract contains flavonoids, tannins and carotenoids while the betel nut and gambier extract contains tannins and flavonoids. This shows that the three extracts contain substances which are potentially natural color as the dye. Therefore the three extracts are mixed and then applied to color the albasia wood.

Extraction of Dye On Mixed Leaves Betel, Areca Seed and Gambier

Preparation of dye materials that have been floured sifted and then each sample is determined water content. Betel leaf has water content of about 15.83%, 14.47% areca nut, gambier and 20.89% in 2.00 grams of sample.Furthermore, a mixture of all three dyes with the same weight ratio (5g: 5g: 5g) and then extracted with 375 mL aquades, a 30-minute brown extract solution obtained and stored for subsequent procedures. The use of water as the extraction showed that the dyes obtained are polar, may contain hydroxyl groups (-OH) which is a mixture of compounds (meiyanto, 2008: Hermawan 2007).

Staining Dye Natural Wood Surface Without Mordant on Albasia

Dye mixture with a ratio of (5g: 5g: 5g) in the extraction and then used to color wood 4x5x1cm measuring the surface is white. Albasia wood stained weighed mass before (can be seen in Table 4.2.). It aims to determine the magnitude of the absorption of dyes by wood albasia wood so that after the coloring process is weighed again. The process of coloring wood by soaking with a mixture of dyes made in varying time of 15 minutes: 30 minutes: 60 minutes. The amount of dye adsorption on albasia wood can be seen in Table 4.2.

Table 4.2 Data Mass Observation Results Mixed Natural Dye with the variation of immersion time.

Comparison of betel leaf, Areca nut, gambier (g)	Time (menutes)	Code	Before Staining Wood mass (g)	The mass of wood after staining (g)	The amount of adsorption of Wood Fiber (g)	Adsorption Average (g)	Color appearance on Wood Surfaces
5 : 5 : 5	15	A011 A012 A013 A014	3,7209 3,5087 3,7405 3,3989	3,7797 3,5715 3,7915 3,4637	0,0597 0,0628 0,0510 0,0648	0,0595 ± 0,0060	Light brown
	30	A021 A022 A023 A024	3,4273 4,6862 3,8222 3,6102	3,4691 4,7310 3,8672 3,6806	0,0418 0,0448 0,0450 0,0740	0,0514 ± 0,0150	Light brown
	60	A031 A032 A033 A034	4,2212 5,0087 3,6580 3,9073	4,2590 5,0446 3,6969 3,9624	0,0378 0,0359 0,0389 0,0551	0,0419 ± 0,0089	Light brown

Based on the observations in Table 4.2 shows that the ratio of the mixture of dyes with varying soaking time can color the wood fibers. This is proven by the uptake of dyes by wood fiber on each albasia wood. Adsorption process that occurs probably because the group of cellulose found in wood fibers capable of forming hydrogen bonds with the group from the dye. Hydrogen bonds formed are weak and easily broken. This looks after the endurance test by immersion in water 1% detergent, dye attached to the timber to be washed out and the rest of the dishwater brown. Based on the literature of a weak bond and easily broken indicates the formation of a physical bond (Sukardjo, 1985; Osipow, 1962). Judging from the colors produced in each timber with varying immersion time obtained no significant color change is all wood light brown. The data in Table 4.2 can be described in graphical form as shown in Figure 4.1.

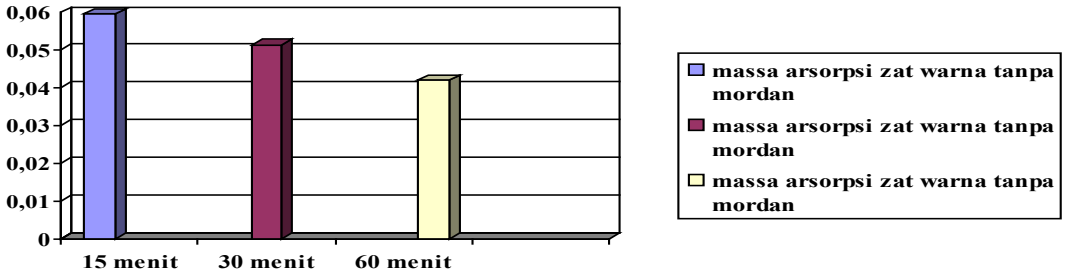

Figure 4.1 Diagram of the mass of natural dyes are adsorbed on the timber albasia

Based on Figure 4.1 shows the maximum adsorption occurs on a comparison of betel-nut seed-gambier 5:5:5 ie 0.0595 ± 0.0060 within 15 minutes of immersion, while the smallest adsorption of 0.0419 ± 0.0089 in 60 minutes immersion. This process is due at time of 15 minutes of absorption occurs at the maximum of natural dyes and is already saturated so it does not absorb and it is precisely the absorption decreased return. Seen on 30 minutes and 60 minutes immersion occurs descent mass adsorption on the surface of albasia wood. The weakness of the natural dyes are easy to wear off, so to clarify the colors revealed in albasia wood as well as strengthen the bond between the dye with the wood fibers so that the

mixture of the dye should be added auxiliary substances (mordant) $KAlSO_4$.

Mass Determination of Optimum Natural Dye adsorbed with Addition Effect of Varying mordan $KAlSO_4$ on Wood Surface Albasia

The mixture of natural dyes between betel leaves, areca seed and gambier ratio (5g: 5g: 5g) was then added to the extraction $KAlSO_4$ shaped white crystals with varying weight of 0.50 g: 0.70 g: 0.90 g, and then heated for 30 minutes. Albasia wood that has been prepared to further marred soaked at room temperature in a time varying over 15 minutes: 30 minutes: 60 minutes in the extract of the dye mixed with varying $KAlSO_4$ mass can be seen in Figure 4.3. The amount of dye adsorption by albasia wood with the effect of adding $KAlSO_4$ can be seen in Table 4.3: Table 4.4: Table 4.5

Table 4.3.Data Mass Observation Results Mixed Natural Dye with varying time 15 min: 30 min: 60 min with a mass of 0.5 grams $KAlSO_4$.

Comparison of betel leaf, Areca nut, gambier (g)	Code	Time (minutes)	Before Staining Wood Mass (g)	The mass of wood after staining (g)	The amount of adsorption Wood Fiber (g)	Adsorption Average (g)	Color appearance on Wood Surfaces
5:5:5	A.111 A.112 A.113 A.114	15	3,5367 3,7377 3,7165 3,8210	3,5991 3,7929 3,7794 3,8888	0,0544 0,0552 0,0629 0,0678	0.0601 ± 0,0064	reddish brown
	A.121 A.122 A.123 A.124	30	3,8397 4,1239 3,5708 4,2686	3,9370 4,1758 3,6688 4,3304	0,0973 0,0519 0,0980 0,0618	0,0772 ± 0,0239	reddish brown
	A.131 A.132 A.133 A.134	60	3,4952 3,5512 3,9576 3,5025	3,5750 3,6285 4,0169 3,5730	0,0798 0,0773 0,0593 0,0705	0,0717 ± 0,00916	reddish brown

Table 4.4 Data Mass Observation Results Mixed Natural Dye with varying time 15 min: 30 min: 60 min with a mass KAlSO4 0.7 grams.

Comparison of betel leaf, Areca seed, gambier (g)	Code	Time (minutes)	Before Staining Wood Mass (g)	The mass of wood after staining (g)	The amount of adsorption Wood Fiber (g)	Adsorption Average (g)	Color appearance on Wood Surfaces
5:5:5	B.111 B.112 B.113 B.114	15	4,0110 3,6961 4,2960 3,6979	4,0089 3,7712 4,3815 3,7490	0,0779 0,0751 0,0855 0,0511	0,0724 ± 0.0092	reddish brown
	B.121 B.122 B.123 B.124	30	3,3633 4,2738 4,4372 3,4998	3,4322 4,3538 4,5300 3,5772	0,0689 0,0800 0,0928 0,0774	0,0789 ± 0,0096	Dark brown
	B.131 B.132 B.133 B.134	60	3,1409 3,8486 3,3719 3,7750	3,1991 3,9403 3,4384 3,8792	0,0582 0,0917 0,0665 0,1042	0,0801 ± 0.0214	Dark brown

Table 4.5.Data Mass Observation Results Mixed Natural Dye with varying time 15 min: 30 min: 60 min with a mass $KAlSO_4$ 0.9 grams.

Comparison of betel leaf, Areca nut, gambier (g)	Code	Time (minutes)	Before Staining Wood Mass (g)	The mass of wood after staining (g)	The amount of adsorption Wood Fiber (g)	Adsorption Average (g)	Color appearance on Wood Surfaces
5:5:5	C.111	15	3,9608	4,0361	0,1023	0,0909 ± 0.0305	reddish brown
	C.112		3,1098	3,2382	0,1283		
	C.113		4,4524	4,5129	0,0605		
	C.114		4,0261	4,0986	0,0725		
	C.121	30	3,4476	3,5379	0,0903	0,1012 ± 0,0257	Dark brown
	C.122		4,7373	4,8764	0,1391		
	C.123		3,6210	3,7033	0,0823		
	C.124		3,1116	3,2047	0,0931		
	C.131	60	3,9096	4,0170	0,1074	0,1111 ± 0,0069	Dark brown
	C.132		3,7461	3,8673	0,1212		
	C.133		3,6045	3,7146	0,1101		
	C.134		3,5821	3,6879	0,1058		

Based on the observations in Table 4.3, Table 4.4 and Table 4.5 show that the mixture of dye extraction by the addition of varying $KAlSO_4$ and soaking time can color the wood fibers with a variety of colors. This can be seen from the mass of dye adsorption and the resulting color of each color varies albasia wood brown and depicted as in Figure 4.3. The ability of absorption of dye on each timber may be caused by a group of cellulose found in wood fibers capable of forming covalent bonds with the dye so that the colors produced sharper because of the effect of adding mordant ($KAlSO_4$). With the presence of auxiliary substances (mordant) $KAlSO_4$ so as to improve adhesion of the dye on the surface of albasia wood fiber, which $KAlSO_4$ is a salt that is able to produce hydrolysis of $[Al(H_2O)_6]_3^+$ and precipitate $Al(OH)_3$ (p) onto the wood fiber and the dye absorbed (adsorp) by $Al(OH)_3$ (p). Adsorption occurs on cellulose compound in accordance with the above sentence can be estimated as follows:

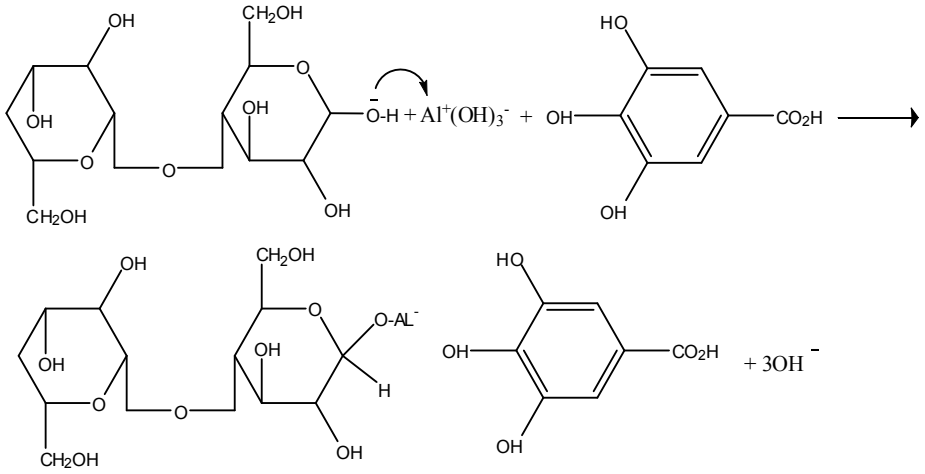

Figure 4.2 Adsorption of natural dyes on cellulose by the addition $KAlSO_4$

So the presence of Al which is amphoteric expected to provide bond securities with different groups that are active in the wood fibers. The more $KAlSO_4$ is added, the greater the dye is absorbed by the $Al(OH)_3$ so that the colors more crisp.

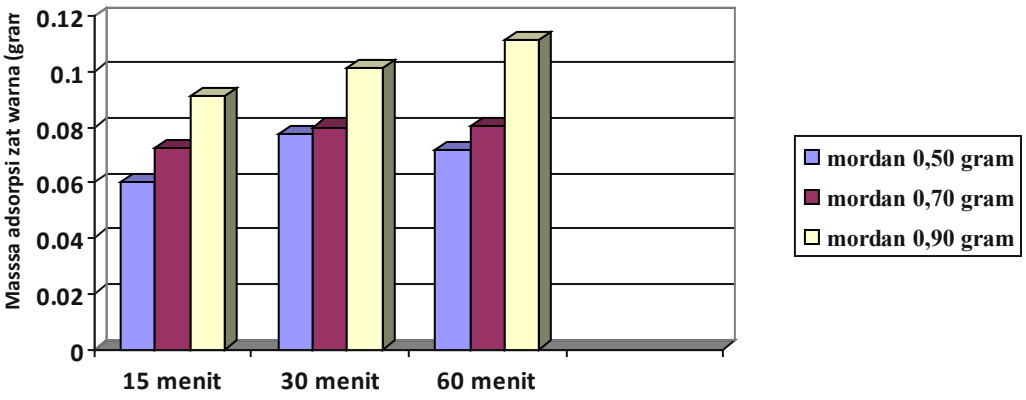

Figure 4.3 Diagram of the amount of adsorption of dyes by wood $KAlSO_4$ albasia with the effect of adding 0.50 g: 0.70 g: 0.90 g.

Based on Figure 4.3 shows that as more additions mordant $KAlSO_4$ with a longer soaking time can increase the mass adsorption, it is seen on the addition of 0.90 grams mordant with soaking time of 60 minutes in the weight ratio of betel leaf, areca nut, gambier 5:5 : 5 turned out to provide optimum absorption of the dye of 0.1111 grams. The resulting difference absorption adsorption on each timber with a ratio of dye mixture, soaking time and mass varying mordant probably caused by the absorption ability of wood to the effect of adding varying mordant of cellulose content in each timber is different. So the greater the addition mordant and the longer soaking time led to the adsorption on the surface of the wood increases. So the addition mordant and times vary greatly influence the absorption of the dye on the surface of wood. Adsorption process is not only influenced by mordant ($KAlSO_4$) but there are several factors that influence, namely: the surface area, type of adsorbate, adsorbate molecular structure, the stirring speed, temperature, contact time (Anonymous, 2009).

Having compared the absorption of the dye without mordant with dyes that are added mordant turns on the absorption of the dye without mordant smaller than the dye is added mordant. This is probably because the dye is adsorbed in the form of multilayer and have weak bonds so that the dye is absorbed less.Evidently the formation of a weak bond of the endurance test visible dye colors were faded and the wood surface looks brown liquid laundry and easily separated from the surface of albasia wood. Based on the nature of the dye without dye adsorption mordant were classified into physical adsorption. In addition, physical adsorption also has a balance of nature occur in reversible adsorption.

In a mixture of dyes with the addition mordant amount of dye absorbed is greater than the dye without mordant. This is probably because the dye is adsorbed in the form of a monolayer, which is only one layer is formed on the surface of albasia wood so that after the endurance test with detergent 1% mass of water absorption is decreased significantly. Adsorption occurs with the addition of chemical adsorption mordant classified. In addition to monolayer adsorption of the chemical nature also has a relatively stronger binding properties than the bonding on the physical adsorption and equilibrium adsorption occurs irreversible.

Judging from the appearance of color in each timber having a difference, both substances without or with mordant mordant, wood staining with dyes without mordant give the same color by varying the immersion time produces the light brown color. In the timber with the addition mordant provide a variety of colors ranging from reddish brown, brown, dark brown. Addition mordant and soaking time varied to give a huge influence that sharpen the resulting color on each albasia wood. To determine the resistance of the resulting color on albasia wood color endurance test is carried out using 1% detergent water.

Endurance test is carried out using 1% detergent water.

Determination of endurance test the dye on the wood aims to find out how much resilience the color that is bound to albasia wood with a 15 minute immersion in 1% detergent water. Endurance test the color on each timber that has been dyed without the addition mordant ($KAlSO_4$) or with the addition mordant ($KAlSO_4$) 0.50 g: 0.70 g: 0.90 g shown by Table 4.6.

Table 4.6. Durability Test Results of the observation data Dye with detergent for 15 minutes Water immersion.

Comparison of betel leaf, areca nut, gambier (g)	Code	KAlSO4 Mass	The mass of wood after staining (g)	The mass of wood with the Water Tested After Detergent (g)	The mass of the Missing After Dye Tested (g)	Color appearance on Wood Surfaces
5 : 5 : 5	A.014	-	3,4637	3,4331	0,0306	Dark brown
	A.024	-	3,6806	3,6503	0,0303	Dark brown
	A.034	-	3,9624	3,9424	0,0200	Dark brown
	A.114	0,50	3,8888	3,8773	0,0115	reddish brown
	A.124	0,50	4,3304	4,3235	0,0049	reddish brown
	A.134	0,50	3,5730	3,5604	0,0126	reddish brown
	B.114	0,70	3,7490	3,7366	0,0124	chocolate
	B.124	0,70	3,5772	3,5668	0,0104	reddish brown
	B.134	0,70	3,8792	3,8696	0,0096	reddish brown
	C.114	0,90	4,0986	4.0818	0,0168	reddish brown
	C.124	0,90	3,2047	3,1894	0,0153	chocolate
	C.134	0,90	3,6879	3,6857	0,0040	chocolate

Based on data in Table 4.6 shows that the extract mixture without the addition $KAlSO_4$ dye absorption value was obtained in the timber without mordant decreased, in which dye is absorbed on albasia wood faded after immersion in water 1% detergent for 15 minutes, This is because the hydrogen bonds formed are weak and easily broken.

The surface of the dye with the addition mordant ($KAlSO_4$) that characterizes the timber after the endurance test of the dye by soaking in water 1% detergent for 15 minutes lees dye not significant, visible from the decrease in absorption obtained is not too large, as well as the color of the wood has not changed . This can be seen from the water color detergent that does not significantly change from initial color detergent water. This shows that with the addition mordant ($KAlSO_4$) highly influence which can strengthen the bond between the dye with wood fiber. The more mordant added the bonding that occurs in the dye to the wood fibers become stronger and sharpen the color of the wood produced.

CONCLUSION AND SUGGESTIONS

Conclusion

Based on the results of research and discussion can be summarized as follows:

1. The yield of each material used as a dye which is betel leaf 28.60% 40.10% areca seed; gambier 30.20%
2. Betel leaf extract contains flavonoids, tannins and carotenoids, whereas extracts areca seed and gambier containing tannins and flavonoids.
3. Optimum mass natural dyes are adsorbed by the addition of 0.90 g $KAlSO_4$ and soaking time of 60 minutes is obtained for 0.1111 g on a comparison of betel leaf – areca seed - gambier (5g: 5g: 5g).
4. The color of the wood produced after the coloring process that varies from light brown, reddish brown, to dark brown.
5. The results of endurance test color using 1% detergent water-soaked for 15 minutes in which the dye without $KAlSO_4$ easily fade, while the dye with the addition $KAlSO_4$ not easily fade.

Suggestion

From the research results obtained, it can be recommended:

1. Soaking temperature variations need to be done with the addition of the same mordant.
2. Needs to be done adding an alkaline mordant on the mass of a mixture of different dyes.
3. Needs to be done by the method of dyeing process premordanting and postmordanting.

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THE RELATIONSHIP BETWEEN REARING SYSTEM AND PRODUCTION PROFILE OF *ETAWAH* CROSS BRED GOATS IN SOME GOAT FARM IN BALI

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ABSTRACT

Little up to dated information is known about milk production profile of *etawah* cross bred goats which is related to its rearing system in Bali. Therefore, this research is important to be conducted to search for the solution by interviewing twenty *etawah* cross bred goat farmers in five regencies in Bali which are chosen purposively based on some considerations. This research is designed as an explanatory research and the result is analysed by qualitative description method. The correlation between rearing system and production profile of *etawah* cross bred goat was analysed by statistical non-parametric that uses the Spearman rank correlation coefficient. The result showed that farmers tended to apply traditional rearing system up to semi rational system. Only few farmers apply rational rearing system. The correlation between rearing system and milk production profile tended to be positive. This is shown on the final marketing goal that farmers only sell alive-goats but not their milk production neither the meat/milk products.

Keywords: *rearing system, traditional, rational, farmer profile, production profile*

THE DEVELOPMENT OF LATICIFER ON CALLUS CULTURE of *Catharanthusroseus* (L) G Don INDUCED IN ZENK MEDIUM WITH COMBINATION OF BAP + NAA

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ABSTRACT

Research on developments of laticifer in the callus culture of *Catharanthusroseus* (L) G. Don was done using 2nd leaf from shoot apex as explants. Explants were grown on Zenk medium supplemented by combination of plant growth regulators BAP + NAA. Laticifer developments were evaluated using descriptive analysis method of anatomical callus at 4-14 weeks old cultures. Laticifer percentage was determined by counting the number of laticifer and the average number of cells other than laticifer under a microscope. The results showed that the induction of laticifer can be observed in the callus *C. roseus* in the early growth stages i.e. at 6 weeks old callus. The characteristics of laticifer are thicker cell wall is thicker and largere cell size compared to the nearby cells. Laticifer elongation started at 7-week-old callus, then very long laticifer was found on the anatomy of callus at age of 12 weeks. The percentage of the maximum amount of laticifer induced by BAP + NAA was 0.74% at the age of 12 weeks old callus.

Keywords: callus, explants, descriptive, medium, laticifer, BAP, NAA

CELLULASE AND PECTINASEACTIVITIES OF *Fusarium oxysporum* f.sp. *vanillae* EXPOSED TO THE EXTRACT OF *Aglaophenia* sp., A MARINE ANIMAL

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ABSTRACT

The purpose of this research was to evaluate the effect of *Aglaophenia* extract on the activities of cellulase and pectinase of *Fusarium.* The treatments were done *in-vitro* with concentration of extract 0.2, 0.1, 0.05, 0.025, 0.0125, 0.00625, and 0% (w/v). The result exhibited that at higher concentration of the extract, activity of both enzymes were low. Cellulase activity was depressed on 0,0016% of extract (with activity of 908.86 µg/mL/minute) and was significantly lower than control (1946.74µg/mL/minute). Whilethe pectinase activity was affected at the concentration of extract much higher i.e. at 0.025%. Pectinase activity at that concentration was 389.98 µg/mL/minute, whereas in the control was 3930.89 µg/mL/minute. This proved that cellulase was more sensitive than pectinase in responding *Aglaophenia*extract.

Keywords: Cellulase, pectinase, *Fusarium*, *Aglaophenia* sp.

INTRODUCTION

Once a pathogen get infected plant cell, the pathogen use its pathogenecity factors to overcome the barriers of infection. The barriers are cuticule, cell wall, and chemically self defence system. The main barier is cell wall because of its complex structure and the pathogen must degrade it using appropriate enzymes. Therefore, the enzymes are very important as infection factor of a pathogen. Decreasing activity of enzymes is one of the main goals of any fungicides application.

Polygalacturonase and cellulase are enzymes that are able to degrade pectin and cellulose of cell wall respectively (Dickinson, 2003). The two enzymes were producedprior other mechanisms when one pathogen penetrate into plant cell (Mahalingam *et al.*, 1999). Polygalacturonase is an endo-polygalacturonase degraded polygalacturonane ring randomly and resulted to short chain oligogalacturonane. While exo-polygalacturonase degrade the molecule terminally, resulted in monomer molecule such as galacturonic acid (Bateman and Basham, 1976).

Production of pectolityc and cellulolityc enzymes as main agent of patogenecity have been identified on some species of *Pythium* including *P. aphanedermatum* (Sutton *et al*., 2006). Polygalacturonase secretion is one of the key factors for infection processes on plant host (Clausen and Reen, 1996), even the differences on total enzymes excretion correlate significantly to its virulent (Wei-Chen *et al*., 1998; Ohazurike and Arinze, 1999; Owen-Going *et al.,* 2004). Many species of *Fusarium* are known be able to produce cellulase and pectinase along infection processes. While Ugwuanyi and Obeta (1997) stated that *F. oxysporum* was able to produce pectin degrading enzymes such as hydrolase, lyase, and pectin esterase.

Cellulases are one group of enzymes that degrading cellulose into glucose. The enzymes group including endo-B-1,4- glucanase, exo-1,4 glucanase (celobiohydrolase), and B-1,4-glucosidase. The two type of exoglucanase are the enzymes that degrade glucose unit from non reduction terminale of cellulose chains (Onuh and Ohazurike, 2008). Pectinolityc enzymes have important role in maceration process of cell wall to degrade pectin on middle lamella of primary cell wall into separated cell (Onuh and Ohazurike, 2008). The hydrolysis is started by pectin esterification of pectin methyl esterase become methanol and polygalacturonic acid. The enzyme activity provide away for other enzymes activity. The activity of the next enzymes are divided into two enzymes based on its mode of action, those are: (1) endo- cutting mode, those are the enzymes cut pectin randomly into olygomer, for example, endo polygalacturonase that hydrolysis glycocide bond, (2) exo cutting mode, degrade from polymer terminal into separated monomer/dimer, for example exopectate lyase that degrade polygalacturonan into olygigalacturonide with B-elimination (Reignault *et al*., 2008).

The purpose of this research was to know the activity of two main enzymes functioned on *Fusarium* pathogenecity influenced by *Aglaophenia* sp. extract .

MATERIALS AND METHODS

The research was done in Laboratory of Biotechnology Faculty of Agriculture Udayana University. SDS-PAGE profile was examinedat Shinshu University Japan. Marine animal *Aglaophenia* sp. was collected at Tukad Abu Karangasem. The research was started on October 2008 until February 2009.

Strain of fungi and marine biota samples

Fusarium (*Fusarium oxysporum* f.sp. *vanillae, Fov*) was isolated and identified in laboratorium of Agricultural Biotechnology, Udayana University from infected vanilla plant collected from farmer garden in Tabanan, Bali. Single spore isolation was applied to the pathogen in order to obtain single strain of *Fusarium*. Marine biotas samples were collected at intertidal zone and in the depth of 1-7 metres of sea water of Tukad Abu beaches ofKarangasem Regency, Bali Province.

Medium and culture conditions

The medium used to isolate *Fusarium* was Matuo medium (per litre: 1.00 g K_2PO_4, 0.50 g KCl, 0.50 g $MgSO_4.7H_2O$, 0.01 g Fe-Na-EDTA, 2.00 g L-Asparagine, 20.00 g D-Galactose, and 1.00 litre aquadest) sterilized at 121°C for 20 minutes, then added antimicrobe substances i.e.: 1.00 g PCNB 75% WP, 0.50 g Oxgall, 1.00 g $Na_2B_2O_7$, 0.30 g Streptomycin sulphate; and then shaked well) (Matuo, 1972). Culture medium for *Fusarium* bioassay were potato dextrose agar (PDA, Nissui) and potato dextrose broth (PDB, Difco) with a little modifications with pH 4.5.

Determination of mycelial protein

Experiment to determine mycelial protein was done on PDA medium using Bradford method (Dunn, 1992). Bradford method counts soluble protein of the cell. One hundred gram of mycelia (fresh materials) obtained from the experiment (done as experiment to measure colony diameter, above description) were ground on mortar with liquid nitrogen medium. The ground mycelia was added by 0.5 ml buffer (0.1 M Tris-HCl pH 8.0 and 1 mM EDTA), centrifuged on 12,000 rpm for 10 minutes, then its supernatans were diluted by the same buffer (1:5). Twenty microlitres samples were added by 1 ml 1/5 Bradford reagent, vortexed, and incubated for 30 minutes before its absorbant spectrophotometricized at A595 nm. Standard curve was determined by BSA on the same condition.

Enzyme extract and protein analysis. Thirty mililitre PDB (*potato dextrose broth*) pH 4,5 in the 250 mL Erlenmeyer tube poured with *Aglaophenia* sp. Extract until its concentration were 0.2, 0.1, 0.05, 0.025, 0.0125, 0,00625, and 0% (w/v). Into each tube was inoculated with Ɵ5 mm fungi colony plug of *Fov* 7 days culture. The culture incubated 28°C and shaked 125 rpm. After incubation time, the culture was sieved with Whatmann paper number 2 and its filtrate considered as enzyme extract. The mycelia got was used for protein analysis.

Activity of cellulase enzyme. Cellulase enzyme activity was determined using DNS method (Wu *et al.,* 2008). Recation mix was consisted of 0.1 mL enzyme extract1.9 mL 1% (w/v) CMC-Na (*Carboxymethylcellulose*) in phosphate buffer, incubated for 50°C for 20 minutes. 1.5 mL DNS was added into the mix and then vortexed well. The mix then boiled 100°C for 10 minutes and cooled. The volume was adjusted into 25 mL with destiled water. The reduction sugar spectrum was determined in 520 nm wave length. One unit of enzyme activity was amount of reduction sugar produced by 1 mg enzyme per minute. Glucose (Sigma, St. Louise, Mo., USA) was used for established standard curve.

Activity of pectinase enzyme. Pectinase enzyme activity especially polygalacturonase was determined using DNS method (Wu *et al.,* 2008). Reaction mix was consisted of 2 mL of enzyme extract, 2 mL solution of 0.4% (w/v) pectin (*poly-D-galacturonic acid ethyl ester*, Sigma Aldrich), diluted in 0.2 M buffer ethyl acetic pH 4.4, and shaked well. The mix reacted in 45°C in water bath for 30 minutes, added with 1.5 mL DNS (*3,5-dinitrosalicylic acid*) then boiled for 5 minutes. The volume was adjusted to 25 mL with destiled water. The reduction sugar spectrum was determined in 520 nm wave length. One unit of enzyme activity was calculated by amount of *ß*-galacturonic acid produced by 1 mg enzyme per minute. Standard curve established from 0.1% (w/v) *ß*-galacturonic acid (Sigma, St. Louise, Mo., USA).

DNS Reagent. Quatitative determination of reduction sugar was done using*3,5-dinitrosalicylic acid* (DNS). A reaction between DNS and reduction sugar resulting*3-amino-5-nitro salicylic acid* with yellow

color and its concentration can be determined by spectrophotometer.DNS Reagent formulated by: DNS 0.2 g, phenol 0.04 g, Na_2SO_4 0.01 g, potassium sodium (+)-tartrate tetrahydrate (*Rochelle salt*) 4 g; diluted in 100 mL water. Standard curve was costructed by: 0.5 mL sugar solution with concentration of 0-800 µg/mL), 1.5 mL DNS reagent boiled for 5 minutes, cooled and then added water until volume of 25 mL. Its absorbant was quantified by spektrophotometer on 540 nm wave length.

Protein quantification

Lowry method. A part of mycelia from above preparation was dried in oven in80°C until its weight fixed, and then powdered in mortar.Amount of 25 mg of hypha powder were added with 1 mL 2 N natrium hydroxide, pH 7,shaked and boiled 80°C for 10 minutes. The mix then, centrifugated 5000 rpm for 10 minutes and its supernatans absorbants determinedspectrophotometrically in wave length of 750 nm. Standard curve was costructed by Bovin serum albumin (BSA) in concentration of 0-100 µg/100 µL natrium hydroxide.

Bradford method. The mycelia at amount of100 g in liquid nitrogen was directly ground and then added by 0.5 mL buffer (0.1 M Tris-HCl pH 8,0 and 1 mM EDTA) centrifugated at 12,000 rpm for 10 minutes and then its supernatans were diluted by the same buffer (1:5). As much as 20 µL of the samples were added by 1 mL 1/5 reagent Bradford, vortexed. The absorbants was spectrophotometrically determined at 595 nm after 30 minutes incubation. Standard curve was constructed by Bovin serum albumin at the test condition.

Electrophoresis

Protein extraction. The protein for electrophoresis was prepared by grinding the mycelia in mortar after cooled by liquid nitrogen.Fifty mL micelial extract added with 500 µL buiffer extraction (20 mM Tris, 10 mM sodium bicarbonate, 10 mM magnesium cloride, 0.1 mM $Na_2EDTA.2H_2O$, 10 mM β-mercaptoethanol, 100 gL^{-1} sucrose and 1 mol L^{-1} Triton X-100, pH 8 (HCl). The extract was centrifugated at 20.000 rpm for 20 minutes at 4°C and supernatantput at new eppendorf.

Protein separation. The protein was separated using polyacrilamide gelelectrophoresisin electrophoresis instrument (10x8 cm; SE 250, Hoefer Scientific Instruments). *Stacking gel* (3,75 *acryl amide-bisacrylamide* 30:0.8 v/v) in 125 mM buffer Tris/HCl pH 6.8 and*separating gel* (7.5% *acryl amide-bisacrylamide* 30:0.8 v/v) in 375 mM Trsi/HCl, pH 8.8 as buffer. Gel was run with AC 110 V, 15 mA for 75 minute inelectrode buffer, Tris *glycine*contained25 mM Tris and 192 mM *glycine*wih pH of 8.3.

RESULTS AND DISCUSSION

Effect of the extract against extracellular enzymes of Fov

Generally the extract was able to depress both extracellular enzymes studied, the higher the concentration of extract given, the lower activities of both enzymes. Cellulase is more sensitive than pectinase. Cellulose activities was depressed at lowest concentration, while pectinase was depressed at 0.025% of extract (Table 1).

Table 1. The influence of the *Aglaophenia* extract against activity of extracellular enzymes

Extract concentration(%)	Activities of enzyms (µg/mL/minutes)	
	Cellulase	Pectinase
0	1946.74 A	3930.89 a
0.0016	1908.86 B	3914.23 a
0.0031	1885.38 B	3908.17 a
0.0063	1817.20 C	3891.50 a
0.0125	1806.59 C	3806.65 a
0.025	183.87 D	389.98 b
0.05	166.44 D	361.20 b
0.1	164.92 D	358.17 b
0.2	0.00 E	0.00 e

Numbers followed by same letters in one column are not significantly different according to Duncan multiple ring test of 5%. Data were analyzed after transformed by √(x+0,5).

Because of the two enzymes had important role on patogenisityof the pathogen (deVries and Visser, 2001, Dickinson, 2003), therefore the influence of the extract would decreasing the *Fov* penetration on vanilla plant.

Influence of the extract against protein of mycelia

Aglaophenian extract was significantly decreasing protein content of *Fov.* The higher concentration of the extract were given, protein content was lower on the both methods (Table 2).

Table 2. Influence of the extract against mycelium protein content of *Fov*

Extract concentration (%)	Content of protein mycelium (µg/g)	
	Lowry method	Bradford method
0	208.71 a	208.60 a
0.0063	205.37 a	187.52 a
0.0125	195.76 b	170.24 a
0.025	179.95 c	163.88 a
0.05	159.88 d	142.79 b
0.1	127.79 e	110.00 c
0.2	93.02 f	62.95 d

Numbers followed by same letters in one column were not significantly different according to Duncan multiple ring test of 5%. Data were analyzed after transformed by √(x+0.5).

Based on Lowry method, protein content of mycelia was had influence by extract concentration of 0.0125%, while based on Bradford method the depression was just happened at concentration of 0.05%. The suppression was supported by Sudana (1997) research that exhibited the secondary metabolite as phenolic substance (a common substance produced by antagonistic/antibiotic organism) could suppress nucleic acid and protein synthesis of *Ceratocystis paradoxa*. The suppression was caused by suppression of glucose-6-fosfat dehydrogenase and succinic dehydrogenase due to decrease on respiration. According to Bruinenberg (1982 cited by Sudana, 1997) the two enzymes had important role in respiration process.

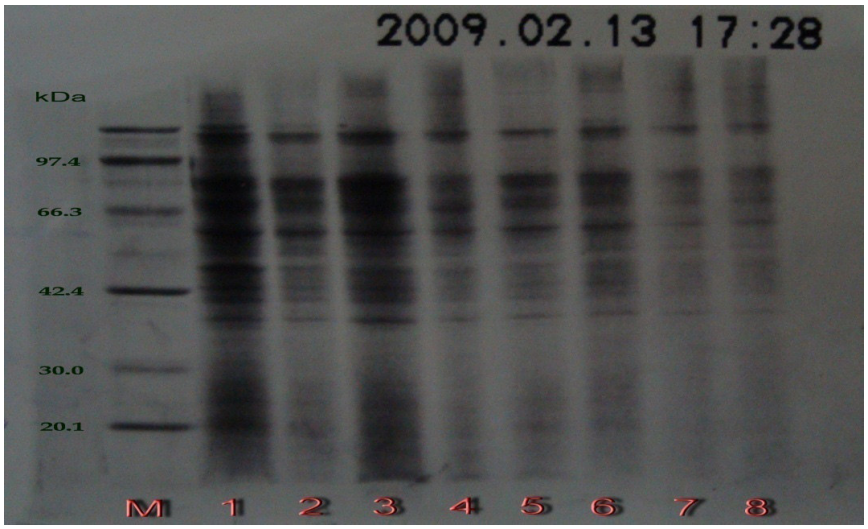

Figure 1. Profile of SDS-PAGE mycelia proteinof *Fov*on various extract concentration of *Aglaophenia* sp. M: Marker; Line 1-8: protein band on concentration of 0, 0.0016, 0.0031, 0.0063, 0.0125, 0.05, 0.1, and 0.2% (w/v) respectively.

The influence of the extract against protein also appeared on SDS-PAGE gel as exhibited on Figure 1. The higher concentration of extract given, the lower content of protein in mycelium. Total protein on high extract concentration (0.1% and 0.2%) were lower than that of lower concentration. This results were indicated by its protein bands on those concentration were thinner than the others. The suppression of protein produced caused onto the low ability of mycelia to grow and forming colony as indicated by former research.

CONCLUSION

Enzyme activity was one important factor in the pathogen infection process on plant. To suppress its activities therefore, some fungicide were discovered including marine sources. *Aglaophenia* sp. was a marine animal being investigated for its ability to suppress enzyme activityof *Fusarium* on vanilla. The research exhibited that the increase of extract concentration given, the decrease of the two enzyme activity. The cellulase activity has been suppressed on 0.0016% of extract (the activity of 908.86 µg/mL/minute) and significantly lower compared to control (1946.74µg/mL/minutes). While the pectinase activity was just influenced on higher concentration i.e. 0.025%. The enzyme activity was 389.98 µg/mL/minute, while on control was 3930.89 µg/mL/minute. Those data indicated that cellulase was more sensitive than pectinase against extract given.

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THE EFFECT OF CONCENTRATE SUPPLEMENTATION ON NITROGEN BALANCE OF BALI CATTLE FED RICE STRAW BASED DIET

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Faculty of Animal Science Udayana University

ABSTRACT

An experiment that aim to determine the nitrogen balance of Bali cattle fed rice straw and different level of concentrate was conducted for twelve weeks at Penatih village Denpasar regency and Animal Nutrition laboratory Faculty of Animal Science Udayana University of Bali. The Completely Randomized Block Design (CRBD) was used in this experiment consisted of three treatments and three block (replicates). So there are nine experiments unit. In this experiment, each experiment unit consisted of a head of cattle. So the total number of cattle used in this experiment was nine heads with initial body weight of 223 ± 0.8 kg. The three treatments were concentrate supplementation at level of 1.0; 1.5; and 2.0 % of body weight respectively for treatment P1, P2 and P3.

POSTER PRESENTATION: AGRITECH AND FOOD

INCREASING ANTIOXIDANT ACTIVITY OF MILLET (*PENNISETUM SP)* ON SPRAGUE DAWLEY RATS.

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ABSTRACT

Millet is cereal crops that would be useful as alternative sources in food diversification programme. It has protein content more than rice and has the prospect to be developed in Indonesia. Recently, demand of food industries are not only nutritional values but also health aspects. The result of many prior research showed that millet had bioactive compounds that function in health, such as to decreased degenerative disease risk. The objective of this research was to study the biological potency of millet liver antioxidant capacity in rats. Three groups of rats including control, rats fed diet containing 50% or rats fed diet containing 100% millet as sources of carbohydrate. Results of this research showed that all rats 50% or 100% of millet showed decreased liver malondialdehyde (MDA) by 13% and, 14% respectively, increased liver super dioxide dismutase enzyme activity (SOD) by 85% and 88% respectively, increased liver catalyse enzyme activity (CAT) by 14% and 16% respectively, and increased liver glutathione peroxides enzyme activity (GPx) by 29% and 33% respectively. The effective result was from rats fed diet containing 100% of millet as source of carbohydrate. The conclusion of this research was the millet was good for health.

Keywords : millet, source carbohydrate, antioxidant activity, liver, rats

INTRODUCTION

Millet is cereal crop that became sixth food source in world. In Indonesia, it was not popular as food source but popular as bird feed. Recently food source from cereals are good for healthy. One of food of cereal that famous is oatmeal. Oatmeal is food instant from wheat. Recently the import wheat is very high and government has programme to decrease import wheat and diversification programme. Millet is potential as another carbohydrate source beside rice and potential to decrease the import of wheat. The performance of millet could see in Figure 1.

Although millet was popular as bird feed but it has a prospect to be explored as functional food such as oatmeal. In Africa or dry place such as Gunung Kidul (central Java), millet has become an important food source. It contain carbohydrate almost much as rice. Carbohydrate of millet and rice were 75% and 79% respectively. On the other hand, it had 8% protein but rice just 7%. Structure component of bioactive compounds that benefit health were very much same as structure protein. The bioactive compound of millet is known as phenolic compound such as ferulic acid. One of benefit to health is bioactive compound which function as antioxidant.

To improve the use of millet as functional food needs information on the benefit of millet to health. One benefit of millet to health is antioxidant source. Antioxidant is compound that is used to fight oxidation that can result free radical. Free radical is one component that has negative effect for health. The objective of research is to determine antioxidant activity of millet by in vivo on Sprague Dawley.

(a)

(b)

Figure 1. millet plant (a)and millet crop (b)

MATERIALS AND METHOD

The research was done at Biochemistry Nutrition Laboratory of Agriculture Technology Faculty of Bogor Agriculture Institute, Animal Model Laboratory of SEAFAS Centre of Bogor Agriculture Institute. Materials of research were millet from bird market in Bogor, that consist kuali variety, male spargue dawley from BPOM-Jakarta, standard animal food. *xanthin oksidase* (grade IV, Sigma X-4875), *xanthin* (Sigma, X-7375), NaEDTA, H_2O_2, glutation tereduksi (Sigma), NADPH (Sigma), KH_2PO_4 H_20, Na_2HCO_3 H_20. Eguipemnt of research were vortex, spektrofotometer, spektrofotometer UV-Vis (Ishimatsu, UV- 160) kuvet, pH-Meter (Orion, 210 A), *water bath* (GFL 1083), toples and tools of determination rats.

The experiment used three group of rats. Every group consist of seven rats. Group of treatments were 1) control group; 2) 50 % rat fet of millet carbohydrate source and 3) 100% rat fet of millet carbohydrate source. Analysis of variabel were antioxidant activity of enzim antioxidant of superokside dismutase (SOD), catalase (CAT) and Glutation peroxidase (GPx) on liver of sprague dawley. Flow Chart of research could be seen at Figure 2.

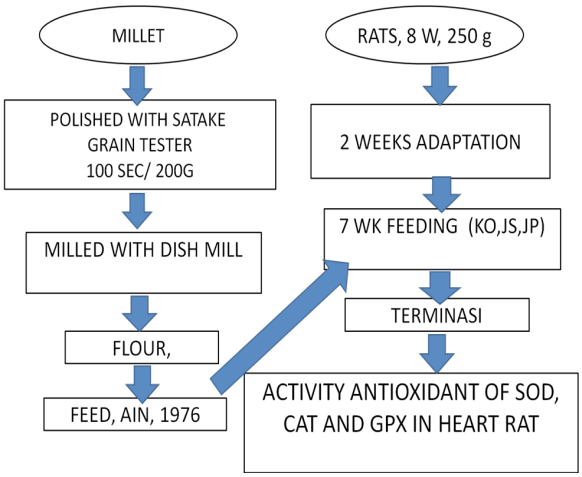

Figure 2. Flow chart of research

RESULT AND DISCUSSION

Malondialdehyde (MDA)

The chart show treatments of 50% and 100% milet significant influence with treatment control on MDA value. Average amount of MDA value could see on Figure 3

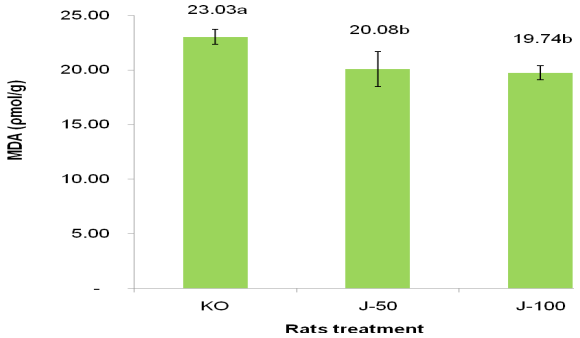

Figure 3.MDA value

Figure 3 shows MDA value. The highest value was at control treatment which was 23.03% and the lowest on 100 % millet treatment which was 19.74%. The higher the levels of millet, the lower the MDA levels. However, between 50% and 100 % millet, had no significant effect on MDA value. This

condition could be caused by the content of millet which was bioactive compound such as phenolic acid that function as antioxidant that could fight free radical or lower of MDA value. Treatment 50 % and 100% millet could decrease MDA value from control treatment such as 13% and 14%.

Superdioxide dismutase enzyme activity (SOD)

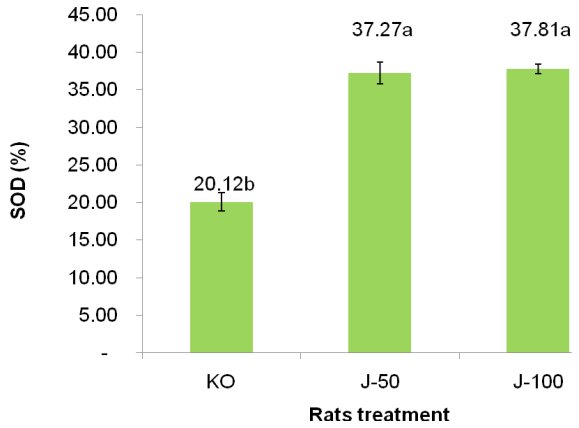

Figure 4. Superdioxide dismutase enzyme activity (SOD)

Figure 4 shows that the highest of SOD activity was on 100% millet with value of 37.81% and the lowest was on control treatment with amount of 20.12%. Concentration between 50% and 100% had no significant effect on SOD activity. This condition could be caused by bioactive compound of millet that can maintain SOD enzyme cell from broken cell that caused free radical. Moreover activity of SOD enzyme could flow well. Treatment 50% and 100 could increased SOD activity from control treatment suc as 85% and 88%

Catalyse enzyme activity (CAT)

The chart shows treatments of 50% and 100% milet had significant influence with control on CAT activity. Average amount of CAT activity could see on Figure 5

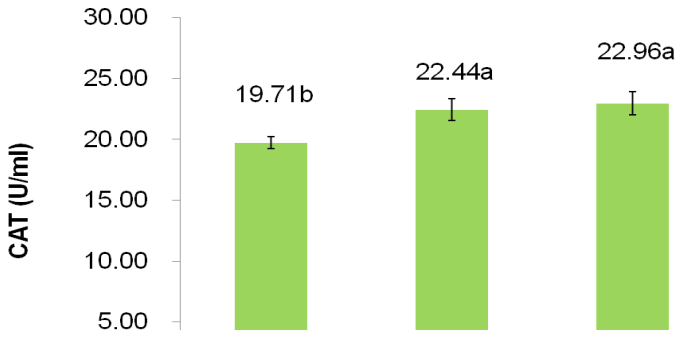

Figure 5. Catalyse enzyme activity

Figure 5 shows the highest of CAT activity was on 100% millet with amount 22.96% and the lowest was on control with amount 19.71%. Treatments between 50% and 100% had no significant effect on CAT activity. This condition could be caused by bioactive compound of millet that could be maintain CAT enzyme cell from broken cell that caused free radical. Moreover activity of CAT enzyme could flow well. Treatment 50% and 100 could increased CAT activity from control treatment suc as 14% and 16%

Glutathione peroxides enzyme activity (GPx)

The chart shows treatments of 50% and 100% milet significant influence with treatment control on GPx activity. Average amount of GPx activity could see on Figure 6

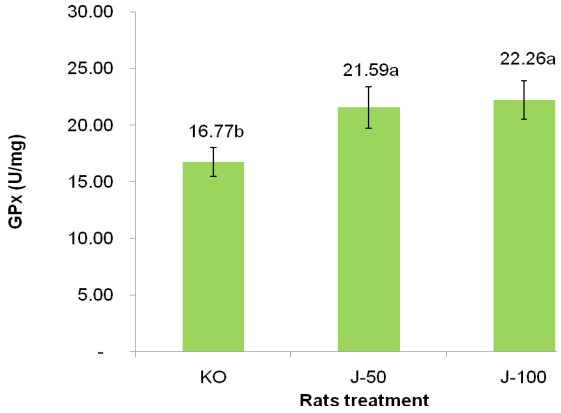

Figure 6. Glutathione peroxides enzyme activity

Figure 6 shown the highest of GPx activity on 100% millet with amount 22.26% and the lowest on control treatment with amount 16.77%. Between 50% and 100% no significant effect on GPx activity. This condition could be caused bioactive compound of millet could be maintenance GPx enzyme cell from broken cell that caused free radical. Moreover activity of GPx enzyme could flow well. Treatment 50% and 100 could increased of GPx activity from control treatment suc as 29% and 33%.

CONCLUSION

Consumption of carbohydrate source from millet decreased MDA level and increased enzyme antioxidant activity such as SOD, CAT and GPx. Consumption of millet 50% and 100% had no significant effect on MDA, SOD, CAT and GPx. Decreasing of MDA, and increasing of SOD, CAT and GPx on millet 50 % and 100 % such as: 13% and 14%; 85% and 88%; 14% and 16 %; 29% and 33% respectively. the best result on millet 100 %

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THE EFFECT OF YELLOW CASSAVA FLOUR (*MANIHOT ESCULENTA* CRANTZ.) AS SUBSTITUTE FOR WHEAT FLOUR AT DIFFERENT CONCENTRATIONS ON THE WET NOODLE'S PRODUCTION

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ABSTRACT

The purpose of this study was to observe the effect of yellow cassava flour (*Manihot esculenta* Crantz.) as substitute for wheat flour at different concentrations on the wet noodle's and to determine the right concentration of yellow cassava flour as the substitute of wheat flour to obtain wet noodle with the best characteristic. This study used block random design, substitution treatment of wheat flour with yellow cassava flour on different concentration namely : 0%, 9%, 19%, 28%, 38%, and 48%. Each treatment was done three times grouping based on processing time, in order to obtain 18 units of experiment. The obtained data was analysis with analysis of variance, followed with Duncan's test. Determination of the best treatment by the effectiveness test.
The substitution of wheat flour with yellow cassava flour in the wet noodle's production was highly significant effect on moisture content, ash content, protein content, total carotene, colour, flavor, texture, taste, and overall acceptance. Wet noodle's with the best treatment in the treatment of yellow cassava flour concentration of 19% with the following characteristics : water content (24.05%), ash content (0.45%), protein (5.42%), total carotene (146.64 µg/100g), color of 5.33 (somewhat like), flavor of 4.67 (normal), texture of 4.07 (chewy), taste of 6.07 (like), and overall acceptance of 6.07 (like).

Keywords : Substitution, yellow cassava (*Manihot esculenta* Crantz.) flour, wet noodle's.

INTRODUCTION

Noodle is a kind of wheat flour's product. Wheat flour was still imported, till February 21th 2011 the price of wheat flour was Rp 7.602/kg (Tragistina, 2011). On 2010, consumtion of imported wheat flour was 762.515 ton, increased from last year 645.010 ton and it probably will increase 6% for this year (Ariffianto, 2011).

Alternate flour was needed to decrease consumtion of wheat flour, one of them was yellow cassava flour. Yellow cassava was high potential local product. Production of cassava in Bali on 2010 was 169.761 ton and the price was Rp.2000,00 / Kg (Anon, 2011).

Yellow cassava was selected as substitute for wheat flour on wet noodle production for the reason that cheap, high carbohydrate content (37,9 g / 100 g), and contain vitamin A (385 SI) also for long term it was desired to decrease dependency of wheat flour.

The purpose of this study was to observe the effect of yellow cassava flour (*Manihot esculenta* Crantz.) as substitute for wheat flour at different concentrations on the wet noodle's and to determine the right concentration of yellow cassava flour as the substitute of wheat flour to obtain wet noodle's with the best characteristics.

MATERIAL AND METHOD

Material

Wheat flour, salt, and chicken egg was bought in Hardy's Supermarket, sodium tripolyphosfate (STPP) and sodium carbonat from Bratachem, Denpasar and yellow cassava (*Manihot esculenta* Crantz.) from Gianyar Market, and water.

Method

This study used block random design, substitution treatment of wheat flour with yellow cassava flour on different concentration namely : 0% (S0), 9% (S1), 19% (S2), 28% (S3), 38% (S4), and 48% (S5). Each treatment was done three times grouping based on processing time, in order to obtain 18 units of experiment.

Preparation of yellow cassava flour

Yellow cassava flour was prepared based on Astawan was modified (1999) i.e. : yellow cassava was selected, then peeled manually by knife, cutted , washed and grated. Then, yellow cassava was dried with oven, temperature of 70 ^{o}C for two and half hours, thickness of 0.5 cm. After dried, it was smashed and finally sift with 60 mesh sifter therefore, yellow cassava was obtained. Analysed of water content, protein contain and carotene total was done.

Wet Noodle Making

Formula on wet noodle making was showed on Table 1.

Tabel 1. Formula on wet noodle making with substitution treatment of wheat flour with yellow cassava flour on different concentration

Material	Treatment					
	S0	S1	S2	S3	S4	S5
Wheat flour (g)	105	95	85	75	65	55
Yellow cassava flour (g)	0	10	20	30	40	50
Sodium Tripolophosfate (g)	0.25	0,25	0,25	0,25	0,25	0,25
Salt (g)	1	1	1	1	1	1
Water (g)	38	38	38	38	38	38
Sodium Carbonate (g)	0.4	0.4	0.4	0.4	0.4	0.4
Chicken Egg (g)	8	8	8	8	8	8

Lit. : Astawan was modified (1999)

Wet noodle making was done derived from modified Astawan (1999) as follow :

1. Salt, sodium carbonate, and STPP was dissolved by egg (after beat) in bowl.
2. Wheat flour and yellow cassava flour was added (depand on the treatment) then added water litle by litle while mixed by hand until became dull dough and let it for 15 minute.
3. The dough was pressed by using dough sheet roller (until dough sheet thickness of 1.5 mm).
4. Dough sheet was spreaded by 5 g wheat flour, input to the roll press to get noodle with the length of 20 cm and the wide of 2 mm.
5. Acquired noodle was boiled in the oil – contained water for 1.5 minute at temperature of 100 ^{o}C while slowly stired, drained, and then warmed.

Analysis

Chemical Analysis included water content by oven method (Sudarmaji et *al*., 1997), ash content by glowing method (Sudarmaji et *al*., 1997), protein content by Kjeldahl method, and carotene content (Lestariana et *al*., 1988 dan Muchtadi, 1989). Organoleptic test integrated color, flavor, taste dan overall acceptance by hedonic test while texture by score test (Soekarto, 1985). The obtained data was analysed with analysis of variance, followed with Duncan's test (Gomez dan Gomez, 1995). Determination of the best treatment by the effectiveness test (de Garmo, *et al*., 1984).

RESULT AND DISCUSSION

Chemical Characteristic

Based on the Analysis of Variance, substitution treatment of wheat flour with yellow cassava flour on different concentration had highly significant effect on moisture content, ash content, protein content, and total carotene. The data was presented on Table 2.

Table 2. Average of water content, ash content, carotene content, and protein content of wet noodle with substitution treatment of wheat flour with yellow cassava flour on different concentration

Treatment	Yellow Cassava Flour Concentration (%)	Water Content (%)	Ash Content (%)	Carotene Content (mg/100g)	Protein Content (%)
S0	0	20.36 f	0.20 f	61.70 f	8.05 a
S1	9	22.59 e	0.33 e	102.86 e	6.25 b
S2	19	24.05 d	0.45 d	146.64 d	5.42 c
S3	28	27.09 c	0.48 c	185.19 c	4.44 d
S4	38	33.43 b	0.56 b	231.62 b	4.11 d
S5	48	36.53 a	0.63 a	264.45 a	3.45 e

Note : the same characters behind average showed not significant effect (P>0.05).

Table 2 demonstrated that increasing of yellow cassava flour concentration enhanced water content, ash content and carotene content however decreased protein content for the reason that yellow cassava flour contained water content, ash content, and carotene content higher than wheat flour. Water, ash, and carotene content of yellow cassava flour was 12.42%, 0.7%, 348.11 µg/100gram, respectively. Nevertheless, water and ash content of wheat flour was 7.0%, 0.4%, respectively and without carotene content. On the other hand, protein content of yellow cassava flour was 3.07% lower than wheat flour (11,20%) (preresearch).

Based on quality standard of wet noodle (SNI No. 01-2987-1992), requirement of wet noodle's water content was 20% - 35%. In this research, water content of wet noodle on the treatment of yellow cassava flour concentration of 0%, 9%, 19%, 28%, and 38% was appropriate with quality standard of wet noodle but wet noodle on the treatment of yellow cassava flour concentration of 48% not appropriate with quality standard of wet noodle.

Ash content, based on quality standard of wet noodle (SNI No. 01-2987-1992), maximum wet noodle's ash content regulation was 3%. In this research, all treatment was suitable with quality standard of wet noodle.

Carotene that was found on the yellow cassava flour as vitamin A source, it was important for eye, helped scar healing, and also gave natural yellow color with no synthetic color needed (Setiawan, 2008).

Protein content, based on quality standard of wet noodle (SNI No. 01-2987-1992), requirement of minimum wet noodle's protein content was 8%. In this research, almost all of treatment had protein content lower than standard except wet noodle on the treatment of yellow cassava flour concentration of 0% was 8.05%.

Organoleptic Characteristic

The Analysis of Variance showed that substitution treatment of wheat flour with yellow cassava flour on different concentration had highly significant effect on colour, flavor, texture, taste, and overall acceptance. The data was presented on Table 3.

Table 3. The result of statistical analysis of organoleptic test of wet noodle with substitution treatment of wheat flour with yellow cassava flour on different concentration

Treatment	Yellow Cassava Flour Concentration (%)	Color	Flavor	Texture	Taste	Overall acceptance
S0	0	4.47 d	3.53 e	4.47 a	3.67 d	3.40 b
S1	9	5.13 c	4.13 de	4.20 a	4.27 c	5.60 a
S2	19	5.33 bc	4.67 cd	4.07 a	6.07 a	6.07 a
S3	28	5.40 bc	5.20 bc	3.53 b	5.67 a	5.80 a
S4	38	5.93 ab	5.40 ab	2.93 c	5.07 b	5.47 a
S5	48	6.20 a	6.07 a	2.07 d	4.80 bc	5.40 a

Note : the same characters behind average showed not significant effect (P>0.05).

Table 3 demonstrated that panelis gave average for color of 4.47 to 6.07 (neutral to like). The highest value was S5 of 6.20 (like) while the lowest was S0 of 4.47(neutral). Color distinction on wet noodle because presence of carotene in yellow cassava flour (348.11 g/100g) (pre research) that gave natural yellow color on wet noodle. Increasing of yellow cassava flour concentration caused increasingly yellow.

Flavor average of 3.53 to 6.07 (rather dislike to like). The highest value was S5 of 6.07 (like) while the lowest was So of 3.53 (rather dislike) (Table 3).

Texture average was 2.07 to 4.47 (tender to chewy) (Table 3). The flour's protein content (especially gluten) effected product texture. Anon (2009) said that the more protein content will give the stronger gluten character and difficult to break. Increasing of yellow cassava flour yield noodle more tender because yellow cassava had low protein content than wheat flour.

Table 3 demonstrated that average of taste was 3.67 to 6.07 (neutral to like). The highest average of taste was treatment of yellow cassava flour concentration of 19% and the lowest was without yellow cassava flour addition. Table 3 also presented that Overall acceptance average of obtained wet noodle was 3.40 to 6.07 (rather dislike to like).

Effectiveness Test

Effectiveness Test was done to find out the best treatment achieved by calculating effectiveness value and result value. Based on the effectiveness test, the highest result value was 0.672 (treatment of yellow cassava flour concentration of 19%) therefore, the best treatment was yellow cassava flour (*Manihot esculenta* Crantz.) as substitute for wheat flour at concentration of 19%.

CONCLUSION

1. The substitution of wheat flour with yellow cassava flour in the wet noodle's production was highly significant effect on moisture content, ash content, protein content, total carotene, color, flavor, texture, taste, and overall acceptance.
2. Wet noodle's with the best treatment in the treatment of yellow cassava flour concentration of 19% with the following characteristics : water content (24.05%), ash content (0.45%), protein (5.42%), total carotene (146.64 µg/100g), color of 5.33 (somewhat like), flavor of 4.67 (normal), texture of 4.07 (chewy), taste of 6.07 (like), and overall acceptance of 6.07 (like).

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RE-FERMENTATION PROCESS BASED ON POLYPHENOL OXIDASE ENZYME TO IMPROVE THE QUALITY OF COCOA BEAN FROM FARMERS PRODUCTS

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ABSTRACT

The objective of this research was to determine the influence of re-fermentation based on temperature and pH of polyphenol oxydase (PPO) activity conditions on the quality characteristics of dried cocoa bean from farmer products. The levels of temperature and initial pH used as the treatments were adopted from the optimum temperatures and pH for the activity of PPO enzyme. Group Randomized Design with two factorials was applied for the experiment. The first factor was the temperature of water soaking the bean with three levels: 44°C , 54°C, and 64°C, and the second factor was the initial pH with three levels: 4.4, 5.4, and 6.4. The soaking process was 90 minutes. Observation was conducted for the quality characteristic of dried cocoa bean quality based on the SNI. The results indicated that both temperature, and pH, as well as their interactions provided significant influences on various cocoa quality parameters: moisture content, unfermented bean, molded bean, pH, and fermentation index. The combination among temperature range of 54-64°C, and pH range of 5.4-6.4 during the re-fermentation process was recommended to produce good bean quality.

Keywords *: cocoa, re-fermentation, PPO, bean quality*

INTRODUCTION

The total area of cocoa plantation in Indonesia reached 1.19 million ha producing 794 thousand tons dried cocoa bean in 2007 (Anon., 2008). Ninety percents of the bean was produced by farmers, while the rest was produced by state and private cocoa estates. Farmers commonly did not implement fermentation process, thus resulted in low quality of cocoa bean with high content of foreign matter, acidity, and slaty beans; low quality taste since the precursor for cocoa taste was not formed; and un-uniformity of color.

One effort to improve the quality of farmer's cocoa bean was by introducing re-fermentation process applying temperature and pH optimal conditions for the activity of poly phenol oxydase (PPO) during soaking the cocoa bean in water. The enzyme could hydrolyze polyphenol in the cocoa bean so it would reduce bitter and smoky taste of the cocoa, and provide uniform color (Lopez, 1986). In general, the cocoa bean produced by farmers indicated relatively high content of poly phenol oxydase as much as 122.98 U/mg protein (Permana, 1992). However, without optimum condition for fermentation it did not work as expected. The application of 53.4°C temperature, and 5.4 pH for 81 minutes on the isolated enzyme from farmer's cocoa bean, resulted in the enzyme activity of 157.5 ± 58.0 U/min/g dry basis, enzyme kinetics parameters - V_{max} = 595.2 U/min/g dry basis, and K_m = 0.2 M (Ganda-Putra *et al*., 2009a).

Rehydration during re-fermentation might help the dried cocoa bean re-adsorbed water to activate the polyphenol oxydase. Lopez (1986) recommended the fermentation temperature in a range of 40 – 60°C, and the substrate moisture content of 40 - 10 % for the activity of PPO.

The objective of this research was to determine the influence of the re-fermentation based on temperature and pH of activity conditions of polyphenol oxydase (PPO) on the quality characteristics of dried cocoa bean from farmers products. The outcome was expected to be implemented by the processors buying the farmer's cocoa bean for further manufacturing of food products.

MATERIALS AND METHODS

Dried cocoa bean was sampled from farmers of the central production in Jembrana District. Chemicals used in the experiments covered methanol, HCl, NaOH, anhydrate glucose, Nelson A, and Nelson B reagent, H_2SO_4, phenol red, $AgNO_3$, Felin-ciocalteaus 2N reactive, Na-carbonate, and galic acid standard. Equipments used were water bath, pH meter, spectrophotometer, vacuum filter, and reverse cooler.

Group Randomized Design with two factorials was applied for the experiment. The first factor was

the temperature of water soaking the bean with three levels : 44°C, 54°C, and 64°C, and the second factor was the pH with three levels : 4.42, 5.42, and 6.42. The combinations of factorials were repeated three times.

Three kg of samples for each treatment was soaked in a water bath at the stated conditions for the combination of temperature and pH levels for 90 minutes. Samples were then drained and sundried until the moisture content reached 7.5 %. Observations were taken for the quality of dried cocoa bean (SNI 01-2323-2002) : moisture content, 2) shell, 3) nib, 4) flat beans, 5) bean counts per 100 g, 6) slaty beans, 7) foreign matter, 8) molded beans, 9) pH, and 10) fermentation index.

RESULTS AND DISCUSSION

The results of Anova (Table 1) indicated that the increasing re-fermentation temperature increased the bean count and the fermentation index significantly, and reduced the cocoa bean moisture content, the slaty beans, and the flat beans. However, it also increased the molded beans. Temperature of 64°C and pH of 5.4 obtained the lowest bean moisture content of 6.04 %, slaty bean of 5.67 %, and flat beans of 3.44 %. However the molded beans was the highest with a level of 9.89 %. Farmer's cocoa bean which was not going through re-fermentation produced 15 % moisture content, over than 30 % slaty beans, and over than 20 % foreign matter (Ganda-Putra *et al*., 2009b).

Table 1. The Quality carachteristic of re-fermented cocoa beans

Treat-ments		Quality Parameters									
Tem-pe-rature (°C)	pH	Moist-ure Content (%)	Shell (%)	Nib (%)	Bean Counts per 100 g	Slaty Beans (%, per 300 beans)	Molded Beans (%, per 300 beans)	Flat Beans (%, per 300 beans)	Fermentation Index (abs. 460/ 530)	Nib pH	Foreign Matter (%)
44	4.4	6.48 abc	14.05 a	85.95 a	103.67 d	8.67 ab	4.33 c	6.56 ab	0.77 cd	6.41 ab	6,00 a
	5.4	6.71 abc	12.77 a	87.23 a	102.67 d	9.67 a	5.44 bc	8.44 a	0.73 e	6.43 a	5,92 a
	6.4	6.81 ab	13.71 a	86.29 a	102.33 d	8.67 ab	4.89 c	6.33 ab	0.76 d	6.25 ab	7,92 a
54	4.4	6.72 abc	14.97 a	85.03 a	104.00 c	8.33 abc	5.56 bc	6.33 ab	0.77 cd	6.18 b	6,53 a
	5.4	7.02 a	12.78 a	87.22 a	107.00 bc	6.89 cd	8.11 a	6.11 ab	0.80 ab	6.35 ab	7,45 a
	6.4	6.34 abc	13.98 a	86.02 a	105.67 cd	9.67 a	7.67 ab	6.67 ab	0.80 ab	6.23 ab	5,91 a
64	4.4	6.13 bc	13.31 a	86.69 a	114.00 a	7.67 bc	8.33 a	5.44 bc	0.82 a	6.22 b	4,78 a
	5.4	6.04 c	14.70 a	85.30 a	109.0 abc	5.67 d	9.89 a	3.44 c	0.79 bc	6.23 ab	5,19 a
	6.4	6.38 abc	15.31 a	84.69 a	111.67 ab	6.67 cd	8.89 a	4.22 bc	0.81 ab	6.21b	6,06 a
LSD 5%		0.76	2.73	2.69	5.05	1.70	2.74	2.50	0.02	0.20	5.25

CONCLUSIONS

1. Both temperature, and pH, as well as their interactions provided significant influences on various cocoa quality parameters : moisture content, unfermented bean, molded bean, pH, and fermentation index.
2. The combination among temperature range of 54-64°C, and pH range of 5.4-6.4 during the re-fermentation process was recommended to produce good bean quality.

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THE INFLUENCE TYPE OF SOLVENT AND EXTRACTION TIMES ON THE YIELD AND CHARACTERISTICS ESSENTIAL OIL OF SANDALWOOD FRANGIPANI FLOWER (*Plumeria alba)*

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ABSTRACT

This study aims to 1) determine the influence of solvent of type, extraction time and their interaction on the characteristics of essential oil of sandalwood frangipani flower 2) find out the right type of solvent and extraction time to produce the best yield and characteristics of essential oil of sandalwood frangipani flower and 3) determine kind and the composition of compounds in the essential oil of sandalwood frangipani flower. This research used ramdomized block design with factorial pattern. The first factor (type of solvent) consisted of 2 types namely: n-hexane and ethyl acetate, the second factor (extraction time) was comprised of 4 extents: 1, 2, 3, and 4 hours. Each treatment was done twice. The best treatment was measured with effectiveness test.
The results showed that the type of solvent and extraction time had significant effect on the yield and characteristics of essential oil of sandalwood frangipani flower. N-hexane solvent and extraction time 4 hours is an appropriate treatment to produce essential oils of sandalwood frangipani flower and yield the best characteristics, with yield of 0.78%, the aroma preference of 5.45 (between like- very like) and aroma strength 6.90 The essential oil of sandalwood frangipani flower containing 46 types of compounds. Constituent compounds essential oil of sandalwood frangipani flowers that have the highest relative concentration values derived from the group of alcanes and alcohols.

Keyword : sandalwood frangipani flower, n-hexane, ethyl acetate, essential oils.

INTRODUCTION

Frangipani flower is currently very popular in Bali because it has a fragrant aroma and is widely used as fragrances, incense and air freshener. The smell fragrant frangipani flowers due to the volatile oil content in the frangipani flower. Essential oils are volatile oils and are mixture of several compounds. Essential oils in the plant material can be extracted by solvent extraction method in certain circumstances.

The quality and quantity of essential oil compounds derived from natural materials is strongly influenced by the methods used in the separation process and the process operating conditions. The results Wartini (2007), indicating that the bay leaf extraction using n-hexane and ethyl acetate extracts with different characteristics and produces good color, flavor and chemical composition of the extract constituent. Similarly, the results shown by Saputra (2010) on the extraction of flavor compounds in pandan leaf fragrance, extracts obtained characteristics are influenced by the type of solvent and extraction time.

The solvent extraction process, influenced by the nature of the solvent used and the selection of solvent is determined by the solubility of volatile material and the ease of solvent separation. Therefore the selection of the solvent extraction process will determine the success (Ojha et al., 1995; Guenther, 1987). This study used the solvent n-hexane and ethyl acetate as the solvent hexane (non polar) has a different polarity with ethyl acetate (semi-polar) so that the alleged use of these solvents will produce essential oils with different quality addition to the price is relatively cheap and easily obtainable in the market. Besides the types of solvents, extraction time of essential oils affect the compounds can be extracted from the raw material. It has been proven in materials with time of ginger extraction 4, 6, 8 hours (Ma'mun et al., 2008), garlic bulbs with extraction time of 0.5, 1, 2, 3, 4 hours and the highest yield obtained the extraction time of 4 hours (Agung et al., 2005). Saputra (2010), to extract fragrant pandan leaf flavor extract for 1, 2, 3 and 4 hours and fragrant pandan leaf extract the best flavor is produced in the extraction of 3 and 4 hours. In this study extraction with n-hexane and ethyl acetate with the time extraction of 1, 2, 3, and 4 hours. Based on these things and in the development of essential oil in Indonesia, in-depth and detailed research on the effect of solvent type and extraction time on yield and composition of compounds in the essential oil of frangipani flowers sandalwood needs to be done.

MATERIALS AND METHODS

Materials

Materials used in this study consisted of raw materials and chemicals. The raw material is fresh from the plant frangipani flowers frangipani sandalwood (*Plumeria alba*) is directly obtained from the area around Denpasar with the criteria that is white with yellow and the inside of the florescence score of 2-3 (little bloom - bloom). Chemicals used include : Technical organic solvent (n-hexane and ethyl acetate), distilled water, anhydrous MgSO4, nitrogen gas is obtained at the Laboratory of Food Analysis Faculty of Agricultural Technology, Udayana University.

Experimental design

This experiment is a two factor factorial experiment, using a randomized block design (RGD). Factor I are the type of solvent (P) consists of two types, namely: P1 = n-hexane, P2 = ethyl acetate. Factor II is composed of four extraction time are : L1 = 1 hour, L2 = 2 hours, L3 = 3 hours, L4 = 4 hours. Thus obtained 2 x 4 = 8 treatment combinations. Each treatment performed 2 times to obtain 16 units of the experiment. Objective data were analyzed varieties and followed by Duncan's test (Gomez and Gomez, 1995), whereas subjective data were analyzed by Friedmen test (Meilgaard et al., 1999). Determination of the best treatments using the test of effectiveness (de Garmo et al., 1984).

Extraction of essential oil of sandalwood frangipani flower

Frangipani flower was sliced of size of approximately 0.1 cm in order to expand the surface at the time of the extracted material. 50 grams of frangipani flowers that have been sliced weighed and then inserted into the Soxhlet extraction tool that pumpkin is filled by solvent n-hexane or ethyl acetate solvent of 250 ml (appropriate treatment). The process of extraction is carried out for 1, 2, 3, and 4 hours (according to treatment) at a temperature corresponding by boiling point solvent (n-hexane 65 ° C, 78 ° C ethyl acetate) to obtain the essential oil mixed with solvents.

Essential oils mixed solvent was added anhydrous $MgSO_4$ (1%) to absorb water in the extract, then filtered using Whatman filter paper No.1. Essential oils that have been filtered inserted into the flask and evaporated at 40 ° C. Then put in a dark glass bottle, the gas flowing nitrogen to remove residual solvent and prevent oxidation. The products were stored in a refrigerator before analysis.

Variables analysis

Essential oil was analyzed its characteristics, namely yield (AOAC., 1990), the profile of essential oil compounds (Wijaya, 1995 is modified), preference of aroma and aroma strength (Meilgaard et al., 1999), determining the best treatment to test the effectiveness (de Garmo et al., 1984). Essential oil of the best treatment identified constituent chemical compounds (Wijaya, 1995 modified).

RESULTS AND DISCUSSION

The yield and characteristics of essential oil of sandalwood frangipani flowers and the amount compounds in essential oils of sandalwood frangipani flowers.are presented in Table 1.

Table 1. The yield and characteristics of essential oil of sandalwood frangipani flowers

Treatments (type of solvent, extraction time)	Yield (%)	Aroma preference	Aroma strength	The amount compounds
n-hexane, 1 h	0.43 f	4.95 a	6.5 a	86
n-hexane, 2 h	0.64 e	4.80 a	6.2 a	103
n-hexane, 3 h	0.67 e	5.05 a	6.4 a	97
n-hexane, 4 h	0.78 d	5.45 a	6.9 a	86
ethyl acetate, 1 h	1.06 c	4.20 a	2.3 b	39
ethyl acetate, 2 h	1.84 b	4.35 a	2.7 b	40
ethyl acetate, 3 h	1.90ab	4.85 a	2.9 b	37
ethyl acetate, 4 h	1.96 a	3.95 a	2.1 b	30

*) The different letters following the average values mean significant differences (P <0.05)

The yield of essential oil of sandalwood frangipani flowers influenced by the type of solvent and extraction time. Polar solvent levels determine the type and number of compounds that can be extracted from the material. The higher level of polar solvent (ethyl acetate) and the longer the extraction, the resulting yield is also higher. This is because the solvent will extract the compounds that have the same or a similar polarity to the polar solvent is used. Table 1 shows that the average yield of essential oil of frangipani flowers between 0.43 to 1.96%. Treatment using the solvent ethyl acetate can produce the essential oil yield of frangipani flowers more than the solvent n-hexane. This suggests that in terms of producing high yield, the use of ethyl acetate solvent is better than the use of n-hexane solvent. Extraction time also affects the yield of essential oil of sandalwood frangipani flowers. The longer the extraction process, the more compounds that are extracted in the material (Vogel, 1978). It can be seen that extraction time 3 and 4 hours produces a higher yield compared to the extraction time 1 and 2 hours.

The essential oil of sandalwood frangipani flowers is extracted with n-hexane solvent indicate the number of compounds more than the solvent extraction of ethyl acetate at the same time. Extraction with both types of solvent extraction of 2-hour long show produced the highest number of compounds. These results are likely after 2 hours of extraction of essential oils to be altered by the process of hydrolysis and oxidation.

The strength aroma of the essential oil of sandalwood frangipani flowers by treatment with n-hexane solvent and extraction time 4 hours had the highest compared with other treatments but not significantly different from essential oil to the treatment of type n-hexane solvent and extraction time of 1, 2, and 3 hours. The essential oil of frangipani flowers are produced by using the solvent n-hexane has a stronger aroma than the solvent ethyl acetate, this is due to a compound that gives the scent of frangipani flowers including most non-polar, so it can be perfectly extracted with n-hexane. This is also supported by the results of profile analysis that shows the number of compounds that appear in n-hexane solvent treatment more than the solvent extraction of ethyl acetate at the same time. The effectiveness test showed that the best treatment is n-hexane and extraction time for 4 hours has the highest value is 0.87.

The results of the analysis by gas chromatography mass spectrometry (GCMS) on a frangipani flower essential oil of sandalwood frangipani flower with n-hexane solvent and extraction time of 4 hours (best treatment) shows the essential oil is composed of 46 kinds compounds (Figure 1). Types of compounds, molecular formula, retention time and relative concentrations of the constituent compounds of sandalwood essential oil of frangipani flowers using n-hexane solvent and extraction time of 4 hours are presented in Table 2.

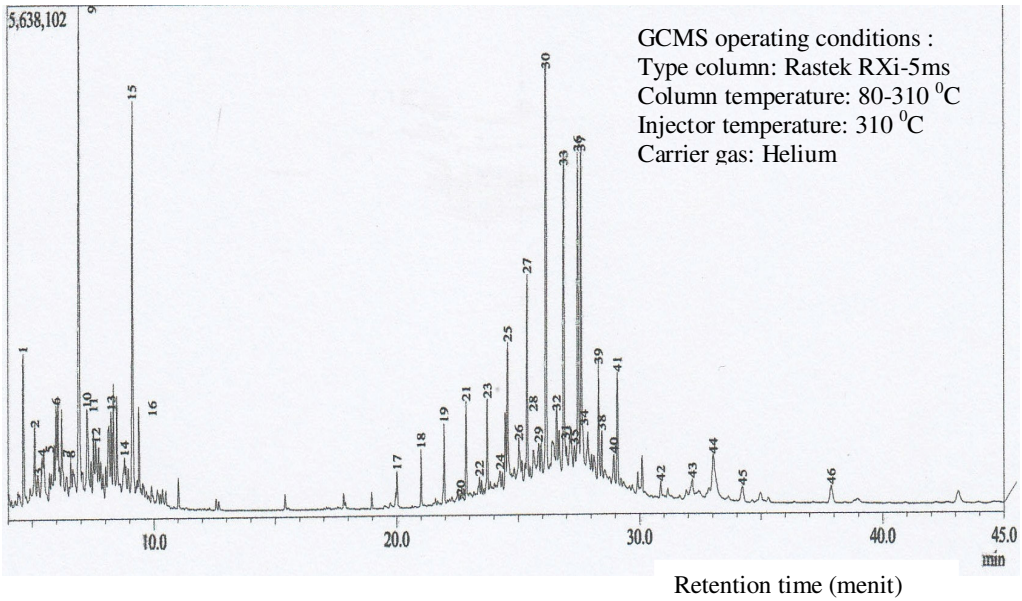

Figure 2. Chromatogram of essential oil of sandalwood frangipani flowers with the solvent n-hexane and and the extraction time of 4 hour.

Table 2. Compounds of sandalwood essential oil of frangipani flowers on the treatment of n-hexane solvent and entraction time 4 hours.

No.	Retention time (menit)	Compound	Relative concentration (%)
1	4.633	decane	2,68
2	5.108	4-methyl-decane	1,34
3	5.233	tridecane	0,51
4	5.408	2- methyl propyl-sikloheksane	2,32
5	5.708	1-butil-2-ethyl-siklopentane	0,69
6	5.983	3,3-dimethyl-oktane	3,59
7	6.417	cyclololoheksaneethanol	0,46
8	6.592	1-methyl-2-penthyl-cyclohexsane	0,65
9	6.933	Undecane	10.35
10	7.258	decahydro-2-methyl-napthalin	2,96
11	7.525	2,3,6,7-tetramethyl-octane	1,07
12	7.625	trans-dehydrocarvon	0,92
13	8.242	4-methyl-1-undecene	3,46
14	8.792	2 hexyl-1-decanol	1,20
15	9.133	dodecane	7,68
16	9.392	2,6,7-trimethyl-decane	1,47
17	20.017	hexadekane	0,77
18	21.008	heptadecane	0,85
19	21.958	oktadecane	1,38
20	22.625	3-metil-dodecane	0,30
21	22.867	heneikosane	1,46
22	23.408	11-butil-l-docosane	0,56
23	23.733	nonadecane	1,52
24	24.25	unidentified	0,76
25	24.567	docosane	4,23
26	25.033	linalool	1,64
27	25.375	pentatriacontane	3,65
28	25.633	tritetracontane	0,67
29	25.842	11-decyl-tetracosane	0,64
30	26.158	nonacosane	8,46
31	26.408	unidentified	0,91
32	26.592	11-desyl-docosane	1,01
33	26.9	heptacosane	6,32
34	27.175	thiogeraniol	1,33
35	27.325	unidentified	0,36
36	27.475	1-eikosanol	6,08
37	27.617	tetratetracontane	5,62
38	27.883	trans-geraniol	0,82
39	28.325	unidentified	1,97
40	28.942	1-docosanol	0,71
41	29.092	unidentified	1,98
42	30.875	unidentified	0,35
43	32.167	oktadesyl acetate	0,59
44	33.029	methyl kommate b	2,21
45	34.225	octahydro-1-2-octadesyl-pentalin	0,70
46	37.892	oktadesyl hexadecanoat	0,82

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ANALYSIS OF ARTIFICIAL SWEETENERS IN MIX ICE DRINK SOLD BY ROADSIDE TRADERS IN SOUTH DENPASAR DISTRICT

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ABSTRACT

This study aimed to obtain artificial sweetener and to determine level of artificial sweeteners in mix ice drinks that sold by roadside traders in District South Denpasar. The method used simple random sampling method, by taking 30% sample of the total population. The results showed that the type of artificial sweetener used cyclamate artificial sweetener. Levels of cyclamate in mix ice drink which meet the standards of using of artificial sweeteners 10mg/kg-400mg/kg, as much as 71.4% and that do not meet the standard 600 mg/kg-800 mg/kg as much as 28,6%.

Keywords: *mix ice drink, roadside traders, Denpasar Selatan, artificial sweetener, cyclamate.*

INTRODUCTION

Since mid-20th century, use of food additive increased along with the increase of production technology of synthetic food additive. The varieties of food additive and their availability will increase the individual consumption (Cahyadi & Wisnu, 2008).

Food additive consist of antioxidant, acidity regulator, artificial sweetener, bleaching, flour, emulsion, seasoning, flavor, hardener, natural and synthetic coloring sequestran. One most used food additive is artificial sweetener, which is cheap and easy to find on market and used widely in commercial food. Artificial sweeteners is sweeter than sucrose, in the range of 30 to 5000 times. Amount of artificial sweeteners added on food product is very little which mean that it is low or non-calorie component. Sucrose had high calorie content. Consumption on high calorie content food and drink unbalancing with other food nutrition will cause metabolism disease (Usmiati & Yuliani, 2004). This condition makes the using of sucrose getting decline.

At the beginning, artificial sweetener was used by diabetic patients. However, nowadays, it is also applied on many varieties of food product. Many artificial sweetener can be used by direct adding to the food or drinks by the customer as substitute of sucrose. Propaganda on artificial sweeteners using generally concerning of healthy issues: body weight regulation, preventing teeth damages, controls blood glucose for diabetic patients. However, artificial sweeteners are synthetic materials and are produced using chemical reaction which products may not be safe for health in case of over-usage (Ambarsari et al, 2009).

Research show that many artificial sweeteners potentially cause tumors and carcinoma. WHO has standard for daily consumption of artificial sweeteners without risk which is called as Acceptable Daily Intake (ADI).

Mix ice is a type of drink that are easily found sold by roadside traders. Some are made with artificial sweetener. The first health symptom after consuming mix ice drink are sore throat. BP POM Indonesia found that many food traders at primary schools in Malang, West Java using artificial sweeteners at unsafe level (Indriasari & Lusiana,2009)

In South Denpasar district, 47 mix ice roadside traders (30.7%) was found, whereas in North Denpasar 35 traders (22.87%), East Denpasar 39 traders (25.4%) and West Denpasar 32 traders (20.9%). This study was conducted in South Denpasar as the biggest percentage of mix-ice road side traders was found in this area .

MATERIALS AND METHOD

The research was conducted in Food Biochemistry Laboratory, Faculty of Agriculture, Udayana University, Bukit Jimbaran Bali. Samples of mix ice drinks were collected in South Denpasar during February-March 2011

Materials and Methods

The materials used in this research were mix ice drinks sold by roadside traders in South Denpasar and chemicals for laboratory analysis.

Equipments used on this research were sample bottles, spectrophotometer, waterbath, 250 ml erlenmeyers, 500 ml flasks, vacum ball, test tubes, 1 ml-10 ml volumetric pipettes, separator flask, measuring cup, analytic balance, litmus paper, funnel, electric stove, beakers.

Sampling Method

The method used for sampling was simple random method. Determining of volume sample by 30 % random (Jarwanto and Subagyo, 1981). Number of population and sample were shown in Table 1.

Table 1. Number of population and samples

District	population	**Number of sample**
South Denpsar	47	14

Laboratory Analysis

Solvent of mix ice drink was been laboratory analysis on type and level of the artificial sweetener (saccharin and cyclamate). Type of artificial sweetener was using Qualitative Test (Munir *et al*. 2000) and level of artificial sweetener using Spectrophotometer (Simatupang 2009).

RESULT AND DISCUSION

Population and Research Area Description

The Research area is South Denpasar District which has the highest number of mix ice Drink roadside traders. The research population is permanent mix ice drink roadside traders or at least more than 3 month in this job.

Sample Description

Mix ice drink samples in this research was directly bought from roadside traders in South Denpasar. 14 samples was taken which is equivalent to 30 % of 47 mix ice drink roadside traders in.

Mix ice drink samples were analyzed qualitative and quantitatively on artificial sweetener content in solution, excluded fruit and other food contents. Volume of solution was 200 ml per sample.

Artificial sweetener qualitative test result (Saccharin and Cyclamate)

Table 2. Saccharin and cyclamate qualitative test result

Sample	**Artificial sweetener**	
	Saccharin	**Cyclamate**
1.	-	√
2.	-	√
3.	-	√
4.	-	√
5.	-	√
6.	-	√
7.	-	√
8.	-	√
9.	-	√
10.	-	√
11.	-	√
12.	-	√
13.	-	√
14.	-	√

Table 2 shown from the 14 samples which is qualitative tested found cyclamate artificial sweetener and not found using saccharin artificial sweetener. Economic factors, sweetness level and easy to find on market is the reasons why roadside trader using cyclamate artificial sweetener as substitute of sucrose . Usually cyclamate artificial sweetener use for diabetic patient as substitute of sucrose. In long term and over dosage usage cause diseases bladder tumor, damage on pulmonary and lymph dysfunction (Yuliarti & Nurheti 2007). Research result on 14 samples not found using saccharin artificial sweetener . This may cause by saccharin has bitter taste effect and hard to find in market..

Cyclamate Quantitative Test Result

Quantitative test was only carried out on cyclamate content. Standard solutions of cyclamate was used. Absorbance of cyclamate solution standard was shown in Table 3. Cyclamate content of samples is shown in Table 4.

Table 3. Absorbance result cyclamate solution standard from many concentration

Concentration **(X)**	Absorbance **(Y)**
0 ppm	0.000
10 ppm	0.003
20 ppm	0.005
40 ppm	0.007
60 ppm	0.010
80 ppm	0.011
100 ppm	0.013

Table 4. Absorbance result cyclamate solution and cyclamate artificial sweetener content

Sample	**Cyclamate Absorbance**	**Cyclamate content (mg/kg) Material**	**cyclamate solution standard**
1	0.005	20	500 mg/kg
2	0.010*	600	
3	0.006**	600	
4	0.005	20	
5	0.012	90	
6	0.005**	400	
7	0.006	30	
8	0.011*	800	
9	0.004*	300	
10	0.004	15	
11	0.005	20	
12	0.012	90	
13	0.006**	600	
14	0.003	10	

remarks: * 10 times dilution
** 20 times dilution

Table 3 shown absorbance of cyclamate solution standard, more higher the cyclamate absorbance impact more higher cyclamate concentration. Table 4 shown from 14 samples of mix ice drinks has range absorbance value 0.003 ppm-0.012 ppm. Cyclamate content from 14 samples of mix ice drinks between 10 mg kg – 800 mg kg, From 14 samples of mix ice drinks found 4 samples has cyclamate content more than 500 mg kg, the rest 10 samples below 500 mg kg. According to BPOM (2004) maximum addition on standard on artificial sweetener 500 mg kg (SNI 01-6993-2004). It mean that from 14 samples found 10

samples (71.4%) appropriate and 4 samples (28.6%) not appropriate to maximum addition on standard on artificial sweetener. Using cyclamate artificial sweetener over the maximum standard due to want to have more higher profit, although (according Yuliarti & Nurheti 2007) in long term and over dosage usage cause diseases bladder tumor, damage on pulmonary and lymph dysfunction.

CONCLUSION

1. Type of the artificial sweetener on Mix ice drink roadside traders in South Denpasar cyclamate.
2. Cyclamate content on Mix ice drink roadside traders in South Denpasar still in range of appropriate from maximum addition on standard on artificial sweetener between 10 mg kg – 800 mg kg. From 14 samples of mix ice drinks found 4 samples has cyclamate content more than 500 mg kg, the rest 10 samples below 500 mg kg. According to BPOM (2004) maximum addition on standard on artificial sweetener 500 mg kg (SNI 01-6993-2004). It mean that from 14 samples found 10 samples (71.4%) appropriate and 4 samples (28.6%) not appropriate to maximum addition on standard on artificial sweetener.

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ENZYMATICALLY LIQUEFACTION OF SWEET POTATO (*IPOMEA BATATAS* L) TO BIOETHANOL PRODUCTION USING *SACCHAROMYCES CEREVISIAE.*

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ABSTRACT

Sweet Potato (*Ipomea batatas* L) were hydrolyzed by enzymatically liquefaction process and fermented by *Saccharomyces cerevisiae* to produce bioethanol. The objective of this research was to determine (1) the optimum conditions of enzymatically liquefaction process, (2) the optimum conditions of fermentation process using *Saccharomyces cerevisiae*. These studies were designed by factorial randomized block design with two factors. The liquefaction process consists of 2 factors, namely: the concentration of α-amylase enzyme and temperature. The concentration of α-amylase enzyme consists of 3 levels, namely 0.8; 1 and 1.2 ml/kg of starch. The temperature of hydrolysis consists of 3 levels, namely: 90°C, 95°C and 100°C. The fermentation process consists of 2 factors, namely the pH of the substrate and temperature. The pH of the substrate consists of 3 levels: 4.5; 5.0 and 5.5, while, the temperature consists of 3 level, namely: 30°C, 35°C, 40°C. The best result on liquefaction process was the enzyme concentration 1.2 ml/kg of starch with a temperature of 100°C. Liquefaction process was produced glucose with a concentration of 57.56 g/L. The best fermentation process carried out at a pH of 4.5 with a temperature of 35 °C and produce bioethanol with a concentration of 5.32 %(v/v) and fermentation efficiency of 70.16 % of theoretical ethanol production.

Keywords : Liquefaction, sweet potato, bioethanol, *Saccharomyces cerevisiae*

INTRODUCTION

An efficient method for conversion of biomass into fuel is by bioethanol production because bioethanol is an economical as well as environmentally friendly fuel. Bioethanol has the advantages of being renewable, cleaner burning and produces no greenhouse gases (Altýntas et al., 2002). Sweet potato is one source of starch which is widely used in industry, one of them was for the production of bioethanol. Bioethanol production from starch raw materials requires hydrolysis process, because yeast are unable to consume raw starch and hence, the starch must first be hydrolysis into simple sugars (Birol *et al*., 1998). Hydrolysis process can be carried out enzymatically or chemically (Taherzadeh and Karimi, 2007). The hydrolysis of starch to products with low molecular weight, catalyzed by α-amylases is one of the most important commercial enzyme processes. Amylase enzyme used to break down the polymer to smaller sugar units, which is eventually converted to the individual basic glucose units (Delphine *et al*., 2000; Baskar *et al*., 2008).

Yeast cells *Saccharomyces cerevisiae* are facultative anaerobes and under anaerobic conditions can ferment glucose to bioethanol. *S. cerevisiae* is ideal for bioethanol production due to several properties including fast growth rates, efficient glucose repression, efficient ethanol production and a tolerance for environmental stresses, such as high ethanol concentration and low oxygen levels.

In this study, the conversion of starchy materials into bioethanol had two steps, enzymatically liquefaction of starch, and fermentation of sugar to etanol. Batch fermentation runs were performed to produce bioethanol using strains of *Saccharomyces cerevisiae.*

MATERIALS AND METHODS

Raw material

Sweet potato was collected from Tabanan. *Saccharomyyces cerevisiae* was collected from microbiology laboratorium Bogor University. Sweet potato was analysed to determine chemical compotition before it is used in liquefaction and fermentation process.

Inoculum culture *S. Cerevisiae*

The strain *S. cerevisiae* were maintained on potato dextrose agar (PDA). The medium composition for cultivation was as follow: yeast extract 5 g/L, malt 5 g/L, glocose 10 g/L and peptone 5 g/L. Cultivation was conducted in 250 ml Erlenmeyer flask for 24 hours at 30 °C and agitation 125 rpm.

Liquefaction

Sweet potato slurry was hydrolyzed enzymatically. The suspension sweet potatoes made with a concentration of 30 % (w/v), pH was adjusted to 6.5 with NaOH. Gelatinization process carried out at a temperature of 90 °C. The enzyme concentration and temperature liquefaction process was conducted in accordance with the treatment for 1 hour.

Fermentation

Batch fermentation process carried out for 4 days in 250 ml erlenmeyer with 200 ml work volume. Substrate was sterilized at 121 ° C for 15 minutes and cooled, then cultured *S. cerevisiae* with a concentration of 10% (v/v) was added aseptically to the substrate. The pH and temperature of the process carried out in accordance with the treatment.

Experimental design

This study was designed by factorial randomized block design with two factors. The liquefaction process consists of 2 factors, namely: the concentration of α-amylase enzyme and temperature. The concentration of α-amylase enzyme consists of 3 levels, namely 0.8; 1 and 1.2 ml/kg of starch. The temperature of hydrolysis consists of 3 levels, namely: 90^{o}C, 95^{o}C and 100^{o}C. In the fermentation process consists of 2 factors, namely the pH of the substrate and temperature. The pH of the substrate consists of 3 level: 4.5; 5.0 and 5.5, while, the temperature consists of 3 level, namely: 30^{o}C, 35^{o}C, 40^{o}C.

Analysis methods

Glucose concentration was assayed by DNS reagent (Miller, 1959). The pH of substrate was measured using pH-meter. Ethanol concentration was analysed by gas chromatography (GC) The ethanol concentration was analised at the end of fermentation process. Fermentation efficiency was measured according to formula: Fermentation efficiency (%) = (ethanol actual/ethanol teoritic) x 100% (Caylak and Fazilet, 1998).

RESULTS AND DISCUSSION

Proximate analysis showed that the contents of sweet potato flour was 67,94 ± 1,07% moisture, 0,91 ± 0,03 % ash, 16,07 ± 1,15 % fat, 0,04 ± 0,03 % protein, 2.69 ± 0.04 % crude fiber and 15,05 ± 2,25% carbohydrate. The moisture content of the substrate has a major impact on how long it can keep in the storage and still remains nutritional.

In liquefaction process, the analysis variance of the concentration of glucose showed that the difference of treatment liquefaction enzyme concentration and temperature significantly affect the concentration of glucose. The highest concentration of glucose that is 57.56 ± 4.81 g/L generated from the process liquefaction using an enzyme concentration 1.2 ml/g with a temperature of 100 °C and this treatment was significantly different from other treatments. The lowest concentration of glucose that is 22.96 ± 11.20 g/L resulted from treatment using enzyme concentrations of 0.8 ml/g with a temperature of 100 °C and this treatment was also significantly different from other treatments. The average value of the glucose concentration in the liquefaction process showed in the Table 1.

Table1. The average value of the glucose concentration in the liquefaction process

Treatment		Temperature (T ^{o}C)		
		T1 (90 ^{o}C)	T2 (95 ^{o}C)	T3 (100 ^{o}C)
Concentration (α-amylase)	C1 (0,8 ml/g)	28,99 ^{ab}	32,64 ^{ab}	22,96 ^{c}
	C2 (1 ml/g)	40,10 ^{b}	26,21 ^{ab}	24,87 ^{ab}
	C3(1,2 ml/g)	29,87 ^{ab}	24,15 ^{ab}	57,56 ^{a}

** Notation with different letters behind the averages indicate a significant difference between treatments ($p<0.05$)

The difference results of the concentration of glucose in liquefaction process caused by specificity of α-amylase in time, temperature, pH, concentration and substrates. The rate of hydrolysis can be controlled

by regulating the enzyme concentration and hydrolysis time, so that the enzymatic reaction can be controlled and can be terminated if the degree of conversion has been reached. Lee *et al*. (1995) studied ethanol production by fermentation using tapioca starch. They reported that liquefaction and saccharification of tapioca starch resulted in a glucose-maltose mixture containing approximately 92 % glucose and 8 % maltose.

In fermentation process, analysis of variance showed that the interaction of temperature and pH in the fermentation process significantly affect for concentration of ethanol ($P<0.01$). The highest ethanol concentration of 5.320% (v/v) produced from the fermentation process using a temperature of 35 ° C with pH 4.5 and this treatment was significantly different from other treatments. The lowest ethanol concentration of 0.030% (v/v) resulted from treatment using a temperature of 45 ° C with a pH of 5.5 and this treatment was also significantly different from other treatments. The average of ethanol concentration (% v/v) in fermentation processes showed in the Table 2.

Table 2. The average of ethanol concentration (% v/v) in fermentation processes

Treatment		Temperature (T °C)		
		T1 (30 °C)	T2 (35 °C)	T3 (40 °C)
pH	P1 (4,5)	4,650[b]	5,320[a]	3,127[d]
	P2 (5,0)	3,887[c]	3,927[c]	2,353[e]
	P3 (5,5)	3,090[d]	3,147[d]	1,163[f]

** notation with different letters behind the averages indicate a significant difference between treatments ($p <0.05$)

Table 3. The fermentation efficiency (% of theoretical ethanol) in the fermentation process

Treatment		Temperature (T °C)		
		T1 (30 °C)	T2 (35 °C)	T3 (40 °C)
pH	P1 (4,5)	66,364[b]	70,159[a]	42,062[d]
	P2 (5,0)	52,437[c]	52,523[c]	30,022[e]
	P3 (5,5)	40,636[d]	40,584[d]	15,087[f]

** Notation with different letters behind the averages indicate a significant difference between treatments ($p <0.05$)

Efficiency of fermentation is the percentage concentration of ethanol produced to ethanol obtained theoretically. Ethanol is obtained from the theoretical stoichiometric ratio of the fermentation process in which one mole of glucose will produce 2 moles of ethanol and 2 moles of CO_2 (Caylak and Fazilet, 1998). Analysis of variance on the efficiency of fermentation showed that the interaction of temperature with a pH significantly affect to the fermentation efficiency. Highest fermentation efficiency of 70.159% of the theoretical ethanol production and resulting from the interaction of temperature of 35 °C with a pH of 4.5. Fermentation efficiency of 70.159% indicate that the glucose is used as the substrate is not fully utilized for the formation of ethanol. The fermentation efficiency (% of theoretical ethanol) in the fermentation process showed in the Table 3.

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OXIDATION OF CASSAVA STARCH WITH H_2O_2 TO IMPROVE *BAKING PROPERTIES*

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ABSTRACT

To support food tenacity, an effort to use local product as import substitution is required. Cassava has the potential to be developed as semi finished product, so it has the potency as substitution of wheat as main material to make loaf and others. One of common way to modify cassava starch is by using chemical modification. This research began with optimization of baking expansion with chemical modification using oxidizer solutions: 0.2; 0.4; 0.6; 0.8; 1.0 until 2.0% H_2O_2 for 30 minutes. The highest expansion of modified starch using oxidation, character comparison between modified starch and unmodified starch was done. The result shows that the best modification for cassava starch by using 0,6 % H_2O_2 was 11,54 ml/g, which was higher than unmodified starch 7,32 ml/g, with analysis results include: amylose content of 31.54 %, pH 2.95 and paste viscosity peak of 684.80 cP. Observation of starch granule structure with Scanning Electron Microscopy indicated reduction of granule size compared with manufactured starch before modification, carbonyl group content of 0.088%, carboxyl group content of 0. 86% and paste clarity of 70.43%.

Keywords: cassava starch, oxidized starch, H_2O_2, baking expansion

INTRODUCTION

To reduce imported products dependency, need to concern the substitution of wheat to alternative carbohydrates sources, in particular cassava starch is considerable. Cassava is abundant food stuff in Indonesia. Cassava starch as main stuff in bakery industry including bread that involve baking process have not been done commonly in Indonesia. It is due to single use of cassava starch is considered not giving perfect expansion as wheat, a substitution to other source of grain instead of wheat is need to be concerned. Cassava has potential to be developed as semi finished product, so it has potency as substitution of wheat as main material to make loaf and others. One of common way to modify cassava starch is by using chemical modification.

This research aimed to identify effect of chemical modification with oxidation by H_2O_2 on cassava starch to improve baking expansion so cassava starch usage as wheat substitute can be increased.

MATERIALS AND METHOD

Materials

Main material used in this research was Gunung Agung manufactured cassava starch, produced by Sungai Budi in Lampung, obtained from its distributor in Yogyakarta. The material was treated as modified starch. Chemical material used was H_2O_2 . Material used to chemical analysis was Pro-Analysis grade.

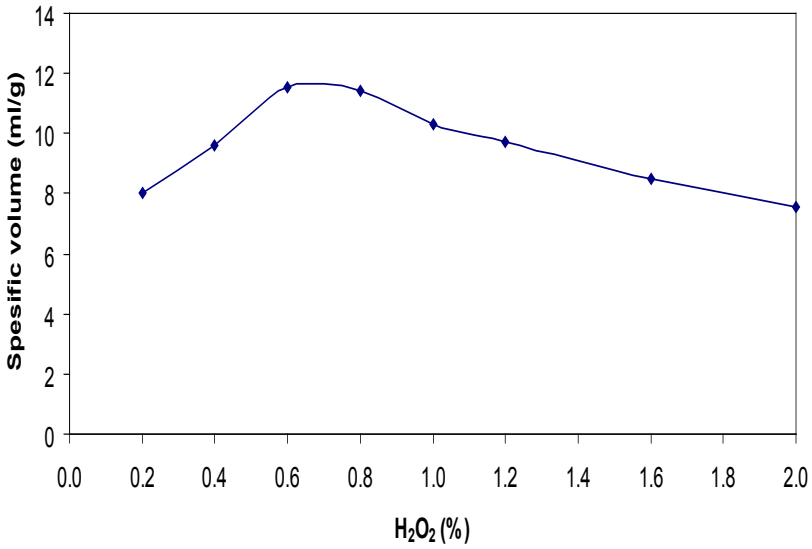
Methods

This research was begun with expansion optimization in baking with oxidation of manufactured starch with various H_2O_2 oxidants in different concentrations. Early, 100 g manufactured starch (dry basis) was suspended into H_2O_2 solution (0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4, 1.6 and 2 %) for 30 minutes and agitated in room temperature. Then, oxidation was stopped by adding sodium metabisulfite for 5 minutes. To eliminate possibility of residue, washing was done three times. Reagent-free manufactured starch was dried in oven at 50^0C, grilled with mortar and sieved with 60-mesh sieve. Oxidized manufactured starch was tested on expansion level in baking process. Based on the highest baking expansion, chemical analysis were done including pH, amylose percentage, paste viscosity, starch granule microscopic, paste clarity and carbonyl and carboxyl group contents, which then was compared with unmodified manufactured starch.

Baking expansion rate measurement is done based on the method used by Demiate *et al*. (2000) with few modifications. Manufactued-starch weighed 10 gram stirred with 30 ml aquadest is processed in the waterbath until the gelatinitation is occurred. Later, the sample is divided in 3 portions to be baked in 200 ^{o}C temperature for 25 minutes. After the baking process, we could measure the baking expansion rate defined in specific volume (ml/g).

RESULTS AND DISCUSSIONS

Baking Expansion Value Measurement



The research indicated that oxidant concentration giving the highest baking expansion level was 0.6% H_2O_2 solution. Increase in expansion rate in baking process occurred along with increase in oxidant solution concentration up to 0.6% that giving baking expansion of 11.54 ml/g higher than unmodified starch (7.32 ml/g). However, the baking expansion tended to decrease when oxidant concentration was increased. Decrease in expansion rate in baking process was expected due to excessive oxidation process in cassava starch.

For starch with modification treatment, the lowest pH was in treatment of 0.6% H_2O_2 oxidation of 2.95. It was proved with baking expansion of starch modified with H_2O_2 was higher than usage of other oxidants. Increase in carboxyl content after oxidative treatment has been discussed by some researchers. Carboxylate content of starch modified can relate directly to molecular fragmentation due to oxidative treatment. So more oxidized starch molecule lead to decrease in pH. Modification starch having highest expansion had higher amylose content than unmodified starch. Starch was composed of mix of two polysaccharides, amylose and amylopectin.

Amylose is polymer having straight structure with α(1,4)-glycosidic bond and forms compact crystalline part, while amylopectin is branched chain polymer with α-(1,4)-glycosidic bond and α-(1,6)-glycosidic bond in its branching site that form amorphous part of the starch so it may be entered by enzyme water and chemical matter. Due to amylopectin structure is amorphous, the site was presumed easier to oxidize by oxidant at C atom no.2 and 3 that form carbonyl and carboxyl groups or depolymerization of 1,4 and 1,6 glycosidic bond.

The Best Modified Starch Characteristic

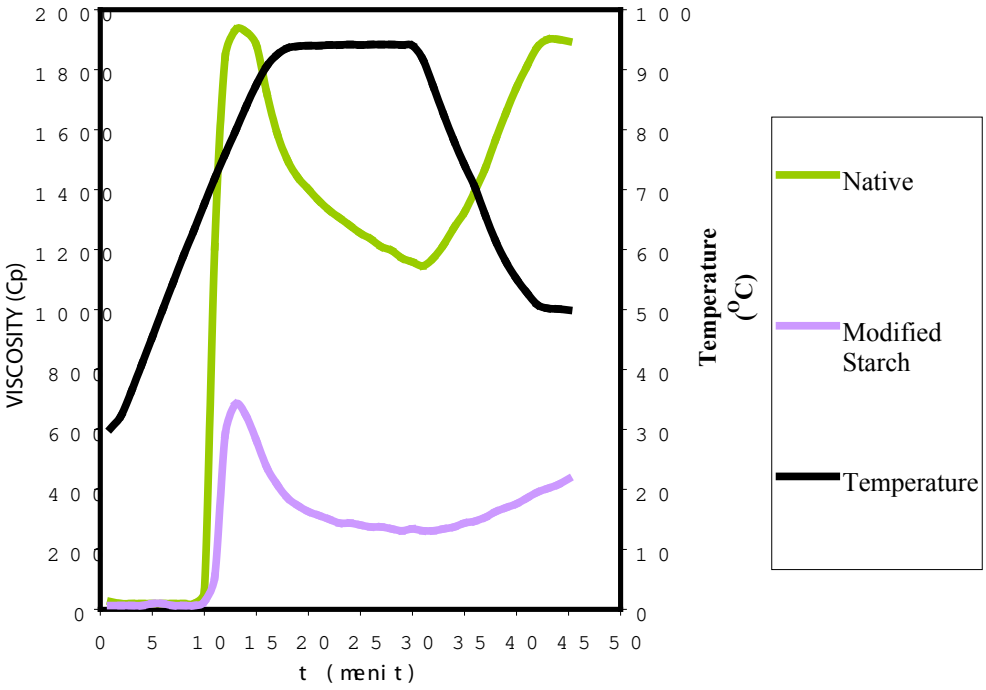
Physical and Chemical Properties Native and Modified Starches

Analysis	Native	Oxidation strach (H_2O_2 0,6 %)
Spesific Volume (ml/g)	7,32	11.54
pH	4,39	2,95
Amilose (%)	25,92	31.54
Carbonyl (%)	nd*	0.088
Carboxyl (%)	0,55	0. 86
paste clarity (%)	60,63	70.43

***nd=** not detected

Increase in clarity of modified starch was expected due to oxidation of OH group resulting carbonyl and carboxyl groups that block hydrogen bond fulfill polymer chain due to anionic carboxyl group that cause electrostatic repulsion between near starch molecules and prevent retrogradation that facilitate increase in transmittance.

The low peak paste viscosity in modified starch indicated that oxidation process due to UV irradiation at starch will be able to depolymerize starch chain fermented in 1,4 and 1,6 glycosidic bond that lead to low paste viscosity that affect baking expansion. The best result of starch modified with 0.6% H_2O_2 oxidation has viscosity of 684.80 cP that was lower than manufactured starch of 1932.80 cP



Viscoamilograms of native and modified starches with H_2O_2 0.6 %

CONCLUSION

Conclusion from this research was that starch modification with oxidation that have greatest expansion rate has characteristic of increasingly little starch granule size due to depolymerization, higher acidity level, lower starch paste viscosity, higher amylose content, higher carbonyl and carboxyl group content, and higher paste clarity than unmodified starch.

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THE EFFECT MALTODEXTRIN CONCENTRATION ON THE CHARACTERISTICS OF GRAPES' SKIN AND SEED MICROCAPSULE (*Vitis vinifera L.*)

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ABSTRACT

The aim of this study is to determine the effect of maltodextrin concentration on the characteristic of grapes' skin and seed microcapsules and to find the right maltodextrin concentration to produce microcapsule with the best characteristic. This research is using Block Randomized Design with maltodextrin concentration of: 3, 5, 7, 9 and 11 % and it repeated three times. The variables observed in this study are: the yield of microcapsule, water content, solubility and the antioxidant activity (DPPH method). The result showed that maltodextrin concentration has significant effect on the yield of microcapsule and on the water content. Concentration of 11% maltodextrin produce the best characteristics of microcapsule withmicrocapsule yield of 3,57%, water content 8,78 %, solubility70,80 %, and antioxidant activity 45,45 %.

Keywords: microcapsule, maltodextrin, antioxidant, grapes' skin and seed.

INTRODUCTION

The antioxidant activity of red wines is associated with the content of polyphenols such as flavonoids, phenolic acids, stilbenes, coummarines, and lignoids (Radovanovic and others 2009). Phenolic compounds in grape seeds and skins include catechins, epicatechins, epicatechin-3-O-gallate, phenolic acids, caffeic acid, quercetin, myricetin, proanthocyanidins, and resveratrol. Resveratrol, quercetin, and rutin are generally found in grape skin extracts, while catechin and epicatechin are found in the seeds. (Brewer, 2011). Grape seed extract has been shown to inhibit both lipid hydroperoxide and propanal formation in an emulsion system (Hu and Skibsted 2002).

Wine industry produced 20-25% waste of grape seeds and skin (Sirait, 2004).Wedananta (2008) reported that grapes' skin from wine waste industry contains 38.50% - 60.20% antioxidant, and the seed contains 69.87% - 87.42% antioxidant. Based on that research the skin and seeds of grape waste from wine industry have a potential as a source of antioxidants. Liquid antioxidant is easy to get damaged by the environment such as from light exposure, temperature, pH and oxidation, so it cannot be stored for a long period (Mardawati, 2008). Microencapsulation is one way to provide protection against antioxidant compounds and to make it becomes more stable. Madene et al. (2006) reported that encapsulation can be used to retain aroma in food products during storage period, protect the flavor from undesirable interactions with food, minimize flavor interactions, protect from light-induced reactions and/or oxidation, increase shelf-life flavors and/or allow controlled release.

Maltodextrin is a good compromise between cost and effectiveness, as it is bland in flavor, has low viscosity at a high solid ratio and is available in different average molecular weights (Apintanapong & Noomhorm, 2003). Madene *et al.* (2006) reported that many factors such as the kind of wall material (Imagi et al., 1992), ratio of the core material to wall material (Minemoto et al., 1999), encapsulation method (Minemoto et al., 1997), and storage conditions (Minemoto et al., 1997; Yoshii et al., 1997) affected the anti-oxidative stability of encapsulated flavor. Maltodextrin concentration of 5% is an appropriate to obtain microcapsules antioxidant of mangosteen extract (Yusrista, 2010).

The first aim of this study is to determine the effect of maltodextrin concentration on the characteristic of grapes' skin and seed grape microcapsule from wine *waste* industry. The second aim is to find out the right maltodextrin concentration to produce the best grape skin and seed microcapsules characteristics.

METHOD

Skin and seed grape extraction: grapes' skins and seeds are purchased from PT. Arpan Bali Utama, which produces 'Hatten Wines'. It then washed, drained and crushed with blender. Take 100 g and extracted

with 700 ml ethanol 80% by maceration for 60 minutes then filtered. The filtrate evaporated with rotary vacuum evaporator at a 55°C for ± 3 hours, flow chart presented in Fig. 1

Encapsulation Process: encapsulan solution is started by diluted process of maltodextrin (3, 5, 7, 9 and 11) g with aquabides until 100 ml, left for 12 hour than added with 10 ml of skin and seed grape extract then homogenized for 5 minutes in amplitude 19. Dried the emulsion with vacuum oven with at temperature 30°C until dry, it is indicated with cracks on the surface and the material is easy to remove from the container.

Experimental Design: Research is using Block Randomized Design with 5 different concentration of maltodextrin (3; 5; 7; 9 and 11) % w/v. The experiment is repeated three times. Variables observed in this research are: yield (AOAC, 1975), water content (Sudarmadji *et al*., 1997), solubility and antioxidant activity with DPPH method (Yun, 2001).

RESULTS AND DISCUSSION

Yield of Microcapsules

The result shows that the concentration of maltodextrin has a significant effect on the yield of microcapsule. The highest yield 3.57% was produced by concentration maltodextrin of 11%. As encapsulant, maltodextrin has a function to protect antioxidants on the grape skin and seed. The increasing of maltodextrin concentrations has affected the viscosity of encapsulation solution and cause the yield of microcapsules. According to Hustiany *in* Setyaningsih (2009), the greater amount of the coating, the greater the yield of the microcapsule product.

Table 1. The average value of yield microcapsules skin and seed grape

Maltodextrin Concentration (%)	Yield of Microcapsules **(%)**
3	1.02e
5	1.74d
7	2.38c
9	2.94b
11	3.57a

Water Content

The result shows that the concentration of maltodextrin has a significant effect on the microcapsules water content. The lowest value, 8.8% was produced by maltodextrin concentration of 11%. The higher concentrations of maltodextrin mean the higher of soluble material and it causes the lowest microcapsules water content.

Tabel 2. The water content of microcapsules of grape skin and seed

Maltodextrin Concentration (%)	Water content (%)
3	9.54 a
5	9.05 b
7	8.84 c
9	8.78 c
11	8.80 c

Solubility

The result shows that maltodextrin concentrations have no significant effect on the microcapsules solubility. The solubility of maltodextrin is determined by a Dextrose Equivalent (DE). The concentration of maltodextrin has no effect on the solubility of microcapsules. Maltodextrin DE value will affect

the hygroscopicity, plasticity, sweetness, solubility, and osmolality (Lynn A. Kuntz, 1997 *in* Chafid & Kusumawardani, 2010).

Tabel 3. The solubility of microcapsules of grape skin and seed

Maltodekstrin Concentration (%)	Solubility (%)
3	65.04 a
5	66.22 a
7	67.18 a
9	68.91 a
11	70.80 a

Antioxidant Activity

The result shows that the concentrations of maltodextrin have no significant effect on the microcapsules antioxidant activity. The properties and viscosity of encapsulan has effect on the retention of microcapsules. The high viscosity tends to limit the occurrence of the thermal convection in the capsule. The high viscosity limits the heat convection in the capsule. Hustiany (2006), Soottitantawat (2004) and Bhandari *et al*. (1992) in Setyaningsih 2009) reported that the increased in the encapsulan concentration will lead to increased retention of coated materials. The high concentrations of encapsulan will protect the antioxidants from drying damage. The result shows that the low concentration of maltodextrin is unable to protect the antioxidants, so even though the concentrations are high but it has no significant effect on the microcapsules antioxidant activity.

Table 4. The antioxidant activity of microcapsules of grape skin and seed

Maltodextrin concentration (%)	Mean DPPH (%)
3	41.63a
5	41.40a
7	44.93a
9	46.01a
11	45.45a

CONCLUSION

1. maltodextrin concentrations have a significant effect on the yield and water content of the grapes' seed and skin microcapsules
2. Concentration 11% of maltodextrin produce the best characteristics of microcapsule with yield 3.57%, water content 8.78%, solubility 70.80%, and antioxidant activity 45.45%.

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THE ACTIVITY OF *ASPERGILLUS PARASITICUS* AND THE POTENT TO PRODUCE AFLATOXIN B1 ON WHEAT FLOUR DURING STORAGE

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ABSTRACT

Strains of *A. parasiticus* can produce aflatoxins B1, B2, G1, G2, and M1, contaminating a number of agricultural products such as peanuts, corn, cereal grains and the products. Aflatoxin contamination occurs by colonization of the fungus on susceptible crops, or may arise during harvesting, drying, storage, or processing.

The research was aimed to know the activity of *A. parasiticus* in wheat flours and their ability to produce aflatoxin B1 during storage. *A. parasiticus* was inoculated on wheat flour and growing on the period of storage 0,5,10 and 15 days.

Result of research showed that the activity of *Aspergillus parasiticus* were susceptible to grow on wheat flour. The population *A. parasiticus* in wheat flour during storage 0,5,10 and 15 days were 9,8 x 10^5 cfu/g, 2,3 x 10^8 2,6 x 10^8 cfu/g., 3,7 x 10^8 cfu/g and 3,68 x 10^8 cfu/g respectively.

A. parasiticus is a potent to produce aflatoxin B1 in wheat. The production of aflatoxin B1 in wheat flour during storage 5, 10, 15 days are 78,8, 76,0, 77,2 ppb and none aflatoxin B1 with incubation 0 days..

Keywords: *Aspergillus parasiticus*, growth., cereals flour and Aflatoxin B1

INTRODUCTION

Flour is the solid particles in the form of fine grains or very subtle depending on usage. Usually used for research purposes, domestic, and industrial raw materials. Flour is produced from grains and cereals are common in the market. Besides flour is the main raw material in processed food products such as baby food, pastries, noodles, vermicelli and others.

Wheat flour is the main ingredient in the manufacture of bread produced from wheat grain processing. Wheat grass is included in the group of plants with good quality wheat flour has a dry nature, does not clot when pressed, white, free from particles of skin, no foreign smelling like rotten, containing mouse droppings, contamination of foreign materials and contaminated with fungus / mold (Bankole. and Mabekoje, 2004).

Aspergillus parasiticus is a pathogenic fungus that is often found as a contaminant on the type of nuts and cereals including processed products. A. Contamination parasiticus can occur since these commodities are in the field, during harvesting and after harvesting, especially in tropical regions (Lopez-Garcia *et al*., 2001). *A. parasiticus* is the producer of aflatoxin such as aflatoxin B1, B2, G1 and G2, while *A. favus* as a producer of aflatoxin B1 and B2 (Sinha, 1993). Among the four types of aflatoxin is aflatoxin B1 have the toxic effects of the most high. Aflatoxins are carcinogenic, mutagenic and hepatotoxic (Saad, 2001; Herman and Walker, 2001),. So that to the attention of the World Health Organization (WHO) and categorized as group 1 carcinogens cause. However aflatoxins can also be immunosuppressive (Boutrif, 1997).

Although lethal cases are rare, acute diseases caused by mycotoxins, particularly aflatoxins (aflatoksikosis), have been reported in some parts of the world. Aflatoxin contamination often occurs in countries of tropical and humid climates such as Africa, Asia and South America. Aflatoxin contamination cases known to occur in the early 1960s with the deaths of more than 100,000 young turkeys chickens at the farm the UK since imports of feed used brazil nuts contain high levels of aflatoxin. These events are known as "Turkey X disease". In 1967 three people died from eating Taiwanese rice contaminated with aflatoxin, and in 1974 more than 100 Indians died from consuming corn contaminated with aflatoxin.

Mold contamination in the flour material escaped the attention of consumers because white flour is almost like the color of mold mycelium growing on wheat, but the contamination of mycotoxins (aflatoxin B1) becomes very serious and dangerous. Based on this then do research on the activity of *A. parasiticus* on various types of flour and its potential in producing aflatoxin B1.

MATERIALS AND METHODS

PLACE AND TIME RESEARCH

The research was conducted at the Laboratory of Microbiology, Laboratory of Food Analysis Faculty of Agricultural Technology, Udayana University, Laboratory of Microbiology and Center for Veterinary Bali.

Materials and Equipment

Powdery material that is used in this study were wheat flour,. Materials used for the analysis: Potato Dextrosa Agar (PDA), Chloramphenicol, distilled water, Aquabidest, Aquatridest, Methanol, 0.8% Nacl, Alcohol

The tools used in this study as follows: petri dish, tube, micro pipette 100-1000 mL, fast Ridasscreen testkit aflatoxins, ELISA reader (Biored).

Research Methods

The study used descriptive methods to display data in the form of images, tables and photographs. Materials tested as a growth medium *A. parasiticus*, wheat flour and with an storage period of 0, 5, 10 and, 15 days. Works carried out include: preparation of spore suspension, treatment of samples, determination of population *A. parasiticus*, and the determination of the concentration of aflatoxin B1

Spore Suspension

A. parasiticus rejuvenated pure cultures on Petri dishes containing PDA medium and storage for 3-5 days at room temperature. *A. parasiticus* that grow subsequently taken one oze and put into test tubes already containing PDA medium agar. Subsequently incubated for 5 days at room temperature. The parent culture *A. parasiticus* are then taken one oze to put in 10 ml of sterile water. A. total of 1 ml of each of these further diluted into sterile water for dilution series was made up to 10-4 dilution. From these dilution series, prepared for the 10-4 dilution was inoculated into the sample to be tested each of 5 ml

SampleTreatment

Each sample of 50 g flour put in a Petri dish and sterilized in an autoclave. Furthermore, the sample was cooled and inoculated in 5 ml x 104 CFU / ml *A. parasiticus* into a petri dish that already contains the sample. Each sample was then incubated in an incubator with a temperature of 30 ° C for 0, 5, 10, and 15 days. Observations made on population of the colony A. parasiticus and aflatoxin concentration formed after 0, 5, 10, and 15-day incubation period.

Population Determination of *Aspergillus parasiticus*

Each sample 10 g was weighed and wrapped in gauze, then successively dipped into 70% alcohol and sterile water for 1-3 minutes. The sample was added 90 ml of sterile water, then do filtering and the filtrate stored in 250 ml glassware. The filtrate is then diluted from 10-1 to 10-5 as much as 0.1 ml of suspension from each dilution were grown in Petri dishes containing the media who have PDAs. These cultures were incubated for 5-7 days at 30 ° C in the incubator. Observations made on population *A. parasiticus* is to calculate each of the colonies that grow

Determination of Concentration of Aflatoxin B1

Aflatoxin B1 testing used an ELISA method (enzyme linked Immunosorbant Assay) (Ridascreen Fast aflatoxin B1 Test Kit).

Material on the Petri dish that has been stirred taken 10 g of each was added methanol: water (70:30), homogenized with blended for 3 minutes. Then filtered with filter paper wahattman No. 2. Accommodated as much as 1 ml of the filtrate with microtube and and added 1 ml of sterile water.

Aflatoxin standard solution of each (0, 0.1; 0.2, 0.5; 1.0 and 2.0) and pipetted samples of 50 ul, is

inserted into the respective wells. Wells which already contains the standard and each sample was added to each well konjugate enzymes as much as 50 ul and mixed well and incubated for 30 minutes at room temperature and dark room. Fluid in the next microwells thrown out by dumping on clean tissue paper and washed with sterile water. This work was repeated 4 times.

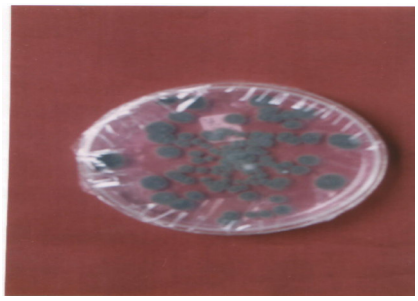
A substrate containing 3.3 '5, 5'-tetramethylbenzidine 0.4 g / l in the organic base and substrate B containing 0.02% H2O2 in a citric acid buffer. Substrates A and B mixed 30 minutes earlier. Then added as many as 100 ul into the wells is mixed well and incubated for 5 minutes at room temperature and dark room.

Reaction stopper solution containing 0.01 M phosphate acid is then added as many as 100 ul into each of the wells. The next activity absorbance readings on microwells by ELISA reader at a wavelength of 450 nm.

RESULTS AND DISCUSSION

Characteristics of Aspergillus parasiticus

Microscopically according to King et al., (1979) mold *A. parasiticus* can be viewed on the color of colonies grown on media Dextrosa Potato Agar (PDA). Colony color *A. parasiticus* is a blackish green as can be seen in figure 1 and figure 2 shows A. parasiticus has visikel a rounded shape (globular) with a set of sterigmata with spores rough surface (serrated) with a large spore size



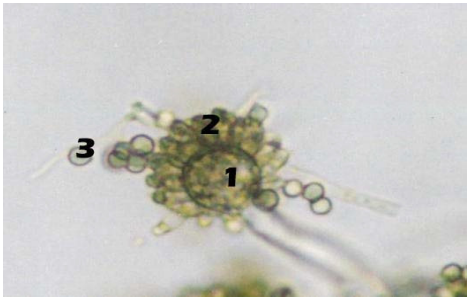


Figure 1. Colonies of *A. parasiticus* Figure 2. Fruit body A. parasiticus on PDA medium 5 days after inoculation (1. Viskel 2. Sterigmata 3. Spore) 400x

Aspergillus parasiticus population

The results showed *Aspergillus parasiticus* populations carried varies in mediums wheat flour during the storage period of 0, 5, 10 and, 15 days at an incubation temperature of 30 ° C. In Table 1 shows the variation of population *A. parasiticus* grown on different medium during the storage period.

Table 1. Populasi *Aspergillus parasiticus (* cfu/g)

storage (days)	Wheat flour				
	I	II	III	Total	Everage
0	11,0 x 10^5	8,5 x 10^5	9,0 x 10^5	28,5 x 10^5	9,5 x 10^5
5	1,8 x 10^8	2,5 x 10^8	2,6 x 10^8	6,9 x 10^8	2,3 x 10^8
10	2,2 x 10^8	2,9 x 10^8	3,0 x 10^8	8,1 x 10^8	2,7 x 10^8
15	3,5 x 10^8	3,9 x 10^8	3,7 x 10^8	11,1 x 10^8	3,7 x 10^8

Aspergillus parasiticus population in Wheat Flour

Population of *A.parasiticus* in wheat flour during the storage period of 0, 5, 10 and 15 days increased (Fig. 5). At 0-day storage period the growth of *A. parasiticus* 9.8 x 105 cfu / g. The incubation period of up to 5 days is known that the population of A. parasiticus by 2.3 x 108 and the population in the 10-day incubation period reached 2.6 x 108 cfu / g. At the time of incubation reached 15 days, the population of *A. parasiticus* to 3.7 x 108 cfu / g as can be seen in Table 1.

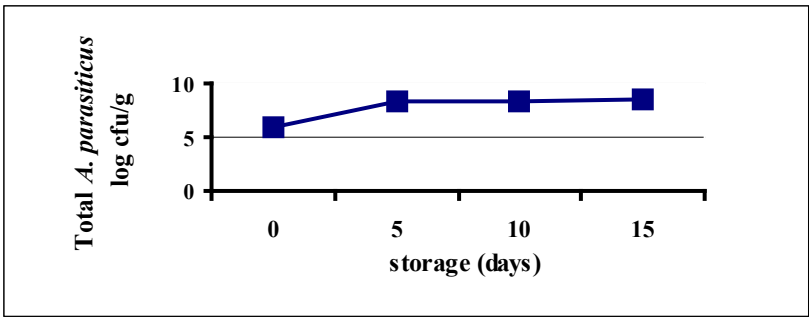


Figure 5.Populasi *A. parasiticus* On Wheat Flour

The existence of population variation on the wheat flour is influenced by the availability of nutrients during the incubation period. The content of nutrients in the flour will support the growth of *A. parasiticus*. (Abbas, 1994).

Nutrition are the most dominant in the flour are carbohydrates, but it is also protein and fat. Carbohydrates contained in the medium to grow wheat flour by 78.03 g/100 g of material, protein 8.3 g/100 g of fat and 0.86 g/100 g of material.

Fairly complete nutrition such as carbohydrates, proteins, fats, water and some minerals like zinc, iron and magnesium is needed for the growth of molds (Winarno, 1987). Medium growing wheat flour contains one of which is an iron mineral that most of the two other growing medium that is equal to 7.32 mg / 100g ingredients.

Production of Aflatoxin B1

The results showed that the growth medium contained different concentrations of aflatoxin B1 variations produced during the incubation period of 0, 5, 10 and, 15 days. The concentration of aflatoxin B1 in each type of flour as a growing medium ranged from a maximum of 78.80 ppb in the wheat flour and a low of 32.40 ppb pada rice flour. The concentration of aflatoxin B1pada each type of flour are in Table 2.

Table 2. Aflatoxin B_1 Wheat Flour (ppb).

Storage (days)	Aflatoxin (ppb)				
	I	II	III	Jumlah	Rataan
0	0	0	0		
5	77,70	79,30	79,40	236,40	78,80
10	75,50	76,20	76,30	228,00	76,00
15	77,70	76,90	77,00	23160	77,20

Production of Aflatoxin B1 in Wheat Flour

Production of aflatoxin B1 wheat flour during the incubation period 0, 5, 10 and 15 days are very varied. During incubation 0 days in the wheat flour is not yet produced aflatoxin B1. At the time of incubation up to 5 days aflatoxin B1 production of the wheat flour of 78.80 ppb and in the storage period of up to 10 days decreased the production of aflatoxin B1 of about 2.8% to 76.00 ppb.

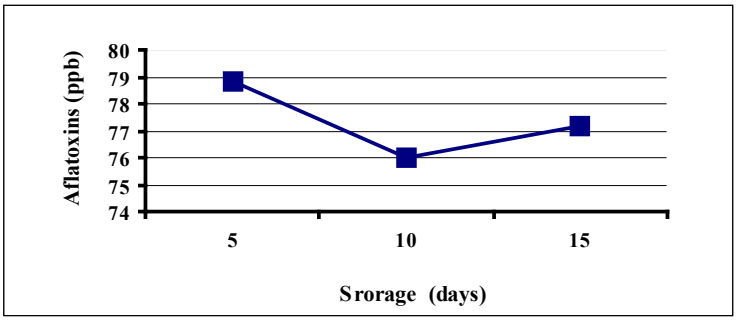


Figure 8. Concentration Afatoksin B1 on Wheat Flour

Aflatoxin B1 production started to increase again to reach the 15-day incubation period that is equal to 77.20 ppb (Table 2). In Figure 8 can be seen the production of aflatoxin B1 in wheat flour during the storage.

Production of aflatoxin on the wheat flour are highly variable it is determined by several factors, among others, fungi genetic potential, environmental conditions (substrate, humidity, temperature, pH) and duration of contact between the fungi with the substrate (Donatus, 1990). The results Duniaji (2002) states that the moisture content of materials that do not consistently show variable effect on aflatoxin production. This is also evidenced in the flour where the water content of the material is 8%, but reached 77.20 ppb aflatoxin production during the incubation period of 15 days in which the relatively low water content materials contain high levels of aflatoxin.

Production of aflatoxin in foods other than due to the low water content and high temperature environments can also be caused by lack of nutrients and the bio competitive of various microbes that grow and grow on food (Duniaji, 2002).

This is consistent with research Heathcote and Hibbert (1978) who found that the presence of carbon compounds in foods and mineral components such as molybdenum, zinc and iron can affect the production of aflatoxins. Bio competition microbes that grow on food also affects the stress level of *A. parasiticus* as aflatoxin producers.

CONCLUSION AND SUGGESTIONS

Aspergillus parasiticus growth in the wheat flour is highest 3.68 x 108 cfu / g during 15 days incubation. Production of aflatoxin B1 in the highest wheat flour is 78.80 ppb during the 5 days of storage.

From the results of this study can be recommended as follows supervise the content of aflatoxin on food in the market, especially the various types of flour as the main raw material on processed foods need to be done.

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EFFECT OF EARLY TREATMENT ON STORAGE LIFE OF ROSE CUT FLOWER (*Rosa damascena Mill*).

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ABSTRACT

The main objective of this research was to evaluate the effect of early treatment in maintaining the storage life of rose cut flower (*Rosa damascena* Mill). Early treatment was pre-cooling for 3, 4 and 5 hours and the use of citrate acid solution concentration of 0, 200, 400 and 600 ppm. This research applied with randomized block design with two factors. The first factor was duration of pre-cooling which were 3, 4 and 5 hours with the temperature used 0 ± 3 ˚ C. The second factor was the concentration of citrate acid solution with a concentration of 0, 200, 400 and 600 ppm. Each treatment combination was made into two groups. Therefore there were 24 experimental units. The result showed that the pre-cooling treatment at a temperature of 0 ± 3 º C for 4 hours and soaked in concentration of citrate acid solution of 400 ppm can maintain the life storage of rose cut flower for 5 days.

Keywords: pre-cooling, citrate acid, storage life, rose cut flower

INTRODUCTION

Indonesia as a country with a tropical climate has the potential to develop the cultivation of horticulture, such as fruits, vegetables and cut flowers (Anon., 1999). Some countries even rely on foreign exchange earnings from the sale or export of cut flowers. Horticultural crops incorporated into food crops, so that now there are food crops and horticulture. To plant flowers that are typical of a commodity, needs special attention because it requires the ability to cultivate based on specific skills in terms of mastery of the art technology and trading it.

Rose (*Rosa damascena* Mill) is one commodity that popular ornamental plants and has long been cultivated and grown in Indonesia because it has high economic value (Anon, 2002). Roses are also useful as food and beverages, pharmaceuticals, fragrances (Rukmana, 1995). Rose cutting flower as horticultural commodities are easily damaged so easily degraded flowers. To keep the cut flower fresh and beautiful, attempt is made to extend the preservation of fresh flowers or other plant parts that have been cut (Murtiningsih and Satuhu, 2005).

Preservation efforts can be done to extend the fresh cut flowers by cooling and the use of soaking solution during storage. Cooling is done by using temperatures near freezing temperatures (at a temperature of 0 ± 3 º C), the lower the temperature used, the slower the chemical reactions of the enzyme activity due to the growth of microorganisms (Wijandi, 1981).

The treatment of cooling does not becomes a major factor in cut flowers. The cut flower quickly changes color and can not maintain the freshness due to the heat carried from the field on plants at harvest and this causes the respiration process continues so that the flowers quickly wither (Murtiningsih and Satuhu, 2005). The pre-cooling with a solution of citrate acid concentration of 400 ppm can extend the storage life of cut flowers, pleasant evening for 5-8 days (Suciati, 2002).

Based on the above information, research was conducted to try the precooling of cut flowers and roses with a time of immersion in a solution of citrate acid with different concentrations to store long known rose cutting flower. The purpose of this study was to determine the effect of early treatment in maintaining the storage life of rose cut flowers (*Rosa damascena* Mill.). Early treatment includes the pre-cooling for 3, 4 and 5 hours, and the use of citrate acid solution concentration of 0, 200, 400 and 600 ppm. The benefits of this research are expected to provide scientific information for the community, about the conduct of the pre-cooling time and the concentration of citrate acid solution as a marinade in maintaining and extending the storage life of rose cut flowers (*Rosa damascena* Mill.)

MATERIALS AND METHOD

Materials and EquipmentS

Materials used in this study ARE the rose with the criteria of fresh, strong red petals and strong stems, which are picked directly from the flower planter on Jl. Bedugul Botanical Garden Flora, aquades, ice and citrate acid.

Equipment used in this study is a bucket, knife (stainless steel), flower scissors (stainless steel), jars, measuring cups 1000 ml (Pyrex), thermometers, scales, plastic boxes and styrofoam box.

Research Design

Experimental design used in this study was randomized block design with two factors. The first factor was the length of the pre-cooling which consists of three levels of treatment:
P1 = pre-cooling duration (ice) for 3 hours
P2 = pre-cooling duration (ice) for 4 hours
P3 = pre-cooling duration (ice) for 5 hours

The second factor was the concentration of citrate acid solution, which consists of four levels of treatment :
A0 = concentration citrate acid solution of 0 ppm (aquades)
A1 = concentration citrate acid solution of 200 ppm
A2 = concentration citrate acid solution of 400 ppm
A3 = concentration citrate acid solution of 600 ppm

Each treatment combination was made into two groups so obtained 24 experimental units. The data obtained and analyzed by analysis of variance and if there are real effects, the analysis followed by Duncan test (Steel and Torrie, 1995).

RESEARCH IMPLEMENTATION

Sortation

Sortation was done to earn interest with a clean and homogeneous state of florescence 50% of the number of flowers already in full bloom and throwing a third of the leaves at the base of the old diseases and pests. At the time of taking flowers, flower stalks cut to uniform length of 50 cm stalk then cut sloping base of the flower stalk with a uniform slope of 30 ˚.

Precooling

Roses were cooled to a temperature of 0 ± 3 ˚ C with a cooling duration treatment was 3, 4, and 5 hours. Cooling rate prior to the display of the transport process by using a plastic box that is inserted into a Styrofoam box filled with ice.

Transportation to the Place Display

This process is done after pre-cooling and cultivated with the proper delivery to reach the destination in a fresh condition and undamaged.

Soaking in Solution Citrate Acid

Media immersion is a solution of citrate acid with a concentration of 0 ppm (aquades), 200 ppm, 400 ppm and 600 ppm. Cut rose flowers were dipped in a solution of citrate acid. Immersion performed on the base of the flower stalk with the same volume of immersion for each 1000 ml container with a long handle which is submerged approximately 10 cm for each treatment. The number of flower stalks soaked in each container was up to four rods. Observations were made every day. Flowchart of research can be seen in Figure 1.

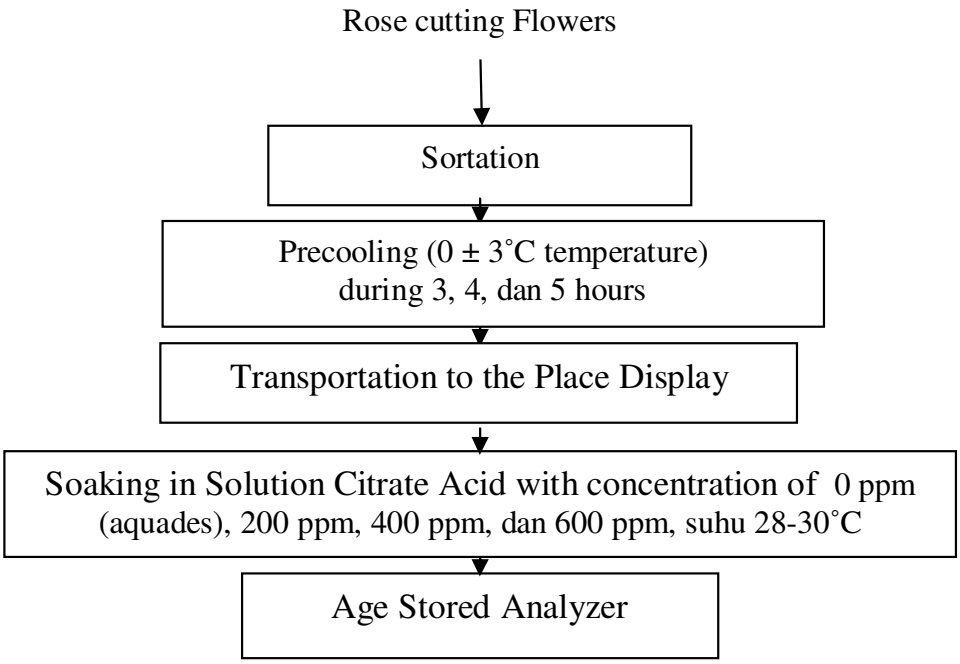

Figure 1. Flow Chart of Implementation Research

Broken flowers criteria: (Pandji *et al*., 1998) :
1) The color of the petals turned into dark
2) Petal fall
3) Stalk slimy
4) The shaft lined
5) Petals wilt

Calculation:
Storage life = broken flower day is calculated starting from day 0 to minus one day broken flowers.

RESULTS AND DISCUSSION

Storage Life

Based on the results obtained, the pre-cooling treatment and soaking in a solution of citrate acid had significant effect ($P < 0.01$), while the interaction of the two treatments was significant at $P < 0.05$. Treatment of pre-cooling and soaking in a solution of citrate acid produce flower with storage life that varies with the range of 4 to 8.5 days. The average value of the storage life of rose cut flowers roses that were cooled and soaked in a solution of citrate acid can be seen in Table 1.

Table 1 also shows that the average age stored of the roses with the pre-cooling of 0 ± 3 º C for 4 hours and immersion in a solution of citrate acid concentration of 400 ppm has the longest age stored. This is because citrate acid with a concentration of 400 ppm can optimally absorb water and is able to inhibit the growth of slime-causing microbes on the surface of the flower stalk, so that water uptake by the flower stalk is not disrupted (Halevy and Mayak, 1979).

Treatment of immersion in concentration of citrate acid solution of less than 400 ppm will form a higher layer of mucus on the flower stalks that can cause blockage in the stems, so the absorption is inhibited. As a result, the cut flower has short storage life. Inhibition of absorption of the solution will produce a low florescence and flower become quickly wither due to lack of water. Soaking in a solution of citrate acid concentration of more than 400 ppm can cause the solution is too acidic and lead to plasmolisis which ultimately resulted in delayed absorption poses (Halevy and Mayak, 1979).

Table 1. The average value of the age stored of rose cutting flowers with cooling treatment and soaking in concentration of citrate acid solution

Treatment		Age Stored Average (days)
Cooling	Citrate Acid (ppm)	
Precooling 0 ± 3 ° C During 3 hours	P_1 A_0 (0)	3,0 a
	P_1 A_1 (200)	3,0 a
	P_1 A_2 (400)	4,0 a
	P_1 A_3 (600)	4,0 a
Precooling 0 ± 3 ° C During 4 hours	P_2 A_0 (0)	3,0 a
	P_2 A_1 (200)	3,0 a
	P_2 A_2 (400)	5,0 a
	P_2 A_3 (600)	4,0 a
Precooling 0 ± 3 ° C During 5 hours	P_3 A_0 (0)	3,0 a
	P_3 A_1 (200)	3,0 a
	P_3 A_2 (400)	5,0 a
	P_3 A_3 (600)	4,0 a

Description: The average value followed by the same letter showed no significant effect (P> 0.05).

CONCLUSIONS

Pre-cooling followed with soaking in concentration of citrate acid solution affected the storage life of rose cut flowers. The best pre-cooling treatment at a temperature of 0 ± 3 ° C for 4 hours and soaking in concentration of citrate acid solution of 400 ppm. This treatment can extend the storage life of cut flowers roses for 5 days. Further research on other types of immersion solution, or a combination of temperature and different times needs to be done.

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THE EFFECTS OF PACKAGING MATERIALS ON SENSORY CHARACTERISTICS AND NUTRITION FACT OF INSTANT *LEDOK* DURING STORAGE

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ABSTRACT

Ledok is a traditional food from Nusa Penida, Bali, in form of non-rice porridge. The main ingredients of *ledok* were corn and cassava, and other ingredients were peanut, red bean and spinach. Instant *ledok* is product developed from traditional *ledok*. The objective of this research was to investigate the effects of packaging materials on the sensory characteristics and nutrition fact of instant *ledok* during storage. Experiments was conducted by completely randomized design, with three kind of packaging materials as a treatment and replicated three times. The kind of packaging materials such as: (1) polyprophylene, (2) polyethylene and (3) aluminium foil. Instant *ledok* were stored eight weeks in room temperature. The results of the study showed that after eight weeks storage, polyprophylene, polyethylene and aluminium foil have a similar effect on the sensory characteristics, but aluminium foil better than polyprophylene and polyethylene in protect the stability of nutrient content of instant *ledok*.

Keywords: Instant *ledok*, polyprophylene, polyethylene and aluminium foil

INTRODUCTION

Ledok is one of traditional food from Nusa Penida, Klungkung regency which uses corn and cassava as main ingredient without using rice. Others ingredient such as long bean, read bean and *kemangi* are used as additional ingredient. Instant *ledok* is product developed from *ledok* traditional. Study to improve the nutritive value instant *ledok* added with white tuna (tenggiri) has reported by Sugitha, *et al*. (2007) and added with seaweed and frigate mackerel has reported by Suter, *et al*. (2009).

Packaging is intended to preserve food against spoilage and contamination and extend its shelf life (Vaclavik, 1998 and Morris, *et al*., 2004,). Three kinds of packaging materials include polyprophylene (PP), polyethylene (PE) and aluminium foil (AF) common used in food (Syarief, *et al*., 1989), but if its used to packed instant *ledok*, the effect of its packaging materials to instant *ledok* during storage did not known yet. The aim of this study to find out the effects of packaging materials on the sensory characteristics and nutrition fact of instant *ledok* during storage.

MATERIALS AND METHOD

Materials

The *ledok* materials consisted of white corn, yellow cassava, red bean, peanuts, spinach, *kemangi*, *salam* leaf, onion, chilli, salt, and lime. The materials for analysis consisted of H_2SO_4, NaOH, tablet Kjeldhal, boric acid, HCL, alcohol, petroleum benzene, methyl red, and methyl blue, PP, PE and AF.

Design Experiment and Formulation

Experiments was conducted by completely randomized design, with three kind of packaging materials as a treatment and replicated three times. The kind of packaging materials such us: PP, PE and AF. Instant *ledok* were storage eight weeks in room temperature

The formulation of raw materials was white corn (110 g), cassava (55 g), red bean (55 g), peanuts (55 g), spinach (9,0 g), *kemangi* (3,0 g), *salam* leaf (5,0 g), lengkuas (5,0 g), water (4 x total weight of raw materials), salt (1 g), and spices (15 g)

Experimental Steps

a. Preparation and Storage of instant *ledok*

The preparation of instant *ledok* included: 1) yellow cassava was firstly pealed, then washed and chopped into size of 0,5 x 0,5 x 0.2 cm. Then the chopped cassava was steamed at 100 ^oC for 35 minutes and finally after cooling the steamed cassava was dried at 70^oC in oven dryer; 2) Peanuts and red beans

was boiled at 100°C, and then dried at 70°C in oven dryer. The ratio of peanuts or red bean and water used in boiling was 1:3; 3) White corn was boiled at 100°C, for 37 minutes and then dried at 70°C in oven dryer. The ratio of white corn and water used in boiling was 1:3; 4) Spinach, *kemangi* and *salam* leaf blanched at 85°C for 5 minutes, and then continued by drying at 70°C in oven dryer until dried; 5) Lengkuas was minced and then dried in oven drier at 70°C until dried. Preparation for spices involved the activities as follows: firstly the raw materials of spices such as onion, red chili, salt, and lime were weighed as formulation, and then blended using mixer. The blended spices were dried into the oven drier at 70°C until dried. Finnaly, all of materials weight as formulation and mix together to produce instant *ledok*. Instan ledok (50 g) package by AF, PP and PE , storage in room temperature during eight weeks. Instant *ledok* (50 g) were filled by the boiling water (200 ml) and stirred continuously until ready to serve and then analysis.

b. Analysis

The analysis performed into the instant *ledok* included: 1) Sensory analysis (color, aroma, taste, texture, and overall preference) using Hedonik Test (Larmond, 1977) and 2) Analysis of nutrition fact (carbohydrate, protein, fat, ash, and water content) using the *Proxymate analysis* methods (Apriyantono, *et al.*, 1989).

RESULTS AND DISCUSSION

1. Fresh Instan *Ledok*

The nutrition fact of ready to serve instant *ledok* before storage (fresh) was water 80,68 %, ash 0,91 %, protein, 7,32 %, fat 1,81 % and carbohydrate 9,29 %, and the results of sensory test was : color 5,6 (like slightly), aroma 5,6 (like slightly), taste 6,0 (like moderately), texture 5,6 (like slightly) and overall preference 6,0 (like moderately).

2. Instan *Ledok A*fter Eight Weeks Storage

a. Nutrition fact of instant *ledok.*

The kinds of packaging materials non significantly affected the water, protein, fat and carohydrate content, but significanly affected the ash content of instan *ledok* after eight weeks storage (Table 1). It can be seen from Table 1 that water content varied from 79,76 % to 80,08 % , protein content varied from 2,22 % to 2,80 %, fat content varied from 2,59 % to 3,40 % and carbohydrate varied from 13,66 % to 13,94 %. Ash content of instant *ledok* packed by AF higher than packed by PP, but insignificanly different with PE. Based on nutrition content, especially ash content, AF is a better packaging material for instant *ledok*.

Table 1.Nutrition fact of instan *ledok* after eight weeks storage

Packaging materials	Water (%)	Ash (%)	Protein (%)	Fat (%)	Carbohydrate (%)
AF	79,76 a	0,88 a	2,22 a	3,40 a	13,75 a
PP	80,08 a	0,82 b	2,80 a	2,65 a	13,66 a
PE	79,92 a	0,85 ab	2,69 a	2,59 a	13,94 a

b. Sensory characteristics of instant *ledok*

The kinds of packaging materials not significantly affecting the preference on color, aroma, texture, taste and over all preference of instant *ledok* after eight weeks storage (Table 2). It can be seen from Table 2 that the preference of the panelis to the color varied from 6,0 (like moderately) to 6,1 (like moderately), aroma varied from 5,8 (like slightly) to 6,1 (like moderately), texture varied from 5,7 (like slightly) to 6,0 (like moderately), taste varied from 5,6 (like slightly) to 6,0 (like moderately) and over all preference varied from 5,4 (like slightly) to 5,8 (like slightly). After eight weeks storage, PP, PE and AF could protect the sensory quality in the same way.

Table 2. The average score of sensory test of instan *ledok* after eight weeks storage

Packaging materials	Color	Aroma	Texture	Taste	Over all preference
AF	6,1* a	5,8 a	6,0 a	5,7 a	5,4 a
PP	6,1 a	6,1 a	5,8 a	6,0 a	5,8 a
PE	6,0 a	5,8 a	5,7 a	5,6 a	5,7 a

*) Score 1 = dislike very much; 2 = dislike moderately; 3 = dislike slightly; 4 = neither like nor dislike; 5 = like slightly; 6 = like moderately and 7 = like very much.

During eight weeks storage the preference of the panelis to the over all preference of instant *ledok* decrease from average score 6,0 (like moderately) to average score 5,6 (like slightly). Water, ash, and protein content were decrease, but fat and carbohydrate content increase. Loss of water might be caused by transpiration process.

CONCLUSION

The results of the study showed that after eight weeks storage, PP, PE and AF have a similar effect on the sensory characteristics, but AF better than PP and PE in protect the stability of nutrient content of instant *ledok*. Until eight weeks storage instant *ledok* promise to consume.

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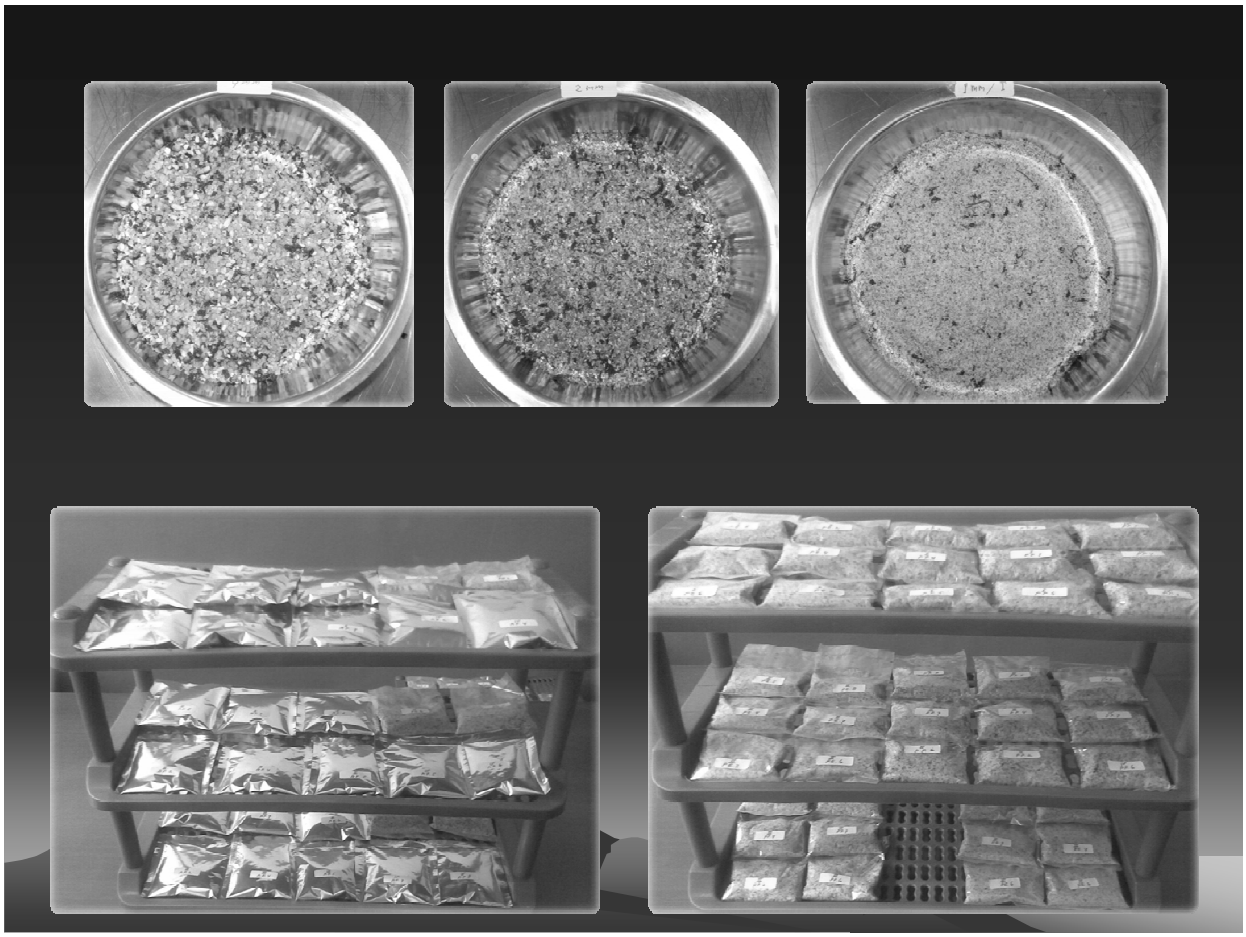

Figure 1. Particles size and instant *ledok*

THE EFFECTS OF SOYBEAN AND *EUCHEUMA SPINOSUM* SEAWEED RATIO ON CHARACTERISTICS OF TEMPEH

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ABSTRACT

The study was conducted in order to find out the best ratio of soybean and seaweed to produce the best characteristics of tempeh. The experiment was carried out by randomized block design, with five soybean and seaweed ratio as treatments and three replications. The treatments were ratio of soybean and seaweed : (1) F1 = 100 %: 0 %, (2) F2 = 95 % : 5 %, (3) F3 = 90 % : 10 %, (4) F4 = 85 % : 15 % and (5) F5 = 80 % : 20 %. The results of the study showed that the best ratio of soybean and seaweed was F3 = 90 % : 10 %. The nutrition fact of this tempeh was iodine content 0.11 %, protein content 19.28 %, water content 63.75 %, and ash content 1.15 %. Texture and color of raw tempeh 3.45 (somewhat hard) and 3.65 (slightly white), texture and fried tempeh (somewhat hard) and 3.65 (slightly brown), taste 3.65 (like), and overall acceptance 4.05 (between normal and like).

Keywords : tempeh, soybean, seaweed and iodine.

INTRODUCTION

Seaweed has a high content of iodine and fiber. The iodine content in seaweed is 0.1 – 0.8 % in brown seaweed and 0.1 – 0.15 % (100 – 1500 µg/g) in red seaweed. Even though in small amount, iodine is highly needed by the human body (Winarno, 1996). The use of sea weeds in overcoming GAKI (*Gangguan Akibat KekuranganGizi* – malnutrition disorder) can be done by fortifying high-iodine seaweed into tempe products, because tempeh is a fermented food product favored by all level of community from all ages, contains high nutrient values and relatively affordable. Moreover, it is expected that with the fermentation process in tempeh making, polysaccharide contained in sea weed will be easier to digest in the body. This research aims to find out the best ratio that can produce tempeh with best characteristic.

MATERIALS AND METHOD

The research was conducted in randomized block design with treatment such as the ratio between soybean (%) and seaweed (%). The treatments consist of five levels, which are: F1: soybean 100% seaweed 0%;F2: soybean 95% seaweed 5%;F3: soybean 90% seaweed 10%;F4: soybean 85% seaweed 15%; and F5: soybean 80% seaweed 20%

The treatments were replicated three times. Data obtained was then analyzed by statistic, and then continued by Duncan test when treatment influence occurred (Steel and Torrie, 1993). Variable observed are: water content, iodine content, protein content, and ash content. Organoleptic test covers the color and texture of raw tempeh, meanwhile score test was performed to fried tempeh on its color and texture, and preference test was performed on the taste, aroma and acceptance in general.

RESULTS AND DISCUSSION

1. Water content, iodine, protein, and ashes.

The average values of water content, iodine, protein and ashes can be seen onTable 1.

Tablel 1. Average values of water content, iodine, protein and ashes.

Treatments	Water (%)	Iodine(%)	Protein(%)	Ash(%)
F1	61,57 d	0,01 a	20,52 a	0,94 cd
F2	62,18 d	0,09 b	19,74 ab	0,98 cd
F3	63,75 c	0,11 c	19,28 bc	1,15 bc
F4	63,90 b	0,13 d	18,65 bc	1,32 ab
F5	65,56 a	0,14 e	17,57 c	1,48 a

Table 1 shows that the highest water contents on tempeh is obtained from soybean 80% and seaweed 20% (F5); which is 65.56 %. And the lowest water content is obtained from soybean 100% and seaweed 0% (F1) which is 61.57%. The more seaweed added to tempeh, the higher water content becomes. This is due to the raise in seaweed concentration so the tempeh becomes more absorbent to water.

The highest iodine content on tempeh is obtained from soybean 80% and seaweed 20% (F5); which is 0.14 %. And the lowest iodine content is obtained from soybean 100%and seaweed 0% (F1) which is 0.012%.

The highest protein contents on tempeh is obtained from soybean 100% and seaweed 0% (F1); which is 20.52 %. And the lowest protein content is obtained from soybean 80% and seaweed 20% (F5) which is 17.57%. This is due to seaweed having very low content of protein mean while tempeh has a high content of protein, 35%. According to SNI 3144:2009, the protein content in tempeh is minimum 16%.

The highest ash contents on tempeh is obtained from soybean 80% and seaweed 20% (F5); which is 1.49 %. And the lowest ash content is obtained from soybean 100% and seaweed 0% (F1) which is 0.94%. It shows that the increase of seaweed addition has caused the increase of ash content in tempeh.

2 Organoleptic test

2.1 Texture and color of raw tempeh.

Adding seaweed until a certain level can improve texture and color of tempeh Table 2). Texture with the highest score is obtained from tempeh made of soybean 90% and seaweed 10% (F3) which is 3.45 (between slightly hard and hard). Mean while texture with the lowest score is coming from tempeh made from soybean 80% and seaweed 20% (F5) which is 2.00 (between soft and slightly hard). The color of tempeh changed from 4.00 (between cream and slightly white) into 2.95 (between sliglty brown and cream).

Table 2. The result of score test on texture and color of raw tempeh.

Treatments	Variable	
	Texture	Color
F1	2,40 c	4,00 a
F2	3,00 ab	3,80 ab
F3	3,45 a	3,65 ab
F4	2,95 ab	3,45 b
F5	2,00 c	2,95 c

2.2. Texture and color of fried tempeh

Texture with the highest score obtained in tempeh that is made of soybean 90% and seaweed 10% (F3) which is 3.65 (between slightly hard and hard). Mean while the lowest score comes from tempeh made of soybean 100% and seaweed 0% (F1) which is 2.3 (between soft and slightly hard) as shown in Table 3.

Table 3. The result of test score on the texture and color of fried tempeh.

Treatments	Variable	
	Texture	Color
F1	2,3 c	2,3 c 4,15a
F2	3,15 b	3,15 b 3,85 ab
F3	3,65 b	3,65 a 3,5 bc
F4	3,1 b	3,1 b 3,45 bc
F5	3 c	3 b 3,05 c

The highest color score comes from tempeh that is made from soybean 100% and seaweed% (F1) which is 4.15 (between slightly brown and brown). The lowest color score comes from tempeh made from soybean 80% and seaweed 20% (F5) which is 3.05 (between cream and slightly brown).

2.3. Aroma, taste, and general acceptance of the fried tempeh.

The highest taste score comes from tempeh made from soybean 90% and seaweed 10% (F3) which is 3.65 (between normal and like). Meanwhile for the lowest taste score comes from tempeh made from soybean 80% and seaweed 20% (F5) which is 2.90 (between dislike and normal) (Table 4). Aroma test shows that there was no effect after adding seaweed in the making of tempeh.

Table 4. The results of score and hedonic tests on the aroma, taste and general acceptance on the fried tempeh.

Treatments	Variable		
	Aroma	Taste	General acceptance
F1	3.1 a	3.10 bc	3.10 bc
F2	3.15 a	3.50 ab	3.50 b
F3	3.35 a	3.65 a	4.05 a
F4	3.05 a	3.50 ab	3.30 bc
F5	2.9 a	2.90 c	2.70 b

In the test of general acceptance, the highest score comes from soybean 90% seaweed 10% (F3) which is 4.05 (between normal and like). Meanwhile the lowest score comes from soybean 80% and seaweed 20% (F5) which is 2.70 (between dislike and normal).

CONCLUSION

The best ratio for adding seaweed in tempeh making is soybean 90% and seaweed 10%. The tempe characteristic produced has iodine content: 0.11 %, protein content: 19.28%,water content 63.75%, ash content 1.15% , test on texture and color results in 3.45 (slightly hard) and 3.65 (slightly white), texture and color of fried tempe results in 3.65 (slightly hard), and 3.65 (slightly brown), taste 3.65 (like) and general acceptance 4.05 (between normal and like).

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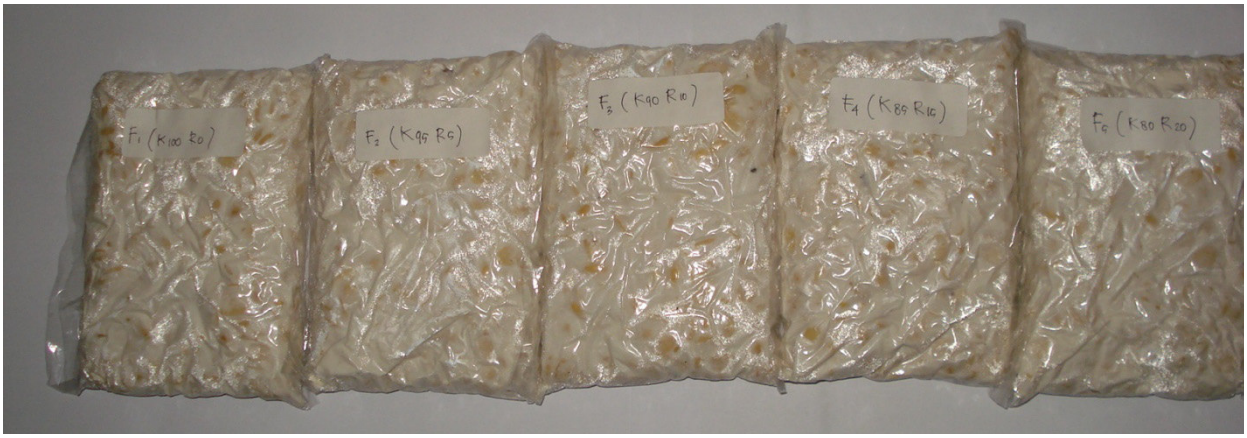

Photo 1. Tempeh made from soybean and seaweed

EFFECT OF BOILING TIME IN SUGAR SOLUTE ON CHARACTERISTIC OF EGGPLANT DRY CANDY

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ABSTRACT

Objectives of the research is to investigate the effect of boiling time of sugar solute on characteristic of eggplant dry candy and boiling time of sugar solute that produce the best of characteristic of eggplant dry candy. The research was conducted using randomized block design with six treatments, i.e. 15, 30, 45, 60, 75 and 90 minute of boiling time of sugar solute with three replication, resulted in 18 units of experiment. Results show that boiling times treatment gave significant effect on characteristic of eggplant dry candy such as water value, vitamins C, sugar total, texture, color and odor and not significant effect on taste and overall acceptance. The boiling time at 45 minute gave the best characteristic of eggplant dry candy, i.e. water value 20.9%, total sugar 51.7%, vitamins C 0.7 mg/100 g, texture was rather hard, texture, odor and overall acceptance were rather like, color and taste were neutral.

Keyword: boiling time, sugar solute, characteristic, eggplant dry candy

INTRODUCTION

Eggpalant (*Solanum melongena L*) is one of the horticulture commodity which is easily spoil. It is not handle in appropriate. In Indonesia eggplant spread widely as a main stay in low land. Eggplant prow well in any area with variety elevated well as in conditions, in low land as in highland up to 100 m above sea level.

Most of the people used eggplant as part of their menus or in daily meal, such as in making eggplant curry, salad and other product, but it is not process in many varieties of processed as 'dodol'. It is product made of sticky nice, coconut milk and palm sugar.

Processing eggplant in to variety of processed product is as an alternative to be carried out. Producing eggplants candy were including boiling in sugar solution and drying. If the eggplants were boiled in short time, the eggplants candy will characterized by its hard texture and when it is boiled for long time, it's texture will be soft .

The aimed of this research were ; to develop a method in boiling eggplant in sugar solution and to improved the quality and appearance of eggplant candies with best characteristic.

MATERIALS AND METHOD

Place of Research

The research was carried out at Agriculture Product Technology, Faculty of Agriculture Technology, Udayana University, Denpasar.

MATERIAL AND EQUIPMENT

Material

Material used in making dried eggplant candies were dried purple eggplant flour from Sanglah market, water, citric acid (Gajah brand), sugar cane and lime flour from Nirmala supermarket- Jimbaran.

Material for analysis were aquadest, HCL (Merk), NaOH (J.T. Baker), PP (J.T. baker), luff schoold solution, (Na2CO3 – J.T. Baker), citric acid (J.T. Baker), CuSO4 (J.T. Baker), H2SO4 (Merk), KI (Merk), Na2S2O3 (J.T. Baker), HPO3 (Merk), Acetic acid (Merk), 2.6 dichlorphenolindo-phenol natrium salt 2-dihydrat (Merk), NaHCO3 (J.T Baker), filter paper and alumunium foil.

Equipment

Equipment were stain steel knife, chopping board, wash basin, gas stove, cabinet oven dryer, balance, stirring spoon, thermometer, measuring cylinder, analytic balance, aluminum dish, oven, desicator, brass mortar, measuring flash pipet, inject, water batch, Erlenmeyer, biuret

Methods

Research design as following random block design with boiling time in sugar solution treatment by 6 level are :

P1 = boiled for 15 minutes
P2 = boiled for 30 minutes
P3 = boiled for 45 minutes
P4 = boiled for 60 minutes
P5 = boiled for 75 minutes
P6 = boiled for 90 minutes

Treatment wer repeated 3 times, therefore there were 18 units of experiment. Datas ole obtained were analyzed by ANOVA, if there were an significant effect on variable observed analysis to be continued with DMRT (Steel and Torrie, 1993).

Product of Research

1. Purple eggplants were grading, washed and drained
2. Eggplant were cut longitudinally with 4 pieces, every piece then cut at an angle about 5 cm length, 2 cm width and 2 cm thick
3. Eggplant were soaked in 0.53 lime solution for 4 hours completely
4. After soaking then washed with clean water
5. Sugar solution (60%), contained by citric acid were boiled up to 100 °C and then took eggplant boiled (500 g) in boiled solution the time of boiling is counted when the solution boiling again. The time of boiling according to the treatments
6. The eggplant then drained after treated
7. Dried in oven (50 °C) far 24 hours (see the diagram)

Figure 1. Digrams of dried eggplant candy process

Variable observed

Variable observed were moisture content (AOAC, 1984), Vitamin C (AOAC, 26D, 1984), Sugar total (Sudarmaji et al. , 1984),

RESULTS AND DISCUSSION

Table 1. mean of moisture content of dried eggplant candies

Product	Treatment (boiling time)	Mean value
P1	15 minute	19.9 d
P2	30 minute	20.4 cd
P3	45 minute	20.9 c
P4	60 minute	21.2 bc
P5	75 minute	22.0 b
P6	90 minute	23.2 a

Mean not followed by he same letter are significant different (P>0.05)

Table 1 shown the highest was moisture content of dried eggplant candy which was boiled far 90 minutes while the lowest moisture content was dried eggplant candy which was boiled far 15 minutes. Overall moisture content of dried eggplant candy meet the Indonesia standar No:01-37190-1995 which maximum moisture content is 31%

Vitamin C Content

The vitamin C of dried eggplant candy with different treatment were presented in Table 2

Table 2. Mean of vitamin C content of dried eggplant candies

Product	**Treatment (boiling time)**	**Mean value**
P1	15 Minute	1,426 a
P2	30 MINUTE	1.424 b
P3	45 MINUTE	0.711 c
P4	60 MINUTE	0.711 c
P5	75 MINUTE	0.710 cd
P6	90 MINUTE	0.708 d

Mean not followed by he same letter are significant different (P>0.05)

Table 2 shown the highest vitamin C content of dried eggplant candy which boiling processed far 15 minutes, which dried eggplant candy with the lowest vitamin C content was boiled far 90 minutes. According to Poedjiadi (1994) and Winarno (2002), that vitamin C lost easily during boiling, steaming and roasted process and easily dissolved in water

Total Sugar

Total sugar of dried eggplant candy produced with different treatment were presented in Table 3

Table 3. Mean of sugar total content of dried eggplant candies

Product	**Treatment (boiling time)**	**Mean value**
P1	15 minute	48.923 c
P2	30 minute	50.380 d
P3	45 minute	51.683 c
P4	60 minute	52.897 b
P5	75 minute	54.600 a
P6	90 minute	55.427 a

Mean not followed by he same letter are significant different (P>0.05)

Table 3 shown the highest sugar total content of dried eggplant candy was product treated by boiling far 90 minutes which was not significant difference with boiled far 75 minutes. The lowest sugar total content of dried eggplant candy was boiling far 15 minutes

CONCLUSION

1. Treatments with different boiling time on sugar solution affect significantly on moisture content, vitamin c content and sugar total
2. The longer the boiling time: moisture content, sugar total increased, but vitamin C content decreased

CHANGES IN POLYPHENOL OXIDASE ENZYME (PPO) IN THE FRESH-CUT BAMBOO SHOOTS DURING ROOM TEMPERATURE STORAGE

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ABSTRACT

Bamboo shoots soon after harvested and peeled, experienced browning discoloration due to PPO activity. Research was conducted to find out the changes of Polyphenol Oxidase Enzyme (PPO) activity in fresh-cut bamboo shoot stored in room temperature. The research was carried out with completely randomized design. Storage duration consisted of 4 treatments, each treatment was 0,1, 2, and 3 days storage. Observation was done on the brightness, polyphenol oxidase enzim activity/ PPO and total phenols. Polyphenol oxidase activity increased in fresh-cut bamboo shoots from 0,03 unit/g at 0 days storage to 0,07 unit/g at three days of storage. Total phenols decreased from 0.18 % at 0 days of storage to 0.10 % at 3 days storage. There was a decrease in brightness from 0 days of storage with a value of 68.00 to 47.45 at 3 days of storage.

Keywords: Bamboo shoot, Fresh-cut, PPO, Phenol, Brightness

INTRODUCTION

Bamboo shoots quickly get damaged if not handled properly after harvesting. One of the damage that arises is the color change to brown. Brown color on the bamboo shoots indicates a low quality of bamboo shoots. The change to brown color is due to the activity of polyphenol oxidase enzyme with phenol and oxygen as substrates. To prevent the formation of brown color on fresh-cut bamboo shoots, bamboo shoots should be stored at low temperature to inactivate enzyme or soaked in water after peeling to prevent contact with oxygen. The optimum temperature of activity of the enzyme polyphenol oxidase is at room temperature. Based on that information the changes in the PPO enzyme activity were monitored in fresh-cut bamboo shoot during storage at room temperature.

MATERIAL AND METHODS

The main plant materials are bamboo shoots of bamboo species *Gigantochloa nigrociliata* Buese Kurz. Bamboo shoots with bright white color were peeled and cut of length 10 cm,. Bamboo shoots that have been peeled and cut were put in jar packaging, then stored at room temperature. Observations made every day ranging from 0 to 3 days of storage. The brightness of bamboo shoots were measured using Minolta color reader Chromatometer based systems methods Hunter / L *, a *, b *. Analysis of the activity of polyphenol oxidase (PPO) was done based on method of Gardjito (2003). Determination of total phenols was conducted using method of Andarwulan *et al*. (1990)with modification..

RESULTS AND DICUSSION

The brightness of fresh-cut bamboo shoots

Brightness value of fresh-cut bamboo shoots decreased during storage at room temperature (Figure 1). According to Whitaker and Lee (1995) the occurrence of injuries on fresh plant material produced oxygen easily, lead to contact with phenolic compounds. With the help of PPO enzyme, oxidation of phenolic compounds will occur.

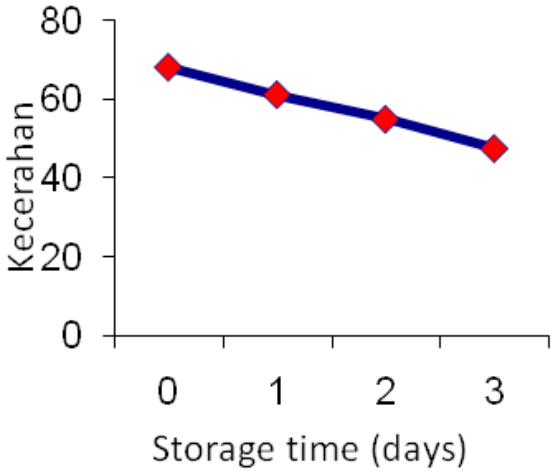

Figure 1. Graph showing the relationship of storage time against the value of fresh-cut bamboo shoots brightness

The decline in the value of brightness during storage, presumably because of oxygen penetration into the bamboo shoot higher with increasing duration of storage, so that more oxygen is available. The availability of oxygen, causing oxidation process is catalyzed by the enzyme phenol PPO will run fine until a process of browning. The availability of oxygen, lead to oxidation process which is catalyzed by the enzyme phenol PPO. The process will run until a process of browning.

PPO activity of fresh-cut **bamboo shoots**

Longer storage of bamboo shoots at room temperature showed increasing PPO enzyme activity (Figure 2).

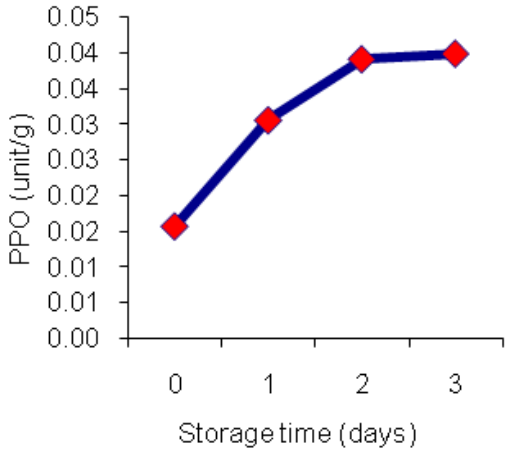

Figure 2. Graph of storage time and PPO activity of fresh-cut bamboo shoot

PPO in fresh-cut bamboo shoots increased significantly along with the storage time is from 0 to 3 days. Fresh-cut bamboo shoots during storage for 3 days showed the highest PPO activity that is equal to 0.07 g / unit, while the lowest PPO activity is shown by the fresh-cut bamboo shoots in storage for 0 day that is equal to 0.03 g / unit. According to Laurila (1998) enzymatic browning occurs when there are raw materials that contain phenolic compounds as substrates, oxygen and oxygen catalyzed by enzymes such oxydoreductase poliphenolxydase or phenolase. If one of these three components does not exist, then the browning reaction does not occur.

Whitaker and Lee (1995) mentions the formation of quinone which is the initial browning reactions was affected by the enzyme polyphenol oxidase, and is also influenced by the availability of oxygen in the material.

Total phenol fresh-cut bamboo shoots

Fresh-cut bamboo shoots with highest total phenol seen in the 0-day storage time is equal to 0.18 % and the lowest obtained in fresh-cut bamboo shoots in a 3-day storage time is equal to 0.10 % (Figure 3).

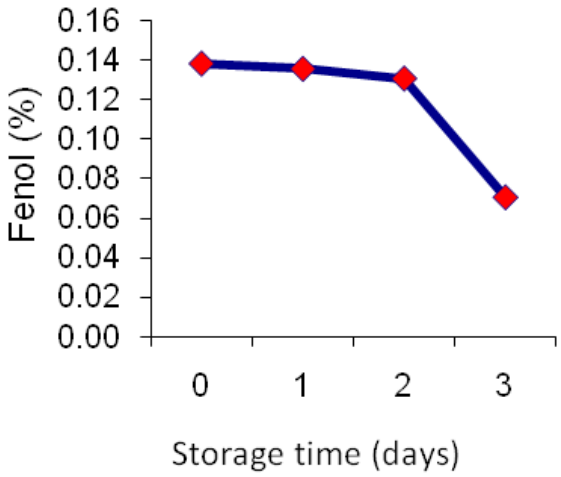

Figure 3. Graph of total phenols fresh-cut bamboo shoots during storage room temperature

Decrease in total phenol fresh-cut bamboo shoots is linked storage period. The longer the storage, the more penetration of oxygen into the product, so that the phenol is oxidized, and subsequently with the enzyme, in this case the PPO as a catalyst can change the polyphenol compounds into quinone compounds, thus decreasing phenol .

According to Whitaker and Lee (1995), when the substrate concentration is high, then all the enzyme molecules can form a complex bond with the substrate so that the maximum rate of reaction that increase the substrate concentration will have no effect on the rate of reaction. According to Watada (1999) browning caused by injuries occur in fruits and vegetables after harvested, where the oxygen will penetrate the product. When oxygen is in the network, the PPO enzyme in chloroplasts immediately oxidizes the phenol group that is naturally available in the network. This oxidation process changes the phenol group into o-quinone, which serves as a precusor formation of brown color. O-Quinones then produce brown color.

CONCLUSION

During storage of fresh-cut bamboo shoots at room temperature conditions, the activity of the enzyme polyphenol oxidase of 0.03 g / unit on a storage 0 days, increased to 0.07g/unit after the storage for 3 days, while the total phenol and brightness values decreased during storage.

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THE EFFECT OF SUGAR CONCENTRATION AND THE USE OF *Saccharomyces bayanus* EC 118 ON TOTAL POPULATION OF FUNGI IN PURPLE SWEET POTATO´S "BREM"

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ABSTRACT

The objective of this research was to determine the effect of sugar concentration and the use of *Saccharomyces bayanus* EC 118 on total population of fungi in purple sweet potato´s "brem". This research used Randomized Completed Design with two treatments i.e. the use of sugar in different level concentration (0%, 10 %, 12%, 14%, 16%, 18%, and 20%) and the use of *Saccharomyces Bayanus* EC 118 (with, without of *Saccharomyces Bayanus* EC 118 and *Saccharomyces cereviseae*). Parameters observed in this research were total population of fungi and reducing sugar content. The result indicated that addition of 20% sugar and use of *Saccharomyces bayanus* EC 118, produced purple sweet potato´s "brem" with the lowest population of fungi by 1,9 x 10^4 CFU/ml and reducing sugar content by 2,31 %.

Keywords : *Saccharomyces bayanus* EC 118, sugar, purple sweet potato´s "brem".

INTRODUCTION

"Brem" is balinese tradisional drink that famous in Indonesia also at the other country (Sudjatha, 1997). This drink was from formerly until now had used as completion in religious ceremony at balinese, beside that during the year it more like by local society also regional because of the fixed processing. Bali island progress in tourism also made local food product became exported product.

"Brem" is one of balinese tradisional drink that was exported to the international market and many tourist likes Japanese, Korean, China, Vietnam, Malaysian, Thailand and several Europe countries with another asia enjoy "brem". Many customer from abroad dislike "brem" that has taste of sweet, rather sour and low alcohol content.

Basic material of "brem" was rice. Now adays, production of rice was limited in Indonesia. Therefore it was needed to find out alternate material. One of them was purple sweet potato's. Purple sweet potato's had high carbohydrate content (37,9-40 percent), beside that purple sweet potato's also contain several minerals likes calcium and phosphor that important for bone growth and another body organ repair (Suliantari and Rahayu, 1990). Manipulation of purple sweet potato's "brem" making by using *Saccharomyces bayanus* and addition of sugar on liquid "tape" during fermentation process was desired to increase purple sweet potato's brem quality.

The objective of this research was to determine the effect of sugar concentration and use of *Saccharomyces bayanus* EC 118 on total population of fungi in purple sweet potato´s "brem".

MATERIALS AND METHOD

Materials and equipment. That used in this research was purple sweet potato's was bought at Kumbasari market, "tape" inoculum (merk NKL/Na Kok Liong), sugar, was bought at Sanglah market, *Saccharomyces bayanus* EC 118 from Lavin-Canada, *Saccharomyces cereviseae,* filters paper, aquadest, luff schoorl, H_2SO_4 20%, KI 20%, natrium thiosulfat 0,1 N, potato dekstrose agar, filters cloth, bowl, pan, buret bottle, scales, erlenmeyer, autoclave, reaction tube, coloni counter, mikropipet tip, petridish, oven

Method. This research used Randomized Completed Design with two treatments i.e. the use of sugar (G) in different level concentration (0% (G1), 10 % (G2), 12% (G3), 14%, (G4), 16% (G5), 18% (G6), and 20% (G7)) and the use of *Saccharomyces Bayanus* EC 118 (R) (with (R2) , without of *Saccharomyces Bayanus* EC 118 (R0), *Saccharomyces cereviseae* (R1)). Repeated twice. Parameters observed in this research were total population of fungi and reducing sugar content. The data was analysed by Analysis of Variance Continue with Duncan Test (Steel and Torrie, 1983)

Brem Making :

1. Basic commodity preparation, purple sweet potato's.
2 Peel and wash. First, puple sweet potatos were peeled and cut with thickness 1-3 cm and then cleaned with water. After that, they were steamed until the texture of rather soft. Then purple sweet potatos were cooled for 10-15 minute.
3. Addition or inoculation of "tape" inoculum (NKL)
   For 1 kg ingredient was inoculated 5 g "tape" inoculum. Before inoculation, the "tape" inoculums was crushed by using blender. "Tape" inoculum was spread evenly on ingredient surface that provided with niru and overlayed banana leaf.
4. Fermentation
   The ingredient that was given "tape" inoculum has been wrapped up with banana leaf and putted into container and closed tightly. Then it was fermented during 3 day.
5. Separation
   After fermentation process, during 3 days, obtained "tape" was very soft and yield liquid. This liquid was separated from the "tape".
6. Pressing
   The separated "tape" from it liquid was wrapped by filter cloth then pressed.
7. Liquid of "tape" was gave sugar treatment and *Saccharomyces bayanus* EC 118 depand on the each treatment.
8. Storage for 1 month
9. Parameters observed in this research were total population of fungi and reducing sugar content..

RESULTS AND DISCUSSION

1. Reducing Sugar Content

The result showed that the use of *Saccharomyces cereviseae* and *Saccharomyces bayanus* EC 118 had no significant effect ($p < 0,05$) to reducing sugar content of "brem". But sugar concentration treatment high significant effect ($p > 0,01$) to reducing sugar content. The data was presented on Table 1.

Table 1. Reducing sugar content of "brem" (%)

Treatment	G0	G1	G2	G3	G4	G5	G6	Average
R0	2.90	3.28	3.51	3.46	3.35	3.12	3.00	3.23 a
R1	3.84	3.43	2.90	3.19	2.63	2.09	1.82	2.84 b
R2	3.80	3.75	2.89	2.90	2.49	2.65	2.31	2.97 ab
Average	3.51 a	3.48 a	3.10 ab	3.18 ab	2.82 b	2.62 b	2.37 b	

Table 1 presented that reducing sugar in "brem" was 1,82- 3,80 %. In young "brem", beside dextrin, also contain sucrosa. Sucrosa was broke by invertase enzyme became glucose and fructosa

2. Total population of fungi

The result showed that the use of *Saccharomyces cereviseae*, *Saccharomyces bayanus* EC 118 and sugar concentration treatment had high significant effect ($p>0,01$) to total population of fungi. That was interaction between *Saccharomyces cereviseae*, *Saccharomyces bayanus* EC 118 and sugar concentration treatment. The data was showed on Table 2.

Table 2 showed that treatment R1G6 (the use *saccharomyces bayanus* EC 118 and addition of sugar concentration 20 %) obtained lowest total population of fungi was $1,9 \times 10^4$ cfu/ml. The highest was R0G0 (treatment without S*accharomyces bayanus* EC 118 and without sugar increasing) $4,6 \times 10^5$ cfu/ml, because "tape" liquid (young 'brem") was the result of fermentation of yeast/mold during fermentation process. Temporary the use of *Saccharomyces bayanus* EC 118 and sugar concentration 20% showed that the total population of fungi was very low because high sugar concentration could inhibite microbe

growth, and also because during fermentation process was formed etanol and organic acid that could depress activity of fungi.

Table 2. Total population of fungi of "brem" (cfu/ml)

Treatment	Total population of fungi (cfu/ml)
R0G0	4.6×10^5 a
R0G1	2.3×10^5 ef
R0G2	5.8×10^5 a
R0G3	4.9×10^5 a
R0G4	2.7×10^5 cde
R0G5	1.9×10^5f
R0G6	1.2×10^5 g
R1G0	4.6×10^5 a
R1G1	3.3×10^5 bcd
R1G2	3.4×10^5 bc
R1G3	3.2×10^5 bcd
R1G4	5.0×10^4 i
R1G5	4.0×10^4 i
R1G6	2.2×10^4 k
R2G0	3.5×10^5 bc
R2G1	3.9×10^5 b
R2G2	2.5×10^5 de
R2G3	1.9×10^5 f
R2G4	6.9×10^4 h
R2G5	3.8×10^4 ij
R2G6	1.9×10^4 a

CONCLUSION

The result indicated that addition of 20% sugar and use of *Saccharomyces bayanus* EC 118, produced purple sweet potato´s "brem" with the lowest population of yeast by $1,9 \times 10^4$ cfu/ml and reducing sugar content by 2,31 %.

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BIOASSAYOF *BROTOWALI* (*TINOSPORACRISPA* (L) MIERS) LEAVES CRUDE EXTRACT ON GRAM POSITIVE BACTERIAL, GRAM NEGATIVE BACTERIAL AND *ARTEMIASALINA* L. LARVAE

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ABSTRACT

The main objective of this research was to investigate the toxicity of crude extract of leaves of *Tinosporacrispa* (L) Miers on several Gram positive bacterial species (*Staphylococcus aureus*, *Streptococcus pyogenes* and *Bacillus cereus*), several Gram negative bacterial species (*Salmonellathypi*, *Escherichia coli*, and *Vibrio colerae*), and larva of *Artemiasalina* L. The project was conducted at the Laboratory of Microbiology, Organic Chemistry, and plant physiology, Faculty of Mathematics and Natural Sciences, University of Udayanafrom November 2003 to February 2004.

The result showed that the crude extract of leaves of this plant inhibited the growth of both Gram positive and Gram negative bacterial species when exposed at the concentration of 100 ppm or more, in vitro. The lethal concentracion (LC_{50}) of the extract on *Artemiasalina* L was 50.137 ppm, indicating that this extract has the potential to be developed into an alternative anti cancer compound.

Keywords : Leave of *Tinosporacrispa* (L) Miers, Kirby-Bauer method, brine shrimp lethality bioassay dayusuryanti@yahoo.co.id

COMPARATIVE CONTENT ANALYSIS OF CADMIUM (CD) AND LEAD (PB) IN LOCAL APPLE AND IMPORTED RUM BIUTY FUJI IN DENPASAR

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ABSTRACT

Apple is a very popular crop worldwide. There are many varieties of apples, some of which are local apple type rum biuty and Manalagi. There are so many varieties of imported Rum Biuty types, i.e. Fuji apples, New Zealand, Fuji PRC, etc. The results of the community survey showed that imported apples are preferred. Beside rich in nutrient content, apples are also easily contaminated by heavy metals, such as cadmium (Cd), lead (Pb), Arsenic, Ag, Hg, etc., which were derived from the growth environment. Cd, for example, can originated from fertilizers and pesticides. The purpose of this study was to determine the content of Cd and Pb in local apples and apple rum imported biuty fuji found in the city of Denpasar. The research was conducted at the Analytical Laboratory, Udayana University. The results showed that the content of Cd and Pb in imported fuji type and local types of rum biuty were 4.84; 3:12 (µg/g) and 5:13; 7:08 (µg/g), respectively. Both Pb and Cd exceeds the provisions of the WHO (2.0 (µg/g).

Keywords: apples, heavy metals Cd, Pb heavy metals.

INTRODUCTION

Apple crop is a plant that is very popular in the world. The species has many varieties of apples, of which there are local apple types and kinds of rum biuty Manalagi and to import apples there are so many varieties like fuji apples, New Zealand, fuji PRC and others. Based on the results of the community survey is currently imported apples are very loved in the community. Because it has several advantages such as the appearance of fruit such as size and color is very attractive, sweet taste. Local apples tend to under-emphasized because it tends to taste sour and the fruit less attractive appearance. Apples are the fruit of a very vaporit in the community. Often used as a fruit dessert and as a refresher when thirst. Judging from the nutritional content of apples contain carbohydrate, fiber, vitamins and minerals (Almatsier, 2001).

Beside rich nutrient content, apples are also easily contaminated by heavy metals such as cadmium (Cd), lead (Pb), Arsenic, Ag, Hg, etc.. derived from the growth environment. Metal Cd for example, can come from fertilizers, pesticides and industrial. Likewise, other metals such as Pb can be derived from motor vehicle fumes, fertilizers, pesticides and industrial (Saeni, 2000). If heavy metals are taken into the body or cause a variety of dams on the health pack. The purpose of this study was to determine the content of heavy metals Cd and Pb in local apples and apple rum imported rum biuty fuji circulating in the city of Denpasar.

MATERIALS AND METHOD

The research was conducted at the Analytical Lab at Udayana University. Material in the form of import types fuji apples and apple rum biuty local species. Samples of small apples cut into small each of 0.5 kg is inserted into a paper bag. Then dried in an oven 110 ° C, until constant weight. Further samples of ground and weigh a sample of 0.3 g inserted into the pumpkin destruction. Then sulfuric acid is added carefully and hati.Larutan heated slowly until the solution is black, and then etched with concentrated nitric acid solution for 10 to 20 drops to a solution of clear-colored (yellow). Then the solution was cooled and diluted to 25 ml aquades then shaken. Then the solution is filtered to obtain filtrate. The filtrate was analyzed by AAS. After measuring the concentration is known then the actual content of the dried sample can be determined by the formula $M = (\underline{C \times V \times F})/B$. M = Pb or Cd content in the sample (ug / g), C = concentration obtained from the calibration curve (ug / g / ml), V = volume of sample solution (ml); F = dilution factor; B = weight of sample (g). The data were presented in tabular form, to see a comparison of heavy metal contents of Pb and Cd on imported apples and apple fuji type of local types of rum biuty shown by histogram.

RESULTS AND DISCUSSION

Results showed an average content of heavy metals Pb and Cd in fuji apples and apples imported types of local species rum biuty.are presented in table.1

Table 1. Average Heavy Metal Content of Pb and Cd on the type of fuji apple imports and local apples rum biuty no

no	type of heavy metal	type fuji apple (ug / g)	Type rum biuty (ug / g)
1	Pb		
	Deuteronomy I	5,05	7,09
	Deuteronomy II	5,10	7,10
	Deuteronomy III	5,14	7,05
	Average	**5,13**	**7,08**
	Cd		
	Deuteronomy I	4,79	3,10
	Deuteronomy II	4,80	3,6
	Deuteronomy III	4,91	3,18
	Average	**4,84**	**3,12**

Table 1. indicates that the content of heavy metals Pb for both types of local and imported apples both showed greater yield of 5.13 for the import of ug / g and to 7.08 for local microg / g, when compared with heavy metals Cd for the import value of 4.84 g / g, while for the local 3.12 ug / g. High content of Pb in both types of apples suggests that apples already contaminated by pollutants from the environment. Heavy metal Pb from many sources such as household appliances, industrial, workshop or from which gasoline accounted for a large enough Pb to the environment as well as pesticides and fertilizers (Darmono, 1995).

Figure 1. indicate heavy metal content of Pb to the type of local apple rum biuty higher (7.08 / ug / g) than other types of imported apples fuji (5.13 ug / g). This shows that our country more environmentally polluted by heavy metals Pb, especially the area around planting apple rum biuty type, when compared with the State of origin of country fuji apples Japan. This suggests that the source of Pb metal such as gasoline, workshops and other equipment more in Indonesia compared with Japan State. Both local apples and apple fuji apple imports in particular have been contaminated with heavy metals are all Pb is already exceeded the WHO quality standard (2.0 ug / g).

Besides the high content of heavy metals Pb at a local apple rum biuty skin texture caused by apple rum biuty more coarse compared with fuji apple imports. Rough skin texture / coarse will facilitate pollutant substances into the fruit, and substances that accumulate pollutants are also much more. While the slippery skin of the fruit is more difficult to be penetrated by substances pollutants because there are layers of wax that coats the surface of the skin of the fruit, and substances that accumulate contaminants in fruit are also fewer (Kabelan.K, 2006). Cd heavy metal content for the type of fuji apple imports is higher (4.84 ug / g) compared with other types of local apples rum biuty (3.12 ug / g) (Figure 1). This suggests that the type of content fuji apples imported pesticides are more than the local type of apple rum biuty. Or the many factories that use heavy metals Cd is close to fuji apple plantations. Because heavy metals Cd found in many pesticides, fertilizers and factories that use heavy metals Cd ..

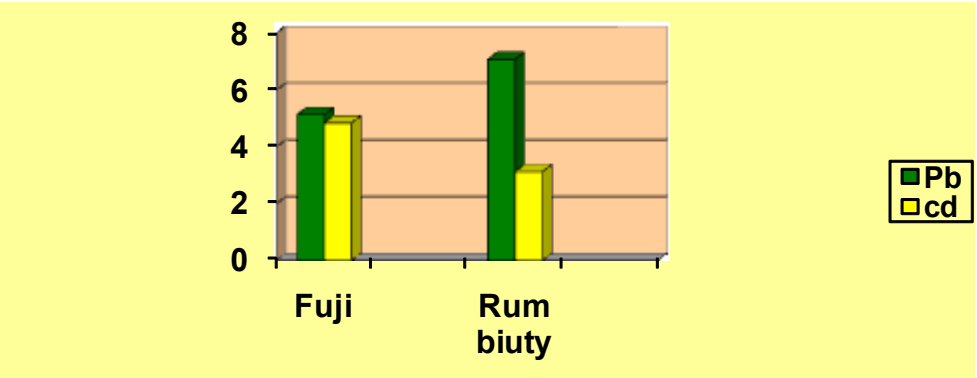

Figure 1. Diagram Comparison Average Heavy Metal Content of Pb and Cd on import types fuji apples and apple rum biuty local species

Heavy metal content of Cd when compared with the Pb content of heavy metals in both types of import fuji apples and apples on the type of local rum biuty showed a larger value (6.11 ug / g) than the heavy metal content of Cd (3.98 ug / g) (figure.1). This showed that heavy metal pollution Pb are more than the heavy metal Cd. The high value of heavy metal Pb can be derived from motor vehicle fumes, pesticides, such as textile mills and other factories that use heavy metals Pb (Suriani, 2008). When compared with the quality standard of the WHO (2.0 mg / g) whether the heavy metal content of heavy metals Pb and Cd in both types of imported apples fuji apples and types of local rum biuty both already exceed the quality standard.

Wachjadim et al (2003), states that at a young apple Manalagi already contain residues of pesticides benomil at 6 mg / kg. Due to the high use of pesticides and chemical fertilizers on land other than the high residues on apples apples also have an impact on the ground. Stone apple farms in poor already damaged, so many apple farmers are turning to the livelihood lainn (Anonymous, 2011). Lukitaningsih E et al (2002) states that the pesticide residues found in apples can be reduced by washing the apples with a solution of surfactant.

CONCLUSION

1. Pb content of heavy metals on the type of local apple rum biuty higher when compared with the type of fuji apple imports. 2. Heavy metal content of Cd on the type of local apple rum biuty lower than the type of imported apples fuji. 3. The content of heavy metals Pb for both types of apples is higher than the content of heavy metals Cd . 4. The content of heavy metals Pb and Cd heavy metal content for both types of apples have exceeded the quality standard WHO Suggestion.

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CHANGE OF CHEMICAL PROPERTIES AND CAROTENE DEGRADATION OF REFINED BLEACHED DEODORIZED PALM OIL (RBDPO) DURING FRYING OF TOFU

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ABSTRACT

The aim of this research was to know the change of chemical properties and total carotene of used deep-frying oil (RDBPO) during frying of tofu. This research was done in several steps i.e.: chemical analysis of fresh oil, five cycles of frying at 160°C and chemical analysis of used deep-frying oil. Comparison between tofu and fresh oil was used for frying 1 : 4, and the end of each frying cycle, used pan-frying oil was collected for analysis. Data was analysed using descriptive method and presented on graph. Result showed that moisture content, free fatty acid value, peroxide value and degradation of carotene total increased during frying of tofu. Based on this results, it could be suggested that it was important to add fresh oil at the end of each frying cycle to regenerate used deep-frying oil so that the quality of this oil would be better.

Keywords: RBDPO, frying, tofu, carotene degradation

INTRODUCTION

Frying is done in homes, restaurants (food services), and at large industrial operations. Pan frying or griddle frying is done mostly at homes or at the restaurants. In this process, a thin layer of oil is heated on a skillet or a griddle. The food is fried in a layer of oil and fried until completion. Oil plays a great role in determining the storage stability quality of the fried product. However, oil is also prone to oxidation, which leads to rancidity of the product in storage (Gupta 2005).

Carotenoids is one of minor compounds in palm oil. Carotenoids are the precursors of vitamin A, with β-carotene having the highest provitamin A activity. Palm oil has 15 times more Retinol Equivalents than carrot and 300 times more than tomato (Sundram 2007). The structures of carotenoids confer on them many important physiological properties, such as antioxidant activity. They, therefore, play an essential role in protecting cells and organisms against lipid peroxidation. The highly conjugated polyene systems are extremely efficient quenchers of singlet molecular oxygen and free radical species in lipid phases and have been associated with decreased risks of cancer, atherosclerosis and cataract (Bonnie dan Choo 1999).

Carotenoids are widely used in food applications. Their highly unsaturated nature makes them susceptible to degradation by oxidation and thermal processes, especially under severe processing and storage conditions. The oxidation products formed are mixture of epoxides, apocarotenal and hydroxy compounds. Isomerization, oxidation and breakdown of the carotenoid molecule occur as a result of thermal degradation. Two types of thermal degradation products are formed: volatile and non-volatile (Bonnie dan Choo 1999).

Tofu is one of food products that can be consumed as side dish and used widely in our society. Tofu has high water content, it´s about 86 %. This condition cause hydrolysis reaction during frying so the quality of oil will be decrease. Based on this fact, it is important to conduct research in order to know the change of chemical properties in cooking oil (RBDPO) and to examine the level of carotene degradation during frying process.

The aim of this research was to know the change of chemical properties and carotene total of used pan-frying oil (RDBPO) during frying of Tofu until five cycle of frying.

MATERIALS AND METHODS

Materials

RBDPO was purchased at the supermarket, Tofu was purchased at the local market, NaOH, ethanol 95%, Starch solution, phenolftalein, acetic acid glassial, chloroform, saturated KI, aquadest, $Na_2S_2O_3$ 0.1 N, and Hexsan.

Methods

This research was divided into two step i.e. :

1. Chemical analysis of RBDPO properties (moisture content, FFA value, peroxide value and total of carotene)
2. Chemical analysis of used deep-frying oil after 5 cycles of frying includes moisture content, FFA value, peroxide value and total of carotene.

Comparison between tofu and fresh oil was 1 : 4, and the end of each frying cycle, used deep-frying oil was collected for analysis. Data was analysis using descriptive method and presented on graph.

RESULTS AND DISCUSSION

Tofu Maturity Index

Tofu maturity index is yellowish brown. The change of color which used as maturity index will be presented in Figure 1. In the frying process, food, such as vegetables, meat, or seafood, is brought in direct contact with hot oil. The food surface becomes golden yellow to dark brown in color and develops a pleasant fried food flavor (Gupta 2005).

Moisture Content

Result (Figure 2) showed that moisture content of used deep – frying oil (RBDPO) was increased non significantly during frying of tofu. This result indicated that moisture content of RDBPO was below of maximum level of SNI 01-3741-2002 by 0,1 % for quality I and 0,3 % for quality II. Low moisture content of used deep frying oil caused by temperature of frying (160^{o}C) was higher than temperatur of water boil point (100^{o}C). Gupta (2005) reported that in this process, the hot oil supplies the heat to the product being fried. Heat turns the internal moisture of the food product into water vapor. The water vapor comes out of the product through the outer surface.

1a

1b

Figure 1. Raw tofu (1a); Tofu after cooking (1b)

Free Fatty Acid (FFA) Content

Free fatty acid is indicator of hydrolytic degradation process that usually used as control parameter of fresh oil and used deep-frying oil. In many countries, critical level of free fatty acid content were 2,5 % and 4,5 % (Rossell, 2001). Result in Figure 3 showed that free fatty acid content of used deep frying oil would enhance after frying of tofu. This condition caused by hydrolytic process which is exposure between hot oil and water in foodstuff was occurred for long time during processing. Based on analysis, tofu had high moisture content by 83,90 %.

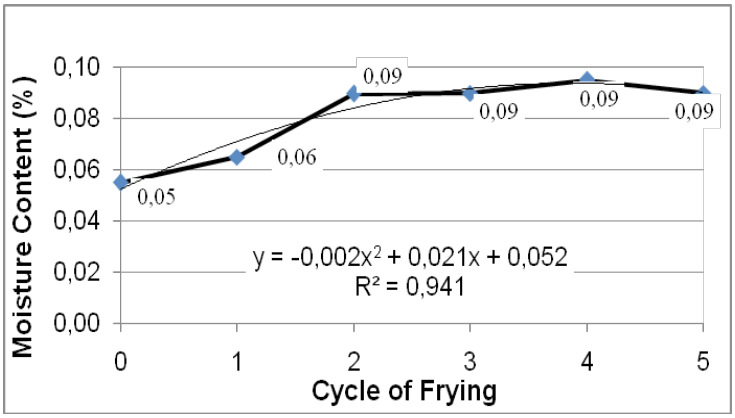

Figure 2. Graph of increasing moisture content (%) in used deep-frying oil after five cycles frying of tofu.

Berger (1984) reported that hydrolytic process could be stimulated by water and mostly occurred when wet products were fried. Gupta (2005) reported that the oil undergoes the following chemical reactions during frying process, such as : hydrolysis, autoxidation, oxidative polymerization, and thermal polymerization. In hydrolysis process, an oil (triacylglycerol, also known as triglyceride) molecule reacts with a molecule of water, releasing a molecule of fatty acid, commonly known as free fatty acid (FFA), and a molecule of diacylglycerol (DG, also called diglyceride).

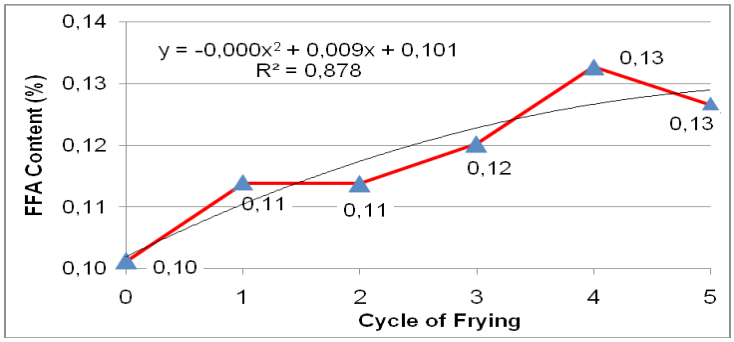

Figure 3 Graph enhancement of free fatty acid content (%) in used depp-frying oil after five cycles frying of tofu.

Peroxide value

Result in Figure 4 indicated that peroxide value of used deep-frying oil was increased. Enhancement of peroxide value in used deep-frying oil after frying of tofu caused by thermal process where expose between oil and oxygen in the air during frying would form hydroperoxide.

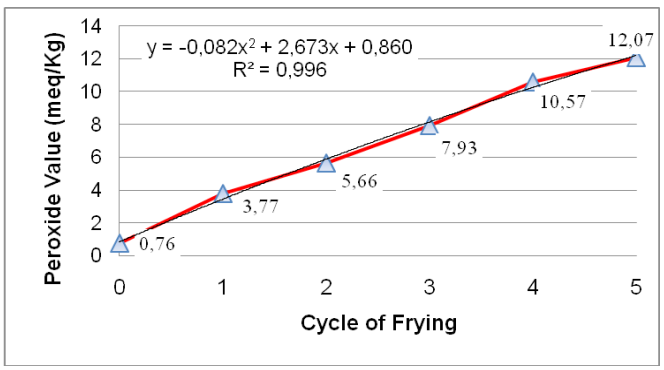

Figure 4. Graph enhancement of peroxide value (meq/kg) in used deep-frying oil after five cycles frying of tofu.

Autoxidation is one of the major reactions taking place during frying. Autoxidation of unsaturated fatty acids is initiated by a free radical, which is formed in the oil when an unsaturated fatty acid is exposed to oxygen in the presence of a metal initiator, such as iron, nickel, or copper. Heat generally accelerates the process of free radical formation and the subsequent reaction steps forming a molecule of hydroperoxide (Gupta 2005).

Carotene Content

Based on result in Figure 5 showed that high degradation of carotene total was occur during frying. This caused by the temperature of frying was high (160°C) and exposed between oil and oxygen during frying leads to increasing of hydroperoxide and accelerates degradation of carotene. Carotenes are sensitive to oxygen and light. The oxidation of carotenes is accelerated by hydroperoxides generated from lipid oxidation, leading to discoloration and bleaching (Sundram 2007).

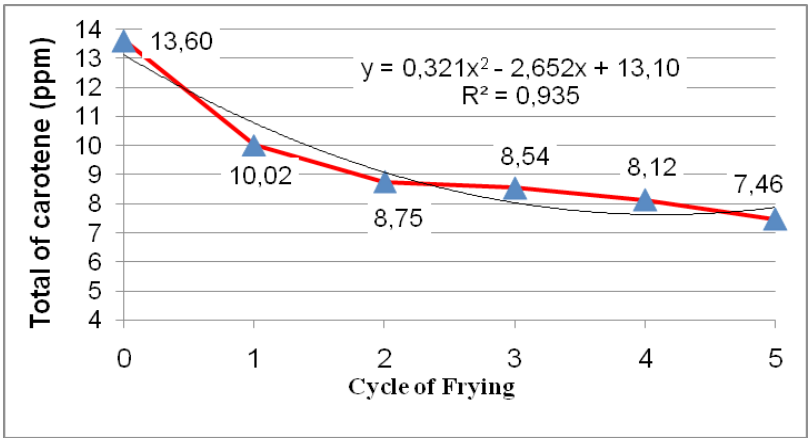

Figure 5. Graph of carotene total value (ppm) in used deep-frying oil after five cycles frying of tofu.

CONCLUSION

Results conclude that moisture content of used deep-frying oil was increase nonsignificantly after five cycles frying of tofu. Free fatty acid and peroxide value were increase and high degradation of carotene was found in used deep frying oil.

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IMPROVING THE NUTRITION OF PURPLE SWEET POTATO (*Ipomoea batatas L)* THROUGH BIOFERMENTATION OF *Aspergillus niger* AS FEED SUBSTANCE CONTAINING ANTIOXIDANTS

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ABSTRACT

The study aimed to determine the increasing of purple sweet potato (*Ipomoea batatas* L) nutrients through biofermentation of *Aspergillus niger* as feed substance that contains antioxidants. The study used a completely randomized design (CRD) with two treatments, which were purple sweet potato without *Aspergillus niger* fermentation (U1) and purple sweet potato with *Aspergillus niger* fermentation (U2). Each treatment had five replicates. Variables observed were the concentration of crude protein (CP), gross energy (GE), ether exctract (EE), crude fiber (CF), calcium, phosphorus, tannins, anthocyanins, and antioxidant capacity. The data were analyzed by T-test (Sastrosupadi, 2000). The results showed that the purple sweet potato that fermented by *Aspergillus niger* has increased nutrient value of protein, gross energy, phosphorus, anthocyanins, and antioxidant capacity, but the energy extract, crude fiber, calcium, and tannins were decreased significantly ($P < 0.05$) compared with the nutrient value of unfermented purple sweet potato. It can be concluded that the fermentation of purple sweet potato (*Ipomoea batatas* L.) with *Aspergillus niger* could improve the nutrient content of feed substance that contain antioxidants.

Keywords: purple sweet potato, biofermentation, content of nutrients, anthocyanins, and antioxidant capacity.

INTRODUCTION

Sweet potato (*Ipomoea batatas* L.) is one of the food crops which can grow throughout Indonesia. Sweet potato is one of fourth highest sources of non-rice carbohydrate after rice, maize, and cassava, as well as to increase availability and diversification of food. As a source of food, sweet potato contain of energy, β-carotene, vitamin C, niacin, riboflavin, thiamin, and minerals. Therefore, these commodities have important role, both in the supply of foodstuffs and industrial raw materials and feed (Ambarsari *et al.,* 2009).

Sweet potato productivity is affected by the environmental growth and also the adaptability of varieties to the environment (Trisnawati *et al*, 2005). Among types of them are white, yellow and purple. Tubers of purple potatoes have distinctive chemical content. Susilawati and Medikasari (2008) found that purple sweet potato meal contain of protein, fiber, and fat were 2.79%, 4.72%, 0.81%., while tuber of purple sweet potato contains of chemical composition of vitamin C, crude ptotein and crude fiber were levels 17.13%, 1.64% and 8.61% (Trisnawati *et al*, 2005).

Improving the nutrition of purple sweet potato could be done with mold and amoniated (Wydianto *et al*., 1995) such as *Aspergillus niger* and urea. *Aspergillus niger* produced enzyms such as selulase, glucoamylase. pectin lyase, and alpha-amylase (Mushtadi *et al*., 1992) which could degrade the crude fiber into simple sugars, and urea as a source of amino groups, the presence of deamination and transamination, forming protein, thus increasing the fermentable material by *Aspergillus niger*.

Abidin (2009) has examined the fermentation with the lees tapioca solid media could increase the protein content from 0.96% to 19.63%. Yadnya *et al.* (2007) have been tried to give sawdust biofermentation with Lactobacillus complex and amoniated by urea that increased crude protein from 0.90% to 9.1% and decreased crude fiber content of 83.0% to 48.5%. Palinka (2011) reported that oil sludge fermentation with *Aspergillus niger* can boost the crude protein content of crude fiber decreased from 16.3% to 13.8%. Further they've tried to give oil sludge fermentation (LSF) with cedar 0; 5%, 10% and 15% and indicated that increased the conversion ration, and increased consumption of dry matter significantly in broilers. Roeswandy (2006) reported that the utilization of sludge fermentation of *Aspergillus niger* SAIT in the ration at 0%, 10%, 20%, 30%, did not affect the weight of cattle, carcass weight and carcass percentage, abdominal fat while on a decline with increasing high content of LSF in the ration.

Purple sweet potato (*Ipomoea batatas* L) in addition to protein, fat, and crude fiber, also contains anthocyanins. Aripnur (2010) reported that total crop anthocyanin content range from 20 mg/100 g to 600 mg/100 g fresh weight. Suprapta *et al*. (2004) have been examined the levels of anthocyanin in

tubers of purple sweet potatoes quite high as 110-210 mg per 100 g. Hasim and Joseph (2008) states that anthocyanins play a role in preventing the onset of aging, decrease of memory, and dementia, polyp, stomach pain suffer (stomach acid), coronary heart disease, cancer and degenerative diseases, such as atherosclerosis. In addition, anthocyanins are also have the ability as antimutagenic and acarsinogenic. Almost all of the nutrients content in the purple sweet potato could support the ability to fight against coronary heart attack.

Consumers have a tendency to choose higher quality of meat, especially meat which content of low cholesterol and healthy, because in rations containing tubers purple sweet potatoes (*Ipomoea batatas* L), rich in anthocyanins premises contain of flavonid that are antioxidants and can reduce LDL cholesterol (low density lipoproteins) and raise HDL (high density lipoprotein) (Marilyn Sterling, 2011).

Based on the above fact, so the research held which entitled: "Improving nutrition of purple sweet potato (*Ipomoea batatas* L) through biofermentation of *Aspergillus niger* as feed that contain of antioxidants".

MATERIALS AND METHOD

The proximate analysis of tubers from purple sweet potatoes (*Ipomoea batatas* L) including crude protein (CP), gross energy (GE), ether extract (EE), crude fiber (CF), calcium and phosphorus were carried out at Animal Nutrition Laboratory, Faculty of Animal Husbandry, Udayana University. Test for anthocyanins, tannins, and antioxidant capacity conducted at the Laboratory of Chemistry and Microbiology, Faculty of Agricultural Technology, Udayana University. The research was conducted beteween 1 May – 6 September 2011.

Materials and Equipment

Tubers of purple sweet potatoes (*Ipomoea batatas* L) obtained in the Banyuwai (Agus Gunawan). *Aspergillus niger* used in this study were obtained from the Institute for Agricultural Technology (BPTP) Denpasar.

The chemicals used in this study include sulfuric acid, Sodium Hydroxide 50%, 2% Borax acid, hydrochloric acid 0,, 1N, catalyst tablets, etahanol, acetone, Follin-Denis reagent, sodium carbonate, pH 1 buffer solution and pH 4.5.

The equipment used in this study were weight scale, oven, dessicator, test tubes, burette, erlenmeyer, bomb calorimeter, spectrophotometer, pipette, measuring glass, beaker glass, centrifuge, and termometer.

Methods

Experimental design used in this study was Completely Randomized Design (CRD) with two treatments were purple sweet potato without *Aspergillus niger* fermentation (U1) and purple sweet potato with *Aspergillus niger* fermentation (U2). Each treatment with five replications.

Fermentation purple sweet potato (*Ipomoea batatas* L)

Before the fermentation process takes place purple sweet potato implemented first activation process *Aspergillus niger*.

The activation process require tools include a clean plastic tub, aerator, whereas the necessary ingredients are sugar, and urea each 1% NPK as much of the volume of water. Water that used well water that contains no chlorine. To maintain sterility boiled water first reach the temperature of 100^{o}C, then cooled, last entered *Aspergillus niger* seed as much as 1% (Guntoro, 2008). The activation process of *Aspergillus niger* as in Figure 1 ..

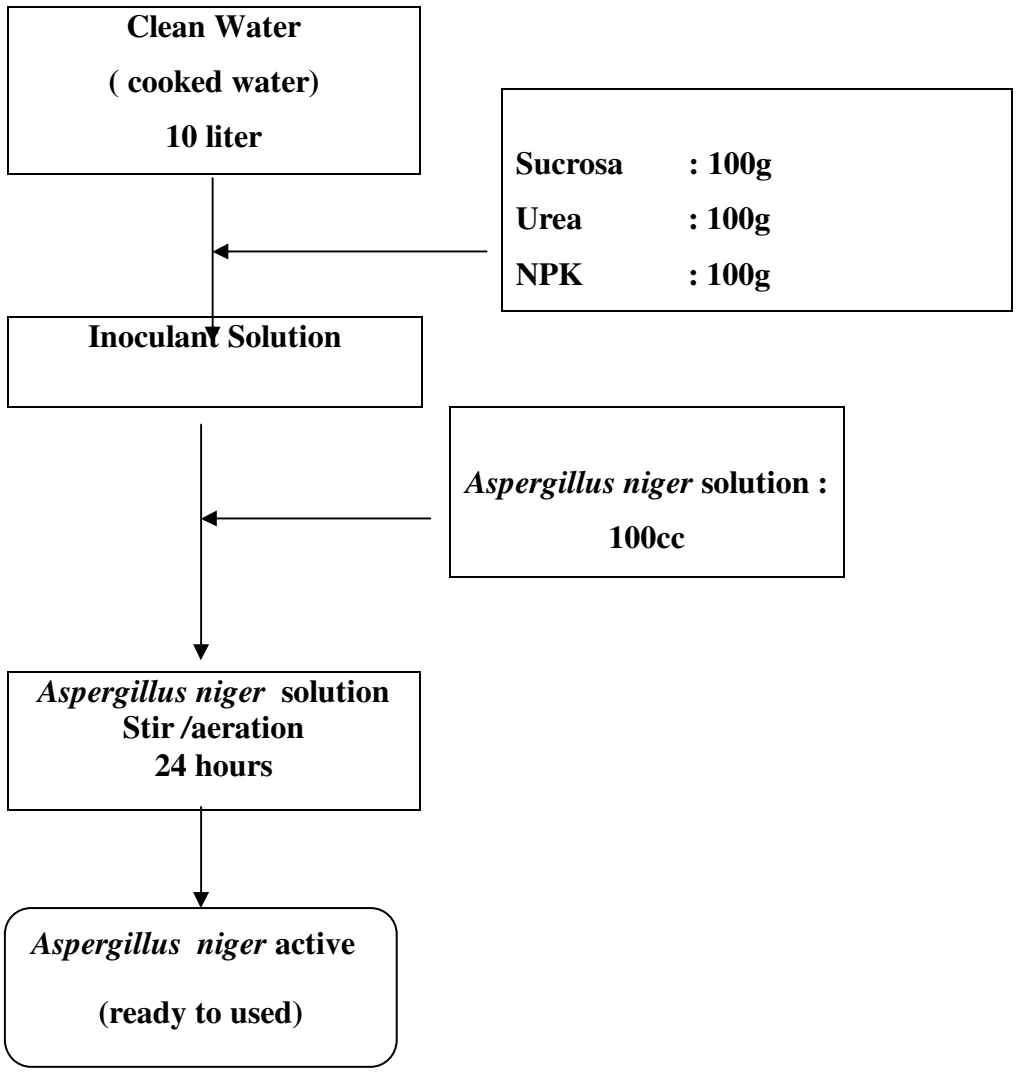

Figure 1. Activation *Aspergillus niger* (Guntoro, 2008)

Tubers of purple sweet potatoes (*Ipomoea batatas* L) are already in the form of flour sack placed on a bed of placed on top of the bamboo strips, then sprayed with *Aspergillus niger* that has been activated until the water solution 50% (when fists are not broken). The cover then covered by a sack and left for 6 days after fermentation for 6 days and then dried in the sun, so it is ready to be analyzed in the laboratory.

Variables Observed:

Variables observed were crude protein (CP), gross energy (GE), ether exctract (EE), crude fiber (CF), levels of anthocyanins, tannins, and antioxidant capacity.

Statistics Analysis

The obtained data were analyzed by T test (Sastrosupadi, 2000).

RESULTS AND DISCUSSION

Proximate Analysis

Tubers of purple sweet potatoes (*Ipomoea batatas* L) without fermented by *Aspergillus niger* (U1) showed that the value of proximate analysis were lower ($P<0.05$) than tubers of purple sweet potatoes that fermented by *Aspergillus niger* (U2) (Table 1).

Table 1. Nutrient of Purple Sweet Potato (*Ipomoea batatas* L) with and without *Aspergillus niger* Biofermentation

Treatments	Proximate Analysis				
	CP(%)	GE(Kcal/kg)	EE(%)	CF(%)	Ca(mg/kg)
U1*	3.97	3466.04	0.69	4.53	690.93
U2*	6.47	3817.50	0.31	2.99	799.8

Note : * Means in the row with different superscript significantly differrent (P< 0,05).

Based on Table 1, showed that the tubers purple sweet potatoes fermentation (U2) could increase the crude protein. This may be due to the presence of alkaline compounds such as urea could apart lignoselulose bond, so that the enzymes would be easier to degrade the compounds of purple sweet potato crude fiber (Wydianto *et al.,* 1995). The existence of enzymes that produced by *Aspergillus niger*, such as cellulase, glucoamylase, pectin lyase, alpha-amylase (Muschtadi *et al*., 1992), which could apart crude fiber into simple compounds called glucose, so the fiber will decrease from 5.85% to 2.99%. The presence of urea as a source of amino groups, so the premises of deamination reactions, and transamination (Soeharsono, 1984), is formed of proteins, so the result of tubers purple sweet potatoes fermentation may increase the levels of protein and decrease the crude fiber significantly. The results are similar with those obtained by Palinka (2011) who obtained that the result of fermentation in the oil sludge could increase the levels of crude protein from 13.25% to 35.43% and decrease the crude fiber content from 16.3% to 13.8%.

The fermented tubers of purple sweet potatoes generate gross energy (GE) is higher than the tubers purple sweet potatoes without fermented (Table 1). So, there some crude fiber that could be digested by enzymes contain of *Aspergillus niger*, more crude fiber are digested and will be produced more simple compounds, so that more energy is generated through Crebs cycle or glycolysis (Murray *et al*., 2009). Fermentation is an attempt to get energy, so more a process rather than fat oxidation, so that the fat content in the fermented tubers of purple sweet potatoes decreased significantly (P<0.05). The same thing ever done by Yadnya *et al.* (2007) which examined biofermentation of sawdust as a source of fiber with complex *Lactobacillus* bacteria that could increase the levels of protein, energy, and decrease crude fiber and fat significantly (P<0,05).

The effect of fermentation in tubers of purple sweet potatoes, reduce the biological calcium from 3.52% to 2.44%. This may be due to fermentation of calcium used as a catalyst in the process so much calcium metabolism is used causing calcium content in tubers of purple sweet potatoes were decrease, whereas the increase of phosphorus occurs 690.03 mg/kg to 799.81 mg/kg. This is due to the fermentation process as acompound phytate phosphorus partially hydrolyzed so that will be released, so that the phosphorus concentration is significantly.

Tanin Concentration, Anthocyanins and Antioxidant Capacity

Tuber of purple sweet potato that fermented by *Aspergillus niger* (U2) reduce the concentration of tannins, and increase the content of anthocyanin and antioxidant capacity (Table 2).

Table 2. Tannins Concentration, Anthocyanins, and Antioxidant Capacity in Tuber of Purple Sweet Potato Fermented by *Aspergillus niger*

Treatments	Variables		
	Tanin (%)	Anthocyanin(mg/100g)	Antioxidant(mg/100g)
U1*	19.68	20.25	0,57
U2*	8.30	52.47	3.06

Note : * Means in the row with different superscript significantly differrent (P<0,05).

Apparently, concentration tannins in treatment U1 is decrease from 19.68% to 8.30%. This may be due to enzymes released by *Aspergillus niger,* so most of the tannins degraded into simple compounds, so the levels of tannins in tubers of purple sweet potatoes fermentation may decrease. The process of fermentation in tubers of purple potatoes (as a source of carbohydrate) will form a simple compounds with

enzymes from *Aspergillus niger* which produce ethanol compound (and suspected of compounds will be turned into ethanol as a basis for the formation of polyphenol compounds Anthocyianin). The increasing of anthocyanin content in tubers of purple sweet potatoes is caused by the significantly increase of antioxidant capacity (Table 2). The presence of antioxidants substances could be reduce the effect of free radicals, thus play a role in preventing the onset of aging, cancer and degenerative diseases such as atherosclerosis. In addition, anthocyanins are also have own ability as antimutagenic and mutagenic and carcinogenic content in foodstuffs and processed products, to prevent liver disfunction, antihypertensi, and lowers blood sugar levels (antihyperglisemic) (Yusuf *et al.,* 2008).

CONCLUSION

From the results of this study, it can be concluded that the tubers of purple sweet potato (*Ipomoea batatas* L) that fermented by *Aspergillus niger* could increase the nutrients and substances, including anthocyanins and antioxidant capacity in fermented purple sweet potato tuber.

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THE DETERMINATION OF ETHANOL LEVELS IN *ARAK* BY GAS CHROMATOGRAPHY TECHNIQUES

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ABSTRACT

Ethanol is an alcohol allowed in alcoholic beverages at a certain concentration. Drinking of ethanol at a certain amount is safe as its metabolism can produce energy in the form of adenosine triphosphate (ATP). However, drinking alcoholic beverage excessively for long periods can be toxic to the human body which can leads to disease called alcoholism. One of Balinese famous alcoholic beverage is *arak* which ethanol concentration varies depend on how it is produced. In anticipation of excessive alcohol consumption, it is necessary to investigate the levels of ethanol content in *arak* which is readily available in the market. The aim of this research is to determine the ethanol content in *arak* that sold in some villages in the Regency of Badung. The determination is carried out using gas chromatography technique. The results show that the ethanol content in the samples varied between 20.09 and 70.09% w/v.

Keywords: ethanol, *arak*, gas chromatography

INTRODUCTION

Alcohol is a clear liquid that evaporates easily, have a distinctive smell and feel cool when on the skin. Alcohol is formed from fermentation of plant sugars containing a carbohydrate that is assisted by the plant organism that serves leaven the sugars into alcohol compound. Types that can be consumed alcohol is ethanol. Ethanol has the chemical formula CH_3-CH_2-OH or C_2H_5OH, which boils at 78.3°C (172.9 °F) and freezes at -117.3°C (-243.1 °F) (Anomin, 2010).

Nowadays a lot of use of liquor with alcohol content exceeding 55% on the market. One of them is *arak* with a high ethanol content. Lately many deaths occur from consuming liquor oplosan with a sufficiently high concentration of ethanol (Adiprabowo, 2008).

Arak is a typical Balinese alcoholic beverages. *Arak* is usually consumed as body warmers. But the *arak* with high levels of ethanol can be used as fuel. *Arak* is distilled from the sap of coconut with ethanol content varying depending on the sap and fermentation duration (Yeliana, 2005; Suaniti, 2009).

Consuming excessive amount of ethanol may cause liver damage. Ethanol is chemical that can cause various effects on the body because it will go through the process of detoxification. Liver is an important organ of the body to detoxify chemicals that harm the body, including ethanol.

This study aims to determine the ethanol content in *arak* on the market.

MATERIAL AND METHODS

The study is conducted in Denpasar Branch Police Forensic Laboratory. Tools are volumetric flask, micro pipette, measuring pipette, a glass beaker, gas chromatography GC-6890-N Technologies aligent Network GC System, carrier gas He (Helium). Materials are methanol, ethanol, propanol, buthanol, acetic acid, distilled water. The samples are *arak* and are taken on the market randomly.

Preparation of standard solution 1000 ppm

Pipette solution 12.6 mL of each standard compounds (ethanol, methanol, propanol, butanol, acetic acid) then dilute each standard with aquades in 10 mL volumetric flask, to obtain a solution of 1000 ppm.

Preparation of standard solution in the mixture

Each standard solution 1000 ppm prepare from a solution of methanol, ethanol, butanol, acetic acid in the mixture with a ratio of 1: 1: 1: 1. Furthermore, the solution pipette respectively 0.25 mL, 0.5 mL:

1.0 mL, 2.0 mL, 3.0 mL thus obtain solution with a concentration of 25ppm; 50ppm; 100ppm: 200ppm; 300ppm.

Arak sample

Preparing *arak* samples are diluted 1000 times using aquades. Selectivity of test is done first by injecting standard solutions into the chromatographic instrument in a concentration of 10 ppm as many as 1.0 mL. subsequently mixture solution of 1.0 mL is injected into the chromatographic instrument. Chromatogram will show the retention time before and after mixing together. Determination of ethanol content in *arak* samples by as much as 1.0 mL sample is injected into the chromatographic instrument. Chromatograms obtain from the measurement results and calculate of the peak area.

RESULTS AND DISCUSSION

The results of measurements of standard solution of ethanol obtain from a different area each concentration. Having obtain outside the area of each concentration is done of validation linearity which serves to prove the linear relationship between concentration and the area. Furthermore, the regression equation is determined calibration curve of ethanol. While the linear regression line equation of ethanol that is: y = 0.2247 x + 1.3967 with a correlation coefficient (r) = 0.9624 (Figure 1). The correlation coefficient values indicate that the detector gives a linear response between concentration and the area.

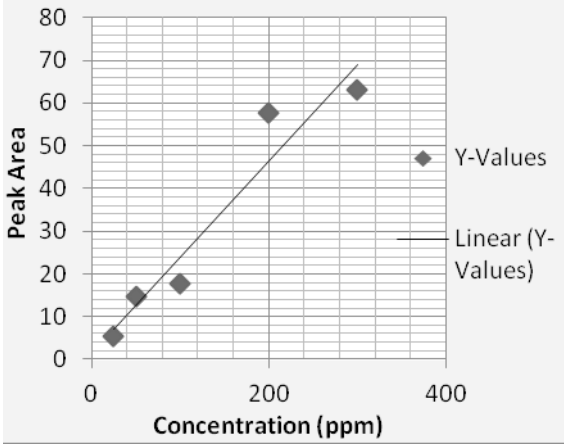

Figure 1. Calibration Curve

Arak samples are obtained from the Markets, which is taken at random. Samples of *arak* that had been diluted 1000 times injected into gas chromatograph equipment. By using the data area of the sample chromatogram and the equation of the regression line of standard compounds, it can be determined levels of ethanol is contained in the sample. Ethanol levels and peak area in the *arak* samples are show in Figure 2.

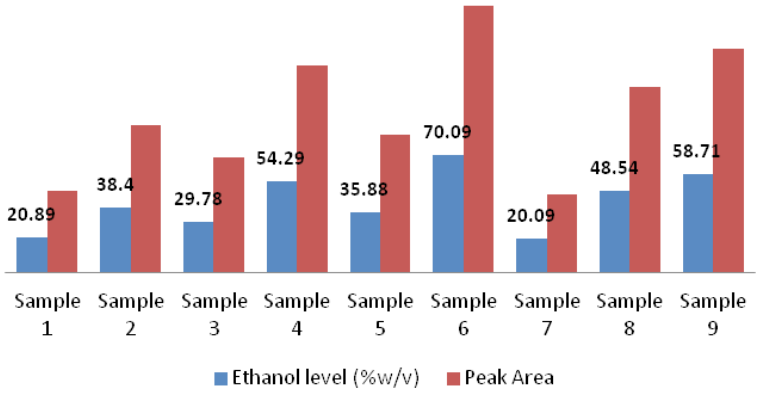

Figure 2 Ethanol levels in *arak*

According to the legislation the Minister of Health of Indonesia Number 1516/A/SK/V/81 and Number 86/Menkes/Per/IV/77, levels of alcohol in liquor or alcoholic beverages is expressed in units of percent by volume or proof (Goldfrank, et al ., 2002). Ethanol content in beverages classified into three namely: class A (beer, 1-5%), group B (wine / wine, l5-20%), and group C (whiskey, brandy, genever, cognac, *arak*, gin, rum , and vodka, 20-55%). Based on research results, which acquired the bulk of ethanol content in *arak* that circulate in the markets in some village in Badung regency levels are obtained between 20-55% are categorized into the category C so it is worth as a beverage. Although this also required a control, should drink adequate amounts of just warm body, because alcohol metabolism occurs primarily in the liver. Drinking alcohol with a low dose can Produce energy in the form of adenosine triphosphate / ATP (Almatsier, 2006). Ethanol content is also supported previous research that the ethanol content in coconut and palm domestic industrial output, respectively, 31.20% and 46.45% (Suaniti, 2009).

Levels that exceeded also obtained on two samples each of six samples obtained 58.71% and 70.09% of samples obtained 9, so it needs to be done periodically research alcohol content in *arak* before consuming or circulating in the community. *Arak* with levels above the permitted content can be as an alternative fuel materials (Yeliana and Wirawan, 2005).

CONCLUSION

Ethanol standard solution gives good results both qualitatively and quantitatively are obtained from the linear regression equation of ethanol that is: $y = 0.2247 x + 1.3967$ with a correlation coefficient (r) = 0.9624. Ethanol level in *arak* in some villages are obtained vary levels between 20.09 and 70.09% (w/v).

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FOOD SAFETY BY VEGETARIAN LIFE STYLE

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INTRODUCTION

Human body consist of unlimited (billions) of cells which form tissues or organs of the body such as muscles, heart, lever, intestine, brain, skin, etc. Every organ has different function to support this life. To maintain and protect the body in order to function normally, nutrient which is come from food is needed. Food not only for satisfy our tongue and stop our hungry, but also for the spirit soul. Vedanta divides food into three categories or three types of food: *satvika* (goodness) food, *rajasika* (passion) food, and *tamasika* (ignorance) food.

SATVIKA FOOD healthy fruits

Vegetarian is food of *satvika* category, that able to bring people to the higher level of spiritual realization.

Food play an important role for continuation the life of living being, because in the food consist of nutrient that is necessary for the cells of the body in order to running their function. Many people are vegetarian, that mean this life style must be very healthful and useful. All over the world now are going to promote and encourage people to become vegetarian. Many slogans such as "safe our planet, stop global warming" etc. How to safe our planet and stop global warming? By vegetarian life style, peaceful and be happy. Non violent of life style is life without eating everything that resulted from killing animal including chicken, fish, and egg. Life style now already changes; mostly people are eating meat just to satisfy the tongue, for prestige, social status etc. Many diseases caused by food factors such as heart diseases, cancer, hyper tension, and so on. Food also very much influent the emotional, ones who eat meat very easily get angry due to unable to control their emotion. General statement said: "what you eat, that you are".

Many kind of fruits produced by land (mother *Bhumi*)

COMPARISON BETWEEN MEAT AND VEGETABLES

To maintain this life, our body is basically need protein, carbohydrate, fat and salt. Food that contain a lot of protein: peas, cheese, milk, and beans. Contain a lot of carbohydrate: wheat, corn, rice, fruits, and almost all of vegetables.

Because all of substances that needed by the body are available in the plant or crops, therefore meat actually not very important and not needed by our body. It is wrong, if we think that meat is the only one of our protein source, and never be substitute by other type of food. Vegetables rich of fiber, while meat not consist of fiber, fiber is very useful for maintain good digestion and absorb water (Wardhana, 2010).

If we seriously observe every weight of plant or vegetables, it has food value which is very amazingly and more useful than that of food staff resulted from animal. Let we see the comparison among the vegetarianism and meat eater. Food that resulted from meat is not essential for the human body. One whose habitually eat meat is not better physically and mentally as compared to that of vegetarianism. Fruits and vegetables are very important for the development of the body, therefore for the children who are in the stage of development is better to consume fruits and vegetable than meat.

Many type of diseases come from meat. For example we can find heart worm in the cow and buffalo meat, and also in the gout and might be also in the pig meat. In the human body meat will produce a rubbish or an extract that cause poison. Our kidneys will work harder to remove this poison, and as a result the kidneys become sick or kidneys failure. Actually, no one in this world wants to be infected by any type of diseases.

There is no physiologist will not agree that human must be life upon vegetarian food (vegetable and fruits). Anatomy proved that human body is similar to animal fruits eater, is formed to eat fruits, grain and plant which is contain starch. Natural food for human from composition point of view is consisting of fruits, roots, and vegetables. Eating meat mean we should kill animal to get meat. Killing is violent and considered sinful activity that will give bad karmic reaction, especially the killing of the cow. Cow is very nice animal because she produces milk which is rich of protein and very useful for our health. Therefore, cow is considered one of our mothers.

IF WE **LOVE** HER, SHE WILL **GIVE US MORE** THAN WHAT WE NEED FOR MAINTENANCE OF OUR LIFE

Vegetarian food is very healthy and strongly recommended for pregnant mother. Many mothers think that food they eat just for themselves. But actually it is not. If mother want to safe both soul and health of mother and baby, it is suggested to be very careful selecting menu, and recommended just take vegetarian food (Dharmayasa, 1989).

May be it is said that man who is vegetarian weaker than that who is meat eater. But the fact is different. History from the past has recorded that vegetarianism is stronger, such as bike racing in Germany, all the top rank were vegetarian. Olympic game champion many is vegetarianism. The strength and ability of man who meat eater is quickly decrease, due to meat is immediately becoming rot in the intestine, and is needed quickly new energy supply (Dharmayasa, 1984).

THESE PREPARATIONS VERY HEALTHY, DELICIOUS, FIRST OFFER TO SRI KRISHNA, THAN IT BECOMES "***PRASADAM***", ALL PREPARATIONS WERE MADE BASED ON **LOVE** AND **DEVOTION**

Everyone will agree that vegetarian food is cheaper than meat. Vegetables and roots are very cheap. Vegetarian food actually is giving many benefits, not only for saving money but also more acceptable to the environment. It can be concluded that vegetarian food is the best food for human being.

Rice is a gift from Krishna. Rice, one of the oldest grains known to man, has throughout history been a staple in the diet of nearly three fourths of the world's people, and it remain so today (Visaka, 1982).

PHILOSOPHY

The role of man in nature is to inquire about the meaning of the Absolute Truth, the higher knowledge and in this connection man will be able to see clearly the web of life and balance of nature (Singh, 2011).

'Patram puspam phalam toyam, yo me bhaktya prayacchati, tad aham bhaktya-upahrtam, asnami prayatatmanah' If one offers Me with love and devotion a leaf, a flower, fruit or water, I will accept it (BG 9.26). If one wishes to reach the goal of life – the transcendental loving service of God – then he should find out what the Lord desires of him. One who loves Krishna will give Him whatever He wants, and avoids offering anything which is unasked for. Sri Krishna explains that only the remains of sacrifice are purified

and fit for consumption by those who are seeking advancement in life and release from the clutches of the material entanglement (BG 3.13). Preparing nice, simple vegetable dishes, offering them before the picture or Deity of Lord Krishna and bowing down and praying for Him to accept such a humble offering, enable one to advance steadily in life, to purify the body, and to create fine brain tissues which will lead to clear thinking. Above all, the offering should be made with an attitude of love (Prabhupada, 1971).

If one offers Me with love and devotion a leaf, a flower, fruit or water, I will accept it (BG 9.26)

CONCLUSION

There are some conclusions can be drawn as follows:

1. To maintain life we need food.
2. There are three types of food: *satvika* (goodness), *rajasika* (passion) and *tamasika* (ignorance) food.
3. Vegetarian is *satvika* (nonviolent or goodness) food category.
4. Vegetarian is natural food for human being, if it is offering to Lord Krishna it become *prasadam* (free karmic reaction diet).
5. Vegetarian safe our future food resource.

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THE MODEL OF WEIGHT LOSSES ON POTATOES DISTRIBUTION CHAIN FROM FARMERS IN BATURITI, TABANAN REGENCY UNTIL RETAILER IN DENPASAR

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ABSTRACT

This study aims to determine the model of weight losses on potatoes distribution chains from producer (farmers) in Baturiti until retailer in Denpasar.

The method used in this research is survey method by using questionnaires given at each stage of potatoes distribution chain from farmers in Baturiti, Tabanan regency, to the retailer in Denpasar with using a purposive sampling method. The data obtained made mathematical models with form of linear, quadratic and exponential. Models of each distribution chain is selected based on the largest correlation coefficient.

The results showed that there are 4 chains of potatoes distribution from farmers in Baturiti, Tabanan regency until retailer in Denpasar. The distribution chain I, consisted of farmers, collectors, wholesalers, and retailers in Denpasar with the weight losses model of $Y = -0.007x^2 + 0.021x + 0.036$. The distribution chain II, consisted of farmers, collectors, wholesalers, and retailers in Baturiti with a model of $Y = 0.001x^2 - 0.024x + 0.079$. The distribution chain III, consisted of farmers, collectors, wholesalers, suppliers and retailers in Denpasar with a model of $Y = 1.182e^{-1.89x}$. The distribution chain IV, consisted of farmers, collectors and wholesalers with a model of $Y = 0.012x^2 - 0.065x + 0.110$. On the distribution chain IV, the total of potatoes weight losses showed the lowest that was 11,49%.

Key words: the potatoes, distribution chain, weight losses and model

INTRODUCTION

Potato (Solanum tuberosum L.) is one of the priority development of horticultural commodities today. Potato production in Indonesia is quite high, and from year to year tends to increase. Potato production in 2007 reached 1,003,732 tons and in 2008 rose to 1,071,543 tons. In 2010 in the province of Bali, the potato became one of the horticultural products of vegetables that proclaimed to be developed in addition to onion (Harsojuwono, *et al*., 2010).

Agribusiness development in potato commodities have good prospects, because the potato could support the diversification program (diversification) of non-rice food is highly nutritious, increase farmers' income as fast potato yield (cash crop), as an export commodity and food industry raw materials.

Development of potatoes to the province centered in Tabanan Bali spread at 5 locations namely : Candi Kuning Village, Baturiti, Baturejo and Antapan, and the District Baturiti (Anonymous, 2002). Tabanan is an area with a predominantly agrarian livelihoods have in agriculture (Tabanan regency has an area of 893.33 km2 (14.9% of the island). According to statistics Tabanan in 2010 showed that the total area of horticultural crops of potatoes reached 200 ha with an area of 300 ha of land development, and productivity reach 18-20 tones / ha and the total average production reaches 7600 tons. Potatoes of the Tabanan Regency the next will be distributed to various areas in Bali, one of which is to Denpasar (Harsojuwono, *et al*., 2010).

Marketing potatoes follow some distribution chains before it reaches the consumer, such as collectors, wholesalers, small traders, retailers, traditional markets and supermarkets. At each distribution chain, potato experience that enables the handling and treatment of potatoes have lost weight. Weight losses occurs because the potatoes continue to make the process of respiration during the distribution. In addition, weight losses can also occur due to handling distribution so that potatoes are not good having damaged / defective and is discarded in the process of sorting. Until now, there is still no definitive information about the model to lose weight in the distribution of potatoes Baturiti District to the City of Denpasar. Therefore it is necessary to do research on models of the potatoes in the path of losing weight distribution.

METHOD

Research conducted by survey method using questionnaire as a data collection tool. Questionnaires distributed at each stage of distribution chains of potato Baturiti district, Tabanan regency, to the city of Denpasar, later created a mathematical model of weight losses.

Location

The research was conducted in the District Baturiti, Tabanan, Bali Province and the City of Denpasar. The basic consideration the location decision of this research is due largely Baturiti District residents are farmers and agribusiness entrepreneurs to develop horticulture, one of which is a commodity potatoes.

Population and Sample

Populations observed in this study were all farmers, collectors, wholesalers, small traders and retailers potatoes in Bali. Based on field surveys in mind that there are 4 groups of farmers in District Baturiti, but only 3 of the farmers' group that developed the potato commodity. Each farmer group consisted of 30 farmers, so many potato farmers in the district population is 90 people Baturiti.

The sample of this study was determined by purposive sampling, with the consideration that the sampling done by following the distribution chain through which the distribution of potatoes from a potato farmer in the subdistrict Baturiti to the consumer in the city of Denpasar.

This study sample consisted of:

a. Potato farmers, a swab of 30 potato growers.
b. Collectors potatoes on distribution chain through which these potatoes.
c. Wholesalers in the distribution chain through which these potatoes.
d. Retailers through which the distribution chain.
e. Potato distributor through which distribution chain.
f. Traders in traditional markets in the district in the city of Denpasar Baturiti and through which distribution chain such potatoes.

Data Collection

The data was collected after randomly distributing questionnaires to the respondents who had been assigned. The questionnaire was read by the researcher, so that respondents understand the meaning of questions compiled by researchers. Answers are recorded by the researchers in the questionnaire (Harsojuwono, 2002). The data sought in this study are:

a. Potato marketing distribution chain
b. Volume sales of all potato harvest (kg)
c. The volume of potatoes that are damaged and discarded at each stage of distribution (kg)

Determination of the distribution chains

The study looked at and studied the chains and distribution stages through which the potato by farmers in the village of Baturiti up to the consumer. Potato marketing distribution chain obtained through questionnaires given at each stage of the distribution of potatoes.

Mathematical Modeling of Weight Losses

This study was conducted to obtain a mathematical model of the relationship between levels of the distribution by percentage weight losses of potato in each distribution chain. Potatoes are harvested by farmers in the district of Tabanan Baturiti, then distributed to the consumers. The distribution of potatoes from farmers in vegetable Baturiti District is divided into several distribution chains. At each respondent at each stage of the path distribution of potatoes to the Denpasar District Baturiti administered questionnaire. The questions in the questionnaire that supports the creation of mathematical models is the degree distribution, the weight of potatoes are bought and sold and thus lose weight potato

unknown variable weight loss (losses) at each stage of distribution chain potatoes.

Data of the questionnaires were tabulated in the next created a mathematical model between the tiers of distribution with weight losses using linear regression model, quadratic regression models and exponential regression. Having obtained three mathematical models of potatoes every distribution chain, then look for the largest correlation coefficient of correlation of these 3 models, to be elected as a weight losses model.

Linear Regresion Model

The equation of the linear model (Harsojuwono, 2002) :

Y = a + bX

Description:
Y = dependent variable, as the weight losses
a, b = Constants
X = independent variable, as the distribution levels

To find the value of (a) and (b) may be done by using formulas as follows:

$a = \sum Y : n$

$b = \sum XY : \sum X^2$

Jika $\sum X = 0$

Quadratic Model

Equations of quadratic model (Harsojuwono, 2002):

$Y = a + bX + CX^2$

Description:
Y = dependent variable, as the weight losses
a, b, c = Constants
X = independent variable, as the distribution levels

To find the coefficient obtained by the formula :

$a = (\sum Y - c\sum X^2) : n$
$b = (\sum XY / \sum X^2)$
$c = (n\sum X^2 Y - (\sum X^2)(\sum Y)) : (n\sum X^4 - (\sum X^2)^2)$
Jika $\sum X = 0$

Exponential Model

Exponential regression model has the function of the equation is as follows :

$Y' = a.X^n$

Which can be simplified into the form of logarithmic function, namely :

log Y '= log a + n (log X)

Description:
Y = dependent variable, as the weight losses
a = Constant
X = independent variable, as the distribution levels.
n = Number of data

If Σx = 0, then the coefficients a and b can be searched by the formula

$$\log a = (\sum \log Y) : n$$

The Correlation Coefficient

Formula used for calculating the correlation coefficient is as follows (Harsojuwono, 2002).

$$r = \frac{n \sum XY - \sum Y \sum Y}{\sqrt{[n \sum X^2 - (\sum X)^2][n \sum Y^2 - (\sum Y)^2]}}$$

Description:
r = correlation coefficient
Y = dependent variable, as the weight losses
X = independent variable, as the distribution levels
n = number of data

If r > 0, then there is a relationship between a dependent variable with independent variables.
r = 0, then there is no relationship between the two independent variables and bound.

Variables observed

The variables observed in this study include distribution chains, weight losses, and distribution levels.

Distribution chains

The study looked at or studied the distribution chain through which the potatoes are produced by manufacturers in the village Baturiti up into the hands of consumers in the city of Denpasar.

The weight losses

The weight losses is the weight difference is acceptable to the weight of potatoes are sold by every level of distribution chains.

The assumptions

1) Assume the distribution levels in potatoes from the distribution chains to the city of Denpasar District Baturiti moves follow a mathematical pattern above the third.
2) Activities conducted within each level of distribution at the same level of distribution assumed to be equal.
3) When switching from one distribution level to the next level is assumed to equal distribution.
4) Depth distribution for each restricted model can not be used if more than specified.

RESULTS AND DISCUSSION

Based on the results of the survey, obtained the distribution chain in the District of potatoes from farmers in Baturiti until retailers in Denpasar through four distribution chains. The distribution chain I, consisted of farmers, collectors, wholesalers, and retailers in Denpasar. The distribution chain II, consisted of farmers, collectors, wholesalers, and retailers in Baturiti. The distribution chain III, consisted of farmers,

collectors, wholesalers, suppliers and retailers in Denpasar. The distribution chain IV, consisted of farmers, collectors and wholesalers.

Mathematical models of Weight Losses on Potatoes Distribution

Based on field research has been conducted and followed by data analysis, so we get a weight losses mathematical model on distribution of potatoes in each chains. The mathematical model on each distribution chain is explained as follows.

Mathematical Model on the Distribution Chain I

Based on the analysis of mathematical models using linear regression, quadratic, and exponential distribution of level relationships with weight losses, then the resulting three mathematical models as shown in Figure 1.

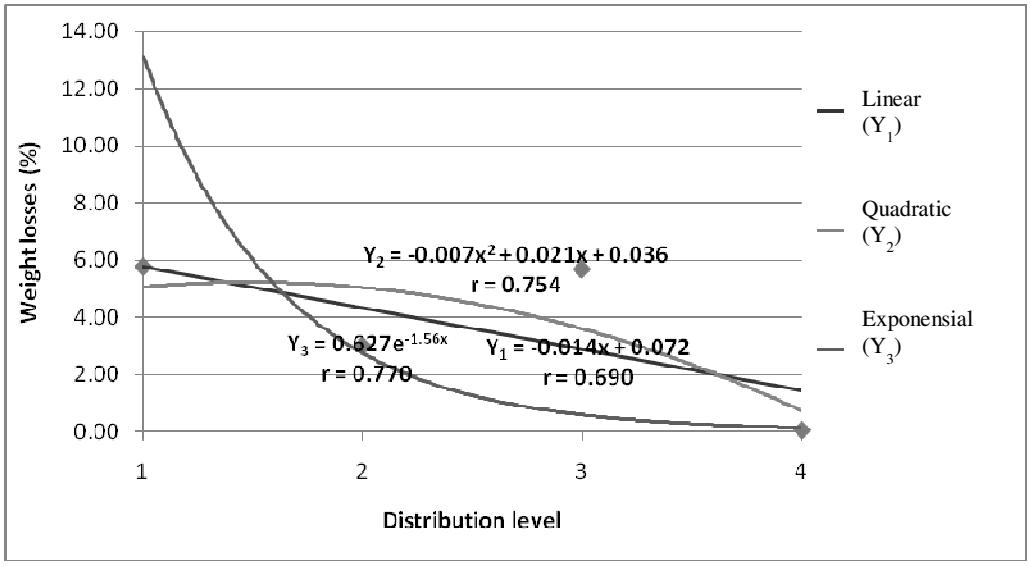

Figure 1. Mathematical model and the correlation coefficient between the weight losses with the distribution level on the distribution chains I.

Figure 1 shows that weight losses model obtained is:

1) $Y1 = -0.014x + 0072$
2) $Y2 = -0.021x + 0036 + 0.007x2$
3) $Y3 = 0.627e^{-1.56x}$

Each of these equations has a significance value and correlation coefficient values which can be seen in Table 1.

Table 1. F-count value, significance and correlation coefficients of each equation at distribution chain I

No	Equation	F-count value	Significance value	Correlation coefficient
1	$Y_1 = -0.014x + 0.072$	1.8247102	0.30928691	0.754
2	$Y_2 = -0.007x^2 + 0.021x + 0.036$	1.070309	0.409577	0.770
3	$Y_3 = 0.627e^{-1.56x}$	0.217223	0.223993	0.690

Through the equation is known that there is a correlation between weight losses of potatoes with distribution levels, and note also that the distribution is one of the factors causing the loss of weight of potatoes in the distribution chain I.

Based on the Figure and Table 1 were known that weight losses model of potatoes to follow the equation of $Y = -0.007x^2 + 0.021x + 0.036$ because the correlation coefficient (r) was a largest with value of 0.770. That was caused the handling on the farmers and retailers levels a worse than the collectors and wholesalers level (Harsojuwono, 2003).

Mathematical Model on the Distribution Chain II

Based on the analysis of mathematical models using linear regression, quadratic and exponential models of the relations of distribution levels and weight losses, then the resulting three mathematical models as shown in Figure 2.

On distribution chain II, to be known 3 weight loss models obtained were:

1) Y1 =-0.018x + 0072
2) Y2 = 0.001x2 - 0.024x + 0079
3) $Y3 = 1.102e^{-1.99x}$

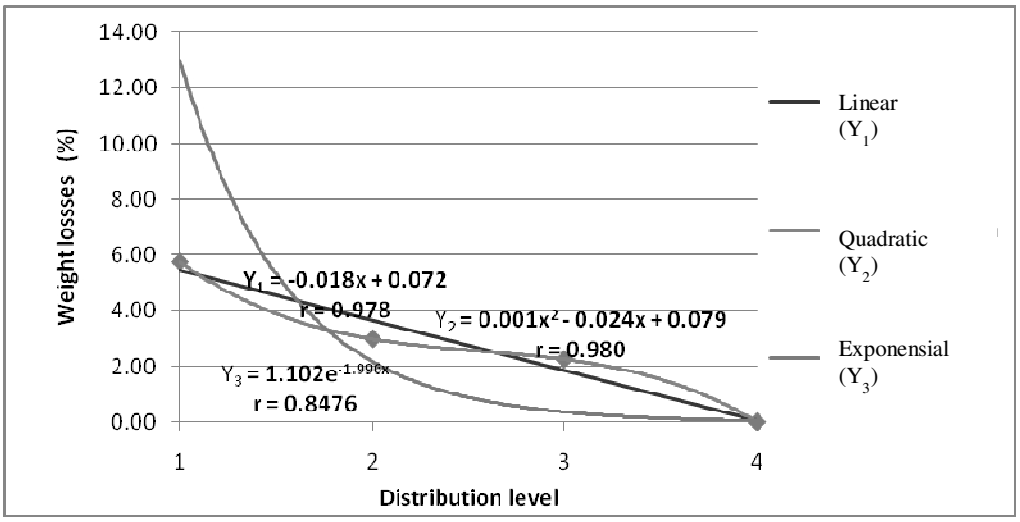

Figure 2. Mathematical model and the correlation coefficient between weight loss with the distribution level on the distribution chain II.

Table 2 shown the equations models, significance and correlation coefficient values of each distribution chain. Based on Table 2, to be known that there is a correlation between weight losses potatoes and distribution levels on each distribution chain.

Table 2. F-count value, significance and correlation coefficients of each equation at distribution chain of II

No	Equation	F-count value	Significance value	Correlation coefficient
1	$Y_1 = -0.018x + 0.072$	46.10371735	0.021009105	0,978
2	$Y_2 = 0.001x^2 - 0.024x + 0.079$	9.243004	0.093296	0,980
3	$Y_3 = 1.102e^{-1.99x}$	3.133212	0.218732	0,847

By looking at the largest correlation coefficient, it can be determined that the weight losses models on distribution chains II tend to follow the mathematical model Y = 0.001x2 - 0.024x + 0079 with a value of r = 0.980. That was caused the handling on the farmers and retailers levels a worse than the collectors and wholesalers level (Harsojuwono, 2005).

Mathematical Model on the Distribution Chain III

Analysis of mathematical models using linear regression, quadratic, and exponential models of the relationship of distribution levels and weight losses. The resulting model as shown in Figure 3.

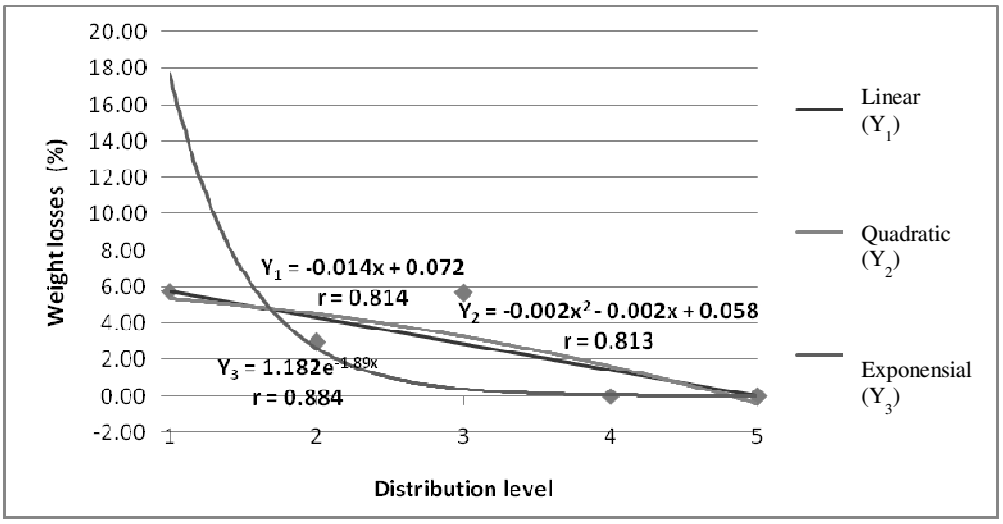

Figure 3. Mathematical model and the correlation coefficient between distribution levels and weight losses on the distribution chain III.

Based on mathematical modeling of the distribution chains III as listed in Figure 3, shows that weight loss model obtained is:
1) Y1 =-0.014x + 0072
2) Y2 =-0.002x2 - 0.002x + 0058
3) Y3 = $1.182e^{-1.89x}$

Each of these equations has a significance and correlation coefficient values which can be seen in Table 3.

Table 3. F-count value, significance and correlation coefficients of each equation at distribution chain III

No	Equation	F-count value	Significance value	Correlation coefficient
1	$Y_1 = -0.014x + 0.072$	5.427352838	0.102178729	0,814
2	$Y_2 = -0.002x^2 - 0.002x + 0.058$	3.122551	0.175382	0,813
3	$Y_3 = 1.182e^{-1.89x}$	3.077328	0.177666	0,884

Table 3 shown that there were correlation between the distribution level dan weight losses of potatoes on the distribution chain IV. By looking at the largest correlation coefficient, it can be determined that the weight losses model on the distribution chain III to follow the model : $Y = 1.182e^{-1.89x}$ with a value of r = 0.884. It was shown that on the distribution level from farmers until retailer, occured decreasing of weight losses. It mean the distribution levels on distribution chain III occured increasing the good handling (Harsojuwono, 2008)

Mathematical Models of Distribution Chain IV

Based on the analysis of mathematical models using linear regression, quadratic, and exponential models of the relationship between distributin levels and weight losses, shown in Figure 4.

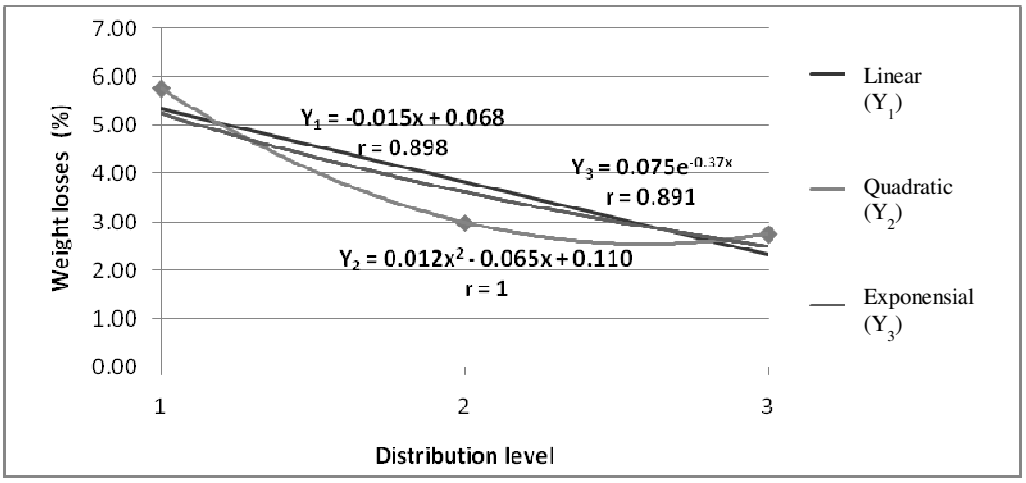

Figure 4. Mathematical model and the correlation coefficient between distribution level and weight losses on the distribution chain IV

Figure 4 shows that the weight losses model obtained is :

1) Y1 =-0.015x + 0068
2) Y2 = 0.012x2 - 0.065x + 0110
3) $Y3 = 0.075e^{-0.37x}$

Each of these equations has a significance and correlation coefficient values which can be seen in Table 4.

Table 4. F-count value, significance and correlation coefficients of each equation at distributon chain IV

No	Equation	F-count value	Significance value	Correlation coefficient
1	$Y_1 = -0.015x + 0.068$	4.20788	0.28876564	0,898
2	$Y_2 = 0.012x^2 - 0.065x + 0.110$	3.744257	0.303663	1
3	$Y_3 = 0.075e^{-0.37x}$	5.104197	0.265282	0,891

Table 3 shown that there were correlation between the distribution level dan weight losses of potatoes on the distribution chain IV. By looking at the largest correlation coefficient, it can be determined the weight losses model on the distribution chain IV to follow a mathematical model of Y = 0.012x2 - 0.065x + 0110 with a value of r = 1. It was shown that the potatoes handling on the collectors level was better than the farmers and retailers level (Harsojuwono, 2000).

Based on the equation model and graphic on the Figure 1 until 4, to be known that the highest of potatoes weight losses, occurred on farmers and retaileer level. Based on the potatoes weight losses model of distribution chain I to IV, to be known that the distribution chain IV occured the lowest weight losses, it was 11,49%.

CONCLUSION

Based on the research can be summarized as follows:

1) The distribution chain of potatoes from the Denpasar District Baturiti through the 4 chains, as follows:
   a. Distribution chain I: Farmers -> collectors -> Wholesalers -> Retailers in the Baturiti District -> Consumer.
   b. Distribution chain II: Farmers -> collectors -> Wholesalers -> Retailers in Denpasar -> Consumers.
   c. Distribution chain III: Farmers -> collectors -> Wholesalers -> Retailers 1 in Denpasar -> Retailer 2 in Denpasar.

d. Distribution chains IV: Farmers -> collectors -> Wholesalers -> Consumer.

2) Each distribution chain has different of the weight losses model. The weight losses model at distribution chain I is $Y = -0.007x^2 + 0.021x + 0.036$ ($r = 0,770$). At distribution chain II is $Y = 0.001x^2 - 0.024x + 0.079$ ($r = 0,980$). At distribution chain III is $Y = 1.182e^{-1.89x}$ ($r = 0,884$). At distribution chain IV is $Y = 0.012x^2 - 0.065x + 0.110$ ($r = 1$)

3) The distribution chain IV was the lowest weight losses, it was 11,49%.

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EFFICATION OF ETHANOL EXTRACT OF LILIGUNDI LEAF (*VITEX TRIFOLIA* L) AS ADULTICIDE FOR *AEDES AEGYPTI* MOSQUITO

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ABSTRACT

Dengue Hemorrhagic Fever is an infectious disease caused by dengue virus and *Aedes aegypti* mosquito as a principal vector. Unavailability of anti-viral drugs and effective vaccines for dengue virus, the way we can do to prevent Dengue Hemorrhagic Fever is by vector control. Resistance and negative impact of chemical insecticides to the environment, has led to search of alternative materials for vector control. Plants can be an alternative for mosquito vector control. The leaf of *V. trifolia* contains essential oil with composition of sequisterpenes, terpenoids, etc. This research aimed to assess the efficacy of ethanol extract of *V. trifolia* leaf as a adulticide for *Ae. aegypti* mosquito. This research was a laboratory experimental research using posttest only one group control design. The research subject were *Ae. aegypti* mosquito and in every treatment were used 25 *Ae. aegypti* musquito. Adulticide test was done according to WHO bioassay using tube. Adulticide test using 7 concentration of ethanol extract of leaf *V.trifolia* and 1 control in 4 replicates. The results of adulticide test showed value LC_{50}, LC_{90}, LC_{95} i.e 6.18 % b/v, 11.16 % b/v, 13.19 % b/v respectively. In conclusion ethanol extract of leaf *V trifolia* has an efficacy as adulticide for *Ae. aegypti* mosquito.

Keywords: Adulticide, Liligundi, *Aedes aegypti*

PRODUCTION OF CRUDE ENZYME AMYLOGLUCOSIDASE FROM "ONGGOK" BY *ASPERGILLUS NIGER*

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ABSTRACT

The crude enzyme amyloglucosidase was produced from "Onggok" (by-product tapioca factory) by *Aspergillus niger*. The experiments were carried out in two phases. The first phase was the cultivation *A. niger* to determine the stationary phase of maximum spore yield. The second phase was the determination of the production time needed to give maximum crude enzyme activity. Enzyme assay was conducted by measuring the amyloglucosidase activities. The results showed that the stationary phase of maximum spore yield occurred after 7 days of cultivation *A. niger* with maximum spore number of $1.26x10^9$ spores mL^{-1}. Optimum activity of the crude enzyme obtained after 7 days of fermentation with the amyloglucosidase activities of 62.77 ± 4.49 U mL^{-1}.

Keywords: *Onggok*, Amyloglucosidase, *Aspergillus niger*

EFFECT OF CONCENTRATION OF LIQUID FOOD DYES AND TIME OF SOAKING ON THE QUALITY OF TUBEROSE (*POLIANTHES TUBEROSE*L.)

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Faculty of Agriculture Technology, Udayana University

ABSTRACT

This research was carried out in the purpose of knowing the influence of concentrate solution of yellow liquid food coloring and time of soaking on the quality of the colored Tuberose, the treatment that produces the best quality colored Tuberose and to determine the colored Tuberose price. This research was using complete random design with factorial pattern with 2 factors. The first factor was the concentration of dark yellow liquid food coloring (P) consisting of 3 (three) levels of concentration of 4%, 6%, and 8%. The second factor was the time of immersion (W) consisting of 3 (three) levels of 240, 480, and 720 minutes. Variable observed include self-life, fluorescence, flower damage, the total solution was absorbed and organoleptic tests (yellow color, aroma, and overall acceptance).The treatment was repeated twice to obtain 18 trial units. The data obtained was analyzed using analysis of variance followed by Duncan test.

POSTER PRESENTATION: HEALTH

THE ROLE OF HEMOPURIFIER-COATED WITH HERBAL ANTIBODIES TO CLEAN INFECTED BLOOD IN REDUCTION VIRAL LOAD IN HIV INFECTED PATIENTS

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ABSTRACT

HIV/AIDS tends to be the most dangerous disease in the last decade, especially in the developing country. In Indonesia, more than 200.000 persons suffering HIV/AIDS. The main problem facing patient with HIV/AIDS appeared when the viral load grows too much and the CD4+ decreases significantly. While CD4 is less than 500, lot of opportunistic infections might have been appeared and be the serious problem. Nowadays, scientists begin to develop many drugs and instruments to compress the number of viral load. A new instruments, Hemopurifier, is believed able to reduce viral load. The aims of this study were to understand positive effects of Hemopurifier, analyze the work-mechanism of Hemopurifier, and compare the output of the infected blood. The method was as follow; trials were searched in computerized general databases (PUBMED,SCIENCEDIRECT,etc) by checking bibliographies and searching the keywords.Trials were eligible if they were: (1)RCT or controlled clinical trials; and (2)including the analysis and neutral point-of-view in the results of Hemopurifier. Results showed that blood infected with HIV was flowed into Hemopurifier's tube at velocity 0.7–10 ml/min. The proportion of viral elimination were measured by ELISA and PCR. This prcoedure successfully eliminate 98% of HIV particles from leukocyte culture supernatants. Affinity hemopurifier effectively trap this virus from blood plasma (90%). Variations in temperature (77–98°F), blood type, cell-matrix linkage, membran-pores diameter, and antibody-antigen complex made non-significant effects. An adult man typically contains approximately five liters of blood. If all of the blood flow through the Hemopurifier, it will spend time about 12 minutes. The procedure is repeated until none of the toxin nor pathogen are found in the blood. Nevertheless, recent study and experiment should be done to understand these long-time effects.

Keywords: *Hemopurifier, Clearance Rate, Plant-Antibodies, Viral-Load*

INTRODUCTION

HIV/AIDS is a major global health problem in developing country that our duty to control this infection is the first priority. Nowadays, it is likely that the discovery of an efficient and effective device (instrument, drugs, vaccine) consider to the clinical and patient safety to cure HIV infected patient, to prevent HIV pandemic and HIV-related disease will be necessary and important to achieve this goal successfully [1].

Hemopurifier is believed has vital role as a medical filtration instrument and as a theraupetical equipment served as an artificial adjunct to the immune system by removing all circulating pathogens like infectious HIV, including those strains making antiviral drug therapy fail to cure the patient. Not only purifying blood from pathogens, Hemopurifier is also believed able to help patient to increase their natural immune system and repair their immune response with the removing of pathogens and toxic proteins shed by HIV that potent to destroy immune cells [2].

Figure 1 – Hemopurifier Device. Source : *Manjula Puthenedam. 2010. Hepatitis C Research and News*

Hemopurifier is placed in the table while a tube is inserted to brachial artery. Then, the infected blood flows toward the hemopurifier and begins to be detoxified. This machine has toxin filters named colander that made from a thermoplastic polymers called polysulfone $[OC_6H_4OC_6H_4SO_2C_6H_4]_n$. This colander allows small particles like viruses pass-through this filter, whereas hug particles like erythrocytes can't. This colander is also covered with special antibodies taken from herbs medicine and some leaves that resists the pathogens quickly, thus ensuring that any pathogen doesn´t penetrate into the bloodstream. The extract of this filter is a purified blood which flows back to the blood-vessel via another tube connected into artery. After that, the purified blood is observed by electrone microscope (TEM or SEM) or analyzed by PCR and ELISA [3].

RESULTS

HIV pandemic and the development to become AIDS is a classical problem in the world population that can be a big trouble. The patophysilogy of this illness is the dropping of CD4+ T cells dramatically, which consequence is lackness of the immune system, thus the patients are unable to defend against opportunistic infections [4]. However, the mechanism of why HIV can cause AIDS isn't understood clearly [5]. But, some cohort studies show that both of the infected T cells and uninfected T cells are destroying and dying due to HIVderived envelope proteins (gp120). gp120 tends to play a significant role in AIDS dementia and can be measured in high level in the cerebrospinal fluid even in the early stages of HIV [6].

The most difficult obstacle in the treatment of patient with HIV/AIDS by using Highly Active Anti Retroviral (HAART) drugs is that some resting memory CD4 T lymphocytes in harbore chromosome still being integrated latent [7,8]. Even though scientist expected that total numbers of the CD4 T cells in human's body are not too much (approximately 1-10 infected cells per million total CD4 T lymphocyte), these highly stable cells serve as a life-long reservoir for HIV in infected individuals, despite long-term effective therapy [9]. The goal of the curing of patient infected with HIV/AIDS, if ever achieveable, is to eliminate every cells that is infected by the virus. This is an eventual method believed success for curing HIV, which still extremely challenging. Even though it is unclear how that can be achieved, recent developments show tendency to get there. One of the new development believing in curing patient with HIV/AIDS is Hemopurifier. This new device is introduced by Aethlon, a company working in the medical theraupetic device.

THE WORK-MECHANISM OF HEMOPURIFIER JUST LIKES HEMODIALYSIS

Scientists designed hemopurifier working likes a hemodialysis. The components of hemopurifier are similar to hemodialysis, but extra using colander with thin fibers filter and plant-derived antibody covering the fiber to catch and remove viruses and bacteries from the purified blood. The blood flows into the machine and give an image about blood in the vessels, which is sent from the tube into the machine, then return to the artery. This device is believed can be used successfully for treatment some infectious diseases, e.g. HIV/AIDS and Hepatitis or chronic disease, e.g. End-Stage Renal Disease.

A clinical data from an experiment held by Tullis in 2004 shows that Hemopurifier works like Hemodialysis machine in purifying blood from infected agents, like virus, microorganisms, and bactery. However, the clearance rate of Hemopurifier has been observed by Tullis who has already been comparing the clearance rate of pathogens between Hemopurifier and Hemodialysis.

This experiment has documented the impact of Hemopurifier / Hemodialysis to the clearance rate of HIV and gp120 steady-state levels until after colonization. As the researchers expected in the steady-state expressions, the clearance rate of both HIV and gp120 are shaped like parabolic curve. For the viral load shown in the left figure, there is a continuous decrease of the virus in human blood. For the viral envelope shown in the right figure, there is one momment that gp120 levels decreasing rapidly. In this figure, the abscissa of this curve shiws an intermitten dialysis time 8 hours every day using 54 Watt Hemodialysis machine.

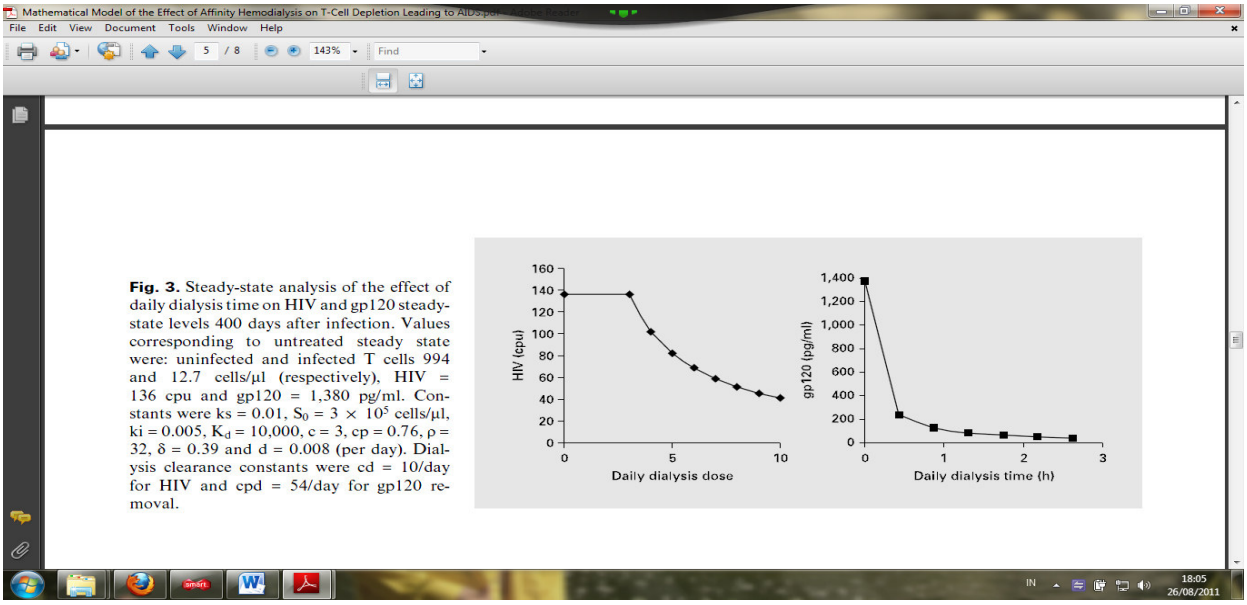

Figure 2 – Analysis of the effect of the daily dilaysis in the clearance of viral load and their glycoprotein (gp120). Source : *Tullis H. 2004. Blood Purif 2004;22:84–91*

The results of this daily dialysis shows a lowering level of viral load and viral envelope (gp120) in human plasma. The dropping of gp120 concentration in human plasma, due to the proportional using Hemopurifier continuously, tends to affect the recovery level of human T-cells. Proportional dialysis using Hemopurifier / Hemodialysis in duration and frequency makes the viral load of HIV and gp120 decline dramaticaly to undetectable levels. Therefore, the daily dialysis schedule gives an opportunity to decrease approximately 5-fold in gp120 levels and increase T-cell levels about 25%. In every experiment held by Tullis, the level of T cells showing a tendency to be increased slightly that wil be very helpful in HIV/AIDS recovery. Tullis has also compared at least two different techniques of Hemopurifer / Hemodialysis using intermitten daily schedule (4-hour and 8-hour alternate-day dialysis). This experiment began with proportional dialysis in the separation of viral load and viral envelope (gp120) with the purified. There is no significant different between these two different techniques in declining the viral load and increasing human T-cells [10].

DISCUSSION

HIV-derived envelope glycoprotein (gp120) has been understood to be the major factor that is contributing to trigger HIV status leads to AIDS status long time ago. These glycoproteins (gp120) have an affinity that able to stick with T-cells membran and have contributions in the destruction of both infected and uninfected T-cells that accelerate the conversion from HIV to AIDS in a process known as the 'bystander effect' [11].

Hemodialysis and Hemopurifier has been tested by clinician and researchers, give a decision that they could be effective in lowering viral load, defends against viral toxins [12]. Both devices were measured by Perelson Formula with deterministic calculation models and both of them show a significant and continuous decreasing in gp120 concentration in the purified blood. In this model, the affinity of Hemopurifier / Hemodialysis machine only created little different associated with the exact number of increasing mature T-cell levels released from lymphatic tissues to recover the immune system [10]. The mathematical models were used to support the hypothesis that Hemopurifier / Hemodialysis treatment are very helpful adjunctive therapy, for recovering the immune system and cure the HIV-infected patients combined with drug therapy. For HIV-infected patients that resistant or unable to get HAART and another anti-retroviral drugs due to their contraindications of these drugs, Hemodialysis / Hemopurifier treatment may be the solution [12].

Several experiments were reported showing that clinical data successfully supported the hypothesis whether both of Hemodialysis and Hemopurifier make significant change in lowering viral load in the blood [13]. Hemopurifier works similar to Hemodialysis, with an extra herbal-coated antibody covering the thin fibers. Study using pre-clinical human blood sample have shown the effectiveness of the Hemopurifier in capturing HIV, HCV, and Orthopox Viruses. The mechanism of colander in Hemopurifier that able to capture HIV and another virus or bactery has been shown using the polysaccharide chains that adhere on the surface of viral envelope made by glycoproteins (in HIV, we know it as gp120).

The plant-derived antibody, which coated the colander, is made from polysaccharide portions that can attached easily to the viral glycoprotein envelope in the infected host cell or uninfected host cell whose functions were not controlled by virus. Those herbal-antibodies which coated the colander are very invariant. Since the high concentration of antibody allowed virus to escape immune surveillance, thus while patient's blood contains a lot of viral loads with different viral proteins in their structure, the polysaccharide chains in the herbal antibody coated in the colander should be invariant, so that the Hemopurifier is given higher ability to capture more strains of different type of viruses. Some scientists predicted that lectin, the herbal antibody coated the thin fibers, has a great affinity to the agents and has ability to immobilized pathogens, so that this machine able to inhibit the replication and the growth of every tested strains of HIV, FIV, HCV, Measles, Mumps, Influenza, Ebola, Marburg, and Orthopox viruses [14]. Due to their abilities and capabilities, Hemopurifier is believed able to solve the world's problem about Infectious Disease which have been a Global Pandemics (e.g. HIV/AIDS), Chronic Disease (e.g. Hepatitis C), and pathogens weaponized in bioterrorism that potentially attack civilian community [14].

Nevertheless, Hemopurifier has several limitations and weakness. Hemopurifier is expensive since this machine is a new development in biotechnology. Local bleeding is a relative contraindication in Hemopurifier / Hemodialysis using arteriovenous catheter. This contraindication is a simple problems that mainly related to the vascular access. Another complications predicted in using Hemopurifier and Hemodialysis that have been considered little harmful are septic shock associated with catheter-related infection, thrombosis of the catheter or tubing and dialyzer, and overactive systemic hemorraghic [15].

CONCLUSION

The Hemopurifier® made by Aethlon Medical Corporation (AEMD) is a new phenomenon of the colander devices for daily dialysis which has been developed by researchers. The Hemopurifer is a potential device that able to decrease the number of pathogens, bacteries, rickettsia, paracytes, until small infectious viruses in the bloodstream rapidly. Developed by Aethlon, the Hemopurifier® is a colander which has thin filtering covered by plant-derived antibody that able to recover the human T-cells and increase the ability of the immune system to defend from pathogen's attack and to fight against infections by isolating bactery / virus genom and removing the virus's capsule made by glycoprotein. Clinical data that has been verified by researcher shows that Hemopurifier can effectively and high-selectively capture immunosuppressive exosomes and virus genomic that can make the immune system getting worse. At this moment, more than 65 treatment of patient using daily Hemopurifier (with approximately 260 hours of treatment time) have been inspected in longitudinal studies in India to check the positive effect and side-effect of using Hemopurifier for a long time [16].

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EFFECT OF TEMU PUTIH (*CURCUMA ZEDOARIA* (BERG.) ROSCOE.) RHIZOME EXTRACT ON WEIGHT BODY AND REPRODUCTIVE ORGANS OF MALE MICE (*MUS MUSCULUS* L.)

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ABSTRACT

The present study was undertaken to evaluate the effect of *temu putih* (*Curcuma zedoaria* (Berg.) Roscoe.) extract on body weight and reproductive organs (testis, epididymis, seminal vesicle and prostate gland) of male mice (*Mus musculus* L.). The mice were divided into 4 groups of 6 animals each; one control group which was given distilled water (M_0 = control group) and three treatment groups (M_1 = group that was given 100 mg extract/kg b.w.; M_2 = group was given 200 mg extract/kg b.w.; M_3 = group was given 300 mg extract/kg b.w). The extract was given orally once a day for 35 days with a dose of 0.5 cc/mice. Testis, epididymis, seminal vesicle, prostate gland and body weight were determined. Data were analysed using ANOVA analysis which showed that the body weight of mice were not decreased, but reproductive organs were decreased significantly ($p< 0.05$) after receiving *temu putih* (*Curcuma zedoaria* (Berg.) Roscoe.) extract for 35 days.

Keywords: *Temu putih* (*Curcuma zedoaria* (Berg.)*, mice* (*Mus musculus* L.)*, reproductive organs.*

INTRODUCTION

According to Nri (2004) temu putih rhizome contains saponins, flavonoids and polyphenols. Other compounds that are also found are tannins, glycosides, triterpenoids and alkaloids (Anonymous, 2007). The content of flavonoid compounds that have estrogen-like activity could be expected to suppress the function of the anterior pituitary to secrete FSH and LH (Middleton et al., 2000 in Suartha, 2005). Research conducted by Handajani (2003) shows that treatment with rhizome extract of temu putih continuously for 33 days causing a decline in the number of mitotic cells and decreased number of spermatogenic spermatogenic cells lining the seminiferous tubules of the testis.

The male reproduction organs consist of testis, epididimis, vas deferens, vesicular seminalis gland, prostate gland and Bulbourethal glands. Testis are organs that produce sperm cells. The testis also produces hormones, including testosterone, which stimulates the production of sperm cells and facilitates male maturation. (Johnson & Everitt, 1988)

Based on Handajani (2003), it is important to carry on a further study about the effect of temu putih rhizome extract on weight body and reproductive organs in male mice.

MATERIALS AND METHODS

Temu putih were rhizome thinly sliced and dried. Dried rhizomes were then blended to form powders. The powder was macerated with methanol for 72 hours, then filtered with Whatman paper. Fluid obtained then evaporated with Rotary Vacuum Evaporator with temperatures 40°C until the final results obtained in the form of crude extract (crude extract). Extracts of temu putih obtained, at each dose (100 mg, 200 mg and 300 mg) was dissolved first in 2 ml of distilled water before being given to test animals. The sample in this study was fertile male mice, Balb-C strain. The samples were divided into 4 groups of 6 animals each, namely a control group (M0= were given 0 mg extract/kg b.w.) and 3 treatment groups (M1= 100 mg extract/kg b.w. , M2=200 mg extract/kg b.w. and M3=300 mg extract/kg b.w.). Provision made during the day every day, once daily orally for 35 days.

Observed variables were body weight and reproductive organs (testis, epididymis, seminal vesicles and prostate gland). Data were analyzed using ANOVA (Minitab 15 statistical software).

RESULTS AND DISCUSSION

Effect on Weight body and Reproduction Organs

Administration of temu putih rhizome extract caused no change in body weight when compared

with the controls. That showed on the confidence intervals for body weight of mice treated with M0 overlap with other treatments (Figure 1). However, reproductive organs weight were decreased significantly ($p < 0.005$), when compared with the control groups (Table 2). Significantly decrease obtained in M3, while the M1 to M2 is not significantly different, where the M3 shows the lowest weight of all reproductive organs are analyzed (Figur 3).

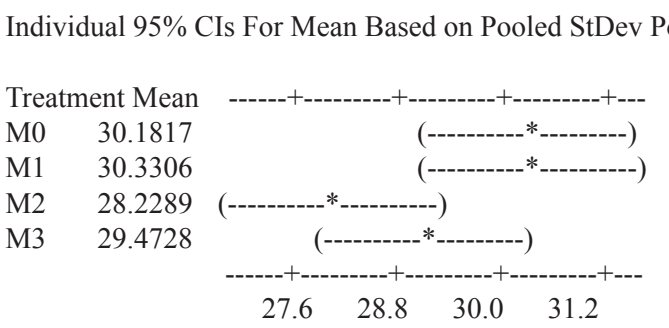

Figure 1. The picture above shows that the confidence interval for body weight of mice treated with M1, M2 and M3 were overlap with confidence interval of M0.

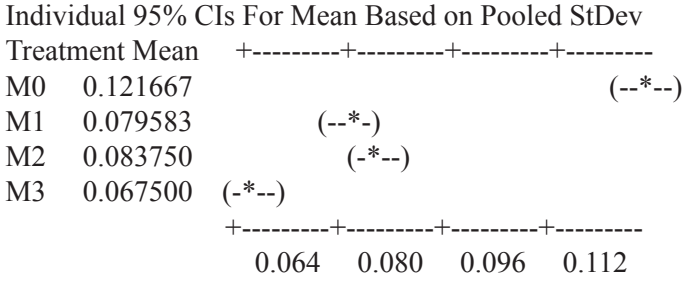

Figure 2. The picture above shows that the confidence interval for reproductive organs weight of mice treated with MI, M2 and M3 were not overlap with confidence interval of M0.

The decline in testicular weight caused the temu putih rhizome extract acts directly on germ cells in early spermatogenesis. This is supported by research conducted Handajani (2003), that temu putih extracts administered orally for 33 days in male mice, can decrease the number of mitotic cells and decreased number of spermatogenic cells lining the seminiferous tubules of the testis. Even disruption of spermatogenesis will cause atrophy of the testes (Adnan and Halifah, 2000). Impaired spermatogenesis will cause a decrease in production and quality of sperm in the seminiferous tubules in the testes (Elfira et al., 2008).

Decrease in testicular weight are also causes a decrease in epididymal weight because of the epididymis serves to store sperm and the maturation of sperm produced in the testes. Ashok and Meenakshi (2004) research on Wistar rats revealed that the epididymal weight decreased significantly after being given turmeric extract (*Curcuma longa* L.). This is caused by the content of flavonoid compounds contained in turmeric are also present in temu putih. Flavonoids compounds give estrogenic effect, because it can stimulate the formation of estrogen in the body (Cambie and Brewis, 1995; Robinson, 1995 in Sumapta, 2005). Increased estrogen levels will give negative feedback to anterior pituitary, to not release FSH and LH. Decreased levels of LH cause the disruption of testosterone secretion by Leydig cells. Disruption of testosterone secretion affects the accessory glands such as the seminal vesicles and prostate gland. In this study the weight of the seminal vesicle and prostate gland decreased significantly between the controls and treated mice, because the accessory gland is an organ that depends on the hormone testosterone.

CONCLUSION

The extract of temu putih (*Curcuma zedoaria* (Berg.) Roscoe.) rhizome did not decrease the body weight of male mice, however it causes severe weight reduction in reproductive organs on male mice (*Mus musculus*).

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HISTOLOGICAL STRUCTURE OFRESPIRATORYORGANS OF MICE (*MUSMUSCULUS*L.) AFTERBEING EXPOSED TOELECTRIC MOSQUITO REPELLENTVAPOR

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ABSTRACT

Electric mosquito repellent is one of the chemical substance which used daily by people. The main purpose of the research is to study the influence of electric mosquito repellent vapor on trachea and lung histological structure of mice (*Musmusculus* L.) at different ages. Twelve male mice DDI strain are divided into two groups of treatment (control and exposed by vapor) and also 2 different ages (1 and 4 months). The vapor were exposed for 6 hours everydayduring 30 days of treatment. At the end of treatment, trachea and lung were taken for histological observation using paraffin method with HE and AB-PAS staining. The data obtained were analyzed qualitatively. The result showed that the electric mosquito repellent vapor influence the respiratory organs structure in the form of erosian cilia (coherent crossed cilia), mucus hypersecretion, metaplasia, existence of alveolar makrofag, alveolar septal thickening and emphysema.

Keywords: *vapor, electric mosquito repellent, respiratory organs, metaplasia*

INTRODUCTION

In his life, many people use chemicals for purposes of household activities such as food additives, cosmetics, cleaning the room, insect repellent and other houses. The use of chemicals, in accordance with instructions and functions will benefit users, but uncareful and wrong usage will disturb human health and pollute the surrounding environment. One of the chemicals commonly used by the public is anti-mosquito repellent that has a primary function to repel, disrupting mosquitoes to find their target, inhibited bite, and eventually kill other insects.Indirectly negative effect can also affect people and environment. Vapor arising from the anti-mosquito repellent will be inhaled and over time will cause a disruption intherespir atorytract(Supriyatno,2002).

Electric mosquito repellent use colorless clear solution that can be vaporized by heating. It is considered the safest way to use because it does not cause smoke, does not produce ash, smell nice, safe and efficient. Generally, the active ingredient of anti mosquitorepellent is rapidly unfolding and high-power poison that kills mosquitoes quickly. Based on research that has been done on anti-mosquito mat showed the side effects on the respiratory system (Mulyati, 1997 and Widjajanto, 1997).

This research was conducted to determine the effect of electric mosquito repellent vapor on the histological structure of the respiratory tractsof mice (*Musmusculus*L.) including the luminal and epithelium of trachea and alveoli.

METHODS

This research used strains of DDI male mice (*Musmusculus* L.) with two groupsof different age (1 and 4 months, the beginning age when the treatment starts) and two groups of treatment (control and exposed by vapor), with six individuals (three replicates) in each group. The materials of this research are : electric mosquito repellent (active ingredient propoxur and praletrin), NaCl, Bouin fixative solution, alcohol, toluol, xylol, paraffin, Meyers' albumin, aquadest, Haematoxylin andEosin (HE) staining, Alcian Blue – Periodic Acid Schiff (AB-PAS) staining, Canada balsam, pellets and tap water. The tools used in this research is the cage of mice (as the vapor exposed room) made of stainless steel (Tokiwa, size 13.4 x 24 x 16.5 cm), glass objects, glass covers, microscopes, and microanatomy preparation tools.

Animals exposed to electric mosquito repellent vapor for 6 hours per day for 30 days. Mice were divided into control group (without being exposed) and treatment group (exposed to electric mosquitorepellent vapor) with age replicates (Wilson, 1988). After 30 days, mice were dissected to collect respiratory organs

(trachea and alveoli). The organs were fixed in Bouin solution for approximately 15 hours. Histological preparation used paraffin method and stained with Hematoxilin-Eosin (HE) and Alcian Blue-Periodic Acid Schiff (AB-PAS) (Handari et al., 1983).The study used Completely Randomized Design and the data were analyzed qualitatively. The air analysis in the location of electric mosquito repellent vapor exposure resulted the amount of chemical compounds content such as NO_2, NO, particles (TSP), CO_2, and CO. Each level of the substances is still below the air quality standard so it is still considered safe for health, but the level of ammonia (NH_3) exceed the permissible air quality standards (BTKL, 2004).

RESULTS AND DISCUSSION

It is necessary to the analyze the respiratory organs histological structure to identify the toxicity of electricmosquito repellent vapor against respiratory organs of mice. The function of an organ system heavily influenced by the condition of the organs that carry out these functions.

1. Trachea

Histological preparation of mice trachea at different ages in both treatments showed different results. There was no visible damagefound in micewith the treatmentat theearly age of1 month of the control group. The columnar stratified epithelial cells with the nuclei clearly visible in the basal and cilia on the cell surface (Fig. 1.A.).The group exposed to vapor (Fig.1.C.), showedciliarycolumnar epithelial cells storey lower than the control. The cilia of the epithelial cells attached to each other, mucous covered the surface, the lamina propriadiminished and its core unclear. Micewith the treatmentat theearly age of4 months (Fig.2.A and 2.C.), still have numerous cilia on the surface of the epithelium to dissipate the incoming particles, but these cell attach to each other and produce a lot of mucus (mucus hypersecretion) in the cavity of the trachea. This is the body's efforts to remove foreign objects that enter through the respiratory tracts.

Trachea of mice exposed to vapor showed some damages because of the influence of foreign gases and particles inhaled by the mice. Electric mosquito repellent vapor caused negative effect on the epithelial surfaces such as hypersecretion of mucus and cilia attached or missing, but did not affect the part below the epithelial cells. This is indicated by the discovery of inflammation in the trachea. This condition illustrates that the pollutant of electricmosquito repellent vapor that are inhaled by mice can be secreted directly on the surface and did not damage the bottom part of the columnar epithelial cellsof the trachea.

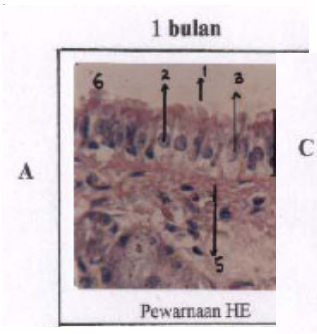

Fig.1.A.
Control 1 Month

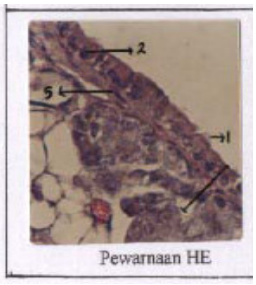

Fig.1.C.
Exposed 1 Month

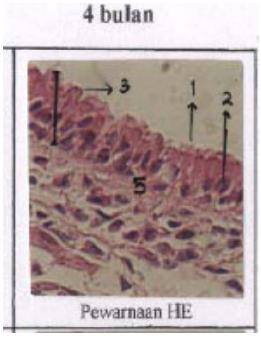

Fig.2.A.
Control 4 Months

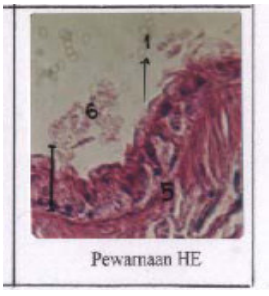

Fig. 2.C.
Exposed 4 Months

2. Alveoli

The exchange of oxygen and carbondioxyde between the air and red blood cellstake place in the alveoli. The structure of the wall is devoted to facilitate and accelerate the diffusion between the outer and inner environment. Histological preparations showed emphysema of some alveoli cells in miceexposed by vapor with the treatmentat theearly age of1 month (Fig.3.C.)characterized by enlargement of alveolar spaces. The inhaled electric mosquito repellent vapor that reach the alveoli is expected to reduce elasticity of the pulmonary connective tissues. This situation still allows air to enter the alveoli but hard to get out. It will cause air space enlargement of the alveoli (emphysema). There is the presence of macrophages

containing particles in this emphysema condition. In all treatments,alveolar wall thickening do not occur.

Pollutant are not entirely derived from inhaled electric mosquito repellent vapor. It also expected from many other particles in the research location such as ammonia, house dust, smoke and others. The particle size determines its place in the respiratory tracts. The particles inhaled into the respiratory tracts will be phagocyted by macrophages. Murine alveolar showed a similar condition between the controlandtreatmentgroupexposed tovapor. In both treatments, there are emphysema of alveoli cells and also macrophages containing particles (Fig.4.A. and Fig.4.C.). The alveoli of mice exposed by vaporwith the treatmentat theearly age of4 months, showed alveolar septal thickening and widening of the alveolar space.

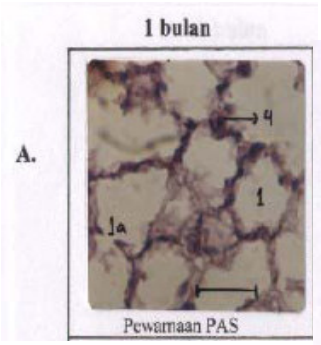

Fig.3.A.
Control 1 Month

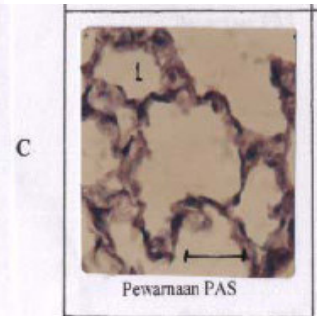

Fig.3.C.
Exposed 1 Month

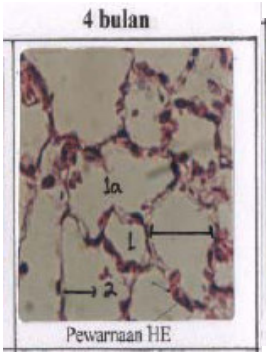

Fig.4.A.
Control 4 Months

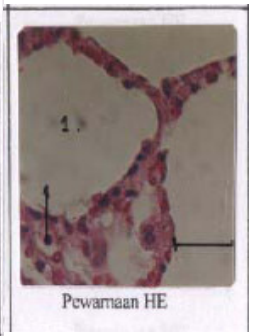

Fig.4.C.
Exposed 4 Months

Based on histological preparations, the macrophages present in all treatments including the control and in both groups of different age. This explains that many type and size of particles exist at the study site. Particles phagocyted by murine macrophages not only derived from the electric mosquito repellent vapor but also other particles that exist around the cage, such as ammonia which are very numerous in the research site (the air analysis of electric mosquito repellent vapor). Ammonia particles derived from the feces and urine secreted by the animals in the study site.

According to Junqueira et al. (1998), NO_2 inhalation may result in the destruction of most of the cells lining the alveoli (type I and type II cells). Effect of toxic compounds or substances increased type II cell mitotic activity. In all treatments in each age group, alveolar emphysema (a condition of alveolar wall destruction followed by the reduction of respiration) occurs due to air pollutants such as NO and NO_2that enter into the alveoli and diffuses into the blood to form MetHb. The existence of MetHb will reduce the amount of oxygen that can be carried by the blood. Condition of oxygen deprivation causes the destruction of the cells lining the alveoli (emphysema). Emphysema also occurs in the alveoli of the control mice, because in the location, there is also other sources of pollutant affect the respiratory system of mice. Ammonia and a lot of smoke containing particles that cause odor,reduced the amount of oxygen in the air so that the amount of free oxygen that can be inhaled by testing animals is limited. These conditions affect the alveoli of the control mice (Wichaksana, et al. 2002).

Macrophages containing particles can also be found in the alveoli. Foreign particles can reach the alveoli due to erosion in the tracheal cilia so that the process of clearing (removing particles) is disturbed. This condition causes a lot of dust particles can enter into the body and will soon phagocyted by the macrophages and then deposited inside the alveoli. Penetration of particles into the respiratory tracts depending on the size of the particle itself. Generally, more than 90% of deposits in the mucus can be cleared within 1 hour. When the particles reach the bronchioles, alveolar macrophages and fluids will push them into the ciliated area,and these particles will be removed away. This process takes one to several days depending on the nature and type of the toxicant (Anonymous, 1996).

Electricmosquito repellent vapor only affects the surface of the luminal respiratory tracts or the part that directly contact with particles and foreign substances that can reach the tracts.The epithelium of the lower respiratory tractswill not be affected. The evidence is, there is no inflammation occurs in the bottom of tracheal epithelial cells. There is also no cells proliferation happen in the lamina propria. Based on the

results of this study, compared with previous studies of the respiratory system of mice exposed to various forms and types of anti-mosquito, electric mosquito repellent (liquid) is relatively safe and has a smaller negative effect than the anti-mosquito coils or anti-mosquito mat.

CONCLUSION

Electric mosquito repellent vapor causes changes in histological structure of the respiratory organsin micewith the treatmentat theearly age of4 months. The structural changes are the erosion cilia or cilia attached to each other in the luminal epithelium of the trachea, mucus hypersecretion, and metaplasia of epithelium of trachea and bronchioles. In the alveoli, there are alveolar macrophages containing particles, alveolar septal thickening and emphysema.

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IMPROVEMENT OF PAPAIN STABILITY THROUGH MICROENCAPSULATION USING ALGINATE-CHITOSAN FOR ORAL DELIVERY

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ABSTRACT

Papain is a therapeutic protein andis commonly administered through oral route. As a protein, papain is degraded in gastric compartment by pepsin and acidic environment. To solve this problem, a specific approach is necessary to protect papain from this condition so that papain will be effective for local therapy in gastric or able to cross biological membrane for systemic therapy. The aim of this study is to improve papain stability through microencapsulation for oral delivery purpose.

Mikroencapsulation was prepared using combination of alginate-chitosan with emulsification/internal gelation technique. Variables studied in this work were stirring speed (200, 300, 400, 500 rpm), water/oil ratio (20 : 80, 30 : 70, 40 : 60), and acid/$CaCO_3$ molar ratio (3 : 1, 6 : 1 dan 9 : 1). To obtain hard texture of microcapsule surface, the microcapsules were incubated in chitosan solution (0.3%) with different time of incubation. The properties of papain microcapsule consisted of particle size distribution, microcapsule morphology, entrapment efficiency of papain in the microcapsule and release study. The release study was performed in various pHs with or without proteolitic enzyme. The proteolitic activity of papain was measured using RP-HPLC with C_{18} coloum, flow rate 0.5 ml/minutes, UV detector 220 nm. The release profile of papain was analyzed using several models.

Narrow particle size distribution with average of 315-560 μm was obtained from microcapsule prepared with water/oil ratio of 30 : 70, acid/Ca molar ratio of 6 : 1 and agitation speed of 400 rpm. Papain microcapsule with that composition has entrapment efficiency of 95.79%. The in vitro release studies showed that alginate-chitosan complex formation reduce erosion of alginate-chitosan matrix at pH 6.8. The release of papain from the alginate-chitosan microcapsule took place trough diffusion and relaxation of polymer at pH 1.2-4.5 Both pepsin and pancreatin increased release of papain.

Alginate-chitosan polyelectrolyte complex occurred on the surface of papain microcapsule controlled the release of papain from microcapsule. The presence of chitosan on the surface of microcapsule retained the release of papain in undesired site (gastric). In conclusion, papain microcapsule developed in this study is suggested to be appropriate approach for oral delivery of papain.

Keywords: papain, microcapsule, stability, pH, proteolytic enzyme

INTRODUCTION

Active compound such as peptide and protein drugs are increasingly becoming a important class of therapeutics agents as a result of rapid advanced in biotechnology. Peptide and protein normally administered by the parenteral route. However complication such as the trombophlebitis or tissue necrosis and poor patient compliance have simulated the investigation af alternative nonparenteral route. Among this, the most convenient nonparenteral route for patient remains the peroral application. Unfortunately, the oral bioavailability of peptidic drugs is generally very low, owing the acidic conditions of stomach, the proteolytic activity of the gastrointestinal tract, poor permeability across the intestinal mucosa, rapid clearance after absorption as well as chemical and physical instability during the process of manufacture and storage (Silva , CM et al, 2006).

Papain is a protease with sulfidril group derived from Carica papaya which degraded by pepsin at pH 1.2 and temperature 37^0C into small peptides (Huet, J., et al 2006). To minimize protein denaturation and the loss of its biological activity, a mild microencapsulation methods, avoiding exposure to elevated heating and organic solvent, should be adapted (Coppi, et all, 2001). Among this emulsification/internal gelation method could be considered proper for protein encapsulation (Poncelet, et all, 1992).

Howefer, entrapment efficiency of internal gelation method is very low caused by several things, such as alginate microcapsules surface is not homogeneous, the protein diffuses from the internal phase to external phase and the loss of microcapsules in the collection phase. Moreover, the cation cross-linked alginate network can degraded by removal of the calcium ions by chelating agents such as lactate, citrate and phosphate. As calcium ions are removed, the cross-lingking in the gel decreases and tge gels are destabilized, leading to fast drug delivery rates(Coppi et al, 2001).

To overcome the weaknesses of the method emulsification-internal gelation above, is necessary to modify the surface texture of alginate microparticles. Chitosan, a polysaccharide known to be used to achieve this goal through the formation of complex polyelectrolytes modified with Ca-alginate. Polyelectrolyte complex chitosan-alginate microcapsules will strengthen the surface structure and reduce porosity so as to prevent the release of proteins in unwanted places (stomach).

Chitosan is a polysaccharide derivative of chitin which has been widely used to strengthen the structure of alginate microparticles through the interaction between amino group of chitosan and carboxylic groups of alginate. Alginate-chitosan complex is broken slowly in phosphate buffer so that it can withstand the release of the drug in large quantities (Peniche, C., et al, 2003). Moreover, the alginate-chitosan complex was noted to be stable to pH values ranging from 3.7 to 4.7. (Coppi et al, 2001). Based on the expected interaction between alginate and chitosan can produce a stable surface structure in acidic media.

Thus, the aims of this work was to investigatethe feasibility of emulsification/internal gelation method to produce alginate and chitosan in order to obtain an oral formulation of papain which is physically and chemically stable.

MATERIALS AND METHODS

Sodium alginate (Algin Kimica) purchased from PT. Pafa Mandiri Sakti. Papain (with 3.08 U/ mg proteinenzymatic activity (Sigma-Aldrich). Chitosan ≥ 95% deacetylation (Sigma-Aldrich). Casein from cow's milk (Sigma-Aldrich). Pepsin derived from bovine gastric mucosa, 800-2500 units/mg protein (Sigma-Aldrich). Pankreatin 4x USP specifications (Sigma Aldrich). Cysteine hydrochloride monohydrate (Sigma Aldrich). Potassium dihydrogen phosphate, calcium carbonate, paraffin liquid , glacial acetic acid, Span 80, Tween 80, citric acid, trichloroacetic acid, sodium hydroxide, hydrochloric acid, trifloro acetic acid, sodium acetate, sodium chloride, acetonitrile, and water

Microspheres preparation

Microspheres are made using the method of emulsification/internal gelation. (Ponselet et al., 1992). Briefly, a 2% (w/v)sodium alginate solution was dispersed into phosphate buffer pH 6.8 containing 1.5% (w/v) papain. A suspention of 5% (w/v) ultrafine$CaCO_3$ was added to the polymer solution to obtain a Ca/alginate ratio (w/w) of 25% and, after a homogenization, the mixture was dispersed into the paraffin oil containing 1% (v/v) Span® 80 with varying stirring speeds: 200, 300, 400, 500 rpm using the IKA ®-Werke Eurostar mixer (IKA, Staufen, Germany) with paddle-shaped impeller .Comparison between alginate and oil mixture was varied: 20: 80, 30: 70 and 40: 60 (v/v). After 15 minutes of emulsification, 20 ml of paraffin oil containing glacial acetic acid was added to the w/o emulsion to obtain an acetic acid/calcium molar ratio of 3, 6 and 9 and stirring continued to permit $CaCO_3$ solubilization. Gelled microcapsules were recovered from oily phase by using1% (v/v) Tween®. Microcapsules was then washed again until no residual paraffin in suspension. Microcapsules was then dried in a oven vacuum at 30^0C until constant weight is obtained.

Preparation of chitosan-coated alginate microspheres

Chitosan-coating of microspheres obatained by internal gelation was performed based on Ribeiro, A.J et al. Chitosan solution prepared by dissolving 300 mg of chitosan with 200 mL of glacial acetic acid. 0.1 M NaOH was added into chitosan solution to obtain pH 5.6. The solution is then filtered, put into a glass and added a volume of 100 ml distilled water. This solution was then added to the microcapsules that have been washed and made stirring with a speed of 200 rpm for a certain time (30, 60 and 120 minutes).

Morphological and particle size analysis

The particle size distribution of microcapsules was determined by a sieve method with various mesh. The shape and surface texture of microcapsules was examined by scanning electron microscopy (SEM) JEOL JSM-6360LA, 10 kV

Determination of encapsulation efficiency

A number of microcapsules equivalent to 50 mg of papain crushed in a mortar and then dissolved in magnetically stirred phosphate buffer pH 6.8 for 1 hour. The resulting solution was centrifuged at 2500 rpm for 10 min and supernatant was assayed (n = 3) for enzyme content by casein digestion method

Determination of Proteolytic Activity Papain by Casein Digestion Method

The ability of papain proteolysis of casein substrate performed by incubating a certain amount of papain was dissolved in phosphate buffer-cysteine in a substrate buffer containing 2.0 ml of casein (12 mg/ml, pH 7.2) at a temperature of 40^0C for 60 minutes.Enzymatic reaction was stopped by adding 3.0 ml of a mixture of trichloroacetic acid (18 g trikloro acetate, 3 g of sodium acetate and 39 ml of glacial acetic acid in 1000 mL distilled water) and re-incubated at 40^0C for 30 minutes. The solution was then filtered using Whatman paper No. 42. Proteins are soluble in trichloroacetic acid were analyzed using HPLC. HPLC column used was C_{18} (250x4, 6 mm), 5 µm, flow rate 0.5 mL / min, UV detector 220 nm with a column temperature of 25^0C. The mobile phase used was a gradient elution using aquabidestilata/acetonitrile (each acidified with 0.1% v / v trifloroasetat acid) gradient was applied under the following conditions: 100% water, 2 min; 0-70% acetonitrile, 10 min; 70% acetonitrile, 4 min, 70% acetonitrile-100% water, 4 min; 100% water, 10 min (equilibration). The injection volume of the samples was 20 µL. The total peak area of the peptides was used to quantify the extent of papain digestion. (Harshadrai et al, 1998).

Effect of pH and proteolytic enzyme on release profile

To study the effect pH and enzyme proteolytic on papain release profile 'in vitro' dissolution study was carried out using USP XXVI dissolution apparatus 2 (Erweka DT 6) in 500 ml of different pH media (buffer pH 1.2, buffer pH 4.5, buffer pH 6.8, buffer pH 4.5 plus pankreatin, simulated gastric fluid (USP) and simulated intestinal fluid (USP) on optimized batch of at 37 $\pm 0.1^0$ C with paddle speed of 75 ppm each for 2 hours. Accurately weighed samples (n =3) equivalent to 50 mg of papain were introduced to dissolution media and samples of 5.0 ml, were collected at 0, 5, 10, 15, 30, 60, 90, and 120 minutes respectively for the analysis of proteolytic activity of papain as before.

Curve fitting

The in vitro release pattern was evaluated to check the goodness of fit to the zero-order release kinetics, first-order release kinetics, Higuchi's square root of time equation, Korsmeyer-Peppas'power law equation and Hixson-Crowell's cube root of time equation. The goodness of fit was evaluated using the r (correlation coefficient) values.

Statistical Analysis

The digestion and analysis were repeated three times and analyzed by means of standard deviation (SD). Statistical difference were analysed by using one-way analysis of variance (ANOVA) followed by a Bonferroni post test. For a value of P less than 0.05 the difference was considered significant.

RESULTS

Encapsulation efficiency

Papain-dissolved alginate aqueous phase was emulsified into paraffin oil and the ionized alginate carboxylic groups were cross-linked by an internal source of calcium resulting in oil-dispersed gelled alginate microspheres. A high encapsulation efficiency, above 95.79%. was obtained for uncoated microsphere (Table 1). In respect to formulation parameters, values of encapsulation efficiency varied from 61.27 to 95.79% and especially affected by parameters influencing stirring speed, water/oil ratio and acid/$CaCO_3$ molar ratio.

Table 1. Encapsulation efficiency values of formulations

Code	Stirring speed (rpm)	Water/oil ratio (v/v)	Acid/$CaCO_3$ molar ratio	Encapsulation Efficiency
I	200	30 : 70	6 : 1	61,27± 0,37
II	300	30 : 70	6 : 1	80,83 ± 2,81
III	400	30 : 70	6 : 1	95,79 ± 2,21
III1	400	20 : 80	6 : 1	93,12 ± 10,81
III2	400	40 : 60	6 : 1	63,85 ± 1,48
IIIA	400	30 : 70	3 : 1	70,10± 0,74
IIIB	400	30 : 70	9 : 1	66,02 ± 2,91

Morphological and size characteristics

Scanning electron micrograph of alginate microsphere showed spherical microspheres with a smooth external surface (Fig. 1A) while clumping observed during coating of microspheres as can see through the existence of alginate microsphere surrounded by chitosan coaservate (Fig. 1B). Papain-alginate microspehes which produsedwith emulsification/internal gelation method more porous that can be seen in the Fig.1D. Clumping on coated microspheres indicated the presence of electrostatic interactions between alginate-chitosan which is a two polyelectrolytes of opposite charge.

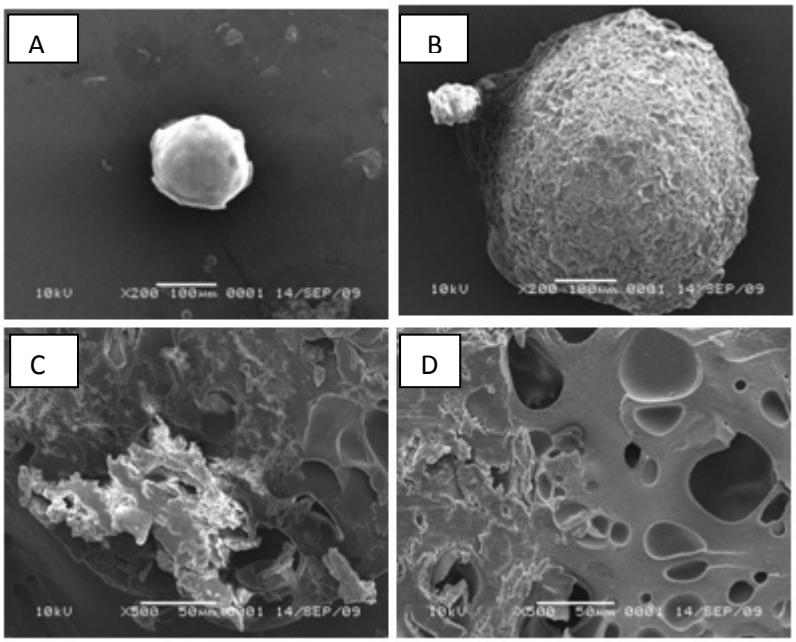

Fig 1.Scanning electron migrograph of : (A) papain loaded alginate microsphere (B) papain loaded alginate microsphere and reinforced with chitosan for 60 min; (C) papain was entrapment within the alginate microsphere; (D) large pore in alginate microsphere which produced by internal gelation

Microspheres diameter ranged from 200-800 μm and particle size distribution at various stirring speeds are shown Fig. 2. Narrow particle size distribution with a peak of about 315-560 μm obtained from the microcapsules prepared with 30: 70 water/oil ratio, 6: 1 acid/$CaCO_3$ molar ratio and 400 rpm stirring speed. A broader size was seen at microspheres prepared by stirring speed 200 and 300 rpm.

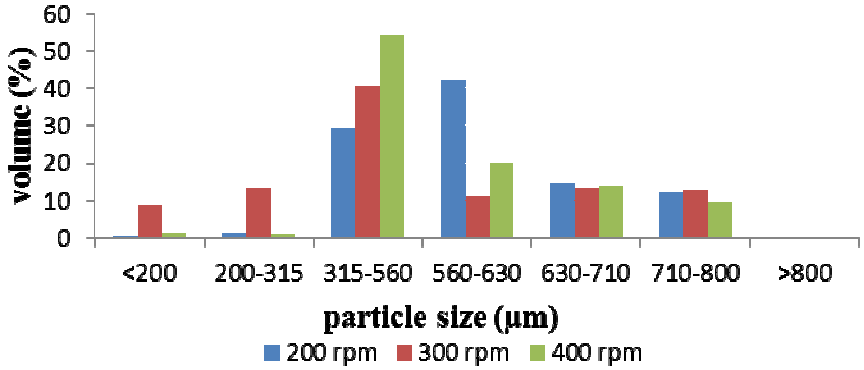

Fig. 2. Particle size distribution of microspheres which formulated by 30: 70 water / oil ratio, 6: 1 acid/$CaCO_3$molar ratio at various stirring speeds.

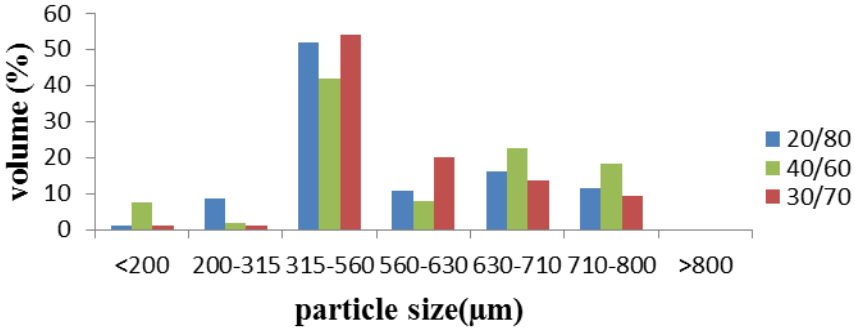

Fig 3. Particle size distribution of microspheres which formulated by 6 : 1 acid/$CaCO_3$ molar ratio, 400 rpm stirring speed at various water/oil ratio

Increase on the water/oil ratio during the emulsification step from 20 to 40%, while keeping totalliquid volume constant, led to a decrease in microspheres mean size (Fig. 3).

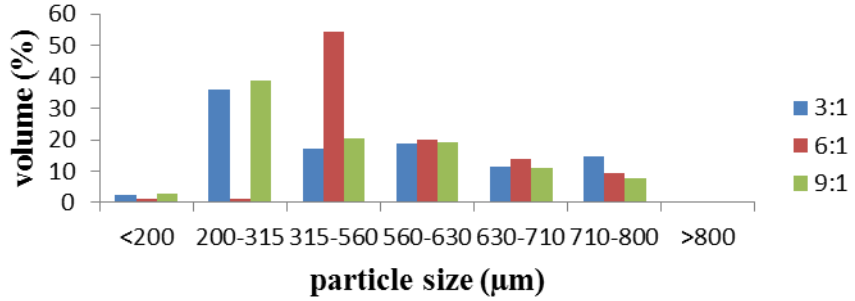

Fig 4. Particle size distribution of microsphereswhich 30: 70 water/oil ratio, 400 rpm stirring speed at various acid/$CaCO_3$molar ratio

Acid/$CaCO_3$ molar ratio, parameter influencing the gelation characteristics of the polymer, influence particle size distribution of microsphere. A broader size distribution was seen when acid/$CaCO_3$ ratio increased from 3: 1 to 6: 1 (Fig. 4).

Papain release study

Papain release profile from microspheres, in gastrointestinal simulated pH conditions, is plottes in Fig.6. Both microspheres presented a low protein release at pH 1.2, reaching 7.72% and 7.06%. In other hand, it showed that rapid release of papain in the initial minutes (5 min) followed by a decline which indicated degradation of papain at pH 1.2 (Figure 5.A). Papain is stable at pH 4.5 as indicated by the constancy of the percentage amount of papain during study. The increaseof papain release from

the alginate microspheres which reached 75.03%. Howefer, papain release from of alginate-chitosan microspheresat pH 4.5 is still low at 17.23% for 2 hours of testing (Fig. 5.B). At pH 6.8, papain-alginate release at 9.77% and reached 100% after 2 hours of testing.Lower results occur in alginate-chitosan microspheres, the number of papain release into media was 4.96% at 5 minutes early and reached 85.38% at 2 hours of testing (Fig. 5.C).

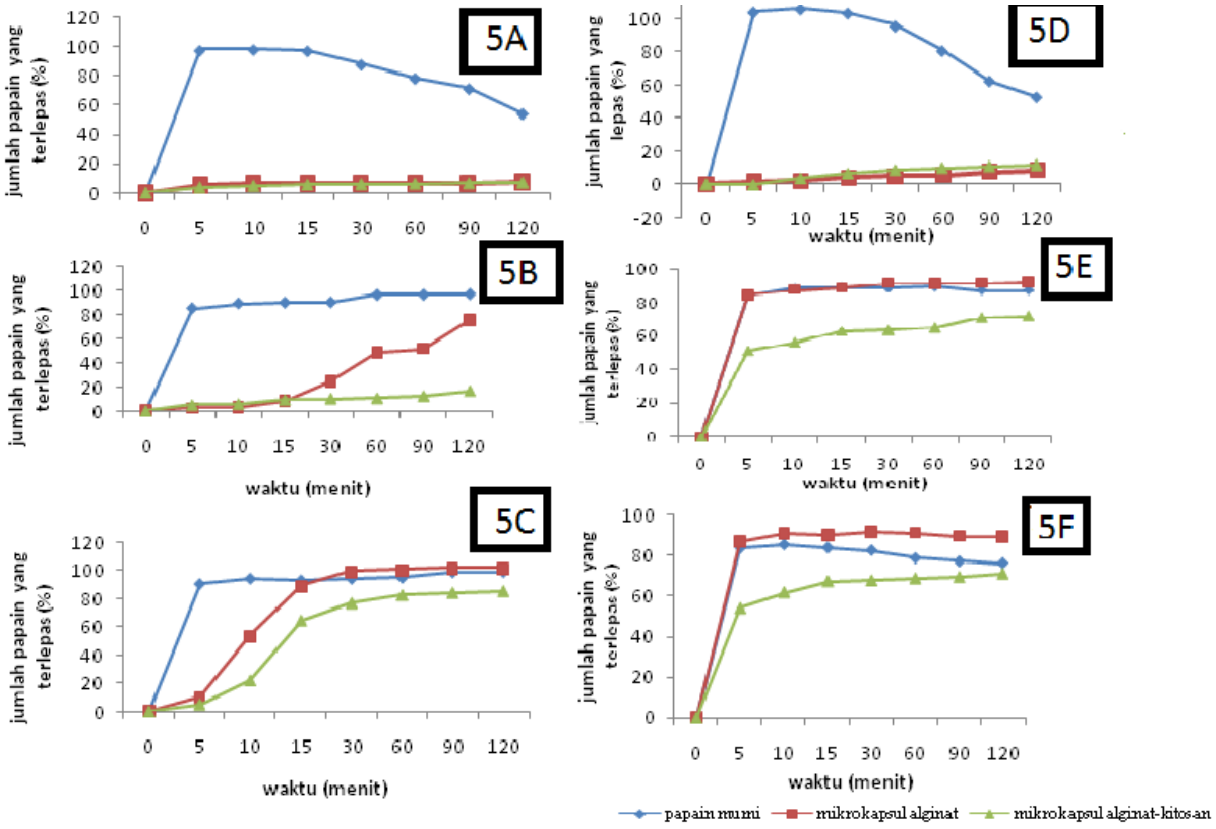

Fig 5. Papain release profile in various pH (pH 1.2 (5A), pH 4.5 (5B), pH 6.8 (5C) and in the dissolution media with proteolytic enzymes (simulated gastric fluid (5D), buffer pH 4 ,5 + pankreatin 0.25% (5E), pankreatin buffer pH 6.8 + 0.25% (5F)

Under simulated gastric fluid, in the presence of pepsin, papain release from alginate-chitosan is greater than the alginate microspheres respectively for 12.07% and 8.44%. When compared with the amount of papain release on the dissolution media pH 1.2 without pepsin, protein release from alginate-chitosan microspheres showed a significant increase ($p \leq 0.05$). When compared with alginate, alginate-chitosan complexes provide weaker protection to the activity of pepsin in increasing the percentage release of papain into the media. Chitosan which is a cation can compete with Ca^{2+} ions thus weakening the bond Ca-alginate and caused papain releases into (Silva et al, 2006)

Then when tested in higher pH, at pH 4.5 with pankreatin, showing the amount of papain that release in to media was also higher. At 5 minutes beginning, papain releases from alginate microspheres and alginate-chitosan respectively 84, 10% and 50. 96% and reached 91.67% and 71, 54% after 2 hours of testing. Papain release from the alginate microspheres showed a significant increase when compared with the release in media pH 4.5 without pankreatin. This shows that alginate microspheres matrix erosion is increasing. Different profile was seen at release profile of alginate-chitosan microspheres. At pH 4.5 with and without pankreatin showed similar profile. These results confirm the research results of Coppi et al stating that the alginate-chitosan complex damaged slowly at pH 3.7 to 4.7.

Curve fitting and release mechanism

The in vitro dissolution profile of the optimized batch is shown in Fig. 5. The values of the release exponent (n) and the kinetic constant (k) were derived using zero order kinetics, first order kinetics, Higuchi's square root of time equation, Korsmeyer-Peppas' power law equation and Hixson-Crowell's cube root of time equation and these are presented in Table 2.

Table 2. Comparison of different dissolution kinetics models

Dissolution media		*Release models*										
		Orde 0		Orde 1		Higuchi		Korsmeyer-Peppas			Hixson-Crowell	
		k_0	r_0	k_1	r_1	k_H	r_H	k_k	r_k	n	k_s	r_s
pH 1.2	P	-0.189	0.904	-0.002	**0.905**	-2.472	0.892	-0.169	0.835	2.154	0.006	0.905
	A	0.006	0.860	0.001	0.848	0.082	0.881	0.074	0.905	0.710	-0.001	0.852
	AC	0.016	0.828	0.000	0.786	0.221	0.881	0.175	0.908	0.493	-0.002	0.800
pH 4.5	P	0.046	0.799	0.000	0.794	0.643	0.853	0.040	0.895	1.905	-0.001	0.796
	A	0.314	0.980	0.012	0.884	4.201	0.978	1.174	0.985	0.505	-0.020	0.930
	AC	0.044	0.900	0.002	0.844	0.593	0.903	0.374	0.906	0.422	-0.005	0.866
pH 6.8	P	0.031	0.889	0.000	0.885	0.416	0.897	0.025	0.885	1.940	-0.001	0.886
	A	0.257	0.650	0.005	0.552	3.946	0.750	0.575	0.778	0.974	-0.010	0.587
	AC	0.435	0.737	0.005	0.627	6.500	0.827	0.782	0.840	0.503	-0.015	0.670
pH 1.2 + pepsin	P	-0.249	0.993	-0.003	0.992	-3.254	0.976	-0.209	0.894	2.227	0.007	0.993
	A	0.028	0.910	0.007	0.818	0.390	0.933	0.647	0.909	-0.377	-0.006	0.855
	AC	0.052	0.821	0.003	0.748	0.755	0.893	0.414	-0.007	0.018	-0.012	0.586
pH 4.5 + pankreatin (0.25%)	P	0.000	0.377	0.000	0.379	0.078	0.403	0.006	0.433	-0.012	0.000	0.369
	A	0.023	0.720	0.000	0.716	0.347	0.803	0.025	0.881	1.914	-0.001	0.717
	AC	0.092	0.836	0.001	0.814	1.290	0.882	0.099	0.923	1.771	-0.003	0.821
pH 6.8 + pankreatin (0.25%)	P	-0.040	0.902	0.000	0.904	-0.538	0.908	-0.036	0.867	1.962	0.001	0.787
	A	0.000	0.495	0.000	0.497	0.036	0.590	0.005	0.690	1.947	0.000	0.496
	AC	0.059	0.659	0.000	0.643	0.868	0.703	0.075	0.746	1.705	-0.002	0.648

The enzyme release data in dissolution media (pH 1.2, 4.5 and 6.8) show a good fit to the Korsmeyer-Peppas'power law release kinetics which can confirmed by comparing the values of the correlation coefficient (*r*) with those of the other models. The values of the Korsmeyer-Peppas' release exponent (*n*) determined for the various formulations studied ranged from 0.442-0.503, suggesting probable release by diffusion and polymer relaxation.

DISCUSSION

The goal of this study was to develop papain-loaded microspheres prepared by emulsification/internal gelation capable retaining the protein in the mastrix during the gastric passage and sustaining the papain release at simulated intestinal pH. The strategies was chitosan coating of alginate microspheres. The necessary conditions for successful encapsulation of papain chitosan coated alginate microspheres prepared by emulsification/internal gelation with high encapsulation efficiency have been established.

A previous study comparing the encapsulation efficiency of bovine serum albumin in alginate microspheres showed that internal gelation of proteins may result in reduced encapsulation efficiency when compared to external gelation due to important loses during formulalation (Ribeiro et al, 2005). Microspheres formed via internal gelation are more homogeneous but more porous which lead to higher protein losses during manufacturing of DNA microspheres (Quong et al, 1998). Macromelecular

interaction between negatively alginate and positively papain were used to increase protein retention in alginate matrices during production and microspheres isolation. Specially, given papain 's isoelectric point of approximately 8.75 and hence a net positive charge at pH below 7.0, its complexes with alginate were explored. The goals were avoid papain loss during emulsification by stabilizing the compelx and during recovery steps by creating the appropriate environment, namely pH. The high encapsulation efficiency of papain is consistent with the view that stronger protein-polyanion complex, can retain protein within the alginate matrix (Ribeiro, et al, 2005). Higher papain losses in chitosan-coated alginate microspheres may be explained by an incomplete recovery of miscrospheres during phase partitioning and by the agitation destabilizing effect during emulsification in both microspheres networks.

Increasing the stirring speed increases the efficiency of papain entrapment in microcapsules. Stirring speed affects the gelation process. The higher the speed of stirring, the calcium ions is more homogeneously distributed, so the more papain can be hooked into the microcapsules. The increase in viscosity produces emulsion droplet sizes are larger but the same amount of Ca ions resulted in fewer gelation so that encapsulation efficiency is lower. One mole of $CaCO_3$ reacted with 2 moles of acetic acid. Increasing acid/ CaCO3 molar ratio, more calcium release to form a stronger microspheres that can prevent the losses of papain. Howefer, Very high on this parameter resulting a decrease in encapsulation efficiency of papain.

The size distribution of the recovered microspheres obtained by emulsification/internal gelation is correlated with the size distribution of the emulsion droplet. The average size of droplets is determined by various parameters such as apparatus design, viscosity of two immisible phases and speed of mixing (Silva, C.M., et al, 2006). In This study, parameters related to the vessel and stirrer characteristics were made constant.

The effect of higher stirring speed on microspheres mean diameter, contributing to a higher heterogeneity of the batch may caused by the distribution of turbulent forces through-out the emulsion (Silva, C.M. et al, 2006). Since, shear stress is higher at the tip of the propeller that at the centre, a faster stirring speed increased this difference, thereby providing a less uniform distribution of energy and giving rise to microspheres of a wider size distribution. Thus , strring speed of 400 rpm was chosen to allow the achievement of small microspheres with a narrow size distribution. Moreover, protein drugs are susceptible to denaturation when they are submitted to high shear forced (Shively, 1997).

Increasing water/oil ratio from 20 to 40% led to increased on microspheres mean size where it was verified that a higher proportion of dispersed phase produce an overall increase in mean size, justified by an increase in the frequency of collisions during emulsification, resulting in the formation of aggregates (Bahukudumbi et al, 2004). Thus 30 : 70 ratio was chosen for the standard formulastions, because the effect of water/oil ratio on encapsulation efficiency was considered more important comparatively to the mean size of microspheres.

Parameters directly related the gelation chsracteristics of the polymer, such us acid/$CaCO_3$ molar ratio influenced on the mean size of microspheres. Acid is responsible for calcium release. Considering the reaction between acetic acid and calcium carbonate, each mole of $CaCO_3$ react with 2 mol of CH_3COOH. When an acid/$CaCO_3$ molar ratio of 3 : 1 was used the amount of acetic acid was insufficient to dissolve all the amount of $CaCO_3$ added to alginate solution. But in acid/$CaCO_3$ ratio is too high (in this study (9: 1) causes the shringking of alginate gel so that the particle size will be smaller (Yotsuyanagi et al, 1987).

Carbon dioxide was liberated from calcium carbonate in the presence of an acid. The above observation could be due to the carbon dioxide that was rapidly liberated. This might result in a porous microspheres matrix (Chan, L.W et al, 2002). Scanning electron micrograph observation of microspheres confirms aggregation phenomena during chitosan-coating which can explained by strong electroctrolytes interaction between alginate and chitosan, two polyelectrolytes of opposite charge. The spherical shape of microspheres may be attributed to a high degree of cross-lingking occurring in each case.

Papain release profile of microcapsules depends on the pH. Alginate shrinks at low pH and the encapsulated drugs are not released (George, M. 2t al, 2006). Microspheres incubation at pH 1.2, causes calcium ions to be displaced from the polymer network and the calcium-alginate gel is converted to the unionized form of alginic acid. At neutral pH (pH 6.8) alginate carboxylate groups almost entirely deprotonased, adhesion intermoleculer is reduced which causes increased water adsorption and swelling of alginate polymer and then papain was released into the dissolution media.

Release of papain from the microcapsules can occur through a process of diffusion or matrix relaxation. At a acid pH, erosion does not occur in the matrix because the alginate microspheres are stable in the environment so that the release of papain in the least amount of both alginate microsphere and chitosan coated alginate microspheres may be caused by diffusion. Whereas at pH 6.8, in addition to due process of diffusion of phosphate ions in the dissolution media that has a high affinity for Ca^{2+}ions cause the erosion of the matrix so that the papain release. This study showed that chitosan can increase the strength of microspheres through ionic bonding (NH^{3+}) with alginate carboxy ion (COO-).

The level of deacetylation is an important factor that determines the strength of alginate-chitosan complex. The interaction may be weak or strong depending on the magnitude of residual amide groups on chitosan. The higher level of deacetylation the amide residues will be smaller so that the power complex will be increasingly weaker (Lawrie et al, 2007). In this study deacetylation degree of chitosan was 95%. The level of deacetylation causes the residual amide group which is bonded to the carboxylic group alginate is limited.

In conclution, papain loaded alginate microspheres formulation was optimized in order to prevent papain release at gastric pH. Coated alginate microspheres wih chitosan achieving sustained release of papain at simulated gastric. Nevertheless, chitosan-coated alginate microspheres may good candidates for oral delivery of papain, since the presence of chitosan may promote papain absorption through intestinal epithelia and this needs further confirmation by in vivo studies

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IDENTIFICATION OF ANTICANCER COMPOUNDS AGAINST MYELOMA AND HELA CELLS FROM WHITE TURMERIC (*CURCUMA ZEDOARIA* (BERG.)ROSCOE) CHLOROFORM EXTRACT

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ABSTRACT

Cancer is an abnormal and uncontrolled of cell growth. White turmeric (*Curcuma zedoaria* (Berg.) Roscoe) is a plant that can be used for treating various diseases such as cancer. Identification of anticancer compounds against myeloma and HeLa cells from white turmeric chloroform extract were carried out. Toxicity test towards *Artemia salina* L. was used as a prescreening to determine anticancer compounds. The compounds from white turmeric were extracted by maceration method. Yellowish solids were obtained on chloroform extract. The solids were then separated by column chromatography with chloroform: ethyl acetate (7:3) as an eluent. There were 6 fractions obtained in which the third fraction was the most active. The toxic isolate (F_3) was analyzed by Gas Chromatography Mass Spectroscopy (GC-MS). Three compounds (spathulenol, β-eudesmol, and dioctyl hexanedioic) can be identified based on their similarity with the GC-MS database. These compounds were suspected as anticancer agents. *In vitro* test toward myeloma cells showed that toxic isolate (F_3) chloroform extract of the white turmeric cannot inhibit the growth of myeloma cells. While i*n vitro* test toward HeLa cells showed that isolate could inhibit the growth of HeLa cells with LC_{50} of 12,58 ppm.

Keywords: *Curcuma zedoaria*, anticancer activity, myeloma cells, and HeLa cells.

INTRODUCTION

Biodiversity of Indonesia provides the potential to be developed as a traditional medicine that can inhibit the growth of cancer cells. One plant that is believed to overcome the development of cancer cells are white turmeric (*Curcuma zedoaria* (Berg.) Roscoe). The part usually used for the treatment is rhizome (Hembing *et al*., 1999).

Some research suggested that white turmeric rhizome (*Curcuma zedoaria*) has a lot of benefits of which have antimicrobial activity (Bugno *et al*., 2007; Wilson *et al*., 2005; Ficker, *et al*., 2003). Seo *et al*., (2005) reported that water extract of rhizomes of white turmeric have role in inhibiting the spread of B16 melanoma cells, while Kim *et al*., (2005) stated that the water extract of the rhizome can be used for the treatment of chronic liver disease.

The white turmeric has been used to cure patients with abdominal bloating and indigestion. In China, it has long been used in the treatment of cervical cancer and is believed can increase the effect to kill cancer cells while doing chemotherapy. In addition, it can cure other diseases such as toothache, sore throat, and cough. Recently it is known as an antitumor promoter inhibiting tumor growth (Anonymous, 2009).

White turmeric (*Curcuma zedoaria* (Berg.) Roscoe) rhizomes chemical contents consists of curcuminoid (diarilheptanoid), essential oils, and other groups such as flavonoids, saponins, triterpenoids, alkaloids, tannins, starch, and lipids (Kardian *et al*., 2003).

Murwati (2004) has observed that the ethanol extract of white turmeric was able to inhibit the growth of lung tumors in mice. In addition, Seo *et al*. (2005) reported that water extract of white turmeric rhizomes played a role in inhibiting the spread of B16 melanoma cancer cells. The fresh rhizome at concentrations of 50, 100, 150, and 200 mg/mL was able to inhibit a cancer cell that was above 50 percent (Gklinis, 2004).

The preliminary toxicity test was conducted towards *Artemia salina* L. larvae on ethanol, n-hexane, and chloroform extracts. Based on these preliminary tests found that the chloroform extract has the highest toxicity compared with the other extracts. The LC_{50} value of those chloroform, ethanol, and n-hexane extracts was 28.2 ppm 35.48 ppm, and 125.9 ppm respectively (Rita, 2009).

Myeloma cells are lymphocytes B cancer cells derived from mice, whereas HeLa cells are human epithelial cells derived from cervical cancer. HeLa cells were named according to the name cervical cancer patient that her cancer cells were taken, that was Henrietta Lacks. HeLa cells were taken and then

reproduced by cell culture and they have been widely used in research (Anonymous, 2010).

Considering the potential of the white turmeric rhizome as an anticancer agent, the high toxicity of chloroform extract, and no studies on the anticancer activity of chloroform extracts of white turmeric rhizome against myeloma and HeLa cells, it is worth to study the anticancer activity of chloroform extract of white turmeric rhizome *in vitro* against both the cells.

The aims of this study are as follows: 1) To identify compounds contained in active isolates of white turmeric rhizome chloroform extracts and 2) to determine the activity of these isolates in inhibiting the growth of myeloma and HeLa cells.

MATERIALS AND METHOD

The research was conducted at the Laboratory of Marine Genetic Resources Development and Genetic Engineering Udayana University. GC-MS analysis was performed at the Laboratory of Organic Chemistry of Natural Sciences, Gadjah Mada University in Yogyakarta. Anticancer test was conducted at the Laboratory of Veterinary Virology University of Udayana and Integrated Research and Testing Laboratory, Gadjah Mada University in Yogyakarta.

Materials

Materials used in this study were white turmeric rhizome (*Curcuma zedoaria* (Berg.) Roscoe). Biological materials as a toxicity test was the larvae of *Artemia salina* Leach, materials for anticancer study were mice myeloma cell types P3UI and human cell line cervical

Chemicals

The chemicals used in this research were n-hexane, chloroform, ethanol, ethyl acetate, methanol (technical and pro analysis), distilled water, silica gel GF254, silica gel 60, dimethyl sulfoxide (DMSO), yeast, aquadest, and phytochemical reagents. Powdered culture medium RPMI 1640 containing HEPES and $NaHCO_3$, FBS (Fetal Bofine Serum) serum, PBS (Phospate Buffer Saline), trypan blue, MTT (3 - (4,5-dimetiltiazol-2-yl) -2.5-difeniltetrazolium bromide), trypsin, streptomycin, penicillin, fungison, amphicillin and sodium dodecyl sulfate (SDS) 10% in 0.05 N HCl.

Equipments

Equipments used in this study were a set of glassware, desiccator, analytical balance, separating funnel, flask, measuring pipette, pipette volume, micro pipettes, petri dishes, a set of tools column chromatography and thin layer chromatography, UV lamp, rotary vacuum evaporator, gas chromatography and mass spectrometry (GC-MS), ELISA reader, hemocytometer, multiwell plates (24 and 96 well), culture flasks (25 cm), centrifuge, culture flasks (75 cm), pipette tips (1 mL), and pipette tips (10 mL).

Methods

The compounds from white turmeric rhizome were extracted with ethanol 96% by maceration method. The ethanol extract was concentrated by rotary vacuum evaporator and then partitioned with chloroform. The compounds in chloroform extract were separated using thin layer chromatography and column chromatography techniques. From the chromatography column were obtained several fractions. The fractions are then tested its toxicity. Toxicity tests using *Artemia salina* larvae used method that had been described by Meyer (1982).

Identification of the most toxic isolate was carried out by phytochemical screening and gas chromatography-mass spectroscopy (GC-MS) methods.

In vitro anticancer test of the toxic isolate against mice myeloma cells was conducted following the method that has been reported by Hidayat (2002). While *in vitro* anticancer test of that against HeLa cells was carried out following the method that has been described by Amalia (2008).

Result and Discussion

About 20 g ethanol extract was yielded from 1700 g white turmeric rhizome dry powder. The partition

of 7.17 g ethanol extract with chloroform produced 5.83 g of a yellow chloroform extract. Rita (2009) has been reported that the chloroform extract of white turmeric rhizome was toxic (LC_{50} = 29.51 ppm).

Before the separation process using column chromatography techniques, the selection of the best mobile phase was performed using a thin-layer chromatography (TLC) with various mixed solvents with different polarity (Table 1).

Table 1 Thin Layer Chromatography of Chloroform Extract of white turmeric rhizome

Mobile phase	The number of spots	Rf
Chloroform	6	0,16; 0,25; 0,31; 0,37; 0,41; 0,66
Ethyl acetate	6	0,53; 0,60; 0,70; 0,78; 0,86; 0,93
Chloroform - Ethyl acetate (4:6)	6	0,25; 0,44; 0,60; 0,77; 0,80; 0,84
Chloroform - Ethyl acetate (3:7)	8	0,25; 0,44; 0,56; 0,71; 0,74; 0,77; 0,83; 0,88

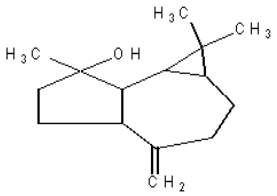
From the Table 1, it can be seen that chloroform: ethyl acetate (3:7) was the best mobile phase. Thus, the mobile phase can be used in the separation using column chromatography and gave six fractions (F1 - F6). The fractions were then tested its toxicity against the larvae of *Artemia salina* Leach.

The number of dead larvae at each concentration can be seen in Table 2. Based on the Table 2 showed that all fractions were toxic. According to Meyer (1982), an ingredient was said to be toxic if it has LC_{50} <1000 ppm. Among all the fractions, fraction 3 had the highest toxicity against the larvae of *Artemia salina* L with LC_{50} of 31.62 ppm. Since F3 was only composed of a stain, then the fraction was further tested purity.

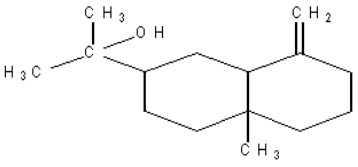
Furthermore, the compounds in this fraction were identified by Gas Chromatography-Mass Spectroscopy (GC-MS) technique. Gas chromatography-mass spectroscopy analysis of the active isolates showed 12 peaks as shown in Figure 1.

From the analysis by mass spectroscopy, it was identified three compounds based on the suitability of the database fragmentation. There were sesquiterpen alcohol (spathulenol and β-eudesmol) and the ester (dioctyl hexadioic).

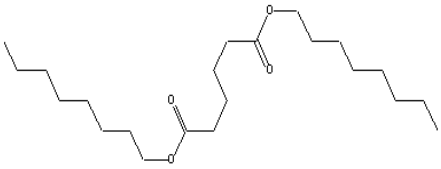
The next stage was the anticancer activity test of fraction 3 against the myeloma cells of mice and the HeLa cells. Myeloma cell pictures are presented in Figure 2.



Spathulenol



β-eudesmol



dioctyl hexadioic

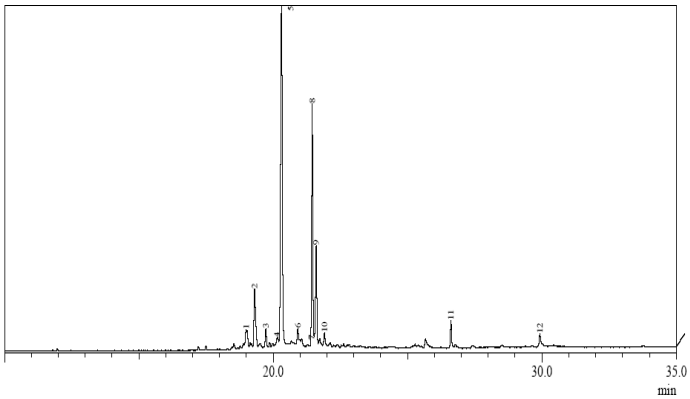


Figure 1. Chromatogram analysis of fraction 3 by gas chromatography

Table 2. The toxicity test of fractions from column chromatography against larvae of *Artemia salina* Leach

fraction	Concentration (ppm)	The number of dead larva			% Mortality	LC_{50} ppm
		1	2	3		
F1 (1-10)	0	0	0	0	0	257,04
	10	1	0	1	3,59	
	100	2	1	1	18,75	
	1000	10	10	10	100	
F2 (11-25)	0	0	0	0	0	33,11
	10	1	0	1	6,09	
	100	10	9	8	90,63	
	1000	10	10	10	100	
F3 (26-36)	0	0	0	0	0	31,62
	10	2	0	1	6,48	
	100	9	9	9	90,91	
	1000	10	10	10	100	
F4 (37-64)	0	0	0	0	0	33,11
	10	0	0	0	5,58	
	100	9	9	8	92,86	
	1000	10	10	10	100	
F5 (65-94)	0	0	0	0	0	63,10
	10	0	0	0	4,10	
	100	4	4	5	60,47	
	1000	10	10	10	100	
F6 (95-229)	0	0	0	0	0	165,96
	10	0	0	0	3,65	
	100	3	2	2	37,48	
	1000	10	10	10	100	

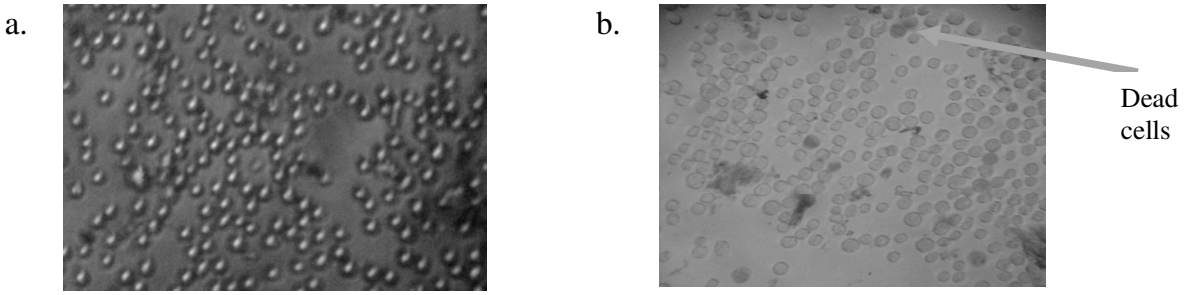


Figure 2. Myeloma cells under the microscope: a) the healthy cells; b) after given toxic isolates (F3)

The graph comparison of concentration by the percent viability of dead myeloma cells can be seen in Figure 3.

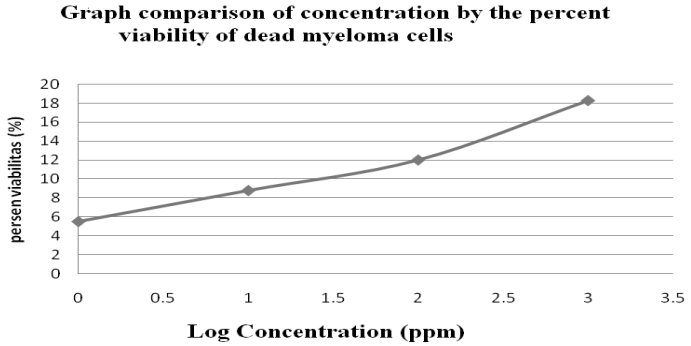


Figure 3. graph comparison of concentration by the percent viability of dead myeloma cells

The picture of HeLa cells before and after being given toxic isolates are presented in Figure 4. While the graph comparison of concentration by the percent viability of dead HeLa cells can be seen in Figure 5.

a. b.

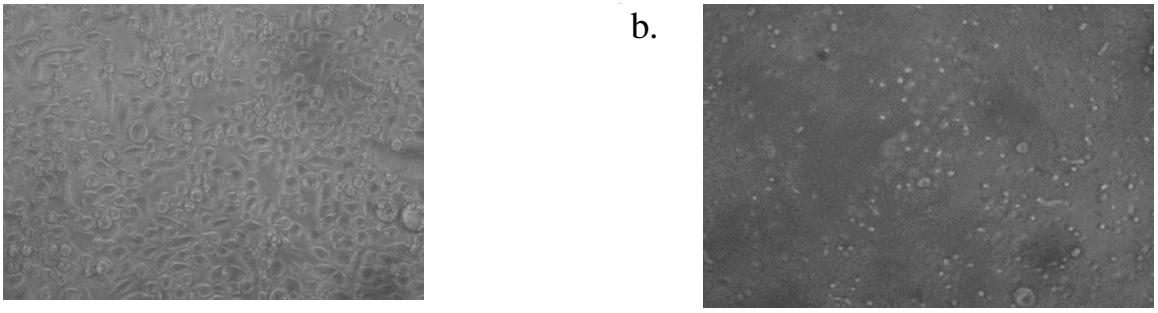


Figure 4 HeLa cells before and after being given toxic isolates: a) before; b) after

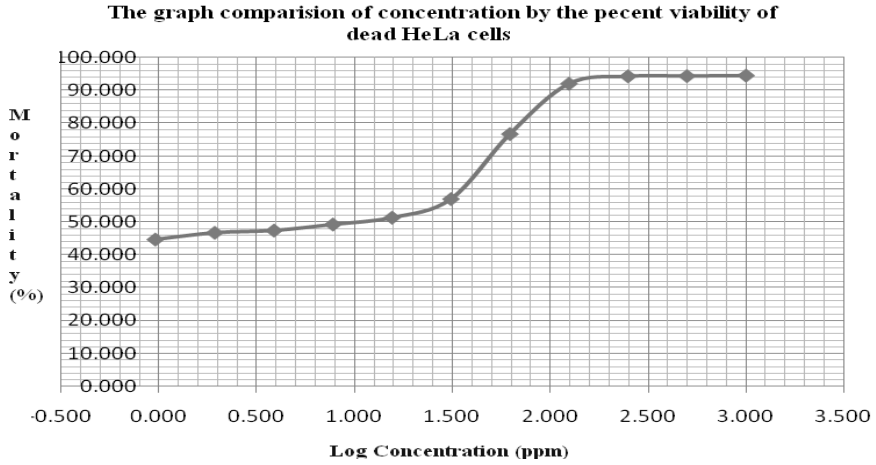


Figure 5 the graph comparison of concentration by the percent viability of dead HeLa cells

The toxic isolate (F_3) chloroform extract of the white turmeric cannot inhibit the growth of myeloma cells. While *in vitro* test toward HeLa cells showed that isolate could inhibit the growth of HeLa cells with LC_{50} of 12,58 ppm.

CONCLUSION

The compounds contained in toxic isolate (F3) derived from the chloroform extract of white turmeric rhizomes can be identified as sesquiterpene alcohol (spathulenol and β-eudesmol) and the ester (dioctyl heksadioic). The Toxic isolate (F3) cannot inhibit the growth of myeloma cells, but it can highly inhibit the growth of HeLa cells with LC_{50} of 12.58 ppm.

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IN VITRO MIGRATION OF MURINE LYMPHOKINE-ACTIVATED KILLER (LAK) CELLS

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ABSTRACT

The motility and deformability of lymphokine-activated killer cells, purified by their adherence to plastic (A-LAK cells), was investigated *in vitro*. *In vitro*, A-LAK cells were observed as intrinsically motile cells. They continuously change their shape while forming protopods or pseudopods and crawled over the culture surface. A-LAK cells were able to migrate across micropores of 3 μm diameter, which was three times smaller than the average diameter of an A-LAK cell. Even in the absence of serum factors and of interleukin-2 (IL-2), more than half of the inoculated cells migrated across such micropore membranes within 18 h. Electron microscopic examination of these micropore membranes showed that the A-LAK cells were highly deformable. A-LAK cells also migrated across confluent monolayer of endothelium-like 10T1/2 cells. After 24 h incubation on the mono layers, about 20% of the A-LAK cells were found underneath the monolayer. There, they actively moved in the narrow space between the monolayer and the bottom of the culture dish. This observation represents a very important aspect determining the efficiency of A-LAK in immunotherapy.

Keywords: lymphokine-activated killer cells ● migration

INTRODUCTION

The use of cytolytic lymphocyte in adoptive immunotherapy has recently attracted wide attention as a potential form of therapy against cancer. Clinical trials in patients with cancer have already been initiated with infusion of Lymphokine-Activated Killer (LAK) cells, i.e. lymphocytes that have been activated and expanded in cultures in the presence of highly concentration Interleukin-2. Exposure of lymphocytes to IL-2 results in their proliferation and the development of enhanced cytotoxicity against neoplastic cells. These activated lymphocytes are derived from non B (because they lack surface immunoglobulin) and non T cell (because they lack T cell receptor) precursor found in spleen, bone marrow and the peripheral blood.

IL-2 stimulated lymphocytes have proven to be effective antitumor effectors in a variety of animal immunotherapy models, and clinical trials are in progress. However, the therapeutic benefits have been limitated and there have been considerable problems with side effects associated with the current treatment protocols. The regulation of lymphocytic traffic through tumors is poorly understood. The endothelium of tumor vasculature may have selective lymphocyte binding properties. Soluble chemotactic factors, locomotion inhibitor factors, and substrate-bound adhesion gradients probably all coexist and interact in the tumor microenvironment.

Lymphocyte must have the capability to migrate throughout the organism they are to defend, not only by passive transportation in the blood and lymph, but also active movement into three dimensional lattices of tissues (Verschueren, *et al*, 1987) and extra cellular matrix during extravasation and infiltration into inflamed tissues. *In vitro* models can be valuable tools for analyzing the lymphocyte properties and micro environmental variables. The possibility that lymphocytes reach extra vascular sites by responding to chemotactic stimuli has prompted a number of investigations using filter assays. Most of the work on leukocyte motility has been done with neutrophils and monocytes, and much less is known about lymphocyte motility.

MATERIALS AND METHODS

Lymphocyte Culture

Murine splenic cultures (8-12 wk-old C57Bl/6 mice) were stimulated with human recombinant IL-2 by a procedure modified from that of Vujanovic *et al*, 1988. Cell suspension was depleted of B-cells and macrophages by passing through a nylon wool column and incubated for 45 min at 37°C, 5% CO_2 , 95% humid air. The nonadherent cells were recovered from the column. The cells were washed in Phosphate Buffered Saline (PBS) pH 7.4 and resuspended in complete medium (RPMI 1640 supplemented with 0.2 mM L-Glutamine, 100 U/ml penicillin, 100 µg/ml streptomycin, 10% inactivated fetal calf serum FCS)

with 50 µm 2-Mercaptoethanol and 1000 U/ml hrIL-2. Lymphocyte suspension of 15–20 ml (2x10^6/ml) culture were incubated at 37°C, 5% CO_2, 95% humid air, in 75 cm^2 tissue culture flasks. After 2-4 days the floating cells were removed. These adherent cells were further cultured for another 3-5 days. At the end of the culture period there were adherent (A-LAK cells) which are adherent to the plastic and non adherent which are not adherent to the plastic but derived from adherent ones (Gunji *et al*, 1989)

Cytology

Morphological analysis was performed in cytocentrifuge preparations. Lymphoid cells were resuspended at 0.5x10^6 /ml of complete medium, prepared in a Cytospin 2 , centrifuged at 900 rpm for 7 min. The slides were then stained with May-Grünwald-Giemsa.

The cells were observed with a high power objective 63x, 100x, microscope and the cells were identified on the basis of the size, of nuclear shape, and of the presence of typical granules in the cytoplasm.

Monolayer Invasion Assay

The 10T1/2 fibroblast cells were used for monolayer and cultured in Basal Medium Eagle (BME) with Earle salts, supplemented with antibiotics and Fetal Calf Serum (FCS, 10% during growth phase, 2% after confluence was reached). Sub cultures were made using Trypsin (0.2%) and EDTA (0.005%) in Phosphate Buffered Saline (PBS). For control BW-O-Lil cells (invasive to hepatocytes *in vitro* and highly metastatic in vivo) were used.

Confluent 10T1/2 monolayer in 25 cm^2 flask or 10 cm^2 dishes were changed to RPMI 1640 medium with 2% FCS. A-LAK (effector) cells of 2x10^6 were added per cm^2. 24 hr later the dishes were vigorously shaken, the medium was withdrawn, and fresh medium added.

Using phase-contrast optics, the underlying effector cells could easily be identified. The flattened effector cells were counted in 10 microscope fields of 0.6 mm^2 (20x objective). (Verschueren *et al,* 1987).

Migration and Locomotion Assay

In vitro migration of lymphoid populations was assayed by a technique using Transwell-24 plates. The lower wells of the chamber were filled with 300 µl of control medium or chemo attractant. The upper compartment of the micro chamber was separated by polycarbonate filter with 3 µm pore size. A-LAK cells (10^6) in 200 µl medium were seeded in the upper wells and were incubated for 4 hr, 7 hr, or 18 hr at 37°C in 5% CO_2 humid air incubator. Subsequent tests were done in serum free conditions. The cells that migrated through the membrane to the lower compartment were counted with counting chamber. Percentage of migration was measured from the number of cells in the lower compartment as a fraction of the total cell number. At the end of incubation, filters were removed, fixed, dehydrated, embedded in epon for transmission electron microscopy or were critical-point dried and gold sputtered for scanning electron microscopy.

Microscopy

For scanning electron microscopy (SEM), cells grown on cover glass were fixed with glutaraldehyde and postfixed with 1% OsO_4 in Cacodylate buffer, dehydrated, critical point dried, and gold sputtered. Microscopic analysis was done with a Philips SEM 50 S electron microscope. For transmission electron microscopy (TEM), cells grown on 24 wells were fixed with glutaraldehyde and postfixed with 1% OsO_4 in Cacodylate buffer, dehydrated, dehydrated, embedded in Epon, removed from plastic wells with liquid nitrogen. Ultra thin sections were made perpendicular to the monolayer or culture substrate. Microscopic analysis was done with a Philips TEM 400 electron microscope.

RESULTS

Morphology of A-LAK cells

Adherent -LAK cells will proliferate faster and express the homogeneous morphology, with hand mirror shape, protruding protopodia, anterior nucleus and tail (uropod). The cells moved actively crawling on plastic surface, and have locomotory shape with the leading protopodia followed by the nucleus, in its turn followed by the granules, and other organelles. Cytospin preparations showed that the cells are homogeneous and 99% with azurophilic granules. The nucleus is slightly indented and eccentric cytoplasm is abundant. In old cultures, cells were appeared with a bigger size, abnormal granules and sometimes more than one nucleus. These cells are probably senescent cells. They have no locomotory shape and did not crawl over the plastic but are firmly attached to it and needed more time in cold PBS to be attached.

Monolayer Invasion Assay (MIA)

Adherent -LAK cells were found to interact with a monolayer of 10T1/2 fibroblast cells. The interaction of A-LAK cells was compared to BW-O-Lil cells which are highly invasive in this assay. As seen in phase contrast microscopy, 10T1/2 cells formed a smooth layer that was monocellular in the sense that nuclear overlap did not occur. The cytoplasmic overlapping can be seen, indicating that the monolayer was completely confluent, leaving no part of the culture substrate uncovered. The cells were large and extremely flattened, and could easily be distinguished from the smaller A-LAK cells.

A-LAK cells seeded onto 10T1/2 monolayer expressed a locomotory morphotype with protopodia and uropodia, some with strong halo and they were easily distinguishable from the cells that were flattened under the monolayer. The monolayer looked intact in the vicinity of most of the flattened cells, where the 10T1/2 cytoplasm was visible.

It is clear that BW-O-Lil cells and A-LAK cells are able to penetrate through the monolayer, make contact with substrate underneath, and become located between the substrate and a resealed monolayer. After 24 hr there were approximately 35-47 underlying LAK cells/mm2 and 95-130 BW-O-Lil cells/mm2. Infiltrating A-LAK cells were also seen to migrate underneath the monolayer.

Micropore Migration Assay

The migration of A-LAK and bulk LAK was compared in different concentrations of FCS. The result shows that A-LAK cells always give higher migration in every FCS concentration tested, as compare to bulk LAK.

Microscopy

Adherent -LAK cells resemble larger granular lymphocytes in morphology with size of 9 μm, which are larger than smaller lymphocytes (5-7 μm) but smaller than big LAK cells (11 μm). They have high cytoplasm/nucleus ratio and containing azurophilic granules. Nuclear shape frequently eccentric and reniform. Under electron microscopy, the cytoplasm contains a large number of mitochondria and granules. These granules contain an amorphous dense matrix in the center, surrounded by vesicular material in the periphery. As seen in the light microscopy, A-LAK cells on the filter actively moved, they formed the uropod and protopods. In the TEM, the penetration of 9 μm diameter LAK cells through 3 μm pore could be seen, leading with the protopods, followed by the nucleus which was squeezed across the pore and followed by the uropod with the organelles. The cells dropped into the medium of the wells and some adhered to the bottom of the filter.

DISCUSSIONS

Certain spleen cells, upon incubation with IL-2, developed the ability to adhere to plastic. These plastic adherent cells could be separated from non-adherent cells and expanded in the presence of IL-2, they were termed A-LAK cells. MIA and transwell assay can be used to analyze the interaction of A-LAK cells with monolayer of 10T1/2 fibroblast like cell line. The cells migrated actively through 3 μm pores polycarbonate filter. This is an active process; suggest that the tumor cells secrete soluble factors that

increase the motility of A-LAK cells. They are functional assays to provide direct evidence for deformability and migratory capacity of the cells. IL-2 stimulated lymphocytes have proven to be effective antitumor effectors in *in vitro* immunotherapy models.

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CONCENTRATION OF 20 % IN THE EXTRACT OF *ARECA CATECHU* L. MAY REDUCETHE NUMBER OF COLONIESOF *CANDIDAALBICANS* ON THE HEAT CURED ACRYLIC RESIN

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ABSTRACT

Removable acrylic denture cleaning is very important to prevent inflammation of the oral mucosa under the acrylic denture base that is called Denture Stomatitis. Denture Stomatitis can be caused plaques and microorganisms particularly *Candida albicans* that attach to the acrylic denture base. The prevention of denture stomatitic is by keeping clean and preventing mouth and denture from contamination of *Candida albicans*, by submerging the denture using denture cleanser.This research is aimed to determine the effectiveness of the extracts of *Areca catechu* L. as denture cleanser to the presence of *Candida albicans* on acrylic resin denture base. In a research, there was an acrylic resin plate10x10x1mm's immersion in a solution of the extracts of *Areca catechu* L. of 20 % for 2 hours.The results of this study is that it can be concluded that by submerging the extracts of *Areca catechu* L. at the concentration of 10% is effective way to reduce the number of colonies of *Candida albicans*, and submerging the extracts of *Areca catechu* L for 2 hours effective way to reduce the number of colonies of *Candida albicans*.

Keywords: *Denture stomatitis*, *Areca catechu L.*, *Candida albicans*

INTRODUCTION

Human teeth have many roles; loss of teeth from the mouth of a person will result in changes in anatomical, physiological and functional and sometimes it also causes psychological trauma.

In 2007, Health Research Association (RISKESDAS) of Ministry of Health of Republic of Indonesia reported that loss of teeth in the age group of 45-54 years were 1.8%, 5.9% in 55-64 years old, and in the age group of 65 years and olderreached 17.6%, where dentures usage is needed[1]. The use of dentures that can continuously cause some reaction to the network because the mucosa under the dentures will be covered in a long time, thereby blocking the oral mucosal surface cleaning and dentures by the tongue and saliva resulting in the attachment of microorganisms suchas *Candida albicans*, resulting in denture stomatitis.[2,3]

Prevention of denture stomatitis is by maintaining oral hygiene and cleanliness of dentures from the contamination of *Candida albicans.* One way to prevent denture stomatitis is by soaking the dentures with a cleaning solution/denture cleanser. Cleaning solutions which are often used are varying, and most are based of cleaning agents of chemicals with a relatively expensive price. One alternative material that can inhibit the growth of fungus can be found in *Areca catechu*L.

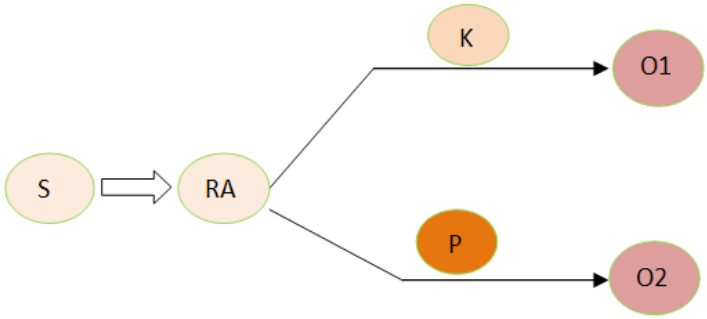
PURPOSE

The purpose of this study is to prove that the methanol extract of *Areca catechu* L. may inhibit the growth of colonies of *Candida albicans* in vitro to heat cured acrylic resin plate.

MATERIALS AND METHODS

The design of this study is experimental laboratory, using a control group using the design of posttest only control group design.[4]

The instrument used in this study is vibrator, cuvette, hydraulic press, incubator, sterile petri, Bunsen, tweezers, incubator, autoclave, reaction tubes, spreader, Whatman filter paper, Erlenmeyer, micropipettes, tally counters. This research material is acrylic resin, stock *Candida albicans* (ATCC 10 231) of the Section of Microbiology Faculty of Medicine, University of Gajah Mada, extracts of *Areca catechu* l. Sabouraud's dextrose agar, Phosphate Buffer Saline solution/PBS pH 7.0, 100 cc of sterile saliva, aquades, alcohol 95%, NaCl.



S : Samples
RA : Random allocation
K : control (sterile distilled water)
P : Treatment, the extract of *Areca catechu l.* concentration of 10%

The procedure of this study is the acrylic resin plate (10x10x1) is washed under running water for 48 hours to reduce the residual monomer, then is sterilized using an autoclave 121 ^{0}C for 18 minutes.[2,3] Acrylic plate is immersed in saliva for 1 hour, then PBS-rinsed twice[5]. Furthermore, heat cured acrylic resin plate is inserted into a test tube containing a suspension of *Candida albicans* and then incubated for 24 hours at 37^0 C. The acrylic resin plate after contaminated by putting it into the test tube containing methanol extract of *Areca catechu* L. 10% with 8 hours of soaking time, is to control the use of acrylic resin plate that distilled and rinsed twice with PBS to remove the residual methanol extract of *Areca catechu* l. that are still lagging behind in plat. Acrylic resin plate was put in 10 ml RPMI medium, then it was vibrated with a vortex for 30 seconds to release the *Candida albicans* attached to the acrylic plate. Taking 0.1 ml suspension of *Candida albicans* in RPMI media, and incorporated into Sabouraud's dextrose agar, underwent incubation spreading for 48 hours at 37 ^{0} C, and counting the number of colonies of *Candida albicans* in CFU/ml.[2,3]

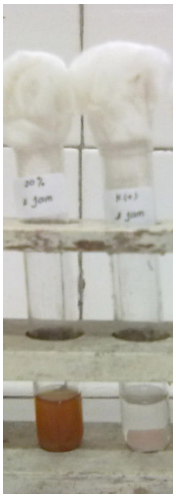


Figure 1. The Soaking of the 20% *Areca catechu* L. acrylic plate for 2 hours and distilled water

RESULTS

The results from the difference between treatments with control were tested by One Way Anova. The mean number of *Candida albicans* after a given treatment were significantly different ($p < 0.05$). The results of the analysis are shown in the table below.

Subject Group	n	Mean Number of *Candida albicans*	SB	*F*	P
Control (Aquadest)	3	15200,00	1430,52	43,06	0,001
Extract of Areca Catechu L. 20%	3	7080,00	385,75		

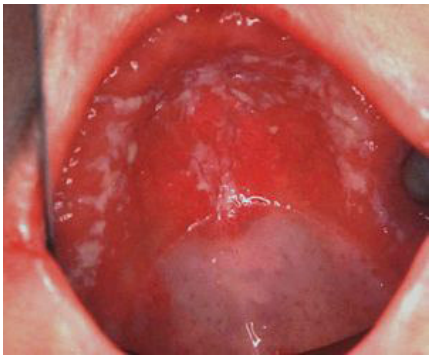


Figure 2 Denture Stomatitis

DISCUSSION

The Immersion of heat cured acrylic resin plate in a solution of *Areca catechu* L. methanol extract with a concentration of 20% with 2 hours soaking can decrease the number of colonies of *Candida albicans* to 7080.00 CFU/ml out of distilled water control with the number of colonies 15200.00 CFU / ml (reduced to 53,42%).

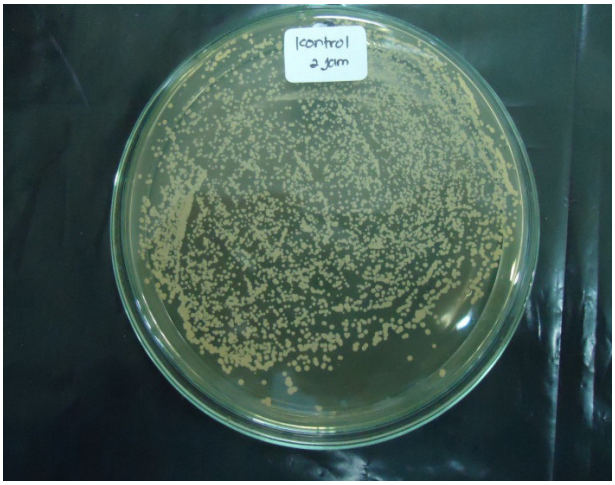


Figure 3.The number of Colonies of C.albicans in Sabouraud medium, s dextrose agar.The threshing result of acrylic resin plate after immersion in distilled water for 2 hours

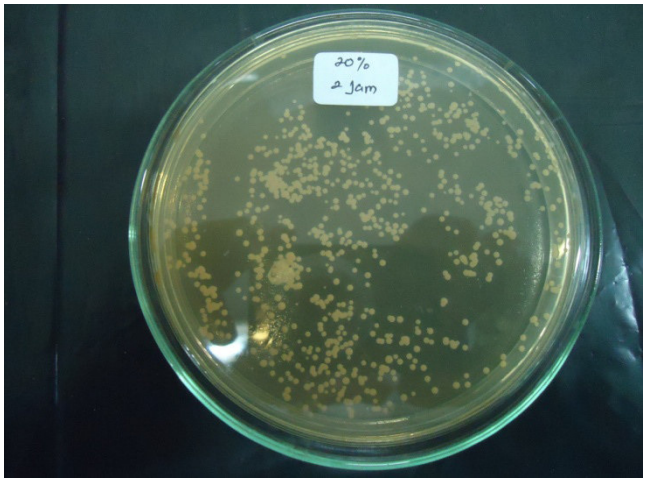


Figure 4. Number of Colonies of *C.albicans* in Sabouraud medium, s dextrose agar.The threshing result of acrylic resin plate after immersion in 20% of *Areca catechu* L. extract for 2 hours

Anti-fungal effect on the extract of *Areca catechu* L. due to a chemical compound in *Areca catechu* L. The chemical class of compounds including tannins, saponins, phenolics, flavonoids, terpenoids, steroids and alkaloids. The effect of phenolic compounds against C. *albicans* is by denaturizing the binding protein on the cell membrane, so that the cell membrane can be analyzed and the possibility of phenol to penetrate into the nucleus. With the entry of phenol into the cell nucleus, it will cause the fungus of *Candida albicans* does not develop. *Candida albicans* is a species thas is highly sensitive to phenolic compounds and phenols are widely used as a disinfectant. Efficacy of anti-fungal compounds have been reported due tothe saponins and flavonoids.[6-9]

Alkaloids are compounds that have antimicrobial activity, which inhibits esterase as well as DNA and RNA polymerase, also inhibits cell respiration and plays a role in DNA intercalation.Flavonoid compounds have been reported as an anti-fungal. As an anti-fungal, flavonoids can inhibit fungal growth as in vitro. [10,11] Flavonoids also can interfere with the process of diffusion of food into the cells so that the fungal growth is halted or until the fungi are dead. While saponin will be as a surfactant in polar form, breaking up the fat layer on the membrane causing disruption germ cell membrane permeability, resulting in inclusion of materials or substances that are needed may be disrupted and eventually the cell will swell and burst.[12]

The research data of LSD test , shows that the control group (sterile aquades) have significant differences in all treatment groups. That is because sterile aquades does not have anti-fungal effect against *Candida albicans.* The data also shows that immersion in distilled water as control of the longer trend immersion occurs, and there are more number of colonies of *Candida albicans* in acrylic resin plate. These results are probably due to an increase in the number of colonies of *Candida albicans* and immersion in sterile distilled water, derived from *Candida albicans* that multiplies in a row with the accretion of immersion time, because distilled water is not inhibiting the growth of *Candida albicans* colonies[2].

CONCLUSION

Based on the research results of giving extract of *Areca catechu* L. the conclusions are as follow: The extracts of *Areca catechu* L may inhibit the growth of colonies of *Candida albicans*.Soaking in the concentration of extract of *Areca catechu* L. 20%, with 2 hours of immersioncan reduce the number of colonies of *Candida albicans*.

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THE EFFECT OF INFUSION PURPLE ROSELA FLOWER (*HIBISCUS SABDARIFFA VAR SABDARIFFA RUBBER*) ON THE LEVELS OF SGOT (*SERUM GLUTAMATE OXALOACETATE TRANSAMINASE*) AND SGPT (*SERUM GLUTAMATE PYRUVATE TRANSAMINASE*) IN HYPERLIPIDEMIA RATS

Lastdes Cristiany Friday[1] Fatma Zuhrotun Nisa[1] Umi S Intansari[2]

Illness results from bad consumption increasing day by day. Bad consumption is inadequate intake both the quality and quantity. One of example of bad consumption is high fat with low fiber diet which is causing hyperlipidemia and furthermore causing destroys liver function. Many methods to solve that problem, one of the ways are with consuming Purple Rosella (Hibiscus sabdariffa var sabdariffa rubber) as an alternative treatment. The aim of this study is to know the effect of purple rosella (Hibiscus sabdariffa var sabdariffa rubber) to SGOT and SGPT serum in white rats (Rattus norvegicus) with hyperlipidemia condition. The research was done with experimental pre and post test group control design. The research used 28 rats at the age of ± 60 days were given high fat diet in one week after that it given Purple Rosella in 28 days. Statistic analysis was used One-way ANOVA with tukey post hoc test. SGOT level in group A was decreased 28.79%, group B was decreased 52.23%, group C was decreased 60.12%. SGPT level in group A decreased 15.30%, group B decreased 46.76%, and group C decreased 58.95%. Purple rosella consumption can decreased SGOT and SGPT serum level significantly. The highest decreasing of SGOT and SGPT level was showed by dose 0.09 gr/ml/250 gr BB

Keywords: Hyperlipidemia, purple rosella, SGOT, SGPT

INTRODUCTION

Illness due to consumption patterns that one day becoming increasingly the case. Consumption is one of the intake is not adequate both in terms of quality, type and number. One common misconception is that the consumption patterns of food consumption patterns of high-fat and low in fiber, resulting in hyperlipidemia. Hyperlipidemia is a general term for any or all of the increase in plasma lipids (1). Hiperlipid can cause disturbances in the liver due to fat metabolism occurs in the liver. Many ways in which to tackle these problems one of which is to take an active substance in a plant or herb is thought to have anti-hyperlipidemic effect. One type of herbs that have been studied are rosella flowers. Efficacy rosella flower varieties have been widely studied but are consumed and have limited dietliti red rosella flowers. Active ingredient in the anthocyanin rosela can maintain liver function (2). Rosela purple has the highest anthocyanin content compared with the other two varieties rosella (3). Therefore, researchers wanted to find out more about the effect of steeping water roselle purple petals treated high-fat diet.

MATERIALS AND METHODS

This study is purely experimental research. The design of this study used a completely randomized design (CRD). Studies over 42 days old consisting of 7 days of adaptation, 7 days a feed high in fat, and 28 days of treatment administration rosela purple. The materials used are dried purple flower petals rosella, a standard feed that is feed from Japfa Comfeed Br II, high-fat food is a standard feed mixed with pork fat 10%, and the white rat male *Sprague Dawley* age ± 2 months with a weight of ± 250 gr. Rats were divided into 4 groups, the first group was a control group given aqua, the second group is the group given infusion rosella purple petals with a dose of 0.0225 gr gram/ml/250 g w, the third group is the group of rats given infusion rosella flower petals purple with a dose of 0.045 g/ml/250 g w, the fourth group is the group of rats given infusion Effect of Rosella Purple Flower petals against SGOT and SGPT levels steeping rosella purple petals with a dose of 0.09 gram/ml/250 g w. Tools used in this study are the individual cages, glass capillary tubes (mikrohematokrit), Canula Syringes, centrifuges, and spectrophotometers. The data are grouped based on the measurement results of the study period after 7 days of adaptation, feeding high-fat, 7 days, and after 28 days treatment rosella purple. Data delivery rosella, number of data grouped mice with body weight, weight of feed, food remains, the data content of SGOT, SGPT, batch number and date of measurement data analysis in this research is to use parametric statistical methods namely normality using the Shapiro-Wilk, and then test ANALISYS of

Variance (ANOVA) to see is there any treatment effect is significant difference of the observed variables, then followed by Post hoc Tukey

RESULT AND DIISCUSSION

SGOT Levels

Increased levels of SGOT and has significance based on test results Paired T-tests indicated by the value of P = 0.000. SGOT data are shown in Table 1 and decreased levels of SGOT can be seen in Figure 1.

Table 1. SGOT levels after infusion of Purple Roselle Flower in *Sprague Dawley*

Group	Adaptation (IU/L)	Hyperlipidemia (IU/L)	Treatment (IU/L)
Control	18.61±0.30	46.18±0.82	46.27±0.80
Dosage 0.0225 gr/ml/250 gr w	18.53±0.51	46.87±0.68	33.38±0.45
Dosage 0.045 gr/ml/250 gr w	18.45±0.42	46.14±0.83	22.04±0.30
Dosage 0.09 gr/ml/250 gr w	18.13±0.28	46.59±0.88	18.58±0.29

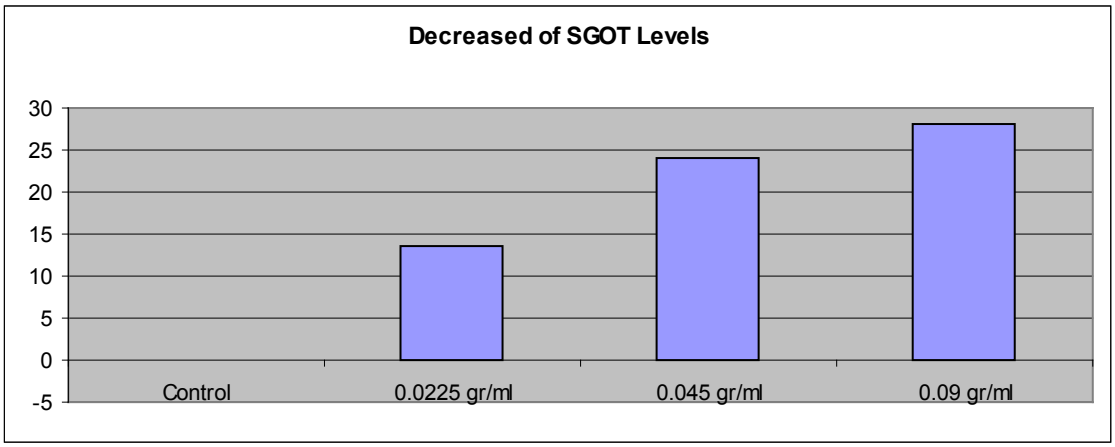

Figure 1. Decreased of SGOT Levels after infusion of Purple Roselle Flower in *Sprague Dawley*

The end of the study in the control group did not occur due to decreased levels of SGOT in the control group not given steeping rosella flower petals purple. In the dose group BB 0.0225 g/ml/250g significant decline from an average of 46.87 IU / L to 33.38 IU / L 0 with a decrease of 28.79%. In the dose group g/ml/250g BB 0.045 significant decrease from an average of 46.14 IU / L to 22.04 IU / L with a decrease of 52.23%. At the 0.09 dose group BB g/ml/250g significant decrease from an average of 46.59 IU / L to 18.58 IU / L with a decrease of 60.12%. Post hoc Tukey test based on the known sequence of the best dose in lowering levels of AST in rats is dose group g/ml/250g BB 0.09, then the dose of 0.045 g/ml/250g BB and the last is the dose groups 0.0225 g/ml/250 g BB.

SGPT Levels

SGPT can be seen from the data that an increase in alanine amino transferase levels and has a significance test based on the results of Paired T-tests are indicated with a P value = 0.000. SGPT data can be seen in Table 2 and decreased levels of SGPT can be seen in Figure 2.

Table 2. SGPT levels after infusion of Purple Roselle Flower in *Sprague Dawley*

Group	Adaptation (IU/L)	Hyperlipidemia (IU/L)	Treatment (IU/L)
Control	20.92±0.29	54.96±0.98	55.36±1.05
Dosage 0.0225 gr/ml/250 gr w	20.65±0.26	54.99±1.21	46.58±0.42
Dosage 0.045 gr/ml/250 gr w	21.00±0.32	54.24±0.68	28.88±0.65
Dosage 0.09 gr/ml/250 gr w	21.17±0.39	54.50±1.00	22.37±0.36

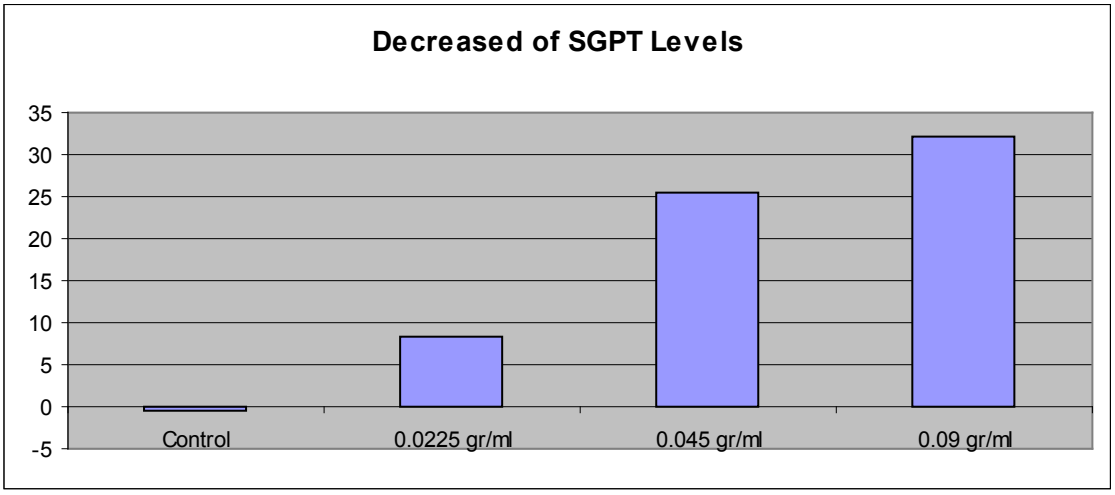

Figure 2. Decreased of SGPT Levels after infusion of Purple Roselle Flower in *Sprague Dawley*

At the end of the study in the control group did not occur due to decreased levels of alanine aminotransferase in the control group not given steeping rosella flower petals purple. In the dose group g/ml/250g BB 0.0225 alanine aminotransferase levels decreased significantly from an average of 54.99 IU / L to 46.58 IU / L with a decrease of 15.30%. In the dose group g/ml/250g BB 0.045 significant decrease from an average of 54.24 IU / L to 28.88 IU / L with a decrease of 46.76%. At the 0.09 dose group BB g/ml/250g significant decrease from an average of 54.5 IU / L to 22.37 IU / L with a decrease of 58.95%. Post hoc Tukey test based on the known sequence of the best dose in reducing ALT levels in rats is dose group g/ml/250g BB 0.09, then 0.045 g/ml/250g BB dose group and the last is the dose groups 0.0225 g/ml/250 g BB.

SGOT and SGPT levels after infusion petals Given Rosela Purple Flower (Hibiscus sabdariffa var sabdariffa rubber)

Decreased levels of SGOT and SGPT in Sprague Dawley rats do not escape from this cholesterol-lowering mechanism presumably because the active compounds contained by purple petals rosella (Hibiscus sabdariffa var sabdariffa rubber). Phenol compounds are organic compounds that have at least one organic ring with one or more carboxyl groups. Isoflavones are phenolic compounds that have powerful natural antioxidant activity because of its ability to stabilize free radicals. In rosela purple purple dyes are compounds of flavonoids that have antioxidant activity of natural (4). Substance delphinidin-3-sambubioside an anthocyanin-containing antioxidants can prevent oxidation of LDL (5). The mechanism of reduction of antioxidants on lipid oxidation in rosela stage serves as a barrier to oxidation and decrease the speed of reaction initiation (6). Substance 3-hydroxymethylglutaril-Co-A reductase (HMG-Co-A) is an enzyme of cholesterol biosynthesis pengambat that helps cleanse the liver so that the speed of the rest of chylomicrons cholesterol synthesis in the liver depends on this enzyme. Rosella role is to increase the amount of chylomicrons is clean so the loss of many of triglycerides affect the lipid profile that liver function as lipid metabolism maintained (7). The content of vitamin C in purple rosela accelerate beta

oxidation resulting in lower levels of triglycerides in the blood by stimulating the synthesis of carnitine on track so that it can decrease the concentration of triglycerides in the blood (8). Vitamin E is chain breaker peroxide on the membrane of fat and Low Density Lipoprotein (LDL). Mechanism of action of vitamin E in donating hydrogen ions to neutralize or reduce blood fat levels peroxide work begins with α-tocopherol radical which is then transformed into α-tocopherol peroxide, α-tocopherol radical tocoferol transformed into α-α dimers and eventually became a by-tocoquinone Vitamin C can be regenerated back into α-tocoferol (9). Decreased levels of SGOT and SGPT in the suspect can be caused by pectin contained in rosella flower petals purple. Pectin has a binding effect of organic substances such as bile acids and cholesterol, thus lowering the amount of fatty acids in the digestive tract (10). Niacin contained by rosella purple can also lower cholesterol levels. Mechanism of action of niacin is a form molecule with endogenous cholesterol by binding cholesterol in the intestine and is excreted through the feces (11).

CONCLUSION

Purple Flower petals Rosela infusion significantly influences the lower levels of SGOT and SGPT white rats fed a diet high in fat. From the Tukey test in the know that the levels of steeping roselle purple petals of the greatest lower levels of SGOT and SGPT was 0.09 gr/ml/250 grBB dose group.

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CARBAPENEM RESISTANT-*KLEBSIELLA PNEUMONIAE* INSANGLAH HOSPITAL: DISTRIBUTION AND SUSCEPTIBILITY PATTERN

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ABSTRACT

Carbapenem resistance in *Klebsiella pneumoniae* is increasing. Most of these bacteria are involved in nosocomial and systemic infection. This issue is a serious challenge in the treatment of infectious diseases because these bacteria are resistant to almost all kind of antibiotics and the mortality either morbidity due to infection of these bacteria are high. The purpose of this study was to determine the prevalence and susceptibility profiles of *K. pneumoniae* resistant to carbapenem from January-June 2011 at Sanglah Hospital. Antimicrobial susceptibility of all isolates was performed by disk diffusion method and interpreted by Clinical and Laboratory Standard Institute (CLSI) 2011. A total of 110 clinical isolates of *K. pneumonia* were found. About 10.9% (12/110) isolates of *K. pneumoniae* resistant to carbapenem (meropenem or ertapenem or imipenem). Most isolates of *K. pneumoniae* resistance to carbapenem were obtain from surgery department 33.3% (4/12) and most collected from urine specimens 33.3% (4/12). Further study to confirm phenotype and genotype is needed for early detection of *Klebsiella pneumonia* resistance to carbapenem.

Keywords: Carbapenem-Resistant *Klebsiellapneumoniae*, antibiotic susceptibility pattern.

INTRODUCTION

Klebsiella spp. commonly infect the patients who are hospitalized and suffer from severe underlying diseases such as diabetes mellitus, chronic pulmonary obstruction, urinary tract infection, pneumonia and intra abdominal infections. Carbapenems are the widest spectrum of antibacterial activity and frequently used as the last choice in treating serious infection caused by multidrug resistant microorganism. Unfortunately, the emergence of carbapenem resistance *Klebsiellapneumoniae*make a serious problem in infection control worldwide. The data about *K. pneumoniae*resistance to carbapenem in our country is very limited. Therefore in this study we conducted to evaluate the prevalence distribution and antibiotic susceptibility pattern of *K. pneumoniae*from clinical specimens.

MATERIAL AND METHODS

Clinical isolates

The total 110 spesimens*Klebsiella pneumoniae* for this study were collected from inpatient of Sanglah Hospital during January-June 2011. Infections caused by more than one microorganism and the contamination were excluded.

Antimicrobial susceptibility test

Antimicrobial susceptibility testing of the isolates was perform on Mueller Hinton agar plate by disk diffusion method and interpreted by Clinical and Laboratory Standard Institute (CLSI) 2011.*Escheriachia coli* ATCC 25922 used as control organism.

RESULT

This study found 12 isolates (12/110) of *Klebsiella pneumoniae* resistance to carbapenems.

Most carbapenem resistance isolates were from surgical department and ICU and most found in urine isolates.

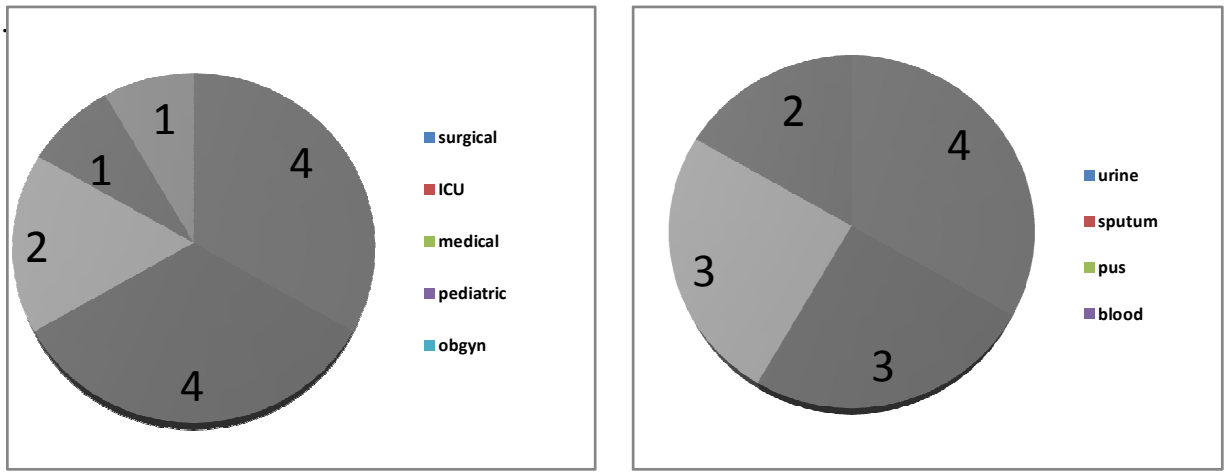

Figure 1. Distribution of KPC producing*Klebsiellapneumoniae*

Isolates that were resistant to ampicillin, aztreonam, cefotaxime, cefuroxime was 91.7% (11/12), resistant to gentamicin and cephalothin was 83.3 % (10/12); resistant to ceftazidime was 75% (9/12); resistant to amikacin, chloramphenicol, piperacill in/ta zobactam, trimethoprim/sulfamethoxazole was 66.7% (8/12); resistant to ciprofloxacin and tigecyclin was 58.3 % (7/12).

DISCUSSION

Most carbapenem resistance isolates were from surgical department and ICU. A lot of patients are transferred to the ICU from other healthcare facilities, where they have acquired resistant pathogens. As well as Patients in surgery and ICU are usually treated with antibiotic combination, undergo invasive procedures and exposure by other patient with multiple drug resistant pathogens.[1,2]

Mechanism in carbapenem resistant include production of carbapenemase (most in Enterobacteriaceae), porin loss, upregulated efflux and combination of cephalosporinase and porin loss. Combination ofporin OmpK35/36 insertion inactivation and down regulation of the phosphate transport porin and chanes in penicillin-binding proteins caused high-level carbapenem resistance in *K. pneumoniae*[2,3].

Klebsiella pneumonia carbapenemases (KPC) can inactivates all penicillins, cephalosporins, aztreonam and carbapenem and the most common mediated carbapenem resistance in *Enterobacteriaceae.* Other Gram-negative resistance mechanism such as ESVL, fluoroquinolone and aminoglycoside can co-exist with this enzyme. The gene for KPC can spread easily among the species or even among different genera because the genes are usually on the plasmid[2,4,5].

CONCLUTION

In this study found 12 isolates resistance to carbapenem from disk diffusion suseptibility test. This result need further phenotype and genotype confirmation test.

Carbapenems are commonly used to tread infections cause by multidrug resistance microoganism and the emergence of carbapenem resistance *Klebsiella pneumoniae*make a serious problem in infection control worldwide.

Further study to confirm phenotype and genotype is needed for early detection of *Klebsiella pneumoniae* resistance to carbapenem.

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DETECTION OF TOXOPLASMA GONDII ANTIBODIES FROM SERA OF EXPERIMENTALLY INFECTED MICE BY ELISA METHOD

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ABSTRACT

Toxoplasma gondii is an obligate intracellular parasite that infects a wide range of wam-blooded animals and humans causes the diseases toxoplasmosis. In particular, there is a high prevalence in animals and humans varies in different part of the world.. Mice as an animal model of toxoplasmosis which experimentally inoculated chickens inoculant. The purpose of this study detection an increase mice Toxoplasma's antibody response by Elisa methods. The results obtained that an increase in mice Toxoplasma's antibody from prior in comparison with two weeks first and two weeks last of infections. Conclusion that the chicken inoculant causes an increase in mice Toxoplasma's antibody after 4 weeks infection.

Keyword : *Toxoplasma gondii*, Elisa method, Mice, Antibodies

INTRODUCTION

Toxoplasmosis is a zoonotic disease caused by Toxoplasma gondii. Toxoplasma gondii is an obligate intracellular parasite that infect a wide range of warm-blooded vertebrate and causes diseases in agricultural animal and nearly one-third of the human population (Dubey *et al.,* 1988). Prevalence of Toxoplasmosis varies in defferent part of the world and this variation related to, life style, age, climatic, condition, nutritional habits and other sociocultural factor (Spalding *et al*.,2005). Cases of toxoplasmosis in animals have been reported to be 50-80% and in humans 40-85% (Subekti *et al*.,2005). ELISA test based on GRA1 antigen to detect the mice toxoplasma′s antibody. Gra1 is a part of Toxoplasma gondii protein GRA. GRA1 is an antigen protein which is important as the principal components vacuoles in tachyzoite and cyst wall bradyzoite (Lebrun et al., 2007). ELISA (Enzyme Linked Immunosorbent Assay) as an enzyme immunoassay (EIA) is a biochemical technique used mainly in immunology to detect the presence of an antibody or antigen in sample. ELISA use to determine how much of particular antibody is present in an individual blood. The basic principle of an ELISA is to use an enzyme to detect the binding of antigen antibody. The enzyme converts a colorless substrate to a colored product, indicating the presence of antigen : antibody binding (Ma *et al*., 2006).

Purpose in this study is to determine response mice antibodies in sera which experimentally infected chicken inoculant for prior until 4 week infection.

MATERIALS AND METHOD

Main material of this study consisted of 40 Balb/c mice, chicken inoculant (the digestion of heart and brain local chicken), ELISA Equipment, the recombinant protein of Toxoplasma gondii Gra1 (provided by Prof Artama of UGM), Anti-Mouse IgG Alkaline Phosphatase Conjugate (Sigma) and Elisa reader.

Chicken inoculant is the product of digestion from brain and heart local chicken come from 4 district in Bali (prepared as described by Apsari et al., 2011). Balb/c mice were injected by intraperitoneal route with chicken inoculant. Before inoculation, the mice were taken a blood serum as preinoculation serum. The two weeks first and two weeks second of the infection, mice were collected blood serum as a serum sample.

Detection of the mice immune response was determined by Indirect Elisa Technique with secondary antibodies anti-mouse alkaline phosphatase conjugate and read on Elisa reader with a wavelength of 405 nm.

RESULT AND DISCUSSION

The results of 40 mice infected with chicken inoculant from 4 districts in Bali shows in Table 1.

Tabel 1. Mouse Antibodies Titer Four Weeks Infection

Provided Chicken Inoculant	Pre inoculation (Elisa Unit)	Two Weeks First (Elisa Unit)	Two Weeks Second (Elisa Unit)
Badung : 01	32	Control 1 : 32	Control 1 : 128
02	32	Control 2 : 32	Control 2 : 128
03	128	DJ,I.1 : 256	DJ.I.1 : 256
04	128	DJ.I.2 : 256	DJ.I.2 : 512
05	128	DJ.II.1 : 256	DJ.II.1 : 1024
06	128	DJ.II.2 : 128	DJ.II.2 : 64
07	128	DO.I.1 : 256	DO.I.1 : 256
08	128	DO.I.2 : 1024	DO.I.2 : 256
09	64	DO.II.1 : 1024	DO.II.1 : 512
10	64	DO.II.2 : 1024	DO.II.2 : 128
Denpasar 01	64	Control 1 : 128	Control 1 : 256
02	64	Control 2 : 256	Control 2 : -
03	64	DJ.I.1 : 256	DJ.I.1 : 1024
04	32	DJ.I.2 : 256	DJ.I.2 : 1024
05	32	DJ.II.1 : 128	DJ.II.1 : 128
06	32	DJ.II.2 : 128	DJ.II.2 : 64
07	64	DO.I.1 : 256	DO.I.1 : 256
08	32	DO.I.2 : 128	DO.I.2 : 128
09	16	DO.II.1 : 128	DO.I.1 : 128
10	16	DO.II.2 : 64	DO.II.2 : 64
Tabanan 01	16	Control 1 : 256	Control 1 : 256
02	64	Control 2 : 128	Control 2 : 256
03	32	DJ.I.1 : 512	DJ.I.1 : 32
04	16	DJ.I.2 : 512	DJ.I.2 : 256
05	16	DJ.II.1 : 128	DJ.II.1 : 256
06	32	DJ.II.2 : -	DJ.II.2 : 64
07	16	DO.I.1 : 256	DO.I.1 : 256
08	-	DO.I.2 : 128	DO.I.2 : 1024
09	-	DO.II.1 : 256	DO.II.1 : 1024
10	-	DO.II.2 : 256	DO.II.2 : -
Karangasem 01	16	Control 1 : 64	Control 1 : 64
02	32	Control 2 : 64	Control 2 : 32
03	64	DJ.I.1 : 64	DJ.I,1 : 256
04	64	DJ.I.2 : 64	DJ.I.2 : 256
05	16	DJ.II.1 : 512	DJ.II.1 : 64
06	16	DJ.II.2 : 64	DJ.II.2 : -
07	64	DO.I.1 : 64	DO.I.1 : 128
08	32	DO.I.2 : 64	DO.I.2 : 128
09	64	DO.II.1 : 64	DO.II.1 : 1024
10	32	DO.II.2 : 64	DO.II.2 : 256

DJ.I.1 : Mice first infected by Heart Chicken inoculant pooling I
DJ..I.2 : Mice second infected by Heart Chicken inoculant pooling I
DJ.II.1 : Mice first infected by Heart Chickeninoculant pooling II
DJ.II.2 : Mice second infected by Heart Chicken inoculant pooling II
DO.I.1 : Mice first infected by Brain Chicken inoculant pooling I
DO.I.2 : Mice second infected by Brain Chicken inoculant pooling I
DO.II.1 : Mice first infected by Brain Chicken inoculant pooling II
DO.II.2 : Mice second infected by Brain Chicken inoculant pooling II

In Table 1 appears there an increase in antibody titers on chicken inoculant Badung DJ.I.2 and DJ.II.1; on chicken inoculant Denpasar DJ.I.1 and DJ.I.2; on chicken inoculant

Tabanan DO.I.2 and DO.II.1 as well as in chicken inoculant Karangasem, DJ.I.1 DJ.

I.2; DO.I.1, DO.I.2 and DO.II.1. The results of the cut-off analysis titer antibody that titer is at 256, meaning that mice antibodies titers above the cut-off value showed a positive Toxoplasma gondii (Biorad, 2000).

Increasing antibody titers in mice that showed positive results detected Toxoplasma gondii that is Mice First infected by a heart chicken inoculant pooling I (DJ.I.2) and Mice First infected by a heart chicken inoculant pooling II (DJ.II.1) came from Badung. Similarly, in Mice First infected by a heart chicken inoculant pooling I (DJ.I.1) and Mice Second infected by a heart chickent inoculant pooling I (DJ.I.2) came fromDenpasar. Furthermore, in Mice Second infected by a brain chicken inoculant pooling I and Mice First infected by a brain chicken inoculant pooling II came from Tabanan. The last, Mice First infected by a brain chicken inoculant pooling II came from Karangasem, Toxoplasma gondii was detected positive in mice sera. Appropriate the results of previous studies found that the cysts of Toxoplasma gondii is more widely available in local chicken heart and brain (Suwanti et al., 2005). Using the digestion method to isolate Toxoplasma gondii from local chicken heart and brain were found to contain 50% cysts in both organs (Apsari et al., 2011 in press). In this study of 16 heart chicken inoculant experimentally infected to mice which was detected there are 4 positive Toxoplasma gondii antibodies in the mice sera. While of the 16 brain chicken inoculant that were 3 positive detected Toxoplasma gondii antibodies in the mice sera. Isolation of Toxoplasma gondii from the extract meat first introduced by Dubey (1998) is an accurate method to obtain inoculant as a bioassay test in mice.

CONCLUSION

During the four weeks of treated chicken inoculant infection in mice increased antibody response in the four mice inoculated by chicken heart inoculant, whereas in the three mice inoculated with chicken brain inoculant

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THE EFFECT OF ISOFLAVONE TEMPE ON THE SUPEROXIDA DISMUTASE (SOD) AND MALONDIALDEHYDE (MDA) OF FEMALE RATS AS ANIMAL OSTEOPOROSIS MODEL

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ABSTRACT

Isoflavones tempe have become widely used as a dietary supplement, particularly among postmenopausal women and promising dietary agents for prevention of osteoporosis may variably act as antioxidation and estrogenic effect. The present study was conducted to observe the effect of isoflavone tempe on the superoxida dismutase (SOD) and malondialdehyde (MDA) in the liver and erytrocyte of female rats as animal osteoporosis model. A total of twenty female Spraque Dawlly rats two month and body weight average 200 g were used for this study. They were sub-divided into four groups; (1) a control group (K), (2) a isoflavone group (ISF), was treated by isoflavone only, (3) a osteoporosis model group, treatment by ovariectomy (OV), (4) a osteoporosis model group, treatment by ovariectomy followed by treatment with isoflavone (OV+ISF). Isoflavone-riched tempe flour was orally administrated isoflavone dose of 4 mg/200g bw/day for four month. The result showed that the administration of isoflavones on the OV+ISF and ISF group can increased SOD activity in the rat liver of 157.11 and 146.00 U/mg protein and were not significantly different (P>0.05), when compared with OV group (142.30 U/g protein), whereas SOD activity in the erythrocyte i.e. of 34.89 and 23.78 U/mg protein, respectively and significantly more higher (P <0.05), when compared with of OV group (20.07 U/g protein). Otherwise, MDA levels in the rat liver on OV+ISF and ISF group more lower i.e. of 40.68 and 45.97 picomol/g, when compared with of OV group (47.04 picomol/g), as well as MDA levels in the erythrocyte were also lowered, i.e. of 13.79 and 12.15 U/g protein, respectively when compared with of OV group (14.50 U/g protein) but both were not significantly different (P> 0.05).

Keywords: Isoflavone, tempe, superoxida dismutase, malondialdehyde, osteoporosis

INTRODUCTION

Oxidative stress may play critical roles in the pathogenesis of various features of the metabolic syndrom, diabetes mellitus, menopause, and reduction in oestrogen secretion from ovarium cells. Increased oxidative stress may also underlie the pathophysiology of menopause. Therefore, the reduction of oxidative stress may be a useful target for new therapies for patients with the metabolic syndrome and menopause (Furukawa S *et al*., 2004).

Oxidative stress is thought to contribute to the pathogenesis of degenerative diseases. Oxygen free radicals have been implicated in ovarium cell dysfunction and apoptosis associated with menopause. Oxidative defence decreases with age because the defence systems may be compromised after the menopause (azadbakht *et al*., 2007). A reduced antioxidant defence is also seen specifically in postmenopausal due to the lack of oestrogen. Therefore, postmenopausal and with the metabolic syndrome are at high risk of oxidative stress.

Soy isoflavonoids are natural dietary compounds widely marketed and consumed for their potential health benefits. In recent years soy isoflavonoids have become increasingly popular as dietary supplements, particularly for postmenopausal women seeking a safe natural alternative to traditional hormone therapies (azadbakht *et al*., 2007).

Isoflavones are known to be estrogen analogues and bind to estrogen receptors. Thus, it is logical that if the isoflavones possess actions similar to estrogens, they could influence several biological processes including lipid and bone metabolism. Because postmenopausal women are at risk for health problems related to estrogen deficiency, such as osteoporosis, consumption of soy products containing isoflavones might affect risk factors for these diseases (Potter SM *et al*., 1998)

Several studies have focused on the effect of diet on oxidative stress, especially the effect of soy isoflavones consumption (Mahn *et al*., 2005. The antioxidant properties of soy isoflavones may protect against lipid oxidation (malondialdehyde= MDA) and improve plasma antioxidant status, ie superoxide

dismutase (SOD), and catalase (Vega-Lopez S *et al*., 2005).

The present study was conducted to observe the effect of isoflavones tempe on the superoxida dismutase (SOD) and malondialdehyde (MDA) in the liver and erytrocyte of female rats as animal osteoporosis model.

MATERIALS AND METHOD

Preparation of animal experiments

A total of twenty female Spraque Dawlly rats two month and body weight average 200 g were used for this study. They were sub-divided into four groups; (1) a control group (K), (2) a isoflavone group (ISF), was treated by isoflavone, (3) a osteoporosis model group, treatment by ovariectomy (OV), (4) a osteoporosis model group, treatment by ovariectomy followed by treatment with isoflavone (OV+ISF). Isoflavone-riched tempe flour was orally administrated isoflavone dose of 4 mg/200g bw/day for four months.

Determination of MDA activity

MDA activity was determined using the method according to Capeyron *et al* (2002). As much as 1 ml of liver or erythrocytes homogenates adding 4 ml of 0.25N HCl in cold conditions containing 15% TCA, 0.38% TBA and 0.5% BHT. The mixed solution was heated 80°C for 1 hour. After cooling, the mixed solution was centrifuged 5000 rpm for 10 minutes. Abosorbance was measured at λ 532 nm. TEP (1,1,3,3-tettraethoxy propane) was used as the standard solution.

Determination of SOD activity

SOD enzyme activity was analysis using the method according to Nebot *et al* (1993). A total of 400 µ l solution of chloroform/ethanol cold of 37.5/62.5 (v/v) was added to 150 µl of liver or erythrocytes lysates and then homogenized for 3 seconds and centrifuged at a speed of 5000 rpm 4°C for 10 minutes. A total of 50 µl sample or standard solution (control) adding of 2.9 ml of solution A (mixture of xanthine and solution of cytochrome c) and homogenized slowly. Furthermore adding 50 µl solution B (xanthine oxidase) and homogenized slowly. Then the absorbance was measured using a spectrophotometer at λ 550 nm.

Statistical analysis

The experimental design used was Completely Randomized Design. The data obtained were analyzed by analysis of variance (ANOVA). Statistical differences between the treatment group were determined by the Duncan test.

RESULTS AND DISCUSSION

The observations in the Table 1 shows the groups of rat treated with isoflavones (ISF-4) MDA levels of liver lowest, ie of 40.68 picomol/ml, but not significantly different (P> 0.05) compared with other groups, ie groups ovariectomy and given isoflavones (ISF-4+OV) of 45.97 picomol/ml, control group of rat of 46.15 picomol/ml, and the group of rat ovariectomy (OV) of 47.03 picomol/ml. Similarly, MDA levels of erythrocyte, the lowest levels is owned by the isoflavone treatment group of rats (ISF-4) of 13.79 picomol/ml, but not significantly different (P> 0.05) when compared with the other three treatment groups of rat.

Results of analysis of Malondialdehyde levels during the experiment are presented in Table 1.

Table 1. The average MDA levels of liver and erythrocytes on rat during the experiment

Group	Malondialdehyde levels (MDA)(picomol/µl)	
	Liver	erythrocytes
K(-)	46.15±6.46ba	14.44±5.4a
ISF-4	40.68±5.05a	13.79±2.69a
OV	47.03±6.33a	14.50±2.28a
OV+ISF-4	45.97±6.27a	14.15±2.81a

Value of followed by different letters in the same row indicate significantly different test results (P <0.05). K (-) = negative control (normal rats), ISF = group of isoflavones, OV = group of ovariectomi, OV + ISF = group of ovariectomi were given isoflavone 4mg/200gbb/day

The results of analysis of levels of the enzyme superoxide dismutase (SOD) liver and erythrocytes on rat are presented in Table 2.

Table 2. The average SOD levels of liver and erythrocyte on rat during experiment

Group	SOD levels (U/mg protein)	
	Liver	erythrocyte
K(-)	149.70±15.49ba	27.48±8.28ab
ISF-4	157.11±33.13a	34.89±10.14b
OV	142.30±33.64a	20.07±8.28a
OV+ISF-4	146.00±28.08a	23.78±13.09ab

Value of followed by different letters in the same row indicate significantly different test results (P <0.05). K (-) = negative control (normal rats), ISF = group of isoflavones, OV = group of ovariectomi, OV + ISF = group of ovariectomi were given isoflavone 4mg/200gbb/day

The results of the analysis of SOD enzyme levels in Table 2, can be seen that the group of rats given with isoflavone (ISF-4) has the highest SOD levels of 157.11 U/mg protein, but not significantly different (P>0.05) compared with the three other treatment groups, ie groups of rat treated by ovariectomy of 142.30 U/mg protein, group of rat ovariectomy and given isoflavones of 146.00 U/mg protein, and the control group of rat of 149.70 U/mg protein. The same was seen on SOD levels in erythrocytes. Erythrocyte of SOD levels are highest in isoflavone treatment groups of rat, ie of 34.89 U/mg protein and significantly different (P <0.05) with groups of rats ovariectomy of 20.07 U/mg protein, but with groups of rats ovariectomy and given isoflavones of 23.78 U/mg protein and the control group of rat of 27.48 U/mg protein is not significantly different (P> 0.05).

Malonaldehyde (MDA) is a volatile product formed from the peroxidation of certain polyunsaturated fatty acids and is considered an index of lipid peroxidation (Tug *et al*., 2007). MDA has been implicated in aging, mutagenesis and other degenerative diseases. MDA is a highly reactive compound and it is well documented that it undergoes reactions with lipid, amino acids, proteins and nucleic acids. Isoflavones can inhibit of lipid peroxidation through a mechanism of direct capture of free radicals (free radical scavenger) and then prevents the amplification of the radical becomes more stable product or prevent a chain reaction of free radicals (Lee *et al*. 2004) and the same was reported by Yen and Lai (2003), about the ability of isoflavones inhibit lipid peroxidation so that MDA generated will be reduced.

Superoxide dismutase (SOD) is an enzyme that catalyzes the dismutation of two superoxide anions ($O2^-$) into hydrogen peroxide and molecular oxygen. Superoxide dismutase protects the tissue to a certain degree from the harmful effects of superoxide radicals (Karabulut *et al*., 2002). The activity of these enzymes plays an important role in the progress of the disease and the care of osteroporosis

Isoflavones are phenolic compound antioxidants may protect cells by its ability as a scavenger of free radicals. The role of isoflavones as an antioxidant thought through his ability as a hydrogen ion donor and scavenger of free radicals formed during lipid peroxidation (Nijveldt, 2001). According to Heim *et al*, (2002), the ability of isoflavones are powerful antioxidants, can not be separated from the chemical

structure, in which the hydroxyl group configuration on the B ring of isoflavone compound was reported act as free radical scavenger.

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GRANT OF MUCUS SNAIL (*ACHATINA FULICA*) TOPICALLY HEAL CALCULUS GINGIVITIS GRADE 3 FASTER THAN 10% POVIDONE IODINE

I Gusti Agung Ayu Putu Swastini
I Dewa Made Sukrama
I Wayan Putu Yasa Sutirta

ABSTRACT

Gingivitis is a common illness which can occur anytime after onset of new tooth, the gingiva appears red. The cause of gingivitis is bacteria plaque found on the surface of tooth. The incidence of gingivitis is nearly as high as 50% in rural communities. People are looking for a cheaper alternative these days by switching to traditional medicine, one of which is snail mucus. The purpose of this study was to determine whether the snail slime cure gingivitis grade 3 faster than 10% povidone iodine.

This study was carried out experimentally with pre-posttest control group design using 16 subjects with a diagnosis of gingivitis grade three as samples. On the jaw of each subject, snail slime was applied on one site while povidone iodine 10% was applied on the opposite site, which was done randomly for the measurement of gingival index. Based on the result of statistical analysis with Wilcoxon test result 0.72 ± obtained mean gingival index Mucus group of snails (LB) was 0.38 0.82 there is a difference ± group of 10% povidone iodine (PI) 1.00 gingival index for PI 0,62 higher than the LB. Analysis of significance by the Wilcoxon test showed that the value Z = 2.89 and p-value = 0.004, which means that the average gingival index in two groups was significantly different ($p < 0.05$). The group with snail mucus appliance reduce the gingival index faster than 10% povidone iodine.

Keywords: Gingivitis, snail slime, povidone iodine 10%, gingival index

INTRODUCTION

Gingivitis is an inflammation of the gingiva, causing bleeding accompanied by swelling, redness, exudate, change in normal contour, gingivitis is a common illness and can occur anytime after the onset of new tooth, gingiva appears red. Inflammation of gum can occur in one or two teeth, but also can occur in all teeth. Gingival bleeding easily occurs because small stimuli such as brushing teeth, or even without any stimulation, bleeding of the gum can occur at any time (Ubertalli, 2008).

The buildup of bacterial plaque on tooth surface is the main cause of periodontal disease. Periodontal disease begins from gingivitis, if left untreated it could develop into periodontitis where there is a damage to periodontal tissue destruction in fibers, periodontal ligament and alveolar bone (Wahyukundari, 2008).

People are looking for a cheaper alternative nowadays by switching to traditional medicine derived from the natural surrounding. Tropical country like Indonesia has enormous natural potential, one of which is the use of flora and fauna for health. Remote rural communities do not depend entirely on modern medicine due to geographical factor that does not allow the availability of drug. They inherited the hereditary traditional medicine, natural ingredients that are believed efficacious as antimicrobial agent, one of which is snail slime (Grahacendikia, 2009).

Result of a study conducted by Priosoeyanto in 2005 from Laboratory of Parasitology Faculty of Medicine, Bogor Agricultural Animal Institute proves that snail mucus or Achatina fulica able to heal wound two times faster than the wound treated with normal saline solution (Ali, 2010)

Snail (*Achantina fulica*) as one of the traditional medicine from animal material, needs further research and developed. Traditionally, snail is used as a new wound healing drugs. Scientifically there isn't any study conducts towards the fraction ability of the separation for snail slime as an anti-microbial (Ernawati and Sunari, 1994).

Snails are soft animals (molluscs), the division molluscs, gastropods that are classified into classes, snail's slime is an antimicrobial peptides that may affect viability of the ultrastructure of gram-negative and gram-positive through changes in cell ultrastructure (Berniyanti, 2007).

Snail mucus has a high biological value in the healing and inhibition of inflammatory processes (Ernawati and Sunari, 1994).

Researcher interested in studying about topical administration of snail's slime cure gingivitis faster than povidone iodine 10%. The result of this study is to expect the use of snail's slime by public, especially due to the use of traditional medicine to speed up the healing process of gingivitis.

The general objective of this study was to determine appliance of mucus of snail (*Achatina fulica*) topically would cure gingivitis grade 3 faster than povidone iodine 10%.

The specific objective of this study was to find out the mucus of snail (*Achatina fulica*) topically may reduce gingival index gingivitis grade 3 due to calculus.

RESULTS

This study used 16 subjects, nine male and seven female subjects, aged between 21 to 46 year, with a diagnosis of gingivitis grade three. In each jaw of sample subjects, it is applied with 10% povidone iodine (PI) on one side and the side opposite applied snail mucus (LB)

Table 1 Characteristics of Subject

Age (year)	Male f (%)	Female f (%)	Total f (%)
21-30	3 (50%)	3 (50%)	6(100%)
31-40	3(75%)	1(25%)	4(100%)
41-50	3(50%)	3(50%)	6(100%)

Table 2 Result of Normality Data Test of gingival index in each group

Group		Mean	P
Povidone iodine 10% day 2	1,	1,00	0,004
Povidone iodine 10% day 3		0,12	0,001
Snail slime day 2		0,38	0,001
Cure *Povidone iodine 10%*		2,81	0,002
Cure snail's slime		2,25	0,000

Based on result in Table 2, advanced test is used to determine the reduction in gingival index in each treatment group using Friedman Test, whereas to know the difference between two groups Wilcoxon Sign Rank Test test is being used because the samples were matched.

Analysis of treatment effect tested based on gingival index between before (day 1) with the treatment given after (day 2 and day 3). The result of significance analysis by Friedman test is presented in Table 3.

Table 3 The mean gingival index between Prior to After application with Snail Slime

Variable	Snail's slime			χ^2	P
	Day 1	Day 2	Day 3		
Gingival Index	3,00±0,00	0,38±0,72	0,00±0,00	30,15	0,001

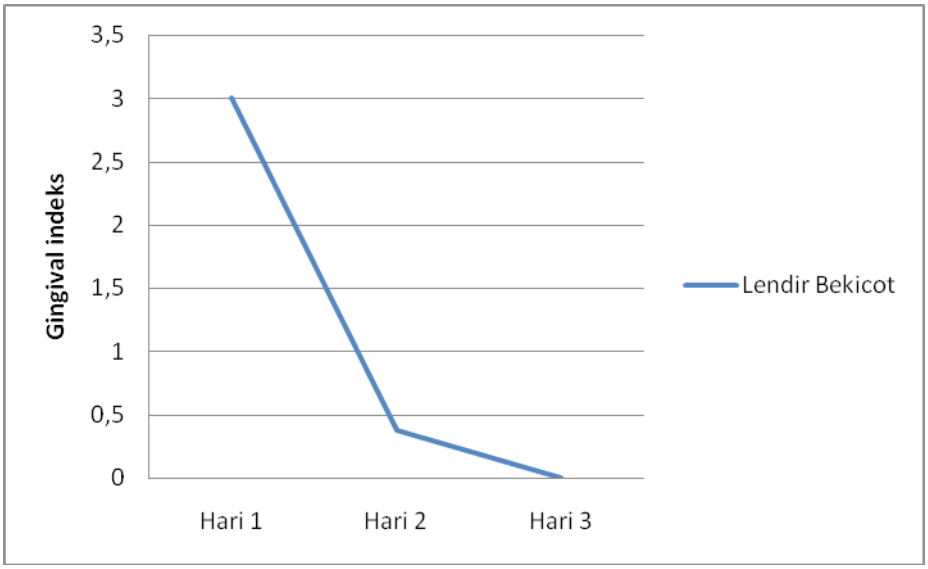

Figure 1 Decreasing ***Gingival*** Index in Snail's slime group

Table 4 Wilcoxon Test towards decreasing *gingival* index in applicated snail's slime group

Examination day	Mean difference
Day 1 and 2	2,62
Day 1 and 3	3
Day 2 and 3	0,38

Table 5 Mean *Gingival* Index between prior and after application with *Povidone iodine* 10%

Variable	*Povidone iodine* 10%			χ^2	P
	Day 1	Day 2	Day 3		
Gingival Index	3,00±0,00	1,00±0,82	0,12±0,34	30,14	0,001

The result of the above analysis is also presented in line graph

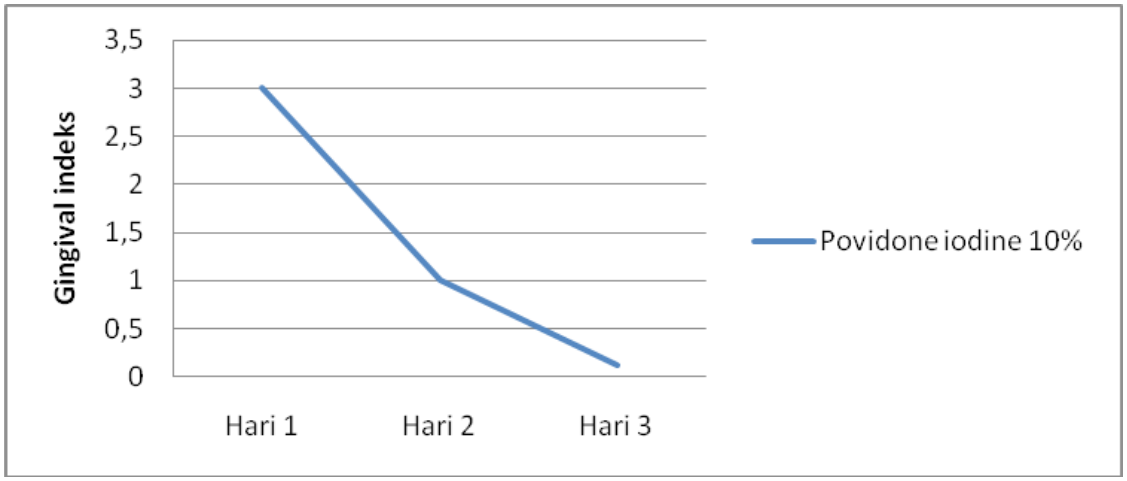

Figure 2 The decrease in gingival index in 10% povidone iodine group.

To assess the difference between groups using Wilcoxon test. The test result is presented in Table 6.

Table 6 Wilcoxon test gingival index decreased in the group that was applied with 10% povidone iodine

Examination day	Mean difference
Day 1 and 2	2,00
Day 1 and 3	2,88
Day 2 and 3	0,88

Analysis of comparability tested based on the mean gingival index between groups. Predictive analysis of test result with Wilcoxon Sign Rank Test is presented in Table 7.

Table 7 Mean gingival index between treatment groups on Day One

Subject group	**N**	**Mean *Gingival* Index**	**SB**	***Z***	**P**
Snail's slime (LB) *Povidone*	16	3,00	0,000	0,00	1,00
iodine 10% (PI)	16	3,00	0,000		

Table 8 Mean gingival index between treatment groups on Day Two

Subject group	**N**	**Mean *Gingival* Index**	**SB**	***Z***	**P**
Snail's slime (LB)	16	0,38	0,72	2,89	0,004
Povidone iodine 10%(PI)	16	1,00	0,82		

Analysis of treatment effects were tested based on the mean gingival index between groups on the third day. Predictive analysis of test results with Wilcoxon Sign Rank Test are presented in Table 9.

Table 9 Mean gingival index between treatment groups on Day Three

Subject group	**N**	**Mean *Gingival* Index**	**SB**	***Z***	**P**
Snail's slime (LB) *Povidone*	16	0,00	0,00	1,41	0,157
iodine 10% (PI)	16	0,12	0,34		

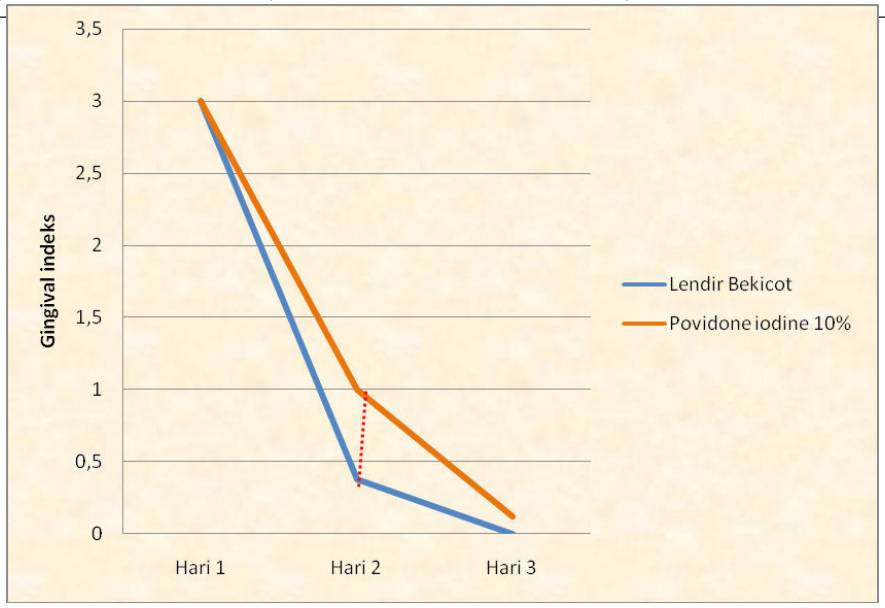

Figure 3 Comparison of gingival index Given Before and After Treatment

The third day cure rates in subjects who applied with the mucus of snails (LB) is 100%, whereas in 10% povidone iodine (PI) 87.5%, cure rates are slower than PI LB. Analysis of treatment effects were tested based on the mean gingival index between groups after the third day. Predictive analysis of test results with Wilcoxon Sign Rank Test are presented in Table 10.

Table 10 Mean healing time between treatment groups

Subject group	N	Mean Healing Time	SB	*Z*	P
Snail's slime (LB) *Povidone iodine* 10%(PI)	16 16	2,25 2,81	0,45 0,66	3,00	0,003

Result from analysis of healing time in each treatment is presented in graphical form.

Figure 4 Comparison of Cumulative Prognosis
Figure 4 Review LB on day 2 reached 75% and day 3 showed full recovery (100%).

DISCUSSION

In this study, we used 16 subjects with gingivitis grade three. Gingival index normality data both before treatment and after treatment in each group tested using the Shapiro-Wilk test. The result showed that all data is not normally distributed, so nonparametric test is used, Friedman Test and Wilcoxon Sign Rank Test.

There was a decreasement in gingival index 6.2 after application of snail mucus and povidone iodine 10% . Based on analysis of group that used snail mucus, it was found that during average first day of the gingival index was 3.00 ± 0.00, the average second day was 0.38 ± 0.72, and mean gingival index was 0.00 ± third day 0.00. Analysis of significance with Friedman test showed that gingival index decreased significantly in group applied with snail mucus ($p < 0.05$), whereas to aim the group's difference, need to be tested further with Wilcoxon test, result obtained average groups differed significantly during day 2 (day 1 group mean is higher than the average group during day 2). Day 1 meant significantly different compared to day 3 (day 1 group mean is higher than the average group during day 3). and average group did not differ from day 2 day 3 (mean group day 2 higher than the average group day 3).

The average gingival index in group using povidone iodine 10% during first day was 3.00 0.82, and mean gingival index was ± 0.00, the second day was 1.00 ±, the third day was 0.34 ± 0.12.

Analysis of significance using Friedman test showed the gingival index decreased significantly in the group with applicaton of povidone iodine 10% ($p < 0.05$), whereas to aim the difference between groups, need further testing using Wilcoxon test, result obtained significantly different from day 1 to day 2 (mean group day 1 higher than the average group day 2), the average group significantly different from day 1 to day 3 (mean group day 1 higher than the average group day 3), mean 2 group significantly different during day 3 (mean group day 2 was higher than average group on day 3). Further analysis comparability between treatment groups was tested based on mean gingival index of both groups. Analysis of significance using Wilcoxon test showed the average gingival index during first day between the two groups did not differ, whereas mean gingival index on the second day, third day, and the rate of recovery after third day there was a difference between groups was significant($p<0.05$). . The decrease in gingival index after being applied with snail mucus which was an antimicrobial peptide, might affect viability ultrastructure of gram-negative and gram-positive through changes in cell's ultrastructure (Berniyanti, 2007). Snail mucus had a high biological value in healing and inhibition of inflammatory process (Ernawati and Sunari, 1994).

Our study was supported by similar study result by Priosoeyanto Bambang Pontjo which was conducted at the Laboratory of Parasitology Faculty of Medicine, Bogor Agricultural Institute of animals during 2005, which stated that mucus Achatina fulica snail capable to heal the wound two times faster than normal saline solution (Ali, 2010). Snail (Achantina fulica) as one of the traditional medicine of animal material is now being regarded as a new wound healing. Ernawati and Sunari (1994), state that fraction separation ability of snail slime as an anti-microbial. Snails were used as a cure towards light wound, jaundice, skin diseases, as well as its mucus is used to relieve toothache. Snail's slime relieve pain by inhibiting pain mediator, so pain does not occur, as pain mediator that stimulate pain receptor is blocked, so the pain is not being forwarded to pain center. Snail mucus can also be used to relieve toothache, i.e by attaching snail slime on sore tooth (Mutiarawati, 2009). Similarly, study by Ibrahim et al (1995) which states that there is positive effect from snail mucus against open wound healing.

Study by Tripurnomorini et.al (2000), snail's slime as anti-inflammatory agent has relatively similar strength compared to asetosal. Application with povidone-iodine 10% can reduce gingival index gingivitis grade 3. This is due bacteriostatic and bactericid effect from povidone-iodine. Povidone-iodine has low toxicity, but its detergent in cleaning solution will increase its toxicity further. In 10% povidone iodine containing 1% iodide is capable of killing bacteria in 1 minute and kill spores dams within 15 minutes (Ganiswara, 1995).

Our study found that snail mucus was able to cure gingivitis faster compared to 10% povidone-iodine, it could be seen from the result during the second day after tooth is applied with snail slime and povidone-iodine 10%, there was a significant difference, with a mean gingival index snail slime group is lower than povidone-iodine group 10%. The cure rate after the third day was significantly different, the snail slime recovery rate was 100%, whereas 87.5% in povidone iodine.

CONCLUSION

Based on the result of our study in patients with gingivitis grade 3, we obtained the following conclusions:

1. Provision of snail's slime (*Achatina fulica*) topically might reduce gingival index gingivitis grade 3 because of calculus.
2. Provision of 10% povidone iodine topically could reduce the gingival index gingivitis grade 3 because of calculus.
3. Provision of snail's slime topically decreased gingival index gingivitis grade 3 in a faster period rather than povidone iodine 10%
4. Provision of snail's slime (*Achatina fulica*) topically might reduce gingival index gingivitis grade 3 because of calculus after the first day, second and third.
5. Provision of 10% povidone iodine topically could reduce the gingival index gingivitis grade 3 because of calculus after the first day, second and third.

6. Gingivitis cure time grade three because of the calculus after topical administration of snail's mucus was faster than the provision of 10% povidone iodine.

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THE ROLE OF PROTEIN 53 AND PROTEIN BAX IN THE CHEMORADIATION RESPONSE OF CERVICAL SQUAMOUS CELL CARCINOMA STAGE III

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ABSTRACT

Cervical cancer is the second most common cancer in women worldwide and the most frequent cause of death related to woman cancer in Indonesia. Radiochemoresistance remains a major problem in the clinic, so molecular examinations as diagnostic methods are needed for earlier diagnostic and rapid detection of resistance. A case control study was performed in post chemoradiation patients with cervical squamous cell carcinoma stage III. The total number of samples were 44 consisted of two groups, 22 samples for the case with positive viable malignant cells in pap smear evaluation after chemoradiation, and another 22 samples for the control with negative result. The expression of p53 and bax were studied by immunohistochemistry in the biopsy specimens using monoclonal antibodies specific for these protein. Analysis was done using the logistic regression for odds ratio and the p-value was calculated using the chi-square test, $\alpha = 0.05$. Protein 53 was expressed strongly in 50 % of malignant cells in one sample of case and one of control. Six samples of case and four of control showed weak to medium expression in less than 10% of malignant cells (OR = 5; p = 0.368). Protein bax were expressed strongly in 80-90% of malignant cells in five samples of case and three of control. Eight samples of case and seven of control showed weak to medium expression in 10-90% of malignant cells (OR = 0.160 ; p = 0.110). High expression of p53 and bax were not significant increase the risk of chemoradiation resistance. The results suggest that chemoradiation induced cells death in malignant cells may cause by many mechanisms, not only by apoptosis through the p53-bax pathway.

Keywords: p53, bax, chemoradiation, response, cervical carcinoma

INTRODUCTION

Cancer is still as health problem worldwide and is being diagnosed more and more frequently in the developing countries. One of this cancer is cervical cancer. Cervical cancer is the second most common cancer in women worldwide and the most common cancer in women in Indonesia, as in most developing countries. From hospital-based data, it accounts for 28.6% of female cancers in Indonesia. More than 80% of women dying from cervical cancer usually in late stage, live in developing countries. Without urgent action, deaths due to cervical cancer are projected to rise by almost 25% over the next 10 years. Prevention of these deaths are by adequate screening and treatment.[1,2,3]

High-risk *human papillomavirus* (HVP) as major risk factor play a critical role in the pathogenesis of most cervical cancer and their precursor lesions, supported by a large number studies of epidemiologic, clinicopathologic and molecular. Other minor risk factors that have classically been associated with cervical cancer play a much less important role, like number of sexual partners, sexual activity less than 16 years of age, early age of first pregnancy, parity, low socioeconomic class, cigarette smoking, vitamin deficiencies, oral contraceptive use and others.[4] Ninety per cent of cervical cancer cases are squamous cell carcinomas arising from the metaplastic squamous epithelium of the transformation zone, the other 10% are cervical adenocarcinomas arising from the columnar epithelium of the endocervix.[2]

The three basic treatment of squamous cell carcinoma including surgery, radiotherapy and chemotherapy.[4] FIGO stage IIB-IV of cervical carcinoma is treated by chemotherapy and/or radiotherapy.[5] The cure rate for invasive cervical cancer is closely related to the stage of disease at diagnosis and the availability of treatment. If left untreated, cervical cancer is almost always fatal. In radiotherapy, the tumour is treated with ionizing radiation. Radiation can damage and destroy cancer cells by damage deoxyribonucleic acid (DNA). Radiation also cause apoptotic of cancer cells.[2,6] Chemotherapy may be used concurrently with surgery or radiation to treat bulky tumours and to increase radiation effect.[2,7] TP53 tumor supressor gene can exert antiproliferative effects and regulates apoptosis. The p53 protein encoded by TP53 gene plays an important role in protecting cell by inducing growth arrest and or cell suicide (apoptosis) in DNA damage induced by mutagen such as ionizing radiation. The proapoptotic effects of TP53 seem to be mediated by up-regulation of bax synthesis.[8] The mutant and thereby dysfunctional of this gene might alter radiosensitivity or chemosensitivity of tumor cells.[9,10] Radioresistance remains a major problem in the clinic, so molecular examinations as diagnostic methods are needed for earlier diagnostic

and rapid detection of resistance. The aim of this study is to know the role of protein 53 and protein bax in the chemoradiation response of cervical squamous cell carcinoma Stage III.

MATERIALS AND METHOD

A case control study was performed at several departments, included The Department of Obstetrics and Gynecology, The Department of Radiology and Department of Anatomical Pathology Sanglah Hospital Denpasar. Samples origin from patients who diagnosed as cervical squamous cell carcinoma stage III and prepared to treated by chemoradiation. The chemotherapy regimen consists of bleocyn, oncovin and mitomycin with procedure of treatment according to procedure at Department of Obstetrics and Gynecology Sanglah Hospital Denpasar. This treatment followed by radiotherapy according to procedure at Department of Radiology Sanglah Hospital Denpasar. Each patient received two GY everyday, five days a week. The total dose was 60 Gy in 6 week. Pap smear was done in all of these patients, six week after the last of radiation at Department of Anatomical Pathology Sanglah Hospital Denpasar. Twenty two samples were use as case if there were positive viable malignant cells in pap smear evaluation after chemoradiation and another 22 samples for the control with negative result. The expression of p53 and bax were studied by immunohistochemical strepavidin biotin method in the biopsy specimens using monoclonal antibodies specific for these protein at Department of Anatomical Pathology Sanglah Hospital Denpasar.

Analysis was done using the logistic regression for odds ratio and the p-value was calculated using the chi-square test available in SPSS for windows 16,0 version, with $\alpha = 0.05$.

RESULTS AND DISCUSSION

The results of the study showed in table 1-4, consist of age distribution, histopathological type and expression of p53 and bax from immunohistochemical examination.

Table 1. Age distribution of case and control group

Age group (year)	Case		Control	
	n	%	n	%
21-30	2	9.1	2	9.1
31-40	5	22.7	4	18.1
41-50	6	27.3	6	27.3
51-60	8	36.4	8	36.4
61 +	1	4.5	2	9.1
Total	22	100.0	22	100.0

Table 1 reveal the most prevalence of cervical squamous cell carcinoma according to age distribution was 51-60 years old, 36.4% each for case and control group.

Table 2. Histopathological type of case and control group

	Histopathological type					
	NKSCC*		KSCC^		Total	
	n	%	n	%	n	%
Case	0	0	22	100	22	100
Control	3	13.6	19	86.4	22	100

*Non Keratinizing Squamous Cell Carcinoma
^Keratinizing Squamous Cell Carcinoma

Table 2 reveal the most prevalence type of cervical squamous cell carcinoma according to histopathological examination was keratinizing squamous cell carcinoma, 100% and 86.4% in case and control group respectively.

Table 3. p53 expression of case and control group

	p53 expression							
	positif		negatif		total		OR	p
	n	%	n	%	n	%		

Table 3 reveal protein 53 expressed strongly in 50 % of malignant cells in one sample (4.5%) of case and one of control. Six samples of case and four of control showed weak to medium expression in less than 10% of malignant cells (OR = 5; p = 0.368; α = 0.05). High expression of p53 was not significant decrease the risk of chemoradiation response.

Table 4. Bax expression of case and control group

	bax expression							
	positif		negatif		total		OR	p
	n	%	n	%	n	%		

Table 4 reveal that protein bax were expressed strongly in 80-90% of malignant cells in five samples of case (22.7%) and three (13.6%) of control. Eight samples of case and seven of control showed weak to medium expression in 10-90% of malignant cells (OR = 0.160 ; p = 0.110; α = 0.05). High expression of bax was not significant decrease the risk of chemoradiation response.

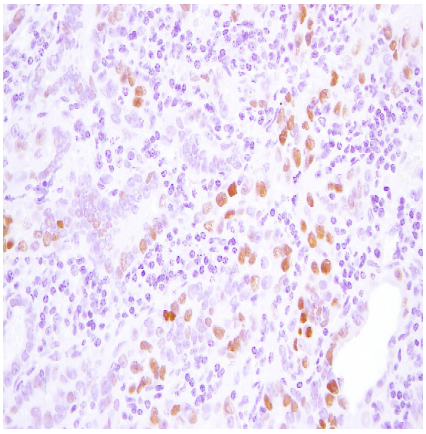

Figure 1. Intranuclear
Strong Expression of p53 (IHC, 400 x)

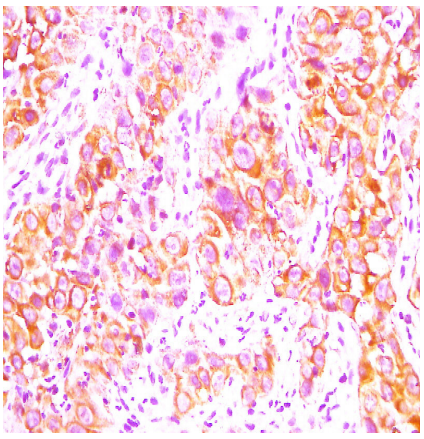

Figure 2. Intracytoplasmic
Strong Expression of bax (IHC, 400 x)

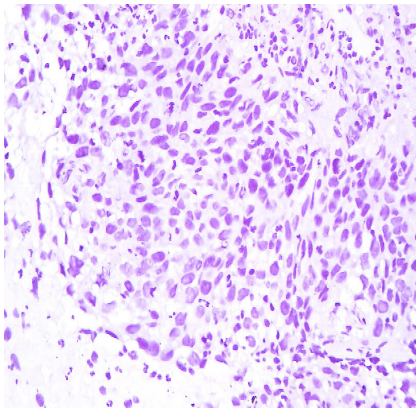

Figure 3 Negative Expression of p53
(IHC, 400x) x)

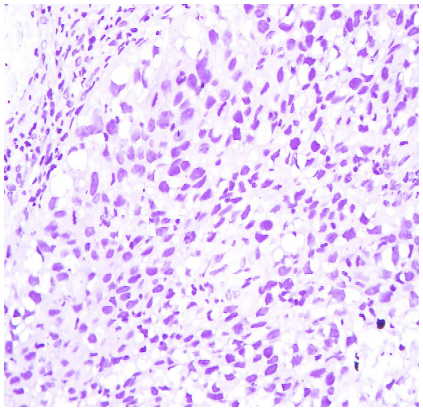

Figure 4 Negative Expression of bax
(IHC, 400x) x)

Cervical squamous cell carcinoma is highly correlated with HPV infection. Because the viral oncoprotein E6 binds and inhibits the function of p53 protein, inhibition by HPV may be one cause of chemoresistance in cervical cancer. However, in cervical cancer the relationship between apoptosis through the p53-bax pathway and chemosensitivity is not clear. More than one mechanism may be involved in the initiation of radiation-induced genomic instability in survival cells. Many mechanisms have been postulated to explain chemoresistance, including decreased drug accumulation inside tumor cells, increased cellular detoxification, and increased DNA repair activity.[7,10]

CONCLUSIONS

The results suggest that chemoradiation induced cells death in malignant cells may cause by many mechanisms, not only by apoptosis through the p53-bax pathway.

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ETHANOL EXTRACT OF PURPLE SWEET POTATO TUBERS *(IPOMOEA BATATAS L)* DECREASES BLOOD GLUCOSE AND INCREASE TOTAL ANTIOXIDANT LEVEL IN RATS WITH HIGH GLUCOSE INTAKE.

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ABSTRACT

High blood glucose level can increase the production of free radicals and the AGEs. Flavonoids from fruits and vegetables can prevent that events because they have antioxidant and hypoglycemic effects. The aim of this study was to examine possible hypoglycemic and antioxidant effect of the ethanol extract of purple sweet potato tubers. Subject of this study were 20 male adult rats divided into 4 groups with randomized pre-test and post-test control group design. Before treatment, blood samples were collected from retro-orbital sinus of all rats for blood glucose, MDA and total antioxidant measurement as pre-test data. After pre-test, group 1 (control group) rats were given normal diet without ethanol extract of purple sweet potato tubers. Group 2, 3, and 4 rats were given normal diet and ethanol extract of purple sweet potato tubers 1 cc/day, 2 cc/da,y 4 cc/day, respectively, for one week. After one week of treatment, treatment groups were given single dose of high glucose intake. After 1, 2, and 3 hours of treatment with high glucose, the blood samples were collected for post-test examination. The data were then analysed by one way anova and paired-t test, and the result showed a significant increase of blood glucose and MDA in group 1 ($p<0,01$), significant decrease of total antioxidant in group 1($p<0,01$). In group 3, all of the parameters were different with group 1 and group 2 ($p<0,01$). In group 4, MDA decreased significantly after treatment and the total antioxidant was increased significantly ($p<0,01$). From this findings, it can be concluded that ethanol extract of purple sweet potato tubers can maintain the blood glucose level within normal limit, prevent the increase of MDA, and increase total antioxidant of the blood after high glucose intake in rats. The effective dose of the exctract in this research was medium dose.

Keyword: Purple sweet potato tubers, blood glucose, total antioxidant, rat.

INTRODUCTION

Diabetes mellitus (DM) is one of the health problems faced by all the countries in the world, including Indonesia. Common agents used in the therapy of DM includes insulin and oral antidiabetics (OAD) (Srinivasan, 2007). Sulfonylurea (one of the many kinds of OAD) increases the secretion of insulin, but can also cause adverse effects to diabetic patients. There is common failure in the administration of sulfonylurea to maintain the blood glucose level within normal limit, because this agent can only stimulate insulin secretion in a relatively same amount (Ghosh, 2007), hence it contributes to body weight increase on the patients (Jayaprakasam, 2004). The use of alternative agent is needed to overcome this problem in the management of DM. Studies showed that daily dietary pattern and food composition affect the occurence of DM. Fruits and vegetables high in polyphenol/flavonoid was proved to influence the blood glucose level via pancreatic beta cells insulin production stimulation (Jayaprakasam, 2005), and could decrease the insulin resistance (Ghosh, 2007). Tsuda which is quoted by Ghosh (2007), found that food high in flavonoid, such as purple corn (containing *cyanidin 3-glicoside*) could prevent the genesis of insulin resistance caused by high fat intake in mice. Dietary pattern also contribute to postprandial blood glucose level in diabetic patients. Increase in postprandial blood glucose level is highly associated to the oxidative stress, which is showed by the increase of MDA (*malondialdehyde*) in the blood, and this can increase the risk of cardiovascular complications. Oxidative stress can be decreased by the administration of antioxidant or foods high in flavonoids (Ghosh, 2007). In uncontrolled DM, postprandial blood glucose level commonly increased, hence it also increase the risk of cardiovascular complications.

Anthocyanin-rich foods can control blood glucose level and decrease free radicals in streptozotocin-induced diabetic rats (Chambers, 2003). Beside its antioxidant effects, anthocyanin also has hypoglycemic effects in some researchs (Ghosh, 2007), and *in vitro*, some kinds of anthocyanin can increase the insulin secretion by beta cell of the pancreas (Jayaprakasam, 2004).

Purple sweet potato tubers from Bali are high in antocyanin (Suprapta, 2004), and has antioxidant effects on blood and various organs of mice suffering from oxidative stress (Jawi, 2008). Purple sweet potato tubers has been developed as food product, so it effects should be studied, especially its potentials on controlling postprandial blood glucose and oxidative stress in diabetic patients. To prove its effects,

research was done on rats that were given ethanol extract of purple sweet potato tubers and oral glucose load, and then blood samples were obtained to measure the level of blood glucose every hour, total antioxidant, and MDA.

MATERIALS DAN METHOD

This research is a laboratoric experimental research with randomized control group pre and post-test design. The research is done in 1 month in Animal Laboratory Unit of Pharmacology Department, Medical School, Udayana University. The population of this research was male *wistar* rats, 3-4 months old, weighing 200-225 gram. Sample size was 20 rats (determined from Fiderer's equation) and were grouped into 4 groups (5 rats in each group). Before the research was done, all rats were treated so for 2 weeks to make them adapted to the laboratory setting. After 2 weeks, blood samples were obtained to measure the level of MDA, total antioxidant, and fasting blood glucose as pre-test data. Then, Group 1 rats were treated as control group as they were given high oral glucose load only. The other groups (group 2, 3, and 4) were given 1cc, 2cc and 4 cc ethanol extract of purple sweet potato tubers, respectively, on a daily basis for 1 week. The extracts were given orally via *sonde*. Group 2, 3, and 4 were then treated with high oral glucose load. The control group was treated with water via *sonde* to adjust for the stress level faced by the treatment groups.

Ethanol extracts of purple sweet potato tubers were made through special procedure. Harvested purple sweet potato tubers were soaked in clean water and peeled. The purple sweet potato tubers then cut cross-sectionally (2-2,5 cm wide) and made into powder. Ethanol (70%) then added to this powder (1 L of ethanol for 1 kg of tubers). This mixture was filtrated by using three layers of fine cloth. The filtrate was evaporated to get viscous extract. This extract then dissolved again in 1 liter of water, and heated until boiling. The anthocyanin content of the end product was 119 mg/mL.

After 1 week of treatment, all of the rats were given 50% glucose orally with the dose of 2 g/rat. The blood samples were then collected in the 30th, 60th, 120th and 180th minute to measure the level of blood glucose, MDA, and total antioxidant. Glucotest, TBARS, and spectrophotometer were used to measure the level of blood glucose, MDA, and total antioxidant level, respectively. The data were then analyzed with T test and Anova.

RESULTS

Study results showing pre-test and post-test average level of blood glucose, MDA, and total antioxidant, in all of the trial animal groups can be seen in and Figure 1-3.

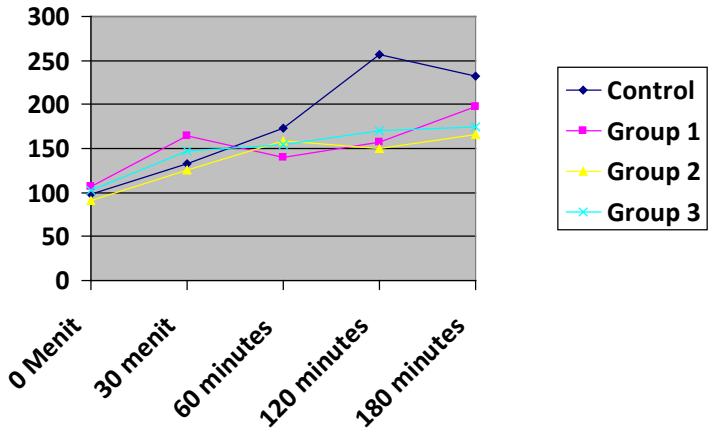

Figure 1. The comparison of average blood glucose level in all trial animal groups

The blood glucose level in the initial phase of this study was not significantly different in all of the treatment groups (p>0,01). In the end of the study (after 3 hours), there was significant blood glucose level increase (p<0,01), in the control group. In group 3 and 4, the blood glucose level increase was

significantly lower than group 1 (p<0,01), meanwhile in group 2 the blood glucose level did not differ significantly compared to group 1 (p>0,01). The most intersting thing was the blood glucose level 2 hours of observation. All of the treated grous showed statistically significant lower blood glucose level increase (p<0,01). All of the treatment groups showed statistically significant lower blood glucose level relative to blood glucose level of the control group (p<0,01). Only group 2 showed statistically different result compared to the control group (p<0,01). Figure no. 1 shows that there was blood glucose level change during observation. Blood glucose level was higher in control group compared to that of control group from 60th to 180th minute.

Pre-test MDA level in all of the rats in relatively same (p>0,01). MDA level was increased one hour after the administration of high glucose intake, and the level was still high until three hours. This increase was statistically significant (p<0,01). Increase in MDA level was also observed in group 2 rats (treated with 1 cc/day extract), which was significantly differ with pre-test MDA level (p<0,01). If compared with control group, this MDA increase was significantly lower (p<0,01).

In group 3 (treated with 2 cc/day extract), MDA level did not change if compared with pre-test MDA level (p>0,01). MDA level in group 4 showed significant decrease in 3 hours of observation (p<0,01). The change in MDA level during 3 hours of observation can be seen in Figure 2.

Total antioxidant level in the control group rats was significantly decreased after the administration of high glucose intake (p<0,01). Meanwhile, in the treatment groups (which were treated with the ethanol extract of purple sweet potato tubers) the total antioxidant level change was dose-dependent. Total antioxidant level decrease was relatively same between group 2 rats (treated with 1 cc/day dose) and group 1 rats (control group). Total antioxidant level did not change in group 3 rats (treated with 2 cc/day dose) (p>0,01). Significant increase in total antioxidant level was observed in group 4 rats (treated with 4 cc/day dose) if compared with pre-test data and the other groups (p<0,01). This data can also be seen in Figure 3.

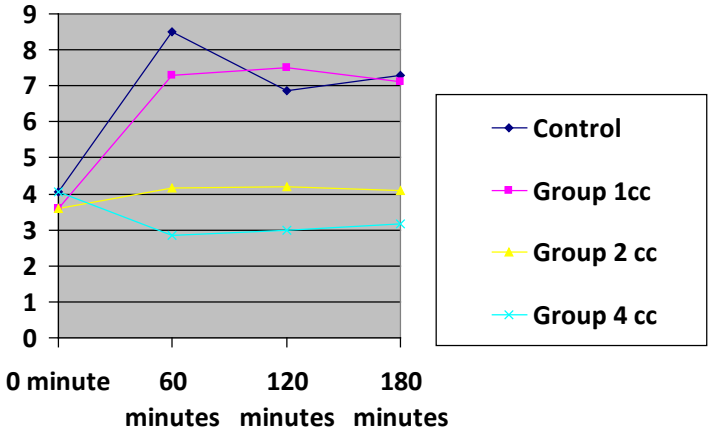

Figure 2: The comparison of average MDA level in the blood of all of the treatment groups

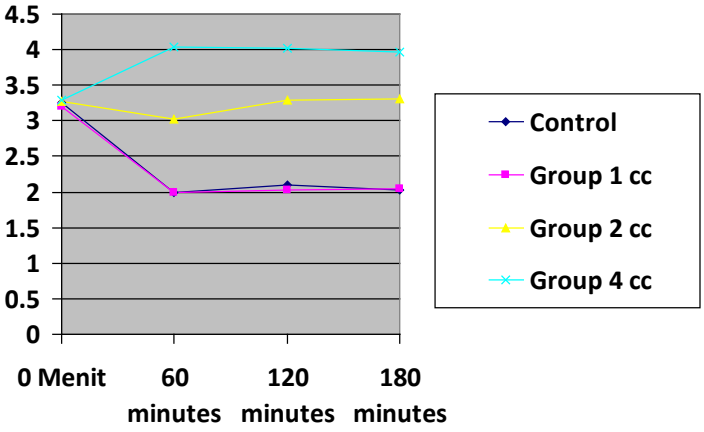

Figure 3: The comparison of average total antioxidant level in the blood of all of the treatment groups

DISCUSSION

There were two important things in this study. First, there was significant change in blood glucose level in rats with high oral glucose load, which could be treated by the administration of various doses of ethanol extract of purple sweet potato tubers. Second, there was significant change in blood MDA and total antioxidant level the after high oral glucose load. The blood MDA increase and total antioxidant decrease could also be treated by the administration of the extract. Significant blood glucose level was observed due to high oral glucose load. Compared to pre-test data, the increase differ significantly ($p<0,01$), in group 1. In the treatment groups, which were given ethanol extracts of purple sweet potato tubers for 2 weeks, the blood glucose level increased before high oral glucose load. This increase was observed to be significantly lower than in control group. The difference was significant, in 60th and 180th minute ($p>0,01$). Anthocyanin is a member of flavonoids that is contained within the flesh of purple sweet potato tubers (Huang, 20004). This agent inhibits glucose absorption through the gastrointestinal tract via the inhibition of alpha-glucosidase, hence anthocyanin has hypoglycemic effect (Suda, 2003; Han, 2007). Anthocyanin also inhibits glucose absorption via various mechanisms (Han, 2007).

MDA level increase was a marker of oxidative stress in rats given high oral glucose load. Total antioxidant level was also decreased in these rats. In the treatment groups, the level of blood MDA and total antioxidant increased significantly despite being given extract with the dose of 1 cc/ day, if compared to the control group and before treatment. The administration of the anthocyanin-rich extract (Suprapta, 2004), will function as exogenous antioxidant, hence it can increase the total antioxidant level. Anthocyanin is a natural pigment with *phenolic* structure, so that this molecule can transfer hydrogen atom from hydroxyl free radical. The bluish colour of plants is an indicator of high anthocyanin content (Prior, 2003), such as in purple sweet potato tubers. This is the rationale of giving purple sweet potato to increase the total antioxidant level in oral glucose-induced hyperglycemic rats. This effect is certainly due to antioxidant property of the extract, which decrease the use of endogenous antioxidant during oxidative stress. The antioxidant effect of purple sweet potato tubers was proved via DPPH method (Huang, 2004).

The effective dose in this research was 2cc/rat/day. This dose could prevent blood sugar level increase, MDA level increase, and total antioxidant level decrease.

CONCLUSION

From this study, it can be concluded that

1. The administration of ethanol extract of purple sweet potato tubers can prevent the increase of blood sugar level in rats with high oral intake of glucose.
2. Ethanol extract of purple sweet potato tubers can decrease blood MDA and increase blood total antioxidant level in rats with high oral intake of glucose.
3. The effective dose of the ethanol extract of purple sweet potato tubers to prevent blood glucose level increase is moderate dose (2 cc/rat/day).
4. The effective dose of the ethanol extract of purple sweet potato tubers to prevent the increase of blood MDA and the decrease of blood total antioxidant level is moderate dose (2 cc/rat/day).

Suggestions

In order to get clinically applicable study results, the same study should be done on healthy humans as volunteers. More study on dose variance should also be done to get the optimal dose of the ethanol extract of purple sweet potato tubers in treating hyperglycemic conditions.

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WATER EXTRACT OF PURPLE SWEET POTATO LEAVES IMPROVED BLOOD LIPID PROFILE AND SOD CONTENT OF RATS WITH HIGH CHOLESTEROL DIET

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ABSTRACT

Food stuffs with high flavonoids content are believed to prevent various diseases caused by oxidative stress because of its antioxidants effect. Purple sweet potato leaves have been proved containing high flavonoids, and can be developed very easily. To investigate the antioxidant and hypolipidemic properties of the water extract of purple sweet potato leaves, one study was conducted in the Department of Pharmacology, Faculty of Medicine, Udayana University. The study design was randomized control group pre- and post-test. Twenty adult male wistar rats were divided into two groups of 10 rats. Both groups of rats were given high-cholesterol diet for three months to induce dyslipidemia. Control group of 10 rats were given only high-cholesterol diet alone, whereas the treatment group also treated with water extract of purple sweet potato leaves with a dose of 6 cc per day divided into two doses. Before treatment and after treatment, lipid profile and blood SOD levels were measured. The results showed a decrease in total cholesterol, triglycerides and LDL cholesterol significantly in the treated group (P = 0.0001). In the treatment group there was also an increase in HDL cholesterol and blood SOD which was significantly differerent than the control group (P = 0.0001). From the results of this study, it can be concluded that administration of water extract of purple sweet potato leaves can improve the lipid profile and increase blood SOD of rat given high-cholesterol diet**.**

Keywords: Purple sweet potato leaf, lipid profile, high-cholesterol, rat.

INTRODUCTION

Hypercholesterolemia and oxidative stress are still become major health problem until this time. It is related with the incidence of cardiovascular diseases and its complications. Dyslipidemia will cause elevation and activation of NADH/ NAD (P) H oxidise, that makes the increase of anion superoxide production, that is one of the free radical that cause oxidative stress. Oxidative stress can cause endothelial disfunction, that initiate the increase of molecule adhesion like VCAM-1 that is the starting point of aterosklerosis[1].

Food stuff that contain flavonoid like vegetables, fruits, and tubers, are believed to have ability to prevent many kind of diseases that are related to oxidative stress. Flavonoid has antioxidant properties by catching the free radical, so it is so important in keep the balance between oxidant and antioxidant in our body[2]. Flavonoid can repair the function of artery endothelia, can decrease the sensitivity of the LDL to the effect of free radicals[3,4] and can be hypolipidemic, anti inflammation and also as antioxidant[5,6].

Atherosclerosis is a chronic inflammation in the artery wall that can cause many complication and symptom[7,8]. Oxidative stress that is accompanied by the increased of cholesterol will initiate LDL oxidation that worsen the inflammation and atherosclerosis. The administration of red wine that contain flavonoid can decrease the level of molecule adhesion like ICAM-1 and VCAM-1, that have role in the inflammation and atherosclerosis. The administration of flavonoid can decreased the level of proinflammation mediator by inhibition of nuclear factor kB[9]. The water of aronia melanocarpa that is rich of flavonoid can decrease hyperlipidemia incidence in the rats[11]. Anthocyanin extract of soya bean (*Glycine max*. L) can improve the lipid profile significantly.[(12)]. These effects were occur because the flavanoids is antioxidant and can decrease the formation of interleukin proinflaamtion[13].

Flavonoid is an exogen antioxidant that is proved to be able to prevent the cell damage that is caused by oxidative stress. Mechanism of action of the flavanoid as antioxidant can be directly or indirectly. Flavonoid as direct antioxidant acts as donor its hydrogen ion, so it can prevent the toxic effect of the free radical. Flavonoid as indirect antioxidant acts by increasing the expression of endogen antioxidant gene through several mechanisms. One of the antioxidant gene expression mechanism is by activation nuclear factor erythroid 2 related factor 2 (Nrf2) so there is an increase of the gene that has role in the antioxidant enzyme synthesis like SOD (*superoxide dismutase*) gene[14].

The leaf of sweet purple potato contain high flavonoid and can catch in vitro free radical[15,16]. Purple sweet potato tubers that are rised in Bali also contain high flavonoid anthocyanin[17], and has antioxidant effect and hypolipidemic effect in rat and rabbit that were suffer oxidative stress [18,19]. We need at least 4

months if we want harvest the purple sweet potato tuber, on the other hand we need fewer times to harvest the leaves and can be harvested in many times. The leaf of purple sweet potato is easier to be prepared for consumption and have not been studied its effect as antioxidant and hypolipidemic in vivo.

This study is conducted to investigate the hypothesis that the water extract of the purple sweet potato leaves can increase and improve the blood lipid profile. This study is done to the rats that are given high cholesterol diet and water extract of sweet purple potato leaves for 3 month.

MATERIALS AND METHODS

This study was a laboratory experimental with randomized pre and post-test control group design. Duration of the study was 3 months, that take time between June until August 2010 at Pharmacology Laboratory and Bio Molecular Laboratory of Medical Faculty, Udayana University.

The population of this study are male white rat, age 4-5 month that were breed at Animal Laboratory Unit of Pharmacology Department, Medical Faculty, Udayana University. Samples were determine by Fiderer formula, each group consist of 10 rats with 150-200 gram body weight. Independent variable in this study is water extract of sweet purple potato leaves. Dependent variables are blood SOD level and blood lipid profile. Controlled variables are sex, health status, body weight, diet, age, and environment. After the pre test, control group were given high cholesterol food for 3 month. Treatment group were given high cholesterol food and also 3 cc water extract of sweet purple potato leaves in the morning and evening everyday for 3 months. After 3 months, blood specimens were taken from the rat for blood lipid profile and SOD examination. High cholesterol food is special mixed food that was obtained from food study centre of Gajah Mada University, Jogyakarta.

Water extract of sweet purple potato leaves was the extraction product from sweet purple potato leaves that was produced by smoothen young sweet purple potato leaves, cut 30 cm from the tip. One kg of the leaves was smoothen and mixed with 1 litre of water and then filtrated. Filtrated water then boiled for 10 minutes. The water extract then cooled down and ready for use in this study. The data were analysis by T test.

RESULTS

The result of this study, that include pre-test and post-test of blood lipid profile and SOD are presented in table 1

Table 1. Lipid profile and level of SOD in the blood

Variable	Group	Pre-Test (Mean±SD)	Post-Test (Mean±SD)
Cholesterol	Control	119,39±19.9	299,59±4,5*
	Treatment	114,37±18.7	128,48±2,5
Triglyceride	Control	131,52±25,2	143,02±2,5
	Treatment	128,84±22,6	76,58±3,3*
HDL	Control	47,36±9,4	61,69±2,3
	Treatment	49,62±10,5	86,83±1,9*
LDL	Control	45,72±24,3	209,27±5,0*
	Treatment	38,96±24,0	26,25±3,3
SOD	Control	618,64±109	351,65±8,0*
	Treatment	653,63±100	663,01±11,7

The results showed a significant increase (* symbol in Table 1) of total cholesterol, triglycerides and LDL cholesterol in the control group (high cholesterol diet), and decrease of SOD significantly

DISCUSSION

The result of this study show that water extract of sweet purple potato leaves has hypolipidemic effect and increase SOD. The blood lipid profile examination on this study show the ability of the water extract of sweet purple potato leave in maintaining lipid profile in normal reference. Total cholesterol in the control group after given high cholesterol diet for 3 months were increase from 119,39mg/dl become 299,59 mg/dl. In the treatment group, that were given high cholesterol diet and water extract of sweet purple potato leaves, the total cholesterol almost the same (128,48 mg/dl become 128,84 mg/dl).

The increase of total cholesterol in the control group were very significant (P=0,0001). This result was similar to the LDL and triglyceride, where there was significant increase in the control group and in the treatment group was almost constant.

The contrary result was occurring in HDL, which was increase in the treatment group and decrease in the control group. The role of water extract of sweet purple potato leaves in this study is clearly as hypolipidemic agent, or can maintain the lipid profile in the normal reference even given high cholesterol diet. High concentration of flavonoid in the sweet purple potato leaf[15] can decrease cholesterol, LDL, and triglyceride and increase HDL. The result of this study is similar with study in rabbits that are given water extract of sweet purple potato tuber to maintain lipid profile[19]. This result is associated with the flavonoid content of the test material. The result of this study is match with the theory that flavanoid has benefit effect on human cells because has antioxidant, antimutagenic, hepatoprotective, antihypertensive and affect blood lipid level[14]. The result of this study also matches with some studies like: administration of water of *aronia melanocarpa* fruit that rich of anthocyanin can decrease hyperlipidemia on the rat[11]. Anthocyanin extract from soya bean (*Glycine max.)* can improve lipid profile, because can decrease triglyceride and total cholesterol significantly and can increase the HDL[12].

The result of SOD examination in this study show that the water extract of sweet purple potato leaves has properties in maintaining SOD level. SOD level in the beginning of the study were almost similar, 618,68 U/g Hb in control group and 653,63 U/g Hb in the treatment group (P=0,465). SOD level after the treatment for 3 months is decrease significantly in control group (P=0,0001), the result in the treatment group also almost similar. The result of this study show that water extract of sweet purple potato leaves has antioxidant properties. High flavonoid[15] has ability to catch free radical or increase the activation of Nrf2 so the production of SOD enzyme increase[14]. The result of this study is match with the study on the patients that were given black rice for 6 months can increase SOD so can increase total antioxidant[21]. The result of this study is match with the theory that flavonoid like anthocyanine can accumulates in the cells and has function as antioxidant that can prevent cell damage because of oxidative stress[20].

The weakness of this study is the cages of the rat are not comfortable, because the cages are located in the same building with the class that could make the rat become discomfort and stress. The other weakness is there is no negative control in this study, but can be diminished by doing pre-test examination.

CONCLUSION

Water extract of sweet purple potato leaves can improve white rat's blood lipid profile that was given high cholesterol diet. Water extract of the sweet purple potato leaves also can increase white rat's blood SOD level that was given high cholesterol diet.

To get better study result and can be applied, we recommend that clinical trial should be done with healthy people and sick patient.

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DETECTION OF EXTENDED-SPECTRUM BETA LACTAMASE (ESBL) PHENOTYPE FROM *Escherichia coli* AND *Klebsiella spp* ISOLATES RESISTANCE CEPHALOSPORIN AT SANGLAH HOSPITAL DENPASAR

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ABSTRACT

Increased use of antibiotics leading to increased bacterial resistance to antibiotics. The use of beta-lactam antibiotics, especially second and third-generation cephalosporins is increasing to treat infections caused by bacteria, both Gram positive and Gram negative. This gives the impact of increased bacterial resistance to beta-lactam antibiotics in both the community and in hospitals. ESBL β-lactamase is an enzyme that makes the bacteria resistant to penicillin; first, second and third generation cephalosporins and aztreonam by hidrolyse all three types of antibiotics. However, the bacteria is not resistant to cephamycin or carbapenem. The aim of this study was to determine the phenotype of ESBL in isolates of *Escherichia coli* and *Klebsiella sp* in Sanglah Hospital in 2010. The research is conducted by culturing and identification, followed by phenotyping test using Double Disc Synergy Test (DDST). During the period January- December 2010, 134 isolates were found resistant to ceftazidime and cefotaxim, which were 80 *E. coli* isolates and 54 *Klebsiella sp.* isolates from clinical specimens. DDST test resulted in 29 (63%) *E.coli* isolates and 17 (37%) *Klebsiella sp.* isolates that showed a positive ESBL phenotype. The clinical specimens that contained positive ESBL were 22 urine isolates (47.8%), followed by 10 isolates from blood specimens (21.7%), pus 5 isolates (10.9%), sputum 4 isolates (8.8%), tissue 2 isolates (4.3%), stool 2 isolates (4.3%) and peritoneal fluid 1 isolate (2.2%). It can be concluded that ESBL-producing bacteria in Sanglah Hospital is spread throughout the hospital and from various clinical specimens. Therefore, a wise use of antibiotics should be considered.

Keywords: ESBL, *Extended Spectrum Beta Lactamase, Double Disc Synergy Test*

BACKGROUND

Cephalosporin resistance in Enterobacteriaceae in particular caused the emergence of strains that produce extended-spectrum enzyme beta-lactamase (ESBL), where this enzyme can hydrolyze cephalosporin antibiotics. (Grover, et al 2006). ESBL enzymes are encoded by plasmids that increase due to a point mutation in the gene TEM-1, SHV-1 and OXA beta-lactamase. This plasmid is very easy to switch to other bacteria. ESBL mostly produced in *Escherichia coli* and *Klebsiella spp* where the bacteria are numerous in the hospital environment, so that nosocomial infections caused by both bacteria are widely reported (Nijssen et al., 2004).

A total of 26.2% E coli isolates producing ESBL (Nijssen et al., 2004). Several studies have shown 27.8% (North America), 61.9% (Latin America) and 75% (Western Pacific) isolates suspected of having a confirmed ESBL producing ESBL phenotype (Winokur et al., 2001). Nijssen et al study showed prevalence of ESBL in *E. coli* and *Klebsiella spp* was 4.5% while Jones et al demonstrated the prevalence of ESBL in *E. coli* and *Klebsiella spp* was 3.8% (Jones et al., 2000; Nijssen et al., 2004). Although ESBL prevalence data has been widely reported but for hospitals in Indonesia have not been widely reported, including at the hospitals in Bali, especially Sanglah Hospital. By knowing the prevalence of ESBL it can suppress the use of antibiotics in the hospital so that cases of antibiotic resistance can be reduced.

RESEARCH AIM

In general, the aims of this study is to determine the prevalence of ESBL in isolates of *E. coli* and *Klebsiella* sp. in Sanglah Hospital, Denpasar.

METHODS

Target population of this study is all isolates of *E. coli* and *Klebsiella sp.* obtained from clinical specimens in cases of infection in the Clinical Microbiology Laboratory of the Faculty of Medicine, University of Udayana/Sanglah Hospital in 1 year period. Materials for the isolation of *E coli* and *Klebsiella spp* bacteria from clinical specimens are MacConkey and identification by Microgen Kit (Pronadisa). While

materials for the detection of ESBL phenotype by Muller Hinton, NaCl, antibiotics disc are (cefotaxime (30 g), ceftazidime (30 g), cefepime (30 g) aztreonam (30 mg) and amoxicillin 20 mg plus 10 mg clavulanic acid).

Method

The detection of ESBL phenotype isolates of *E. coli* and *Klebsiella spp* was performed by using the method of Double-Disc Synergy Test (DDST). The first step is the preparation of the solution according to 0.5 McFarland inoculum, 4 to 5 colonies of bacteria were diluted in NaCl and then vortexed until turbidity reached 0.5 McFarland. Then the solution was inoculated onto 2 plates of Muller Hinton agar medium using swabs. In Plate I, amoxicillin 20 mg plus 10 mg clavulanic acid disc was placed in the middle which had been inoculated with bacteria to be tested, then cefotaxime (30 g), ceftazidime (30 g), cefepime (30 g) aztreonam (30 g) discs were placed radially at a distance of 30 mm from amoxicillin 20 mg plus 10 mg clavulanic acid disc. While in the second plate only cefotaxime, ceftazidime and aztreonam disks were placed. Both plates were incubated in parallel on aerobic incubator at 37 °C for 24 hours. The strain was considered as ESBL producer, if the zone size around any of the discs increased by ≥ 5 mm towards disc amoxicillin 20 mg plus 10 mg clavulanic acid compared to that produced in absence of amoxicillin 20 mg plus 10 mg clavulanic acid disc.

RESULT AND DISCUSSION

ESBL-producing bacteria on DDST test will show the addition of a diameter greater than or equal to 5 mm compared with a routine sensitivity test. During the period January - December 2010 obtained 134 isolates that were resistant to ceftazidime and cefotaxim divided into 80 isolates of *E. coli*, 54 isolates of *Klebsiella sp* from clinical specimens. Of DDST test conducted found 29 (63%) *E coli* isolates and 17 (37%) *Klebsiella sp* isolates that showed a positive ESBL phenotype. Distribution of clinical specimens containing positive ESBL most were 22 urine isolates (47.8%) followed by a specimen of blood 10 isolates (21.7%), pus 5 isolates (10.9%), sputum 4 isolates (8.8%) , tissue 2 isolates (4.3%), stool 2 isolates (4.3%) and peritoneal fluid 1 isolates (2.2%).

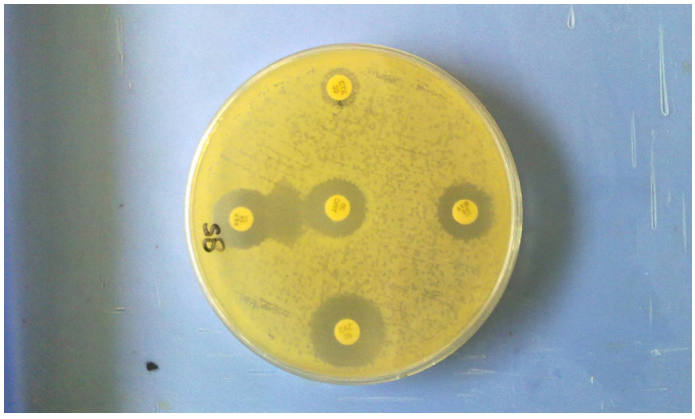

Figure 1. Culture Phenotype ESBL Positive

Based on these results it can be concluded that there ESBL-producing bacteria in the hospital Sanglah whose distribution is spread throughout the hospital and from various clinical specimens. So that should be considered a wise use of antibiotics.

Screening tests by viewing data of ceftazidime and cefotaxime resistant obtained 134 *E coli* and *Klebsiella sp* isolates from clinical specimens, after continued by confirmation test found only 46 (34.3%) ESBL-producing isolates positive Resistance detection. Screening by using either ceftazidime or cefotaxime sensitivity for the detection of ESBL-producing strains, failed to establish the diagnosis in 15—20% of cases. When compared with research in the hospital Soetomo Surabaya there are differences in the proportion of ESBL-producing E. coli (29%) and of ESBL-producing Klebsiella (36%). this is caused by

the distribution of these bacteria in hospitals that varied. Sanglah Hospital more isolated the bacteria *E coli* compared with *Klebsiella sp.*

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BLOOD LEVELS OF INTERLEUKIN-6 AND TUMOR NECROSIS FACTOR-ALPHA OF PRE-TERM NEWBORNS

Trisna Windiani IGA

ABSTRACT

Cytokines interleukin-6 and tumor necrosis factor alpha is an important mediator of host response to stress and infection, but little is known about their levels in the blood and activity during early life, especially in a population of premature newborns. The results of previous studies about the ability of cells from healthy newborns to produce cytokines have been contradictory, showing a decline, normal, or increased IL-6 and TNF alpha decreased or normal. The aims were to determine levels of cytokines interleukin-6 and tumor necrosis factor-alpha in the blood of premature newborn. The study design was cross sectional, population under study consisted of twelve healthy preterm neonates. Blood samples were obtained from preterm neonates at the time before 24 hours of delivery. Samples were taken with consecutive from all preterm born. Inclusion criteria: preterm infants (ages birth <37 weeks) with stable medical condition or experiencing mild stress based on the criteria of the Neonatal Infant Scale stressors, 1500-2499 g birth weight, Appropriate gestational age (Lubchenco curve). Exclusion criteria: preterm infants with comorbidities, major congenital anomalies, asphyxia, with a history of mothers suffer from immunodeficiency diseases, mothers with premature rupture of membranes, amniotic fluid turbid and smelly, fever before the birth. Medical records of these preterm neonates were reviewed and coded without knowledge of cytokine results. The data included gestational age, birth weight, gender, Apgar scores, type of delivery. Blood samples were collected aseptically in 1.0 ml glass blood collection tubes. The method used for examination of IL-6 and TNF-alpha is the Enzyme Linked Immunosorbent Assay (ELISA); Double Sandwich Streptavidine Biotin Antibody Test. During the study period a total of 12 subjects acquired neonatal prematurity, sex boy 7 and girl 5, the average gestational age 33.83 weeks (SD: 1:58), mean birth weight 2091, 67 (SD: 285.11), the average age of mothers at gave birth to 24.8 years (SD: 5.78), mean levels of IL-6 8.69 (SD: 5.48) and mean levels of TNF-alpha 13.04 (SD: 2.56). Levels of proinflammatory cytokine IL-6 and TNF alpha in healthy preterm neonates is in accordance with the normal range.

Keywords: IL-6, TNF alpha, preterm neonates

INTRODUCTION

The most prominent cytokines tumor necrosis factor alpha (TNF alpha), interleukin (IL)-1, and IL-6. Various studies have shown that in response to sepsis, immune system initiates a cytokine cascade characterized by sequential production of TNF alpha, IL-1, IL-6.[1] TNF alpha is first to appear with its primary response being upregulating vascular adhesion molecules, activating neutrophils and stimulating monocytes to secrete IL-1 and IL-6.[1,2]

The inflammatory response is mediated by cytokines that are used as neonatal infection markers, especially interleukin-6 (IL-6). IL-6 is an inducer of hepatic protein synthesis, promotes production and liberation of C-reactive protein, and can be detected early when there is bacterial bloodstream invasion. It acts as a signal for T-cell activation, promotes antibody secretion by B cells and differentiation of cytotoxic T cells, and stimulates liberation of other cytokines, particularly TNF-alpha.[1,3]

Cytokines interleukin-6 and tumor necrosis factor alpha is an important mediator of host response to stress and infection, but little is known about their levels in the blood and activity during early life, especially in a population of premature newborns. The results of previous studies about the ability of cells from healthy newborns to produce cytokines have been contradictory, showing a decline, normal, or increased IL-6 and TNF alpha decreased or normal.[1-4]

The aims of this study were to determine levels of cytokines interleukin-6 and tumor necrosis factor-alpha in the blood of premature newborn.

MATERIALS AND METHODS

The study design was been accepted by the ethical commission of the School of Medicine, Udayana University and Sanglah Hospital, all patients gave informed consent. The population under study consisted of 12 subjects premature neonatal. The study design was cross sectional, population under study consisted of twelve healthy preterm neonates.

Blood samples were obtained from preterm neonates at the time before 24 hours of delivery. Samples were taken with consecutive from all preterm born. Inclusion criteria: preterm infants (ages birth <37 weeks) with stable medical condition or experiencing mild stress based on the criteria of the Neonatal Infant Scale stressors, 1500-2499 g birth weight, Appropriate gestational age (Lubchenco curve). Exclusion criteria: preterm infants with comorbidities, major congenital anomalies, asphyxia, with a history of mothers suffer from immunodeficiency diseases, mothers with premature rupture of membranes, amniotic fluid turbid and smelly, fever before the birth.

Medical records of these preterm neonates were reviewed and coded without knowledge of cytokine results. The data included gestational age, birth weight, gender, Apgar scores, type of delivery. Blood samples were collected aseptically in 1.0 ml glass blood collection tubes. The method used for examination of IL-6 and TNF-alpha is the Enzyme Linked Immunosorbent Assay (ELISA); Double Sandwich Streptavidine Biotin Antibody Test.

RESULTS

During the study period a total of 12 subjects acquired neonatal prematurity, sex boy 7 and girl 5, the average gestational age 33.83 weeks (SD: 1.58), mean birth weight 2091, 67 (SD: 285.11), the average age of mothers at gave birth to 24.8 years (SD: 5.78) in table 1.

Table 1 Characteristics preterm neonatal

Characteristic	
Sex, n (%)	Boy 7 (58.3)
Mean estational age, weeks (SD)	33.8 (1.58)
Mean Birth weight, gram (SD)	2091.6 (285.11)
Age of mother, years (SD)	24.8 (5.78)

The values of the laboratory markers of inflammation with mean levels of IL-6 8.69 (SD: 5.48) and mean levels of TNF-alpha 13.04 (SD: 2.56).

DISCUSSION

Relatively little is known concerning the circulating concentrations or production of chemokines (with the exception of IL-8) in preterm newborns. Our observations support the results of recent studies indicating that serum concentrations of chemokines in noninfected preterm neonates were either similar to or higher than those measured in term infants and adults.[1,3,5-7] Our findings also suggested that circulating cytokine and chemokine concentrations of IL-6 and TNF alpha in healthy preterm neonates is in accordance with the normal range. More importantly, the relatively small sample size in this studies, the lack of a reference cut off for most diagnostic markers.

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PATTERN OF INFLUENZA VIRUS IN DENPASAR DURING INFLUENZA-LIKE ILLNESS SURVEY IN 2009-2011

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ABSTRACT

Background

Influenza is considered one of the most important infectious diseases. Complicating the global burden is emergence of new strain of virus during H1N1 outbreak, we now experiencing of the 1st pandemic in this century. Although influenza illness is usually mild, but changes in their genetic material will create more pathogenic virus than its origin. The aim of this study is to investigate the seasonal pattern of influenza in Denpasar during actively Influenza-like Illness surveys in 2009-2011. Subject of this study was categorized in two groups, for the surveillance cases, samples were collected from Southern Denpasar I Primary Health Care Center, whereas for the outbreak, samples were collected from Sanglah Hospital Denpasar. Samples were collected from nasal and throat specimens. The specimens were transported using Hank medium. In the laboratory, specimens were extracted using Invitrogen Extraction Kit. PCR used was Superscript III Platinum One-Step qRT/PCR System Real-time PCR. For the surveillance specimens, we determined Influenza A and Influenza B type and H1 seasonal, H3 and H5 subtype. While for the outbreak we only determine the H5 subtype for Avian Influenza Outbreak and H1 novel for Swine Flu Outbreak.

During August 2009-June 2011 period, 832 specimens were collected. In the period of August 2009-June 2010, a total 381 specimens were collected, among them 25 (7%) was positive for Flu A, 8 (2%) positive for Flu B and 13(3%) positive for H3 while 335 (88%) were categorized in non-subtype. On the other hand, during July 2010-June 2011, a total of 451 specimens were collected, 17 (3,8%) were flu A positive, 25 (5,5%) flu B positive, 2(0,4%) H3 positive and 7 (1,6%) positive for H1. During 2009-2010, Flu A occurrence was fluctuated, while flu B was only detected in June and H3 was highest in April 2010. During 2010-2011, Flu A was detected in January, 2011, Flu B was highest in September 2010 and Swine flu (H1) was highest in March,2011. H1 and H5 was not detected in this survey period.

We can conclude that during this 2 years period of survey Flu B was found increased during 2010-2011, and Swine flu (H1) only found during outbreak in 2010-2011.

Keywords: ILI, Flu A, Flu B, Denpasar.

INTRODUCTION

The emergence of pandemic influenza, whether bird flu (H5N1) or swine flu (H1N1) in Indonesia in 2006 and 2009, making the government and health department increasingly recognized that influenza is not just a common disease but also can be very deadly.

Influenza is a disease caused by a virus belonging to the family of Orthomyxoviridae (Murphy,*at all*.,2001). Based on differences in the matrix protein (M protein), influenza viruses can be divided into several types of influenza type A, B and C. Influenza type A is further subdivided into several subtypes based on antigenic differences in the external glycoprotein that called Haemagglutinin (HA) and Neuraminidase (NA). Until now there are at least 15 types of HA and 9 different NA types have known. However, only a few subtypes of influenza A, that cause disease in humans such as H1-, H3- and H5- subtype. The N subtypes that usually fatal such as N1-and N21. Indonesia has an experience of Influenza outbreak in 2006 and 2009 that cause by H5N1 and H1N1 subtype.

Clinical manifestations of influenza that is often similar to respiratory diseases caused by other pathogens, causing the disease was difficult to diagnose when only based on clinical symptoms. To overcome this difficulty, several methods have been developed, such as viral genetic isolation, antigenic examination, and molecular biology. Each of these diagnostic methods has its own drawbacks and advantages as can be seen in Table 1.

Tabel.1. Comparison of Influenza virus diagnostic method.

Method	Target	Specimen	Sensitivity	Specificity	Time
RT-PCR	RNA	Swab, Tissue	+++++	+++++	< 3 day
Virus Isolation	Virus	Swab, Tissue	+++++	+++++	2-5 day
Antigen Examination	Antigen	Swab, Tissue	++	++	1 day

Rapid and accurate method of diagnosis would help the clinician to perform the right and appropriate therapy and also help the health officer to monitoring and surveillance of ILI cases in community. This is supported by the program that is currently developed by the Agency for Health Research and Development (Balitbangkes) Ministry of Health Republic of Indonesia by pointing to several laboratories in the region as Regional Influenza Laboratory. Since 2007, Section of Microbiology Faculty of Medicine, Udayana University was appointed as the Regional Influenza Laboratory for Bali and Nusa Tenggara. By forming the Regional Laboratory, the government expects to have sufficient data for the spread of influenza cases in Indonesia.

The purpose of this study was to determine the frequency of influenza viruses from specimens that was collected from the patient with ILI symptom (Fever > 100F, with cough and/or Shore throat) who comes to the Denpasar Selatan 1 Primary Health Centre and RSUP Sanglah Denpasar.

METHODS

This study is a descriptive study. The study was conducted at the Molecular Biology Laboratory Faculty of Medicine, Udayana University from August, 2009 until June 2011. The specimens were collected from Denpasar Selatan 1 Primary Health Centre and Sanglah Hospital. The samples were divided into 2 groups: samples derived from surveilance cases and samples from outbreak cases. For the case of surveillance, samples derived from patients who has ILI symptoms and went to Denpasar Selatan 1 Primary Health Centre and for the case of an outbreak, a sample derived from Sanglah Hospital Denpasar. The specimen was taken from the nose and throat swabs, then placed into transport medium of Hank's media and further processed in the laboratory.

RNA ISOLATION

For surveillance specimens, specimens were extracted using the QIAmp ® Viral RNA Mini Kit (Qiagen) using outbreak specimens while the Pure Link ™ Viral RNA / DNA Mini Kit (Invitrogen).

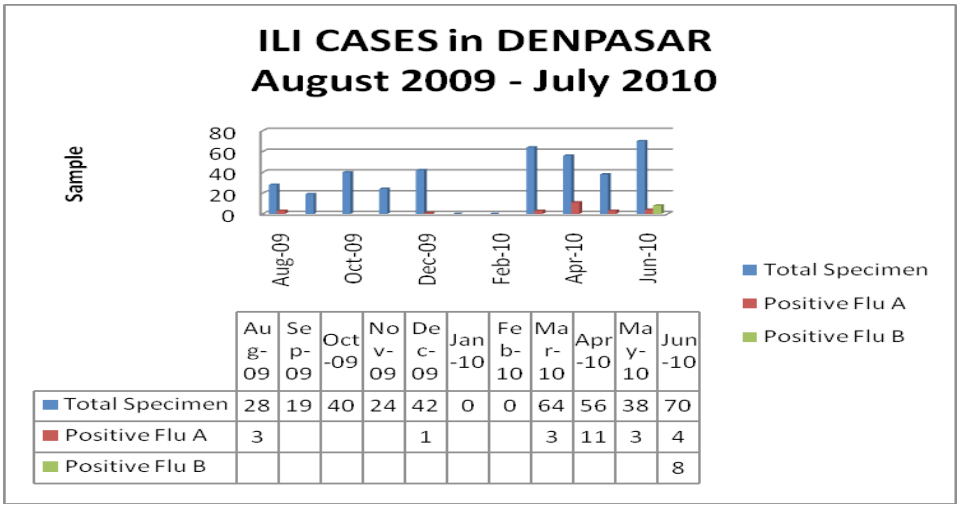
Real-time PCR

Primers used for PCR process was provide by Balitbangkes according to CDC Atalanta protocol. For surveillance specimens Real-time PCR was used AgPath-ID ™ One-Step RT-PCR Kit (Ambion), while for outbreak specimens used Superscipt III Platinum One-Step qRT / PCR System Real-time PCR Kit (Invitrogen). Differences reagents used for each of these specimens affect the cycle time used in the PCR process. In surveillance specimens, PCR tube already containing 25μL PCR mix and mold incorporated into real-time PCR machine IQ-5 (Biorad) with a cycle of reverse transcriptase (RT) at 50 ° C for 30 minutes, pre-denaturation at 95 ° C for 10 seconds and proceed with the process of denaturation and annealing respectively at 95 ° C for 15 sec and 55 ° C for 30 seconds as many as 45 cycles. The difference lies in the process of pre-cycle denaturation where for the outbreak specimen took 2 minutes within the same temperature.

For examination of Surveillance specimens, we determined the type of Influenza A and Influenza B, followed by examination of seasonal subtype H1, H3 and H5. As for the specimens' outbreak, we just check for the H5 subtype of bird flu outbreaks and the H1 subtypes for novel swine flu outbreak.

RESULTS AND DISCUSSION

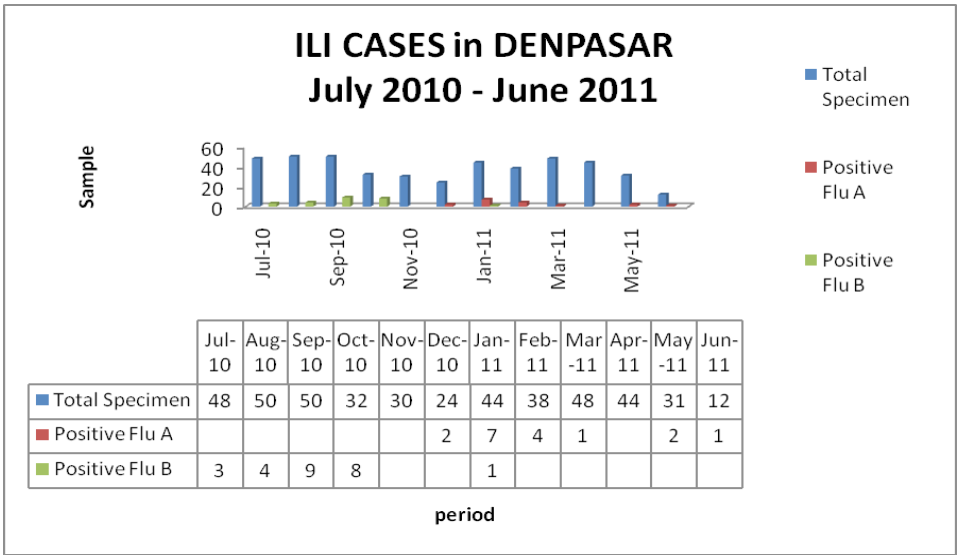
During the surveillance period (August 2009-june 2011), there are a total of 851 specimens was collected. Three hundred eighty one specimens were collected during August 2009- June 2010, while 451 specimens collected during July 2010 – June 2011. Among them total of 40% was female and 60% male, which age range was from 1 month – 49 years old.



	Aug-09	Sep-09	Oct-09	Nov-09	Dec-09	Jan-10	Feb-10	Mar-10	Apr-10	May-10	Jun-10
Total Specimen	28	19	40	24	42	0	0	64	56	38	70
Positive Flu A	3				1			3	11	3	4
Positive Flu B											8

Graph 1. The number of ILI Surveillance Specimen August 2009-July 2010

Among 381 specimen was collected, during surveillance of ILI cases in August 2009-July 2010, 25 (6,6%) positive Flu A and 8 (2,1%) positive for flu B. the high number of specimen was collected in first four month of 2010. Increasing the number of cases was due to in this month was rainy season. In January and February 2010, there is no samples was collected, this is not due to no cases of ILI during this 2 month, but because of technically problem.



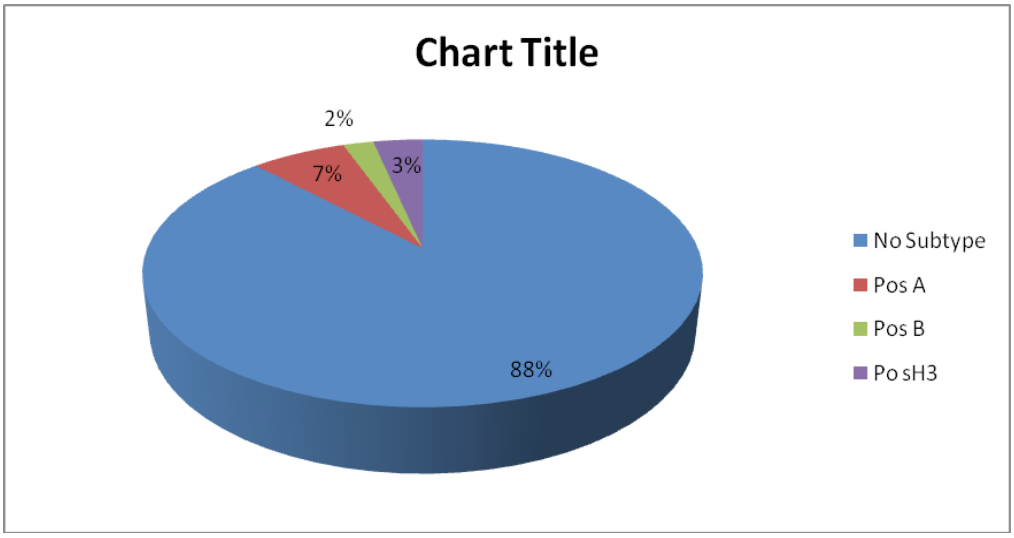
	Jul-10	Aug-10	Sep-10	Oct-10	Nov-10	Dec-10	Jan-11	Feb-11	Mar-11	Apr-11	May-11	Jun-11
Total Specimen	48	50	50	32	30	24	44	38	48	44	31	12
Positive Flu A						2	7	4	1		2	1
Positive Flu B	3	4	9	8			1					

Graph.2. The Number of ILI Surveillance Specimen July 2010 – June 2011

Seventeen (3,8%) of 451 specimen collected during July 2010 – June 2011 was positive Flu A and as much as 25 (5,5%) specimen was positive Flu B. One case of Flu B was isolated in 2011. Data of flu B in 2011 isn't a representative of Flu B in a year, because fluctuation of cases is always changes. The interesting data is a pattern when the Flu A isolated there is no Flu B was found and vice versa. This situation indicating that in a year one of them will dominant.

During this two year surveillance, specimen positive for Flu A was found all the year, most often isolated in March to June each year. On the other hand specimen which is positive for flu B, was found in June – October 2010. The association between season and pattern of Influenza A and B hasn't understood yet, because we don't analysis that association. This result is in line with data obtained in Jakarta and surrounding areas. Djoko *et al* (2009), using an different antisera and hemaglutination inhibition found that influenza A was dominantly, and small number of influenza B. From research conducted by Agrawal *et al* (2009), data from surveillance of children in India acquired 11.09% of influenza A and influenza B 5.41% of total 1091 samples.

Hadzhiolova et al (2006) in Bulgaria in 2004/2005, obtained 77 clinical samples consisting of 13% of positive influenza A subtype H3N2 and 24.6% of positive influenza subtype H1N1. But to note, that in this research in Bulgaria, Hadzhiolova still using the conventional PCR method by using gel electrophoresis in the examination.



Graph 3. Influenza Surveillance Data in Bali the period August 2009-June 2011

From 851 specimen that we have had analysis, among them 7% positive for Flu A, 3% positive for H3, and 2 % positive for Flu B and 88% was no-subtype. The high number for no-genotype can be due to the sensitivity of the method that can use to detect, the reagent that use was in appropriate. Upper respiratory infection can be caused by bacteria, virus or fungi. Most of them is virus. In this research we just identified for Influenza virus, in negative specimen there is might be virus other than influenza virus (Rhinovirus, parainfluenza virus, and respiratory sinctial virus) or mutated influenza virus.

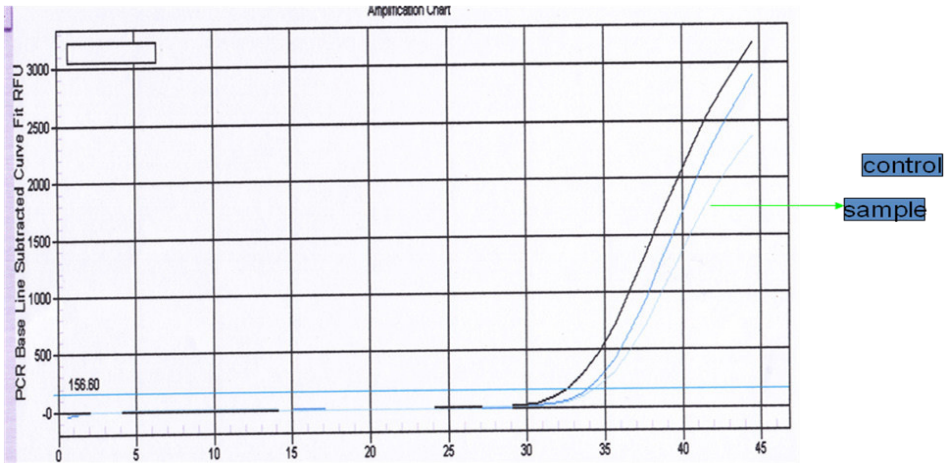


Figure.1. Real Time PCR result

Over the past two decades, virus isolation and serology have been the mainstay of the clinical laboratory for diagnosing respiratory virus infections. A variety of serological test including the hemagglutination inhibition (HAI) test, complement fixation, and enzyme immunoassay (EIA) were used for testing paired acute- and convalescent-phase sera for diagnosing viral infections. Currently,viral culture usually in combination with immunofluorescence (IF) is the "gold standard" for laboratory diagnosis. However, it is not a rapid diagnostic test, and therefore, its clinical value is limited. Now days Nucleic Acid based diagnostic was use to detected respiratory viral infection (Mahony et al.,2008). Real-Time PCR methode in combination with cell culture increase the sensitivity to detected respiratory viral infection (Strorch.,2003)

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THE DIFFERENT OF ABATE 1G® KILLING ABILITY WITH SUPPORTING SUBSTANCE AND WITHOUT SUPPORTING SUBSTANCE TO *AEDES AEGYPTI* MOSQUITO LARVAE

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ABSTRACT

Dengue Haemorragic Fever (DHF) still health problem for society in Indonesia. Especially vector of DHF is mosquito *Ae. aegypti*. Methode precisely to fight against mosquito *Ae. aegypti* is fight against larvae in breeding places by larvacide abate 1G®. Abate 1G® usage methode and abate 1G® durability in the water must be the attentive factors in larvacide usage. The research aimed to detect abate 1G® killing ability with supporting substance and without supporting substance to *Ae. aegypti* mosquito larvae at various containers. The research type is an experiment with device of research post test only control group design. Dose abate 1G® used 1 gram/ 10 of litre water. The supporting substance used spume. The containers consist of plastic, clay and cement. Every type container made three replicate. As control of every container one replicate. Sum up sample of larvae test to every container are 25 tails. Research done during 5 week and gift of larvae done every week. After observation 48 hours counted larva's death. Result of research indicate that abate 1G® with supporting substance and without supporting substance effective to kill *Ae. aegypti* larvae at various containers. *Kruskal-Wallis* analysis result show that no difference which significant between killing ability abate 1G® with supporting substance and without supporting substance at various containers (p>0,05). Need existence of similar research to using supporting substance and also type of other container.

Keywords : Killing ability, abate 1G®, supporting substance, *Ae. aegypti* , mosquito larvae

INTRODUCTION

Disease Dengue Hemorrhagic Fever (DHF) or dengue haemorrhagic fever (DHF) is a disease caused by dengue virus is transmitted through the bite of Aedes aegypti and Aedes albopictus.[1)] Based on the fact that there are generally the least role in the transmission of Ae. aegypti because of his life in the house is being Ae. albopictus in the garden or fields so that the less frequent contact with human.[2)]

In Indonesia dengue disease was first reported in Surabaya in 1968 with the number of people at that time as many as 58 cases, 24 of them died (CFR 41%). In 2000 recorded 33.443 cases with 472 deaths, in 2001 recorded 45.904 cases with 497 deaths, in 2002 recorded 39.158 cases with 489 deaths.[3)] From January to March 2004 total dengue cases has reached 26.015 with 389 deaths.[1]

There is an interesting phenomenon behind the recent outbreak of dengue cases, namely a shift in the age of the patient. If the first dengue fever is more common in children, but in recent decades seen a trend increase in the proportion of patients with dengue disease in adult.[4)]

Likewise, the increasing spread of the disease, which saw that the disease was originally found only in major cities only, then spread to almost all major cities in Indonesia and even to the rural areas with dense population in a relatively brief.[5)] Increasing the number of cases and the increase in the affected region, due to the population gets better means of transport, the existence of new settlements, the lack of people's behavior toward cleaning mosquito breeding, the presence of mosquito vectors in almost all corners of the ground water and the presence of four cell types of the virus circulating throughout years.[1)]. Various mitigation efforts have been made by the government since 1969, among others, by extension, the mobilization of communities in the Eradication of mosquitoes nest (PSN) and spraying insektisida.6) prevention efforts have been successful in reducing the mortality rate (CFR) of DHF and pain in Pelita V numbers (incidence) can be restricted below 10 per 100,000 population, but the area affected by the widespread.[7)] Thus the number of cases and death is expected to continue to increase along with the ever-expanding area of deployment [3)]

At this time eradicating the mosquito Ae. aegypti is the main way being done to eradicate dengue fever, because a vaccine to prevent and cure to eradicate the virus is not yet available[2)] Right way to combat the mosquito Ae. aegypti is to eradicate larvae in breeding sites can be done by sprinkling powder on ® 1G abate water reservoirs that are difficult drained or in areas difficult to obtain clean water so it needs to accommodate the rain water.[8)] How to combat the larvae of Ae. aegypti larvae using a repellent insecticide

(larvasida) is known as abatisasi[2)] Abatisasi preventative activities carried out before reaching the peak of transmission. Abate 1G ® used is a highly effective insecticide against the mosquito-borne diseases, safe for humans and orther warm-blooded animals.[9)] How to use and the ability to abate 1G ® ® 1G abate survive long in water is a factor that must be considered. How to use 1G ® abate it is to pour into clean water all the shelters are thought to be a nest of mosquitoes inside and outside the home. In this way according to the WHO study (1967) in Bangkok turned out to abate 1G ® have residual effects for 2.5 to 3 months.

Based on the results of research conducted, I Nyoman Nuidja (1982) in Jembrana (Bali) shows that to extend larvasida residues in water can be done by mixing it with buffer material larvasida. Materials commonly used buffers include: foam, sawdust, coconut fiber, rice straw and cork. By mixing the buffer material residual effects can be extended up to 3.5 month.[10)] It is not yet known whether the mixing of buffer material will affect the power to kill abate 1G ® against larvae of Ae. aegypti. Therefore, further research needs to be done about it.

MATERIALS AND METHOD

This study includes a research experiment with the method quasi-experimental (quasi-experimental research) and experimental lapangan. Use this quasi-experimental method because the design does not have strict restrictions on the randomization. The study design used in this study is posttest only control group design. The population in this study is the mosquito larvae of Ae. aegypti whereas the sample in this study were third and fourth instar larvae start hatching eggs of the mosquito Ae. aegypti with the calculation:

25 x 21 container tail X 5 repetitions of the experiment larvae = 2625 tail

Research Procedure

1. Preparation abate ® 1G:
   a. Abate 1G ® with a buffer created by the way: according to abate 1G ® measuring a dose of 1 gram / 10 liters of water mixed with foam and then wrapped using plastic bags that have been perforated. Created as many as 9 units.
   b. Abate 1G ® without a buffer created by the way: according to abate 1G ® measuring a dose of 1 gram / 10 liters of water and then wrapped using plastic bags that have been perforated. Created as many as 9 units.
2. Preparation of the experiment consisted of seven containers of plastic containers, 7 containers of clay, 7 containers of cement. Each container is filled as much as 10 liters of water. Each treatment in each type of container as much as three replicates. As a control each type of container as much as a fruit.
3. Preparation of mosquito larvae of Ae. aegypti early instar III and IV as many as 2625 birds.

How it works :

1. Abate 1G ® with buffer (foam) is inserted into a container that consists of three plastic containers, three containers of clay and three containers of cement as well as abate 1G ® without buffering. As a control used 3 containers consist of a plastic container, a clay container and a container of cement without the rated 1G ® abate.
2. Inserted into the container as much as 25 tail larvae of Ae. aegypti early instar III and IV.
3. Do observations and the number of dead larvae are lying and are calculated with observation time 1 hour, 2 hours, 4 hours, 6 hours, 24 hours, 48 hours with a measured pH and water temperature. After 48 hours the number (percentage) mortality rates are recorded and larvae are still alive and the dead taken by using a pipette and discarded.
4. Every 6 days later (day-to-6, 12, 18, 24, 30) the addition of 25 tail larvae per container. Calculation of larvae that were carried out by the observation time 1 hour, 2 hours, 4 hours, 6 hours, 24 hours, 48 hours while still measured pH and water temperature. The death rate of test larvae were recorded after 48 hours.

Counted dead larvae and larval mortality in a number of replications are combined then calculated the average and the percentage of his death. If the number of larvae in the control group mortality of less than 5% are ignored, but if more than 20% must be retested and if the mortality of larvae in the control group between 5% - 20%, then the actual percentage of larval mortality was calculated using the formula Abbot Correction to the formula :

$$\frac{\text{\% mortality treatment- \% mortality control}}{\text{100\% - \% mortality control}} \text{ X 100 \%}$$

The data obtained were analyzed in the form of descriptive tables and charts. Analytic analysis using SPSS version 10. Probit analysis is used to determine the lethal time 90 mosquito larvae of Ae. aegypti after being abate 1G ® with buffer and no buffer in various containers. If data are normally distributed and homogeneous then the different test to detect a significant difference in mortality between the mode of administration 1G ® abate with buffer and no buffer against mosquito larvae of Ae. aegypti in a variety of containers carried by analysis of variance (ANOVA), but if the data are not normally distributed (distributed free) and do not homogeneous then the data was tested using the Kruskal-Wallis.

RESULTS AND DISCUSSION

Based on observations and measurements of water temperature, water pH, water type, the type of container and the death of larvae during the study obtained the following results:

A. Water temperature

Water temperature measurements performed in the morning and afternoon. Results of measuring the temperature of water used in this study is the average temperature of the water weekly. The result is the lowest temperature of -25 while the maximum temperature is 26oC with an average temperature of 25.2 ° C, as shown in Table 1.

Table 1. Average water temperature in the container experiment during the observation week to week I through V

Treatment	Type of container	Observation week :				
		I	II	III	IV	V
Abate 1G ® with buffer	Plastic	26	25	25	25	25
	Clay	26	25	25	25	25
	Cemen	26	25	25	25	25
Abate 1G ® without buffer	Plastic	26	25	25	25	25
	Clay	26	25	25	25	25
	Cemen	26	25	25	25	25
Control	Plastic	26	25	25	25	25
	Clay	26	25	25	25	25
	Cemen	26	25	25	25	25

Source : Results of the study in June-July 2005

B. Water pH

Water pH measurements made before larvae of Ae. aegypti is inserted into the container experiment. The results of measurement used is the average pH of the water weekly. The results obtained lowest water pH 6.2 and pH 7.5 with the highest average water pH of 7.02, as shown in Table 2.

Table 2.The average pH of the water in the container experiment during the observation week to week I through V

Treatment	Type of container	Observation week :				
		I	II	III	IV	V
Abate 1G ® with buffer	Plastic	6,2	7	7,5	7,4	7
	Clay	6,2	7	7,5	7,4	7
	Cemen	6,2	7	7,5	7,4	7
Abate 1G ® without buffer	Plastic	6,2	7	7,5	7,4	7
	Clay	6,2	7	7,5	7,4	7
	Cemen	6,2	7	7,5	7,4	7
Control	Plastic	6,2	7	7,5	7,4	7
	Clay	6,2	7	7,5	7,4	7
	Cemen	6,2	7	7,5	7,4	7

Source : Results of the study in June-July 2005

C. Type of water

This type of water used in this study is piped water. During the study did not do addition and subtraction of water volume.

D. Types of container

Types of containers used in this study is made of plastic materials, clay and cement are placed randomly in a single room.

E. Probit Analysis

F.

Tabel 3.LT90 values 1G ® abate group with a buffer on a variety of containers based on the observation week to week I through V

Type of Container	Obeservation week :	LT_{90} (hour)	Range (hour)
Plastic	I	2,55	2,03<LT<4,75
	II	2,68	2,33<LT<3,27
	III	2,64	2,14<LT<4,50
	IV	2,49	1,83<LT<21,31
	V	3,16	2,44<LT<5,78
	Average	2,70	
Clay	I	3,75	2,76<LT<8,18
	II	4,35	3,88<LT<5,01
	III	2,86	2,23<LT<5,75
	IV	2,62	2,15<LT<4,00
	V	2,72	2,24<LT<4,10
	Average	3,26	
Cement	I	2,46	1,86<LT<6,64
	II	3,36	2,82<LT<4,45
	III	2,55	2,24<LT<3,11
	IV	2,02	1,69<LT<2,72
	V	2,85	2,50<LT<3,42
	Average	2,65	

In Table 3 shows that the value of 1G ® LT90 abate group with an average buffer in a plastic container obtained 2.70 hours, 3.26 hours a clay container and container cement 2.65 hours.

Table 4. LT90 values abate group 1G ® without buffers at various containers based on the observation week to week I through V

Type of Container	Obeservation week :	LT_{90} (hour)	Range (hour)
Plastic	I	2,00	1,50<LT<5,66
	II	1,27	0,47<LT<1,90
	III	2,13	1,64<LT<4,83
	IV	2,30	1,68<LT<33,27
	V	2,26	1,78<LT<4,38
	Average	1,99	
Clay	I	2,22	1,74<LT<4,42
	II	2,55	2,23<LT<3,12
	III	2,08	1,60<LT<5,02
	IV	2,37	2,04<LT<3,00
	V	3,05	2,27<LT<5,54
	Average	2,45	
Cement	I	2,44	2,09<LT<3,12
	II	1,98	1,65<LT<2,70
	III	1,54	1,14<LT<2,25
	IV	1,57	1,17<LT<2,28
	V	2,16	1,83<LT<2,85
	Average	1,94	

In Table 4. seen that the value of the group LT90 1G ® abate without buffer averaging at 1.99 hours obtained plastic containers, containers of clay 2.45 hours and 1.94 hours of cement containers.

G. Death of larvae of Ae. Aegypti.

Based on survey results revealed that the death of larvae of Ae. aegypti to abate 1G ® with buffer and no buffer during the observation week I to week between container type V is very diverse. More detail can be seen in Figure 1 through Figure 12.

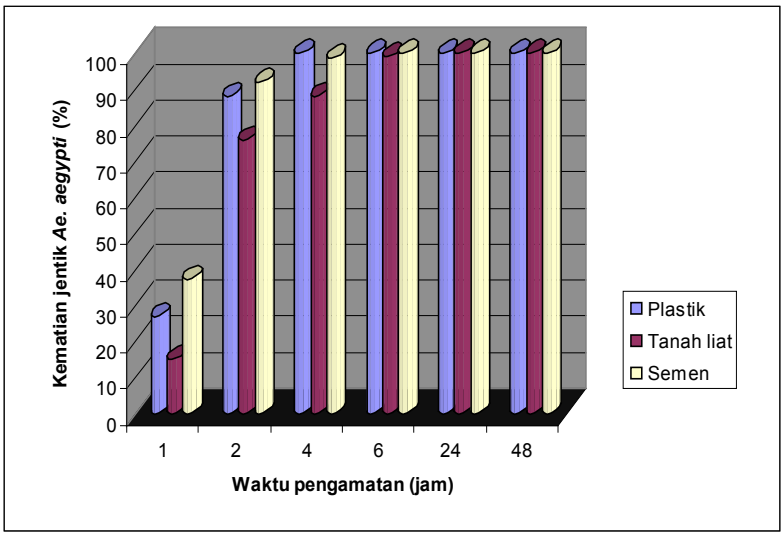

Figure 1. Graphs the percentage of larvae mortality of Ae. aegypti in various containers after being given a 1G ® abate with time-based buffer to the I-week observation.

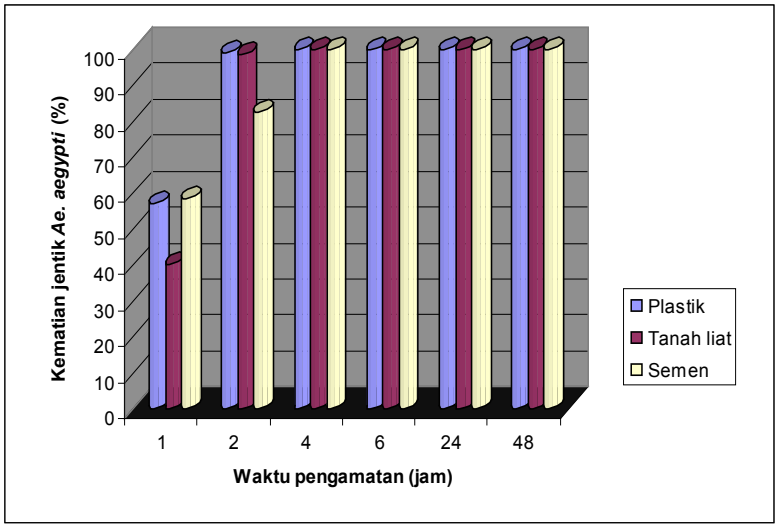

Figure 2. Graphs the percentage of larvae mortality of Ae. aegypti in various containers after being given a 1G ® abate without a buffer based on the observation time week I.

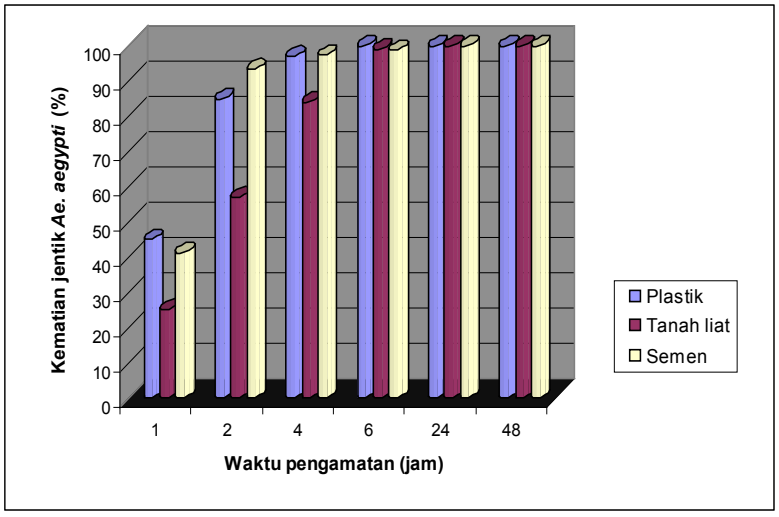

Figure 3. Graphs the percentage of larvae mortality of Ae. aegypti in various containers after being given a 1G ® abate with time-based buffer into the second week of observation.

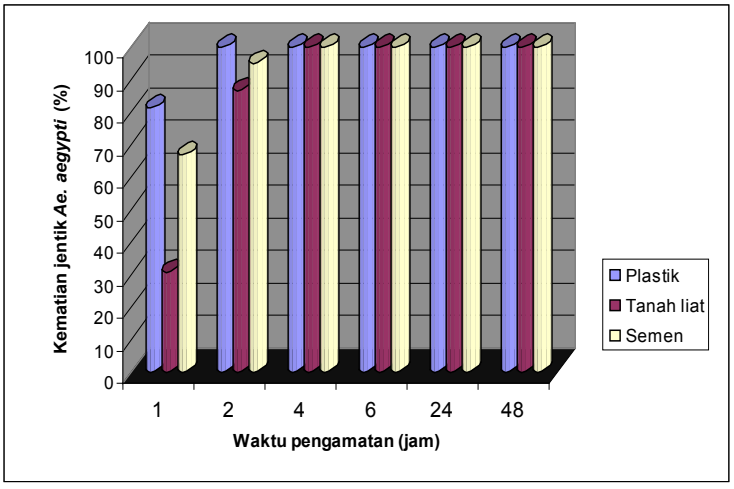

Figure 4. Graphs the percentage of larvae mortality of Ae. aegypti in various containers after being given a 1G ® abate without a buffer based on the observation time to the second week.

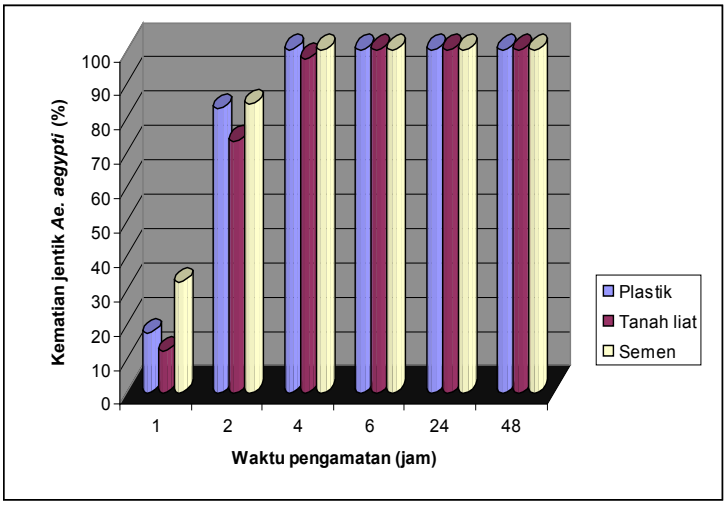

Figure 5. Graphs the percentage of larvae mortality of Ae. aegypti in various containers after being given abate ® 1G with a buffer based on the observation time to the third week.

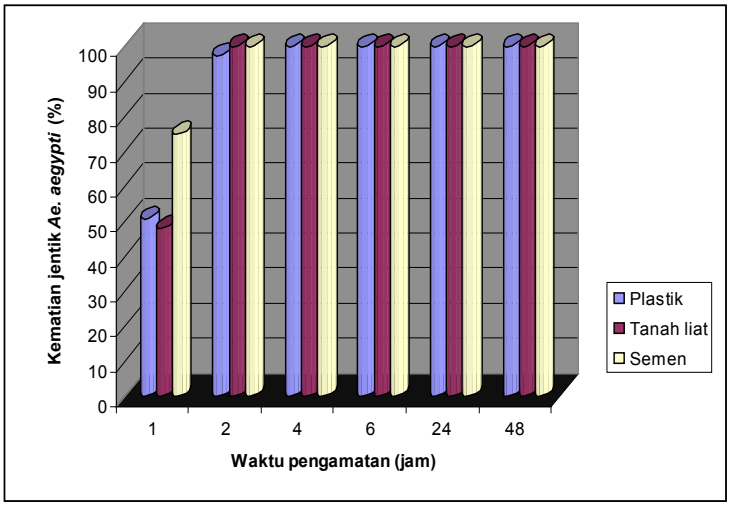

Figure 6. Graphs the percentage of larvae mortality of Ae. aegypti in various containers after being given a 1G ® abate without a buffer based on the observation time to the third week.

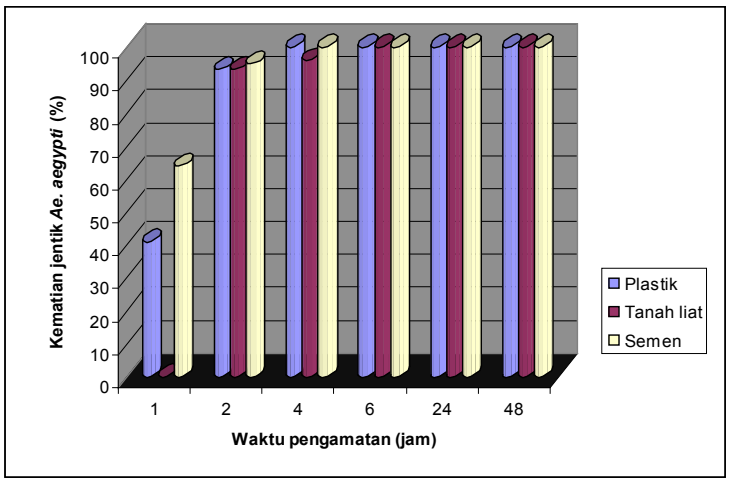

Figure .7 Graph the percentage of deaths larvae of Ae. aegypti in various containers after being given abate ® 1G with a buffer based on the observation time to the fourth week.

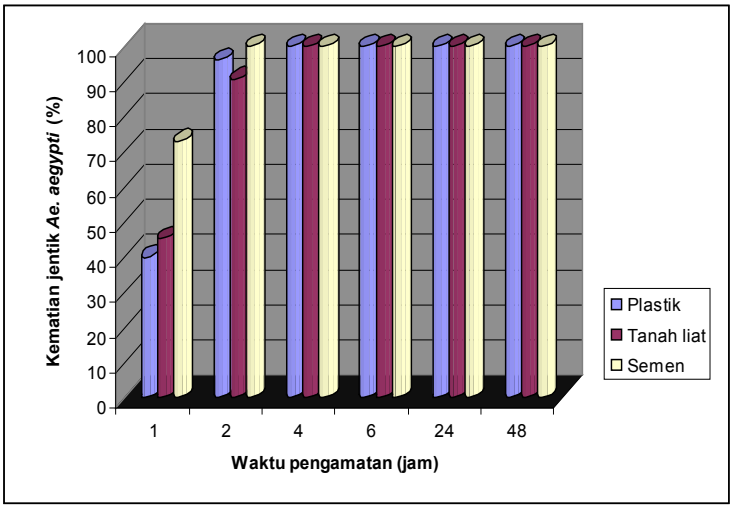

Figure 8. Graphs the percentage of larvae mortality of Ae. aegypti in various containers after being abate 1G ® without a buffer based on the observation time weeks IV.

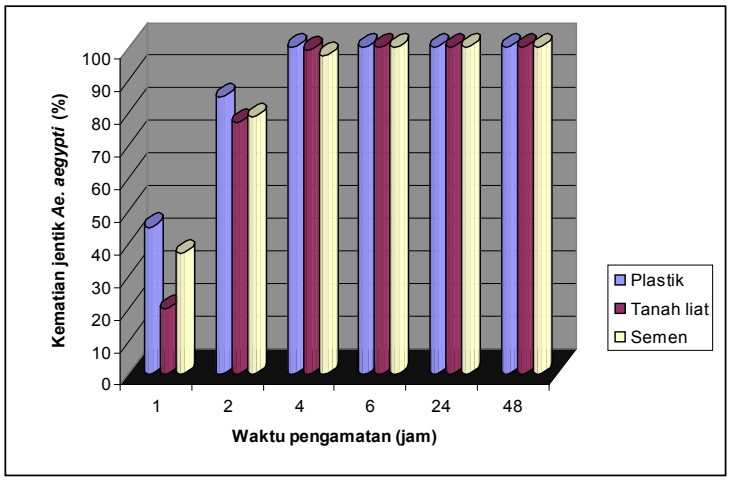

Figure 9. Graphs the percentage of larvae mortality of Ae. aegypti in various containers after being given a 1G ® abate with time-based buffer-week observation to V.

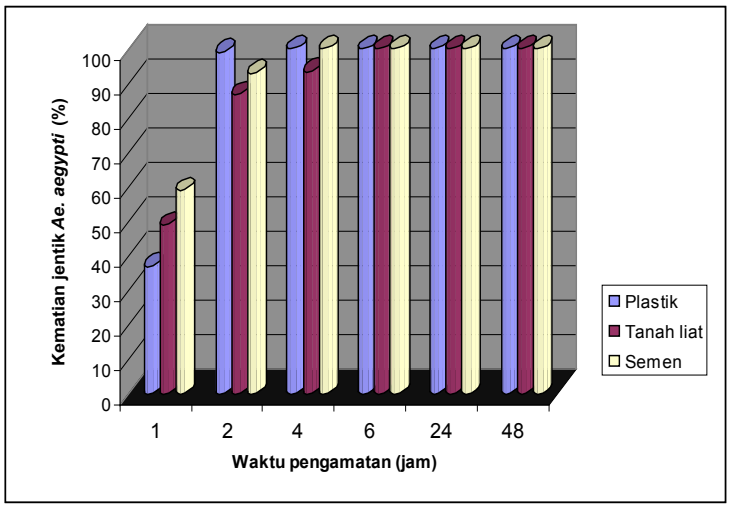

Figure 10 Graph of the percentage of dead larvae of Ae. aegypti in various containers after being given a 1G ® abate without a buffer based on the observation time week to V.

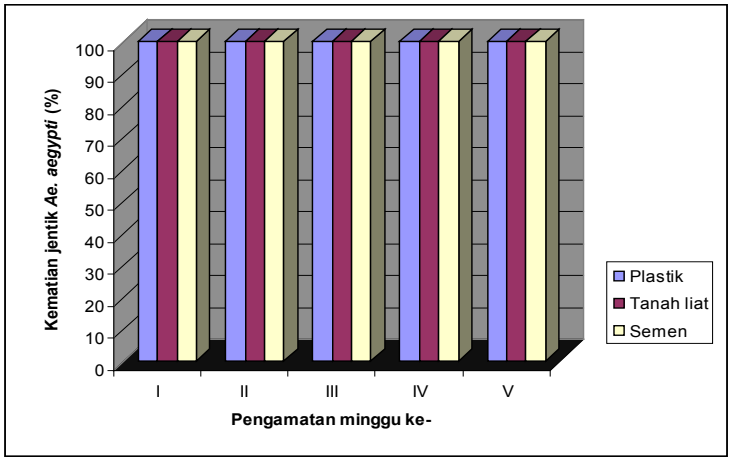

Figure 11 Graph of the percentage of dead larvae of Ae. aegypti in various containers after being given abate ® 1G with a buffer during the observation week to week I through V.

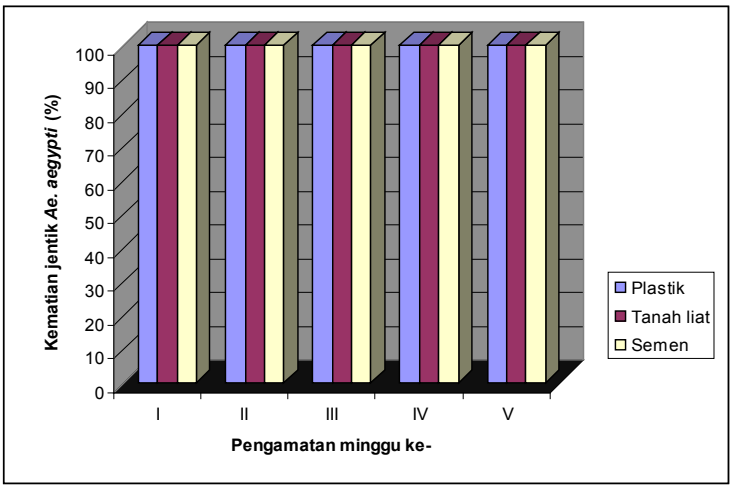

Figure 12 Graph of the percentage of dead larvae of Ae. aegypti in various containers after being given a 1G ® abate without a buffer during the observation week to week I through V.

Based on Figure 1 and Figure 12 is known that during the observation week to week I to V larvae mortality of Ae. aegypti in containers that have been given various 1G ® abate with buffer and without a buffer showed a 100% mortality after 48 hours of observation. This means abate 1G ® with buffer and no buffer effectively kills the larvae of Ae. aegypti in various containers. Larvasida said to be effective if the larvae mortality rate reach at least 90% within 48 hours.The results of probit analysis shows that the time required to abate 1G ® buffer and without buffers at various containers to kill larvae of Ae. aegypti also varied by 90%. Abate 1G ® who put in the water would soon break down and be active toxins out of the granules and attached to the pore walls of the container. Habits of larvae of Ae. aegypti pick up food at the edge of the container allow the contact with 1G ® abate. The faster the occurrence of contact between the larvae of Ae. aegypti with 1G ® abate the death of larvae of Ae. aegypti will also be more rapid.

The results of Kruskal-Wallis analysis showed that there was no difference in the death of larvae of Ae. aegypti after being abate 1G ® with buffer and no buffer at various containers ($P > 0,05$). This means that the mode of administration 1G ® abate with buffer (foam) had no effect on the solubility of 1G ® abate and not block the release of toxins. According to the theory of "Slow release" stated that a solid polymer formulation larvasida in both rubber and plastic when placed in water larvasidanya will escape slowly from the polymer surface. If the concentration larvasida on the surface has been exhausted by diffusion within the polymer larvasida going out again to the surface, thus the release

of the active ingredient will run continuously until the reserves run out larvasida in the polymer.[10] The different types of containers in this study were also not affect the power to kill either abate 1G ® buffer provided with or without buffering. This means abate 1G ® with buffer and no buffer can be applied to various types of containers that exist in society.

CONCLUSION AND SUGGESTION

Based on the results of measurements, observations during the study and discussion in the previous chapter the conclusions and suggestions can be taken as follows:

1. Abate 1G ® with buffer and no buffer effectively kills the mosquito larvae of Ae. aegypti in various containers.
2. 2. LT90 values for group 1G ® abate with the average buffer in a plastic container obtained 2.70 hours, 3.26 hours a clay container, cement and container 2.65 hours. To abate the 1G ® without buffer averaging at 1.99 hours obtained plastic container, clay containers and containers of cement 2.45 hours 1.94 hours.
3. 3. There was no difference in killing power 1G ® abate with buffer and no buffer at various containers until the observation week to V ($P > 0,05$).
4. 4. Usage 1G ® abate with the same buffer and without the same buffer effectively kills the larvae of Ae. aegypti in a variety of containers to the observation week to the V.

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THE EXTRACT OF MORINDA CITRFOLIA FRUIT *(MORINDA CITRIFOLIA)* LOWER BLOOD PRESSURE MALE WHITE RATS WISTAR *(RATTUS NORVEGICUS)* WITH HYPERTENSION

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ABSTRACT

High blood pressure or hypertension is a medical condition where increase of blood pressure are chronic and resulted in high risk factor for stroke, heart attack, and also chronic heart failure. *Morinda Citrifolia* is a traditional medicine which can decrease the high blood pressure. Its have pharmacological effects such as anti-hypertensive, diuretic, increase blood circulation. The study is a true experiment using *Pre-test* and *Post test* control group design which consist of 4 (four) groups. Sample size is 32 male white rats aged 3 months with body weight 180-200 grams. Research was carried out for 8 weeks, the negative control group (PO) was treated by aquadest 0.5 ml, positive control group (PI) was treated by oral captopril 0,25 mg/200 gr BW male white rats, the group II (P2) treated by 30 mg/200 gr BW male white rats, the Morinda extract group II (P3) treated by Morinda extract 60 mg/200 gr BW male white rats. One way anova showed that there were significant differences ($P<0.05$) of systolic and diastolic blood pressure between the control group (PO) and treatment group (PI), (P2), and (P3). It is concluded that the extract of *Morinda citrifolia* dose of 30 mg/gr BW male white rats and 60 mg/gr BW male white rats can lower blood pressure of male white rats with hypertension. Extract of *Morinda citrifolia* with the dose of 60 mg/gr BW male white rats are more effective than dose of 30 mg/200gr BW male white rats. It is recommended to do further research using higher doses to obtain a more optimal doses in lowering blood pressure.

Keywords: Extract *Morinda citrifolia* fruit, blood pressure, white albino male rats, wistar strain hypertensive

INTRODUCTION

Hypertension is a medical condition of chronically elevated blood pressure. Blood pressure more than 140/90 mmHg, including hypertension. High blood pressure which is always a risk factor for stroke, heart attack and chronic heart failure. High blood pressure can be reduced by taking the medicine regularly throughout the life of the patient *Morinda citrifolia* fruit is one of the traditional medicines that has long been used to reduce high blood pressure. This research aims to determine the benefits of *Morinda citrifolia* fruit extract to reduce blood pressure male white rats with hypertension.

MATERIALS AND METHOD

Materials used in this study is the drained *Morinda citrifolia* fruit for 72 hours. Extracted with 95% ethanol and evaporated until ethanol-fres extracts obtained, then the test preparation is divided into 2 doses a dose of 500 mg/kg BW and doses of 1000 mg/kg BW. This study is a true experiment with Pre-test and post-test control group design.

White male rats by 32, i.e., wistar strain, weight 180-200 g, aged 3 months, and then acclimatized for 1 week to adjustment with the environment. Then, the white male rats created hypertensive 170 mmHg-200 mmHg and random. After 2 weeks, blood pressure of male rats which created hypertensive, measured with *blood pressure analyzer.* Then the sample was divided into 4 groups, each group consisted of 8 male white rats.

Negative control group (PO) given 0.5 ml distilled water; positive control group(P1) given oral captopril 3 mg/kg BW; treatment group II (P2) given *Morinda citrifolia* fruit extract dose of 500 mg/kg BW; treatment group III (P3) given doses *Morinda citrifolia* fruit extract 1000 mg/kg BW for 2 weeks. After 2 weeks, blood pressure measurements were taken of male rats in each group, with blood pressure analyzer. Conducted data analysis with Shapiro-Wilk test for normality test and homogeneity test with Levene's test Then carried out test with one way ANNOVA comparability.

RESULTS AND DISCUSSION

Based on normality test with Shapiro Wilk, data are normally distributed, whereas the homogenity test with the Levene test gives a homogeneous data distribution. The comparison test between the four groups with oneway Annova indicates that the differences in blood pressure (systolic and diastolic) was significant ($p<0.05$). Furthermore, it was also found that there are significant differences between the control group (PO), which *mean* is 178.62 ± 8.37 mmHg and with treatment group I (PI), the *mean* is 125.38 ± 8.32 mmHg, the treatment group II (P2) *mean* is 157.38 ± 5.21 mmHg and treatment group III (P3) *mean* is 138.50 ± 10.27 mmHg (P O.05). That means, systolic and diastolic blood pressure of male white rats in all four groups are significantly different.

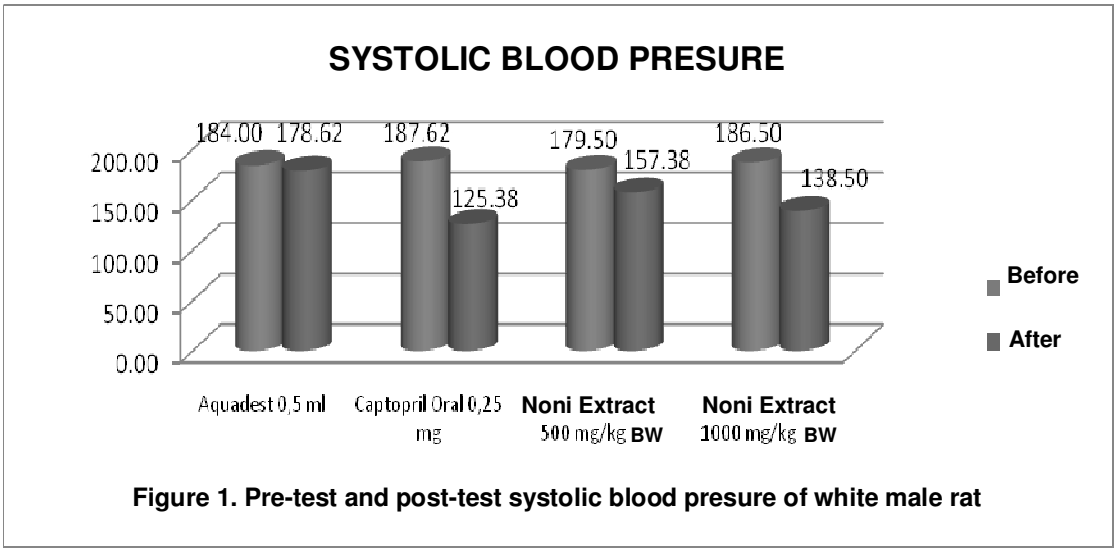

Figure 1. Pre-test and post-test systolic blood presure of white male rat

In this research, it was found that the systolic and diastolic blood pressure of male white rats in the treatment group, which given the extract Morinda fruit, decreased significantly. This is due to Morinda fruit has a pharmacologist effects as anti-hypertensive, diuretic and also increasing blood circulation. Morinda contains scopoletin that serves as vasodilator, works by dilating blood vessels and xeronine as a diuretic, works by releasing storage of sodium from the body, which decreases the blood pressure.

CONCLUSION

It is concluded that giving Morinda extract at 500 mg/kg BW and 1000 mg/kg BW can reduce blood pressure of male white rats with 1000 mg/kg BW is more effective than the 500 mg/kg BW dose.

Further research using larger doses in order to obtain the optimal dose to reduce blood pressure of male rats is recommended.

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THE EFFECT OF MENGKUDU EXTRACT IN HAMPERING THE GROWTH OF *STREPTOCOCCUS MUTANS*AS THE SOURCE OF DENTAL PLAQUE

I G A AyuDharmawati, I WayanPutuSutirtaYasa, I Dewa Made Sukrama

ABSTRACT

Sixty percent of Indonesian suffers from dental disease initiated by dental plaque, caused by bacteria*Streptococcus mutans*. The dental plaque can be prevented usingchlorhexidin mouth-wash which creates discolouration of teeth and tongue. *Mengkudu* is a traditional plant that contains antibacterial substance. The current study is aimed at identifying the effects of *mengkudu*'s extract in hampering the growth of *Streptococcus mutansin vitro*.

This research is carried out using*completely randomized posttest only control group design*. The subject of study is *Streptococcus mutans* ATCC 35668. The material used is *mengkudu*'sextract with 50%, 75%, and 100% concentration. Effect of the ectract can be observed from the appearance of hampering zone diameter. The difference of effects of *mengkudu*'s extract hampering power to *Streptococcus mutans*is tested using *One Way Anova* and *Chi Square.*

The results show, the average of the hampering zone diameter on the control group is 0,00±0,00, 15,57±0,79 on the 50% concentration, 17,36±1,35 on the 75% concentration, and 18,00±0,76 on the 100% concentration, that there is a significant difference between the *mengkudu'*s extract effects and the control group($p<0,05$). The *mengkudu*'s extract with 100% concentration is better than the 50% one ($p<0,05$). It means that *mengkudu*'s extract has powerful hampering power.

In short, *mengkudu*'s extract has the hampering effectsto the growth of *Streptococcus mutans* in vitro. The results can be used as an initial step for a clinical trial on human before it is publically consumed in society.

Keywords: *mengkudu extract, Streptococcus mutans, growth hampering effect*

INTRODUCTION

Oral health has an important role for the health and welfare of the general body. Oral health also affects the quality of one's life for the function of speech, chewing and self-confidence. Disorder that occurs in both the hard tissue and the tissue supporting the teeth will have an impact on one's productivity.

In Indonesia, dental and oral diseases, especially caries and periodontal diseases are still suffered by a lot of children - both children and adults. Household health survey of national health surveys (SKRT-SURKESNAS) (2001), states that dental disease is complained by60% of the population of Indonesia. International Dental Journal (2002) states that dental disease and mouth disease is the fourth most expensive cost recovery in many countries (Suhartono, 2008). The occurrence of caries and tooth abnormalities buffer network begins with the formation of dental plaque. Dental plaque is a soft deposit which is firmly attached to the tooth surface made up of microorganisms that proliferate in an intracellular matrix if one ignores the teeth and oral hygiene (Forest, 1995). Plaque firmly attached to the tooth surface and gingiva have considerable potential against the occurrence of dental hard tissue disease (dental caries) and its supporting tissues (periodontitis) (Meganandaet al., 2009).

The main types of bacteria found in plaque are several strains of *Streptococcus mutans* , especially with the ability to form extracellular polysacharida (Willet, 1991; Jawetz, 2008).

Oral health problems can be prevented. Miller (1989) stated that based on the theory of chemico parasitic, caries can be prevented by antibacterial to reduce and prevent plaque formation. Mouthwash can kill the germs that are in the area between the teeth and gums and can control the formation and slow the process of maturation (ripening) of dental plaque (Astoeti, 2010). Chlorhexidin has long been used as a drug with side effects such as discoloration of teeth and tongue (Prijantojo, 2010), therefore the traditional materials of interest to be selected (Waspodo, 2000).

Mengkudu is a tropical plant that humans used to treat various diseases. Mengkudu contains anti-bacterial and anti fungal, among others: antraquinon, saponins, flavonoids, volatile oil and alkaloids (Suhidayat, 1991). The study by Salomon (1994) reported that more than 78% of patients who have disorders of the throat and mouth can be cured (Goretti, 2000), research byRaiyanti et al. (2004), rinsing withmengkudu juice may decrease the plaque score of 0.47 to 0.32. Research by Dewi (2010) about the antibacterial activity of mengkudu extract note that the extract of mengkudu has a strong inhibitory power

against Gram-positive bacteria.

This study aims to determine whether the extract of Mengkudu has the in vitro inhibiting effect of the of *Streptococcus mutans*growth. And to determine whether differences in the concentration effect on the inhibiting effect of mengkudu extracts to the growth of *Streptococcus mutans*.

MATERIALS AND METHODS

This study is an experimental study with a design completely randomized posttest only control group design. Research sample is taken from one serotype that causes dental plaque, *Streptococcus mutans*ATCC 35 668. *Streptococcus mutans*had grown on plates with Mueller-Hinton culture medium plus 5% sheep blood, were divided into four quadrant ,each plate according to treatment groups: control with ethanol, treatment with the extract concentration of 50%, 75% and 100%, with seven times replication. By using disk diffusion and soaking for 15 minutes at different concentrations of mengkudu extract, then the disk diffusion paired on Mueller Hinton bred on appropriate treatment quadrant. After 18-24 hours of regional measurable inhibition zones appear around the disk diffusion.

RESULTS

Normality test to the inhibition zone diameter of *Streptococcusmutans*datas used the Shapiro-Wilk test. The results indicate normally distributed data (p> 0.05). Homogenity test to the inhibition zone diameter of*Streptococcus mutans*datas usedLevene's test trials, indicating heterogeneous data (p <0.05).

Analysis of treatment effects were tested based on the mean diameter of *Streptococcus mutans*'s inhibition zone between groups after the treatment is given. The results of the analysis of significance by One Way Anova test are presented in Table 5.3 below.

Table 5.3 The result of one way Anova test on treatment effect of mengkudu on *Streptococcus mutans*

Subject Group	n	Mean	SB	*F*	p
Control	7	0.00	0.00	679.60	0.001
Extract Mengkudu 50%	7	15.57	0.79		
Extract Mengkudu 75%	7	17.36	1.35		
Extract Mengkudu 100%	7	18.00	0.76		

Table 5.4 The Differences of Inhibition Zone Diameter of *Streptococcus mutans*With and Without Mengkudu Extracts Between Two Groups by Tamhane Test.

Subject Groups	Mean	p
Control and concentration 50%	15.57	0.001
Control and concentration 75%	17.36	0.001
Control and Concentration 100%	18.00	0.001
Concentration 50% dan 75%	1.79	0.076
Concentration 50% dan100%	2.43	0.001
Concentration 75% dan100%	0.64	0.881

The mean of inhibition zoneby advancedTamhane test was differentbetween the control group with treatment group. Significant differences occurred between the control group with the treatment group with p = 0.001 (p <0.05). While at a concentration of 50% to 75% no significant differences occurred, where p = 0.76 (p> 0.05), as well as between concentrations of 75% and 100% not happening significant differences p = 0.88 (p> 0.05).

Analysis of inhibitory power quality tested by Chi-Square based on 4 x 2 cross table. The strength of inhibitory bacteria were categorized according to Davis and Stout (1971) to be: very strong (> 20mm), strong (10 - 20mm), medium (5 - 10 mm), weak (<5mm). The results of the analysis of significance by Chi-Square test are presented in Table 5.5 below.

Table 5.5 Inhibitory Power Quality

Subject Group	Quality		χ^2	p
	Strong	Not detected		
Control	0	7	28.00	0.001
Mengkudu Extract 50%	7	0		
Mengkudu Extract 75%	7	0		
Mengkudu Extract 100%	7	0		

Table 5.5 above, indicates that mengkudu extract 50%, 75% and 100% had a strong inhibitory power on the growth of *Streptococcus mutans*, whereas controls had no inhibitory power. (χ 2 = 28.00, p = 0.001).

DISCUSSION

The results showed that was different significantly between control group with mengkudu extract given treatment group. Inhibiting effect occurs because mengkudu contains anti-bacterial substances are compounds antraquinon, flavonoids, alkaloids, and alizarin acubin that can kill*Stahpylococcusaureus, Bacillus subtilis, Protensmorganii, Pseudomonas, Escherichia coli*. Bacterial growth inhibiting effect allegedly associated with phenolic compounds that are contain in mengkudu extractVolk and Wheeler (1984), Pelczar and Reid (1988) states that the phenols are able to migrate from the liquid phase to the lipid phase contained in the cell membrane that causes voltage drop in the surface membrane cell (Rahayu, 2000). Next proteins denaturation can be disrupted cell membrane functions as a selective layer, so that the cell becomes lysis (Jawetz et al., 2008). Phenol compounds contained in mengkudu include antraquinon, acubin and alizarin. All three of these compounds contain antibiotic substances (Bangun and Sarwono, 2002).

The content of flavonoids in mengkuduis very effective to inhibit growth of Gram-positive bacteria. Order part peptidoglycan in Gram-positive bacteria would be very sensitive to antiseptic ingredients (Jawetz, 2008; Radji, 2009). Flavonoids are the most antibacterial compounds found in mengkudu (Djauhariya, 2003). Flavonoids are polar making it easier to penetrate the peptidoglycan layer which are also polar in Gram-positive bacteria than the nonpolar lipid layer. In addition to the Gram positive cell walls contain polysaccharides (terikoat acid) is a polymer that dissolves in water, which serves as the transformation of positive ions to exit entry. Soluble nature is showing that Gram-positive cell walls are more polar. Inhibitory activity of mengkudu extract on Gram-positive bacteria cause the disruption of cell wall functions as a conduit of cell shape and protects cells from osmotic lysis. With the disruption of the cell wall that cause the cell lysis (Dewi, 2010).

Ascorbic acid contained in the mengkudu is a source of vitamin C, anti-oxidants, and also in defense mechanisms against microorganisms. (Goretti, 2000; Hariana, 2007; Kusuma et al., 2003). This activity has occurred by its ability to form soluble complexes with proteins, extracellular proteins and a complex form with bacterial cell walls, so it can function as an antibacterial (Kresnawaty, 2009). Increased concentrations of mengkudu extract from 50% to 100% there is a significant difference in inhibitory power, this is showed that the extract of mengkudu 100% had inhibitory power is better than 50%. This is consistent with the statement of Pelczar and Chan (1988) that the higher concentration of an antibacterial material is more powerful antibacterial activity.

CONCLUSION

Based on the results of research about themengkudu extract on *Streptococcus mutans* colonies obtained the following conclusion: Extracts mengkudu has the *in vitro*inhibiting effect of the *Streptococcus mutans* growth, with strong category inhibitory power quality.Mengkudu extract concentration of 100% has the inhibiting effect of the*Streptococcus mutans*growth that is stronger than a concentration of 50%.

RESIDUE OF SMOKE MOSQUITO COIL MADE OF LEAVES of *LEGUNDI* (*Vitex Trifolia* L.) ACTIVE INGREDIENT ON THE LUNGS OF MICE

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ABSTRACT

The research about analysis of residues of the active ingredient of smoke mosquito coil made of leaves of *legundi* (*Vitex trifolia* L.) in the lungs of mice by gas chromatography-mass spectroscopy (GC-MS) has been done. Mosquito coil made from the leaves of l*egundi* (*Vitex trifolia* L.) burned, the smoke is exposed to male mice (*Mus muculus*) for 20 days (acute exposure). Mice that had been exposed to mosquito coil smoke of *legundi* (*Vitex trifolia* L.) anesthetized with cotton that has been moistened with a solution of chlorofom, then mice were dissected abdominal organs taken transversely to her lungs. Lungs of mice macerated with ethanol 96% for 1x24 hours. Then extract the lungs of mice were analyzed by GC-MS. The results of GC-MS of mosquito coil smoke of *legundi* (*Vitex trifolia* L.) identified 15 compounds, including: methenamine; 2-methoxy-4-vinylphenol; 2, 6-dimethoxyphenol; 4-hydroxy-3-methoxy-benzoic acid; 2-methoxy-4-(1-propenyl) phenol-E; 5-tert-butylpyrogallol, 1, 3, 7, 7-tetramethylbicycloheptan-2-one, 2, 3, 5, 6-tetrafluoroanisol; diethyl pthalate, 2, 6 - dimethoxy-4-(2-propenyl) phenol; N-N-dimethyl methanesulfonamide; 4-hydroxy-3-nitrocoumarin; citenamide; 1-methyl-anthraquinone; and hexamethyilcyclotrisiloxane. The result of the analysis of lungs of mice with GC-MS found a compound which is a mosquito coil smoke residue of *legundi* (*Vitex trifolia* L.) are compound hexamethylcyclotrisiloxane.

Keywords: *Vitex trifolia* L., *Mus musculus*, gas chromatography-mass spectroscopy (GC-MS)

EFFECTIVENESS OF *CENTELLA ASIATICA* EXTRACT THE ORIGINAL BALI PLANT TO STIMULATE TUMOR NECROSING FACTOR ALPHA SECRETION ON MICE INFECTED BY *SALMONELLA TYPHI*

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ABSTRACT

Tumor necrosing factor alpha is a cytokine produced by macrophages and other mononuclear cells, is a good antibacterial agent against *Salmonella spp*, especially *Salmonella typhi*. *Centella asiatica* is an alternative drug that is expected as an immunostimulant in patients with typhoid fever. Comparing the effectiveness of *Centella asiatica* extract the original Bali as an immunostimulant and without stimulants in mice infected *Salmonella typhi* in terms of tumor necrosing factor alpha secretions. This study is an experimental study with a post test only with control group design. A total of 20 mice were divided into 4 groups. The first and second groups each given *Centella asiatica* extract 75 mg/20g bw (0.5 cc) and without a given extract for 4 weeks. Both groups were inoculated orally *Salmonella typhi* 10^6 per ml of bacteria in the second week. The third and fourth groups were given thiamphenicol with *Centella asiatica* extract 75mg/20g bw (0.5 cc) and thiamphenicol without any extract for 4 weeks respectively. Both groups were inoculated orally *Salmonella typhi* 10^6 per ml of bacteria in the first week. All groups terminated on fourth week and examination levels of tumor necrosing factor alpha and gall culture. The mean levels of tumor necrosing factor alpha in groups (1-4) is 68.10 ± 26.79 pg/ml, 22.01 ± 15.76 pg/ml, 39.87 ± 7.19 pg/ml and 19.21 ± 2.19 pg/ml respectively. Based on examination of the gall cultures showed positive results in the first and second groups, while a negative result on the third and fourth groups. Based on the Oneway ANOVA analysis on levels of tumor necrosing factor alpha, there are significant differences between the first group with the second group ($p<0.05$), while between the third and fourth groups found no significant differences ($p> 0.05$). There was an increased levels of tumor necrosing factor alpha in mice given *Centella asiatica* extract with *Salmonella typhi* infection.

Keywords: *Centella asiatica* extract, *Salmonella typhi*, tumor necrosing factor alpha.

COMPARISON OF RESIDUE ACTIVE INGREDIENT SMOKE MOSQUITO COIL FROM LEAVES OF *VITEX TRIFOLIA* AND MOSQUITO COIL BRANDED IN LUNGS OF MICE

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ABSTRACT

Residual ingredient of smoke active compound of mosquito coil made from leaves of *Vitex trifolia* and mosquito coils branded in lungs of mice were analyzed using gas chromatography-mass spectroscopy. This is an experimental study starting with identification active compounds on both smoke of *Vitex trifolia* mosquito coil and branded mosquito coil. Then, study on exposure of these two mosquito coils on Gas chromatograph used was GC Agilent Technologies 6890-N with capillary column HP-5 MS (30 m x 0.25 mm) stationary phase phenylmethylsiloxane, MS detector, carrier gas helium, injector temperature 250, flow rate 1 mL per minute, column temperature programmed (gradient) is the initial temperature of 70 º C for 5 minutes and then increased by 10 º C per minute to 270 ^{0}C.

Identification of smoke mosquito coil made from leaves of *Vitex trifolia* with gas chromatography-mass spectroscopy produces 15 compounds and smoke mosquito coil branded produce 27 compounds. Maceration lungs of mice with 96% ethanol solvent produced colorless clear filtrate, analyzed by gas chromatography-mass spectroscopy. After compared with the results of the analysis of smoke mosquito coil made from leaves of *Vitex trifolia* and smoke mosquito coils branded discovered the same compound that is Hexamethylcyclotrisiloxane.

Keywords: Smoke, Mus musculus, Gas chromatography-mass spectroscopy, Lungs

POLYMORPHISMS OF EXON-1 insl3 GENE IS MARKER FOR THE OCCURRENCE OF CRYPTORCHIDISM IN BOYS

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ABSTRACT

Cryptorchidism is an abnormality of the genitourinary tract manifested as incomplete descend of the testes into the scrotum, the testis may be located along the normal descension tract, but did not move to its intended normal place in the scrotum. In premature infants, the incidence of cryptorchidism is found to be 30%, this incidence decreases to 3-5% in infants born at term, and at the age of 3 months the incidence becomes 1-3% and at 1 year the incidence is only 0,8%. The complications of cryptorchidism include infertility, malignancy. In experimental mice, deletion of insl3 gene causes cryptorchidism. In human, insl3 gene polymorphism is often found, but its relationship with cryptorchidism remains unclear. The aims this study to explain polymorphisms of exon-1 insl3 gene is marker for the occurrence of cryptorchidism in boys.

This study is observational case control study, at the Pediatric Policlinic of RSUD Wangaya, RSUP Sanglah, and RB Permata Hati from September until December 2010. Cases were boys with cryptorchidism who came to the policlinic and for every case a healthy boy came to the policlinic was appointed as the control and matched by age in years and history of gestational age. Calculation number of cases' was 31 boys and number of controls was 31 boys. Polymorphisms of exon-1 insl3 gene check by sequencing of PCR product at YAYASAN GENNEKA Eijkman Molecular Biology Institute Jakarta with forward primer (1F): tgggagaaaggctctggcac. The analysis of polymorphisms with paired odds ratio and hypothesis with (α) and $p < 0.05$.

The results of this study polymorphisms frequency of exon-1 insl3 gene were M1M (case= 3,23% , control = 0%), A9A (case = 19,36%, control = 6,45%), L42L (case = 3,23%, control = 0%), L42P (case = 3,23%, control = 0%), V43M (case = 3,23%, control = 0%), T60T (case = 51,61%, control = 16,13%), T60A (case = 25,81%, 48, 39%). Polymorphisms T60A (ACC[]GCC) more in control, polymorphisms A9A (GCG[]GCN/GCA) more in case and polymorphisms T60T (ACC[]NCC) more significant in case.

Conclusion of this study polymorphisms T60T exon-1 insl3 gene is marker for the occurrence of cryptorchidism in boys. Hope of this study can use choice of therapy, prognosis and base of next study.
Keywords: Polymorphisms exon-1 insl3 gene, cryptorchidism, boys

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100	Manurung, M.	PA9	240	Utama, S.	PH18
101	Marumoto, Takuya	KP2, KP8	241	Utami, I.A.P.	OA8
102	Matsuura, Hideyuki	PA6	242	van den Hondel, C.A.M.J.J.	OH20
103	Maududi, Allay	OH15	243	Wahjuni, Sri	OH2
104	Mayun, I Gusti Ngurah	OH8	244	Wahyuniari, I.A.I	OH7
105	Meyer, Christopher	OBE2	245	Wande, I Nyoman	OH8, PH24
106	Miwada, I N.S.	OA9	246	Warmadewi, D.A.	OA8
107	Mudita, I M.	PA7	247	Wartini, Ni Made	PAF4
108	Mulyani, Sri	PAF8	248	Wiadnyani, Anak Agung Istri Sri	PAF7
109	Muzakhar, Kahar	OAF1	249	Wibawa, A.A.P. P.	PA7
110	Narayani, Inna	PH6	250	Wibowo, Indra	OH19
111	Nelson, Matthew	OA2	251	Widarta, I Wayan Rai	PAF18
112	Nguyen Thi Minh	KP8	252	Widhiartini, Ida Ayu Alit	OH8, PH24
113	Nindhia, Tjokorda Sari	OBE2	253	Widjajaseputra, Anna Ingani	OAF2
114	Nisa, Fatma Zuhrotun,	OH1, PH8	254	Widyastuti, N.P.	PAF21
115	Nitta, Takenori	OH15	255	Wijana, I W.	PA1
116	Nocianitri, Komang Ayu	OH11, OAF4, PAF11	256	Wijaya, Hendy	OH16
117	Nugraha, Ricardo Adrian	PH1	257	Winaya, Ida Bagus Oka	PH10
118	Nurdeviyanti, N	OH12	258	Windiani, IGA Trisna	PH17
119	Nursini, W.	OAF4	259	Wipradnyadewi, Putu Ari Sandhi	PAF15
120	Oetari, Ariyanti	OAF3	260	Wirajaya, A.	PAF3
121	Ohga, Shoji	KP9, PBE2	261	Wirapartha, I M.	PA1
122	Oka, A.A.	PA10	262	Wiratmini, Ngurah Intan	PH2
123	Oka, I G.L.	OA6	263	Wirawan, I G.P	OH17
124	Oka, Tjokorda Gede	PH24	264	Wirawan, I W.	PA7
125	Okabe, Hiroaki	KP12	265	Wirya, Gusti Ngurah Alit Susanta	OA4
126	Pamudji, Jessie Sofia	PH4	266	Wisaniyasa, Ni Wayan	PAF11
127	Pardede, E.	PAF6	267	Wiyana, I K. A.	PA1
128	Partama, I.B.G.	OA6, PA13	268	Yadnya, Tjokorda Gede Belawa	PAF20
129	Parwanayoni, Ni Made Susun	PAF17	269	Yamaguchi, J.	KP3
130	Parwati, Tuti	PH18	270	Yamamoto, Kazuo	KP7
131	Pharmawati, Made	PA3	271	Yamashiro, Yasuhiro	OH15

132	Pinto-Teixeira, Filipe	OH19	272	Yasa, I W. P. Sutirta	OH18, PH12, PH14, PH21, PH24
133	Prasetyo, Afiono Agung	OH3, OH4, OH5, OH6	273	Yogisutanti, Gurdani	OH9
134	Pratiwi, S.U.T.	OH20	274	Yokoyama, Kazuhira	KP8
135	Priosoeryanto, Bambang Pontjo	PH11	275	Yukihiro, Yada	KP6
136	Probosuseno	OH1	276	Yulihastuti, Dwi Ariani	PH3
137	Pudja, Ida Ayu Rina Pratiwi	PAF23	277	Yunianta	OAF2
138	Purwantoro, Azis	OA3	278	Yusa, Ni Made	PAF11, PAF12
139	Puspani, E.	OA6	279	Yusasrini, Ni Luh Ari	PAF15, PAF18
140	Puspawati, GAK. Diah	PAF1, PAF5			