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The Effect of Extracellular Protein Isolated from *Streptococcus bovis* 9A as A Biopreservative in Beef Meat by Means of pH Change

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Abstract. Lactic acid bacteria (LAB) are a microorganism that has non-toxic properties and capable of producing antimicrobial compounds namely bacteriocin. Bacteriocin is defined as a protein compound that has a small molecular weight with antibacterial activity. The study aimed to determine the ability of extracellular protein isolated from *Streptococcus bovis* 9A as a biopreservative in beef. The study started by cultivation of isolate on to MRS medium, folowed by production of extracellular protein, preparation of meat samples and application of extracellular protein as biopreservative by means of changing in pH value. As many as 18 experimental combinations consist of periods of extracelluler protein immersion and periods of storage at 4°C were applied in the study. The experiment was repeated 3 times, and pH values of each treatment were observed. Results of the study showed that the extracellular protein of the strain capable of inhibiting the pH increase in the meat and also prolong the meat storage at 4ºC for 10 days. This result concluded that the extracellular protein of *Streptococcus bovis* 9A is effective to be used as a biopreservative.

***Keywords: Streptococcus bovis 9A*, extracelluler protein, beef, pH change**

I. INTRODUCTION

Meat is one of the main commodities in Indonesia. In line with the increasing of population and public awareness, the demand for safety meat for consumption as a source of protein is also increase [1]. In accordance with the national nutritional standard that confirmed the need of Indonesian people for animal protein originated from livestock is predicted 6.0 grams/capita/day. This fact indicated that meat with high quality is needed [2]. On the other hand, the meat quality that are trade in the market especially in the traditional market often not well secured [3].

In order to determine the quality of meat, the researcher usually view with two factors, namely the physical and chemical factors. The physical factor including pH value, water holding capacity (WHC), cooking loose, and texture, whereas the chemical factor can be determined by the alteration of chemical components such as water content, protein and fat content, etc [4, 5].

Lactic acid bacteria (LAB) is a group of Gram-positive, non-spore, round or stem shape bacteria and can convert carbohydrates into lactic acid [6]. Lactic acid bacteria include safe microorganisms when added in food because they are non-toxic and do not produce toxins, so-called food grade microorganisms or known as Generally Recognized As Safe (GRAS). LAB are microorganisms that are not risk to health, and even some types are useful for health. Lactic acid bacteria can be used as food preservatives as a result of its produce organic acids, reduce their environmental pH and express compounds such as H_2O_2, diacetyl, CO_2, acetaldehyde, d-isomers, amino acid and bacteriocin [7]. Bacteriocin is defined as a protein compound that have small molecular weights and have activity as antibacterial or bacteriostatic [8].

According to the previous study by Widyadnyana *et al*. [9] who found *Streptococcus bovis* isolate 9A able to inhibit the growth of pathogenic bacteria such as *Staphylococcus aureus* with inhibition zone

ranged from 0.77 - 1.26 cm. Their results indicated the strain has potency to be used as biopreservative, therefore the study about it is interesting to be revealed.

II. RESEARCH METHODS

The sample used in the study was rump section (*upper thigh*) of bali cattle beef (*Bos sondaicus*) that was purchased at Pesanggaran Slaughterhouse and isolate of *Streptococcus bovis* 9A which was stored in 30% glycerol at -20ºC. This study used a randomized block design 3 x 6 factorial pattern with 3 treatments as factor: treatment I, beef without soaking into the extracellular protein as a control; treatment II, beef was soaked into the protein for 5 minutes, and treatment III beef was soaked into the protein for 10 minutes. Furthermore, factor II was 6 periods of storage at 4ºC: H0 (0 day), H2 (2^{th} day), H4 (4^{th} day), H6 (6^{th} day), H8 (8^{th} day), and H10 (10^{th} day 10), respectively. Totally, the study use 3 x 6 x 3 = 54 experimental unit.

Lactic acid bacteria (LAB) isolate 9A which have been grown for 24 hours were centrifuged at 7.000 rpm for 10 minutes. The supernatant was precipitated by adding ammonium sulphate until 70% saturation. The supernatant was re-centrifuged at 10.000 rpm for 10 minutes. The precipitates was collected then added with NaCl physiological solution at 1:1 (v/v) [10].

One kg of rump section was aseptically cut to 5 x 5 x 5 cm size and divided into 30 packs. All samples were previously immersed in NaCl physiological solution to harmonize microbial contamination. Furthermore, the samples were divided into 3 groups of treatment: (I) beef was not treated with the protein as a control; (II) beef was soaked in the protein for 5 minutes; and (III) beef was soaked in the protein for 10 minutes, respectively. After treatment, all samples were wrapped in a plastic clip and then stored in a refrigerator at 4ºC. Each treatment was replicated 3 times and the pH value was observed on the 0^{th}, 2^{nd}, 4^{th}, 6^{th}, 8^{th}, and 10^{th} days of preservation. The data were then analyzed descriptively.

III. RESULTS AND ANALYSIS

Results of study in term of the effect of extraceluler proteine treatment isolated from *Streptococcus bovis* 9A to the change of beef pH are presented in Table 1.

TABLE 1.
THE PH VALUE OF BEEF MEAT WITH AND WITHOUT TREATMENT WITH EXTRACELLULAR PROTEIN AT THE DIFFERENT PERIOD OF STORAGE AT 4°C.

Immersion time (minute)	Replication	Period of storage (Days)					
		0	2	4	6	8	10
0	1	6.0	6.2	6.5	6.6	8.5	8.6
	2	6.0	6.1	6.2	6.4	8.1	8.3
	3	6.0	6.3	6.3	6.5	8.2	8.4
Averages		6.0	6.2	6.33	6.5	8.27	8.47
5	1	6.0	6.3	6.3	6.4	7.0	7.5
	2	6.0	6.1	6.1	6.2	6.9	7.3
	3	6.0	6.1	6.2	6.2	7.1	7.6
Averages		6.0	6.17	6.20	6.27	7.00	7.46
10	1	6.0	6.1	6.2	6.4	7.3	7.6
	2	6.0	6.2	6.3	6.5	7.0	7.5
	3	6.0	6.2	6.3	6.3	7.2	7.4
Averages		6.0	6.17	6.27	6.4	7.17	7.5

Based on the data in Table 1, it showed the different pH value of the treated and untreated meat. A rapidly increased of pH value was observed in the untreated (control) meat in comparison to the treated beef meat. The result of Duncan test are sumarized in Table 2.

TABLE 2.
DUNCAN RANGE TEST OF BEEF PH VALUE AMONG TREATMENTS WITH AND WITHOUT IMMERSION OF EXTRA CELLUER PROTEIN OF *STREPTOCOCCUS BOVIS* 9A

Immersion time of extracelluler protein	Averages	Notation
0 minute	6,956	a
5 minutes	6,517	b
10 minutes	6,583	b

Note: letters in different columns show significantly different (P <0.05), otherwise the values with the same letter show no significant difference (P> 0.05).

The results of Duncan Multiple Range Test (DMRT) indicated that there are significant difference (P <0.05) between immersion of beef with protein 0 minute and immersion with 5 and 10 minutes. While the immersion between 5 and 10 minutes was no significant difference (P> 0,05). The averages of pH values for each treatment based on the time of immersion were 6.956, 6.517 and 6.583, respectively. Furthermore effects of time storage on pH values are summaried on Table 3.

TABLE 3.
DUNCAN MULTIPLE RANGE TEST OF TIME STORAGE OF BEEF WITH AND WITHOUT TREATMENTS AGAINST PH VALUE OF BEEF

Time storage	pH averages	Note
0 day	6,000	a
2 day	6,178	b
4 day	6,267	b
6 day	6,389	c
8 day	7,478	d
10 day	7,800	e

Note: letters in different columns show significantly different (P <0.05), otherwise the values with the same letter show no significant difference (P> 0.05).

The pH averages in Table 3 showed that beef pH between 0 day and 2nd day is significantly different (P <0.05), while on the 2nd day and 4th day is no significant difference (P> 0.05). The pH change of beef showed difrence (P <0.05) starting from 6th day , 8th day, and 10th day of observation. The results were due to the decomposition process or the gradual decomposition during storage. Decomposition process is believed will raise the pH value of the beef gradually. This condition resulted by the process of autolysis and protein decomposition by microbes contained in the meat. Low pH value of beef predict inhibit the bacterial growth in beef [11].

Interaction between immersion time of extracelluler protein and time storage against pH change are presented in Table 4.

TABLE 4.
DUNCAN MULTIPLE RANGE TEST INTERACTION BETWEEN IMMERSION TIME OF EXTRACELLULER PROTEIN AND TIME PERIOD OF PRESERVATION ON 4^{O}C AGAINST PH CHANGE

Interaction	Averages	Notation
B0 H0	6,000	a
B0 H2	6,200	b
B0 H4	6,333	b
B0 H6	6,500	c
B0 H8	8,267	d
B0 H10	8,433	e
B5 H0	6,000	a
B5 H2	6,167	b
B5 H4	6,200	b
B5 H6	6,267	b
B5 H8	7,000	c
B5 H10	7,467	d
B10 H0	6,000	a
B10 H2	6,167	b
B10 H4	6,267	b
B10 H6	6,400	b
B10 H8	7,167	c
B10 H10	7,500	d

Note: letters in different columns show significantly different (P <0.05), otherwise the values with the same letter show no significant difference (P> 0.05).

According to the data in Table 4 showed significat interaction of pH value between 0 day and 2nd day of observation, but for control showed more rapidly change characterized by significantly interaction between the 4th day and 5th day of observation. On the ather hand between immersion of beef with extracelluler protein for 5 minutes and 10 minutes did not show significantly different (P>0.05). The results indicate that there are interaction between the treatments with extracelluler protein time and time storage to the decrease of pH.

The equation of the regressian correlation between the immersion of beeft with extracelluler protein and the time of storage

against pH change for control was Y = 5,63 + 0,26x with correlation coefficient R2 = 0,810. Furthermore for immersion with 5 min was Y = 5,81 + 0,14x with correlation coefficient R2 = 0, 807 and 10 min was Y = 5,82 + 0,15x with correlation coefficient R2 = 0,868 (Figure 2).

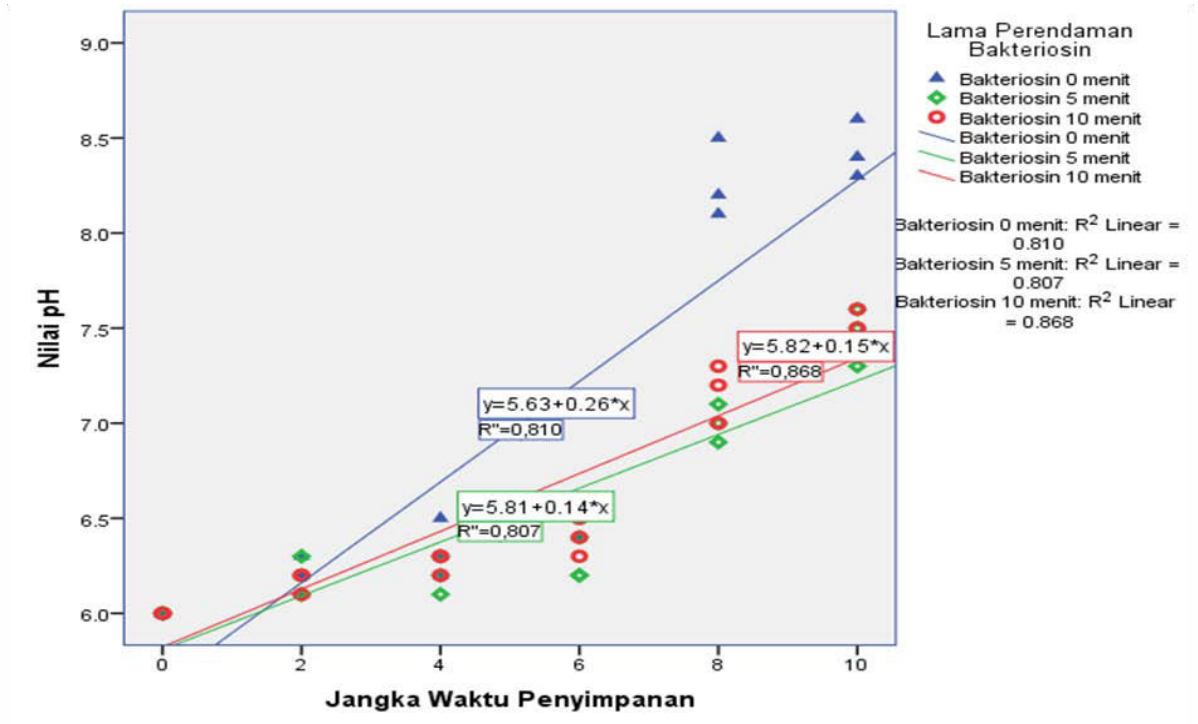

Figure 1. Linear curve of pH value based on the period of immersion in extracellular protein at different period of storage at 4ºC.

Based on the regression line equation in Figure 1 it was shown that the increase of pH value at the protein treatment at 0 minutes (control) was faster than 5 and 10 minutes of immersion. The results confirmed the significantly effect of the treatment with different protein immersion time. Beef is rich in nutrient matrix which is very suitable for the growth of bacterial decomposition and bacterial pathogens. The fact recomended it is required the right method to maintain the security and quality of beef.

Generally, It is known several parameters of food decay were color change, smell (smell), texture, shape, mucus formation, gas, pH change and liquid accumulation. Decay of food by microbes occurs faster than decay due to intracellular and extracellular enzymes. Raw and processed foods contain a variety of molds, yeasts, and bacteria that have the ability to multiply and cause decay [6].

Decomposition of meat can be caused by the activity of enzymes in meat (autolysis), chemicals (oxidation) and microorganisms. This decay mechanism is very complex. Factors affecting the growth of microorganisms in meat are: the type and number of early microorganisms (pollutants) as well as their distribution, the physical properties of meat, the chemical nature of meat, the availability of oxygen, and the temperature. The concentration of these components in meat and their use by certain types of microbes will determine the timing of onset and type of decay [12, 13]. In general, the lower pH of product will increase the shelf life of the product resulted by the bacteria will be difficult to live at low pH unless the bacteria are resistant to low pH (Achidophilic) [4,5]. Most microorganisms are known can grow in the pH range of 6.0-8.0 and pH values outside of the 2.0-10.0 range are usually destructive. Some microorganisms in certain foods such as yeast and lactic acid bacteria grow well in the range of pH values from 3.0 to 6.0 and are often referred to as acidophils [13].

Result of study indicated the potency of *Streptococcus bovis* 9A as a candidate of

biopreservatif, and also confirm the prime potency local isolates. This result is also inaccordance with previous study that found potency of lactic acid bacteria isolated from gastric fluid of bali cattle had potency as biopreservatif [14].

IV CONCLUSSION

The study showed extracelluler protein producing *Streptococcus bovis* 9A is potential to be used as a candidate of biopreservative according to their potency to decrcrease of pH change while periode of storage at 4^{o}C.

REFERENCES

[1] Harmini, Asmarantaka, R.W., and Atmakusuma, J. (2011). A Systemically Dynamic Model of Nationally beef availability. J. Ekonomi Pembangunan 12(1): 128-146.

[2] Khasrad, and Ningrat, R.W.S. (2010). Improving Carcass Quality of Indigenous Cattle of West Sumatera Fed Local Feed Resources. Pakistan. J of Nutrition. 9(8): 822-826.

[3] Priyanto R, Fuah, A.M., Aditia, E.L., Baihaqi, M., and Ismail, M. (2015). Increasing Productivity and Meat Quality Traits of Local Beef Cattle through Fattening Using Cereals Based Concentrate Containing Different Energy Level. J Ilmu Pertanian Indonesia. 20 (2): 108-114.

[4] Soeparno, (2005). Meat Science and Technology. 4thEd. Gadjah Mada University Press.Yogyakarta.

[5] Suardana, I W., and Swacita, I.B.N. (2009). Food Hygiene. 1st Ed. Udayana University Press. Denpasar.

[6] Frazier, W.C. and Westthoof, D.C. (1998). Food Microbiology, McGrawhill, New York, chap.9. In ray, B. and M. Daeschel, eds. Food Biopreservatives of Microbial Origins. CRC Pr.

[7] Kusmiati and Amarila, M. (2002). Bacteriocin Activity of Leuconostoc mesenteroides Pbac1 Bacteria on Several Media. Makara Journal. 16(1): 1-7.

[8] Diop, M.B., Dubois-Dauphin, R., Tine, E., Ngom, A., Destain, J., and Thonart, P. (2007). Bacteriocin Producer from Traditional Food Products. Biotechnol. Agron. Soc. Environ. 11 (4): 275-281.

[9] Widyadnyana D.G.A., Sukrama, I.D.M., and Suardana, I.W. (2015). Studies on Lactic Acid Bacteria Isolate 9A from Bali Cattle' Colon as a Probiotic Through 16S rRNA Gene Analysis. J. Sain.Vet. 33(2): 228-233.

[10] Sudirman I, F., Mathiau, M., Michael, and Lefebvre, G. (1993). Detection and Properties of Curvaticin 13, a Bacteriocin Like Substance Produced by *Lactobacillus curvatus* SB 13. *Current Microbiol.*27 : 35-40.

[11] Besung, I.N.K., Wulandari, N.M.D.A., and Swacita, I.B.N. (2013). Effect of Spices and Pork Storage Time on Total Plate Count Bacteria. Bul. Vet. Udayana. 6(1): 29-34.

[12] Aberle, E.D., J.C. Forrest, H.B. Hendrick, M.D. Judge and Merkel, R.A. (2001). Principles of Meat Science. W.H. Freeman and Co., San Fransisco.

[13] Buckle, K. A., Edwards, R.A., Fleet, G.H., and Wootton, M. (1987). Meat Sciences. UI-Press. Jakarta.

[14] Suardana, I.W., I.N. Suarsana, I.N. Sujaya dan K.G.Wiryawan. 2007. Isolation and Identification of Acid Lactic Bacteria from Bali Cattle's Gastric Fluid as a Potential Candidate of Biopreservative. *J.Vet.* Vol 8(4): 155-159.