

Full Length Research Paper

## Fermented milk enriched with passion fruit peel flour (*passiflora edulis*): Physicochemical and sensory aspects and lactic acid bacteria viability

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This study aimed to evaluate the physicochemical parameters, total and thermotolerant coliforms, lactic acid bacteria viability, instrumental color, and sensory analysis of fermented milks added to passion fruit peel flour (PFPF), throughout 29 days of storage, except composition. Four fermented milk treatments were prepared as follows: 1, fermented milk without addition of PFPF; 2, fermented milk added with 1% PFPF; 3, fermented milk added with 2% PFPF; 4, fermented milk added with 3% PFPF. According to the results obtained, acidity and pH values were inversely proportional, and microbiological analyses of coliforms showed no contamination, lactic bacteria were viable up to the 15<sup>th</sup> day of storage, treatment 3 showed the highest water holding capacity and syneresis decreased by raising the levels of PFPF. Fermented milk with the lowest level of addition of PFPF showed better scores and was the most preferred among panelists.

**Key words:** Whey, milk, pH, acidity, viable lactic acid bacteria.

### INTRODUCTION

Passion fruit (*Passiflora edulis*) belongs to the family *Passifloraceae* (Sebrae, 2005) and is a fruit of tropical and subtropical climates; the fruit consists of approximately 52% peel, 34% pulp and 14% seeds and cultivation is aimed at the juice and pulp industry (Zeraik

et al., 2010). Environmental care and waste reduction are increasing concerns of the food industry; therefore, viable alternatives to the use of food waste in the development of new products for human consumption must be proposed (Garmus et al., 2009).

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**Table 1.** Formulations of fermented milk drinks enriched with passion fruit peel flour (PFPF).

Ingredients	Treatments with PFPF (%)			
	0	1	2	3
Whole milk (%)	60	60	60	60
Milk whey (%)	40	40	40	40
Sucrose (%)	10	10	10	10
PFPF (%)	-	1	2	3
Starter culture (mg/L)	400	400	400	400

Passion fruit by-products (peel and seeds) may have technological and biological characteristics of interest to the food industry (Martinez et al., 2012). According to Ambrosio-Ugri and Ramos (2012), after drying, passion fruit peel is ground to obtain passion fruit flour or passion fruit fibre. The passion fruit peel is composed of flavedo, which corresponds to the outer layer of yellow-green color, rich in insoluble fibre and albedo, corresponding to the white inner layer rich in soluble fibre, in particular pectin, with small amounts of mucilage (Janebro et al., 2008).

Dietary fibres helps the bowel function and are considered prebiotic; soluble fibres retard intestinal passage, gastric emptying and glucose uptake, helping to reduce blood cholesterol, insoluble fibres accelerate intestinal transit, increasing the fecal volume, slowing down glucose hydrolysis, contributing to the reduction of some colon diseases (Pereira, 2002) and can serve as a substrate for beneficial microorganisms such as probiotics (Gallina, 2009).

According to the World Health Organization (FAO, 2002), probiotics are defined as live microorganisms that when administered in adequate amounts, confer a health benefit to the host. Awaisheh et al. (2005) reports that bacteria belonging to the genus *Lactobacillus*, which colonize the small intestine of humans and combat pathogens such as *Salmonella* spp., and those of the genus *Bifidobacterium*, which colonize the large intestine of human and inhibits the growth of *Escherichia coli* and *Candida* spp., are major microbial species with probiotic properties. Lee et al. (1999) claims that products containing *L. acidophilus* and *B. bifidum* have the capacity of improving the peristaltic movements of the intestine, increasing absorption of nutrients, controlling or preventing intestinal infections by blocking the receptors of pathogens, inactivating the effects of enterotoxin and favoring the development of microorganisms resistant to pathogens, especially against *Escherichia coli*.

Gallina et al. (2012) have reported that the main technological challenge for the processing industry is the viability and stability of probiotic cultures and that probiotic foods should contain specific strains of probiotic microorganisms and maintain adequate levels of viable cells during product storage without interfering with flavor

and texture.

Since the functional foods take an important place in the daily meal of the consumers, new studies must be carried out to: test ingredients, explore more options of food matrix that have not yet been industrially utilized, reengineer products and processes (Coman et al., 2012).

The aim of this work was to evaluate physical, chemical and microbiological parameters, sensory analysis and morphological structure of each fermented milk enriched of passion fruit peel flour (PFPF).

## MATERIALS AND METHODS

### Development of Fermented milks

Refrigerated milk and milk whey were obtained from enzymatic coagulation of Mozzarella cheese in a dairy industry located in the city Rio Verde (GO, Brasil).

The processing of fermented milks was conducted at the Laboratory of Products of Animal Origin - Instituto Federal Goiano, Rio Verde Campus, GO, Brazil (IF Goiano). Milk and whey were filtered to eliminate physical contamination. Four treatments consisting of 4 L were prepared, with proportion of 60% milk and 40% milk whey added with 10% sucrose; subsequently, the mixture was submitted to heat treatment at 90°C for 3 min. After pasteurization, fermented milk was cooled to 42°C for the addition of the Bio Rich<sup>®</sup> lyophilized culture (*Lactobacillus bulgaricus*, *Lactobacillus acidophilus*, *Streptococcus thermophilus* and *Bifidobacterium*) and incubated at 42°C up to pH 4.5.

After coagulation, fermented milks were removed from the oven and cooled to reach 20°C, then clot breaking was performed using glass rod in circular movements for 1 min. After clot breaking, passion fruit peel flour was added in the following proportions: 1, fermented milk without addition with passion fruit peel flour (0%); 2, fermented milk added with 1% passion fruit peel flour; 3, fermented milk added with 2% passion fruit peel flour and 4, fermented milk added with 3% passion fruit peel flour. The formulations for each drink are presented in Table 1. After the addition of PFPF, fermented milks were packaged in aseptic polypropylene packages, identified and stored at 5°C for sensory evaluation, scanning electron microscopy (SEM), pH, acidity, syneresis, water holding capacity, viable lactic acid bacteria, and instrumental color during the 29 days of storage.

### Physicochemical analyses

All analyses were performed in triplicate, except for the sensory analysis that was performed only once on the eighth day of storage.

**Milk**

Milk samples were collected for chemical composition evaluation at the Laboratory of Milk Quality, Research Center, School of Veterinary, Food and Animal Science - Federal University of Goiás, using MilkScan 4000 equipment (Foss Electric A/S. Hillerød, Denmark) to obtain fat, protein, lactose and non-fat solid (NFS) results, expressed in percentage (%).

**Milk whey**

Analyses of pH, acidity, fat and protein were performed in Ecomilk equipment (Cap Lab) milk analysis.

**Fermented milks****Fat**

Fat was analyzed using the Gerber method as IAL (2005). About 10 ml of sulfuric acid were transferred to Gerber butyrometer and 11 ml of sample and 1 ml of isoamyl alcohol were added, sealed and centrifuged at  $1200 \pm 100$  rpm for 15 min.

**Protein determination**

For crude protein analysis, total nitrogen was determined by the micro-Kjeldahl method according to official method No.960.52 of AOAC International (1997). Total nitrogen was converted into crude protein using factor 5.95 (Alencar; Alvarenga, 1991). The equipment used was digester block (Tecnal, TE-0070) and nitrogen distiller (Tecnal, TE-0363).

**Titrateable acidity determination**

For titration of samples, 10 ml of diluted fermented milk were added to 10 ml of distilled water with five drops of 1% phenolphthalein solution, followed by titration with 0.1 N sodium hydroxide solution up to the appearance of a persistent pink color for approximately 30 seconds (Brazil, 2006). Acidity was determined according to the following equation: lactic acid (%) =  $(V \times f \times 0.9) / m$  (IAL,2005).

**pH**

For pH assessment, bench Bel Engineer digital potentiometer was used. The electrode was inserted into the sample after homogenization without touching the bottom or the sides of the package, so the reading was carried out (Brazil, 2006).

**Microbiological analyses**

Analyses of total coliforms, *Escherichia coli* and estimation of viable lactic acid bacteria were performed at the Laboratory of Food Microbiology (IF Goiano) at storage times of 1, 8, 15, 22 and 29 days.

About 25 g of fermented milk were weighed and added to 225 ml of sterile peptone water and after homogenization, the solution was diluted to concentrations of  $10^{-2}$ ,  $10^{-3}$ ,  $10^{-4}$ ,  $10^{-5}$  and  $10^{-6}$ . The enrichment step for total coliform count with difference for *Escherichia coli* used 1 ml aliquots of concentrations of  $10^{-1}$ ,  $10^{-2}$  and  $10^{-3}$ , which were transferred into test tubes containing 10 ml of lauryl sulfate broth (LST) and incubated at 35°C for 24 h. Then, the presence of coliforms was confirmed using Brilliant Green Bile Broth (BG) incubated at 35°C for 24 h and in *E. coli* broth (EC) incubated for 24 h in water bath at 45°C.

For viable cell count, the method used was "pour plate" in-depth plating, using MRS agar (Kasvi). Serial dilutions were made from

$10^{-1}$  to  $10^{-6}$  and plating performed in triplicate and incubated at 35°C for 48 h (Macedo, 1997). The results were presented as  $\text{Log}^{-1}$  CFU/ml.

**Syneresis determination**

To determine syneresis, 30 g of yogurt were filtered in funnel and distributed on filter paper and after 5 h of draining, the supernatant was removed and weighed, and the syneresis rate was expressed in percentage (%), which was obtained by the ratio between supernatant weight and the total sample weight multiplied by 100 (Riener et al., 2010).

**Water holding capacity**

The water holding capacity (WHC) was determined in triplicate according to the modified method of Parnell-Clunies et al. (1986), being expressed as percentage (%), according to the following Equation:

$$\text{WHC (\%)} = 100 \times (\text{initial sample weight} - \text{supernatant weight}) / \text{sample weight}$$

**Color parameters**

Instrumental color parameters ( $L^*$ ,  $a^*$  and  $b^*$ ) of fermented milk samples were analyzed in triplicate in colorimeter (HunterLab, 1998) at the Post-Harvest Laboratory of Plant Products (IF Goiano).

**Sensory evaluation**

This study has been submitted and approved by the ethics research committee of Instituto Federal Goiano, with number 20/2013.

Analyses were performed at the Laboratory of Sensory Analysis (IF Goiano). Fifty-one untrained panelists aged 18-56 years, 64.3% females and 35.7% males, participated at the sensory analysis. Fermented milk samples were coded with three-digit numbers and presented under white light in 50 ml white cups ( $\pm 20$  g fermented milk) to each of the panelists.

The sensory analysis used acceptance and ordination tests: for acceptance, evaluation was based on scores awarded by panelists through a nine-point hedonic scale, where value one (1) represented "dislike extremely" and nine (9) "liked extremely", assessing flavour, aroma, texture and colour. The ordination test was analyzed, in which panelists put on an increasing order the samples they liked the most and those they liked less (IAL, 2005).

**Scanning electron microscopy**

Fermented milk samples were lyophilized in lyophilizer equipment (Enterprise II / Terroni®). Then, samples were defatted and analyzed in scanning electron microscope (JSM - 6610 / Jeol®) for the acquisition of images.

**Statistical analyses**

The experimental design adopted in the analysis was a completely randomized design (CRD) and syneresis, water holding capacity and were presented by means of regression while pH, acidity, viable lactic acid bacteria values, color parameters results were analyzed by comparison among treatment means using the Tukey's test. Analyses were performed using SISVAR and Sigma Plot 11.0 software.

**Table 2.** Average results of fat and protein of milk drinks enriched by increasing levels of passion fruit peel flour.

Parameter	PFPF (%)			
	0	1	2	3
Fat (%)	1.43 ±0.15a	1.40 ±0.4a	1.40 ±0.17a	1.23 ±0.15a
Protein (%)	2.19 ±0.03c	2.44 ±0.11bc	2.59 ±0.02b	3.24 ±0.17a

\*Small letters in the line do not differ from each other according to Tukey's test at 5% significance level.

## RESULTS AND DISCUSSION

The composition of the milk used in the manufacture of fermented milks (fat 3.6% ± 0.04; acidity 0.16 ± 0.02, density 1.030 g/100 ml; cryoscopy -0.530<sup>o</sup>H; NFS 8.4% ±0.03; protein 3.12% ± 0.15 and pH 6.70) shows that milk was in accordance with Normative Instruction N<sup>o</sup>. 62/2011, which establishes the following minimum physicochemical parameters: fat, 3.0 g/100 g; acidity from 0.14 to 0.18 g of lactic acid/100 ml; relative density from 1.028 to 1.034 g/ ml; Cryoscopic index from -0.512 °C at 0.531 °C; NFS at least 8.4%; protein 2.9%; whey showed fat 0.31% ± 0.16; acidity 0.10 ± 0.07; density 1.026 g/ 100 ml; cryoscopy of -0.500 °H; NFS 0% ± 6.25; protein 1.10% ± 0.08 and pH 6.54.

The inclusion of increasing levels of PFPF in the preparation of fermented milks did not affect the fat content (Table 2); however, there was an increase of the protein content of fermented milks with the addition of PFPF, which was due to its average protein content of 15.4%, considered high.

The fat and protein values of the present study are lower than those observed by Gallina et al. (2011) in fermented milks with and without addition of probiotics and prebiotics (fat 2.8% and protein from 4.03 to 4.28%) by Toledo et al. (2013) in yogurts added of pulp and passion fruit flour (*Passiflora edulis*) (fat from 2.42 to 2.88% and protein from 2.98 to 4.20%) and Gerhardt et al. (2013) in fermented milks added of ricotta whey and collagen hydrolysate (fat from 2.90 to 3.10% and protein from 2.99% to 4.44%).

The titratable acidity and pH results of fermented milks throughout the storage period were inversely proportional. Fermented milk with 0% PFPF showed lower acidity while pH was the highest on the fifteenth day of storage. In this period, fermented milk with 3% PFPF showed high acidity and the lowest pH; at the end of the twenty-ninth day of storage, titratable acidity decreased and pH increased, and fermented milk with 0% PFPF showed the lowest acidity and fermented milk with 3% PFPF showed the highest pH (Table 3).

The titratable acidity results of this study were higher than those reported by Gallina et al. (2012), who worked with fermented milk produced from symbiotic fermented milk added of guava pulp and found variations from 0.41

to 0.42%, but the pH values were lower to those reported by the author who observed values from 4.40 to 4.42. Results similar to those of this study were observed by Gerhardt et al. (2013) in fermented milks using whey ricotta and collagen hydrolysate, which ranged from 0.72 to 0.91%; pH results corroborate those found by Toledo et al. (2013) in yogurts added with passion fruit pulp and flour (*Passiflora edulis*), ranging from 4.49 to 3.63.

Similar values were observed by Costa et al. (2013) in a study with fermented milk made with different stabilizers / thickeners (titratable acidity from 0.55 to 0.61% and pH from 3.95 to 4.07), and those reported by Gonçalves and Leão (2013) in yogurts added of mixed flours from apple, passion fruit and grape waste (titratable acidity from 0.84 to 0.89% and pH from 4.20 to 4.60).

The 29th day of storage the samples of fermented milk showed a decrease in acidity. This can be explained by the fact that the 29th day of storage the samples fermented milk showed incidence fungus samples, which caused decrease in acidity. Coelho et al. (2009), when evaluating shelf life in yogurt for 60 days there were decreased acidity for the high count of yeasts and molds. Franco and Langraf. (2003) reported that some species of yeasts and molds using lactic acid, leading consequently to an increase in the pH.

Regarding the count of coliforms, none of the samples showed typical colony formation, which results are similar to those reported by Tebaldi et al. (2007) in 20 samples of fermented milks commercialized in southern Minas Gerais and by Araújo et al. (2012) in passion fruit-flavored sundae-type yogurt, where microbiological results for analysis of coliform bacteria showed no turbidity with acidification (turning) and gas production, indicating absence of this microorganism in the samples analyzed. Paula et al. (2012) observed presence of <10 CFU ml<sup>-1</sup> of coliforms at 30°C and 45°C in fermented milk.

The viable cell count results of Figure 2 shows that 3% PFPF concentration up to the fifteenth day of storage showed the highest number of CFU/ml, followed by concentrations of 2 and 1%, which is in accordance with the current legislation that establishes minimum number of viable bacteria per milliliter (10<sup>6</sup> CFU) during the validity period (Penna, 2002). On the fifteenth day of storage, it was observed that the 0% PFPF showed

**Table 3.** Acidity (%) and pH fermented milk with increasing levels of flour passion fruit peel during storage.

Treatment (%)	Storage period (Days)									
	1		8		15		22		29	
	Acidity	pH	Acidity	pH	Acidity	pH	Acidity	pH	Acidity	pH
0	0.63±0.01Ab	4.36±0.09Aa	0.68±0.2Bb	4.23±0.01Aab	1.12±0.16Aa	4.20±0.01Aab	1.32±0.04Aa	4.10±0.01Bb	0.56±0.06Ab	4.11±0.01ABb
1	0.74±0.03Abc	4.31±0.05ABa	0.87±0.07ABbc	4.20±0.05Aa	1.05±0.01Aab	4.14±0.05ABb	1.27±0.03Aa	4.00±0.01Bb	0.70±0.06Ac	4.16±0.02ABb
2	0.73±0.02Ab	4.26±0.09 Ba	1.09±0.3Aa	4.25±0.01 Aa	1.16±0.07Aa	4.06±0.05ABb	1.36±0.04Aa	4.02±0.01Bb	0.70±0.06Ac	4.02±0.01ABb
3	0.72±0.02Ab	4.18±0.02 Bb	1.19±0.1Aa	4.12±0.05 Ab	1.19±0.03Aa	3.98±0.05Bb	1.34±0.03Aa	3.97±0.01Bb	0.80±0.09Ab	4.21±0.2 ABb

\* Capital letter on same column do not differ from each other, same lowercase letters on the same lines do not differ according to Tukey's test at 5% significance level. 0% = no addition of PFPF (0%); Treatment 1%= 1% of PFPF; Treatment 2% = 2% of PFPF; Treatment 3% = 3% of PFPF.

**Table 4.** Viable lactic acid bacteria (CFU/mL) in fermented milks with increasing levels of passion fruit peel flour during storage.

Treatment (%)	Storage period (Days)				
	1	8	15	22	29
0	8.62x10 <sup>6</sup> ±0.07Aa	9.05 x10 <sup>6</sup> ±0.11Aa	5.05 x10 <sup>6</sup> ±0.10Bb	4.67 x10 <sup>6</sup> ±0.8Ab	0±0Ac
1	7.56 x10 <sup>6</sup> ±0.30Aa	9.30 x10 <sup>6</sup> ±0.12Aa	8.46 x10 <sup>6</sup> ±0.01Aa	1.00 x10 <sup>6</sup> ±0.1Bb	0±0Ab
2	7.26 x10 <sup>6</sup> ±0.20Aa	9.27 x10 <sup>6</sup> ±0.15Aa	8.50 x10 <sup>6</sup> ±0.5Aa	1.00 x10 <sup>6</sup> ±0.1Bb	0±0Ab
3	9.27 x10 <sup>6</sup> ±0.12Aa	9.96 x10 <sup>6</sup> ±0.03Aa	8.41 x10 <sup>6</sup> ±0.6Aa	0±0Bb	0±0Ab

\*Capital letter on same column do not differ from each other, same lowercase letters on the same lines do not differ according to Tukey's test at 5% significance level. 0% = no addition of PFPF (0%); Treatment 1%= 1% of PFPF; Treatment 2% = 2% of PFPF; Treatment 3% = 3% of PFPF.

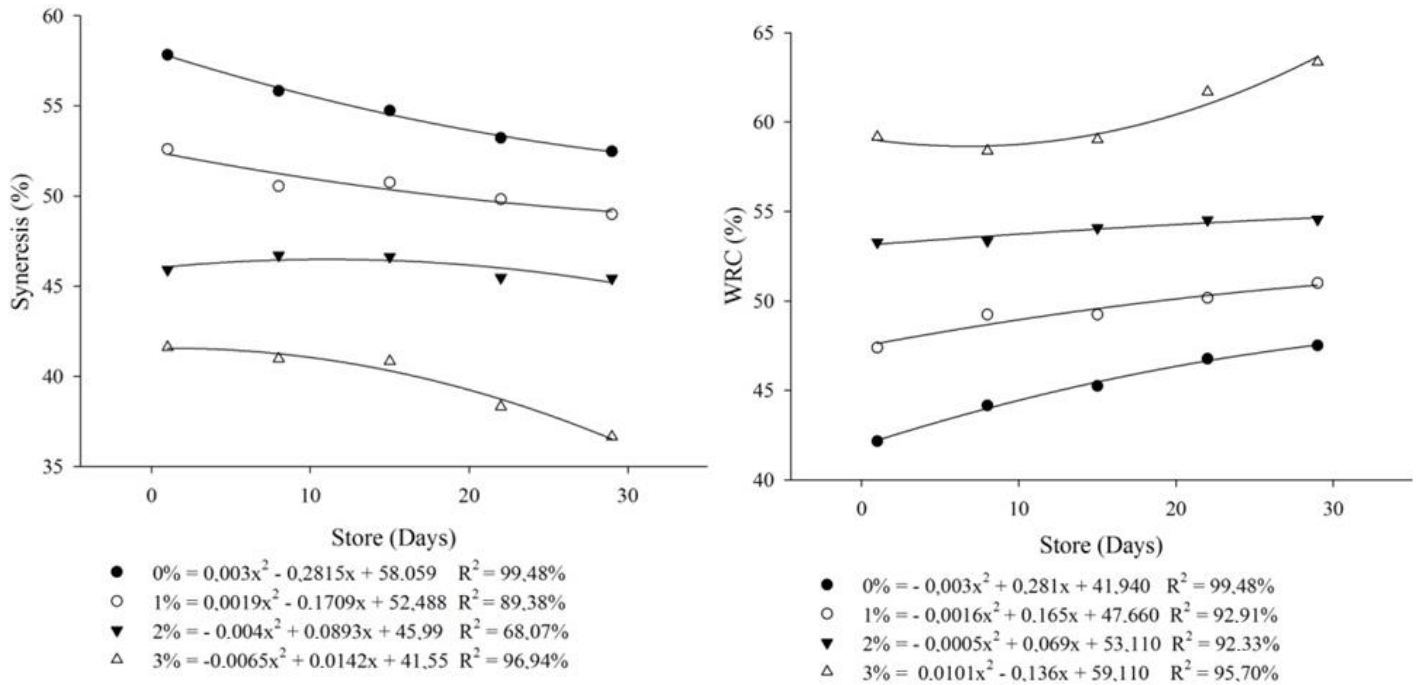
values lower than 10<sup>6</sup> CFU/ml. On the twenty second day of storage 3%, 2% and 1% PFPF drastically decreases while the 0% PFPF concentration remained constant, showing a decrease in CFU/ml only on the twenty-ninth day of storage (Table 4).

The lactic acid bacteria results corroborate those found by Gallina et al. (2011) assessing the viability of lactic acid and probiotic bacteria during shelf-life, which remained within adequate levels

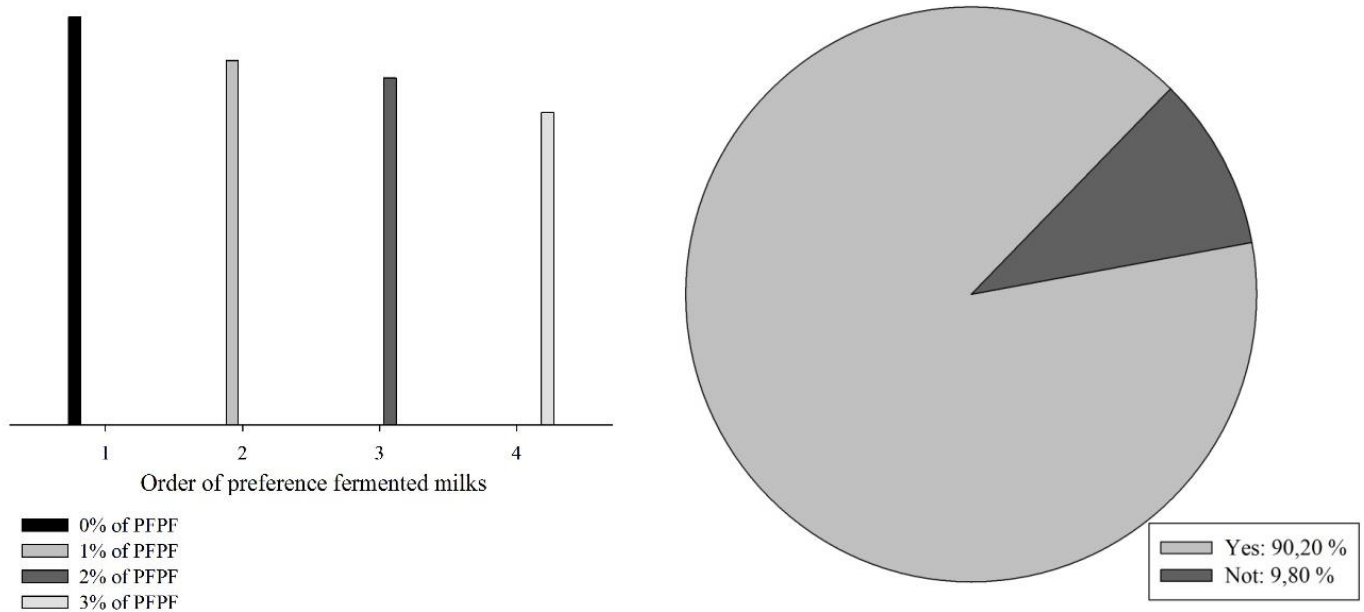
(10<sup>6</sup> CFU/ml) up to 15 days. Coman et al. (2012), while working with fermented milk added with different percentages of wheat flour and oat bran in concentrations (control, 2, 4 and 6%) with 2 types of sepas (*L. rhamnosus* IMC 501®, *L. paracasei* IMC 502®, SYN BIO®) for each treatment; up to 28 days of storage all treatments showed lactic acid viable with levels above 10<sup>6</sup> CFU/ml. Higher values were found by Matta et al. (2012) in symbiotic rice-based drink after 22 days

of storage which showed from 10<sup>9</sup> to 10<sup>10</sup> CFU/ml and those reported by Ribeiro et al. (2014) with fermented milk made with *Camellia sinensis* that after 30 days of storage showed 10<sup>7</sup> CFU/ml.

The increase in PFPF levels led to a decrease in syneresis values over a period of twenty nine days of storage, and fermented milk with the highest concentration (3% PFPF) resulted in the lowest syneresis value, while fermented milk with 0% PFPF showed the highest syneresis value,



**Figure 1.** Syneresis and water holding capacity of dairy drinks with increasing levels of passion fruit peel flour during storage.



**Figure 2.** Hedonic scale of preference and purchase intent of fermented milks with increasing levels of passion fruit peel flour.

indicating that there was a better whey release. WHC showed increased values in fermented milk with 3% PFPF and decreased values for 0% PFPF, these results show that syneresis and WHC have an inverse relationship (Figure 1).

Similar results were observed by Toledo et al. (2013) in

a study with yogurt added of passion fruit pulp and flour (*Passiflora edulis*), who found that samples with lower PFPF content showed higher whey release and consequently higher syneresis values. The same behavior was observed by Antunes et al. (2007), who studied nonfat probiotic yogurt in combination with starter

**Table 5.** Mean L\* coordinate values of milk drinks with increasing levels of passion fruit peel flour during storage.

Treatment (%)	Storage period (days)				
	1	8	15	22	29
0	89.86±0.02Abc	89.04±0.06Ad	89.42±0.06Ac	90.87±0.04Aa	90.50±0.02Aab
1	72.22±0.50 Bc	72.21±0.19Bc	73.11±0.11Bab	73.45±0.16Ba	72.46±0.17Bbc
2	65.34±0.77Cb	65.80±0.72Cab	66.13±0.37Ca	66.19±0.16Ca	65.36±0.80Cb
3	62.67±1.13 Db	62.67±0.26Db	64.95±0.37Da	62.51±0.14Db	62.38±0.13Db

\*Capital letter on same column do not differ from each other, same lowercase letters on the same lines do not differ according to Tukey's test at 5% significance level. 0% = no addition of PFPF (0%); Treatment 1%= 1% of PFPF; Treatment 2% = 2% of PFPF; Treatment 3% = 3% of PFPF.

**Table 6.** Mean a\* coordinate values of milk drinks with increasing levels of passion fruit peel flour during storage.

Treatment (%)	Storage period (Days)				
	1	8	15	22	29
0	1.99±0.04Dc	1.87±0.04Dab	1.99±0.02Da	1.71±0.02Cb	2.06±0.01Da
1	4.40±0.16Cb	4.01±0.04Cb	4.31±0.14Cb	4.33±0.10Cb	4.51±0.14Cb
2	6.39±0.14Ba	5.72±0.19Bb	5.89±0.14Ba	6.26±0.08Ba	6.11±0.25Bb
3	7.01±0.21Aa	6.69±0.13Aa	6.44±0.13Aa	6.93±0.03Aa	7.03±0.04Aa

\*Capital letter on same column do not differ from each other, same lowercase letters on the same lines do not differ according to Tukey's test at 5% significance level. 0% = no addition of PFPF (0%); Treatment 1%= 1% of PFPF; Treatment 2% = 2% of PFPF; Treatment 3% = 3% of PFPF.

**Table 7.** Average b\* coordinate values of milk drinks with increasing levels of passion fruit peel flour during storage.

Treatment (%)	Storage period (Days)				
	1	8	15	22	29
0	12.45±0.08Db	11.60±0.06Dc	13.25±0.06Ca	13.34±0.01Da	13.32±0.02Ca
1	19.64±0.29Cbc	19.27±0.12Cc	19.26±0.04Bc	20.04±0.07Cab	20.79±0.16Ba
2	22.93±0.18Bbc	22.26±0.12Bcd	21.81±0.19Ad	23.05±0.06Bb	24.27±0.29Aa
3	24.08±0.43Aa	24.24±0.08Aa	22.49±1.29Ab	24.24±0.04Aa	24.72±0.08Aa

\*Capital letter on same column do not differ from each other, same lowercase letters on the same lines do not differ according to Tukey's test at 5% significance level. 0% = no addition of PFPF (0%); Treatment 1%= 1% of PFPF; Treatment 2% = 2% of PFPF; Treatment 3% = 3% of PFPF.

culture and milk whey protein concentrate and found lower syneresis levels and increased whey retention capacity in formulations added of protein concentrate, and by Gerhardt et al. (2013), who prepared 11 treatments of fermented milks varying formulations with ricotta whey and collagen hydrolysate and observed that samples containing less collagen (0.65 and 0.5%) showed higher syneresis values. The results indicate that the addition of solids to fermented milks had an effect on syneresis.

The L\* parameter (Table 5) indicates brightness and can determine values between zero (0) and one hundred (100), called black and white, respectively. The inclusion of increasing levels of PFPF in the preparation of

fermented milks influence the brightness parameter (L\*), and with increasing proportion of flour in treatments, there was a decrease in L\*, corroborating results by Toledo et al. (2013) in the characterization of yogurts added of passion fruit pulp and flour (*Passiflora edulis*).

Parameter a\* (Table 6) showed significant difference ( $P<0.05$ ) among treatments, and increasing flour levels led to an increase in a\* values. Treatment with 0% was negative (-a\*) towards green while treatments with 1, 2 and 3% flour were positive (+ a\*) towards red.

Parameter b\* (Table 7) showed the addition of increasing levels of PFPF in fermented milks increased the b\* chromaticity coordinate values. According to Caldeira et al. (2010), b\* values greater than zero go

**Table 8.** Average acceptance of milk drinks with increasing levels of passion fruit peel flour after 8 days of storage.

Treatment (%)	Aroma	Flavour	Acidity	Viscosity	Appearance	Color
0	7.42±1.31a	7.85±1.41a	7.31±1.28a	5.04±1.50c	7.35±1.30 a	7.22±1.49a
1	5.60±1.93b	5.10±2.22b	5.54±2.04b	5.80±2.40 a	6.08±1.96b	6.22±1.61b
2	4.96±2.02cb	3.86±2.17c	4.58±1.78cb	4.52±2.29b	5.22±2.27cb	5.76±1.78cb
3	4.36±1.99c	3.20±2.21c	4.22±2.16c	4.38±2.51b	4.96±2.15c	5.28±1.99c

\*Means with same letters in the column do not differ by the Tukey's test at 5% significance level.

towards yellow and b\* values less than zero go towards blue. Yellow coloration is related to the use of milk whey.

The test results are presented in Table 8; four types of fermented milks presented significant differences ( $P < 0.05$ ) in the aroma, flavour, acidity, viscosity, appearance and colour, presented notes between ranging from 4.4 to 7.4 (disliked slightly to liked moderately), from 3.20 to 7.85 (disliked moderately to liked moderately), from 4.22 to 7.31 (disliked slightly to liked moderately), from 4.38 to 5.04 (disliked slightly to indifferent), from 4.96 to 7.35 (disliked slightly to liked moderately), from 5.28 to 7.22 (indifferent to liked moderately).

However, fermented milk without addition of PFPF obtained higher score in relation to parameters of fermented milks added of PFPF.

Fermented milks added with PFPF showed sand-like texture; description corroborated by Espírito Santo et al. (2013) when studying probiotic yogurt enriched with passion fruit fibre. According to Gonçalves and Leão (2013), yogurt added with mixed flour containing passion fruit peel and apple bagasse showed acceptability between 5 (not liked nor disliked) and 6 (liked slightly).

Figure 2 shows the preference of consumers regarding the addition of PFPF to fermented milks, in which panelists evaluated from the most preferred to the least preferred fermented milk, and it was observed that among the 50 panelists, the control drink (without PFPF) was the most preferred, followed by fermented milks added of 1% PFPF and 2% PFPF and the least preferred fermented milk was that added of 3% PFPF.

According to Figure 2, it was observed that fermented milks added with passion fruit peel fibre were rejected, and according to Espírito Santo et al. (2013), this rejection is explained by the fact that panelists were unfamiliar with the consumption of yogurt added of fibre, which is corroborated by Ribeiro et al. (2010), when reporting that the Brazilian yogurt market is dominated by yogurt with fruit flavor (about 95% of the market), and colorful and sweet yogurts are preferred by consumers.

However, some sensory properties of fermented milks such as aroma, flavor, acidity, viscosity, appearance and color may have been underestimated by panelists because they are not used to consume fermented milks added of fibre. When the 51 panelists were asked if they would buy the fermented milk they liked the most, 90.2%

responded yes and only 9.8% responded they would not buy.

Fermented milks added with passion fruit peel flour were intended for fast freezing in Ultrafreezer (Terroni<sup>®</sup>) at  $-80^{\circ}\text{C}$  and then freeze-dried in lyophilizer equipment (Enterprise II/Terroni<sup>®</sup>). After lyophilization, samples were degreased by soxhlet method, stored in plastic bags and placed in desiccator with silica gel and then transported to the Laboratory of Electron Microscopy (LabMic) at the Federal University of Goiás. Samples were mounted on stubs and covered with gold plating. At the end of this procedure, stubs were examined in scanning electron microscopy (JSM - 6610 / Jeol<sup>®</sup>). Figure 3 shows the scanning electron microscopy images of fermented milk drinks added with passion fruit peel flour at 500x magnification

The images at Figure 3 show the microstructure of a protein matrix and with the addition of flour passion fruit peel showed incidence of surface holes, which are called pits (Martins et al., 2009) and are more present the fermented milk added with 3% PFPF (Figure D), with higher porosity compared to control treatment. It was observed that fibres do not show a smooth and homogeneous surface, but rather quite irregular surface covered by recesses and protrusions.

## Conclusion

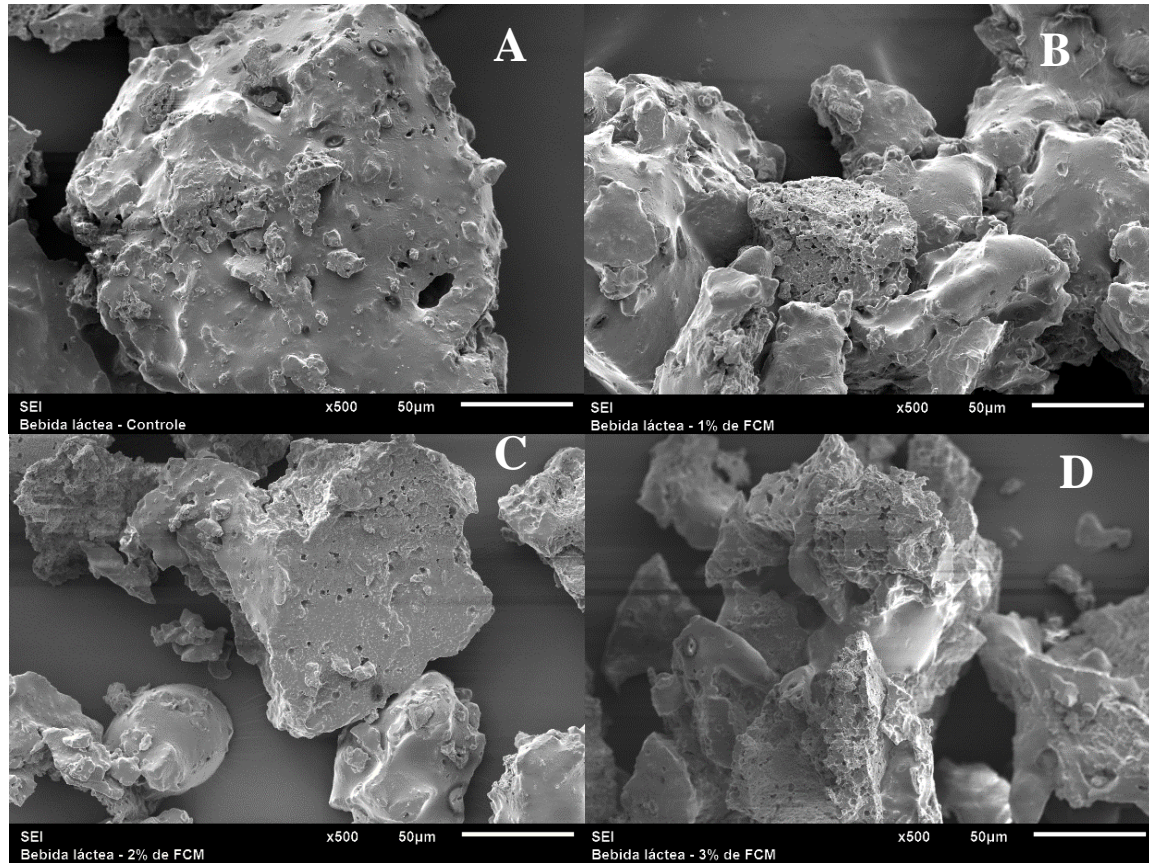
The results allowed concluding that the use of passion fruit peel for the production of flour is an alternative for the reuse of this product, as it is rich in nutrients. Fermented milks produced achieved physical and chemical parameters established by Brazilian law, with no contamination by total and thermotolerant microorganisms throughout the 29 days of storage.

The viability of fermented milks showed efficiency up to the fifteenth day of storage; and in relation to acceptance, the results demonstrated that fermented milks added of PFPF reached satisfactory sensory acceptance.

## Conflict of interest

Authors declare there is no conflict of interest for this research.





**Figure 3.** Scanning electron microscopy. (A) fermented milk drink with no addition of PFPF, (B) fermented milk drink with 1% PFPF, (C) fermented milk drink with 2% PFPF, (D) fermented milk drink with 3% PFPF.

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