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Acerola nectar with added microencapsulated probiotic

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ABSTRACT

The aim of this study was to evaluate the survival of a probiotic microorganism microencapsulated in cellulose acetate phthalate, added to acerola nectar. The changes in pH, Brix, organic acid content and color of the product during its shelf-life were evaluated. A total of 3 processing runs were carried out on a semi-industrial scale, each consisting of a 15-liter batch of acerola nectar with added prebiotics and a microencapsulated probiotic culture. The physicochemical characteristics of the samples remained stable throughout storage. After 30 days storage the acerola nectar samples containing microencapsulated probiotic microorganisms exhibited counts above 8 log CFU per 200 mL, within the limits set by the Brazilian regulation for functional foods. On the other hand, the samples containing free *Bifidobacterium animalis* cells showed counts of 5.9 log CFU per 200 mL after the same storage time. It was concluded that microencapsulation was a suitable technique for improving the viability of probiotic microorganisms in acerola nectar during cold storage.

1. Introduction

Fruit juice is considered to be a healthy food product, and is currently consumed frequently and loyally by a large percentage of the global consumer population (Luckow & Delahunty, 2004a). Significant growth in the fruit market has attracted the attention of fruit growers, fruit distributors and processors to meet the demands (Renuka, Kulkarni, Vijayanand, & Prapulla, 2009). Although most of the current probiotic foods are mainly dairy based, there is a growing interest in the development of non-dairy probiotic products. Non-dairy products could be helpful for cases where lactose intolerance, allergy to milk proteins and hypercholesterolemia are drawbacks, and for others where people refuse to ingest dairy products or where dairy products are inaccessible (Granato, Branco, Nazzaro, Cruz, & Faria, 2010; Ranadheera, Baines, & Adams, 2010; Rivera-Espinoza & Gallardo-Navarro, 2010).

A prebiotic is a non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon which can improve the host's health (Gibson & Roberfroid, 1995). On the other hand, probiotics are defined as live microorganisms that, when administered in adequate amounts, provide health benefits to the host (FAO/WHO, 2001). According to Luckow and Delahunty (2004b), there is a genuine interest in developing fruit-based functional beverages, fortified with probiotic and prebiotic ingredients. Fruit juices are rich in nutrients and do not contain starter cultures that compete with probiotics for nutrients (Ding & Shah, 2008; Sohail, Turner, Prabawati, Coombes, & Bhandari, 2012). Yoghurts and fermented milks are the main vehicles for probiotic cultures (Cruz, Antunes, Sousa, Faria, & Saad, 2009). However, beverages such as fruit and vegetable juices could be the next food category where healthful probiotic bacteria will make their mark (Prado, Parada, Pandey, & Soccol, 2008).

Dairy products containing *Bifidobacterium* and *Lactobacillus* strains, sugar fortified with FOS (fructo-oligosaccharides) or inulin have been emerging on the food market for more than 10 years

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(Saad, Delattre, Urdaci, Schmitter, & Bressolier, 2013). There are currently two probiotic-carrying fruit juice blends on the Canadian market, containing between 1 and 3 billion probiotic cells per 250 mL portion (Champagne & Gardner, 2008).

The survival of *Lactobacillus* and *Bifidobacterium* strains has been studied in the following fruit juices: orange, pineapple, apple, green apple, cranberry, pear, grape, passion fruit, lemon, peach, strawberry, mango, kiwi, pomegranate and cashew apple (Champagne & Gardner, 2008; Cruz et al., 2009; Ding & Shah, 2008; Nualkaekul & Charalampopoulos, 2011; Nualkaekul, Lenton, Cook, Khutoryanskiy, & Charalampopoulos, 2012; Pereira, Maciel, & Rodrigues, 2011; Rodrigues et al., 2012; Saarela et al., 2011; Saarela, Virkärvi, Alakomi, Sigvart-Mattila, & Mättö, 2006, Saarela, Virkajärvi, Nohynek, Vaari, & Mättö, 2006; Sheehan, Ross, & Fitzgerald, 2007; Sohail et al., 2012; Vinderola, Costa, Regenhardt, & Reinheimer, 2002;). The viability of probiotic microorganisms is different depending on the fruit matrices and is also strain dependent. Even if fermented milks are the preferred base to deliver probiotics there is a trend to use exotic fruits as an ingredient (Santo, Perego, Converti, & Oliveira, 2011).

Viability of probiotic bacteria is of paramount importance in the marketability of probiotic-based food products (Kailasapathy, 2006). In Brazil functional foods are regulated by Anvisa – Brazilian Agency of Sanitary Surveillance, and the present regulation states that the minimum viable quantity of probiotic culture should be between 10^8 and 10^9 CFU per daily portion of the product and that the probiotic population should be stated on the product label (Anvisa, 2002).

Adding probiotics to fruit juices is more complex than formulating in dairy products. In part this is due to the low pH of fruit juices and insufficient quantities of some of the small peptides and free amino acids necessary for probiotics. In addition, acid and bile tolerance is better in cells added to milk than in those added to juice, and the probiotics should not only survive in the food product, but should reach the intestine alive (Champagne & Gardner, 2008; Saarela, Virkärvi, Alakomi,et al., 2006). Microencapsulation technology can promote probiotic viability in fruit juices, expanding the application of probiotics because microcapsules may provide a more favorable anaerobic environment for the sensitive probiotic bacteria, as well as representing a physical barrier from the harsh acidic conditions of the fruit juice (Ding & Shah, 2008). Microencapsulation is the process of applying a shell to sensitive microorganisms to protect them from their external environment, thus reducing cell injury (Capela, Hay, & Shah, 2007; Kailasapathy, 2006). Natural polymer-based materials represent promising matrices as controlled delivery systems due to their biodegradability, compatibility food-grade nature and wide availability (Doherty et al., 2011). Anekella and Orsat (2013) studied the microencapsulation of probiotics in raspberry juice by spray drying and Doherty et al. (2011) evaluated the efficacy of whey protein isolate as an encapsulation matrix.

The acerola fruit is native to Central America and very well adapted to cultivation in Brazil, which, in turn, has become the major worldwide producer, consumer and exporter (De Rosso & Mercadante, 2005). This fruit is well known as an excellent food source of vitamin C and also contains phytochemicals such as carotenoids and polyphenols (Mezadri, Villaño, Fernández-Pachón, García-Parrilla, & Troncoso, 2008). Vitamin C can be beneficial to probiotic survival during storage, presumably because it is an oxygen scavenger, thus promoting a more favorable anaerobic environment (Dave & Shah, 1997).

The aim of this study was to develop an acerola nectar with added inulin and *Bifidobacterium animalis* microencapsulated in cellulose acetate phthalate by a spray-drying technique, evaluating the survival of the probiotic microorganism and monitoring changes in the pH value, Brix, organic acid content, vitamin C content and color of the product during the shelf-life.

2. Material and methods

For the microencapsulation processes a culture of *B. animalis* subsp. *lactis* BB-12 (Chr. Hansen, Valinhos/Brazil) was used and the following encapsulating agents: cellulose acetate phthalate - CAP (Index Pharmaceutical, India), glycerol (Difco), Hi-maize, maltodextrin (Mor-Rex), Tween 80 (Synth), trehalose (Sigma–Aldrich), inulin (Clariant, Orafit) and reconstituted milk (Molico, Nestle).

Acerola pulp (De Marchi) plus inulin (Orafti), refined commercial sugar and citric acid were used to manufacture the fruit nectars.

For the tests, the culture was reactivated by subculturing in sterile milk (12 g of milk powder diluted in 86 mL of water) supplemented with 1 g glucose and 1 g yeast extract and incubated at 37 °C for 24 h. The active cultures in sterile supplemented milk were centrifuged (6000 × *g* for 10 min at 4 °C), the supernatants discarded and the suspensions washed twice in 0.85 g/100 mL saline solution (20 mL), centrifuging as above and resuspending in sterile NaCl solution.

2.1. Microencapsulation of the probiotic bacteria by spray drying

The *B. animalis* subsp. *lactis* BB-12 culture was immobilized in cellulose acetate phthalate following the methodology described by Fávaro-Trindade and Grosso (2002) with some alterations. Each 100 mL of the microencapsulation solution consisted of the following combination of ingredients: 7.5 g CAP, 3.5 g glycerol, 2.0 g maltodextrin, 0.8 g tween 80, 3.0 g reconstituted milk, 1.0 g hi-maize and 2.0 g trehalose. The cell suspension was added to the microencapsulation solution and the mixture submitted to spray drying in a Büchi model B-290 mini spray dryer with a 1.5 mm diameter atomizing nozzle and an evaporating capacity of 1.0 L h^{-1}. The operational parameters of the equipment were as follows: air entrance temperature of 110 °C, air flow of 439 L h^{-1} and outflow of 6 mL min^{-1}.

2.2. Characterization of the acerola pulp

The batches of acerola pulp were characterized according to the pH value, Brix and total titratable acidity. The pH value was determined using a digital pH-meter (Micronal B-375). The total titratable acidity was determined by titration associated with the use of a pH-meter, expressing the results in grams citric acid per 100 g sample. The soluble solids content was measured directly using a digital refractometer (Atago/PAL-1) and the results expressed in °Brix.

2.3. Processing of the acerola nectar

Three separate 15 L batches of acerola nectar with added prebiotics were processed in the Pilot Plant, and microencapsulated *B. animalis* added to one, free *B. animalis* cells to another and no probiotic bacteria to the third (control). Fig. 1 shows the flow diagram for the processing of the products. The acerola nectars were formulated to result in products with a ratio of 40 (°Brix/titratable acidity ratio), containing 30 g/100 g of fruit pulp. Thus the formulations were calculated to result in products with a total titratable acidity of 0.29 g citric acid per 100 g product with 11.6 °Brix. Inulin was also added at a rate of 7.5 g per kg of product to conform to the Brazilian regulation that establishes 1.5 g of prebiotics per 200 mL portion of nectar.

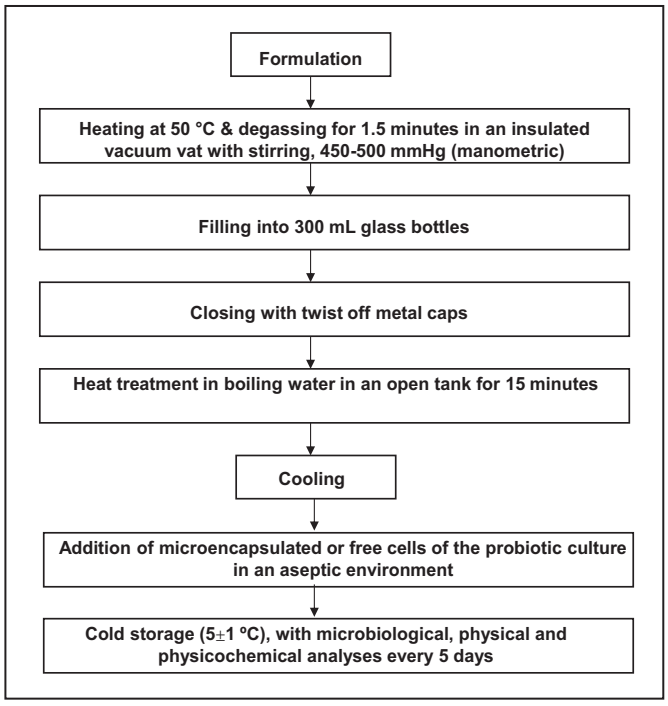

Fig. 1. Flow diagram of the processing and analysis of the acerola nectar samples.

The acerola nectars were manufactured with fruit pulp (De Marchi/Brazil), filtered water, commercial refined sugar, inulin (kindly provided by Orafti) and citric acid. Processing consisted of heating and degassing in an insulated vacuum vat with stirring (50 °C, 450–500 mmHg manometric pressure), followed by filling into glass 300 mL bottles, closing with metallic twist-off caps and heat treatment in boiling water in an open tank for 15 min. Cooling was carried out in two stages: first with water at 60 °C to avoid heat shock of the glass bottles, followed by water at room temperature. After cooling the *B. animalis* cultures, either in the form of microparticles or DVS (Direct Vat Set) freeze dried free cells, were added to the appropriate bottles in an aseptic environment.

After processing, the nectars were stored in a cold chamber at a temperature of 5 ± 1 °C. The three batches were evaluated every 5 days for a total of 35 days of storage, determining the ascorbic acid content, soluble solids, pH value and color, and also carrying out an electronic microscopy examination. The microbiological evaluation consisted of *B. animalis* and yeast and mold counts for up to 35 days of storage.

2.4. Bifidobacterium animalis count

The stability of the probiotic culture during storage in the fruit nectar was determined by counting the viable cells after freeing them from the particles by shaking in a shaker at 150 rpm in a solution of phosphate buffer (0.2 mol/L) at pH 7.5 for 3 h. The counts were carried out in MRS (de Man, Rogosa and Sharpe, Difco) agar supplemented with lithium chloride (0.1 g/100 mL), sodium propionate (0.3 g/100 mL) and L-cysteine (0.05 g/100 mL) using the pour plate technique (Liserre, Ré, & Franco, 2007). Incubation was done under anaerobic conditions (Anaerogen, Oxoid/England). Serial decimal dilutions were prepared in 0.1 mL/100 mL peptone water. The results were expressed in log CFU mL^{-1}.

2.5. Determination of yeasts and molds

The yeasts and molds in the fruit nectar were determined by the spread plate technique on Rose-Bengal Chloramphenicol Agar (Difco), incubating for 5 days at 25 ± 1 °C. The results were expressed in log CFU mL^{-1}.

2.6. Total aerobic psychrotrophic microbial count

The total aerobic psychrotrophic microbial count in the fruit nectar was determined by inoculation onto PCA agar (Plate Count Agar, Difco) and incubation at 7 ± 1 °C for 7 days. The results were expressed in log CFU mL^{-1}.

2.7. Physical and chemical analyses

The samples of acerola nectar were analyzed for pH, total titratable acidity and soluble solids content every 5 days.

The ascorbic acid content was determined by HPLC with a mobile phase of acetonitrile plus deionized water in the proportion of 3:1, filtered and degassed according to the methodology of Maeda, Yamamoto, Owada, Sato, and Masui (1989). The chromatographic conditions used were the following: Shimadzu model class 10 chromatograph; model SPD-M6A diode array detector with a wavelength of 245 nm; mobile phase of KH_2PO_4 (Synth), heptanesulfonic acid, sodium salt (Acros), triethylamine (Acros) and methanol (Tedia), with a flow rate of 0.8 mL min^{-1}.

2.8. Color analysis

A Konica Minolta CR-400 portable colorimeter was used to analyze the color of the fruit juice using the CieLab system with illuminant C. The measurements of L*, a* and b* were analyzed throughout storage and the results expressed as the total color difference (ΔE^*_{ab}) between each sample at a determined storage time and the color of the just-processed sample, using the following equation:

$$\Delta E^*_{ab} = \left[\left(\Delta L^*\right)^2 + \left(\Delta a^*\right)^2 + \left(\Delta b^*\right)^2\right]^{1/2} \quad (1)$$

where Δ = the difference in the color parameter between the stored and just-processed samples.

2.9. Optical microscopy of the microencapsulated particles

The capsules added to the fruit juice were analyzed from images captured using a Carl Zeiss model Axiocam ICC 1 digital scientific camera (Germany), coupled to a Zeiss model Axio Scope A1 microscope.

2.10. Analysis of the results

The results obtained were analyzed statistically using ANOVA and the means of the treatments compared using Tukey's test, with a level of significance of $p < 0.05$. Mean values and standard deviations were calculated from the data obtained with triplicate trials for the physical and chemical analyses and duplicate trials for the microbiological analyses. The data were then compared by Statsoft Inc, Version 5.5. (2000).

3. Results

The viability of the probiotic culture added to the acerola nectar in the form of microcapsules was accompanied during refrigerated storage, and the results compared with those obtained for the addition of the same culture in the form of free cells (Fig. 2). The results were calculated considering a 200 mL portion of nectar, based on the Brazilian regulation (Anvisa, 2003). The control

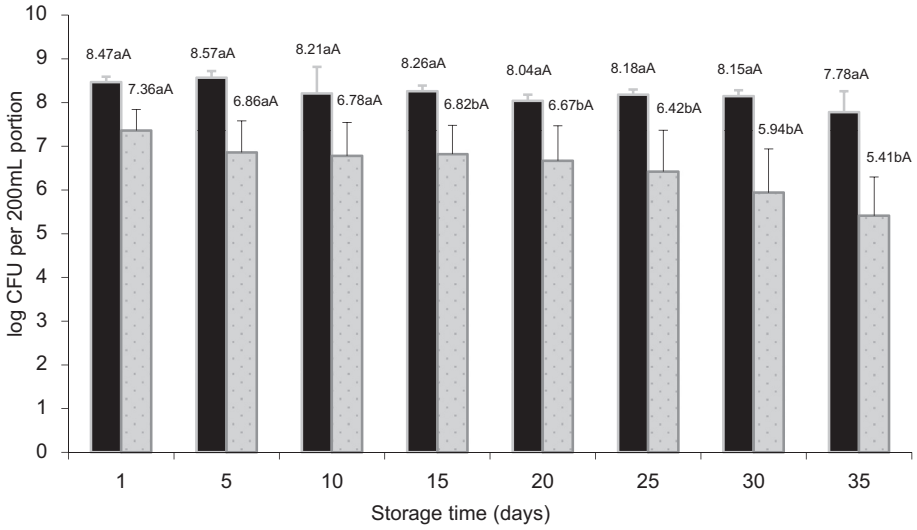

Fig. 2. Mean populations (log CFU mL^{-1}) of *B. animalis* in the form of microencapsulated cells (■) and free cells (▩) in acerola nectar with the addition of 1.5% inulin per portion, during refrigerated storage (5 ± 1 °C). Obs.: Results followed by the same small letters do not differ at the 5% level with respect to the treatment. Results followed by the same capital letters do not differ at the 5% level with respect to the time.

samples (with no added probiotic bacteria) were also analyzed under the same conditions, but no colonies were found in the plates containing culture medium selective for bifidobacteria. In a complementary way, the decrease in the microencapsulated and free cell probiotic culture counts during 35 days of storage, as compared to the respective first day of storage acerola nectar samples, were also calculated, and the results can be seen in Table 1.

The counts for the total psychrotrophic aerobes and for yeasts and molds were determined in the acerola nectar samples, giving counts below the detection limits of the analyses (<1 log CFU mL^{-1}), indicating good hygienic-sanitary quality of the samples during the 35 days of refrigerated storage.

Table 2 shows the mean results obtained for the pH values of the 3 batches of acerola nectar. The pH values of the control samples and of those with added *B. animalis* in the free cell form did not differ significantly throughout the whole storage period. On the other hand, the sample with added microparticles differed from the others showing higher pH values, probably due to the wall material of the particles.

The results of the color parameters were interpreted according to the total difference in color between the samples stored under refrigeration and the initial samples (zero time). Table 3 shows the total difference in color (ΔE^*_{ab}) throughout the storage of the acerola nectar samples. No statistically significant difference was found between the treatments when the samples analyzed at each stage were compared. This signifies that the addition of probiotics, be it in the form of free cells or microencapsulated cells, did not interfere with the color of the product. In addition, no statistically significant differences were observed when each treatment was analyzed throughout the storage period up to the 30th day. Such results showing total color maintenance for each treatment are in agreement with the ascorbic acid content of the samples. The ascorbic acid contents were very similar for the 3 treatments, showing means of 189 mg, 187 mg and 189.5 mg per 100 mL sample during refrigerated storage, for the control samples and for those with the addition of probiotic bacteria in the microencapsulated and free cell forms, respectively.

The results for total titratable acidity did not differ significantly, independent of the treatment and refrigerated storage time. The mean results for the titratable acidity throughout 35 days of storage were 0.28 ± 0.01; 0.29 ± 0.01 and 0.28 ± 0.00 g 100 g^{-1}, respectively, for the control sample and for those with probiotics added in the form of microparticles and free cells. The mean soluble solids contents for the three treatments also remained constant, presenting a mean value of 11.9 ± 0.08 °Brix throughout 35 days of storage.

Fig. 3 shows the results obtained in the microscopy of the samples. The size of the microcapsules containing *B. animalis* was found to vary between 0.11 μm and 65.51 μm, with the mean for the majority of the particles being about 20.2 μm.

4. Discussion

The samples containing the microencapsulated *B. animalis* culture maintained counts of about 8 log CFU per portion (200 mL

Table 1
Decrease in the *B. animalis* counts (log CFU mL^{-1}) during 35 days of storage as compared to the just-processed samples of acerola nectar containing the microencapsulated (M) probiotic cells in the free form (F).

Time (days)	M	F
5	−0.10	0.50
10	0.26	0.58
15	0.21	0.54
20	0.43	0.69
25	0.29	0.94
30	0.32	1.42
35	0.69	1.95

Table 2
Results for pH obtained during storage of acerola nectar samples with added inulin at 1.5 g per portion in the control (C), and with added microencapsulated (M) or free (F) *B. animalis* cells.

Time (days)	C	M	F	MSD
0	3.46 ± 0.02 b	3.62 ± 0.03 a	3.45 ± 0.03 b	0.061
5	3.45 ± 0.02 b	3.60 ± 0.04 a	3.43 ± 0.03 b	0.081
10	3.43 ± 0.02 b	3.58 ± 0.01 a	3.43 ± 0.02 b	0.145
15	3.44 ± 0.03 b	3.60 ± 0.02 a	3.44 ± 0.03 b	0.069
20	3.41 ± 0.02 b	3.56 ± 0.01 a	3.40 ± 0.03 b	0.052
25	3.71 ± 0.04 b	3.83 ± 0.03 a	3.71 ± 0.03 b	0.081
30	3.49 ± 0.04 b	3.62 ± 0.02 a	3.47 ± 0.03 b	0.068
35	3.44 ± 0.02 b	3.60 ± 0.02 a	3.44 ± 0.02 b	0.047

Obs.: Samples (mean ± standard deviation) followed by the same small letters in the same line do not differ at the 5% level. Means and standard deviations for $n = 3$. MSD − minimum significant difference.

Table 3
Total difference in color (ΔE^*_{ab}) throughout storage of the acerola nectar samples with added 1.5 g of inulin per portion in the control (C), and with added microencapsulated (M) or free (F) *B. animalis* cells.

Time (days)	C	M	F	MSD
5	0.24 ± 0.05 b B	0.51 ± 0.29 ab B	0.74 ± 0.14 a BC	0.470
10	0.41 ± 0.14 a B	0.38 ± 0.09 a B	0.43 ± 0.31 a C	0.502
15	0.83 ± 0.19 a AB	0.93 ± 0.12 a B	0.99 ± 0.26 a BC	0.488
20	0.71 ± 0.11 a AB	0.80 ± 0.16 a B	0.63 ± 0.35 a BC	0.577
25	0.60 ± 0.06 a AB	0.57 ± 0.10 a B	0.69 ± 0.07 a BC	0.188
30	1.36 ± 0.51 a AB	1.97 ± 0.64 a AB	1.69 ± 0.72 a B	1.571
35	2.20 ± 1.41 a A	2.93 ± 1.34 a A	2.89 ± 0.57 a A	2.929
MSD	1.603	1.611	1.128	

Obs.: Samples (mean ± standard deviation) followed by the same small letters in the same line do not differ at the 5% level. Samples (mean ± standard deviation) followed by the same capital letters in the same column do not differ at the 5% level. Means and standard deviations for $n = 3$. MSD – minimum significant difference.

nectar) for 30 days of refrigerated storage. This count conforms to the value recommended by the Brazilian regulation for products alleging functional properties (Anvisa, 2002). Comparing the two forms of addition (microencapsulated and free cells) on the thirtieth day, the survival of the probiotic culture in the form of free cells decreased by about 1.5 log CFU per portion of acerola nectar. After 35 days of refrigerated storage, the viable count of the microencapsulated probiotic culture in the nectar had decreased to an extent that the product no longer conformed to the Brazilian regulation. When compared with the first day of storage, a difference of 0.69 and 1.95 log CFU mL^{-1} was observed, respectively, for the microencapsulated and free cells of the probiotic culture. According to Table 1, the decrease in viability of the probiotic culture was less than 0.5 log CFU mL^{-1} up to 30 days of storage of the acerola nectar. On the other hand the bifidobacteria added to the product in the form of free cells showed a reduction of 0.5 log CFU mL^{-1} in the first 5 days of storage. Thus encapsulation of the probiotic culture was shown to be efficient in increasing the viability of the culture in the acerola nectar, which presented a shelf life of 30 days with respect to its functional allegation.

According to Saarela, Virkajärvi, Nohynek, et al., 2006, a decrease of about 3 logarithmic cycles was observed in the count of *B. animalis* subsp. *lactis* after the storage of commercial fruit juices (orange, grape and passion fruit) with the addition of this culture and stored at 4 °C for 6 weeks. The final probiotic counts in the juices were about 4 log CFU mL^{-1}. The culture used in the trial was the same as that used in the present study, but it was not microencapsulated, although a cryoprotector was added.

Champagne and Gardner (2008) evaluated the viability of nine probiotic lactobacilli strains in a commercial fruit drink stored at 4 °C for up to 80 days. The results pointed to considerable variability in the stability between the different species and strains. Data from that study also suggest that 35 days of storage (at 4 °C) of the fruit drink would not affect the sensitivity of the probiotics to bile or pancreatic enzymes.

The survival of *Lactobacillus* (*Lactobacillus salivarius* ssp. *salivarius* UCC118, *L. salivarius* ssp. *salivarius* UC500, *Lactobacillus paracasei* ssp. *paracasei* NFBC 4338, *Lactobacillus rhamnosus* GG, *L. rhamnosus* GG, *Lactobacillus casei* DN-114 001) and *B. animalis* ssp *lactis* Bb-12, in orange juice (OJ), pineapple juice (PJ) and cranberry juice (CJ) was monitored by Sheehan et al. (2007). All the screened strains survived longer in OJ and PJ as compared to CJ. Of the evaluated strains, *L. casei* DN-114 001, *L. rhamnosus* GG and *L. paracasei* NFBC 43338 displayed greater robustness, surviving at levels above 10^7 CFU mL^{-1} in OJ and above 10^6 CFU mL^{-1} in PJ, for at least 12 weeks.

The survival of free and microencapsulated probiotic bacteria in orange and apple juices, using eight different strains of probiotic bacteria, was investigated by Ding and Shah (2008). Encapsulated probiotic bacteria survived in the fruit juices throughout six weeks of storage ($>10^5$ CFU mL^{-1}), whereas free probiotic bacteria lost their viability within five weeks (no viable bacteria remaining).

Vinderola et al. (2002), studied the effect of natural fruit juices (strawberry, peach, pineapple, kiwi and green apple) on the growth of lactic acid starter and probiotic bacteria. Among the juices assessed, strawberry showed the highest inhibition capacity, since it inhibited strains of all the species used except for the *L. casei* group strains. *Lactobacillus acidophilus* CNRZ was inhibited by pineapple juice and kiwi juice.

The use of chitosan coated alginate beads for the survival of microencapsulated *Lactobacillus plantarum* in pomegranate juice was evaluated by Nualkaekul et al. (2012). The study indicated that a multi-layer coating was more protective than a single coated bead. After 6 weeks of storage in pomegranate juice at 4 °C the probiotic viability was 5.7 log CFU mL^{-1} and 6.6 log CFU mL^{-1}, respectively, for single and double coated beads.

Orange juice fortified with probiotic cultures was subjected to heat pasteurization at 76 °C for 30 s and at 90 °C for 1 min in addition to high pressure treatment at 400 MPa for 5 min (Sheehan et al., 2007). The results indicated that no strain was capable of withstanding the treatments necessary to achieve a stable juice at levels of $>10^6$ CFU mL^{-1}. As a consequence, the addition of cultures post processing is necessary. The flex aseptic dosing machine developed by Tetra Pak allows for the addition of probiotic cultures to liquids just before they are filled into the cartons. This innovation is expected to significantly boost the market for probiotic beverages, which have so far been restricted by the delicate nature of the ingredient (Prado et al., 2008).

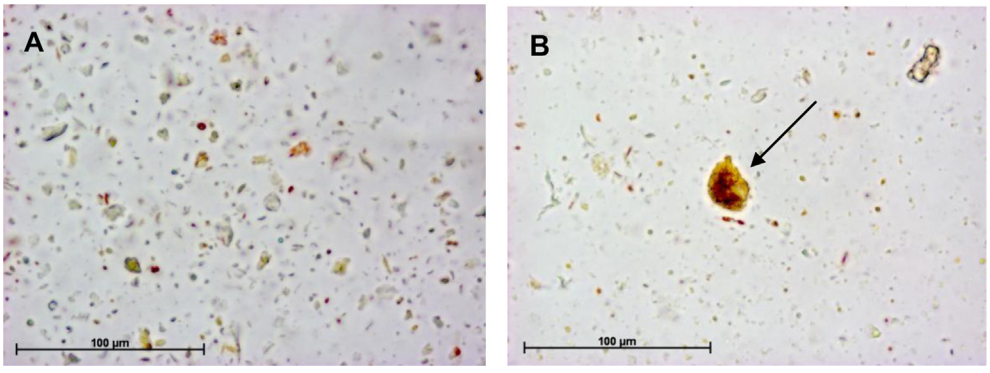

Fig. 3. Photomicrographs of the control acerola nectar (A) and of that with the addition of particles (B) containing microencapsulated *B. animalis* as indicated by the arrow (X400).

Champagne and Gardner (2008), studying the storage stability at 4 °C of 9 probiotic lactobacilli in a drink composed of 10 fruit and dairy ingredients, observed that the pH of the juice samples remained unchanged after 28 days of storage, indicating weak metabolic activity of the probiotic lactobacilli at 4 °C.

In another work carried out by the same group (Champagne, Raymond, & Gagnon, 2008), in which *L. rhamnosus* R0011 was inoculated into an apple-pear-raspberry juice blend at 4.5×10^9 CFU per 250 mL portion, the pH of the samples varied between 3.63 and 3.90. The inoculated juices showed pH readings less than 0.1 unit lower than the non-inoculated juices, a difference that was not statistically significant ($P = 0.45$). Therefore, the presence of the lactobacilli did not affect the pH of the product.

In the present study, no significant decrease in pH and/or increase in total titratable acidity was observed throughout the refrigerated storage of the samples (5 ± 1 °C), indicating that even the free cells of the probiotic culture showed low metabolic activity. On the other hand, Ding and Shah (2008), observed an average pH decrease from 2.81 to 2.57 in orange juice containing free probiotic bacteria after six weeks of storage, whereas the pH remained almost the same in the orange juice containing encapsulated probiotics after the complete storage period. When they evaluated apple juice, the probiotic culture tested reduced the pH of the juice regardless of whether it was in the free or encapsulated state. However, at the end of six weeks storage the final pH of the apple juice inoculated with encapsulated probiotic bacteria was higher than that inoculated with free probiotic bacteria.

Sohail et al. (2012), investigated the effect of microencapsulation on the survival of *L. rhamnosus* GG and *L. acidophilus* NCFM and their acidification in orange juice. Encapsulation of these two bacteria did not significantly enhance survivability. However, the pH of orange juice containing encapsulated probiotics was significantly higher than that of orange juice containing free probiotic bacteria after 12 days of storage at 25 °C or after 35 days of storage at 4 °C.

L. paracasei L26 cultures encapsulated in alginate, chitosan-coated alginate or dextran sulfate-coated alginate and added to orange and peach juices were evaluated by Rodrigues et al. (2012). Independent of the type of microcapsule, a ΔpH of between 0.44 and 0.48 was observed in peach juice and a variation of between 0.29 and 0.39 in orange juice.

5. Conclusion

The production of acerola nectar containing a culture of *B. animalis* microencapsulated by spray drying is feasible, obtaining a culture with viability within the values stipulated by the Brazilian regulation for functional foods. Microencapsulation of the probiotic culture improved its viability throughout cold storage of the product for up to 30 days, as compared to the addition of freeze dried free cells. The physicochemical characteristics of the samples remained stable throughout storage up to 35 days, with the exception of the color parameter (ΔE^*_{ab}), which presented a statistically significant difference at the end of the storage period. Based on the microbiological and physiochemical results, it was concluded that the shelf life of the acerola nectar with added prebiotic and microencapsulated probiotic would be 30 days under refrigerated storage at 5 ± 1 °C.

To the best of our knowledge there are no previous studies that elaborated fruit juice/nectar on a semi-industrial scale to evaluate the addition of microencapsulated probiotic bacteria. The vast majority of the studies found employed commercial samples of juice/nectar, with the exception of one in which 1 L of a model juice was elaborated (Shah, Ding, Fallourd, & Leyer, 2010), and another which was obtained by pressing the peduncles of cashew apples (Pereira et al., 2011). The present study presents the differential of studying the application of microencapsulated probiotics and prebiotics to a matrix of acerola nectar processed on a semi-industrialized scale in a standardized way. In this way the conditions of industrialization are simulated better, obtaining a more reliable result for the shelf life of the product. The study could be applied in the food industry under the concept of healthfulness, since it proposes a product that aggregates the benefits of pre and probiotics in acerola nectar, which is one of the richest fruits in vitamin C. However, simulated gastro-intestinal studies are recommended to truly evaluate the acerola nectar as an effective vehicle to deliver *B. animalis*.

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