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Spray drying of potentially probiotic lactobacilli incorporated in pineapple (*Ananas comosus*) juice: survival and storage stability

Secado por aspersión de lactobacilos potencialmente probióticos incorporados a jugo de piña (*Ananas comosus*): sobrevivencia y estabilidad en el almacenamiento

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Abstract

Usually, probiotics are consumed with dairy products, which are not suitable for people with lactose intolerance and milk protein allergies, so the objective of this work was to evaluate the effect of spray drying temperature on survival of a probiotic (*Limosilactobacillus reuteri*) and a potentially probiotic (*Lactobacillus helveticus*) bacteria in spray dried pineapple juice and their stability during storage at 4 °C to produce a non-dairy functional food. Cells of *L. helveticus* ATCC 27558 or *Lmb. reuteri* NRRL B-14171 collected at the stationary phase were resuspended in pineapple juice at 9 log CFU/mL. Maltodextrin (DE 10) was added as a spray drying carrier (2:1 w/w). Subsequently, the physical and chemical qualities of dehydrated fruit juice were evaluated. *L. helveticus* survival after spray drying was higher than *Lmb. reuteri* with the highest values (92 % and 72 % respectively) at Tin=150 and Tout=70 °C. Process variables did not significantly affect on moisture content, a_w, pH, total phenols, and antiradical activity. *L. helveticus* showed more stability than *Lmb. reuteri* and remained in numbers >7 log CFU/g during a storage period of four months at 4 °C. Results show that it is possible to produce a nondairy functional product with pineapple juice and potentially probiotic bacteria by spray drying.
Keywords: *Lactobacillus*, probiotic, spray drying, pineapple juice.

Resumen

Los probióticos suelen consumirse en productos lácteos, que no son aptos para personas con intolerancia a la lactosa y alergias a las proteínas de la leche, por lo que, el objetivo de este trabajo fue evaluar el efecto del secado por aspersión sobre la supervivencia de bacterias potencialmente probióticas (*Limosilactobacillus reuteri* NRRL B-14171 y *Lactobacillus helveticus* ATCC 27558) en jugo de piña secado por aspersión y su estabilidad durante el almacenamiento. Las bacterias recolectadas en fase estacionaria fueron resuspendidas en jugo de piña (9 log UFC/mL) con maltodextrina (DE 10) como material de soporte (2:1 p/p) y deshidratadas a dos temperaturas de entrada (150 y 170 °C) y dos de salida (70 y 80 °C). Después de la deshidratación, la supervivencia en *L. helveticus* (92 %) fue mayor que en *Lmb. reuteri* (72 %) con los valores más altos a Te=150 y Ts=70 °C. No se observaron efectos significativos sobre el contenido de humedad, a_w, pH, fenoles totales y actividad antirradical. *L. helveticus* mostró más estabilidad (>7 log UFC/g) que *Lmb. reuteri* durante cuatro meses de almacenamiento a 4 °C. Los resultados muestran que es posible obtener un producto funcional con jugo de piña y bacterias potencialmente probióticas mediante secado por aspersión.
Palabras clave: *Lactobacillus*, probióticos, secado por aspersión, jugo de piña.

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1 Introduction

The interest of the population to take care of their health and improve their quality of life has stimulated research for the formulation of foods that provide special benefits for the consumer, functional foods enhance bioregulation such as stresses, appetite, and absorption; biodefence, such as immunity and suppression of allergies; can prevent diseases, including diarrhea, constipation, cancer, high cholesterol levels and diabetes (Mitsuoka, 2014).

Functional food can contain components such as vitamins, minerals, fatty acids, antioxidants, or probiotics (Vicentini *et al*., 2016). The FAO defines probiotics as living microorganisms that confer a health benefit when administered in adequate quantities (FAO, 2016). Functional foods with probiotics are reported to help maintain the intestinal microbiota balance, increase resistance against various pathogens, lower blood cholesterol, and alleviate symptoms related to lactose intolerance (Tripathi and Giri, 2014).

Generally, probiotics are related to foods of dairy origin, however, due to problems such as lactose intolerance, allergy to milk protein, and cholesterol content, an increasing number of non-dairy food matrices exhibit potential for delivery of probiotics (Min *et al*., 2018). The incorporation of probiotics in spray-dried fruit juices like raspberry (Anekella and Orsat, 2013), lychee (Kalita *et al*., 2018; Kingwatee *et al*., 2015), *orange* (Barbosa *et al*., 2017), blackberry (Colín-Cruz *et al*., 2019), passion fruit (Dias *et al*., 2018; Santos *et al*., 2020), offers an alternative to traditional sources. Another alternative may be pineapple juice. Pineapple is the second most important tropical fruit after Mangoes with a world production of 25 842.4 thousand tons (FAO 2019). Pineapple juice has high nutritional value, it is rich in vitamins and minerals, and its antioxidant activity has been reported (Jittanit *et al*., 2010), it is generally consumed as fresh fruit, however, the application of spray drying process allowed obtaining pineapple juice powder with good quality attributes (Cardona *et al*., 2023), so it is an attractive option to subject it to processes that increase its useful life as a probiotic functional food.

On the other side, spray drying has been widely studied as a technique to preserve foods since it is fast, continuous, and inexpensive, it tries to preserve most of the nutritional compounds of the original food, securing the chemical and microbiological stability of the product (Peighambardoust *et al*., 2011; Archacka *et al*., 2020; Borras-Enríquez *et al*., 2023, Carpintero-Tepole *et al*., 2024). The biggest challenge during the processes of making food added with probiotics, especially by spray drying, is to avoid losses in viability and protect the integrity of the cells during storage, since it has been recommended that for greater effectiveness of a probiotic food it must contain at least 7 log CFU/g or mL of viable cells at the time of consumption (Barbosa *et al*., 2015).

Various stress factors can affect the viability of probiotic microorganisms added to foods, such as temperature, the food matrix composition, and storage conditions (Tripathi and Giri, 2014). Therefore, the aim of this work was to evaluate the effect of spray drying temperature on the survival of a probiotic (*Limosilactobacillus reuteri* NRRL B-14171) and a potentially probiotic (*Lactobacillus helveticus* ATCC 27558) bacteria in spray dried pineapple juice and their stability during storage at 4 °C.

2 Materials and methods

2.1 Materials

Pineapple fruits (*Ananas comosus* v. cayena) with maturity organoleptic scale 3 (M3=25-75 % of yellow color on the surface of the fruit) were purchased in a local market of Veracruz city. Lactic acid bacteria (LAB) strains *Lmb. reuteri* NRRL B-14171 and *L. helveticus* ATCC 27558 were proportionated by The Agricultural Research Service USDA (United States Department of Agriculture) and the American Type Culture Collection, respectively. Maltodextrin (MD DE10) (CPI Ingredientes S.A. de C.V., Toluca, Mexico) was used as carrier material for spray drying. The culture media were purchased from DIFCO co. The reagents used to determine total phenols content and antiradical activity were purchased from Sigma-Aldrich Corp. (St. Louis, Mo, USA).

2.2 Lactobacilli growth conditions

Lactic acid bacteria (LAB) strains were reactivated by 3 propagation steps in MRS broth. Growth was followed by absorbance measurements at 640 nm in MRS broth to identify growth phases. All the cultures were prepared with 1% inoculums and incubated at 37 °C. Cells were collected in stationary phase by centrifugation at 7000 x g for 10 min at 4 °C in an Eppendorf® Centrifuge 5810R Rotor F-34-6-38 (Golowczyc *et al*., 2011). After carefully removing the supernatant, the sediment was resuspended and washed twice by centrifugation in sterile NaCl (0.85 % w/v) to eliminate MRS broth residues.

2.3 Preparation of pineapple juice

Pineapple fruits were washed by immersion in NaClO solution (250 ppm) for 5 min. The juice was extracted using a domestic juicer, and the juice was vacuum filtered with Whatman No. 3 to eliminate the solids in suspension, preventing the obstruction of the spray dryer atomizer. The filtered juice was collected in a glass flask, heat-treated by immersion in a water bath at 90 °C for 60 s, and after cooled by immersion in an ice bath to reduce the microbial charge. The juice was analyzed to assess the absence of spoiling microorganisms through spread plating on PCA, PDA and MRS agar.

2.4 Evaluation of LAB stability in pineapple juice

To corroborate that the composition of the juice does not affect cell viability, the stability of lactic acid bacteria incorporated into thermally treated pineapple juice was evaluated. Juice samples with LAB added (9 log CFU/mL) were stored at 25 ºC, and the viability of LAB was evaluated at 0, 2, and 4 h by the pouring plate method on MRS agar.

2.5 Preparation of pineapple juice with incorporated LAB

The carrier material MD DE10 was added to 100 g of thermally treated juice at a concentration of 2:1 (g/g), under sterile conditions by magnetic stirring until complete dissolution. The cell pellet was added (1 g /100 mL of juice) to reach 9 log CFU/mL (previously standardized by the pouring plate method for each LAB), and the mixture was immediately spray-dried.

2.6 Spray drying of pineapple juice with incorporated LAB

The drying process was carried out in a BUCHI B-190 mini-spray Dryer with a co-current open cycle. The spray dryer was conditioned before the drying experiments; it was treated with sterile water for 20 min with the inlet air temperature (T_{in}) adjusted to 180 °C and outlet air temperature (T_{out}) of 100 °C. The collecting vessel was sterilized by UV light for 15 min (Páez *et al*., 2013)

For the drying of pineapple juice with each LAB culture two Tin (150 and 170 °C) and two Tout (70 and 80 °C) levels were applied according to $2x2^2$ factorial completely random design. Spray-dried juice was stored in vacuum-sealed aluminum bags. Physicochemical and microbiological analyses were performed immediately after drying and during storage at 4 ºC.

2.7 Drying yield for spray-dried powders

After each spray drying process, the drying yield (%) was determined as the percentage of the powder collected from the collecting vessel attached to the bottom of the cyclone, compared to the initial value of solids contained in the liquid feed (Juice solids + drying agent solids + cell pellet solids all in dry weight), using the following equation:

$$Yield = \frac{Weight\ of\ the\ solids\ in\ spray\ dried\ product}{Dry\ weight\ of\ solids\ in\ the\ liquid\ feed} \times 100 \quad (1)$$

2.8 Evaluation of LAB viability

The enumeration of *L. plantarum* and *Lmb. reuteri* was determined before spray drying, immediately after spray drying and at regular intervals throughout storage. Survival was evaluated by rehydration of each dried sample to their initial solids' concentration in peptone water according to Jittanit *et al*. (2010). The appropriate serial decimal dilutions were made, and colonies were enumerated in duplicate by the pouring plate method using MRS agar. The plates were incubated at 37 °C for 24-36 h.

2.9 Physicochemical analysis of thermally treated and dehydrated juice

Fresh, thermally treated and dehydrated pineapple juice added with LAB was analyzed for pH, antiradical activity and total phenols content. Powder samples were rehydrated as described in section 2.8. The total and soluble solids content was analyzed only on fresh and thermal-treated pineapple juice. Water activity (a_w) and moisture content were evaluated only on powders.

2.9.1 Total and soluble solids

The juice's total soluble solids (°Brix) content was measured using a digital refractometer Leica mark II, according to the AOAC 983.17 method. Total solids were evaluated by the AOAC 920.151 method; 25 g of fresh or heat-treated pineapple juice was dried until the weight variation was less than 3 mg (AOAC, 2003).

2.9.2 pH

Fresh, thermally treated and rehydrated pineapple juice added with LAB was analyzed for pH using a potentiometer Science Med model PHS-W, according to the EN 1132 (1994) method.

2.9.3 Antiradical activity

The antiradical activity was measured by the DPPH (2,2-diphenil-1- picrylhydrazyl) method according to Saikia *et al*. (2016) with some modifications. A sample volume (100 μL) was added to 3.9 mL of DPPH (25 mg/L) dissolved in methanol; the mixture was incubated in the dark for 5 min at 25 ºC. Absorbance was measured at 517 nm. Standard calibration was made with ascorbic acid. Results were expressed as ascorbic acid equivalents (AAE) on dry base (AAE mg/g d.b.) and antiradical activity retention (%) was calculated using the following equation:

$$Antiradical\ activity\ retention\ (\%) = \frac{Antiradical\ activity\ in\ spray-dried\ juice\ \left(\frac{mgAAE}{100\ g\ d.b.}\right)}{Antiradical\ activity\ in\ fresh\ juice\ \left(\frac{mgAAE}{100\ g\ d.b.}\right)} \times 100 \quad (2)$$

2.9.4 *Total phenolic content*

Total phenolic content was evaluated by the Folin-Ciocalteu method. A mixture of 100 μL of Folin-Ciocalteu reagent (2 M), 20 μL of sample and 1580 μL of distilled water was vortexed for 10 seconds and incubated for 5 min, subsequently 300 μL of 20 % Na_2CO_3 was added and incubated in the dark at 40 °C for 30 min more. Absorbance was measured at 765 nm. Standard calibration was made with gallic acid. Results were expressed as gallic acid equivalents (GAE) on dry base (GAE mg/g d.b.) using the following equation:

$$Total\ Phenols\ Retention\ (\%) = \frac{Total\ phenols\ in\ spray-dried\ juice\ \left(\frac{mgGAE}{100\ g\ d.b.}\right)}{Total\ phenols\ in\ fresh\ Juice\ \left(\frac{mgGAE}{100\ g\ d.b.}\right)} \times 100 \quad (3)$$

2.9.5 *Determination of water activity and moisture content*

The water activity (a_w) was measured in triplicate at 25 ± 1 °C, using an Aqualab, Decagon, Devices, model Serie 3 hygrometer. Water activity was evaluated by the AOAC 925.10 (32.1.03) method.

2.9.6 *Storage stability evaluation*

Stability during storage was evaluated at 4 °C each month for 4 months to the spray-dried juice with the highest cell viability of LAB. The dehydrated product was packaged in vacuum-sealed aluminum bags. The viability of LAB was evaluated as mentioned in 2.8.

2.9.7 *Statistical analysis*

Spray drying of pineapple juice was performed according to $2x2^2$ factorial completely random design for two inlet (150 and 170 °C) two outlet (70 and 80 °C) temperatures and two strains (*Lactobacillus helveticus* ATCC 27558 and *Limosilactobacillus reuteri* NRRL B-14171), with three replicates by treatment. The response variables were moisture content (% d.b.), water activity and retained viability (% of log(CFU)/mL). These variables were fitted to the model presented in following equation,

$$y_i = \beta_0 + \beta_1\ x_{i1} + \beta_2\ x_{i2} + \beta_{12}\ x_{i1}x_{i2} \quad (4)$$

where the independent variables are the inlet (x_{i1} [150,170]) and outlet temperatures (x_{i2} [70,80]); and y_i are the response variables. The values of the parameter estimators (β_i) were calculated by linear regression. Analysis of variance was performed on the parameter estimators to determine the significant effect of the independent variables of spray drying on the response using the Minitab 16 software; values of $p < 0.05$, indicated a significant difference.

3 Results and discussion

3.1 *Characteristics of fresh and heat-treated pineapple juice*

The Physical and chemical characteristics evaluated of fresh and heat-treated pineapple juice (Table 1) were not statistically different (ANOVA and Tukey's multiple range test $p > 0.05$) indicating that heat treatment by immersion in a water bath at 90 °C for 60 s, did not affect the fresh fruit juice characteristics. These results are in agree with those reported by Jittanit *et al*. (2010) about spray-dried pineapple juice, but unlike with results reported by Wu *et al*. (2021) about the juice treatment at 95 ºC/3 min where the color, bioactive components, antioxidant capacity and relative content of volatile compounds in pineapple juice were affected, probably due to the time and temperature conditions applied, which were higher.

Table 1. Physical and chemical characteristics of fresh and heat-treated pineapple juice.

Variable	Fresh juice	Heat-treated juice*
pH	3.490 ± 0.274	3.590 ± 0.130
Soluble solids (°Brix)	13.267 ± 0.526	13.611 ± 0.674
Total solids (g/g of juice)	0.142 ± 0.008	0.149 ± 0.002
Antiradical Activity (mg AAE/g d.b.)	2.488 ± 0.015	2.574 ± 0.014
Total phenols (mg GAE/g d.b.)	3.380 ± 0.058	3.289 ± 0.034

Means ± SD of triplicates values. *Immersion in a water bath at 90 °C for 60 s.

Table 2. Lactic acid bacteria viability in spray-dried pineapple juice.

Drying temperature (°C)	Viability (Log CFU/mL)	
Tin / Tout	*L. helveticus* ATCC 27558	*Lmb. reuteri* NRRL B-14171
150 / 80	8.07 ± 0.39^{a}	2.16 ± 0.05^{bc}
170 / 80	8.12 ± 0.36^{a}	2.96 ± 0.55^{c}
150 / 70	8.73 ± 0.08^{a}	7.50 ± 0.57^{a}
170 / 70	8.56 ± 0.25^{a}	5.57 ± 0.24^{ab}

Means ± SD. The significant difference was evaluated by ANOVA and Tukey test ($p \leq 0.05$). Different letters show the significant difference between group means in the column ($p < 0.05$).

Table 3. Parameter estimators (βi) for viability in spray-dried pineapple juice.

βi	*Lmb. reuteri* NRRL B-14171	*L. helveticus* ATCC 27558
β_0	1493.1900	204.7570
β_1	-7.2900	-0.4570
β_2	-18.9800	-1.5110
β_{12}	0.1000	0.0060

3.2 *LAB stability in thermal-treated pineapple juice*

To standardize the LAB concentration in the feed mixture, different amounts of pellets were resuspended in 100 g of thermal-treated juice. The amount of pellet with which the recommended cell concentration was obtained (> 8 log CFU/mL) for spray drying was 1 g in 100 g of juice, which led to reaching 9 log /mL. Juice composition did not affect cell viability since both strains were stable during 4 h incorporated in the thermally treated pineapple juice, with cell concentrations of 9.57 log CFU/mL for *Lmb. reuteri* and 9.62 log CFU/mL for *L. helveticus.*

3.3 *Effect of spray-drying temperatures on LAB viability added to pineapple juice*

During drying LAB cells are exposed to stress by contact with heat which can induce dehydration and physiological problems (Guergoletto *et al*., 2017), cells are in contact with large amounts of air too, promoting lipids oxidation (Teixeira *et al*., 1996) and are exposed to osmotic stress with loss of metabolic activity, cell injury and death (Liu *et al*., 2015). Each strain possesses different critical conditions and then the injury observed could be different at the same conditions of spray drying.

The highest viability after spray drying was observed on *L. helveticus* ATCC 27558 with 86-92 % of survival in all conditions (Figure 1). No significant effect ($p > 0.05$) of temperature was observed on this *L. helveticus* parameter with less than one log-unit of reduction on viability (Table 2). Inlet and outlet temperatures had a significant effect ($p < 0.05$) on survival of *Lmb. reuteri* NRRL B-14171 (Table 3). A reduction of 6 log units was observed at Tout = 80 °C with 32 and 25 % of viability at Tin = 150 and 170 °C, *Lmb. reuteri* respectively (Table 2). Less reduction of viability was observed at Tout = 70 °C with a 2 log-units reduction at Tin = 150 °C and 4 log-units at Tin = 170 °C with viability of 72 and 59 % respectively (Figure 1).

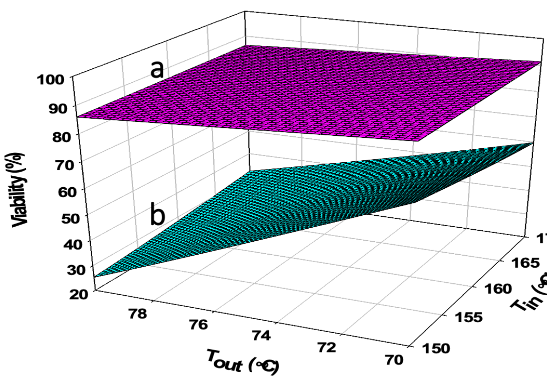

Fig. 1. Effect of spray-drying temperatures on viability of a) *Lactobacillus helveticus* ATCC 27558 and b) *Limosilactobacillus reuteri* NRRL B-14171 incorporated to pineapple juice.

Teixeira *et al*. (1996) reported that cells exposed to lower temperatures than critical conditions showed cell membrane injury, while the exposition to temperatures higher than critical induce to protein and ribosome denaturalization as well as cell wall injury and death. In this work, results showed that *Lmb. reuteri* NRRL B-14171 present low resistance to Tout higher than 70 °C, this could indicate a possible critical Tout for this strain close to 70 °C.

The viability results obtained for *L. helveticus* (8.73 ± 0.08 log CFU/mL) and for *Lmb. reuteri* (7.50 ± 0.57 log CFU/mL), after spray drying at 150/70 °C, in the reconstituted juice at the initial solids content, are considered enough to classify the dried juice as a probiotic functional food and are comparable with that reported by Kingwatee *et al*. (2015) and Barbosa

Table 4. Parameter estimators (βi) for a_w and moisture content in spray-dried pineapple juice.

Lmb. reuteri NRRL B-14171	*L. helveticus* ATCC 27558			
βi	Moisture Content (%)	a_w	Moisture Content (%)	a_w
β_0	0.3107	4.6362	-1.0857	-4.4687
β_1	-0.0016	-0.0259	0.0071	0.0293
β_2	-0.0037	-0.0581	0.0144	0.0657
β_{12}	0.00002	0.0004	-0.00009	-0.0004

Table 5. Total phenols content and antiradical activity of spray-dried pineapple juice added with *Lmb. reuteri* NRRL B-14171 or with *L. helveticus* ATCC 27558.

Drying Temperature (°C)	*L. helveticus* ATCC 27558		*Lmb. reuteri* NRRL B-14171	
Tin / Tout	Total Phenols (mg GAE/g d. b.)	Antiradical activity (mg AAE/g d. b.)	Total Phenols (mg GAE/g d. b.)	Antiradical activity (mg AAE/g d. b.)
150 / 80	1.298 ± 0.201	1.175 ± 0.181	1.128 ± 0.008	1.290 ± 0.044
170 / 80	1.026 ± 0.146	1.133 ± 0.153	1.193 ± 0.103	1.179 ± 0.107
150 / 70	1.165 ± 0.062	1.174 ± 0.085	1.291 ± 0.151	1.182 ± 0.0003
170 / 70	0.979 ± 0.049	1.120 ± 0.092	0.957 ± 0.052	1.095 ± 0.093

Means ± SD of triplicate values. ANOVA and multiple range Tukey test ($p \leq 0.05$) showed no significant difference between treatments.

Table 6. Retention percentage of total phenols content and the antiradical activity in spray-dried pineapple juice added with *Lmb. reuteri* NRRL B-14171 or with *L. helveticus* ATCC 27558.

Drying Temperature (°C)	*L. helveticus* ATCC 27558		*Lmb. reuteri* NRRL B-14171	
Tin / Tout	Total phenols retention (%)	Antiradical activity retention (%)	Total phenols retention (%)	Antiradical activity retention (%)
150 / 80	38.418 ± 5.967	47.23 ± 7.30	33.396 ± 0.262	51.85 ± 1.79
170 / 80	30.369 ± 4.320	45.55 ± 6.16	35.309 ± 3.067	47.40 ± 4.33
150 / 70	34.485 ± 1.839	47.21 ± 3.44	38.208 ± 4.483	47.52 ± 0.01
170 / 70	28.962 ± 1.463	45.03 ± 3.73	28.317 ± 1.567	44.01 ± 3.75

Means ± SD of triplicate values. ANOVA and multiple range Tukey test ($p \leq 0.05$) showed no significant difference between treatments.

et al. (2015) for litchi, and orange juice respectively. These authors reported viability values higher than 7 log CFU/g in spray-dried product. Santos *et al*. (2020), reported that the atomization air flow rate coefficient had a significant effect on the concentration of viable cells of *Limosilactobacillus reuteri* after the spray drying process, they also reported that viability could be improved by modifying the droplet size. This agreed with the results obtained in this work at Tin = 150 ºC and Tout = 70 ºC for *Lmb. reuteri* since, under these conditions, the bacteria showed a higher viability of 7.50 ± 0.57 log CFU/mL.

3.4 Physicochemical properties of LAB-added pineapple juice after spray-drying

Spray-drying temperatures evaluated in this work had no significant effect (Table 4) on the a_w and moisture content values ($p > 0.05$) of the powder of pineapple juice incorporated with LAB. The juice powders had pH values from 3.7 to 3.9. Values of a_w of the products were between 0.3 and 0.4 (Figure 2). Moisture content

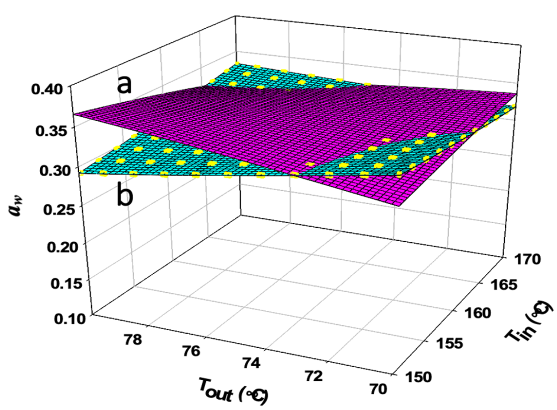

Fig. 2. Effect of spray-drying temperatures on the a_w of pineapple juice incorporated with a) *Lactobacillus helveticus* ATCC 27558 and b) *Limosilactobacillus reuteri* NRRL B-14171.

varied from 3.6 to 3.9 % (Figure 3). Other authors have already reported concordant values of moisture content and a_w in spray-dried pineapple juice at similar conditions (Jittanit *et al*., 2010; Saikia *et al*., 2015). The mentioned values comply with the a_w

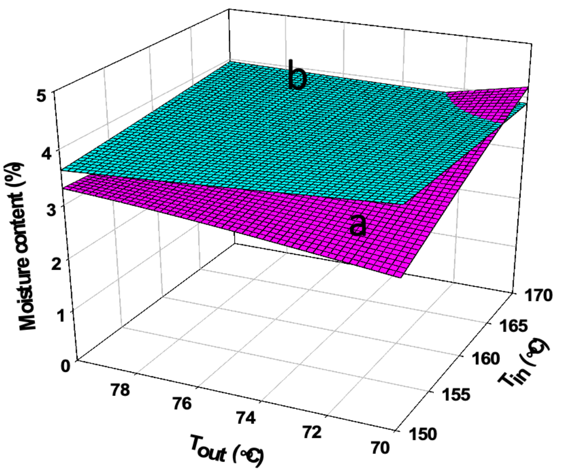

Fig. 3. Moisture content of pineapple juice incorporated with a) *Lactobacillus helveticus* ATCC 27558 and b) *Limosilactobacillus reuteri* NRRL B-14171 after spray-drying.

(< 0.6) and moisture content (4-7 %) characteristics recommended to guarantee the microbiological and chemical stability of spray-dried products (Jantzen *et al*., 2013).

The total phenols content of spray-dried pineapple juice incorporated with LAB was 1-1.2 mg GAE/g (Table 5) and the retention percentage varied from 28 to 38 % (Table 6). The antiradical activity was 1-1.3 mg AAE/g d.b. (Table 5) and the retention percentage remained between 44 and 51% (Table 6).

The ANOVA showed that the inlet and outlet temperatures evaluated had no significant effect ($p > 0.05$) on total phenols content and the antiradical activity of spray-dried pineapple juice added with *Lmb. reuteri* NRRL B-14171 or with *L. helveticus* ATCC 27558 between the different treatments. These results may be due to the outlet temperatures evaluated and the protector effect of maltodextrin used as carrier material. Nevertheless, compared with the fresh juice a decrease of about 70 % in total phenols content and 50 % in the antiradical activity was observed, the observed decrease may be related to their individual sensibility to heath and conformational changes of phenolic compounds present in pineapple juice (Saikia *et al*., 2015b).

3.5 Drying yield

The drying yield (%) of pineapple juice added with LAB was determined as the % weight fraction of the total solids contained in the liquid feed that could be recovered from the collecting vessel attached to the bottom of the cyclone. With all the drying conditions evaluated, the yields obtained, ranged between 32 and 40 % without significant difference between all treatments ($p > 0.05$). The yields obtained in this work were like the results reported for probiotic orange juice (Barbosa *et al*., 2016). It has been recommended a yield upper than 50 % to consider a laboratory scale spray drying process as efficient. The yield of spray-dried products could be affected by different parameters like inlet temperature, feed flow rate and the concentration of drying agents, in addition, the presence of low molecular weight sugars like glucose, fructose and sucrose in the feed mixture causes higher adherence of the product to the walls of the drying chamber (Barbosa *et al*., 2015). In this sense, the soluble solids content (like sugars) of pineapple Juice (13.6 °Brix) could affect the yields obtained in this work.

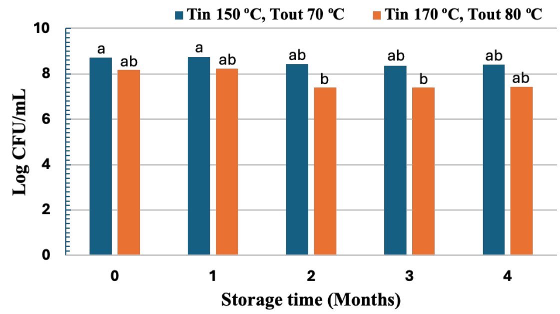

Fig. 4. Stability during storage at 4 °C of *Lactobacillus helveticus* ATCC 27558 after spray drying incorporated into pineapple juice. The significant difference was evaluated by ANOVA and Tukey test ($p \leq 0.05$). Different letters show a significant difference ($p < 0.05$).

3.6 Stability of LAB during the storage of spray-dried pineapple juice

As mentioned before (3.3), inlet and outlet temperatures of spray drying evaluated in this work did not affect the viability only of *L. helveticus* ATCC 27558 ($p > 0.05$) in spray-dried pineapple juice, therefore, the stability of BAL was evaluated on products with *L. helveticus* during storage at 4 °C by 4 months. The viability of *Lmb. reuteri* during the storage of spray-dried pineapple juice was not evaluated since CFU/g in products with this LAB was lower and close to the minimal cellular concentration required to be considered as a functional probiotic product (7 log CFU/g o mL). The viability of *L. helveticus* remained stable during the 4 months of storage at 4 °C in the two spray drying treatments (T1 y T2), without significant difference ($p > 0.05$) with respect to the storage time (Figure 4). It has been demonstrated that the storage at low temperatures of spray-dried products added with LABs, helps to maintain the viability of LABs. The microorganisms could adapt their metabolism at low temperatures, their cytoplasm viscosity could increase by the accumulation of salts (Lapsiri *et al*., 2012).

Conclusion

The results of the present study showed that spray drying temperatures (Tin 150 and 170 °C and Tout 70 and 80 °C) evaluated did not have a significant effect on *L. helveticus* viability, unlike these spray drying conditions caused a significant reduction in *Lmb. reuteri* viability. The physicochemical characteristics observed in Pineapple juice spray dried with *L. helveticus* added were acceptable to guarantee the chemical and microbiological stability of the powder, and the product shows the characteristics required to be considered a functional food (> 7 log CFU/g o mL) almost during four months of storage at 4 ºC. Therefore, spray-dried pineapple juice could be a good vector for the distribution of lactic acid bacteria with probiotic potential.

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