J. Dairy Sci. 96:96–100
http://dx.doi.org/10.3168/jds.2012-5576

Short communication: Effects of different whey concentrations on physicochemical characteristics and viable counts of starter bacteria in dairy beverage supplemented with probiotics

W. F. Castro,*[1] A. G. Cruz,* D. Rodrigues,* G. Ghiselli,* C. A. F. Oliveira,† J. A. F. Faria,* and H. T. Godoy*
*Universidade Estadual de Campinas (UNICAMP), Faculdade de Engenharia de Alimentos (FEA), Cidade Universitária Zeferino Vaz, 13083-862, Campinas, São Paulo, Brazil
†Universidade de São Paulo (USP), Faculdade de Zootecnia e Engenharia de Alimentos Campus da USP (FZEA), Pirassununga, 13635-900, São Paulo, Brazil

ABSTRACT

Fermented dairy beverages supplemented with the probiotics *Lactobacillus acidophilus* and *Bifidobacterium lactis* containing different concentrations of whey in their formulas (0, 20, 35, 50, 65, and 80%, vol/vol) were processed and checked for pH; proteolysis; levels of glucose, lactose, ethanol, acetic acid, lactic acid, diacetyl, and acetaldehyde; and lactic bacteria and probiotic counts. The results allowed the effect of whey concentration on the dairy beverages to be observed for each of the different parameters analyzed. The degree to which the whey concentration was useful for the microbial cultures, particularly probiotic cultures, appeared to have a limit. In general, dairy beverages processed with different levels of whey in their formulation exhibited good potential as a food matrix for supplementation with probiotic bacteria, with production of characteristic compounds of fermented milk products, such as volatiles and organic acids.
Key words: dairy beverage, metabolite, probiotic, whey

Short Communication

The growth of the market for fermented milks constitutes an opportunity for the development of whey-based beverages of high nutritional and sensory value, along with a considerable reduction in processing costs (Gallardo-Escamilla et al., 2005), to the extent that the levels of lactose and other nutrients essential for microbial growth naturally present in whey provide better conditions for growth and viability of the microorganisms (Panesar et al., 2007; Magalhães et al., 2011).

In addition to the capacity to generate antihypertension peptides (Madureira et al., 2010), whey alone intrinsically imparts a series of human health benefits (Madureira et al., 2007). It has been used as a raw material for developing and manufacturing edible antimicrobial films (Ramos et al., 2012) and microcapsules (Rodrigues et al., 2011). Recently, the consumption of whey has been suggested as a possible reason for the longevity of the Portuguese people (Tavares and Malcata, 2012). With respect to processed food products, dairy products, including cheeses (Madureira et al., 2011) and dairy beverages (Dragone et al., 2009; Pescuma et al., 2010) containing whey in their formulation, have shown potential for supplementation with probiotic bacteria.

However, the effect of different levels of added whey on the survival and metabolism of probiotic bacteria has not been evaluated so far. Within this context, the objective of this study was to assess the survival and metabolism of probiotic bacteria in dairy beverages formulated with different levels of whey.

The probiotic beverages were formulated with pasteurized milk [pH 6.63, 3% (wt/vol) fat content] from the São José Dairy Farm located in Santo Antônio de Posse, São Paulo, Brazil, and whey [pH 6.26, 1.24% (wt/vol) protein content] obtained during the manufacture of Minas Frescal cheese by the enzyme coagulation process, with the whey drained off before salting. In total, 6 different beverages were manufactured, containing 0, 20, 35, 50, 65, and 80% whey (vol/vol), respectively, with the remaining percentage volume being completed with milk, taking into account the volume of inoculum. Sugar (Caravela, Ariranha, São Paulo, Brazil) was added at a concentration of 10% (wt/vol) of the probiotic beverage, and the resulting mixtures were subjected to a heat treatment of 83°C for 15 min. Upon conclusion of the heat treatment, the mixtures were cooled to 46°C and the following were added: (1) 1% (wt/vol) of a ready-to-use fruit preparation (Industrial Duas Rodas, Jaraguá do Sul, Santa Catarina, Brazil) consisting of strawberry pulp and colorant to prepare the beverages; (2) 1% starter culture inoculum (vol/vol); and

Received March 27, 2012.
Accepted September 1, 2012.
[1]Corresponding author: wellfreitas@gmail.com

(3) 2% (vol/vol) of the amount of the probiotic culture, proportional to the product volume. The inocula were prepared from reconstituted skim milk powder (Molico, São Paulo, Brazil; 11% wt/vol), with addition of direct vat set (DVS) cultures of *Streptococcus thermophilus* (TA-40; DuPont, Copenhagen, Denmark) and *Lactobacillus delbrueckii* ssp. *bulgaricus* (LB-340; DuPont), plus a probiotic culture consisting of a blend of *Lactobacillus acidophilus* (La-14; DuPont) and *Bifidobacterium lactis* (Bl-07; DuPont). The inocula media were prepared using DVS cultures at concentrations of 0.1 g of probiotic and 0.05 g of starter culture (each per liter).

The resulting samples were then kept at 45°C during fermentation. Once pH 4.7 ± 0.01 was reached, fermentation was interrupted by cooling to 8°C, and the test samples were stored under refrigeration (5 ± 1°C) in 300-mL plastic polyethylene terephthalate bottles.

The probiotic dairy beverages were submitted to the following analyses at 7 d of refrigerated storage, using previously published methodologies: pH (Marshall, 1993); proteolytic activity using the *o*-phthaldialdehyde (OPA) method (Church et al., 1983); glucose, lactose, lactic acid, and acetic acid contents (Donkor et al., 2005); and diacetyl and acetaldehyde (Condurso et al., 2008). The biochemical analyses were carried out in triplicate. For the microbiological analyses, the following analytical methodologies were used: *Strep. thermophilus* (M17 agar; Difco, Detroit, MI), pour plate, incubated aerobically for 2 d at 37°C; *Lactobacillus bulgaricus* counts [de Man, Rogosa, Sharpe (**MRS**) agar; Oxoid, Basingstoke, UK], pH 5.2, pour plate, incubated anaerobically for 3 d at 45°C (Cruz et al., 2012b); *Bifidobacterium lactis* count (MRS agar; Oxoid), supplemented with 0.6% LiCl (wt/wt; Synth, Diadema, Brazil); and 0.9% sodium propionate (wt/wt; Sigma-Aldrich, St. Louis, MO), pour plate, incubated anaerobically (GasPak, Oxoid) for 3 d at 37°C (Zacarchenco and Massaguer-Roig, 2004); and *Lactobacillus acidophilus* count (MRS agar; Oxoid), supplemented with 0.15% bile salt (wt/wt; Oxoid), pour plate, incubated aerobically for 3 d at 37°C (Mortazavian et al., 2007). All the culture media were tested previously, aiming to guarantee their selectivity regarding the microorganisms. The microbiological analyses were carried out in duplicate.

The experiments were repeated 2 times. The data were submitted to one-way ANOVA, with the probiotic whey beverage considered the source of variation. When needed, the calculations were followed by Tukey's test ($P < 0.05$). All analyses were carried out using Assistat software (version 7.6 β, 2011; Paraíba, Brazil).

Table 1 depicts the results of the biochemical and microbiological analyses of the probiotic dairy beverages produced with different levels of whey. In general, the effect of the increase in whey concentration on the dairy beverage could be observed in the various parameters assessed ($P < 0.05$). However, the extent to which the whey concentration could be used (i.e., further converted) by the microbial cultures beyond a certain point did seem to have a limit, particularly for the probiotic cultures. On the one hand, this should be seen as a potential advantage because it demonstrates the possibility of developing products with high whey concentrations and high microorganism counts that are beneficial to human health. On the other hand, it makes it absolutely necessary to conduct consumer tests of the product to improve the beverage formulation so as to yield a final product that is both functional and acceptable to consumers.

Proteolysis in fermented milk occurs from the metabolism of proteins to obtain essential AA during the growth of microorganisms and contributes to the development of their sensory properties in the fermented milks, such as aroma, flavor, and texture (Savijoki et al., 2006). Proteolysis increased as the concentration of whey increased to 65% of whey (vol/vol; $P < 0.05$), suggesting a limited use of the cheese whey nutrients by the lactic and probiotic bacteria.

The *Strep. thermophilus* and *L. bulgaricus* counts ($P > 0.05$) were higher than 8 log cfu/mL even after 7 d of refrigerated storage, in agreement with other recent

Table 1. Microbiological viable counts and physicochemical analyses of probiotic dairy beverages after 7 d of storage at 5°C[1]

Whey (%)	pH	Proteolysis	ST	LB	LA	BL	Lactose	Glucose	Acetic acid	Lactic acid	Diacetyl	Acetaldehyde	Ethanol
0	4.09	0.39^{bc}	8.77^{a}	8.31^{a}	8.83^{a}	4.24^{a}	25.70^{b}	25.00^{a}	0.45^{a}	8.89^{a}	31.05^{d}	3.83^{e}	40.41^{d}
20	4.07	0.29^{c}	8.66^{a}	8.28^{a}	8.69^{a}	4.15^{a}	26.30^{b}	26.40^{a}	0.59^{a}	8.74^{a}	63.13^{c}	14.53^{cd}	73.67^{a}
35	4.14	0.46^{b}	8.82^{a}	8.02^{a}	8.70^{a}	4.15^{a}	30.70^{ab}	29.40^{a}	0.52^{a}	8.45^{a}	44.00^{cd}	8.55^{de}	70.15^{ab}
50	4.08	0.47^{b}	8.63^{a}	8.05^{a}	8.77^{a}	3.00^{b}	35.20^{a}	28.90^{a}	0.45^{a}	8.06^{ab}	109.18^{b}	26.22^{b}	52.52^{bcd}
65	4.11	0.64^{a}	8.82^{a}	8.02^{a}	8.78^{a}	4.00^{a}	36.50^{a}	32.50^{a}	0.54^{a}	7.14^{ab}	37.60^{d}	21.14^{bc}	47.02^{cd}
80	4.07	0.48^{b}	8.73^{a}	8.08^{a}	8.69^{a}	4.54^{a}	35.50^{a}	30.30^{a}	0.50^{a}	6.08^{b}	256.00^{a}	43.32^{a}	62.80^{abc}

[a–e]Different superscript letters within the same column indicate a statistical difference among treatments ($P < 0.05$).

[1]Proteolysis is expressed in absorbance at 340 nm. *Streptococcus thermophilus* (ST), *Lactobacillus bulgaricus* (LB), *Lactobacillus acidophilus* (LA), and *Bifidobacterium lactis* (BL) are expressed in log colony-forming units per milliliter. Lactose, glucose, acetic acid, and lactic acid are expressed in milligrams per milliliter. Ethanol, diacetyl, and acetaldehyde are expressed in micrograms per milliliter.