Journal of Functional Foods 111 (2023) 105883

Contents lists available at ScienceDirect

Journal of Functional Foods

journal homepage: www.elsevier.com/locate/jff

Araticum (*Annona crassiflora)* juice is an excellent matrix for *Lactobacillus paracasei* LBC-81 with high antioxidant potential

Luiza Coêlho Midlej [a], Sandra Fernandes Arruda [a], Maria Carolina Mesquita [a], Márcio Antônio Mendonça [b], Eliana dos Santos Leandro [a,*]

[a] Department of Nutrition, College of Health Sciences, University of Brasília, Brasília, DF 70910900, Brazil
[b] College of Agronomy and Veterinary Medicine, University of Brasília, Brasília, DF 70910900, Brazil

ARTICLE INFO

Keywords:
Araticum
Lactobacillus paracasei LBC-81
Survival
Antioxidant activity
In vitro gastrointestinal conditions

ABSTRACT

This study aimed to develop an araticum (*Annona crassiflora)* juice supplemented with a probiotic strain *L. paracasei* LBC-81, and evaluate the probiotic strain viability during refrigerated storage period and under *in vitro* gastrointestinal conditions. Three juices formulations were elaborated containing 20 % (F1), 30 % (F2) and 40 % (F3) of araticum pulp, with and without *L. paracasei* LBC-81. Changes in the physiochemical properties in the formulations during storage were also evaluated. The viability of *L. paracasei* in the three formulations during 28 days of storage was significantly greater ($P < 0.05$) than the recommended minimum (6 log CFU mL^{-1}) for a product to be considered a probiotic. *L. paracasei* showed significantly ($P < 0.05$) greater viability in the formulation made with 40 % araticum pulp after 28 days of refrigerated storage. The araticum juices enriched with *L. paracasei* showed a significantly ($P < 0.05$) marked decrease and increase in pH and acidity, respectively. The addition of *L. paracasei* in the araticum juice formulations provided a significant increase ($P < 0.05$) in the total phenolic content (F1: 1926; F2: 3338.6; F3: 4390.5 and F1:1584; F2: 2629; F3: 3139 μmol galic acid/L juice with and without bacteria, respectively) and greater stability of the antioxidant activity (F1: 1492; F2: 2305; F3: 2618 and F1: 902; F2: 1461; F3: 1450.2 μmol TROLOX/L juice with and without bacteria, respectively). The araticum juice proved to be an excellent matrix for *L. paracasei* by providing a high percentage of survival during the *in vitro* gastrointestinal conditions.

1. Introduction

In recent years, the pursuit for new food matrices has been the subject of research in order to assess the potential of these foods as matrix of probiotic microorganisms. For decades, dairy products (e.g., yogurt and other fermented products) were the main matrix of probiotic microorganisms, they have proven to be a matrix capable of providing excellent growth and survival of different probiotic strains. However, the increasing number of lactose intolerant individuals and/or hypercholesterolemic, milk protein allergy, and the expansion of veganism followers, demanded changes (Nguyen et al., 2019; Pimentel et al., 2021). Thus, matrices such as fruits and vegetables have been evaluated by several researchers as a potential vehicle of probiotics (Andrade et al., 2019; De Oliveira et al., 2021; Fonseca et al., 2021).

Fruit juices have been considered an ideal medium for functional foods because of their high nutritional value due to the presence of various phytochemicals, vitamins, minerals, dietary fiber, etc. (Granato et al., 2010; Yoon et al., 2004). The high content of sugars and other nutrients has stood out as one of the desirable characteristics for the growth and survival of probiotic microorganisms. On the other hand, the fruit pH and the antimicrobial action of some antioxidant compounds, represent a great technological challenge to provide a suitable medium for probiotic microorganisms growth and survival (Shah, 2001; Shori, 2016). Da Costa et al. (2017) demonstrated that orange juice is a suitable matrix for probiotic supplementation, the viability of *Lactobacillus paracasei* spp. *paracasei* was higher than 10^6 CFU/mL during 28 days of cold storage, despite the acidic conditions. The authors attributed the high survival of the probiotic in orange juice to vitamin C content, which may promote the reduction of the dissolved oxygen in the medium, improving probiotic survival, and also to its fiber content. Another study observed loss of viability of the probiotic *Lactobacillus paracasei* ssp. culture in white grape juice during 28 days of storage (12 Log CFU/200 mL on day 1 to 7.3 Log CFU/200 mL on day 28). However, according to the authors, white grape juice could be considered probiotic for 21 days,

* Corresponding author.
E-mail address: elianantunb@gmail.com (E. dos Santos Leandro).

https://doi.org/10.1016/j.jff.2023.105883
Received 30 April 2023; Received in revised form 14 October 2023; Accepted 30 October 2023
Available online 7 November 2023

since the count *Lactobacillus paracasei* ssp. in the juice at 21 days of refrigerated storage was 9.1 Log CFU/200 mL. The acidity of the juice, the presence of oxygen in the medium or the low number of nitrogenous compounds and dietary fibers may be related to the loss of viability of the probiotic culture (Okina et al., 2018).

Araticum (*Annona crassiflora*) is an exotic fruit typical of the Brazilian Cerrado region (Arruda, Pereira, & Pastore, 2017). It is much appreciated by the local population, and is among the 20 species most used in regional food, consumed *in natura* or processed (ice cream, jellies, jams and juice) (Almeida et al., 1987; Arruda et al., 2017, 2018). It contains a great diversity of phytochemical compounds, such as carotenoids, phenolic compounds, tocopherol and some vitamins, minerals and oligosaccharides (Arruda et al., 2017, 2018; De Morais Cardoso et al., 2013). Araticum has been the subject of studies involving chemical characterization and identification of biological and pharmacological properties of extracts obtained from different parts of the fruit (Machado et al., 2015; Pimenta et al., 2014; Vilar et al., 2008). Despite the great functional potential of araticum, there have been few studies that evaluate its potential in developing new food products.

The development of araticum juice supplemented with probiotic can be considered an interesting strategy to add greater functionality to the product. *Lactobacillus paracasei* subsp *paracasei* LBC-81 is a commercially available culture that has already been studied in several food matrices during the storage period. Such matrices are: fermented milk enriched with green banana biomass (Vogado et al., 2018), cottage cheese supplemented with oatmeal, green banana or chickpea flour (De Medeiros et al., 2021), chickpea and coconut vegetable beverage (Mesquita et al., 2020) and passion fruit juice (Fonseca et al., 2021). However, the physiological behavior of *L. paracasei* LBC-81 in araticum juice is still unknown.

Thus, the objective of this study was to develop an araticum juice supplemented with the probiotic strain *L. paracasei* LBC-81, and evaluate the pulp percentage effect on the probiotic strain viability during storage period and under *in vitro* gastrointestinal conditions. In addition, chemical changes in the formulations during storage were also evaluated.

2. Material and methods

2.1. Araticum juice preparation

The araticum (*Annona crassiflora*) fruit was purchased from a local street market in Brasilia city, Federal District, Brazil. The fruits were first washed, sanitized and subsequently peeled and pulped. The pulps were vacuum packed in a plastic bag and immediately stored in at −80 °C.

Three base formulations of araticum juice were elaborated: Formulation 1 (F1): araticum pulp (20 %) + mineral water (80 %); Formulation 2 (F2): araticum pulp (30 %) + mineral water (70 %); Formulation 3 (F3): araticum pulp (40 %) + mineral water (60 %). The control formulations were not enriched with probiotic strain *L. paracasei* LBC-81, while the test formulations were enriched with a probiotic strain *L. paracasei* LBC-81.

The formulations were homogenized in a blender (Walita, São Paulo, Brazil) at speed 6 for 2 min at a room temperature. Then, the formulations were sieved in voile to eliminate residues. A volume of 150 mL of each formulation was distributed in glass vials and then submitted to batch pasteurization heat treatment at 65 °C for 15 min in a water bath. After pasteurization, the formulations were stored in a refrigerator (4 °C) for 28 days.

2.2. Juices preparation with L. paracasei cell concentrate

2.2.1. L. paracasei stock culture

The commercial lyophilized culture of *L. paracasei* subsp *paracasei* LBC-81 (Danisco, Barueri, São Paulo, Brazil) was used in this study. 50 μL of the frozen culture (9.0 Log CFU/mL) was activated in 5 mL of De Man Rogosa Sharpe broth (MRS) (Acumedia-Neogen, Michigan, USA) and incubated in a bacteriological oven (Ethik Technolgy, Vargem Grande, SP) at 37 °C for 24 h. The culture was stored at −80 °C in a suspension containing MRS broth and 20 % glycerol (Mesquita et al., 2020). All experiments were carried out using this stock culture.

2.2.2. Cell concentrate preparation

50 μL of the frozen stock culture was activated in 5 mL of De Man Rogosa Sharpe broth (MRS) (Acumedia-Neogen, Michigan, USA) and incubated in a bacteriological oven (Ethik Technolgy, Vargem Grande, SP) at 37 °C for 24 h. 750 μL of the activated stock culture of *L. paracasei* LBC 81 was mixed with 75 mL of MRS broth (1 % inoculum (v/v)) and then incubated at 37 °C for 24 h. The cell pellet was obtained by centrifugation at 2655*g* for 5 min at 4 °C. The pellet was washed twice in 0.85 % saline solution and added to flasks containing 150 mL of araticum juice formulations. The control araticum juices were not fortified with *L. paracasei* cell concentrate (Fonseca et al., 2021; Pimentel et al., 2015). The initial Colony Forming Units (CFU) counting was carried out in araticum juices formulations.

2.3. Determination of the centesimal composition of the araticum pulp and araticum juices formulations

Araticum pulp and araticum juices formulations (F1, F2 and F3 without the addition of probiotic – control formulations) were analyzed regarding centesimal composition. The moisture content was determined according to the Analytical Standards of Adolfo Lutz Institute (IAL, 2008). Protein concentration was performed by the Kjeldahl 991.22 (AOAC, 2005) method. The araticum juices and pulp total lipid content was determined by the Am 5–04 extraction method, using a lipid extractor (model ANKOM XT15 by Ankom Technology, New York, United States of America) (AOCS, 2005). The ashes quantification was performed using the 945.45 method (AOAC, 2005). The crude fiber quantification was determined by the AOAC 978.10 methodology (2005). The total carbohydrates content present in the araticum pulp and araticum juices formulations was determined by calculating the difference between 100 % and moisture, protein, lipids and ashes content, according to 986.25 method (AOAC, 2005).

2.4. Chemical and viability characterization of L. paracasei in araticum juice before and during storage

Araticum juices with and without the addition of *L. paracasei* cell concentrate were evaluated before and during storage (7, 14, 21 and 28 days) at 4 °C for chemical characteristics (acidity, pH, total polyphenol and antioxidant capacity) and *L. paracasei* LBC-81 viability (for juice formulations with cell concentrate).

2.4.1. L. paracasei viability analysis

The araticum juice formulations containing *L. paracasei* were submitted to serial decimal dilutions in saline solution, and then were spread plated on Petri dishes containing MRS agar and incubated at 37 °C for 48 h. The *L. paracasei* viability was estimated from the Colony Forming Units (CFU) counting, and the results were expressed as Log CFU mL^{-1}.

2.4.2. Acidity and pH

A pH meter (PHS – 3E, Birigui, Araucária-PR, Brazil) was used for araticum juices's pH measurements. The araticum juices total acidity was determined according to the method described by Adolfo Lutz Institute (IAL, 2008).

$$\text{Titratable acidity} = V \times F \times M \times 100/P$$

Which:
V = volume of NaOH solution used in the titration (mL).

M = NaOH molarity.
P = sample mass (g).
F = NaOH solution correction factor.

2.4.3. Total phenolic content and antioxidant activity of araticum juices

Total phenolic content: The total phenolic content was determined using *Folin-Ciocalteu* assay according to the method established by Singleton and Rossi (1965), and the results were expressed as μmol of gallic acid /L juice.

Fe-reducing Antioxidant Potential (FRAP) assay: The ferric reducing/antioxidant power (FRAP) of araticum juices was measured using the procedure described by Benzie and Strain (1996). Antioxidant activity was expressed as μmol TROLOX/L juice.

DPPH● (2,2-diphenyl-1-picrylhydrazyl) scavenging capacity assay: The antioxidant potential of araticum juices was determined using the DPPH● scavenging capacity assay according to the method described by Cheng et al. (2006). For each sample, a curve was plotted using the variables % DPPH quenched × sample volume, and the radical scavenging capacity (RDSC) expressed as mol TROLOX equivalent/L juice.

2.5. L. paracasei survival under gastrointestinal in vitro simulated digestion

The araticum juices added with *L. paracasei* LBC-81 cell concentrate, stored for 7 days at 4 °C, were subjected to the following conditions: (i) artificial gastric juice exposure; (ii) artificial intestinal juice exposure; and (iii) sequential exposure to gastric and intestinal juices.

Artificial gastric juice: the viability of *L. paracasei* to simulated gastric juice digestion was performed according to Bautista-Gallego et al. (2013) with modifications. Gastric juice was prepared with NaCl (0.205 g/L), KH_2PO_4 (0.06 g/L), KCl (0.037 g/L) and distilled water. The pH was adjusted to 2.0 with 1 mol/L HCl solution, and then the solution was autoclaved at 121 °C for 15 min. Pepsin solution (0.0133 g/L) (Dinâmica®, Idaiatuba-SP, Brazil) was added to the autoclaved solution. Araticum juice and gastric juice solution were mixed in a 1:1 ratio and incubated at 37 °C for 90 min. The exposure time to gastric juice was in accordance with that established by Calabuig-Jiménez et al. (2019). After the incubation period, the samples were centrifuged at 7871*g*, 4 °C, for 5 min. The obtained pellet was washed twice with saline solution and then centrifuged under the same conditions. The pellets were submitted to serial dilutions, cultivated on MRS agar medium, and then incubated at 37 °C for 48 h. The CFU number was determined after the incubation period and expressed in Log CFU/mL.

Artificial intestinal juice: the viability of *L. paracasei* in simulated intestinal juice digestion was performed according to Bautista-Gallego et al. (2013) with modification. The exposure time to the intestinal juice was in accordance with that established by Calabuig-Jiménez et al. (2019). Intestinal juice was prepared by dissolving Na_2HPO_4 (50.81 g/L), NaCl (8.50 g/L), bile salts (3.00 g/L) in distilled water. The solution pH was adjusted to 8.0 with 1 mol/L NaOH and autoclaved at 121 °C for 15 min. Filtered pancreatin solution (0.1 g/L) (Sigma-Aldrich, St. Louis, Missouri, USA) was added to the autoclaved solution. Araticum juice and intestinal juice solution were mixed in a 1:1 ratio and incubated at 37 °C for 90 min. The conditions for centrifugation and samples plating on MRS agar were carried out according to the procedure described for simulated gastric juice.

Sequential exposure to gastric and intestinal juices: araticum juice and gastric juice solution were mixed in a 1:1 ratio and incubated at 37 °C for 90 min. Subsequently, the samples were centrifuged under the same conditions mentioned above. The obtained pellet was suspended in 1 mL of intestinal juice and incubated at 37 °C for 90 min. The same conditions of centrifugation and samples plating on MRS agar were carried out as described for the individual treatment with gastric or intestinal juice.

The Survival percentage was calculated as Eq. (1):

$$\text{Survival}\ (\%) = \text{final}\ (\text{CFU/mL}\ N_t)/\text{initial}\ (\text{CFU/mL}\ N_0) \times 100 \quad (1)$$

Where N_t represents CFU/mL at time t = 7 days of storage and N_0 represents CFU/mL at time t = 0 days.

2.6. Experimental design and statistical analysis

The experimental design of the study includes three araticum juice formulations and five different measures of storage time intervals (except for centesimal composition analysis and survival *in vitro* gastrointestinal conditions). All experiments were performed three times, and the results were presented as mean ± standard error. Variance analysis (ANOVA) and Tukey's test were used to calculate significant differences at $P < 0.05$. The data analyzed at storage time intervals were also subjected to regression analysis. Statistical analyzes were performed using SAS software (SAS Institute Inc., Cary NC, version 9.4).

3. Results and discussion

3.1. Centesimal composition of the araticum juice formulations and araticum pulp

In orde to characterize the main nutrients of araticum the centesimal composition of the araticum juices and the araticum pulp are shown in Table 1. The araticum pulp presented higher percentages of carbohydrates and crude fiber, and lower percentages of proteins and lipids. Similar to that obtained by De Lima et al. (2016). According to Bates et al. (2001), fruits are poor in lipids and have variable and scarce protein content. As expected, the concentration of carbohydrates (3.1–5.8 %), protein (0.2–0.42 %) and lipids (0.3–0.8 %) in the different formulations were proportional to the percentage of araticum pulp added. There was no significant difference ($P > 0.05$) in fiber and ashes content in the different formulations, despite the increase in the percentage of pulp. Therefore the araticum juices formulations may have suffered a greater reduction in the crude fiber and ashes content, since the formulations had to be passed through a voile to remove solid residues.

3.2. Probiotic viability, pH and titratable acidity in araticum juice formulations throughout storage at 4 °C

L. paracasei viability in the three araticum juice formulations during storage at 4 °C for 28 days is shown in Fig. 1. Before storage (day 0), the three araticum juice formulations showed no significant difference ($P > 0.05$) regarding the viable population of *L. paracasei* (9.5, 9.3 and 9.4 Log CFU/mL for F1, F2 and F3 formulations, respectively; Fig. 1A and 1B), which indicates that the same amount of *L. paracasei* concentrate was added. The *L. paracasei* population in juices made with 20 (9.5 and

Table 1
Centesimal composition of araticum juice formulations and araticum pulp.

Variables (%)	Araticum juice formulations (F)			Araticum pulp
	F1	F2	F3	
Moisture	95.86 ± 0.05a	93.81 ± 0.05b	92.00 ± 0.05c	73.08 ± 0.05d
Carbohydrates	3.18 ± 0.05d	4.74 ± 0.05c	5.85 ± 0.05b	18.92 ± 0.05a
Proteins	0.20 ± 0.05c	0.29 ± 0.05bc	0.42 ± 0.05b	1.37 ± 0.05a
Lipids	0.30 ± 0.05d	0.50 ± 0.05c	0.80 ± 0.05b	2.30 ± 0.05a
Fiber	0.34 ± 0.05b	0.45 ± 0.05b	0.64 ± 0.05b	3.55 ± 0.05a
Ashes	0.11 ± 0.05b	0.19 ± 0.05b	0.26 ± 0.05b	0.76 ± 0.05a

F1 = Araticum juice (20 %); F2 = Araticum juice (30 %); F3 = Araticum juice (40 %).
Means followed by the same letters on the line do not differ from each other ($P > 0.05$) by the Tukey test.

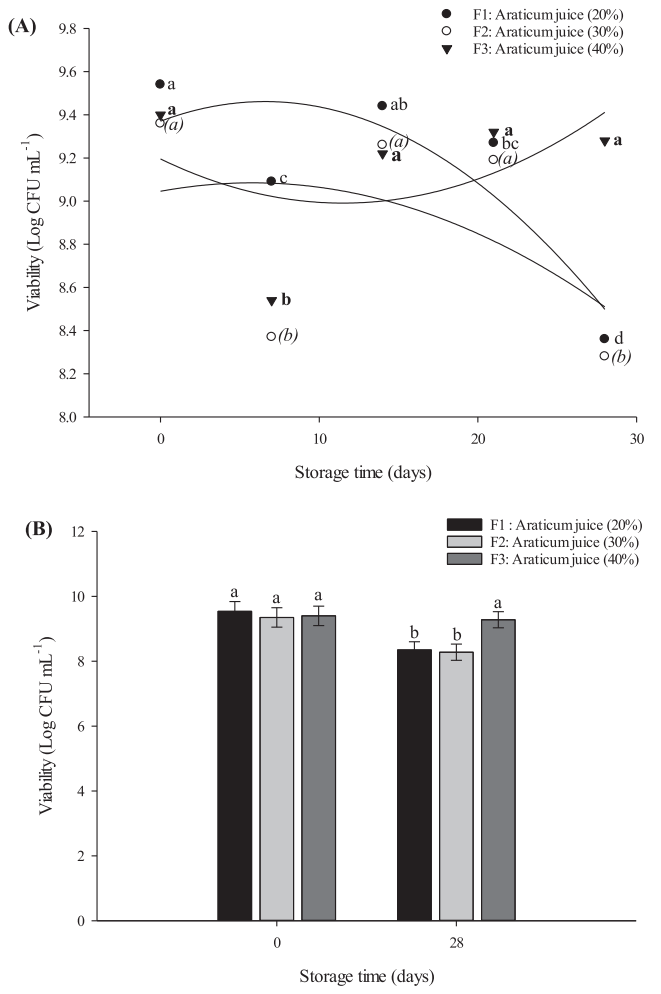

Fig. 1. (A) Changes in the log counts of *L. paracasei* LBC-81 in different araticum juice formulations (F1, F2, and F3) during 28 days of refrigerated storage. (B) Comparison between the log counts of *L. paracasei* LBC-81 on days zero and 28 of different araticum juice formulations (F1, F2 and F3). F1 = Araticum juice (20 %); F2 = Araticum juice (30 %); F3 = Araticum juice (40 %). Different letters within the same araticum juices formulation indicate statistical differences throughout storage time ($P < 0.05$). Columns with different superscript letter are significantly different ($P < 0.05$).

8.4 Log CFU/mL on days 0 and 28, respectively) and 30 % (9.4 and 8.3 Log CFU/mL on days 0 and 28, respectively) of araticum pulp, decreases according to the quadratic equation as shown in Fig. 1A and Table 2. However, in juices made with 40 % of araticum pulp, a decrease was observed initially (9.4–8.5 Log CFU/mL on days 0 and 7, respectively), but around the 14th day an increase (9.2 Log CFU/mL) took place in the *L. paracasei* population. Such behavior is described by the quadratic equation (Table 2). On the 28th day of storage, the juice with 40 % of pulp, showed a significantly higher *L. paracasei* population than the others (8.4, 8.3 and 9.3 Log CFU/mL for F1, F2 and F3, respectively). These results suggest that araticum juices containing 20–40 % of araticum pulp may be considered probiotic for 28 days, since a probiotic product should have counts of probiotic culture higher than 6 Log CFU mL^{-1} (Sheehan et al., 2007). The higher concentration of carbohydrates and proteins in the juice with 40 % of araticum pulp in relation to those with 20 and 30 % of araticum pulp may explain the higher *L. paracasei* viability of this formulation on day 28, since probiotic bacteria requires these nutrients to maintain its viability (Oneca et al., 2007; Rodrigues et al., 2012).

The results of *L. paracasei* viability in the araticum juice formulations can be correlated with the pH (Fig. 2A and 2C) and titratable acidity

Table 2
Regression equations of the viability of *L. paracasei*, pH, titratable acidity, total phenolics, FRAP and DPPH during storage of araticum juice formulations.

Variables	Formulations (F)	Regression Equation	r^2	SEM
Viability	F1	$\hat{Y} = 9.3703 + 0.0276x - 0.0021x^2$	0.71	0.35
	F2	$\hat{Y} = 9.045 + 0.0135x - 0.0012x^2$	0.20	0.65
	F3	$\hat{Y} = 9.1954 - 0.0356x + 0.0015x^2$	0.22	0.43
pH	F - With bacteria	$\hat{Y} = 4.0949 - 0.00562x + 0.0010x^2$	0.97	0.07
	F - Without bacteria	$\hat{Y} = 4.5246 - 0.0046x$	0.62	0.04
Titratable acidity	F - With bacteria	$\hat{Y} = 4.1280 + 0.1434x$	0.92	0.53
	F - Without bacteria	$\hat{Y} = 2.6203 + 0.0067x - 0.0007x^2$	0.20	0.44
Total phenolics	F1 - With bacteria	$\hat{Y} = 2288.7666 - 13.9071x$	0.87	65.66
	F1 - Without bacteria	$\hat{Y} = 2287.2000 - 29.6977x$	0.88	139.85
	F2 - With bacteria	$\hat{Y} = 4331.1000 - 36.1452x$	0.84	198.29
	F2 - Without bacteria	$\hat{Y} = 4096.3333 - 58.7000x$	0.91	227.30
	F3 - With bacteria	$\hat{Y} = 4298.3190—5.8340x + 0.5199x^2$	1.00	0.00
	F3 - Without bacteria	$\hat{Y} = 4751.5333 - 55.7405x$	0.82	3.31
FRAP	F1 - With bacteria	$\hat{Y} = 1901.1673 - 15.7500x$	1.00	0.00
	F1 - Without bacteria	$\hat{Y} = 2046.3817 - 99.6568x + 2.0991x^2$	1.00	0.00
	F2 - With bacteria	$\hat{Y} = 3050.7240 - 28.2540x$	0.91	109.16
	F2 - Without bacteria	$\hat{Y} = 2955.4835 - 123.0825x + 2.6393x^2$	0.91	253.33
	F3 - With bacteria	$\hat{Y} = 2922.8886 - 10.4682x$	1.000	25.10
	F3 - Without bacteria	$\hat{Y} = 3212.4426 - 66.2658x$	0.82	3.66
DPPH	F - With bacteria	$\hat{Y} = 20.7836 - 0.4205x + 0.0093x^2$	0.94	0.66
	F - Without bacteria	$\hat{Y} = 21.2608 - 0.2937x$	0.90	1.24

F1 = Araticum juice (20 %); F2 = Araticum juice (30 %); F3 = Araticum juice (40 %).
SEM: standard error of the mean.

(Fig. 2B and 2D) results. The araticum juice pH with *L. paracasei* concentrate added, decreases until approximately on the 21st day of storage accordingly the quadratic equation (4.1–3.3 on days 0 and 21; Table 2). From the 21st day on, a tendency towards pH stability was observed (3.3 and 3.4 on days 21 and 28; Fig. 2A). The araticum juice titratable acidity with the cell concentrate showed an increase that is described by the linear equation (3.9–7.7 % on days 0 and 28; Fig. 2B and Table 2). The pH (Fig. 2C) and acidity (Fig. 2D) showed significantly ($P < 0.05$) lower and higher values, respectively in the araticum juice with the bacteria concentrate (3.6 ± 0.02 and 6.1 ± 0.2 %, respectively) compared to araticum juice without bacteria (4.5 ± 0.02 and 2.5 ± 0.2 %, respectively).

Therefore, the pH decrease and the acidity increase of araticum juice with bacteria demonstrate the occurrence of fermentation during

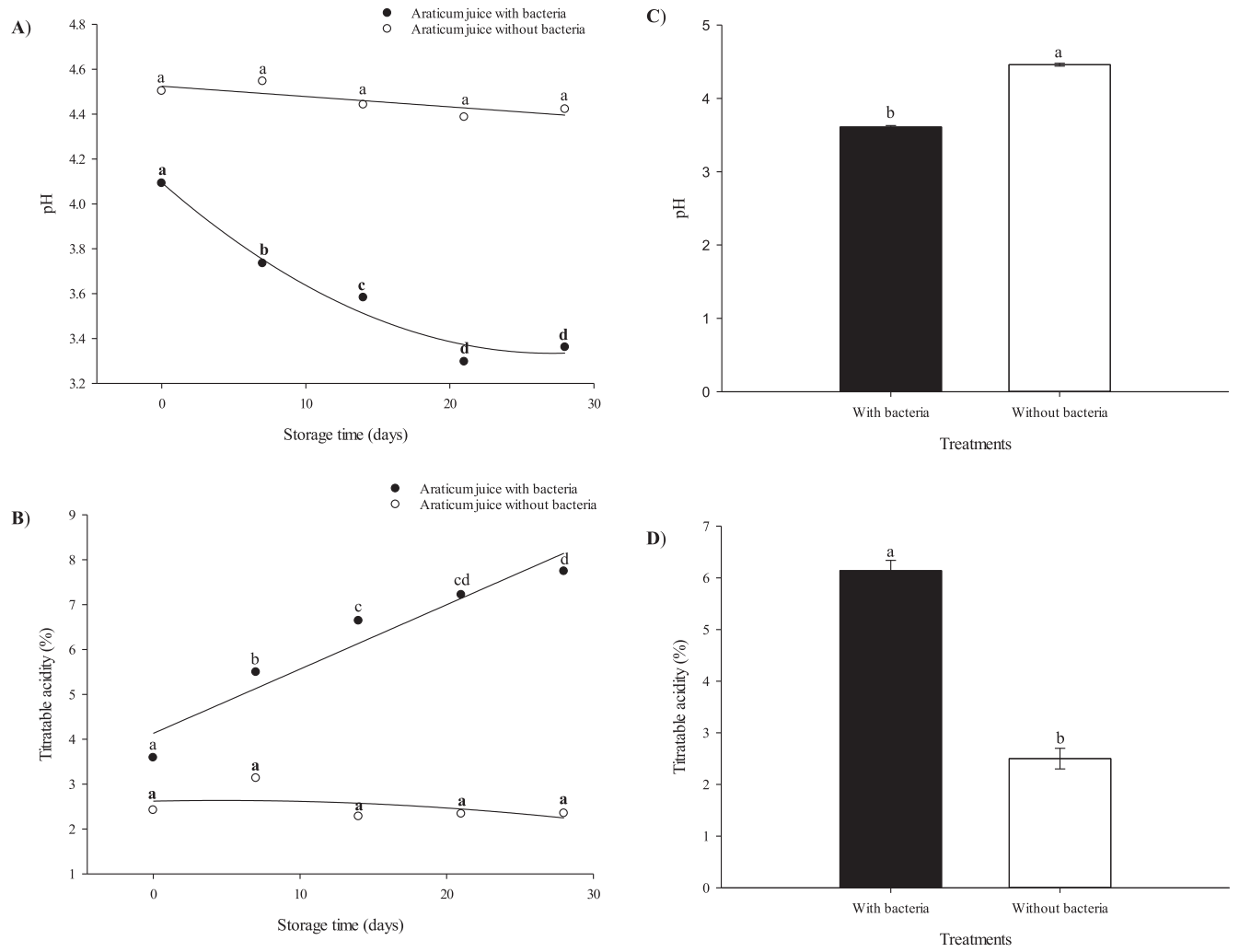

Fig. 2. Behavior of pH (A) and titratable acidity (B) in the formulation of araticum juice with and without addition of *L. paracasei* during 28 days of storage at 4 °C and comparison by Tukey test of pH (C) and titratable acidity (D) in araticum juice with and without addition of *L. paracasei*.

storage. The *L. paracasei* viability decrease in juices with 20 and 30 % araticum pulp may be related to the decrease in pH and increase in acidity (Fig. 2C and 2D). The pH decreases during storage of araticum juices with *L. paracasei* concentrate added was quite similar (4.1–3.4 on days 0 and 28) to the pH decrease (4.0–3.5) of blueberry juice fermented at 37 °C for 48 h (Liao et al., 2023).

Some studies on unfermented fruit juices supplemented with probiotics have indicated that changes in pH and acidity vary depending on the strain and the type of fruit used. Gumus and Demirci (2022) found that the grape juice enriched with the probiotic strains *Lactobacillus fermentum* CECT5716 or *Lactobacillus acidophilus* DSM20079, during storage, provided a slight decrease in pH and an increase in acidity when compared to the control sample (without addition of probiotic). Unfermented apple juice enriched with probiotic cultures (PRO-G, SYNB-G, PRO-P and SYNB-P formulations) caused a slight increase in titratable acidity and decrease in pH during 28 days of storage (Pimentel et al., 2015). Unfermented beverage (banana, strawberry and juçara blend) enriched with probiotics (*Bifidobacterium animalis* subsp *lactis* BB-12; *Lactobacillus acidophilus* LA-5; *Lactobacillus casei* BGP93; *Lactobacillus plantarum* CNPC003) showed pH and titratable acidity stability of beverages prepared with individual probiotic cultures during 90 days of storage (Ribeiro et al., 2020).

Probiotic lactic acid bacteria can metabolize simple sugars from fruit juice, and dead cells from probiotics can release enzymes that hydrolyze the medium's sugars, increasing the final product's acidity (Ding & Shah, 2008; Rodrigues et al., 2012). Simple sugars contained in juices can be converted into organic acids by the *Lactobacillus* bacteria. Although, the aim of this study was to obtain an unfermented beverage, the organic acids derived from fermentation contribute to the taste and palatability of fruit juices, the higher acidity of probiotic products can protect the juices from the development of food spoilage microorganisms, thus increasing their shelf life. Such changes are desirable as long as they do not affect the sensory or technological characteristics of the products (Pimentel et al., 2015).

Although during the 28-day storage period decreases or increases in the population of *L. paracasei* was observed, it was noted that over time the population remained above the minimum required (6 log CFU mL^{-1}) for a food to be considered probiotic, and able to provide health benefits to the individual (Sheehan et al., 2007). The results obtained in this study corroborate reports of the efficiency of fruit juice as a matrix for probiotics in probiotic-supplemented (Gumus & Demirci, 2022; Ribeiro et al., 2020) and fermented beverages (Lan et al., 2023; Liao et al., 2023).

The results obtained for the total phenolic content in the araticum juices can be correlated with the viability of *L. paracasei* during storage. The decrease in total phenolic content in juices with 20 and 30 % araticum pulp, with (2246 ± 408 to 1926 ± 408 and 4200 ± 408 to 3338 ± 143 μmol galic acid/L juice on days 0 and 28, respectively) or without (2360 ± 408 to 1584 ± 408 and 4336 ± 408 to 2629 ± 143 μmol galic acid/L juice on days 0 and 28, respectively) the addition of bacteria

concentrate, is described according to linear and quadratic equations (Fig. 3A, 3B and Table 2). This behavior has also been observed by other authors.

Nematollahi et al. (2016) found that the total phenolic content of unfermented cherry juice supplemented with *Lactobacillus rhamnosus* ATCC 7469 decreased after 28 days of refrigerated storage. In a pineapple juice beverage fermented by probiotic strains (*Bifidobacterium lactis* Bb12, *Lactobacillus plantarum* 299 V and *Lactobacillus acidophilus* La5) a reduction in the total phenolic content was also observed during storage at 4 °C for 28 days (Nguyen et al., 2019). The presence of dissolved oxygen in juices due to the homogenization process, as well as the activation of endogenous polyphenoloxidase enzyme during juice storage or the presence of metal ions such as iron and cupper can result in the oxidation of phenolic compounds. Total phenolic content determination by the Folin-Ciocalteu method is based on a redox reaction, therefore the reduction capacity of oxidized polyphenols is weakened, resulting in lower result values (Millet et al., 2017). Patthamakanokporn et al. (2008) observed that in the absence of light and oxygen, the phenolic compounds content has not considerably changed during the period of refrigerated storage. In the present study, as the juices were not stored in bottles that block the entry of light, and no conditions have been created to prevent the entry of oxygen, it is likely that these factors may have contributed to the reduction in total phenolic during storage. In the juice made with 40 % araticum pulp, the phenolic content showed a marked decrease only for the juice without added bacteria concentrate (4941 ± 143 to 3139 ± 143 μmol galic acid/L juice on days 0 and 28, respectively).

The values of total phenolic content in the juice with 40 % of pulp and bacteria concentrate remained constant during storage time (4343 ± 143 to 4390 ± 143 μmol galic acid/L juice on days 0 and 28, respectively. Fig. 3C), however higher than that obtained for araticum juice with 40 % of pulp without bacteria (4390 ± 143 μmol galic acid/L juice and 3139 ± 143 μmol galic acid/L juice, respectively on day 28). The highest content of the total phenolic content in the araticum juice with bacteria can be correlated with the increase in the population of *L. paracasei* at the end of storage (Fig. 1A). Liao et al. (2023) observed an increase in the total phenolic content during fermentation of blueberry juice by probiotic bacteria (*Lactobacillus fermentum* and *Lactobacillus plantarum*) at 37 °C for 24–48 h, suggesting that probiotic bacteria increase phenolic compounds in juices by the fermentation process. In our study araticum juice formulations were not fermented juices, however, during storage time, occurred fermentation of araticum juice by *L. paracasei* LBC-81, since pH and titratable acidity significantly decreased and increased (Fig. 2A and 2B), respectively in formulations with bacteria. Therefore, the highest phenolic content obtained on day 28 in araticum juices with 30 and 40 % of pulp and bacteria compared to those formulations without bacteria (Fig. 3B and 3C) may be attributed to the fermentation process. Gumus and Demirci (2022) also suggested that the increased phenolic content in grape juice fermented by *Lactobacillus fermentum* may be due to the ability of enzymes such as β-galactosidase and α-amylase, produced by this bacterium, to hydrolyze phenolics bound to sugars into free phenolic in the juice. It has already been stated that a strain of *Lactobacillus paracasei* was effective in phenolic bioconversions due to its high β-glucoside and β-galactoside content (Bontsidis et al., 2021; Zhao & Shan, 2014).

It is known that phenolic compounds can act as protective or antimicrobial agents (Succi et al., 2017). Some authors suggest that the decrease in total phenolics content during juice storage allows a high population of *L. paracasei* in the juices until the end of storage, due to phenolics antimicrobial activity. However, in the juice with 40 % araticum pulp, the phenolic content remained high and the viability of *L. paracasei* increased at the end of storage (28 days; 9.3 Log CFU/mL. Fig. 1). Therefore, it is possible that with fermentation less toxic phenolics were formed allowing the growth of *L. paracasei* in the juice with 40 % araticum pulp. The high percentage of phenolic in the juice with 40 % araticum pulp, may have hindered the adaptation of *L. paracasei* at

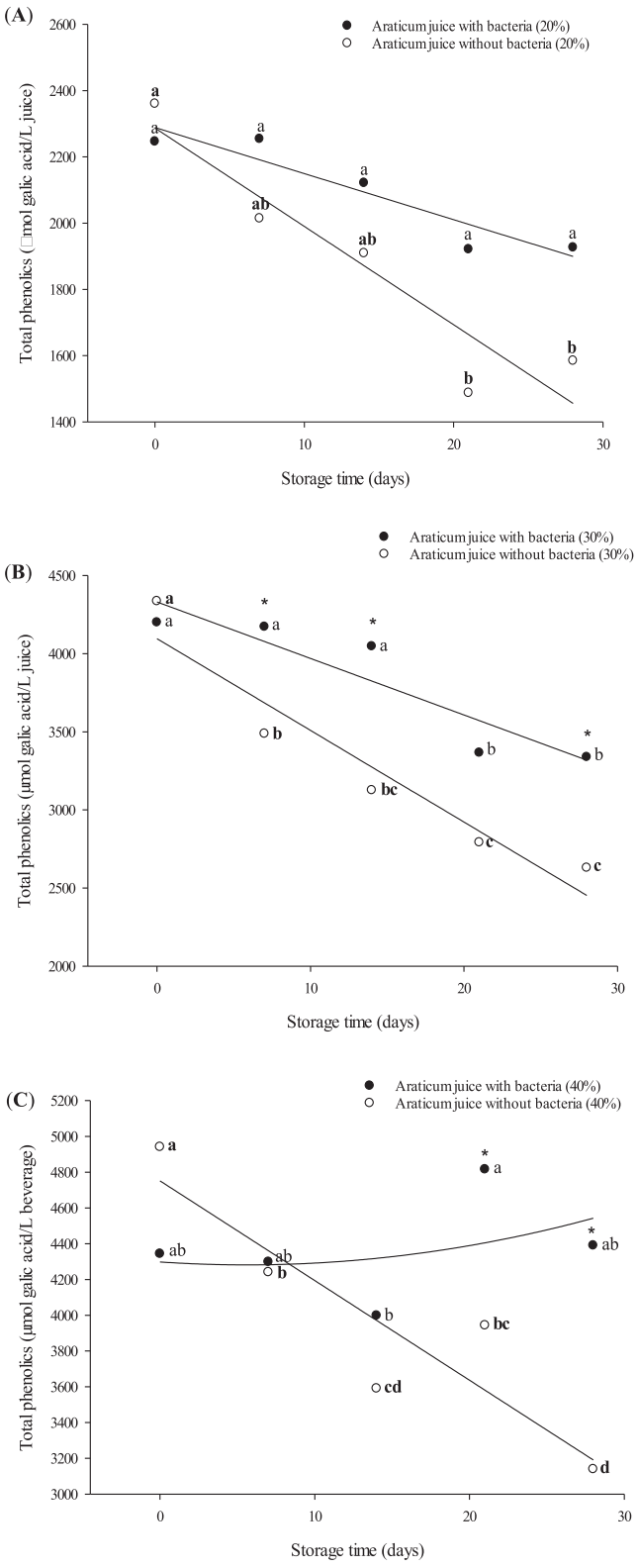

Fig. 3. Total phenolics content in araticum juice formulations made with 20 % (A), 30 % (B) and 40 % (C) of araticum pulp, with and without addition of *L. paracasei* LBC-81, during 28 days of storage at 4 °C. * Statistical differences comparing araticum juices formulations with and without bacteria in the same storage time. Different letters within the same araticum juices formulation indicate statistical differences throughout storage time.

the beginning of storage. In addition, other intrinsic characteristics of the juice with 40 % araticum pulp may have been altered, thus being able to provide growth in the population of *L. paracasei*.

The antioxidant potential of araticum juice formulations measured as FRAP assay (Fig. 4B and 4C) was significantly higher in formulations with 30 and 40 % of pulp with bacteria (2606 ± 92 and 2806 ± 92 on day 14, respectively) in relation to formulations without bacteria (1727 ± 92 and 2043 ± 92 on day 14, respectively) from the 14 st days of storage onwards. In the juice with 40 % araticum pulp and bacteria, FRAP values remained constant from day 0 to day 28 of storage time (2927 ± 92 and 2618 ± 92, respectively). Considering that FRAP values showed a similar behavior to the results obtained for total phenolic compounds, concerning to greater value stability over storage time and greater values for formulations with bacteria against without bacteria, and that phenolic compounds contribute highly to the antioxidant potential of plant foods (Da Silva et al., 2006), we suggest that phenolics are the main compounds responsible by the antioxidant potential of araticum juices.

Regardless of the percentage of the juice pulp, both the juice with (21.07 ± 0.44 to 15.9 ± 0.44 mol TROLOX/L juice on days 0 and 28, respectively) and without (22.6 ± 0.44 to 13.7 ± 0.44 mol TROLOX/L juice on days 0 and 28, respectively) bacteria showed a decrease in antioxidant activity measured by radical scavenging capacity assay (DPPH●) during storage (Fig. 5A). It is observed that before storage antioxidant activity (DPPH●) of the juice without bacteria (22.6 ± 0.44 mol TROLOX/L juice), was significantly ($P < 0.05$) higher than that of the juice with bacteria (21.07 ± 0.44 mol TROLOX/L juice; Fig. 5B). On the 28th day of storage, the juice antioxidant activity with bacteria became significantly ($P < 0.05$) higher (15.9 ± 0.44 mol TROLOX/L

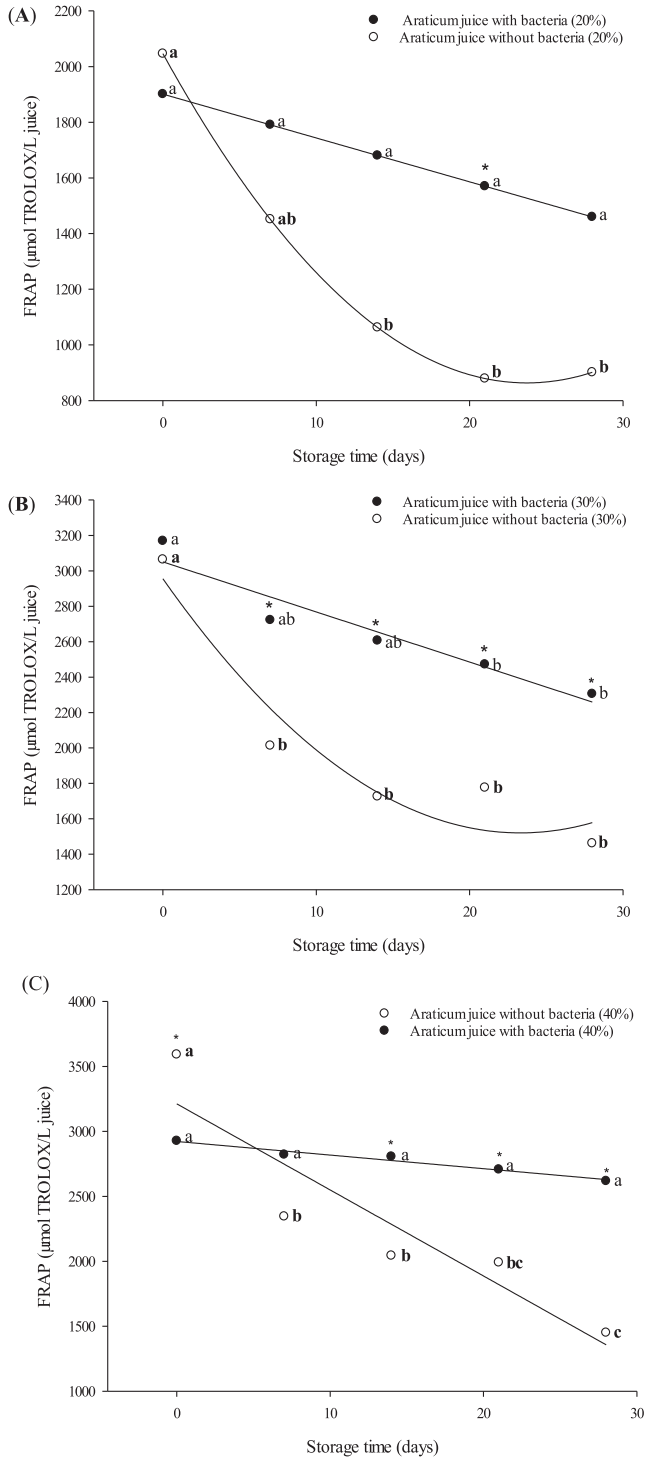

Fig. 4. Antioxidant activity determined by FRAP assay in araticum juice formulations made with 20 % (A), 30 % (B) and 40 % (C) of araticum pulp, with and without the addition of *L. paracasei* LBC-81, for 28 days storage at 4 °C. * Statistical differences comparing araticum juices formulations with and without bacteria in the same storage time. Different letters within the same araticum juices formulation indicate statistical differences throughout storage time.

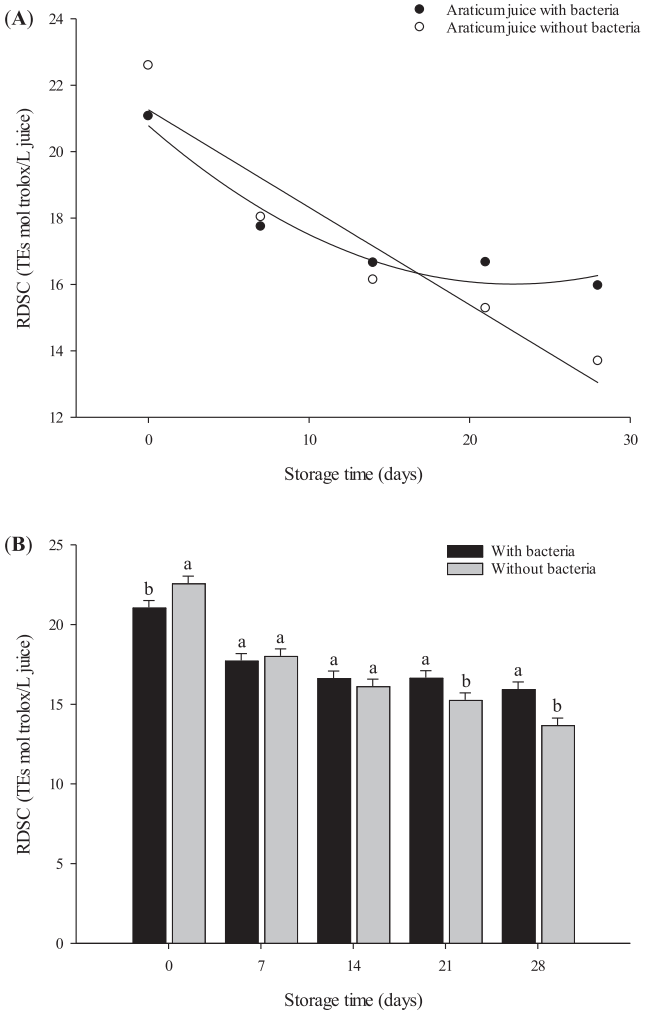

Fig. 5. Antioxidant activity estimated by DPPH● *scavenging capacity assay* in araticum juice formulations, with and without *L. paracasei* LBC-81, during 28 days of storage at 4 °C (A). Antioxidant activity in araticum juice formulations, with and without *L. paracasei* LBC-81, at the different storage times (B). Columns with different superscript letter are significantly different ($P < 0.05$).

juice) compared to juice without bacteria (13.7 ± 0.44 mol TROLOX/L juice).

The different behavior observed in the antioxidant potential of araticum juice formulations measured by the FRAP and DPPH● assays, such as a significant interaction effect obtained for formulation × FRAP but not for formulation × DPPH●, may be associated to the principle of the methods, as the FRAP assay measures ferric reducing ability (Benzie & Strain, 1996) while the DPPH● measures the radical scavenging capacity (Cheng et al., 2006). Thaipong et al. (2006) suggested that FRAP assay showed the highest correlation with phenolic compounds than DPPH●, ABTS● and ORAC assays.

The antioxidant activity of araticum juices can be attributed to their total phenolic content. The decrease in the phenolic content is proportional to the reduction in the antioxidant activity in the juices, while the increase in the phenolic content in the juices added with bacteria is also proportional to the increase in the antioxidant activity of the juices added with bacteria.

The accumulation of phenolic compounds during fermentation is one of the main reasons for higher antioxidant capacity (Wang et al., 2021). In blueberry juice fermented by probiotic strains (*Lactobacillus plantarum* and *Lactobacillus fermentum*), the authors observed that the increase in flavonoid compounds provided an increase in antioxidant capacity (Liao et al., 2023). Furthermore, depending on the probiotic strain, metabolites with antioxidant activity (glutathione, folate, vitamins, etc.) can be released into the fruit juice.

3.3. Survival of *L. paracasei* in vitro gastrointestinal digestion conditions

The viability and survival of *L. paracasei* in araticum juice formulations after exposure to simulated *in vitro* gastrointestinal conditions are shown in Fig. 6. Viability (Fig. 6A) and survival (Fig. 6B) of *L. paracasei* were significantly higher ($P < 0.05$) when exposed to gastric juice, when compared to exposure to intestinal juice and sequential exposure to gastric and intestinal juice. The authors believe that *L. paracasei* may have developed an adapted response due to the presence of organic acids in the araticum pulp.

Srisukchayakul et al. (2018) found that pretreatment of *Lactobacillus plantarum* NCMIB8826 with citric acid (pH 3–6) is able to increase survival in various highly acidic fruit juices, such as pomegranate (pH 3.5), lemon juices and lime (pH 2.8). These authors believe that the increase in survival is associated with a decreased membrane fluidity and permeability, which prevent protons influx into cells during exposure to gastric juice.

It was observed that the percentage of pulp in the juices did not provide significant difference ($P > 0.05$) when *L. paracasei* was exposed to different conditions simulated *in vitro* (Fig. 6C). This result is important because it shows that it is possible to prepare a more concentrated araticum probiotic beverage.

4. Conclusion

The araticum juice proved to be an excellent matrix for *L. paracasei* LBC 81 as it provided a high percentage of survival to simulated *in vitro* gastrointestinal conditions, regardless araticum pulp concentration. The shelf life of araticum juices with probiotic, based on the minimum recommended amount of probiotics would be 28 days. Although, fermentation occurred during storage, this proves how well the strain adapted to the araticum juice. The addition of *L. paracasei* in the three formulations provided an increase in the content of phenolic compounds and greater stability of the antioxidant activity during storage.

Ethics statement

The research was not carried out with animals and humans.

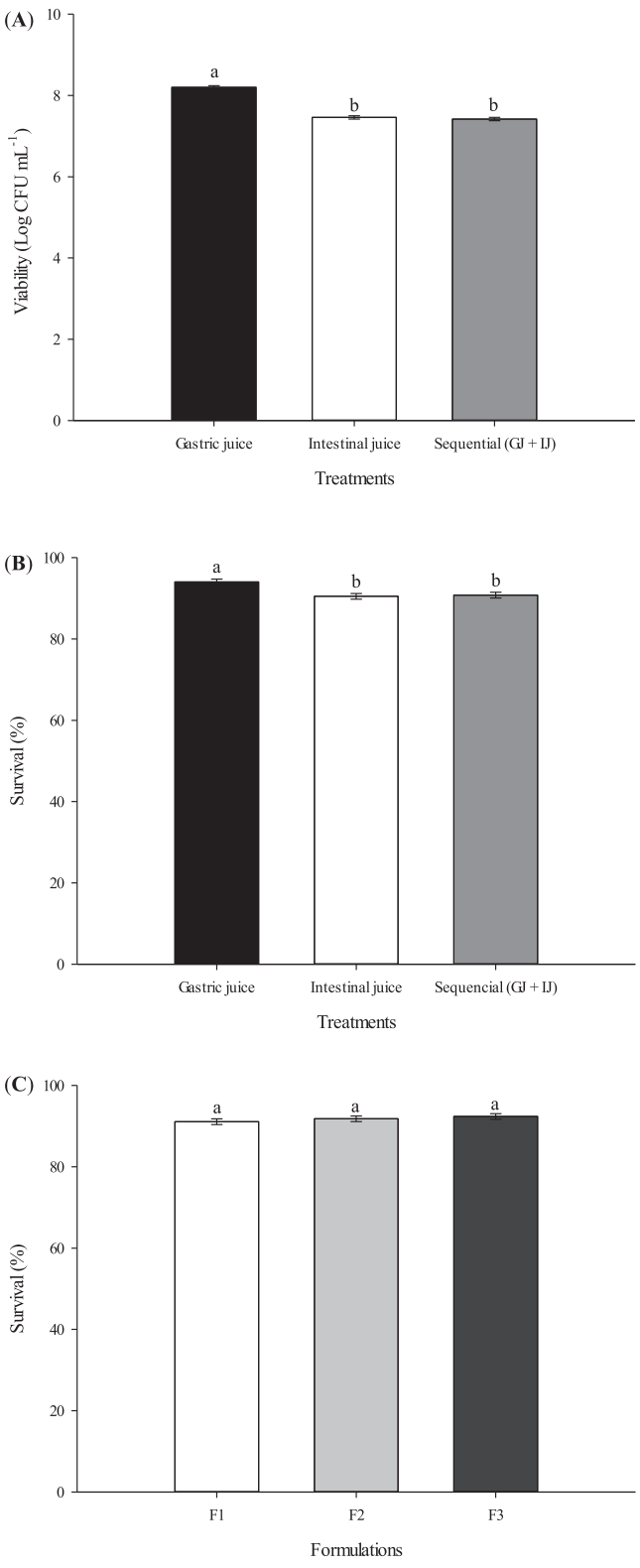

Fig. 6. Viability (A) and percentage survival rate (B) of *L. paracasei* LBC-81 independent of the araticum juice formulation after an *in vitro* digestion and (C) effect of the formulation on the survival rate of *L. paracasei* LBC-81 independent of the gastrointestinal tract stress condition. F1 = Araticum juice (20 %); F2 = Araticum juice (30 %); F3 = Araticum juice (40 %). Columns with different superscript letter are significantly different ($P < 0.05$).

CRediT authorship contribution statement

Luiza Coêlho Midlej: Conceptualization, Investigation, Data curation, Writing – original draft. **Sandra Fernandes Arruda:** Validation, Resources, Methodology, Writing – review & editing. **Maria Carolina Mesquita:** Investigation, Data curation, Writing – review & editing. **Márcio Antônio Mendonça:** Resources, Data curation. **Eliana dos Santos Leandro:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgment

The authors acknowledge the financial support of the Fundação de Amparo a Pesquisa do Distrito Federal (FAPDF) – Process: 00193-00000104/2019-86 and for complementary financial resources from Decanato de Pesquisa e Inovação (DPI) and the Decanato de Pós-Graduação (DPG) of the University of Brasília.

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