



## Influence of different content of cheese whey and oligofructose on the properties of fermented lactic beverages: Study using response surface methodology

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### ABSTRACT

Lactic beverages have been used as an important vehicle for probiotics, and the utilization of cheese whey and oligofructose would contribute even more to the functional properties of this product. However, because of the short lifespan of probiotics, studies have been carried out aiming at the evaluation of the contribution of the prebiotics in the improvement of the viability of these microorganisms. The technological properties (fermentation time, acidity and syneresis index) and the population of probiotic bacteria in fermented lactic beverages manufactured with different content of cheese whey and oligofructose were evaluated. The results showed that oligofructose, at the concentrations evaluated in this study, did not show any significant influence on the response variables, whereas the content of cheese whey only influenced the syneresis index of lactic beverages.

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### 1. Introduction

Cheese whey, a byproduct of the cheese-making industry, shows great potential for the development of dairy products due to its nutritional value, since it is not only a source of the most biologically valuable proteins, but also rich in minerals and vitamins, mainly riboflavin (Ha & Zemel, 2003). However, in spite of being a nutritive product, whey is still little used in human diet (Drgalic, Tratnik, & Bozanic, 2005). It has been noted that while in developed countries about 95% of the total whey is used in food production, in Brazil only 50% is utilized (Silva, Ferreira, Costa, & Magalhães, 2001). The partial substitution of milk for liquid cheese whey in the manufacture of fermented milk could be a rational alternative in order to incorporate this byproduct in human nutrition.

Fermented lactic beverage can be defined as a type of fermented milk, resultant from the mixture of milk and cheese whey containing lactic culture and other dairy products (Gallardo-Escamilla, Kelly, & Delahunty, 2007). Due to its low acidity level, as well as its

consistency and commercial value, this type of drink shows great acceptability by Brazilians (Almeida, Bonassi, & Roça, 2001; Iglécio, 1995). Moreover, the image of health associated with yoghurt and other dairy products, such as lactic beverages, has led to an increase in the consumption of these foods (Achanta, Aryana, & Boeneke, 2007). However, the ratio between milk and whey to be used in the manufacture of lactic beverage has not been well defined yet (Almeida et al., 2001; Penna, Sivieri, & Oliveira, 2001).

Alteration in the composition of lactic beverages can affect syneresis, that is, the spontaneous appearance of whey on the surface of a gel during storage. In fermented milks, syneresis is considered a primary defect and a limiting factor in consumers' acceptability of the product (Amatayakul, Sherkat, & Shah, 2006; Kailasapathy, 2006; Lucey, 2001; Lucey & Singh, 1998). Another aspect that can compromise acceptability is acidity, which can also affect the viability of probiotic bacteria in lactic beverages (Talwalkar & Kailasapathy, 2004; Thamer & Penna, 2006; Vinderola, Bailo, & Reinheimer, 2000; Vinderola, Prosello, Ghiberto, & Reinheimer, 2000).

Lactic beverages have also been used as an important vehicle for probiotics (Almeida, Tamime, & Oliveira, 2008; Drgalic et al., 2005). Probiotics are live microorganisms which when administered in

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adequate amounts confer a health benefit on the host (FAO/WHO, 2002) by improving microbial balance in the host's gut flora and defenses against pathogenic microorganisms. Other benefits attributed to probiotics include prevention of cancer, stimulation of the immune system, lowering of serum cholesterol levels, and improvement of vitamin synthesis (Heenan, Adams, Hosken, & Fleet, 2004; Saad, 2006). The species which are most frequently used as probiotics belong to the genera *Lactobacillus* and *Bifidobacterium* (Gomes & Malcata, 1999; Isolauri, 2004). However, due to the short lifespan of probiotics, studies have been carried out to evaluate the contribution of prebiotics to the viability of these microorganisms (Aryana & McGrew, 2007; Huebner, Wehling, & Hutkins, 2007; Losada & Olleros, 2001; Rao, 2001; Rycroft, Jones, Gibson, & Rastall, 2001).

Prebiotics are non-digestible dietary components, generally oligosaccharides, with bifidogenic activity; that is capable of stimulating the proliferation and/or activity of some desirable bacteria in the intestine (Gibson & Roberfroid, 1995; Vinderola, Bailo, et al., 2000). Amongst them, oligofructose has been widely used; not only due to its important nutritional benefits, but also for the functional properties it shows (Sangeetha, Ramesh, & Prapulla, 2005; Thamer & Penna, 2005). Amounts of fructans between 4 and 5 g per day are considered effective in stimulating the growth of bifidobacteria of the colonic microbiota, thus resulting in the prebiotic effect (Rao, 2001; Roberfroid, 1999).

The experimental design in conjunction with the response surface methodology makes it possible to carry out, through a small number of experiments, a simultaneous investigation of the main effect of the experimental variables and the effect of their interaction on the desired response (Teófilo & Ferreira, 2006). In this study, the levels of variables were selected in accordance with the data available in literature for similar products. Almeida et al. (2001) manufactured lactic beverages containing 30, 40, and 50 mL/100 mL liquid whey. Thamer and Penna (2005, 2006) used 1, 2, and 3 g/100 mL of oligofructose in lactic beverages whereas Fuchs, Tanamati, Antonioli, Gasparello, and Doneda (2006) used 5 g/100 mL oligofructose. According to Coussement (1999), oligofructose can be added in foods to allow a specific nutritional claim such as that regarding the bifidogenic activity. In these foods, typical levels are from 1 to 6%.

The addition of cheese whey, probiotic bacteria, and prebiotics to a lactic beverage could result in a functional food, serving as a new alternative for the dairy industry and for consumers interested in a healthy, nutritious diet; it also has new sensorial characteristics (Thamer & Penna, 2005; Vinderola, Bailo, et al., 2000). Little work has been done on measuring the characteristics of lactic beverages combining pro- and prebiotic ingredients. Therefore, the objective of this study was to evaluate the technological properties (fermentation time, acidity and syneresis index) and the population of probiotic bacteria in fermented lactic beverages manufactured with different content of cheese whey and oligofructose.

## 2. Materials and methods

### 2.1. Experimental design

The Central Composite Design (CCD) for two independent variables was used in this study. The codified levels of the variables (whey content –  $X_1$  and oligofructose concentration –  $X_2$ ) and their real values are shown in Table 1. The experimental design was composed of 13 assays; four factorial (combination of levels –1 and +1), four axial (a variable at the level  $\pm\alpha$  and to another one at zero) and five repetitions in the central point (two variables at level zero). Experiments in the centre of the design were performed to make the estimation of possible pure error. Due to systematic

**Table 1**

Central Composite Design (CCD) for two variables for manufacture of lactic beverages.

Assay	Codified		Reals	
	$X_1$	$X_2$	Whey content (mL/100 mL)	Oligofructose concentration (g/100 mL)
1	–1	–1	30	2
2	1	–1	40	2
3	–1	1	30	5
4	1	1	40	5
5	$-\alpha$	0	27.93	3.5
6	$\alpha$	0	42.07	3.5
7	0	$-\alpha$	35	1.379
8	0	$\alpha$	35	5.621
9	0	0	35	3.5
10	0	0	35	3.5
11	0	0	35	3.5
12	0	0	35	3.5
13	0	0	35	3.5

$\alpha = \pm 1.414$  for two independent variables.

$X_1$  = whey content variable.

$X_2$  = oligofructose concentration variable.

errors, all the experiments were carried out at random to minimize the effect of unexplained variability on the responses observed. The dependent variables (responses) were fermentation time (hours), acidity (g/100 g lactic acid), syneresis index (mL) and count of probiotic viable cells (log cfu/mL). The initial concentration of culture (6 log cfu/mL) and fermentation temperature (40 °C), indicated by the manufacturer (Chr Hansen®), were kept constant.

### 2.2. Manufacture of lactic beverages

Lactic beverages were manufactured by using methodology adapted from the one developed by Almeida et al. (2001). A mixture of commercial pasteurized milk (3 g fat per 100 mL, Tirol, Treze Tílias, Brazil) with commercial sucrose (União, Limeira, Brazil, 5 g/100 mL of the total volume of beverage) was submitted to thermal treatment at 95 °C for 5 min, whereas the mixture of liquid cheese whey (obtained from the manufacture of fresh Minas cheese) with oligofructose (Raftilose, Beneo P95®, Orafiti, Oreye, Belgium) was heated at 65 °C for 30 min. The temperature of the mixtures was lowered to 40 °C. After that, the beverages were manufactured in accordance with the experimental design proposed in this study. In each assay, 1000 mL of lactic beverage was manufactured. The lactic culture (ABT-4®, Chr Hansen, Hønsholm, Denmark) used was the freeze-dried commercial culture for direct vat set (DVS), which consisted of *Lactobacillus acidophilus* LA-5, *Bifidobacterium* BB-12 and *Streptococcus salivarius* subsp. *thermophilus*, and was added to the lactic beverages at 8.3 mg/100 mL, where fermentation occurred at 40 ± 1 °C. The fermentation time (hours) of lactic beverages was defined as being the time required to reach pH around 4.6. After fermentation, the beverages were cooled at 4 ± 1 °C, gently stirred, packaged in plastic pots and stored at this temperature until the analyses were done.

### 2.3. Evaluation of the technological and microbiological properties

The acidity, expressed in g/100 g lactic acid (AOAC, 2005), was determined in duplicate. The syneresis index was determined in accordance with 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 (determined in duplicate) was considered as being

the amount of liquid (mL) per 100 mL of sample after 2 h of draining ( $4 \pm 1 \text{ }^\circ\text{C}$ ).

For the enumeration of *L. acidophilus* LA-5, MRS agar (Merck, Darmstadt, Germany) modified with the addition of 0.15 g/100 mL of bile (bile-MRS) was used; while in the counting of *Bifidobacterium*, MRS agar modified with addition of 0.2 g/100 mL of lithium chloride and 0.3 g/100 mL sodium propionate (LP-MRS) was used (Vinderola & Reinheimer, 2000). The plates were incubated in anaerobic jars containing AnaeroGen® (Oxoid, UK) at  $37 \pm 1 \text{ }^\circ\text{C}$  for 72 h. After this incubation period, the counting of probiotic viable cells was carried out, expressed as log colony-forming units per mL (log cfu/mL).

2.4. Statistical analysis

The regression coefficients for linear, quadratic, and interaction terms were determined by using multiple linear regression (MLR). The significance of each regression coefficient was judged statistically by computing the *t*-value from pure error obtained from the replicates at the central point of this experiment. The analysis of variance (ANOVA) was used to validate the model. The regression coefficients were then used to generate response surfaces. All calculations and graphics were performed by using the Statistica 6.0 (2001) (StatSoft Inc., Tulsa, OK, USA) software. A difference was considered statistically significant when  $P < 0.05$ .

3. Results

The effect of the combinations between the variables whey content and oligofructose concentration on the manufacture and properties of lactic beverages was evaluated. The responses obtained from the 13 assays are shown in Table 2.

The variables whey content and oligofructose concentration at the levels studied did not show any influence ( $P > 0.05$ ) on the following response variables: fermentation time, acidity, and count of probiotic viable cells; whereas the syneresis index was influenced ( $P < 0.05$ ) by the whey content used in lactic beverages (Table 3), where only the linear effect of this variable was observed. However, the concentration of oligofructose, at the levels studied, did not influence the syneresis index ( $P > 0.05$ ); neither was the interaction between the variables significant ( $P > 0.05$ ). The results show that the higher the whey content used, within the levels studied, the greater the syneresis index of fermented lactic beverages.

Table 2 Evaluation of the technological and microbiological properties of fermented lactic beverages (according to the assays described in Table 1).

Responses				
Assay	Fermentation time (h)	Acidity (g/100 g lactic acid)	Syneresis index (mL)	Probiotic total (log cfu/mL)
1	4.50 ± 0.00	0.76 ± 0.00	39.00 ± 0.00	6.30 ± 0.00
2	4.70 ± 0.00	0.76 ± 0.01	48.50 ± 0.70	6.61 ± 0.00
3	4.80 ± 0.00	0.78 ± 0.00	39.00 ± 0.00	6.37 ± 0.00
4	4.50 ± 0.00	0.72 ± 0.00	45.50 ± 0.70	6.09 ± 0.00
5	4.50 ± 0.00	0.73 ± 0.00	37.50 ± 0.70	6.26 ± 0.00
6	5.10 ± 0.00	0.79 ± 0.01	48.00 ± 0.00	6.81 ± 0.00
7	4.70 ± 0.00	0.76 ± 0.00	41.50 ± 0.70	6.19 ± 0.00
8	4.50 ± 0.00	0.73 ± 0.00	44.00 ± 0.00	6.28 ± 0.00
9	4.50 ± 0.00	0.78 ± 0.00	43.00 ± 0.00	6.50 ± 0.00
10	5.20 ± 0.00	0.82 ± 0.00	41.50 ± 0.70	6.28 ± 0.00
11	4.80 ± 0.00	0.76 ± 0.00	41.00 ± 1.41	6.12 ± 0.00
12	4.80 ± 0.00	0.78 ± 0.00	39.50 ± 2.12	6.84 ± 0.00
13	4.50 ± 0.00	0.74 ± 0.00	43.00 ± 1.41	6.52 ± 0.00

Values are expressed as mean ± standard deviation of duplicate determinations.

Table 3 Analysis of variance of the values of syneresis index of fermented lactic beverages.

Source	Sum of squares	DF	Mean square	F value	P value
Linear					
X <sub>1</sub>	118.960	1	118.960	58.115	0.0001*
X <sub>2</sub>	0.036	1	0.036	0.017	0.898
Quadratic					
X <sub>1</sub> <sup>2</sup>	2.827	1	2.827	1.381	0.278
X <sub>2</sub> <sup>2</sup>	2.827	1	2.827	1.381	0.278
Interaction					
X <sub>1</sub> X <sub>2</sub>	2.250	1	2.250	1.099	0.329
Error	14.329	7	2.047		
Total model	140.577	12			

\*Values significantly different ( $P < 0.05$ ).

A graphic indication of the quality of the model can be seen in Fig. 1A and B. The predicted versus the observed values plot for syneresis index shows a linear behavior with correlation coefficient of 0.95 (Fig. 1A). The plot of residuals versus values observed (Fig. 1B) shows that the assumptions of randomness of the residuals were satisfied.

The model built for the syneresis index (Y) is in Eq. (1), and Fig. 2 shows the contour plot elaborated from this regression model.

$$Y = 15.38941 + 0.77129X_1 \tag{1}$$

This contour plot shows that there was a linear increase in syneresis due to whey content, where higher syneresis indices were observed when higher whey contents were used in lactic beverages.

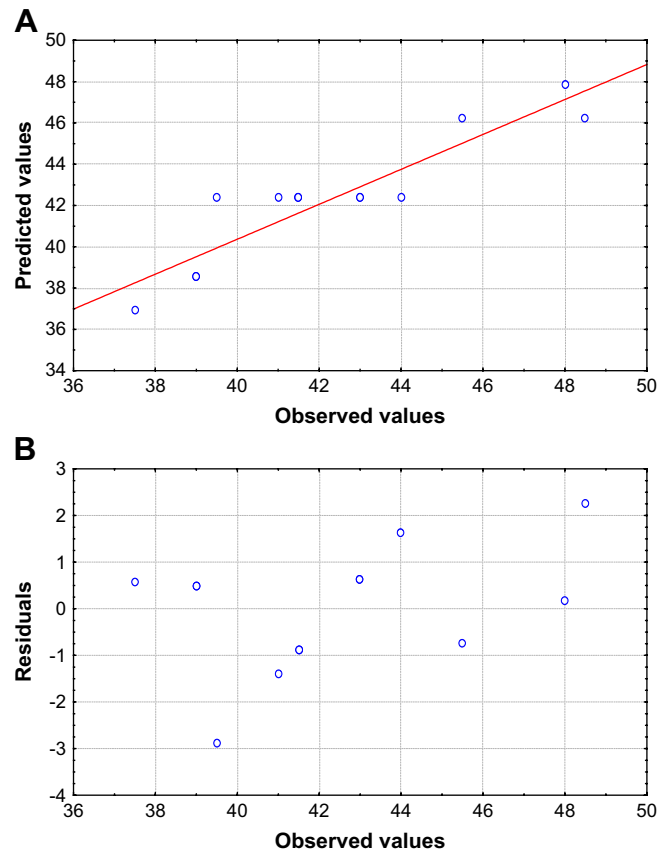


Fig. 1. (A) Plot of the predicted versus observed syneresis index (mL). (B) Plot of residual versus observed syneresis index of the fermented lactic beverages samples.

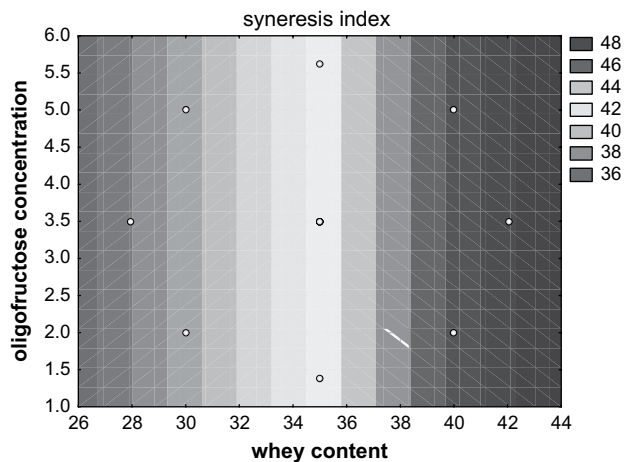


Fig. 2. Contour plot for the syneresis index (mL) of the fermented lactic beverages as a function of their whey content (mL/100 mL) and oligofructose concentration (g/100 mL).

#### 4. Discussion

The fermentation time observed for lactic beverages containing probiotics and oligofructose (Table 2) was different from the results obtained by Dave and Shah (1998), for fermented milk with *Streptococcus thermophilus*, *L. acidophilus* and *Bifidobacterium* BB-12, whose variation was of 7.5–9 h. However, these results were similar to those obtained by Thamer and Penna (2006) and Almeida et al. (2001), whose fermentation times were between 3 and 4.25 h, for lactic beverages containing probiotic and prebiotics (1–3 g/100 mL); and 4–4.5 h, for fermented lactic beverages, containing 30–50 mL/100 mL of whey in substitution to milk, respectively. The same was verified by Fuchs et al. (2006) in yoghurts, containing *S. thermophilus*, *Lactobacillus bulgaricus*, *Lactobacillus casei* and prebiotics (1 g/100 mL of inulin and 5 g/100 mL of oligofructose), in which the average fermentation time was 6 h.

According to Penna, Baruffaldi, and Oliveira (1997) factors such as temperature and the lactic culture used could be responsible for the variation in the fermentation time. Thamer and Penna (2005) state that probiotic cultures grow slowly in milk producing little acid and therefore resulting in a prolonged fermentation time. These authors relate that the utilization of *S. thermophilus* would increase growth rate of probiotics, reducing fermentation time, while Dave and Shah (1998) indicate that the fermentation time would be influenced by the composition of fermented milk. Therefore, all these factors could be responsible for the shorter fermentation time obtained in this work, which is an advantage for the dairy industry, as long as the characteristics and quality of the product are maintained.

The acidity values of the different lactic beverage formulations (Table 2) were lower than those found by Fuchs et al. (2006) (1.15%) and Akalin, Tokusoglu, Gönç, and Aycan (2007) (between 1.21 and 1.33%), for probiotic yoghurts supplemented with prebiotics. According to Thamer and Penna (2006), acidity is related to the type of solid used, milk-based or not, and with the activity of the culture responsible for fermentation. The behavior of the acidity of beverages was similar to the one obtained by Zhu (2004) and Drgalic et al. (2005), who verified the stability of acidity in yoghurts with 4 g/100 mL of oligofructose, and fermented lactic beverage manufactured with reconstituted cheese whey and containing a prebiotic (inulin), respectively.

The influence of the increasing whey content utilized to increase the syneresis index was also observed in yoghurts by González-Martínez et al. (2002) and in probiotic yoghurts by Penna, Gurram,

and Barbosa-Cánovas (2006). These authors reported that the increase of whey content in stirred products contributes to the formation of acid gels with an open structure, due to reduction in the intermolecular interactions and, therefore, are more susceptible to syneresis. However, as was verified in probiotic yoghurts containing oligofructose P95 (1.5 g/100 mL), by Aryana and McGrew (2007) and Aryana, Plauche, Rao, McGrew, and Shah (2007), the syneresis index was not influenced by oligofructose at the concentrations used in this work.

Although this study showed no influence of the whey content and oligofructose concentration used in the counting of the probiotic bacteria evaluated, all the lactic beverages were considered probiotics (Table 2). Boylston, Vinderola, Ghoddsi, and Reinheimer (2004) and Gomes and Malcata (1999) state that fermented bio-products must contain a satisfactory minimum number of viable cells of at least 6 log cfu/mL, because the daily minimum therapeutic dose recommended is around 8–9 log of viable cells per 100 mL of the product.

Results obtained by some authors are divergent as to the viability of probiotics in the presence of whey and/or prebiotics. However, behaviors similar to those found in manufactured beverages were observed by Castro, Pinheiro, Hoffmann, and Penna (2001), who used concentrations from 1 to 3 g/100 mL oligofructose, and by Fuchs et al. (2006), who used 1 g/100 mL inulin and 5 g/100 mL oligofructose; both studies were on yoghurts. The same was verified by Thamer and Penna (2005) and Drgalic et al. (2005), by using 1–3 g/100 mL of oligofructose and 1 g/100 mL of inulin, respectively, and whey in probiotic fermented lactic beverages, while Akalin, Fenderya, and Akbulut (2004), Aryana and McGrew (2007), Aryana et al. (2007) and Capela, Hay, and Shah (2006) obtained improvement in the viability of probiotic bacteria in yoghurts containing oligofructose.

According to Donkor, Nilmini, Stolic, Vasiljevic, and Shah (2007), this discrepancy could be attributed to a strain-dependant response of probiotics to prebiotics. Huebner et al. (2007) compared the extension where different commercial prebiotics stimulate the selective growth of *Lactobacillus* and *Bifidobacterium* and they also concluded that the fermentation of prebiotics is dependent mostly on the bacterial strain, rather than based on species or genera. Moreover, prebiotics may exert a protective effect on selected probiotic bacteria by improving their survival and activity during storage of the product as well as on their passage through the upper parts of the gastrointestinal tract (Donkor et al., 2007).

In conclusion, oligofructose, at the concentrations evaluated, did not show any significant influence on the response variables, whereas whey content only influenced the syneresis index of lactic beverages. Therefore, we can suggest that the usage of whey in the production of fermented probiotic lactic beverages can improve its utilization and consumption in human nutrition. Since 4–5 g of fructans per day is enough to confer the prebiotic effect (Rao, 2001; Roberfroid, 1999), the oligofructose concentration used in this study would be recommended, assuming a daily consumption of 200 mL of the lactic beverages. However, evaluations of specific combinations of pro- and prebiotics are still necessary to verify the viability of these *in vivo*, as well as during the storage of lactic beverages.

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