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Bioactive compounds of pulp powder of tarumã fruits (*Vitex megapotamica*) at two maturity stages

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Abstract

This study aimed to characterize the pulp of the tarumã fruit, harvested at different stages of maturation (immature and mature), in terms of physicochemical parameters and mineral content, and also to evaluate the bioactive compounds of the powdered pulp of the fruits, through analysis of phenolic compounds, antioxidant capacity (DPPH and ABTS) and anti-inflammatory activity. There was an increase ($p \leq 0.05$) in the pH, in the *Ratio* (soluble solids/titratable acidity) and in the sugar levels during the maturation of the tarumã fruits. The powdered pulp showed a more intense red color (a*) when was mature, due to the degradation of chlorophyll and the synthesis of anthocyanins that occur during fruit ripening. Among the minerals evaluated, relevant concentrations of potassium were detected in the fruit pulp, and in ripe fruits, the concentration was even higher ($p \leq 0.05$). The values of total phenolic compounds, antioxidant activity (for both evaluated methods) and anti-inflammatory activity were higher in aqueous extracts of immature fruits. The tarumã can be a promising source of bioactive compounds, mainly the immature fruits, which showed the best results, while the ripe fruits can be used as raw material for fermentation processes, or for the production of natural dyes due to their intense purple color.

Keywords: *Vitex megapotamica*. Maturation. Minerals. Antioxidants. Anti-inflammatory.

Compostos bioativos da polpa em pó do tarumã (*Vitex megapotamica*) em dois estádios de maturação

Resumo

Este estudo teve como objetivo caracterizar a polpa do fruto do tarumã, colhidos em diferentes estádios de maturação (imaturo e maduro), quanto aos parâmetros físico-químicos e teor de minerais, e também avaliar os compostos bioativos da polpa em pó dos frutos, através das análises de compostos fenólicos, capacidade antioxidante (DPPH e ABTS) e

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atividade anti-inflamatória. Observou-se um aumento ($p \leq 0,05$) do pH, da relação (sólidos solúveis/acidez titulável) e dos teores de açúcares durante a maturação dos frutos do tarumã. A polpa em pó apresentou coloração vermelha (a*) mais intensa quando estava madura, devido a degradação da clorofila e a síntese das antocianinas que ocorrem durante o amadurecimento de frutos. Dentre os minerais avaliados, foram detectadas concentrações relevantes de potássio na polpa do fruto, sendo que, nos frutos maduros, a concentração foi ainda maior ($p \leq 0,05$). Os valores de compostos fenólicos totais, atividade antioxidante (para os dois métodos avaliados) e atividade anti-inflamatória foram maiores nos extratos aquosos dos frutos imaturos. O tarumã pode ser uma fonte promissora de compostos bioativos, principalmente os frutos imaturos, que apresentaram os melhores resultados, enquanto os frutos maduros podem ser utilizados como matéria-prima para processos fermentativos, ou para a produção de corantes naturais devido a sua coloração púrpura intensa.

Palavras-chave: *Vitex megapotamica*. Maturação. Minerais. Antioxidante. Anti-inflamatório.

Introduction

Tarumã (*Vitex megapotamica*), also known as "azeitona do mato", "copiúba" among other popular names, belonging to the Lamiaceae family, has occurrence in various Brazilian regions. The fruits are classified as drupes and are green when immature turning purple/black when ripe, with juicy pulp and sweet flavor. Although they are edible and can be consumed *in natura*, they have been used only as a raw material for the manufacture of jams and liqueurs (Caldeira et al., 2004; Cosmo et al., 2009).

Studies on tarumã leaves have shown the antioxidant capacity and the presence of medicinal compounds such as allopurinol when infused (Onofre et al., 2016). Most of the studies on tarumã are related to the health research area and focused only on leaves (Hamann et al., 2016).

Fruit maturity phenomenon involves a series of physiological, biochemical, and organoleptic changes, including the degradation of chlorophylls a and b, degradation of pigments, synthesis of new pigments (carotenoids), changes in flavor, decreased acidity, higher soluble solids levels, and changes in texture (Domingues; Ono; Rodrigues, 2001). The advancement of maturity leads to the oxidation of phenolic compounds, which contributes to the biosynthesis of anthocyanins that accumulate in this period (Castrejón et al., 2008; Belwal et al., 2019). Therefore, it is important to study tarumã at different maturity stages to understand the possible interference of the fruit maturity on the bioactive compounds.

Phenolic compounds are the main bioactive compounds in fruits and have health benefits, such as antiallergic, anti-inflammatory, and antimicrobial properties, with emphasis on antioxidant activity (Balasundram; Sundram; Samman, 2006). Research has been carried out to search for fruits with antioxidant capacity, as reported by Resende, Franca e Oliveira (2019). In this context, the present study aimed to characterize tarumã fruit pulp harvested in different maturity stages for the physicochemical properties, minerals content, phenolic compounds, and determination of antioxidant and anti-inflammatory properties.

Materials and Methods

Materials

The Folin-Ciocalteau reagent, 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), 2,2-diphenyl-1-picrylhydrazyl (DPPH), gallic acid, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), sulfanilamide, sodium nitroprusside, acarbose, α-amylase, and N-(1-naphthyl) ethylenediamine dihydrochloride (NED) were purchased from Sigma-Aldrich (Brazil). Sodium hydroxide, zinc acetate, and potassium ferrocyanide were purchased from Synth (Brazil). Hydrochloric acid, sulfuric acid, neutral lead acetate, copper sulfate, potassium sulfate, sodium and potassium tartrate, acetone, ethyl alcohol, methyl alcohol, potassium persulfate, and sodium carbonate from Dinâmica (Brazil). Sodium phosphate monobasic was obtained from Cinética Reagentes e Soluções (Brazil); sodium phosphate dibasic from Reatec (Brazil), dinitrosalicylic acid (DNS) from Inlab (Brazil). The other reagents used in the determinations were of analytical grade.

Harvesting and preparation of fruits

Tarumã fruits were harvested in 2020, in the plantation belonging to the Federal Institute of Education, Science and Technology of Rio Grande do Sul - IFRS Campus Sertão, in the city of Sertão/RS, Brazil (Latitude 28° 2' 26" S, Longitude 52° 16' 25" O), in two maturity stages, determined by calculating the *Ratio* (total soluble solids/titratable acidity).

After collection, the fruits were selected, cleaned, and sanitized with sodium hypochlorite solution (200 $mg.kg^{-1}$) for 15 minutes, washed in running water, and placed in sieves to remove excess water. The fruits were divided into two parts and vacuum packaged in polyethylene bags (20 cm x 25 cm), placed in metalized zipper pouch bags, and kept refrigerated (~ 5°C) in a conventional refrigerator (Electrolux, Brazil) until analysis.

Physicochemical characterization of fruits at different maturity stages

After cleaning, the fruits selected for the analysis were pulped and crushed in a high-speed industrial

blender at 36.288 g force (Spolu, Brazil) for 5 minutes. The samples were characterized according to AOAC methodologies for moisture (method 925.45b); ether extract by the Soxhlet method (method 920.39c); protein (method 920.152) using a conversion factor of 5.75; ash (method 940.26); total dietary fiber (method 985.29); total soluble solids (°Brix) (method 932.14c); reducing sugars in glucose) (method 925.36); non-reducing sugars (method 925.35); pH (method 981.12); and total titratable acidity (method 942.15a) (AOAC, 2016). The carbohydrate content was calculated by difference.

Obtaining the fruit pulp powder

The whole fruits *in natura*, not used in the characterization analyses, were punctured with the aid of a 2 mm diameter needle, frozen in an ultra-freezer (ULT 335/710 D Vertical, Indrel, Brazil) for 24 hours at -86°C, dehydrated at -60°C under the vacuum pressure of 0.05 mTorr in a freeze dryer (TFD 5503, IIShin, Netherlands) for 24 hours, and then subjected to pulping. The pulps were again frozen in the ultra-freezer (-86°C /24 hours) and subjected to freeze-drying (-60°C /0.05 mTorr /24 hours) until reaching moisture content between 14% and 15%. Then, the pulps were crushed in a high-speed industrial blender at 36.288 g force (Spolu, Brazil) for 5 minutes, homogenized in a sieve (32 mesh), vacuum packaged in metalized bags, and stored under freezing (-86°C) until the analyzes.

The color of the tarumã pulp powder was evaluated by reflectance using a Minolta colorimeter (CR-410, HunterLab, Brazil), according to the methodology defined by the manufacturer, using the CIELAB system. The values of L* (brightness) varying from black (0) to white (100), a* from green (-a *) to red (+ a *) and b* from blue (-b*) to yellow (+ b*) were used to define a three-dimensional color space. The equipment was previously calibrated using white and black standards. Mineral contents were also evaluated according to AOAC method 985.35 (2016), with adaptations to determine the Ca, Ti, Fe, Zn, K, Mg, Cd, Ni, Pb, Cu, and Na levels, using a flame atomic absorption spectrometer (SpectrAA 220) and hollow cathode lamps 44 (VARIAN®).

Determination of phenolic compounds and antioxidant activity

The extraction was carried out according to the methodology described by Rufino et al. (2007a), with modifications. For that, 1 g of tarumã pulp powder was weighed in a 100 mL beaker, 40 mL of 50% methanol was added, and the mixture was vortexed (Vortex Mixer K45-2810, Kasvi, Brazil) and kept at rest for 60 minutes at room temperature. Then, the mixture was transferred to tubes and centrifuged (centrifuge SL-700, Solab, Brazil) at 25.200 g force for 15 minutes. The supernatant was transferred to a 100 mL volumetric flask. Then, 40 mL of 70% acetone was added to the residue from the first extraction, homogenized, kept at rest (60 minutes), and centrifuged (25.200 g force for 15 minutes). The supernatant was then transferred to the volumetric flask containing the first supernatant and the volume was made up to 100 mL with distilled water. The extracts were frozen at -86°C in the ultra-freezer (ULT 335/710 D Vertical, Indrel, Brazil) for the determination of the antioxidant activity by DPPH, ABTS, and total phenolic compounds.

The content of total phenolic compounds was determined according to the Folin-Ciocalteu method. After preparation, the samples were kept in the dark, at room temperature, and absorbance readings were performed, in triplicate, on a spectrophotometer (Cirrus 80 SA, Femto, Brazil) at 760 nm, as described by Roesler et al. (2007), with modifications. The quantification of total phenolic compounds was performed using the standard curve of gallic acid, and the results were expressed in gallic acid equivalent per 100 g of sample (mg GAE.100 g^{-1}).

The analyses of antioxidant activity by the DPPH and ABTS radical scavenging method were performed as described by Rufino et al. (2007b), respectively. A DPPH standard curve was constructed, and the absorbance readings of the samples were performed at 515 nm, in triplicate, using a spectrophotometer (Cirrus 80 SA, Femto, Brazil). For the calculation of EC_{50}, the final absorbance reading was performed after absorbance stabilization. The results were expressed in $mg.L^{-1}$, which corresponded to the sample concentration required to reduce in 50% the initial concentration of DPPH (EC_{50}). For the ABTS analyze, a Trolox standard curve was constructed, and the absorbance readings of the diluted extracts were performed at 734 nm, in triplicate, using a spectrophotometer (Cirrus 80 SA, Femto, Brazil). The results were expressed in μM Trolox.g^{-1} of sample.

Determination of anti-inflammatory activity

The sample extract was prepared according to the method of Wang et al. (2019). The nitric oxide radical scavenging activity was determined as reported by Hazra, Biswas e Mandal (2008). Absorbance readings of the extracts were performed on a spectrophotometer (Cirrus 80 AS, Femto, Brazil) at 540 nm. A Trolox standard curve was constructed, and the results were expressed in μM Trolox.g^{-1} of sample.

Statistical analysis

The results were analyzed by analysis of variance (ANOVA) and Tukey's average comparison test at a 5% significance level, using the STATISTICA 14 Trial Software (Statsoft). Data were presented as mean values and standard deviation of three measurements.

Results and Discussion

Physicochemical composition of tarumã fruits from two maturity stages

The tarumã fruits presented high moisture levels, in both degrees of ripening (Table 1), as observed for most fruits and vegetables. The immature fruit showed higher moisture content, due to the moisture transfer from the peel to the pulp during the maturity stage. It is noteworthy that climatic conditions, periods of intense rain or extreme drought, may interfere with the moisture of the fruits, which may have occurred in this study.

No significant difference was observed for the ash contents, ether extract, total dietary fiber, and proteins (Table 1) between the two maturity stages ($p \leq 0.05$). The pH of the fruits increased during maturity, possibly due to the decrease in total organic acids, since they underwent degradation during ripening (Ayour et al., 2017). Martineli et al. (2018), reported a lower pH for grapes of the cultivar Vitria (4.05), when compared to tarumã, and a decrease in acidity with increasing pH, as also observed in our study.

Table 1 – Physicochemical composition (wet basis) of tarumã fruits from two maturity stages

Parameters	**Maturity stages**	
	Immature	**Ripe**
Moisture (%)	84.53 ± 0.38[a]	81.05 ± 0.74[b]
Ash (%)	1.40 ± 0.13[a]	1.28 ± 0.17[a]
Ethereal Extract (%)	1.13 ± 0.03[a]	1.17 ±0.02[a]
Protein (%)	0.97 ± 0.06[a]	0.87 ± 0.06[a]
Total Dietary Fiber (%)*	3.44 ± 0.02[a]	3.01 ± 0.31[a]
Carbohydrates (%)	8.54	12.20
pH	5.61 ± 0.11[b]	6.02 ± 0.06[a]
Soluble Solids (°Brix)	12.93 ± 0.35[b]	18.77 ± 0.31[a]
Total Titratable Acidity (%)	1.43 ± 0.07[a]	1.11 ±0.11[b]
Reducing sugars (%)	40.66 ± 2.78[b]	53.49 ± 5.09[a]
Non-reducing sugars (%)	41.27 ± 0,84[b]	43.80 ± 1.24[a]
*Ratio***	9.34	16.15

Values expressed as mean ± standard deviation; *Fraction included in carbohydrates; ***Ratio*: soluble solids / total titratable acidity; Averages followed by the same letter, on the same line, do not differ statistically by the Tukey test at the 5% level of significance.

The total titratable acidity was significantly higher in the immature fruits when compared to the ripe fruit, corroborating the pH results (Table 1). Organic acids are used as substrates for respiratory processes and consumed during the ripening metabolism, leading to the release of sugars, resulting in a lower acidity (Chitarra; Chitarra, 2005; Pimentel et al., 2010) with a consequent increase in pH values.

A significant difference ($p \leq 0.05$) was observed for the soluble solids (°Brix), reducing sugars, and non--reducing sugars, with higher values for the ripe fruits (Table 1). The increase in these levels with maturity is due to the biosynthesis and degradation of polysaccharides in simple sugars, evidencing the maturation of the fruits (Sehn et al., 2021).

The sugars contents tend to increase during maturity and are higher for ripe fruits, which are related to the soluble solids content (Monteiro et al., 2018). According to Maieves et al. (2015), the higher sugar contents (reducing and non-reducing) show the predominant sugar in the fruit, which was observed in the present study for glucose and fructose (reducing sugar), while non-reducing sugars such as sucrose were not predominant. With these results it is possible to indicate that the mature tarumã can be a good raw material for fermentation processe.

Color and mineral of tarumã pulp powder from two maturity stages

The color variation between the powders is given by the values of the coordinates L*, a* and b* (Table 2), which are shown in Figure 1. The reduction in lightness (L*) observed ($p \leq 0.05$) is related to degree of ripeness of the fruits, with a darker color when ripe and low brightness intensity. As the L* axis reflects the lightness (values range from 0 to 100), values far from 100 (total white) indicate the darker samples. An increase in the coordinate a* was observed for the powder of ripe fruits, with positive results, therefore indicating a tendency to red color. In contrast, a reduction in the b* coordinate was observed from the immature to the mature stage, due to the fruit's development and consequent ripening.

Positive b* values indicate a tendency to yellow color at the end of ripening.

The loss of the green color of the fruits is a common alteration due to the degradation of pigments such as chlorophyll present in plant cells. The pH is one of the factors responsible for this degradation, leading to the release of organic acids, and therefore the oxidation of this pigment. From these reactions, other compounds are formed, such as anthocyanins that are responsible for the purple color of fruits (Chitarra; Chitarra, 2005; Maieves et al., 2015).

The major mineral (Table 2) determined in this study was potassium, for both maturity stages, with a higher content found in the ripe fruit. Gomes et al. (2019), studied the mineral content of Niagara and Bordo grape pulp, and reported potassium, calcium, and magnesium levels of 433.69 and 623.89 mg.100 g^{-1}; 12.01 and 8.72 mg.100 g^{-1}; and 10.45 and 5.90 mg.100 g^{-1}, respectively.

Table 2 – Color, minerals and total phenolic compounds, antioxidant and anti-inflammatory activities of tarumã pulp powder, from two maturity stages

Parameters	**Stages**	
	Immature	**Ripe**
Color		
L*	27.55 ± 0.37[a]	21.59 ± 0.22[b]
a*	3.15 ± 0.12[b]	7.15 ± 0.11[a]
b*	21.80 ± 0.16[a]	18.63 ± 0.14[b]
Minerals (mg.100 g^{-1})		
Potassium	118.90 ± 4.61[b]	158.04 ± 5.65[a]
Calcium	9.15 ± 0.90[a]	9.13 ± 0.96[a]
Magnesium	8.75 ± 0.13[a]	8.60 ± 0.30[a]
Zinc	0.27 ± 0.08[a]	0.24 ± 0.06[a]
Iron	0.06 ± 0.03[a]	0.03 ± 0.01[a]
Total Phenolic Compounds	474.91 ± 10.15[a]	383.16 ± 4.44[b]
ABTS	319.98 ± 0.29[a]	287.74 ± 1.70[b]
DPPH	1019.25 ± 17.25[b]	2583.5 ± 36.5[a]
Anti-inflammatory	35.58 ± 0.16[a]	33.73 ± 0.31[b]

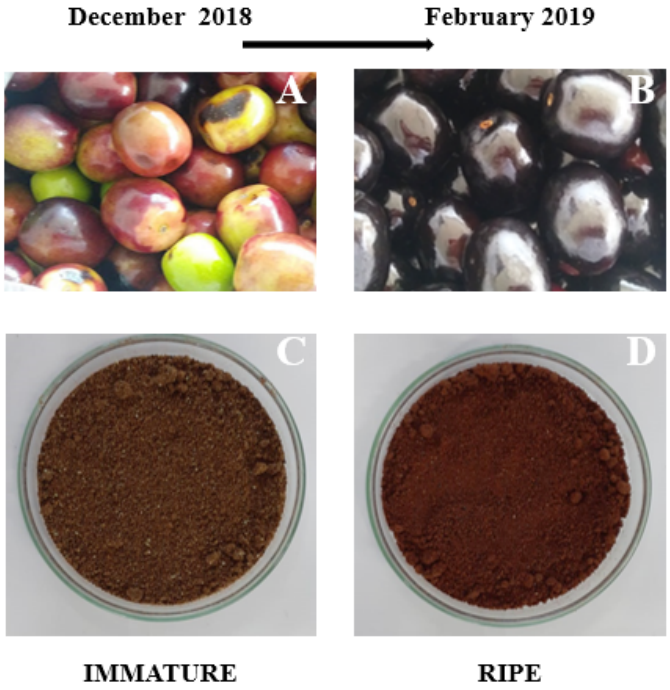
Values expressed as mean ± standard deviation. Total Phenolic Compounds (mg EAG.100 g^{-1} sample); Antioxidant activity by the ABTS radical (μM TROLOX.g^{-1} of sample); Antioxidant activity by the radical DPPH, expressed as EC50 (mg.L^{-1}); Anti-inflammatory (μmol TROLOX.g^{-1} of sample). Averages followed by the same letter, on the same line, do not differ statistically by the Tukey test at the 5% level of significance.

The cultivar Niagara presented higher levels for all minerals, while the cultivar Bordo showed lower calcium and magnesium levels when compared to the tarumã pulp of the present study. Gordon et al. (2012), studied açaí fruits at different degrees of ripening and reported higher potassium, calcium, and magnesium levels when compared with this study, with values of 4271 and 930 mg.100 g^{-1}; 962 and 930 mg.100 g^{-1}; and 397 and 172 mg.100 g^{-1}, for immature and ripe fruits, respectively. The zinc and iron levels of tarumã pulp were higher than the values obtained by Gomes et al. (2019), for Bordo grape pulp, with values of 0.016 and 0.019 mg.100 g^{-1}, respectively. The Niagara cultivar showed 0.08 and 0.18 mg.100 g^{-1} for these minerals, respectively, and only the iron content was higher than that found for the tarumã pulp powder.

Total phenolic compounds, antioxidant activities (DPPH and ABTS), and anti-inflammatory properties of tarumã pulp powder

The total phenolic compounds (Table 2) were higher for the powder from the immature fruits, probably due to the advance of fruit ripening, in which the conversion of soluble into insoluble compounds occurs due to the binding of polysaccharide network in the fruit cell walls. In fruits, in addition to acting against pests, these compounds affect the nutritional value and the sensory quality, providing distinct color, texture, and flavor (Everette et al., 2010; Benchikh et al., 2014).

Figure 1 – Immature (A), ripe (B) fruits and immature (C), ripe (D) tarumã pulp powder.



Casarin et al. (2016), studied blackberry flour and reported 344.94 mg EAG.100 g^{-1} of sample, which is lower when compared to the tarumã pulp powder from both maturity stages. These differences are due to several factors, including the diversity of phenolic compounds, the nature of the compound, the extraction method, the extraction efficiency, sample size, extraction time, and storage conditions. The solubility of these compounds can also be influenced by the polarity of the solvent used, the degree of polymerization, and the interactions between other compounds (Angelo; Jorge, 2007).

Regarding the antioxidant activity determined by the ABTS and DPPH assays, the immature fruits showed higher ABTS values and lower DPPH values when compared to the ripe fruits, indicating that the extract from immature fruit pulp powder presented greater antioxidant capacity, with a significant difference ($p \leq 0.05$) when compared with the extract from the ripe fruit pulp powder (Table 2).

Macharek e Hanchi (2017), studied lemon fruits at different degrees of ripening and reported higher DPPH values for immature fruits when compared to ripe fruits, probably due to the higher total phenolics levels in these fruits, which may also have occurred in the present study, once the extract from immature pulp powder showed higher total phenolic compounds and antioxidant activity values. The results reported by Gordon et al. (2012), in "açaí" fruits at different ripening stages, showed antioxidant activity by the ABTS assay of 17 and 2.78 μM *Trolox.100* g^{-1} dry sample for immature and ripe fruits, respectively. Although the results found for the tarumã fruit pulp were lower when compared with the results reported by those authors, a similar behavior was observed, with a reduction of antioxidant activity with an increase in the degree of ripening.

Similar behavior was also observed by other authors, such as Castrejón et al. (2008), who reported significant differences in the phenolic compounds and the antioxidant activity of blueberries (*Vaccinium corymbosum* L.), which reduced throughout the maturity. A certain amount of phenolic compounds is accumulated in immature fruits, which protects the fruits from diseases that can be transmitted during pre-maturity. The advancement of maturity leads to the oxidation of phenolic compounds, contributing to the biosynthesis of anthocyanins that accumulate during this period (Belwal et al., 2019).

Greater anti-inflammatory activity was observed for the extract from the immature fruit pulp powder when compared to ripe fruits. Gómez-Maqueo et al. (2019), investigated the anti-inflammatory activity by the nitric oxide radical scavenging activity (NO%) for two varieties of prickly pear (*Opuntia ficus-indica* L.) and reported values of 300.6 and 273.6 mmol Trolox.g^{-1} for the varieties Pelota and Sanguinos, respectively, which was higher than the results found for the tarumã fruits of this study.

Gómez-Maqueo et al. (2019), used high hydrostatic pressure for pear extracts under controlled conditions, which may have contributed to the antioxidant potential. Another factor that may have influenced the results is the solvent used in the extraction, methanol, which can be considered more effective for the extraction of compounds that inhibit the production of the NO radical. In addition, the characteristics of each fruit can interfere

with the results, due to the interaction between each species and the extraction solvent. It is noteworthy that the extracts of the tarumã pulp powder, for analysis of anti-inflammatory activity, were obtained using water as a single solvent.

Conclusion

Significant differences were observed for the physicochemical properties of tarumã fruits evaluated at different stages of maturity. The reduction of acidity levels, the increase in soluble solids, pH levels, and sugar contents confirmed the fruit ripening in the periods evaluated. The major minerals in the fruit pulps were potassium at both maturity stages, which was more relevant in the mature pulps, followed by calcium and magnesium. The best results of antioxidant activity, total phenolic compounds, and anti-inflammatory activity were observed for the pulp powder from the immature fruit, which presented the highest total phenolic compounds content. The characterization of little-known fruits, such as tarumã, can launch further studies on its properties, mainly concerning the sugar contents, the antioxidant activity, and the phenolic compounds, aiming at the later application in products both in the food and the pharmaceutical industries.

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Authors contribution

LKM: Conceptualization, methodology, validation, formal analysis, research and writing. **SVS**: Research. **CF**: Research. **AOL**: Research. **ER**: Visualization and writing. **GARS:** Conceptualization, visualization and writing. **DC:** Project management, supervision, data curation, visualization and writing.

Full Disclosure

The authors also declare that there is no conflict of interest in the research and publication of the manuscript.

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