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New functional non-dairy mixed tropical fruit juice microencapsulated by spray drying: Physicochemical characterization, bioaccessibility, genetic identification and stability

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ABSTRACT

This study shows the development of a powdered non-dairy probiotic 40:60% (w/w) acerola:siriguela mixed juice. The mixed juice enriched with the probiotic *Lactobacillus rhamnosus* LPAA 01, *Lactobacillus casei* LPAA 02 and *Lactobacillus plantarum* LPAA 03 strains was microencapsulated by spray drying using 140 °C air inlet temperature, 0.60 L/h feed flow rate and 10% (w/w) 5 dextrose equivalent maltodextrin. Microcapsules were analyzed in terms of physicochemical characteristics, chemical composition, *in vitro* bioaccessibility, microbial viability and stability at 5 and 25 °C for 45 days. Viable cell counts were >6 log CFU. g^{-1} for up to 20 days at 5 °C and 14 days at 25 °C, and physicochemical properties of microparticles were shown to be in acceptable ranges. Each specific primer pair showed unique amplification, confirming the survival of all the three probiotics. The levels of phenolic compounds, with the exception of quercetin, as well as antioxidant activity by ORAC method increased after exposure to simulated gastrointestinal conditions.

1. Introduction

Over the past ten years, more than 500 new functional foods and beverages have entered the market, given the growing consumer interest in the various health benefits they can bring (Arepally & Goswami, 2019; Ramos, Cerqueira, Teixeira, & Vicente, 2018). This motivated researchers to develop new functional products based on probiotics, which are defined as microorganisms that provide beneficial effects to consumers when administered in adequate quantities (FAO & WHO, 2002). In this context, non-dairy food matrices have been studied as potential carriers for these microorganisms due to the growing number of individuals suffering from lactose intolerance, milk protein allergy, galactosemia and hypercholesterolemia (Silanikove, Leitner, & Merin, 2015).

There is currently a growing market for juices composed of more than one fruit and this tendency is most observed in products that use tropical fruits including are acerola (*Malpighia emarginata* D.C.) and siriguela (*Spondias purpurea* L.). Acerola is a fruit native to Central America and northern South America, with some of the largest plantations in Brazil, which has high contents of ascorbic acid, carotenoids and phenolic compounds. Its high ascorbic acid content can be exploited in iron absorption, reduction of oxidative stress, synthesis of collagen and tyrosine, and increase in immunity of the human body (Almeida et al., 2011; Boonpangrak, Lalitmanat, Suwanwong, Prachayasittikul, & PrachayasittikuL, 2016). Siriguela is a tree native to Central America but widespread in all tropical countries, mainly in the Caatinga biome of northeastern Brazil, which produces a small yellow fruit with a pleasant flavor and aroma bearing the same name. From a nutritional point of view, it is a fruit rich in carbohydrates, which represent about 19% of its weight and are responsible for its high calorific value (74 kcal/100 g), calcium, phosphorus, iron, vitamin B, vitamin C and pro-vitamin A (Augusto, Cristianini, & Ibarz, 2012; Engels et al., 2012; Kohatsu et al., 2011).

However, the high water content of fruit juices makes them highly

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perishable products and entails high transport costs. In this sense, the development of powdered probiotic fruit juices would represent an interesting alternative for the food industry, resulting in more stable products, with reduced volumes and, consequently, easier to transport and store (Shishir & Chen, 2017). Microencapsulation has been successful in protecting the probiotic culture from environmental conditions (Ghasemnezhad, Razavilar, Pourjafar, Khosravi-Darani, & Ala, 2017), increasing the product shelf life and masking changes in the flavor, aroma and color (Granato, Nazzaro, Pimentel, Esmerino, & Cruz, 2019).

Spray drying is the most used technique in the production of powdered juices. Nevertheless, the high contents of low molecular weight sugars and organic acids in the compositions of acerola and ciriguela juices can affect their drying, creating problems of adherence to the chamber wall and resulting in low process yield as well as operational difficulties (Cano-Chauca, Stringheta, Ramos, & Cal-Vidal, 2005). To overcome these limitations, different encapsulating agents, mainly polysaccharides, can be used to microencapsulate probiotic juices (Arslan, Erbas, Tontul, & Topuz, 2015; Barbosa et al., 2015). Among them, maltodextrins with low dextrose equivalent (5, 10 and 15) are the most suitable for spray drying microencapsulation thanks to their excellent encapsulating properties and low moisture diffusivity (Kavitake, Kandasamy, Davi, & Shetty, 2018; Ribeiro et al., 2019).

Several studies have reported the addition of a probiotic in different fruit juices to produce functional beverages (Kalita, Saikia, Gautam, Mukhopadhyav, & Mahanta, 2018; Kingwatee et al., 2015; Lascano et al., 2020; Paim, Costa, Walter, & Tonon, 2016; Vivek, Mishra, & Pradhan, 2020). However, there is no report in the literature on the use of multiple probiotic microorganisms in mixed beverages.

The aims of this study were a) to develop a non-dairy probiotic product, composed of two native and exotic fruits from the northeastern Brazil, enriched with three *Lactobacillus* strains (*L. rhamnosus* LPAA 01, *L. casei* LPAA 02 and *L. plantarum* LPAA 03) and microencapsulated by spray drying, b) to characterize the resulting powders for their physicochemical properties, chemical composition, *in vitro* bioaccessibility of phenolics and antioxidant activity and c) to check the presence of the three probiotics in the microcapsules by the multiplex PCR method as well as their viability and stability.

2. Materials and methods

2.1. Materials

Ripe fruits of acerola and siriguela were purchased from the Supply and Logistics Center of Pernambuco (Recife, PE, Brazil). *Lactobacillus rhamnosus* LPAA 01, *Lactobacillus casei* LPAA 02 and *Lactobacillus plantarum* LPAA 03 were obtained from the stock cultures of the Department of Rural Technology of the Federal Rural University of Pernambuco (Recife, PE, Brazil). Maltodextrin with 5 dextrose equivalent (DE) was donated by Ingredion (São Paulo, SP, Brazil) to be used as carrier agent.

2.2. Activation of microbial cultures

The cultures of lactobacilli were maintained in De Man, Rogosa and Sharpe (MRS) medium containing mineral oil and kept under refrigeration at 4 °C. After static activation for 24 h at 35 °C in 250-mL Erlenmeyer flasks containing 100 mL of MRS broth, they were reactivated by transferring them to the same fresh medium in a proportion representing 10% (v/v) of the initial fermentation volume. The stock culture was prepared by adding 15% (v/v) of sterile glycerol to the reactivated culture and stored at −20 °C in sterile screw cap tubes.

2.3. Fermentation of acerola and ciriguela mixed juice

Probiotic acerola and siriguela juice was prepared with 60% (w/w) acerola and 40% (w/w) siriguela. The juice was inoculated with 1% (v/v) of each microbial culture up to a total of 10^{10} colony forming units per gram of solid (CFU.g^{-1}) and incubated at 35 °C for 24 h under anaerobiosis. The fermented mixed juice was then added to an equal volume of fresh mixed juice, which underwent the same fermentation process as the previous portion.

2.4. Microencapsulation of fermented probiotic mixed juice

The fermented mixed juice was mixed in the 1:1 (w/w) ratio with 10% (w/w) 5 DE maltodextrin prepared in sterile distilled water and the resulting mixture homogenized with a homogenizer, model TE-102 (Tecnal, Piracicaba, SP, Brazil). Microencapsulation was carried out in a mini spray dryer, co-currently, model MSD 1.0 (Labmaq, Ribeirao Preto, SP, Brazil), with a nozzle of 1.2-mm diameter. The mixture was fed into the chamber with a peristaltic pump at a 30 m^3/h air flow rate and a 0.6 bar air pressure. The air inlet temperature and feed flow rate were set at 140 °C and 0.60 L/h, respectively, according to Ribeiro et al. (2019).

2.5. Microbial viability

The samples either of the probiotic juice or of the microencapsulated product were analyzed for viable *Lactobacillus* sp. counts by 10^{-7} serial dilutions in 0.1% sterile peptone (Merck, Darmstadt, Germany). After plating the last dilutions on MRS agar, the plates were incubated anaerobically at 35 °C for 72 h. The colonies were counted with the help of a colony counter with magnifying glass and their number was expressed as log CFU. g^{-1}.

2.6. In vitro bioaccessibility of phenolic compounds and antioxidant activity

The *in vitro* gastrointestinal digestion procedure mimicking physiological gastrointestinal conditions was assessed in three sequential fractions, namely the gastric fraction and the non-dialyzed (OUT) and dialyzed (IN) fractions after intestinal digestion, and the extracted phenolic compounds were determined according to the method proposed by Dutra et al. (2017).

2.6.1. Quantification of phenolic compounds

Phenolic compounds were analyzed with a high-performance liquid chromatograph (HPLC) (Acquity H-Class, Waters, Milford, MA, USA) equipped with a C18 column (BEH 2.1 × 100 mm, 1.7 μm particle size) (Waters) and coupled to a mass spectrometer with single quadrupole mass detector (SQ Detector 2, Waters). The mobile phase (A) was an aqueous solution containing 2% MeOH, 5 mM ammonium formate and 0.1% formic acid, while the mobile phase (B) a methanolic solution of 0.1% formic acid fed at a flow rate of 0.3 mL/min. The gradient elution was programmed as follows: a) initial fixed proportion of 98% A and 2% B for 0.25 min, b) linear increase in B proportion up to 99% in 8.5 min, c) maintenance for 1 min, d) immediate decrease to 2% B, and e) maintenance for up to 11 min. The sample injection volume was set at 10 μL, and column and auto-injector temperatures were maintained at 40 and 10 °C, respectively. Data acquisition was performed in Single Ion Recording (SIR) mode, searching for masses of the following compounds: gallic acid (170.0215 Da), syringic acid (198.0528 Da), *p*-coumaric acid (164.0473 Da), kaempferol (286.0477 Da), quercetin (302.0426 Da) in negative ionization. Chromatograms and mass spectra were acquired by the MassLynx software (Waters, Canada). Standard curves with concentrations in the range 5–100 μg/mL were used to quantify each compound, by relating the areas of peaks to those of standards by means of the corresponding curves' equations. Curves and quantifications were done using the TargetLynx tool of MassLynx software (Waters, Canada).

2.6.2. Determination of antioxidant activity

The antioxidant activity was determined by both the Oxygen Radical Absorbance Capacity (ORAC) and the 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assays. The ORAC assay was carried out on a multilabel counter equipped with a fluorescence filter (Fluostar Omega, BMG Labtech, Ortenberg, Germany) according to the procedure described by Zulueta, Esteve and Strígoro (2009). The ABTS assay was performed using a spectrophotometer (UV–1650PC, Shimadzu, Kyoto, Japan) according to the method described by Re et al. (1999).

2.7. Multiplex PCR method

The preparation of samples for PCR detection started with the identification and quantification of the three strains of *Lactobacillus* sp. after microencapsulation. The powder was reconstituted in 1:10 (w/v) peptone water and the suspension was kept at 25 °C for 30 min to release the cells. Aliquots (1 mL) of dilutions were spread on plates containing MRS Agar and 0.02% bromophenol blue and incubated at 35 °C for 72 h. After that, the multiplex PCR assay was carried out according to the method proposed by Kwon, Yang, Yeon, and Kim (2004). The samples were analyzed under UV radiation on 1% agarose gel containing SYBR® green (BioRad, Hercules, CA, USA) as dye. Primer sequences and size of the amplicons are displayed in Table 1.

2.8. Physicochemical analyses

2.8.1. Hydrogenionic potential

The powder was diluted in distilled water up to 1:5 (w/v) and submitted to measurement of the hydrogenionic potential (pH) using a previously-calibrated pHmeter with glass electrode (TEC-5, Tecnal, Piracicaba, SP, Brazil).

2.8.2. Soluble solids content

The content of total soluble solids was determined at 20 °C using a digital refractometer and the results were expressed in °Brix (model r2i300, Reichert, New York, USA).

2.8.3. Titratable acidity

The titratable acidity was determined by titration according to methodology described by AOAC (2016) and the results were expressed as g of citric acid 100 g^{-1}.

2.8.4. Color

The color either of pulp or powder was determined using a colorimeter, model CR-400 (Konica Minolta®, Tokyo, Japan), according to the L*a*b* (CIE Lab) system (AOAC, 2016). The total difference in color (ΔE*) between *in natura* pulp and powder has been calculated as color average according to Equation (1):

$$\Delta E^{*} = \left[(L^{*} - L_0)^2 + (a^{*} - a_0^{*})^2 + (b^{*} - b_0^{*})^2 \right]^{1/2} \quad (1)$$

where L_0*, a_0* and b_0* are luminosity, red/green color intensity with green in negative and red in positive directions, and yellow/blue color intensity with blue in negative and yellow in positive directions, of *in natura* pulp, while L*, a* and b* are those of the powder, respectively.

Table 1
Multiplex PCR primers used in this study.

Target bacteria	Primer	Sequence (5′ to 3′)	Size
Lactobacillus rhamnosus	*rhamnosus*	TGCATCTTGATTTAATTTTGAA	1.119 pb
Lactobacillus rhamnosus	IDL03R	CCACCTTCCTCCGGTTTGTCA	
Lactobacillus casei	IDL11F	TGGTCGGCAGAGTAACTGTTGTCG	733 pb
Lactobacillus casei	IDL03R	CCACCTTCCTCCGGTTTGTCA	
Lactobacillus plantarum	IDL62R	CTAGTGGTAACAGTTGATTAAAACTGC	648 pb
Lactobacillus plantarum	IDL04F	AGGGTGAAGTCGTAACAAGTAGCC	

Source: Kwon et al. (2004).

2.8.5. Total carotenoid

The total carotenoid contents of pulp and powder were determined according to the methodology described by Rodriguez-Amaya (1999) and quantified using the mathematical expression described by Gross (1987, p. 303).

2.8.6. Ascorbic acid

The ascorbic acid contents of pulp and powder were determined by the 2,6-di-chlorophenol-indophenol titrimetric method (AOAC, 2016).

2.8.7. Rehydration time

The rehydration time was determined by adding 2 g of the dry powder to 50 mL of distilled water at 26 °C. The mixture was agitated in a 100-mL glass beaker (MA 089, Marconi, Piracicaba, SP, Brazil) at 800 rpm using a magnetic stir bar. The time required for complete powder rehydration was recorded (Goula & Adamopoulus, 2010).

2.8.8. X-ray diffraction

X-ray diffraction analysis of microparticles was performed on an X-ray diffractometer (D8 Advance, Bruker, Karlsruhe, Germany) using a graphite crystal as the monochromator with a filter radiation of Cu-Kα1 at 40 kV and 40 mA. Samples were analyzed at angles from 4 to 40° in 2θ with a 0.02° (1°/min) step.

2.9. Chemical compositions

The ash, lipid and protein contents of pulp and powder were quantified according to AOAC (2016), and the results expressed in g.100 g^{-1}. The moisture content of powder was determined on an infrared balance, model ID50 (Marte Científica, Santa Rita do Sapucaí, MG, Brazil), at 105 °C for 30 min, while that of pulp at 105 °C for 45 min, and the results were expressed in % (w/w). The carbohydrate content was calculated as the difference between the total sample mass and the contents of moisture, ash, lipids, and proteins, and expressed in g.100 g^{-1}.

2.10. Stability tests

The powdered probiotic juice samples were placed in airtight glasses with a saturated solution of lithium chloride at refrigeration (5 °C) and room (25 °C) temperatures. The water activity, moisture, content of total phenolic compounds, cell viability and color were evaluated over the storage time (0, 3, 10, 14, 20 and 45 days) in order to understand the relationships between probiotic survival and product physicochemical parameters. All determinations were performed as described in section 2.8. Phenolic compounds were quantified according to Wettasinghe and ShahidI (1999) by reaction with the Folin Ciocalteau reagent (Merck, Darmstadt, Germany) and subsequent absorbance measurement at 725 nm, and the results expressed in mg of gallic acid equivalent per 100 g of sample.

2.11. Data analysis

The results of tests on microencapsulated powders were performed in triplicate and values of the dependent variables in the stability study were evaluated by two-way RMANOVA test and compared with the Duncan test at 5% probability ($p \leq 0.05$) by the StatScoft Statistica program, version 7.0 (SAS Institute, Cary, NC, USA).

3. Results and discussion

3.1. Microbial viability

According to Table 2, spray drying caused a loss in probiotic viability of about 2 log CFU. g^{-1} compared to the mixed juice of acerola and siriguela before microencapsulation (10.39 ± 0.21 log CFU. g^{-1}). Nonetheless, the viable cell count was higher than the minimum value (6.0 log CFU. g^{-1}) recommended for probiotics in food products to ensure therapeutic benefits (FAO & WHO, 2002), thus demonstrating that the atomization conditions adopted were suitable for guaranteeing the viability of probiotics in the microencapsulated mixed juice. Such counts are close to those reported for microencapsulation by spray drying of litchi (Kalita, Saikia, Gautam, Mukhopadhyay, and Mahanta, 2018), jussara (Paim et al., 2016) and passion fruit (Dias et al., 2018) probiotic juices, while lower probiotic counts were reported by Kingwatee et al. (2015) for the first probiotic juice.

3.2. In vitro bioaccessibility of phenolic compounds and antioxidant activity

The results of *in vitro* simulation of powdered probiotic mixed juice digestion showed a increase in both the content of phenolic compounds, with the exception of quercetin and the antioxidant activity by ORAC method (Table 3 and Fig. 1). The levels of gallic acid, rutin, *p*-coumaric acid and syringic acid in the dialyzed fraction after intestinal digestion (IN fraction) and in the non-dialyzed fraction after intestinal digestion (OUT fraction) were increased by 109.43, 49.10, 20.50 and 63.04%, respectively, compared to the gastric fraction. This same increase was observed by Dutra et al. (2017) for umbu-cajá pulp, in a study bioaccessibility of phenolic compounds exposed to simulated gastrointestinal conditions, reported an increase in the compounds rutin, gallic acid, quercetin and *p*-Coumaric acid after intestinal digestion (IN and OUT) in relation to gastric fraction. A previous study reported that the increase of phenolic compounds in the intestinal fraction (Bouayed, Hoffmann, & Bohn, 2011) may be due the action of intestinal and digestive enzymes (e.g., pancreatin) that facilitate the release of phenolics bound to the matrix (Dutra et al., 2017). The gastrointestinal tract can be considered as an efficient extraction device, where part of the compounds contained in food matrices are extracted and made available for absorption in the intestine (Tagliazucchi, Verzelloni, Bertolini, & Conte, 2010). The increase in rutin after exposure to simulated gastrointestinal conditions may also be related to the fact of rutin is generated from quercetin, which is its aglycone form. Consequently, during the exposure to acidic conditions, rutin could be generated from quercetin through rupture of the linkage with the sugar (Celep, Charehsaz, Aküz, Acar, &).

Table 2
Physicalchemical and chemical characterization of mixed juice of acerola and siriguela probiotic powder.

Determinations	Mean ± Standard Deviation
Microbial viability (log CFU.g^{-1}) pH	8.48 ± 0.03
	3.74 ± 0.02
Soluble solids (° Brix)	8.06 ± 0.11
Titratable acidity (g of citric acid 100g^{-1})	0.45 ± 0.01
Color (L*)	86.42 ± 0.38
(a*)	4.14 ± 0.07
(b*)	29.25 ± 0.36
Difference of color (ΔE*)	39.47 ± 0.40
Total carotenoid (μg/g)	10.47 ± 0.30
Ascorbic acid (mg.100g^{-1})	1060.46 ± 100.23
Rehydration time (s)	135.66 ± 1.52
Protein (g.100 g^{-1})	3.40 ± 0.09
Lipid (g.100 g^{-1})	5.53 ± 0.68
Ash (g.100 g^{-1})	4.55 ± 1.05
Moisture (%)	3.74 ± 0.01
Carbohydrate (g.100 g^{-1})	82.98 ± 0.45

Table 3
Results of *in vitro* bioaccessibility tests of phenolic compounds and antioxidant activity of powdered probiotic mixed juice of acerola and siriguela.

Phenolic compounds and antioxidant activity	Gastric fraction	OUT fraction[a]	IN fraction[b]
Gallic acid (μg/g)	65.06 ± 5.78	53.08 ± 4.08	83.18 ± 17.48
Rutin (μg/g)	192.40 ± 48.60	170.45 ± 17.19	116.43 ± 7.63
Quercetin (μg/g)	25.60 ± 2.00	7.23 ± 1.12	ND[c]
p-Coumaric acid (μg/g)	142.80 ± 10.20	91.93 ± 4.75	80.15 ± 15.50
Syringic acid (μg/g)	423.20 ± 110.87	367.15 ± 13.89	322.87 ± 25.98
Kaempferol (μg/g)	<5.00	<5.00	<5.00
ORAC (μmol TEAC/100 g)	4945.94	3236.57	2077.13
ABTS (μmol Trolox/100 g)	5649.71	3172.83	1945.52

[a] Non-dialyzed fraction after intestinal digestion.
[b] Dialyzed fraction after intestinal digestion.
[c] Not determined.

The antioxidant capacity was determined both in the initial sample of mixed probiotic powder (ORAC method: 6024.83 μmol TEAC/100 g and ABTS method: 7806.40 μmol Trolox/100 g) and at all the stages of digestion. Compared to the initial sample before digestion, the antioxidant activity in the IN fraction determined by these methods was reduced by 65.5 and 75.1%, respectively, while in the OUT fraction there were reductions of 46.3 and 59.4%, respectively. Similar loss of antioxidant capacity determined by different methods (51–78%) was reported after *in vitro* gastrointestinal digestion of juçara fruit (Schulz et al., 2017). The antioxidant capacity by ORAC method in the dialyzed fraction after intestinal digestion (IN fraction) and in the non-dialyzed fraction after intestinal digestion (OUT fraction) was increased while, by ABTS method was decreased, compared to the gastric fraction. It is noteworthy that the comparison among *in vitro* studies is rather difficult due to differences not only in the starting material but also in the digestion procedure and antioxidant activity assays (Silva, Costa, Branco, & Branco, 2016).

3.3. Identification of probiotic species by multiplex PCR

The multiplex PCR assay was performed to identify each of the three *Lactobacillus* species, namely *Lactobacillus casei, Lactobacillus rhamnosus* and *Lactobacillus plantarum*, after microencapsulation. As expected, each specific primer pair showed unique amplification, confirming their survival. To quantify their relative abundance in powdered mixed juice, microbial viability was checked by colony counts on plates containing MRS Agar and bromophenol blue. It was observed that *L. casei* had the greatest ability to survive the atomization conditions adopted in this study, showing 50% cell survival, followed by *L. rhamnosus* (30%) and *L. plantarum* (20%). These results not only confirm the effectiveness of maltodextrin as an encapsulating matrix, but also suggest that the sugars contained in the juice may have contributed to cell survival by acting as thermoprotectants. Moreover, maltodextrin itself, which is considered a moderate prebiotic, may have played a role not only in the survival of probiotics (Kalita, Saikia, Gautam, Mukhopadhyay, & Mahanta, 2018; Lascano et al., 2020) but also in reducing the moisture content and water activity of microparticles, thus increasing their stability (Anandharamakrishnan & Padma, 2015).

3.4. Physicochemical properties

Soluble solids (8.06 ± 0.11° Brix), total titratable acidity (0.45 ± 0.01 g of citric acid 100 g^{-1}), and pH (3.74 ± 0.02) of reconstituted probiotic juice (Table 2) exhibited favorable characteristics for marketing and sensory acceptance. In particular, such a pH value is expected

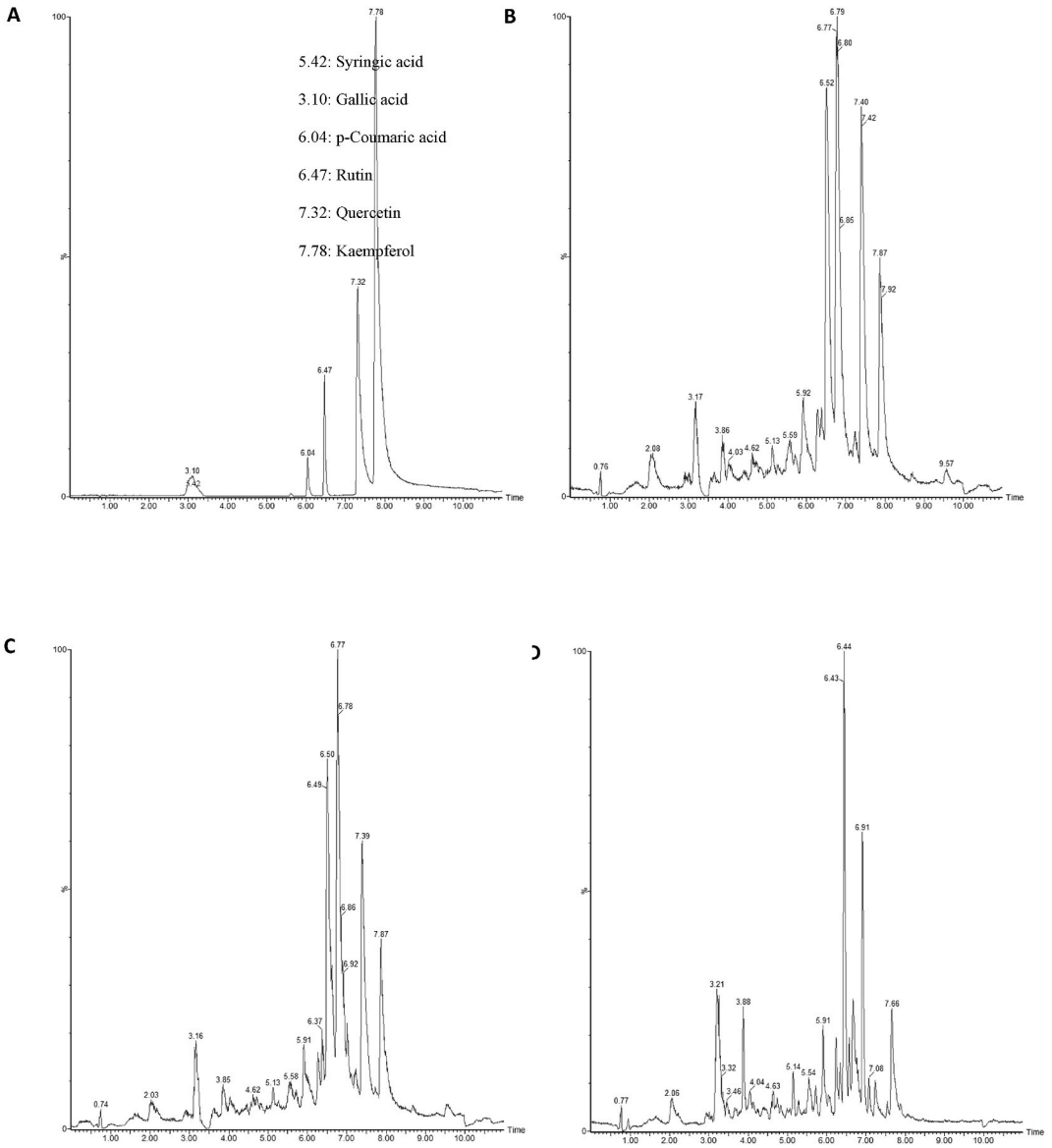

Fig. 1. HPLC-MS chromatograms of simulated gastrointestinal digestion of powdered probiotic mixed juice of acerola and siriguela. Control (A), Gastric fraction (B), Non-dialyzed fraction (OUT) after intestinal digestion (C), Dialyzed fraction (IN) after intestinal digestion (D).

to have a less severe effect on probiotic organisms than pH 2 (Bajaj, Survase, Bule, & Singhal, 2010). Moreover, the values of acidity and pH obtained in this work were close to those reported by Kalita, Saikia, Gautam, Mukhopadhyav, and Mahanta (2018) for synbiotic spray dried powder of litchi juice.

The color is an important indicator for food since it reflects sensorial quality and influences attractiveness, being one of the main attributes that drive consumption. The luminosity value (L* = 86.42 ± 0.38) of powders was significantly higher than that obtained for the *in natura* pulp (54.38 ± 0.40), whereas the values of red/green (a* = 4.14 ± 0.07) and yellow/blue (b* = 29.25 ± 0.36) coordinates were lower than those of pulp (22.34 ± 0.14 and 43.39 ± 0.48, respectively). Since the reductions were likely due mainly to the addition of white maltodextrin that acted as a "diluent" of pigments in the powders (Ferrari, Ribeiro, & Aguirre, 2012), it can be inferred that the atomization operating conditions were suitable for the appearance of microparticles as they did not drastically alter the color (Table 2). In evaluating the color coordinates of mixed juice of pineapple and juçara fermented by *L. rhamnosus* GG, Campos et al. (2019) observed no interference in luminosity with respect to unfermented juice taken as a control, while both a* and b* were significantly increased. Costa et al. (2017) reported that the addition of *L. paracasei* caused browning in orange juice, with a consequent loss of yellow color, thereby affecting the acceptability of the product by consumers.

It has been reported that several factors are responsible for the degradation of ascorbic acid and phenolic compounds including the presence of oxygen, high temperature, exposure to light and low pH (Porto, Okina, Pimentel, Garcia, & Prudencio, 2018). Despite being a good source of ascorbic acid, the powdered probiotic mixed juice showed low values of total carotenoids (Table 3) due to the presence of a significant fraction (40%) of siriguela. Despite being considered highly heat-sensitive compounds, both ascorbic acid (1144.08 ± 27.52 mg.100 g^{-1}) and total carotenoids (16.56 ± 0.18 μ/g) had their contents only slightly reduced by the microencapsulation process compared to the *in natura* pulp. This result was likely due to the efficacy of maltodextrin as a coating agent in the atomization process as well as the inactivation of some heat-sensitive oxidative enzymes (Cano-Higuita, Villa-Vélez, Telis-Romero, Váquira, & Telis, 2015).

The relatively short rehydration time of the powder (Table 2) may have been the result not only of the high maltodextrin solubility in

water, but also of the high temperature used for microencapsulation (140 °C). The effectiveness of maltodextrin in rehydrating powders could also be due to the reduced stickiness of particles, which improved their solubility in water thanks to their increased surface of contact with water. Furthermore, according to Goula and Adamapoulos (2010), an increase in drying air temperature leads to larger and more porous particles, which tend to settle instead of floating in water like the smaller ones, thus shortening the time required to rehydrate them. Alves, Messaoud, Desobry, Costa, and Rodrigues (2016) reported a similar reduction in rehydration time for a powdered probiotic orange juice.

X-ray diffraction is commonly used to confirm the crystalline or amorphous state of powdered dry products. In general, crystalline materials exhibit a series of sharp peaks, while the amorphous ones produce a broad background pattern (Caparino et al., 2012). The diffractogram of powdered probiotic mixed juice showed amorphous characteristics and no crystalline peak formation (Fig. 2).

The chemical composition of foods is essential for assessing their safety and nutritional value. The low average moisture content of the powdered probiotic mixed juice (Table 2) shows that it lost more water (95.76%) than the *in natura* pulp (88.19 ± 0.28%), which means that the product is more stable and protected against the development of deteriorating or even pathogenic microorganisms. The contents of ash, protein, lipid and carbohydrate were higher than those in the *in natura* counterpart (0.44 ± 0.03, 1.85 ± 0.12, 0.72 ± 0.18 and 8.80 ± 0.22 g.100 g^{-1}, respectively), due to the concentration of these compounds resulting from drying. The particularly high carbohydrate content was certainly due to the use of maltodextrin as an encapsulating agent for drying (Phisut, 2012).

3.5. Stability study

Temperature proved to be a very important parameter for product stability, since the cell survival profile of the powder stored at 5 °C was better than that stored at 25 °C throughout the whole storage period (Fig. 3). A reduction in the storage temperature from 25 to 5 °C did in fact extend from 14 to 20 days the time during which viable cell counts in the microencapsulated product kept above the minimum level (6.0 log UFC. g^{-1}) recommended for probiotics in food products to produce therapeutic benefit (FAO & WHO, 2002). Nonetheless, the results can be considered satisfactory up to 20 days of storage at 25 °C and 45 days of storage at 5 °C. Lascano et al. (2020), studying the viability of spray-dried probiotic cells in passion fruit juice powder at different storage temperatures (4, 25, and 37 °C), observed a temperature-dependent log reduction in the number of live cells.

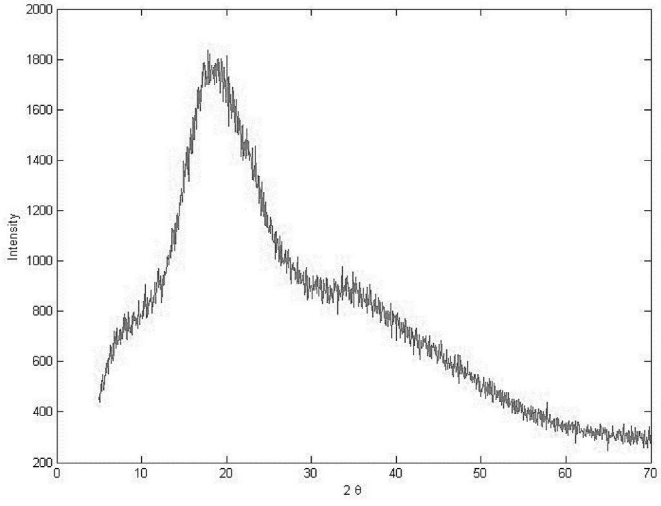

Fig. 2. Diffractogram of powdered probiotic mixed juice of acerola and siriguela.

Spray-dried passion fruit juice supplemented with *L. plantarum* S20 stored at 4 °C showed the best performance among treatments.

The moisture content of the powdered probiotic mixed juice varied during storage, getting values up to 4.40% at 5 °C and 4.05% at 25 °C (Fig. 3). Such a moisture content increase may have been due to both the presence of sugars in fruits and the use of maltodextrin as a carrier agent (Molina, Clemente, Scapim, & Vagula, 2014). With statistically significant difference ($p < 0.05$) only on the 20 day at 5 °C and 25 °C. The values obtained were low enough to consider the powder stable and safe from a microbiological point of view. A similar increase in moisture content during storage time has been reported in stability studies of atomized tamarind juice (Muzaffar & Kumar, 2016) and probiotic Sohiong fruit powder containing *L. plantarum* (Vivek et al., 2020).

The water activity of the powdered probiotic mixed juice varied significantly ($p < 0.05$) between days 3–10, 14–20 and 20–45 at 5 °C. No significant difference ($p \geq 0.05$) was observed at 25 °C (Fig. 3), all conditions ensured values of this parameter (0.16–0.25 at 5 °C and 0.18–0.21 at 25 °C) below the maximum value (0.30) considered microbiologically safe for food. For most food systems, the critical water activity below which no microorganism can grow commonly ranges from 0.6 to 0.7, with the critical value for the growth of pathogenic bacteria set at 0.85–0.86. Since the water activity of the powdered product obtained in the present study was quite below this critical threshold value, we can believe it would be safe in terms of development of pathogenic bacteria (Lascano et al., 2020). These values are also close to those reported by Kingwatee et al. (2015) for lychee juice with *L. casei* 01 microencapsulated by spray drying using different encapsulating agents and by Lascano et al. (2020) for powdered passion fruit juice with *L. plantarum* S20.

The content of phenolic compounds in the powdered probiotic mixed juice stored at 5 °C differ significantly ($p < 0.05$) between the different storage times 10–14 and 20–45 days. The powder stored at 25 °C differ significantly ($p < 0.05$) between the days 10–14, 14–20 and 20–45 (Fig. 3). There was a significant increase in the content of phenolic compounds at the end of the shelf-life for both temperatures (at 5 °C 4775.00 ± 33.07 mg_{GAE} 100 g^{-1} and 25 °C 3333.33 ± 7.21 mg_{GAE} 100 g^{-1}) when compared to the initial time (2637.50 ± 12.50 mg_{GAE} 100 g^{-1}). As expected, different profiles were observed in non-microencapsulated fermented juices, suggesting an important role of atomization in the content of phenolic compounds. For instance, Hashemi et al. (2017) observed a significant reduction in the concentration of total phenolics in sweet lemon juice fermented by *L. plantarum* LS5 as a likely result of their metabolization by the microorganism. Conversely, some studies reported an increase in total phenolic compounds during fermentation, which may be related to the ability of lactic acid bacteria to convert the bioactive compounds present in the juice into their metabolites, resulting in the production of new phenolic compounds (Kumar & Kumar, 2016).

The values of color coordinates of the powdered probiotic mixed juice differed significantly ($p < 0.05$) throughout the storage period at both temperatures (Fig. 3). The luminosity parameter (L*) showed statistically significant difference ($p < 0.05$) in time 0 compared to the other days of storage at 5 °C and 25 °C. The observed changes in luminosity may have been due to the physical changes and chemical reactions during storage (Chang, Karim, Abdulkarim, & Ghazali, 2018). Vivek et al. (2020) observed a significant decrease in the L* value of spray-dried probiotic Sohiong powder extending the storage period from 20-24 days to 24–28 days.

The intensity of red/green color component (a*) showed statistically significant differences ($p < 0.05$) among storage times at both temperatures, a generalized progressive decrease of a* could be detected up to 20 days and a final increase at the end. On the other hand, Vivek et al. (2020) observed a marked a* increase after 16-day-storage of probiotic Sohiong powder, likely due to the increase in powder moisture content during storage.

Significant variations ($p < 0.05$) in the intensity of yellow/blue color

Fig. 3. Stability study of powdered probiotic mixed juice of acerola and siriguela at 5 °C and 25 °C.

component (b*) were observed during storage at both temperatures, except for days 14 and 20 at 25 °C. Considering that carotenoids have many conjugated double bonds (Phisut, 2012) absorbing in a wide wavelength range, the simultaneous and specular decreasing trend of a* and increasing trend of b* during storage up to 20 days are indicative of reductions of the red component towards the green one and of the blue component towards the yellow one, respectively. This observation may be attributed to carotenoids' oxidation reactions (Granalto et al., 2010) with consequent formation of different degradation products with different absorption profiles, which are very common in products

subjected to storage. Whereas a different ratio between carotenoids and degradation products may justify the a* increase and b* decrease observed at the end of storage, the dissolution in the lithium chloride solution of some microcapsule component may have been responsible for the significant increase in a* and decrease in b* at the start of storage test compared to the dry microparticles (Table 2). Color and stability of anthocyanins are well known to depend on pH, light, temperature, and structure. Most of them are highly stable under acidic conditions, while degradation occurs under alkaline conditions (Khoo, Azlan, Tang, & Lim, 2017; Wahyuningsih, Wulandari, Wartono, Munawaroh, & Ramelan, 2017), leading to a color change from red to blue. Based on this, the results obtained for the coordinate *a can be justified by the fact that, under more acidic conditions, anthocyanins are present in greater quantities in their ionic form, which is responsible for the red color.

4. Conclusions

The present investigation showed that it is possible to obtain dry probiotic foods using different fruit juices as matrices that already contain beneficial nutrients such as minerals, vitamins, dietary fibers, and antioxidant compounds. The mixed juice of acerola and siriguela containing three different species of lactobacilli was successfully microencapsulated by spray drying, and the resulting microcapsules showed physicochemical characteristics and chemical composition favorable to marketing. Powdered mixed acerola and siriguela juice proved to be a suitable medium to incorporate three probiotics belonging to the genus *Lactobacillus*, namely *Lactobacillus rhamnosus* LPAA 01, *Lactobacillus casei* LPAA 02 and *Lactobacillus plantarum* LPAA 03, with counts higher the minimum recommended value for probiotics in food (6 log CFU. g^{-1}). All parameters evaluated during storage for 45 days of powdered probiotic juice at two different temperatures (5 and 25 °C) differed significantly ($p < 0.05$). Although the physicochemical characteristics and the content of phenolic compounds of the powder were satisfactory under all storage conditions, those that ensured viable cell counts above 6.0 log CFU. g^{-1} were 20-day storage at 5 °C and 14-day storage at 25 °C.

The multiplex PCR assay was performed to identify each of the three *Lactobacillus* species in the microcapsules. Each specific primer pair showed unique amplification, confirming the survival of all of them. However, *L. casei* was able to survive in greater numbers, thus demonstrating the suitability of the atomization conditions adopted in this study. The contents of phenolic compounds, except that of quercetin, as well as the antioxidant activity by ORAC method increased after exposure to simulated gastrointestinal conditions.

Declaration of competing interest

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

CRediT authorship contribution statement

Michelle Souza: Conceptualization, Data curation, Methodology, Visualization, Writing – original draft. **Amanda Mesquita:** Methodology, Investigation. **Paulo Souza:** Validation, Formal analysis. **Graciele Borges:** Supervision, Formal analysis. **Túlio Silva:** Formal analysis. **Attilio Converti:** Writing – review & editing. **Maria Inês Maciel:** Resources, Project administration, Funding acquisition, Visualization, Investigation, Formal analysis, Writing – review & editing.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.lwt.2021.112271.

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The five key references are mentioned below (with asterisk mark). These references are key references, as they are studies that use a probiotic microrganism in fruit juice and subsequently microencapsulation by spray dryer.

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