Heliyon 10 (2024) e37270

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

Room temperature storage of myrtle (*Eugenia gracillima* Kiaersk.) tropical juice: Effects of physical and chemical preservation methods

Bruno Fonseca Feitosa [a,b,g,*], Leidiana Elias Xavier [b], Maria Silvanete Benedito de Sousa Lermen [c], Monica Correia Goncalves [b], Tiago Augusto Lima Cardoso [b], Joao Vitor Fonseca Feitoza [d], Adriano Sant'Ana Silva [b], Emanuel Neto Alves de Oliveira [e], Marcella Camargo Marques [a], Lilian Regina Barros Mariutti [a], Monica Tejo Cavalcanti [b,f]

[a] *University of Campinas, 13056-405, Campinas, SP, Brazil*
[b] *Federal University of Campina Grande, 58840-000, Pombal, PB, Brazil*
[c] *Family Farmers' Association of Serra dos Paus Dóias, 56230-000, Exu, PE, Brazil*
[d] *Federal University of Ceará, 60020-181, Fortaleza, CE, Brazil*
[e] *Federal Institute of Education, Science and Technology of Rio Grande do Norte, 59900-000, Pau dos Ferros, RN, Brazil*
[f] *National Institute of the Semiarid Region, 58434-700, Campina Grande, PB, Brazil*
[g] *University of the State of Amapá, 68950-000, Amapá, AP, Brazil*

ARTICLE INFO

Keywords:
Heat treatment
Myrtaceae
Native fruit
Non-alcoholic beverage
Potassium sorbate
Sodium benzoate

ABSTRACT

Tropical fruit juices produced from native fruits have been widely marketed by small agribusinesses in the Brazilian semiarid region, necessitating a deeper understanding of the impact of preservation methods on quality parameters. This study aimed to prepare myrtle (*Eugenia gracillima* Kiaersk.) tropical juice and investigate the effects of physical preservation (90 °C for 60 s) and chemical preservation (potassium sorbate and sodium benzoate) methods. Tropical juice formulations were evaluated after preparation and every 15 days during 60 days of storage in high-density polyethylene bottles at room temperature (25 ± 2 °C). Microbiological parameters, optical microscopy, physicochemical and bioactive parameters, antioxidant capacity, and color parameters were determined. Heat-treated tropical juice showed low counts of all microbiological parameters, but optical microscopy revealed the presence of filamentous fungi after 60 days of storage. Combined use of potassium sorbate and sodium benzoate effectively prevented the development of total yeasts and molds up to 28 days of storage. Bioactive compounds in myrtle pulp contribute to storage stability, mainly total phenolics, estimated at 855.86 mg gallic acid equivalents 100 g^{-1}. The results suggest that it is possible to harness the economic and agro-industrial potential of *E. gracillima* Kiaersk. fruits for the production of tropical juices, but it is recommended that other technologies be explored, such as aseptic processing or the combined use of physical and chemical methods.

* Corresponding author. University of Campinas, School of Food Engineering, Monteiro Lobato 80, 13056-405, Campinas, SP, Brazil.
E-mail addresses: brunofonsecafeitosa@live.com (B.F. Feitosa), leidiana_elias@hotmail.com (L.E. Xavier), netelermen@yahoo.com.br (M.S.B.S. Lermen), mnygoncalves@gmail.com (M.C. Goncalves), tiagoipj@yahoo.com.br (T.A.L. Cardoso), joaovitorlg95@hotmail.com (J.V.F. Feitoza), adriano_santana@yahoo.com.br (A.S. Silva), emanuel.oliveira@ifrn.edu.br (E.N.A. Oliveira), marques.marcella@gmail.com (M.C. Marques), lilianma@unicamp.br (L.R.B. Mariutti), monicatejoc@yahoo.com.br (M.T. Cavalcanti).

https://doi.org/10.1016/j.heliyon.2024.e37270
Received 6 May 2023; Received in revised form 27 August 2024; Accepted 29 August 2024
Available online 31 August 2024

Abbreviations

$+a^*$	Redness
$+b^*$	Yellowness
ΔH^*	Hue difference
ΔE^*	Total color difference
$ABTS^{\bullet+}$	2,2′-Azinobis(3-ethylbenzothiazoline-6-sulfonate) radical cation
AC	antioxidant capacity
AsA	Ascorbic acid
a_w	Water activity
BI	Browning index
C^*	Chroma
CFU	Colony-forming units
CI	Color index
GAE	Gallic acid equivalents
h°	Hue angle
INS	International Numbering System
L^*	Lightness
MP	Myrtle pulp
MPN	Most probable number
^{ns}	Not significant
r	Correlation coefficient
R^2	Coefficient of determination
TBC	Total aerobic mesophilic bacterial count
TE	Trolox equivalents
TI	Technological index
JA	Tropical juice treated by a chemical method
JB	Tropical juice treated by a physical method
JC	Control tropical juice
RSD	relative standard deviation
TPC	Total phenolic content
TS	Total sugars
TSS	Total soluble solids
Trolox	6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid
TTA	Total titratable acidity
TYMC	Total yeast and mold count.

1. Introduction

In Brazil, the fruit production sector reached a record value of US$ 1 billion in exports in 2021, equivalent to 1.2 million tonnes, with an 18 % increase in production by weight compared with 2020 [1]. Brazilian tropical fruit farms have high bioeconomic potential but are subject to environmental, biological, climatic, and seasonal effects. Research exploring differentiated marketing strategies for biodiversity products is crucial to strengthen family agriculture and agribusinesses in the Brazilian semiarid region [2,3].

A prominent component of the Brazilian flora, the genus *Eugenia* (*Myrtaceae*: Myrteae) comprises about 400 species recorded in Brazil and has great economic and pharmacological potential [4]. The tropical species *Eugenia gracillima* Kiaersk., locally known as myrtle, is native and endemic to Brazil. It attracts commercial and agroindustrial interest for its nutritional composition, sensory characteristics, and presence of bioactive and functional compounds in leaves and fruits [5–7], which can be used to produce medicines and food products, such as sweets, alcoholic beverages, and juices [8,9].

There has been a growing number of studies assessing the production of tropical juices from native Brazilian fruits in the scientific and biotechnological literature. Supermarket shopping analyses showed rising demand for fresher and healthier products with higher nutritional quality to the detriment of sales of soft drinks and fruit juices containing artificial flavors [10,11]. In Brazilian legislation, tropical juices are defined as unfermented beverages obtained by dissolving fruit pulps of tropical origin in drinking water or clarified tropical fruit juice. The color, flavor, and aroma must be characteristic of the fruit, and juices may be treated by physical and/or chemical methods to ensure preservation until consumption [12,13].

Thermal processing is commonly applied in the food industry. Processing temperatures should preferably be mild to preserve the fresh product's physicochemical, bioactive, and color characteristics [14,15]. Chemical preservatives such as sorbate and benzoate salts are approved by Brazilian legislation, as scientific evidence confirms the safety of these substances within the maximum permitted concentrations [16,17]. Studies assessed the effects of physical and/or chemical preservation methods on pomegranate juice [18], orange juice with pepper [19], guava juice [20], and açaí [15]. However, there are no reports of the effects of preservation methods on

myrtle tropical juice.

This study investigated preservation treatments that meet the technological access requirements of family farms and small agribusinesses in the Brazilian semiarid region. The objective was to prepare myrtle tropical juices and determine the effects of physical preservation by heat treatment (90 °C for 60 s) and chemical preservation by adding food additives (potassium sorbate and sodium benzoate). Also, to evaluate the microbiological quality, optical microscopic characteristics, physicochemical and bioactive parameters, antioxidant capacity, and color parameters of myrtle juices during 60 days of storage at room temperature (25 ± 2 °C).

2. Material and methods

2.1. Plant material and registration

Myrtle (*E. gracillima* Kiaersk.) fruits were manually collected at stage V (advanced maturation), characterized by being 100 % purple [21]. The collection took place between September and November 2020 at the Lermen family farm (7°21′S 39°53′W, 884 m elevation), located in Serra dos Paus Dóias, Chapada do Araripe, Exu, Pernambuco, Brazil. At least 5,000 units were collected at different locations during the week, on even days.

Authorization for carrying out scientific activities using *E. gracillima* Kiaersk. was obtained from the Chico Mendes Institute for Biodiversity Conservation (ICMBio) of the Brazilian Ministry of Environment (MMA) through the Biodiversity Authorization and Information System (SISBIO, Authentication No. 0768030120201127). The species is registered in the National System for the Management of Genetic Heritage and Associated Traditional Knowledge (SisGEN) of the Genetic Heritage Management Council (CGen) and MMA under accession number A4E391A.

2.2. Processing of myrtle pulp and tropical juices

Myrtle pulp (MP) and tropical juices were processed in two batches, according to Brazilian regulations [12,16,22,23]. Myrtle fruits were subjected to the following unit operations: manual selection (performed in two steps, first in the field and then in the agro-industry), crushing, pressing through 15- and 18-mesh sieves (two stages with subsequent repetitions), and sieve filtration through 18-mesh sieves for pulp extraction. The yield was calculated using Eq. (1), and the technological index (TI) was determined using Eq. (2) [24].

$$\text{Yield}\ (\%) = \frac{\text{PW}}{\text{FW}} \times 100 \tag{1}$$

$$\text{TI}\ (\%) = \frac{\text{Yield} \times \text{TSS}}{100} \tag{2}$$

where PW is the pulp weight (kg), FW is the fruit weight (kg), and TSS is the total soluble solids content (°Bx).

Preliminary tests were performed using serially diluted pulp by successive addition of 10 % drinking water. The optimum ratio of MP to drinking water was determined to be 1:1 (v/v) based on cost/benefit calculations, yield, and sensory evaluations of taste, acidity, visual color, and overall appearance (data not shown).

The final products did not contain added sugar and were under Brazilian regulations [12]. Three formulations of myrtle juices were prepared, as follows: JA, myrtle juice added with potassium sorbate (INS 202, $C_6H_7KO_2$) and sodium benzoate (INS 211, $C_7H_5NaO_2$) at a total concentration of 0.1 g 100 mL^{-1}, according to the International Numbering System (INS) [16]; JB, myrtle juice subjected to heat treatment at 90 °C for 60 s, followed by hot filling and cooling in an ice bath; and JC, control formulation without chemical or physical preservation.

2.3. Room storage and sample characterization

After the respective treatments, tropical juices were stored in 500 mL, square, high-density polyethylene bottles immediately closed with plastic screw caps. Samples were maintained at room temperature (mean 25 ± 2 °C) for 60 days. Stability analyses were performed at least in two replicates per treatment at time 0 (before storage) and every 15 days. MP samples were analyzed only before storage (t_0).

All analytical parameters and techniques were performed under the microbiological standards for food products set by the Brazilian Health Regulatory Agency (ANVISA) [25] and the Manual of Methods of Analysis of Beverages and Vinegars – Non-Alcoholic Beverages Section, published by the Ministry of Agriculture, Livestock, and Food Supply (MAPA) [26].

2.3.1. Microbiological parameters

Microbiological quality was evaluated using the following parameters: *Enterobacteriaceae*, determined by the Petrifilm (3M™ Petrifilm™, Poland) plate technique, with incubation at 35–37 °C for 24 h; total coliforms, thermotolerant coliforms, *Escherichia coli*, total aerobic mesophilic bacterial count (TBC), total yeast and mold count (TYMC), coagulase-positive *Staphylococcus aureus*, lactic acid bacteria, and *Salmonella* spp [27]. Coliform results were expressed as the most probable number (log MPN mL^{-1}). *Salmonella* spp. results were dichotomized as presence or absence in 25 mL of sample. The other microbiological parameters were expressed in colony-forming units (log CFU mL^{-1}).

2.3.2. Optical microscopy

Optical microscopy examinations were performed at t_0 and after 60 days of room temperature storage (t_{60}). Sample aliquots were directly applied on microscope slides, covered with coverslips, and analyzed under an ABM 102i optical microscope at 50/60 Hz (PHYSIS, China). Microphotographs were recorded using a 10 × eyepiece and a 10 × objective, resulting in a total magnification of 100 × . For better visualization, some samples were magnified using a 10 × eyepiece and a 40 × objective, equivalent to a total magnification of 400 × .

2.3.3. Physicochemical parameters

Total ash was determined by incineration in a muffle furnace (Quimis®, São Paulo, Brazil) at 550 °C for 6 h; total sugars (TS) by the Lane–Eynon method, using Fehling A and B solutions; total soluble solids (TSS) by using a digital benchtop refractometer (RTD-95, Instructherm®, São Paulo, Brazil) with a measuring range of 0–95 °Bx; total titratable acidity (TTA) by titration with a 0.1 N sodium hydroxide (NaOH) solution containing 1 % phenolphthalein indicator ($C_{20}H_{14}O_4$); and pH by using a digital pH meter (DM-22, Digimed®, São Paulo, Brazil) calibrated with pH 4.0 and 7.0 buffer solutions [28,29]. Water activity (a_w) was measured using a water activity meter (Aqualab 3 TE, Decagon Devices®, Albufeira, Portugal) at 25 °C. The TSS/TTA ratio was also determined [30].

2.3.4. Bioactive parameters

Ascorbic acid (AsA, $C_6H_8O_6$) was quantified by a spectrophotometric method with adaptations [31]. Samples were weighed (1.5–2.0 g), diluted in 35–55 mL of 0.4 % oxalic acid ($C_2H_2O_4$), and centrifuged at 1,500 rpm for 1 min in the dark. 2,6-Dichlorophenol-indophenol ($C_{12}H_7Cl_2NO_2$), 0.4 % oxalic acid, and a 0.1 % AsA standard solution were used to construct an analytical curve ($y = 0.1011x - 0.1403$, $R^2 = 0.9355$). Decolorization of solutions in test tubes was achieved using 0.005–0.007 g of AsA P.A. Absorbance was measured at 520 nm in the dark on a UV–Vis digital spectrophotometer with a working range of 190–1100 nm (IL-593-S-BI, Kazuaki®, Paraná, Brazil). Results were expressed in mg AsA (mg AsA 100 g^{-1}).

Total phenolic content (TPC) was determined by the Folin–Ciocalteu method [32]. Samples were weighed (0.5 g) and diluted in 10–20 mL of distilled water in the dark. The analytical curve was constructed using 20 % sodium carbonate (Na_2CO_3) and a standard solution of gallic acid ($C_7H_6O_5$) ($y = 0.0542x + 0.0228$, $R^2 = 0.9956$). Absorbance readings were taken at 765 nm in the dark on a UV–Vis spectrophotometer (IL-593-S-BI, Kazuaki®, Paraná, Brazil). Results are expressed in milligrams of gallic acid equivalents (mg GAE 100 g^{-1}).

Anthocyanins were identified and quantified in a 1200 Series High-Performance Liquid Chromatograph (HPLC, Agilent®, California, USA) coupled to a Diode Array Detector (DAD). Samples (0.5–1.0 mL) were diluted in 10 mL of distilled water with 5 % formic acid (CH_2O_2) and filtered through a 0.45 μm membrane. Separation was achieved on a C18 Zorbax column (5 μm, 250 mm × 4.6 mm) at 29 °C, using a mobile phase gradient of (A) ultrapure water with 5 % formic acid and (B) methanol (CH_3OH) with 5 % formic acid, from 90:10 (A:B) to 60:40 (A:B) in 20 min, followed by 20:80 (A:B) in 15 min, and maintaining this ratio for another 5 min [33]. Quantification was performed using 0.0015 and 0.021 mg mL^{-1} of cyanidin 3-*O*-glucoside analytical curve (RANGE, LOD = 0.00018 mg mL^{-1}, and LOQ = 0.0006 mg mL^{-1}; $y = 368716.91x - 153.28$, $R^2 = 0.9994$). The results were expressed in milligrams of cyanidin 3-*O*-glucoside (mg Cy3G 100 mL^{-1}).

2.3.5. Antioxidant capacity

Antioxidant capacity was estimated by the 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonate) ($ABTS^{\bullet+}$, $C_{18}H_{18}N_4O_6S_4$) assay, with modifications [34,35]. Samples (0.5 g) were diluted in 15–30 mL of 50 % methanol and centrifuged at 3,000 rpm for 5 min in the dark. 6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox, $C_{14}H_{18}O_4$) was used as standard. Absorbance readings were performed at 734 nm in a UV–Vis spectrophotometer (IL-593-S-BI, Kazuaki®, Paraná, Brazil). Inhibition percentages were calculated using Eq. (3), and a calibration curve constructed by linear regression ($y = -0.0002x + 0.5349$, $R^2 = 0.9937$) was used to express the results in mM TE g^{-1}.

$$\text{Inhibition } \% = \left[\frac{\left(\text{Absorbance}_{\text{ABTS}} - \text{Absorbance}_{\frac{\text{Sample}}{\text{Trolox}}}\right)}{\text{Absorbance}_{\text{ABTS}}}\right] \times 100 \quad (3)$$

2.3.6. Color parameters

Color analyses were carried out at room temperature (25 ± 2 °C) before (t_0) and after 60 days of storage (t_{60}). Measurements were made directly on a portable colorimeter (CM-600d, Konica Minolta Sensing Americas®, Inc., Japan). Results were expressed in Commission Internationale de l'Éclairage (CIE) color space coordinates, with D_{65} illuminant and an observation angle of 10°. According to the manufacturer's instructions, calibration was carried out using standard white and black reference plates. Readings were performed using a set of Petri dishes (35 × 10 mm).

In the CIE $L^*a^*b^*$ system, which expresses colors in rectangular coordinates, the parameters evaluated were L^*, lightness, ranging from white ($L^* = 100$) to black ($L^* = 0$); a^*, a range encompassing redness ($+a^*$) to greenness ($-a^*$); and b^*, a range encompassing yellowness ($+b^*$) to blueness ($-b^*$). In the CIE L^*C^*h color space, which represents color intensity in cylindrical coordinates, the parameters evaluated were chroma (C^*), ranging from 0 (impure color) to 60 (pure color), and hue angle (h°), which corresponds to a color appearance range where 0 and 360° = red, 90° = yellow, 180° = green, and 270° = blue, calculated using Eqs. (4) and (5) [36].

$$C^* = \left[(a^*)^2 + (b^*)^2 \right]^{\frac{1}{2}} \tag{4}$$

$$h^\circ = \text{arctg}\left(\frac{b^*}{a^*}\right) \tag{5}$$

From these primary data, we calculated color change parameters (dL^*, da^*, db^*, and dC^*) between juice formulations and MP (untreated sample), hue difference (ΔH^*), total color difference (ΔE^*), browning index (BI), and color index (CI), according to Eqs. (6)–(10) [37–39].

$$\Delta H^* = \left[(\text{d}a^*)^2 + (\text{d}b^*)^2 - (\text{d}C^*)^2 \right]^{\frac{1}{2}} \tag{6}$$

$$\Delta E^* = \left[(\text{d}L^*)^2 + (da^*)^2 + (\text{d}b^*)^2 \right]^{\frac{1}{2}} \tag{7}$$

$$X = \frac{a^* + 1.75L^*}{5.64L^* + a^* - 3.01b^*} \tag{8}$$

$$\text{BI} = \frac{100 \times (X - 0.31)}{0.172} \tag{9}$$

$$\text{CI} = \frac{2000 \times a^*}{L^* \times \left[(a^*)^2 + (b^*)^2 \right]^{\frac{1}{2}}} \tag{10}$$

2.4. Statistical analysis

Microbiological parameters with non-constant behavior were analyzed by exponential regression using Microsoft Excel 2013. Graphs show the equation of the trend line and the coefficient of determination (R^2). The Shapiro–Wilk test was applied to assess the normality of the other data. Assistat software version 7.7 beta [40] was used for the two-way analysis of variance (ANOVA).

A completely randomized experimental design was used in a 5 × 3 factorial arrangement (physicochemical and bioactive parameters and antioxidant capacity) and a 2 × 3 factorial arrangement (color parameters). Both arrangements were performed with at least three replications. The studied factors were storage time (0, 15, 30, 45, and 60 days) and tropical juice treatment (JA, JB, and JC). In the case of non-significant interaction effects, data were unfolded, and statistical analysis was carried out considering the main effects. The results of bioactive parameters and antioxidant capacity were unfolded to investigate the significance of effects at each storage time. Parameters with negative values were transformed by $X = x/K$, with $K = -1$. When ANOVA revealed a significant effect, means were compared by Tukey's test at $p < 0.05$ and $p < 0.01$.

Simple correlations between variables (bioactive parameters, antioxidant capacity, and color parameters) were determined by estimating Pearson's correlation coefficient, as described in Eq. (11). Data at the initial storage time were selected for estimation. We considered the final means of each formulation and the simple arithmetic mean (without weights between terms), totaling four replications of each variable. Means were compared by Student's t-test at $p < 0.05$ and $p < 0.01$.

$$r = \frac{\sum_i (x_i - \bar{x})(y_i - \bar{y})}{\sqrt{\sum_i (x_i - \bar{x})^2 \sum_i (y_i - \bar{y})^2}} \tag{11}$$

where i is the number of replications ($i = 1, \ldots, n$), x_i represents the values of the dataset ($x_1, x_2, \ldots, x_n$), $\bar{x}$ is the mean of the dataset, y_i represents the values of another corresponding dataset ($y_1, y_2, \ldots, y_n$), and $\bar{y}$ is the mean of the other corresponding dataset.

3. Results and discussion

MP yield (27.60 ± 3.76 %) and TI (7.87 ± 1.07 %) were low. Differences between the batches may be attributed to variations in water availability in agroforestry systems, which affects the juiciness of fruits. The potential of myrtle (*E. gracillima* Kiaersk.) for pulp and whole juice production may be limited by low yields, high labor costs, and high energy consumption. A large amount of agro-industrial waste (e.g., peel and whole seeds) was generated from pressing [41]. Fruit sanitization and additional crushing or freezing operations are not recommended, as they may negatively alter sensory characteristics and promote tissue disruption, degradation by direct contact with enzymes, microorganisms, and oxygen, and leaching of bioactive constituents into the percolating fluid.

3.1. Microbiological parameters

MP and juice formulations had low scores of *Enterobacteriaceae* (<1.0 log CFU mL^{-1}), total coliforms at 35 °C (<0.50 log NMP mL^{-1}), thermotolerant coliforms at 45 °C (<0.50 log NMP mL^{-1}), *E. coli* (<1.0 log CFU mL^{-1}), and coagulase-positive *S. aureus* (<1.0

log CFU mL^{-1}) after processing and 60 days of room storage (25 ± 2 °C). *Salmonella* spp. was not detected in any sample (per 25 mL) in compliance with Resolution No. 60/2019 [25]. These results demonstrate that the unit processing operations were performed adequately [22] and Good Manufacturing Practices concerning hygiene and sanitary conditions. Juice formulations and MP had lactic acid bacteria counts lower than 1.0 and 2.85 log CFU mL^{-1}, respectively. This group of microorganisms is tolerant to acidic medium but somewhat resistant to heat treatment [42].

Exponential models showed a good fit to the data ($R^2 > 0.9$), with good representativeness for TBC and TYMC. The results of TBC and TYMC are depicted in Fig. 1A and B.

All samples had a TBC lower than 1.0 log CFU mL^{-1} at initial storage time (Fig. 1A). The efficiency of the physical preservation method (JB) was evident after 60 days of storage. The period of microbial adaptation (lag phase) was higher in JA, and the presence of the preservatives delayed multiplication. The optimal temperature range for aerobic mesophilic bacteria is 25–40 °C, including most foodborne pathogens [43]. On day 15 of storage, no differences were observed between JA and JB. According to the model, TBC increased with storage time for JC and JA, reaching 4.5–5.5 log CFU mL^{-1} at 60 days of storage (Fig. 1A), and characterizing metabolic processes of fermentation. Outbreaks of foodborne illnesses due to juice consumption may result from the lack of heat treatment to inhibit pathogens such as *E. coli*, *Salmonella* spp., *S. aureus*, and *Shigella* spp [17]. Mena et al. [18] observed a substantial reduction in TBC in pomegranate juice after heat treatment (65–95 °C, 30–60 s).

MP had an expressive diversity of yeasts and molds (3.55 log CFU mL^{-1}), with colonies of different sizes, shapes, colors, and mycelial characteristics. The TYMC of MP was lower than the upper regulatory limit [25]. Both preservation treatments were effective in reducing TYMC by 3 log units (Fig. 1B). In the study of Xu, Lin, Wang, and Liao [19], pasteurization (110 °C, 8.6 s) of orange juice with pepper led to a 4-log reduction in TYMC. The physical preservation method ensured the stability of JB during 60 days of storage. According to the model, JA exceeded the maximum TYMC limit allowed by legislation [25] after about 28 days of storage. Chemical

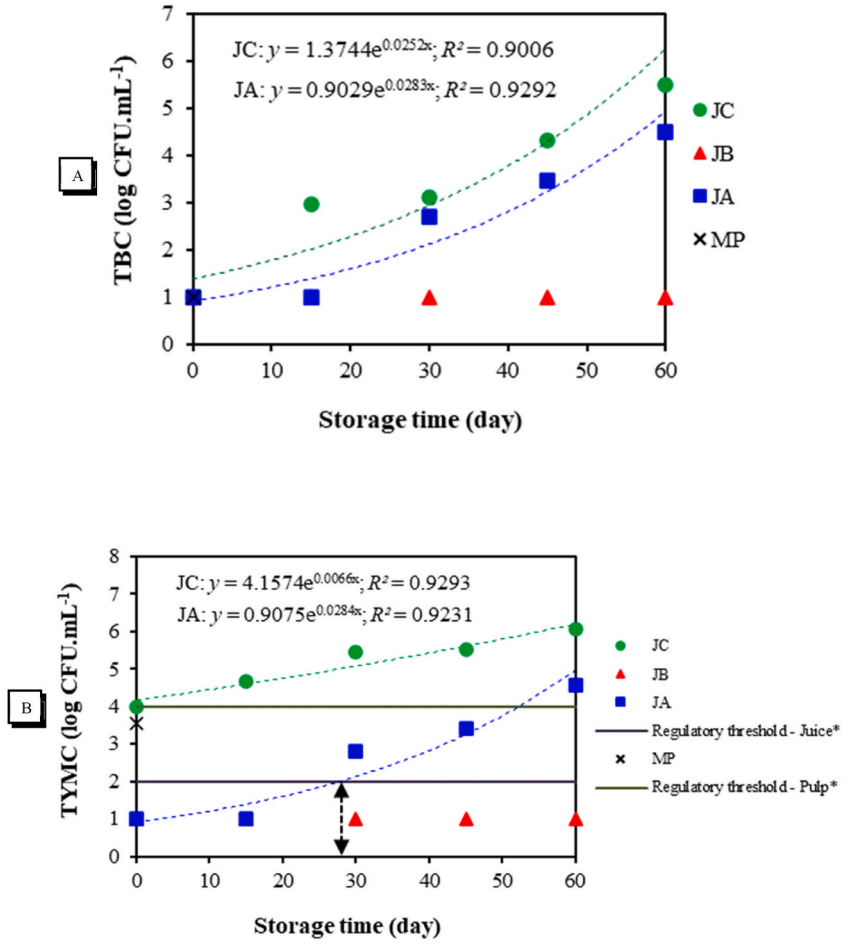


Fig. 1. TBC (A) and TYMC (B) in MP and tropical juice formulations during 60 days of storage at room temperature. TBC, total aerobic mesophilic bacterial count; TYMC, total yeast and mold count; CFU, colony-forming units; MP, myrtle pulp; JA, myrtle tropical juice subjected to a chemical preservation method; JB, myrtle tropical juice subjected to a physical preservation method; JC, myrtle tropical juice not subjected to preservation processes (control). *The continuous green line represents the maximum limit for juice (4.0 log CFU mL^{-1}) and the continuous purple line represents the maximum limit for pulp (2.0 log CFU mL^{-1}) [25].

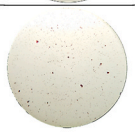
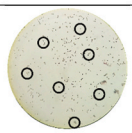
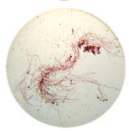
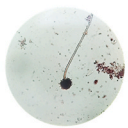
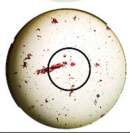
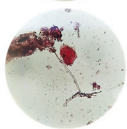
preservatives would probably be more efficient at refrigeration temperatures or in samples with a lower initial microbial count since the preservative concentration used here corresponds to the maximum allowed by legislation [16]. High TYMC values and increasing yeast and mold multiplication were observed in JC, reaching 6.05 log CFU mL^{-1} at 60 days of storage (Fig. 1B). However, multiplication rates were lower than those observed for TBC (Fig. 1A). Films, swollen bottles, and unpleasant fermentation odors were observed, as yeast and mold were favored by temperature, pH, a_w, oxygen, and nutrient availability.

3.2. Optical microscopy

Table 1 and Fig. 2 show the results of the optical microscopy.

Microphotographs revealed a high concentration of pigments in MP, with several fragments resulting from fruit processing. Adding drinking water (50 %) to MP allowed a higher passage of light through the slide–cover slip system. All juice samples had lower concentrations of pigmented fragments before storage (Table 1). JC had numerous agglomerates after 60 days of storage, which were likely budding yeast-like fungi (Fig. 2A), in agreement with Fig. 1B. The most common yeasts found in juices are *Saccharomyces*

Table 1
Optical microscope images at 100 × and 400 × magnification of MP and tropical juice formulations during 60 days of storage at room temperature.

Time (day)	Sample	Matrix	Total magnification	
			100×	400×
0	MP			
	JC			
	JB			
	JA			
60	JC			
	JB			
	JA			
				

MP, myrtle pulp; JA, myrtle tropical juice subjected to a chemical preservation method; JB, myrtle tropical juice subjected to a physical preservation method; JC, myrtle tropical juice not subjected to preservation processes (control).

cerevisiae and *Zygosaccharomyces bailii* [17]. Although physical preservation afforded good results, filamentous fungi were detected in JB (Fig. 2B). The heat resistance of these microorganisms, the presence of vegetative cells, or spore germination can explain this result.

Other molds with well-defined traits were observed in JA, showing fruiting bodies characteristic of *Aspergillus* spp., possibly *Aspergillus niger* (Fig. 2C), and *Penicillium* spp. (Fig. 2D), such as *Penicillium spinulosum*. These genera are known as storage molds, responsible for producing mycotoxins, and commonly found in tropical climates and fruit products [43,44]. *Aspergillus* sp. has a non-septate conidiophore, dilated at the top in the form of a vesicle, in which cells that originate spores are formed. *Penicillium* sp. has verticillate conidiophores with phialide formation. Molds of the genera *Fusarium* and *Penicillium* were not controlled by osmotic or thermal treatment in seeds of the family *Myrtaceae* [45].

3.3. Physicochemical parameters

Table 2 shows the results of the physicochemical analyses.

Significant interaction effects of storage time and treatment were observed on all parameters ($p < 0.01$) except for total ash and a_w. A non-significant ($p \geq 0.05$) effect of treatment was only observed for total ash content. The preservation technique significantly influenced TS, TSS, TTA, pH, and aw. Lactic acid bacteria count and TYMC were likely favored by total ash (0.39 %), acidic pH (3.26), high a_w (0.974), and high levels of TS (20.95 %) and TSS (28.50 °Bx) in MP, influencing the characteristics of juice formulations.

At the initial storage time, TS, TSS, pH, and TSS/TTA ratio were significantly higher ($p < 0.05$) in JB than in JC. During physical preservation, the sample underwent boiling and water evaporation, resulting in the concentration of the solid matrix, breakdown of substances, and disaccharide hydrolysis. The higher TSS, pH, and TSS/TTA ratio in JA may be attributed to the combined use and solubilization of preservatives potassium sorbate and sodium benzoate, and microbial multiplication (Figs. 1 and 2), which may influence TSS content.

The physicochemical parameters of JC varied during the 60 days of storage ($p < 0.05$), indicating decomposition by oxidation and fermentation. Other factors likely responsible for the change in physicochemical parameters of JC were enzymatic reactions; metabolic activity of microorganisms, causing changes in density by degradation of carbohydrates and other chemical components (Figs. 2 and 3); and permeability of plastic packaging, allowing passage of substances and fluids through the pores of the polymer matrix.

The effects of packaging differ according to the permeability, morphology, crosslinking degree, and molar mass of polymers [46,47]. Similar physicochemical behavior was observed in JA but at significantly smaller proportions ($p < 0.05$). JB was the most physicochemical stable sample, although TS, TSS, TTA, pH, and TSS/TTA ratio were probably affected by chemical and enzymatic reactions, microbial multiplication, and constant gas exchange [48].

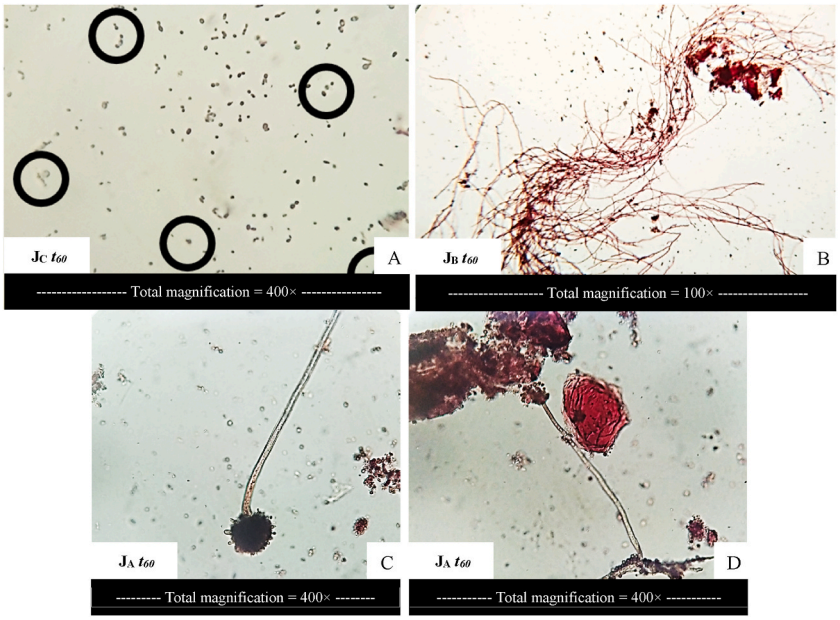


Fig. 2. Representative microphotographs of yeast (A) and molds (B–D) in myrtle tropical juice formulations at 100 × and 400 × total magnification after 60 days of storage at room temperature. JA, myrtle tropical juice subjected to a chemical preservation method; JB, myrtle tropical juice subjected to a physical preservation method; JC, myrtle tropical juice not subjected to preservation processes (control).

Table 2
Physicochemical characteristics of MP and tropical juice formulations during 60 days of storage at room temperature.

Parameter	MP	Time (day)	Formulation			RSD (%)	*p*-value[1]		
			JC	JB	JA		S	T	S × T
Total ash (%)	0.39 ± 0.05	0	0.37	0.32	0.34	8.52	ns	ns	ns
		15	0.39	0.40	0.39				
		30	0.33	0.39	0.34				
		45	0.34	0.36	0.37				
		60	0.37	0.35	0.38				
TS (%)	20.95 ± 0.96	0	9.22^{aY}	9.53^{aX}	9.20^{aY}	1.34	**	**	**
		15	2.48^{bZ}	8.90^{bX}	8.20^{bY}				
		30	1.92^{cZ}	8.47^{cX}	7.86^{cY}				
		45	1.46^{dZ}	8.11^{dX}	6.85^{dY}				
		60	0.30^{eZ}	7.37^{eX}	5.40^{eY}				
TSS (°Bx)	28.50 ± 0.44	0	12.70^{aZ}	13.20^{aY}	13.50^{aX}	1.26	**	**	**
		15	6.37^{bZ}	12.10^{bY}	13.03^{cX}				
		30	5.10^{cZ}	12.07^{cY}	12.40^{cX}				
		45	4.00^{dZ}	11.10^{cY}	11.77^{dX}				
		60	3.63^{eZ}	10.87^{cAX}	9.87^{eY}				
TTA (%)	1.35 ± 0.01	0	0.45^{eX}	0.44^{eY}	0.44^{eY}	1.25	**	**	**
		15	0.54^{dY}	0.47^{dZ}	0.61^{dX}				
		30	0.70^{cY}	0.62^{cZ}	0.79^{cX}				
		45	0.85^{bX}	0.79^{bZ}	0.82^{bY}				
		60	1.14^{aX}	0.85^{aZ}	0.89^{aY}				
pH	3.26 ± 0.10	0	3.68^{aY}	3.73^{aXY}	3.80^{aX}	1.26	**	**	**
		15	3.31^{bZ}	3.75^{aX}	3.51^{bY}				
		30	3.29^{bZ}	3.72^{aX}	3.40^{cY}				
		45	3.23^{bY}	3.34^{bX}	3.27^{dXY}				
		60	2.73^{cY}	3.19^{cX}	2.81^{eY}				
TSS/TTA ratio	21.04 ± 0.48	0	27.95^{aZ}	30.34^{aY}	31.02^{aX}	1.45	**	**	**
		15	11.70^{bZ}	26.02^{bX}	21.51^{bY}				
		30	7.29^{cZ}	19.53^{cX}	15.71^{cY}				
		45	4.72^{dY}	13.99^{dX}	14.36^{dX}				
		60	3.19^{eZ}	12.80^{eX}	11.04^{eY}				
a_w	0.974 ± 0.006	0	0.990^{X}	0.990^{X}	0.993^{X}	0.24	ns	**	ns
		15	0.993^{X}	0.988^{Y}	0.994^{X}				
		30	0.994^{X}	0.988^{Y}	0.992^{XY}				
		45	0.995^{X}	0.990^{Y}	0.991^{XY}				
		60	0.995^{X}	0.991^{X}	0.992^{X}				

[1]Statistical significance of ANOVA: **($p < 0.01$); [ns](not significative). S – storage time, T – juice treatment; S × T – interaction between factors. MP, myrtle pulp; JA, myrtle tropical juice subjected to a chemical preservation method; JB, myrtle tropical juice subjected to a physical preservation method; JC, myrtle tropical juice not subjected to preservation processes (control); RSD, relative standard deviation; TS, total sugars; TSS, total soluble solids; TTA, total titratable acidity; a_w, water activity. [a–e]: different superscript lowercase letters in the same column for same juice at different storage time denote difference ($p < 0.05$; Tukey's test); [A–B]: different capital letters in the same line for different juices at same storage time denote difference ($p < 0.05$; Tukey's test).

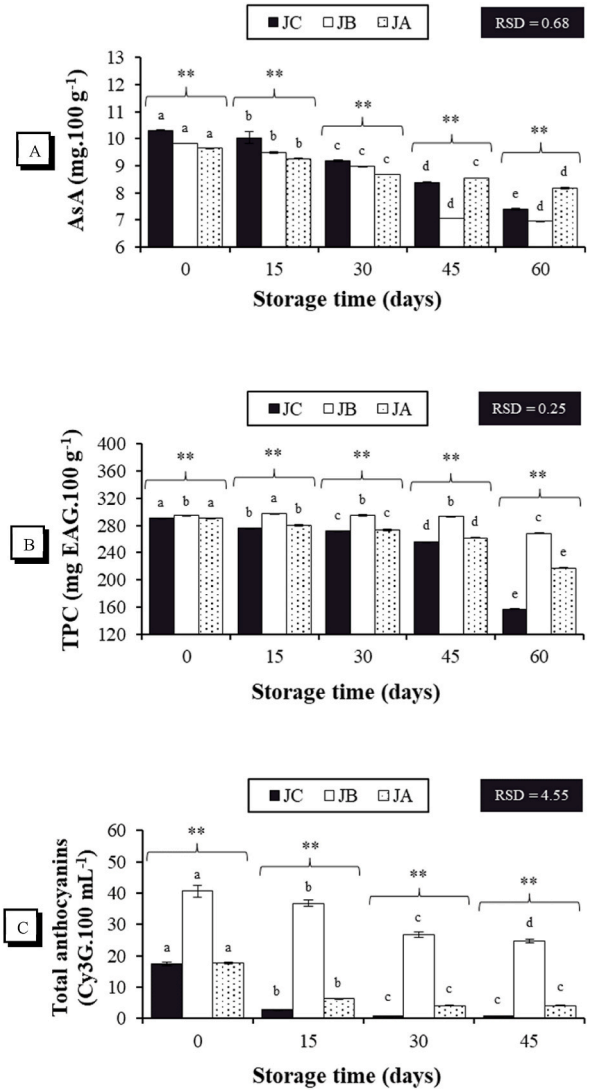


Fig. 3. Bioactive parameters (A, AsA; B, TPC; C, total anthocyanins) of myrtle tropical juice formulations during 60 days of storage at room temperature. AsA, ascorbic acid; TPC, total phenolic content; JA, myrtle tropical juice subjected to a chemical preservation method; JB, myrtle tropical juice subjected to a physical preservation method; JC, myrtle tropical juice not subjected to preservation processes (control); RSD, relative standard deviation. Different letters indicate significant differences between storage times ($p < 0.05$). Asterisks indicate significant differences between treatments, $^{**}p < 0.01$ (ANOVA).

3.4. Bioactive parameters

Fig. 3A, B, and 3C depict the bioactive parameters of myrtle juice formulations. Juice samples were a relevant source of bioactive compounds, such as phenolics. However, AsA, TPC, and total anthocyanins were 33 %, 65 %, and 60 % lower in juice samples than in MP, which had an AsA content of 15.24 mg 100 g^{-1}, TPC of 855.86 mg GAE 100 g^{-1} and total anthocyanins of 100.91 mg Cy3G 100 mL^{-1}. These findings agree with the characteristics and changes in Tables 1 and 2. Treatment of juice formulations significantly influenced ($p < 0.05$) bioactive parameters at all storage times.

At the initial storage time, treated juice formulations had lower AsA levels than the control (Fig. 3A), probably because the former samples were exposed to risk factors such as oxygen and light during processing. In the case of JB, these factors might have been further potentiated by room temperature (25 ± 2 °C) and heat treatment (90 °C for 60 s). Silva et al. [20] observed a decrease in AsA content (29.2–26.8 mg 100 g^{-1}) over 180 days of storage in sweetened guava juice with and without preservatives, subjected to heat treatment (90 °C for 60 s) and hot filling.

There was a significant reduction ($p < 0.05$) in AsA content in all juice formulations up to 30 days of storage, reaching 8.67–9.18 mg 100 g^{-1} (Fig. 3A). Such a reduction might be associated with the permeability of plastic packaging, oxidative processes, or the sensitivity of AsA to degradation and volatilization. The highest rate of AsA degradation or activity loss was detected in JB, even

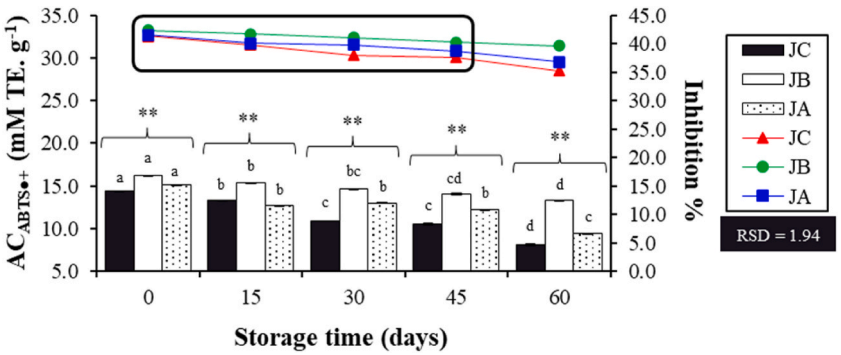


Fig. 4. Antioxidant capacity of myrtle tropical juice formulations during 60 days of storage at room temperature. AC, antioxidant capacity; TE, Trolox equivalents; JA, myrtle tropical juice subjected to a chemical preservation method; JB, myrtle tropical juice subjected to a physical preservation method; JC, myrtle tropical juice not subjected to preservation processes (control); RSD, relative standard deviation. Columns refer to the primary axis and rows to the secondary axis. Different letters indicate significant differences between storage times ($p < 0.05$). Asterisks indicate significant differences between treatments, $^{**}p < 0.01$ (ANOVA).

though the sample had the highest TPC stability and the highest rate of preservation of total anthocyanins throughout storage. JC and JA were unstable concerning TPC and total anthocyanins, not surpassing 220 mg GAE 100 g^{-1} and 5 mg Cy3G 100 mL^{-1} after 60 days, respectively, as neither sample underwent heat treatment (Fig. 3B and C).

The major anthocyanin found in the myrtle pulp and juices was cyanidin 3-*O*-glucoside, corresponding between 85 % and 95 % of total anthocyanins, followed by minor amounts of delphinidin 3-*O*-glucoside (Supplementary Table 1 and Supplementary Fig. 1). Similar anthocyanin profiles were observed in fruits from the *Myrtaceae* family, such as purple pitanga [49] and jabuticaba [50], in which only non-acylated anthocyanins are found. It is also not uncommon in this family to find only mono-glycosylated anthocyanins [51]. The behavior during storage showed higher ($p < 0.05$) anthocyanin content in JB (Fig. 3C and Supplementary Fig. 2).

3.5. *In vitro antioxidant capacity by ABTS$^{•+}$ method*

The results of antioxidant assays are presented in Fig. 4.

The high TPC of myrtle juice formulations (Fig. 3B) may be correlated with the *in vitro* biological activity of the samples, as evidenced by their high antioxidant capacity. This biological potential might have contributed to the delay in oxidative degradation throughout room storage. MP showed an antioxidant capacity of about 60 % of inhibition. There was a significant influence ($p < 0.01$) of tropical juice treatment on antioxidant capacity at all storage times. Antioxidant activity by the ABTS$^{•+}$ method was similar to TPC

Table 3
Color parameters of MP and tropical juice formulations during 60 days of storage at room temperature.

Parameter	MP	Time (day)	Formulation			RSD (%)	*p*-value[1]		
			JC	JB	JA		S	T	S × T
d*L**	–	0	-9.07^{aX}	-9.37^{aY}	-9.16^{aXY}	1.25	**	*	**
		60	-10.14^{bZ}	-9.43^{aX}	-9.71^{bY}				
d*a**	–	0	3.14^{bY}	2.76^{bZ}	3.47^{bX}	1.93	**	**	*
		60	4.07^{aY}	3.89^{aZ}	4.30^{aX}				
d*b**	–	0	1.91^{Y}	2.02^{Y}	2.38^{X}	3.94	ns	**	ns
		60	1.94^{Y}	2.13^{Y}	2.42^{X}				
d*C**	–	0	3.67^{bY}	3.42^{bZ}	4.21^{bX}	1.50	**	**	**
		60	4.50^{aY}	4.43^{aY}	4.93^{aX}				
Δ*H**	–	0	0.11^{bX}	0.03^{bY}	0.06^{bXY}	17.20	**	**	ns
		60	0.26^{aX}	0.18^{aY}	0.18^{aY}				
Δ*E**	–	0	9.79^{bY}	$9.97b^{XY}$	10.08^{bX}	1.11	**	**	**
		60	11.10^{aX}	10.42^{aY}	10.89^{aX}				
BI	4.05 ± 0.48	0	95.56^{bY}	100.73^{bY}	116.24^{bX}	3.85	**	**	**
		60	135.15^{aX}	118.97^{aY}	142.75^{aX}				
CI	111.74 ± 0.98	0	313.09^{bX}	314.70^{bX}	308.23^{bX}	2.17	**	**	**
		60	410.55^{aX}	343.62^{aZ}	361.50^{aY}				

[1]Statistical significance of ANOVA: $^{*}p < 0.05$, $^{**}p < 0.01$; ^{ns}, not significant. S – storage time, T – juice treatment; S × T – interaction between factors. MP, myrtle pulp; JA, myrtle tropical juice subjected to a chemical preservation method; JB, myrtle tropical juice subjected to a physical preservation method; JC, myrtle tropical juice not subjected to preservation processes (control); RSD, relative standard deviation; d*L**, d*a**, d*b**, and d*C**, change in color; Δ*H**, hue difference; Δ*E**, total color difference; BI, browning index; CI, color index. $^{a–e}$: different superscript lowercase letters in the same column for same juice at different storage time denote difference ($p < 0.05$; Tukey's test); $^{A–B}$: different capital letters in the same line for different juices at same storage time denote difference ($p < 0.05$; Tukey's test).

results. Almeida et al. [52] demonstrated that the $ABTS^{\bullet+}$ assay is more directly associated with TPC than with AsA content when investigating 11 tropical fruits from northeastern Brazilian.

We grouped storage times at which similar antioxidant capacity were observed. $ABTS^{\bullet+}$ values were similar from days 0–45 of storage (37.64–42.33 % inhibition). JB was more stable (13.31–16.25 mM TE g^{-1}), with significantly higher values ($p < 0.05$) than other formulations. After 60 days of storage, JC and JA had a significantly lower ($p < 0.05$) antioxidant capacity, reaching 8.14–9.41 mM TE g^{-1}. These findings can be explained by the significant reduction ($p < 0.01$) in AsA, TPC, and total anthocyanins (Fig. 3A, B, and 3C, respectively). Similar results were reported by Silva et al. [20] in evaluating guava juice added with potassium sorbate (8.60 mM TE g^{-1}); a significant reduction in antioxidant capacity was observed over 180 days of storage.

3.6. Color parameters

The results of color measurements are described in Table 3.

Interaction effects were non-significant ($p \geq 0.05$) for db^* and ΔH^*. Juice treatment showed a significant effect ($p < 0.05$) on all parameters. There was no significant influence ($p \geq 0.05$) of storage time except on db^*. At the initial storage time, juice samples were darker (d$L^* < 0$), redder (d$a^* > 0$), yellower (d$b^* > 0$), and more saturated and neutral (d$C^* > 0$) than MP. BI and CI values confirmed these findings, given that the indices were at least 23.5 and 2.75 times higher in juice samples than in MP, respectively.

Treated juice samples had smaller da^* and dC^* color changes ($p < 0.05$) than Feitosa, Lima, Figueirêdo, Queiroz, and Amadeu [53] found in a formulation of MP, distilled water, and maltodextrin (1:1 + 30 % maltodextrin) (MOR-REX®). The latter had a darker color and higher BI than MP alone. Similarly, Osorio, Forero, and Carriazo [54] reported lower L^* values for ripe guava puree than aqueous guava extract.

The ΔE^* of juice samples was classified as very distinct ($\Delta E^* > 3$), according to Adekunte, Tiwari, Cullen, Scannell, and O'Donnell [55]. However, this parameter can be misleading as to the actual color of the material if evaluated individually. Visual determination of acceptable color combined with other quality parameters is recommended [56]. Color change and difference parameters were significantly higher ($p < 0.05$) after 60 days of storage, except db^*.

During storage, there were changes in microbiological, physicochemical, and bioactive parameters, which might have affected the color parameters. Enzymatic browning, resulting from increased enzyme activity, contact with substrates and induced by application of heat in JB (90 °C for 60 s); multiplication of microorganisms that synthesize pigments (Figs. 2 and 3); and chemical oxidation of AsA with dark pigment formation (Fig. 3A), possibly occurred and influenced BI and CI values of myrtle juice formulations.

3.7. Pearson's correlation

Pearson's correlation coefficients are presented in Table 4. Only very positive and very negative correlations were considered ($r > 0.9$ or $r < -0.9$).

As expected, L^*, a^*, b^*, and C^* showed a significant positive correlation ($p < 0.01$) with color change parameters (dL^*, da^*, db^*, and dC^*), with $r \geq 0.999$. Significant positive correlations ($p < 0.01$) were observed between BI and the values of b^* and db^* ($r = 0.9999$), demonstrating the influence of browning on the spectrum of yellowness (closer to neutrality and less blue hues). CI was negatively correlated ($p < 0.01$) with a^*, C^*, and dC^* ($r < -0.95$). A significant negative correlation ($p < 0.05$) of L* and dL^* with TPC and $ABTS^{\bullet+}$ values indicated that darker-colored juice formulations exhibit higher bioactive compound content and activity.

A positive correlation between $ABTS^{\bullet+}$ scavenging activity and TPC was expected because both methods are based on the same reaction mechanism, electron donation; therefore, high TPC content implies high antioxidant capacity. Souza, Vieira, and Putti [57] and Rigolon, Barros, Vieira, and Stringheta [58] also observed this positive correlation in assessing grape peels and phenolic extracts of blackberry, blueberry, and jabuticaba, respectively. It is important to note that AsA content showed a non-significant correlation ($p \geq 0.05$) with TPC and antioxidant capacity, corroborating the results of the current study. According to Guo et al. [59] and Souza et al. [57], the contribution of AsA to the antioxidant capacity of fruits is variable, not always showing a significant positive correlation.

4. Conclusions

The data obtained for MP and juice formulations may contribute to the future definition of standards of identity and quality by competent bodies. Myrtle tropical juices can be tested as a potential substrate for isolating or cultivating yeast or probiotic bacteria. Under the conditions of the present experiment, the combined use of preservatives potassium sorbate and sodium benzoate was effective against yeast and mold for up to 28 days of room storage (25 ± 2 °C). Chemical preservation was less efficient in delaying and controlling crobial multiplicationthan the physical method. The physical preservation method did not result in a completely safe product for consumption, given the presence of filamentous fungi, changes in physicochemical quality parameters, and AsA oxidation. It was not possible to determine tolerance and acceptance limits from instrumental color data. Exploring aseptic processing technologies, combined use of physical and chemical methods, cold preservation, or rigid glass packaging is recommended, contributing to product visibility, reduced permeability and porosity during storage.

Funding

This study was partially supported by the Federal University of Campina Grande (UFCG, Brazil), the National Institute for the Semi-Arid (INSA, Brazil), the Paraíba Technological Park Foundation (PaqTcPB, Brazil); the Federal Institute of Education, Science, and

Table 4
Pearson's correlation between bioactive parameters, antioxidant capacity, and color parameters of myrtle tropical juice formulations before storage.

	*L**	+*a**	+*b**	*C**	*h*°	d*L**	d*a**	d*b**	d*C**	Δ*H**	BI	Δ*E**	CI	AsA	TPC	$AC_{ABTS\bullet+}$
L*	1.0000	0.7058^{ns}	0.0013^{ns}	0.5276^{ns}	−0.9202^{ns}	0.9999**	0.7112^{ns}	0.0013^{ns}	0.5156^{ns}	0.9322^{ns}	−0.0156^{ns}	−0.4215^{ns}	−0.4514^{ns}	0.5114^{ns}	−0.9646*	−0.9980**
a*		1.0000	0.7094^{ns}	0.9742*	−0.3721^{ns}	0.7058^{ns}	0.9999**	0.7094^{ns}	0.9709*	0.4016^{ns}	0.6973^{ns}	0.3449^{ns}	−0.9507*	−0.2478^{ns}	−0.8677^{ns}	−0.6591^{ns}
b*			1.0000	0.8502^{ns}	0.3903^{ns}	0.0013^{ns}	0.7039^{ns}	1.0000**	0.8575^{ns}	−0.3607^{ns}	0.9999**	0.9063^{ns}	−0.8929^{ns}	−0.8587^{ns}	−0.2652^{ns}	0.0626^{ns}
C*				1.0000	−0.1529^{ns}	0.5276^{ns}	0.9724*	0.8502^{ns}	0.9999**	0.1844^{ns}	0.8412^{ns}	0.5480^{ns}	−0.9962**	−0.4602^{ns}	−0.7330^{ns}	−0.4722^{ns}
***h**°*					1.0000	−0.920 ^{ns}	−0.3792^{ns}	0.3903^{ns}	−0.1390^{ns}	−0.9995**	0.4058^{ns}	0.7429^{ns}	0.0660^{ns}	−0.8070^{ns}	0.7843^{ns}	0.9433^{ns}
d*L**						1.0000	0.7112^{ns}	0.0013^{ns}	0.5156^{ns}	0.9322^{ns}	−0.0155^{ns}	−0.4215^{ns}	−0.4514^{ns}	0.5114^{ns}	−0.9646*	−0.9980**
d*a**							1.0000	0.7039^{ns}	0.9690*	0.4086^{ns}	0.6918^{ns}	0.3377^{ns}	−0.9483^{ns}	−0.2404^{ns}	−0.8715^{ns}	−0.6648^{ns}
d*b**								1.0000	0.8575^{ns}	−0.3607^{ns}	0.9999**	0.9063^{ns}	−0.8929^{ns}	−0.8587^{ns}	−0.2652^{ns}	0.0626^{ns}
d*C**									1.0000	0.1706^{ns}	0.8487^{ns}	0.5597^{ns}	−0.9973**	−0.4726^{ns}	−0.7234^{ns}	−0.4598^{ns}
Δ*H**										1.0000	−0.3764^{ns}	−0.7211^{ns}	−0.0978^{ns}	0.7878^{ns}	−0.8037^{ns}	−0.9534*
BI											1.0000	0.9133^{ns}	−0.8852^{ns}	−0.8672^{ns}	−0.2488^{ns}	0.0794^{ns}
Δ*E**												1.0000	−0.6189^{ns}	−0.9948**	0.1673^{ns}	0.4786^{ns}
CI													1.0000	0.5360^{ns}	0.6708^{ns}	0.3934^{ns}
AsA														1.0000	−0.2665^{ns}	−0.5653^{ns}
TPC															1.0000	0.9457^{ns}
$AC_{ABTS\bullet+}$																1.0000

± 0.90 ▲ ▲ ± 1.00
Significant | *r* | value

Statistical significance of ANOVA: $^{*}p < 0.05$, $^{**}p < 0.01$; ^{ns}, not significant. *L**, lightness; +*a**, redness; +*b**, yellowness; *C**, chroma; *h*°, hue angle; d*L**, d*a**, d*b**, and d*C**, change in color; Δ*H**, hue difference; BI, browning index; Δ*E**, total color difference; CI, color index; AsA, ascorbic acid; TPC, total phenolic content; AC, antioxidant capacity.

Technology of Rio Grande do Norte (IFRN, Brazil), and University of Campinas (UNICAMP, Brazil). LRBM thanks National Council for Scientific and Technological Development (CNPq, Brazil) for the grant.

Ethical statements

Participants gave informed consent via the statement "I am aware that my responses are confidential, and I agree to participate in this survey" where an affirmative reply was required to enter the survey. They were able to withdraw from the survey at any time without giving a reason. The products tested were safe for consumption.

Data availability statement

The data that support the findings of this study are available from the corresponding author (Bruno Fonsêca Feitosa, brunofonsecafeitosa@live.com), upon reasonable request.

CRediT authorship contribution statement

Bruno Fonseca Feitosa: Writing – review & editing, Writing – original draft, Validation, Methodology, Investigation, Formal analysis, Conceptualization. **Leidiana Elias Xavier:** Writing – review & editing, Methodology, Investigation. **Maria Silvanete Benedito de Sousa Lermen:** Writing – review & editing, Validation, Investigation. **Monica Correia Goncalves:** Writing – review & editing, Supervision, Resources, Project administration. **Tiago Augusto Lima Cardoso:** Writing – review & editing, Methodology, Formal analysis. **Joao Vitor Fonseca Feitoza:** Writing – review & editing, Formal analysis, Conceptualization. **Adriano Sant'Ana Silva:** Writing – review & editing, Validation, Methodology. **Emanuel Neto Alves de Oliveira:** Writing – review & editing, Funding acquisition, Conceptualization. **Marcella Camargo Marques:** Writing – review & editing, Validation, Investigation. **Lilian Regina Barros Mariutti:** Writing – review & editing, Resources, Project administration, Funding acquisition. **Monica Tejo Cavalcanti:** Writing – review & editing, Resources, Project administration, Funding acquisition.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:Lilian Regina Barros Mariutti reports financial support was provided by Cell Press. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

To the Family Farmers' Association of Serra dos Paus Dóias (AGRODOIA Agroindustry), represented by the Lermen family.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e37270.

References

[1] Brazilian Association of Producers and Exporters of Fruits and Fruit Products, Brazil bate recorde e alcança mais de um bilhão de dólares em exportação de frutas, Available at: https://abrafrutas.org/2022/01/Brazil-bate-recorde-e-alcanca-mais-de-um-bilhao-de-dolares-em-exportacao-de-frutas/, 2022. (Accessed 14 January 2023).
[2] A.F.A.A. Gerum, G.S. Santos, M.A. Santana, J.S. Souza, C.E.L. Cardoso, Tropical fruit growing: potential risks and their impacts. Documents 232. Cruz das Almas, BA: Embrapa Mandioca e Fruticultura. 28p, 2019. Available at: https://ainfo.cnptia.embrapa.br/digital/bitstream/item/197322/1/Documento232-AureaGerum-Ainfo.pdf.
[3] L.A.B.M. Mendonça, R. Matias, D.F.P. Zanella, K.R.A. Porto, J.F. Guilhermino, D.L. Moreira, A.R. Roel, A. Pott, C.M.E. Carvalho, Toxicity and phytochemistry of eight species used in the traditional medicine of sul-mato-grossense, Brazil, Braz. J. Biol. 80 (2020) 574–581, https://doi.org/10.1590/1519-6984.216406.
[4] N.M.P. Araújo, H.S. Arruda, D.P. Farias, G. Molina, G.A. Pereira, G.M. Pastore, Plants from the genus Eugenia as promising therapeutic agents for the management of diabetes mellitus: a review, Food Res. Int. 142 (2021) 110182, https://doi.org/10.1016/j.foodres.2021.1101.
[5] M.G.V. Sampaio, C.R.B. Santos, L.C.S. Vandesmet, B.S. Santos, I.B.S. Santos, M.T.S. Correia, A.L.B. Martins, L.C.N. Silva, I.R.A. Menezes, M.C.V. Gomez, M. V. Silva, Chemical composition, antioxidant and antiprotozoal activity of *Eugenia gracillima* Kiaersk. leaves essential oil, Nat. Prod. Res. 35 (2019) 1914–1918, https://doi.org/10.1080/14786419.2019.1644506.
[6] R.M. Feitosa, R.M.F. Figueirêdo, A.J.M. Queiroz, W.P. Silva, I.S. Moreira, Assessment of myrtle powder dried by spray drying, Int. J. Food Eng. 16 (2020), https://doi.org/10.1515/ijfe-2019-0211.
[7] R.M. Feitosa, R.M.F. Figueirêdo, A.J.M. Queiroz, R.C. Silva, E.N.A. Oliveira, Evaluation of lyophilized myrtle pulp powder stability during storage, J. Agric. Stud. 8 (2020), https://doi.org/10.5296/jas.v8i1.15447.
[8] B.F. Feitosa, E.N.A. Oliveira, F.B.F. Freitas, J.O. Oliveira Neto, R.M. Feitosa, T.L.S. Lima, M.L.S. Medeiros, J.V.F. Feitoza, H.D.M. Coutinho, Beverage composed of fruits and soy: microbiology, colorimetry and effects of refrigerated storage on physical-chemical parameters, Food Biosci. 49 (2022) 101863, https://doi.org/10.1016/j.fbio.2022.101863.
[9] B.F. Feitosa, R.M. Feitosa, E.N.A. Oliveira, R.M.F. Figueirêdo, A.J.M. Queiroz, R.C. Silva, I.S. Moreira, N. Piovesan, Myrtle (*Eugenia gracillima* Kiaersk.) as a fermented alcoholic beverage alternative to wine: preliminary study, Food Biosci. 54 (2023) 102830, https://doi.org/10.1016/j.fbio.2023.102830.
[10] Ó. Rodríguez, W.F. Gomes, S. Rodrigues, F.A.N. Fernandes, Effect of indirect cold plasma treatment on cashew apple juice (*Anacardium occidentale* L.), LWT - Food Sci. Technol. (Lebensmittel-Wissenschaft -Technol.) 84 (2017) 457–463, https://doi.org/10.1016/j.lwt.2017.06.010.
[11] J.F. Fundo, F.A. Miller, A. Tremarin, E. Garcia, T.R. Brandão, C.L. Silva, Quality assessment of Cantaloupe melon juice under ozone processing, Innovat. Food Sci. Emerg. Technol. 47 (2018) 461–466, https://doi.org/10.1016/j.ifset.2018.04.016.
[12] Brazil, Ministry of Agriculture, Livestock and Supply. Normative Instruction No. 12, of September 4, 2003. To Approve the Technical Regulation for Setting General Identity and Quality Standards for Tropical Juice, Official Diary of the Union, Brasília, DF, 2003. https://www.gov.br/agricultura/pt-br/assuntos/inspecao/produtos-vegetal/legislacao-1/biblioteca-de-normas-vinhos-e-bebidas/instrucao-normativa-no-12-de-4-de-setembro-de-2003.pdf/view. Available at:.
[13] Brazil, Ministry of Agriculture, Livestock and Supply. Decree No. 6871, of June 4, 2009. Regulates Law No. 8918, of July 14, 1994, Which Provides for Standardization, Classification, Registration, Inspection, Production and Supervision of Beverages, Official Diary of the Union, Brasília, DF, 2009. http://www.planalto.gov.br/ccivil_03/_ato2007-2010/2009/decreto/d6871.htm. Available at:.
[14] E. Rifna, S.K. Singh, S. Chakraborty, M. Dwivedi, Effect of thermal and non-thermal techniques for microbial safety in food powder: recent advances, Food Res. Int. 126 (2019) 108654, https://doi.org/10.1016/j.foodres.2019.108654.
[15] M.F.D. Linhares, E.G. Alves Filho, L.M.A. Silva, T.V. Fonteles, N.J. Wurlitzer, E.S. Brito, F.A.N. Fernandes, S. Rodrigues, Thermal and non-thermal processing effect on açai juice composition, Food Res. Int. 136 (2020) 109506, https://doi.org/10.1016/j.foodres.2020.109506.
[16] Brazil, Ministry of Health. National Health Surveillance Agency. RDC Resolution No. 8, of March 6, 2013. Provides for the Approval of the Use of Food Additives for Fruit and Vegetable Products and Mocotó Jelly, Official Diary of the Union, Brasília, DF, 2013. https://www.gov.br/agricultura/pt-br/assuntos/inspecao/produtos-vegetal/legislacao-1/biblioteca-de-normas-vinhos-e-bebidas/resolucao-rdc-no-8-de-6-de-marco-de-2013.pdf/view. Available at:.
[17] R.A. Rego, A. Vialta, L.F.C. Madi, Series: Industrialized Foods 2030. Industrialized Juices and Other Non-carbonated Beverages, 1th Edition, ABIR/ITAL, São Paulo, 2020. ISBN 978-65-991416-2-1.
[18] P. Mena, S. Vegara, N. Martí, C. García-Viguera, D. Saura, M. Valero, Changes on indigenous microbiota, colour, bioactive compounds and antioxidant activity of pasteurised pomegranate juice, Food Chem. 141 (2013) 2122–2129, https://doi.org/10.1016/j.foodchem.2013.04.118.
[19] Z. Xu, T. Lin, Y. Wang, X. Liao, Quality assurance in pepper and orange juice blend treated by high pressure processing and high temperature short time, Innovat. Food Sci. Emerg. Technol. 31 (2015) 28–36, https://doi.org/10.1016/j.ifset.2015.08.001.
[20] N.K.V. Silva, L.B.S. Sabino, L.S. Oliveira, L.B.V. Torres, P.H.M. Sousa, Effect of food additives on the antioxidant properties and microbiological quality of red guava juice, Rev. Cienc. Agron. 47 (2016), https://doi.org/10.5935/1806-6690.20160009.
[21] D.R. Araújo, E.M.P. Lucena, J.P. Gomes, R.M.F. Figueiredo, C.P. Silva, Characterization of ripening stages of myrtle fruit, Rev. Bras. Frutic. 38 (2016), https://doi.org/10.1590/0100-29452016712.
[22] Brazil, National Health Surveillance Agency. RDC resolution No. 216, of September 15, 2004. Provides for the Technical Regulation of Good Practices for Food Services, Official Diary of the Union, Brasília, DF, 2004. https://bvsms.saude.gov.br/bvs/saudelegis/anvisa/2004/res0216_15_09_2004.html. Available at:.
[23] Brazil, Ministry of Agriculture, Livestock and Supply. Normative Instruction No. 49, of September 26, 2018. Identity and Quality Standards for Fruit Juice and Pulp, Edition 187, Official Diary of the Union, 2018. Section 1. Available at: https://www.in.gov.br/web/guest/materia/-/asset_publisher/Kujrw0TZC2Mb/content/id/42586576/do1-2018-09-27-instrucao-normativa-n-49-de-26-.
[24] M.I.F. Chitarra, A.B. Chitarra, Postharvest of Fruits and Vegetables: Physiology and Handling, 2th Edition, 2005. Lavras: *Editora UFLA*. 783pp.
[25] Brazil, Ministry of Health. National Health Surveillance Agency. RDC Resolution No. 60, of December 23, 2019. Establishes the Microbiological Standards for Foods and Their Application, Edition 249, Official Diary of the Union, Brasília, DF, 2019. Section 1. Available at: https://www.in.gov.br/web/dou/-/instrucao-normativa-n-60-de-23-de-dezembro-de-2019-235332356.
[26] Brazil, Ministry of Agriculture, Livestock and Supply. Normative instruction No. 24, of September 8, 2005. Approves the operating manual for beverages and vinegars. Cad 07 Non-alcoholic, Official Diary of the Union, Brasília, DF, 2005. https://www.gov.br/agricultura/pt-br/assuntos/inspecao/produtos-vegetal/legislacao-1/biblioteca-de-normas-vinhos-e-bebidas/instrucao-normativa-no-24-de-8-de-setembro-de-2005.pdf/view. Available at:.
[27] N. Silva, V.C.A. Junqueira, N.F.A. Silveira, N.H. Taniwaky, R.A.R. Gomes, M.M. Okazari, Manual of Methods of Microbiological Analysis of Food and Water, fifth ed., São Paulo: Blucher, 2017.

[28] Adolfo Lutz Institute, Physico-chemical methods for food analysis, 4th Edition, 1st Edition. Digital, São Paulo. 1020pp. Available at: https://wp.ufpel.edu.br/nutricaobromatologia/files/2013/07/NormasADOLFOLUTZ.pdf, 2008.
[29] Association of Official Analytical Chemists, Official Methods of Analysis, twentieth ed., 2016. Washington, D.C. ISBN 0935584870.
[30] Brazil, Ministry of Agriculture, Livestock and Supply. Ordinance No. 76, of November 27, 1986. Approves the Analytical Methods that Become Official Standards for the Analysis of Beverages and Vinegars, Official Diary of the Union, Brasília, DF, 1986.
[31] D. Pearson, Técnicas de laboratório para el análises de alimentos, Acribia, Zaragoza, 1976.
[32] A. Waterhouse, Folin–Ciocalteu Micro Method for Total Phenol in Wine, vols. 3–5, America Journal of Enology and Viticulture, 2006, 2006. Available at: https://waterhouse.ucdavis.edu/folin-ciocalteau-micro-method-total-phenol-wine.
[33] A.F. Faria, M.C. Marques, A.Z. Mercadante, Identification of bioactive compounds from jambolÃ£o (Syzygium cumini) and antioxidant capacity evaluation in different pH conditions, Food Chem. 126 (2011) 1571–1578, https://doi.org/10.1016/j.foodchem.2010.12.007.
[34] R. Re, N. Pellegrini, A. Proteggente, A. Pannala, M. Yang, C. Rice, Antioxidant activity applying an improved ABTS radical cation decolorization assay, Free Radic. Biol. Med. 26 (1999) 1231–1237, https://doi.org/10.1016/S0891-5849(98)00315-3.
[35] M.S.M. Rufino, R.E. Alves, E.S. Brito, S.M. Morais, C.G. Sampaio, J. Pérez-Jiménez, F.D. Saura-Calixto, Metodologia científica: Determinação da atividade antioxidante total em frutas pela captura do radical livre ABTS●+, Embrapa Agroindústria Tropical. Comunicado Técnico 128 (2007). https://ainfo.cnptia.embrapa.br/digital/bitstream/CNPAT/10224/1/Cot_127.pdf. Available at:.
[36] K.M. Sensing, Color accurate communication, in: AEBDPK®, Sakai –, vol. 59p, Minolta Co., Ltd., Osaka, Japão, 1998.
[37] A.F. Camelo, P.A. Gómez, Comparison of color indexes for tomato ripening, Hortic. Bras. 22 (2004) 534–537, https://doi.org/10.1590/S0102-05362004000300006.
[38] P.B. Pathare, U.L. Opara, F.A. Al-Said, Colour measurement and analysis in fresh and processed foods: a review, Food Bioprocess Technol. 6 (2013) 36–60, https://doi.org/10.1007/s11947-012-0867-9.
[39] L.E. Ordóñez-Santos, J. Martínez-Girón, M.E. Arias-Jaramillo, Effect of ultrasound treatment on visual color, vitamin C, total phenols, and carotenoids content in Cape gooseberry juice, Food Chem. 233 (2017) 96–100, https://doi.org/10.1016/j.foodchem.2017.04.114.
[40] F.A.Z. Silva, C.A.V. Azevedo, The assistat software version 7.7 and its use in the analysis of experimental data, Afr. J. Agric. Res. 11 (2016) 3733–3740, https://doi.org/10.5897/AJAR2016.11522.
[41] B.F. Feitosa, R.L.J. Almeida, N.C. Santos, E.N.A. Oliveira, M.S.B.S. Lermen, S.S. Monteiro, E.G. Lima, M.T. Cavalcanti, J.S.F. Araújo, G.S.C. Borges, Effects of different temperatures on electric oven drying of myrtle (*Eugenia gracillima* Kiaersk.) seeds modified by High Hydrostatic Pressure (HHP), LWT 189 (2023) 115554, https://doi.org/10.1016/j.lwt.2023.115554.
[42] Suárez, C., Espariz, M., Blancato, V. S., & Magni, C. Expression of the agmatine deiminase pathway in Enterococcus faecalis is activated by the AguR regulator and repressed by CcpA and PTS (Man) systems. PLoS One, 8, e76170. https://doi.org/10.1371/journal.pone.0076170.
[43] B.D.G.M. Franco, M. Landgraf, Food Microbiology, 2th Edition, Atheneu, São Paulo, 2008.
[44] A. Bisconsin-Junior, B.F. Feitosa, F.L. Silva, L.R.B. Mariutti, Mycotoxins on edible insects: should we be worried? Food Chem. Toxicol. 177 (2023) 113845 https://doi.org/10.1016/j.fct.2023.113845.
[45] C.F. Françoso, C.J. Barbedo, Osmotic and heat treatments on control of fungi associated with seeds of Eugenia Braziliensis and E. pyriformis (Myrtaceae), Journal of Seed Science 38 (2016), https://doi.org/10.1590/2317-1545v38n3159481.
[46] F.C. Morelli, A. Ruvolo Filho, Nanocomposites of polypropylene and organophilic clay: X ray diffraction, absorption infrared spectroscopy with fourier transform and water vapor permeation, Polímeros 20 (2010) 121–125, https://doi.org/10.1590/S0104-14282010005000014.
[47] T.M.C. Pereira, E. Domingos, E.V.R.D. Castro, W. Romão, G.L.D. Sena, Effect of organic solvents on the physical-chemistry behavior of high density polyethylene (HDPE) and polypropylene (PP), Polímeros 24 (2014) 300–306, https://doi.org/10.4322/polimeros.2014.045.
[48] P. Subramaniam, The stability and shelf life of confectionery products, in: The Stability and Shelf Life of Food, vol. 2, Woodhead Publishing, 2016, pp. 545–573, https://doi.org/10.1016/b978-0-08-100435-7.00019-8. ISBN 978-0-08-100435-7.
[49] A.L. Tambara, L. de Los Santos Moraes, A.H. Dal Forno, J.R. Boldori, A.T. Gonçalves Soares, C. de Freitas Rodrigues, C.C. Denardin, Purple pitanga fruit (Eugenia uniflora L.) protects against oxidative stress and increase the lifespan in Caenorhabditis elegans via the DAF-16/FOXO pathway, Food Chem. Toxicol. 120 (2018) 639–650, https://doi.org/10.1016/j.fct.2018.07.057.
[50] M. Plaza, Â.G. Batista, C.B.B. Cazarin, M. Sandahl, C. Turner, E. Östman, M.R. Maróstica Júnior, Characterization of antioxidant polyphenols from Myrciaria jaboticaba peel and their effects on glucose metabolism and antioxidant status: a pilot clinical study, Food Chem. 211 (2016) 185–197, https://doi.org/10.1016/j.foodchem.2016.04.142.
[51] K.A. Reynertson, H. Yang, B. Jiang, M.J. Basile, E.J. Kennelly, Quantitative analysis of antiradical phenolic constituents from fourteen edible Myrtaceae fruits, Food Chem. 109 (2008) 883–890, https://doi.org/10.1016/j.foodchem.2008.01.021.
[52] M.M.B. Almeida, P.H.M. Sousa, A.M.C. Arriaga, G.M. Prado, C.E.C. Magalhães, G.A. Maia, T.L.G. Lemos, Bioactive compounds and antioxidant activity of fresh exotic fruits from northeastern Brazil, Food Res. Int. 44 (2011) 2155–2159, https://doi.org/10.1016/j.foodres.2011.03.051.
[53] R.M. Feitosa, F.C.S. Lima, R.M.F. Figueirêdo, A.J.M. Queiroz, L.T.S. Amadeu, Maltodextrin: effects caused the physicochemical properties of myrtle pulp, Revista Brasileira de Agrotecnologia 7 (2017) 104–109. https://www.gvaa.com.br/revista/index.php/REBAGRO/article/view/5126. Available at:.
[54] C. Osorio, D.P. Forero, J.G. Carriazo, Characterization and performance assessment of guava (Psidium guajava L.) microencapsulates obtained by spray drying, Food Res. Int. 44 (2011) 1174–1181, https://doi.org/10.1016/j.foodres.2010.09.007.
[55] A. Adekunte, B. Tiwari, P. Cullen, A. Scannell, C. O'Donnell, Effect of sonication on colour, ascorbic acid and yeast inactivation in tomato juice, Food Chem. 122 (2010) 500–507, https://doi.org/10.1016/j.foodchem.2010.01.026.
[56] B.F. Feitosa, B.L.A. Decker, E.S. Brito, S. Rodrigues, L.R.B. Mariutti, Microencapsulation of anthocyanins as natural dye extracted from fruits – a systematic review, Food Chem. 424 (2023) 136361, https://doi.org/10.1016/j.foodchem.2023.136361.
[57] A.V. Souza, M.R.S. Vieira, F.F. Putti, Correlations between the phenolic compounds and antioxidant activity in the skin and pulp of table grape, Braz. J. Food Technol. 21 (2018) e2017103, https://doi.org/10.1590/1981-6723.10317.
[58] T.C.B. Rigolon, F.A.R. Barros, É.N.R. Vieira, P.C. Stringheta, Prediction of total phenolics, anthocyanins and antioxidant capacity of blackberry (Rubus sp.), blueberry (Vaccinium sp.) and jaboticaba (Plinia cauliflora (Mart.) Kausel) skin using colorimetric parameters, Food Sci. Technol. 40 (suppl. 2) (2020) 620–625, https://doi.org/10.1590/fst.34219.
[59] C. Guo, J. Yang, J. Wei, Y. Li, J. Xu, Y. Jiang, Antioxidant activities of peel, pulp and seed fractions of common fruits as determined by FRAP assay, Nutr. Res. 23 (2003) 1719–1726, https://doi.org/10.1016/j.nutres.2003.08.005.