Research Article

Evaluation of *Lactiplantibacillus pentosus* Probiotic Fermented Buffalo Milk with Citrus Juice

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Abstract | Functional food is a type of food or drink that can benefit people's health. Fermented milk is a functional food derived from the fermentation of milk with probiotic lactic acid bacteria, specifically the *Lactiplantibacillus pentosus* HBUAS53657. This study aims to determine the antioxidant activity, total phenol, and organoleptic characteristics including the taste, aroma, and texture of fermented milk with orange juice added (*Citrus nobilis* L.). The study was based on a Factorial Completely Randomized Design (CRD), with starter concentrations of 4 (A1), 5 (A2), and 6% (A3), as well as orange juice concentrations of 10 (B1), 15 (B2), and 20% (B3). The results showed that fermented milk with orange juice revealed antioxidant activity ranging from 25.04 to 37.71%, total phenol 38.32-67.20 mg GAE/gr, taste organoleptic test 3.20–4.08 (somewhat liked), aroma 3.26-3.76 (slightly liked), and texture 2.70–3.40 (rather liked). The study concluded that fermented milk with orange juice has higher antioxidant activity and is preferred by consumers, allowing it to be recommended as a functional food.

Keywords | Antioxidants, Fermented milk *Lactiplantibacillus pentosus,* Oranges, Organoleptic, Total phenols

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INTRODUCTION

Functional foods can be derived from a variety of dairy products. Milk is a product from livestock with good nutritional value for the body but is susceptible to damage if not handled and processed correctly. Probiotics are live microorganisms that, when consumed in sufficient quantities by humans, have a beneficial effect on health (FAO/WHO, 2006). Probiotics are widely associated with gut health, as they promote the balance of intestinal microbiota (Pundir et al., 2013). Probiotics must be GRAS (generally recognized as safe) approved (Lin et al., 2006), adhere to the host's intestinal epithelium to survive and colonize the gastrointestinal tract (Kesen and Aiyegoro, 2018), and can inhibit the growth of pathogenic bacteria such as *E. coli, Staphylococcus aureus*, and *Listeria monocytogenes* (Melia et al., 2019; Melia et al., 2017).

Fermented milk can be made from various milk products, including cow's milk, buffalo milk, and goat's milk, by adding probiotic bacteria. Melia et al. (2021) and Melia et al. (2020) used the probiotic *Pediococcus acidilactici* BK1 to ferment goat's milk and whey, Jelita (2017) used *Lactobacillus Plantarum* and *Streptococcus thermophilus* to ferment cow's milk, and Chairunnisa (2006) used a lactic acid bacteria starter to ferment milk products. Buffalo milk contains higher nutrients than cow milk (Becskei et al., 2020), as well as the lipid, protein, energy, and nutritional content contents are higher than that of cow milk and the latter might be attributed to higher fat. Fermented buffalo milk produced by probiotic *Lactiplantibacillus pentosus* HBUAS53657 and orange juice contains protein ranged from 5.81-6.33%, fat 6.14-6.35%, and total LAB colonies from 4.67×10^9 to 9.0×10^9 CFU/mL (Susmiati et al, 2022). Buffalo milk has high lactose, protein, calcium, and zinc contents which can help children overcome stunting problems and act as an anti-inflammatory (Licata et al., 2012).

Milk is processed by fermentation with lactic acid bacteria (LAB) isolated from Dadih from Jorong Tanjung Modang Nagari Tanjung Bonai, North Lintau Buo District, Tanah Datar Regency, with the type *Lactiplantibacillus pentosus* HBUAS53657, to extend the shelf life of dairy products. This dadih contains good quality nutrients (Alzahra et al., 2021). When consumed by the body, it lasts longer and provides better benefits. In this regard, consuming probiotic dairy products benefits the body by lowering blood cholesterol, and preventing diabetes, cardiovascular disease, and strokes (Hill et al., 2014).

Product diversification in functional food can be accomplished by including sources of antioxidants from plant extracts, fruits, and probiotic bacteria in fermented milk. Furthermore, it was explained that adding fruits could effectively improve plain yogurt's taste and therapeutic properties. (Zainoldin and Baba, 2009). Estrada et al. (2011) made strawberry yogurt. Boycheva et al. (2011) flavored goat's milk yogurt with Aronia and blueberry juice. The medicinal plant sweet orange (*Citrus nobilis* L.) contains antioxidant compounds that can be used to prevent and treat various diseases. Sweet orange (*Citrus nobilis* L.) contains chemical compounds such as flavonoids, tannins, phenols, terpenoids, vitamin C, and steroids (Rauf et al., 2014). This study aimed to determine the antioxidant activity, total phenol content, and organoleptic activity (the taste, aroma, and texture) of *Lactiplantibacillus* fermented milk with orange juice (*Citrus nobilis* L.).

MATERIALS AND METHODS

Buffalo milk and citrus fruits were sourced from Jorong Tanjung Modang Nagari Tanjung Bonai, North Lintau Buo District, Tanah Datar Regency, West Sumatra. The experimental method was used with a Completely Randomized Design (CRD) Factorial 3 x 3 with three replications; the treatment was the addition of starter concentrations (factor A) of 4, 5, and 6%, as well as the addition of orange juice concentrations (factor B) 10, 15, and 20% with antioxidant activity, total phenol, and organoleptic parameters measured. This research has been accepted by the ethics committee of the Faculty of Nursing, Andalas University No. 019.laiketik/KEPKFKEPUNAND/2021

Starter preparation

The fermentation of the milk starter was based on a modification of Ishmayana et al. (2015) that used curd to isolate lactic acid bacteria. Buffalo milk was pasteurized at 65°C for 30 min before being cooled to 43°C. Isolate 1 mL of lactic acid bacteria from curd that has been enriched in MRS Broth medium (Merck, Germany) for 24 h at 37°C. The lactic acid bacteria culture was centrifuged at 14,000 rpm for 10 min at 4°C (micro centifuge 5417 R, Germany) . Centrifugation yielded pellets, which were inoculated with pasteurized milk and incubated at 37°C for 12 hours.

Fermented milk preparation

Sugiarto (2010) made fermented milk with the addition of modified orange juice. About 2700 mL of milk were pasteurized at 65°C for 30 min, cooled to 43°C, and divided into 27 bottles. The starter was inoculated at 4, 5, and 6%, and orange juice was added at 10, 15, and 20% of the amount of milk, and incubated at 37°C in for 15 h. Each group contains three samples, for a total of 27 samples.

Antioxidant Activity

1 mL of each sample was mixed with 1 mL of a methanol solution containing 80 ppm DPPH (Diphenylpicryl-hydroxyl) to test antioxidant activity after Huang et al. (2005). After stirring, the liquid was placed in a dark room for 30 min. A spectrophotometer (Shimadzu UV-1800 series) was set to 517 nm absorbance and was used to take measurements. As a control, methanol was employed. The calculations were carried out according to the following formula:

$$\text{Inhibition } (\%) = \frac{\text{control absorbance} - \text{sample absorbance}}{\text{control absorbance}} \times 100\%$$

Total Phenol

One gram of sample was enriched with 10 ml of methanol and vortexed. One ml of the sample was pipetted into a test tube. Fill the test tube halfway with distilled water. Then, the folin solution (1:10 mL distilled water) and 1 mL Na2Co3 (7.5%) were added and vortexed. Allow for one hour. Then, the readings were recorded by spectrophotometery at a 735 wavelength. The calculations were carried out the following formula:

$$\text{Total fenol (mg GAE/gr)} = \frac{X \ x \ FP}{\text{sample weight}} \times 100\%$$

Where; X = standard equation (Y = 0.0232 X + 0.1227) and FP = Dilution factor

Organoleptic Examination

Organoleptic quality properties are product quality properties that can only be measured by organoleptic testing, according to the Rahayu (2001) method. The hedonic test is an organoleptic test commonly used to assess food product preferences (liking). The preference responses are presented in the form of levels known as hedonic scales. This hedonic scale (1-5) can be ranged from very much like to dislike when evaluated by fifty panelists.

Statistical Analysis

The data was analyzed using an experimental method that included a 3 x 3 factorial, with *Lactiplantibacillus pentosus* HBUAS53657 (Factor A) 4, 5, and 6% and the orange juice concentrations (factor B) 10, 15, and 20%. Completely Randomized Design (CRD) with three replications and additional tests using Duncan's Multiple Range Test (DMRT). Furthermore, IBM SPSS statistical v.22 (IBM Corp., NY, USA) was used to process the data.

RESULTS AND DISCUSSION

Antioxidant Activity

The A3B3 treatment (Table 1) has the highest antioxidant value (37.71%), while the A1B1 treatment has the lowest (25.04%). On average, the antioxidant value of fermented dairy ranged from 25.04 to 37.71%. The addition of 20% orange juice (B3) significantly (P< 0.05) increased the antioxidant activity of fermented milk. This is possible because orange juice contains phenolic and flavonoid compounds. Flavonoid compounds are antioxidants that can protect against free radicals because they have a hydroxyl group, which can donate hydrogen atoms to free radicals and stabilize reactive oxygen compounds (ROS) (Reynertson et al., 2005).

Table 1: Antioxidant activity of fermented milk (%)

Starter	Orange juice			Mean
	B1	B2	B3	
A1	25.04^{e}	30.17^{d}	35.22^{b}	30.14
A2	25.27^{e}	30.17^{d}	37.25^{a}	30.90
A3	25.19^{e}	32.73^{c}	37.71^{a}	31.88
Mean	25.16	31.02	36.73	

Note: The mean of superscripts with lowercase letter (^{abcdef}) showed a significant difference (P<0.05)

Orange juice also contains bioactive antioxidant compounds such as vitamin C, carotenoids, and phenolic compounds (Khan et al., 2019). Meanwhile, different protein fractions are thought to be the primary cause of the antioxidant activity of milk and milk products, in addition to vitamin and mineral contents. According to Barba et al. (2013), the combination of orange juice and milk produces products rich in antioxidant compounds and nutrients because orange juice and milk can be fermented by LAB with the help of various LAB strains (L. Brevis POM, L Plantarum TR-71, and L. L Plantarum TR-14). This is in line with Sharoba et al. (2019) the addition of probiotic cultures and prebiotic substances having a substantial influence on antioxidant activity. In line with other lactic acid bacteria, *Lactobacillus brevis* or *Lactobacillus plantarum* fermentation of milk and orange juice can increase antioxidant capacity compared to controls (de la Fuente et al, 2021). The antioxidant content of dairy products can be altered by using natural additives in animal feed or at the milk processing stage. The most common ingredients are medicinal herbs, fruits, waste from the fruit and vegetable industry (Stobiecka et al, 2022)

Total Fenol

Table 2 showed that the A3B3 treatment had the highest total fermented milk phenol concentrations (67.20 mg GAE/gr), while the A3B1 treatment had the lowest (38.32 mg GAE/gr). The total phenol contents of fermented milk ranged from 38.32 to 67.20 mg GAE/gr. Because orange juice contains phenolic compounds and flavonoids, adding 20% orange juice (B3) significantly (P<0.05) affects the total phenol of fermented milk. Rao et al. (2021) proved that oranges contain antioxidant components such as essential oils, fatty acids, alkaloids, coumarins, limonoids, carotenoids, ascorbic acid, tocopherols, amino acids, and flavonoids. The addition of 20% citrus fruit (B3) had the highest antioxidant activity and the highest total phenol value. Huang et al. (2005) showed that antioxidant activity is proportional to total phenol.

Table 2: Total fenol of fermented milk (mgGAE/gr)

Starter	Orange juice			Mean
	B1	B2	B3	
A1	40.75^{de}	43.65^{c}	51.70^{b}	49.74
A2	44.49^{d}	57.79^{b}	60.82^{b}	54.36
A3	38.32^{e}	57.47^{b}	67.20^{a}	54.33
Mean	41.19	54.73	62.52	

Note: The mean of superscripts with lowercase letter (^{abcdef}) showed a significant difference (P<0.05)

Organoleptic Test

Flavor: According to Figure 1, the average organoleptic value of fermented milk tastes ranges from 3.20 to 4.08 (neither like nor dislike – like slightly). The A3B3 treatment, with the addition of 6% LAB starter and 20% orange juice, had the highest organoleptic taste value of 4.08. The A1B1 treatment with 4% LAB starter and 10% orange juice had the lowest organoleptic taste value of 3.20. The panelists preferred fermented milk products as more orange juice was added. As a result, the addition of starter and orange juice has a preferred value of range from 3.20–4.08. This is related to the metabolism of lactic acid bacteria, which produce lactic acid and other organic acids during the fermentation process, influencing the taste of fermented milk preferred by the panelists. Costa and Conte-Junior (2016) reported that lactose fermentation produces lactic acid, which is derived from the conversion of carbohydrates in proportion to the presence of lactic acid produced by LAB during the fermentation process and results in a distinct taste of fermented milk that is still acceptable to the panelists. Orange juice fermented by *L. paracasei* had a more appealing aroma and received the highest sensory score (Quan Q et al., 2022)

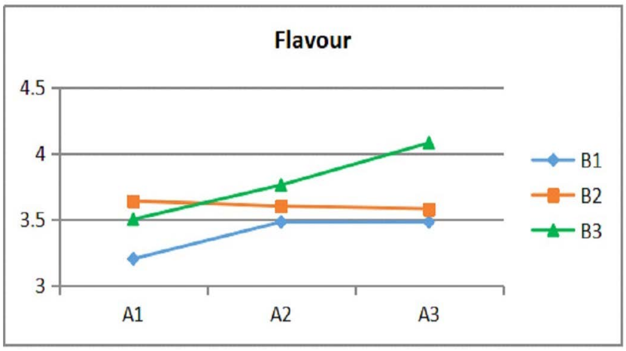

Figure 1: Organoleptic value of fermented milk taste

The addition of orange juice has a significant (P< 0.05) impact on the organoleptic flavor of fermented milk. This finding was supported by Ranadheera et al. (2012), who found that adding fruit juice improves the flavor of goat's milk yogurt and positively affects the natural sugars found in fruit. Consumers prefer fermented drinks that are less sour but still taste sweet. As a result of the acidity of fermented milk influencing taste preferences, the taste of orange juice and the LAB mixture produced can increase the panelists' attractiveness.

Aroma: The A2B2 treatment (Figure 2) with the addition of 5% LAB starter and 15% orange juice had the highest organoleptic aroma value of up to 3.76. The A3B2 treatment with the addition of 6% BAL starter and 15% orange juice had the lowest organoleptic taste value of up to 3.26. As a result, the addition of starter and orange juice has a preferred value of 3.26-3.76 (neither like nor dislike).

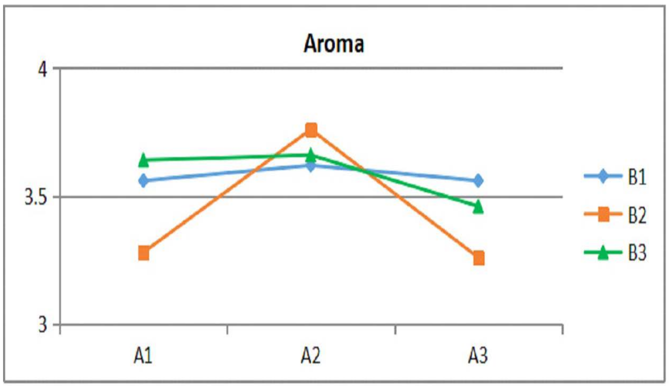

Figure 2: The organoleptic value of fermented milk aroma

Aroma is an organoleptic factor influencing the panelists' preference for a product. According to Widodo (2002), LAB-produced substances such as secondary metabolites of acetaldehyde compounds and volatile components can contribute to the characteristic aroma of fermented milk products. Acidity is essential in product taste evaluation, according to Davidson (2000). According to Adhikari et al. (2002), organic acids are indicators of metabolic activity produced by bacteria added to fermented milk products. This acid is a natural preservative that contributes to distinctive sensory properties.

Texture: The A2B1 treatment (Figure 3) with the addition of 5% LAB starter and 10% orange juice had the highest organoleptic texture value of up to 3.40. The A1B3 treatment, with the addition of 4% LAB starter and 20% orange juice, had the lowest organoleptic texture value of up to 2.70. As a result, the addition of starter and orange juice has a preferred value of 2.70–3.40 (dislike slightly - neither like nor dislike). Manab (2008) revealed that the structure of fermented milk products is formed due to the presence of casein in coagulated milk and will create a gel-like design due to bacterial activity. The type and number of microorganisms in the fermented milk starter had a significant (P<0.05) impact on the formation, taste, and texture of the fermented milk. When the fermentation process occurs, LAB clumps the milk, causing the pH value to fall. The more starter added, the faster the clotting process in the milk occurs, affecting the texture of the fermented milk.

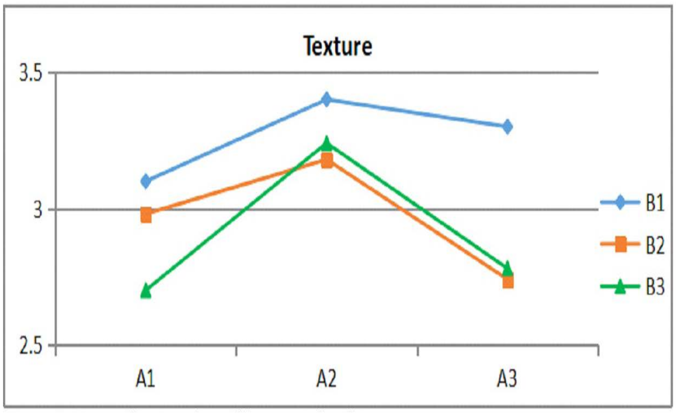

Figure 3: Organoleptic value of fermented milk texture

CONCLUSION

According to the findings of the study, fermented buffalo milk *Lactiplantibacillus pentosus* HBUAS53657 had antioxidant activity ranging from 25.04 to 37.71%, total phenol 38.32 - 67.20 mg GAE/gr, organoleptic taste 3.20-4.08 (neither like nor dislike – like slightly), aroma 3.26-3.76 (neither like nor dislike), and textures 2.70-3.40. (dislike slightly - neither like nor dislike). The fermentation of buffalo milk was much more successful with 6% starter and 20 % orange juice.

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CONFLICT OF INTEREST

The authors report no conflicts of interest.

NOVELTY STATEMENT

The processing of fermented milk from buffalo milk using lactic acid bacteria as a result of probiotic *Lactiplantibacillus pentosus* HBUAS53657 with the addition of orange juice (*Citrus nobilis* L.) as a source of antioxidants.

AUTHORS CONTRIBUTION

All authors are involved in conducting research, data analysis, and writing the articles.

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