

Two-Phase Buffalo Milk Yogurt with Pineapple and Beetroot Jam Using Products from Brazilian Amazon

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Abstract

This study aimed to develop a two-phase yogurt based on buffalo milk incorporating pineapple-beetroot jam and to evaluate its physicochemical properties and technological feasibility. Buffalo milk was processed and fermented using standard yogurt cultures, and a fruit-vegetable jam was prepared with pineapple pulp and beetroot. The final product was analyzed for pH, titratable acidity, and total soluble solids. The flavored yogurt presented pH (4.43 ± 0.04), titratable acidity ($1.13 \pm 0.03 \text{ g} \cdot 100 \text{ g}^{-1}$), and total soluble solids (26.20 ± 0.06 °Brix), indicating physicochemical stability and appropriate fermentation. The incorporation of the fruit-vegetable blend contributed to increased soluble solids and product differentiation. These results demonstrate that the proposed formulation is

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technologically viable and represents a promising value-added dairy product. Further studies on sensory acceptance and functional properties are recommended.

Keywords: Dairy. Functionality. Flavored food. Fruit-vegetable blend. Product development.

INTRODUCTION

Buffalo milk has a creamy and heavy texture (Juvinal et al. 2023), due to the absence of carotenes (provitamin A), and is always whiter when compared to cow's milk (Emakpor et al. 2024) with the addition of the blue-green pigment called biliverdin (El-Salam and El-Shibiny 2011). It also has a higher percentage of constituents, mainly fat, protein, lactose, and total solids, with fat and protein being particularly relevant, as they are present in twice the amount found in cow's milk (Liao et al. 2025). Additionally, buffalo milk contains higher levels of minerals, such as calcium, potassium, iron, manganese, and selenium, than cow's milk (Brohi et al. 2026). The production and demand for buffalo milk and its derivatives have been increasing and gaining significant importance both nationally and internationally (Shiddiqui et al. 2024). Currently, intensive efforts are being made to establish buffalo farming definitively, as buffalo is a triple-purpose animal that produces meat, milk, and energy (Badpa and Saghir 2014). In this context, buffalo milk is an extremely important source of dairy and meat products, accounting for about 15% of global production (Luo et al. 2020) and experiencing continuous growth in demand (FAO 2021).

According to Manoharan et al. (2020), yogurt is a food made from clotted milk produced by lactic fermentation, mainly by homofermentative lactic acid bacteria (LAC) such as *Lactobacillus bulgaricus* and *Streptococcus thermophilus* (Ge et al. 2023), in pasteurized or concentrated milk, with or without powdered milk. These bacteria are grown together or separately and then inoculated into the milk in set proportions. During fermentation, the milk's pH favors the growth of *Streptococcus thermophilus* (Salmazo et al. 2023). As acidification increases, *Lactobacillus bulgaricus* thrives (Yunita et al. 2023). Yogurt consumption has shown steady growth, with an estimated annual rate of 4.4% between 2024 and 2029. This increase is driven by yogurt's functional and traditional characteristics, which provide sensory and nutritional appeal (Cardoso et al. 2025), as well as the potential for higher demand. The growth is mainly due to flavor and aroma diversification, the potential to enrich yogurt with vitamins and minerals, which enhances its healthy image, and the association of its sensory properties (Gonçalves et al. 2018; Siqueira et al. 2009) with functional benefits such as antioxidant activity (Stobiecka et al. 2022). Furthermore, yogurt stands out as a trend in functional beverages, fueled by growing awareness of food composition and nutrition (Gupta et al. 2023).

It is important to highlight that the cooling process can influence yogurt classification, especially when fruits are added. One-phase yogurt is low-viscosity and made by stopping fermentation below 10 °C. Two-phase yogurt cools rapidly from below 20 °C to 5 °C, resulting in higher viscosity and limited syneresis (Sfakianakis and Tzia 2014; Tamime and Robinson 2007; Walstra et al. 1999).

Pineapple is high in sugar when ripe and rich in key nutrients. Per 100 g of fresh pulp, it has about 87.3% water, 0.5% protein, 0.5% lipids, 8.66% sugars, 0.3% ash, and 1.2% fiber. It also contains notable amounts of potassium, magnesium, phosphorus, calcium, zinc, copper, and vitamins such as ascorbic acid, folates, niacin, and thiamine (Sharma et al. 2024).

Beetroot has high nutritional value due to its antioxidant properties (Farhan et al. 2024), and pineapple has excellent sensory quality, with characteristic flavor and aroma, and shows significant acceptance profiles across varieties (Freitas et al. 2024).

This study aimed to formulate a two-phase yogurt from buffalo milk with pineapple and beetroot jam, using a fruit-vegetable blend, and to assess its impact on human nutrition and health.

MATERIAL AND METHODS

The study was conducted at the Federal Rural University of the Amazon (UFRA), Belém Campus, in the Biochemistry Laboratory. The milk from the Murrah breed water buffalo used in the experiment was obtained from females on Marajó Island, PA, via mechanical milking under appropriate hygienic conditions. The pineapple pulp and beetroots were purchased at the local market in Belém-PA.

Figure 1 - (a) Buffalo cow and calf from Marajó Island, Brazil. (b) Buffalo milk production in Northern Brazil. (c) Illustrative photo of the pineapples. (d) Whole and sliced beetroots.



Source: (a) Federal Rural University of the Amazon (UFRA, 2022); (b) Pará Government (SECOM, 2021); (c) Freepik (2026); (d) Freepik (2026).

Yogurt Processing

For the yogurt formulation, these steps were followed: 10% sugar was added to the milk. The mixture was heated to 85 °C for 15 minutes to reduce microbial load and cause partial concentration. Afterward, the milk was cooled to 45 °C, which is the optimal temperature for coagulating microorganisms. Next, 1% microbial culture was inoculated into the milk to make plain yogurt. The mixture was incubated until the pH reached about 4.6 and a firm texture was achieved. The yogurt was cooled, the curd was broken, and mechanical liquefaction was performed. Finally, the yogurt was stored at 8°C (Sfakianakis and Tzia 2014; Tamime and Robinson 2007; Walstra et al. 1999).

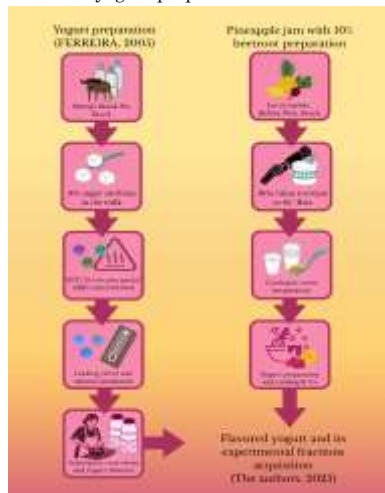
Jam Processing

In the formulation of the pineapple and beetroot jam (10% beetroot pulp was added to the pineapple pulp), the soluble solids content was increased to 65 °Brix. The jam was poured into disposable cups immediately and kept at room temperature. Subsequently, the yogurt was poured over the jam, and the product was kept under refrigeration at 8°C. It is important to note that no citric acid was added during the processing of this formulation, and the acidity obtained originated solely from its ingredients.

Physicochemical Analysis

The analyses of the raw milk, the pineapple and beetroot jam, and the formulated yogurt were performed in triplicate. Titratable acidity was determined by neutralization volumetric using a standardized 0.1 N sodium hydroxide solution and a 1% alcoholic phenolphthalein solution as an indicator (Brazil 2006). The determination of pH in the samples was carried out by direct reading using a bench-top potentiometer (Quimis), and the soluble solids content was determined according to the methodology described by the Association of Official Analytical Chemists (AOAC 2019).

Figure 2 - Flavored yogurt preparation and methodology scheme.



Source: The authors (2025).

RESULTS AND DISCUSSION

The following table (Table 1) shows the values obtained for the raw materials and final product are presented in Table 1.

Table 1. Physicochemical analysis of raw materials and finished product.

Formulation	pH	TSS (°Brix)	TA (g.100 g ⁻¹)
Raw buffalo milk	6.22 ± 0.05	12.43 ± 0.40	0.27 ± 0.03
Pineapple and beetroot jam	4.80 ± 0.10	66.08 ± 0.11	0.22 ± 0.07
Natural yogurt	4.40 ± 0.07	20.03 ± 1.93	0.83 ± 0.04
Flavored two-phase buffalo milk yogurt with pineapple and beetroot jam	4.43 ± 0.04	26.20 ± 0.06	1.13 ± 0.03

TSS: Total soluble solids; TA: Titratable Acidity.

The buffalo milk showed the mean acidity value is not in compliance with the legislation for bovine milk, which stipulates a titratable acidity of 0.14-0.18 g.100 g⁻¹ (Brazil 2018). However, this legislation does not establish pH parameters for raw milk. Mousa et al. (2025) reported a pH of 6.68 and titratable acidity of 0.14 g.100 g⁻¹ for buffalo milk, with the pH value agreeing with the findings of the present study (6.22). The higher acidity observed in this study (0.27 g.100 g⁻¹) may be explained by the metabolism of lactose into lactic acid by bacteria, which can contribute to the sensory characteristics of yogurt, such as its tangy taste (Abidin et al. 2024). Buffalo milk naturally has a higher fat content than other breeds, which can influence its acidity due to higher levels of casein and phosphate. Siregar et al. (2021) found titratable acidity values ranging from 0.50% to 0.58% (5.0 to 5.8 °D) and pH between 5.9 and 6.4 in buffalo milk from Indonesia. The pH of the raw buffalo milk analyzed in this study (6.22) falls within the expected range for buffalo milk, confirming its alignment with the expected parameters. With increased acidity, the pH of buffalo milk is typically lower than that of bovine milk, establishing unique characteristics for buffalo milk from the Marajó island.

Pineapple can be blended with various fruits and vegetables to create new innovative products, enhancing its acceptability (Salam et al. 2020) and addition of value. In the present study, the pineapple and beetroot jam presented a pH of 4.80. This value is higher than the range reported by Haider et al. (2024) for sugar beet (*Beta vulgaris L.*) jams, which varied between 3.26 and 3.44, and by Talchisa et al. (2022), who observed pH values ranging from 2.92 to 3.31 in pineapple jams produced with different concentrations of sugar, pectin, and temperature. In both studies, the lower pH values were attributed to the addition of citric acid, which was not used in the present formulation. The absence of citric acid may contribute to the higher pH observed, which could influence the preservation and shelf-life of the product. Regarding total soluble solids, the values obtained in this study were within the range reported by the mentioned authors. However, titratable acidity values were lower than those found in the literature, which may also be explained by the absence of citric acid in the formulation.

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The pH of the natural yogurt produced in this study (4.40) is consistent with Hamed et al. (2024), who reported a pH of 4.61 for control buffalo milk yogurt with essential oils, and with Siddique et al. (2025), who found titratable acidity values close to 0.97 g.100 g⁻¹, similar to this study's value (0.83 g.100 g⁻¹). In Brazil, Pinto et al. (2018) developed buffalo yogurt with açai pulp (*Euterpe oleracea*) and reported a total soluble solids content of 11.80 °Brix, lower than that observed here (20.03 °Brix). This difference likely results from varying raw materials and formulations.

The innovative final product, a flavored two-phase yogurt, presented pH values (4.43) within the range observed (4.61 to 4.63) in the study by Mousa et al. (2025) for yogurts produced from Egyptian buffalo milk fortified with ZnO micro- and nanoparticles. In contrast, the titratable acidity parameters found in that study (0.77 to 0.88 g.100 g⁻¹) were lower than those obtained in the present study (1.13 g.100 g⁻¹), a difference that may be related to variations in buffalo species and specific sample characteristics. Regarding total soluble solids (°Brix), this study reported values (26.20 °Brix) above the post-filtration range (11.0 °Brix) from Rebak, Cantero, and Obregón (2025) in the production of pineapple-flavored buffalo yogurt whey. This discrepancy likely reflects the fact that sugar in the cited study was added solely to enhance microbial growth, whereas the present formulation incorporated jam into the product.

CONCLUSION

The developed two-phase buffalo milk yogurt with pineapple and beetroot jam demonstrated suitable physicochemical properties, indicating technological feasibility. The formulation represents a promising alternative for value-added dairy products. Further research should focus on sensory evaluation, shelf-life studies, and functional characterization.

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