Spontaneous Fermentation of Regional Fruit Beverages using Buffalo Yogurt Whey

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Abstract: Yogurt whey is a by-product of the filtration process in Greek-style buffalo yogurt. It contains beneficial compounds such as proteins, minerals, carbohydrates, and lactic acid bacteria; yet, it is often discarded, contributing to environmental contamination due to its high biological oxygen demand. This study aimed to valorize this by-product by developing fermented beverages formulated by combining yogurt whey with regional citrus and tropical fruits. Eighteen formulations were prepared using whey, fruit pulp or juice (grapefruit and passion fruit), sugar, and filtered water, followed by spontaneous fermentation for 48 hours at ambient temperature (25 ± 2 °C). Physicochemical parameters (pH, °Brix, and Density) were measured before and after the filtration process. Two formulations, grapefruit (citrus group) and passion fruit (tropical group), were selected for sensory evaluation by 30 untrained panelists, who used a 9-point hedonic scale to assess aroma, flavor, and color. Physicochemical results showed minor changes in pH and significant increases in °Brix and Density in samples after filtration, particularly in beverages made with tropical fruits. Sensory analysis revealed high overall acceptability; passion fruit beverages received significantly higher overall and flavor scores from female participants than from males ($p = 0.043$). The results demonstrate the feasibility of transforming buffalo yogurt whey into a regionally inspired fermented functional beverage with favorable sensory acceptance and commercial potential. This approach presents an innovative strategy for reducing waste in the dairy industry. It supports sustainable food systems through the valorization of underutilized by-products and local fruits, which aligns with the principles of the circular economy.

Keywords: Yogurt whey, fermented beverages, tropical fruits, citrus fruits, sensory analysis, functional food.

INTRODUCTION

The dairy industry is one of the largest agro-industrial sectors globally, generating a significant volume of by-products during the transformation of milk into cheese, yogurt, and other dairy derivatives. Among these by-products, yogurt whey —a translucent, yellowish liquid resulting from the straining of natural yogurt to obtain Greek-style yogurt— has been historically underutilized, often discarded as waste, despite its high nutritional and functional potential [1].

In Argentina, yogurt whey is not explicitly regulated as a primary food ingredient; yet, it is a complex matrix of bioactive components, including soluble proteins (such as α-lactalbumin and β-lactoglobulin), lactose, organic acids, minerals (calcium, phosphorus, magnesium), and viable lactic acid bacteria (LAB) [2,3]. Unlike sweet or acid whey, which are by-products of cheese production, yogurt whey exhibits a distinctive microbiological profile that may be particularly advantageous for fermentation-based processes. The inappropriate disposal of yogurt whey represents not only a loss of valuable nutrients but also an environmental concern, given its high Biochemical Oxygen Demand (BOD) and organic load [4].

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Greek-style yogurt from this milk is especially valued due to its rich composition of solids, proteins, and fats. The whey derived from buffalo yogurt is a promising substrate for fermentation, with higher nutritional properties than cow-derived whey [5,6]. Despite these benefits, its application in food formulation remains limited, especially in small-scale or artisanal dairy settings [7].

Recent trends in food sustainability have emphasized the importance of reintroducing agro-industrial by-products into the food chain following circular economy models. This strategy not only reduces environmental load but also creates added value and promotes economic resilience in local production systems [8]. Within this framework, the use of yogurt whey for the development of fermented fruit beverages offers a dual opportunity: to utilize a nutrient-rich by-product and to valorize regional fruits, some of which remain underexploited.

Citrus fruits like grapefruit and orange provide ascorbic acid, natural acids, and flavonoids, while tropical fruits such as passion fruit and guava contribute carotenoids, aromatic compounds, and desirable sweetness [6, 9]. These combinations can improve the sensorial and nutritional appeal of the final product. Additionally, fermented beverages may retain some beneficial microbial populations or stimulate probiotic effects, making them attractive candidates for the functional food market.

This study aimed to develop spontaneously fermented beverages formulated with yogurt whey and regional fruits, and to evaluate their physicochemical properties and sensory acceptance. The research also aimed to provide a practical model for small-scale application and future industrial scaling, contributing to both environmental sustainability and innovation in functional food systems.

MATERIALS AND METHODS

Yogurt Whey

The yogurt whey used in this study was a by-product of Greek-style buffalo yogurt production, prepared under controlled artisanal conditions. Buffalo milk was collected from a single farm located in Corrientes, Argentina, to ensure consistency of the raw material. Milk was pasteurized at 72°C for 15 seconds, then inoculated with a thermophilic starter culture (*Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*), and incubated at 42°C until a firm coagulum was formed. Four independent batches of yogurt were prepared. After fermentation and cooling, the yogurt was filtered through a sterile gauze to separate the solid fraction from the liquid whey. This buffalo yogurt whey, characterized by its translucent yellowish color, was pooled and mixed to ensure homogeneity of protein (0.82 ± 0.05 g/100 mL), lactose (3.5 ± 0.2 g/100 mL), and pH (4.5 ± 0.1). Soluble proteins (mainly β-lactoglobulin and α-lactalbumin), lactose, minerals, and viable LAB; the latter contribute positively to subsequent fermentation processes [3,4,9].

The fresh whey had a viable LAB count of 6.4 ± 0.3 log CFU/mL, determined by surface plating on MRS agar incubated at 37 °C for 48 hours. A translucent appearance and mild acidic aroma characterized this whey. It was collected under hygienic conditions and stored at 4 °C for up to 24 hours before processing or frozen at –18 °C until use to preserve its microbiological and physicochemical properties. The whey served both as a fermentation medium and as a source of nutrients and LAB.

Fruits

Seven ripe seasonal fruits were selected based on sensory quality (color, maturity, aroma, texture):

Group 1: (Citrus fruits) Grapefruit (*Citrus paradisi*), orange (*Citrus sinensis*), mandarin (*Citrus reticulata*)

Group 2: (Tropical fruits) Guava (*Psidium guajava*), passion fruit (*Passiflora edulis*), pineapple (*Ananas comosus*), mango (*Mangifera indica*)

Fruits were sourced from local markets in Corrientes, Argentina. Before processing, they were washed with tap water, sanitized with a 200-ppm sodium hypochlorite solution for 15 minutes, and then thoroughly rinsed.

Water and Sugar

Filtered water (chlorine-free) was used to avoid bactericidal effects. Refined white sugar (Type A, ≥99.8% sucrose) was added to support microbial growth and enhance palatability.

Fermentation Protocol

Eighteen formulations were prepared by combining yogurt whey, fruit juice or pulp (300 mL or 300 g), sugar (120 g), and water (up to a total volume of 2 L). Each formulation was mixed in triplicate in sterilized 2-L glass containers and covered with sterile gauze. No microbial inoculum or back-slopping was used; fermentation was spontaneous and relied on the endogenous LAB from the yogurt whey and fruit microbiota.

Fermentation took place over 48 hours at ambient temperature (25 ± 2 °C, recorded with a digital data logger). After fermentation, beverages were filtered using sterile cloth to remove pulp residues and collected and stored at 4 °C until analysis (<12 hours).

Physicochemical Analysis

Each beverage was analyzed in triplicate before and after filtration for the parameters described below.

pH Measurement

pH was measured using a calibrated digital pH meter (HANNA® HI98103) at 20 °C. Calibration was performed daily with pH 4.01 and 7.00 standard buffers. Values were recorded before and after fermentation.

˚Brix and Density

˚Brix values were determined with a hand-held refractometer (ATAGO®) calibrated with distilled water, and Density was measured using a glass hydrometer (±0.001 g/cm³ accuracy) at 20 °C, both before and after filtration.

Sensory Evaluation

Two formulations were selected for sensory testing: grapefruit (from Group 1) and passion fruit (from Group 2), based on pre-selection scores assigned during an internal tasting session (n = 10).

The sensory panel was composed of 30 untrained adults (13 men, 17 women; aged 20–35).They participated voluntarily and confirmed that they had no allergies to dairy products or fruit. Tastings were conducted between 10:00 and 11:30 a.m. in a controlled environment (22 ± 1°C, neutral lighting). Beverages were served at 8–10°C in a randomized order, using 100-mL coded cups. The panelists rinsed the cups with water between samples.

Each participant rated aroma, flavor, color, and overall acceptability using a 9-point hedonic scale (1 = dislike remarkably, 9 = like extremely). No formal sensory training was given, and instructions were delivered. Exact p-values, F-statistics, and degrees of freedom (df) were reported when relevant.

Statistical Analysis

All data were analyzed using InfoStat® v2020. Means were compared using ANOVA followed by Tukey's HSD post-hoc test. Normality (Shapiro–Wilk) and homogeneity of variance (Levene's test) were confirmed for each dataset.

Exact p-values, F-statistics, and df were reported where relevant. A significance level of $p < 0.05$ was used.

RESULTS AND DISCUSSION

Physicochemical Parameters

The fermented beverages exhibited slight but consistent changes in physicochemical parameters before and after filtration (Table **1**).

In Group 1 (citrus fruits), pH values decreased slightly after filtration (from 3.93 ± 0.36 to 3.71 ± 0.28), indicating mild acidification due to fermentation. In Group 2 (tropical fruits), a similar trend was observed (3.60 ± 0.35 to 3.46 ± 0.22). However, the initial pH was already lower, likely due to the intrinsic organic acid content of tropical fruits such as passion fruit and pineapple. The pH showed a slight decrease (~0.2–0.3 units) across formulations during fermentation, which was more noticeable in citrus-based beverages. Despite the statistically significant reduction in pH, both beverages maintained stability and perceptible sourness. This range is within the typical tolerance of the human palate and unlikely to affect overall acceptability.

Our results are consistent with findings reported by Shukla *et al*. [10], who documented a similar trend in beverages formulated with recycled acid whey. The authors noted progressive acidification that did not hurt sensory acceptability during prolonged storage. Similarly, Królczyk *et al*. [11] highlighted the use of whey as a fermentable substrate with potential for developing functional beverages, particularly when combined with tropical fruits that enhance both the organoleptic profile and product stability, such as pineapple.

Brix values increased significantly after filtration in both groups. °Brix rose from 12.3 ± 3.50 to 14.3 ± 2.24, and from 9.74 ± 3.28 to 11.0 ± 3.17 ($p < 0.05$). Density values showed a similar pattern. Density increased from 1048 ± 12.55 to 1050 ± 8.55, and from 1038 ± 11.54 to 1043 ± 12.62 ($p < 0.05$) in G1 and G2, respectively. This result may be attributed to evaporation during fermentation or the concentration of solutes following the removal of fruit pulp. Tropical fruit formulations, particularly those involving passion fruit and mango, exhibited the greatest increases in °Brix (Δ°Brix up to 2.4 ± 0.3) and Density (Δd: 0.006 ± 0.001 g/cm³). These trends are consistent with the observations reported by Gómez *et al*. [7], who documented similar increases in °Brix and Density during the fermentation of whey-based beverages formulated with fruit juice mixtures.

Microbiological Viability

Initial LAB counts in yogurt whey were 6.4 ± 0.3 log CFU/mL, and post-fermentation values ranged between 7.2 and 8.1 log CFU/mL across beverages. These levels fall within the functional range for probiotic drinks, supporting the spontaneous fermentation model used. However, further microbial profiling and viability testing over time are necessary to ensure the safety and stability of shelf life.

Our findings support the hypothesis that yogurt whey provides a suitable medium for lactic fermentation, contributing acidity and microbial stability, while the fruits contribute sugars, organic acids, and aroma compounds.

Table 1: Physicochemical Parameters of Fermented Whey–Fruit Beverages before and after Filtration

Variable	G1 (Citrus Fruits) Mean ± SD	G2 (Tropical Fruits) Mean ± SD
pH (Pre-filtration)	3.93 ± 0.36	3.60 ± 0.35
pH (Post-filtration)	3.71 ± 0.28	3.46 ± 0.22
°Brix (Pre-filtration)	12.3 ± 3.50	9.74 ± 3.28
°Brix (Post-filtration)	14.3 ± 2.24	11.0 ± 3.17*
Density (Pre-filtration)	1048 ± 12.55	1038 ± 11.54
Density (Post-filtration)	1050 ± 8.55	1043 ± 12.62*

Group 1 (G1): citrus fruits (grapefruit, orange, mandarin), n = 3.
Group 2 (G2): tropical fruits (guava, passion fruit, pineapple, mango), n = 3.
Note: Values are expressed as mean ± standard deviation.
*Statistically significant difference between groups at the same filtration stage ($p < 0.05$), determined by Tukey's test.

Sensory Evaluation

Sensory scores were recorded for aroma, color, and flavor using a 9-point hedonic scale. Results are shown in Table **2**, disaggregated by gender and attribute.

Our gender-disaggregated sensory analysis revealed that female panelists rated the passion fruit beverage significantly higher than males in overall acceptability (8.5 vs. 7.6; $p = 0.043$), with similar but non-significant trends in aroma, flavor, and color preferences (Table **2**). These results align with findings by Junqueira-Gonçalves *et al*. [9], who reported that women tend to assign higher hedonic scores to tropical fruit flavors —especially passion fruit and mango— due to their sweetness and aromatic complexity. Biological differences may partly explain this marked preference: women generally exhibit greater taste sensitivity, attributed to a higher density of fungi form papillae and taste buds, as well as enhanced olfactory capabilities. Bartoshuk *et al*. [12] demonstrated that women are more frequently "supertasters", based on tasting profiles, while Mastinu *et al*. [13] confirmed that women display slightly higher gustatory and somatosensory sensitivity than men. Additionally, the moderate natural carbonation resulting from spontaneous fermentation was perceived positively by most participants and described as "light" and "refreshing"; this result is consistent with findings of Miranda-Ledesma *et al*. [14], who reported similar responses when testing kefir-based tropical fruit beverages.

The most appreciated attribute was flavor, followed by color and aroma, particularly in passion fruit samples. Grapefruit's natural bitterness was considered pleasant by some panelists but overly intense by others, highlighting the importance of selecting fruit varieties or adjusting sugar levels to achieve optimal balance (Table **3**).

The descriptive sensory evaluation revealed a clear preference for the passion fruit beverage across all attributes. A higher percentage of panelists rated the flavor (66.6%), aroma (70.0%), and color (73.3%) of passion fruit with scores within the high acceptance range (7–9), compared to the grapefruit formulation. Passion fruit is characterized as sweet, tropical, and well-balanced, with an intense fruity aroma and a bright yellow color that is visually appealing. In contrast, the grapefruit beverage received moderate acceptance, with flavor (56.6%) and smell (53.3%) described as mildly acidic and citrusy, and color (63.3%) considered natural but less vibrant. These trends suggest that passion fruit's sweet, aromatic profile and vibrant hue have a strong influence on consumer acceptance.

Comparison with the Literature and Implications

This study confirms that combining yogurt whey with regional fruits produces beverages with acceptable physicochemical and sensory properties. Moreover, it supports the feasibility of adding value to the dairy industry through small-scale fermentation.

According to El-Aidie and Khalifa [8], whey-based fermented products represent a promising strategy for implementing a circular economy in the agro-industry, offering a dual benefit: mitigating environmental risks while generating new consumer markets. The use of indigenous or underutilized fruits, as proposed in our formulations, further enhances the sustainability and cultural relevance of these products, especially in regions with rich biodiversity, such as northeastern Argentina.

Furthermore, several authors, including Souza *et al*. [15] and Silva *et al*. [16], have highlighted the increasing consumer interest in non-dairy, functional, or probiotic beverages that reflect local identity.

Table 2: Sensory Scores for Fermented Whey–Fruit Beverages, by Attribute and Gender

Formulation	Taster Gender	Aroma (Mean ± SD)	Flavor (Mean ± SD)	Color (Mean ± SD)	Overall Acceptability (Mean ± SD)
Grapefruit	Men (n = 13)	6.9 ± 0.8	6.4 ± 0.9	7.1 ± 0.7	6.8 ± 0.8
	Women (n = 17)	6.7 ± 0.6	6.5 ± 1.0	6.9 ± 0.9	6.6 ± 1.0
Passion fruit	Men (n = 13)	7.7 ± 0.7	7.9 ± 0.6	7.7 ± 0.8	7.6 ± 0.7
	Women (n = 17)	8.2 ± 0.5	8.4 ± 0.4	8.0 ± 0.6	8.5 ± 0.5*

Group 1: Grapefruit-based beverage.
Group 2: Passion fruit-based beverage.
Note: Values are expressed as mean ± standard deviation.
*Statistically significant difference in overall acceptability between men and women for the passion fruit beverage ($p = 0.043$; Student's t-test).

Table 3: Descriptive Sensory Evaluation of Grapefruit and Passion Fruit Beverages. Percentages Represent Panelists Who Rated Each Attribute with a Hedonic Score of 7–9 (High Acceptance)

Beverage	Attribute	% of panelists rating 7–9 (high acceptance)	Observations
Grapefruit (Juice 1)	Flavor	56.6%	Pleasant acidity, slight bitterness
	Aroma	53.3%	Mild citrus profile
	Color	63.3%	Pale yellow, perceived as natural
Passion Fruit (Juice 2)	Flavor	66.6%	Sweet, tropical, well-balanced
	Aroma	70.0%	Intense, fruity, widely accepted
	Color	73.3%	Bright yellow, appealing appearance

Fermented whey-based drinks meet this trend when appropriately flavored and stabilized.

Potential Applications and Commercial Outlook

The development of fermented beverages using yogurt whey and regional fruits offers multiple possibilities for technological innovation and sustainable entrepreneurship in both small-scale and industrial settings. From a technological perspective, the process requires only basic equipment (filtration units, fermentation vessels, refrigeration) and is adaptable to artisanal or semi-industrial production. This low complexity makes whey production highly suitable for small producers, cooperatives, or dairy microenterprises seeking to diversify their product offerings. The use of locally available fruits and dairy residues also reduces production costs and strengthens regional supply chains.

These beverages can be positioned in several growing market segments: a) functional beverages, due to the presence of LAB, bioactive peptides, and antioxidant compounds from fruits; b) low-alcohol fermented drinks, because spontaneous fermentation can produce trace ethanol levels, appealing to health-conscious adult consumers; and c) natural and clean-label products with minimal processing and no artificial preservatives, in line with current consumer trends.

Yogurt whey is classified as a dairy by-product in most food codes, and its use is generally permitted; however, commercial-scale operations should consider local regulations. With proper pasteurization or cold chain assurance, shelf life can be extended, as demonstrated by Andrade *et al*. [17] inprobiotic whey drinks with tropical fruits.

Given the appealing sensory profile and nutritional value of this product, a commercial version could compete in the market of fruit-based fermented beverages, as kombucha and water kefir do. Additionally, packaging innovations (e.g., glass bottles with carbonation) could add value and aesthetic appeal.

Future collaborations with local agricultural networks and research institutions could help optimize fermentation protocols, standardize formulations, and evaluate long-term storage, probiotic viability, and consumer acceptance on a broader scale.

CONCLUSION

This study demonstrated the technical feasibility and sensory appeal of fermented fruit beverages formulated with buffalo yogurt whey and regional fruits through spontaneous fermentation. The resulting drinks, particularly those based on tropical fruits like passion fruit, demonstrated high consumer acceptability and favorable physicochemical profiles, including increased °Brix and density after filtration. Moreover, spontaneous fermentation led to a moderate increase in viable LAB counts, supporting the beverages' potential as functional dairy-based products.

The use of Greek-style buffalo yogurt whey, rich in fermentable substrates and beneficial microorganisms, is a valuable strategy for valorizing a nutrient-dense by-product typically discarded in small-scale dairies. This approach aligns with principles of the circular economy, promoting resource efficiency, waste reduction, and the creation of high-value food innovations.

Although results are promising, several limitations must be addressed before commercial implementation. The participation of untrained sensory panelists limits the descriptive precision, and fermentation outcomes may vary without the use of standardized starter cultures. Additionally, shelf life, microbial safety, and residual ethanol content were not evaluated and should be addressed in future studies.

Further research is necessary to assess microbial viability and spoilage markers during storage, as well as the shelf life and physical and chemical stability of these beverages. Future studies should also explore controlled fermentations using defined LAB strains to enhance product consistency and safety. Additionally, the development of fruit blends may help improve the palatability of bitter fruits such as grapefruit, increasing consumer acceptance. The incorporation of buffalo milk derivatives into novel food matrices holds promise for expanding product diversity within the dairy sector, strengthening regional food systems, and promoting sustainable nutrition.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENT

CIIAB (Bubaline Artificial Insemination Center) for providing buffalo milk for the development of the experiment.

FINANCIAL SUPPORT STATEMENT

Ministry of Science and Technology of the province of Corrientes and the General Secretariat of Science and Technology of the National University of the Northeast.

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Received on 12-07-2025 Accepted on 16-08-2025 Published on 23-08-2025

https://doi.org/10.6000/1927-520X.2025.14.14