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Physical and chemical characteristics, total phenolics and antioxidant potential of bacupari at different stages of maturation

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Resumo. The objective of this work was to characterize fruits of bacupari at different maturities regarding the physical, physical-chemical, phenolic compounds and antioxidant capacity. Fruits at different maturities and color patterns of the peel were characterized by weight, diameters, pH, titratable acidity, total soluble solids, reducing and non-reducing sugars, ascorbic acid content, phenolic compounds (acetonic 70%, ethanolic 95% and methanolic extracts) and antioxidant potential by the 1,1 diphenyl-2-picrilhidrazil (DPPH) assay. The fruits showed variations from 4.14 to 7.24 g, 2.11 to 2.36 cm, 1.94 to 2.32 cm, 3.29 to 5.65 mg 100 g^{-1}, 1.80 to 8.04 g 100 g^{-1}, 0.76 to 2.40 g 100 g^{-1}, 2.43 to 3.63 g 100 g^{-1}, 2.70 to 2.82, 8.06 to 13.18º Brix for medium weight, longitudinal diameter and transversal diameter, ascorbic acid, reducing sugars, non-reducing sugars, titratable acidity, pH and total soluble solids, respectively. Maturation resulted in a reduction in ascorbic acid and acidity and an increase in sugars, pH and soluble solids. Concentrations of phenolic compounds in acetonic, methanolic and ethanolic extracts were 149.95 to 326.38, 147.04 to 364.00 and 108.22 to 407.51 mg GAE 100g^{-1}, respectively. The antioxidant capacity (EC_{50}), varied between 56.91 to 84.00 µg mL^{-1}. The ripening of the fruits resulted in a decline in total phenols and an increase in the antioxidant capacity. It was found that the ripeness positively influences the quality of the bacupari fruits. There was a negative correlation between the concentration of phenolic compounds extracted by different solvents and the antioxidant capacity.
Keywords: functional foods, bioactive compounds, characterization, ripening, *Garcinia brasiliensis*.

Introduction

Having a cultivation area that exceeds 2 million hectares, Brazilian fruit growing is considered one of the most diversified in the world (PNDF, 2018). Individuals who consume sufficient amounts of fruit decrease the risk of chronic diseases, since fruits are rich sources of vitamins, minerals, fibers and a wide variety of phytochemicals that act as antioxidants, anticarcinogenics and immunomodulators (Yahia, 2019). The increase in health problems has led people to seek well-being and improved quality of life in food, acquiring a healthy eating habit.

Many fruits can help in disease prevention and are therefore known as functional foods which can be defined as food products used in the standard diet to provide additional beneficial health effects in addition to traditional nutritional effects (Donno et al., 2018). Prevention is a more effective strategy than treatment for chronic diseases, a constant supply of vegetables containing beneficial phytochemicals to health, in addition to basic nutrition, it is essential to provide a defense mechanism that reduces the risk of chronic diseases in humans (Vieira et al., 2011). In this context, we highlight the antioxidant properties of many phytochemicals that maintain the balance between the production and the elimination of reactive oxygen species, which attack key biological molecules and trigger degenerative diseases, such as cancer, inflammation, atherosclerosis and aging (Patthamakanokpornet al., 2008; Rufino et al., 2009).

Among the promising fruits as a functional food, there is the bacupari*Garcinia brasiliensis*, belonging to the Clusiaceae family, also known as Guttiferae, which belongs to the class of angiosperms. It presents itself as a fruit tree of medium arboreal size, pyramidal crown and that blooms from August to September (Santa-Cecília et al., 2013); It is of Amazonian origin and is dispersed throughout the north of South America, covering the entire Brazilian territory up to Paraguay (Souza& Gentil, 2012).

Bacupari is commonly used in folk medicine to treat numerous diseases, being chemically constituted by a wide diversity of metabolites; Chemical studies have shown various structural classes, such as polyisoprenylated benzophenones, flavonoids, xanthones and coumarins (Naves, 2019), chemical derivatives that are of great interest in the pharmaceutical and food industry (Ferreira et al., 2012). Studies have demonstrated several beneficial properties of bacupari to health, its compounds isolated from extracts of leaves and epicarp showed activities, antispasmodic (Coelho et al., 2008), anti-inflammatory and antioxidant (Martins et al., 2008; Santa-Cecília et al., 2011), antiproteolytic and leishmanicidal (Pereira et al., 2010; Gontijo et al., 2012).

The knowledge of the physical, chemical and physical-chemical aspects are essential factors for maintaining the nutritional assessment of a product and quality control of foods aiming at commercialization, in addition to assessing the consumption and formulation of new products (Schneider, 2020).

The point of harvest and the maturation stage of the fruits at the time of collection act directly on the quality of the ripe fruits (Chitarra; Chitarra, 2005). The maturation process is marked by biochemical and physiological transformations that promote the development of aroma, flavor and placement, defining the point of harvest and its post-harvest validity (Dantas, 2015). Several criteria have been used to determine fruit maturity, based on appearance, such as size, diameter and color, as well as chemical composition, analyzing the content of soluble solids, titratable acidity, among other components of the product presented at the time of harvest (Silva et al., 2009). Bacupari has several substances with or without functional properties, which can be found in different concentrations according to the degree of ripeness at which the fruit appears. Thus, the physicochemical characterization of fruits is a tool that will help in monitoring the nutritional composition, sensory quality and functional characteristics during the ripening process in order to define the best point for harvest according to what is proposed.

Material and Methods

The samples of Bacupari fruits (*Garcinia brasiliensis*) were collected in a rural area in the municipality of Sinop - MT (-11.9205763 and -55.45900296) and submitted to identification by the Herbário Centro Norte Mato-Grossense (CNMT 9328).

The harvest included differentiated fruits in four stages of maturation according to the skin tones: totally green coloring of the fruit peel(G), slightly yellowish green color of the fruit peel(YG), slightly greenish yellow color of the fruit peel(GY) and totally yellow and / or orange coloration of the fruit peel(Y), selecting those that presented no visible damage. The fruits were characterized in terms of physical aspects and subsequently immersed in sodium hypochlorite at 200 ppm for 15 minutes, followed by washing in running water. After this procedure, the fruits of each stage were submitted to the pulp extraction process.

Physical and physical-chemical methods for fruit and pulp analysis

The whole fruits were first characterized in terms of their mass, obtaining the results by means of individual weighing of the fruit on an analytical scale and the values of the transverse and longitudinal diameter were obtained, in centimeters, using a caliper. For each stage of maturation, the physical analysis was performed in four repetitions, each containing 20 fruits. The pulps were determined for pH using a digital pH measurement (Tecnal, HMCDB-150), previously calibrated. For titratable acidity analysis (TA), a titrometric method was used using 0.1M NaOH as the titrant and 1% phenolphthalein alcoholic solution as an indicator. For the determination of total soluble solids (TSS), the sample was homogenized, adding a drop in the prism of the Abbé refractometer, performing a direct reading in ºBrix at a temperature of 20ºC. The reducing and non-reducing sugars in glucose were determined using the Lane-Eynon titrometric method (Instituto Adolfo Lutz, 2008). The ascorbic acid content was determined by the Tillmans titrometric method using Dichlorophenolindophenol solution as the titrating agent (AOAC, 1997).

Evaluation of the functional properties of the pulp

The analysis of total phenolic compounds was carried out according to the Folin-Ciocalteau method (Singleton& Rossi, 1965), based on the extractions and reactions proposed by Rocha et al. (2011) using three different solvents for cold extraction: 70% acetone, 95% ethanol and metanol P.A. The absorbances were obtained at 760 nm in a spectrophotometer (Bioespectro, SP - 220) using glass cuvettes. The total phenol content (FT) was determined by interpolating the absorbance of the samples against a calibration curve constructed with gallic acid standards and expressed as mg of gallic acid equivalent (GAE) $100g^{-1}$. The antioxidant potential of the pulp was evaluated by the assay with 1,1 diphenyl-2-picrilhhydrazyl (DPPH), according to

Brand-Willians et al. (1995), with extraction and reaction standardized by Rufino et al. (2007). The reduction of the DPPH radical was measured in a spectrophotometer (Bioespectro, SP - 220) at 515 nm just after 30 minutes of rest. The sequestration of free radicals with a decrease in the absorbance values of the samples was correlated with the control and the percentage of discoloration of the DPPH radical was established. The results were expressed as EC_{50} (mg mL^{-1}), antioxidant capacity equivalent to ascorbic acid (AEAC) and antioxidant capacity equivalent to Trolox (TEAC) in dry matter mass.

Statistical analysis

The experiment consisted of a completely randomized design with 4 treatments (stages of maturation of the samples) and 4 repetitions containing about 1 kg of fruits each. The data were statistically evaluated using the ASSISTAT 7.7 Beta software (Statistical Assistance). The results obtained were subjected to analysis of variance (ANOVA) and the treatment means were compared using the Tukey test ($p < 0.050$). To assess the correlation between factors, Pearson's correlation was applied.

Results and discussion

Physical and physical-chemical characteristics of fruits

As for the physical aspects, it can be observed that the weight and the longitudinal and transverse diameter showed statistically higher values in the most mature stages, with the values for the longitudinal diameter (LD) standing out slightly over the values of the transverse diameter (TD) in all stages of maturation (Table 1). The LD / TD ratio is a characteristic indicator of the shape of the fruit, because as the coefficient obtained approaches 1, more rounded is the appearance of the fruit (Souza, 2007). The results obtained from the LD / TD ratio for bacupari fruits were 1.09 for G stage, 1.07 for YG, 1.05 for GY and finally, 1.02 for Y. It can be observed that as the fruit ripens, the LD / TD variation decreases, with G stage characterized by the presence of more oval fruits and Y stage by slightly rounded fruits (table 1)

Table 1. Physical parameters of bacupari pulp at four maturation stages

Parameter	Peel color				CV%
	G	YG	GY	Y	
Weight (g)	4.14 c	5.18 bc	5.98 b	7.24 a	9.23
Longitudinal Diameter (cm)	2.11 b	2.22 ab	2.29 a	2.36 a	3.29
Transversal Diameter (cm)	1.94 c	2.07 bc	2.18 ab	2.32 a	3.64
Ratio (LD/TD)	1.09	1.07	1.05	1.02	-

Different letters on the same line indicate significant differences using the Tukey test ($p < 0.05$); G - totally green coloring of the fruit peel; YG - slightly yellowish green color of the fruit peel; GY - slightly greenish yellow color of the fruit peel; Y - totally yellow and or orange coloration of the fruit peel; CV - coeficiente of variation; LD - longitudinal diameter; TD - transverse diameter.

The results of the physical-chemical characteristics demonstrate changes in all parameters along the ripening of the fruit, similar to the results of Magalhães et al. (2019) who observed changes in the red pulp pitaya during its development.

Regardless of the maturity stage of bacupari, low pH values were found, compatible with the characteristics of acid to sweet-acid taste that the fruit presents in fresh consumption, only G fruits showed significantly lower average values in relation to the other ripeness classifications (Table 2). At the same time, it can be observed that the values of TA in citric acid gradually decreased from G to Y fruits, presenting a slight increase in the result found in the ripe fruit (Table 2). The increase in pH during the advance of the maturation stages is directly related to the decrease in the titratable acidity in the fruit, due to the conversion or oxidation of organic acids into sugars that will be used by the cells as an energy source (Agostini, 2012; Sartori et al., 2012). When analyzing bacupari fruits, Pinto (2013) also found a reduction in the TA values as the ripening progressed.

Table 2 - Physico-chemical parameters of bacupari pulp at four different maturation stages

Parameters	Peel color				CV%
	G	YG	GY	Y	
pH	2.70 b	2.80 a	2.82 a	2.82 a	1.26
Titratable acidity (g citric acid $100g^{-1}$)	3.63 a	3.11 b	2.43 c	2.69 bc	7.66
Total Soluble Solids (%Brix)	8.06 c	8.11 c	10.58 b	13.18 a	2.98
Glucose-reducing sugars (g $100g^{-1}$)	1.80 d	3.16 c	5.80 b	8.04 a	4.68
Non-reducing sugars in sucrose (g $100g^{-1}$)	0.76 d	1.28 c	2.04 b	2.40 a	9.53
Ascorbic acid (mg $100g^{-1}$)	5.65 a	3.87 b	3.81 b	3.29 b	11.75
Ratio (TSS/TA)	2.24 c	2.61 c	4.36 b	4.92 a	7.33

Different letters on the same line indicate significant differences using the Tukey test ($p<0.05$); G - totally green coloring of the fruit peel; YG - slightly yellowish green color of the fruit peel; GY - slightly greenish yellow color of the fruit peel; Y - totally yellow and or orange coloration of the fruit peel; CV - coeficiente of variation; TSS - total soluble solids; TA - titratable acidity.

The content of total soluble solids differed statistically between the ripening stages (Table 2), not differing in the initial stages, G and YG, following an increasing behavior in the other ripenings, being possible to verify an increase as the fruit ripened, as observed by Bezerra et al. (2017) in breadfruit. There was an increase in reducing sugars during maturation, with greater concentration in the fruit of the most mature stage. Likewise, similar behavior, but to a lesser extent, was observed in non-reducing sugars. Monteiro et al. (2018) found that the concentrations of reducing and non-reducing sugars in sapota-do-Solimões, increased in the most advanced stages of maturation; the same behavior was also observed in studies with pomegranate (Fawole& Opara, 2013); 'Champagne' oranges (AgostinI, 2012) and star fruit (Zainudin et al., 2014).

In the bacupari fruits, it was verified that the ratio, the relationship between TSS and TA, differed statistically between three stages of maturation (Table 2), obtaining an increase in the values according to fruit maturation. According to studies by Schneider et al., (2020) carried out with bacuparis from the cerrado, it was found that the ratio (TSS / AT) increased considerably according to the increase in the degree of fruit maturation. According to Lima et al. (2015), the ratio indicates the degree of sweetness of a fruit or its product, showing which flavor is predominant, sweet or acid, or whether there is a balance between them.

The levels of ascorbic acid showed a decreasing and significant behavior between stage G for YG, remaining with a stable behavior during the other stages (Table 2). The same behavior was observed in abiu fruits (Sanches et al., 2016), 'Champagne' oranges (Agostini, 2012) and sweet orange (Beber, 2013). According to Xu et al. (2020) the ascorbic acid content decreases according to fruit ripeness. This decrease is associated with the action of the enzyme called ascorbic acid oxidase (ascorbate oxidase), whose enzymatic activity in ripe fruits is greater than in immature (Nogueira et al., 2002).

When correlating the parameters with each other, the variable of TA was negatively correlated with the variables of pH, reducing sugar and non-reducing sugar showing that as the TA decreases these variables presented negatively tend to increase in the same proportion. The results obtained demonstrate a significant simple correlation at the level of $p<0.05$ when comparing the values of soluble solids with the pH and a significant simple correlation at the level of $p<0.01$ for the other parameters (Table 3).

Table 3. Simple correlation matrix between physicochemical parameters of bacupari fruits at four stages of maturation

Parameters	(R^2)				
	pH	TA	TSS	Reducingsugars	Non-reducingsugars
pH	1	-0.8030	0.6056	0.6980	0.8065
TA	**	1	0.6653	-0.7753	-0.8380
TSS	*	**	1	0.9668	0.9158
Reducingsugars	**	**	**	1	0.9642
Non-reducingsugars	**	**	**	**	1

*significant simple correlation at the level of $p<0.05$; ** significant simple correlation at the level of $p<0.01$; Correlation: r = 0.10 to 0.30 (weak); r = 0.40 to 0.6 (moderate); r = 0.70 to 1 (strong) according to Dancey and Reidy (2005); TSS - total soluble solids; TA - titratable acidity.

Functional characteristics of fruits

All solvents tested showed efficiency in the extraction of phenolic compounds present in the bacupari pulp (Table 4). It was found that the efficiency of each type of solvent depends on the maturation stage, since for the YG and Y stages, the types of solvents used did not differ in the extraction efficiency, in G stage, Ethanol and Methanol were more efficient, while in GY, it was acetone. A reduction in the phenolic composition was observed as the maturation progressed, verifying that in the acetone extract, the concentration of total phenols was constant until the GY stage, significantly reducing when passing to stage Y. In the extractions carried out with ethanol and methanol, the significant reduction occurred from the YG to the Y stage.

Table 4. Total phenolic compounds in bacupari pulp at four maturation stages using three types of extracting solvents

Parameter	Peel color				CV%
	G	YG	GY	Y	
Total phenols (mg GAE $100g^{-1}$) (Acetonic extract)	282.58 aB	326.38 aA	285.98 aA	149.95 bA	11.11
Total phenols (mg GAE $100g^{-1}$) (Ethanolic extract)	364.00 aA	301.18 abA	239.08 bAB	147.04 cA	9.80
Total phenols (mg GAE $100g^{-1}$) (Methanol extract)	407.51 aA	281.14 bA	195.16 bcB	108.22 cA	18.78

Means in the same column followed by equal capital letters and means in the same line followed by equal lower letters are equivalent, according to Tukey's test ($P<0.05$); G - totally green coloring of the fruit peel; YG - slightly yellowish green color of the fruit peel; GY - slightly greenish yellow color of the fruit peel; Y - totally yellow and or orange coloration of the fruit peel; CV - coeficiente of variation.

Likewise, there was a reduction in the concentration of phenolic compounds in ethanolic extracts during the maturation of sapota-do-Solimões, whose values obtained were 11.07 mg AGE. $100g^{-1}$ for immature fruits and 9.22 mg AGE. $100g^{-1}$ for fruits mature (Monteiro et al., 2018). In contrast, buriti fruits at different maturities presented a tendency to increase phenolic compounds in ethanol extracts, with averages of 10.57 mg GAE $100g^{-1}$ and 20.65 mg GAE$100g^{-1}$ for immature and ripe fruits, respectively (Milanez et al., 2018).

When analyzing phenolic compounds, it should be noted that several factors can influence the results obtained, such as the nature of the compound, the extraction method used, the sample size, the time, storage conditions, the pattern used and also the presence of interfering. The solubility of phenolics varies according to the polarity of the solvent used and the degree of polymerization of the phenolics (Angelo &Jorge, 2007).

The antioxidant potential of bacupari was evaluated for its ability to inhibit oxidation of the DPPH radical. It was found that the advance of ripening resulted in a greater antioxidant potential (lower EC_{50} values and higher AEAC and TEAC), that is, greater antioxidant capacity in more mature fruits (GY and Y), with significant differences in these compared to the G and YG fruits. The ripe fruits showed excellent characteristics of antioxidant potential even though the concentration of ascorbic acid and total phenolic compounds decreased during ripening, indicating that the antioxidant potential may be related to othercompounds (table 5).

Table 5. Antioxidant potential (DPPH) of bacupari pulp at four different stages of maturation.

Unity	Peel color				CV%
	G	YG	GY	Y	
EC_{50} (µg mL^{-1})	74.50 a	84.00 a	56.91 b	57.00 b	6.68
AEAC (mg $100g^{-1}$)	388.12 b	345.03 b	510.93 a	509.99 a	7.80
TEAC (µmol g^{-1})	35.07b	31.11b	45,91a	45.84a	6.68

Different letters on the same line indicate significant differences using the Tukey test ($p<0.05$); G - totally green coloring of the fruit peel; YG - slightly yellowish green color of the fruit peel; GY - slightly greenish yellow color of the fruit peel; Y - totally yellow and or orange coloration of the fruit peel; AEAC - ascorbic acid equivalent antioxidant capacity; TEAC - trolox equivalent antioxidant capacity.

Other studies have shown that ripe fruits of *Garcinia brasiliensis* and *Eugenia brasiliensis* presented EC_{50} of 2.79 and 0.47 mg mL^{-1}, respectively (Infante, 2013), therefore the antioxidant capacity of the pulp of *G. brasiliensis* was lower than the present study. The EC_{50} observed in olives showed a similar behavior with that of bacupari fruits, with higher values (lower antioxidant capacity) being observed in the initial stages of maturation, where the fruits were still in the G stage, followed by a slight increase in antioxidant potential during advancing maturation, indicating that the antioxidant capacity was higher in the most mature stage of the fruits (Sousa et al., 2015). Similar behavior was also observed in juçara fruit (Schulz et al., 2015).

The correlation between the phenolic compounds extracted by methanol and ethanol resulted in higher coefficients when compared with the correlations of any of them with acetone, indicating that methanol and ethanol have similar effects and responses in the extraction of these compounds. As for the correlation between the phenolic components extracted with different extraction solutions and the antioxidant potential (TEAC), it is noted significant and negative correlation, in other words, the high content of phenolic compounds correlates with lower TEAC values, that is, less antioxidant potential (Table 6).

Table 6. Simple correlation matrix between phenolic compounds at different extracts and antioxidant potencial (DPPH) in bacupari fruits in four stages of maturation

Parameters	(R^2)			
	Total phenols (Acetonicextract)	Total phenols (Ethanolicextract)	Total phenols (Methanolicextract)	TEAC
Total phenols (Acetonicextract)	1	0.7995	0.6454	-0.58083
Total phenols (Ethanolicextract)	**	1	0.8835	-0.6718
Total phenols (Methanolicextract)	**	**	1	-0.6147
TEAC	*	**	*	1

* significant simple correlation at the level of $p<0.05$; ** significant simple correlation at the level of $p<0.01$. Correlation: r = 0.10 to 0.30 (weak); r = 0.40 to 0.6 (moderate); r = 0.70 to 1 (strong) according to Dancey and Reidy (2005); ; TEAC - trolox equivalent antioxidant capacity.

The results obtained demonstrate a significant positive correlation and a significant negative correlation at the level of $p<0.05$ when the TEAC values are compared with the concentration of phenolic compounds extracted with 70% acetone and methanol and a significant negative correlation at the level of $p<0.01$ when extracting with ethanol 95%. As the correlations were negative due to the observed characteristics of reduced phenolic compounds and increased antioxidant potential during fruit ripening, it can be suggestive that the ripening process is influenced by other compounds that contain antioxidant activity, or compounds that prevent the extraction of phenols during sample processing, since, despite the performance obtained showing a significant simple correlation at the level of $p<0.01$, they present an average adjustment between the behavior of phenols with the antioxidant potential present.

Conclusion

The physical-chemical quality of bacupari varied according to the stage of maturation, with a significant increase in the amount of sugars, soluble solids and pH, with a reduction in acidity and ascorbic acid, thus, the fruits in the most mature stages the most indicated for consumption.

In general, the efficiency of extraction of phenolic compounds by different solvents was influenced by the stage of fruit maturation.

During ripening, a decrease in the concentration of total phenolic compounds was observed, while the antioxidant potential (DPPH) had its values increased. The amount of phenolic compounds present in the samples did not correlate positively with the antioxidantepotencial.

Future studies should be carried out in order to identify the types of phenolic compounds present in the bacupari pulp and its relationship with the ripening process, since the bacupari pulp has a high antioxidant potential, and a study is needed to evaluate the application of this fruit in the industry pharmaceutical, cosmetic and food.

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