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A functional and probiotic approach: mixed fruit juice powder with addition of lactobacilli

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Abstract

The aim of this study was to evaluate changes in a mixture of acerola and ciriguela juice (40/60%, respectively) and the juice mixture supplemented with *Lactobacillus rhamnosus* LPAA 01, *L. casei* LPAA 02, and *L. plantarum* LPAA 03, ratio 1:1, both powders obtained by spray drying, during storage. The stability of acerola and ciriguela mixed powder without the addition of microorganisms was evaluated for 90 days at 25°C in different water activities (Aws) (0.11, 0.23, and 0.34). Aw that most effectively maintained the stability of mixed powdered juice was used for the evaluation of the fermented mixed juice powder containing probiotics for 45 days at 5 and 25°C, respectively. Samples were characterized by physicochemical properties, bioactive compounds, and cell viability. The physicochemical properties of the powdered mixed juice without the addition of probiotics remained stable when stored at 25°C at an Aw of 0.11 for 60 days. Ascorbic acid, carotenoids, and total phenolics showed favorable characteristics for the commercialization and bioactive properties of the powders, with potential use as functional ingredients in food. Probiotic powders showed viable cell counts above 6.0 log colony-forming units (CFU)/g after storage for up to 20 days at 5°C and up to 14 days at 25°C.

Keywords: *Malpighia emarginata* D.C.; *Spondias purpurea* L.; mixed juice powder stability; probiotics; spray dryer.

Practical Application: The powders represent a good alternative for new food products for individuals, mainly those who are affected by lactose intolerance, milk protein allergies, galactosemia, and hypercholesterolemia.

1 INTRODUCTION

The northeast region of Brazil is known for the production of native and exotic fruits with nutritional properties for humans. Two of the fruits produced in this region are acerola (*Malpighia emarginata* D.C.), which is characterized by high levels of bioactive compounds in its composition, such as a high content of ascorbic acid (AA), levels of amino acids, flavonoids, lipids, terpenoids, and polyphenolics (Xu et al., 2020), and ciriguela (*Spondias purpurea* L.), which contains nutrients such as minerals, carbohydrates, and phenolic compounds (PCs) (Todisco et al., 2015).

The addition of probiotics and prebiotics to non-dairy fruit juice products is important for consumers with lactose intolerance or milk protein allergies, as well as vegans (Szutowska, 2020). Probiotics exert beneficial effects on host health through various mechanisms such as immune response modulation, production of organic acids and antimicrobial compounds, interaction with resident microbiota, and interface with the host, thereby improving intestinal barrier integrity and enzyme production (Sanders et al., 2019). Studies on the inoculation of probiotic microorganisms in fruit and vegetable juice have shown promise and have been gaining ground as an option for developing new products (Jeon et al., 2022; Lascano et al., 2020; Santos Filho et al., 2019; Souza et al., 2021).

Probiotic foods should be safe and contain sufficient probiotic microorganisms during the shelf life of the product. Therefore, selected probiotic strains should be suitable for large-scale industrial production and have the ability to survive and retain their functionalities during food processing and storage (Tripathi & Giri, 2014). Several strains of *Lactobacillus plantarum*, *L. acidophilus*, and *L. casei* can grow in fruit matrices due to their tolerance to acidic environments (Peres et al., 2012). Probiotics have been encapsulated for decades using different encapsulation methods to maintain their viability during processing, storage, and digestion and to provide health benefits (Misra et al., 2022).

In this context, microencapsulation of probiotic cells, which protects cells from unfavorable environments, has been widely studied. Microencapsulation can provide numerous benefits to the encapsulated material, as it can protect it from oxidation, degradation, and volatilization reactions (Betored et al., 2020).

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Among the microencapsulation techniques, the *spray dryer* stands out.

In addition, fruits are a highly perishable food, so it is important to choose the right processing technique to extend the shelf life and maintain the nutritional and functional stability of the product. Among the various processing techniques, spray drying involves subjecting a liquid product to hot air; the droplets formed lose moisture, resulting in a powdered product with more stable physical properties and improved ease of handling, transport, and storage (Tontul & Topuz, 2017).

The food industry uses spray drying because of its rapid processing and drying times, low water activity (Aw), low energy requirements, simplicity of transport, flexibility, high process yield, simple storage, homogeneous distribution throughout the product, and favorable applications in the production of functional foods (Sharma et al., 2022). Based on all these considerations, the aim of this study was to evaluate the stability of spray-dried acerola and ciriguela mixed juice and their relationship with physicochemical properties and bioactive compounds, as well as to assess the cell viability of probiotic lactobacilli in powders.

2 MATERIAL AND METHODS

2.1 Materials

Acerola and ciriguela fruits were purchased from the Supply Center of Pernambuco located in the city of Recife, PE, Brazil. Maltodextrin 5 DE (Ingredion, São Paulo, Brazil) was used as an encapsulating agent during the spray-drying process. *L. rhamnosus* LPAA 01, *L. casei* LPAA 02, and *L. plantarum* LPAA 03 were obtained from stock cultures and maintained at the Universidade Federal Rural De Pernambuco (UFRPE).

2.2 Microencapsulation of mixed juice with and without probiotic lactobacilli

To obtain probiotic mixed juice, a microbial culture was initially inoculated in the mixed juice to a concentration of 10^{10} colony-forming units (CFU)/g. The juice containing the inoculum was fermented at 35°C for 24 h in anaerobiosis.

The juices of acerola and ciriguela fermented and not fermented were mixed with 10% (w/w) maltodextrin dextrose equivalent DE 5. A stirrer, model TE-102 (Tecnal, Piracicaba, SP, Brazil), was used to homogenize the mixture. Spray drying was performed with a mini spray dryer (LM, model MSD 1.0, SP-Brazil) that was operated with an inlet temperature of 140°C, a liquid feed rate of 0.60 L h^{-1}, an injector nozzle 1.2 mm in diameter, an air flow rate of 30 m^3 h^{-1}, and an air pressure of 0.6 bar; the process was optimized previously by Ribeiro et al. (2018).

2.3 Storage stability evaluation

Hermetic containers with different Aw conditions of 0.11, 0.23, and 0.34 were prepared using saturated solutions of lithium chloride, potassium acetate, and magnesium chloride, respectively (Greenspan, 1977). Samples of spray-dried acerola and ciriguela mixed juice powder without probiotics were stored at 25 ± 1°C in each Aw condition mentioned earlier and protected from light; evaluations were performed at 30, 60, and 90 days. The samples of the powdered product containing the probiotic bacteria were placed in airtight glasses with a saturated lithium chloride solution (Aw = 0.11) and evaluated after 3, 10, 14, 20, and 45 days of storage at refrigeration and room temperature (5 and 25°C, respectively).

2.4 Carotenoid content

The carotenoid (CA) content was determined according to the method described by Rodriguez-Amaya (2001); 2 g of the powder was used, and the reading was performed at 450 nm in a spectrophotometer (Shimadzu, model UV 1650PC, Japan). The mathematical expression used to determine the CA content was described previously by Gross (1987), considering the absorption coefficient of 2,500. The results were expressed in micrograms of β-carotene equivalent (β-CE) g^{-1} on a dry basis.

2.5 AA content

The AA was determined by the titration method used by Tillmans based on the reduction of the dye 2,6-dichlorophenol indophenol (DCFI), which is reduced by the AA in the sample (AOAC, 2006). For determination, 2 g of the powdered product was used, and the results were expressed as milligrams of AA per 100 g sample on a dry basis (mg AA 100 g^{-1}).

2.6 Phenolic compounds

The total PC content was determined by using Folin–Ciocalteu phenol reagent according to the method described by Wettasinghe and Shahidi (1999); 2 g of powder was used, and the absorbance was recorded at 725 nm in a spectrophotometer (Shimadzu, model UV 1650PC, Japan). The PC content was calculated using a standard curve prepared from aqueous solutions of gallic acid (0–120 μg mL^{-1}), and the result was expressed in milligrams of gallic acid equivalent (GAE) 100 g^{-1} on a dry basis.

2.7 Radical 2,2-diphenyl-1-picrylhydrazyl assay

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity was determined according to the methodology described by Brand-Williams et al. (1995) and modified by Sanchez-Moreno et al. (1998). For extraction, 2 g of the powdered product was used, and the extracting solvent was distilled water. Readings were performed at a 515 nm wavelength in a spectrophotometer (Shimadzu, model UV 1650PC, Japan). The antioxidant capacity was expressed in μg mL^{-1} as half-maximal effective concentration (EC50), which is defined as the effective concentration of juice extract that can decrease the initial concentration of DPPH by 50%.

2.8 Radical 2,2-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) assay

The 2,2-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical cation scavenging activity was determined

according to the methodology described by Re et al. (1999). For extraction, 2 g of the powdered product was used, and the extracting solvent was distilled water. The antioxidant capacity was assessed at different concentrations of aqueous extract of mixed juice powder (0.08, 0.21, and 0.44 g L^{-1}). All readings were performed after 6 min at a wavelength of 734 nm, and the results were expressed in µM Trolox equivalent (TE) g^{-1} on a dry basis.

2.9 Aw, moisture content, and soluble solids determination

Water activity (aw) was measured by direct reading in an electronic meter (Aqualab Dew Point 4TEV, Pullman, WA, USA) after samples were stabilized at 25°C. The moisture content (MC) was determined using an infrared moisture analyzer (Marte, ID50, Piracicaba/SP) at 105°C for 30 min; the results were expressed as a percentage (%). The soluble solids (SS) were determined using a refractometer (Reichert® r2i300, New York, USA) that gave direct readings of the diluted sample (1 g in 10 mL of distilled water), and the results were expressed in °Brix.

2.10 pH and titratable acidity

The pH of the samples was analyzed by direct measurements of the sample after reconstituting 1 g of powder in 100 mL of distilled water, according to AOAC (2006), using a glass electrode pH meter (Tecnal® TEC-3 MP, Piracicaba/SP). The titratable acidity (TA) content was determined by diluting 1 g of powder in 50 mL of distilled water and titrating with a standard 0.1 N NaOH solution; phenolphthalein solution was used as an indicator as described in AOAC (2006). The results were expressed as grams of citric acid per 100 g on a dry basis.

2.11 Colorimetric analysis

The color of the powders was measured using a colorimeter (Konica Minolta® CR-400, Tokyo, Japan) according to the Commission Internationale de l´Eclairage (CIELAB) system (L*, a*, and b*), where L* indicates light (0 = black and 100 = white) and a* and b* are coordinates for green (-a*)/red (+a*) and blue (-b*)/yellow (+b*). The variation in the color (ΔE) of the juice obtained by the atomization process was calculated by Equation 1 (Appendix 1) (Kha et al., 2015), where the zero-time analysis result was considered the reference color.

2.12 Evaluation of mixed juice powder containing probiotic microorganisms

2.12.1 Microbial viability before and after microencapsulation

Before microencapsulation, the mixed juice containing the probiotic microorganisms was serially diluted to 10^{-7} with sterile peptone water. Aliquots of 1 mL of dilution were plated in triplicate on plates containing de Man, Rogosa and Sharpe (MRS) agar (pour plate method), and plates were incubated at 35°C for 72 h. After this, plates containing between 25 and 250 typical colonies of lactobacilli were counted. After microencapsulation, the powder was reconstituted in peptone water (1:10 w/v), and the suspension was kept at 25°C for 30 min to release the cells. Then, the number of lactobacilli cells was counted, as described earlier. The colony counts were expressed as the log of colony-forming units per gram of solid (log CFU/g).

2.13 Statistical analysis

All determinations were performed at least in triplicate, and the measures of the dependent variables were evaluated by analysis of variance (ANOVA) and compared with the Tukey test by the Statistical Analysis System (SAS) program, version 9.0, at a significance level of 5%. The results are expressed as the mean ± standard deviation.

3 RESULTS AND DISCUSSION

3.1 Stability of spray-dried acerola and ciriguela mixed juice powders subjected to various Aw conditions at 25°C

The stability of total CAs, AA, PCs, and antioxidant capacity in spray-dried acerola and ciriguela mixed juice powder were evaluated 0, 30, 60, and 90 days after storage at Aw values of 0.11, 0.23, and 0.34 and maintained at 25°C.

At the end of storage (90 days), with regard to time zero, the CA, AA, PC, and ABTS contents of the acerola and ciriguela mixed juice powder decreased, as shown in Figures 1 and 2. As shown in Figure 1A, the CA content in acerola and ciriguela mixed juice powder stored at Aw of 0.11 after 90 days was significantly reduced ($p < 0.05$) compared to that at the initial time; the CA content at 0 days remained almost constant up to 60 days and then decreased after 90 days of storage, with a loss of 30.3% at Aw values of 0.23 and 0.33. The same behavior was observed with a loss of 35.6 and 22.8%, respectively.

After 90 days, the AA and PC contents of the mixed juice powder stored at Aw values of 0.11, 0.23, and 0.34 showed a significant reduction ($p < 0.05$) with respect to the initial time (Figures 1B and 1C), with losses of ~31, 35, and 34% with respect to AA and 26, 22, and 24% with respect to PC, respectively.

The effect of the extrinsic moisture factor favored the degradation of the compounds, accompanied by changes in color caused by oxidation (Pavlovska & Tanevska, 2013; Porto et al., 2017; Udomkun et al., 2016). González et al. (2019) studied the stability of spray-dried grapefruit powder under different relative humidities (RH) for up to 9 months at 20°C and observed a decrease in AA at RH higher than 33% during the storage period. A reduction in PC during storage of hog plum juice powder was also reported by Mishra et al. (2017).

The scavenging concentration of each sample at 50% (EC50) was used to compare their antioxidant capacity. Lower EC50 values indicate that the required amount of a sample needed to reduce the initial DPPH concentration by 50% is lower, indicating a higher antioxidant capacity of the sample (Brand-Williams et al., 1995; Sanchez-Moreno et al., 1998). Therefore, the lower the EC50 value, the higher the antioxidant capacity of the analyzed extract (Vieira et al., 2011).

As shown in Figure 2A, the EC50 at 90 days increased ($p < 0.05$) compared to time 0; therefore, there was a reduction in the antioxidant capacity at all Aw values evaluated. At the end of

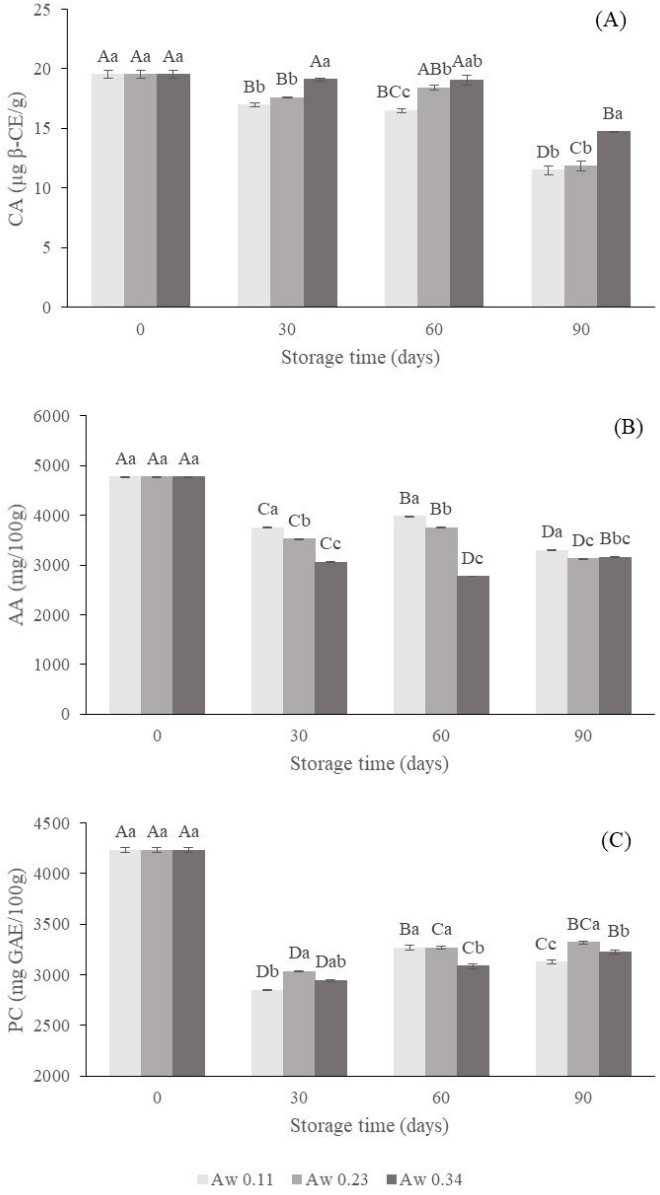

Figure 1. (A) Carotenoids (CAs) content, (B) ascorbic acid (AA) content, and (C) phenolic compounds (PCs) content of spray-dried acerola and ciriguela mixed juice powder were stored at 25°C in different water activities (Aws) for 90 days. Results are expressed as mean ± standard deviation (n = 3). Equal lowercase letters do not differ significantly ($p > 0.05$) at the same storage time between Aw by the Duncan test. Equivalent capital letters do not differ significantly ($p > 0.05$) between the storage times in the same Aw by the Duncan test.

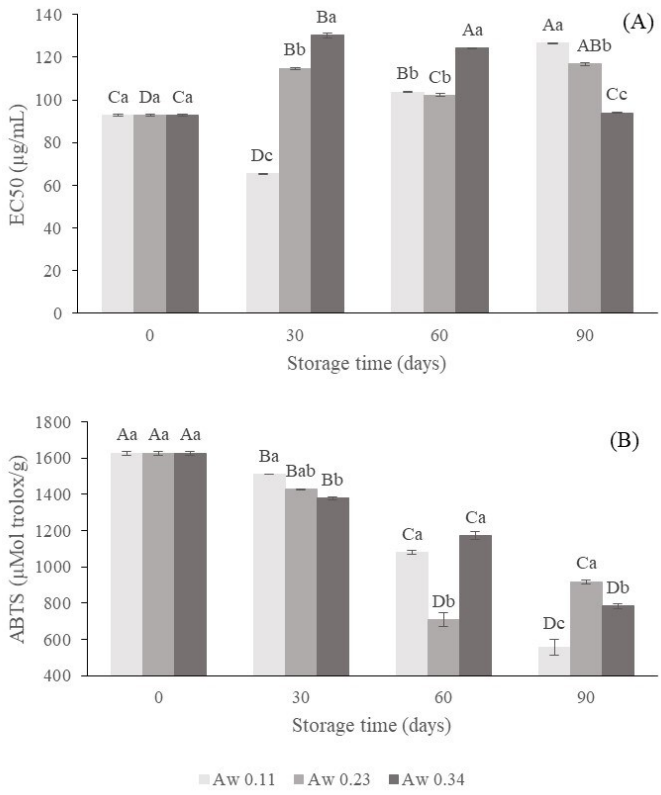

Figure 2. Antioxidant capacity was determined by (A) radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) and (B) radical 2,2-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) of spray dried acerola and ciriguela mixed juice powder stored at 25°C in different water activities (Aws) for 90 days. Results are expressed as the mean ± standard deviation (n = 3). Equal lowercase letters do not differ significantly ($p > 0.05$) at the same storage time between Aw by the Duncan test. Equivalent capital letters do not differ significantly ($p > 0.05$) between the storage times in the same Aw by the Duncan test.

the storage period, the EC50 values increased by 136, 134, and 101% at Aw values of 0.11, 0.23, and 0.34, respectively; the higher the EC50 values, the lower the retained antioxidant capacity.

According to the ABTS assay (Figure 2B), the antioxidant capacity of mixed juice powder also decreased ($p < 0.05$) as the storage period increased at the three Aw values tested. Similar results were observed by Fang and Bhandari (2011) in bayberry powder and González et al. (2019) in grapefruit powder; that is, the antioxidant capacity decreased with increasing storage period.

A decrease in the levels of bioactive compounds and in the antioxidant capacity was observed, confirming the relationship between the levels of these compounds and the antioxidant capacity of spray-dried acerola and ciriguela mixed juice powder.

3.1.2 Physicochemical properties

3.1.2.3 Aw, MC, and SS

According to Figure 3A, the Aw of the spray-dried acerola and ciriguela mixed juice powder increased in the first 30 days of storage at an Aw of 0.11 and then decreased ($p < 0.05$) after 60 and 90 days of storage. This behavior may have been due to the water migration into the powder reaching equilibrium after 90 days of storage (AquaLab, 2015). At Aw values of 0.23 and 0.34, there was an increase after 90 days of storage ($p < 0.05$) compared to the initial time. Under all storage conditions, regardless of the storage time, the product was microbiologically safe since the Aw was less than 0.6. In general, food powders with an Aw of < 0.6 are considered to be safe (Arepally et al., 2020).

The MC (Figure 3B) of the mixed juice powder increased ($p < 0.05$) at 30, 60, and 90 days of storage for all Aw values evaluated

with respect to the initial time. The MC of the mixed juice powder did not exceed 7% under any of the conditions studied.

In Figure 3C, the SS of the mixed juice powder was 8.50°Brix at an Aw of 0.11, and no significant differences ($p > 0.05$) were observed for the SS during the 90 days of storage. At Aw values of 0.23 and 0.34, there was a slight reduction in the SS at 30, 60, and 90 days compared to day 0. The reduction in SS may be due to increased water content during storage, as the proportion of solids in the total mass was changed. This reduction in SS during storage was also reported by Silva et al. (2005) in umbu-cajá powder stored for 60 days in different types of packaging. Reis et al. (2017) also observed this same behavior in acerola flour.

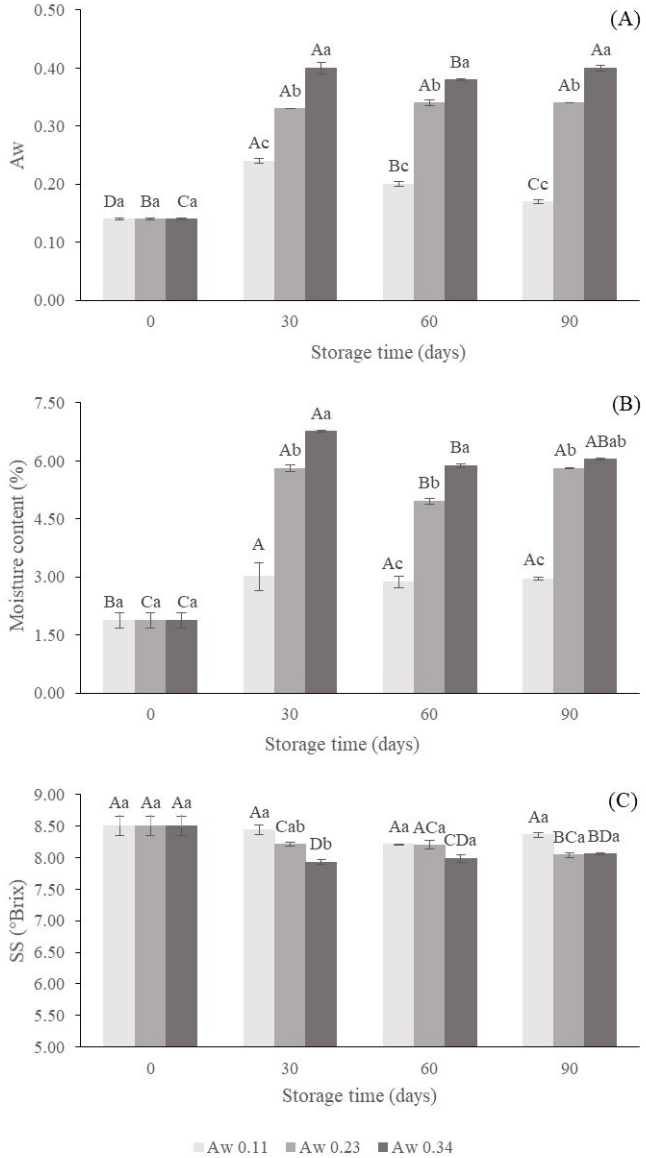

Figure 3. (A) Water activity (Aw), (B) moisture content, and (C) soluble solids (SS) of spray-dried acerola and ciriguela mixed juice powder stored at 25°C in different Aws for 90 days. Results are expressed as the mean ± standard deviation ($n = 3$). Equal lowercase letters do not differ significantly ($p > 0.05$) at the same storage time between Aw by the Duncan test. Equivalent capital letters do not differ significantly ($p > 0.05$) between the storage times in the same Aw by the Duncan test.

3.1.2.4 pH and TA

The pH values in spray-dried acerola and ciriguela mixed juice powder increased during storage at different Aw values. The pH of the mixed juice powder reached 3.15, which is in the range of acidic foods and is considered restrictive for the development of bacteria. The TA of the acerola and ciriguela mixed powder during storage at an Aw of 0.11 increased ($p < 0.05$) compared with that at time 0, with an initial value of 0.42 g of citric acid 100 g^{-1} to 0.46 g of citric acid 100 g^{-1} at the end of storage.

However, at Aw values of 0.23 and 0.34, the TA decreased ($p < 0.05$) at 90 days of storage compared to the initial time. The acidity is related to the pH of the mixed juice powder; an increase in the acidity corresponds to a decrease in the pH or otherwise. These changes in pH and TA (Figure 4) could be attributed to the reaction of basic amines to form compounds of lower basicity and to the degradation of sugars into acids during the Maillard reaction (Liu et al., 2010).

3.1.2.5 Colorimetric analysis

Table 1 shows the values of L*, a*, b*, and ΔE of the spray-dried acerola and ciriguela mixed juice powder during storage.

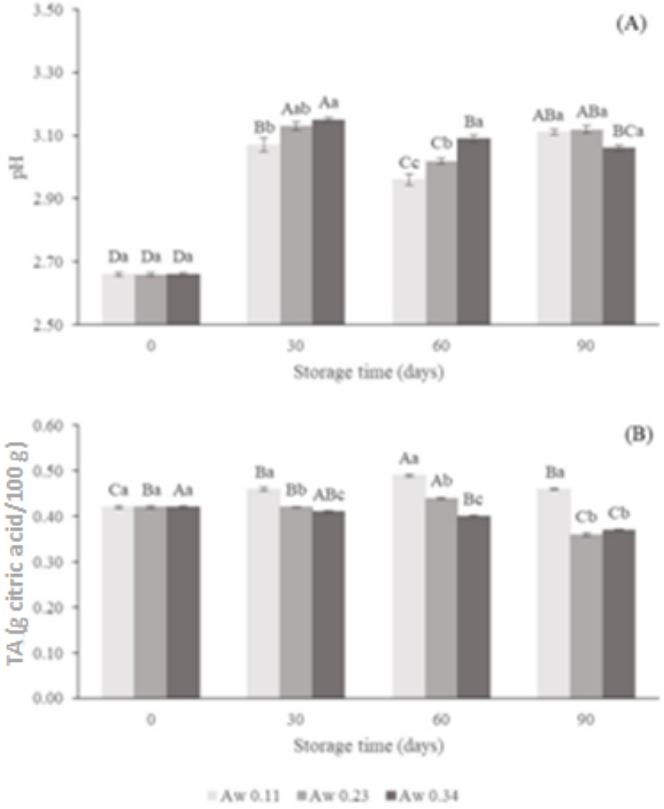

Figure 4. (A) pH and (B) titratable acidity (TA) of spray-dried acerola and ciriguela mixed juice powder stored at 25°C in different water activities (Aws) for 90 days. Results are expressed as the mean ± standard deviation ($n = 3$). Equal lowercase letters do not differ significantly ($p > 0.05$) at the same storage time between Aw by the Duncan test. Equivalent capital letters do not differ significantly ($p > 0.05$) between the storage times in the same Aw by the Duncan test.

The L* value is the brightness on the surface and determines the position of the point on the vertical axis, which varies from 0 (black) to 100 (white). At an Aw of 0.11, the value of L* showed no difference ($p > 0.05$) at different storage times, as the luminosity was stable with respect to the initial time. However, at an Aw of 0.23, compared to the initial time, at the end of the storage time, the luminosity of L* increased ($p < 0.05$). The same behavior was observed at an Aw of 0.34. In both cases (Aw values of 0.23 and 0.34), a decrease in a* (less red color intensity) and an increase in b* (more yellow color intensity) were observed. These results were clearly confirmed in Figure 5, which shows photos of the powders, confirming that the color changed during the storage period under different Aw values (0.11, 0.23, and 0.34) at 25°C.

The results obtained for the values of a* and b* (Table 1) show that all data were located in the first quadrant (+a*, +b*), indicating a tendency to red and yellow. At an Aw of 0.11, the value of a* decreased ($p < 0.05$), and the value of b* remained statistically stable ($p > 0.05$) at 90 days of storage compared to that at the initial time. Compared to the initial time, at Aw values of 0.23 and 0.34, the red intensity (a*) was significantly reduced ($p < 0.05$) and the yellow intensity (b*) was increased ($p < 0.05$) during the period of storage.

Table 1 also shows that the storage period and Aw values in powders were different from those in the control ($\Delta E > 1.5$) except for an Aw of 0.11 at the end of storage. The acerola and ciriguela mixed juice powder showed the lowest value ($\Delta E < 1.5$) at the end of the 90-day storage. This low value is desirable because it indicates that the color of the powder was maintained and no visual differences were observed (Silva et al., 2013).

The color change due to time can be attributed to moisture absorption and the degradation of AA and CAs (Kha et al., 2015; Muzaffar & Kumar, 2016). A color change during storage was reported in tomato powder studies (Liu et al., 2010), spray-dried tamarind pulp powder studies (Muzaffar & Kumar, 2016), and spray-dried beetroot extract studies (Carmo et al., 2018).

Based on the results obtained, the powder stored at an Aw of 0.11 for 60 days at 25°C presented better physicochemical quality, bioactive compounds, and antioxidant capacity.

3.2 Stability of spray-dried acerola and ciriguela mixed juice powders containing probiotic microorganisms maintained at 25 and 5°C at an Aw of 0.11

3.2.1 Microbial viability

The initial probiotic count of the mixed juice before microencapsulation was 10.02 ± 0.40 log CFU/g. According to the results (Figure 6), spray drying reduced the probiotic viability by

Figure 5. Visual records of spray-dried acerola and ciriguela mixed juice powder stored at 25°C in different water activities during 90 days.

Table 1. Color parameters (L*, a*, b*, and ΔE) of spray-dried acerola and ciriguela mixed juice powder stored at 25°C in different water activities for 90 days.

Condition	Parameters	Storage time (days)			
		0	30	60	90
Aw 0.11	**L***	82.78 ± 0.06^{Aa}	83.06 ± 0.10^{Ab}	82.94 ± 0.05^{Aa}	83.04 ± 0.21^{Ab}
	a*	10.16 ± 0.08^{Ca}	11.80 ± 0.23^{Ba}	12.58 ± 0.06^{ABa}	8.81 ± 0.14^{Da}
	b*	23.77 ± 0.19^{Aa}	21.17 ± 0.43^{Cc}	21.20 ± 0.05^{BCc}	23.48 ± 0.11^{Ac}
	ΔE	—	3.14 ± 0.28^{Ac}	3.54 ± 0.01^{Ac}	1.43 ± 0.18^{Bc}
Aw 0.23	**L***	82.78 ± 0.06^{Aa}	84.77 ± 0.12^{Ba}	81.54 ± 0.14^{Db}	84.19 ± 0.12^{BCa}
	a*	10.16 ± 0.08^{Aa}	5.90 ± 0.21^{Cb}	6.90 ± 0.33^{Bb}	1.45 ± 0.17^{Dc}
	b*	23.77 ± 0.19^{Da}	26.44 ± 0.56^{Cb}	27.72 ± 0.10^{BCb}	30.77 ± 0.29^{Ab}
	ΔE	—	5.47 ± 0.18^{Bb}	5.28 ± 0.25^{Bb}	11.27 ± 0.17^{Ab}
Aw 0.34	**L***	82.78 ± 0.06^{Aa}	77.48 ± 0.10^{Cc}	78.43 ± 0.10^{BCc}	72.65 ± 0.06^{Dc}
	a*	10.16 ± 0.08^{Aa}	3.66 ± 0.08^{Dc}	6.12 ± 0.23^{Bbc}	4.96 ± 0.15^{Cb}
	b*	23.77 ± 0.19^{Da}	36.39 ± 0.11^{Aa}	31.88 ± 0.21^{Ca}	36.37 ± 1.07^{ABa}
	ΔE	—	15.15 ± 0.12^{Ba}	10.08 ± 0.32^{Ca}	17.02 ± 0.73^{Aa}

Results are expressed as mean ± standard deviation ($n = 3$). Equal lowercase letters do not differ significantly ($p > 0.05$) at the same storage time between the aw by the Duncan test. Equivalent capital letters do not differ significantly ($p > 0.05$) between the storage times in the same aw by the Duncan test.

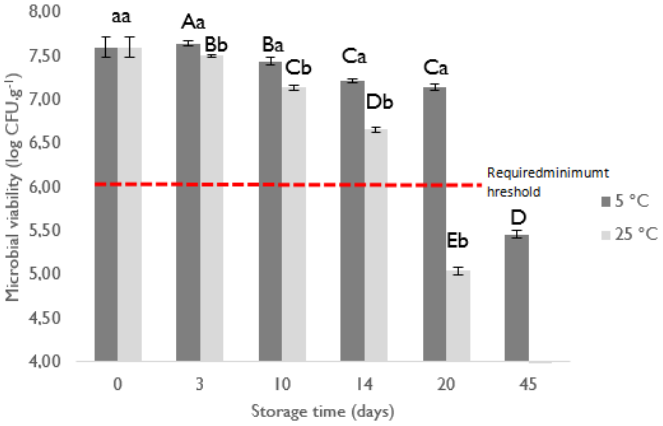

Figure 6. Microbial viability of spray-dried acerola and ciriguela mixed juice with probiotic powder stored at 25 and 5°C in water activity (Aw) = 0.11. Results are expressed as the mean ± standard deviation (n = 3). Equal lowercase letters do not differ significantly ($p > 0.05$) by the Duncan test.

3 log circles, showing that this technique is feasible and relatively inexpensive for the protection of probiotic cells. The powdered probiotic mixed juice showed viable cell counts above 6.0 log CFU/g after up to 14 days of storage at 25°C and after up to 20 days of storage at 5°C; considering that 6.0 log CFU/g is the minimum recommended level of probiotics in food products needed for their therapeutic benefits (FAO, 2002).

Temperature was the most relevant parameter in the study of stability, since powders stored at 5°C presented higher cell survival rates compared to powders stored at 25°C during the entire storage period. Pereira et al. (2014) evaluated the viability of *L. casei* in microencapsulated cashew juice after 35 days of storage at 4 and 25°C with different concentrations of carrier agents by atomization; these authors obtained viable cell counts above 6.0 log CFU/g after 7 days at 25°C and 21 days at 4°C, which was close to the survival rate presented in this study. Lascano et al. (2020) studied the viability of spray-dried probiotic cells in passion fruit juice powder after storage at different temperatures (4, 25, and 37°C) and observed a log reduction in the microbial cells each week at varying temperatures. The best treatment in this previous study involved the storage of passion fruit with spray-dried *L. plantarum* S20 at 4°C. Vivek et al. (2020) observed a significant decrease in the probiotic viability of spray-dried Sohiong after 16 days of storage. The probiotic viability decreased below 6 log CFU/g after 36 days of storage at 25°C and 50% RH. The significant damage caused to cells during dehydration, together with the limited availability of water and nutrients, significantly decreases the content of microorganisms within a few days of storage (Betored et al., 2020).

Storage at room temperature (25°C) is still a challenge. However, promising results were obtained in this study, with satisfactory survival rates over 20 days of storage at 25°C. According to Mestry et al. (2011), fermented juice powder is highly desirable because the dried product potentially has both a longer shelf life and a lower transportation cost. Moreover, probiotic fruit and vegetable juice powders can be used in probiotic drinks, probiotic ice creams, syrups, and prepared soups.

4 CONCLUSION

The results showed that better preservation of the physicochemical quality and minimization of changes in the bioactive compounds of spray-dried mixed juice powder without probiotic lactobacilli were observed until 60 days of storage at 25°C at an Aw of 0.11. Better probiotic viability was observed for powders obtained by spray drying and stored at an Aw of 0.11 for up to 20 days at 5°C and 14 days at 25°C, with a viability above 6.0 log CFU/g. The results show that it is possible to maintain the viability of probiotic bacteria and good functional antioxidant characteristics in mixed microencapsulated juice by atomization; this allows the formulation of new products in other foods in addition to the dairy products normally available on the market without the limitations presented by them. Probiotics can be incorporated into non-dairy food matrices as potential carriers for microorganisms, representing a good alternative for new food products for individuals who are affected by lactose intolerance, milk protein allergies, galactosemia, and hypercholesterolemia.

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Appendix 1. The variation in the color (ΔE).

$$\Delta E = \sqrt{(L_0^* - L^*)^2 + (a_0^* - a^*)^2 + (b_0^* - b^*)^2} \quad (1)$$

Were L_0^* , a_0^*, and b_0^* are the sample values at zero storage time.