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Physicochemical and microbiological properties of fermented milk using lactic acid bacteria isolated from *dangke*

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Abstract. *Syah SP, Mukhlisah AN, Ningtiyas WD, Irfan M, Ananda N, Amalia A, Tasmin. 2024. Physicochemical and microbiological properties of fermented milk using lactic acid bacteria isolated from* dangke. *Biodiversitas 25: 3876-3883.* This study aimed to investigate the effect of milk and starter culture type (commercial and lactic acid bacteria (LAB) isolated from *dangke*). Two strains of LAB (*Lactobacillus fermentum* A323L and B111K) and commercial culture were tested for physicochemical and microbiological properties in milk fermentation. The results showed that the type of culture had a significant effect ($p<0.05$) on pH, titratable acidity, WHC, syneresis, viscosity, and total number of LAB, while the type of milk had a significant effect ($p<0.05$) on almost all variables, except for protein content and L* (lightness) of fermented milk. Two strains of LAB isolate from *dangke* produce pH values, titratable acidity, and viscosity that were relatively good compared to commercial cultures and even tended to be better, specifically in terms of the total number of LAB from fermented milk produced. Skimmed milk produced the best-fermented milk in terms of composition, color, pH value, acidity, and viscosity, but was not very good as a medium for LAB growth due to the low number of microbes compared to the other two types, specifically UHT (Ultra High Temperature) low-fat. However, the number of LAB in skimmed milk met the quality standards for fermented milk products. In conclusion, both strains of LAB isolated from *dangke* could be used as starter cultures in the manufacture of fermented milk with good physicochemical and microbiological properties.

Keywords: *Dangke*, fermented milk, lactic acid bacteria, microbiology, physicochemical

INTRODUCTION

Milk is considered a nearly perfect food due to the complete nutritional composition and high level of digestibility. Although it has less protein compared to meat and eggs, milk provides almost all the essential amino acids required for human growth (Walstra et al. 2006). Milk is not a favorite food among Indonesians despite the high nutritional value. This is because in addition to the relatively high price, many Indonesians experience difficulty in digesting carbohydrates and proteins, a condition known as lactose and protein intolerance. One solution to improve milk utility, acceptability, shelf life, and economic value is through fermentation technology, using beneficial microbes that can be isolated from various food sources (Widodo 2002). Fermentation process can create products with higher nutritional value, easier digestibility, and a unique taste more appealing than fresh milk while remaining safe to consume (Legarová and Kouřimská 2010; Nemati et al. 2023; Yang et al. 2023b).

Fermented milk is a food group that is currently developing very rapidly with widely reported influence on health and wellness trends (Savaiano and Hutkins 2021; Hadjimbei et al. 2022), specifically the probiotic effects (Sakandar and Zhang 2021). Furthermore, lactic acid bacteria group (LAB) is widely used in the production of fermented milk. LAB has been widely used as a starter microbe in the manufacture of various dairy products, which are generally fermented milk including yogurt, kefir, acidophilus milk, *Lactobacillus casei* milk, Bifidus milk, and others (Widodo 2002; Surajudin et al. 2008; Lin et al. 2017; Nemati et al. 2023; Yang et al. 2023b; McGovern et al. 2024). This group of bacteria can reduce the number of pathogenic microbes (Luo et al. 2022; Jensen et al. 2023; Wang et al. 2023b) and the use in fermentation process produces products having high nutritional value, with a unique product taste (Nemati et al. 2023; Yang et al. 2023b). LAB could also produce fermented products that have functional benefits for health (probiotics), by decreasing the number of pathogenic microbes in the digestive tract to balance the growth of beneficial microflora in the intestines (Fatmarani et al. 2018; Aini et al. 2021; Mgomi et al. 2023; Kim et al. 2024).

A strain of LAB with great potential in the production of fermented milk is *dangke* isolate. *Dangke* is a traditional dairy product from Enrekang District. It is a cheese product made traditionally and passed down from generations by residents and considered a local wisdom (Sabil et al. 2017; Yusuf et al. 2022). As a dairy product, *dangke* naturally contains numerous LAB. Various studies have examined and successfully isolated LAB from *dangke* (Nur et al. 2017; Zakariah et al. 2019; Zakariah et al. 2022) using molecular identification techniques (Nur et al. 2017; Zakariah et al. 2022). In this study, different LAB strains were successfully isolated from *dangke*, specifically two strains, namely *Lactobacillus fermentum* A323L and B111K which have probiotic potential (Syah et al. 2017a, 2017b). Generally, the production of probiotic fermented milk products using both strains has not been studied. The use of indigenous BAL from *dangke* is considered an alternative local starter culture in producing innovative probiotic fermented milk products. There is currently a high demand in the market for products that offer health benefits (Alsubhi et al. 2023; Joshi et al. 2024). The production of food and beverages fermented by LAB is increasing because of the bacterial activity and metabolites produced that offer product-specific characteristics (de Souza et al. 2023; Joshi et al. 2024). Therefore, this study offers insights into the production of functional fermented milk products based on LAB strains. The products isolated were subjected to physicochemical (composition and color, pH, titratable acidity, whey expulsion, and viscosity) and microbiological examinations.

MATERIALS AND METHODS

Materials

Materials used include skimmed milk, UHT (Ultra High Temperature) full cream milk, UHT low-fat milk, commercial culture (Lactina®, Bulgaria), and LAB isolated from *dangke* (*Lactobacillus fermentum* strain A323L and B111K) (Syah et al. 2017a, 2017b).

Experimental design

This study used an experimental method and a completely randomized design with a 3×3 factorial arrangement and 5 replications, resulting in a total of 45 experimental units. The first factor was the type of milk (skimmed milk, UHT full cream and UHT low-fat obtained from supermarkets), while the second factor was the type of starter culture (commercial culture, *L. fermentum* strain A323L, and *L. fermentum* strain B111K). The treatment combinations were as follows; P0S1: Skimmed milk + 3% commercial culture, P0S2: UHT full cream milk + 3% commercial culture, P0S3: UHT low-fat milk + 3% commercial culture, P1S1: Skimmed milk + 3% *L. fermentum* strain A323L, P1S2: UHT full cream milk + 3% *L. fermentum* strain A323L, P1S3: UHT low-fat milk + 3% *L. fermentum* strain A323L, P2S1: Skimmed milk + 3% *L. fermentum* strain B111K, P2S2: UHT full cream milk + 3% *L. fermentum* strain B111K, P2S3: UHT low-fat milk + 3% *L. fermentum* strain B111K.

Starter culture activation

Starter culture activation was conducted in several stages based on the method described by Dai et al. (2016). These included culture propagation, stock culture preparation, mother culture, and bulking culture preparation.

Culture propagation

Culture propagation was conducted with three incubation periods. Each stock of dried bacterial culture from the three LAB strains (commercial starter as well as indigenous LAB strains A323L and B111K from *dangke*) was removed from the packaging or ampoule, then introduced into MRS broth media and homogenized using a vortex mixer. Subsequently, the mixture was incubated at 37ºC for 24 hours and this process was repeated three times to ensure optimal LAB growth.

Stock culture preparation

The propagated LAB was centrifuged at 4,000 rpm for 15 minutes then the cell pellet was collected. About 600 µL was taken and mixed with 400 µL of 20% glycerol in a 1.5 mL vial, followed by homogenization using a vortex mixer. The mixture was subsequently stored at -20ºC and the stock culture can be preserved for up to 6 months (Lewis and Fleming 1995)

Mother culture and bulking preparation

Mother culture was prepared by growing LAB in skimmed milk at a concentration of 12% (w/v), achieved by mixing 120 g with 1 liter of distilled water. The reconstructed skimmed milk was sterilized using an autoclave at 121ºC and 1 atm for 15 minutes, then cooled to 40ºC. A 3% inoculum of LAB culture from the stock was added to the sterile reconstructed skimmed milk, which was incubated for 24 hours at 37ºC. The culture was quantified to determine the initial amount of LAB used. About 1 mL of the mixture was taken to calculate the number of microbes that grew before being used as a starter for fermented milk production. The quantification was performed using the plate count method according to the method used in a previous study (Yousef and Carolyn 2023). The bulking culture was prepared to increase the quantity of starter cultures used each time in the production of fermented milk. A 3% inoculum of the culture was added to 100 m of skimmed milk (20% w/v) and then homogenized. The mixture was subsequently incubated at 37ºC for 24 hours and ready to be used for making fermented milk (Nemati et al. 2023).

Fermented milk preparation

Fermented milk was prepared using the yogurt preparation method based on Nemati et al. (2023) with slight modifications. The primary ingredients for making fermented milk included skimmed, UHT full cream, and UHT low-fat milk with a 3% LAB starter. A total of 350 mL milk (skimmed, UHT full cream, and UHT low fat) was placed into a glass container, sterilized in an autoclave at 121ºC and 1 atm for 15 minutes, then allowed to cool at 45ºC. Milk was supplemented with commercial cultures and LAB isolated from *dangke* according to the treatment protocol. The mixture was homogenized, incubated at 45ºC until a pH of 4.6 to 4.7 was reached (approximately 12 hours), and stored at 4ºC.

Composition and color analysis

Composition and color analysis were carried out after the preparation of fermented milk. Protein and fat were analyzed using Lowry and Kjedahl methods (AOAC 2010), while total solid (TS) was analyzed using oven-drying methods (Badan Standardisasi Nasional Indonesia 2009). L* (lightness), a* (red/greenness), and b* (yellow/blueness) values were measured using a digital colorimeter (NAKEAP Portable Colorimeter Digital, CS-220, China).

pH and Titratable acidity (TA) measurement

A milk pH meter (HM Digital PH-80, USA) was used to measure pH changes in fermented milk products. Before use, pH meter was calibrated with a 7.00 buffer standard at a temperature of 22-24°C. Titratable acidity (TA) was measured according to the method described by Nemati et al. (2023).

Whey expulsion analysis

Water Holding capacity (WHC)

WHC measurement was carried out using the centrifugation method according to Rahman et al. (2024). A total of 10 g fermented milk was centrifuged at a temperature of 20ºC at 5000 rpm for 10 minutes. WHC value was calculated using the formula:

$$\text{WHC (\%)} = \frac{\text{Sample weight - Supernatan weight (g)}}{\text{Sample weight (g)}} \times 100$$

Syneresis

Syneresis in fermented milk was measured following a method proposed by Rahman et al. (2024). A 10 g sample of fermented milk was centrifuged for 10 minutes at 4ºC and 5,000 rpm then the resulting supernatant was weighed. The syneresis value was calculated as the weight of the supernatant divided by the initial weight of the sample.

Viscosity measurement

Viscosity measurement was conducted according to Nemati et al. (2023). A total of 250 mL fermented milk sample was prepared in a container, and viscosity was measured using a NDJ-8S Digital Rotary Viscometer (Shanghai Yueping Scientific Instrument, Shanghai, China). The end of viscotester was inserted into the container with fermented milk and allowed to rotate until the indicator needle stabilized at a specific scale. Measurements were taken for 30 seconds at a speed of 20 rpm and the recorded scale indicated viscosity of the sample, measured in millipascal seconds (mPas).

Microbiological analysis

LAB were enumerated using a standard plate count method according to Yousef and Carolyn (2023). About 1 mL sample of fermented milk was diluted in 9 mL of 0.1% buffered peptone water (Merck, Germany). The dilution series was prepared up to 10^{-8} (10^{-6}, 10^{-7}, and 10^{-8}). A total of 1 mL from each dilution was transferred into a sterile petri dish and then combined with 12-15 mL of de Man, Rogosa, and Sharpe Agar (Merck, Germany), followed by homogenization. The mixed media was allowed to solidify before being incubated at 37ºC for 24 to 48 hours. Plates with 25 to 250 colonies were counted, and the results were expressed as colony-forming units per milliliter (CFU/mL) of the inoculated sample.

Data analysis

All obtained data were subjected to statistical analysis using analysis of variance (ANOVA) with general linear model procedures in IBM SPSS Statistics® version 25. The Tukey HSD test was used for multiple range comparisons (p<0.05) to identify significant differences among the measured parameters.

RESULTS AND DISCUSSION

The statistical analysis showed that the type of culture had a significant effect on pH, titratable acidity, WHC, syneresis, viscosity, and total lactic acid bacteria (LAB) count, while the type of milk had a significant effect on all variables except for protein content and color (L* - lightness) of fermented milk.

Composition and color

Based on the results, the composition, and color of fermented milk are presented in Table 1. The highest TS and fat values were observed in skimmed milk and lowest in UHT low-fat milk treatment, ranging from 34.40 to 38.72%, and 2.41 to 2.57% respectively. On the other hand, the lowest TS and fat values were found in UHT low-fat milk, ranging from 33.49 to 35.05% and 2.41 to 2.57%, respectively. The highest color values for both a* (red-green) and b* (yellow-blue) were observed in skimmed milk treatment, with values of 0.35 to 0.71 and 13.71 to 15.02, while the lowest color values were observed in UHT low-fat and full cream treatments, with a* (-0.68) and b* (10.05), respectively.

Table 1. Composition and color of fermented milk

Samples		TS (%)	Fat (%)	Protein (%)	Color		
					L*	a*	b*
KK	SS	34.40±10.84[b]	0.07±0.09[a]	12.33±1.41	81.04±0.50	0.39±0.33[c]	14.31±0.50[b]
	FC	35.06±0.79[a]	2.57±0.53[b]	10.72±3.15	86.93±1.44	-0.40±0.17[b]	10.22±0.90[a]
	LF	35.06±0.87[a]	0.18±0.12[a]	13.53±0.66	83.46±0.52	-0.68±0.21[a]	11.22±0.55[a]
KA	SS	35.19±1.06[b]	0.08±0.07[a]	9.91±1.64	83.21±2.99	0.35±0.78 [c]	13.71±2.77[b]
	FC	38.55±6.54[a]	3.23±0.65[b]	12.01±6.75	87.48±0.51	-0.62±0.39[b]	10.05±0.37[a]
	LF	33.49±3.23[a]	0.42±0.03[a]	10.69±0.71	84.51±1.86	-0.53±0.34[a]	11.57±1.13[a]
KB	SS	38.72±6.71b	0.12±0.07[a]	7.56±0.69	81.33±0.71	0.71±0.53[c]	15.02±0.91[b]
	FC	37.84±6.51[a]	2.41±0.58[b]	10.17±2.11	84.19±7.47	-0.20±0.30[b]	10.20±0.23[a]
	LF	34.26±2.57[a]	0.28±0.14[a]	11.91±3.66	84.07±0.43	-0.83±0.26[a]	11.52±0.43[a]

Notes: KK: Commercial culture; TS: Total solid; KA: *L. fermentum* strain A323L; KB: *L. fermentum* strain B111K, SS: Skimmed milk; FC: UHT full cream; LF: UHT low fat. Means values in the same column with different superscript letters are significantly different (p<0.05)

pH and titratable acidity

pH and titratable acidity values are presented in Figure 1 where the highest titratable acidity was shown by skimmed milk across all types of microbial starter cultures used, specifically in *L. fermentum* A323L and B111K cultures, peaking at 2.30% and 2.26%. On the other hand, UHT full cream milk showed the lowest stable acidity, with titratable acidity ranging around 1.38 to 1.41%. This indicates that skimmed milk can produce fermented milk with a high lactic acid content, while full cream milk produces low lactic acid content. THE skimmed milk had a higher pH value (around 4.5-4.3) compared to UHT full cream and low-fat milk. The results also showed that UHT full cream milk tended to have the lowest pH value (around 4.0), suggesting milk with a higher fat content tends to produce lower pH. UHT low-fat milk showed moderate pH and acidity values.

Expulsion of serum

As shown in Figure 2, WHC value of fermented milk produced from UHT full cream milk with commercial cultures and *L. fermentum* A323L culture had the highest values, at 52.22% and 48.32%, respectively. Meanwhile, the lowest WHC value was shown by fermented milk made from UHT low-fat milk at 29.03%. In line with WHC values, syneresis of fermented milk also showed similar results (Figure 2), where the lowest value was observed in fermented milk produced from UHT full cream milk, with the lowest syneresis value shown by *L. fermentum* B111K culture at 47.46%.

Viscosity

As shown in Figure 3, The viscosity values of fermented milk ranged from 280.40 to 13,548.00 mPa.s, equivalent to 0.28040 to 13.548 Pa.s, with the highest viscosity observed in skimmed milk fermented with *L. fermentum* A323L. The lowest viscosity values were produced from UHT low-fat milk fermented using commercial culture and *L. fermentum* B111K culture, with values of 304.20 and 280.40 mPa.s equivalent to 0.30420 to 0.28040 Pa.s, respectively. However, the results of this study showed a lower viscosity than the research conducted by Nemati et al. (2023), who obtained a yogurt viscosity value of 70.9 to 71.4 Pa.s, equivalent to 70,900 to 71,400 mPa.s. Similarly, the research conducted by Vicent (2024) indicated that the viscosity values of soy yogurt range from 500 to 3,500 Pa.s, which is equivalent to 500,000 to 3,500,000 mPa.s.

Number of lactic acid bacteria

The *L. fermentum* B111K starter culture showed a more significant increase in the number of LAB across all types of milk (Figure 4), although skimmed and UHT full cream milk yielded almost similar results. The commercial culture had the lowest LAB count in all types of milk. This indicates that the commercial culture is less effective in fermenting milk compared to the other cultures. UHT low-fat milk showed more variation in LAB counts, but UHT low-fat milk had the highest microbial count, with microbial growth ranging from 3.4×10^7 CFU/mL to 2.1×10^9 CFU/mL.

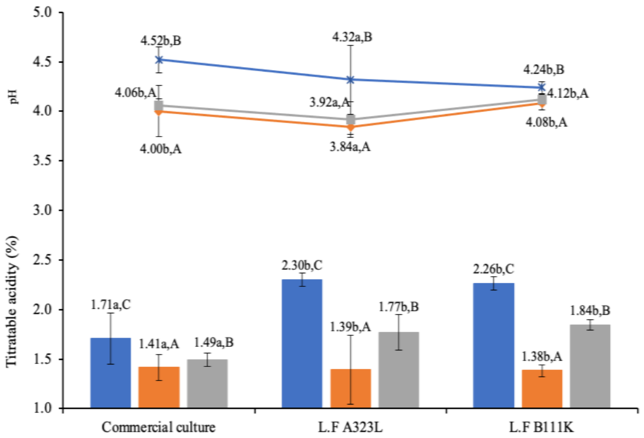

Figure 1. pH and titratable acidity of fermented milk. Values are expressed as mean ±SD. L.F: *L. fermentum.* The number with letters a, and b showed a significant difference (p<0.05) among starter culture treatments. The number with letters A, and B showed a significant difference (p<0.05) among milk type treatments. (■ - ✱): Skimmed milk; (■ - ◆): UHT full cream; (■ - ■): UHT low fat

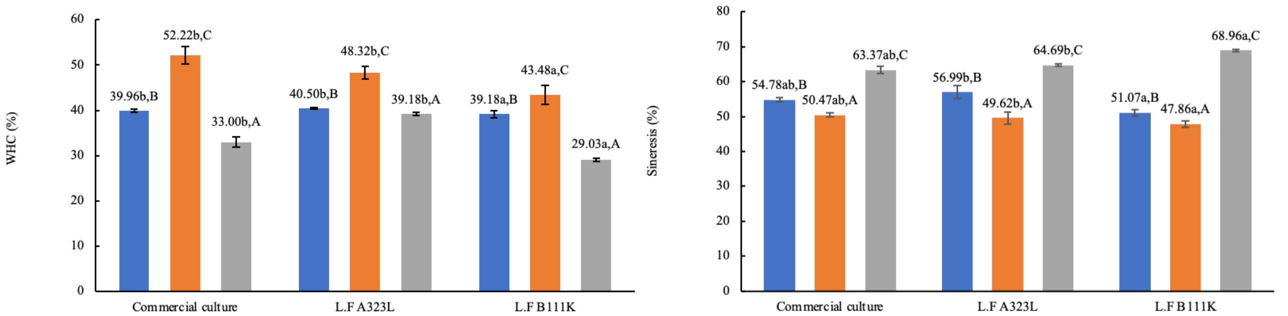

Figure 2. WHC and syneresis of fermented milk. Values are expressed as mean ±SD. L.F: *L. fermentum.* The number with letters a,b, and c showed a significant difference (p<0.05) among starter culture treatments. The number with letters A, B, and C showed significant differences (p<0.05) among milk-type treatments. (■): Skimmed milk; (■): UHT full cream; (■): UHT low fat

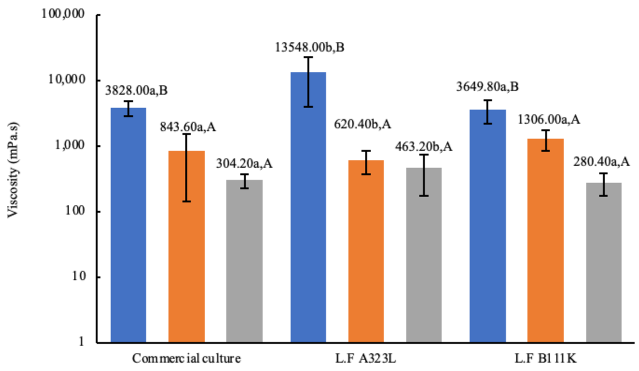

Figure 3. Viscosity of fermented milk. Values are expressed as mean ±SD. L.F: *L. fermentum*. The number with letters a, and b showed a significant difference (p<0.05) among starter culture treatments. The number with letters A, and B showed significant differences (p<0.05) among milk type treatments. (■): Skimmed milk; (■): UHT full cream; (■): UHT low fat

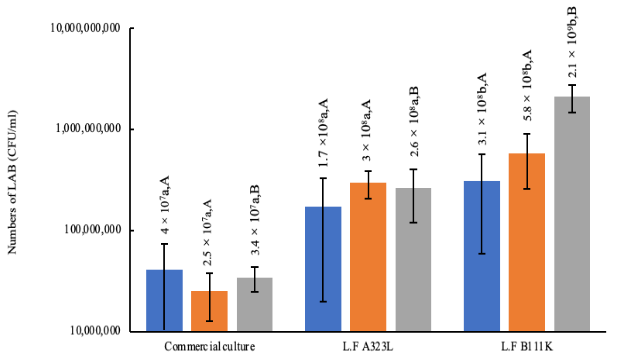

Figure 4. The amount of LAB in fermented milk. Values are expressed as mean ± SD. L.F: *L. fermentum*. The number with letters a, and b showed a significant difference (p<0.05) among starter culture treatments. The number with letters A, and B showed significant differences (p<0.05) among milk type treatments. (■): Skimmed milk; (■): UHT full cream; (■): UHT low fat

Discussion

The three LAB starter cultures showed the same effect on TS, fat, and protein content. This indicates that *L. fermentum* A323L and B111K cultures have the same effect as the commercial culture in fermenting milk. The highest TS value was observed in skimmed milk, with the highest value being 38.72%. The fat content was influenced by the type of milk used as the raw material for making fermented milk (Wang et al. 2023a). The highest fat content was found in UHT full cream milk, containing high fat. The percentage of protein content across all samples did not show any difference, indicating that the protein content produced was the same. In general, composition of fermented milk produced from the two LAB isolates had the same quality as the commercial culture. TS, fat, and protein values also met the quality standards set by the Codex Alimentarius for fermented milk (CODEX STAN 234-2003) and SNI 2981:2009 with a minimum of 8.2% for TS (BSN 2009), less than 10% for fat, and a minimum of 2.7% for protein (FAO-WHO Committee 2012).

The TS is a solid fraction of fermented product derived from a mixture of ingredients and nutrients contained in milk. The elevated TS in fermented milk may be caused by the nutritional composition of the ingredients used, which increases as fermentation process progresses. According to Wirdayat and Rosida (2024), the residual total sugar, lactic acid, and organic acids formed are calculated as TS. Fermentation process leads to bacteria activity breaking down milk substrate, resulting in the hydrolysis of carbohydrates and proteins into simpler compounds which causes TS to increase (Chen et al. 2022). The three LAB cultures used were highly effective in fermenting skimmed milk, as evidenced by titratable acidity (Figure 1) and viscosity values (Figure 3). Several studies have also reported that adding skimmed milk to fermented products could increase TS of the product (Sintasari et al. 2014; de Souza et al. 2021; Wirdayat and Rosida 2024).

Color appearance plays a very important role in consumer preference for food products (Dai et al. 2016). L* value represents the brightness (100) to darkness level (0) and is a representation of the whiteness level of food (Zare et al. 2011). As shown in Table 1, L* values for all fermented milk were not significantly different. This indicated that the type of LAB and milk result produced the same brightness level. In another study conducted using skimmed milk, low-fat, and full cream, fermented milk with the same color was also produced (Dai et al. 2016). a* and b* values represent red-green (positive-negative) and yellow-blue (positive-negative) colors respectively. Fermented skimmed milk showed redness (positive a* value), while UHT full cream and low-fat milk had greenness (negative a* value). All types of fermented milk showed a yellowish color (positive b* value), suggesting the total fat does not affect the color of fermented milk. Skimmed milk produced the highest a* (redness) and b* (yellowish) values due to the Maillard reaction during the sterilization process of milk at 121°C for 15 minutes using an autoclave. Dai et al. (2016) reported that yogurt supplemented with glucomannan showed an increase in a* and b* values due to pasteurization, triggering the Maillard reaction and the addition of brown-colored konjac glucomannan powder. Furthermore, fermentation process by LAB can cause changes in color, texture, taste, and aroma in fermented milk products (de Souza et al. 2023; Nemati et al. 2023).

The pH and titratable acidity are two important factors in the success of product fermentation (Yu et al. 2023). Titratable acidity of fermented milk represents the total amount of lactic acid (Dai et al. 2016). Generally, fermented products produce a low pH value along with an increase in titratable acidity, which represents the total amount of lactic acid (Xu et al. 2022; Nemati et al. 2023; Rahman et al. 2024). This study showed contradictory results, where a high titratable acidity in skimmed milk did not lead to the lowest pH value. Conversely, milk with a high-fat content (UHT full cream) produced a lower pH and acidity. This may be due to the influence of milk fat, which can act as a barrier to the diffusion of nutrients including lactose to LAB, causing microbial growth and activity to be slower.

Since fermentation occurs more slowly in high-fat milk, pH typically remains higher for a longer period compared to low-fat or skimmed milk. Fat effectively reduces the rate of acidity increase, leading to a slower decline in pH. According to a previous study, (Aljewiczet al. 2016), the substitution of milk fat with palm oil fat led to decreased acidification activity of starter culture. Furthermore, de Souza et al. (2023) states that, other factors that can inhibit the production of metabolites such as lactic acid include bacteria strain and culture conditions, including media composition, incubation period, temperature, initial pH, and aeration. This is also consistent with the total LAB produced (Figure 4), where the three LAB cultures tested grew slowly in UHT full-cream milk and rapidly in UHT low-fat milk.

An important aspect of a milk gel is whey separation, which refers to the appearance of liquid on the surface of the milk gel. It is a common defect in fermented milk products, such as yogurt (Vareltzis et al. 2016). Gel stiffness and WHC are considered factors in determining the stability of milk against syneresis. The increase in gel properties and WHC can reduce the formation of syneresis in fermented milk (Arab et al. 2023). Based on the results, high-fat milk (UHT full cream) produced a high WHC and a low syneresis value (Figure 2). This is due to fat content of milk. High-fat content helps reduce syneresis, maintaining a more stable gel structure in fermented products (Arab et al. 2023) by reducing whey separation through increased WHC, and enhancing product stability (Su et al. 2022; Zhao et al. 2023; Tan et al. 2024). Furthermore, high-fat fermented products including Greek yogurt (Cândido de Souz et al. 2020) or cream cheese (Wang et al. 2023a) tend to be more stable and less prone to whey separation because fat helps maintain the structure of the gel formed during fermentation. Low-fat or fat-free fermented products often experience more whey separation because the protein matrix is less stable without the presence of fat (Kew et al. 2020; Ningtyas et al. 2021). Syneresis values show differences among LAB cultures, where the lowest was found in *L. fermentum* B111K culture. The significant reduction in syneresis was due to the increased percentage of fat content and the use of different starter cultures, which had a significant effect (Tavakoli et al. 2019; Brodziak et al. 2020). LAB can also modify the protein structure of milk into a gel that consists of exopolysaccharides, thereby increasing WHC value in fermented milk and reducing syneresis value (Ge et al. 2022; Li et al. 2022; Yang et al. 2021).

The increased viscosity may be caused by lactic acid produced that can precipitate proteins, leading to thicker fermented dairy products (Wu et al. 2021). The highest value was found in the treatment of skimmed milk fermented with the *L. fermentum* A323L culture, which is consistent with the highest titratable acidity value observed in skimmed milk fermented with *L. fermentum* A323L culture (Figure 1). Additionally, fat can enhance the firmness and adhesion of milk (Su et al. 2022). In this study, the type of milk significantly affects viscosity of the resulting fermented dairy products. Skimmed and UHT full-cream milk had the highest viscosity values compared to UHT low-fat milk (Figure 3). In high-fat milk, the interaction between fat and casein (the main protein in milk) produces a smoother, gel-like consistency (Chakraborty et al. 2021). Consequently, whole milk tends to have a thicker and creamier consistency compared to low-fat milk. Increasing the fat (Sfakianakis and Tzia 2014) and protein content of milk (Arab et al. 2023) led to improved consistency and viscosity. The increased viscosity in fermented milk produced from the three LAB cultures used may be due to the ability to produce gels in the form of exopolysaccharides that can enhance product viscosity (Yang et al. 2021; Ge et al. 2022; Li et al. 2022). In this study, the highest viscosity value was found in *L. fermentum* A323L culture across all types of milk used (Figure 3).

Many factors influence the growth of LAB in milk, including bacteria strain of starter culture used, starter culture conditions, milk media composition, incubation period and temperature, initial pH, as well as aeration (de Souza et al. 2023). In this study, fermented milk made from UHT low-fat and skimmed milk tends to have better LAB growth compared to UHT full-cream milk (Figure 4). This is due to the low-fat content in UHT low-fat and skimmed milk, which allows for higher microbial activity, leading to an increase in LAB population. UHT full cream milk contains high fat, which can inhibit the activity of LAB culture used (Aljewicz et al. 2016). *L. fermentum* A323L culture can grow substantially in all three types of milk, particularly in UHT low-fat milk. The three cultures tested have good microbiological quality by meeting the standard LAB counts in accordance with regulations, both based on the national standard SNI 2981:2009 (BSN 2009) and the international standard CODEX STAN 243-2003 (FAO-WHO Committee 2012), which is a minimum of 10^7 CFU/mL. The increase in LAB count will enhance the quality of fermented product, specifically in terms of taste, aroma, and texture (Mukhlisah et al. 2017; Nemati et al. 2023), providing health effects (Icer et al. 2023; Sharma et al. 2023; Zhang et al. 2023), inhibiting the growth of pathogenic bacteria (Fatmarani et al. 2018; Kalhoro et al. 2023; Mgomi et al. 2023), and supporting the immune system (Yang et al. 2023a; Yeboah et al. 2023).

In conclusion, both LAB isolate cultures from *dangke* (*L. fermentum* A323L and B111K) demonstrated similar or better physicochemical and microbiological quality compared to commercial cultures. Both also had enhanced LAB growth in the three types of milk media tested. Among the media, skimmed milk proved to be the most effective for producing fermented milk. Although skimmed milk was slightly less efficient in promoting LAB growth compared to UHT low-fat milk, the total number still meets the required quality standards. Based on the results, *L. fermentum* A323L and B111K cultures could be used as starter cultures for producing fermented milk, given the excellent physicochemical and microbiological properties.

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