



Buffalo milk increases viability and resistance of probiotic bacteria in dairy beverages under in vitro simulated gastrointestinal conditions

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ABSTRACT

Probiotic dairy beverages prepared from buffalo and cow milks with different levels of whey (0, 25, and 50%) were evaluated for kinetic fermentation parameters, protein and fat contents, post-acidification profile, viability of *Streptococcus thermophilus*, *Lactobacillus bulgaricus*, and *Lactobacillus acidophilus* during 21 d of refrigerated storage, and resistance to in vitro gastrointestinal conditions. Progressive acidification that occurred during storage of all dairy products was reduced in the presence of whey. Lactic acid bacteria showed viable cell counts at the end of shelf life, with the highest values (7.33 to 8.83 log cfu/mL) detected in buffalo dairy products. Compared with fermented cow milk products, those made with buffalo milk showed better bacterial viability during in vitro simulated gastrointestinal digestion, which suggests a beneficial protective effect on human microbiome.

Key words: buffalo milk, dairy beverage, probiotic, gastrointestinal stress, in vitro simulation

INTRODUCTION

Dairy beverages are products made by blending milk (in natura, pasteurized, sterilized, reconstituted, concentrated, powdered, whole, semi- or partially skimmed, or skim milk) with whey (liquid, concentrated, or powdered whey), vegetable fat, fermented milk or milks, selected milk ferments, and other dairy products (Ministério da Agricultura e do Abastecimento, Brazil, 2005). Whey is a by-product of the cheese industry with high nutritional value, which has serious environmental effects when improperly disposed of (Magalhães et al., 2011; Silva, 2015); therefore, its reuse is highly hoped for. With this aim in mind, when employed to

produce dairy beverages, whey not only provides them with more fluidity but also reduces their cost.

Formulation of buffalo milk beverages may expand the range of products with different qualities than those traditionally made with cow milk. Compared with cow milk, buffalo milk has, in fact, nutritional characteristics that stand out, such as higher contents of fat, protein, lactose, and some minerals (calcium, iron, magnesium, and phosphorus), lower cholesterol level, and almost double the content of CLA (Ahmad et al., 2013; Guimarães et al., 2014). In addition, its higher contents of casein and fat are able to provide the final products with better gel consistency and more creaminess, respectively (Sfakianakis and Tzia, 2014).

The so-called probiotic dairy-based beverages contain probiotic bacteria, which are known to be “live microorganisms that, when administered in adequate amounts, confer a health benefit on the host” (FAO/WHO, 2002, p. 8). Consumption of probiotics contributes to the maintenance of health through benefits resulting from their direct action on the human gastrointestinal tract, stimulating the immune system and even enhancing the host’s emotional health (de Vos and de Vos, 2012; Parker et al., 2018). To obtain potential health benefits, probiotics should be consumed regularly and must be present in sufficient viable quantities in products at the end of shelf life (about 10^6 to 10^8 cfu/mL; Ertem and Çakmakçi, 2017). Probiotic viability in products can be influenced by several factors, such as the food matrix consistency, acidity of the food matrix, and the interaction between probiotic and starter culture (Mishra and Mishra, 2013; Casarotti and Penna, 2015). Therefore, product characterization, viability of probiotics in the product at the end of shelf life, and even their ability to survive in vitro simulated gastrointestinal stress conditions are essential to check compliance with their recommended intake (Casarotti and Penna, 2015).

Based on this background, the present study aims to compare the main attributes of probiotic dairy beverages prepared with buffalo milk and cow milk, using

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different proportions of whey during refrigerated storage. Dairy beverages were evaluated in terms of kinetic parameters, protein and fat contents, post-acidification profiles, and survival of probiotics through both shelf life and in vitro simulated gastrointestinal conditions.

MATERIALS AND METHODS

Ingredients and Cultures

Dairy beverages were formulated with buffalo milk and cheese whey supplied by the Fazenda Montenegro (Sorocaba, SP, Brazil), which breeds Murrah buffaloes. Commercial pasteurized cow milk (Fazenda Bela Vista, Tapiratiba, SP, Brazil) was also used to prepare dairy beverages. Cow milk was obtained from retail stores and the corresponding whey from the handicraft preparation of Minas Frescal type cheese. The following microorganisms were used to ferment milks: *Streptococcus thermophilus* TA040 (DuPont Danisco, Sassenage, France), *Lactobacillus bulgaricus* LB340 (DuPont Danisco), and *Lactobacillus acidophilus* La5 (Christian Hansen, Hørsholm, Denmark).

Experimental Design

Dairy products were prepared according to the central composite experimental design described in Table 1, whereby they were divided depending on the milk type and the whey content used for their production. The design consisted of 6 different formulations of dairy products, 3 of which were prepared with buffalo milk (BM) and the other 3 with cow milk (CM). For the formulation of dairy drinks, cheese whey, at 0, 25, and 50% (vol/vol) proportions, was added after milk fermentation.

Fermentation and Preparation of Dairy Products

The milk base used, buffalo or cow milk, was heated at 90°C for 5 min using a Thermomix device (Vorwerk and Co. KG, TM31, Wuppertal, Germany) and subsequently transferred to sterile Schott flasks, cooled in an ice bath, and stored under refrigeration at 4°C for 24 h. For fermentation, Schott flasks containing 500 mL of buffalo or cow milk were inoculated with *Strep. thermophilus*, *L. bulgaricus*, and *L. acidophilus* cultures up to an initial count of approximately 5.0 log cfu/mL each. Flasks were transferred to a water bath at 42°C and coupled with the Cinétique d'acidification system (Ysebaert, Frépillon, France), through which we could continuously monitor pH and acidification kinetic parameters such as the acidification rate (V_{\max}) expressed in 10^{-3} pH units/min, the time and pH at

Table 1. Central composite experimental design used to prepare probiotic dairy beverages

Treatment ¹	Milk	Dairy base	
		Whey (%)	Milk (%)
BM1	Buffalo	0	100
BM2		25	75
BM3		50	50
CM1	Cow	0	100
CM2		25	75
CM3		50	50

¹BM = buffalo milk; CM = cow milk.

which V_{\max} was reached, and the times required to reach pH 5.5, 5.0, and 4.7. Fermentation was monitored via the CINAC system until pH 4.7 was reached, after which fermentation was stopped in an ice bath, and the matrix of the fermented product was broken using vertical movements of a steel rod with a perforated disk for about 60 s. The fermented dairy bases were mixed with whey in the earlier defined proportions using the Thermomix device. Subsequently, the dairy products were placed in plastic pots with metal lids and stored at 4°C for 21 d.

Determination of Fat and Protein Contents

To determine the fat content in dairy products, the Gerber method was used, which is based on the breakdown of milk emulsion. Briefly, 11 mL of the product was added slowly, with the aid of a volumetric pipette, to 10 mL of 95% sulfuric acid in a butyrometer. After addition of 1.0 mL of pure isoamyl alcohol, the butyrometer was capped with a cork stopper, carefully stirred until complete dissolution, centrifuged for 15 min at $161 \times g$, and heated in a bath at 65°C for 3 min. The lipid portion of milk was expressed as percentage of fat present in the sample (Instituto Adolfo Lutz, 1985).

The protein content in dairy products was determined via the Kjeldahl method for total nitrogen. For this purpose, 2.0 g of potassium sulfate plus 12 to 14 mL of copper sulfate used as a catalyst were added to 1.0 g of sample previously placed in a digestion tube (50 × 250 mm). After further addition of 20 mL of sulfuric acid, the tubes were routed to the digester block (TE040, Tecnal, Piracicaba, SP, Brazil), where digestion was carried out at a temperature of 350 to 420°C until the samples achieved a translucent light green color (about 2 h). After cooling, the tubes were placed in a distiller connected with 250-mL Erlenmeyer flasks containing 50 mL of boric acid, and phenolphthalein was added as an indicator. Distillation was performed after addition of 60 mL of 40% NaOH and considered complete when

the distillate volume reached 150 mL. The distillate was then titrated with 0.1 N HCl, whose consumed volume allowed calculation of the percentage of nitrogen in the sample using a conversion factor of 6.38, as indicated by the official method of the Instituto Adolfo Lutz (1985). All analyses were performed in duplicate 7 d after the fermentation and elaboration of dairy products.

Post-Acidification Profile

The post-acidification profile of dairy products was determined by measuring the pH after 1, 7, 14, and 21 d of refrigerated storage (4°C) using a digital pH meter (Mettler-Toledo, Schwerzenbach, Switzerland) duly calibrated with buffer solutions (Merck-Millipore, Darmstadt, Germany) at pH 7.0 and 4.0 (Instituto Adolfo Lutz, 1985).

Counts of Viable Bacteria

Streptococcus thermophilus and *L. bulgaricus* were counted on M17 and de Man, Rogosa, and Sharpe agar (MRS; Difco, Le Pont de Claix, France), respectively, at pH 5.4 and incubated at 37°C for 72 h. The cultivation media were prepared according to Saccaro et al. (2011). *Lactobacillus acidophilus* was enumerated using modified MRS agar medium supplemented with 50% maltose solution after incubation at 37°C for 72 h in an anaerobic jar (Dave and Shah, 1997). Anaerobic conditions were established using GasPack (BD GasPack EZ, Dublin, Ireland). Plates containing from 30 to 300 colonies were chosen for enumeration, which was expressed in colony forming units per milliliter of product (cfu/mL). Analyses were performed in triplicate over 21 d of refrigerated storage.

In Vitro Gastrointestinal Stress in Static Mode

The in vitro gastrointestinal stress resistance of microorganisms was checked in the dairy beverages on the seventh day of refrigerated storage through a static digestion model adapted from Bedani et al. (2013). This model is divided into 3 phases: (i) the gastric phase (stomach), (ii) the first enteric phase (small intestine), and (iii) the second enteric phase (large intestine). At the end of each phase, aliquots of the samples were taken for viable bacteria counting.

For the analysis, 10 mL of a solution made with 25 mL of milk beverage samples and 225 mL of saline (NaCl 0.85%) were transferred to 500-mL bottles. In the gastric phase (i), the pH of samples was adjusted to a range of 2.4 to 2.7 using 1.0 N HCl. Pepsin (Henri-farma Chemicals and Pharmaceuticals, São Paulo, SP,

Brazil) and lipase (Amano lipase G, from *Penicillium camemberti*, Sigma-Aldrich, St. Louis, MO) solutions were then added at concentrations of 3.0 and 0.9 mg/L, respectively. The flasks were then incubated in a metabolic bath (Maxi-Shake, Heto-Holten, Allerød, Denmark) at 37°C under agitation of 150 rpm for 2 h. In the first enteric phase (ii) the pH was increased to 6.0 to 6.3 using a 1.0 N NaOH solution in phosphate buffer. Subsequently, solutions of 10 g/L of bile (porcine bile extract, Sigma-Aldrich) and 1.0 g/L pancreatin (Pancreatin 3 NF, Henrifarma Chemicals and Pharmaceuticals, Cambuci, SP, Brazil) were added before the flasks were again incubated in a bath at 37°C and 150 rpm for 2 h. In the second enteric phase (iii) the pH of the same bile and pancreatin solutions used for the first enteric phase was adjusted to 7.2 to 7.4 with the previously described 1.0 N NaOH solution in phosphate buffer. Finally, the flasks were again incubated at 37°C and 150 rpm for 2 h.

Statistical Analysis

Data were treated by multivariate ANOVA and mean comparison tests using the Minitab 17.0 program (Minitab Inc., State College, PA) to determine the statistical significance of differences among means. Mean values were compared using the Tukey's test at $P \leq 0.05$.

RESULTS AND DISCUSSIONS

Fermentation Kinetics

Both buffalo and cow milks were fermented by a mixture of the *Strep. thermophilus* and *L. bulgaricus* starter culture and the probiotic bacterium *L. acidophilus*. The kinetic parameters of these fermentations are listed in Table 2.

The maximum rate of acidification (V_{max}) in cow milk (17.45×10^{-3} upH/min) was 8.2% higher than in buffalo milk, which led to significant differences in all kinetic parameters and, in particular, to 17.1, 7.5, and 5.3% reductions in the values of the times to reach pH 4.7, V_{max} , and pH 5.0, respectively (Table 2). This result may be associated with relevant differences in cow and buffalo milk compositions. Particularly, the 22% higher total solid content in buffalo milk (16.54 g/100 g) compared with cow milk agrees with the observations of other authors (Ahmad et al., 2013; Khedkar et al., 2016). According to Kristo et al. (2003), the lower the total solid content, the poorer the buffering capacity, which in turn leads to a higher pH decrease for a given amount of acid produced.

Table 2. Kinetic parameters of fermentation of buffalo and cow milks by *Streptococcus thermophilus*, *Lactobacillus bulgaricus*, and *Lactobacillus acidophilus*¹

Milk	Initial pH	$t_{pH5.5}$ (h)	$t_{pH5.0}$ (h)	$t_{pH4.7}$ (h)	V_{max} (10^{-3} upH·min ⁻¹)	t_{max} (h)	pH _{max}
Buffalo	6.65 ± 0.05 ^a	2.87 ± 0.12 ^a	3.80 ± 0.04 ^b	4.67 ± 0.04 ^b	16.13 ± 0.15 ^a	2.70 ± 0.04 ^b	5.57 ± 0.06 ^a
Cow	6.57 ± 0.03 ^a	2.73 ± 0.12 ^a	3.60 ± 0.02 ^a	3.87 ± 0.06 ^a	17.45 ± 0.31 ^b	2.50 ± 0.03 ^a	5.41 ± 0.08 ^a

^{a,b}Different superscript letters in the same column indicate statistically significant difference ($P \leq 0.05$).

¹Values are expressed as means ± SE (n = 3). $t_{pH5.5}$ = time required to reach pH 5.5; $t_{pH5.0}$ = time required to reach pH 5.0; $t_{pH4.7}$ = time required to reach pH 4.7; V_{max} = acidification rate, 10^{-3} pH units/min; t_{max} = time at which V_{max} was reached; pH_{max} = pH at which V_{max} was reached.

Almeida et al. (2009), who investigated the influence of total solid content on whey fermentation by *Strep. thermophilus* in coculture with *L. bulgaricus*, observed a 34% increase in the maximum acidification rate and an 18% decrease in the time needed to complete fermentation when the total solids content of whey was increased from 8 to 12 g/100 g. Similar values of acidification kinetic parameters were found by other authors for cow milk fermentation by different probiotic bacteria (Florence et al., 2012; Oliveira et al., 2012; Casarotti and Penna, 2015).

Fat and Protein Contents

The results of fat and protein contents of dairy products and milks are listed in Table 3. The fat contents of milks used to prepare dairy products were significantly different ($P \leq 0.05$), with that of buffalo milk (5.15 g/100 g) about 58% higher than that of cow milk. As expected, fat content in dairy products decreased when whey was added to their formulation. In particular, fermented buffalo (FBM) and cow milks (FCM) without added whey (FBM1 and FCM1) showed no significant difference ($P \geq 0.05$) compared with their respective controls (BM and CM). However, compared with controls, the fermented dairy beverages with 25% whey in their compositions (FBM2 and FCM2) showed reductions of about 28 and 21% in the fat content, whereas those with 50% whey showed reductions as large as 44% (FBM3) and 47% (FCM3). Higher fat content (9.60 g/100 g) was reported by Blasi et al. (2008) for milk of Italian buffalo.

The addition of whey also reduced the protein content in dairy products. The fermented cow milk enriched with 50% whey (FCM3) exhibited the lowest protein content (2.09 g/100 g), representing a 27% reduction compared with the same fermented milk without any whey addition (FCM1), whereas FBM3, FCM1, and FCM2 did not show any significant differences among them ($P \geq 0.05$). By contrast, the protein content of the control buffalo milk (3.53 g/100 g) was about 10% higher than that of cow milk ($P \leq 0.05$), likely due to its higher thermoresistance (Akgun et al., 2016).

Higher protein contents were reported for buffalo milks from different breeds and countries (4.54 to 4.92 g/100 g; Yadav et al., 2007; Zhou et al., 2018), whereas those of cow and buffalo milk yogurts (Guimarães et al., 2014; 2.61 to 2.71 g/100 g) were close to the values obtained in the present study for the fermented products without any whey addition (FBM1 and FCM1). It has been proposed that regional variations may greatly influence the chemical composition of buffalo milk, especially in terms of fat and protein contents (Akgun et al., 2016).

Post-Acidification Profile

As expected, pH decreased similarly throughout storage in all dairy products due to progressive lactic acid formation ($P \leq 0.05$), even though the extent of post-acidification of buffalo milk products was lower than that of cow milk products (Table 4). In fact, whereas the pH of fermented buffalo milk products ranged from 4.37 to 4.48 during refrigerated storage, consistent with observations by Akgun et al. (2016) (4.35 to 4.38), that of fermented cow milk products was 4.32 on average.

Table 3. Fat and protein contents of dairy products and milks¹

Dairy product or milk ²	Fat (g/100 g)	Protein (g/100 g)
FBM1	5.10 ± 0.08 ^a	4.28 ± 0.03 ^a
FBM2	3.68 ± 0.12 ^b	3.89 ± 0.03 ^b
FBM3	2.87 ± 0.01 ^{cd}	2.71 ± 0.01 ^c
FCM1	3.52 ± 0.14 ^b	2.88 ± 0.03 ^e
FCM2	2.56 ± 0.13 ^d	2.71 ± 0.01 ^c
FCM3	1.70 ± 0.17 ^e	2.09 ± 0.03 ^f
BM	5.15 ± 0.07 ^a	3.53 ± 0.07 ^c
CM	3.25 ± 0.21 ^{bc}	3.21 ± 0.11 ^d

^{a-f}Different letters in the same column indicate statistically significant difference ($P \leq 0.05$).

¹Values are expressed as means ± SE (n = 3).

²FBM = fermented buffalo milk product. FBM1 = FBM containing 0% whey, 100% milk; FBM2 = 25% whey, 75% milk; FBM3 = 50% whey, 50% milk. FCM = fermented cow milk product. FCM1 = FCM containing 0% whey, 100% milk; FCM2 = 25% whey, 75% milk; FCM3 = 50% whey, 50% milk.

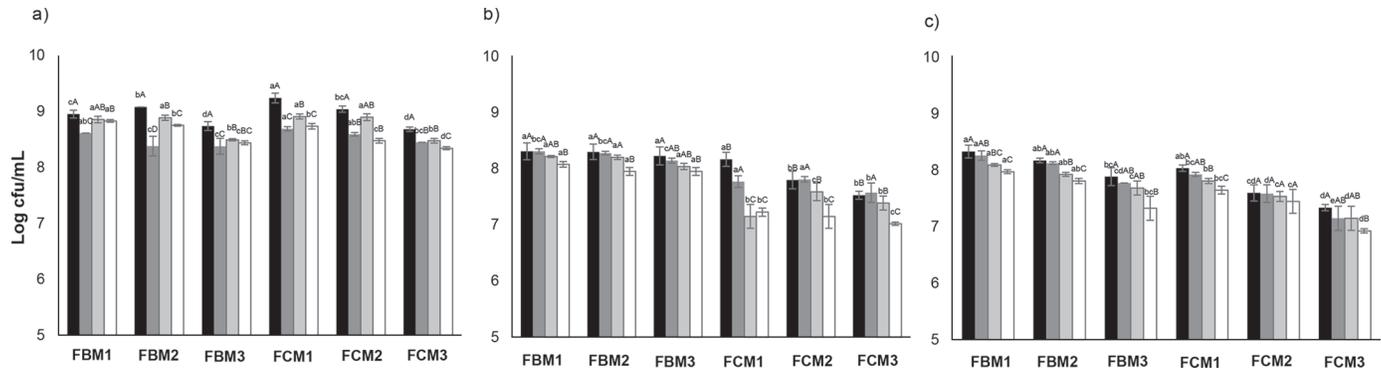


Figure 1. Viable counts of *Streptococcus thermophilus* (a), *Lactobacillus bulgaricus* (b), and *Lactobacillus acidophilus* (c) in dairy products after 1 (black bars), 7 (dark gray bars), 14 (light gray bars), and 21 (unshaded bars) d of refrigerated storage. Different lowercase letters indicate significant differences ($P \leq 0.05$) among dairy products on the same day. Different capital letters indicate significant differences ($P \leq 0.05$) for the same product throughout the storage period. Fermented buffalo milk product containing 0% whey, 100% milk (FBM1); 25% whey, 75% milk (FBM2); and 50% whey, 50% milk (FBM3). Fermented cow milk product containing 0% whey, 100% milk (FCM1); 25% whey, 75% milk (FCM2); and 50% whey, 50% milk (FCM3).

Counts of Viable Bacteria

Several factors are reported to influence viability of bacteria in dairy products, among them pH, milk matrix composition, product formulation, microbial interactions, presence of food additives, hydrogen peroxide and dissolved oxygen levels, concentration of acidic metabolites, and product buffering capacity throughout shelf life (Karimi et al., 2011; Mousavi et al., 2019; Tavakoli et al., 2019).

Although *Strep. thermophilus* enumerations were significantly different ($P \leq 0.05$) between fermented buffalo and cow milks, all they remained quite high throughout the 21-d refrigerated storage, being as high as 8.83 ± 0.02 log cfu/mL in FBM1 and 8.34 ± 0.03 log cfu/mL in FCM3 at the end of shelf life (Figure 1a). Such a stability of *Strep. thermophilus* counts in dairy

products was also observed by Casarotti and Penna (2015) and Mousavi et al. (2019).

In contrast, *L. bulgaricus* showed better viability in fermented beverages made with buffalo rather than cow milk. In particular, FBM1 showed the least decrease of *L. bulgaricus* enumeration, 8.31 ± 0.15 log cfu/mL after 1 d and 8.08 ± 0.05 log cfu/mL after 21 d of storage (Figure 1b). Compared with dairy products made with buffalo milk, those made with cow milk suffered a more significant loss in *L. bulgaricus* viability throughout storage, especially in the case of the wheyless product (FCM1; 1.02 log cfu/mL).

A gradual decrease also occurred in *L. acidophilus* counts throughout storage (Figure 1c), with the lowest value (6.93 ± 0.04 log cfu/mL) detected in FCM3. It is likely that the higher fat content in fermented buffalo milk preserved the viability of *L. bulgaricus* and *L.*

Table 4. pH values of dairy products during shelf life at 4°C for 1, 7, 14, and 21 d¹

Dairy product ²	Shelf life (d)			
	1	7	14	21
FBM1	$4.58 \pm 0.04^{b,A}$	$4.46 \pm 0.03^{c,B}$	$4.41 \pm 0.05^{b,B}$	$4.37 \pm 0.04^{b,B}$
FBM2	$4.57 \pm 0.04^{b,A}$	$4.49 \pm 0.02^{d,B}$	$4.45 \pm 0.03^{b,B}$	$4.39 \pm 0.02^{b,C}$
FBM3	$4.67 \pm 0.03^{a,A}$	$4.58 \pm 0.01^{a,B}$	$4.50 \pm 0.03^{a,C}$	$4.48 \pm 0.02^{a,C}$
FCM1	$4.45 \pm 0.02^{c,A}$	$4.41 \pm 0.02^{c,B}$	$4.33 \pm 0.01^{d,C}$	$4.26 \pm 0.01^{d,D}$
FCM2	$4.46 \pm 0.02^{c,A}$	$4.41 \pm 0.02^{c,B}$	$4.37 \pm 0.01^{d,C}$	$4.33 \pm 0.03^{c,D}$
FCM3	$4.50 \pm 0.05^{b,A}$	$4.53 \pm 0.01^{b,A}$	$4.41 \pm 0.03^{b,B}$	$4.39 \pm 0.01^{b,B}$

^{a-d}Different lowercase letters in the same column indicate significant differences ($P \leq 0.05$) among different dairy products after the same length of shelf life.

^{A-D}Different capital letters in the same row indicate significant differences ($P \leq 0.05$) for the same product after different lengths of shelf life.

¹Values are expressed as means of 3 determinations ($n = 3$) \pm SD.

²FBM = fermented buffalo milk product. FBM1 = FBM containing 0% whey, 100% milk; FBM2 = 25% whey, 75% milk; FBM3 = 50% whey, 50% milk. FCM = fermented cow milk product. FCM1 = FCM containing 0% whey, 100% milk; FCM2 = 25% whey, 75% milk; FCM3 = 50% whey, 50% milk.

acidophilus by increasing their acidity resistance during shelf life (Verruck et al., 2015). However, Akgun et al. (2016) reported that *L. bulgaricus* population was not significantly affected in buffalo milk yogurts during a 20-d storage period.

Bearing in mind that counts of probiotic bacteria in dairy products should range between 10^6 and 10^8 cfu/mL during shelf life to provide health benefits to the host, and that these microorganisms must survive through the digestive tract (Granato et al., 2010; Ertem and Çakmakçı, 2017), the results obtained in the present work are in agreement with what is recommended for a probiotic product.

Survival of In Vitro Gastrointestinal Stress

The simulation of gastrointestinal stress under the typical conditions of the human digestive system, such as pH and bile salts, allows evaluation of the influence of these conditions on bacterial viability (Uriot et al., 2017). Figure 2 illustrates the viability of lactic bacteria used in this study to produce dairy products throughout each phase of the in vitro gastrointestinal test.

In general, bacteria suffered a progressive reduction of their viability in dairy products, due to the strongly acidic conditions simulating the digestive tract (Verruck et al., 2015). The viability of microorganisms, whose counts before the simulation varied from 8 to 9 log cfu/mL, did in fact decrease significantly ($P \leq 0.05$) in all fermented products as soon as after the gastric phase. Particularly, viable counts of *Strep. thermophilus*, *L. bulgaricus*, and *L. acidophilus* were more

affected in dairy drinks made with cow milk, especially those containing 50% whey (FCM3), which suffered reductions as high as 3.66, 4.11, and 3.12 log cfu/mL, respectively.

In the first enteric phase, *L. bulgaricus* had its viability completely suppressed, no viable cells being detectable in FCM3, whereas *Strep. thermophilus* and *L. acidophilus* exhibited generalized decrease in viability, especially in FCM3, where counts fell to 2.22 ± 0.13 and 1.93 ± 0.02 log cfu/mL, respectively. As proposed by Bedani et al. (2013), the action of bile in this simulation phase may have affected bacterial phospholipids and membrane proteins, making cell homeostasis difficult. Ziar et al. (2014) also observed high sensitivity of *L. bulgaricus* and *Strep. thermophilus* to bile action.

In the second enteric phase, no viable cells of any of the microorganisms under investigation were detected in FCM3, whereas only *L. acidophilus* showed viability in FCM2 (2.42 ± 0.13 log cfu/mL). Indeed, *L. acidophilus* remained viable in all dairy products, its counts being especially high in FBM1 (4.85 ± 0.05 log cfu/mL) and FBM2 (4.19 ± 0.06 log cfu/mL). These results confirm the high survival capacity already shown by *L. acidophilus* in strongly acidic environments as well as in fermented milks with different fruit flours after in vitro gastrointestinal stress (Casarotti and Penna, 2015). In this same phase, *Strep. thermophilus* viability remained high in both FBM1 (4.65 ± 0.21 log cfu/mL) and FCM1 (2.41 ± 0.11 log cfu/mL). Although it has long been believed that this bacterium would be unable to survive the digestive tract, several studies have reported its resistance to drastic gastrointestinal conditions (Boke et al., 2010; Fang et al., 2013; Ziar et

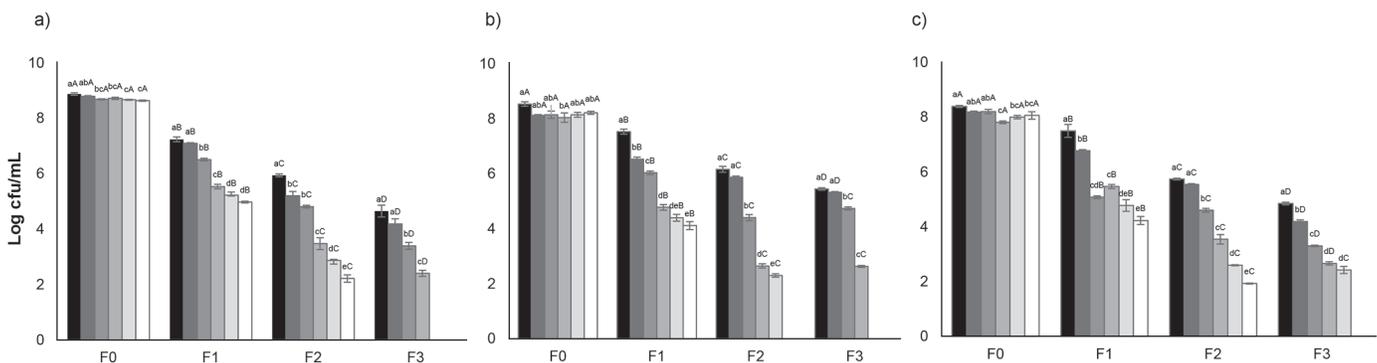


Figure 2. Survival of *Streptococcus thermophilus* (a), *Lactobacillus bulgaricus* (b), and *Lactobacillus acidophilus* (c) during in vitro simulated gastrointestinal stress tests. Fermented buffalo milk product containing 0% whey, 100% milk (black bars, FBM1); 25% whey, 75% milk (dark gray bars, FBM2); and 50% whey, 50% milk (medium-dark gray bars, FBM3). Fermented cow milk product containing 0% whey, 100% milk (medium-light gray bars, FCM1); 25% whey, 75% milk (light gray bars, FCM2); and 50% whey, 50% milk (white bars, FCM3). F0 = beginning of the test, F1 = gastric phase, F2 = enteric phase 1, F3 = enteric phase 2. Different lowercase letters indicate significant differences ($P \leq 0.05$) among dairy products in the same phase. Different capital letters indicate significant differences ($P \leq 0.05$) for the same product in different phases.

al., 2014; Junjua et al., 2016). This capacity seems to depend not only on the ability of the strain to produce extracellular polysaccharides but also on the type of carbon source available in the medium (Boke et al., 2010; Uriot et al., 2017). Ziar et al. (2014) did in fact observe greater resistance of *Strep. thermophilus* to the action of bile in media containing lactose as the only carbon source.

Although the bacteria used in this study had their populations significantly diminished throughout the gastrointestinal simulation test, they remained more viable in dairy products made with buffalo rather than cow milk. The higher fat content in buffalo milk may have exerted a protective effect on cultures from the gastrointestinal stress by increasing their resistance to both acidic conditions and bile action (Verruck et al., 2015). Likewise, Ranadheera et al. (2012) observed higher survival of *L. acidophilus* in ice cream and goat milk yogurt containing 10% fat submitted to *in vitro* gastrointestinal stress.

CONCLUSIONS

Streptococcus thermophilus, *L. bulgaricus*, and *L. acidophilus* were used to produce fermented products from buffalo and cow milks. Dairy products made with buffalo milk showed lower post-acidification during shelf life than those made with cow milk. In addition, the viability of lactic acid bacteria used to prepare the fermented products was better preserved in fermented buffalo milk for up to 21 d of refrigerated storage. In particular, the viability of *L. acidophilus* was so high as to guarantee the number of viable cells recommended for probiotic products. Likewise, during *in vitro* simulation of gastrointestinal stress, the bacteria used to produce milk beverages displayed better survival in buffalo milk, which could then be used as a promising alternative milk matrix for the delivery of probiotics. Future studies will be performed with whey derived from buffalo milk products, to evaluate the differences with those derived from local cheese production using cow milk.

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