BIODIVERSITAS
Volume 25, Number 1, January 2024
Pages: 269-275

ISSN: 1412-033X
E-ISSN: 2085-4722
DOI: 10.13057/biodiv/d250131

Changes in physicochemical, microbiological, and sensory quality of fermented milk with *Lactiplantibacillus pentosus* during cold storage

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Manuscript received: 20 November 2023. Revision accepted: 26 January 2024.

Abstract. *Susmiati, Melia S, Khairina I, Alzahra H. 2024. Changes in physicochemical, microbiological, and sensory quality of fermented milk with* Lactiplantibacillus pentosus *during cold storage. Biodiversitas 25: 269-275.* Fermented milk is a probiotic milk product created through a fermentation process that includes the addition of lactic acid bacteria, specifically *Lactiplantibacillus pentosus*. Enhancing the product's organoleptic qualities and extending its shelf life are the primary objectives of milk fermentation. This study aimed to determine the levels of protein, fat, water, pH value, TTA value, total lactic acid bacteria (LAB) colonies and sensory quality like taste, aroma and texture of fermented milk at 1, 7, 14, 21 and 28 days of storage. The results showed that protein content, fat content, water content, pH value, TTA value, and total colony LAB ranged from 5.30-6.96%, 6.01-7.49%, 72.03-88.89%, 4.78-5.17, 0.96-1.30%, 6.73-11.06 x10^9 CFU/mL, respectively . The overall organoleptic quality level was highest in the samples with the shortest storage time.

Keywords: Fermented milk, *Lactiplantibacillus pentosus*, probiotics, storage time

Abbreviations: LAB: Lactic Acid Bacteria

INTRODUCTION

Fermented milk is a suitable probiotic carrier widely used worldwide as an essential part of the human diet to support health. The purpose of making fermented milk is to increase the shelf life of milk (Khorshidian et al. 2020). Systematic studies suggest that fermented milk products have been found to have health benefits such as gastrointestinal health, lactose intolerance, diarrhea, constipation, cardiovascular health and disease, hypertension, blood lipids, cancer, weight and body composition, diabetes risk, metabolic syndrome, and bone health. National nutritional guidelines should encourage the consumption of these products (Savaiano and Hutkins 2021). Lactic acid bacteria are used in many food fermentations and are one of the most common and well-known methods of food preservation. It also intends to improve sensory and product quality (Ayivi et al. 2020).

Lactobacillus, Streptococcus, Leuconostoc, Pediococcus, and *Lactococcus* are commonly used to ferment milk and dairy products. The Lactic Acid Bacteria (LAB) species is useful and produces bioactive compounds and metabolites that vary depending on incubation time and other processing conditions (Sharma et al. 2023). More research on specific lactic acid bacteria is required. *Lactiplantibacillus pentosus*, formerly known as *Lactobacillus pentosus*, is a newly reclassified species that contains strains recovered from settings ranging from fermentation products to mammalian gut microbiota. Several *Lactobacillus pentosus* strains demonstrate health-promoting features such as immunomodulatory and antiproliferative activity and are considered potential probiotic strains. *Lactobacillus pentosu*s and two probiotic strains of *Lactobacillus plantarum* have solid probiotic potential, but more research is needed to determine the degree of their biological capabilities (Saxami et al. 2017). The antimicrobial and biopreservation potential of probiotic LAB (*Lactobacillus paraplantarum* and *Lactobacillus pentosus)* derived from buffalo milk (Kalhoro et al. 2023).

Lactic acid bacteria can play various roles in the food industry, including improving the taste of fermented foods, increasing food nutrition, reducing harmful substances, increasing shelf life, and acting as probiotics to enhance body health (Wang et al. 2021). Previous research has discovered the properties of LAB isolates from Lintau curd (fermented buffalo milk) prevalent in probiotics, notably *Lactiplantibacillus pentosus*. This bacteria is then utilized as a starter in producing fermented buffalo milk, with oranges added as a prebiotic to improve taste and antioxidant levels. Fermented milk products with 6% *L. pentosus* starter and 20% orange juice produced the best results. Studies showed that protein content ranged from 5.81-6.33%, fat content 6.14-6.35%, water content 81.63-85.78%, pH 3.57-4.23, TTA value 1.30 -1.85 and the number of LAB colonies ranges 4.67×10^9 to 9.0×10^9 CFU/mL (Susmiati et al. 2022). This product has also been tested for antioxidant content and organoleptic properties. Antioxidant activity ranges from 25.04 to 37.71 percent, while total phenols range from

38.32 to 67.20 mgGAE/gr (Alzahra et al. 2022) and the micronutrients had higher iron and zinc content than fermenting raw cow's milk (Susmiati et al. 2023). This product is suited for use as a probiotic drink.

The main goal of milk fermentation is to extend the product's shelf life and improve its organoleptic properties. Raw materials, processing, fermentation, and storage heavily influence the quality of fermented milk. Changes in the length of storage of dairy products undoubtedly affect their physical, chemical, and microbiological quality. As a result, additional research is required to evaluate the durability of milk at different storage times so that the nutritious content remains helpful to health. The aim of present study was to assesses the physicochemical and microbiological properties of buffalo milk fermented with *Lactiplantibacillus pentosus* at various storage durations.

MATERIALS AND METHODS

This study was conducted at the Animal Products Technology Laboratory, Faculty of Animal Husbandry, Universitas Andalas, Padang, Indonesia. The lactic acid bacterial isolates were isolated from curd (fermented buffalo milk). The Ethics Commission of the Faculty of Medicine, Universitas Andalas, granted permission for this study under the number 346/UN.16.2/KEP_FK/2023.

Culture propagation

Lactiplantibacillus pentosus was obtained from the Laboratory of Animal Product Technology, Faculty of Animal Husbandry, Universitas Andalas, as a culture collection from Lintau curd. The culture was placed under anaerobic conditions for 72 hours at 37°C after being spread on De Man Rogosa and Sharpe (Neogen, USA). Ten milliliters of De Man Rogosa and Sharpe (Neogen, USA) were used to inoculate one colony, then incubated for twenty-four hours at 37°C. The bacterial cells underwent two successful transitions before being separated by centrifugation at 5000×g for 10 min at 4°C. The cells were washed twice with sterile physiological saline (PS) (0.85% NaCl). The cell pellet was re-suspended in PS to obtain approximately 10^9 CFU/mL and used as a starter culture to produce fermented milk.

Milk fermentation

The buffalo milk was collected from a local farm in Tanjung Boani Lintau, Tanah Datar. Milk was heated in a water bath for 15 minutes at 90°C with agitation, cooled to 40°C, inoculated with 2% (v/v) *Lactiplantibacillus pentosus* suspended in physiological saline, and incubated at 37°C for 24 hours. The fermented milk was cooled to 4°C and kept for four weeks. The tests were performed after 1, 7, 14, 21, and 28 days of storage.

Protein content

Protein content was measured based on AOAC (2005) using the Kjeldahl method. The sample was weighed to one gram and placed in a Kjeldahl flask. The flask is heated with one gram of selenium and one mL of H_2SO_4. The process of destruction continues until the solution is clear or colorless. Volumetric flask (500 mL) was filled halfway with the solution, then dilute to the line mark with distilled water. Then, in a distillation flask, mixed 25 mL of the sample solution, 25 mL of 30% NaOH, and 150 mL of distilled water. The solution was heated (2/3 distilled) until all of the nitrogen in the flask's liquid was collected by 0.05N H_2SO_4, which was then combined with three drops of methyl red in an Erlenmeyer first. In Erlenmeyer, the distillate was titrated with 0.01N NaOH (Z mL). As a blank, 25 mL of 0.05N H_2SO_4 and three drops of methyl red indicator were added to another Erlenmeyer and titrated with 0.1N NaOH until the color changed from pink to yellow (Y mL)

$$\text{Protein content (\%)} = \frac{\text{(Y -Z) x NNaOH x 0.014 x 6.38}}{\text{Sample weight}} \text{ x 100\%}$$

Note: Y = blank titration volume (mL); Z = sample titration volume (mL); N = normality NaOH; 6,38 = convertion factor of total N into protein

Fat content

The pumpkin fat oven was used for 30 minutes at 100-105°C to test the fat content according to the AOAC method (2005). The fat flask should next be dried in a desiccator and weighed (A). Weigh the dry sample up to 1 gram (B). Wrap it in greaseproof paper and place it in the soxhlet linked to the fat flask. The extraction was carried out for 5-6 hours or until the fat solvent that drops into the flask became clear. The used fat solvent was distilled and collected. Dry the obtained fat extract in the flask in an oven at 100-105°C for 1 hour. Cool the flask in a desiccator, then weigh it (C).

$$\text{Fat content (\%)} = \frac{C - A}{B} \text{ x 100\%}$$

Note: A = weight of base flask (gram), B = sample weight (gram), C = weight of base flask and extracted fat (gram) (AOAC 2005)

Moisture content

The cup was roasted at 110°C for one hour to test the water content according to the AOAC method (2005). The cup was weighed after being cooled in a desiccator to eliminate moisture (A). The sample was as much as 5 grams in a cup dry (B) and baked for 8 hours at 105°C. The sample was weighed after cooling in a desiccator for 30 minutes (C). This procedure was done until a constant weight was obtained the AOAC method (2005).

$$\text{Moisture content (\%)} = \frac{B - C}{B - A} \text{ x 100\%}$$

Note: A = weight of base flask (gram), B = weight of flask + sample weight (gram), C = weight of flask + dry sample (gram)

pH value

Using a pH meter calibrated with a buffer solution with pH values of 4 and 7, the degree of acidity of the sample was determined using the AOAC method (2005). Add 5 grams of sample in 10 mL of distilled water, and mixed for 5 minutes. Transfer the sample to a measuring cup, then dip the pH meter into the sample. The pH value can be established by reading the scale the pointer indicates.

Total Titratable Acidity (TTA)

Five milliliters of fermented milk and three drops of phenolphthalein were added in each Erlenmeyer. Each Erlenmeyer flask was titrated with 0.1 N NaOH until it formed a consistent pink color when agitated. After recording the amount of NaOH solution used, the overall acidity of the fermented milk was computed (AOAC 2000).

$$\text{Acidity} = \frac{\text{titration volume x 0.009}}{\text{sample (mL)}}$$

Total colonies of lactic acid bacteria

The technique of Purwati et al. (2005) was used to test for total LAB colonies. For this 1 gram of material was diluted in 9 mL of MRS Broth solution. 100 µL of the diluted sample (10-7) was spread on a petri dish containing MRS Agar, and flatten it with a sterile L- shaped spreader. Petri dishes were incubated in an anaerobic jar for 48 hours at 37°C. The Quebec colony counter was used to count the growing colonies. The CFU/mL formula was used to compute the findings.

$$\frac{\text{CFU}}{\text{mL}} = \text{number of colonies} \times \frac{1}{\text{dilution factor}} \times \frac{1}{\text{sample weight}} \times 10$$

Organoleptic test

Organoleptic quality attributes were determined by 20 people of trained panelists, according to Rahayu's technique (Rahayu 2001). The hedonic (liking) test was an organoleptic test often used to assess food product preferences. The preferred response was expressed in the form of levels known as the hedonic scale. This hedonic scale can range from very much to dislike, based on the opinions of multiple panelists with a range of 1-5. Consumer acceptability ratings on a 5-point hedonic scale (1 = strongly dislike, 2 = slightly dislike, 3 = neither like nor dislike, 4 = slightly like, and 5 = extremely like). The panelists then fill out the form supplied. The organoleptic test findings were summarized in a table for further analysis.

Statistical analysis

All tests were carried out in triplicate, and the results were expressed as mean values SD. Statistical Product and Service Solutions (SPSS) software was used to perform analysis of variance (ANOVA) and Tukey's test for pairwise comparisons. Pearson correlation analysis was utilized to investigate the link between variables.

RESULTS AND DISCUSSION

Protein content

The protein content of fermented milk with *Lactiplantibacillus pentosus* and prolonged fermentation processes A (1 day), B (7 days), C (14 days), D (21 days), and E (28 days) indicates the presence of actual effect on the protein content of fermented milk.

According to Table 1, the average protein content of fermented milk ranged from 5.30 to 6.96%. Treatment A (1 day) had the highest mean protein content (6.96%), while treatment E (28 days) had the lowest (5.30%). The analysis of variance revealed that the fermented milk storage period had a significant ($p<0.05$) effect on the protein content of fermented milk. LAB can produce proteolytic enzymes both inside and outside the cell, resulting in the hydrolysis of peptides into free amino acids and the utilization of amino acids (Wedajo 2015). The optimal pH for proteolytic bacteria formation and proliferation in milk is 7-7.5. Low temperatures inhibit bacterial growth and development and reduce enzymatic activity (Kieliszek et al. 2021). The extend of proteolysis is significantly increased during cold storage, particularly throughout the last two weeks of storage (Abdel-Hamid et al. 2019). Since fermented milk creates a pH in the isoelectric point range during storage, protein content might be a reduced when milk is stored for an extended period. The health benefits of fermented milk depend not only on the presence of live microbes (mainly lactic acid bacteria; LAB), but also on Pro-Health Molecules (PHM) originating from microbial conversion of food compounds. The main PHMs are Bioactive Peptides (BPs) released from milk proteins through microbial proteolysis. Bioactive peptide functions include antihypertensive, antioxidant, immunomodulatory, and antimicrobial activities (Tagliazucchi et al. 2019).

According to SNI (2009), the minimum protein content for products made from fermented milk is 2.7%. Melia et al. (2020) reported that shelf life of fermented goat milk for 28 days is about 4%. Although the protein content of present fermented milk up to 28 days of storage was 5.30 percent, which was still high compared to the Indonesian national standard. The reasons for this variation may be the sources of fermented milk. Buffalo milk has more protein than the milk from cows, goats, camels, horses, and donkeys (Nayak et al. 2020).

Table 1. Protein content of fermented milk

Storage period (days)	Protein content %
1	6.96 ± 0.39^{a}
7	6.33 ± 0.30^{ab}
14	6.04 ± 0.52^{abc}
21	5.59 ± 0.41^{bc}
28	5.30 ± 0.80^{c}

Note: Superscripts with different letters (abc) in the treatment show significantly different ($P<0.05$)

Fat content

The fat content of fermented milk with *Lactiplantibacillus pentosus* was significantly affected when the milk goes through a lengthy fermentation process.

The average fat level of fermented milk with *Lactiplantibacillus pentosus* is ranged from 6.01 to 7.49% (Table 2). The mean fermented milk fat content ranged from 6.01% in treatment E (28 days) to 7.49% in treatment A (1 day). According to the analysis of variance, the amount of time fermented milk was stored had a significant ($p<0.05$) impact on its fat content. This aligns with the findings of Magalhães et al. (2011), who hypothesized that microorganisms could be responsible for the decline in fat content by creating lipase enzymes. As a result, milk has less fat because the lipase enzyme hydrolyzes fat. The decomposition of lactose into lactic acid, hydrolysis of casein into peptides, degradation of protein into amino acids, and breakdown of milk fat into free fatty acids are the three major reactions that bacteria will experience in breaking down milk components during the fermentation process (Wang et al. 2021). The milk produced by fermented *Lactiplantibacillus pentosus* was accepted as appropriate for consumption if it was compared to the minimum 3% fat level required by the Indonesian National Standard 2981:2009 for fermented milk.

Moisture content

The water content of fermented milk was significantly affected when the milk goes through a lengthy fermentation process.

Table 3 shows that the water content of fermented milk containing *Lactiplantibacillus pentosus* ranged from 72.03 to 88.89%. The mean fermented milk water content ranged from 72.03% for treatment E (28 days) to 88.89 percent for treatment A (1 day). The results of statistical analysis showed that the amount of time that fermented milk was stored had a significant ($p<0.05$) impact on how much water it contained. According to Bawinto et al. (2015), the length of storage affects the water content of food ingredients, where the longer the storage time, the less water is present in food products. According to Krisnaningsih et al. (2021), storing yogurt for 28 days reduces the water content. A fall in the pH level of fermented milk during storage may have caused a reduction in water-retaining capacity. The drop in pH of soured milk during storage causes a reduction in water holding capacity. A lower pH value affects the reduction in water retaining capacity.

Value of pH and Total Titratable Acidity (TTA)

The pH value of fermented milk was dramatically impacted when citrus fruit juice was added after a lengthy fermentation process.

The pH range of fermented milk enriched with *L. pentosus*, as shown in Table 4, was 4.78 to 5.17. The mean fermented milk pH value decreased from 5.17 (days 1) to 4.78 (days 28). According to the variance analysis, the pH level of fermented milk decreased significantly ($p<0.05$) during storage. The typical TTA value of fermented milk ranged from 0.96 to 1.30%. The average TTA value of the fermented milk in Treatment E (28 days) was the highest, at 1.30%, whereas that in Treatment A (1 day) was the lowest, at 0.96%. The results of the analysis of variance showed that the amount of time that fermented milk was stored had a significant ($p<0.05$) impact on its TTA value. These results agree with those of Abdel-Hamid et al. (2019), Titratable Acidity percentage (TA%) and pH values of fermented milk manufactured by *Lb. casei* ATCC 393 during storage for 21 days at 4°C. A similar trend was reported by Dimitrellou et al. (2016) who found that TA% was increased from 0.7 to 0.9, whereas the pH values were reduced from 4.69 to 4.05 during 28 days of storage at 4°C of fermented milk produced by yogurt culture enriched with *Lb. casei* ATCC 393.

The pH value of fermented milk decreases with time due to the activity of lactic acid bacteria, which turn glucose, fructose, and sucrose into organic acids. Furthermore, diffusion of some acid phenols, and decomposition of other phenolic compounds into acids, contribute to the pH decrease (Kiai and Hafidi 2014). The TTA value of fermented milk increased with prolonged storage. This occurs when LAB grows and consumes sugar from fermented milk, producing lactic acid and other by products. Lactic acid is broken down more readily in fermented milk with greater acidity values. LAB can multiply and produce lactic acid metabolites.

Table 2. Fat content of fermented milk

Storage period (days)	Fat content %
1	7.49 ± 0.33^{a}
7	7.79 ± 0.67^{a}
14	7.24± 0.74^{a}
21	6.88± 0.13^{ab}
28	6.01± 0.31^{b}

Note: Superscripts with different letters (ab) in the treatment show significantly different (P<0.05)

Table 3. Water content of fermented milk

Storage period (days)	Water content %
1	88.89 ± 0.15^{a}
7	84.00 ± 0.78^{b}
14	81.61 ± 0.82^{c}
21	76.25 ± 1.11^{d}
28	72.03 ± 1.15^{e}

Note: Superscripts with different letters (abcde) in the treatment showed significantly different (P<0.05)

Table 4. pH value of fermented milk

Storage period (days)	pH	Total titratable acidity
1	5.17± 0.03^{d}	0.96± 0.03^{e}
7	5.06 ± 0.04^{c}	1.05± 0.03^{d}
14	4.95± 0.04^{b}	1.14± 0.04^{c}
21	4.91± 0.05^{b}	1.21± 0.04^{b}
28	4.78± 0.03^{a}	1.30± 0.01^{a}

Note: Superscripts with different letters (abcd) in the treatment show significantly different (P<0.05)

The ability of lactobacillus bacteria to produce organic acids, which increase the acidity value of milk fermentation, led to the percentage of acidity value of fermented milk using *Lactobacillus casei* 393 culture having a TTA range of 0.73-0.90% (Abdel-Hamid et al. 2019). This process lowers the pH value of fermented milk when it is stored for an extended period. The yogurt's pH and acidity decreases due to the lactic acid bacteria using the existing carbohydrates to produce lactic acid (Sandra et al. 2019). The pH decreases during storage due to the culture's metabolic activity, and the longer the product is stored, the more lactic acid is produced, lowering the pH (Kurnia et al. 2021).

Total colonies of lactic acid bacteria

The total lactic acid bacteria value of fermented milk with *L. pentosus* and land lengthy fermentation treatment was related to the total lactic acid bacteria value of fermented milk.

Total LAB colonies in fermented milk enriched with *L. pentosus* ranged from 6.73 to 11.06 x10^9 CFU/mL (Table 5). Treatment A (1 day) had the highest mean total LAB colonies in fermented milk with 11.06 × 10^9 CFU/mL, whereas treatment E (28 days) had the lowest with 6.73 x 10^9 CFU/mL. The diversity investigation found a significant (p 0.05) relationship between the total number of LAB colonies of fermented milk and its storage length. This outcome is consistent with Melia et al. (2020), who found that at 28 days of storage, the total amount of fermented LAB goat milk containing *Pediococcus acidilactici* decreased (9.106 log CFU/mL). Rossi et al. (2021) reported that after 28 days of cold storage, there was a significant decrease in the amount of *Lactobacillus plantarum* VP-3.3 and *Streptococcus thermophilus* in yogurt (10.08-9.98 log CFU/mL). The viability of *Lactobacillus Casei* subsp. *casei* R-68 (LCR-68) and *Lactobacillus casei* strain Shirota (LCS) decreased significantly with cold storage time (Pato et al. 2020). In contrast, during the 21 days of cold storage of probiotic yogurts, Dimitrellou et al. (2016) showed a considerable increase in *Lb. casei* ATCC 393. These varying trends in the viability of lactic acid bacteria throughout storage could be brought about by variations in the fermented milk type, volume of the starter inoculation, incubation temperature, and duration of storage.

Lactic acid bacteria grow less quickly when fermented milk is stored for an extended period because the resources necessary for their growth are depleted. Furthermore, the bacterial growth phase, which included the lag phase, log phase, static phase, and death phase, impacted the decline in the overall number of LAB colonies. In ideal conditions with sufficient energy sources, LAB grow more; if nutrients are in short supply, the number of lactic acid bacteria decline (Abedin et al. 2023). A lack of additional nutrients for LAB growth during cold storage is responsible for the total decrease in LAB. The cells exhaust their stored energy. Additionally, lactic acid bacteria could die during storage because they lower the pH of fermented milk and prevent the growth of lactic acid bacteria under conditions when the acidity is too high. In the present study, the count of *Lactiplantibacillus pentosus* during the storage period of 28 days was LAB 10^9 CFU/mL. This means that after 28 days, fermented milk could still provide health benefits. To achieve probiotic effects, live probiotic bacteria counts must be at least 10^9 log CFU /mL during fermentation and storage time (Palanivelu et al. 2022).

Organoleptic test

Results of long term storage for organoleptic test of fermented milk reveal significant effect on organoleptic test value of milk.

The sensory evaluation of fermented milk containing *Lactiplantibacillus pentosus* revealed a significant effect (P < 0.05) in the taste, aroma, and texture after 28 days of storage (Figure 1). Table 6 and Figure 1 shows that the average fermented milk flavor ranged between 3.08 and 3.51.

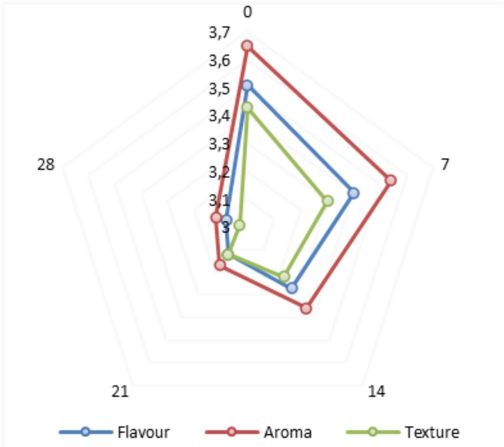

Figure 1. Panelists' acceptance score for fermented milk containing *Lactiplantibacillus pentosus*

Table 5. Total lactic acid bacterial colonies of fermented milk

Storage period (days)	Total lactic acid bacteria ($x10^9$ CFU/mL)
1	11.06 ± 1.48[a]
7	9.33 ±0. 85[ab]
14	8.50 ± 1.13[bc]
21	7.40 ± 0.79[bc]
28	6.73 ± 1.00[c]

Note: Superscripts with different letters (abc) in the treatment show significantly different (P<0.05)

Table 6. Sensory analysis of fermented milk containing *Lactiplantibacillus pentosus*

Storage period (days)	Flavor	Aroma	Texture
1	3.51 ± 0.18[a]	3.65± 0.24[a]	3.43 ± 0.12[a]
7	3.40 ± 0.17[ab]	3.54± 0.17[a]	3.30 ± 0.14[ab]
14	3.27± 0.04[bc]	3.36± 0.20[ab]	3.22 ± 0.04[bc]
21	3.12± 0.06[c]	3.17± 0.11[b]	3.12 ± 0.06[bc]
28	3.08± 0.09[c]	3.12± 0.08[b]	3.03 ± 0.05[c]

Note: Superscripts with different letters (abc) in the treatment show significantly different (P<0.05)

Treatment A (1 day) had the highest mean fermented milk taste organoleptic test (3.51), while treatment E (28 days) had the lowest (3.08). The panelists' preference for fermented milk decreases when it is stored for an extended period of time, which is due to an increase in the acidity of the milk. The taste is correlated to sweetness and inversely related to acidity. Although there were variations in sensory profiles, they were generally acceptable, indicating that all products during storage were accepted by the panelists. The fermented milks were categorized into five groups based on their primary sensory attribute: milky, creamy, cheesy, fermented, and miscellaneous (Liu et al. 2022). The pH of fermented milk decreased as milk was stored for more extended period of time. The lactic acid bacteria, which turned lactose into lactic acid, were responsible for pH reduction. This is in line with the assertion made by (Wang et al. 2022) that LAB not only reacts with glucose to produce acids, but it also produces specific enzymes such as protease and lipase, which break down proteins, fats, and carbohydrates, and some metabolites react with one another to produce flavorful substances. The role of LAB in flavor formation is determined by three factors: their metabolic ability to convert food components into aroma compounds, aroma-producing activity at the site, and other microorganisms or enzymes that are active in the food at the same time (Thierry et al. 2015). The variability and diversity of LAB in traditional fermented foods are essential in the flavor formation mechanism (Wang et al. 2023).

The average fermented milk containing *Lactiplantibacillus pentosus* aroma ranged between 3.12 to 3.65. Treatment A (1 day) had the greatest mean aromas 3.65, while treatment E (28 days) had the lowest, 3.12. The diversity analysis revealed that the storage period of fermented milk had a significant effect ($p<0.05$) on the organoleptic value of the aroma of fermented milk. Long-term storage of fermented milk reduces panelists' liking for fermented milk. Aroma is an organoleptic factor influencing a panelist's preference for a product. In microbial metabolism, the processes of glycolysis, proteolysis, and lipolysis result in flavor enzymes that promote the synthesis of aroma compounds (Hu et al. 2022). However, biodiversity contributes to microorganisms' capacity to produce a variety of aromas (Chen et al. 2021). Aldehydes, lactones, and esters are aroma compounds that can produce pleasing flavors and alter how people perceive flavors. Chemicals produced by LAB, such as secondary metabolites of acetaldehyde compounds and volatile components, can impart a distinct scent to fermented milk products (Niimi et al. 2014).

The average organoleptic value of fermented milk containing *Lactiplantibacillus pentosus* texture ranged from 3.03 to 3.43. Treatment A (1 day) had the greatest mean organoleptic value of fermented milk texture (3.43), while treatment E (28 days) had the lowest (3.03) (Table 1 and Figure 1). The diversity analysis revealed a significant ($p<0.05$) effect of fermented milk storage duration on the organoleptic value of fermented milk texture. Long-term storage of fermented milk decreased panelists' preference for the mouthfeel of fermented milk. Fermentation thickens the texture of fermented milk. This may be possible because lowering the pH of fermented milk might impact the hydrolysis of fermented milk protein. Sandra et al. (2019) stated that the acidic conditions in yogurt are affected by the activity of lactic acid bacteria in yogurt; decreasing the pH of yogurt can cause coagulation of yogurt protein, which forms more lumps or coagulants, causing the texture of yogurt to thicken.

In conclusion, the physicochemical, microbiological, and sensory quality of fermented milk with *Lactiplantibacillus pentosus* were evaluated during 1, 7, 14, 21, and 28 days of refrigerated storage. Protein content, fat, moisture, pH, TTA, total lactic acid, and sensory quality were significantly affected by 28 days of storage. Protein values, fat content, water content, pH values, TTA values, and total LAB colonies varied from 5.30-6.96%, 6.01-7.49%, 72.03- 88.89%, 4.78-5.17, 0.96-1.30%, and 6.73-11.06 x 10^9CFU/mL, respectively. The overall organoleptic quality level was highest in the samples with the shortest storage time. Fermented milk can be stored for 28 days and meet physicochemical and microbiological standards.

ACKNOWLEDGEMENTS

Under the research implementation contract for Riset Publikasi (RP) No. 106/SPK/PTN-BH/FKep/Unand-2023, Dr. dr. Susmiati, M. Biomed, this study sponsored the UP2M Faculty of Nursing, Universitas Andalas. The authors are grateful to the Laboratory of Animal Product Technology, Faculty of Animal Husbandry, Universitas Andalas, Padang for the facilities used during the research.

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