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# MICROBIOLOGICAL AND PHYSICOCHEMICAL CHANGES OF GREEN COFFEE (Coffea arabica) FERMENTATION IN KINTAMANI, BANGLI, BALI

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*ABSTRACT*

*The excellent quality of the fermentation process is closely related to microbes involved in the process fermentation. This study aimed to compare the microbiological changes and physicochemical quality of green coffee during 16 hours fermentation. Lactic acid bacteria and yeast were the predominant microorganisms found throughout the fermentation process. The total lactic acid bacteria and yeast reached the highest value of 9.31 log10 CFU/g and 9.10 log10 CFU/g respectively on the coffee bean and 9.30 log10 CFU/g and 9.10 log10 CFU/g respectively on liquid of fermented coffee bean. The quantity of microflora on the coffee bean and liquid of fermented coffee bean increased and reached its maximum value after twelve hours and decreased after exceed its peak, while fungi was no detected during fermentation. Sixteen hours fermentation significantly (P<0.05) influenced the bulk density, moisture content and bean weight/100 beans, bean amount/10 g and cause defect to green coffee such us "broken beans", "brown beans" and "partly black beans" yet were not significantly affected (P>0.05) bean size and color of the green coffee. Throughout coffee fermentation, the acids on the green coffee increased from 5.33 ± 0.144 to 9.99 ± 0.144% and the caffeine on the green coffee decreased from 3.70 ± 0.017 to 1.66 ± 0.003%. Phenol content on the green coffee and its brews also decreased successively from 1381.58 ± 8.40 to 980.16 ± 10.07 mg GAEs/100 g and 922.25 ± 17.961 to 812.06 ± 12.660 mg GAEs/100 g. While for the antioxidants capacity, green coffee was more stable and its brews increased successively from 11564.28 ± 255.345 to 112 34.428 ± 174.377 mg GAEAC/L and 32326.24 ± 22.744 to 38658.62 ± 180.107 mg GAEAC/L. It can be concluded that coffee fermentation had more a positive effect on microbiological changes and physicochemical quality of green coffee.*

***Keywords :*** *: coffea arabica, green coffee, fermentation, microflora*

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## INTRODUCTION

Fermentation is one of critical wet coffee processing stage for green coffee which affects the end quality (Correa et al., 2014). Coffee fermentation is useful to degrade the pectin-containing mucilage adhering firmly to coffee beans by pectinase. The mucilage which can be eliminated by fermentation is made of saccharide, enzymes, pectin lipids and protopectin (Masoud and Jespersen, 2006). The composition of mucilage layer has high nutritional value consisting of 84.2% of water; 4.1% sugar; 8.9% protein; 0.91% concentrated acid and 0.7% ash (Clifford and Ramirez-Martinez, 1991); or in 20.25% dry matter consist of 11.5% of total sugars, 15.5% protein; 2.8% fat; 5.5% total pectin; 28.5% acid detergent fiber; 12.0% lignin; 16.2% cellulose and 8.0% ash (Silva et al., 2013), which has potential as a medium for microbial growth.

Bacteria, yeast and filamentous fungi have been detected during coffee fermentation (Silva et al., 2013; Nasanit and Satayamut, 2015; Feng et al., 2016). The research of coffee Arabica fermentation in Thailand and China using wet method has found that Enterobacteriaceae and Klebsiella pneumonia are predominant bacteria, Weissellasp, Leuconostocsp., Lactobacillus sp. and Lactococcussp. are the predominant lactic acid bacteria, Bacillus sp. is the predominant Gram-positive bacteria, whereas Candida, Pichia, Debaryomyces, Kluyveromyces and Saccharomyces are most common yeast. Filamentous fungi are less found during the fermentation and Penicillium is the predominant fungi and most of them are pectinase-producing microorganisms (Leong et al., 2014; Nasanit and Satayamut, 2015; Feng et al., 2016).

During fermentation, a large number of microorganisms can produce pectinolytic enzymes, proteolytic enzymes and metabolites such as organic acids which increase acidity, the pH may be reduced from 6.3 to 4.0, and pectin-containing mucilage is hydrolyzed, and also the caffeine may be reduced by microbes for 12-36 hours up to 48 hours. Moreover, some microorganisms have the ability to produce a special flavor and aroma. The green coffee beans are then extensively washed, polished and sun-dried and air-dried (Farah, 2012; Farida et al., 2013; Silva et al., 2013; Yusianto and Widyotomo, 2013; Correa et al., 2014; Nasanit and Satayamut, 2015; Feng et al., 2016).

Some treatment significantly influenced physical quality of Arabica green coffee are fermentation container, fermentation agents, and temperature of fermentation yet duration of fermentation does not significantly influenced bulk density, moisture content, bean amount/10 g, bean size, color and cause defect to green coffee (Yusianto and Widyotomo, 2013). On the other hand, green coffee bean is also well known as a good source of antioxidant. The presence of antioxidant will slow or prevent the oxidation of other chemical. Oxidation can be simplified as gain of oxygen and loss of electron. Antioxidants may be either present in two forms which are natural antioxidants and synthetic antioxidants. Natural antioxidants include flavonoids, phenolic, tocopherol and also ascorbic acid. About the caffeine content, Arabica coffee generally has less amount of the caffeine, which is from 0.6-1.9% while Robusta has 2.2% of caffeine. The fermentation affects on the chemical composition of green coffee particularly in water-soluble components \such us acids, caffeine and the other components like phenolic (Belay, 2011; Siva et al., 2016).

Until now, there has been no report on the microbial communities and physicochemical parameters present during green coffee fermentation process in Kintamani, Bangli-Bali. Therefore, the current study aimed to describe, quantitatively and qualitatively, the

microbiological changes and physicochemical quality of Arabica green coffee fermentation in Kintamani, Bangli, Bali.

## MATERIALS AND METHODS

### Sampling

Coffee cherries (Coffea arabica) were handpicked at the mature stage and collected from many different sites of each coffee plantation on Central Batur Village, Kintamani District, Bangli Regency, Bali Province, Indonesia in July, 2017. The external mesocarp was mechanically eliminated immediately after harvesting by wet pulping. Coffee beans were then conveyed at a water stream to fermentation box and left to ferment for 16 hours. The fermentation began at 6:00 pm, outside temperature was decreased, and ended at 10:00 am. Every 4 hours, samples of coffee beans and liquid of fermented coffee beans were collected by collecting the samples from five different points at the middle depth of the fermentation box and mixing.

### Measurement of pH and Temperature

Measurement of the pH outside coffee beans was done by taking 5 g coffee beans and gently swirled in 5 mL distilled water (1:1) and the pH of the supernatant was measured. Measurement of the pH inner coffee beans was done by crushing 5 g coffee beans using blender and mixed in 5 mL distilled water (1:1) and the pH of the supernatant was measured, while the pH of liquid of fermented coffee beans could be measured directly (Avallone et al., 2001; Nasanit et al., 2015). The temperature at each collection point was measured using a portable electronic thermometer.

### Microbiological Analysis

Fermented coffee beans (10 g) and liquid of fermented coffee beans (10 mL) were homogenized in 90 mL of sterile distilled water (0.85% NaCl) (10-1) by using vortex. The suspension was taken 1 mL and diluted in 9 mL of sterile distilled water (10-2) and so on until dilution 10-7. Lactic acid bacteria (LAB) were counted on de Man, Rogosa and Shape Agar (MRSA), yeast and fungi on Potato Dextrose Agar (PDA), aerobic microorganisms were counted on Plate Count Agar (PCA), bacteria on Nutrient Agar (NA). Plates were incubated in incubator at 37°C for 48 hours for bacteria and lactic acid bacteria, at 30°C for 4-6 hours for aerobic microorganisms, yeast and fungi. Colony-forming units (CFU) were counted and data were expressed as the mean of the decimal logarithm of the CFU/mL or CFU/g of fermented coffee beans or liquid of fermented coffee beans. Several isolates were purified and stored at -80°C in 40% glycerol (v/v) for further research (Avallone et al., 2001; Nasanit et al., 2015).

### Physical Analysis

Wet-coffee parchments were washed and artificial-dried (50-60°C, ± 40 hours until moisture content < 12% (wb). The dried parchments were hulled and examined for the bean quality. Physical analysis included bulk density, moisture content, bean weight/100 beans, bean number/10 g, bean size, color and defect “broken beans”, “brown beans” and “partly black beans” (SNI 01-2907-2008/ Indonesian National Standards on the quality of green coffee beans, BSN, 2008).

### Measurement of Total Acid Content

Green coffee beans were done by destroying 10 g of green coffee then mixed and stirred evenly with 90 mL of distilled water then filtered. If the sample color was still dark then the sample was diluted again. Samples were taken 25 mL to be titrated. Sample mixed with 3 drops of phenolphthalein (PP) 1% before titrated, after which the samples was titrated with 0.01 N

NaOH until the pink color was constant and then measured the total acid content (Ranggana, 1987).

$$\text{Lactic acid} = \frac{\text{mL titration x N NaOH x Dilution factor x BM lactic}}{\text{mL sample x 1000 mg}} \text{ x } 100\%$$

**Caffeine Content**

Approximately 1 g of ground green coffee mixed with 50 mL hot distilled water, and filtered by using the Buchner's funnel. The filtrate was added 1 g of calcium carbonate (CaCO3) and was extracted until 3 times by using 25 mL of chloroform (CHCl3). The extract was taken by using separating's funnel and was evaporated by using rotary evaporator. The caffeine extract was added 5 mL distilled water and was diluted 10 times. The absorbance was measured by using spectrophotometer at 275-285, while caffeine standard solution used optimum at 278 nm. A calibration curve was made from caffeine standard solution (100, 200, 300, 400 and 500 mg/L) and the blank was prepared with distilled water. The total caffeine content was expressed as percent w/w (Fitri, 2008).

**Total Phenolic Content**

Total phenolic content in spent ground coffee and its brews were determined by using the Folin-Ciocalteu reagent according to the colorimetric method. Approximately 10 mg of ground green coffee or its brews mixed with 10 mL of methanol 85% (v/v), and filtered. The standard and filtrate were taken 0.4 mL and were added 0.4 mL of Folin-Ciocalteu reagent and were waiting for 30 minutes at room temperature. The absorbance was measured by using spectrophotometer at 760 nm. A calibration curve was made from gallic acid standard solution (2, 4, 8, 12 and 20 mg/L) and the blank was prepared with distilled water. The total phenolic content was expressed as milligram gallic acid equivalent (GAEs) per 100 g sample (Chien and Ho, 1995).

**Antioxidant Capacity**

Antioxidant capacity in spent ground coffee and its brews were determined by using the DPPH (1.1-diphenyl-2-picrylhydrazyl) method (Subagio and Morita, 2001). Approximately 0.1 g of ground green coffee mixed with 5 mL of methanol 100%, and for its brew, approximately 0.1 g of ground green coffee mixed with 50 mL of the distilled water, and centrifuged at 3000 rpm for 15 minutes and then filtered. The filtrate were taken 0.1 mL (50 µL sample + 50 µL methanol 100%) and were added 700 µL DPPH, vortex and were waiting for 30 minutes at room temperature. The absorbance was measured by using spectrophotometer at 517 nm. A calibration curve was made from gallic acid standard solution (5, 10, 15, 20, 25 and 30 mg/L), the control was prepared with 100 methanol 100%, and was added 700 µL DPPH and the blank was prepared with methanol 100%. The antioxidant content was expressed as milligram GAEAC per liter sample.

**Data Analysis**

All data obtained were analyzed by using SPSS 17.0 for the analysis of variance (ANOVA), one-way, where Duncan's test was used to determine the significance difference ($P<0.05$) between different treatments. Results were expressed as means ± standard deviation (SD) of duplicate analyses, unless otherwise stated.

## RESULTS AND DISCUSSION

### pH and Temperature during Coffee Fermentation

Measurement of pH and temperature during fermentation was undertaken every 4 hours during 16 hours fermentation. The pH values and temperatures during coffee fermentation were determined as shown in Figure 1. As can be seen, the pH outside/mucilage coffee beans and pH inner coffee beans decreased gradually during 16 hours fermentation from 4.5 to 3.9 and from 5.8 to 5.4, respectively. However, the pH of liquid of fermented coffee beans increased from 3.8 to 4.7. In previous studies, the coffee fermentation microflora significantly could produce metabolites such as ethanol and organic acids. Some of ethanol is oxidized to acetic acids and then organic acids, where oxalic was the highest, followed by acetic acids and lactic acids made the pH outside/mucilage of coffee beans decreased or acidity increased. Ethanol and some of the organic acids outside coffee beans will penetrate into the coffee beans and pH inner coffee beans decreased (Silva et al., 2013; Yusianto and Widyotomo, 2013; Correa et al., 2014; Nasanit and Satayamut, 2015; Feng et al., 2016).

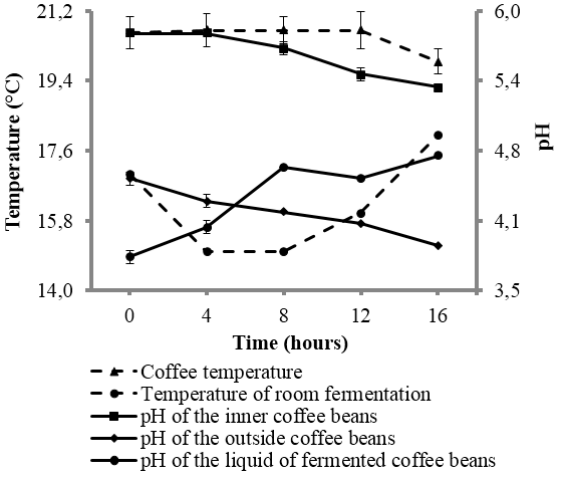


Figure 1. Change of pH and temperature during 16 hours wet fermentation of green coffee beans

These result were in opposition to the pH of the liquid of fermented coffee beans that was increased, it is probably because at the 0 hours of the fermentation, there was only a little sugar at liquid of fermented coffee beans that are metabolized by microflora (Nurhayati, 2012) and citric acids in liquid of fermented coffee that had been overhauled became ethanol and CO2 by yeast (Hamadi et al., 2014; Sisbudi and Fauzi, 2015) or 8.9% of protein in mucilage layer that had been overhauled became free amino acids that is alkaline (Arnold and Ludwig, 1996; Kinyua et al., 2017).

The temperature on the coffee fermentation was ± 20.7°C and air bubbles existed even though the temperature did not increased, because the outside temperature was rather cold (15°C), and the temperature decreased on the last fermentation period. The temperature also affect the quality of fermentation (Masoud et al., 2006), because it affects life and death of microorganisms (Sisbudi and Fauzi, 2015). In the current study, the temperature of fermented coffee beans was ± 20.7°C and air bubbles were also exist even though the temperature did not increased, which have already reported by Yusianto and Widyotomo (2013); Leong et al. (2014); Yusianto and Nugroho (2014); Nasanit and Satayamut (2015); Feng et al. (2016), that the fermentation process was characterized by an increase in the temperature of the coffee beans in the sack (dry fermentation) and the optimum temperature of fermented coffee beans was 15-30°C or air bubbles even though the temperature did not increased (wet/semi-wet fermentation).

### Microbial Trend during Coffee Fermentation

The quantity of bacteria, aerobic bacteria lactic acid bacteria, yeasts and fungi

determined on the coffee beans and liquid of fermented coffee beans are reported at a logarithmic scale as shown in Figure 2. In previous studies, bacteria, aerobic microorganisms, lactic acid bacteria, yeast and fungi were detected during coffee fermentation (Silva et al., 2013; Leong et al., 2014; Nasanit and Satayamut, 2015; Feng et al., 2016). The results showed that lactic acid bacteria and yeast were the predominant microorganisms found from the 0 hours of fermentation, whereas aerobic microflora and bacteria also growth because water was extensively used in the process. At the end of the fermentation, yeasts, bacteria and lactic acid bacteria were predominant microorganisms found when the pH was low because of their higher resistance to acid conditions. Yeasts and lactic acid bacteria could be responsible for flavor and aroma of the coffee brew (Farah, 2012; Leong et al., 2014; Nasanit and Satayamut, 2015).

Bacteria, lactic acid bacteria and yeast were predominant microorganisms, some of which could produce pectinolytic enzymes, proteolytic enzymes and organic acids (Silva et al., 2013). Enterobacteriaceae was predominant bacteria species during coffee fermentation, Leu. mesenteroides, Lb. brevis, Lb. plantarum and Enterococcus casseliflavus were the predominant lactic acid bacteria. Furthermore, some lactic acid bacteria for example Ln. mesenteroides would play a role in the solubilization of pectin substances (Nasanit and Satayamut, 2015; Feng et al., 2016). Several strain of lactic acid bacteria as lactic acids producer as described for fermented goat milk (Martharini and Indratiningsih, 2017; Wang et al., 2017), resulting in a decreased pH during coffee fermentation, which leads to optimum conditions for yeast to grow until the end of the fermentation up to 16 hours. Most of these lactic acid bacteria have the ability as biopreservative agents and can produce antimicrobial or antifungal compounds especially organic acids, H2O2 and bacteriocin (Yang et al., 2012). Nocianitri et al. (2017) has reported that these lactic acid bacteria also had the ability as probiotics which could give functional effect for human healthy.

Yeasts were predominant microorganisms during coffee fermentation period. The major yeast genera had already reported in previous studies of coffee fermentation by the wet process such us Candida, Pichia, Debaryomyces, Kluyveromyces and Saccharomyces such as P. kluyvery, P. fermentans, Hanseniasporauvarum, P. kudriavzevii, Issatchenkiaorientalis, Clavisporalusitaniaeand P. guilliermondiiwere most common yeast (Hamadi et al., 2014; Nasanit and Satayamut, 2015; Feng et al., 2016). Most of these yeasts have the ability to produced pectinolytic enzymes and organic compounds. S. cereviseae UFLACN727, P. guilliermondii UFLACN731 and C. parapsilosis UFLACN448 had the ability to produced pectin lyase (PL) and 1.2-propanediol, hexanoic acid, decanoic acid, nonanoic acid and ethyl acetate isolates were promising candidates as coffee starter cultures (Silva et al., 2013). S. cereviseae CAT1, P. anomala, P. kudriavzevii, Issatchenkiaorientalis, Clavisporalusitaniaeand P. guilliermondii potential for bioethanol production from wet coffee processing waste (Menezes et al., 2013; Hamadi et al., 2014; Sisbudi and Fauzi, 2015; Woldesenbet et al., 2016). Some yeast species such as S. cereviseae mixed with Lb. rhamnosus NRRL B-442 resulted in the inhibition of the growth of fungi (A. flavus producer of aflatoxin) (Sheikh-Ali et al., 2015). Inoculation of S. cerevisiae UFLA YCN727, S. cerevisiae UFLA YCN724, Candida parapsilosis UFLA YCN448 and P. guillermondii UFLA YCN731 in a semi-dry coffee had been recently reported to be able to produce a special aroma of caramel flavor in coffee that was not detected in control

(Yusianto and Widyotomo, 2013; Evalingelista et al., 2014).

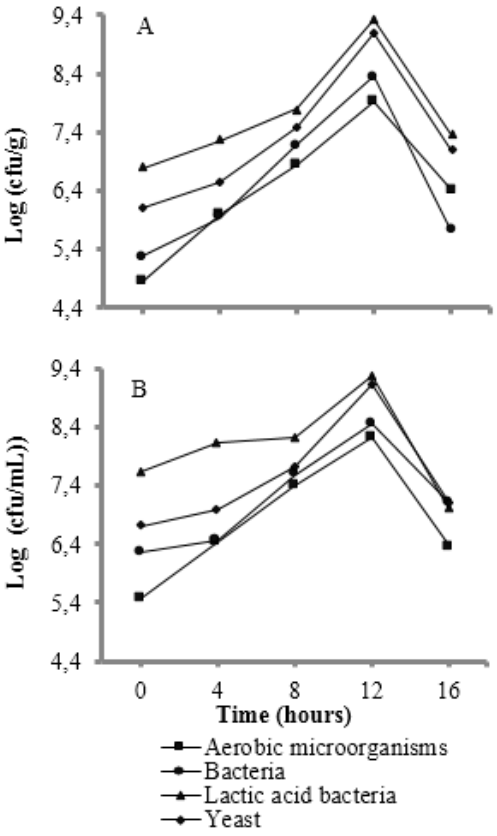


Figure 2. Coffee fermentation microflora at the sampling of the coffee beans (A) and the liquid of fermented coffee beans (B)

The quantity of bacteria, aerobic bacteria, lactic acid bacteria and yeast on the coffee beans and the liquid of fermented coffee beans increased gradually from the 0 hours of fermentation and reached its maximum level up to 12 hours, and decreased after exceed the peak. Much sugar on mucilage layer may be consumed by microflora until 12 hours fermentation (Nasanit and Satayamut, 2015). The microflora consume sugar on mucilage layer and firstly consume substrate that was easily metabolized, such as a monosaccharide before hydrolyzing a polysaccharide (Clifford and Ramirez, 1991; Silva et al., 2013), and citric acids at liquid of fermented coffee beans that have been overhauled by yeast to be ethanol and CO2 (Hamadi et al., 2014; Sisbudi and Fauzi, 2015) until 12 hours fermentation and sugars run out after exceeds it pick. Some of the metabolites such as organic acids, bacteriocin, hydrogen peroxide from lactic acid bacteria caused some microbes died and be inhibited for their growth (Yang et al., 2012; Leong et al., 2014). It was probably the reason that the quantity of microflora at the sampling increased gradually from the 0 hour fermentation and reached its maximum level up to 12 hours and decreasing after exceed it peak.

Fungi were no detected microorganisms on the wet fermentation process on Kintamani, Bangli, Bali. However, it has been reported that the wet fermentation process was diverse in Brazil and Thailand, especially Penicillium and Aspergillus which were the most common genus in the current study and their pectinolytic activity has been reported (Silva et al., 2008; Nasanit et al., 2015). It was probably due to water activity and the growth of other microbial groups e.g. bacteria, lactic acid bacteria and yeasts that were very high. These microflora significantly produced metabolites which increased acidity and pH decreased which inhibited the growth of fungi (Silva et al., 2008; Silva et al., 2013; Nasanit and Satayamut, 2015; Feng et al., 2016). These differences found in the number and diversity of fungi in the coffee beans could be related with the long period of fermentation and drying (15-25 days) (Silva et al., 2008) and period for coffee beans fermentation in Kintamani, Bangli was only 16 hours. However, some fungi species including Penicillium and Aspergillus detected were be able to produce mycotoxins such as ochratoxin A, aflatoxin B1, citrinin, which could affect consumer health because mutagenic, carcinogenic, nephrotoxic and pathogenic (Hsuuw et al., 2013; Klaric et al.,

2013; Ostry et al., 2013; Kumar et al., 2014; Sheikh-Ali et al., 2014; Aaraj et al., 2015; Carvajal-Moreno, 2015; Gan et al., 2015; Madrigal-Bujaidar et al., 2015; Park et al., 2015; Bovdisova et al., 2016).Therefore, this matter needs to be considered in coffee production. Fortunately, these were not found in this study.

**Physical Analysis of Green Coffee**

Sixteen hours fermentation significantly influenced the bulk density, moisture content, bean weight/100 beans, bean number/10 g and cause defect on green coffee beans such us “broken beans”, “brown beans” and “partly black beans”, as shown in Table 1 and Table 2. As can be seen, the bulk density, moisture content and weight/100 beans decreased gradually during the 16 hours fermentation period, successively from 0.656 ± 0.01 to 0.645 ± 0.01 g/mL, from 8.344 ± 0.01to 7.661 ± 0.03% and from 19.175 ± 0.25 to 18.139 ± 0.13 g. However, the bean number/10 g increased from 52.330 ± 1.00 to 57.330 ± 1.16. The physical characteristics and the flavor of the green coffee was highly dependent on genetic, environmental, fruit maturity, and technological factors processing, and are very important inside determined the price of coffee in the market. The flavor characteristics depends on the combination of several factors, among them chemical composition and physical characteristics of green coffee (Yusianto and Widyotomo, 2013; Tarigan and Towaha, 2017).

Sixteen hours for green coffee fermentation was too high, thus significantly more causing defective bean compared to twelve hours fermentation, particularly the brown beans and partly black beans. In this study, the broken beans, the brown beans and partly black beans increased gradually during the fermentation successively from 2.109 ± 0.00 to 2.190 ± 0.00, from 2.040 ± 0.06 to 3.594 ± 0.16 and from 0.200 ± 0.01 to 1.087 ± 0.08. Specialty Coffee Association of America (SCAA) (2009) state that the brown beans and partly black beans was referred to as "full sour beans", was a physical disability first category which had directly effect to the taste of coffee. The physical disabilities in first category are black beans, brown beans, which were attacked by moulds, heavy insects and dried beans. Examples of coffee containing bean defects in the first category cannot include quality requirement for specialty coffee. Besides that, according to Ilao et al. (2017), the taste and aroma are the important product attributes in latte drinks.

Fermentation was not significantly affected bean size and color of the green coffee as shown in Table 1 and Table 2. According to SNI 01-2907-2008, it was known that Arabica Kintamani Coffee included small beans. Beans size can be seen from the length, width and thickness and weight of 100 green coffee beans (Randriani et al., 2014). In this study, it has been reported that the length, width and the thickness of coffee beans in sequence were 9.436-9.742 mm; 6.436-7.155 mm and 4.213-4.305 mm, respectively. Furthermore, it had also been reported the, and thickness of several cultivar of Arabica coffee beans in Cikandang, Garut, West Java in sequence of 10.83-13.03 mm; 7.32-8.78 mm and 3.87-5.21 mm, respectively (Randriani et al., 2014). Beans size much more affected by varieties and land altitude than its processing (Yusianto and Widyotomo, 2013; Qadry et al., 2017). Beans size related to consumer demand and visuals on downstream industries, because beans size significantly affected to roasting shrinkage (roasting rendement), bulk density of roasted coffee, brewed pH, total acidity and body of brew, yet no significantly affected to the essence content, optical density, aroma, taste, and pleasure level (Sulistyowati et al., 1996).

The color of green coffee was a very important physical criterion for determining the quality of green coffee. Color

measurements based on L*, a* and b* can be used as an indicator to estimate the chemical composition of coffee beans and flavor (Cavaco-Bicho et al., 2008; Saath et al., 2012). This study is in accordance with reported by Yusianto and Widyotomo (2013) that fermentation period was not significantly affected color of the green coffee, but yet storage duration significantly influenced the color, particularly for the storage at room temperature and humidity (Yusianto et al., 2007). SCAA (2009) states that the color of coffee beans that qualify for specialties were blue-green, green-bluish or bluish-green and green.

Table 1. Influence of fermentation period on bulk density, moisture content, bean number/10 g, weight of 100 beans and bean size of Arabica green coffee

| Fermentation period (hours) | Bulk density (g/ml) ± SD | Moisture content (%) | Bean number/ 10 g | Weight of 100 beans | Bean size (mm) | | |
|---|---|---|---|---|---|---|---|
| | | | | | Long | Wide | Thick |
| 0 | 0.656 ± 0.01$^{ab}$ | 8.344 ± 0.01$^{d}$ | 52.330 ± 1.00$^{a}$ | 19.175 ± 0.25$^{b}$ | 9.465 ± 0.42$^{a}$ | 7.068 ± 0.14$^{a}$ | 4.271 ± 0.05$^{a}$ |
| 4 | 0.663 ± 0.01$^{b}$ | 7.972 ± 0.02$^{c}$ | 53.000 ± 1.53$^{a}$ | 19.049 ± 0.26$^{b}$ | 9.436 ± 0.51$^{a}$ | 7.059 ± 0.21$^{a}$ | 4.275 ± 0.08$^{a}$ |
| 8 | 0.672 ± 0.00$^{b}$ | 7.854 ± 0.01$^{b}$ | 54.330 ± 2.01$^{a}$ | 19.029 ± 0.23$^{b}$ | 9.742 ± 0.41$^{a}$ | 7.155 ± 0.17$^{a}$ | 4.254 ± 0.08$^{a}$ |
| 12 | 0.646 ± 0.01$^{a}$ | 7.777 ± 0.09$^{b}$ | 57.000 ± 1.73$^{b}$ | 18.794 ± 0.09$^{b}$ | 9.657 ± 0.42$^{a}$ | 7.081 ± 0.19$^{a}$ | 4.305 ± 0.05$^{a}$ |
| 16 | 0.645 ± 0.01$^{a}$ | 7.661 ± 0.03$^{a}$ | 57.330 ± 1.16$^{b}$ | 18.139 ± 0.13$^{a}$ | 9.538 ± 0.75$^{a}$ | 6.938 ± 0.30$^{a}$ | 4.213 ± 0.15$^{a}$ |

Mean value ± standard deviation with different lowercase letter in the same column have a significant difference (P<0.05).

Table 2. Influence of fermentation period on color and defect on green coffee

| Fermentation period (hours) | Color | | | | | Defect, bean number/ 100 g | | |
|---|---|---|---|---|---|---|---|---|
| | L | a* | b* | C* | H* | Broken beans | Brown beans | Partly black beans |
| 0 | 29.073 ± 2.21$^{a}$ | 13.677 ± 1.28$^{a}$ | 17.973 ± 0.680$^{a}$ | 22,600$^{a}$ ± 1,14 | 52,780$^{a}$ ± 2,29 | 2.109 ± 0.00$^{a}$ | 2.040 ± 0.06$^{a}$ | 0.200 ± 0.01$^{a}$ |
| 4 | 29.820 ± 1.00$^{a}$ | 14.207 ± 1.26$^{a}$ | 17.480 ± 0.468$^{a}$ | 22,567$^{a}$ ± 1,15 | 50,963$^{a}$ ± 1,69 | 2.176 ± 0.00$^{bc}$ | 2.127 ± 0.08$^{a}$ | 0.403 ± 0.03$^{b}$ |
| 8 | 30.360 ± 0.22$^{a}$ | 12.763 ± 2.78$^{a}$ | 17.997 ± 1.865$^{a}$ | 22,100$^{a}$ ± 3,11 | 54,970$^{a}$ ± 3,34 | 2.144 ± 0.00$^{b}$ | 2.899 ± 0.02$^{b}$ | 0.500 ± 0.02$^{c}$ |
| 12 | 30.577 ± 0.96$^{a}$ | 13.403 ± 2.70$^{a}$ | 17.563 ± 0.335$^{a}$ | 22,167$^{a}$ ± 1,27 | 52,903$^{a}$ ± 6,29 | 2.177 ± 0.00$^{bc}$ | 2.991 ± 0.01$^{b}$ | 0.660 ± 0.02$^{d}$ |
| 16 | 30.667 ± 0.83$^{a}$ | 11.477 ± 3.59$^{a}$ | 17.320 ± 2.017$^{a}$ | 29,833$^{a}$ ± 3,59 | 57,143$^{a}$ ± 5,93 | 2.190 ± 0.00$^{c}$ | 3.594 ± 0.16$^{c}$ | 1.087 ± 0.08$^{e}$ |

Mean value ± standard deviation with different lowercase letter in the same column have a significant difference (P<0.05).
Minolta Chroma Meter CR 300 with color value of L*a*b* (CIELAB method); L* = lightness; a* and b* = chromacity coordinate, a* = red/ green direction; b* = yellow/ blue direction.

**Total Acids and the Caffeine Content**

The relationship between the concentrations of organic acids to changes in caffeine suggests that acids concentration increased, yet the caffeine decreased, as shown in Figure 3. The 0 hour fermentation, total acid was 5.33 ± 0.144% and the caffeine was 3.70 ± 0.017%. At 16 hours of fermentation, acids total was 9.22 ± 0.144% and the caffeine 1.60 ± 0.003%. That is because organic acids are the metabolites produced by the microflora during fermentation (Hamadi *et al*., 2014; Sisbudi and Fauzi, 2015), so that the increase in total acid shows that the amount of microflora contained in the substrate is also getting bigger, and some of this microflora are capable of degrading caffeine, so that the caffeine on the green coffee decreased (Gokulakrishnan *et al*., 2005; Farida *et al*., 2013). The reactions occurs is as follows:

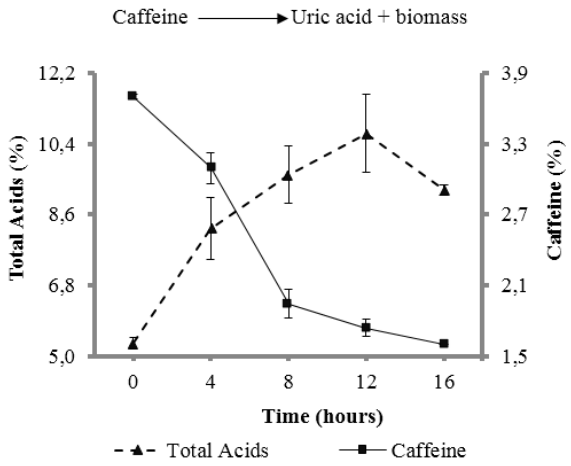


Figure 3. The relationship of total acids and caffeine content of green coffee during fermentation

This study is in accordance with reported by Farida et al. (2013) that fermentation decrease caffeine of the green coffee to be 46.88 mg/100 mL. The longer the fermentation time the less concentration of caffeine in the coffee. Fermentation process can degrade caffeine into uric acid, 7-methilxanthine, and xanthine (Todar, 2010). Caffeine compounds become free with smaller size and smaller molecular weight so that it is easy to move and diffuse through the cell wall and subsequently dissolved in the solvent/water. The caffeine presents in the cytoplasm. Moreover, caffeine is a water-soluble component (Siva et al., 2016). Degradation process of caffeine to be uric acid began at 12 - 36 hours fermentation (Gokulakrishnan et al., 2005), and optimum at 18 hours fermentation (Farida et al., 2013), thus wet fermentation is optimum to lowers caffeine levels for > 12 hours.

**Total Phenolic Content and Antioxidant Capacity**

Changes in total acids of coffee beans were also related to changes in polyphenols of coffee beans during fermentation. The results of this study showed that total acids of coffee beans increased, yet total polyphenols of coffee beans decreased (Figure 4). Organic acids, especially acetic acid is an organic acid that diffuses inwardly bean pieces so that polyphenol oxidase that oxidized polyphenols compounds can be activated. At the beginning of fermentation, the polyphenol from the ground of green coffee and its brews in sequence was 1381.58 ± 8.40 mg GAEs/100 g; 922.25 ± 17.96 mg GAEs/100 g. At 16 hours the fermentation, polyphenols from ground coffee and its brews in sequence decreased to 980.16 ± 10.07 mg GAEs/100 g; 812.06 ± 12.660 mg GAEs/100 g. A decrease in total polyphenols and increase in organic acid concentration is in accordance with the results obtained by Apriyanto and Rujiah (2017), on the addition of microbial inoculum including S. cerevisiae, Lc. lactis and A. aceti on fresh cocoa beans fermentation. Decrease in total polyphenols during fermentation can be thought to be caused by oxidation of enzymatic and non-enzymatic activity after bean drying.

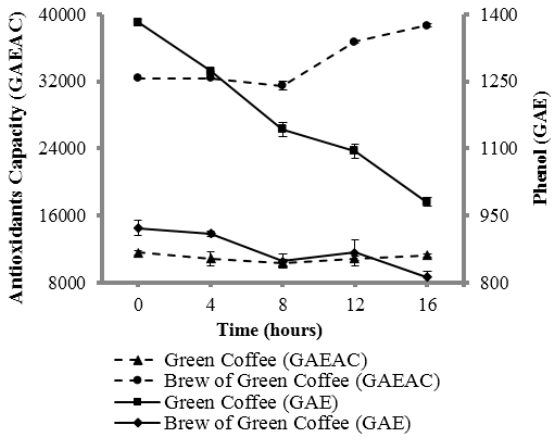


Figure 4. The relationship of antioxidant capacity and total phenol of green coffee and the brew of green coffee during coffee fermentation

Changes in antioxidant capacity of the

ground of green coffee and its brews are also related to changes in polyphenols the ground of green coffee and its brews during fermentation. The results of this study showed that as the antioxidant capacity increased, the total polyphenols decreased. At the beginning of fermentation, the antioxidant capacity from the ground of green coffee and its brews in sequence was 11564.28 ± 255.34 mg GAEAC/L; 32326.24 ± 22.744 mg GAEAC/L. At 16 hours the fermentation, antioxidant capacity from ground coffee was stable, yet its brews in sequence were 11234.43 ± 174.38 mg GAEAC/L; 38658.62 ± 180.107 mg GAEAC/L. Dewi et al. (2014) has reported that in the process of coffee fermentation, the phenolic compounds and organic acids are easily hydrolyzed so that the solubility in coffee beans increased causing antioxidant activity increased as well. In addition, ethanol was also produced during coffee fermentation. The ethanol has no antioxidant activity, but yet can increase the solubility of phenolic compounds in the water. The increase of antioxidant capacity is strongly suspected more closely related to the increase in total acids during coffee fermentation. Citric acid in mucilage is naturally antioxidative. Organic acids produced by lactic acid bacteria is known to contain a-hydroxiacids that act as antioxidants (Pramurdia and Kusnadi, 2014).

LAB also produce other secondary metabolites that were also antioxidative such as vitamins C and vitamin E. In addition, during the biochemical process of sugar reshuffling into lactic acid, there were antioxidative intermediate compounds (Madigan and Martinko, 1997). These compounds will penetrate into the beans resulting in the green coffee antioxidant capacity increased. Apriyanto and Rujiah (2017) state that green coffee brews are known to have higher antioxidant capacity than the green coffee it self, since most of the organic acids, vitamin C and some other organic compounds are water-soluble, so that when the green coffee brewed with water, antioxidant capacity will be higher. In contrast to the polyphenol content of green coffee brews, which brewed by hot water, the phenol will be oxidized to quinon so that the levels will be lower than green coffee.

A decrease in total polyphenols and increase in antioxidant capacity of the ground of green coffee and its brews is similar with data obtained by Ribeiro et al. (2017), as increased antioxidant activity can be observed after processing expecially roasting. Although some natural antioxidants are eliminated during the heating, the antioxidant properties can be maintained or improved by the formation of new antioxidants (Ribeiro et al., 2014).

The most abundant phenolic compounds of coffee beans are chlorogenic acids (CGA) (Bicho et al., 2013). Ribeiro et al. (2017) has reported that heating especially roasting, the contents of 3-CQA and 4-CQA did not vary significantly between golden coffee/GC (the grinded green coffee after dried in a Heraeus oven at 140°C for 10 minutes, a process that changed the samples into a golden tone and robusta roasted coffee/RRC (the grinded Robusta coffee was obtained by a medium roasting level 220 ± 10°C for 9 minutes), whereas 5-CQA significantly decreased in RRC. Therefore, 5-CQA was the CQA with higher reduction rates triggered by roasting. Due to such large decreased, total CQA significantly decreased as well in RRC. Due to such large decreased, total CQA significantly decreased as well in RRC. In RRC the contents of 3-FQA and 4-FQA were significantly increased by 195% and 214%, respectively. The total FQA was only slightly increased (11%) in RRC. It is probably the reason of the decrease in total polyphenols and increase in antioxidant capacity of the ground of green coffee and its brews.

## CONCLUSION

Aerobic microorganisms, bacteria, lactic acid bacteria and yeast were detected during the fermentation process of green coffee by using the wet method in Kintamani, Bangli, Bali. The predominant microflora was lactic acid bacteria and yeast. This microflora could produce metabolites increasing acidity and decreasing pH which was suitable for inhibiting fungi's growth, because in this study fungus was no detected during coffee fermentation. It was probably due to water activity and the growth of other microbial groups that was very high. Sixteen hours fermentation significantly ($P<0.05$) influenced the bulk density, moisture content and bean weight/100 beans, bean amount/10 g and defect of "broken beans", "brown beans" and "partly black beans" of green coffee yet were not significantly affected ($P>0.05$) bean size and color of the green coffee. The bulk density, moisture content and weight/100 beans decreased during the fermentation period, successively from 0.656 ± 0.01 to 0.645 ± 0.01 g/mL, from 8.344 ± 0.01 to 7.661 ± 0.03% and from 19.175 ± 0.25 to 18.139 ± 0.13 g. However, the bean amount/10 g increased from 52.330 ± 1.00 to 57.330 ± 1.16. The broken beans, the brown beans and partly black beans increased during the fermentation successively from 2.109 ± 0.00 to 2.190 ± 0.00, from 2.040 ± 0.06 to 3.594 ± 0.16 and from 0.200 ± 0.01 to 1.087 ± 0.08. Throughout coffee fermentation, the acids on green coffee increased from 5.33 ± 0.144 to 9.99 ± 0.144% and the caffeine on green coffee decreased from 3.70 ± 0.017 to 1.66 ± 0.003 % bb. The phenol content on green coffee and its brews also decreased successively from 1381.58 ± 8.40 to 980.16 ± 10.07 mg GAEs/100 g and 922.25 ± 17.961 to 812.06 ± 12.660 mg GAEs/100 g. For the antioxidants capacity, green coffee was more stable and its brews increased successively from 11564.28 ± 255.345 to 112 34.428 ± 174.377 mg GAEAC/L and 32326.24 ± 22.744 to 38658.62 ± 180.107 mg GAEAC/L.

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