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**Research Article**

# Potential of Milk Freeze Concentration for the Production of Functional Fresh Cheeses

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**Abstract:** The aim of this study was to evaluate the milk freeze concentration process, choosing the best concentrated milk for the manufacture of functional fresh cheeses. These cheeses were evaluated in relation to their chemical, physical, microstructural and/or microbiological properties. Since that the second stage concentrated from the milk freeze concentration process showed the highest total solids content mass transference, it was used for the manufacture of the functional fresh cheeses. Therefore, *Bifidobacterium* BB-12 addition was notable to modify the cheeses properties. However, as expected the fresh cheese with inulin addition showed the highest total solids content, reflecting on the increase of total carbohydrates content and on the tendency toward a green color. Inulin addition and whey presence contributed to the firmness increase, resulting in a less breakable fresh cheese and thus, with a less fragile and more compact structure. For the fresh cheese with bifidobacteria, the viable cells count was above than those number recommended for a product to be classified as probiotic. *Bifidobacterium* BB-12 and inulin addition resulted in a symbiotic fresh cheese.

**Keywords:** Freeze concentration, fresh cheese, prebiotic, probiotic, symbiotic

## INTRODUCTION

From the food industry's point of view, the freeze concentration process is a very suitable technology because is able to retain the nutritional quality of liquid foods. As stated by Petzold *et al*. (2015), the industrial future of freeze concentration process has been associated with the configuration of one-step systems, such as block freeze concentration. According to these authors, the basis of block freeze concentration is as follows: a food liquid solution is completely frozen, the whole frozen solution is thawed and then the concentrated fraction is separated from the ice. The use of freeze concentration to replace the traditional technologies that use heat to concentrate liquid foods, such as milk, can reduce the loss of compounds (Aider and de Halleux, 2009). This feature is attributed to the low temperatures used in freeze concentration, thereby preventing the degradation of components, such as heat sensitive proteins. Thus, the use of freeze concentration in milk would make it possible to obtain products with high nutritional quality and prevent undesirable physical and chemical changes. Aider and Ounis (2012) described that a traditional concentration procedure can be carried out in multistage evaporators at a temperature around 75°C in the first section of the evaporator. However, it has been established that heating milk at 70°C can cause irreversible aggregation of heat sensitive proteins (Aider and Ounis, 2012). Among the processes employed by the industry to concentrate milk compounds, membrane technologies, such as ultrafiltration, are quite prominent. Despite these processes using temperatures capable of preserving milk constituents, they require the use of quality water for the cleaning step and it is often necessary to replace the membranes due to fouling (Chabarov and Aider, 2014).

With respect to the freeze concentration process, Aider and Halleux (2009) and Chabarov and Aider (2014) point out that it is still necessary to know the behavior of the raw material that is to be subjected to this technology, providing the necessary conditions for its application. Considering that milk consists of approximately 88% of water, many processes involved in its transformation into its derivatives could be improved if it were firstly concentrated (Abd El-Gawad and Ahmed, 2011).

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Although Karimi *et al*. (2012) have produced a fresh cheese from ultrafiltered milk and claimed its functional properties, studies on fresh cheese made from concentrated milk by freeze concentration processes are non-existent. It should be emphasized that foods with claims of functional properties are all those foods or ingredients that, in addition to the basic nutritional functions, when consumed as part of the usual diet, produces beneficial health effects. In the past few decades, this concept has also applicable to foods containing probiotics and/or prebiotics (Kip *et al*., 2006).

Probiotics are live microorganisms which when administered in appropriate amounts confer benefit to host health (FAO/WHO, 2006), improving the balance of the host's intestinal microbiota and defense against pathogens (Saad *et al*., 2013). Meanwhile, prebiotics can be defined as non-digestible food ingredients, which are also consumed regularly and in suitable amounts, benefit the host by the selectively stimulate proliferation and/or activity of probiotic bacteria (Gibson and Roberfroid, 1995).

Kask *et al*. (2003) reported that the survival of probiotic bacteria in products such as fresh cheese is higher when compared to matured cheeses, which are stored for a longer time because they have high sodium chloride content, lower moisture content and consequently, lower water activity. Thus, these conditions are limiting for probiotic bacteria survival. It is noteworthy that the viability and the maintenance of adequate amounts of probiotic bacteria in fresh cheeses, such as from genera *Lactobacillus* and *Bifidobacterium*, have been observed by Buriti *et al*. (2005), Fritzen-Freire *et al*. (2010a, 2010b), Souza and Saad (2009) and Verruck *et al*. (2015a, 2015b). Among the prebiotics, inulin is one of the most used in foods (Kolida *et al*., 2002; Shoaib *et al*., 2016), offering resistance to digestive enzymes, to remain intact until reaching the intestine to be fermented by bifidobacteria (Bouhnik *et al*., 2007; Roberfroid, 2007). The synergistic potential between probiotics and prebiotics is defined as a symbiosis, being able to further benefit the regular consumer of this type of product (Casiraghi *et al*., 2007).

Karami *et al*. (2009) and Ferrão *et al*. (2016) reported that when the processing of a cheese is modified and microorganisms and/or others ingredients are added, its properties can be altered. Similarly to most solid foods, cheese exhibitboth elastic solid and viscous fluid properties (Messens *et al*., 2000). In the specific case of cheese, these properties depend on its composition, microstructure, the arrangement of components in the protein matrix (Everett and Auty, 2008) and the physical-chemical state of its components, as well as its pH and acidity value (Lucey *et al*., 2003). Taking into account that there is still a significant lack of information in the scientific literature on the milk freeze concentration process and its applications in the development of dairy products, the aim of this study werefirst to evaluate the milk freeze concentration process. Therefore, the best concentrated milk from freeze concentration process was chosen to the manufacture of functional fresh cheeses, which were investigated for their chemical, physical, microstructural and microbiological properties.

## MATERIAL AND METHODS

**Material:** Commercial pasteurized milk (3 g lipid 100 $g^{-1}$, Tirol, TrezeTílias, Brazil), lactic acid (PuracSíntesys, Rio de Janeiro, Brazil), calcium chloride (Vetec, Rio de Janeiro, Brazil), probiotic culture composed of *Bifidobacterium animalis* ssp. lactis (BB-12®, Chr. Hansen, Hónsholm, Denmark), prebiotic composed by inulin (Orafti® HPX, Orafti, Tienen, Belgium) with degree of polymerization (DP) ≥ 23 and commercial rennet with a chymosin produced by *Aspergillus niger var. awamorii* (Ha La®, Chr. Hansen, Valinhos, Brazil) were used in the manufacture of fresh cheeses. MRS Agar (Difco, Sparks, USA), sodium propionate (Fluka, Neu-Ulm, Germany), lithium chloride (Vetec, Rio de Janeiro, Brazil) and AnaeroGen® (Oxoid, Hampshire, UK) were used for the microbiological analysis. All reagents were of analytical grade.

**Protocol of the milk freeze concentration:** 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 Milk (CM) and ice (I) (Fig. 1). Initially, a volume of 30 L of commercial pasteurized milk was separated into batches of 1 L.Each 1 L was fractionated in plastic pots of 30 mL and frozen at -40±2°C in a plate freezer (Frigostrella, Cotia, São Paulo, Brazil). Once the pasteurized milk was frozen, 50% of the initial volume was defrosted at 20±2°C. The defrosted liquid constituted the CM1, 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 (CM and I) remaining from freeze concentration stages was stored at -20±2°C until chemical analysis.

At each freeze concentration stage, the concentration factor (CF) was calculated as a function of the increase of total solids content, according to the methodology proposed by Aider and Ounis (2012), using the following Eq. (1):

$$CF(\%) = \frac{TS_i}{TS_0} x100 \quad (1)$$

where, $TS_i$ is the total solids content (g 100 $g^{-1}$) of the concentrated milk in each freeze concentration stage and $TS_0$ is the total solids content (g 100 $g^{-1}$) of the initial pasteurized milk.

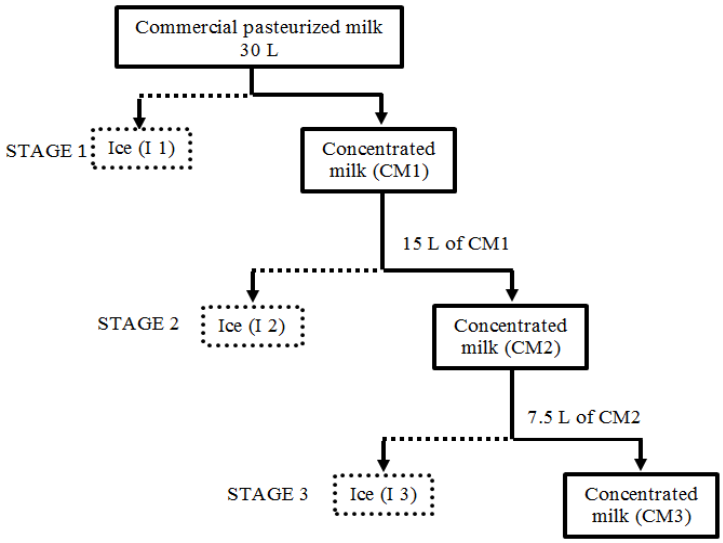


Fig. 1: Diagram of pasteurized milk block freeze concentration process

**Validation of results:** As recommended by Petzold *et al*. (2016), to validate the obtained experimental results, a mass balance of each freeze concentration stage was made and compared to the theoretical value. Therefore, the mass balance was calculated for each freeze concentration stage from the results obtained for the total solids content of milk, concentrated milk (CM) and ices (I). The mass balance of the first stage of freeze concentration (CM1) was calculated in relation to total solids content of the pasteurized milk, the second stage (CM2) in relation to total solids content of CM1 and the third stage (CM3) in relation to total solids content of CM2.

The concentrated milk used in the manufacture of the fresh cheese was chosen from the evaluation of the results obtained for CF and mass balance.

**Manufacture of the fresh cheese:** Three different fresh kinds of cheese formulations, denoted as Control, C1 and C2, were manufactured according to the procedures of Jacobsen Neto (2006), with modifications. The Control was produced without the addition of probiotic culture and inulin. The C1 fresh cheese was produced with the addition of *Bifidobacterium* BB-12 and no addition of inulin. The C2 fresh cheese was prepared with the addition of *Bifidobacterium* BB-12 and 5 g 100 $mL^{-1}$ of inulin, as suggested by Solowiej *et al*. (2015). Each fresh cheese formulation was produced in 2.5 L vat from Concentrated Milk (CM), with or without inulin, heated to 37±1°C. The culture employed was a commercial freeze-dried culture for direct vat inoculation and was added at 0.20 g/L of concentrated milk. Lactic acid (0.225 g/L of concentrated milk), calcium chloride (0.225 g/L of concentrated milk) and commercial rennet with a ratio of 1:3000 (0.9 mL/L of concentrated milk) were added to the fresh cheese (in all the tree formulations) with posterior coagulation period at 37±1°C for 40 min. After coagulation, the fresh cheeses (Control, C1 and C2) were thermo-sealed (Sulplack SPO-150, Caxias do Sul, Rio Grande do Sul, Brazil) with a lid multilayer aluminum and polyethylene and stored at 5±1°C. Each fresh cheese (Control, C1 and C2) production was performed in triplicate.

**Physicochemical analysis:** The pasteurized milk, the Concentrated Milks (CM), the ices (I) and the fresh cheeses (Control, C1 and C2) were analyzed for total solids content (g 100 $g^{-1}$), by the drying of the samples until reaching constant weight at 105°C as described by the Analytical Norms of Adolfo Lutz Institute (Instituto Adolfo Lutz, 2008). The fresh cheeses were also analyzed for moisture (g 100 $g^{-1}$), protein (g 100 $g^{-1}$) by the Kjeldahl method (N x 6.38) (Horwitz, 2005) and lipid content (g 100 $g^{-1}$) by the extraction of lipids with petroleum ether, using the Soxhlet device after denaturation of proteins with hydrochloric acid (Instituto Adolfo Lutz, 2008). The total carbohydrate content (g 100 $g^{-1}$) of the fresh cheeses was calculated by difference, according to Horwitz (2005). The titratable acidity (g 100 $g^{-1}$ lactic acid) of fresh cheeses was determined according to the Analytical Norms of the Adolfo Lutz Institute (Instituto Adolfo Lutz, 2008), while the measurements of pH were carried out with a pH meter (PHS-3 BW, BEL, Piracicaba, São Paulo, Brazil). All analyses were carried out in triplicate.

The color analysis of the fresh cheeses (Control, C1 and C2) was 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 $L^*$, $a^*$ and $b^*$ parameters were used the CIELab color scale. The $L^*$ parameter ranges 0 to 100 and indicates luminosity (variation from black to white), the $a^*$ axis shows the variation from red (+$a^*$) to green (-$a^*$) and the $b^*$ axis is the variation from yellow (+$b^*$) to blue (-$b^*$) (Verruck *et al*., 2015a).

The value of Hue angle ($h^*$) and Chroma ($C^*$) were determined using Eq. (2) and Eq. (3), respectively (Masoud and Jakobsen, 2003). All analyses were carried out in triplicate.

$$h^* = tan^{-1}\, a^*/b^* \quad (2)$$

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

**Physical properties:** The texture analysis, uniaxial compression and dynamic whey release of the fresh cheeses samples (Control, C1 and C2) were carried out using the TA-XT *plus* texture analyzer (Stable Micro Systems Ltd, Surrey, UK), fitted with a 50 kg load cell and a 25 mm diameter aluminum probe. The samples were prepared by removing cylindrical pieces from the fresh cheeses (19 mm diameter; 20 mm height) which were then kept under refrigeration (5±1°C) until testing, without the addition of any lubricants. The data were obtained using the Texture Exponent 32 software version 4.0.13.0 (Stable Micro Systems Ltd, Surrey, UK).

**Texture analysis:** The cylindrical fresh cheese samples were compressed with a test speed of 1.0 mm/s and distance of 10.0 mm, according to the methodology described by Buriti *et al*. (2008). The force data as a function of time were obtained for the two compression-decompression cycles. The following parameters were obtained: firmness (N), cohesiveness, adhesiveness (N.s) and elasticity. All the measurements were carried out five times.

**Uniaxial compression:** The cylindrical fresh cheese samples were compressed to 50% of their height at a cross-head speed of 1 mm/s. The stress ($\sigma$) was calculated through Eq. (4), as proposed by Calzada and Peleg (1978):

$$\sigma_{(t)} = \frac{F_{(t)}}{A_{(t)}} \quad (4)$$

where,
$\sigma_{(t)}$ = The stress at the time (t)
$F_{(t)}$ = The force at the time (t)
$A_{(t)}$ = The area at the time (t)

The strain ($\varepsilon$) was also calculated in accordance with Calzada and Peleg (1978), as shown in Eq. (5):

$$\varepsilon = \ln \frac{H_0}{H - \Delta H} \quad (5)$$

where,
$\varepsilon$ = The strain
$H_0$ = The original height
$\Delta H$ = The change in height

The young module was also calculated through the fracture stress ($\sigma$) and the fracture strain ($\varepsilon$) parameters to determine the rigidity of the fresh cheeses.

The fracture stress ($\sigma$) and the fracture strain ($\varepsilon$) parameters were derived in relation to the fracture point, which is defined as the local of maximum stress/strain of the curve (Wium and Qvist, 1997).

**Dynamic whey release:** In order to evaluate the expulsion of whey of the fresh cheeses samples during the compression tests, a circular sheet paper (Canson, grammage 300 g $m^2$, Brazil) with 90 $cm^2$ of area and 1 mm thickness was used as the bottom of the samples holder in the texturometer. Before this analysis, each sheet paper was placed in an oven at 105±5°C until constant weight. After drying, the sheet papers were cooled for 45 min in a desiccator and weighed. The cylindrical fresh cheese samples were placed on the sheet paper and compressed to 50% of their height at a cross-head speed of 1 mm/s. After compression, the sheet paper was reweighed to determine the whey mass (g) released. The results were expressed as apercentage of whey released during compression in relation to total mass (g) of the test sample. This determination was carried out in triplicate.

**Scanning Electron Microscopy (SEM):** Scanning Electron Microscopy (SEM) micrographs of the fresh cheese samples were obtained using a Jeol JSM 6390 LV scanning electron microscope (Tokyo, Japan), according to Lobato-Calleros *et al*. (2007), with modifications. The samples were frozen in a plate freezer (Frigostrella PF-5, São Paulo, Brazil) and dried in a freeze dryerTerroni (LD 3000, São Carlos, Brazil). The samples were fractured perpendicular to their long axis, mounted on individual stubs with fracture face upwards and coated with a fine gold layer using a Leica EM SCD 500 sputter coater (Wetzlar, Germany). All the samples were examined using an accelerating voltage of 8 kV at a magnification of 800x (20 μm) and 1000x (10 μm).

**Microbiological analysis:** The viability of the probiotic culture (*Bifidobacterium* BB-12) was evaluated in the probiotic fresh cheeses, i.e., C1 and C2 samples. For such evaluation, 25 g portions of fresh cheese samples were collected aseptically from the center and the surface of one of these cheeses. These portions (25 g) were blended with 225 mL of 0.1 g 100 $g^{-1}$ peptone water in a Bag Mixer 400 (Interscience, t. Nom, France) and submitted to serial dilution with the same diluent. According to Vinderola and Reinheimer (2000), for the

enumeration of probiotic culture, MRS agar modified with the addition of 0.2 g 100 $g^{-1}$ of lithium chloride and 0.3 g 100 $g^{-1}$ of sodium propionate was used. The plates were incubated in anaerobic jars containing Anaerogen® at 37±1°C for 72 h. After the incubation period, the count of probiotic viable cells was carried out and expressed as log colony-forming units per gram of fresh cheese (log CFU/g). All the analyses were performed in triplicate.

**Statistical analysis:** The significance of the differences between the means of the samples was determined by one-way Analysis of Variance (ANOVA) followed by Tukey's test. Linear correlation from regression analysis was performed to verify the relationship between the analyses. Differences were considered statistically significant when $p<0.05$. All statistical analyses were performed using the software STATISTICA version 7.0 (StatSoft Inc., Tulsa, OK, USA).

## RESULTS AND DISCUSSION

**Freeze concentration of milk:** The results for total solids content, Concentration Factor (CF) and mass balance of milk freeze concentration are shown in Table 1. When compared to pasteurized milk, all the CM samples showed an increase ($p< 0.05$) in total solids contents, which were also higher ($p<0.05$) in the I2 and I3 fractions. Besides, this content was higher ($p<0.05$) for the I3 fraction than for the pasteurized milk. According to Aider and Ounis (2012), the retention of total solids in the ice fraction is due to the increase insolution viscosity and the concentration of the recovered solution depends on the viscosity of the concentrate in any freeze concentration method. Raventós *et al*. (2007) reported that the ability to separate the high-purity ice fraction from the concentrate is inversely proportional to the solution viscosity. Blanquet *et al*. (2005) also stated that when the viscosity increases, the ability to obtain pure ice crystals decreases, thus interfering in the crystallization phenomenon. Okawa *et al*. (2009) observed that the rate of the solute concentration captured in ice and the solute present in the initial solution may vary from 1/10 to 1/250, depending on the orientation of the ice crystals. These authors also noted that the orientation of the crystals is another important factor to consider in the disposition of the solid ice fraction. Another reason for the high retention of solids in these ice fractions could be attributed to the type of freezing that was used. It is important to note that in the present work the milk was frozen through a rapid method, using a plate freezer. Robles *et al*. (2016) noted that when a higher freezing temperature is applied, such as in slow freezing, during block freeze concentration a higher number of circular crystals of larger sizes and less tortuous channels are formed. These were the conditions under which the best solute recovery was obtained. Samsuri *et al*. (2015) also stated that large ice crystals contain fewer impurities and lower solids content than the smaller crystals. Thus, the rapid freezing generated by the plate freezer might also have caused the high retention of solids in the ice fraction analyzed in the three stages of milk freeze concentration.

It was possible to note that the CF values increased ($p<0.05$) throughout the freeze concentration stages and this behavior could be related to the increase in total solids content. A similar behavior was observed by Aider *et al*. (2007) and Aider and Ounis (2012) in freeze concentration of cheese whey and skim milk, respectively. Finally, the mass transfer phenomena confirmed the experimental results obtained. Based on these results, the concentrated milk from the second concentration stage (CM2) was used in the manufacture of all the samples of the fresh cheese.

**Physicochemical analysis:** The results for the physicochemical composition of the three fresh cheese samples are shown in Table 2. The addition of inulin in sample C2 contributed to the increase ($p<0.05$) in total solids content and, thus, to the decrease in moisture content. This behavior is due to the increase ($p<0.05$) in

Table 1: Total solids content of the commercial pasteurized milk, Concentrated Milk (CM) and ice (I) samples at each freeze concentration stage (1, 2, 3); and the Concentration Factor (CF) and mass transfer in relation to the total solids content at each freeze concentration stage (1, 2, 3)

| Samples | Total solids (g 100 $g^{-1}$) | CF (%) | Mass transfer (%) |
|---|---|---|---|
| Milk | $10.98\pm0.01^{Dc}$ | - | - |
| CM1 | $14.68\pm0.05^{C3}$ | $134\pm1^{\blacklozenge}$ | 64 |
| I1 | $4.62\pm0.01^{d}$ | | 21 |
| CM2 | $19.17\pm0.06^{B2}$ | $175\pm1^{\dagger}$ | 68 |
| I2 | $12.42\pm0.05^{b}$ | | 44 |
| CM3 | $21.92\pm0.64^{A1}$ | $200\pm6^{*}$ | 57 |
| I3 | $17.56\pm0.27^{a}$ | | 46 |

Results are expressed as a mean±standard deviation, among three batches carried out in triplicate for each freeze concentration stage, with three repetitions for total solids content and CF; $^{A-D}$ Within a column, different superscript uppercase letters denote significant differences ($p<0.05$) between the commercial pasteurized milk and the CM of each freeze concentration stage; $^{a-d}$ Within a column, different superscript lowercase letters denote significant differences ($p< 0.05$) between the commercial pasteurized milk and the I of each freeze concentration stage; $^{1-3}$Within a column, different superscript numbers denote significant differences ($p<0.05$) between the CM of each freeze concentration stage; Different symbols indicate significant differences ($p<0.05$) in the CF of each freeze concentration stage

Table 2: Physicochemical and color parameters (*L*, a*, b*, C*, h**) of fresh cheese samples denoted Control, without *Bifidobacterium* BB-12 and inulinaddition; C1, only with *Bifidobacterium* BB-12 addition; and C2, with *Bifidobacterium* BB-12 and 5 g of inulin per 100 mL of milk

| | Samples | | |
|---|---|---|---|
| Parameters | Control | C1 | C2 |
| Total solids (g 100 $g^{-1}$) | 20.64±0.26$^{b}$ | 20.31±0.37$^{b}$ | 24.30±0.02$^{a}$ |
| Moisture (g 100 $g^{-1}$) | 79.36±0.26$^{a}$ | 79.69±0.37$^{a}$ | 75.70±0.20$^{b}$ |
| Protein (g 100 $g^{-1}$) | 6.53±0.19$^{a}$ | 6.50±0.28$^{a}$ | 6.31±0.25$^{a}$ |
| Lipid (g 100 $g^{-1}$) | 5.33±0.47$^{a}$ | 5.46±0.28$^{a}$ | 5.40±0.25$^{a}$ |
| Total carbohydrates (g 100 $g^{-1}$) | 6.41±0.56$^{b}$ | 6.03±0.39$^{b}$ | 10.35±0.49$^{a}$ |
| pH | 6.25±0.04$^{a}$ | 6.31±0.03$^{a}$ | 6.29±0.01$^{a}$ |
| Acidity (g lactic acid 100 $g^{-1}$) | 0.21±0.02$^{a}$ | 0.21±0.01$^{a}$ | 0.20±0.01$^{a}$ |
| *L** | 85.04±1.13$^{a}$ | 85.32±0.41$^{a}$ | 85.71±0.51$^{a}$ |
| *a** | -4.23±0.08$^{b}$ | -4.20±0.06$^{b}$ | -3.89±0.06$^{a}$ |
| *b** | 21.66±0.16$^{a}$ | 21.68±0.23$^{a}$ | 20.66±0.14$^{b}$ |
| *C** | 22.07±0.16$^{a}$ | 22.08±0.23$^{a}$ | 21.02±0.13$^{b}$ |
| *h** | 78.95±0.17$^{a,b}$ | 79.05±0.05$^{b}$ | 79.34±0.23$^{a}$ |

Results are expressed as a mean±standard deviation, among three batches carried out in triplicate for each type of fresh cheese, with three repetitions for physicochemical composition; and five repetitions for the color parameters; $^{a–c}$ Within a row, different superscript lowercase letters denote significant differences ($p<0.05$) among the cheese samples

the total carbohydrates content noted in sample C2. The samewasobservedby Debon *et al*. (2010), Fritzen-Freire *et al*. (2013) and Srisuvor *et al*. (2013) for fermented milk, ricotta cream and set yogurt, respectively and all such products had been added with inulin. In relation to moisture content, it was also possible to note that the three cheese samples manufactured were in accordance with the Brazilian regulations, which establish that fresh cheeses must have a moisture content higher than 55 g 100 $g^{-1}$ (Brazil, 2004). However, in the present work, the moisture contents obtained for all the cheese samples were higher than those noted by Sant'Ana *et al*. (2013). It is noteworthy that none of the cheese samples made from CM2 were cut and drained, thus the freeze concentration process increased the amount of milk in the cheese curd, resulting in an increase in moisture and probably in whey protein also.

Still, in relation to the cheese samples, no differences ($p>0.05$) were noted in their protein and lipid contents. Therefore, our findings are in accordance with the results obtained by Ong *et al*. (2006), who noted that probiotic bacteria do not modify the chemical properties of cheese. Karami *et al*. (2009) also obtained results for ultrafilteredFeta cheese protein content that were similar to those obtained in our study. Meanwhile, Mahmoudi *et al*. (2012) studied the influence of probiotic bacteria on the properties of Iranian white cheese and, as was noted in this present study, they also noted that the addition of probiotic adjunct cultures did not alter the lipid content of the cheese. Moreover, Karimi *et al*. (2015) stated that when inulin is added to food in concentrations equal to or lower than 5%, as is the case of the long chain inulin used in our experiment, the physicochemical properties of cheese will not be strongly affected because inulin is neutral.

The addition of bifidobacteria and/or inulin did not affect ($p>0.05$) did it affect the pH and the titratable acidity values of all the cheese samples. Karimi *et al*. (2015) also reported that the addition of different amounts of inulin had no influence on the pH of cheese, including cheese incorporated with whey during manufacture. Kiliç *et al*. (2009) manufactured Turkish Beyaz cheese added with probiotic strains and noted that the bifidobacteria were not able to induce lactose fermentation and thus there was no production of lactic acid. According to these findings, probiotic bacteria have a weaker acidifying activity compared with the starter culture and are not able to affect the acidity of cheese. The stability in the pH and acidity values of the cheese added with inulin (C2) is probably due to the inulin that was used in this study. According to Muganga *et al*. (2015), the use of inulin with a greater polymerization degree, as the one used in our work, probably contributed to this behavior. These authors stated that prebiotics with longer chains have no influence on the pH and acidity values of dairy products.

Table 2 also shows the color parameters for the three fresh cheese samples. It was possible to note that for sample C2 the parameters a* and h* increased ($p<0.05$), while b* and C* decreased ($p<0.05$). The values obtained for the parameter a* indicate that the Control sample and sample C1 tend toward a slightly more greenish color than sample C2, i.e., added with inulin, which is a reducing sugar. The greenish color in the cheese occurred because of the presence of riboflavin in milk, which is attributable for its slightly green coloration, as described by Nozière *et al*. (2006). On the other hand, Debon *et al*. (2012) stated that the decrease in the greenish color and the increase in the reddish color may be attributed to the kind of inulin used and may be associated with the decrease in the moisture content, as was noted in our study. These authors also state that these reducing carbohydrates may contribute to the change in color of dairy products. The addition of inulin probably also resulted in the increase ($p<0.05$) of the hue angle (h*) of sample C2. However, Wadhwani and McMahon (2012) reported that small variations in composition can cause a large change in the hue angle of cheese. Moreover, Juan *et al*.

Table 3: Texture analysis, uniaxial compression, Young's modulus and dynamic whey release parameters of the fresh cheeses samples denoted Control, without *Bifidobacterium* BB-12 and inulinaddition; C1, only with *Bifidobacterium* BB-12 addition; and C2, with *Bifidobacterium* BB-12 and 5 g of inulin per 100 mL of milk

| | Samples | | |
|---|---|---|---|
| Parameters | Control | C1 | C2 |
| Firmness (N) | 2.30±0.12[b] | 2.36±0.36[b] | 3.33±0.17[a] |
| Cohesiveness (-) | 2.25±0.72 [a] | 2.19±0.18 [a] | 2.22±0.36 [a] |
| Adhesiveness (N.s) | -1.36±0.22[a] | -1.46±0.34[a] | -1.81±0.27[a] |
| Elasticity (-) | 1.05±0.02[a] | 1.10±0.03[a] | 1.21±0.24[a] |
| Fracture stress ($\sigma$) (kPa) | 3.61±0.45[b] | 4.20±0.16[b] | 5.03±0.19[a] |
| Fracture strain ($\varepsilon$) (-) | 0.47±0.01[a] | 0.48±0.01[a] | 0.49±0.02[a] |
| Young module ($\gamma$) (kPa) | 658.78±60.45[b] | 715.99±31.51[b] | 1,001.48±19.75[a] |
| Dynamic whey release (%) | 9.50±0.52[a] | 10.52±0.19[a] | 6.42±0.17[b] |

Results are expressed as a mean±standard deviation, among three batches carried out in triplicate for each type of fresh cheese, with five repetitions for texture analysis and uniaxial compression; and three repetitions for dynamic whey release; [a–b]Within a row, different superscript lowercase letters denote significant differences ($p$<0.05) among the samples

(2013) reported that, because of its particle nature, inulin can also act as light-scattering centers probably affecting the h* value also.

For all the cheese samples, the parameter b* showed a tendency toward a yellow color. This behavior is due to the presence of carotenoids in milk, which are fat-soluble pigments represented primarily by β-carotene. However, an increase (p<0.05) in yellowness (b*) was noted for the cheese with no addition of inulin. These results were similar to the findings by Solowiej *et al*. (2015) for processed cheeses. This behavior could be also associated with the increase in the total solids content observed in sample C2. The same behavior was noted by Debon *et al*. (2012) for fermented milk added with inulin. As expected, there were also differences (p<0.05) between the Chroma (C*) parameters for sample C2 and the other two cheese samples. Solowiej *et al*. (2015) reported that C* represents color saturation, i.e., it is the combination of the parameters a* and b*, which show the proportions in which the color is mixed with white, black, or gray.

The L* parameter remained unaltered (p>0.05) for all the cheese samples, even so, these values were considered high. Wadhwani and McMahon (2012) stated that this behavior is considered to be common in uncolored cheese. These results are in accordance with those obtained by Fritzen-Freire *et al*. (2010a), Fritzen-Freire *et al*. (2013), Magenis *et al*. (2014) and Verruck *et al*. (2015a) for probiotic Minas Frescalcheese, ricotta cream with*Bifidobacterium* BB-12, commercial Minas Frescal cheese and buffalo Minas Frescal cheese added with bifido bacteria, respectively. Therefore, it was possible to note that this result is typical of cheeses that have a very high moisture content, such as those produced in our study. As was the case in the present work, Mahmoudi *et al*. (2012) and Verruck *et al*. (2015a) also noted that the addition of probiotic adjunct cultures normally should not alter the properties of the cheese. Finally, Wadhwani and McMahon (2012) stated that the influence of color on food acceptability is as important as other characteristics of cheese, such as its physical properties.

**Physical properties:** The results of texture analysis, uniaxial compression, Young's modulus and dynamic whey release of the three samples of fresh cheese are shown in Table 3. As expected, the addition of inulin and thus the solids content in sample C2, resulted in a fresh cheese with greater ($p$<0.05) firmness than that of the Control and sample C1. The same behavior was obtained by Hennelly *et al*. (2006) for imitation cheese containing inulin (5%). Guven *et al*. (2005) stated that cheese curd stability is influenced by the total solids content and by the addition of inulin. A correlation between the values for firmness and total solids content (R = 0.894) was also noted. Chaito *et al*. (2016), Drabińska *et al*. (2016) and Shoaib *et al*. (2016) reported that long chain inulin, such as the one used in our study, is capable of developing a particulate gel in the presence of water and thus it alters the texture of food products. These particulate gels are denoted by Karimi *et al*. (2015) as microcrystals, i.e., insoluble submicron crystalline inulin particles, which interact with each other forming small aggregates in the water phase and encapsulating a great amount of water. Therefore, long chain inulin forms primary non spherical crystals (0.5 to 3 μm) that aggregate including significant amounts of the fluid phase and afterward these aggregates interact to form a gel (Meyer *et al*., 2011). On the other hand, Kip *et al*. (2006) state that inulin can also form parts of the protein structural network by complexing with protein aggregates. Karimi *et al*. (2015) also noted this interaction between inulin and whey protein. Thus, the increase in firmness of sample C2 could be because of the incorporation of whey into the curd since it was not cut, stirred and/or drained. According to Glibowski (2009), solutions containing 1 to 15% inulin caused an increase in the firmness values of the gelled mixtures as a result of forming weak inulin and whey protein complexes.

No differences were noted (p>0.05) between the cheese samples regarding the parameters for cohesiveness, adhesiveness and elasticity. Thus, it was noted that neither inulin nor the bifido bacteria was able to affect these parameters. According to Kiziloz *et al*. (2009), cohesiveness is related to the strength of the

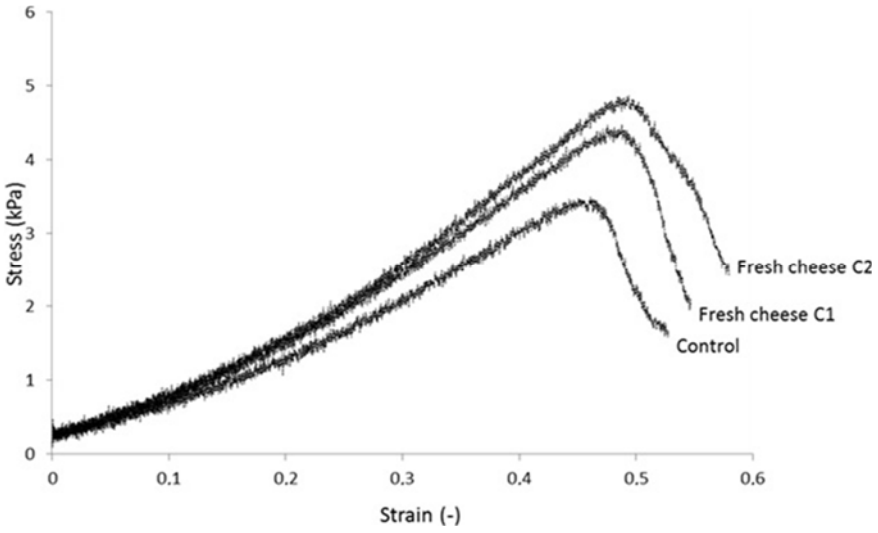


Fig. 2: Stress-strain curves of the fresh cheeses samples denoted Control, without *Bifidobacterium* BB-12 and inulin addition; C1, only with *Bifidobacterium* BB-12 addition; and C2, with *Bifidobacterium* BB-12 and 5 g of inulin per 100 mL of milk

internal bonds in a structure. If it is low, the structure will crumble easily (Kiziloz *et al*., 2009) while by contrast, highly cohesive cheeses adhere to the oral surface (Foegeding *et al*., 2003). Therefore, maintaining cohesiveness in fresh cheese is a quite recommendable practice. Adhesiveness is a tendency of the cheese to resist separation from another material with which it is in contact, i.e., it is an important attribute that affects its sliceability (Kapoor and Metzger, 2008). As also happened in our study, Juan *et al*. (2013) noted that inulin addition had no effect on the cohesiveness of full fat cheeses. In respect to the elasticity parameter, a similar behavior was observed by Meyer *et al*. (2011). These authors only noted pronounced effects on cheese elasticity when it was added with at least 7.5% long chain inulin. Soltani *et al*. (2016) described that cheese elasticity is mostly affected by the pH (near 5.0 or below). It was possible to note (Table 2) that, for all the cheese samples produced, the pH values did not differ and they were higher than 6.25. This behavior could explain the stability in the elasticity parameter between all the cheese samples.

Uniaxial compression was employed to evaluate the mechanical properties of the three cheese samples. When comparing the results for fracture stress ($\sigma$) of sample C2 with those of the other two samples, it is possible to note that its higher ($p<0.05$) numerical value indicate a firmer and less breakable cheese that requires a greater strength in order to fracture. Magenis *et al*. (2014) stated that fracture stress is a point of maximum stress, where the cheese reaches macroscopic failure and that higher values between different cheeses indicate toughness. Therefore, these results confirm the behavior previously observed for the firmness parameter. On the other hand, the high stress fracture of sample C2 could be due to the whey protein and inulin present in the matrix of the cheese. Glibowski (2009) reported that inulin addition in a product with whey protein allows the formation of a gel network. These authors also observed that the whey protein and inulin structure that formed produced the high values in the stress fracture parameter. Meyer *et al*. (2011) highlighted that in these systems inulin probably behaves only as a co-solute that has the ability to bind water molecules, interfering in protein matrix formation due to the dispersion of inulin molecules among casein micelles. On the other hand, the Control sample and sample C1 only showed whey protein interference, which, according to Soltani *et al*. (2016), results in a softer texture and lower fracture stress. The same behavior was observed by Soltani *et al*. (2015) for Iranian white cheese manufactured with ultra filtered milk.

The fracture strain ($\varepsilon$) describes the deformability of cheese (higher numerical values indicate greater deformability). Furthermore, its modulus values may express the stiffness of the cheese, where higher values indicate that the cheese offers a higher resistance to deformation (Juan *et al*., 2013). The data shown in Table 3 indicate that all the cheese samples showed the same deformability before rupturing. Besides, in the present study, the $\varepsilon$values showed a high correlation ($R = 0.977$) with elasticity. This behavior is in accordance with that reported by Gunasekaran and Ak (2002), who state that deformability can express the elasticity properties of cheese. Dantas *et al*. (2016) also associate the fracture strain with the crumbliness of the Minas Frescal cheese. Thus, as mentioned before, the amounts of *Bifidobacterium* BB-12 and of the long chain inulin were not enough to influence on this parameter. However, in order to assess the weakness of the cheese samples, stress-strain curves of the fresh cheeses were plotted (Fig. 2) and it was possible to note that sample C2 was the least fragile. As described by Meyer *et al*.

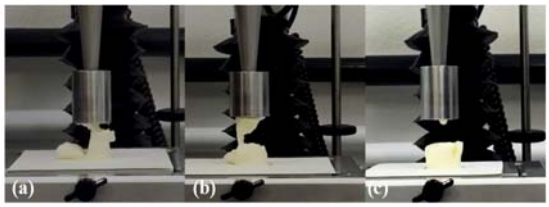


Fig. 3: Images from dynamic whey release during the probe return after the uniaxial compression tests of the fresh cheeses samples denoted by (a): Control, without *Bifidobacterium* BB-12 and inulin addition; (b): C1, only with *Bifidobacterium* BB-12 addition; and (c): C2, with *Bifidobacterium* BB-12 and 5 g of inulin per 100 mL of milk

(2011), the variation of these curves could be explained by factors such as the capacity of inulin to retain water; the interaction of inulin with milk protein, which can lead to an increase in the molar mass; and the formation of small aggregates of microcrystals, which are able to retain water and/or by a higher total solids content.

The highest value obtained from sample C2 for Young's modulus (γ) confirms an increase ($p<0.05$) in its firmness. Felício *et al*. (2016) reported that Young's modulus is the stress to strain ratio at the linear part of the $\sigma$ x $\varepsilon$ curves and expresses the resistance of cheese to axial deformation with respect to a force applied whereas the maximum stress is directly related to the firmness of the cheese. Therefore, as mentioned before, this behavior is also due to the addition of inulin. The addition of inulin is also attributable to the lower ($p<0.05$) dynamic whey release value of sample C2 and according to Srisuvor *et al*. (2013), this could be related to the structures and chemical composition of inulin. Drabińska *et al*. (2016) state that inulin, as well as other long chain fructans, contains a great number of hydrophilic groups, which have the ability to interact with each other forming aggregates. Also, inulin can complex (via H-bridge formation) with protein aggregates and become part of the structural network, contributing to water retention (Srisuvor *et al*., 2013). Srisuvor *et al*. (2013) reported that the better curd stability is reflected by the lower whey release, as observed in our study in sample C2. This phenomenon was observed by these same authors when they used more than 3% inulin. The stability of the cheese from sample C2 can also be seen in Fig. 3, which shows the behavior of the dynamic whey release during the probe return after the uniaxial compression tests, where it was also possible to note a positive effect of inulin on the matrix of the fresh cheese.

**Microstructural properties:** The close relationship between the microstructure and the rheology of cheese is often highlighted and it has also been

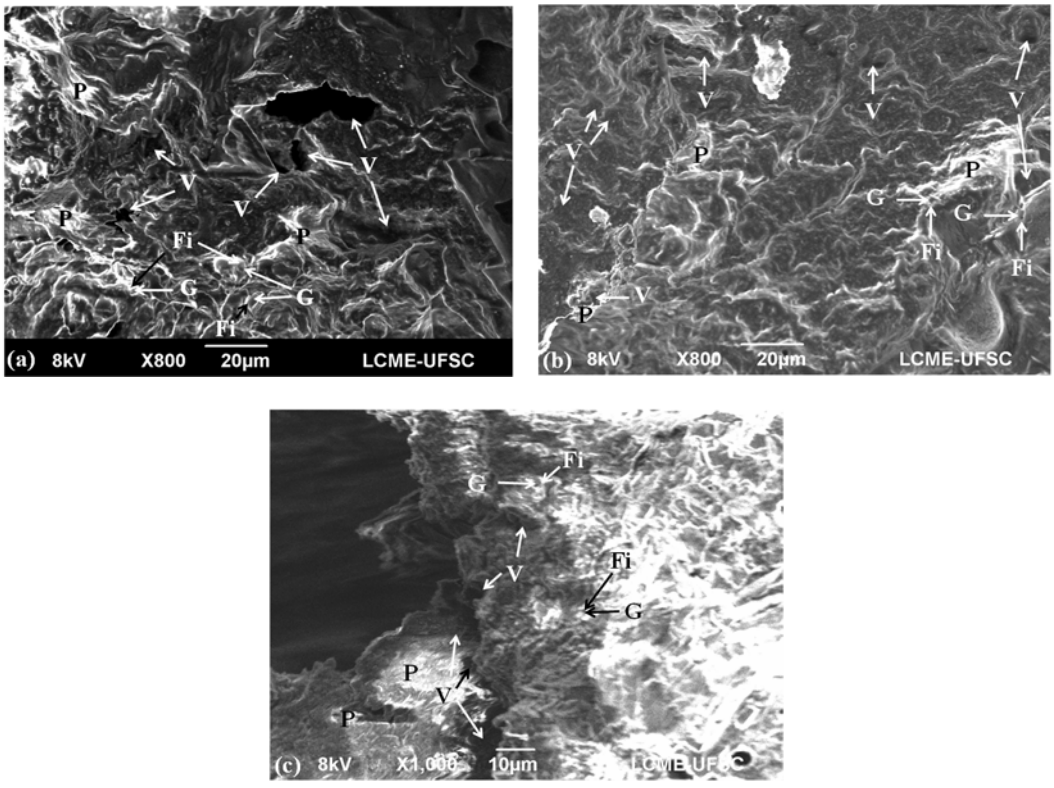


Fig. 4: SEM micrographs of fresh cheeses denoted by (a): Control, without *Bifidobacterium* BB-12 and inulin addition; (b): C1, only with *Bifidobacterium* BB-12 addition; and (c): C2, with *Bifidobacterium* BB-12 and 5 g of inulin per 100 mL of milk. P: Protein matrix; G: Fat globule; V: Void space; Fi: Filamentous structures

noted by Fritzen-Freire *et al*. (2010b), Madadlou *et al*. (2007), Prudêncio *et al*. (2014) and Verruck *et al*. (2015a). The differences detected among the samples during the physical analysis are probably derived from the microstructural properties. These differences are shown in the micrographs of the three fresh kinds of cheese (Fig. 4a to 4c), where for the Control and C1 cheeses the matrix has a more open structure. According to Lobato-Calleros *et al*. (2007), this fact could be attributed to the presence of numerous small void spaces in the protein network, which would affect the interactions between casein chains and favor pore formation. In this case, the addition of bifidobacteria was not able to affect the structure of the cheese. The cheese from sample C2 showed a more compacted structure with fewer void spaces, indicating a closed protein network due to casein alignment. This behavior is in accordance with that observed by Karimi *et al*. (2015). These authors described that cheeses manufactured with ≥3% inulin are characterized by a more compact structure; a denser protein matrix; and a more uniform disposition of protein chains and pores between them compared to other cheeses.

The micrographs of the three kinds of cheese also showed that the fat globules were trapped within the protein matrix. Moreover, filamentous structures were noted on the surfaces of the fat globules. Lobato-Calleros *et al*. (2007) reported that these structures may be composed of chains of casein and/or whey proteins. Besides, these authors stated that the position of these proteins on the surface of fat globules promotes the integration of fat globules in the protein matrix.

**Microbiological properties:** The viable probiotic cell counts for the fresh cheeses added with *Bifidobacterium* BB-12, i.e., C1 and C2, were equal to 7.46 log CFU/g and 7.58 log CFU/g, respectively. Vinderola *et al*. (2011) stated that several factors have to be considered when using probiotics in dairy products such as cheese, in particular, their viability and presence. Vera-Pingitore *et al*. (2016) described that the numbers currently found in probiotic products and the numbers associated with significant outcomes in clinical trials are within the range of 1-10 billion CFU per dose. This number is the same as that recommended by the FAO/WHO (2006). Therefore, the results obtained for both kinds of cheese (C1 and C2) added with bifidobacteria indicate that they can be classified as probiotic products since their viable probiotic cell counts were above the recommended values for a probiotic product. Rutella *et al*. (2016) pointed out that this count value is necessary for probiotics to survive transit through the gastrointestinal tract and thus provide measurable health benefits, which are owed to their ability to modulate the host's immune system and to balance intestinal microbiota. Similar results were obtained by Fritzen-Freire *et al*. (2010a, 2010b), Albenzio *et al*. (2013) and Verruck *et al*. (2015a, 2015b) for Minas Frescal cheese, Scamorza ewe milk cheese and buffalo Minas Frescal cheese, respectively. Another factor that could be related to the survival of probiotic cultures is the high pH values noted in both kinds of cheese (C1 and C2). Ortakci *et al*. (2012) reported that low pH may not maintain sufficient numbers of probiotic bacteria, such as some strains of bifidobacteria. Although Jungersen *et al*. (2014) stated that *Bifidobacterium* BB-12 has an excellent tolerance to pH between 2 and 4, Buriti *et al*. (2005) stated that the Minas Frescal cheese offers excellent conditions for the survival of probiotic strains because of its high water activity, low salt content, absence of preservatives and pH above 5.0. Regarding cheese sample C2, it is important to note that it is a symbiotic product. Karimi *et al*. (2015) reported that a symbiotic product is defined as a mixture of probiotics and prebiotics. Therefore, the fresh cheese classified as probiotic (C1) and principally the fresh cheese classified as symbiotic (C2) may be suitable alternatives aiming to diversify the production of functional foods in a competitive market.

## CONCLUSION

It was possible to verify an enhancement of the total solids content in the concentrated milk obtained through freeze concentration. The highest result for the concentration factor was obtained in the third freeze concentration stage; however, there was also an increase in total solids content in this ice fraction. In contrast, the second freeze concentration stage showed the highest mass transfer in relation to total solids content. Hence, the concentrated milk from the second stage was used in the manufacture of the functional fresh cheeses. In this study, the addition of *Bifidobacterium* BB-12 to fresh cheeses did not affect the properties that were investigated. The fresh cheese added with inulin showed the highest total solids content, reflecting on the increase of its total carbohydrates content and on its lower tendency toward the color green. The addition of inulin and the presence of whey contributed to the increase in firmness and thus resulted in a less breakable fresh cheese, with a less fragile and more compact structure. It was possible to conclude that the viable probiotic cell count for the fresh cheese that was added with bifidobacteria was above the recommended levels for a dairy product to be considered a probiotic. Besides, the addition of bifidobacteria and inulin resulted in the manufacture of a symbiotic fresh cheese. Finally, the use of the concentrated milk, the probiotic culture and the prebiotic agent in the manufacture of fresh cheeses can be highly attractive to the food industry since it offers an opportunity for an entirely new approach regarding the utilization of milk.

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