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Characteristic of Lamb Sausages Fermented by Indonesian Meat-Derived Probiotic, *Lactobacillus plantarum* IIA-2C12 and *Lactobacillus acidophilus* IIA-2B4

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ABSTRACT

Probiotic is a group of microorganism, mainly from lactic acid bacteria (LAB), widely used to increase functionality of various foodstuffs, including lamb which was limited by its goaty odor and short life issue. This study aimed to evaluate the characteristic of lamb sausages fermented by either *Lactobacillus plantarum* IIA-2C12 or *L. acidophilus* IIA-2B4 isolated from local cattle in Indonesia, and stored for 21 days at low temperature (4°C). Fermented lamb sausages were made with the addition of *L. plantarum* IIA-2C12 and *L. acidophilus* IIA-2B4 with three replications. The result showed that pH value, protein, and cholesterol contents of the sausages with addition of *L. acidophilus* IIA-2B4 were higher (P<0.05) than that of *L. plantarum* IIA-2C12. Meanwhile, the sausage fermented with *L. plantarum* IIA-2C12 had higher titratable acid (TA) value, texture, and the content of fat, carbohydrate, tyrosine, lysine, myristoleic (C14:1), pentadecanoic (C15:0), heneicosanoic (C21:0) and cis-11-eicosatrienoic (C20:1) as compared to that of *L. acidophilus* 2C12-2B4. Final population of LAB in the sausage fermented by *L. plantarum* IIA-2C12 was also higher than that of *L. acidophilus* IIA-2B4, yet both can be categorized as a probiotic. The differences between characteristics of the physicochemical traits and microbiological quality of the sausage fermentation associated with the addition of *L. plantarum* IIA-2C12 or *L. acidophilus* IIA-2B4. The 21 days of storage at cold temperatures with probiotics addition could extend shelf life and maintain quality of fermented sausage.

Key words: fermented lamb sausages characteristic, Lactobacillus acidophilus IIA-2B4, Lactobacillus plantarum IIA-2C12, probiotic

ABSTRAK

Probiotik adalah sekelompok mikroorganisme, terutama dari bakteri asam laktat (BAL), yang banyak digunakan untuk meningkatkan fungsi bahan makanan, termasuk daging domba yang dibatasi oleh bau prengus dan masa simpan yang pendek. Penelitian ini bertujuan untuk mengevaluasi karakteristik sosis domba fermentasi dengan penambahan *Lactobacillus plantarum* IIA-2C12 atau *L. acidophilus* IIA-2B4, yang diisolasi dari sapi lokal di Indonesia, dan disimpan selama 21 hari pada suhu rendah (4°C). Sosis fermentasi daging domba dibuat dengan penambahan *L. plantarum* IIA-2C12 dan *L. acidophilus* IIA-2B4 dengan tiga ulangan. Hasil penelitian ini menunjukkan perubahan signifikan nilai pH, kadar protein, dan kolesterol dengan penambahan *L. acidophilus* IIA-2B4 yang mempunyai nilai lebih tinggi dibanding *L. plantarum* IIA-2C12. Selain itu, sosis fermentasi dengan *L. plantarum* IIA-2C12 mempunyai nilai yang lebih tinggi pada total asam tertirasi (TAT), tekstur, kadar lemak, kadar karbohidrat, tirosin, lisin, miristoleat (C14:1), pentadecanoic (C15:0), heneicosanoic (C21:0), dan cis-11-eicosatrienoic (C20:1) dibandingkan dengan *L. acidophilus* 2C12-2B4. Populasi akhir BAL di sosis fermentasi oleh *L. plantarum* IIA-2C12 lebih tinggi daripada *L. acidophilus* IIA-2B4, namun keduanya dapat dikategorikan sebagai probiotik. Perbedaan karakteristik sifat fisikokimia dan kualitas mikrobiologis kedua sosis fermentasi terkait dengan penambahan *L. plantarum* IIA-2C12 atau *L. acidophilus* IIA-2B4. Penyimpanan selama 21 hari pada suhu dingin dengan penambahan probiotik dapat memperpanjang masa simpan dan mempertahankan kualitas sosis fermentasi.

Kata kunci: karakteristik sosis fermentasi daging domba, Lactobacillus acidophilus IIA-2B4, Lactobacillus plantarum IIA-2C12, probiotik

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INTRODUCTION

The use of probiotics for various foodstuffs in Indonesia remarkably increased due to the need of functional foods having high nutritional values and health benefits. Probiotics refer to living microorganisms when administered in adequate amounts results in health benefits, which are mostly belong to lactic acid bacteria (LAB) (Al-Sheraji *et al*., 2013). LAB is usually used as a starter in a controlled fermentation for probiotics purpose. Fermented lamb sausages with the addition of starter culture are considered as manufactured through controlled fermentation. Fermentation aimed to extend the shelf life and to give a distinctive flavor on the characteristics product (Esmaeilzadeh *et al*., 2013).

Fermented sausages are possibly manufactured through spontaneous fermentation process as shown in Urutan, a pork-based fermented sausage from Bali (Darmayanti *et al*., 2014). The traditional fermented sausages made spontaneously using natural starter of raw materials during the maturation process for the purpose of adding flavor (Leroy *et al*., 2006). The probiotic *L. plantarum* IIA-2C12 can be used as starter culture of fermented lamb sausage. The physicochemical, microbiology, and sensory properties of fermented lamb sausages with the addition of *L. plantarum* IIA-2C12 (population 8 log cfu/g) were better than that of control (without probiotic) (Arief *et al*., 2014).

The probiotic strains used in sausage commonly belong to Lactobacilli and Bifidobacteria that are also present in the intestines (De Vuyst *et al*., 2008). Bacteria are considered as probiotics when they are able to survive in the digestive tract, which implies their resistances toward acidic conditions and the presence of bile acids (Sunny-Roberts & Knorr, 2008).

Previously, *L. plantarum* IIA-2C12 and *L. plantarum* IIA-2B4, isolated from meat of Peranakan Ongole cattle collected from traditional markets proved to be a probiotic LAB (Arief *et al*., 2015a). Low temperature storage is widely used to prevent pathogenic contamination risk on fermented lamb sausages. Lindqvist & Lindblad (2009) stated that the population of pathogenic bacteria of the fermented sausages decreased during storage at low temperature (refrigerator) for 20 d. Storage at low temperature might increase the length of the adaptation phase of microbial growth that might affect the shelf life of fermented lamb sausages. This study aimed to evaluate the characteristic of lamb sausages fermented by either *L. plantarum* IIA-2C12 or *L. acidophilus* IIA-2B4 isolated from local cattle in Indonesia, and stored for 21 d at low temperature (4^{o}C).

MATERIALS AND METHODS

Bacterial Strains

LAB used in this study were *L. plantarum* IIA-2C12 and *L. acidophilus* IIA-2B4 isolated from meat of Peranakan Ongole cattle from traditional markets in Indonesia (Arief *et al*., 2015a). Culture of *L. plantarum* IIA-2C12 and *L. acidophilus* IIA-2B4 were growth on media Man Rogoso Sharpe Agar (MRSA) (Oxoid, UK) to determine the initial population. According to Arief *et al.* (2008), the population of the strains used to ferment lamb sausage were over than 10^8 cfu/mL.

Preparation of Fermented Lamb Sausage

Fermented lamb sausage was prepared by following the recipe of Arief *et al.* (2014a). As much as 1000 g of lamb meat was ground first and then inserted sequentially with 2% salt, 2% starter culture of 10^8 cfu/mL *L. plantarum* IIA-2C12 or *L. acidophilus* IIA-2B4, 0.5% spices nutmeg, 2% sugar, 0.5% ginger, 0.5% black pepper, and 0.5% white pepper. The dough was incorporated into the sausage casing with diameter of 12.5 cm, and then conditioning for 24 h at room temperature (27°C). After that, cool smoking was conducted for 5 h for 2 d with a temperature of 28-30°C. Fermented sausages were made with the addition of *L. plantarum* IIA-2C12 and *L. acidophilus* IIA-2B4 with 3 replications. Fermented sausages were packed with polyethylene and stored at refrigerator with a temperature of 4-8℃. Physicochemical and microbiological analysis were carried out at 0, 7, 14, and 21 h according to the research of Afiyah *et al*. (2015).

Physicochemical Analysis

The moisture, protein, fat, and ash contents of the sausages were analyzed using proximate analysis (AOAC, 2005). Carbohydrate content was calculated by difference from the proximate analysis. The pH value was analyzed using a hanna instrument pH meter (USA). Water activity (a_w) was determined using an a_w meter (Novasino). The measurement was conducted according to manufacturer's manual (Ravyts *et al.*, 2010). Total lactic acid was measured by titratable acidity (Afiyah *et al*., 2015). Texture was determined using a texture analyzer Steven-LFRA as kg/cm^2.

Microbiological Analysis

Population of LAB, *E. coli,* and *Salmonella* spp on fermented lamb sausages was determined according to AOAC (2005). Fermented lamb sausage was weighed as much as 25 g and put into 225 mL of buffered peptone water (BPW) and homogenized. LAB was grown on media MRSA (Oxoid, UK) and incubated at 37℃ for 24-48 h. Meanwhile, the total population of *Salmonella* spp and *E. coli* pathogenic bacteria were determined using the Xylose Lysine Deoxycholate Agar (Oxoid, UK) and Eosin Methylen blue Agar (Oxoid, UK), respectively, and incubated at 37^{o}C for 24-48 h.

Amino Acids Composition

The amino acid composition was determined using the method of High-Performance Liquid Chromatography (HPLC) Agilent Technologies. A total of 3 mg fermented lamb sausage samples were hydrolyzed with acid following the method of Osthoff *et al.* (2002). A total of 10 mL sample was added with 25 mL of reagent ortoftalaldehida (OPA). A total of 5 mL was injected into the HPLC column and waited until separation of all the amino acids was complete. The time required was approximately 25 min. The concentration of amino acids in samples of fermented sausage can be calculated.

Fatty Acids Composition

The sausages fatty acid composition was analysed according to the AOAC (2005). The fatty acids were methylated by transesterification. The injector and detector temperatures were set at 250°C and 300°C. Helium was used as the carrier gas with a flow rate of 1.0 mL/min. The results were expressed as mg FFA/g of extracted lipid. All the tests were performed in 175 triplicates.

Analysis of Cholesterol

Cholesterol analysis was conducted by using a method by Lieberman-Buchards method (Kleiner & Dotti, 1962). This method is an analysis of the concentration of cholesterol chemically. A total of 0.1 g of sample was put into the centrifuge tube and added 8 mL alcohol hexane : ether alcohol (3:1) and then centrifuged for 10 min at a speed of 3000 rpm. The remaining residue was dissolved using chloroform as it was poured in a tube scale up volume 5 mL, and added with 2 mL of acetic anhydride and 0.2 mL of concentrated H_2SO_4. The tube was whirled by using vortex and left in the dark for 15 min. The resulting color was a bluish green color that was read at absorbance with 420 nm wavelength.

Statistical Analysis

Data were expressed as means with standard of error of at least 3 independent experiments. Data on the physicochemical and microbiological properties were statistically analyzed using analysis of variance (ANOVA) under completely randomized design based on Steel & Torrie (1995). Post hoc analysis was performed by Duncan test. Data of physicochemical and microbiological properties during storage were analyzed by using factorial completely randomized design.

RESULTS

The physicochemical properties of fermented sausages were shown in Table 1. Addition of *L. plantarum* IIA-2C12 or *L. acidophilus* IIA-2B4 had shown to significantly (P<0.05) contribute to pH values of the sausages. The content (percentage) of titratable acid of fermented sausage with the addition of *L. plantarum* IIA-2C12 was shown to be higher than that of *L. acidophilus* IIA-2B4 (P<0.05). This might explain the significant differences (P<0.05) on the effect of addition of probiotics in the sausage textures found in this study, in which the addition of *L. plantarum* IIA-2C12 produces a texture that was harder than *L. acidophilus* IIA-2B4.

The addition of *L. plantarum* IIA-2C12 produced higher ash content of the products than that of *L. acidophilus* IIA-2B4 (P<0.05) (Table 1). The protein content with the addition of *L. acidophilus* IIA-2B4 was significantly (P<0.05) higher than that of *L. plantarum* IIA-2C12. The carbohydrate content was significantly higher (P<0.05) with the addition of *L. plantarum* IIA-2C12 than that of *L. acidophilus* IIA-2B4. The cholesterol value on the product with the addition of *L. plantarum* IIA-2C12 or *L. acidophilus* IIA-2B4 showed significantly (P<0.05) lower than that of lamb before processing at 162.67 mg/100g.

Further, Table 1 also displayed microbiological quality of fermented sausage. The population of LAB in fermented sausages had significant difference (P<0.05). Tyrosine and lysine showed significant larger (P<0.05) in the fermented sausage products with the addition of *L. plantarum* IIA-2C12 than that with *L. acidophilus* IIA-

Table 1. Physicochemical and microbiological properties of fermented lamb sausages

Variables	Without probiotic	With probiotic	
	Arief *et al*. (2014)	*L. plantarum* IIA-2C12	*L. acidophilus* IIA-2B4
Physicochemical properties			
pH	4.39±0.01	4.18±0.01^{b}	4.20±0.02^{a}
a_w	0.88±0.01	0.86±0.01	0.85±0.01
Titratable acid (%)	2.03±0.14	2.14±0.03^{a}	2.03±0.04^{b}
Texture (kg/cm^2)	0.42±0.01	5.22±0.00^{a}	4.38±0.00^{b}
Ash (%wb)	1.86±0.14	3.46±0.04^{a}	3.27±0.05^{b}
Moisture (%wb)	65.11±0.14	60.75±0.12	59.06±0.25
Crude protein (%wb)	18.87±0.29	18.36±0.01^{b}	18.51±0.01^{a}
Fat (%wb)	7.25±0.18	14.72±0.20^{a}	11.63±0.32^{b}
Carbohydrate (%wb)	6.91±0.26	5.22±0.49^{a}	4.65±0.05^{b}
Cholesterol (mg 100/g)	na	131.22±10.1^{b}	158.13±0.24^{a}
Microbiological properties			
Total lactic acid bacteria (log cfu/g)	6.43±0.03	9.21±0.01^{a}	9.13±0.01^{b}
Total *E. coli* (log cfu/g)	3.58±0.03	0.33±0.57	0.33±0.57
Total *Salmonella* spp	Positive	Negative	Negative

Note: Means in the same row with different superscripts differ significantly (P<0.05); cfu/g= colony forming unit per gram; na: not analyzed.

2B4 (Table 2). The fatty acids of myristoleic, pentadecanoic, heneicosanoic, and cis-11-eicosenoic on fermented sausage by the addition of *L. plantarum* IIA-2C12 were higher than *L. acidophilus* IIA-2B4 (P<0.05) (Table 3). Microbiological properties of fermented lamb sausages during cold storage can be seen in Table 4. The population of LAB in fermented sausages until the 21st day after storaging was 8.95 log cfu/g for fermented sausage with the addition of *L. plantarum* IIA-2C12 and 8.81 log cfu/g for fermented sausage with the addition of *L. acidophilus* IIA-2B4 (P<0.05). Physicochemical properties of fermented lamb sausages during cold storage can be seen in Table 4.

DISCUSSION

As expected, the addition of probiotics used in this study significantly reduces pH values (Table 1). This is due to production of lactic acid as end product of fermentation by *L. plantarum* IIA-2C12 and *L. acidophilus* IIA-2B4 added in the sausages. The pH values of fermented sausage with the addition of *L. plantarum* IIA-2C12 and *L. acidophilus* IIA-2B4 are in the range of pH optimum for BAL to growth (3.5–4.5) and produce antibacterial compounds against pathogenic bacteria. Indeed, Riebroy *et al.* (2008) stated that low pH value was an important factor in the control of bacteria.

This might be due to the ability of *L. plantarum* IIA-2C12 to produce acid compound is relatively higher than that of *L. acidophillus* IIA-2B4. Nevertheless, Afiyah *et al.* (2015) suggested that meat-based substrate (in this study is lamb) was apparently more favorable for biochemical activities of *L. plantarum* IIA-2C12. Noteworthy, titratable acid of fermented sausage with the addition of *L. plantarum* IIA-2C12 was higher from that of lamb sausage with no addition of probiotic culture as reported by Arief *et al.* (2014a). This is plausible since the more LAB presence in the sausage, more acid compounds are produced. This also indicated that the addition of probiotic cultures can be used for specific purposes.

Further, pH value, as well as lactic acid content, was assumed to have some effect on the product texture as proposed by Seo *et al.* (2015). This is because of the dependency of protein structure changes (denaturation and gelation) on the pH value. Riebory *et al.* (2008) clearly showed that apart from water loss, denaturation and gelation of protein in the food systems were indeed related to the texture as it was mainly dealing with shear force or gel strengh.

LAB were known to produce lactic acid associated with the increasing of ash content (Nie *et al.*, 2014). Digestibility of the product with the presence of LAB was mostly found to have higher digestibility as the indegenous proteins were digested into smaller fragments (peptide) by starter LAB (Liu *et al.*, 2011). The differences between the sausages with addition of *L. plantarum* IIA-2C12 and *L. acidophillus* IIA-2B4 reflected the differences on the proteolytic system including activity and specificity.

LAB in both fermented sausages utilized carbohydrates as a source of glucose in fermentation process. The addition of skim milk in fermentation sausage was degraded lactose to glucose and galactose which had

Table 2. Amino acid compositian of sausages with the addition of *L. plantarum* IIA-2C12 and *L. acidophilus* IIA-2B4

Variables	Lamb meat	With probiotic		Mean
		L. plantarum IIA-2C12	*L. acidophilus* IIA-2B4	
Amino acid (w/w) %				
Non polar				
Alanine	1.16	1.20±0.20	1.35±0.60	1.28±0.11
Valine	0.99	0.89±0.11	0.94±0.39	0.92±0.04
Leucine	1.58	1.39±0.18	1.41±0.51	1.40±0.01
Isoluecine	1.08	0.85±0.12	0.83±0.31	0.84±0.01
Methionine	0.70	0.41±0.07	0.39±0.13	0.40±0.01
Phenylalanine	0.88	0.72±0.11	0.74±0.27	0.73±0.01
Glycine	1.02	1.35±0.36	1.69±0.95	1.52±0.24
Polar				
Threonine	0.61	0.79±0.13	0.81±0.27	0.80±0.01
Serine	0.47	0.69±0.10	0.74±0.25	0.72±0.04
Tyrosine	0.55	0.53±0.04[a]	0.45±0.02[b]	-
Aspartic	1.90	1.66±0.21	1.67±0.57	1.67±0.01
Electrically changed (acidic)				
Glutamic	4.18	3.12±0.44	3.14±1.08	3.13±0.02
Electrically changed (acidic)				
Arginine	1.24	1.29±0.22	1.34±0.51	1.32±0.04
Histidine	0.41	0.44±0.10	0.44±0.18	0.44±0.00
Lysine	1.57	1.24±0.02[a]	1.09±0.05[b]	-
Total amino acids	18.33	16.57±2.34	17.03±6.03	16.80±0.33

Note: Means in the same row with different superscripts differ significantly (P<0.05); lamb meat: not analyzed statistically.

Table 3. Fatty acid compositian of sausages with the addition of *L. plantarum* IIA-2C12 and *L. acidophilus* IIA-2B4

Variables	Lamb meat	With probiotic		Mean
		L. plantarum IIA-2C12	*L. acidophilus* IIA-2B4	
Fatty acid (w/w) %				
Total mono-unsaturated fatty acids (SFAs)				
Capric (C10:0)	0.09	0.09±0.01	0.08±0.00	0.09±0.01
Lauric (C12:0)	0.23	0.22±0.02	0.24±0.03	0.23±0.01
Myristic (C14:0)	2.22	2.12±0.16	2.28±0.15	2.20±0.11
Pentadecanoic (C15:0)	0.43	0.46±0.02[a]	0.39±0.01[b]	-
Palmitic (C16:0)	19.43	16.07±0.34	16.34±0.15	16.21±0.19
Heptadecanoic (C17:0)	1.08	0.90±0.05	0.82±0.03	0.86±0.06
Stearic (C18:0)	22.37	18.99±0.49	20.07±1.56	19.53±0.76
Arachidic (C20:0)	0.14	0.15±0.01	0.15±0.01	0.15±0.00
Heneicosanoic (C21:0)	0.04	0.06±0.01[a]	0.04±0.00[b]	-
Behenic (C22:0)	0.04	0.04±0.00	0.03±0.01	0.04±0.01
Total unsaturated fatty acids (UFAs)				
Total mono-unsaturated fatty acids (MUFAs)				
Myristoleic (C14:1)	0.04	0.05±0.01[a]	0.03±0.01[b]	-
Palmitoleic (C16:1)	1.17	1.22±0.11	1.07±0.06	1.15±0.11
Cis-10-Heptadecanoic (C17:1)	0.35	0.37±0.05	0.29±0.03	0.33±0.05
Oleic (C18:1n9c)	22.38	21.01±1.31	18.78±1.36	19.90±1.58
Cis-11-Eicosenoic (C20:1)	0.16	0.39±0.02[a]	0.34±0.01[b]	-
Total poly-unsaturated fatty acids (PUFAs)				
Linoleic (C18:2n6c)	2.49	2.59±0.18	2.44±0.16	2.52±0.11
Cis-11,14Eicosedienoic (C20:2)	0.04	0.04±0.00	0.04±0.00	0.04±0.00
Arachidonic (C20:4n6)		0.17±0.03	0.21±0.15	0.19±0.03
Total fatty acids	72.71	64.65±2.08	63.66±0.35	64.16±0.70
Total mono-unsaturated fatty acids (SFAs)		3.91±0.11	4.04±0.19	3.98±0.09
Total unsaturated fatty acids (UFAs)		3.22±0.21	2.90±0.22	3.06±0.23
Total mono-unsaturated fatty acids (MUFAs)		4.60±0.30	4.10±0.29	4.35±0.35
Total poly-unsaturated fatty acids (PUFAs)		0.93±0.07	0.90±0.11	0.92±0.02
Ratio of total SFAs/UFAs		1.43±0.26	1.41±0.26	1.42±0.01

Note: Means in the same row with different superscripts differ significantly (P<0.05); lamb meat: it was not analyzed statistically.

benefit for brain growth (Zhang *et al.*, 2010). It is interesting that the probiotics used in this study displayed the ability to lower cholesterol concentration. Lye *et al.* (2010) and Wang *et al.* (2014) reported that only some of LAB had ability to do so. Tsai *et al.* (2014) summarized the possible mechanism by which probiotic, including LAB, reduced the cholesterol. Firstly, probiotc may utilise bile-salt hydrolase (BSH) to deconjugate bile salt. Secondly, probiotics may assimilate cholesterol into bacterial cell membrane. Thirdly, probiotics might produce short-chain fatty acids during the growth period. Lastly, cholesterol might be converted into coprostanol.

The population of LAB on fermented sausages was 9.24 log cfu/g with the addition of *L. plantarum* IIA-2C12 and 9.18 log cfu/g with the addition of *L. acidophilus* IIA-2B4. Total population of LAB in this study met the requirement for being considered as probiotics according to Arief *et al.* (2014) stated that the population of the strains used to ferment lamb sausage were more than 10^8 cfu/mL. The LAB population of both sausages were higher than LAB population reported by Arief *et al.* (2008) using *L. plantarum* 1B1 in fermented lamb sausages. The results of this study indicated that in the production of lamb sausages, the cultures of *L. plantarum* IIA-2C12 and *L. acidophilus* IIA-2B4 were considered as better starters to increase the population of LAB. Nie *et al.* (2014) stated that LAB was the main microorganisms that played a role in the sausage fermentation process.

The population of *E. coli* in fermented sausage was 0.33 log cfu/g with the addition of *L. plantarum* IIA-2C12 or *L. acidophilus* IIA-2B4. According to the MLA (2003), population of *E. coli* that was acceptable was 3.6 log cfu/g. *Salmonella* spp was not found in fermented sausages on both products with the addition of *L. plantarum* IIA-2C12 or *L. acidophilus* IIA-2B4. *L. plantarum* produces antimicrobial subtances, called bacteriocin plantaricin, that can inhibit and kill pathogenic bacteria, such as *S. aureus* (Arief *et al.*, 2015b) and *E. coli* (Kia *et al.*, 2016; Arief *et al.*, 2013). According to the MLA (2003) and Arief *et al*. (2014b), with the absence of *Salmonella* spp, the product was considered to be safe for consumption. Accordingly, fermented lamb sausages in this study can be consumed safety because appropriate with MLA (2003).

Amino acids were formed due to proteolysis activity during fermentation process by LAB. Glutamic is an amino acid component which is dominant on both sausages with the addition of bacteria *L. plantarum* IIA-2C12

Table 4. Microbiological and physicochemical properties of fermented lamb sausages at refrigerator temperature storage

Variables	Day of Storage			
	Day-0	Day-7	Day-14	Day-21
LAB (log cfu/g)				
L. plantarum IIA-2C12	9.21±0.01^{a}	9.12±0.02^{b}	9.00±0.03^{cd}	8.95±0.01^{e}
L. acidophilus IIA-2B4	9.13±0.01^{b}	9.03±0.01^{c}	8.97±0.03^{de}	8.81±0.02^{f}
E. coli (log cfu/g)				
L. plantarum IIA-2C12	0.33±0.57	0.00±0.00	0.00±0.00	0.00±0.00
L. acidophilus IIA-2B4	0.33±0.57	0.00±0.00	0.00±0.00	0.00±0.00
Samonella spp (log cfu/g)				
L. plantarum IIA-2C12	Negative	Negative	Negative	Negative
L. acidophilus IIA-2B4	Negative	Negative	Negative	Negative
pH				
L. plantarum IIA-2C12	4.19±0.01^{ab}	4.16±0.04^{abc}	4.15±0.03^{bc}	4.14±0.02^{bc}
L. acidophilus IIA-2B4	4.20±0.02^{b}	4.17±0.01^{abc}	4.15±0.03^{bc}	4.13±0.04^{c}
a_w				
L. plantarum IIA-2C12	0.86±0.00^{a}	0.86±0.00^{ab}	0.85±0.01^{ab}	0.84±0.00^{b}
L. acidophilus IIA-2B4	0.85±0.01^{ab}	0.84±0.01^{b}	0.84±0.02^{b}	0.84±0.01^{ab}
Titratable acid (%)				
L. plantarum IIA-2C12	2.14±0.03^{cd}	2.23±0.03^{abc}	2.28±0.05^{ab}	2.35±0.16^{a}
L. acidophilus IIA-2B4	2.03±0.04^{d}	2.20±0.05^{bc}	2.22±0.03^{bc}	2.28±0.05^{ab}

Note: Means in the same row with different superscripts differ significantly ($P<0.05$); cfu/g= colony forming unit per gram.

and *L. acidophilus* IIA-2B4. Xu *et al.* (2008) suggested that amino acid contributed to the taste of fermentation products, such as glutamic. Protease activity in the meat or the addition of LAB in fermented sausage led to an increase in free amino acids (Candogan *et al*., 2009). Free amino acids also could affect the flavor of fermented sausages (Nie et *al*., 2014).

Tyrosine and lysine showed in the fermented sausage products with the addition of *L. plantarum* IIA-2C12 was larger than that of *L. acidophilus* IIA-2B4. The higher proteolysis activity of culture yielded, the higher amount of free amino acids released (Aro *et al*., 2010). Latorre-Moratalla *et al.* (2014) assumed that the indigenous activity of proteolytic enzymes in the raw material used in this study were similar. Hence, the differences in the profiles of some amino acids attributed to the distinct microbial proteolytic activities, including *L. plantarum* IIA-2C12 and *L. acidophilus* IIA-2B4. The dynamic of amino acid composition in the product is related to the metabolism process covering both catabolism and anabolism. For instance, the decreasing of some amino acid contents found in this study might be due to a high rate of anabolism in which amino acids were used by LAB for growing. This assumption is supported by Marathe & Ghosh (2009) that stated that microorganisms such as lactic acid bacteria utilized amino acids and vitamins for growth.

The sausage with the addition of *L. plantarum* IIA-2C12 had greater fatty acid component as compared with fermented sausage with the addition of *L. acidophilus* IIA-2B4. This difference might be due to the discrepancy on lipolytic activity of probiotics during fermentation. Stearic fatty acid is the dominant component in fermented sausages by the addition of *L. acidophilus* IIA-2B4 and oleic fatty acid is the dominant component in fermented sausages by the addition of *L. plantarum* IIA-2C12.

The addition of *L. plantarum* IIA-2C12 or *L. acidophilus* IIA-2B4 in lamb meat could detect the content of arachidonic fatty acids. Dhanapal *et al.* (2012) stated that the content of arachidonic fatty acid increased following the cooking process. Zhang *et al.* (2010) stated that the oxidation of fat was occurred on fermented lamb sausages and this situation was anticipated by using some seasoning that contained antioxidants to slow down the oxidation of fat.

Total population of LAB at the end of storage period is sufficient for probiotic LAB candidates to provide beneficial effects to the body. Parvez *et al.* (2006) found that probiotics LAB could prevent and had a therapeutic effect against diarrhea, protected against inflammation, and improved the immune system.

The addition of *L. plantarum* IIA-2C12 or *L. acidophilus* IIA-2B4 to sausage on storage day-0 found 0.33 log cfu/g of *E. coli*; while on day-7, 14, and 21, *E. coli* was not detected. Arief *et al.* (2015a) stated that *L. plantarum* IIA-2C12 and *L. acidophilus* IIA-2B4 had antimicrobial activity and good co-aggregation against pathogenic bacteria *E. coli*. According to Astawan *et al.* (2011), the use of culture of *L. plantarum* 2C12 and *L. acidophilus* 2B4 could cause a decrease in the number of *E. coli* populations. *Salmonella* spp bacteria was not found in fermented sausages on both of products with the addition of *L. plantarum* IIA-2C12 or *L. acidophilus* IIA-2B4 during storage. De Vuyst *et al.* (2008) suggested that fermented meat products with certain bacteria could inhibit the growth of pathogenic bacteria.

The pH value is associated with the production of lactic acid by LAB. Low pH values generally related to organic acids produced by LAB (Nie *et al*., 2014).

According to De Vuyst *et al.* (2008), fermented meat products with certain bacteria could inhibit the growth of pathogenic bacteria, indicated by the decrease of pH due to acidity and low a_w. The percentage of lactic acid on both the sausage with the addition of *L. plantarum* IIA-2C12 or *L. acidophilus* IIA-2B4 increased in during storage. Arief *et al.* (2010) stated that the increase of TA was caused by increase of LAB activity that produce lactic acid.

CONCLUSION

Population of *L. plantarum* IIA-2C12 and *L. acidophilus* IIA-2B4, isolated from local cattle in Indonesia, in the sausage could be categorized as a probiotic. The differences between the characteristics of physicochemical traits and microbiological quality of the sausage fermentation associated with the addition of *L. plantarum* IIA-2C12 or *L. acidophilus* IIA-2B4. The 21 days of storage at cold temperatures with probiotics addition could extend the shelf life and maintain quality of fermented sausage.

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