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Shelf Life of a Synbiotic Fermented Dairy Beverage Using Ricotta Cheese Whey

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ABSTRACT

The objective of this study was preparing a synbiotic fermented dairy beverage using ricotta cheese whey, prebiotic and probiotics aiming to reduce the amount of whey incorporated into the dairy wastewate. For this, fermented milk beverages were produced by a full factorial design with 2 independent variables in two equidistant levels, three replicates at the center point and 4 axial points. Ricotta cheese whey concentrations and powder milk were the two variables evaluated in 5 concentrations. The 11 formulations developed were submitted to analysis of pH, acidity, protein, fat, ash and enumeration of specific groups of lactic acid bacteria (*Lactobacillus acidophilus*, *Bifidobacterium* and *Streptococcus thermophilus*). The results obtained in the study show that is possible a preparation of a prebiotic and probiotic fermented dairy beverage prebiotic and probiotic containing till 70% ricotta cheese whey. Thus, the dairy beverage proved to be a viable way to use ricotta cheese whey, reducing the effluent generated at dairy industries, consequently, reducing the costs of treatment and environmental impact generated by inappropriate disposal.

Key words: Fermented dairy beverage, synbiotic, physico-chemical analysis, microbiological analysis, ricotta cheese whey

INTRODUCTION

Ricotta cheese whey is a highly pollutant dairy industry waste characterized by Biochemical Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) values of 50 and 80 g L^{-1}, respectively. Therefore, its improper disposal causes significant environmental issues. Nevertheless, if ricotta cheese whey is disposed into these wastewater industries, it increases the organic matter content of this environment, increasing the costs of wastewater treatment. The dairy industry, therefore, uses most of the ricotta cheese whey as a supplement feed for livestock. Moreover, some papers have presented forms of use for this byproduct (Sansonetti *et al*., 2009, 2010a, b; Pisponen *et al*., 2013). In fact, the fermented dairy beverages have been an alternative to the use of cheese whey. Due to the high content of lactose (near 4-5%) (Sansonetti *et al*., 2009; Pisponen *et al*., 2013), ricotta cheese whey can be easily fermented, constituting a raw material available for the preparation of fermented beverages. Although several authors have studied ways to use biotechnology cheese whey as a raw material (De Castro *et al*., 2009; Castro *et al*., 2013a, b; Costa *et al*., 2013; Lievore *et al*., 2015), the evaluation of potential uses for ricotta cheese whey is limited.

Fermented dairy beverage is a type of fermented milk resulting from the mixture of milk and cheese whey containing lactic culture and other dairy products (De Castro *et al*., 2009). This product has a low production cost, with focus an image of health and has also been used as an important vehicle for probiotics microorganisms to humans (Achanta *et al*., 2007; Drgalic *et al*., 2005).

The term probiotic indicates bacteria associated with beneficial effects for humans and animals. Health professionals have evidenced its beneficial effects, such as a role in immunological, digestive and respiratory functions and a significant effect in alleviating infectious diseases in children. When used in foods, these microorganisms should not only be capable of surviving the passage through the digestive tract but also have the capability of proliferating in the gut. *Lactobacillus acidophilus* (*L. acidophilus*) and *Bifidobacterium animalis* subsp. *lactis* (*B. lactis*) are the lactic acid bacteria most frequently used as probiotics, but they grow slowly in milk, being often used in combination with *Streptococcus thermophilus* (*S. thermophilus*) (Casarotti *et al*., 2014). Besides, according to Shah (2000), it is essential that the counts of viable probiotic bacteria are not less than 6 log CFU g^{-1} so to have the desired therapeutic effects.

Studies have emphasized the probiotics short life span and prebiotics contribution to increasing the viability of microorganisms in the colon (Gibson, 2004). Consequently, prebiotics can be added to formulations in order to improve probiotic's chances of survival in their host. Prebiotics are nondigestible food ingredients that benefit health by modulating microbiota of gastrointestinal tract, in addition to being used in conjunction with probiotics called synbiotic product (FAO/WHO., 2001). Presently, many fibers have been used in the development of fermented dairy products, such the polydextrose (Oliveira *et al*., 2009; Allgeyer *et al*., 2010; Magro *et al*., 2014). Polydextrose is a glucose polymer used as a thickening agent, texturing agent and soluble fiber ingredient in many food products (Raninen *et al*., 2011; Roytio and Ouwehand, 2014). This polymer can increase probiotic survival and, therefore, the beneficial health effects (Allgeyer *et al*., 2010).

The use of ricotta cheese whey prevents the environmental pollution caused by inappropriate disposal and decreases expenditure on wastewater treatment. The fermented dairy beverage is considered a nutritious and inexpensive product, so it is a viable alternative to the ricotta cheese whey disposal. In this context, the aim of this study was to develop a synbiotic fermented dairy beverage using ricotta cheese whey, adding value to this waste product and reducing the amount of cheese whey disposed into the dairy wastewater. Moreover, its shelf life was evaluated through physicochemical and microbiological analysis.

MATERIALS AND METHODS

All the experiments were conducted from January-November, 2014.

Experimental design: A Central Compound Rotational Design was used with trials identified from A to K. Ricotta cheese whey concentrations and powder milk were the two variables evaluated (Table 1). Ricotta cheese whey concentrations ranged from 30-70% (w/w) and whole powdered milk ranged from 6.5-10% (w/w). Even though the use of this type of design leads to the use of Response Surface Methodology (RSM) (Sansonetti *et al*., 2009; Ibarra *et al*., 2012) in this study the design was only used as a scientific basis. Milk powder was used instead of fluid milk in order to increase the formulation with milk proteins, whereas ricotta cheese whey has no significant proteins concentration.

Table 1: Experimental design and levels of factors in coded and real values

	Coded variables		Real variables	
Trials	X_1*	X_2**	X_1*	X_2**
A	-1	-1	7	35.8
B	-1	1	7	64.2
C	1	-1	9.5	35.8
D	1	1	9.5	64.2
E	-1.414	0	6.5	50
F	1.414	0	10	50
G	0	-1.414	8.25	30
H	0	1.414	8.25	70
I	0	0	8.25	50
J	0	0	8.25	50
K	0	0	8.25	50

*X_1: Powder milk concentration expressed in g/100 g beverage, **X_2: Ricotta cheese whey concentration expressed in g/100 g beverage

Table 2: Composition of the fermented dairy beverage formulations

Components	Values
Sacharose (%)	5.0
Powder milk (%)*	6.5-10.0
Polydextrose (%)*	1.5-5.0
Water (%)*	0.0-40.0
Ricotta cheese whey (%)*	30.0-70.0
Inoculum (%)	3.0
Potassium sorbate solution (%)**	0.5
Strawberry pulp (%)	2.5
Fruit mix of strawberry (%)	7.5

*Components that varied according to the experimental design, **0.06 g mL^{-1} sorbate solution

Fermented dairy beverage preparation: The beverages composition is shown in Table 2. The inoculum was prepared by adding 400 mg of lactic culture containing *Lactobacillus acidophilus* LA-5®, *Bifidobacterium animalis* spp. *lactis* BB-12® and *Streptococcus thermophilus* (BioRich, Chr. Hansen, Dinamarca) to 100 g of sterilized reconstituted milk (10% total solids) followed by activation at 37°C for 2 h before use. Eleven fermented dairy beverage trials were carried out (A-K) (Table 1), adapting the procedure described by De Bassi *et al.* (2012) and Castro *et al.* (2013a).

Sugar (5% w/w, Caiano, Brazil), inoculum (3.0% w/w), strawberry pulp (7.5% w/w, Mais Fruta, Brazil), strawberry fruit mix (2.5% w/w, Borsato, Brazil) and potassium sorbate solution (0.5% w/w, Synth, Brazil) were used in equal amounts in all 11 formulations. Powder milk (6.5-10% w/w, Elegê, Brazil), polydextrose (1.5-5% w/w, Sabormax, Brazil), ricotta cheese whey (30-70% w/w, obtained from the local dairy farm) and distilled water (0-40% w/w) were used in amounts established by the experimental design (Table 2). The amount sum of milk powder and polydextrose in all formulations was 11.5% and the sum of water and ricotta cheese whey was 70%.

Sugar, powder milk, ricotta cheese whey and water were mixed and heated at 90°C for 5 min. The inoculum was added after the other ingredients cooled down to a temperature of 43°C (according manufacturer instruction) and the fermentation carried out for approximately 6 h (until it reached a pH value of 4.6). Strawberry pulp (7.5% w/w, Mais Fruta, Brazil), strawberry fruit mix (2.5% w/w, Borsato, Brazil) and 0.06 g mL^{-1} potassium sorbate solution (0.5% w/w, Synth, Brazil) were added after the mix reached 20°C. The samples were stored in sealed bottles under refrigeration at 5°C awaiting analysis.

Analytical determinations: Samples of fermented dairy beverages were subjected weekly to pH and titratable acidity analysis and to ash, total solids, fat and protein analysis on the 1st and 45th storage days of cold storage. The pH levels of the samples were determined using a digital pH meter (Digimed, Brazil). The probe was adjusted by dipping into 7 E4 buffer solutions. The pH values of the samples were recorder after taken average mean from three observed readings. Acidity was determined by titration with 0.1 N NaOH solution and expressed as a percentage of lactic acid. In order to determine ash, samples were weighted on porcelain crucibles and incinerated in a muffle furnace (Marconi, Brazil), with a temperature programmed to reach 550°C. Total solids were determined through vacuum drying (Quimis Q819V, Brazil) at 70±1°C for 24 h. Fat was determined using the Roese-Gottlieb method, by treating the samples with ammonium hydroxide and ethanol to hydrolyze the protein-fat binding. Fat was separated and then extracted with petroleum ether and diethyl ether. Protein content was estimated by the determination of the total nitrogen with the Kjeldahl method using Digestion Blocks (Marconi, Brazil) and a Kjeldahl Distiller (Tecnal, Brazil) and multiplying it by a conversion factor (6.38). The analyses were conducted according to AOAC (2005) and all reagents used were of analytical grade.

Microbiological analysis: For bacteria enumeration 25 g of the samples were homogenized with 225 mL of a sterile 0.1% (w/v) peptone water. Decimal dilutions were prepared in 9 mL of sterile 0.1% (w/v) peptone water and aliquots were plated in duplicate on a variety of media. For enumeration of the lactic acid bacteria, adapted Vinderola and Reinheimer (1999) methodologies were used. *Lactobacillus acidophilus* was enumerated in the Man, Rogosa and Sharp (MRS) agar (BD, Canada) supplemented with bile (Fluka, Germany) and aerobic incubation for 37°C for 72 h. *Bifidobacterium. lactis* was quantified using MRS agar supplemented with lithium chloride (Vetec, Brazil) and sodium propionate (Sigma-Aldrich, USA) by anaerobic conditions provided Anaerobac (Probac, São Paulo, Brazil) at 37°C for 72 h. *Streptococcus thermophilus* were determined on M17 agar (Himedia, India) incubated at 37°C for 48 h.

Statistical analysis of data: The results were submitted to univariate analysis of variance and the significance of the models checked by F-test. The means of the significant models were compared by Tukey test at 5% significance level. Statistical calculations were performed using the Statistica® software, version 7.0.

RESULTS AND DISCUSSION

pH determination: Table 3 shows the values of pH for the fermented dairy beverages during refrigerated storage, varying from 3.69 (trial D) to 4.33 (trial G). Similar results were found by Tonguc *et al*. (2013) in fermented milk and Castro *et al*. (2013b) in probiotic dairy beverage. The pH values suffered several variations during the storage period. According to Lourens-Hattingh and Viljoen (2001) even after the complete fermentation, it continues slowly during cooling, which has been observed by other authors during the storage of fermented dairy beverages and fermented milk using *Lactobacillus acidophilus*, *Bifidobacterium lactis* and *Streptococcus thermophilus* (Kailasapathy *et al*., 2008; Casarotti *et al*., 2014) and in commercial strawberry flavored yogurt containing *B. lactis* (Cruz *et al*., 2010). Ranadheera *et al*. (2012) observed a decrease in pH up to the 7th day and an increase after this period, demonstrating unusual behavior as in the present study and unlike what has been shown in other studies. Kailasapathy *et al*. (2008) obtained a pH decrease in fermented dairy beverages and Castro *et al*. (2013a) observed pH stability for 80 days.

Titratable acidity analysis: According to Table 4, the acidity values of fermented dairy beverage vary from 0.69 (trial E) -0.97 (trial F) g of lactic acid/100 g. These results are in accordance with

Table 3: pH values of fermented dairy beverage during the refrigerated storage

			Days						
Trials	X_1*	X_2**	1	7	14	21	28	35	45
A	7.0	35.8	3.88^{b}	4.09^{a}	4.05^{a}	4.02^{a}	4.00^{a}	4.03^{a}	4.15^{a}
B	7.0	64.2	3.98^{b}	4.17^{a}	4.19^{a}	4.14^{a}	4.04^{b}	4.17^{a}	4.16^{a}
C	9.5	35.8	4.20^{ab}	4.18^{ab}	4.17^{ab}	4.13^{b}	4.07^{c}	4.24^{a}	4.22^{a}
D	9.5	64.2	4.22^{a}	4.18^{ab}	4.12^{c}	4.03^{d}	3.69^{e}	4.14^{bc}	4.16^{bc}
E	6.5	50.0	3.92^{a}	4.06^{a}	4.12^{a}	3.99^{a}	3.81^{a}	4.18^{a}	4.24^{b}
F	10.0	50.0	4.05^{a}	4.12^{a}	4.18^{a}	4.16^{a}	4.15^{a}	4.24^{a}	4.27^{a}
G	8.25	30.0	4.33^{a}	4.27^{a}	4.22^{a}	4.07^{ab}	3.77^{b}	4.14^{a}	4.15^{a}
H	8.25	70.0	4.21^{a}	4.26^{a}	4.23^{a}	4.16^{a}	4.06^{a}	4.25^{a}	4.22^{a}
I	8.25	50.0	4.05^{c}	4.17^{a}	4.14^{a}	4.08^{ab}	4.05^{b}	4.12^{ab}	4.12^{ab}
J	8.25	50.0	4.24^{a}	4.26^{a}	4.22^{a}	4.15^{a}	4.25^{a}	4.19^{a}	4.18^{a}
K	8.25	50.0	4.28^{a}	4.24^{a}	4.15^{ab}	4.13^{ab}	4.11^{b}	4.14^{ab}	4.13^{ab}

Analysis performed in triplicate. Different lowercase letters in the same row in indicate presence of statistical difference ($p<0.05$) among the treatments (fermented dairy beverage) along the storage days, according with the Tukey Test. *X_1: Powder milk concentration expressed in g/100 g beverage, **X_2: Ricotta cheese whey concentration expressed in g/100 g beverage

Table 4: Acidity values of fermented dairy beverage during the refrigerated storage

			Days						
Trials	X_1*	X_2**	1	7	14	21	28	35	45
A	7.0	35.8	0.80^{b}	0.76^{c}	0.76^{c}	0.80^{b}	0.82^{a}	0.70^{d}	0.70^{d}
B	7.0	64.2	0.78^{bd}	0.74^{a}	0.74^{a}	0.76^{ab}	0.79^{cd}	0.77^{abc}	0.76^{ab}
C	9.5	35.8	0.81^{bc}	0.85^{a}	0.85^{a}	0.85^{ab}	0.83^{b}	0.80^{c}	0.79^{c}
D	9.5	64.2	0.87^{ab}	0.82^{c}	0.83^{bc}	0.87^{b}	0.92^{a}	0.85^{bc}	0.84^{bc}
E	6.5	50.0	0.77^{b}	0.70^{d}	0.70^{d}	0.73^{c}	0.81^{a}	0.69^{d}	0.69^{d}
F	10.0	50.0	0.97^{a}	0.84^{c}	0.86^{bc}	0.88^{bc}	0.90^{b}	0.86^{bc}	0.85^{bc}
G	8.25	30.0	0.71^{c}	0.72^{c}	0.79^{b}	0.82^{ab}	0.86^{a}	0.73^{c}	0.72^{c}
H	8.25	70.0	0.83^{ab}	0.81^{bc}	0.81^{bc}	0.82^{ab}	0.84^{a}	0.78^{c}	0.77^{c}
I	8.25	50.0	0.71^{d}	0.72^{d}	0.75^{c}	0.80^{b}	0.85^{a}	0.77^{c}	0.76^{c}
J	8.25	50.0	0.73^{bc}	0.71^{c}	0.71^{c}	0.75^{bc}	0.81^{a}	0.77^{ab}	0.76^{b}
K	8.25	50.0	0.77^{a}	0.74^{bc}	0.74^{bc}	0.72^{c}	0.72^{cd}	0.76^{ab}	0.75^{b}

Analysis performed in triplicate; values expressed in g lactic acid /100 g Different lowercase letters in the same row in indicate presence of statistical difference ($p<0.05$) among the treatments (fermented dairy beverage) along the storage days, according with the Tukey Test. *X_1: Powder milk concentration expressed in g/100 g beverage, **X_2: Ricotta cheese whey concentration expressed in g/100 g beverage

those found by Tonguc *et al.* (2013) in fermented milks and are slightly lower than the results presented in yoghurts produced by Sengupta *et al.* (2014). The decrease in acidity was observed during the 45 days of storage, with some periods in which certain samples showed an increase in this parameter. It is common to observe the increase in acidity (and the consequent reduction in pH) of fermented dairy products caused by the natural process of lactic acid producing and other organic acids from lactose fermentation by the starter and probiotic cultures (Cardarelli *et al.*, 2007). The increase in acidity during storage was observed by Tonguc *et al.* (2013) because lactic acid production is not completely inhibited by low temperatures (Lourens-Hattingh and Viljoen, 2001). However, the results presented in Table 3 and 4 show a decrease in acidity mainly in the period between the 7th and the 28th days. Possibly proteins biodegradation resulting in ammonia generation have caused a decrease in acidity and the different results in this study.

Protein analysis: Table 5 shows that the protein concentration ranged between 2.14% (trial A) and 3.26% (trial F) on the 1st day of shelf life and between 1.88% (trial A) and 3.06% (trial F) on the 45th day. Consequently, all trials are in accordance with the Brazilian legislation (Anonymous, 2005), which requires at least 1% of protein in fermented dairy beverages. The powder milk used enabled protein levels similar to those found in fermented milks produced by Tonguc *et al.* (2013) and Lievore *et al.* (2015) who were, respectively, between 2.34-2.98 and 2.06-2.54%.

Table 5: Centesimal composition of fermented dairy beverage

			Total solids		Ash		Protein		Lipid	
			1	45	1	45	1	45	1	45
Trials	X_1*	X_2**	(Days)							
A	7.0	35.8	19.99^a	20.34^a	0.67^a	0.67^a	2.14^a	1.88^a	0.49^a	0.47^a
B	7.0	64.2	22.37^a	22.88^a	0.82^a	0.84^a	2.65^a	2.24^a	0.54^a	0.50^a
C	9.5	35.8	21.39^b	22.14^a	0.89^b	0.93^a	2.72^a	2.30^a	0.41^a	0.39^a
D	9.5	64.2	20.94^b	21.57^a	0.97^a	0.89^b	2.88^a	2.63^a	0.61^a	0.65^a
E	6.5	50.0	21.32^a	21.19^a	0.69^b	0.70^a	2.44^a	2.03^a	0.65^a	0.63^a
F	10.0	50.0	20.66^a	21.06^a	0.90^a	0.93^a	3.26^a	3.06^a	0.86^a	0.84^a
G	8.25	30.0	23.92^a	20.73^b	0.81^a	0.72^a	2.72^a	2.23^a	0.64^a	0.54^b
H	8.25	70.0	21.75^a	22.08^a	0.89^b	0.92^a	2.95^a	2.40^b	0.80^a	0.82^a
I	8.25	50.0	19.29^b	21.02^a	0.69^b	0.80^a	2.60^a	2.23^a	0.49^a	0.47^a
J	8.25	50.0	21.01^a	21.30^a	0.78^a	0.93^a	2.86^a	2.36^b	0.36^a	0.40^a
K	8.25	50.0	20.34^a	20.74^a	0.78^b	0.81^a	2.85^a	2.24^b	0.74^a	0.79^a

Analysis performed in triplicate; values expressed in g/100 g. Different lowercase letters in the same row in indicate presence of statistical difference ($p<0.05$) among the treatments (fermented dairy beverage) along the storage days, according with the Tukey Test. *X_1: Powder milk concentration expressed in g/100 g beverage, **X_2 : Ricotta cheese whey concentration expressed in g/100 g beverage

In addition, the proteolysis during refrigerated storage is cited by Cruz *et al*. (2010), who obtained an increase in proteolytic activity during 84 days of refrigerated storage of yogurt. This observation reflects the protein results (Table 5), showing a decrease in concentration during storage.

Fat analysis: Lipids levels ranged between 0.36% (trial G) and 0.86% (trial F) on the 1st day of shelf life and between 0.39% (trial C) and 0.84% (trial F) on the 45th day (Table 5). The values are lower than the results found by Tonguc *et al*. (2013) in fermented milk, probably because of the high content of ricotta cheese whey used in the present study.

Ash and total solids analysis: Table 5 shows that total solids ranged between 19.29% (trial I) and 23.92% (trial G) on the 1st day and 20.34% (trial A) and 22.88% (trial B) on the 45th day of shelf life. Despite the high content of ricotta cheese whey, the beverages produced in this study have higher values than those found on fermented milk analyzed by Tonguc *et al*. (2013). The dairy beverage produced in the present study have relatively higher ash content than the ones in other studies (Thamer and Penna, 2006; Cunha *et al*., 2008; Costa *et al*., 2013), which indicates that it has a higher mineral content. This difference is possibly due to the use of milk powder in manufacturing.

Survival of acid lactic bacteria during storage of fermented dairy beverage: The *Streptococcus thermophilus* count fluctuated during shelf life (Table 6), ranging from 6-10.96 log CFU g^{-1}. Similar counts were found by Sarvari *et al*. (2014) in yoghurts and by Castro *et al*. (2013a) in probiotic dairy beverages. Previous studies have reported a slight increase of *S. thermophilus* counts during storage up to 1 week, followed by a decrease in yogurt (Birollo *et al*., 2000; Dave and Shah, 1997a, b). Thamer and Penna (2006) observed, as in this study, a predominance of *Streptococcus thermophilus* over probiotic microorganisms.

According to Table 7, all trials showed a population decline of *B. lactis* on the 28th day of shelf life. The count of *B. lactis* ranged from 6.26-11.48 log CFU g^{-1} (respectively, trials K and F) on the 1st day and from 3.19-6.05 log CFU g^{-1} on the 45th storage day (respectively, trials F and I). Sarvari *et al*. (2014) found *B. lactis* counts between 6.79 and 6.93 log CFU g^{-1} in yoghurts during 21 storage days. Probably the low pH affected the *B. lactis* growth due to its lower acidity

Table 6: Viable *Streptococcus thermophilus* count of fermented dairy beverage during the refrigerated storage

	Days						
Trials	X_1*	X_2**	1	7	14	28	45
A	7.0	35.8	10.37^a	9.51^b	8.69^c	8.10^c	7.79^c
B	7.0	64.2	6.55^d	7.71^c	8.99^a	8.69^b	7.79^c
C	9.5	35.8	7.78^d	7.41^d	8.91^a	8.86^b	8.64^c
D	9.5	64.2	7.68^b	8.35^b	8.80^b	8.86^b	10.36^a
E	6.5	50.0	9.95^a	8.37^b	8.61^b	8.78^b	7.32^b
F	10.0	50.0	8.45^b	9.29^b	10.96^a	8.44^b	6.00^b
G	8.25	30.0	7.93^d	8.10^c	8.35^{bc}	9.13^a	8.51^b
H	8.25	70.0	8.85^c	7.90^c	10.39^a	9.64^b	9.58^b
I	8.25	50.0	7.35^c	8.45^{bc}	8.76^b	8.84^b	9.66^a
J	8.25	50.0	8.93^b	9.31^a	8.62^c	8.91^b	8.67^c
K	8.25	50.0	6.60^d	7.12^d	9.35^a	8.84^b	8.52^c

Microbiological analysis is expressed in log CFU g^{-1} of fermented dairy beverage. Different lowercase letters in the same row in indicate presence of statistical difference ($p<0.05$) among the treatments (fermented dairy beverage) along the storage days, according with the Tukey Test. *X_1: Powder milk concentration expressed in g/100 g beverage, **X_2: Ricotta cheese whey concentration expressed in g/100 g beverage

Table 7: Viable *Bifidobacterium animalis* count of fermented dairy beverage during the refrigerated storage

	Days						
Trials	X_1*	X_2**	1	7	14	28	45
A	7.0	35.8	8.19^a	6.19^b	4.82^b	3.83^b	3.66^b
B	7.0	64.2	8.61^a	7.15^b	7.33^b	4.22^b	4.59^b
C	9.5	35.8	6.92^b	7.30^b	8.42^a	5.77^b	4.74^b
D	9.5	64.2	6.45^a	5.56^b	5.05^{bc}	3.92^c	4.84^c
E	6.5	50.0	8.97^a	6.26^b	5.83^b	3.93^b	3.75^b
F	10.0	50.0	11.48^a	8.32^b	6.61^b	3.93^b	3.19^b
G	8.25	30.0	6.57^b	6.94^a	6.01^c	4.63^d	4.59^d
H	8.25	70.0	6.40^a	6.65^a	7.82^b	5.86^a	5.85^a
I	8.25	50.0	6.33^b	7.72^a	5.29^b	5.98^b	6.05^b
J	8.25	50.0	7.30^b	7.26^b	5.69^a	4.65^a	4.50^a
K	8.25	50.0	6.26^b	6.25^b	7.74^a	5.20^b	4.31^b

Microbiological analysis is expressed in log CFU/g of fermented dairy beverage. Different lowercase letters in the same row in indicate presence of statistical difference ($p<0.05$) among the treatments (fermented dairy beverage) along the storage days, according with the Tukey Test. *X_1: Powder milk concentration expressed in g/100 g beverage, **X_2: Ricotta cheese whey concentration expressed in g/100 g beverage

endurance (Dave and Shah, 1997b; Shah, 2000; Vinderola *et al*., 2000; Kailasapathy, 2006; Kailasapathy *et al*., 2008). Martin-Diana *et al*. (2003) recommend that microorganisms are inoculated with an amount equal to the one desired in the final product, especially in respect of bifidobacteria.

Table 8 shows the counts of *Lactobacillus acidophilus*. The bacteria counts declined after the 7th day of shelf life as occurred in Kailasapathy *et al*. (2008) and Ranadheera *et al*. (2012) in stirred fruit yogurts. Ranadheera *et al*. (2012), as in this study, found lower concentrations of *L. acidophilus* in the samples and a more significant decrease in relation to other microorganisms.

Senanayake *et al*. (2013) affirms that low temperatures may make the organism adaptation difficult, resulting on the decline of bacteria counts. Studies analyzing different product storage temperatures are needed to optimize the survival of probiotic microorganisms. All formulations produced in this study can be classified as fermented up to 45 days, as total count of lactic acid bacteria was greater than 6 CUF g^{-1} as required by Brazilian law (Anonymous, 2005). Moreover, all trials are considered probiotic according to the Brazilian legislation (Anonymous, 2008), which sets the minimum viable amount for probiotics in the range of 8.0-9.0 log CUF in daily recommendation of the product ready for consumption.

Table 8: Viable *Lactobacillus acidophilus* count of fermented dairy beverage during the refrigerated storage

			Days				
Trials	X_1*	X_2**	1	7	14	28	45
A	7.0	35.8	6.38^b	6.66^a	4.29^c	3.62^c	2.40^c
B	7.0	64.2	6.53^b	7.26^a	6.44^b	4.19^c	3.19^c
C	9.5	35.8	6.62^a	4.84^b	4.84^b	4.43^b	4.16^b
D	9.5	64.2	6.93^a	6.69^b	6.30^c	6.15^c	2.60^d
E	6.5	50.0	6.59^b	6.82^a	6.02^c	5.69^{cd}	4.04^d
F	10.0	50.0	6.46^b	6.60^a	6.39^c	5.63^d	2.65^e
G	8.25	30.0	5.81^c	6.07^a	5.92^d	5.62^b	3.69^e
H	8.25	70.0	7.46^a	6.84^b	6.09^c	6.10^c	3.58^c
I	8.25	50.0	7.00^a	6.79^b	6.30^c	5.70^d	4.31^d
J	8.25	50.0	6.70^a	6.06^b	5.96^b	5.94^b	2.48^c
K	8.25	50.0	6.70^a	5.89^b	5.67^b	5.64^b	2.65^c

Microbiological analysis is expressed in log CFU g^{-1} of fermented dairy beverage. Different lowercase letters in the same row in indicate presence of statistical difference ($p<0.05$) among the treatments (fermented dairy beverage) along the storage days, according with the Tukey Test. *X_1: Powder milk concentration expressed in g/100 g beverage, **X_2: Ricotta cheese whey concentration expressed in g/100 g beverage

It was not possible to verify the influence of different polydextrose concentrations on the count of probiotic microorganisms. However, the use of prebiotics is beneficial to the host, benefits such as decreasing fecal pH, increasing residual concentration of short chain fatty acids and increasing the number of bifidobacteria in feces (Jie *et al*., 2000). Thus, its use as a raw material is justified. Moreover, it can be used as a bulking agent for low calorie, aiding in adjusting the viscosity and texture of the product. Moreover, all beverages are classified as prebiotic, according to the Brazilian legislation (Anonymous, 2008), that requires a minimum concentration of 1.5% for semi-solid or liquid food.

CONCLUSION

This study demonstrated that ricotta cheese whey, a high pollution dairy industry waste, can be transformed into raw material in the development of a synbiotic fermented dairy beverage, adding value to this waste and reducing the amount of cheese whey incorporated into the dairy wastewater. The results show that dairy beverages production in this study are classified as probiotic and prebiotic according to Brazilian law. The decline in viable cell counts of *L. acidophilus* and *B. lactis* should be targeted for further study as a way to discover which parameters influenced this result during shelf life. Further studies could analyze different product storage temperatures to maintain stable scores of probiotic microorganisms.

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