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New formulations of fermented milk drinks with fruit pulp added: Physicochemical characteristics during storage and nutritional profile

Nuevas formulaciones de bebida láctea fermentada con adición de pulpa de frutas: Características fisicoquímicas durante el almacenamiento y perfil nutricional

Handray Fernandes de Souza[1,3]. *https://orcid.org/0000-0002-0216-7117*
Lara Aguiar Borges[1]. *https://orcid.org/0000-0001-7013-3819*
Gabriela da Rocha Lemos Mendes[1]. *https://orcid.org/0000-0001-6432-0239*
Carla Adriana Ferreira Durães[1,2]. *https://orcid.org/0000-0002-9808-1034*
Hugo Calixto Fonseca[1]. *https://orcid.org/0000-0001-6410-4253*
Sarah Caroline Oliveira de Souza Boitrago[1,2]. *https://orcid.org/0000-0001-8773-0628*
Jéssica Santos Leal[1]. *https://orcid.org/0000-0002-3030-4967*
Sildimar Rodrigues Ferreira[1]. *https://orcid.org/0000-0002-8596-2254*
William James Nogueira Lima[1,2]. *http://orcid.org/0000-0002-1128-1448*
Eliana Setsuko Kamimura[3]. *https://orcid.org/0000-0002-9686-7519*
Igor Viana Brandi[1,2]. *http://orcid.org/0000-0001-6714-7996*

1. Instituto de Ciências Agrárias (ICA), Universidade Federal de Minas Gerais (UFMG), Campus Regional de Montes Claros, Avenida Universitária, 1000, Bairro Universitário, CEP 39404-547, Montes Claros, Minas Gerais, Brazil
2. Universidade Estadual de Montes Claros (UNIMONTES), Avenida Professor Rui Braga, S/N, Vila Mauricéia, CEP 39401-089, Montes Claros, Minas Gerais, Brazil
3. Universidade de São Paulo (USP), Faculdade de Zootecnia e Engenharia de Alimentos (FZEA), Campus Fernando Costa, Av. Duque de Caxias Norte, 225, CEP 13635-900,Pirassununga, São Paulo, Brazil

*Corresponding author: Igor Viana Brandi
Instituto de Ciências Agrárias (ICA), Universidade Federal de Minas Gerais (UFMG), Campus Regional de Montes Claros, Avenida Universitária, 1000, Bairro Universitário, CEP 39404-547, Montes Claros, Minas Gerais, Brazil
Email: ibrandi@hotmail.com and ibrandi@ica.ufmg.br

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ABSTRACT

The aim of this study was to evaluate the nutritional profile and the physicochemical characteristics during storage of newly developed formulations of fermented milk drinks with added pineapple, mango and passion fruit pulp. The fermented drinks showed a high content of protein, iron, and calcium. The passion fruit milk drink had the lowest pH (4.13) and highest acidity (0.95%, expressed in % of lactic acid), which was significantly different (p<0.05) from the pineapple and mango drinks. As for syneresis and sedimentation, the pineapple milk drink had the highest rates at 14 days storage, with 34.33% and 6.50%, respectively and was significantly different (p≤0.05) when compared to the mango and passion fruit milk drinks. In conclusion, newly developed fermented milk drinks with added fruit pulp were a source of several nutrients. We observed physical-chemical characteristics suitable for a fermented milk product during storage.
Keywords: Milk fermented; Nutritional value; Sedimentation index; Syneresis; Whey.

RESUMEN

El objetivo de este estudio fue el desarrollo de nuevas formulaciones de bebidas lácteas fermentadas adicionadas de piña, mango y maracuyá, para evaluar el perfil nutricional y las características fisicoquímicas durante el almacenamiento. Las bebidas fermentadas mostraron un alto contenido en proteínas, hierro y calcio. En cuanto a las características fisicoquímicas *durante el almacenamiento, la bebida láctea de maracuyá presentó el pH más bajo (4,13) y la acidez más alta (0,95%, expresada en % de ácido láctico), con una diferencia significativa (p < 0,05), en comparación con las bebidas de piña y mango. En cuanto a sinéresis y sedimentación, la bebida láctea de piña presentó los mayores índices a los 14 días de almacenamiento, con 34,33% y 6,50%, respectivamente, y con diferencia significativa (p ≤ 0,05) al compararla con las bebidas lácteas de mango y maracuyá. En conclusión, las bebidas lácteas fermentadas con adición de pulpa de fruta son una fuente de varios nutrientes, y de características físico-químicas adecuadas para un producto lácteo fermentado durante el almacenamiento.*

Palabras clave: Índice de sedimentación; Leche fermentada; Suero; Sinéresis; Valor nutricional.

INTRODUCTION

Tropical fruits have potential for use in food development. In recent years, the production and marketing of these fruits has increased strongly due to their sensory quality, nutritional value, and functional properties[1,2]. In Brasil[3], approximately 44 million tons of fruit are harvested annually, with a total export value of over US$ 1 billion in 2019[4]. According to Alvarez-Rivera et al.[5] (2020), the main species of tropical fruit are Annonaceae (soursop, pine cone), Anacardiaceae (plum, mango, umbu), Bromeliaceae (pineapple) and Passifloraceae (passion fruit). In general, tropical fruits are rich in bioactive compounds (e.g., ascorbic acid, phenolic compounds, carotenoids, lipids and fatty acids) that have high antioxidant activity[6,5]. Such compounds help reduce the incidence of degenerative diseases, such as, aging, cancer, brain dysfunction, inflammation, and heart disease[7,8]. For example, flavonoids are the largest class of polyphenols (considered dietary antioxidants), which, besides showing antioxidant activity, have important anti-inflammatory, antiallergic, anticancer, and antiviral properties, and are used and marketed in pharmaceutical products and food supplements[6]. Other effects that antioxidants provide to human health include protection against arthritis, asthma, and a number of various diseases such as cancer, diabetes mellitus, heart disease, and gastrointestinal inflammation[9].

One of the challenges of the fruit production chain is the high susceptibility after harvest. Technologies and new uses are necessary to reduce the wasting of products, such as storage under appropriate and controlled conditions, minimal processing practices, and packaging application[10,11,12]. The development of products including fruits in their formulation is a way to add value and several products have been reported in the literature, including cereals, juices, pulps, jams, and fibers[13,14,15].

Adding fruit to dairy products, such as yogurts, milk-based beverages, and fermented milk can contribute to product improvement. Fruit pulp can also enhance aroma, sensory acceptance, and the nutritional quality of products[16,17,18]. According to the literature, whey and milk-based drinks with added tropical fruits have been developed and their physicochemical and sensory characteristics evaluated[19,20,21,22]. However, the nutritional profile and physicochemical characteristics related to pH, acidity, whey separation index (syneresis) and sedimentation during storage of fermented milk drinks with the pulp of tropical fruits such as pineapple (Ananas comosus), mango (Mangifera indica) and passion fruit (Passiflora edulis) added have yet to be described. The aim of this study was to evaluate the physicochemical characteristics during storage and the nutritional profile of fermented milk drink formulations, comparing new formations with added pineapple (Ananas comosus), mango (*Mangifera indica*) and passion fruit (*Passiflora edulis*) pulp.

MATERIAL AND METHODS

A commercial lyophilized starter culture Direct Vat Set (DVS) containing mixed culture of *Streptococcus termophilus* and *Lactobacillus delbrueckii* ssp *bulgaricus* were used. Fruit pulp and other ingredients used in formulation were purchased from a local market. All reagents used in this study were analytical grade.

Development of milk fermented drinks

Three formulations were developed according to Reis, et al.[18] (2021), with some modifications. The basis formulation was: 50% milk (v/v), 50% reconstituted whey (in water – 15%, v/v), 10% sugar (w/v), and 0.8% modified starch (w/v). The mixture was heated at 65 °C for 30 min and cooled until 43 °C. Then, starter culture (1% w/v) was added. Fermentation was finished when pH reached 4.6 (6-8 h), after which the formulation was cooled to 5 ± 1 °C for 5 h. The mixture was then subject to agitation and other ingredients were added: fruit pulp (10% w/v – pineapple, mango, or passion fruit), 0.1% potassium sorbate (w/v), and iron amino acid chelate (3 mg/100 mL). The fermented milk drinks were packed in one-liter plastic bottles and stored in a refrigerator at 5 ± 2 ºC until analyses.

Nutritional label development

The nutrition labels of the developed formulations were elaborated according to Brazilian Food Composition Table[23]. The % daily value (DV) was calculated based on Brazilian legislation[24] and dietary reference intake[25].

pH and acidity of milk fermented drink

pH was measured at room temperature (25 ± 2 ºC) by potentiometry and the titratable acidity was performed by titration with 0.1 M NaOH[26]. Results were expressed in g of lactic acid/100 mL.

Syneresis and sedimentation index

Syneresis was used to determine the whey separation index, usually used for fermented milk products such as yogurt and fermented milk drinks with aspects of firm, pasty, semi-solid or liquid consistency[16,27,28]. The syneresis index was determined using 2 g of samples were centrifuged at 8.000 rpm for 10 min at 25 ºC. The supernatant was removed and weighed[16,27]. The syneresis index (%) was obtained according to Equation 1.

$$\text{Syneresis index} = \frac{\text{Amount of supernatant}}{\text{(Total os mass)}} \times 100 \qquad (1)$$

For the sedimentation analysis, we used the method described by Souza et al.,[16] (2020). Ten g of sample was weighed and packed in plastic tubes and refrigerated at 5 ± 1 °C for 72 h. The percent sedimentation index (%) was obtained according to Equation 2.

$$\text{Sedimentation (\%)} = \frac{(V\ sed)}{(V\ total)} \times 100 \qquad (2)$$

V_{sed} = sediment volume (mL)
V_{total} = total sample volume (mL).

Statistical analyses

In this study, a randomized design with three repetitions and triplicates was adopted. The results of the nutritional information of the milk fermented drinks are descriptive. Results of pH, acidity, sedimentation and syneresis were tabulated and compared by fruit type using analysis of variance (ANOVA) and Tukey's post-hoc test using a p-value of ≤0.05. We used R Software (R Development Core Team - 2010)

RESULTS

pH and titratable acidity

During storage, there was an increase in the acidity of fermented drinks prepared with pineapple and mango, however, the fermented drink with passion fruit pulp added did not change (Table 1).

At 7 days of storage we observed a decrease in the pH of mango milk drinks, but not for pineapple and passion fruit drinks. At 14 days of storage there was a significant increase in pH (P<0.05) for all milk drinks prepared. The variation of results can be attributed to differences between the physico-chemical composition of fruits. Studies have reported, for example, that the presence of organic acids in fruit pulps can alter pH and acidity values[29,30].

Syneresis and sedimentation index

The addition of fruits in the formulation also contributed to an increase in the sedimentation index (Figure 1A). The fermented drink with pineapple pulp, as expected, showed the highest sedimentation index at the three times evaluated. Souza et al.[16] (2020) evaluated the physical-chemical stability of dairy fermented drinks with caja-mango pulp and observed higher values of sedimentation (7.2-9.2%). Silva et al.,[31] (2010) found sedimentation index >70% in pineapple pulp up until 10 days of storage.

Syneresis is an important index in the evaluation of fermented milk products such as yogurt and milk drinks because it allows for the separation of components and phases of the mixture, such as whey[16,27]. The fermented drink with pineapple pulp showed the highest syneresis index during the 14 days of storage, an increase 6.4-fold greater than the first day of storage (Figure 1B). The syneresis of fermented drink prepared with mango and passion fruit was lower, a difference of 3.5 and 2.7-fold between 0 and 14 days, respectively.

Table 1. pH and titratable acidity (expressed as lactic acid, %) of fermented drinks developed with fruit pulp at 0, 7, and 14 days storage at 4 °C.

Day	FD + pineapple		FD + mango		FD + passion fruit	
	pH	Acidity	pH	Acidity	pH	Acidity
0	4.53^{aB}	0.67^{bB}	4.49^{aB}	0.66^{cB}	4.13^{bB}	0.95^{aA}
7	4.48^{aB}	0.67^{bB}	4.45^{bC}	0.67^{bB}	4.13^{cB}	0.94^{aA}
14	4.58^{aA}	0.69^{bA}	4.56^{bA}	0.70^{bA}	4.26^{cA}	0.95^{aA}

Notes: Means followed by the same uppercase letter in a column do not differ significantly by Tukey test (p< 0.05) for days of storage. Means followed by the same lowercase letter in the line do not differ significantly by Tukey test (p< 0.05) for different fermented drinks.

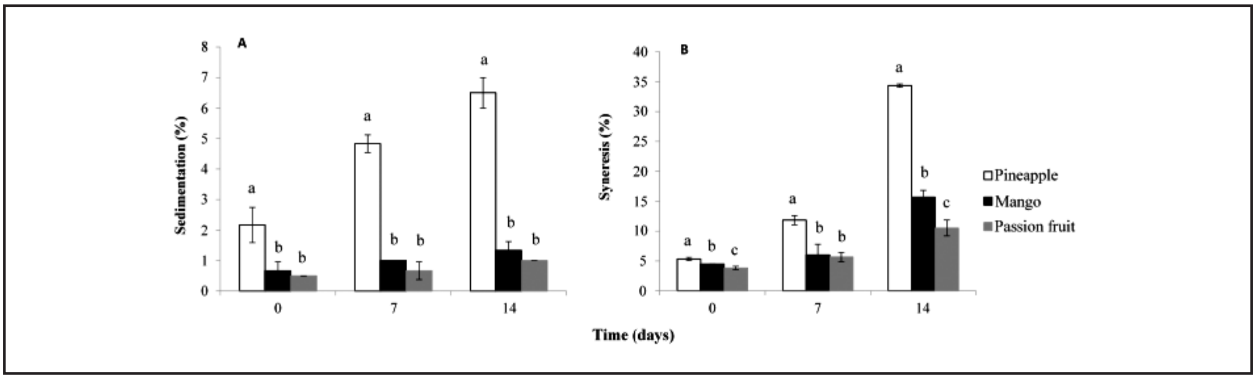

Figure 1: Sedimentation (A) and Syneresis (B) of fermented drink developed with fruit pulp at 0, 7, and 14 days storage at 4 °C. Values represent mean ± standard deviation. Different letters indicate statistically significant differences at α= 0.05.

Nutritional information

Table 2 shows the nutritional label of fermented drinks with the pulp of fruits added and their respective equivalences to daily nutritional values.

All fermented drinks developed showed a protein content of 4 g per portion (200 mL). This value is close to the upper limits found in fermented dairy drinks and can be explained by the whey added in the formulation. Lievore et al.,[32] (2015) developed a beverage fermented with acid whey and reported 2.54% protein in the product, similar to this study.

The formulations showed daily values of Ca equal to 20% and the iron supplementation corresponded to 49% of the recommended dietary intake[24]. With respect to iron, the prepared milk drinks provided a daily value of 49% intake of this micronutrient (Table 2). Thus, the products developed here can be considered as dietary sources of iron.

Table 2. Nutritional information and contribution to recommended daily value of nutrients of the fermented drinks developed with fruit pulp.

	Nutrition information (portion of 200 mL)					
	FD + pineapple	**% DV**	**FD + mango**	**% DV**	**FD + passion fruit**	**% DV**
Energy value	175 kcal= 735 kj	9	179 kcal= 752 kj	9	177 kcal= 743 kj	9
Carbohydrate (g)	34.0	11	35.0	12	35.0	12
Protein (g)	4.0	8	4.0	8	4.1	8.2
Total fat (g)	2.4	3.1	2.4	3.1	2.4	3.1
Saturated fat (g)	1.6	7	1.6	7	1.6	7
Total Fiber (g)	0.1	0.4	0.1	0.4	0.1	0.4
Ca (mg)	198	20	198	20	198	20
Fe (mg)	7	49	7	49	7	49

Notes: Abbreviation: FD, fermented drink; DV (%), Daily Values are based on a diet of 2.000 kcal.

DISCUSSION

In this study, new formulations of fermented milk drinks were evaluated. The increase in acidity during storage of milk products was a result of the post-acidification of the product. This phenomenon is characterized by the continuous metabolic and fermentative activity of microorganisms able to degrade lactose even under refrigeration conditions, although the process is slow. However, other factors such as the type of microorganism, milk ingredients used, fermentation and storage time can also influence acidity[33,34].

According to table 1, there was a significant increase (P≤0.05) in the acidity of pineapple and mango milk drinks and the formulation with passion fruit remained unchanged throughout the 14-day storage period. Regarding pH, there was a significant decrease (P≤0.05) of the values on the 7th day of storage, except for the passion fruit milk drink, which had no pH alteration in this period and presented the lowest value of this parameter. These variations observed between formulations may relate to the different chemical compositions of the fruits. Studies have reported that the presence of organic acids in fruit pulps can alter pH and acidity values[29,30]. A study on the physical-chemical aspects of four different brands of frozen fruit pulps, in the cities of Petrolina (PE) and Juazeiro (BA), Brazil, showed that passion fruit pulps have a lower pH compared to mango and pineapple pulps[35]. Therefore, the difference in the pH and acidity values of the passion fruit milk drink compared to the others can most likely be associated with the factors discussed above.

The reduction in pH and increase in acidity during storage may relate to the continuous production of organic acids, such as lactic acid, from the fermentation of carbohydrates by lactic cultures[36,37]. In this study, a contrary behavior was found at 14 days of storage, this fact can be related to protein degradation, resulting in ammonia generation. Thus, the amino acids in the beverage are the main source of nitrogen for the bacteria, enabling a large amount of nitrogen compounds[38]. In this sense, the degradation of proteins is initiated by the action of enzymes that hydrolyze them into peptides and then into amino acids, since the structure of the intact protein is not able to cross the cell membrane. A study on the physicochemical stability of fermented milk drink with cajá-manga pulp observed a similar phenomena[16]. In their studies, these authors also associated the increase in pH with the amine generation proteolysis, which contributes to increase this parameter.

The results showed low acidifying characteristic of L. bulgaricus in fermented milk content whey and pulp fruit, since the acidity for fermented milk products ranges from 0.7 to 0.9% of lactic acid[39]. According to Vinderola, Bailo and Reinheimer[40] (2000), during the fermentation time, the pH can influence high acidification, possible separation of phases, as well as alterations in the sensory aspects that can make the product undesirable. Therefore, it is important to strictly control pH during fermentation.

Organic acids can affect pH and acidity in fruit pulps[29,30]. Thus, the addition of pulp to formulations can increase the rate of syneresis due to the natural acidity of fruit pulps and decreased total solids[41]. Zhang et al.[42] (2007) describe that citric acid and malic acid are the main organic acids in pineapple. However, Sun et al.[43] (2016) found higher contents for citric acid (corresponding to 62% of total organic acid), followed by malic acid (approximately 14%), tartaric acid, and acetic acid, in different pineapple varieties. On the other hand, mango and passion fruit have lower organic acid contents[44,45,46]. Thus, the higher syneresis rates for the pineapple drink are justified, although, Gallina et al.[47] (2019) also found lower syneresis rates for drink added mango and passion fruit pulp.

About sedimentation (Figure 1A), it was observed that the pineapple milk drink had the highest rate of this parameter during storage, with a significant difference compared to the other drinks. On the other hand, the mango and passion fruit milk beverages had the lowest sedimentation with no significant differences (P≤0.05) between them during storage. According to Modha and Pal[48] (2011), sedimentation in fermented milk beverages is a major hurdle for storage of product. Due to low pH, acidic milk products suffer from protein sedimentation, which leads to whey separation. Furthermore, in products with added fruit pulp and/or cereals, small insoluble particles such as fiber, kernel residues, and peels tend to deposit and form three layers of whey, milk solid mass, and insoluble granules, which favors sedimentation[48]. Souza et al.[16] (2020) studied fermented milk drink with cajá-manga pulp and observed a variation in sedimentation values throughout storage, with higher results than those of this study (sedimentation ranging from 7.16 to 9.16%).

Regarding syneresis (Figure 1B), the pineapple milk drink had the highest rate during storage, with a significant difference (P≤0.05) compared to the other drinks. On the other hand, mango and passion fruit dairy drinks presented a lower syneresis rate. Gallina et al.,[47] (2019) studied the characterization of probiotic fermented beverages and, similar to our results, observed that beverages with added mango and mango/passion fruit pulp showed lower syneresis rate. The increase of syneresis during storage is associated with severe casein network rearrangements that promote whey expulsion[49]. For fermented products, syneresis is associated with high temperatures of incubation, low solids content or inadequate storage temperatures[27]. The use of fruit pulps in the preparation of fermented milk drinks can alter the hydrophilic capacity of casein, as well as the structure of the network of other proteins, thus causing whey separation. One way for solving this problem is to submit the samples to better homogenization, mainly decreased particle size, as suggested by Valoppi et al.[28] (2019).

The potential of whey applications for the development of healthy products and nutrient sources has been highlighted in some studies[16,49,50]. As for the nutrients present in fermented milk drinks, protein is an important macronutrient to physiologic activities. Cho and Jones[51] (2019) stress that protein constitutes an essential nutrient for the development and maintenance of health, moreover, it presents itself as an alternative in the development of food-quality matrices. For calcium (Table 2), the presence of this mineral becomes an interesting aspect as it plays an important role in maintaining the health of bones, teeth, and other normal functions of the body[52]. Fayet-Moore et al.,[53] (2019) corroborate that calcium intake from milk-derived sources is a way to meet the body's needs for this mineral. According to Blanco-Rojo and Vaquero[54] (2019), iron is important for transporting and storing oxygen and plays a key role in metabolic functions such as growth, muscle activity, immunity, nervous system, and bone strength. Furthermore, the scientific literature mentions that in children and adolescents, low iron levels have been related to growth retardation, poor motor and cognitive development[55,56,57], lack of social attention, and low school performance[58].

CONCLUSION

In conclusion, the fermented milk drinks with added fruit pulp formulated as part of the current study were good sources of nutrients, mainly proteins and minerals, providing a daily intake value for calcium and iron of 20% and 49%, respectively. Regarding the physicochemical characteristics during storage, the passion fruit milk drink was more acidic. On the other hand, the pineapple milk drink presented the highest rates of syneresis and sedimentation. Our findings support the development of fermented milk drink with fruit pulp added as a source of nutrients with adequate physicochemical characteristics during storage. However, further studies should focus on the bioavailability of the bioactive compounds of milk drinks during in vitro gastrointestinal digestion as well as on a sensory analysis of the products.

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