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COMMUNICATION

Spray Chilling Microencapsulation of *Lactobacillus acidophilus* and *Bifidobacterium animalis* subsp. *lactis* and Its Use in the Preparation of Savory Probiotic Cereal Bars

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Abstract The use of probiotic microorganisms has been limited by the difficulty of maintaining their viability during processing and throughout the product's shelf life. This study evaluated the viability of microencapsulating *Lactobacillus acidophilus* (LA) and *Bifidobacterium animalis* subsp. *lactis* (BL) using the spray chilling technique to add them to savory cereal bars. The results showed that spray chilling generated a powder that was composed of smooth and continuous spheres with low moisture content and low water activity. The microencapsulated microorganisms exhibited a storage viability at least of 90 days as microparticles and in savory cereal bars, and their counts were superior to those resulting from other methods of adding activated and lyophilized probiotics to savory cereal bars. Thus, microparticles prepared by spray chilling are good vehicles for incorporating probiotics into cereal bars and have the potential to release the probiotics in the consumers' intestines by means of fat digestion. Savory cereal bars that did and did not contain probiotics exhibited no differences in sensorial acceptance or commercial potential.

Keywords Spray cooling · Spray congealing · Viability · Sensorial acceptance · Nutritional facts

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Introduction

Innovation is necessary for finding opportunities to better meet the needs of customers within a market segment. The closer a product is to consumers' needs, the higher the chance that it will be successful and accepted in the market (Rozenfeld et al. 2006). Innovation in the food market is closely linked to the search for safe and nutritional foods that avoid or even treat health-related issues (Gutkoski et al. 2007).

Probiotic products meet the population's requirements for health and well-being. However, the use of probiotic microorganisms in foods remains limited due to the difficulty of maintaining their viability during processing and throughout their shelf life. One potentially feasible solution is microencapsulation. In the food sector, this method has been used to solve problems with the instability of probiotics and to enable new applications of them (Fávaro-Trindade et al. 2011; Bastos et al. 2014).

Microencapsulation techniques such as spray drying, spray chilling, fluidized bed coating, and extrusion have been extensively employed to prepare probiotic microcapsules. However, some studies have demonstrated that the spray chilling method could be an efficient alternative because, although it is similar to spray drying, it is based on injecting cold air to solidify the particles, which allows a larger number of microbes to survive (Heidebach et al. 2012; Okuro et al. 2013a). In fact, the microcapsules produced by spray chilling efficiently protect probiotics as they pass through gastric and intestinal fluids (Pedroso et al. 2012, 2013; Okuro et al. 2013b); in addition, these structures enable the release of cells in the intestinal tract during fat carrier digestion.

On the basis of these considerations, this study focused on microencapsulating *L. acidophilus* (LA) and *B. animalis* subsp. *lactis* (BL) using spray chilling before adding them to

savory cereal bars, thus creating a new functional product with greater sensory appeal for consumers.

Materials and Methods

Materials

Pure and freeze-dried LA and BL (Sacco, São Paulo, Brazil) in direct vat set (DVS) form were maintained at a temperature of −18 °C. A vegetable fat (Tri-HS-48; Triângulo Alimentos, Itápolis, Brazil) with a melting point of 51 °C was used as a carrier.

Microencapsulation

The probiotics were encapsulated using the method described by Okuro et al. (2013b) with some modifications. The lyophilized probiotic (LA or BL) at 4 % (m/m) was mixed with the molten carrier using an Ultra Turrax homogenizer (IKA® T-25; Staufen, Germany) at 7000 rpm for 60 s. These dispersions were maintained under magnetic agitation and heating in a water bath (±55 °C) and were delivered by a peristaltic pump (Masterflex model 77201-62; Illinois, USA) to a spray chiller (Labmaq, Ribeirão Preto, Brazil) for atomization using a double atomizer (Ø 1.2 mm) in a cooled chamber (15 °C ± 2 °C) with an air pressure of 5 bar. The microparticles were stored in closed containers in the presence of oxygen and frozen at −18 °C until use.

Enumeration of LA and BL

Prior to and immediately after atomization, samples were taken to assess the viability of the probiotic microorganisms. The viable cells were counted by pour plating in MRS agar (de Man, Rogosa, and Sharp) using the method described by Grosso and Fávaro-Trindade (2004). Serial dilutions with 2 % sodium citrate heated to 55 °C as the diluent were used to liquefy the lipid particles and release the microorganisms. The plates were incubated at 37 °C for 72 h in jars that contained anaerobic atmosphere generators (Probac of Brazil; São Paulo, Brazil).

Characterization of the Microparticles

The moisture content of the microparticles was determined using an infrared balance (Ohaus® MB35; Halogen, USA), and the water activity was analyzed using an AquaLab meter (Decagon Devices, Pullman, Washington, USA). The particle size was determined using a Shimadzu Sald-201V laser diffraction particle analyzer (Kyoto, Japan) with ethanol for dispersing the particles. The microparticle morphology was analyzed by scanning electron microscopy (SEM). The SEM images were captured using an acceleration voltage of 5 kV and a current of 1750 mA.

Viability of Encapsulated Microorganisms During Storage

To determine the shelf life of the microparticles stored at −18 °C, the probiotics in the microparticles were counted after 0, 30, 60, 90, and 120 days of storage using the enumeration technique described previously.

Preparation of the Savory Cereal Bars

Three formulations of savory cereal bars (A, B, and C) that contained different combinations of ingredients were prepared (Table 1). The formulations were developed on the basis of previous experiments that were conducted using information from the literature (Rodrigues et al. 2011; Gutkoski et al. 2007) and popular knowledge of the ingredients.

The formulations were prepared by individually weighing each ingredient and manually mixing them to formulate the final product. All the ingredients (the dry components and a gelatin solution heated to 45 °C that functioned as a binder) were mixed, a mold inhibitor (0.1 % calcium propionate (m/m)) was added, and the mixture was then poured into a 30 × 20 cm stainless steel mold, which was placed in an oven to dry (at 35 °C for 30 min). After cooling, the cereal bars were removed from the mold and cut into 5 × 5 cm pieces weighing 15 g. Each piece was packaged in a sheet of aluminum foil and kept at room temperature in a sealed plastic container until the sensory analyses were performed.

Table 1 Formulations of the savory cereal bars

Ingredients	Bar A (g/100 g)	Bar B (g/100 g)	Bar C (g/100 g)
Toasted dried garlic	1.0	1.0	1.0
Roasted shelled peanuts	20.0	22.0	20.0
Laminated onion	–	1.0	–
Rice flakes	5.0	–	5.0
Corn flakes	6.0	–	6.0
Real quinoa	10.0	16.0	10.0
Soybean meal	20.0	22.0	20.0
Ground wheat	6.0	10.0	6.0
Flavorless gelatin	10.0	10.0	10.0
Water	15.0	15.0	15.0
Artificial chicken flavor	7.0	–	–
Artificial cheese and fine herb flavor	–	3.0	–
Artificial grilled rump cap flavor	–	–	7.0

A prior sensory analysis was performed to choose the best combination of ingredients using a preference ranking test with a group of 30 untrained panelists in individual booths under red light. Each panelist received three samples (A, B, and C) that were coded with three randomly assigned digits, a glass of water, and a sample evaluation sheet. The probiotic microorganisms were added to the best formulation.

Addition of the Probiotics to the Savory Cereal Bars

The probiotic microorganisms were added to the binding agent (the gelatin solution that was heated to 45 °C) of the bars to produce three types of bars: (1) bars containing lyophilized probiotics (Sacco®), (2) bars containing microencapsulated probiotics, and (3) bars containing activated probiotics (Sacco® lyophilized microorganisms grown in lactose broth at 37 °C for 24 h and centrifuged).

Viability of the Probiotics in the Savory Cereal Bars

The probiotic savory cereal bars were stored at room temperature (±25 °C) or in a refrigerator (±4 °C). The probiotics were counted after 0, 15, 30, 60, 90, and 120 days of storage. The counting was performed using the method described by Grosso and Fávaro-Trindade (2004).

Preference Test

To choose the flavor of the savory cereal bars, a preference test was conducted. In the test, 30 untrained panelists evaluated the three samples (Table 1) in individual booths under red light. The panelists ranked the three samples from 1 to 3 or from least to most preferred.

Acceptability and Purchase Intention Tests

Consumer acceptance tests were conducted to evaluate the overall acceptance of savory cereal bars with and without probiotics. A purchase intention test was also conducted with these samples. A nine-point structured hedonic scale with 1 as "dislike extremely" and 9 as "like extremely" was used in the acceptance test, and a seven-point structured scale with 1 as "would always buy" and 7 as "would never buy" was used in the purchase intention test. In both tests, 100 untrained panelists evaluated the samples in individual booths under fluorescent white light.

To evaluate consumer preferences for the savory cereal bars with or without probiotics, a bilateral paired comparison test was conducted with 25 untrained panelists.

All the applied sensory tests were conducted according to Meilgaard et al. (1999) and approved by the Local Research Ethics Committee of the University of Alto Uruguai (COLEPURI) under number 493.372/2013.

Physicochemical Analysis

To create the nutritional label for the new savory probiotic cereal bar, the carbohydrate content was calculated using the following equation: % carbohydrates = 100 − (% moisture + % protein + % lipids + % ash). The protein content was determined using the Kjeldahl method (AOAC 2002), the fat contents (total, saturated, and *trans*) were determined using capillary-column gas chromatography following AOAC (2002), the dietary fiber content was determined using the enzymatic-gravimetric method of AOAC (2012), the moisture content was determined by drying in an oven at 105 °C until a constant weight was reached following AOAC (2012), the sodium content was determined using atomic absorption spectrometry (AOAC 2002), and the amount of energy was calculated following Merrill and Watt (1973).

Statistical Analyses

Statistical analyses were performed at the 5 % significance level using an analysis of variance (ANOVA), and the comparison of the means was performed using Tukey's test with *Statistica* 10 for Windows (Statsoft Inc., USA).

Results and Discussion

Resistance of Probiotics to the Microencapsulation Process

The microorganisms were highly resistant to the spray chilling process because their cell viability remained unaffected (Table 2). Thus, the conditions that were employed in this process, including those of the homogenization (7000 rpm for 60 s), the temperature of the molten lipid matrix, and the spraying pressure and temperature (5 bar and 15 °C ± 2 °C, respectively) were sufficiently mild and ensured the cell integrity of both probiotic populations.

Table 2 Resistance of probiotics to the spray chilling process, expressed as counts (log CFU g^{-1}), before (dispersion) and after atomization

Microorganism	Dispersions*	Microparticles*
LA	10.6 ± 0.2a	10.2 ± 0.2a
BL	10.7 ± 0.3a	10.4 ± 0.1a

*Mean ± standard deviation followed by the same letters in the same row indicates that there were no significant differences according to Student's *t* test ($p < 0.05$)

Water Activity (Aw), Moisture Content, Particle Size, and Morphological Characterization of the Solid Lipid Microparticles

The microparticles exhibited a water activity and moisture content of less than 0.60 and 5 %, respectively, which favors the stability of the encapsulated microorganisms; when less water is available for biochemical reactions, microorganisms remain in the latent state, which prolongs their shelf life.

The mean sizes of the BL and LA microparticles were 85.9 ± 0.08 μm and 60.9 ± 0.09 μm, respectively; these values are related to the size of the probiotic particles in lyophilized powders. The particles that contained BL exhibited a notably greater difference in size before encapsulation than the particles that contained LA did. A difference in the sizes of BL and LA particles that were microencapsulated by spray chilling was also observed by Pedroso et al. (2012), who found that microparticles that contained BL were 40.4 % larger than ones that contained LA. Diameters smaller than 100 μm are preferred for most applications because of their smaller effect on the product's texture and flavor, which allows probiotic-containing microcapsules to be added directly to foods (Annan et al. 2008).

SEM micrographs (data not shown) revealed that the resulting microparticles were spherical, which may facilitate their incorporation into food products by reducing the surface tension between the microparticles and the food, and also that the particles' surfaces were continuous.

Viability of the Microencapsulated Probiotics During Storage of Solid Lipid Microparticles

Table 3 shows the cell viability of the microcapsules during storage at −18 °C. The viability of LA and BL was maintained for 120 and 90 days, respectively.

Preference Test for Choosing the Savory Cereal Bar Flavor

Table 4 presents the ordination totals and the Friedman test results. The chicken-flavored sample differed significantly from the others ($p > 0.05$) and was preferred by the panelists; it was followed by the grilled rump cap-flavored sample and the cheese and fine herb-flavored sample. Thus, the formulation of the chicken-flavored sample was chosen for the probiotic savory cereal bars.

Probiotic Count in the Savory Cereal Bars

The viable probiotic cell count in the bars was analyzed for two storage temperatures: 22 ± 2 °C and at 4 °C ± 2 °C (Table 5). The bars that were stored at room temperature showed fungal contamination 30 days after being manufactured, even when calcium propionate was used as an antifungal agent. Throughout the period (30 days), the bars that contained the encapsulated and lyophilized forms of LA and BL maintained viable probiotic counts (>10^6 CFU/g). The microorganisms (BL and LA) that were added in activated form lost 4.8 and 8.1 log/CFU, respectively, within the first 15 days and exhibited no viability after 30 days of storage.

Cereal bars contain large quantities of nutrients, especially carbohydrates, that are key factors for fungal growth because they provide high osmotic pressure, which promotes fungal growth (Marini et al. 2007). Additionally, cereals are susceptible to fungal contamination throughout the production process (including during planting, storage, and transport) (Soares and Furlani 1996). Considering the product's low initial water activity (less than 0.6), the packaging must allow for moisture resorption, which facilitates fungal development.

From the perspective of the increased commercial viability of the bars, a second experiment was conducted by storing the bars at 4 °C. These bars remained free of fungal contamination for 120 days. During this period, it was possible to count the probiotics; the results are shown in Table 5.

The activated microorganisms were unstable at both temperatures (22 and 4 °C). The bars that were stored at room temperature (22 ± 2 °C) showed a reduction (<10^6 CFU/g) within 15 days of being stored. In the bars that were refrigerated, the amount of LA also decreased 6 log/CFU within 15 days; however, BL exhibited a higher resistance than the others did when it was activated; its count remained higher than 10^6 for 30 days. According to Rodrigues et al. (2011), the high moisture content of the microorganisms (in activated form) is detrimental to probiotic survival at high temperatures and over long storage periods. The direct use of lyophilized microorganisms in the bars led to greater values (≥10^8 CFU/g)

Table 3 Viability of microencapsulated LA and BL during 120 days of storage at −18 °C

Microparticle	Viable cells (log CFU g^{-1})					
	0	15 days	30 days	60 days	90 days	120 days
LA	10.2 ± 0.2a	10.1 ± 0.3a	10.1 ± 0.3a	10.1 ± 0.1a	10.1 ± 0.1a	8.1 ± 0.2b
BL	10. 4 ± 0.1a	10.0 ± 0.1a	9.4 ± 0.2b	9.1 ± 0.0b	8.6 ± 0.2c	5.8 ± 0.1d

*Mean ± standard deviation followed by the same letters in the same row indicates that there were no significant differences according to Student's t test ($p < 0.05$)

Table 4 Differences in the sum of the total scores of the panelists (preference test) for the savory cereal bar formulations chicken, cheese with fine herbs, and grilled rump cap

Formulation	Difference in sum of the total scores		
	Chicken-flavored bar	Cheese and fine herb-flavored bar	Grilled rump cap-flavored bar
Chicken-flavored bar	–	21*	35*
Cheese and fine herb-flavored bar		–	14^{ns}
Grilled rump cap-flavored bar			–

*The difference is significant ($p < 0.05$) (Friedman Test–Newell and MacFarlane Table, DMS = 19). ^{ns} The difference is not significant ($p > 0.05$)

for up to 30 days for both microorganisms and at both storage temperatures (22 and 4 °C). The lyophilized LA was more stable, with counts of $\geq 10^8$ CFU/g for 60 days at 4 °C.

However, the encapsulated probiotics that were added to the bars were more viable than the lyophilized activated microorganisms were (Table 5). Encapsulated BL remained stable for 105 days at 4 °C ($\geq 10^8$ CFU/g), which is 75 days longer than the lyophilized form of the same microorganism that remained stable when it was added to the bars. Encapsulated LA remained stable for 30 days longer than the lyophilized form did; the counts were $\geq 10^8$ CFU/g for 90 days at 4 °C. This result was expected because the microorganisms inside the lipid matrix were protected from the presence of oxygen, water, and oxidative stress and remained in a latent state. Similarly, recent studies have proven the efficiency of this technique for protecting some bioactives (Sartori et al. 2015; Matos-Jr et al. 2015; Salvim et al. 2015; Consoli et al. 2016; Alvin et al. 2016).

In addition to microencapsulation in lipid matrices, storage at a low temperature (4 °C) promotes higher probiotic stability because the microorganisms remain in a latent state and the low temperature prevents lipid rearrangements of the wall material, which, in turn, prevents inadequate exposure of the microorganisms and promotes a longer shelf life of the microparticles (Albertini et al. 2010).

Sensory Analysis of the Savory Probiotic Cereal Bars

In the preference test, the chicken-flavored savory cereal bar was identified as the best of the three flavored samples that were evaluated (chicken, cheese and herbs, and grilled rump cap); therefore, BL was added to them. BL was selected for addition to the bars because it exhibited higher resistance at 4 °C (105 days) than LA did (90 days).

In the overall acceptability test, the bars with and without probiotics were accepted by 76 and 80 % of the panelists, respectively. Thus, both products were highly acceptable to consumers.

The results of the purchase intention test were 93 and 89 % for the bars with and without probiotics, respectively. Therefore, both products have high potential for commercialization. Similarly, Melo et al. (2010) and Bastos et al. (2014) obtained high purchase intention results for savory cereal bars (92 %) and sweet probiotic cereal bars (94.5 %), respectively.

In the bilateral paired comparison, which was used to evaluate the difference between the bars with and without probiotics, it was not possible to find a significant difference or preference because the minimum number of correct judgements ($p < 0.05$) was not reached (18 samples). The lack of a perceived difference between the cereal bars with and without probiotics leads to the conclusion that adding encapsulated BL to the cereal bars did not affect the product's sensory characteristics. Bastos et al. (2014) evaluated the addition of yeast (*Saccharomyces boulardii*) and bacteria (*Lactobacillus acidophilus*) to cereal bars and observed that the inclusion of these (encapsulated and lyophilized) microorganisms in cereal bars did not affect the bars' sensory and structural qualities, which corroborates the results of this study even though different methods and

Table 5 Probiotic cell counts, expressed as counts (log CFU g^{-1}), in the savory cereal bars stored at 4 °C, over 120 days

	0	15 days	30 days	45 days	60 days	75 days	90 days	105 days	120 days
BL microencapsulated	10.3	10.1	10.1	10.1	10.0	9.6	9.4	8.1	5.2
BL lyophilized	8.9	8.1	8.3	4.7	0	0	0	0	0
BL activated	6.9	6.5	6.8	4.3	0	0	0	0	0
LA Microencapsulated	10.5	9.8	9.7	9.4	9.3	8.6	8.2	7.8	3.1
LA Lyophilized	8.9	8.4	8.9	8.7	8.9	5.2	1.3	0	0
LA Activated	10.5	4.7	2.3	0	0	0	0	0	0

BL—*B. lactis*; LA—*L. acidophilus*

Table 6 Nutritional information for the new probiotic savory cereal bar

Nutrition facts Serving size 25 g		
	Amount per serving	% DV (*)
Energy	340 kJ	4
Carbohydrate	7.1 g	2
Protein	7.1 g	9
Fat, total	3.3 g	6
Saturated fat	0.4 g	2
Trans fat	Nil	
Fiber	0.9 g	4
Sodium	11 mg	0

(*) Percent daily values are based on a 2000-cal diet. Your daily value may be higher or lower depending on your calorie needs

encapsulation matrices (extrusion/calcium alginate and spray chilling/vegetable fat) were used.

Nutritional Characterization of the New Product

Table 6 shows the nutritional information for the new probiotic cereal bar. According to current legislation (ANVISA 2012), this cereal bar can be considered a source of fiber and protein and has a very low sodium content and a low saturated fat content. All of these add value to the product.

A comparison between the nutritional content of the cereal bar with microencapsulated probiotics and the same cereal bar without the probiotics shows that the microencapsulated probiotics only changed the total fat content and saturated fat content, which increased by 19.3 and 9.3 %, respectively; this may be explained by the composition of the lipid matrix of the microcapsules (vegetable fat).

Conclusions

The microencapsulation of lyophilized probiotics (LA and BL) by spray chilling generated a powder with medium-sized particles (from 39 to 126 μm) that were smooth and continuous spheres with a low moisture content (<1.05) and a desirable level of water activity (<0.6). The operational conditions of the microencapsulation (air pressure of 5 bar; chamber temperature of 15 °C ± 2 °C) and the lipid matrix that was used (vegetable fat) did not compromise the viability of the microbes, which demonstrates the technique's efficiency for protection of the analyzed strains.

The microencapsulated microorganisms exhibited high viabilities of 90 and 120 days for BL and LA, respectively, when the microparticles were stored at −18 °C.

The viable cell count in the bars showed the advantage of microencapsulation over other methods that are used to add activated and lyophilized probiotics to savory cereal bars. The encapsulated BL exhibited counts that were higher than 10^8 CFU/g for 105 days of refrigerated storage. The LA maintained the same counts for 90 days. The acceptability of the savory probiotic cereal bar demonstrated that there is a potential market for this new product. The cereal bars with and without probiotics did not exhibit sensory differences, which indicates that the inclusion of microorganisms encapsulated by spray chilling does not affect the products' sensory characteristics.

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