Advance Journal of Food Science and Technology 14(2): 56-68, 2018
DOI:10.19026/ajfst.14.5832
ISSN: 2042-4868; e-ISSN: 2042-4876

Submitted: December 21, 2017 Accepted: February 7, 2018 Published: March 25, 2018

Research Article

Use of Concentrated Whey by Freeze Concentration Process to Obtain a Symbiotic Fermented Lactic Beverage

Maria Helena Machado Canella, Isabella de Bona Muñoz, Stephanie Silva Pinto, Gabriela Rodrigues de Liz, Carmen Maria Olivera Müller, Renata Dias de Mello Castanho Amboni and Elane Schwinden Prudencio
Department of Food Science and Technology, Agricultural Sciences Center, Federal University of Santa Catarina, Rod. Admar Gonzaga, 1346, Itacorubi, 88034-001, Florianópolis, SC, Brazil

Abstract: This study aimed to use the freeze concentration process to concentrate cheese whey to factor equal to 4 at the third concentration stage and choose the best whey concentrated for the manufacture of fermented lactic beverages. Two fermented lactic beverages, both consisting of 70% milk and 30% CW2 and co-cultures of *Lactobacillus acidophilus*, *Bifidobacterium animalis* subsp. *lactis* and *Streptococcus thermophilus*were manufactured with the concentrated whey from the second stage (CW2). The Beverage 1 was produced without the addition of inulin and beverage 2 produced with the addition of 6 g of inulin per 100 mL of the product. Both beverages were evaluated about their physicochemical properties, syneresis index, color parameters and microbiological properties at 4.0±1.0°C while their rheological properties were evaluated at 4.0±0.1°C and 6.0±0.1°C, on day 1 and on day 30 of storage. The total solids content, syneresis index, parameter *a** and apparent viscosity values were influenced by the addition of inulin and the storage time. A thixotropic behavior was noted for both beverages, while, the hysteresis was greater in the beverage with the addition of inulin. The Power Law and Casson models were successfully applied to describe the rheological behavior of the beverages. The Beverage 1 can be classified as a probiotic product and the Beverage 2 as a symbiotic product, respectively.

Keywords: Concentrated whey, fermented lactic beverage, freeze concentration, prebiotic, probiotic, rheological properties

INTRODUCTION

Block freeze concentration technology makes it possible to produce food concentrates with high quality by recovering a food solute based on the separation of pure ice crystals from a freeze-concentrated aqueous phase. When compared with traditional concentration processes, such as evaporation, freeze concentration not only shows some significant potential advantages for the production of a concentrate where no vapor/liquid interface exists but also can protect thermally fragile food compounds (Petzold *et al*., 2015). This technology has highly promising applications, especially, in the production of foods and ingredients that have high nutritive value (Aider and Halleux, 2009). In this technology, a food liquid solution is completely frozen and then, the whole frozen solution is thawed. After that, the concentrate fraction is separated from the ice fraction by gravitational thawing, which sometimes may be carried out assisted by other techniques to enhance separation efficiency (Aider and Halleux, 2008; Petzold *et al*., 2015). Under these conditions, the ice block acts as a solid carcass through which the concentrated fraction passes. However, the efficiency of this process, which is considered to be an environmentally friendly technology, is determined by the purity of the ice formed, i.e., with minimum retention of solutes (Aider and Halleux, 2009). This technique has been used in the concentration of dairy products, such as cheese whey (Chabarov and Aider, 2014; Sánchez *et al*., 2011).

Cheese processing plants generate large volumes of liquid waste including cheese whey, which is the liquid portion produced during cheese-making. According to Mollea *et al.* (2013), cheese whey contains at least half of the total solids present in the original whole milk and therefore it can be considered as a valuable by-product with several applications, especially in the food

Corresponding Author: Elane Schwinden Prudencio, Department of Food Science and Technology, Agricultural Sciences Center, Federal University of Santa Catarina, Rod. Admar Gonzaga, 1346, Itacorubi, 88034-001, Florianópolis, SC, Brazil, Tell.: +55 48 3721 5366 (E.S. Prudencio); Fax: +55 48 37215397

industry. On the other hand, Yadav *et al*. (2015) stated that the components of cheese whey are difficult to degrade and as a result, they create a significant problem to any wastewater treatment plant that treats other effluents. These authors also pointed out that proper management (economic and eco-friendly treatment or re-use) of cheese whey is required before its disposal. Although a significant amount of whey remains unutilized, advanced technologies as freeze concentration could be used to deal with this whey management issue and thus cheese whey could be turned into a valuable functional product. However, the formation and growth of ice crystals can cause alterations in the properties of concentrated whey and consequently, very little attention has been paid to its use. From a valorization point of view, options for the management of concentrated whey, such as its use in the production of fermented lactic beverages, can be taken into consideration.

The image of health associated with functional beverages has led to an increase in their consumption. According to Brazilian regulations, which establish a standard of identity and quality for fermented lactic beverages, these can be defined as a type of fermented food that results from the mixture of milk and cheese whey containing lactic culture and other dairy products, such as freeze-concentrated whey.

Lactic beverages show good potential for increasing the survival of probiotics (Cunha *et al*., 2008, 2009), for the addition of prebiotics (Debon *et al*., 2010, 2012) and for a mixture of probiotics and prebiotics, i.e., symbiotic (De Castro *et al*., 2008, 2009), in the product. Probiotics are living microorganisms that, in adequate amounts and regularly administered, confer health benefits to the host by improving the balance of the host-gut microbiota and the host's defenses against pathogenic microorganisms (FAO/WHO, 2006). The bacteria of the genera *Lactobacillus* and *Bifidobacterium* are used predominantly in probiotic foods (Tripathi and Giri, 2014). Because these bacteria grow slowly in milk, it is recommended that they usually are combined with *Streptococcus thermophilus* (Casarotti *et al*., 2014). Nevertheless, according to Roberfroid *et al*. (2010), the concept of prebiotics is defined as the selective stimulation of growth and activity of one or a limited number of a microbial genus or species in the gut microbiota that provides health benefits to the host. Karimi *et al*. (2015) stated that inulin had been shown to cause a protective effect on some species of *Lactobacillus acidophilus* and *Bifidobacterium* spp., often improving their survival and activity during storage. Inulin, which is a soluble and fermentable fiber, is an example of such prebiotics that, because of their functional and technological properties, has been widely used. Hence, Chaito *et al*. (2016) pointed out that inulin represents a significant ingredient that offers new opportunities to the food industry, which is continually seeking balanced products for the future.

Studies have been conducted to enrich dairy products with supplements to improve the growth of probiotic cultures and thus consequently offer an opportunity for innovation in the food industry. A fermented lactic beverage produced with concentrated whey in association with probiotic bacteria and inulin could result in functional food. Moreover, considering the lack of studies on freeze concentration, such beverage could serve as a new alternative for the dairy industry as well as for consumers interested in health food products. However, few studies have been done on the measuring of the physical and chemical properties of fermented lactic beverages that combine probiotics, prebiotics and different raw materials. Concerning the syneresis index and rheological properties of the beverages, these combinations, as well as storage time, are also capable of causing changes.

Taking the above into consideration, the objective of this study was to evaluate the effects of freeze concentration of cheese whey and then chose the concentrated whey from the best stage performance. After that, fermented lactic beverages were manufactured with co-cultures of *Lactobacillus acidophilus*, *Bifidobacterium animalis* subsp. *Lactis* and *Streptococcus thermophilus* with and without inulin addition. Finally, these fermented lactic beverages were evaluated on their physicochemical properties, syneresis index, color parameters and rheological and microbiological properties on day 1 and day 30 of refrigerated storage.

MATERIALS AND METHODS

Materials: Commercial pasteurized milk (10.98 g total solids 100 g^{-1}, 3.20 g lipid 100 g^{-1}, 2.98 g protein 100 g^{-1}, 0.73 g ash 100 g^{-1} and 4.07 g carbohydrate 100 g^{-1}, Tirol, TrezeTílias, Brazil), commercial rennet at a ratio of 1:3000 (HA-LA®, Chr. Hansen, Valinhos, Brazil), lactic acid (Purac Sínteses, Rio de Janeiro, Brazil) and calcium chloride (Vetec, Rio de Janeiro, Brazil) were used to obtain cheese whey. The fermented lactic beverages were obtained using commercial pasteurized milk, lactic culture (ABT-4®, Chr. Hansen, Hónsholm, Denmark) composed of *Streptococcus thermophilus*, *Bifidobacterium animalis* subsp. *lactis* and *Lactobacillus acidophilus*, prebiotic inulin (Orafti® HPX, Orafti, Tienen, Belgium) with adegree of polymerization (DP) ≥ 23 and sucrose. MRS agar (Difco, Sparks, USA), M17 agar (Fluka, Neu-Ulm, Germany), lithium chloride (Vetec, Rio de Janeiro, Brazil), sodium propionate (Fluka, Neu-Ulm, Germany), bile (Sigma-Aldrich, St. Louis, EUA), lactose (Vetec, Rio de Janeiro, Brazil) and AnaeroGen® (Oxoid, Hampshire, UK) were used for

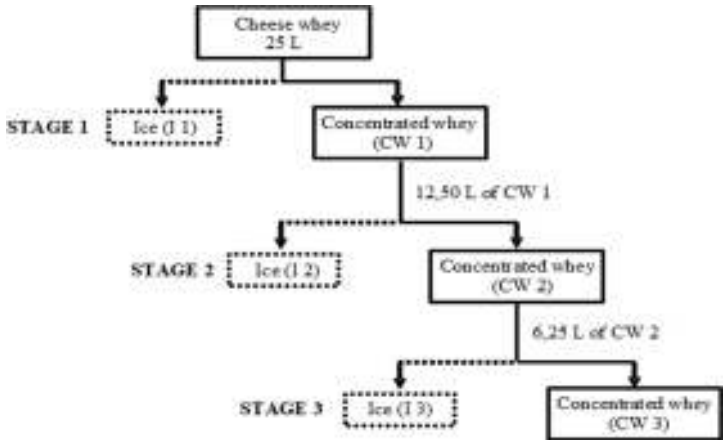

Fig. 1: Diagram of the freeze concentration process in cheese whey

the microbiological analysis. All reagents were of analytical grade.

Manufacture of cheese whey: The cheese whey was obtained from Minas Frescal cheese as described by Souza and Saad (2009), with modifications. The Minas Frescal cheese was produced in a 30 L vat from pasteurized milk heated to 37±1°C, with theaddition of lactic acid (0.25 mL/L of an 85 % lactic acid solution). It was added to the cheese-milk calcium chloride (0.4 mL/L of a 40% calcium chloride solution) and commercial rennet at a ratio of 1:3000 (0.9 mL/L of pasteurized milk) followed by incubation at 37±1°C for 40 min. The resulting gel was gently cut into cubes, allowed to drain and placed in cylindrical perforated containers, each with a capacity of 500 g.

Protocol of the freeze concentration procedure of cheese whey: The freeze concentration method consisted of block freeze concentration following the methodology described by Boaventura *et al*. (2013). In each freeze concentration stage, two fractions were obtained and denoted as Concentrated Whey (CW) and Ice (I) (Fig. 1). Twenty-five liters of cheese whey were divided into 1 L batches and frozen at -40±2°C in a plate freezer (Frigostrella, Cotia, São Paulo, Brazil). Once the cheese whey was frozen, 50% of the initial volume was defrosted at room temperature (20±2°C). The defrosted liquid constituted the CW1, which was frozen at -40±2°C and used as feed solution in the second stage. This procedure was repeated until the third stage. The aliquot samples fractions (CW and I) remaining from freeze concentration stages were stored at -20±2°C until chemical analysis.

At each freeze concentration stage, the Concentration Factor (CF) was calculated according to theme thodology proposed by Aider and Ounis (2012), using the following Eq. (1):

$$CF = \frac{TS_n}{TS_0} \quad (1)$$

where, TS_n is the total solids (g/100g) content of the concentrated whey from each freeze concentration stage and TS_0 is the total solids (g/100g) content of the cheese whey initial.

The Process Efficiency (PE) of the freeze concentration was determined based on the increase of TS in the CW (g/100g) concerning the TS remaining in the I (g/100g) from each freeze concentration stage (n), as described in the Eq. (2):

$$PE(\%) = \frac{\text{TS in the CW}_n - TS\text{in the I}_n}{\text{TS in the CW}_n} x\ 100 \quad (2)$$

The concentrated whey used in the manufacture of fermented lactic beverages was chosen from the evaluation of the results for CF and PE.

Manufacture of fermented lactic beverages: Two fermented lactic beverages, denoted as beverage 1 and beverage 2, were manufactured according to the procedures of Almeida *et al*. (2001), with modifications. The Beverage 1 was produced with no addition of inulin, while the Beverage 2 was elaborated with the addition of 6 g 100 mL^{-1} of inulin, as suggested by González-Tomás *et al*. (2009). The milk (70%) with sucrose (5 g 100 mL^{-1}) mix was submitted to thermal treatment at 95±5 °C for 5 min, while the concentrated whey chosen from the freeze concentration procedure (30%) with or without inulin was heated at 65±2°C for 30 min. The temperature of the mixes was lowered to 40±1°C, the homogenization of these was carried out and then the lactic culture (75 mg 100 mL^{-1}) was added before incubation at 40±1°C until pH 4.6 was reached. After fermentation, the beverages (1 and 2) were cooled to 4±1°C, being gently stirred, conditioned in plastic flasks, thermo-sealed (Sulplack SPO-150, Caxias do Sul, Rio Grande do Sul, Brazil) withmultilayer aluminum and polyethylene lid and stored at this temperature. Each beverage (1 and 2) production was performed in triplicate. Beverages from each batch were used for analysis on day 1 and day 30 of storage.

Physical and chemical analysis: The cheese whey, the concentrated whey (CW 1, CW 2 and CW 3), the ice (I 1, I 2 and I 3) and beverages (1 and 2) were analyzed for total solids content (g/100g). The total solids determination was realized through the drying of the samples until reaching the constant weight at 105°C (IAL(Instituto Adolfo Lutz), 2008). The beverages were also analyzed for protein (g/100g) by the Kjeldahl method (N×6.38) (AOAC, 2005). The lipid content (g/100g) through by extraction of lipids with petroleum ether, using the Soxhlet device after denaturation of proteins with hydrochloric acid and ash (g/100g) through a gravimetric method (IAL (Instituto Adolfo Lutz), 2008). The total carbohydrate content (g/100g) of both beverages were calculated by difference (AOAC, 2005). The titratable acidity (g/100g lactic acid) of beverages was determined according to the Analytical Norms of the Adolfo Lutz Institute (IAL (Instituto Adolfo Lutz), 2008), while the measurements of pH were carried out with a pH meter (PHS-3 BW, BEL, Piracicaba, São Paulo, Brazil). The syneresis index of beverages (1 and 2) was determined following the methodology described by Modler and Kalab (1983); by draining 100 mL of each sample in 100-mesh stainless screen placed on the top of a long-stemmed funnel, which had been introduced in a graduated cylinder to collect the liquid. The syneresis index was considered as being the amount of liquid (mL) per 100 mL of the sample after 2 h of draining (4±1°C). For both beverages (1 and 2) the color analyses were determined using a colorimeter Minolta Chroma Meter CR-400 (Konica Minolta, Osaka, Japan), adjusted to operate with D65 lightning and 10° of observation angle. The colorimeter was calibrated with a white standard plate and to measure the L^*, b^* and a^* parameters was used the CIELab color scale. The L^* parameter ranges 0 to 100 and indicates luminosity (variation from black to white), the b^* axis is the variation from yellow (+ b^*) to blue (- b^*) and thea^* axis shows the deviation from red (+ a^*) to green (- a^*). The total difference of color (ΔE^*) between the measured values in the final time (day 30) and the initial time (day 1) of storage was calculated according to Okpala *et al*. (2010), as described in Eq. (3):

$$\Delta E = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \quad (3)$$

where ΔL^* is the difference of luminosity for the same beverage, between day 1 and day 30 of storage, while Δa^* represents the intensity of the red color and Δb^* the intensity of the yellow color. The value of Chroma (C^*) and Hue angle (h^*) were determined using Eq. (4) and (5), respectively (Masoud and Jakobsen, 2003). All physical and chemical analyses were carried out in triplicate:

$$C^* = \sqrt{(a^*)^2 + (b^*)^2} \quad (4)$$

$$h^* = \tan^{-1}(b^*/a^*) \quad (5)$$

Rheological measurements: The rheological measurements of both fermented lactic beverages (beverage 1 and beverage 2) were carried out with a Brookfield rotational rheometer with a concentric cylinder (Brookfield Engineering Laboratories model DV-III Ultra, Stoughton, MA, USA) using a ULA spindle. These measurements were collected with the Rheocalc® 32 software version 3.2 (Brookfield Engineering Laboratories Inc., Middleboro, MA, USA). The flow curves were generated by a linearly increased shear rate of 4.89 s^{-1} to 75.83 s^{-1} in the first 15 min (upward curve) and returned to 4.89 s^{-1} in the following 15 min (downward curve). The rheometer was thermostatically controlled at temperatures of 4.0±0.1°C and 6.0±0.1°C by a water circulator (TE-184, TECNAL, São Paulo, Brazil). The rotational speed was increased from 2 to 62 rpm, at 2 rpm every 30 s. The flow behavior was described through the Power Law model and the Casson model, according to Eq. (6) and (7), respectively:

$$\sigma = K(\dot{\gamma})^n \quad (6)$$

$$\sigma^{0.5} = \sigma_0^{0.5} + (\eta_c \dot{\gamma})^{0.5} \quad (7)$$

where,

σ = The shear stress (Pa)
$\dot{\gamma}$ = The shear rate (s^{-1})
K = Consistency index (Pa/s)
n = Flow behavior index
σ_0 = Yield stress (Pa)
η_c = CASSON viscosity (Pa s)

Viscosity values in the downward (viscosity/shear rate) curves at a rate of 50 s^{-1} were considered as the apparent viscosity (η) of both beverages (1 and 2). According to Bourne (2002), this rate represents a viscosity approximate which is perceived in the mouth. By calculating the hysteresis loop area between the flow curves (ascending and descending), the thixotropic behavior of the beverages (1 and 2) was obtained. All of the values were obtained in triplicate.

Microbiological analysis: *Lactobacillus acidophilus*, *Bifidobacterium animalis* subsp. *Lactis* and *Streptococcus thermophilus* counts of viable cells were evaluated for both beverages (1 and 2). For such evaluation, 25 g portions of each beverage aseptically collected were blended with 225 mL of 0.1 g/100g peptone water in a Bag Mixer 400 (Interscience, St. Nom, France), followed by decimal dilutions with the same diluent. According to Vinderola and Reinheimer (2000), for *Lactobacillus acidophilus*and *Bifidobacterium animalis* subsp. *Lactis* (probiotic

cultures) counts were used MRS agar modified with the addition of 0.15 g 100 mL^{-1} of bile (bile-MRS) and MRS agar with theaddition of 0.20 g 100 mL^{-1} of lithium chloride and 0.30 g 100 mL^{-1} of sodium propionate (LP-MRS), respectively. The plates were incubated at 37±1°C for 72 h, but for the *Bifidobacterium animalis* subsp. *lactis* count, they were incubated in anaerobic jars containing AnaeroGen®. *Streptococcus thermophilus* count was carried out by pour plate technique using M17 agar with the addition of lactose 10 g 100 mL^{-1}, incubated at 37±1°C for 48 h, as methodology described by International Dairy Federation (IDF) (1997). After the incubation periods, all count of viable cells were carried out and expressed as log colony-forming units per gram of beverage (log CFU/g). All the analyses were performed in triplicate.

Statistical analysis: One-way Analyses of Variance (ANOVA) and Tukey's studentized range (5% significance) were carried out to test for any significant differences between the results. The validity of the Power Law and Casson models was evaluated based on the determination coefficient (R^2). The data were obtained using the software STATISTICA version 7.0 (StatSoft Inc., Tulsa, OK, USA) and expressed as means and standard deviation.

RESULTS AND DISCUSSION

Cheese whey freeze concentration: Table 1 shows the results for the Concentration Factor (CF) of the total solids content and the Process Efficiency (PE) of the freeze concentration of cheese whey. The data in Table 1 show that it was possible to concentrate the total solids of the cheese whey to a factor equal to 4 at the third concentration stage. The PE was higher in the first freeze concentration stage and showed a slight decrease (p<0.05) in the second stage. Miyawaki *et al*. (2016) state that in the first stages of freeze concentration this behavior is expected, i.e., high separation efficiency resulting in a concentrate with high quality. However, the PE obtained in the present study decreased (p<0.05) drastically in the third stage while the total solids content in its ice fraction increased (p<0.05). The same behavior was noted by Aider *et al*. (2007) and Aider *et al*. (2009) during freeze concentration of cheese whey, where it was observed that PE is directly dependent on the total solids content in the ice fraction. Burdo *et al*. (2007) reported that many different factors influence on the separation of the components. Aider and Ounis (2012) noted that the increase in total solids content in the ice fraction could be explained by the high content of total solids entrapped in these fractions in the final stages of freeze concentration processes. Petzold *et al*. (2015) state that this effect is explained by an increase in the initial concentration at each stage, thus implying in a concentrate with higher viscosity and that the concentration of the recovered solution commonly depends on the viscosity of the concentrate in all the different types of freeze concentration processes. Okawa *et al*. (2009) noted that crystal orientation was another significant factor to consider for the elimination of solids from the ice fraction. Moreover, these authors clarified that the ratio of concentration between the solute captured in the ice and the solute present in the mother solution varied from 1/10 to 1/250, depending on the orientation of the ice crystal. On the other hand, Samsuri *et al*. (2015) noted that large ice crystals contain fewer impurities and solids than small ice crystals. Therefore, if ice crystals grow too rapidly, as in the rapid freezing generated by plate freezers, a formation of irregular crystals, could account for the PE decrease in the third stage since irregular crystals are difficult to separate. Based on these results, the concentrated whey from the second stage (CW2) was used in the manufacture of the fermented lactic beverages.

Table 1: The total solids content of the cheese whey, Concentrated Whey (CW) and Ice (I) at each freeze concentration and the concentration factor (CF) and the process efficiency (PE) regarding total solids content

Stages	Samples	Total solids (g 100 g^{-1})	CF	PE (%)
	Cheese whey	5.93±0.02^{dB}	-	-
Stage 1	CW1	10.93±0.02^{c}	2.00±0.02$^{\bullet}$	93.36±0.05*
	I1	0.73±0.01^{D}	-	-
Stage 2	CW2	18.31±0.03^{b}	3.00±0.03^{+}	91.01±0.26^{+}
	I2	1.64±0.05^{C}	-	-
Stage 3	CW3	24.09±0.25^{a}	4.00±0.02*	55.86±0.78$^{\bullet}$
	I3	10.64±0.08^{A}	-	-

Results expressed as a mean±standard deviation, among three batches realized in triplicate for each freeze concentration stage, with three repetitions for total solids, CF and PE; ^{a,b,c} Within a column, different superscript lowercase letters denote significant differences (p<0.05) between the cheese whey and the CW of each freeze concentration stage; $^{A, B, C}$ Within a column, different superscript uppercase letters denote significant differences (p<0.05) between the cheese whey and the I of each freeze concentration stage; Different symbols indicate significant difference (p<0.05) in the CF and PE of each freeze concentration stage

Physical and chemical properties of the fermented lactic beverages: Table 2 shows the results of the physicochemical properties on days 1 and 30 of storage for both fermented lactic beverages, where beverage 1 was produced without inulin and beverage 2 was produced with the addition of 6 g 100 mL^{-1} of inulin. The addition of inulin increased the total solids content, also increasing in an increase in the total carbohydrates (p<0.05). The same behavior was observed by Crispín-Isidro *et al*. (2015) and by Debon *et al*. (2012) for yogurts and fermented milk added with inulin, respectively. These results corroborated those noted by Bot *et al*. (2004), who reported that inulin could affect the composition of dairy products. However, on day 30 of storage, both beverages (1 and 2) showed an increase (p<0.05) in the total solids content. Regardless of the packaging material, Saint-Eve *et al*. (2008) state that the packaging type had a more significant impact on the physicochemical properties of yogurt and that some

Table 2: Physicochemical properties and color parameters (L^*, a^*, b^*, ΔE^*, C^*, h^*) of fermented lactic beverage without inulin (Beverage 1) and fermented lactic beverage with 6 g 100 mL^{-1} of inulin (Beverage 2), on day 1 and on day 30 of storage at 4.0±1.0°C

	Beverage 1		Beverage 2	
Analysis	Day 1	Day 30	Day 1	Day 30
Total solids (g/100g)	16.60±0.01^{bB}	16.87±0.02^{aB}	20.78±0.05^{bA}	21.25±0.18^{aA}
Protein (g/100g)	3.02±0.01^{aA}	3.07±0.21^{aA}	3.10±0.32^{aA}	3.10±0.12^{aA}
Lipid (g/100g)	2.47±0.05^{aA}	2.53±0.01^{aA}	2.43±0.06^{aA}	2.45±0.19^{aA}
Ash (g 100 g^{-1})	1.00±0.08^{aA}	0.91±0.02^{aA}	0.92±0.01^{aA}	0.90±0.02^{aA}
Total carbohydrates (g/100g)	10.12±0.12^{aB}	10.36±0.22^{aB}	13.81±0.44^{bA}	14.83±0.51^{aA}
Titratable acidity (g/100g)	0.72±0.14^{aA}	0.74±0.16^{aA}	0.58±0.01^{bA}	0.63±0.01^{aA}
pH	4.28±0.01^{aA}	4.14±0.01^{bB}	4.28±0.01^{aA}	4.21±0.01^{bA}
Syneresis index (g/100g)	1.59±0.18^{bA}	2.19±0.23^{aA}	0.83±0.04^{aB}	0.68±0.04^{bB}
L^*	76.58±0.22^{bB}	76.98±0.01^{aB}	77.43±0.41^{aA}	77.49±0.24^{aA}
b^*	17.50±0.25^{bA}	18.04±0.05^{aA}	17.59±0.04^{bA}	17.70±0.16^{aB}
a^*	-3.60±0.06^{bA}	-3.72±0.05^{aA}	-3.54±0.08^{aA}	-3.36±0.24^{aB}
ΔE^*	0.69		0.22	
C^*	17.87±0.25^{bA}	18.42±0.05^{aA}	17.94±0.03^{aA}	18.02±0.13^{aB}
h^*	78.39±0.14^{aA}	78.34±0.16^{aA}	78.64±0.28^{aA}	79.26±0.82^{aA}

Results expressed as a mean±standard deviation, among three batches realized in triplicate for each type of the fermented lactic beverage, with three repetitions for each one physical or chemical analysis; ^{ab} Within a row, different superscript lowercase letters denote significant differences (p<0.05) among the different storage time for each one fermented lactic beverage type studied; ^{AB} Within a row, different superscript uppercase letters denote significant differences (p<0.05) among the different fermented lactic beverage type for the same storage time.

changes in these properties may also occur under short-time and low-temperature storage conditions. Furthermore, these authors reported that other factors could influence the properties of yogurts, such as the composition of the food matrices and the external environment (temperature, humidity, among others). On the other hand, neither the addition of inulin nor the storage time influenced (p>0.05) on the protein, lipids and ash contents.

The addition of inulin did not affect (p>0.05) the acidity values compared between beverages 1 and 2. However, on day 30 the acidity showed a slight increase (p<0.05) for beverage 2, i.e., added with inulin. For both beverages, the pH values decreased (p<0.05) at the end of the storage time; however, the decrease was higher (p<0.05) for beverage 1. These behaviors are following the findings of Guggisberg *et al*. (2009), Guven *et al*. (2005) and Paseephol and Sherkat (2009), who reported that the addition of inulin did not affect the pH or the acidity of fermented milk. Kailasapathy (2006) attributes this to the post-acidification that is usually detected in dairy products. During refrigerated storage (between 0 and 5°C), β-galactosidase enzymes, which are produced by bacteria added in fermented milk, are responsible for the catabolism of lactose during the fermentation process, thus resulting in a decrease in pH (Kailasapathy and Sultana, 2003; Resa *et al*., 2007). This catabolism of lactose contributes to the accumulation of lactic acid, acetic acid, citric acid, butyric acid, acetaldehyde and formic acid produced by the starter culture as metabolic by-products (Kailasapathy, 2006). The acidity and pH values for both beverages are in agreement with those recommended as desirable by Gallardo-Escamilla *et al*. (2007) for fermented milk.

As expected, the addition of inulin resulted in a decrease (p<0.05) in the syneresis index, which also could be related to the higher total solids content in beverage 2 than in beverage 1. Rinaldoni *et al*. (2012) stated that a low syneresis index in fermented milk is fundamental because consumers tend to reject the presence of exudates. According to Vargas *et al*. (2008), when the total solids content in dairy products increase, the intensity of the attractive forces between the casein micelles decreases, thereby increasing the water holding capacity while decreasing gel shrinkage, porosity and the syneresis index. Furthermore, Meyer *et al*. (2011) reported that inulin molecules were able to bind water, preventing their free movement. Also, inulin interacts with milk proteins providing stability to the protein network and thus originating a protein matrix less prone to whey expulsion (Meyer *et al*., 2011).

Since beverage 1 was not added with inulin, the increase (p<0.05) in its syneresis index on day 30 of storage could be due to the presence of whey. This result is in agreement with that obtained by Penna *et al*. (2006), who reported that whey contributes to the formation of acid gels that have an open structure due to the decrease in their intermolecular interactions and therefore are more susceptible to syneresis.

The color parameters for beverage 1 and beverage 2 on days 1 and 30 of storage are also shown in Table 2. Despite the differences observed in the parameters b^* and a^*, the Hue angle (h^*) values for both beverages showed no difference (p>0.05), either on day 1 or day 30 of storage. The beverage 2 showed no difference (p>0.05) in whiteness-blackness (L^*). This result complies with that noted by Staffolo *et al*. (2004), who evaluated the effects of different additions of commercial dietary fibers in yogurt. However, between day 1 and day 30 of storage, the parameter L^* value of beverage 1 showed a slight increase (p<0.05), tending toward whiteness. The storage time evaluated in this present study also showed an increase (p<0.05) in the yellow coloration (parameter b^* values) of both

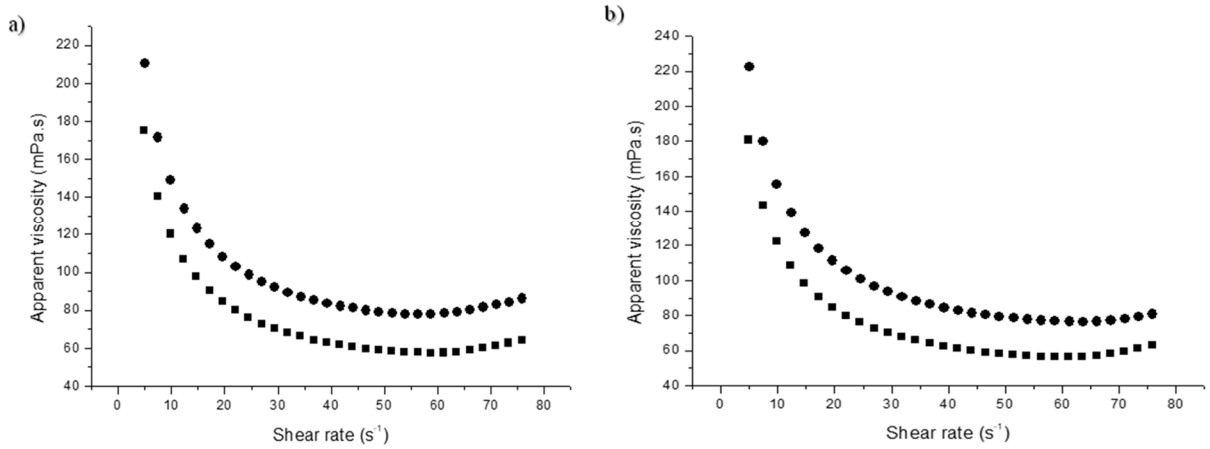

Fig. 2: Apparent viscosity *versus* shear rate at temperatures of (a) 4.0 ± 0.1°C and (b) 6.0 ± 0.1°C for the fermented lactic beverage without inulin (Beverage 1) ( ) and fermented lactic beverage with 6 g 100 mL^{-1} of inulin (Beverage 2) (●) on day 1 of storage

fermented lactic beverages. This increase in *b** may be caused by the increase in the total solids content, primarily by the total carbohydrates content, which may have increased the Maillard reaction. Wang *et al*. (2011) related to the increase of the parameter b * in the intermediate phase of the Maillard reaction, where large amounts of yellow compounds are produced in dairy products, confirming the results obtained in the present study. The values for parameter *a** indicated that both beverages showed a tendency towards the color green. As was noted by Prudêncio *et al*. (2014) for ricotta cheese, these results occurred because cheese whey contains riboflavin, which is attributable to the slightly green coloration of whey. Meanwhile, at the storage times evaluated in the present study, the values noted for parameter *a** indicated that beverage 1 tends toward a more greenish color than beverage 2, which was added with inulin (a reducing sugar). Therefore, the reddish color of beverage 2 may also be attributed to the addition of inulin associated with the higher total solids content. On the other hand, the combination of these color parameters (*L**, *a** and *b**) is relevant because it results in the total difference of color (Δ*E**) values. The beverage 2 presenting ΔE* value lower ($p<0.05$) than beverage 1, confirming what was observed by Staffolo *et al*. (2004), who stated that fermented milk added with inulin had a stable color during storage time.

Calvo (2004) reported that the parameter Chroma (*C**) represents the degree of saturation of visual color. Despite the results obtained for this color parameter, it was possible to note that the *C** value increased ($p<0.05$) only for beverage 1 on day 30 of storage. Rozycki *et al*. (2010) stated that the pigment development in systems as CW, concentrated, depends mainly on the solids concentration. According to these authors, low total solids content may be responsible for the formation of oxidation products that can react with amino groups forming yellow products. This accumulation of oxidation products may be related to the increase in the *C** values observed for beverage 1, at the storage times evaluated.

Rheological measurements: Both beverage 1 (with no inulin) and beverage 2 (with inulin) showed a non-Newtonian fluid behavior (Fig. 2). Therefore, it was possible to note that the addition of inulin did not affect the behavior of the rheological characteristics of beverage 2. For both beverages, there was a reduction in the apparent viscosity along with the increase in shear rate, indicating that the fluid had shear thinning characteristics. This behavior was also observed by De Castro *et al*. (2008), Cunha *et al.* (2008), Debon *et al*. (2010) and Da Silveira *et al*. (2015) for symbiotic lactic beverages, for probiotic fermented milk, for prebiotic microfiltered fermented milk and for probiotic goat dairy beverages, respectively. Debon *et al*. (2010) reported that the reduction in the apparent viscosity with the increase in shear rate in these types of product are related to several factors. Da Silveira *et al*. (2015) reported that changes in viscosity are often associated with structural changes in a dairy beverage. As was stated by these authors, the change in flow behavior suggests the formation of a weak structure that can be caused by an initial formation of inulin aggregates. Therefore, these aggregates would contain inulin crystals with significant amounts of entrapped fluid phase leading to an increase of the volume fraction of the dispersed phase. Inulin aggregates can be relatively stable at low shear rates but, they can be easily disrupted by shearing (Da Silveira *et al*., 2015).

The rheological parameters for beverage 1 and beverage 2 described by the Power Law and Casson models on day 1 and day 30 of storage at 4.0±0.1°C and 6.0±0.1°C are shown in Table 3. The determination coefficient (R^2) for the models ranged from 0.980 to

Table 3: Rheological parameters obtained using Power Law ($\sigma = K(\dot{\gamma})^n$), Casson model ($\sigma^{0.5} = \sigma_0^{0.5} + (\eta_c\dot{\gamma})^{0.5}$), apparent viscosity, and thixotropic indexof fermented lactic beverage without inulin (Beverage 1) and fermented lactic beverage with 6 g 100 mL^{-1} of inulin (Beverage 2), on day 1 and on day 30 of storage at 4.0±0.1°C and 6.0±0.1°C

Samples	T (°C)	Days	Power Law model			Casson model			Apparent viscosity	Hysteresis loop
			K (Pa.s^n)	n	R^2	σ_0 (Pa)	η_c (Pa s)	R^2	η (mPa.s)	area (unit)
Beverage 1	4	1	$0.292^{bB\blacklozenge}$	$0.593^{aB\blacklozenge}$	0.980	$0.266^{bA\blacklozenge}$	$0.030^{aB\blacklozenge}$	0.990	$58.51^{aB\blacklozenge}$	$175.64^{aB\blacklozenge}$
		30	$0.371^{aB\blacklozenge}$	$0.540^{bB\blacklozenge}$	0.990	$0.404^{aB\blacklozenge}$	$0.026^{bB\blacklozenge}$	0.998	$60.67^{aB\dagger}$	$164.97^{aA\dagger}$
	6	1	$0.325^{bB\dagger}$	$0.570^{aB\dagger}$	0.980	$0.298^{bB\dagger}$	$0.028^{aB\dagger}$	0.990	$57.70^{aB\blacklozenge}$	$173.70^{aB\blacklozenge}$
		30	$0.389^{aB\dagger}$	$0.523^{bB\dagger}$	0.988	$0.417^{aB\dagger}$	$0.024^{bB\dagger}$	0.998	$59.00^{aB\dagger}$	$149.92^{bB\dagger}$
Beverage 2	4	1	$0.323^{bA\blacklozenge}$	$0.643^{aA\blacklozenge}$	0.982	$0.253^{bB\blacklozenge}$	$0.046^{aA\blacklozenge}$	0.990	$78.78^{bA\blacklozenge}$	$206.56^{aA\blacklozenge}$
		30	$0.449^{aA\blacklozenge}$	$0.585^{bA\blacklozenge}$	0.992	$0.445^{aA\blacklozenge}$	$0.041^{bA\blacklozenge}$	0.999	$87.00^{aA\blacklozenge}$	$196.56^{aA\dagger}$
	6	1	$0.372^{bA\dagger}$	$0.608^{aA\dagger}$	0.987	$0.336^{bA\dagger}$	$0.041^{aA\dagger}$	0.996	$78.78^{bA\blacklozenge}$	$195.31^{aA\blacklozenge}$
		30	$0.466^{aA\dagger}$	$0.568^{bA\dagger}$	0.992	$0.477^{aA\dagger}$	$0.038^{bA\dagger}$	0.999	$84.67^{aA\dagger}$	$181.66^{aA\dagger}$

Results expressed as a mean±standard deviation, among three batches realized in triplicate for each type of the fermented lactic beverage, with three repetitions for each one rheological parameter; ^{a,b} Within a column, different superscript lowercase letters denote significant differences (p<0.05) among the different storage time for each one fermented lactic beverage type studied, at the same temperature; $^{A, B}$ Within a column, different superscript uppercase letters denote significant differences (p<0.05) among the different fermented lactic beverage type for the same storage time, at the same temperature; Different symbols indicate significant difference (p<0.05) among the different temperatures for the same fermented lactic beverage type and storage time; K, Consistency index; n, flow behavior index; R^2, determination coefficient; σ_0, Casson yield stress; η_c, Casson viscosity; η, apparent viscosity at 50 s^{-1}

0.999, thereby demonstrating the adequate fit of flow curves. The degree of the behavior of shear thinning can be evaluated using the data on the flow behavior index (n). The flow behavior indexes of beverage 1 and beverage 2 had a shear thinning characteristic (n<1), ranging from 0.540 to 0.643 and thus confirming the non-Newtonian behavior. These results are in agreement with those of studies conducted by our research group, who noted that the use of cheese whey with prebiotics or not, results in dairy products with these characteristics. The Power Law model was used to find the flow behavior index (n) as well as the consistency index (K) for both beverages. The latter increased (p<0.05) with the increase in storage time and temperature. Regardless of the storage time and the temperature, the consistency index was higher (p<0.05) for the beverage added with inulin, which is a polysaccharide. Ladjevardi *et al*. (2015) reported that polysaccharides enhance the consistency of products because they can form a stable gel.

The Casson model was used to calculate the Casson yield stress (σ_0) and Casson viscosity (η_c) (Table 3). In general, both beverages (1 and 2) showed an increase (p<0.05) in Casson yield stress on day 30 of storage as well as when the temperature was increased from 4 to 6°C. Ramírez-Sucre and Vélez-Ruiz (2013) and Van Oosten-Manski *et al*. (2009) concluded that the yield stress of stirred yogurt increased because of the concentration of total solids and such conclusion is consistent with the results obtained for both beverages in the present study. The beverage added with inulin (beverage2) showed higher values of Casson viscosity (p<0.05) than the beverage 1, as observed for the values of apparent viscosity. The result for beverage 2 is in agreement with that obtained in a study conducted by Da Silveira *et al*. (2015), who observed that apparent viscosity increased in a dairy beverage supplemented with long-chain inulin (6/g 100g). According to these authors, the variations in the flow behavior of fermented milk added with inulin could be explained by different factors. The capacity of inulin to retain water due to the formation of small aggregates of microcrystals, the interaction of inulin with milk proteins, which can lead to an increase in the molar mass and consequently increase viscosity and also by the higher total solids content in the product are some of the factors. Rinaldoni *et al*. (2012) stated that inulin acts as a thickener that forms complexes through hydrogen bridges with milk proteins, contributing to a lower syneresis index, as was noted for beverage 2 (Table 1) in the present study. The same behavior was observed by Da Silveira *et al*. (2015), who reported that a high total solids content decreases the intensity of attractive forces between casein micelles and thereby increases water retention. Interestingly, on day 30 of storage at 6.0±0.1°C, the apparent viscosity of beverage 2 decreased (p<0.05). According to Gomes *et al*. (2013), this behavior may occur in dairy beverages that undergo a decrease in pH and post-acidification (Table 1). It is also noteworthy that whey protein solution is characterized by the breaking of the disulfide and the Van der Waals bonds and by the ionic and hydrophobic interactions between the protein particles. These characteristics are related to this decrease in viscosity. Therefore, the consequences of post-acidification in the rheological properties in beverage 2 are confirmed by the decrease in apparent viscosity. However, for beverage 1 (without inulin) no differences (p>0.05) were noted for apparent viscosity between the different storage times and temperatures employed.

Toneli *et al*. (2005) reported that the changes in the rheological behavior of a product could be explained by the formation of a hysteresis curve. Oliveira *et al*. (2002) cited that the thixotropy, a phenomenon commonly detected in fragile agglomerated particles, such as those of fermented milk, occurs when these particles are submitted to a shear force. In this case, the tridimensional structure initially formed in the

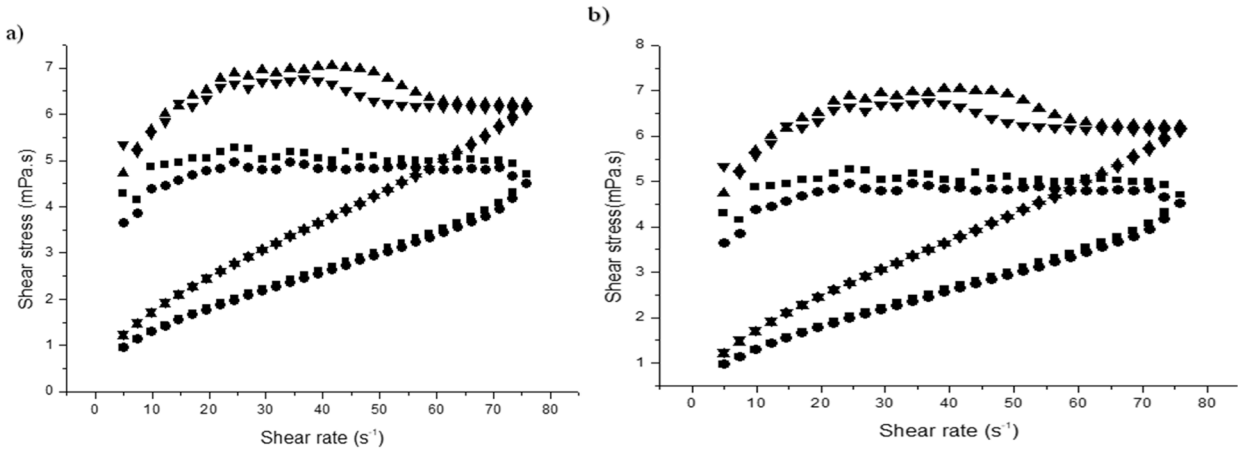

Fig. 3: Flow curves, shear stress *versus* shear rate, for fermented lactic beverage without inulin (Beverage 1) at temperatures 4.0 ± 0.1°C (■) and 6.0 ± 0.1°C (●) and fermented lactic beverage with 6 g 100 mL^{-1} of inulin (Beverage 2) at temperatures 4.0 ± 0.1°C (▲) and 6.0 ± 0.1°C (▲) (a) on day 1 and (b) on day 30 storage

Table 4: Microbiological properties of the fermented lactic beverage without inulin (Beverage 1) and fermented lactic beverage with 6 g 100 mL^{-1} of inulin (Beverage 2), on day 1 and on day 30 of storage at 4.0±1.0°C

Samples	Day	*Lactobacillus acidophilus* (log CFU/g)	*Bifidobacterium animalis* subsp. *lactis* (log CFU/g)	*Streptococcus thermophilus* (log CFU/g)
Beverage 1	1	9.22 ± 0.04^{aA}	9.20 ± 0.02^{aA}	9.27 ± 0.01^{aA}
	30	9.09 ± 0.08^{bB}	9.14 ± 0.01^{bB}	9.14 ± 0.02^{bB}
Beverage 2	1	9.19 ± 0.01^{bA}	9.23 ± 0.01^{bA}	9.22 ± 0.06^{aA}
	30	9.27 ± 0.04^{aA}	9.32 ± 0.01^{aA}	9.24 ± 0.01^{aA}

log CFU/g: log colony-forming units per gram of beverage; Results expressed as a mean±standard deviation, among three batches realized in triplicate for each type of the fermented lactic beverage, with three repetitions for each one microbiological analysis; ^{a,b} Within a column, different superscript lowercase letters denote significant differences ($p<0.05$) among the different storage time for each one fermented lactic beverage type studied; $^{A, B}$ Within a column, different superscript uppercase letters denote significant differences ($p<0.05$) among the different fermented lactic beverage type for the same storage time

fermentation process is lost and can be practically regained after a period of rest. It is possible to note in the reograms shown in Fig. 3 that both beverages showed hysteresis. Following the results obtained in a study conducted by Hernández (1996), in the present work, it was verified that beverage 2, i.e., with higher apparent viscosity values, showed a higher ($p<0.05$) hysteresis area (Table 3), than beverage 1, indicating a change in the system structure, caused by the structural breakdown in dispersion under shear. The increase in thixotropy observed for beverage 2 is a consequence of the higher viscosity and is in agreement with what was noted by Tárrega and Costell (2006) for milk desserts produced with 6g/100g of inulin. This increase in thixotropy can also be attributed to the higher amount of total solids in beverage 2, as shown in Table 2. Taking into consideration the storage times and temperatures evaluated for each beverage, no differences ($p>0.05$) were observed in the hysteresis values.

Microbiological analysis: The counts of the probiotic cultures (*Lactobacillus acidophilus*and *Bifidobacterium animalis* subsp. *lactis*) and of *Streptococcus thermophilus* remained above log 6 CFU/g on day 1 and on day 30 of storage time (Table 4). From the probiotic culture count, it was possible to note that both beverages (1 and 2) were able to be considered as probiotic products. According to Madureira *et al*. (2011), to ensure the health benefits that can be provided by beverages containing probiotics, the standard recommended has been that of a minimum count of viable probiotic cells ranging from 6 to 7 log CFU/g at expiry date. Therefore, the daily minimum therapeutic dose recommended is around 8 to 9 log CFU of viable cells per 100 g of the product for it to cause the desirable effects, provided that the product is regularly consumed (Ng *et al*., 2011). Regarding the count of *Streptococcus thermophilus*, which is a lactic acid bacterium, the results obtained in the present study complies what the Brazilian regulation establishes for fermented lactic beverages. According to Brazilian regulation (Brasil, 2005), the count of this viable lactic acid bacterium should be at least 6 log CFU/g throughout the entire validity period of the beverage. In beverage 2, the inulin content is in agreement with what was recommended by Rao (2001), who indicated a minimal of 5 g of soluble and fermentable fibers per day for the prebiotic effect to be conferred. Allgeyer *et al*. (2010) demonstrated that the addition of 5 g inulin had no impacts on the viability of *Lactobacillus acidophilus*and *Bifidobacterium animalis* subsp. *lactis*

in yogurt throughout 30 days of storage. Moreover, beverage 2 can also be defined as a symbiotic product, because it contains a mixture of probiotics and prebiotics. Prebiotics may aid thesurvival of probiotic bacteria during storage of dairy products, mainly by increasing or, at least, retaining the viability of these bacteria. In the present study, the results showed that inulin at the concentration evaluated influences (p<0.05) on the growth of *Lactobacillus acidophilus*and *Bifidobacterium animalis* subsp. *lactis*, as shown in Table 4. The same behavior was noted by Cunha *et al*. (2009), Fritzen-Freire *et al*. (2010) and Pinto *et al*. (2012) for fermented lactic beverages, Minas Frescal cheese and frozen yogurt, respectively. Donkor *et al*. (2007) also stated that prebiotics could cause a protective effect on selected probiotic bacteria by improving their survival and activity during the storage time of dairy products. One can also interpret that all the three bacteria evaluated (*Lactobacillus acidophilus*, *Bifidobacterium animalis* subsp. *lactis* and *Streptococcus thermophilus*) showed the ability to tolerate the pH and the titratable acidity of both beverages (1 and 2) since they showed high viability in storage times evaluated at 4±1°C. The positive effect on the viability of the probiotic bacteria during refrigerated storage of dairy beverages with cheese whey was cited by Shori (2016). According to these authors, this occurs because the release of sulfur amino acid during thermal treatment of whey may lower the redox potential and thereby cause a positive effect on the survival of probiotics.

The results of this study presented that the use of freeze concentrated whey, the probiotic cultures and the prebiotic inulin in the preparation of fermented lactic beverages could be extremely attractive to the food biotechnology industry. Moreover, this functional dairy product not only can offer the required health benefits to its consumers but is also an alternative to utilize cheese whey, which is an environmental pollutant.

CONCLUSION

The freeze concentration process showed itself to be an alternative possibility for the concentration of cheese whey. The highest result for process efficiency was noted in the first freeze concentration stage. Then, there was a slight reduction followed by a drastic reduction in the second and in the third stages, respectively. Hence, the concentrated whey from the second stage was used in the manufacture of the fermented lactic beverages. The functional beverages 1 and 2 can be classified as probiotic and as symbiotic products, respectively. The addition of inulin and the storage time increased the total solids content, also increasing the total carbohydrates and a decrease in the syneresis index. The addition of inulin did not affect pH value while an increase in the acidity value was noted on day 30 of storage. However, neither the addition of inulin nor the storage time influenced the protein, lipids and ash contents. At the end of the storage time, a lower tendency toward a greenish color was noted for the beverage added with inulin.

The Power Law and Casson models were applied successfully to describe the behavior of the flow of both fermented lactic beverages, which showed a non-Newtonian fluid behavior and characteristics of a shear thinning fluid at both temperatures (4°C and 6°C) at during the storage times evaluated. Moreover, the addition of inulin and the storage time contributed to an increase in apparent viscosity whereas the temperature did not. A thixotropic behavior was verified for both beverages, where the addition of inulin increased the hysteresis.

ACKNOWLEDGMENT

The authors acknowledge financial support from Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Brazil.

REFERENCES

Aider, M. and D. de Halleux, 2008. Production of concentrated cherry and apricot juices by cryoconcentration technology. LWT-Food Sci. Technol., 41(10): 1768-1775.

Aider, M. and D. de Halleux, 2009. Cryoconcentration technology in the bio-food industry: Principles and applications. LWT-Food Sci. Technol., 42(3): 679-685.

Aider, M. and W.B. Ounis, 2012. Skim milk cryoconcentration as affected by the thawing mode: Gravitational vs. microwave-assisted. Int. J. Food Sci. Tech., 47(1): 195-202.

Aider, M., D. de Halleux and A. Akbache, 2007. Whey cryoconcentration and impact on its composition. J. Food Eng., 82(1): 92-102.

Aider, M., D. de Halleux and I. Melnikova, 2009. Skim acidic milk whey cryoconcentration and assessment of its functional properties: Impact of processing conditions. Innov. Food Sci. Emerg., 10(3): 334-341.

Allgeyer, L.C., M.J. Miller and S.Y. Lee, 2010. Sensory and microbiological quality of yogurt drinks with prebiotics and probiotics. J. Dairy Sci., 93(10): 4471-4479.

Almeida, K.E., I.A. Bonassi and R.O. Roça, 2001. Características físicas e químicas de bebidas lácteas fermentadas e preparadas com soro de queijo Minas Frescal. Food Sci. Technol., 21(2): 187-192.

AOAC, 2005. Official Methods of Analysis of AOAC International. 18th Edn., Association Official Analytical Chemists. Gaithersburg, MD, USA.

Boaventura, B.C.B., A.N.N. Murakami, E.S. Prudêncio, M. Maraschin, F.S. Murakami, E.R. Amante and R.D.de M.C. Amboni, 2013. Enhancement of bioactive compounds content and antioxidant activity of aqueous extract of mate (*Ilex paraguariensis* A. St. Hil.) through freeze concentration technology. Food Res. Int., 53(2): 686-692.

Bot, A., U. Erle, R. Vreeker and W.G.M. Agterof, 2004. Influence of crystallisation conditions on the large deformation rheology of inulin gels. Food Hydrocolloid., 18(4): 547-556.

Bourne, M.C., 2002. Food Texture and Viscosity: Concept and Measurement. 2nd Edn., Academic Press, San Diego.

Brasil, 2005. Ministério da Agricultura, Pecuária e Abastecimento. Instrução Normativa n° 16, de 23 de agosto de 2005. Regulamento Técnico de Identidade e Qualidade de Bebida Láctea. Diário Oficial da União, Brasília, Brasil.

Burdo, O.G., E.A. Kovalenko and D.A. Kharenko, 2007. Development of freeze concentration device. Communication of the Odessa State Academy of Food Technologies, Odessa, Ukraine.

Calvo, C., 2004. Optical Properties. In: Nollet, L.M.L., (Eds.), Handbook of Food Analysis. Physical Characterization and Nutrient Analysis. 2nd Edn., Marcel Dekker, New York.

Casarotti, S.N., D.A. Monteiro, M.M.S. Moretti and A.L.B. Penna, 2014. Influence of the combination of probiotic cultures during fermentation and storage of fermented milk. Food Res. Int., 59: 67-75.

Chabarov, A. and M. Aider, 2014. Mathematical modeling and experimental validation of the mass transfer during unidirectional progressive cryoconcentration of skim milk. Innov. Food Sci. Emerg., 21: 151-159.

Chaito, C., K. Judprasong and P. Puwastien, 2016. Inulin content of fortified food products in Thailand. Food Chem., 193: 102-105.

Crispín-Isidro, G., C. Lobato-Calleros, H. Espinosa-Andrews, J. Alvarez-Ramirez and E.J. Vernon-Carter, 2015. Effect of inulin and agave fructans addition on the rheological, microstructural and sensory properties of reduced-fat stirred yogurt. LWT-Food Sci. Technol., 62(1): 438-444.

Cunha, T.M., F.P. de Castro, P.L.M. Barreto, H.D. Benedet and E.S. Prudêncio, 2008. Avaliação físico-química, microbiológica e reológica de bebida láctea e leite fermentado adicionados de probióticos. Semin: Cienc. Agrar., 29(1): 103-116.

Cunha, T.M., E.C. Ilha, R.D.M.C., Amboni, P.L. Barreto, F.P. Castro and E.S. Prudêncio, 2009. The influence of whey and probiotic bacteria on the properties of fermented lactic beverages. Braz. J. Food Technol., 12: 23-33.

Da Silveira, E.O., J.H.L. Neto, L.A. da Silva, A.E.S. Raposo, M. Magnani and H.R. Cardarelli, 2015. The effects of inulin combined with oligofructose and goat cheese whey on the physicochemical properties and sensory acceptance of a probiotic chocolate goat dairy beverage. LWT-Food Sci. Technol., 62(1): 445-451.

De Castro, F.P., T.M. Cunha, P.J. Ogliari, R.F. Teófilo, M.M. Ferreira and E.S. Prudêncio, 2009. Influence of different content of cheese whey and oligofructose on the properties of fermented lactic beverages: Study using response surface methodology. LWT-Food Sci. Technol., 42(5): 993-997.

De Castro, F.P., T.M. Cunha, P.L. Barreto, R.D.M.C. Amboni and E.S. Prudêncio, 2008. Effect of oligofructose incorporation on the properties of fermented probiotic lactic beverages. Int. J. Dairy Technol., 62(1): 68-74.

Debon, J., E.S. Prudêncio and J.C. Cunha Petrus, 2010. Rheological and physico-chemical characterization of prebiotic microfiltered fermented milk. J. Food Eng., 99(2): 128-135.

Debon, J., E.S. Prudencio, J.C.C. Petrus, C.B. Fritzen-Freire, C.M.O. Müller, R.D.M.C. Amboni and C.R. Werneck Vieira, 2012. Storage stability of prebiotic fermented milk obtained from permeate resulting of the microfiltration process. LWT-Food Sci. Technol., 47(1): 96-102.

Donkor, O.N., S.L.I. Nilmini, P. Stolic, T. Vasiljevic and N.P. Shah, 2007. Survival and activity of selected probiotic organisms in set-type yoghurt during cold storage. Int. Dairy J., 17(6): 657-665.

FAO/WHO, 2006. Probiotics in food: Health and nutritional properties and guidelines for evaluation. FAO Food and Nutrition Paper 85, Food and Agriculture Organization of the United Nations and World Health Organization, Rome, pp: 413-426.

Fritzen-Freire, C.B., C.M.O. Müller, J.B. Laurindo and E.S. Prudêncio, 2010. The influence of *Bifidobacterium* Bb-12 and lactic acid incorporation on the properties of Minas Frescal cheese. J. Food Eng., 96(4): 621-627.

Gallardo-Escamilla, F.J., A.L. Kelly and C.M. Delahunty, 2007. Mouthfeel and flavour of fermented whey with added hydrocolloids. Int. Dairy J., 17(4): 308-315.

Gomes, J.J.L., A.M. Duarte, A.S.M. Batista, R.M.F. de Figueiredo, E.P. de Sousa, E.L. de Sousa *et al*., 2013. Physicochemical and sensory properties of fermented dairy beverages made with goat's milk, cow's milk and a mixture of the two milks. LWT-Food Sci. Technol., 54(1): 18-24.

González-Tomás, L., S. Bayarri and E. Costell, 2009. Inulin-enriched dairy desserts: Physicochemical and sensory aspects. J. Dairy Sci., 92(9): 4188-4199.

Guggisberg, D., J. Cuthbert-Steven, P. Piccinali, U. Bütikofer and P. Eberhard, 2009. Rheological, microstructural and sensory characterization of low-fat and whole milk set yoghurt as influenced by inulin addition. Int. Dairy J., 19(2): 107-115.

Guven, M., K. Yasar, O.B. Karaca and A.A. Hayaloglu, 2005. The effect of inulin as a fat replacer on the quality of set-type low fat yogurt manufacture. Int. J. Dairy Technol., 58(3): 180-184.

Hernández, M.J., 1996. Caracterización reológica de hidrogeles de MCCNaCMC + almidón. Tixotropía y sinergismo. Ph.D. Thesis, Universitat de València, Valencia, Spain.

IAL(Instituto Adolfo Lutz), 2008. Métodos físico-químicos para análise de alimentos. 4th Edn., Normas Analíticas do Instituto Adolfo Lutz. São Paulo, Brasil, pp: 1020.

International Dairy Federation (IDF), 1997. Milk and Milk Products-Yogurt-Enumeration of characteristic microorganisms-Colony-count technique at 37 degrees C (*Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus*) are present and viable. IDF Standard 117/B, Brussels, Belgium.

Kailasapathy, K., 2006. Survival of free and encapsulated probiotic bacteria and their effect on the sensory properties of yoghurt. LWT-Food Sci. Technol., 39(10): 1221-1227.

Kailasapathy, K. and K. Sultana, 2003. Survival and [beta]-D-galactosidase activity of encapsulated and free Lactobacillus acidophilus and Bifidobacterium lactis in ice-cream. Aust. J. Dairy Technol., 58(3): 223-227.

Karimi, R., M.H. Azizi, M. Ghasemlou and M. Vaziri, 2015. Application of inulin in cheese as prebiotic, fat replacer and texturizer: A review. Carbohyd. Polym., 119: 85-100.

Ladjevardi, Z.S., S.M.T. Gharibzahedi and M. Mousavi, 2015. Development of a stable low-fat yogurt gel using functionality of psyllium (*Plantago ovata* Forsk) husk gum. Carbohyd. Polym., 125: 272-280.

Madureira, A.R., A.I. Pintado, A.M. Gomes, M.E. Pintado and F.X. Malcata, 2011. Rheological, textural and microstructural features of probiotic whey cheeses. LWT-Food Sci. Technol., 44(1): 75-81.

Masoud, W. and M. Jakobsen, 2003. Surface ripened cheeses: The effects of *Debaryomyces hansenii*, NaCl and pH on the intensity of pigmentation produced by *Brevibacterium linens* and *Corynebacterium flavescens*. Int. Dairy J., 13(2-3): 231-237.

Meyer, D., S. Bayari, A. Tárrega and E. Costell, 2011. Inulin as texture modifier in dairy products. Food Hydrocolloid., 25(8): 1881-1890.

Miyawaki, O., M. Gunathilak, C. Omote, T. Koyanagi, T. Sasaki, H. Take, A. Matsuda, K. Ishisaki, S. Miwa and S. Kitano, 2016. Progressive freeze-concentration of apple juice and its application to produce a new type apple wine. J. Food Eng., 171: 153-158.

Mollea, C., L. Marmo and F. Bosco, 2013. Valorisation of Cheese Whey, a Byproduct from the Dairy Industry. In: Muzzalupo, I. (Ed.), Food Industry, Rijeka, Croatia: InTech, pp: 549-588.

Modler, H.W. and M. Kalab, 1983. Microstructure of yogurt stabilized with milk proteins. J. Dairy Sci., 66(3): 430-437.

Ng, E.W., M. Yeung and P.S. Tong, 2011. Effects of yogurt starter cultures on the survival of Lactobacillus acidophilus. Int. J. Food Microbiol., 145(1): 169-175.

Okawa, S., T. Ito and A. Saito, 2009. Effect of crystal orientation on freeze concentration of solutions. Int. J. Refrig., 32(2): 246-252.

Okpala, C.O.R., J.R. Piggott and C.J. Schaschke, 2010. Influence of high-pressure processing (HPP) on physico-chemical properties of fresh cheese. Innov. Food Sci. Emerg. Technol., 11(1): 61-67.

Oliveira, M.N., I. Sodini, R. Remeuf, J.P. Tissier and G. Corrieu, 2002. Manufacture of fermented lactic beverages containing probiotic cultures. J. Food Sci., 67(6): 2336-2341.

Paseephol, T. and F. Sherkat, 2009. Probiotic stability of yoghurts containing Jerusalem artichoke inulins during refrigerated storage. J. Funct. Foods., 1(3): 311-318.

Penna, A.L.B., S. Gurram and G.V. Barbosa-Cánovas, 2006. Effect of high hydrostatic pressure processing on rheological and textural properties of probiotic low-fat yogurt fermented by different starter cultures. J. Food Process. Eng., 29(5): 447-461.

Petzold, G., J. Moreno, P. Lastra, K. Rojas and P. Orellana, 2015. Block freeze concentration assisted by centrifugation applied to blueberry and pineapple juices. Innov. Food Sci. Emerg. Technol., 30: 192-197.

Pinto, S.S., C.B. Fritzen-Freire, I.B. Muñoz, P.L. Barreto, E.S. Prudêncio and R.D.M.C. Amboni, 2012. Effects of the addition of microencapsulated *Bifidobacterium* BB-12 on the properties of frozen yogurt. J. Food Eng., 111(4): 563-569.

Prudêncio, E.S., C.M.O. Müller, C.B. Fritzen-Freire, R.D.M.C. Amboni and J.C. Cunha Petrus, 2014. Effect of whey nanofiltration process combined with diafiltration on the rheological and physicochemical properties of ricotta cheese. Food Res. Int., 56: 92-99.

Ramírez-Sucre, M.O. and J.F. Vélez-Ruiz, 2013. Physicochemical, rheological and stability characterization of a caramel flavored yogurt. LWT- Food Sci. Technol., 51(1): 233-241.

Rao, V.A., 2001. The prebiotic properties of oligofructose at low intake levels. Nutr. Res., 21(6): 843-848.

Resa, P., T. Bolumar, L. Elvira, G. Pérez and F.M. Espinosa, 2007. Monitoring of lactic acid fermentation in culture broth using ultrasonic velocity. J. Food Eng., 78(3): 1083-1091.

Rinaldoni, A.N., M.E. Campderrós and A.P. Padilla, 2012. Physico-chemical and sensory properties of yogurt from ultrafiltreted soy milk concentrate added with inulin. LWT-Food Sci. Technol., 45(2): 142-147.

Roberfroid, M., G.R. Gibson, L. Hoyles, A.L. McCartney, R. Rastall, I. Rowland, D. Wolvers *et al*., 2010. Prebiotic effects: Metabolic and health benefits. Br. J. Nutr., 104(Suppl 2): S1-S63.

Rozycki, S.D., M.P. Buera, A.M. Piagentini, S.C. Costa and M.S. Pauletti, 2010. Advances in the study of the kinetics of color and fluorescence development in concentrated milk systems. J. Food Eng., 101(1): 59-66.

Saint-Eve, A., C. Lévy, M. Le Moigne, V. Ducruet and I. Souchon, 2008. Quality changes in yogurt during storage in different packaging materials. Food Chem., 110(2): 285-293.

Samsuri, S., N.A. Amran and M. Jusoh, 2015. Spiral finned crystallizer for progressive freeze concentration process. Chem. Eng. Res. Des., 104: 280-286.

Sánchez, J., E. Hernandéz, J.M. Auleda and M. Raventós, 2011. Review: Freeze concentration technology applied to dairy products. Food Sci. Technol. Int., 17(1): 5-13.

Shori, A.B., 2016. Influence of food matrix on the viability of probiotic bacteria: A review based on dairy and non-dairy beverages. Food Biosci., 13: 1-8.

Souza, C.H.B. and S.M.I. Saad, 2009. Viability of *Lactobacillus acidophilus* La-5 added solely or in co-culture with a yoghurt starter culture and implications on physico-chemical and related properties of Minas fresh cheese during storage. LWT-Food Sci. Technol., 42(2): 633-640.

Staffolo, M.D., N. Bertola, M. Martino and A. Bevilacqua, 2004. Influence of dietary fiber addition on sensory and rheological properties of yogurt. Int. Dairy J., 14(3): 263-268.

Tárrega, A. and E. Costell, 2006. Effect of composition on the rheological behaviour and sensory properties of semisolid dairy dessert. Food Hydrocolloid., 20(6): 914-922.

Toneli, J.T.C.L., F.E.X. Mürr and K.J. Park, 2005. Estudo da reologia de polissacarídeos utilizados na indústria de alimentos. Rev. Bras. Prod. Agroind., 7(2): 181-204.

Tripathi, M.K. and S.K. Giri, 2014. Probiotic functional foods: Survival of probiotics during processing and storage. J. Funct. Foods., 9: 225-241.

Van Oosten-Manski, J., S. Hewitt, L. Rutherford and M. Campbell, 2009. Rheology and sensory properties of stirred yoghurts. Proceeding of the 5th International Symposium on Food Rheology and Structure. Zurich, Switzerland, Jun, 15-18, pp: 388-391.

Vargas, M., M. Cháfer, A. Albors, A. Chiralt and C. González-Martínez, 2008. Physicochemical and sensory characteristics of yoghurt produced from mixtures of cows' and goats' milk. Int. Dairy J.,18(12): 1146-1152.

Vinderola, C.G. and J.A. Reinheimer, 2000. Enumeration of *Lactobacillus casei* in the presence of *L. acidophilus*, bifidobacteria and lactic starter bacteria in fermented dairy products. Int. Dairy J., 10(4): 271-275.

Wang, H.Y., H. Qian and W.R. Yao, 2011. Melanoidins produced by the Maillard reaction: Structure and biological activity. Food Chem., 128(3): 573-584.

Yadav, J.S.S., S. Yan, S. Pilli, L. Kumar, R.D. Tyagi and R.Y. Surampalli, 2015. Cheese whey: A potential resource to transform into bioprotein, functional/nutritional proteins and bioactive peptides. Biotechnol. Adv., 33(6): 756-774.