# **BIOACTIVE COMPOUNDS AND ANTIOXIDANT ACTIVITY OF MANGABA**

COMPOSTOS BIOATIVOS E ATIVIDADE ANTIOXIDANTE DE MANGABA

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**ABSTRACT:** The present study aimed to assess the behavior of bioactive compounds and total antioxidant activity of two mangaba varieties (*H. speciosa* var. *gardneri* and *H. speciosa* var. *cuyabensis*) during storage, in two ripening stages. The fruit were harvested from the Germplasm bank of the School of Agronomy, at two ripening stages: "mature green" (mature fruit picked from the tree) and "fallen" (ripe fruit collected from the ground). After collection, they were transported to the laboratory, washed under running water, immersed in chlorine solution at 100 mg L<sup>-1</sup> for 10 minutes, left to dry and stored under ambient conditions ( $22\pm1^{\circ}$ C and  $90\pm5\%$  RH). The fruit were analyzed to determine ascorbic acid content, total extractable polyphenols, yellow flavonoids and antioxidant activity using ferric reducing antioxidant power (FRAP) and ABTS (2,2'-azino-bis-(3-ethylbenzthiazoline-6-sulfonic acid). Analyses were performed daily until the mangaba were unfit for sale, using 3 repetitions with 3 fruit each. The maximum conservation time was eight days for "mature green" and two days for "fallen" fruit. The *cuyabensis* variety exhibited greatest ascorbic acid content at both ripening stages, in addition to higher antioxidant activity, and was therefore found to have the best potential to be marketed as a "superfruit".

KEYWORDS: Antioxidant capacity. Cerrado. H. speciosa var. cuyabensis. H. speciosa var. gardneri.

# **INTRODUCTION**

Certain areas of Brazil enable the cultivation of different types of fruit and are home to native and understudied species with the potential to become "superfruits". The most prominent of these regions is the Cerrado biome, the country's second largest area of vegetation with a wide variety of fruits known for their unique flavor and high nutritional value (LIMA, 2011).

include These the mangabeira tree (Hancornia speciosa Gomes), which produces an aromatic, nutritious and pleasant-tasting fruit (CALDAS et al., 2009), known as the mangaba. The industrial potential of this fruit lies in the fact that it contains around 77% flesh, on average, and only 23% skin and seeds. The mangaba is valued by the local community, but commercially underexplored; it is generally consumed fresh or processed for juice only by the population closest to where it is harvested. Its high nutritional value is due to the mangaba's high levels of vitamins A, B1, B2, and C, iron, phosphorus, calcium and bioactive compounds (SOARES et al., 2004).

Moreover, the consumption of fruit with functional properties has increased, due to the presence of bioactive compounds that exhibit additional physiological effects, even in small amounts (LIMA et al., 2004; MELO et al., 2008). As such, mangaba has the potential to be classified as a "superfruit" and its consumption could provide a variety of health benefits, including a lower incidence of degenerative and cardiovascular diseases (CARDOSO, 2011; PRIOR et al., 2005; XIAO, 2016), thus demonstrating its potential for the consumer market.

However, it is still essentially an undomesticated species and there are almost no organized fruit orchards aimed at its rational exploitation for production (GANGA et al., 2009). In order to reach new markets, it is essential to implement commercial orchards since current production is unable to meet the demands of even local consumers (LEDERMAN et al., 2000).

In this respect, in 2005 the School of Agronomy of the Federal University of Goiás implemented the mangaba germplasm, with seeds from more than 100 mother plants located in the states of Goiás, Tocantins, Bahia, Mato Grosso and Mato Grosso do Sul. Ninety progenies of halfbrothers of these mother plants were planted in the field (GANGA, 2008). In 2010, another collection was compiled, consisting of the progenies of 116 mother plants from different regions in Goiás state (VIEIRA, 2011), enabling studies to be conducted on the domestication of these species (GANGA et al., 2009; COLLEVATTI et al., 2016).

The short shelf life of mangaba may be exacerbated by the fact that the fruit is manually harvested once it has fallen to the ground. This results in mechanical damage to the fragile skin, accelerating metabolism and reducing storage time (MOKADY et al., 1984), in addition to degrading bioactive compounds, which limits the commercial exploitation of the crop for specific markets (VIEIRA et al., 2010).

The first step is to harvest the fruit directly from the plant at a suitable ripening stage for the species (MATTOS; ALMEIDA, 2006), in order to ensure longer storage times and enable distribution. In this regard, Vieira Neto et al. (2002) reported that mangaba fruit harvested directly from the mother tree ("mature green") can be stored until fully ripe, extending the commercialization period to 12 days in relation to those collected from the ground (6 days). This is attributed to the reduction in mechanical injury caused by natural falling (CARNELOSSI et al., 2004; SANTOS et al., 2009; SILVA et al., 2013).

During ripening, fruit undergoes physical, chemical, biochemical and physiological changes that lead to visible modifications in color, flavor, aroma and texture (PRASANNA et al., 2007). Several authors have identified notable differences in the bioactive compound content and antioxidant activity of other types of fruit at different ripening stages (SANTOS, 2011; MORGADO et al., 2010; PARK et al., 2006; ZHANG et al., 2006). However, there are no published studies investigating the metabolism of bioactive compounds during storage of mangaba fruit harvested at different times, and this information is vital to ensuring it can be marketed as a "superfruit" and reach a wider market.

The literature on mangaba does not identify the variety used, and the genetic factor of each variety can influence storage time as well as the behavior of bioactives, since ripening characteristics are affected not only by climate conditions and crop management systems, but genetic characteristics (SOUZA, 2012; LEE; KADER, 2000). Thus, the present study aimed to assess the behavior of bioactive compounds and total antioxidant activity of two mangaba varieties (*H. speciosa* var. gardneri and *H. speciosa* var. cuyabensis) during storage, in two ripening stages.

### MATERIAL AND METHODS

#### Fruit material

Fruit of both varieties (*Hancornia speciosa* var. *gardneri* and *H. speciosa* var. *cuyabensis*) were harvested in the early morning at the germplasm belonging to the School of Agronomy of the Federal University of Goiás, EA-UFG (16°35"39" S latitude, 49°17"07" W longitude and altitude of 733 m). They were collected at two ripening stages: stage 1 - "mature green" (mature fruit picked from the tree) and stage 2 - "fallen" (ripe fruit collected from the ground, after shaking the trees), with 90 mangaba fruit of each variety collected for each stage.

After harvesting, the fruit were stored in plastic crates covered with bubble wrap and transported to the laboratory of the EA-UFG Horticulture Department, where they were selected and standardized, using only intact fruit (no insect damage, visible lesions or symptoms of disease) with uniform coloring for each ripening stage. The mangaba were washed under running water, immersed in a sodium hypochlorite solution at 100 mg L<sup>-1</sup> for 10 minutes, then dried and stored at  $22\pm1^{\circ}$ C and  $90\pm5\%$  relative humidity (RH).

The fruit were chemically and physically characterized at the start of the experiment (Table 1).

### **Experimental design**

A completely randomized design was used, with a double factorial scheme as follows: I - days of storage; II - variety, for each ripening stage ("mature green" and "fallen"). The fruit were assessed daily until they became unfit for consumption, using three repetitions with three mangaba each. The fruit were analyzed to determine ascorbic acid content, total extractable polyphenols, yellow flavonoids and antioxidant activity using ferric reducing antioxidant power (FRAP) and ABTS.

### Assessments

### Initial mangaba characterization

Soluble solid (SS) content was quantified in drops extracted from the ground pulp through direct reading on a handheld refractometer (Instrutemp ITREF 82), with results expressed in percent (AOAC, 2012 - method 932.12).

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Variety	SS*	ТА	SS/TA	FIR		
Ripening stage – "mature green"						
Gardneri	$15.8 \ 3 \pm 0.76^{*}$	$0.64 \pm 0.05$	$25.05 \pm 3.01$	$2.11 \pm 0.39$		
Cuyabensis	$16.67 \pm 0.76$	$0.74 \pm 0.08$	$22.60 \pm 3.05$	$2.46 \pm 0.64$		
	R	ipening stage – "falle	n"			
Gardneri	$19.00 \pm 0.00$	$0.93 \pm 0.06$	$20.40 \pm 1.20$	$0.10 \pm 0.01$		
Cuvabensis	$21.83 \pm 1.04$	$1.05 \pm 0.10$	$20.84 \pm 1.86$	$0.17 \pm 0.03$		

**Table 1.** Mean values for soluble solids (SS), titratable acidity (TA), maturity index (SS/TA), and firmness (FIR) of two *Hancornia speciosa* varieties, harvested at two ripening stages from the germplasm belonging to the School of Agronomy of the Federal University of Goiás.

\*Means followed by standard deviation. Nine fruit were sampled.

Titratable acidity (TA) was quantified in 5 grams of ground pulp diluted in 45 mL of distilled water, by titration with 0.1 mol  $L^{-1}$  NaOH using phenolphthalein as indicator. The results were expressed in percent citric acid (AOAC, 2012-method 942.15).

The maturity index (SS/TA) was calculated by dividing SS values by TA.

Firmness was determined using applanation tonometry in accordance with Calbo; Nery (1995), whereby the force needed to flatten a circular area (A= 0.784 x greatest length x smallest length) of the fruit was calculated by F/A, where F is the force exerted by the glass plate (based on its weight); the result was expressed in Newton (N).

### **Bioactive compound analyses**

Ascorbic acid content was determined by titration, in line with Strohecker; Henning (1967). A 0.5-gram sample of ground pulp was weighed and added with 10 ml of cold 0.5% oxalic acid, followed by titration with a 2.6 dichlorophenolindophenol solution (DFNa) until pink coloring was visible. The results were expressed in mg of ascorbic acid 100 g<sup>-1</sup> of pulp.

Total extractable polyphenol (TEP) levels were measured using the method proposed by Obanda; Owuor (1997). A 4.0-gram sample of ground pulp was weighed, added with 20 ml of 50% methanol and left to stand for an hour. Next, it was centrifuged and 20 ml of 70% acetone was added to the residue and left for one hour. The sample was then centrifuged again and the extract obtained. TEP was quantified using the Folin-Ciocalteu method and the results were expressed in milligrams of gallic acid 100 g<sup>-1</sup> of pulp.

Yellow flavonoids were determined according Francis (1982). A 4.0-gram sample of ground pulp was weighed, added with 30 ml of ethanol (95%)-HCl (1.5 M) at a proportion of 85:15, and mixed for 2 minutes. Next, the volume was completed to 50 ml with ethanol-HCL (1.5 M) and left overnight in a refrigerator. The extract was filtered and absorbance read at 374 nm. The results were expressed in milligrams 100 g<sup>-1</sup> of pulp.

Total antioxidant activity was determined in the same extract used for TEP, by Fe<sup>3</sup>+ reduction (FRAP - ferric-reducing antioxidant power). This method is based on a reduction reaction (in acid) of the ferric tripyridyltriazine complex to the ferrous complex, whose color changes to blue in the presence of an antioxidant (Benzie and Strain, 1996; Pulido et al., 2000). The results were expressed in  $\mu$ M of ferrous sulfate g<sup>-1</sup> of pulp for the FRAP assay and  $\mu$ M of trolox g<sup>-1</sup> of pulp for ABTS free radical scavenging (2,2'-azino-bis-(3-ethylbenzthiazoline-6-sulfonic acid), as described by Rufino (2008).

# Statistical analysis

# Univariate analysis

The results were statistically analyzed using SISVAR software (FERREIRA, 2014). The data were submitted to analysis of variance by the F-test and the means were assessed throughout storage using Tukey's test at 5% significance.

# Multivariate analysis

*Hierarchical clustering.* Hierarchical clustering analysis was performed for both the varieties studied, using the Euclidean distance as similarity coefficient and Ward's algorithm as clustering strategy (HAIR et al., 2009) to identify the differences between varieties at each ripening stage.

Principal components analysis (PCA). The PCA technique generates orthogonal latent variables, centered in the region with the highest variability. To that end, a covariance matrix was calculated and eigenvalues extracted. These generate eigenvectors (principal components or PCs), which are linear combinations of the original variables. The Kaiser criterion was applied considering eigenvalues >1, which produce components containing at least as much information as the original data (KAISER, 1958). Hierarchical clustering and principal component analysis were performed using Statistica software (STATSOFT INC., 2004).

### RESULTS

#### Univariate analysis

#### "Mature green" fruit

The *cuyabensis* variety obtained the highest ascorbic acid concentrations, total extractable polyphenols and total antioxidant activity, measured by FRAP and ABTS. Yellow flavonoid content did not differ significantly between the two varieties studied (Table 2).

**Table 2.** Ascorbic acid content (AA), yellow flavonoids (FLAV), total extractable polyphenols (TEP) and antioxidant activity determined by FRAP and ABTS, for two *Hancornia speciosa* varieties harvested at the "mature green" ripening stage from the germplasm bank of the School of Agronomy of the Federal University of Goiás and stored at 22+1°C and 90+5% RH

Varieties	AA	FLAV	TEP	FRAP	ABTS
Gardneri	140.45 b	3.65 a	32.22 b	12.38 b	5.74 b
Cuyabensis	168.03 a	3.86 a	41.79 a	16.53 a	9.41 a
F-Test	25.60**	1.50 <sup>NS</sup>	37.88**	267.34**	911.29**
LSD	11.06	0.36	3.15	0.52	0.25
Days					
0	136.03 c	3.40 bc	48.91 a	15.88 a	11.05 a
1	151.43 abc	2.79 bc	29.28 d	15.89 a	4.85 e
2	141.34 bc	2.62 c	29.20 d	11.38 c	5.28 e
3	186.72 a	3.26 bc	37.40 bcd	14.09 b	7.20 d
4	153.31 abc	3.79 bc	39.07 abcd	14.96 ab	9.07 b
5	133.66 c	3.86 b	30.66 cd	11.38 c	7.77 cd
6	178.56 ab	3.62 bc	33.50 bcd	15.56 ab	7.22 d
7	187.77 a	3.83 bc	41.11 abc	14.97 ab	8.24 bc
8	119.59 c	6.65 a	43.93 ab	15.99 a	7.52 cd
F-Test	9.14**	19.72**	8.83**	23.40**	104.62**
LSD	38.14	1.23	10.88	1.78	0.85
Interaction (5%)	$2.60^{*}$	1.96 <sup>NS</sup>	5.08**	14.47**	92.35**
CV (%)	12.99	17.21	15.44	6.45	5.89

Interaction (5%) is the result of species-day interaction. Means followed by at least one same letter in a column do not differ according to Tukey's test (p>0.05). \*\* = significant at 1% probability; \* = significant at 5% probability according to the F-test. <sup>NS</sup> = not significant. AA Ascorbic acid (mg ascorbic acid 100 g<sup>-1</sup> of pulp), FLAV yellow flavonoids (mg 100 g<sup>-1</sup> of pulp), TEP total extractable polyphenols (mg gallic acid 100 g<sup>-1</sup> of pulp) and FRAP and ABTS are antioxidant activity by ferric reducing antioxidant power - FRAP ( $\mu$ M of ferrous sulfate g<sup>-1</sup> of pulp) and ABTS free radical scavenging ( $\mu$ M of trolox g<sup>-1</sup> of pulp), respectively.

Ascorbic acid content in the *gardneri* variety fluctuated during storage, with a significant increase on day 7 (54% higher in relation to day zero) and a decline the following day. The highest level for the *cuyabensis* variety was recorded on the third day (39% greater than day zero), followed by a decrease from day 6 until the end of storage (Table 3).

Yellow flavonoid content remained stable for both varieties until day 7; however, there was a significant increase from 3.03 to 7.45 mg 100 g<sup>-1</sup> of pulp for *gardneri* and 3.77 to 5.85 mg 100 g<sup>-1</sup> of pulp for *cuyabensis* on the last day (Table 3).

Total extractable polyphenol content varied significantly during storage of the *gardneri* variety, whereas no significant differences were observed between storage days for *cuyabensis* (Table 3).

Total antioxidant activity was determined by FRAP for both varieties (*gardneri* and *cuyabensis*) and fluctuated during storage; however, at the end of the storage period, differences were not significant when compared to activity on harvesting day. With respect to AA assessed using the ABTS method, both varieties showed a trend toward decreasing antioxidant activity during storage (Table 3).

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Table 3. Behavior of ascorbic acid content (AA), yellow flavonoids (FLAV), total extractable polyphenols (TEP) and antioxidant activity determined by
FRAP and ABTS, for two Hancornia speciosa varieties harvested at the "mature green" ripening stage from the germplasm bank of the School of Agronomy
of the Federal University of Goiás, and stored at 22±1°C and 90±5% RH.

Variety					Days				
-	0	1	2	3	4	5	6	7	8
				Ascorbi	ic acid				
Gardneri	118.9bBC	149.7aABC	121.4bBC	160.6bAB	149.6aABC	100.9bC	154.2bABC	182.7aA	125.5aBC
Cuyabensis	153.1aBC	153.1aBC	161.2aABC	212.7aA	156.9aBC	166.3aABC	202.8aAB	192.3aAB	113.6aC
				Yellow fla	avonoids				
Gardneri	3.03aB	2.27 aB	2.42 aB	3.18 aB	3.58 aB	3.77 aB	3.54 aB	3.62 aB	7.45 aA
Cuyabensis	3.77aB	3.31 aB	2.81 aB	3.35 aB	3.99 aB	3.96 aB	3.71 aB	4.04 aB	5.85 bA
			Т	otal extractab	le polyphenols				
Gardneri	55.97 aA	20.49 bC	20.80 bC	27.69bBC	32.66 bBC	22.46 bC	29.46aBC	38.34 aB	42.10aAB
Cuyabensis	41.86 bA	38.06 aA	37.59 aA	47.11 aA	45.48 aA	38.85 aA	37.54 aA	43.88 aA	45.76 aA
•				FRAP					
Gardneri	14.07bABC	15.89aA	7.62bD	12.06bC	13.20bBC	6.52bD	13.72bABC	13.19bBC	15.15bAB
Cuyabensis	17.69 aA	15.89 aAB	15.14 aB	16.12aAB	16.71 aAB	16.26aAB	17.40aAB	16.76aAB	16.83aAB
•				ABTS					
Gardneri	11.48 aA	3.19 bD	3.13 bD	3.45 bD	7.48 bB	2.87 bD	4.66 bC	8.19 aB	7.26 aB
Cuyabensis	10.62 bB	7.37 aCD	6.57 aD	10.95 aB	10.66 aB	12.67 aA	9.79 aB	8.29 aC	7.78 aC

Means followed by the same letter, upper case in lines and lower case in columns, do not differ according to Tukey's test (p>0.05). Ascorbic acid (mg ascorbic acid 100 g<sup>-1</sup> of pulp), yellow flavonoids (mg 100 g<sup>-1</sup> of pulp), total extractable polyphenols (mg gallic acid 100 g<sup>-1</sup> of pulp) and antioxidant activity by ferric reducing antioxidant power - FRAP ( $\mu$ M of ferrous sulfate g<sup>-1</sup> of pulp) and ABTS free radical scavenging ( $\mu$ M of trolox g<sup>-1</sup> of pulp).

# "Fallen" fruit

The highest ascorbic acid concentrations were recorded in the *cuyabensis* variety, while *gardneri* obtained the highest total extractable

polyphenol levels. With respect to yellow flavonoids and total antioxidant activity (FRAP and ABTS), there were no significant differences

between the two varieties (Table 4).

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**Table 4.** Ascorbic acid content (AA), yellow flavonoids (FLAV), total extractable polyphenols (TEP) and antioxidant activity determined by FRAP and ABTS, for two *Hancornia speciosa* varieties harvested at the "fallen" ripening stage from the germplasm bank of the School of Agronomy of the Federal University of Goiás, and stored at 22±1°C and 90±5% RH.

Varieties	AA	FLAV	TEP	FRAP	ABTS
Gardneri	171.95 b	7.61 a	49.35 a	18.11 a	12.10 a
Cuyabensis	198.98 a	8.12 a	44.51 b	18.02 a	11.41 a
F-Test	23.09**	0.84 <sup>NS</sup>	18.76**	$0.40^{NS}$	1.91 <sup>NS</sup>
LSD	12.26	1.25	2.43	0.31	1.09
Days					
0	161.51 b	8.82 a	41.62 c	18.66 a	13.98 a
1	194.15 a	7.40 a	45.74 b	17.42 c	10.71 b
2	200.74 a	7.38 a	53.44 a	18.12 b	10.58 b
F-Test	18.59**	2.79 <sup>NS</sup>	38.39**	25.67**	19.68**
LSD	18.39	1.88	3.65	0.46	1.64
Interaction (5%)	0.98 <sup>NS</sup>	5.18*	71.56**	7.17**	3.38 <sup>NS</sup>
CV (%)	6.43	15.43	5.05	1.65	9.06

Interaction (5%) is the result of species-day interaction. Means followed by at least one same letter in a column do not differ according to Tukey's test (p>0.05). \*\* = significant at 1% probability; \* = significant at 5% probability according to the F test. NS = not significant. AA Ascorbic acid (mg ascorbic acid 100 g<sup>-1</sup> of pulp), FLAV yellow flavonoids (mg 100 g<sup>-1</sup> of pulp), TEP total extractable polyphenols (mg gallic acid 100 g<sup>-1</sup> of pulp) and FRAP and ABTS are antioxidant activity by ferric reducing antioxidant power - FRAP ( $\mu$ M of ferrous sulfate g<sup>-1</sup> of pulp) and ABTS free radical scavenging ( $\mu$ M of trolox g<sup>-1</sup> of pulp), respectively.

Ascorbic acid content increased significantly during storage, with a 23% and 26% rise in relation to day zero for the *gardneri* and *cuyabensis* varieties, respectively. Yellow flavonoid content showed no significant changes during storage, whereas a significant decline was observed for *cuyabensis* (Table 5).

Total extractable polyphenol levels fluctuated over the storage period; however, there was no difference between them on the last day of storage and harvesting day, with TEP content increasing during storage for the *cuyabensis* variety (Table 5).

Total antioxidant activity for *gardneri*, determined by FRAP, declined by 7% during storage, while *cuyabensis* mangaba exhibited reduced AA on the first day followed by an increase after 2 days (Table 5). When AA was measured by ABTS, both varieties exhibited declining levels during storage, with a 45% and 21% decrease for *gardneri* and *cuyabensis*, respectively.

# Multivariate analysis

Hierarchical clustering was used to assess the behavior of the *gardneri* and *cuyabensis* varieties for the different ripening stages. The dendrogram generated clearly shows the formation of 4 distinct clusters when a Euclidean distance of 5 is used as the cutoff point. The groups formed in accordance with the variety and ripening stage. Thus, three groups of interest were identified among the 4 clusters, with group 1 consisting of *gardneri* mangaba picked in the "mature green" stage, group 2 of ripe fruit from both varieties, and group 3 of "fallen" *cuyabensis* mangaba (Figure 1).

The first two principal components (PC1 and PC2) explained 79.86% of the total variability of the original bioactive compound and total antioxidant activity data (Figure 2). PC1 revealed that all the bioactive compounds (ascorbic acid content, yellow flavonoids and extractable polyphenols) and total total antioxidant activity determined by FRAP and ABTS were positively correlated. The formation of three groups was observed (Figure 2), namely: Group 1: "Fallen", independent of the variety studied (positioned to the left of PC1 - negative side), Group 2: "Mature green", cuyabensis variety (in the center of the biplot) and Group 3: "Mature green", gardneri variety (to the right of PC1 –positive side).

**Table 5.** Behavior of ascorbic acid content (AA), yellow flavonoids (FLAV), total extractable polyphenols (TEP) and antioxidant activity determined by FRAP and ABTS, for two *Hancornia speciosa* varieties harvested at the "fallen" ripening stage from the germplasm bank of the School of Agronomy of the Federal University of Goiás, and stored at 22±1°C and 90±5% RH.

Variety		Days	
	0	1	2
	Ascor	bic acid	
gardneri	152.69 aB	175.67 bAB	187.49 bA
cuyabensis	170.33 aB	212.63 aA	213.98 aA
·	Yellow f	lavonoids	
gardneri	8.41 aA	6.10 bA	8.31 aA
cuyabensis	9.24 aA	8.71 aAB	6.44 aB
	Total extracta	ble polyphenols	
gardneri	53.49 aA	43.67 aB	50.90 bA
cuyabensis	29.76 bC	47.81 aB	55.98 aA
-	FI	RAP	
gardneri	18.99 aA	17.54 aB	17.80 bB
cuyabensis	18.34 bA	17.30 aB	18.43 aA
	Al	BTS	
gardneri	14.53 aA	11.74 aB	10.04 aB
cuyabensis	13.44 aA	9.68 bB	11.11 aB

Means followed by the same letter, upper case in lines and lower case in columns, do not differ according to Tukey's test (p>0.05). Ascorbic acid (mg ascorbic acid 100 g<sup>-1</sup> of pulp), yellow flavonoids (mg 100 g<sup>-1</sup> of pulp), total extractable polyphenols (mg gallic acid 100 g<sup>-1</sup> of pulp) and antioxidant activity by ferric reducing antioxidant power - FRAP ( $\mu$ M of ferrous sulfate g<sup>-1</sup> of pulp) and ABTS free radical scavenging ( $\mu$ M of trolox g<sup>-1</sup> of pulp).

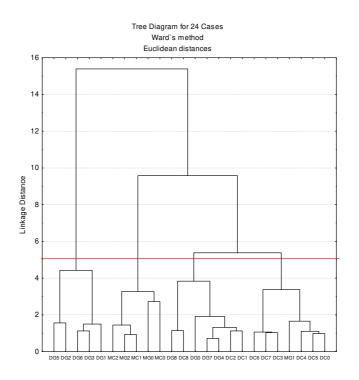


Figure 1. Hierarchical clustering analysis of two "mature green" and "fallen" *Hancornia speciosa* varieties harvested from the germplasm bank of the School of Agronomy of the Federal University of Goiás, and stored at 22°C and 90% RH. The letter D corresponds to the "mature green" stage; M to "fallen" fruit; C to the *cuyabensis* variety and G to *gardneri*.

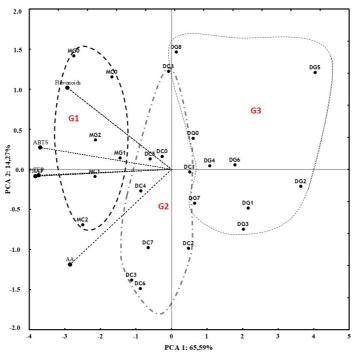


Figure 2. Principal Component Analysis of two "mature green" and "fallen" *Hancornia speciosa* varieties harvested from the germplasm bank of the School of Agronomy of the Federal University of Goiás, and stored at 22°C and 90% RH. The letter D corresponds to the "mature green" stage; M to "fallen" fruit; C to the *cuyabensis* variety and G to *gardneri*. G1, G2 and G3 correspond to groups 1, 2 and 3, respectively.

Thus, principal component analysis (PCA) was performed to visualize the distribution of samples within a two dimensional plane and analyze the power of each parameter based on the vectors formed (Figure 2), in order to better explain the division between the varieties and ripening stages in the dendrogram and indicate which variables interfered in cluster formation (Figure 1).

# DISCUSSION

The shelf life of "fallen" mangaba stored at 22°C was only two days, regardless of variety. When the two varieties were harvested at the "mature green" stage and stored at the same temperature their shelf life increased to eight days. The maximum conservation period for "mature green" fruit was eight days, similar to the 9-day shelf life reported by Campos et al. (2011) for "mature green" mangaba stored under ambient conditions. By contrast, the shelf life of ripe mangaba in this study was only two days, similar to the result obtained by Carnelossi et al. (2004) for ripe fruit stored at 25°C (3-day shelf life) and different from Mariano-Nasser et al. (2018), who found a 6-day storage period for fallen (mature) mangabas, regardless of storage conditions. Harvesting ripe fruit shortened their shelf life due to the difficulties involved in handling them and their low physical resistance (CHITARRA; CHITARRA, 2005).

Given that they were collected from the ground, the ripe fruit had been damaged by the fall from the tree and therefore entered senescence more quickly when compared to "mature green" fruit. Additionally, mangaba collected from the ground had undergone fermentation (because it was impossible to know how many days had passed since they had fallen from the tree), which also contributed to their reaching senescence. These results demonstrate the importance of harvesting at physiological maturity ("mature green") in order to increase the shelf life of mangaba.

# Univariate analysis

The AA levels of mangaba increased during storage (Tables 2 and 4); this is unusual behavior for fruit, which typically show a decline in AA after harvesting. However, ascorbic acid can remain constant or increase during storage for some types of fruit (CAVALINI, 2008; CAVALINI et al., 2006), as observed in the two mangaba varieties studied here. Increased AA in fruit may be related to the action of vitamin C as an antioxidant in response

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to oxidative reactions that occur during ripening (CARNELOSSI et al., 2004).

The ascorbic acid levels recorded for the gardneri and cuyabensis varieties, regardless of ripening stage (Tables 2 and 4), were lower than those reported by Carnelossi et al. (2004) of 274.7 mg ascorbic acid/100 g of pulp for "mature green" fruit and 252.7 mg ascorbic acid 100 g<sup>-1</sup> of pulp for "fallen" fruit. By contrast, Rufino et al. (2010) found similar values (190 mg ascorbic acid 100 g<sup>-1</sup> of pulp) to those recorded here for ripe mangaba. Almeida et al. (2011) found lower values (96.3 mg ascorbic acid 100 g<sup>-1</sup>) than those of this study. However, AA levels of both the varieties analyzed were higher than those found in fruit considered "superfruits" such as blueberries (18.15 mg 100 g<sup>-1</sup>), according to Silveira et al. (2007), and strawberries (43.66 mg ascorbic acid 100  $g^{-1}$ ), in accordance with Cunha Junior et al. (2017).

From a nutritional perspective, high vitamin C levels in fruit are a desirable and important characteristic (SILVEIRA et al., 2007), meaning the mangaba has significant potential to provide consumers with numerous health benefits. Mendes (2015) compiled data on the nutritional composition of native fruit species and found a vitamin C content of 332 mg 100 g<sup>-1</sup> for mangaba, second only to camu camu (1888.2 mg 100 g<sup>-1</sup>). Thus, it shows potential as a "superfruit", since according to Ohr (2012), superfruits have high nutritional value due to their abundant nutrients and antioxidant properties, in addition to consumer appeal and a tendency to be more exotic.

Flavonoids are an important class of natural pigments that frequently occur in nature both in free form and as glycines associated with sugars and tannins, exclusively in plants (BOBBIO; BOBBIO, 2001). These non-nutritious compounds display antioxidant, antimutagenic and anticancer activity in different systems (ANDERSON et al., 2000).

In this study, flavonoid values did not differ between varieties in either ripening stage (Tables 2 and 4). Flavonoid content in "mature green" mangaba increased considerably on the last day of storage, possibly because of the biochemical changes that occur during ripening, such as the enzymatic degradation of chlorophyll and emergence of other colors due to the pigments responsible for fruit coloring (TADESSE et al., 1999). Moreover, yellow flavonoid values were five times higher in the ripe mangaba than "mature green" fruit and similar to those reported by Rufino et al. (2010) of 15 mg 100 g<sup>-1</sup> of pulp. Pedrosa (2018) also found differences in the flavonoid content of mature green mangaba varieties, with an average value of 2.41 mg 100 g<sup>-1</sup> of pulp, which was lower than that obtained here.

Total extractable polyphenols (TEP) in "mature green" fruit were higher in the *cuyabensis* variety (Table 2) because phenolic compound levels vary between fruits. This variation (quantitative and qualitative) is associated with both intrinsic (variety and ripening stages) and extrinsic factors (soil and climate conditions) (MELO et al., 2008).

The PET levels recorded in this study were lower than those recorded by Rufino et al. (2010), who reported 170 mg gallic acid 100 g<sup>-1</sup> of pulp in fresh mangaba, albeit without specifying the variety. By contrast, Rocha et al. (2011) found similar levels to those obtained here for both varieties at the "mature green stage, although the authors did not identify the variety. These differences reinforce the need to study mangaba varieties separately since physiological behavior differs between varieties (GANGA et al., 2009), meaning that research conducted without identifying the variety may produce different results.

Ripe *cuyabensis* fruit exhibited a rise in TEP values during storage, possibly because these compounds are largely responsible for the color and flavor of different fruit (CHEYNIER, 2012), and the biochemical changes that occur during ripening may cause an increase in polyphenol levels.

Antioxidant activity measured by FRAP in "fallen" fruit was similar to that reported by Rufino et al. (2010), although the authors obtained higher values than those observed here for "mature green" mangaba. Antioxidant activity varies according to the variety studied, crop treatments applied in the field, processing method used and the ripening stage at which the fruit was harvested.

Total AA determined by ABTS revealed lower values for both ripening stages and varieties when compared to those observed by Rufino et al. (2010). However, Lima (2011) evaluated the behavior of antioxidant activity in mangaba during storage and corroborated the results obtained here for each variety (Table 3), namely a tendency for values to decline across the storage period.

Results for undomesticated fruit show substantial differences in absolute values, as demonstrated in this study. Since these plants are largely in their natural habitat, factors such as location, pre-and post-harvest management strategies, sun exposure, climate and ripening stage influence the composition of these fruit (MARTINS et al., 2011).

The genetic differences of each mangaba variety are also important factors in the metabolism of antioxidant compounds, as highlighted by Silva et

al. (2015), who studied fruit from different accessions of the Embrapa Tabuleiros Costeiros Mangaba Germplasm, obtaining values of 3.91 to 12.20  $\mu$ M of trolox g<sup>-1</sup>. It is important to underscore that changing the harvesting time, that is, no longer using fallen fruit, results in a four-fold longer shelf life and a fruit that is still rich in these healthy compounds on the eighth day. Both Pedrosa (2018) and Zuninga et al. (2018) found that mangaba exhibit high antioxidant capacity, confirming its potential as a "superfruit".

It is noteworthy that the bioactive compounds and total antioxidant activity of both mangaba varieties were little affected by storage, demonstrating its potential since these components were still present in significant amounts after 8 days of storage ("mature green").

### **Multivariate analysis**

Although univariate analysis proved highly informative, the approach did not make it possible to identify the relationships between quality parameters.

Analysis of the biplot indicated that all the parameters assessed acted positively on the left side. This demonstrates that, regardless of variety, the ripe mangaba collected showed higher ascorbic acid levels, yellow flavonoid content, total extractable polyphenols and total antioxidant activity (Figure 2), confirming the results of the univariate analysis.

The fact that ripe mangaba contained more bioactive compounds and showed greater antioxidant activity when compared to "mature green" fruit can be explained by the chemical and biochemical changes that occur during ripening. Moreover, AA generally increases as ripeness progresses (CARNELOSSI et al., 2004), as observed in this study, where ripe mangaba showed greater antioxidant activity irrespective of variety.

Another important point is the influence of the genetic factor on the quality parameters assessed in the two varieties. In the *cuyabensis* variety, higher ascorbic acid levels, total extractable polyphenols and total antioxidant activity were observed in "mature green" fruit (regardless of the method used) when compared to *gardneri*, since the samples of the latter were positioned on the opposite side to vectors corresponding to each parameter assessed (Figure 2). Furthermore, the genetic factor of each variety may have influenced their division in dendrogram formation (Figure 1) when collected at the "mature green" stage. This can be explained by the findings of Melo et al. (2008), who reported that ascorbic acid content, phenolic compounds and antioxidant activity may vary according to variety and ripening stage.

The results obtained here demonstrate the potential of existing mangaba varieties, with compounds that could diversify the eating habits and nutrition of consumers, supplementing a healthy diet.

# CONCLUSIONS

The "mature green" stage is the most suitable for harvesting mangaba, regardless of variety, because it results in a longer shelf life when compared to ripe fruit.

*Cuyabensis* fruit harvested at physiological maturity ("mature green") showed greater potential, since its bioactive compound content and antioxidant activity were superior to the *gardneri* variety.

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**RESUMO:** O presente trabalho objetivou avaliar o comportamento dos compostos bioativos e da atividade antioxidante total de duas variedades de mangaba (*H. speciosa var. gardneri* e *H. speciosa var. cuyabensis*) durante o armazenamento, em dois estádios de maturação. Os frutos foram colhidos no banco de Germoplasma da Escola de Agronomia, em dois estádios de maturação, "de vez" (frutos maturos colhidos da árvore) e "de caída" (frutos maduros coletados no chão). Após a colheita, foram transportados para o laboratório, onde foram selecionados, lavados em água corrente, imersos em solução de cloro a 100 mg L<sup>-1</sup> por 10 minutos, deixados secar e armazenados sob condição ambiente (22±1°C e 90±5%UR). Os frutos foram analisados quanto aos teores de ácido ascórbico, flavonoides amarelos e polifenóis extraíveis totais, além da atividade antioxidante total determinada pelo método de redução do ferro (FRAP) e ABTS (2,2'-azino-bis-(3-ethylbenzthiazoline-6-sulfonic acid). As análises foram realizadas diariamente até que os frutos se tornassem impróprios para a comercialização, utilizando-se 3 repetições com 3 frutos cada. O período máximo de

conservação foi de oito dias para os frutos "de vez" e dois dias para os frutos "de caída". A variedade *cuyabensis* apresentou maiores teores de ácido ascórbico em ambos os estádios de maturação avaliados, além da capacidade antioxidante mais elevada, mostrando ser a variedade com maior potencial para "superfruta" na comercialização.

**PALAVRAS-CHAVE**: Capacidade antioxidante, Cerrado, *H. speciosa* var. *cuyabensis*, *H. speciosa* var. *gardneri*.

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