Nutritional and Microbiological Quality of Beef Fermented Sausages Using Probiotics *Lactobacillus plantarum* 2c12 and *Lactobacillus acidophilus* 2B4 Isolated from Indonesian Beef

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Fermented sausage is a product of processed meat using lactic acid bacteria as starter culture. *Lactobacillus plantarum*2C12and *Lactobacillusacidhophilus*2B4 were proven as probiotic lactic acid bacteria. *L. plantarum* 2C12 and *L. acidophilus* 2B4 are homofermentatitive lactic acid bacteria that were isolated from Indonesian beef and has been identified by biochemical and molecular technique using 16S rRNA sequencing. The aim of this research wasdetermine the effect of the addition of probiotics to nutritional and microbiological quality of fermented sausages that were made from Indonesian beef (Peranakan Ongole breed). There were three treatments in this research: without addition of probiotic (control)), the addition of *L. plantarum*2C12 and *L. acidophilus*2B4. The result of research in the microbiology quality showed that nutrient content (fat content, crude protein, carbohidrat, ash content and water content) of all treatments of fermented sausagesdid not significantly difference ($p>0,05$). Fermentation by probiotics bacteria : *L. plantarum* 2C12 and *L. acidophilus* 2B4 could increase content of amino acid of fermented sausages than control. It proved that protein quality of fermented sausages with these probiotics were increased. For the microbiological quality, the addition of probiotics*L. plantarum*2C12 and *L. acidophilus* 2B4 product could reduce significantly ($p<0.05$) pathogenic bacteria *Escherichia coli* and *Stapylococcus aureus.Salmonella* was not found because of addition of the probiotics. The addition of probiotic *L. plantarum* 2C12 and *L. acidophilus* 2B4 were adapted to applicate as starter culture on Indonesian beef fermented sausages, while *L. plantarum* 2C12 was better than*L. acidophilus* 2B4.

Key Words: Fermented sausage, Probiotic, *L. plantarum, L. acidophilus*, Beef

INTRODUCTION

Fermented sausages are made from comminuted meat stuffed into casing. Furthermore, fermented sausages can be defined as meat products consisting of a mixture of meat and fat particles, salt, curing agent, spices, etc., which have been stuffed into casing, fermented (ripened) and dried e.g. pepperoni, salami, and chorizo in Europe, Nham and Thai Krak Prew in Thailand, Urutan (a Balinese indigenous fermented sausage) in Indonesia, Lebanon Bologna in Lebanon etc. They are often sub-categorized according to their water activity i.e. dry, semi dry or undried (spreadable).Fermented sausage are those which have been subjected

to the action of microorganisms so that the product characteristics (e.g. flavor, texture and shelf life) alter significantly (Toldra et al., 2001). The development of a strain of microbial flora succeeds, dominating and displacing other undesirable microorganisms, and producing a fermented sausage in which the generated metabolites contribute to the product's appriorite sensory characteristics. One of the microorganism used as starter culture into fermented sausage is lactic acid bacteria.Lactic acid bacteria (LAB) are responsible for lactic acid production, for the ''tangy'' flavour of sausages, and for the small amounts of acetic acid, ethanol, acetoin, also contribute in nutritional value that are produced during fermentation, depending on the starter applied, the carbohydrate used, and the sources of meat proteins and additives. LAB play a defining role in the preservation and microbial safety of fermented foods, thus promoting the microbial stability of the final products of fermentation (Settani and Corsetti, 2008). Because of production of organic acids, carbon dioxide, ethanol, hydrogen peroxide, diacetyl and bacteriocins, LAB is important as food biopreservatives.

Lactobacillus plantarum 2C12 and *Lactobacillus acidophilus* 2B4 were isolated from local beef of Indonesian indigenous cattle (Peranakan Ongole-breed). They were proven as probiotic candidates, had antibacterial activities, could survive in the low pH also could adhere in intestinal cell. They had immunomodulatory capacity and could prevent diarhea caused by enteropathogenic *Eschericia coli* (Arief, 2011). Probiotics can be defined as live microbial food supplements with health benefits to the host by improving the intestinal microbiota.Indigenous probiotics will be important for searching novel probiotic that has high adaptable ability to local food, such as fermented sausage with local beef as main materials. The aims of this research were to apply *L. plantarum* 2C12 and *L. acidophilus* 2B4 as starter cultur of beef fermented sausages and to study the nutritional and microbiological quality of them.

MATERIALS AND METHODS

Production of beef fermented sausages

Beef (4 kg), lard (1 kg), a mix of sodium chloride (25 g), sugar (5 g), nitrite (25 ppm), ginger (2.5 g), pepper (2.5 g) were mixed and filled to edible casings, resulting in 10 cm long and 5 cm diameter fresh sausages. There were three treatments in this research : without addition of probiotic (control)), the addition of *L. plantarum*2C12 and *L. acidophilus*2B4. Starter culture (2% with 10^8 CFU/ml population of *L. plantarum* 2C12 or *L. acidophilus* 2B4) was added into first mixing. The mixed ingredients was ripened as follows : the first stage consisted of one day drying at temperature of 25^0C, then were cold smoked for 4 days, every day was about 4 hours smoking. The ripening was carried out for 6 days at 27^0C.

Nutritional and amino acid analysis

The determination of moisture, ash content, crude protein and fat were performed according to Proximat analysis (AOAC, 2005). Carbohydrat content was calculated by difference

analysis. Amino acid quantity was determined by HPLC with standards from Sigma. Only amino acids with acid hydrolysis were calculated in this experiment.

Microbiological analysis

Beef as raw materials and fermented sausages were determined to microbiological analysis. 25 g of each sample were homogenized into 225 buffer phosphat water (BPW, Oxoid). Further decimal solutions were made and plate count anaylisis were carried out on duplicate agar plates. The analysis were (a) total lactic acid bacteria on MRS agar (Oxoid) incubated at 37^0C for 48 h; (b) *Staphylococcus aureus* on Baird Parker medium (Oxoid) with added egg yolk tellurite emulsion (Oxoid) incubated at 37 ^{0}C for 24–48 h, (c) total *Escherichia coli* on Eosin Methylen Blue agar (Oxoid) incubated at 37^0C for 24-48 h and (d) Salmonella spp was qualitative determined on serial media, including XLD agar (Oxoid) incubated at 37^0C for 48 h. All analysis were according to AOAC (2005). After counting, means and standard deviations were calculated.

Statistical analysis

Completely randomized design was used in this experiments. After verification of the normal distribution of data, ANOVA was used. Tukey's test was used to test for differences between the paramaters (Steel and Torrie, 1995). Experiments were repeated three times and microbiological and nutitional analysis were determined for each sample.

RESULTS AND DISCUSSION

Nutritional quality and amino acid composition of fresh beef and fermented sausages

Table 1 shows that nutritional quality of fermented sausages with addition of *L. plantarum* 2C12 or *L. acidophilus* 2B4 were not signifantly different ($p>0.05$) than control. Comparing with Ferreira et al. (2006), nutritional quality of beef fermented sausage in this experiments were not different, but crude protein of fermented sausageswere higher.

Table 1. Nutritional quality of fresh beef and fermented sausage

Parameters	Fresh beef	Control	Fermented sausage with *L. plantarum* 2C12	Fermented sausage with*L. acidophilus* 2B4	Study of Ferreira et al. (2006)
			--------%--------		
Water content	74.77	55.58±1.51	54.65±2.03	54.36±2.95	43.3-57.2
Ash	2.12	3.37±0.13	3.52±0.15	3.61±0.02	nd
Crude protein	11.53	18.16±0.66	18.56±0.94	19.29±1.13	6.9-15.5
Fat	0.13	11.73±2.08	9.93±0.37	9.20±1.16	10.9-29.6
Carbohydrate	11.45	11.16±2.81	13.35±2.91	13.55±4.44	10.2-20.9

Moisture contentof fermented sausages were lower than fresh beef, because of drying and fermentation process. Ash, crude protein and fat of fermented sausages were higher than fresh

beef. Fat content of fermented sausages were higher than fresh beef, because there was an addition of fat in the sausage-making process.

Table 2. Amino acid composition of fresh beef and fermented sausages

Amino acid	Fresh beef (%w/w)	Fermented sausages				
		Control (a) (%w/w)	*L. plantarum* 2C12 (b) (%w/w)	Improvemen (%)	*L. acidophilu* 2B4 (c) (%w/	Improvemer ** (%)
Aspartate	2.03	1.47	1.53	4.08	1.55	5.44
Glutamate	3.56	2.58	2.68	3.88	2.85	10.46
Serine	0.87	0.63	0.65	3.17	0.65	3.17
Histidine	0.86	0.55	0.58	5.45	0.60	9.09
Glycin	1.14	0.75	0.69	-8	0.62	-17.3
Threonine	0.92	0.69	0.76	10.14	0.73	5.79
Arginine	1.42	1.03	1.06	2.91	1.04	0.97
Alanine	1.32	0.99	1.00	1.00	1.01	2.02
Tyrosine	0.77	0.53	0.58	9.43	0.59	11.32
Methionine	0.61	0.41	0.44	7.32	0.44	7.32
Valine	1.07	0.78	0.82	5.13	0.85	8.97
Phenilalanine	0.91	0.69	0.72	4.35	0.78	13.04
I-Leusine	1.02	0.73	0.79	8.22	0.82	12.33
Leusine	1.63	1.29	1.36	5.43	1.39	7.75
Lysine	1.76	1.38	1.52	10.14	1.61	16.67

* = [(b)- (a)]/(a) x 100% ; ** =[(c)-(a)]/(a) x 100%

Table 2. shows amino acid composition of fresh beef and fermented sausages. Amino acid composition in the fresh beef was higher than fermented sausages. It was because beef was only 80% in the fermented sausages formulation, so that the percentage of amino acid in the whole of fermented sausages was lower than fresh beef. Total value of the highest amino acid content was shown in fermented sausage with addition of *L. acidophilus*2B4, followed by sausage fermented sausage with addition of *L. plantarum* 2C12, and the lowest was control (without LAB addition). Aspartate, glutamate, serine, histidine, threonine, arginine, alanine, tyrosine, methionine, valine, phenylalanine, ileusin, leucine, and aspartate increased in fermented sausages by addition of probiotic culture. Increasing in amino acid compositionsshowed proteolytic activities of these LAB. Toldra *et al.* (2001) stated that the sarcoplasmic and myofibrillar protein hydrolysis were occured in the fermentation. *L. plantarum* and *L. aciophilus* have proteolytic enzyme that plays an important role in the stage of proteolysis. Proteolytic activity contribute to the formation of protein degradation.

Microbiological quality of fermented sausage

Table 3 shows microbial population of fresh beef and fermented sausage. The addition of probiotics had no significantly effect (P> 0.05) to total population of LAB in fermented

sausages. It was because the initial population of LAB in fresh beef was high. *S. aureus* is a pathogenic bacteria that produces a toxin that can cause poisiong in a population of 10^7 CFU/ g. The addition of *L. plantarum* 2C12 could reduce significantly ($p<0.05$) *S. aureus* and *E. coli* population of fermented sausages, while *L. acidophilus* 2B4 had no effect. *S. aureus* population of fermented sausages were lower than minimal population of *S. aureus* that can produce toxin. *L. plantarum* 2C12 produced antimicrobial agents that could inhibit pathogenic bacteria, better than *L. acidophilus* 2B4 (Arief, 2011). Fermented sausages with addition of probiotics were negative of salmonella spp contamination. It showed that *L. plantarum* 2C12 and *L. acidophilus* 2B4 could inhibite *Salmonella* spp on fermented sausages.

Table3. Microbial population of fresh beef and fermented sausages

Bacteria species	Fresh beef	Control	Fermented sausage *L.plantarum* 2C12	Fermented sausage *L.acidophilus* 2B4
	------ Log_{10} CFU/g ------			
LAB	6.67	6.74 ± 0.41	7.84 ± 0.40	6.85 ± 0.89
*S. aureus**	2.86	3.06 ± 0.10^a	1.94 ± 0.77^b	2.88 ± 0.11^a
*E.coli**	0	3.24 ± 0.26^a	0^b	2.84 ± 0.29^a
Salmonella spp	-	+	-	-

*Significantly different ($p<0.05$)

CONCLUSION

Nutritional quality of beef fermented sausages with addition of probiotics *L. plantarum* 2C12 or *L. acidophilus* 2B4 were not different, while amino acid compositions were better than control (without addition of probiotic). Addition of *L. plantarum* 2C12 could reduce pathogenic bacteria *S. aureus* and *E.coli* population in fermented sausages, was better than addition of *L. acidophilus* 2B4. In general, probiotic *L. plantarum* 2C12 was better than *L. acidophilus* 2B4 as starter culture of beef fermented sausages.

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