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Short communications

Survival of *Lactobacillus delbrueckii* UFV H2b20 in ice cream produced with different fat levels and after submission to stress acid and bile salts

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ABSTRACT

The survival of *Lactobacillus delbrueckii* UFV H2b20 in three ice cream formulations (low fat, fat free and high fat) was evaluated after the processing and storage at −16 °C. The survival of *L. delbrueckii* UFV H2b20 was not significantly affected ($P > 0.05$) in three ice cream formulations after processing. The same result was observed during storage for 40 days at −16 °C. Cells of *L. delbrueckii* UFV H2b20 incorporated in three ice cream formulation survived when exposed to acid stress and bile salts. The results demonstrate that *L. delbrueckii* UFV H2b20 has potential for being used in ice cream and capacity to resist acid stress and to grow in the presence of bile salts. This demonstrates that reduction of fat in ice cream does not compromise the viability of *L. delbrueckii* UFV H2B20.

1. Introduction

Probiotic bacteria are defined as living microorganisms which confer health benefits to their hosts (FAO/WHO, 2001), when administered in adequate amounts. Foods containing such bacteria belong to the "functional foods" category. Such foods are described as "foods claimed to have a positive effect on health" (Granato, Branco, Nazzaro, Cruz, & Faria, 2010). Functional foods must contain at least 10^6–10^7 cfu g^{-1} probiotic bacteria at the time of purchase. Probiotic-containing products have become the primary choice for consumers because of their health benefits.

Lactobacillus delbrueckii UFV H2b20 was isolated in the Universidade Federal de Viçosa (Santos, 1984), and studies have demonstrated its probiotic potential (Neumann et al., 1998). Furthermore, these cells exhibited appropriate technological characteristics when this strain was incorporated in symbiotic cottage cheese, it presented good survival rate under conditions simulating those of the gastrointestinal tract (Araújo, Carvalho, Leandro, Furtado, & Moraes, 2010), after 15 days of storage at 5 °C.

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Ice cream is an ideal matrix for delivery of probiotic organisms to the human body compared to fermented dairy products. The pH of ice cream is closer to neutral, whereas that of fermented dairy could be much lower, and low pH may affect the survival and metabolic activity of probiotic bacteria (Akin, Akin, & Kirmaci, 2007). Nevertheless, freezing and thawing may seriously damage the cells, causing death (lethal effect), or inhibition of multiplication and/or interruption of metabolic activity (sublethal effect), which could defeat the potential advantages of probiotics (Santivarangkna, Kulozik, & Foerst, 2008).

In this context, this study aimed to evaluate the survival of *L. delbrueckii* UFV H2b20 in three ice cream formulations, after processing and during storage. Resistance to the production of ice cream will also be assessed by the ability of the cells incorporated in ice cream to survive under acid stress and maintain growth capacity in the presence of bile salts.

2. Materials and methods

2.1. Micro-organisms and growth

The present work used *L. delbrueckii* UFV H2b20, obtained from the culture collection of the Industrial Microbiology Laboratory of the Department of Microbiology, at the Universidade Federal de Viçosa, Minas Gerais, Brazil. Stock cultures in MRS broth were mixed with glycerol (20%, v/v) and stored at −80 °C. *L. delbrueckii* UFV H2b20 was grown at 37 °C for 12 h in cheese whey stabilized and sterilized at 121 °C for 15 min.

2.2. Ingredients for ice cream production

The ice cream mixes were blended using the ingredients and proportions listed in Table 1. For each sample, a 2 L batch of ice cream mix was prepared. Three processing trials were conducted for each fat level. Full fat cream (UFV Funarbe Dairy, Viçosa, Brazil) was used for the ice cream with the highest fat rate. The percentage of fat cream used was 35%. The ingredient inulin was used to replace a portion of the cream used for ice cream production. The ingredients were added to the mixing tank for the preparation of the ice cream mixes, in the following order: water, cream and/or inulin, skim milk powder (Molico Nestlé®, São Paulo, Brazil), granular sugar, stabilizer and emulsifier blends.

2.3. Preparation of culture

The culture containing the probiotic cells was prepared from 1 L of cheese whey which inoculated with 1% of *L. delbrueckii* UFV H2b20. After incubation at 37 °C for 12 h, cells were collected by centrifugation (Mikro 200R, Hettich Zentrifugen) at 8200*g* for 10 min at 4 °C, and washed twice with phosphate buffer saline (PBS, 0.05 M pH 7.2).

2.4. Ice cream production

The ice cream mix was performed then batch was pasteurized at 82 °C for 30 min, cooled to 4 °C and stored for approximately 24 h. The probiotic culture was added after cooling at 4 °C, to keep about 10^8 Colony Forming Units for gram (CFU g^{-1}). Then, the mixtures were frozen/aired, in ice cream maker (Carpigiani, capacity of 5 L). The ice creams obtained were put in rectangular plastic packing, and stored in horizontal freezer to −16 °C.

2.5. Microbiological analysis

The preparation of samples and dilutions for microbiological tests was performed (APHA, 2001). The number of viable cells of *L. delbrueckii* UFV H2b20 was carried out using the *Pour plate* technique on MRS agar. Duplicate plates were incubated microaerofilically at 37 °C for 48 h. After, the CFU g^{-1} of the ice cream was determined and the results expressed on a Log_{10} scale.

2.6. Survival under stress acid and bile salts presence

One gram ice cream was mixed with 9 ml of PBS (pH 7.2). The pH was adjusted to pH 3.0 and 7.0 (control) using a solution of HCl (2 M), respectively. Each mixture was incubated at 37 °C for 3 h (Brashears, Jaroni, & Trimble, 2003; Conway, Gorbach, & Goldin, 1987). After incubation, numbers of viable cells of *L. delbrueckii* UFV H2b20 were determined by plating serial dilutions (with PBS, pH 7.2) on MRS agar at 37 °C for 48 h. The results expressed on a Log_{10} scale.

Effects of bile on the growth of *L. delbrueckii* UFV H2b20 when previously exposed to acid stress were evaluated. After acid (pH 3.0) treatment, the surviving *L. delbrueckii* UFV H2b20 were collected by centrifugation (8200*g*, 4 °C, 5 min) and

Table 1 – Formulations of ice creams.

Ingredients	Fat free (%)	Low fat (%)	High fat (%)
Non-fat milk powder	11	11	11
Fat	0.3	6	12
Inulin[a]	9.0	2.5	
Sugar	15	15	15
Stabilizer[b]	0.1	0.1	0.1
Emulsifier[c]	0.5	0.5	0.5
Vanillin	0.003	0.003	0.003
Total solids	35.9	35.1	38.6

[a] Inulin-based fat replacer (Raftiline®, Orafti, Tienen, Belgium).
[b] Danisc Cultor, Kansas, United States.
[c] Kerry Bio-Science, Norwich, United States.

washed once with PBS (pH 7.2). They were resuspended in 10 ml MRS broth with or without 0.3% (w/v) bile salts and incubated at 37 °C for 12 h. Bile tolerance of the *L. delbrueckii* UFV H2b20 cells was determined by comparing the viable counts on MRS agar after incubation of 37 °C for 12 h. The results expressed on a Log_{10} scale.

2.7. Statistical analysis

The experiment was conducted in a completely randomized factorial design with three repetitions. The results were submitted to analysis of variance (ANOVA) to determine significant differences ($P < 0.05$) between the different types of treatments, using SAS® 9.1 software (SAS Institute Inc., Cary, NC, USA).

3. Results and discussion

3.1. Effect of ice cream manufacturing process and storage on the viability of L. delbrueckii UFV H2b20

Since the effectiveness of the consumption of probiotics on human health is related to their viability, it is of utmost importance not only to minimize cell death during the freezing process but also to ensure minimal loss in the viability of bacteria during storage. The survival of *L. delbrueckii* UFV H2b20 in ice cream was evaluated after processing (Fig. 1). The results show that the three samples of mixes do not differ significantly, which demonstrates that the addition of the biomass used for inoculation was carried out evenly. After processing, the number of viable cells was the same for the three ice cream samples, which reveals that the freezing stages, aeration and formulation did not affect survival. Formulations for ice cream production with different fat and sugar concentrations do not affect the survival of *Lactobacillus johnsonii* La 1 and *Lactobacillus rhamnosus* GG (Alamprese, Foschino, Rossi, Pompei, & Savani, 2002; Alamprese, Foschino, Rossi, Pompei, & Savani, 2005). However, it has been observed that the freezing process caused a significant decrease in the viability of *Lactobacillus acidophilus* La-5 and *Bifidobacterium animalis* Bb-12 in low fat ice cream (Akalin & Eris Ir, 2008). The stability of *L. delbrueckii* UFV H2b20 after the production of ice cream may be associated with low overrun presented by the three ice cream formulations (data not show). Higher values of overrun demonstrably affect the viability of probiotic bacteria (Ferraz et al., 2012).

After 40 days of storage at −16 °C, the ice cream samples did not differ significantly ($P > 0.05$) as for percentage of survival during the storage period (Fig. 2). The number of viable cells in the ice cream samples during the 40 days of storage did not differ significantly ($P > 0.05$) in relation to the number of viable cells after processing. Furthermore, the use of inulin to replace fat partially and totally in the ice cream samples did not affect the microorganism survival during the 40 days of storage. The stability of probiotic bacteria in ice cream during storage has been also observed with strains of *L. acidophilus* and *L. rhamnosus* (Abghari, Sheikh-Zeinoddin, & Soleimanian-Zad, 2011).

3.2. Acid and bile tolerance in L. delbrueckii UFV H2b20 incorporated in ice cream

Gastrointestinal tract is the location where viability of probiotic bacteria is mostly affected. According to Zárate, Morata de Ambrosini, Perez Chaia, and Gonzáles (2002), the stabilities of probiotic bacteria obtained from either *in vivo* or *in vitro* study are similar. For acid tolerance study, PBS or animal gastric liquid could be used. In this study, the viable *L. delbrueckii* UFV H2b20 in each ice cream formulation was determined after 3 h incubation in PBS buffer (pH 3.0). Results from Table 2 show that viable *L. delbrueckii* UFV H2b20 was not significantly affected by acidity in the three ice cream formulations. Acidity in human gastrointestinal content varies from pH 1.5 to 4.5, depending on feeding intervals and food variability. The survival rate of probiotic bacteria in stomach will thus increase in the presence of food, which affects the pH value

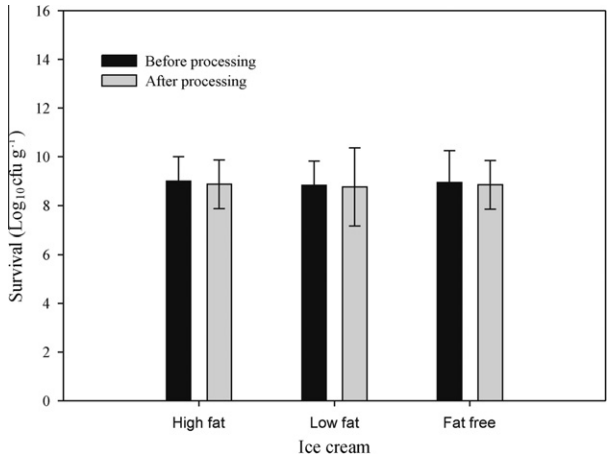

Fig. 1 – Counts of *Lactobacillus delbrueckii* UFV H2b20 strain in mixes and ice creams newly produced. All experiments were performed in duplicate and replicate at least three times.

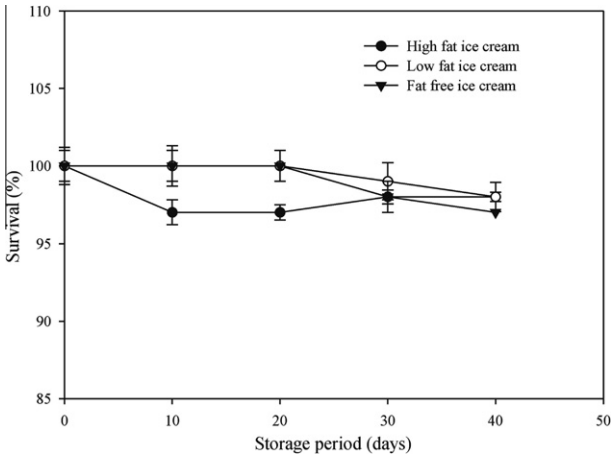

Fig. 2 – Survival rate of *Lactobacillus delbrueckii* UFVH2b20 in ice cream samples after 40 days of storage at −16 °C. All experiments were performed in duplicate and replicate at least three times.

Table 2 – Effects of pH on the survival for Lactobacillus delbrueckii UFV H2b20.

Ice cream	Viable counts ($log_{10}CFU.g^{-1}$)[a]		
	0 h* pH 7.0	3 h pH 3.0	pH 7.0
High fat	8.74 ± 0.06	8.80 ± 0.12	8.81 ± 0.08
Low fat	8.70 ± 0.17	8.80 ± 0.01	8.77 ± 0.12
Fat free	8.72 ± 0.19	8.90 ± 0.10	8.83 ± 0.16

* Cells counts after 30 storage days at – 16 °C.
[a] Each value in the table represents the mean value ± standard deviation (SD) from three trials.

Table 3 – Effects of bile salt on the growth of Lactobacillus delbrueckii UFV H2b20.

Ice cream	Viable counts (log_{10} CFU.g^{-1})[a]		
	Viable counts after acid (pH 3.0) treatment	Counts after 12 h incubation	
		Without bile salt	With bile salt (0.3%)
High fat	8.80 ± 0.12	9.36 ± 0.04	9.09 + 0.01
Low fat	8.80 ± 0.01	9.45 ± 0.11	9.13 + 0.00
Fat free	8.90 ± 0.10	9.36 ± 0.06	9.14 + 0.02

[a] Each value in the table represents the mean value ± standard deviation (SD) from three trials.

and may protect the probiotic bacteria from the effects of pepsin and acid in the stomach (Chen, Cao, Ferguson, Shu, & Garg, 2011).

Bile salts can also greatly affect the viability of lactic acid bacteria (Bustos, Raya, Bru, Valdez, & Taranto, 2011). The composition of human bile juice is not exactly the same as that of the 0.3% bile salts solution. In this study, *L. delbrueckii* UFV H2b20 survived acid treatment (pH 3.0), cultured in MRS broth, with or without 0.3% bile salts, for the assessment of its tolerance to bile. After 12 h cultivation, effects of bile salt on the growth of *L. delbrueckii* UFV H2b20 were observed (Table 3). The growth of *L. delbrueckii* UFV H2b20, incorporated in three ice cream formulations, in MRS broth supplemented with bile salts was not significantly affected. This ability to grow in the presence of bile salts demonstrates that *L. delbrueckii* resists very well to ice cream production process and storage. The use of inulin to replace fat does not affect the protection of these stress conditions. Resistance of *L. delbrueckii* UFV H2b20 to the action of bile salts seems to depend on the physicochemical properties of the cellular envelopes. Alamprese et al. (2002) reported that freezing and thawing, as well as frozen storage, damaged cell membrane, rendering the microorganisms sensitive to bile salts. Therefore, agitation and the consequent physical forces used in the production of ice cream in batch freezer may have caused additional physical damages to the cells and increased the exposure of cell to bile.

4. Conclusion

Use of inulin to replace fat partially and totally in ice cream does not affect the viability of *L. delbrueckii* UFV H2B20 after processing, during storage and under conditions of stress acid and bile salts presence. This demonstrates that reduction of fat in ice cream does not compromise the viability of *L. delbrueckii* UFV H2B20.

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