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The quality of fermented milk produced using intestinal-origin lactic acid bacteria as starters

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Abstract

The aim of this experiment was to evaluate the quality of fermented milk produced using intestinal-origin lactic acid bacteria (LAB) as starters. Fermentation was performed on pasteurised cow milk added with skim milk, constituting a total solid 18%, using a separate single starter of *Lactobacillus casei* strain AP, *Lactobacillus casei* strain AG, and *Pediococcus acidilactici* strain BE. The parameters observed were pH and acidity; nutritional quality, including protein, fat, and lactose content; product's viscosity; and total LAB count. The results showed that the different starter cultures employed did not affect the pH, acidity, fat and lactose contents of the products. The LAB starters affected protein contents and the viscosity of the fermented products. The highest score of viscosity (4.035,66±109.69 cP) was observed in fermented products using *Lactobacillus casei* strain AP as a starter, followed by products obtained using *Pediococcus acidilactici* strain BE (3.109,00±40.00 cP) and *Lactobacillus casei* strain AG (3.052,33±15.27 cP) as starters. Lactose and fat contents, acidity and pH, and total LAB count were not significantly different among fermented products. The average of the total LAB count was not different among products; however, the total LAB count increased during fermentation from 6.98±1.00 $\log_{10}$ CFU/ml to 8.15±0.61 $\log_{10}$ CFU/ml. In conclusion, the use of three strains of human-origin LAB as starters for dairy fermentation partially affected the physicochemical quality of the products, but not the microbiological qualities.

Introduction

Lactic acid bacteria (LAB) are the microorganisms employed as starter cultures for dairy fermentation. This group of bacteria has been used in many dairy fermented products such as cheeses, yogurt, sour milk, and kefirs, and nowadays they are popular as probiotics. As generally regarded as safe (GRAS) microorganisms, LAB are becoming as attractive host for producing desirable metabolites, enzymes, and other proteins for food industries. At present, approximately 400 diverse products derived from milk fermentation are consumed around the world.

Compared to fresh milk, fermented dairy products have higher nutritional value and bioavailability of nutrients due to activities of LAB in degrading macromolecules, resulting in availability of monomers. During fermentation, LAB produce lactic acid and increases acidity, thus inhibiting the growth of spoilage bacteria and conserving milk nutrients. As probiotics, the active cultures of LAB provide distinct health benefits beyond conventional nutrition (Chandan, 2006). Nowadays, probiotic-containing fermented milks such as yogurt and acidophilus milk are gaining increased attention. This is because these products not only provide available nutrients for the body but also offer health benefits beyond food nutrients.

According to Hill *et al*. (2014), probiotics are defined as 'live microorganisms that, when administered in adequate amounts, confer a health benefit on the host". Bacterial strains that are commonly used as probiotics are members of the genera *Lactobacillus* and *Bifidobacterium* (Roberfroid, 2000; Mercenier *et al*., 2003; Grajek *et al*., 2005). These two genera are typically chemoorganotrophic and ferment carbohydrates with lactic acid as a major end product (Fuller, 1989). *Lactobacillus* belongs to the group of LAB with G + C content between 32% and 51%, while *Bifidobacterium* is a part of the *Actinobacteria* phylum and phylogenetically distinct from LAB, with a G + C content ranging from 42% to 67% (Biaviti and Mattarelli, 1991; Gomes and Malcata, 1999; Borriello *et al.*, 2003). Of the 106 species in *Lactobacillus*, 56 are potential probiotics, while of the 30 species in *Bifidobacterium*, 8 are

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potential probiotics (Otieno, 2011).

Health-associated benefits of consuming probiotics have previously been reported such as the capability to reduce blood serum cholesterol (Anderson and Gilliland, 1999), decrease the prevalence of allergies (Parvez *et al*., 2006), reduce risks of certain cancers (Wollowski *et al.*, 2001; Ohashi *et al*., 2002; Xiao *et al*., 2006), and to stimulate the immune system (Gill, 1998; Nagao *et al*., 2000; Pareira *et al*., 2003). To perform as probiotics, bacterial strains must have specific criteria, that is non-pathogenic derived from human intestinal if probiotics would be administered for human consumption (Collins *et al.*, 1998; Dunne *et al*., 1999; Dunne *et al*., 2001). As such, probiotics isolated from human intestines are necessary to be applied as starters for milk fermentation. A number of bacterial strains for probiotics have been isolated from the gastrointestinal track (GIT) of humans (Margolles *et al*., 2009). Widodo *et al.* (2012a, 2012b) have previously reported the isolation and identification of LAB from the faeces of infants consuming breast milk. Widodo *et al.* (2012b; 2014) also reported that some of those isolates were potential probiotics, and identified as *Lactobacillus casei* strains AP and AG and *Pediococcus acidilactici* strain BE.

In Indonesia, a number of LAB species obtained from commercial sources have been used for milk fermentation, for example *Lactococcus lactis* in cheese production and *Lactobacillus casei* for souring milk. LAB species used in Indonesian dairy productions are usually isolated from food products; however, there have been no reports of use of LAB isolated from GIT. In this paper, we report the application of those selected intestinal-origin probiotic strains as starters for milk fermentation and determine the changes produced in milk as a result of fermentation with these bacteria.

Materials and Methods

Fresh milk and bacterial starter cultures for fermentation

Fresh milk was obtained from a local dairy farm in Yogyakarta, Indonesia, and its quality was evaluated prior to being used for fermentation. The human-origin LAB used in this study were *Lactobacillus casei* strains AP and AG and *Pediococcus acidilactici* strain BE, which were obtained from previous experiments (Widodo *et al*., 2012a; 2012b; 2014). Bacterial cultures were grown overnight (~12 hours), and cultures at this stage of growth were used for fermentation.

Milk fermentation

Skim milk powder was added to fresh milk to obtain 18% total solid (TS) and pasteurised at 80°C for 10 minutes. After cooling, 500 ml of heat-treated milk were separately inoculated with: 1) 10% (v/v) culture of *Lactobacillus casei* strain AP, 2) 10% (v/v) culture of *Lactobacillus casei* strain AG, and 3) 10% (v/v) culture of *Pediococcus acidilactici* strain BE. Fermentation was conducted at 37°C for 8 hours, and the fermented products were then stored at 4°C.

Physicochemical analysis of fermented products

Six parameters were measured after fermentation: protein, fat, and lactose contents, acidity, pH, and viscosity. Protein was analysed based on the Micro–Kjealdahl method (AOAC, 1995), fat analysis was carried out based on the Babcock method (Sudarmadji *et al.*, 1997), and lactose analysis was performed using the titration method (Sudarmadji *et al*., 1997). Viscosity measurements were performed according to Tuncturk (2009) by using a Brookfield digital rheometer model DV III (Brookfield Engineering Laboratories, Inc., Massachusetts, USA). All viscosity measurements were expressed in centiPoise (cP), performed in triplicate, and averaged.

pH and titrable acidity assay

Titrable acidity and pH were measured hourly during fermentation. The pH value was measured using a Hanna pH-meter potensiometric method (Hadiwiyoto, 1994). Titrable acidity was measured as percentage (%) lactic acid fermentation by titrating with 0.1 N NaOH using phenolphtalein as an indicator (Lampert, 1975).

Cell viability of LAB

Cell viability of LAB before and after fermentation was measured by measuring total plate count (TPC) on the de Man Rogosa Sharpe (MRS) agar after a series of dilutions of samples with sterilised 0.8% sodium chloride (NaCl). The plates were incubated in a micro-aerobic condition for 48 hours at 37°C, and the colonies that appeared were counted.

Data analysis

Data on pH and acidity were analysed statistically using paired T-test with a statistical significance accepted at $P < 0.05$. Data of physicochemical quality was subjected to analysis of variance (One-Way ANOVA) with statistical significance accepted at $P < 0.05$. Data of cell viability was analysed using completely randomized factorial design and followed by Duncan's new Multiple Range Test, with statistical significance accepted at $P < 0.05$.

Results and Discussion

Quality of fresh milk

The quality of fresh milk was analysed, and the results are presented in Table 1. The quality of cow milk observed here was in agreement with previous studies on the quality of cow milk (Park *et al*., 2007; Widodo *et al*., 2013) and within the range of Indonesian national standards (SNI) for cow milk (Badan Standarisasi Nasional, 1998). The Indonesian national standard for fresh milk requires that the alcohol test (70%) must be negative, have specific gravity at 27.5°C must be minimally 1.0280, 3.0% fat content, 2.7% protein content, and 11% TS (Badan Standarisasi Nasional, 1998). Park et al. (2007) reported a good quality of cow milk with 12.6% TS that consisted of 3.6% fat, 3.2% protein, 4.7% lactose, and 87.4% water content. Good quality cow milk usually has specific gravity at 1.023–1.039, pH at 6.65–6.71, and acidity at 0.22–0.25 (Park *et al.*, 2007).

Table 1. Quality of fresh milk

Parameter observed	Average
Alcohol test at 70%	Negative
pH	6.37±0.07
Acidity (%)	0.23±0.03
Fat content (%)	3.35±0.21
Total solid (%)	12.32±0.02
Lactose content (%)	4.94±0.09
Specific gravity (%)	1.0270±0.01
Protein content (%)	3.51±0.08

n= 3

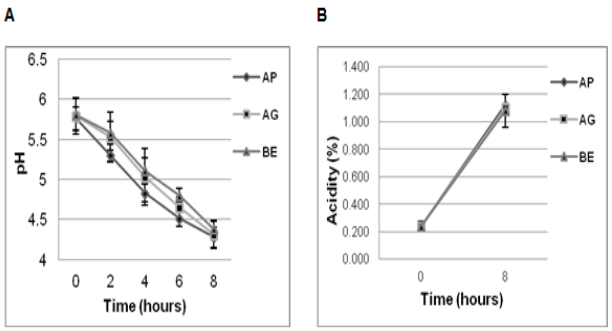

Figure 1. pH (A) and acidity (B) during fermentation; AP: *Lactobacillus casei* strain AP, AG: *Lactobacillus casei* strain AG, BE: *Pediococcus acidilactici* strain BE

Changes during fermentation

Figure 1 shows that the duration of fermentation significantly affected pH ($P<0.05$) (Figure 1A) and acidity (Figure 1B). A decrease in pH was followed by an increase in the acidity during fermentation. The average pH at the beginning of fermentation for *Lactobacillus casei* strains AP and AG and *Pediococcus acidilactici* strain BE was 5.76±0.14, 5.79±0.23, and 5.80±0.20, respectively, whereas after 8 hours of fermentatation it decreased to 4.27±0.13, 4.31±0.17, and 4.37±0.08, respectively (Figure 1A). The titrable acidity of fresh milk before fermentation was 0.24±0.03%, whereas at the end of fermentation it increased to 1.09±0.04% for *Lactobacillus casei* strain AP, 1.05±0.07% for *Lactobacillus casei* strain AG, and 1.05±0.10% for *Pediococcus acidilactici* strain BE (Figure 1B). Differences in the starters did not significantly affect the pH and acidity of the products.

The required minimum pH for fermented dairy products is 4.6 or lower (Chandan, 2006). Figure 1A shows that the final pH of fermented milk was below 4.6, suggesting that the products were within the required standard. Meanwhile, the acidification of fermented milk by those three different starters took 6–8 hours to reach pH 4.5 or below, which is a bit slower compared with the usual 5–6 hours to obtain the same pH. Acidification occurred due to LAB activities in degrading lactose to produce organic acids. LAB strains have the ability to ferment lactose into lactic acids, resulting in the increased acidity and decreased pH of fermented products (Fadela *et al.*, 2010). The decrease in pH was concomitant with the increase in acidity (Figure 1B). The Indonesian national standard (SNI) of acidity of fermented products was 0.5 to 2.0 (Badan Standarisasi Nasional, 1998), suggesting that the acidity of these products was within the range of SNI.

Physicochemical quality of fermented milk

Fermentation was conducted for 8 hours, and the quality of the fermented products was then evaluated for nutritional and chemical qualities. Table 2 shows that the different starters did not affect the lactose, fat, and FFA contents of the products ($P>0.05$). However, the different starters did effected the protein content and viscosity of the products ($P<0.05$). The lactose and fat contents of the fermented products were lower than in fresh milk, suggesting that LAB starters degraded those macromolecules during fermentation. The lactose is known to be fermented enzymatically to produce lactic acid and adenosine triphosphate (ATP) as energy while fat might have been degraded with lipase to produce monomers of fatty acids that are further used for cellular metabolisms. The data showed no differences in fat content among the products fermented with different starters, suggesting that all starters had a similar level of fat degradation, resulting in the same level of FFA (Table 2).

The protein content was also affected by

Table 2. Physicochemical quality of fermented milk

Starter	Lactose (%)^{ns}	Protein (%)	Fat (%)^{ns}	FFA (%)^{ns}	Viscosity (cP)
Lactobacillus casei strain AP	3.97±0.66	4.66±0.03^{a}	2.20±0.40	4.14±1.16	4035.7±109.7^{a}
Lactobacillus casei strain AG	4.05±0.49	4.80±0.10^{b}	2.40±0.34	4.38±2.17	3052.3±15.27^{b}
Pediococcus acidilactici strain BE	4.02±0.62	5.65±0.18^{c}	2.56±0.25	5.04±1.13	3109.0±40.00^{b}
Average	4.02±0.51	5.03±0.47	2.38±0.33	4.45±1.43	3399.0±481.7

^{ns} = not significant (P >0.05)
^{ab} = different superscript on the same column shows significant differences (P < 0.05)

the starters. Fermented products with the starter *Lactobacillus casei* strain AP had the lowest protein content (4.66±0.03%) compared with those fermented by *Lactobacillus casei* strain AG (4.80±0.10%) and *Pediococcus acidilactici* (5.65±0.18%) (Table 2). This suggests that strain AP had the highest protein degradation ability, possibly due to proteinase activity. The final protein content in the fermented products was influenced by the level of skim milk powder added. Since fresh milk was fortified with skim milk powder to obtain 18% TS, these additions might have increased the protein content in the fermented products (Table 2).

Further, the results show that viscosity of the fermented products was affected (P<0.05) by the starter cultures used (Table 2), suggesting the influence of the starter cultures on viscosity. The highest viscosity at 4035.7±109.7 centi Poise (cP) was observed in products fermented by *Lactobacillus casei* strain AP, followed by products fermented by *Lactobacillus casei* strain AG and *Pediococcus acidilactici* strain BE, which have the same viscosity of products (Table 2). Viscosity is the measurement of a fluid's internal resistance to flow that is designated in units of centipoise (cP) or millipascal second (mPa s). One (1) cP is 1 mPa s, while 1 poise is equal to 0.1 Pascal second (Pa s). The viscosity of yogurt is 100–2.825 cP according to the products, while condensed milk has 2600 cP, and milk whey sugar 800–1500 cP (Djurdjević *et al*., 2002). According to Djurdjević *et al.* (2002), a number of factors affect the viscosity of products, including the level of acidity. However, in this experiment, all products showed similar levels of acidity, suggesting that other factors may have influenced viscosity of products.

Total viable lactic acid bacteria

Fermentation increased the total LAB count from 6.98±1.00 (log_{10} CFU/ml) at the beginning of fermentation to 8.15±0.61 (log_{10} CFU/ml) at the end of fermentation (Table 3). Overall, the final population of LAB in the fermented products was within the recommended level to function as probiotics: 7 log_{10} CFU/ml (Kailasapathy *et al*., 2000; Shah, 2000; Birollo *et al*., 2000; Bibiloni *et al*., 2001; Homayouni *et al.*, 2008). Table 3 shows that the total viable LAB was not affected by the different starters used (P>0.05), suggesting that all starters had a similar growth rate during fermentation.

Table 3. Cell viability of LAB

Starter	Fermentation		Average ^{ns}
	before (log CFU/ml)	after (log CFU/ml)	
AP	6.62±0.32	7.89±0.51	7.25±0.89
AG	7.26±1.39	8.08±0.41	7.69±0.58
BE	7.05±1.30	8.47±0.91	7.76±1.00
Average	6.98±1.00^{a}	8.15±0.61^{b}	

^{ns} = not significant (P >0.05)
^{ab} = different superscript on the same row shows significant differences (P < 0.05)

During 8 hours of fermentation, *Pediococcus acidilactici* strain BE increased 1.42 log_{10} CFU/ml viable cells, while *Lactobacillus casei* strain AP increased 1.27 log_{10} CFU/ml viable cells and *Lactobacillus casei* strain AG increased 0.82 log_{10} CFU/ml (Table 3). The highest increase in Pediococcus acidilactici strain BE might be related to its survival ability in organic acids. Ng *et al.* (2010) reported that *L. acidophilus* showed good survival, where low pH caused by the accumulation of organic acids was not a critical factor affecting viability. In another study, Beal *et al.* (1999) explained that the total viable *L. bulgaricus* in yogurt is higher at pH 4.8 than *S. thermophilus*, indicating that *L. bulgaricus* is more resistant to acidic conditions.

Conclusion

The physicochemical quality of the fermented products was partially affected by the starter cultures used for fermentation. The use of different starters affected the protein content and viscosity of the products. The total viable cells after fermentation was not different between *Lactobacillus casei* strains AP and AG and *Pediococcus acidilactici* strain BE,

although the latter starter showed the highest increase during fermentation. This study opens a future application of intestinal-origin LAB as starters for dairy fermentation.

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