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Synbiotic lactose free petit suisse cheese: An alternative product for lactose intolerants

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Abstract
The present study developed a low lactose synbiotic *petit suisse* cheese using ultrafiltration to concentrate the protein and diafiltration to remove the milk lactose. After this, its perspectives were evaluated regarding potential for consumer benefits, instrumental texture during storage and sensory acceptability. Six different trials were made: PCP (probiotic control *petit suisse*); SCP (synbiotic control *petit suisse*); UF-PP (ultrafiltration probiotic *petit suisse*); UF-SP (ultrafiltration synbiotic *petit suisse*); PP-LF (*petit suisse* lactose free) and SP-LF (synbiotic *petit suisse* lactose free). The ultrafiltration combined with diafiltration was efficient in concentrating the milk proteins and removing the lactose. The pH of PP-LF and SP-LF remained higher during storage time compared to the other cheese which can be related with the low lactose content. BB-12 viability remained above 8 log CFU g^{-1} for the entire storage time in all trials. All *petit suisse* formulations had a good matrix to carry the probiotic *Bifidobacterium lactis*, BB-12. However, fructooligosaccharide (FOS) did not influence the BB12 viability during storage. In addition, the UF or UF-DF process did influenced the sensorial analysis when compared with control without membrane process. The processed cheeses presented a brittle matrix considered unpleasant to the judges. The products are a good alternative for lactose intolerants, with excellent prospects for future marketing.

Keywords: synbiotic *petit suisse*, quark cheese, lactose free

Introduction

Dairy products are recognized as excellent food vehicles to carry probiotic bacteria. Several studies with dairy products state the addition of probiotic bacteria in fermented milks and yogurts; however, research has shown that fresh cheese also has great potential for incorporating and carrying these microorganisms due to its physicochemical characteristics (Blaiotta *et al.*, 2016) [5]. Factors such as high pH, low acidity, high buffering capacity, and environment created by the protein-fat matrix improve of the probiotic microorganism viability during the cheese shelf life (Blaiotta *et al.*, 2016) [5]. Quark cheese is an unripened, soft cheese made from skim milk; it has a white color, slightly bitter taste, high moisture content, high-protein content, and low-fat content (Yadav *et al.*, 2019) [41]. In Brazil, quark cheese is used as a base mass to produce Petit Suisse cheese. The Petit Suisse cheese is characterized by the addition of ingredients such as fruit pulp, sour cream, and sugar to the quark cheese mass, giving it characteristics that include light, soft, and creamy, as well as a sweet taste that is well accepted by consumers, especially children (Cardarelli *et al.* 2008, Vieira, 2013) [6, 40].

Probiotics are living microorganisms that, when administered in adequate quantities, beneficially influence the health of their hosts by improving the composition of intestinal microbiota (Hill *et al.*, 2014) [17]. These microorganisms must be able to survive under the various conditions of the human gastrointestinal tract and adhere to mucosal or intestinal epithelial cells to colonize (Kandylis *et al.*, 2016) [20].

Bifidobacterium lactis BB-12 has been found to have beneficial roles to the host's health, such as pathogen inhibition, barrier function enhancement, and immune interactions. In addition, BB-12 has been proven to improve bowel function, to have a protective effect against diarrhea, and to reduce side effects of antibiotic treatment, such as antibiotic-associated diarrhea. In terms of immune function, clinical studies have shown that BB-12 increases the body resistance to common respiratory infections as well as reduces the incidence of acute respiratory tract infections (Jungersen *et al.*, 2014) [19].

It has been reported that the growth of intestinal bifidobacteria and lactobacilli is facilitated when prebiotics are used in association with these microorganisms in dairy products and fruit juices (Shori, *et al.*, 2016) [36]. Prebiotic is a substrate that is selectively utilized as source food by intestinal host microorganisms conferring health benefit (Gibson *et al*, 2017) [14].

The use of synbiotic in dairy products is expanding in the dairy industry (Kandylis *et al.*, 2016) [20]. However, lactose intolerant individuals have limitations in the consumption of these products due to illness. It is estimated that 75% of the population presents some level of intolerance or poor digestion to lactose (Suri *et al*, 2019) [39]. That number can increase significantly because the condition is often not diagnosed. When individuals are unable to digest lactose, this carbohydrate passes into the intestine, where it is fermented by intestinal microbiota causing undesirable symptoms such as cramping, abdominal pain and diarrhea (Suri *et al.*, 2019) [39]. Often individuals stop consuming dairy because of these symptoms, this also impairs the consumption of calcium, as these products are excellent sources of this mineral (Suri *et al.*, 2019) [39]. One alternative is the development of dairy products with lactose-free. The lactose-free dairy market is the fastest growing segment and it is expected to reach a €9 billion turnover by 2022 (Dekker *et al.*, 2019) [10]. In order to serve the lactose intolerant

public, the industry has used new technologies to develop lactose-free dairy products, maintaining or even improving the nutritional quality of the products developed. One of the technologies used in the food industry is membrane separation processes (MSPs) or membrane technology (Das *et al.*, 2016) [9].

Ultrafiltration and diafiltration are membrane processes that have become popular in the food industry, especially the dairy industry. In dairy industries these processes are applied in the production of cheeses with higher yield, in the fractionation of milk and in the concentration and standardization of milk protein (Mistry e Maubois, 2004) [27]. The use of membranes for milk ultrafiltration allows the fractionation, concentration and purification of milk components. When associated with the diafiltration process, UF becomes more efficient in the concentration and purification of proteins. Diafiltration is the sequence of washes of the UF retentate solution, thus allowing a higher protein concentration and purification of the solution of low molecular weight molecules, such as lactose (Baldasso *et al.*, 2011) [3]. In this context, the aim of this study was to develop a synbiotic lactose free *petit suisse* cheese using an ultrafiltration and diafiltration process to remove the lactose of milk.

Materials and methods

Ultrafiltration and Diafiltration

Skimmed ultra-high temperature (UHT) milk (Italac, Brazil) was concentrated and diafiltered in pilot equipment of the TIA (Techniques Industrielles Appliquées) Brazil (Araraquara, SP, Brazil) using an ultrafiltration system (equipped with an organic polyethersulfone spiral membrane with a molecular weight cutoff of 10 kDa, Synder Filtration, USA), Type A skim milk was submitted to UF process. UF and DF process were performed at transmembrane pressure of 5 bar and 12 ◦C. The milk was concentrated in batch mode, i.e., the retentate was recycled back and the permeate was continuously removed until a theoretical concentration factor of 2.0× was reached, as previously described by Sivieri and Pires (2018) [37]. The cleaning procedure was carried out according to the membrane manufacturer's instructions. The retentates milk were kept at 4 °C until quark cheese production and the UF and DF were done in room temperature (± 30 °C). The batch was conducted in triplicate.

Quark cheese production

Three types of Quark cheeses were produced: (1) Control quark cheese (QCC), produced using an UHT milk without membrane process; (2) Ultrafiltrated quark cheese (UF- QC) produced using ultrafiltrated milk; and (3) Diafiltrated quark cheese (UF-DF-QC) produced using diafiltered milk. The composition of the milks was shown in table 1.

All cheeses were processed in triplicate, following the same conditions determined in previous tests. UHT, UF and UF-DF milk were pasteurized in a water bath at 60°C for 30 minutes and then cooled in an ice bath. UHT, UF and UF-DF milks were maintained at 37ºC ± 1°C in a stove, sodium chloride was added at a ratio of 0.250 g/L with pre-activated homofermentative mesophilic starter culture R-704 (Christian Hansen, Valinhos, Brazil) and probiotic culture, *Bifidobacterium lactis* BB-12 (8 log CFU/g), (Christian Hansen, Valinhos, Brazil). As soon as the pH reached values of 6.0-6.2, 1.09 % of rennet Ha-La 1152 (chymosin from *Aspergillus Niger* var. *awaroni*; Christian Hansen, Brazil, 30 mg L^{-1}) previously diluted in water (1/9, v/v) was added in each milk (table 2). Then, the mixture was re-homogenized and maintained at 37°C until a curd was formed with pH of about 4.8 to 5.3. The curd was then carefully cut into cubes, which were placed in sterile cloth sacks to drain off the whey at a temperature of 10°C for 18 h. After drainage, the quark cheese was placed in sterile containers and stored at 4°C until they were mixed with the other ingredients to make the *Petit Suisse* cheese (Vieira, 2013) [40].

Table 1: Composition of the milks used for the production of Quark cheeses (QCC, UF-QC and UF-DF-QC).

Milks	Cheeses	Protein content (%)	Lactose content (%)
UHT milk (control)	Quark Cheese Control (QCC)	3.09[a]	4.30[b]
Ultrafiltrated milk	Ultrafiltrated Quark Cheese (UF-QC)	6.36[c]	3.96[b]
Diafiltrated milk	Ultrafiltrated Diafiltrated Quark Cheese (UF-DF-QC)	5.52[b]	0.91[a]

Determination of protein content, lactose content and yield of quark cheese

The yield of quark cheese was calculated according to equation (A) proposed by Fox *et al.*, (2000) [13]. The protein content was determined by the Kjedahl method (IAL, 2008). To determine the lactose content, two methods were used: Lane-Eynon (IAL, 2008) for cheeses with lactose content > 2% and the Acton method (Acton, 1977) for cheeses with lactose content < 2%. The Lane-Eynon method is based on the reduction of cupric ions to cuprous ions by the reducing sugar in heated alkaline medium. As the present sugar is lactose, the method can be applied to lactose reducing glycids and it can be done to evaluate the lactose content in quark cheese samples. The Acton (1977) method determines the lactose content of cheese by reacting an aqueous cheese extract with phenol and concentrated sulfuric acid.

A. Current Yield (Yc).

$$Yc = 100x\left(\frac{cheese\ weight}{milk\ weight + starter\ cultures}\right)$$

Petit suisse production

Petit suisses were produced using different types of Quark cheese: probiotic control *petit suisse* (PCP) and synbiotic control *petit suisse* (SCP) from the QCC, UF probiotic *petit suisse* (UF-PP) and UF synbiotic *petit suisse* (UF-SP) from the UF-QC, and probiotic *petit suisse* - lactose-free (PP-LF) and synbiotic *petit suisse* - lactose-free (SP-LF) from the UF-DF-QC. *Petit suisse* cheeses were manufactured mixing all the ingredients (Table 2) and homogenized in a multi-processor for 5 min (Walita, São Paulo, Brazil), until a consistent mass was obtained. After homogenization, appropriate containers were filled with cheese and maintained under refrigeration at 4°C for 28 d.

Table 2: Amount and brand of ingredients added in the masses of the cheeses *PCP, SCP, UF-PP, UF-SP, PP-LF and SP-LF*.

Ingredients		Proportion of Ingredients g/100g					
	Brand / country	PCP	SCP	UF-PP	UF-SP	PP-LF	SP-LF
Quark control cheese	Own production	50.0	50.0	-	-	-	-
UF Quark cheese	Own production	-	-	50.0	50.0	-	-
UF-DF Quark cheese	Own production	-	-	-	-	50.0	50.0
Milk cream with lactose	Piracanjuba, Brazil	26.0	23.5	26.0	23.5		
Milk cream lactose free	Piracanjuba, Brazil	-	-	-	-	26.0	23.5
Strawberry pulp	Purapolpa, Brazil	16.0	16.0	16.0	16.0	16.0	16.0
Sucrose	Caravelas, Brazil	8.0	8.0	8.0	8.0	8.0	8.0
Fructooligosaccharide	Infinity pharm, Brazil	-	2.5	-	2.5	-	2.5

(PCP – probiotic control petit suisse; SCP – synbiotic control petit suisse; UF-PP – ultrafiltration probiotic petit suisse; UF-SP – ultrafiltration synbiotic petit suisse; PP – LF – probiotic petit suisse – lactose free; SP – LF – synbiotic petit suisse – lactose free)

***Petit suisses*: centesimal composition and pH**

Moisture, ash, protein and fat content were determined for all *petit suisse* formulations, in triplicate, after 1 day of refrigerated storage (4° ± 1 °C). The moisture content was determined from 1 g of samples by infrared scales at 105 °C for 15 minutes (mod, MOC63u, Unibloc, Shimazdu, São Paulo, Brasil). The ash was determined by heating 3 g of sample at 550 °C until complete burning for 3 to 4 hours. The fat content was determined by the Gerber method and the protein content by the Kjedahl method (IAL, 2008). The carbohydrate content was indirectly estimated by difference according to the following equation: 100 − (% protein +% lipids +% moisture + % ash), and the energy value was calculated using the correction facts for proteins, fats and carbohydrates. The following were considered: 4 Kcal for each gram of protein, 4 Kcal for each gram of carbohydrate and 9 Kcal for each gram of fat (IAL, 2008). The pH was monitored weekly during the storage period (0 to 28 days) for all formulations in triplicate, using a direct reading in the sample, at a temperature of 20 °C with a B500 pH meter (Inolab, São Paulo, Brazil).

Texture profile analysis (TPA)

TPA was determined in triplicate samples after 0, 7, 14, 21, and 28 days of refrigerated storage at 5°C, using a TA. XT plus® Texture Analyzer (Stable Micro Systems, UK) operating in TPA mode. A double penetration test was employed on 12 g samples contained in individual falcon tubes, by a 25 mm diameter acrylic cylinder probe set (P/25P). The probe penetrated to a depth of 10 mm, at a 1 mm s^{-1} speed. The TPA parameters measured were hardness, adhesiveness, springiness, cohesiveness, and gumminess obtained by using the Texture Expert for Windows software version 1.20 (Stable Micro Systems, Godal Ming, UK). The results of TPA (time 0 and 28 days of storage) was submitted to Principal Components Analysis (PCA). The Statistical software, version 12 was used (Cary, USA)

Microbiological analysis

The viable cell counts of *B. animalis BB-12* were monitored weekly during the storage period (0 to 28 days) for all formulations in triplicate. For microbiological analysis, portions of 25 g of duplicate product samples were blended with 225 mL of 0.1 g 100 mL^{-1} peptone water in a Bag Mixer 400 (Interscience, St. Nom, France) and submitted to serial dilutions with the same diluent. *Bifidobacterium animalis BB-12* was counted by drop-plating 100 µL of each dilution on to reinforced *Clostridium* agar (RCA), followed by 48 h of anaerobic incubation at 37 °C (Richter e Vedamuthu, 2001) [34].

Sensory analysis

The *petit suisse* cheese samples were analyzed for texture and taste preference, after one day of process, using the preference ranking test (Associação Brasileira de normas técnicas, 1994) [2]. This analysis was approved by the Ethics and Research Committee of the Faculty of Pharmaceutical Sciences (005707/2018). The Petit suisse cheese samples were evaluated by fifty untrained panelists. The test used 20 g samples of cheeses from each treatment studied, all made on the same day in parallel, served in 50 mL plastic cups at a temperature of 4 ºC ± 1 ºC, coded with random three-digit numbers and presented in a random way. The consumers were instructed to order the samples according texture and taste preference, being value = 1 for the most preferred product and value = 6 for the least preferred product. To determine the result, the positions of the 50 judges for each cheese will be added up, totaling a final value for each formulation. The samples that have the highest value mean that they were least preferred by the 50 judges (example: 5 and 6) and the samples with the lowest value will be those that occupied the most preferred positions (example: 1 and 2).

Statistical Analysis

Data were subjected to ANOVA with the software GraphPad, Prism®, version 5.01 (Graph Pad, software In. La, Jolla, CA, EUA) using a general linear model. Differences between means (formulations and shelf life days) were determined by the post-hoc Tukey's multiple-range test. $p < 0.05$ was deemed significant.

Results and Discussion

Quark Cheese, Protein Content, Lactose Content, and Yield of Quark Cheese.

Table 3 shows the time that each milk needed to reach the following pH ranges: 6.0 to 6.2 and 4.8 to 5.2. Regarding the time to reach the pH 6.2 to 6.0, the milk with higher lactose content (UHT and UF) showed a shorter fermentation time than the DF milk (UF-DF) with now statistical difference. Yet, the coagulation time was statistically higher for DF milk compared with UHT milk and UF milk.

Table 3: Milks, amount of milk, time to reach the values of pH used to produce 800 g of each Quark (QCC, UF-QC and UF-DF-QC).

Cheeses	Amount of milk (L)	Time to reach pH 6.0-6.2 (min)	Time to reach pH 4.8-5.3 (min)
Quark Cheese Control (QCC)	5.0	175.0 ± 25.00[a]	77.0 ± 2.89[a]
Ultrafiltrated Quark Cheese (UF-QC)	3.5	170.0 ± 17.32[a]	85.0 ± 8.66[a,b]
Ultrafiltrated Diafiltrated Quark Cheese (UF-DF-QC)	3.5	210.0 ± 30.00[a]	110.0 ± 17.32[b]

The times of the three independent batches were evaluated. Different lowercase letters (a, b, c) indicate difference ($p < 0.05$) between the values in the same column – difference between the time of each milk).

Cheese is a casein coagulum produced by the action of a rennet on micellar casein. The casein micelles are stabilized by calcium and phosphorus, and the removal of these substances as a result of the UF and DF can cause casein micelles dissociation. The processes of UF and DF cause a buffering effect in the milk, making it difficult to lower the pH during fermentation. On the other hand, the concentration of milk proteins is higher when the coagulation time is faster because the casein micelles are closer. In addition, the decrease in pH causes the precipitation of calcium phosphate from the casein micelles, and lactic fermentation helps to maintain low pH values contributing to the coagulation of milk (Liu *et al*., 2014) [23]. The low-lactose content may have caused the higher fermentation time and coagulation time in the DF milk. Due to the low concentration of lactose and the higher buffering capacity of the DF milk, a longer time for the pH to decrease and the casein to clot was necessary.

Table 4 shows the results of yields (kg/100 kg), lactose content (g/100 g), and protein content (g/100 g) of quark cheese obtained from UHT milk, concentrated milk (UF), and concentrated and DF milk (UF-DF). As expected, the highest yields ($P < 0.05$) and protein content were found in the UF-QC and UF-DF-QC cheese (33.94 and 28.73 kg/100 kg; 17.60 and 18.64 g/100 g, respectively), while QCC showed a yield of 17.46 kg/100 kg and a protein content of 15.52 g/100 g. In addition, when comparing the yields of the processed cheeses, it was observed that the UF-QC had a yield 94.63% higher than the QCC and the UF-DF-QC had a yield 64.55% higher compared with the QCC. When comparing the protein content, the UF-QC had 20.10% higher protein content than QCC, and the UF-DF-QC had a protein content 13.40% higher than QCC.

Due to the pressure and porosity of the membranes used in the milk UF process, the proteins were concentrated. Thus, the increase in the yield of these cheeses can be due to the incorporation of whey proteins that are retained in the 16% to 20% proportion rather than being lost in the desorption process. In UF cheeses, whey proteins may correspond to up to 18% of the protein content, whereas in traditional cheeses they may only correspond to 1% (Faion, 2015) [12].

Increased protein yield and protein concentration has also been reported by other authors using the UF process, such as Cunha *et al.* (2002) [8], that developed fresh Minas cheeses from 3 types of milks concentrated by UF with different concentration factors (2:1; 3:1; 4:1). All cheeses had moisture and protein levels higher than a traditional Minas Frescal cheese.

Table 4: Mean and standard deviations of yields, lactose content and protein content of Quark cheeses obtained from UHT, high protein (UF) and low lactose free (UF + DF) milk.

Quark Cheese	Yield (kg/100 kg)	Lactose Content (g/100 g)	Protein Content (g/100 g)
QCC	17.46 ± 1.17[a]	2.90 ± 0.06[b]	15.52 ± 0.63[a]
UF-QC	33.94 ± 1.73[b]	2.90 ± 0.19[b]	17.60 ± 0.62[b]
UF-DF-QC	28.73 ± 2.24[b]	0.01 ± 0.00[a]	18.64 ± 0.59[b]

(QCC – Quark cheese control; UF-QC – ultrafiltration Quark cheese, UF-DF-QC – Ultrafiltration and diafiltration Quark cheese. Different lowercase letters (a, b, c) indicate difference ($p < 0.05$) between the values in the same column).

The lowest lactose content was observed only in UF-DF lactose-free quark cheese 10 mg/100 g ± 0.01), with a reduction of 99.70% in lactose concentration (Table 4). The DF is used to increase the separation ability of feed material by the addition of a suitable solvent, usually water. The water added aims to promote a retentate dilution by allowing the still retained soluble components to permeate more easily across the membrane (Meena *et al.*, 2017) [25]. According to Solanki and Gupta (2014) [38], when using a UF-DF process, the lactose concentration in skim milk was reduced by 74.64%. Meena *et al.* (2017) [25] showed that the use of UF-DF process contributed to the decrease of lactose and increased the protein purification in retentate milk. Another important point to succeed in lactose reduction is molecular weight cutoff of the membrane. In this study, we used an organic membrane with molecular weight cutoff of 10 kDa. This membrane allowed the passage of low molecular weight substances (<10 kDa) such as water, lactose, and soluble salts into the permeate, while high molecular weight components such as proteins and fat globules were retained and concentrated on retentate (Ng *et al.*, 2017; Meena *et al.*, 2017) [25].

Table 5: Centesimal composition (mean and standard deviations) of *petit suisse* PCP, SCP, UF-PP, UF-SP, PP-LF and SP-LF.

	Formulations					
	PCP	SCP	UF-PP	UF-SP	PP-LF	SP-LF
Ash (g/100 g)	1.02 ± 0.02[A]	0.84 ± 0.02[A]	0.86 ± 0.04[A]	0.85 ± 0.05[A]	0.81 ± 0.03[A]	0.83 ± 0.03[A]
Moisture (g/100 g)	63.25 ± 0.50[A]	62.38 ± 1.56[A]	58.23 ± 3.15[A]	58.67 ± 0.52[A]	64.10 ± 3.03[A]	60.08 ± 3.95[A]
Protein (g/100 g)	7.83 ± 0.01[A]	7.80 ± 0.37[A]	9.54 ± 0.61[B]	9.68 ± 0.78[B]	9.43 ± 0.33[B]	9.36 ± 0.81[B]
Lipids (g/100 g)	6.62 ± 1.16[A]	6.63 ± 1.16[A]	5.75 ± 1.06[A]	5.5 ± 0.71[A]	7.0 ± 0.00[A]	7.75 ± 0.35[A]
Carbohydrates (g/100 g)	21.24 ± 0.27[A]	22.35 ± 1.32[A]	23.75 ± 1.20[A]	22.44 ± 0.66[A]	18.10 ± 3.26[A]	20.27 ±3.53[A]
Energetic value (Kcal)	176.49 ± 0.06[A]	180.28 ± 0.24[A]	182.35 ± 0.11[A]	177.97 ± 2.17[A]	173.13 ± 4.13[A]	187.41 ± 4.21[A]

(PCP – probiotic control petit suisse; SCP – synbiotic control petit suisse; UF-PP – ultrafiltration probiotic petit suisse; UF-SP- ultrafiltration synbiotic petit suisse; PP – LF – probiotic petit suisse – lactose free; SP – LF – synbiotic petit suisse – lactose free. Different capital letters (A, B, C) indicate difference ($p < 0.05$) between the values in the same row

Petit Suisse Evaluation

Centesimal Composition and pH Measurement.

Petit Suisse cheese formulations only showed statistical differences ($P < 0.05$) in relation to the protein content (Table 5). As expected, the Petit Suisse produced with quark cheeses made with milk concentrated by UF resulted in the highest protein content when compared with control Petit Suisse cheeses.

The centesimal composition of Petit Suisse was similar to other studies that developed Petit Suisse (Cardarelli *et al.*, 2008; Vieira, 2013) [6, 40]. The moisture content was in accordance with the Brazilian Technical Regulation on the identity and quality of Petit Suisse cheese. The Brazilian Technical Regulation establishes that Petit Suisse is a cheese with high moisture content, above 55% (MAPA, 2000). This same regulation states that the protein content in Petit Suisse must be above 6%. All Petit Suisse had higher protein content than required by legislation; UF-PP, UF-SP, PP-LF and SP-LF petit suisse showed an increase around 56.0 and 61.3% in protein content when compared with the content required for the commercial product. The high-protein content found was explained by the use of milk UF for the development of the quark cheeses. The tangential filtration technology allowed the highest concentration of proteins and the best incorporation of these proteins during the manufacturing process (Ng *et al.*, 2017) [29].

All Petit Suisse showed a decrease in pH values over time (Table 6). The Petit Suisse obtained from the same milk and the same base mass showed a similar pH decrease throughout the time of storage. The both *petit suisse* lactose-free (PP-LF and SP-LF) showed a higher pH than other formulations (UF-PP, UF-SP, PCP, and SCP). The reduction of pH values is a typical process that occur in cheeses due to the production of lactic acid and other organic acids, resulting from the metabolism of starter cultures and probiotic cultures (Pereira, 2002) [32]. The significant reduction observed in the pH of all Petit Suisse during the 28 d of refrigerated storage time may be due to the fermentation of lactose by starter cultures and probiotic cultures present in the product (Ribeiro *et al.*, 2018) [33]. In addition, the microorganisms present in cheeses can also metabolize other free sugars, such as oligosaccharides and sucrose, which contributes to the decrease in pH (Maruyama *et al.*, 2006) [24]. Petit Suisse formulations with low-lactose content showed the highest pH ($P < 0.05$), but during the refrigerated storage time a pH decrease was observed.

In all formulations, 8 g of sucrose was added to each 100 g of product. The lactic bacteria may have used a specific fermentation pathway in which the sucrose was metabolized and produced lactic acid or others organics acids that acidified the medium and decreased the pH (Bennama *et al.*, 2012) [4]. Moreover, in the current study, the pH values of all formulations were similar to described by Cardarelli *et al.* (2008) [6].

Texture Profile Analysis

Hardness, gumminess, and chewiness values were evaluated during the storage period of 28 d under refrigeration (±4°C). The statistical analysis showed that the storage time influenced the hardness of all formulations of Petit Suisse cheese. When comparing the initial time with the final time, all formulations showed a decrease in hardness values. When comparing the hardness between the formulations, in the initial time (which the sensory analysis performed), the following sequence was given in relation to the best hardness: SP-LF > PCP = PP-LF > SCP = UF-PP = UF-SP. This showed that the SP-LF presented greater hardness in the initial time (Table 7). The Petit Suisse cheeses which showed the smallest difference between the initial and final values were the UF-PP and UF-SP formulations, and the UF-SP remained with a higher value than the other formulations at the end of storage. The hardness is the parameter that evaluates the resistance required to cause the product deformation. Several factors, such as proteolysis, the process of draining the curd, and the concentration of proteins and milk fat can influence the hardness of a product. When referring to cheeses, these factors interfere in the network formed by the caseins, changing the structure of the cheese (O'Callaghan and Guinee, 2004; Fox *et al.*, 2000) [30, 13]. Product hardness stability during the shelf life is desirable because it confirms that the product maintains its initial characteristics such as physical-chemical and sensory properties (Maruyama *et al.*, 2006) [24]. However, the decrease in hardness during cold storage was observed by Buriti *et al.* (2008) [6] among 3 formulations of Petit Suisse (probiotic, symbiotic, and control).

Table 6: pH values and respective standard deviations of PCP, SCP, UF-PP, UF-SP, PP-LF and SP-LF cheeses obtained during the storage period of 28 days under refrigeration (± 4ºC).

	***Petit suisse* cheese**					
Time	**PCP**	**SCP**	**UF-PP**	**UF-SP**	**PP-LF**	**SP-LF**
0	$4.67 \pm 0.06^{B,d}$	$4.69 \pm 0.01^{A,B,d}$	$4.66 \pm 0.08^{A,B,d}$	$4.64 \pm 0.11^{A,a}$	$4.99 \pm 0.01^{C,e}$	$4.98 \pm 0.01^{C,d}$
7	$4.29 \pm 0.01^{A,b}$	$4.32 \pm 0.03^{A,b}$	$4.45 \pm 0.15^{B,c}$	$4.44 \pm 0.20^{B,a}$	$4.91 \pm 0.03^{C,d}$	$4.89 \pm 0.01^{C,c}$
14	$4.38 \pm 0.02^{B,b,c}$	$4.39 \pm 0.02^{B,c}$	$4.30 \pm 0.27^{A,b}$	$4.32 \pm 0.31^{A,B,a}$	$4.80 \pm 0.02^{C,c}$	$4.8 \pm 0.03^{D,c}$
21	$4.07 \pm 0.01^{A,a}$	$4.06 \pm 0.03^{A,a}$	$4.23 \pm 0.12^{B,a}$	$4.23 \pm 0.14^{A,a}$	$4.53 \pm 0.12^{C,b}$	$4.64 \pm 0.01^{D,b}$
28	$4.32 \pm 0.01^{B,c}$	$4.35 \pm 0.00^{C,b,c}$	$4.20 \pm 0.18^{A,a}$	$4.22 \pm 0.18^{A,a}$	$4.47 \pm 0.02^{D,a}$	$4.53 \pm 0.02^{E,a}$

(PCP – probiotic control petit suisse; SCP – synbiotic control petit suisse; UF-PP – ultrafiltration probiotic petit suisse; UF-SP – ultrafiltration synbiotic petit suisse; PP – LF – probiotic petit suisse – lactose free; SP – LF – synbiotic petit suisse – lactose free). Different lowercase letters (a, b, c) indicate difference ($p < 0.05$) between the values in the same column. Different capital letters (A, B, C) indicate difference ($p < 0.05$) between the values in the same row)

Gumminess is the parameter that determines the energy needed to disintegrate a semisolid food into a ready-made food to be swallowed (Nateghi *et al.*, 2012) [28]. At time 0, the order of greatest gumminess is expressed as follows: SP-LF = PP-LF = PCP > SCP = UF-PP = UF-SP. As in the hardness parameter, the gumminess showed the same behavior over the 28 d of cold storage. All Petit Suisse showed a decline in the gumminess values, while at time 7 d, the PCP and SP-LF still showed higher values than the other formulations. At time 14 d, the PCP also showed a higher value than other cheeses, but not statistically different. In times 21 d and 28 d, all formulations showed similar values.

Chewiness is the number of masticates required for a certain amount of sample to satisfactorily decrease the consistency for swallowing (Nateghi *et al*., 2012) [28]. At time 0 d, SP-LF showed the highest value of this parameter ($P < 0.05$), followed by PCP and PP-LF, and the Petit Suisse which showed the lowest values were the SCP, UF-PP, and UF-SP. The order of greatest chewability at the time 0 d was expressed as follows: SP-LF > PCP = PP-LF > SCP = UF-PP = UF-SP. During the time of storage, all Petit Suisse had a decrease in chewiness, and the SP-LF and PCP remained with higher values ($p < 0.05$). As in time 14 d, all Petit Suisse formulations showed statistically similar values, but PCP remained with higher values than the other formulations. At times 21 d and 28 d, there was no statistical difference between any of the formulations. The formulations that had greater stability and less difference between the initial and final values were SCP, UF-PP, and UF-SP.

Principal Components Analysis

The PCA was made to the parameter hardness, gumminess, and chewiness at the initial time (time 0) and final time (28 d). The PCA revealed similarities and differences for the TPA in beginning and end of Petit Suisse storage, explaining 100% of the total variation of the data analyzed (Figures 1 and 2). Principal component 1 explained 98.37% (time 0) and 99.10% (time 28) of data analyzed.

For the time 0 d, the Petit Suisse were divided into 4 groups. Formulation SP-LF was correlated with higher values of hardness. The PP-LF and PCP were positioned in the same quadrant and correlated with higher values of gumminess and chewiness. The other formulations did not correspond with any TPA parameters. For the time 28 d, the Petit Suisse were divided in 3 groups. Formulations UF-PP and SP-LF were positioned into the same quadrant and correlated with higher values of hardness, and UF-SP was correlated with higher values of gumminess and chewiness. The other formulations did not correspond with any TPA parameters.

Low-fat cheeses may have some defects such as a hard and elastic texture as well as a weak melting capacity. These defects in the protein matrix of the cheese change the color and flavor, and result in a more brittle and sandy texture. To improve the texture of the cheeses, improvement technologies can be applied, such as membrane processes and the use of milks with a higher fat content or the addition of creams to provide greater softness (Shafiei *et al.*, 2014) [35]. In this work, we use UF and DF to improve the textures of the cheeses. In fact, the Petit Suisse UF and UF-DF showed better stability over the 28 d, but the results in the sensory analysis showed that its texture became brittle as if a greater homogenization was missing. This non homogeneous texture may have occurred due to the lack of fat in the milks used.

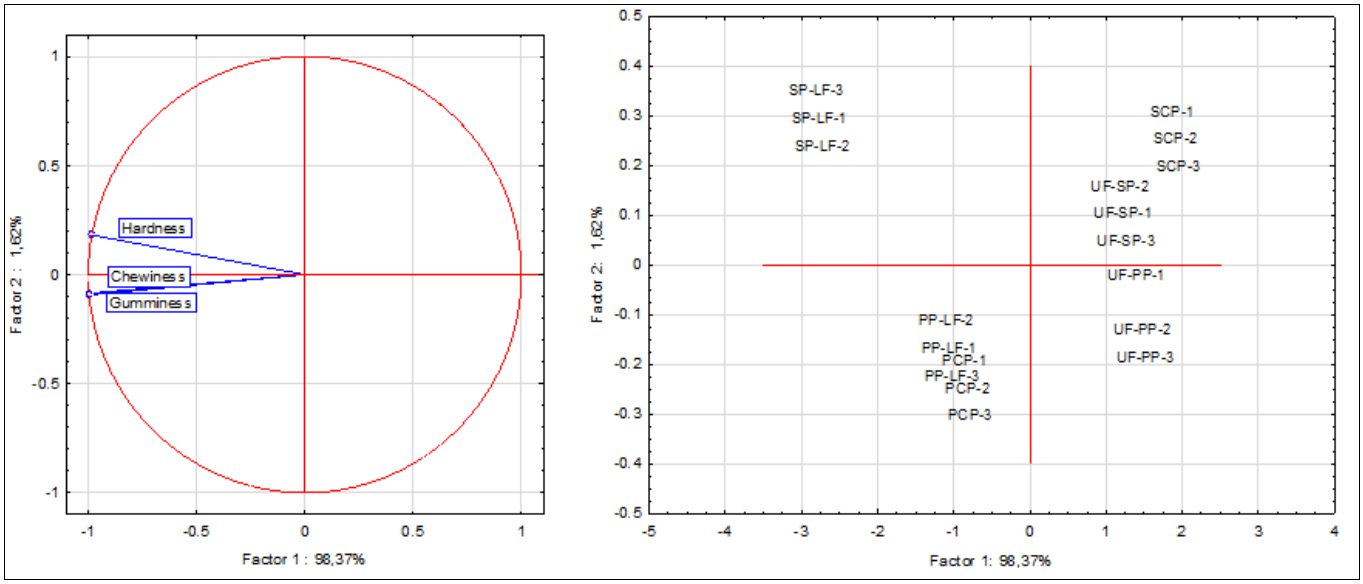
Sensorial Analysis

The judges preferred the control formulations (PCP and SCP) due to the flavor and texture (Table 8). These formulations were not submitted to UF or UF-DF processes and were made with control milk. Petit Suisse formulated with UF processed milk and UF-DF milk had lower preference in relation to flavor and texture. Formulations UF-PP, UF-SP, and SPLF showed no statistical difference; only PP-LF was different ($p < 0.05$) between UF-DF processed milk. According to Pereira (2002) [32], low-lactose yogurts had lower total solids content, changing the consistency and standard characteristics of yogurts. The yogurts with higher lactose content were considered more consistent, while those with low-lactose content were considered "watery." Some judges claimed that the texture of PPLF and SP-LF was brittle, and that this fact influenced their choice of Petit Suisse.

Table 7: Instrumental texture profile of *petit suisse* during storage time of 28 days under refrigeration at 4°C – Hardness, Gumminess and Chewiness.

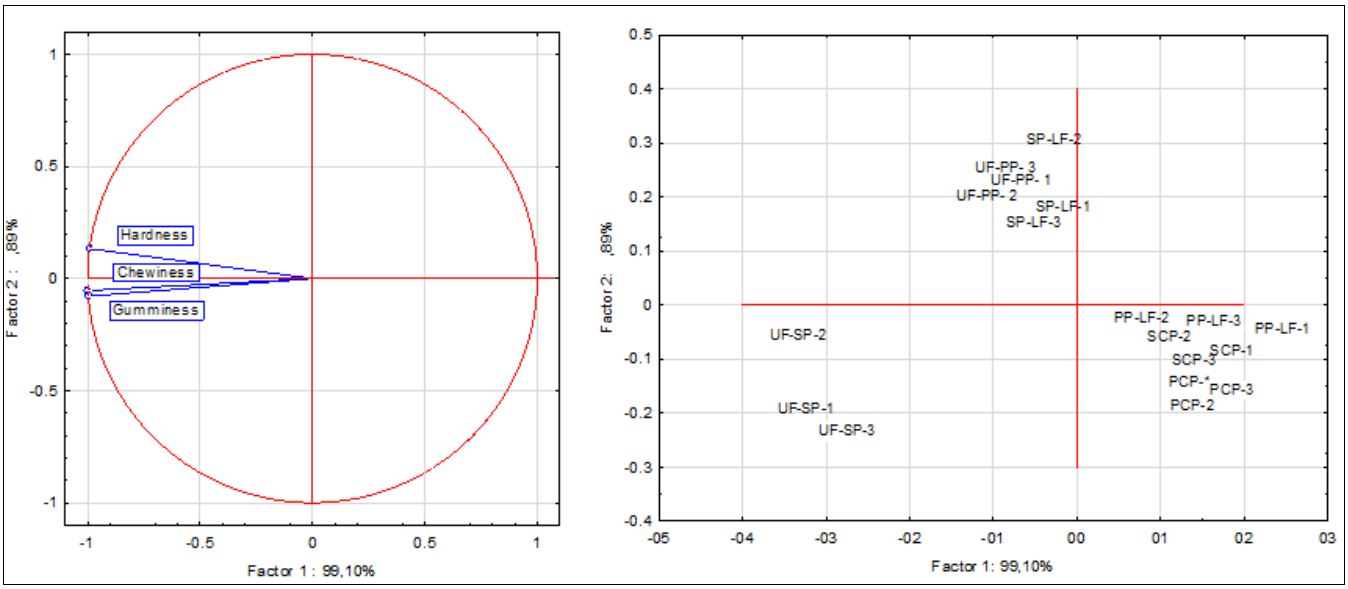
Texture Parameters	Storage time (days)	*Petit Suisse*					
		PCP	**SCP**	**UF-PP**	**UF-SP**	**PP-LF**	**SP-LF**
	0	$0.056 \pm 0.00^{B,c}$	$0.039 \pm 0.00^{A,c}$	$0.039 \pm 0.01^{A,c}$	$0.042 \pm 0.01^{A,b}$	$0.058 \pm 0.01^{B,c}$	$0.078 \pm 0.03^{C,b}$
	7	$0.041 \pm 0.00^{A,b}$	$0.034 \pm 0.00^{A,c}$	$0.038 \pm 0.01^{A,c}$	$0.035 \pm 0.01^{A,a,b}$	$0.037 \pm 0.00^{A,b}$	$0.042 \pm 0.06^{A,b}$
Hardness	14	$0.038 \pm 0.01^{B,b}$	$0.024 \pm 0.00^{A,b}$	$0.030 \pm 0.01^{A,B,b}$	$0.031 \pm 0.01^{A,B,a,b}$	$0.023 \pm 0.01^{A,a}$	$0.031 \pm 0.00^{A,B,a}$
(N)	21	$0.029 \pm 0.09^{A,b}$	$0.022 \pm 0.00^{A,b}$	$0.025 \pm 0.01^{A,a,b}$	$0.026 \pm 0.01^{A,a}$	$0.018 \pm 0.00^{A,a}$	$0.023 \pm 0.00^{A,a}$
	28	$0.014 \pm 0.00^{A,a}$	$0.014 \pm 0.00^{A,a}$	$0.023 \pm 0.01^{A,B,a}$	$0.030 \pm 0.01^{B,a}$	$0.015 \pm 0.00^{A,a}$	$0.021 \pm 0.00^{A,a}$
	0	$3.159 \pm 0.07^{B,c}$	$1.106 \pm 0.59^{A,b}$	$1.569 \pm 1.19^{A,b}$	$1.628 \pm 1.17^{A,b}$	$3.301 \pm 0.83^{B,c}$	$4.230 \pm 0.01^{B,b}$
	7	$2.385 \pm 0.10^{B,a,b}$	$0.968 \pm 0.05^{A,b}$	$1.634 \pm 1.12^{A,B,a,b}$	$1.243 \pm 0.24^{A,B,a,b}$	$1.452 \pm 0.32^{A,B,b}$	$2.047 \pm 1.32^{B,b}$
Gumminess	14	$1.645 \pm 1.53^{A,b,a}$	$0.713 \pm 0.08^{A,a,b}$	$0.788 \pm 0.31^{A,a}$	$0.924 \pm 0.51^{A,a,b}$	$0.576 \pm 0.15^{A,b,a}$	$0.860 \pm 0.19^{A,a}$
(g.sec)	21	$0.768 \pm 0.22^{A,a}$	$0.645 \pm 0.17^{A,a,b}$	$0.811 \pm 0.57^{A,a}$	$0.626 \pm 0.30^{A,a}$	$0.392 \pm 0.10^{A,a}$	$0.637 \pm 0.07^{A,a}$
	28	$0.337 \pm 0.09^{A,a}$	$0.322 \pm 0.13^{A,a}$	$0.485 \pm 0.46^{A,a}$	$0.736 \pm 0.74^{A,a}$	$0.337 \pm 0.15^{A,a}$	$0.445 \pm 0.04^{A,a}$
	0	$2.912 \pm 0.05^{B,c}$	$1.013 \pm 0.59^{A,b}$	$1.448 \pm 1.10^{A,b}$	$1.505 \pm 1.09^{A,b}$	$3.008 \pm 0.75^{B,c}$	$3.854 \pm 1.51^{,c}$
	7	$2.147 \pm 0.09^{B,c,b}$	$0.878 \pm 0.05^{A,b}$	$1.496 \pm 1.06^{A,B,b}$	$1.124 \pm 0.24^{A,a,b}$	$1.332 \pm 0.31^{A,B,b}$	$1.846 \pm 1.17^{B,b}$
Chewiness	14	$1.501 \pm 1.47^{A,b,c}$	$0.628 \pm 0.08^{A,a,b}$	$0.722 \pm 0.28^{A,a}$	$0.838 \pm 0.46^{A,a,b}$	$0.522 \pm 0.14^{A,b,a}$	$0.777 \pm 0.16^{A,a}$
(g.sec)	21	$0.689 \pm 0.20^{A,b}$	$0.563 \pm 0.15^{A,a}$	$0.722 \pm 0.51^{A,a}$	$0.558 \pm 0.28^{A,a}$	$0.344 \pm 0.07^{A,a}$	$0.572 \pm 0.05^{A,a}$
	28	$0.293 \pm 0.08^{A,a}$	$0.276 \pm 0.11^{A,a}$	$0.432 \pm 0.40^{A,a}$	$0.650 \pm 0.66^{A,a}$	$0.294 \pm 0.13^{A,a}$	$0.396 \pm 0.04^{A,a}$

(PCP – probiotic control petit suisse; SCP – synbiotic control petit suisse; UF-PP – ultrafiltration probiotic petit suisse; UF-SP-ultrafiltration synbiotic petit suisse; PP – LF – probiotic petit suisse – lactose free; SP – LF – synbiotic petit suisse – lactose free. Different lowercase letters (a, b, c) indicate difference ($p < 0.05$) between the values in the same column. Different capital letters (A, B, C) indicate difference ($p < 0.05$) between the values in the same row)



(PCP – probiotic control *petit suisse*; SCP – synbiotic control *petit suisse*; UF-PP – ultrafiltration probiotic *petit suisse*; UF-SP-ultrafiltration synbiotic *petit suisse*; PP – LF – probiotic *petit suisse* – lactose free; SP – LF – synbiotic *petit suisse* – lactose free)

Fig 1: Results of Principal Component Analysis of Instrumental texture profile of all *petit suisses* at initial time (time 0): (a) projection of case coordinates on the sample score plot of the Factors 1 and 2; (b) PCA loading plot of Instrumental texture profile of petit suisses selected on the first two factors obtained from PCA.



(PCP – probiotic control *petit suisse*; SCP – synbiotic control *petit suisse*; UF-PP – ultrafiltration probiotic *petit suisse*; UF-SP – ultrafiltration synbiotic *petit suisse*; PP – LF – probiotic *petit suisse* – lactose free; SP – LF – synbiotic *petit suisse* – lactose free)

Fig 2: Results of Principal Component Analysis of Instrumental texture profile of all *petit suisses* at initial time (time 28): (a) projection of case coordinates on the sample score plot of the Factors 1 and 2; (b) PCA loading plot of Instrumental texture profile of petit suisses selected on the first two factors obtained from PCA

Table 8: Mean values of the sensory parameters evaluated in *petit suisses*

	Formulation					
Attribute	PCP	SCP	UF-PP	UF-SP	PP-LF	SP-LF
Taste	135^{A}	89^{A}	197^{B}	201^{B}	228^{B}	200^{B}
Texture	94^{A}	87^{A}	193^{B}	198^{B}	257^{C}	$221^{B,C}$

(PCP – probiotic control petit suisse; SCP – synbiotic control petit suisse; UF-PP – ultrafiltration probiotic petit suisse; UF-SP-ultrafiltration synbiotic petit suisse; PP – LF – probiotic petit suisse – lactose free; SP – LF – synbiotic petit suisse – lactose free. Different capital letters (A, B, C) indicate difference ($p < 0.05$) between the values in the same row).

Cheeses are a network of concentrated proteins that surround fat globules and water molecules to form a gel structure. The integrity of the cheese matrix is maintained by various aggregation systems through interand intra-electrostatic attractions between molecules (O'Callaghan and Guinee, 2004) [30]. In this context, high levels of protein and low fat can cause problems in the texture of the cheese, such as the presence of granules that can be felt during chewing. These manufacturing defects can occur due to the high content of caseins and the high content of calcium

phosphate and free calcium. During the milk UF process, calcium salts are released in the permeate; therefore, the ionic strength increases between the casein molecules, modifying the way the proteins bind and forming a cheese mass that is more brittle or sandy (Fox *et al.*, 2000) [13]. The removal of salts from the milk serum during DF changes the ionic environment of skim milk. This can affect the physicochemical properties of casein micelles and probably affected the base mass of the quark cheese in our study, which may have influenced judges'choices in sensory analysis.

Regarding the texture profile presented by the cheeses and the results of the sensory analysis, it was possible to observe that the mass of the quark cheeses underwent deformation during the process of draining and in the production of the quark cheese. The dough was firm, but due to the low-fat content, it became brittle and not homogeneous, especially for cheeses that had a higher protein content.

Table 9: *Bifidobacterium lactis*, BB-12 counts values in *petit suisse* cheeses during 28 days of refrigerated storage (± 4 ° C).

Petit Suisse						
Storage Time	**PCP**	**SCP**	**UF-PP**	**UF-SP**	**PP-LF**	**SP-LF**
0	$8.40 \pm 0.08^{C,b}$	$8.63 \pm 0.05^{C,a}$	$8.18 \pm 0.17^{B,a}$	$8.33 \pm 0.19^{B,C,b}$	$7.64 \pm 0.18^{A,a}$	$7.65 \pm 0.22^{A,a}$
7	$8.21 \pm 0.13^{A,a,b}$	$8.17 \pm 0.07^{A,a}$	$8.19 \pm 0.11^{A,a}$	$8.77 \pm 0.14^{B,b,c}$	$8.46 \pm 0.30^{A,b}$	$8.26 \pm 0.05^{A,b}$
14	$8.12 \pm 0.00^{A,a}$	$8.22 \pm 0.00^{A,a}$	$8.62 \pm 0.29^{B,b}$	$8.34 \pm 0.20^{A,B,b}$	$8.16 \pm 0.13^{A,a}$	$8.76 \pm 0.17^{B,c}$
21	$7.88 \pm 0.15^{A,a}$	$8.48 \pm 0.37^{A,B,a}$	$8.59 \pm 0.16^{B,b}$	$8.03 \pm 0.04^{A,B,a}$	$8.14 \pm 0.11^{A,B,a}$	$8.53 \pm 0.02^{A,B,b,c}$
28	$8.34 \pm 0.02^{A,b}$	$8.21 \pm 0.13^{A,a}$	$8.65 \pm 0.11^{A,b}$	$8.48 \pm 0.35^{A,b}$	$8.39 \pm 0.18^{A,b}$	$8.55 \pm 0.37^{A,b,c}$

(PCP – probiotic control petit suisse; SCP – synbiotic control petit suisse; UF-PP – ultrafiltration probiotic petit suisse; UF-SP – ultrafiltration synbiotic petit suisse; PP – LF – probiotic petit suisse – lactose free; SP – LF – synbiotic petit suisse – lactose free. Different lowercase letters (a, b, c) indicate difference ($p < 0.05$) between the values in the same column. Different capital letters (A, B, C) indicate difference ($p < 0.05$) between the values in the same row)

Viable Counts of Probiotic Microorganism

Cheeses are good food matrices for probiotics, because some have a high level of fat and protein, and they can have a creamy consistency, a high moisture content, and a high pH. These characteristics contribute to a protective base for probiotics and maintain their viability (Esmerino *et al.*, 2013) [11].

In all products *B. lactis*, BB-12 showed a high level (>8 log cfu/g) at the end of the storage period (28 d) at 5°C (Table 9). Despite the statistical differences observed during incubation, the values between the beginning and the end of storage were maintained higher than 8 log cfu/g, except for the PP-LF and SP-LP formations, which at the beginning of storage had a lower count (7.64 and 7.65 log cfu/g, respectively), but in the end of storage showed high counts (8.39 and 8.55 log cfu/g, respectively, of BB12). It is likely that the addition of sucrose or fructooligosaccharides (FOS) may have helped *B. lactis*, BB-12 to grown during storage. However, it is important to highlight that the FOS addition did not influence the BB-12 viability.

Probiotic bacteria can use different strategies to consume carbohydrates as carbon sources, maintaining its viability in several products. Among these strategies, one of them is to use intra and extracellular enzymes with activity for oligo- and polysaccharides. In this way, *B. animalis* ssp. *lactis* have ability to phosphorylate sucrose and small amounts of oligofructose. Moreover, *B. animalis* ssp. *lactis* are able to synthesize B vitamins such as biotin, cobalamin, folates, nicotinic acid, pantothenic acid, pyridoxine, riboflavin, and thiamine (Kaprasob *et al.*, 2018) [21]. Li *et al.* (2015) [22] evaluated the fermentation capacity of galacto-oligosaccharides (GOS) and FOS in a mixed culture of *Lactobacillus* and *B. lactis*. The FOS and 2 digestible disaccharides (lactose and sucrose) showed significant increase ($p < 0.05$) in the bifidobacteria population during the 24-h fermentation period. However, GOS was the prebiotic carbohydrate that had the greatest influence on the growth of probiotic microorganisms.

Padilha *et al.* (2016) [31] investigated the survival of 2 probiotic strains, *Lactobacillus acidophillus* (La-5) and *B. animalis* (BB-12) incorporated into a probiotic and synbiotic Petit Suisse during 28 d. It was observed that the population of La-5 and BB-12 in the final product was above 7 log cfu/g by the last storage day. In addition, the probiotic bacteria survived during the *in vitro* assay under conditions which were similar to the gastrointestinal tract. The assay showed that Petit Suisse is a probiotic carrier food matrix, and the addition of a prebiotic mixture to this cheese resulted in a protective effect for the survival of probiotic, especially for BB-12. Other study made by Cadarelli *et al* (2008) [7] produced a synbiotic Petit Suisse and the authors observed that the population of *B. animalis* ssp. *lactis* and *L. acidophilus* decreased during storage; however, shelf life counts remained above 10^7 cfu/g.

Conclusions

Lactose-free dairy products are becoming more common and provide excellent opportunities for lactose intolerant people. These people can benefit from the broad palette of different nutritious and delicious products made from milk. We have shown that physicochemical and sensory characteristics are different when compared with control formulations, but the UF and DF processes are a good way to provide consumers with functional food with low-lactose content and high-protein content. All Petit Suisse formulations had a good matrix to carry the probiotic *B. lactis*, BB-12. Finally, the Petit Suisse with low lactose is a good and new alternative product for lactose intolerance, with good prospects for future marketing.

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