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Impact of processing and packages on bioactive compounds and antioxidant activity of Mangaba Jelly

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Abstract

This study evaluated the impact of processing, packages (amber and transparent glass jars), and storage time on the stability of the bioactive compounds (total phenolic compounds and vitamin C), and antioxidant activity of mangaba jelly. Jelly processing, significantly ($p < 0.05$) decreased the bioactive compounds and antioxidant activity. The storage time, significantly ($p < 0.05$) influenced all the variables studied in this work, although they were not affected by packages. A significant decrease of the bioactive compounds and antioxidant activity was observed during the jelly storage. HPLC-DAD/UV-Vis analyses allowed to identify 10 phenolic compounds, including flavonoids and non-flavonoids, and among these, quercetin and catechin were the major compounds. After processing, all these compounds decreased significantly ($p < 0.05$), except for the gallic acid and the *p*-coumaric acid, which presented an increasing tendency. During jelly storage, only gallic acid, caffeic acid, catechin, and rutin were affected. Despite the reduction in the antioxidant activity and bioactive compounds studied, mangaba jelly retained good levels of them and may be considered a potential novel functional food.

Keywords: value addition; cerrado fruit; food preservation; *Hancornia speciosa* Gomes; HPLC-DAD/UV-Vis; shelf-life.

Practical application: Value addition and changes in bioactive compounds throughout storage time.

1 Introduction

In recent years, with the potential health concerns associated with the expected growth of world population from 7.4 billion in 2017 to 9.7 billion in 2050 (United Nations, 2019), there has been an increase in demand for underutilized fruits among consumers, due to their high nutritional value, and many health benefits (Martinović & Cavoski, 2020).

Hancornia speciosa Gomes (family, Apocynaceae), whose fruit is locally known as mangaba or mangareíba is a fruit species found in several regions of Brazil, especially in the Cerrado biome. The ripe fruit presents a yellowish exocarp and red stripes or spots. Its pulp is whitish, fleshy, viscous, sweet and slightly acid (Clerici & Carvalho-Silva, 2011). Recently, mangaba fruit has been attracting much attention not only because of its unique flavor and nutritional properties, but also because of its high content of health-promoting phytochemicals, such as vitamin C, vitamin E, carotenoids, folic acid, and phenolic compounds (Lima et al., 2015a, b; Gonçalves et al., 2019; Reis et al., 2019). Various in vitro, animal, and human clinical trials studies have consistently shown that the intake of mangaba fruit is associated with a wide range of pharmacological activities including antioxidant, and antimutagenic (Lima et al., 2015a), anti-cancer (Araújo et al., 2019), and anti-inflammatory (Bitencourt et al., 2019; Reis et al., 2019; Torres-Rêgo et al., 2016). Indeed, in previous study with male Swiss mice through bone marrow micronucleus test, in vivo gut micronucleus test, comet assay, gut apoptosis cells and oxidative stress was reported a first antimutagenic effect of mangaba fruit pulp (Lima et al., 2015a). Moreover, Araújo et al. (2019) recently evaluated the antitumor effect of mangaba extract fruit adsorbed onto polyethylene glycol microsphere in MCF-7 breast cancer cells co-cultured with blood cells and observed that the cell viability was not affected and the superoxide release increased, which suggest that mangaba can be considered as "*superfruit*" and emphasizes its consumption by women against risk of breast cancer.

Despite its high nutritional value and numerous health benefits, mangaba fruit is still underutilized by the local rural communities and food processing industries due to limited scientific knowledge about its nutraceutical properties and lack of incentive for its commercialization. Apart from this, mangaba is seasonal and highly perishable fruit, therefore has very short shelf-life of about a few days.

Thus, in order to enhance the consumption of mangaba fruit during off-season and in the other regions as well as give it added value and preserve its functional properties, it is important to develop shelf-stable food products such as jellies and jam. However, as for many other food products, thermal processing and storage can lead to a loss of bioactive compounds (Shinwari & Rao, 2020).

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Packaging material has also been appointed as a factor that alters the bioactive compounds during storage as result of oxygen and light incidence through them (Aslam et al., 2019). Polyethylene terephthalate (PET) plastic jars or glass jars are commonly used packaging materials for jellies and jam. Despite scientific evidence on the effect of processing, packaging material and storage on the bioactive compounds of fruit jelly (Cunha et al., 2020) and jam (Aslam et al., 2019), to the best of our knowledge, there is no report in the current literature about the mangaba jelly. Thus, this study aimed to evaluate the functional potential and the impact of processing, packages (amber and transparent glass jars), and storage time (0, 3, 6, 9, and 12 months) on the stability of bioactive compounds and in the antioxidant activity of mangaba jelly.

2 Materials and methods

2.1 Raw material and jelly preparation

The ripe mangaba fruit (*Hancornia speciosa* Gomes) harvested from different trees, were obtained from Curvelo Town, Minas Gerais State, Brazil (latitude 18° 45′ 23″ S, longitude 44° 25′ 51″ Wand altitude 632 m). The seedless pulp was obtained through crushing the fruits in the skin and strain with the use of stainless-steel spoons. The jelly was prepared as described in our previous study (Zitha et al., 2020).

2.3 Preparation of extracts

For vitamin C, 5g of each sample was extracted with 45 mL of aqueous oxalic acid (5%, w/v) and about 0.1 g of Kiesselgur, under mechanical agitation, for 5 min. Then the extracts were filtered using filter paper with 14 µm porosity. For Total phenolics content (TPC) and antioxidant activity, the extracts were obtained according to Gonçalves et al. (2019), with minor modifications. In brief, 2.5 g of each sample was extracted with 20 mL of aqueous methanol (50%, v/v) for 1 h at room temperature in the dark, and then centrifuged at 25,400 x g for 15 min at 4 °C. The supernatant was collected and the residue was re-extracted with an additional 20 mL of aqueous acetone (70%, v/v) and centrifuged under the same condition. Finally, the supernatant of two extract were mixed, filtered through filter paper with 14µm porosity, completed to a final volume of 50 mL with distilled water and stored at -80 °C until analysis.

2.4 Determination of Vitamin C Content

The vitamin C content was performed by a colorimetric method using 2,4-dinitrophenylhydrazine (DNPH) reagent as described by Strohecker et al. (1967). The absorbance was measured at 520 nm using UV-spectrophotometer (Varian, Cary 50 Probe), and the results were expressed in mg of ascorbic acid per 100 g of sample on fresh weight (mg/100g fw).

2.5 Determination of Total phenolics content (TPC)

The TPC of the sample extracts was quantified using Folin-Ciocalteu reagent according to Lou et al. (2020), with minor modifications. Briefly, 2.5 mL of Folin-Ciocalteu reagent (10%, v/v) were mixed with 0.5 mL sample extracts, and then the mixture was mixed with 2.0 mL sodium carbonate solution (4%, w/v) and incubated in the dark at room temperature for 2 h to complete the reaction. The absorbance at 750 nm was measured using UV-spectrophotometer (Varian, Cary 50 Probe). The results were expressed in mg gallic acid equivalents per 100g of sample on fresh weight (mg GAE/100g fw) using the calibration curve of gallic acid.

2.6 Determination of antioxidant activity

The antioxidant activity was determined by DPPH radical scavenging activity (Brand-Williams et al., 1995), ABTS radical scavenging activity (Gonçalves et al., 2019) and β-carotene bleaching (Duarte-Almeida et al., 2006), with minor modifications. For DPPH, an aliquot of 0.1 mL of sample extract was mixed with 3.9 mL of 60 mM DPPH working solution, previously diluted in methanol, and after 80 min of incubation in the dark at room temperature, the absorbance was determined at 515 nm. For ABTS, 7 mM of potassium persulfate was mixed with 2.45 mM of ABTS solution and this mixture was allowed to react for 16 h in the dark at room temperature to get ABTS radical cation ($ABTS^{\bullet+}$). Subsequently, the $ABTS^{\bullet+}$ solution was diluted with ethanol to obtain an absorbance of 0.70 ± 0.05 at 734 nm. Afterward, 30 µL of sample extract was mixed with 3 mL of $ABTS^{\bullet+}$ solution and 6 min after reaction in the dark at room temperature, the absorbance was immediately measured at 734 nm. For both DPPH and ABTS, trolox (0 – 2000 µM) and ascorbic acid (0-20 mg) were used as standards. For β-carotene bleaching, 50 µL of ß-carotene chloroform solution (1mg/mL), 40 µL of linoleic acid and 530 µL of Tween 40 were dissolved in 1 mL chloroform and mixed thoroughly. After that, the chloroform was completely removed under vacuum at 40 °C. The resulting mixture was diluted with aerated distilled water to an absorbance between 0.6 and 0.7 at 470 nm, to obtain ß-carotene linoleic acid emulsion. Then, 0.4 mL of sample extract was added to 5 mL of the emulsified diluent and the absorbance at 470 nm was measured using UV-spectrophotometer (Varian, Cary 50 Probe), after 2h of incubation at 40 °C in water bath. Results were expressed in µmol trolox equivalents (µmol TE/g) and in mg ascorbic acid equivalents (mg AAE/100g) for both DPPH and ABTS; EC_{50} (g g^{-1}) for only DPPH, and in percentage of oxidation inhibition (% O.I) for β-carotene bleaching.

2.7 Analysis of individual phenolic compounds by HPLC-DAD

2.7.1 Extraction

The extract of individual phenolic compounds was carried out according to Gonçalves et al. (2019), with minor modifications. In brief, 2.5 g of each sample was extracted with 20 mL of aqueous methanol (70%, v/v) HPLC grade, vortexed and left for sonication in an ultrasonic bath at room temperature for 1h. Then, the resulting mixture was centrifuged at 1,400 x g for 15 min at 4 °C and filtered through filter paper with 14 µm porosity and stored at −80 °C for further analyses. Before injection into HPLC system, the extracts were filtered again through 0.45 µm membrane filter and transferred to a vial.

2.7.2 Identification and quanatification

High performance liquid chromatography (HPLC) system (Shimadzu Corporation, Kyoto, Japan) with UV-Vis detector, equipped with four high-pressure pumps (LC-20AT), autosampler (SIL-20A), degasser (DGU-20A5), diode array detector (DAD) (SPDM-20A) and column oven (CTO-20AC) was used. The separation of phenolic compounds was carried out in a Shimadzu Shim-pack ODS GVP-C18 column (4.6 × 250 mm, 5 µm) coupled to Shimadzu-pack ODS GVP-C18 guard column (4.6 × 10 mm, 5 µm), using the same protocol and conditions described by Cunha et al. (2020). In brief, acetic acid (2%, v/v) in deionized water (A) and methanol:water:acetic acid (70:28:2 v/v/v) (B) were used as the mobile phase; the system was run at a flow rate of 1.0 mL min^{-1} at 15 °C and the injection volume was set at 20 µL with a gradient elution program and total run time of 60 min. HPLC chromatograms were detected at 280 nm and quantification and identification of individual phenolic compounds were carried out by comparison with retention time and mass spectra of authentic standards under the same conditions and the results were expressed in mg of phenolic compound per 100g of the sample on fresh weight (mg/100g fw).

2.8 Experimental design and Statistical Analysis

Two experiments were carried out. The first one, evaluated the effect of processing, comparing mangaba pulp and mangaba jelly. In the second one, a completely randomized design (CRD) was used, with a double factorial scheme, including packages (amber glass jar and transparent glass jar) and five storage periods (0, 3, 6, 9, and 12 months). In both of the experiments, four replications consisting of 80 g (two glass jars of 40 g) were used. In the first experiment, the means of mangaba pulp and jelly were compared using Student's t-test. The results from the second experiment were subjected to polynomial regression evaluation, and the models were selected according to the significance of the F test and the coefficient of determination. The differences between the groups were assessed by one-way analysis of Variance (ANOVA) using the SISVAR software (Ferreira, 2011). The principal component analysis (PCA) was carried out using Minitab 18 software. Pearson correlation coefficients between bioactive compounds and antioxidant activity were generated using SPSS software. All tests were applied at the significance level of 0.05.

3 Results and discussion

3.1 Vitamin C and Total phenolics content (TPC)

The vitamin C and the TPC were affected significantly ($p < 0.05$) by the processing (Tables 1) and storage (Figure 1), but not by the packages. When comparing the mangaba pulp and jelly, it was observed that processing into jelly significantly ($p < 0.05$) decreased vitamin C and TPC (Tables 1). The vitamin C and TPC also presented a trend of decrease in the jelly during 12 months of storage (Figure 1, $p < 0.05$).

The vitamin C content of mangaba pulp (193.84 mg 100 g^{-1} fw) was lower than that observed by de Lima, Azevedo, et al. (2015) (255.90 ± 34.89 mg 100 g^{-1}), and significantly higher than those reported by Almeida et al. (2011) (96.3 ± 1.7 mg 100 g^{-1}). The vitamin C content decreased about 66% after processing, and 75% during 12 months of storage of mangaba jelly (Table 1 and Figure 1a).

The decrease of vitamin C content might be, mainly, attributed to the thermal treatment. The high-temperature conditions, associated with a long cooking process and storage, led to oxidation of L-ascorbic acid to dehydroascorbic acid (DHAA), followed by hydrolysis to 2,3-diketogulonic acid and later oxidation, dehydration and polymerization reactions. Indeed, these reactions, and especially the hydrolysis of DHAA, are significantly accelerated by high temperature (Gregory, 2008). Moreover, the polymerization reaction leads to formation of nutritionally inactive compounds, such as hydroxymethylfurfural (HMF), responsible for browning after processing and during storage (Chuah et al., 2008).

Our findings were similar to those in a study by Cunha et al. (2020), who reported 26.58 and 52% of reduction in the vitamin C content of curriola jelly after processing and during 12 months of storage, respectively. Tobal & Rodrigues (2019) reported a decrease on the vitamin C content in conventional (58.6%) and diet (38.71%) jams of pitanga, after 140 days of storage at room temperature. According to Ramful et al. (2011), who classified fruits into three groups according to their vitamin C contents: low (<30 mg 100 g^{-1}), medium (30-50 mg 100 g^{-1}), and high (>50 mg 100 g^{-1}), our findings suggest that mangaba fruit may be classified as a high source of vitamin C, considering the results obtained from its pulp.

Even after processing, significant levels of vitamin C were kept in the jelly, the higher the levels, the shorter the storage time. The daily intake recommended of vitamin C to adult men is 90 mg. Thus, about 46 g of mangaba pulp or 137 g of fresh jelly would attend that recommendation. Since jelly is a caloric food, large portions should not be consumed, nevertheless, it could contribute to the daily intake.

Table 1. The effect of the mangaba jelly processing on bioactive and total antioxidant activity.

Bioactive compounds and total antioxidant activity	Mangaba pulp†	Mangaba jelly†
Vitamin C (mg AA 100g^{-1})	193.84 ± 2.95^a	65.61 ± 0.78^b
TPC (mg GAE 100g^{-1})	324.45 ± 3.04^a	161.39 ± 2.97^b
DPPH (EC_{50}, g g^{-1} DPPH)	953.67 ± 5.38^b	1454.07 ± 15.09^a
DPPH (µmol TE g^{-1})	47.93 ± 0.40^a	32.36 ± 0.41^b
DPPH (mg AAE 100g^{-1})	77.88 ± 2.99^a	43.32 ± 1.58^b
ABTS (µmol TE g^{-1})	154.73 ± 17.91^a	103.04 ± 1.01^b
ABTS (mg AAE 100g^{-1})	221.70 ± 5.51^a	102.63 ± 1.13^b
β-carotene bleaching (% O.I)	86.11 ± 1.76^a	73.03 ± 0.97^b

†The data are expressed as means ± standard deviation (n = 4) of fresh weight (fw). Means within a line that are followed by different letters are significantly different, according to the Student's t-test ($p < 0.05$). AA: ascorbic acid; TPC, Total phenolics content; GAE: gallic acid equivalent; DPPH (AAE): antioxidant activity of DPPH radical expressed as ascorbic acid equivalent; ABTS (TE): antioxidant activity of ABTS radical expressed as trolox equivalent; DPPH (TE): antioxidant activity of DPPH radical expressed as trolox equivalent; ABTS (AAE): antioxidant activity of ABTS radical expressed as ascorbic acid equivalent; DPPH (EC_{50}): antioxidant activity of DPPH radical expressed as extract concentration providing half antioxidant activity; O.I: oxidation inhibition.

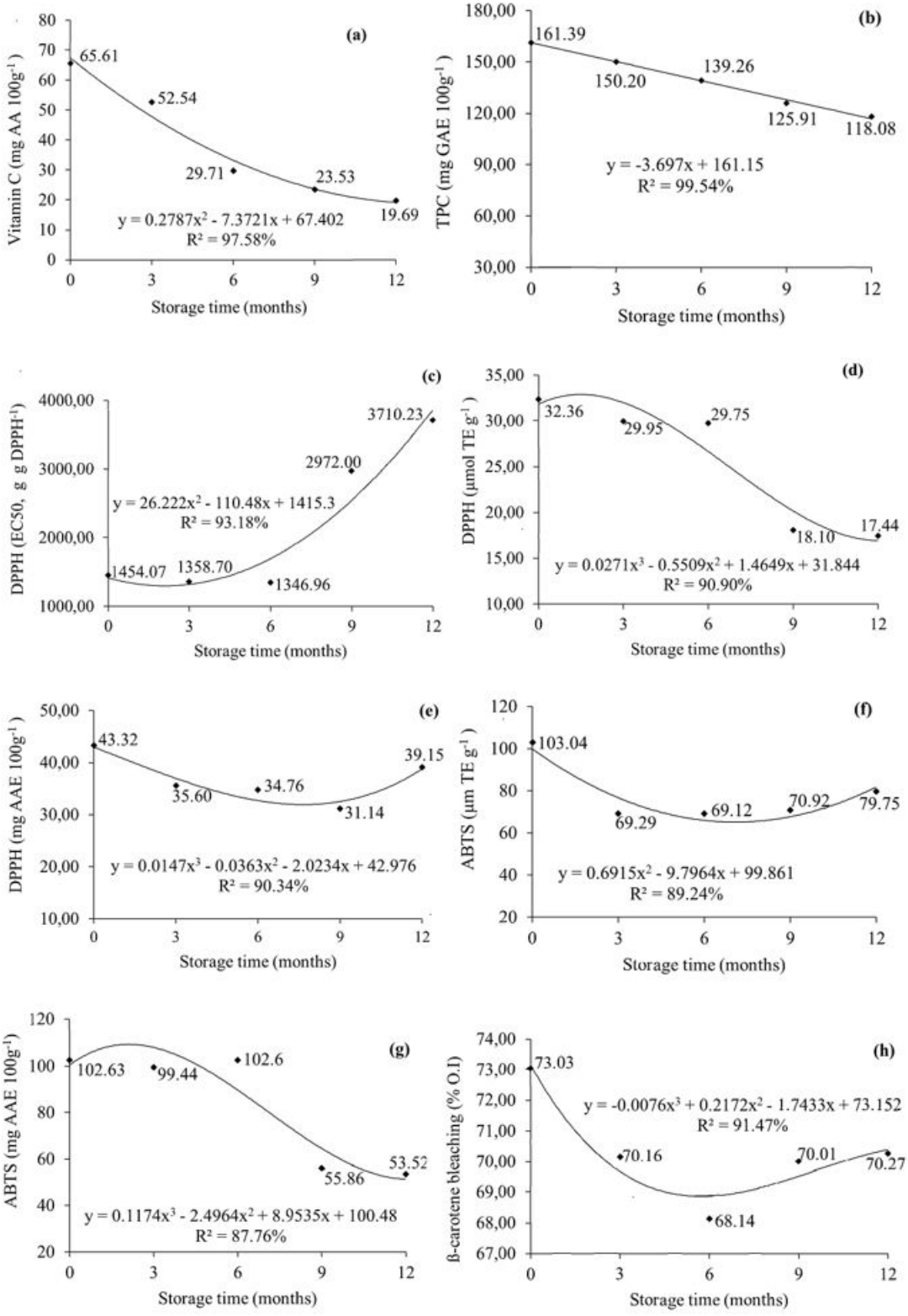

Figure 1. Changes in bioactive compounds and antioxidant activity during 12 months of mangaba jelly storage at room temperature (22 ± 2 °C, 72 ± 11% RH). (a) Vitamin C; (b)TPC; (c) DPPH EC_{50}; (d) DPPH (TE); (e) DPPH (AAE); (f) ABTS (TE); (g) ABTS (AAE); (h) ß-carotene bleaching. Values are means of four replications on a fresh weight basis (fw). AA: ascorbic acid; TPC: Total phenolics content; GAE: gallic acid equivalent; DPPH (AAE): antioxidant activity of DPPH radical expressed as ascorbic acid equivalent; ABTS (TE): antioxidant activity of ABTS radical expressed as trolox equivalent; DPPH (TE): antioxidant activity of DPPH radical expressed as trolox equivalent; ABTS (AAE): antioxidant activity of ABTS radical expressed as ascorbic acid equivalent; DPPH (EC_{50}): antioxidant activity of DPPH radical expressed as extract concentration providing half antioxidant activity; O.I: oxidation inhibition.

The TPC in mangaba pulp was around 324.45 mg GAE 100 g^{-1} fw (Table 1). This value was lower than that reported in the previous study by de Lima et al. (2015b), who observed a range from 490 to 390 mg GAE 100 g^{-1} fw of mangaba fruit during 20 days of storage, and about two-fold higher than those reported by Rufino et al. (2010) (169 mg GAE 100 g^{-1} fw). According to the classification of Vasco et al. (2008), who analyzed 17 fruit, they may be classified into three categories based to TPC: low (<100 mg GAE 100 g^{-1}), medium (100-500 mg GAE 100 g^{-1}) and high (>500 mg GAE 100 g^{-1}). Therefore, the mangaba fruit may be classified as an intermediate source of phenolic compounds. Compared the average of TPC observed in this work to other native fruits from Cerrado, mangaba pulp contain higher levels of TPC than ciruela (55.0 mg GAE 100 g^{-1}), jackfruit (29.0 mg GAE 100 g^{-1}), murici (159.9 mg GAE 100 g^{-1}), sapodilla (13.5 mg GAE 100 g^{-1}, sourop (54.8 mg GAE 100 g^{-1}), sweetsop (81.7 mg GAE 100 g^{-1}), tamarindo (83.8 mg GAE 100 g^{-1}), and umbu (44.6 mg GAE 100 g^{-1}) (Almeida et al., 2011). The TPC decreased significantly ($p < 0.05$), about 50% after processing and 27% during 12 months of storage of the mangaba jelly (Table 1 and Figure 1b). These results were in agreement with those by Wang et al. (2020), who reported a 13.8% decrease of TPC throughout red beetroot jam processing and reduction of 33.9, 41.6 and 51.9%, when the red beetroot jam was stored at 4, 25 and 37 °C for 90 days, respectively. Cunha et al. (2020) reported an average decrease in TPC in curriola jelly by 31% after 12 months of storage. Our findings suggested that although it is believed that concentration has a positive effect to the increase of TPC, thermal treatment accelerated the degradation of phenolic compounds. The reduction of TPC could be attributed to the disruption of cell structure, oxidation reaction, and the non-enzymatic oxidation imposed by thermal degradation (Putriani et al., 2020; Wang et al., 2020).

3.2 Identification of individual phenolic compounds by HPLC-DAD/UV-Vis

The contents of the individual phenolic identified in both products are given in Table 2.

From twelve phenolic compounds tested, altogether ten were identified and quantified in mangaba pulp fruit and in the mangaba jelly, including flavonoids (catechin, rutin, and quercetin) and non-flavonoids acids (gallic, chlorogenic, caffeic, ferulic, trans-cinnamic, *p*-coumaric, and *m*-coumaric acids).

The vanillin and *o*-coumaric acid were not identified. These results are consistent with those of de Lima et al. (2015a), who also reported the presence of flavonoids and non-flavonoids in the ethanolic extract of the mangaba fruit and mangaba pulp. Quercetin (3.13 mg 100 g^{-1} fw) and catechin (1.70 mg 100 g^{-1} fw) were the majority of the phenolic compounds. In contrast, de Lima et al. (2015a) in their study with mangaba fruit pulp, stated that chlorogenic acid (18.09 mg 100 g^{-1} fw) and rutin (10.26 mg 100 g^{-1} fw) were the predominant compounds.

The phenolic compounds decreased significantly ($p < 0.05$) after processing, except for the gallic acid and *p*-coumaric acid that did not change, considering the standard deviation (Table 2), while the storage did not promote a systematic effect on the individual phenolic compounds (Figure 3). Concerning the packages, no significant differences ($p > 0.05$) were observed in jelly individual phenolic compounds. The relative losses of individual phenolic compounds after processing were: catechin (42.35%), chlorogenic acid (25%), caffeic acid (62.90%), ferulic acid (51.85%), *m*-coumaric acid (53.85%), quercetin (47.60%), *trans*-cinnamic acid (83.3%) and rutin (43.90%).

Of the 10 phenolic compounds identified, only gallic acid, caffeic acid, catechin, and rutin changed significantly ($p < 0.05$) over the storage period (Figure 2). The contents of gallic and caffeic acids increased sharply up to 9 months from 0.16 to 0.28 mg 100 g^{-1} fw and 0.13 to 0.19 mg 100 g^{-1} fw, respectively, followed by a decrease to 0.18 mg 100 g^{-1} fw and 0.03 mg 100 g^{-1} fw, respectively, in the last 3 months of storage (Figure 2a and 2b).

The levels of catechin decreased from 0.98 to 0.48 mg 100 g^{-1} fw during 9 months and then increased up to 1.04 mg 100 g^{-1} fw at the end of the storage, while the contents of rutin decreased from 0.46 to 0.17 mg 100 g^{-1} fw over the storage period, with the

Table 2. The effect of the mangaba jelly processing on the profile of phenolic compounds recorded by HPLC-DAD/UV-Vis.

Number	Phenolics compounds	λ (nm)	RT†† (min)	Mangaba pulp† (mg 100g^{-1})	Mangaba jelly† (mg 100g^{-1})
1	Gallic acid	280	6.90	0.12 ± 0.01^{b}	0.16 ± 0.04^{a}
2	Catechin	280	11.40	1.70 ± 0.03^{a}	0.98 ± 0.03^{b}
3	Chlorogenic acid	280	13.50	0.32 ± 0.01^{a}	0.24 ± 0.01^{b}
4	Caffeic acid	280	15.96	0.35 ± 0.18^{a}	0.13 ± 0.01^{b}
5	Vanillin	280	17.34	n.d	n.d
6	*p*-Coumaric acid	280	18.88	0.06 ± 0.00^{b}	0.07 ± 0.00^{a}
7	Ferulic acid	280	23.35	0.27 ± 0.02^{a}	0.13 ± 0.00^{b}
8	*m*-Coumaric acid	280	29.31	0.13 ± 0.00^{a}	0.06 ± 0.00^{b}
9	*o*-Coumaric acid	280	36.63	n.d	n.d
10	Quercetin	280	41.14	3.13 ± 0.05^{a}	1.64 ± 0.06^{b}
11	*trans*-Cinnamic acid	280	52.69	0.06 ± 0.01^{a}	0.01 ± 0.01^{b}
12	Rutin	280	54.23	0.82 ± 0.00^{a}	0.46 ± 0.01^{b}

†The data are expressed as means ± standard deviation (n = 4) on a fresh weight basis (fw). Means within a line that are followed by different letters are significantly different according to the Student's t-test ($p < 0.05$); ††Retention time; $^{n.d}$ Indicate not detected.

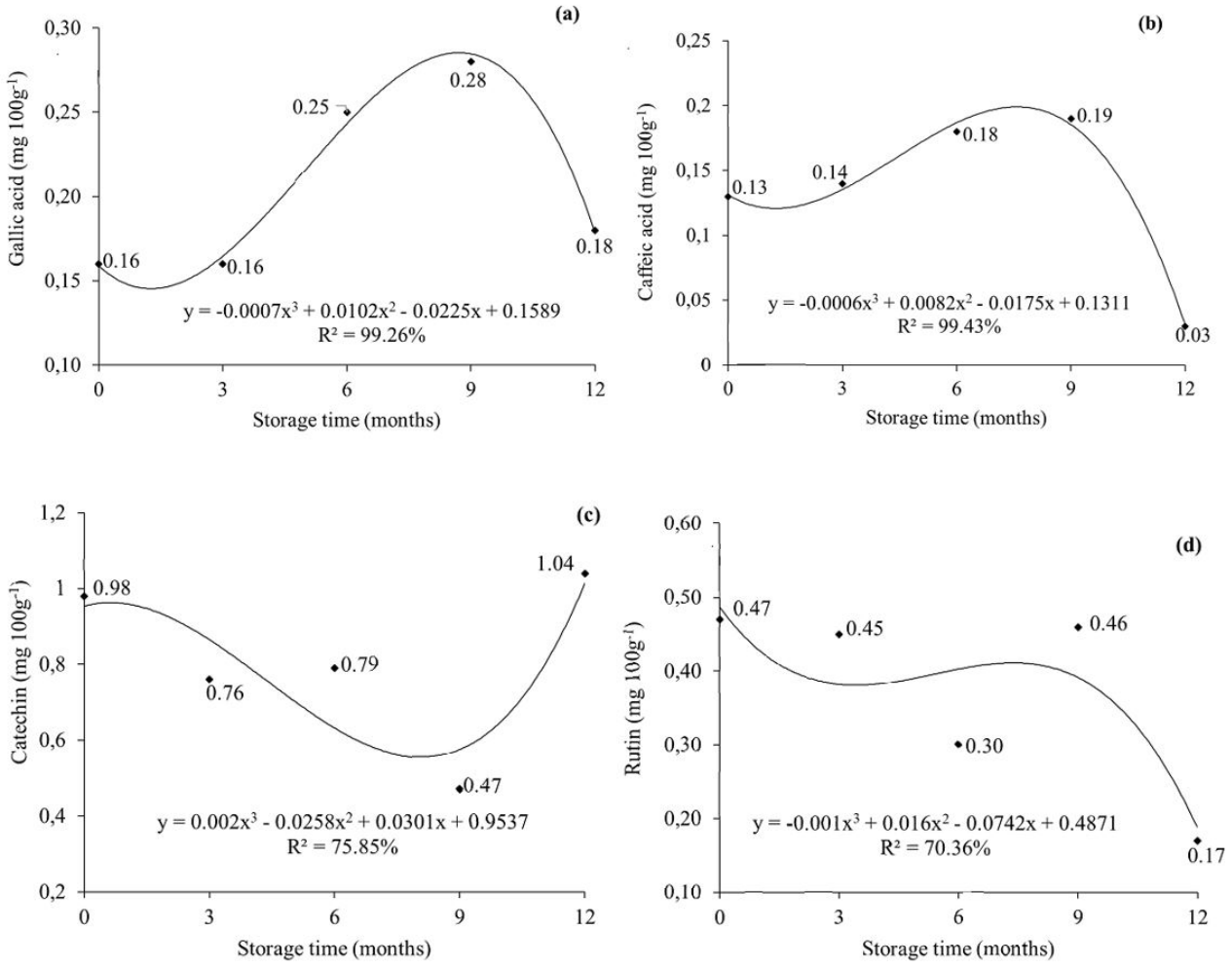

Figure 2. Changes in the profile of phenolic compounds recorded by HPLC-DAD/UV-Vis during 12 months of mangaba jelly storage at room temperature (22 ± 2 ºC, 72 ± 11% RH). (a) gallic acid; (b) caffeic acid; (c) catechin; (d) rutin. Values are means of four replications on a fresh weight basis (fw).

most pronounced decline observed between 9 and 12 months (Figure 2c and 2d).

The losses observed, as previously discussed, may be imputed to the degradation of the phenolic compounds, as a result of high temperatures used during processing (Abolila et al., 2015), as well, as changes in the pH, and in the oxidative process during the processing and storage.

Overall, the variations of the bioactive compounds found in this work could be due to several factors, including fruit maturity, geographic and environmental conditions, cultivar, storage conditions, as well as extraction and identification analytical procedures used. The results suggest that fresh and stored mangaba jelly may be considered as a potential novel functional food.

3.3 Antioxidant activity

The antioxidant activity of the samples was analyzed using DPPH and $ABTS^{\bullet+}$ radical scavenging activity and β-carotene bleaching. The antioxidant activity was significantly affected ($p < 0.05$) by the processing (Table 1) and storage (Figure 1), but not by the packages. The processing, significantly ($p < 0.05$) decreased the antioxidant activity (Table 1) when compared with the mangaba pulp. During 12 months of storage, the antioxidant activity presented a trend of decrease in the jelly (Figure 1, $p < 0.05$).

As shown in Table 1, the total antioxidant activity of fresh mangaba fruit by DPPH expressed as EC_{50} (953.67 g g^{-1} DPPH) was four-fold higher than that reported by Rufino et al. (2010), who found 3385 g g^{-1} DPPH. It is important to highlight that high values of EC_{50} indicate low antioxidant activity.

The antioxidant activities of the mangaba fruit pulp were 77.88 and 221.70 mg AAE 100 g^{-1}, assayed using DPPH and ABTS, respectively (Table 1). These values were lower and higher than those found in the mangaba fruit by Almeida et al. (2011) 118.78 and 162.57 mg AAE 100 g^{-1}, respectively. The antioxidant activities of the mangaba fruit pulp by ABTS and DPPH assay, expressed in TE, respectively (Table 1), were fourteen and seven-fold higher than those reported in the mangaba fruit by Almeida et al. (2011), 10.84 and 6.46 μmol TE g^{-1}, respectively.

The antioxidant activity of the mangaba pulp assayed by β-carotene bleaching method was 86.11% of oxidation inhibition (O.I.) (Table 1). According to Hassimotto et al. (2005), the antioxidant activity by the β-carotene bleaching method is classified as high, intermediate, or low when the levels of oxidation inhibition are >70%, 40 -70% or <40%, respectively. Therefore, following this classification, mangaba pulp has a high antioxidant activity.

The antioxidant activity by DPPH assay expressed as EC_{50} increased by 52% when comparing the mangaba pulp and jelly (Table 1), and 155% during 12 months of storage (Figure 1c). The trend of increase in the DPPH-EC_{50} over the storage period was more pronounced from the 6th month. Considering the higher EC_{50} the lower antioxidant activity, it can be noticed that after processing and also during the storage period, there was a reduction in the antioxidant activity.

The antioxidant activity by DPPH expressed as TE and AAE decreased significantly ($p < 0.05$) after processing about 32% and 44%, respectively (Table 1), and then decreased by about 46% and 10%, respectively, during 12 months of storage (Figure 1d and 1e). Thus, a trend of decrease of DPPH expressed as TE was observed over the storage period, more pronounced from the 6th month, whereas a trend of decrease of DPPH expressed as AAE was observed over nine months of storage, followed by an increase. However, DPPH expressed as AAE of jelly at 12th month was still bellow than that observed in fresh jelly.

The antioxidant activity determined by ABTS and expressed as TE and AAE decreased significantly ($p < 0.05$) about 33% and 54%, after processing (Table 1), and 23% and 48%, over the storage period, respectively (Figure 1f and 1g). When the antioxidant activity was expressed in TE, the most pronounced decrease was observed in the first 3 months, with a slight elevation in the last 3 months, and when it was expressed in AAE, the most pronounced decrease occurred from the 6th month of storage.

The oxidation inhibition measured by the β-carotene bleaching method decreased around 15% after processing, and 3.7% during 12 months of storage (Table 1 and Figure 1h). The trend of decrease of antioxidant activity observed over the storage period was more pronounced in the first 3 months. According to the classification proposed by Hassimotto et al. (2005), the mangaba jelly may be considered as a food with high antioxidant activity.

In general, the results of different antioxidant methods used in this work showed that the antioxidant activity decreased significantly ($p < 0.05$) after processing and also during the storage. These results were in accordance with the results of Tobal & Rodrigues (2019), who reported that the antioxidant activity in pitanga jams significantly decreased after processing and during 320 days of storage. In another study, Cunha et al. (2020) also reported a decrease of antioxidant activity in curriola jelly after processing and during 12 months of storage. The reduction of antioxidant activity could be due to the degradation or destruction of bioactive compounds, such as phenolic compounds and vitamin C, among other compounds, as a result of high temperatures and long cooking process (Cunha et al., 2020; Wang et al., 2020). Indeed, these results were consistent with a significantly decrease of vitamin C and TPC in mangaba jelly after processing and during the storage period.

3.4 Principal component analysis (PCA)

The PCA was performed in order to draw reliable conclusions regarding to the both effects, packages and storage time, on the bioactive compound contents and the antioxidant activity. The PCA scores (Figure 3A) and loadings (Figure 3B) plots show the distribution of each sample on the plan and the contribution of each variable to the PC1 and PC2, respectively. The first two main components accounted for 70.4% of the total variance among the samples and gave eigenvalues of 6.61 and 6.07, respectively (Table 3).

Based on the PCA score plot, it can be noticed that the mangaba jelly samples from both packages (amber and transparent glass jars) shared the same quadrant during each storage time which confirm that no significant differences were observed regarding packages, as previous discussed. The PCA loading plot (Figure 3B) shows that the PC1 (36.7%), was positively related to TPC, Vitamin C, DPPH (AAE), DPPH (TE), ABTS (AAE), quercetin and rutin and negatively with DPPH (EC_{50}). On the other hand, PC2 (33.7%), was mainly contributed by caffeic acid, ferulic acid, *m*-coumaric acid, *p*-coumaric acid, and gallic acid and negatively with DPPH (AAE), ABTS (TE), and Catechin.

Table 3. Eigenvalues, variability, cumulative and contribution of each variables to the factors (PC1 and PC2).

Variable	Loadings	
	PC1	PC2
β-carotene bleaching	0.50	-0.56
Total phenolics content (TPC)	0.99*	-0.07
Vitamin C (VIT C)	0.95*	-0.23
DPPH (EC_{50})	-0.87*	-0.23
DPPH (AAE)	0.42*	-0.87*
DPPH (TE)	0.91*	-0.03
ABTS (AAE)	0.86*	0.03
ABTS (TE)	0.49	-0.65*
Caffeic acid (CA)	0.38	0.87*
Chlorogenic acid (ChA)	-0.13	0.51
Ferulic acid (FA)	0.54	0.74*
m-Coumaric acid (mCoA)	0.24	0.58*
p-Coumaric acid (pCoA)	0.18	0.91*
trans-Cinnamic acid (tCinA)	0.00	0.41
Catechin (Ctn)	-0.13	-0.94*
Quercetin (Qtn)	0.69*	-0.16
Rutin (Rtn)	0.68*	0.41
Gallic acid (GA)	-0.40	0.71*
Eigenvalue	6.61	6.07
Total variance (%)	36.73	33.75
Cumulative variance (%)	36.73	70.48

* Significance level at $p \leq 0.05$; DPPH (EC_{50}), antioxidant activity of DPPH radical expressed as extract concentration providing half antioxidant activity; DPPH (AAE), antioxidant activity of DPPH radical expressed as ascorbic acid equivalent; DPPH (TE), antioxidant activity of DPPH radical expressed as trolox equivalent; ABTS (AAE), antioxidant activity of ABTS radical expressed as ascorbic acid equivalent; ABTS (TE), antioxidant activity of ABTS radical expressed as trolox equivalent.

3.5 Correlation of bioactive compounds and antioxidant activity

Pearson correlation analysis was used to understand the relationships between bioactive compounds and antioxidant activity (Table 4).

TPC was positively correlated (r = 0.887, 0.934 and 0.880, $p < 0.01$) with DPPH (EC_{50}), DPPH (TE), and ABTS (AAE) assays, respectively.

The vitamin C was positively correlated (r = 0.673, 0.735, 0.755, $p < 0.05$ and 0.831, $p < 0.01$) with β-carotene bleaching, DPPH (EC_{50}), ABTS (AAE) and DPPH (TE), respectively (Table 4). Ascorbic acid is an essential cofactor for many enzymes and one of the potent reducing agents and scavenger of free radical in biological systems. It has been suggested as a potential antioxidant that helps protection against human cell damage, as well as inflammation and cancer diseases (Smirnoff, 2018).

Catechin was positively correlated (r = 0.797, $p < 0.01$) with DPPH (AAE), whereas quercetin was positively correlated (r = 0.687, $p < 0.05$) with β-carotene bleaching. The catechin, due to its high antioxidant and antimutagenic properties, has also been reported to be beneficial in preventing and protecting against diseases caused by oxidative stress, including cancer, diabetes, cardiovascular and neurodegenerative diseases (Bernatoniene &

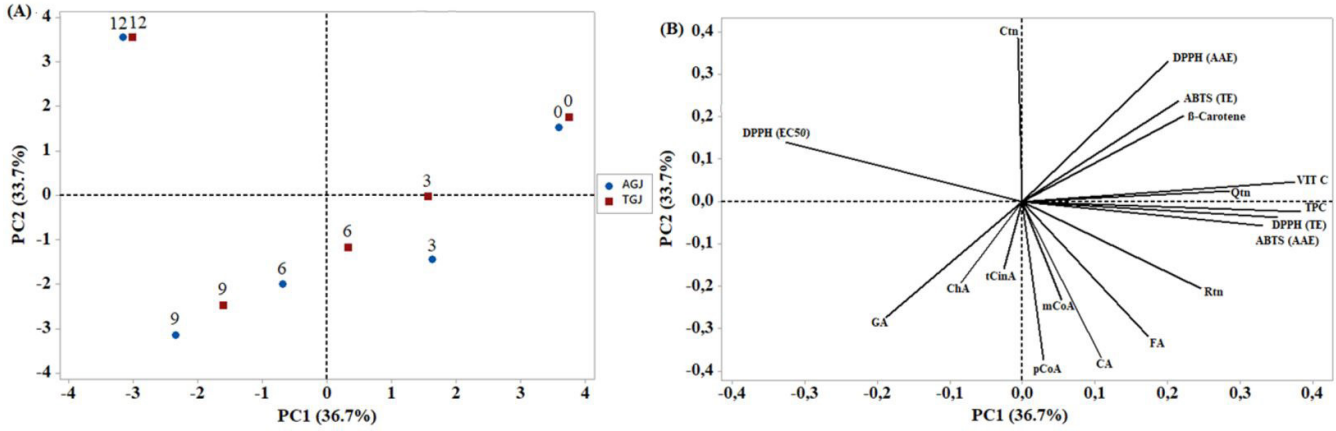

Figure 3. Principal component analysis (PCA) of studied 18 variables (bioactive compounds and antioxidant activity reported in Table 3) in mangaba jelly during storage. (A) PCA scores plot and (B) PCA loadings plot. Abbreviations: AGJ, amber glass jar; TGJ, transparent glass jar; CA, caffeic acid; ChA, chlorogenic acid; FA, ferulic acid; mCoA, *m*-coumaric acid; pCoA, *p*-coumaric acid; tCinA, *trans*-cinnamic acid; Ctn, catechin; Qtn, quercetin; Rtn, rutin; GA, gallic acid; β-carotene bleaching; VIT C, vitamin C; TPC, Total phenolics content; DPPH (AAE), antioxidant activity of DPPH radical expressed as ascorbic acid equivalent; ABTS (TE), antioxidant activity of ABTS radical expressed as trolox equivalent; DPPH (TE), antioxidant activity of DPPH radical expressed as trolox equivalent; ABTS (AAE), antioxidant activity of ABTS radical expressed as ascorbic acid equivalent; DPPH (EC_{50}), antioxidant activity of DPPH radical expressed as extract concentration providing half antioxidant activity.

Table 4. Pearson's correlations coefficients between the bioactive compounds and antioxidant activity of mangaba jelly during 12 months of storage at room temperature (22 ± 2 oC, 72 ± 11% RH).

	β-carotene bleaching	DPPH (EC_{50})	DPPH (AAE)	DPPH (TE)	ABTS (AAE)	ABTS (TE)
Total phenolics content (TPC)	0.499	0.877**	0.480	0.934**	0.880**	0.508
Vitamin C	0.673*	0.735*	0.592	0.831**	0.755*	0.615
Gallic acid	-0.520	0.203	-0.792*	-0.400	-0.331	-0.488
Catechin	0.303	0.231	0.797**	0.015	-0.021	0.503
Chlorogenic acid	-0.079	0.209	-0.425	-0.324	-0.323	-0.146
Caffeic acid	-0.264	-0.524	-0.578	0.317	0.359	-0.305
p-Coumaric acid	-0.266	-0.291	-0.693*	0.069	0.081	-0.374
Ferulic acid	-0.170	-0.683*	-0.353	0.527	0.515	-0.157
m-Coumaric acid	-0.102	-0.208	-0.382	0.082	0.072	-0.253
Quercetin	0.687*	-0.452	0.346	0.497	0.472	0.484
trans-Cinnamic acid	-0.555	-0.314	-0.228	0.236	0.246	-0.264
Rutin	0.421	-0.502	-0.129	0.410	0.383	0.154

* Significance level at $p \leq 0.05$; ** Significance level at $p \leq 0.01$. DPPH (EC_{50}), antioxidant activity of DPPH radical expressed as extract concentration providing half antioxidant activity; DPPH (AAE), antioxidant activity of DPPH radical expressed as ascorbic acid equivalent; DPPH (TE), antioxidant activity of DPPH radical expressed as trolox equivalent; ABTS (AAE), antioxidant activity of ABTS radical expressed as ascorbic acid equivalent; ABTS (TE), antioxidant activity of ABTS radical expressed as trolox equivalent.

Kopustinskiene, 2018). On the other hand, quercetin has gained greater attention for its role in the maintenance of the health of human beings, by lowering the risk of many lifestyle diseases, such as eye diseases, arthritis, allergic disorders, cardiovascular diseases, cancer and many more (Lakhanpal & Rai, 2007). Therefore, these results suggest that vitamin C, catechin, and quercetin may be responsible for presenting the most important contributions to the health-promoting effects of mangaba jelly.

4 Conclusions

The mangaba pulp is a vehicle of bioactive compounds with antioxidant activity. The processing and storage time result in a reduction of the bioactive compounds and antioxidant activity, while there is no significant different effect between amber and transparent packages. In spite of the significant decrease of the bioactive compounds, the mangaba jelly retained good levels of these compounds and high antioxidant activity during 12 months of storage and may be considered as a potential novel functional food

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