Revista Virtual de Química
ISSN 1984-6835

Artigo

Determination of the Content of Ascorbic Acid and Antioxidant Capacity of Fruit *Buchenavia tomentosa* Eichler

Damasceno, E. T. S.; Almeida, R. R.; Pires, B. C.; Dutra, F. V. A.; Borges, K. B.; Guimarães, L. G. L.*

Rev. Virtual Quim., **2019**, *11* (3), 771-784. Data de publicação na Web: 1 de julho de 2019

http://rvq.sbq.org.br

Determinação do Teor de Ácido Ascórbico e Capacidade Antioxidante dos Frutos de *Buchenavia tomentosa* Eichler

Resumo: Neste estudo, cinco soluções extratoras diferentes foram utilizadas para avaliar o teor de ácido ascórbico das polpas *in natura* e liofilizada dos frutos de *Buchenavia tomentosa* Eichler. Também foram avaliadas as capacidades antioxidantes da polpa liofilizada por diferentes ensaios. As concentrações de ácido ascórbico encontrados na polpa dos frutos *in natura* variaram entre 7315,70 e 13272,63, já na polpa liofilizada os teores estiveram entre 1337,67 a 1503,33 mg 100 g^{-1}. O teor de compostos fenólicos totais foi igual a 161,58 ± 24,69 mg de ácido gálico g^{-1} e o de flavonoides igual a 5,93 ± 0,40 mg de quercetina 100 g^{-1} de polpa. As atividades antioxidantes obtidas nos ensaios DPPH e FRAP foram de 50,29 ± 0,61 g de polpa desidratada g^{-1} de DPPH e de 3303,81 ± 50,47 mM de sulfato ferroso g^{-1}, respectivamente. Em contrapartida, para o sistema β-caroteno/ácido linoleico a amostra comportou-se como pró-oxidante, devido ao elevado conteúdo de ácido ascórbico.

Palavras-chave: Mirindiba; Vitamina C; Condições extração; Atividade antioxidante.

Abstract

In this study, five different extractive solutions were used to evaluate the ascorbic acid content of the *in natura* and lyophilized pulps of the *Buchenavia tomentosa* Eichler fruits. In addition, the lyophilized extract was submitted to different tests to determine the antioxidant activities. The fruit pulps presented ascorbic acid contents between 7315.70 and 13272.63 in fresh pulp and between 1337.67 to 1503.33 mg 100 g^{-1} in the lyophilized pulp. The content of total phenolic compounds was 161.58 ± 24.69 mg of gallic acid g^{-1} and of flavonoids equal to 5.93 ± 0.40 mg quercetin 100 g^{-1} of pulp. The obtained antioxidant activities in the DPPH and FRAP assays were 50.29 ± 0.61 g of dehydrated pulp g^{-1} DPPH and 3303.81 ± 50.47 mM ferrous sulfate g^{-1}, respectively. In contrast, for the β-carotene/linoleic acid system the sample behaved as a pro-oxidant because of the high content of ascorbic acid.

Keywords: Mirindiba; Vitamin C; Extraction Conditions; Antioxidant activity

* Universidade Federal de São João del-Rei, Departamento de Ciências Naturais, CEP 36301-160, São João del-Rei-MG, Brazil.
lguimaraes@ufsj.edu.br
DOI: 10.21577/1984-6835.20190056

Volume 11, Número 3 Maio-Junho 2019

Revista Virtual de Química
ISSN 1984-6835

Determination of the Content of Ascorbic Acid and Antioxidant Capacity of Fruit *Buchenavia tomentosa* Eichler

Elisa Tatiana S. Damasceno, Regiamara R. Almeida, Bruna C. Pires, Flavia Viana A. Dutra, Keyller B. Borges, Luiz Gustavo L. Guimarães*

[a] Universidade Federal de São João del-Rei, Departamento de Ciências Naturais, CEP 36301-160, São João del-Rei-MG, Brazil.

* lguimaraes@ufsj.edu.br

Recebido em 3 de setembro de 2018. Aceito para publicação em 24 de maio de 2019

1. **Introduction**
2. **Materials and Methods**
   - **2.1.** Fruits obtention
   - **2.2.** Sample preparation
   - **2.3.** Ascorbic acid extration
   - **2.4.** Ascorbic acid content determination
   - **2.5.** Extract preparation for antioxidant activity determination
   - **2.6.** Total phenolic compounds content determination
   - **2.7.** Flavonoid compounds content determination
   - **2.8.** Determination of antioxidant capacity by the DPPH method
   - **2.9.** Determination of antioxidant capacity by reduction of ferric (FRAP)
   - **2.10.** Determination of antioxidant capacity by inhibition of the oxidation of the β-carotene/linoleic acid system
3. **Results and Discussions**
   - **3.1.** Ascorbic acid content determination
   - **3.2.** Phenolic compounds and flavonoids content determination
   - **3.3.** Antioxidant capacity determination
4. **Conclusion**

1. Introduction

The demand for exotic fruit species of high quality and with high nutrients content is attracting attention. The fruit species high levels of bioactive compounds, with potential to of promoting health and prevent various degenerative diseases. The Cerrado biome is a promising region of exotic fruit species with great potential to better the diet of Brazilian population. However, despite the rich diversity of plants, little is known about the Cerrado fruits species. Therefore, studies that make possible the understanding of the

nutritional value of the existing fruits in Brazilian Cerrado become necessary.[1]

The *Buchenavia tomentosa* Eichler specie, an endemic Cerrado plant, still presents in the literature few reports about its chemical properties. However, in folk medicine the administration of tea from the bark of its stalk is used to treat diabetes.[2-4] This species belongs to the Combretaceae family, is popularly known as "Mirindiba", "Emburidiba" and "Tarumarana", being present in the cerrado, semi-deciduous forest, ciliary forest, gallery forest and dry forest.[2,6] This species has average height ranging from 2 to 6 meters and trunk diameter between 21 and 35 cm, with sapopemas and gray upper branches.[2,3,5]

Although the medicinal properties of *B. tomentosa* are little known, some biological activities have been demonstrated for other species of the *Buchenavia* genus. The anti-HIV and cytotoxic activity of *B. capitata* leaves and the inhibitory activity of the fatty acid synthase enzyme, an important enzyme in the lipid biosynthesis of eukaryotic cells, presented by the ethanolic extract of the *B. parviflora* root, were reported.[7,8] In relation to *B. tomentosa*, some studies describe the antifungal activity of the ethanolic extracts of its fruits against standard strains of *Candida albicans*, *C. parapsilosis*, *C. krusei*, *C. glabrata* and *C. neoformans*.[9,10]

Its fruiting occurs in the months of June, July, August and September. Its fruits are elliptical or spherical drupe with diameter ranging from 2 to 5 cm, fleshy pericarp yellow, sweet when ripe, containing a single seed. They are eatable and eagerly sought by wild animals.[3] Although there were few studies involving *B. tomentosa*, fruit pulp studies demonstrated a high ascorbic acid content. Elanne (2014) found average ascorbic acid levels of 2374.16 mg 100 g^{-1} for the *in natura* fruit pulps of *B. tomentosa*, using colorimetric titration for its determination.[11]

Ascorbic acid, popularly known as vitamin C, is a compound with important nutritional characteristics, being essential for humans. This bioactive compound is involved in several biological processes, including collagen and adrenaline synthesis, formation of bile acids and some neurotransmitters, hormone biosynthesis and in cell division and expansion.[12,13] In addition, ascorbic acid plays an important role in the elimination of reactive oxygen species, which are responsible for cell damage, and which can lead to various abnormalities in the body, such as inflammation, cardiovascular disease, cancer and aging.[12,14] The interest in finding natural antioxidants present in foods has increased considerably, mainly due to their potential nutritional and therapeutic effects.[15]

The ingestion of fresh fruits into the human diet is a simple way to provide the body sufficient amounts of ascorbic acid. Much of this ingested bioactive compound comes from fruits and vegetables, its main sources being citrus fruits, acerola, guava, papaya, tomatoes and vegetables raw leaves.[15] Therefore, the determination of the ascorbic acid content in fruits is of great importance for dietary guidance and attendance of fruit quality.

There are several chemical methods able to evaluate the ascorbic acid content in different foods.[7] Highlighting High-Performance Liquid Chromatography (HPLC), being a reliable and generally simple method. Analysis of ascorbic acid by HPLC is an easy, fast and accurate method. However, for the quantification of this compound, it is initially necessary to extract it from the tissues with acid solutions to prevent its oxidation. Among the acids used in the extractive solutions used are metaphosphoric acid, oxalic acid, acetic acid, trichloroacetic acid and combinations thereof, or the same solutions combined with ethylenediaminetetraacetic acid (EDTA).[15]

In this context, the present study was performed to determine the ascorbic acid content of the fresh and freeze-dried pulp of *B. tomentosa* fruits by HPLC, the evaluating the extraction condition influence. In addition, to evaluate the antioxidant properties of the extract obtained from the pulp of these fruits by the DPPH radical scavenging methods, ferric iron reducing antioxidant power (FRAP) and protection against the oxidation of the β-carotene/linoleic acid system.

2. Materials and Methods

2.1. Fruits obtention

The fruits came from native plants, located in the city of Gurupi-TO. These were harvested manually in the month of August 2015 in the early hours of the morning and packed in plastic bags and frozen at -20 °C. Next, they were transported to the Laboratory of Organic Chemistry of the Department of Natural Sciences of the Federal University of São João del-Rei. The fruits were washed and the pulps were removed manually, and the obtained pulp was divided into two fractions. One of the fractions was sent to the *in natura* fruit analysis and the second fraction was submitted to the lyophilization process.

2.2. Sample preparation

The quantification of the humidity content of the *in natura* sample was performed based on the procedure described by AOCS (1994), adapted by Pimentel *et al.*[16,17] For determination, 5 g of the *in natura* fruit pulp were emerged in 80 mL of cyclohexane (C_6H_{12}) in a 250 mL flask, which was coupled to a dean stark. The system was refluxed for 2 hours. Quantification was performed in quadruplicate.

2.3. Ascorbic acid extraction

To evaluate the best ascorbic acid extraction condition of the lyophilized and *in natura* pulp of the *B. tomentosa* fruits, five extractive solutions were used. These solutions were prepared with a solution based of NaH_2PO_4 (1 mmol L^{-1}) and EDTA (1 mmol L^{-1}) in deionized water, varying only in relation to the acids used and their concentrations. The following solutions were used: solution containing metaphosphoric acid at 1 %,[7] metaphosphoric acid 3 %,[7] oxalic acid 0.5 %,[18] perchloric acid 10 % and metaphosphoric acid 1 %,[19] and acetic acid 8 %, EDTA 2 mmol L^{-1}, sulfuric acid 0.15 mol L^{-1} and metaphosphoric acid 3 %.[20]

The same extraction procedure was used for all extractive solutions. For ascorbic acid extraction, 0.5 and 1.0 g of lyophilized and *in natura* pulp, respectively, were weighed. Next, 10 mL of the extractive solution ware added. The resulting mixture was homogenized, placed in an ultrasonic bath for 5 min and then centrifuged at 3000 rpm. The supernatant was collected and filtered on 0.45 µm PVDF micropore filter for HPLC subsequent analysis.

2.4. Ascorbic acid content determination

The ascorbic acid content of the *in natura* and lyophilized sample was determined by means of high-performance liquid chromatography based on the Cabral *et al.* procedures, followed by small modifications.[21]

The chromatographic analyzes were performed in a HPLC Agilent® 1220 (Palo Alto, CA, EUA) constituted by single module (G4286B), containing an automatic injector model (G1313) and UV-Visc detector. The data were acquired and the instrument controlled by the Agilent Open LAB Chromatography Data System® (CDS) software. Chromatographic separations of ascorbic acid were made using a column Phenomenex® Gemini C18 (150 mm x 4.6 mm, 5 µm) and mobile phase composed of deionized water, conteining NaH_2PO_4 (1 mmol L^{-1}), EDTA (1 mmol L^{-1}), adjusting the pH 3.0 with H_3PO_4 at a flow rate of 1.0 mL min^{-1}. The obtained chromatographic data were acquired at 245 nm. All chromatographic methods were performed at 25 ± 3 °C and the samples injection volume was 20 µL. The obtained ascorbic acid content was expressed in mg 100 g^{-1} of humidity-free fruit.

For the quantification an analytical curve was prepared, based on the procedures described by Valente *et al.*, with ascorbic acid concentrations equal to 75, 100, 125, 150, 175, 200 µg mL^{-1}, being analyzed under the same conditions of the samples.[19] The extractions were done in triplicates. The equation of the line obtained through the analytical curve of ascorbic acid was $y = 2x10^6x + 1x10^7$ with $R^2 = 0.99$. Analyzes of variance were performed to verify the influence of the ascorbic acid extraction condition on lyophilized and *in natura* pulps. The Tukey's test (5 %) was used to compare the mean effects, being all the analyzes performed by the SISVAR program.[22]

2.5. Extract preparation for antioxidant activity determination

The extraction was carried out following the methodology adapted from Pérez-Jiménez & Saura-Calixto (2005).[23] Initially, 10 g of lyophilized pulp was homogenized in 50 % methanol, being this solution kept under stirring for 60 min. Subsequently, the solution was centrifuged for 15 min at 3200 rpm and the supernatant transferred to a 100 mL volumetric flask. In the residue from the first extraction, 70 % acetone was added under stirring, being mixture kept at rest for 60 min at room temperature. Afterwards, the mixture was centrifuged under the same conditions described above and the collected supernatant was transferred to the volumetric flask containing the first supernatant. Finally, the volume of the supernatants was filled with distilled water and the extract packed into vials protected from light and stored at ± 8 °C.[23]

2.6. Total phenolic compounds content determination

For the total phenolic compounds content determination by the Folin-Ciocateau spectrophotometric method, the methodology described by Zhou *et al.* was used, followed by modifications.[24] For the analysis, 500 µL of Folin-Ciocalteu reagent (1 mol L^{-1}) and 500 µL sodium carbonate (20 % m/v) were added to 50 µL of the extract. The readings were performed at 765 nm after 60 min and the results were expressed as mg gallic acid g^{-1} dehydrated pulp.[24]

2.7. Flavonoid compounds content determination

The flavonoid content was determined by the calorimetric method using quercetin as reference, following the adapted methodology described by Bao *et al.*[25] In an aliquot of 0.5 mL of the extract was added 2 mL of distilled water and 0.15 mL of 5 % $NaNO_2$. After 5 min, 0.15 mL of $AlCl_3.6H_2O$ (10 %), leaving the system at rest for 5 min. Then, 1 mL of 1 mol L^{-1} NaOH was added over the reaction mixture, which was stirred and held for 15 min. Subsequently, the readings were performed on a spectrophotometer at 415 nm. Calculations were performed from analytical curve and the results expressed in mg quercetin 100 g^{-1} of dehydrated pulp.[25]

2.8. Determination of antioxidant activity by the DPPH method

The evaluation of the antioxidant activity of the *B. tomentosa* fruits pulp by means of a DPPH free radical neutralization assay was performed according to the methodology adapted from Yepez *et al.*[26] Initially, five different dilutions of the obtained extract (5 to 75 %) were prepared. Then, a methanolic DPPH radical solution was prepared by dissolving 2.4 mg of DPPH in methanol and the volume was made up to 100 mL in a volumetric flask. Finally, the solution was homogenized and transferred to an amber glass bottle. For the analysis, 0.1 mL of each extract dilution was added to 3.9 mL of the DPPH solution, and the readings were performed at 515 nm after 40 min of incubation in the absence of light. For the White one it was used pure methyl alcohol. At

the same time, a control containing 0.1 mL of methanol, acetone and water was prepared in the same concentrations of extract extraction solution with 3.9 mL of the DPPH radical. The antioxidant capacity was expressed in g of dehydrated pulp g^{-1} DPPH.[26]

2.9. Determination of antioxidant activity by *reduction* of ferric (FRAP)

The evaluation of the antioxidant potential of the pulp extracts of the *B. tomentosa* fruits before the FRAP assay was performed by the method described by Pulido *et al.* with modifications.[27] For the analysis, five different dilutions (in triplicate) of the extract in the previously prepared methanol/acetone solvents were used. The FRAP reagent was prepared from the 25 mL acetate plug (0.3 mol L^{-1}), 2.5 mL, of a TPTZ (10 mmol L^{-1}) solution and 2.5 mL of $FeCl_3$ (20 mmol L^{-1}, in aqueous solution). In the assay, in the dark environment 90 µL of each dilution of the extract was homogenized with 270 µL of distilled water and 2.7 mL of the FRAP reagent, and the solution was incubated in a water bath at 37 °C for 30 min. The absorbance was measured at 595 nm using the FRAP reagent as blank. The result was expressed in µM ferrous sulphate g^{-1} dehydrated pulp.[27]

2.10. Determination of antioxidant activity by inhibition of the oxidation of the β-carotene/linoleic acid system

The determination of the antioxidant capacity of *B. tomentosa* fruits pulp in the β-carotene/linoleic acid assay was performed according to the method described by Rufino *et al.*[28] Initially a solution of the β-carotene/linoleic acid system was prepared using 20 mg of β-carotene and 1 mL of chloroform. Then 40 µL of linoleic acid, 530 µL of Tween 40 and 50 µL of the β-carotene solution were added in an erlenmeyer, under stirring. After evaporation of the chloroform, water, previously saturated with oxygen, was added until the resulting solution had an absorbance between 0.6 and 0.7 nm at 470 nm.

For the analysis, four dilutions of the previously prepared extracts (25, 50, 75 and 100 %) of the lyophilized pulp were used. To prepare the reaction mixture, 0.4 mL of each dilution was added in test tubes containing 5 mL of the β-carotene/linoleic acid solution, the tubes were shaken and then kept in a water bath at 40 °C. The absorbance was first read at 470 nm of the reaction mixture after 2 min and then every 15 min up to 120 min. Water was used as white. The reduction of the absorbance of the system without antioxidant was considered as 100 % of oxidation, being determined this way:

$$Absorbance\ reduction\ =\ Abs_{initial} - Abs_{final}$$

The decrease in absorbance values of the dilutions with the system was correlated to establish the oxidation percentage, according to the following equation:

$$\%\ Oxidation = \frac{[(\ Abs\ reduction)_{\text{sample}}\ x\ 100]}{(Abs\ reduction)_{\text{system}}}$$

After the percentage of oxidation of each dilution determination, the obtained values were subtracted from 100, to find the antioxidant activity, which was expressed as percentage of protection, for each dilution of the sample.

3. Results and Discussions

3.1. Ascorbic acid content determination

Fruits are complex samples that contain large amounts of potentially interfering compounds. Therefore, in addition to the chromatographic method, the choice of the sample preparation process is important for an effective quantification of ascorbic acid.[29] The moisture content of the fresh pulp of *B. tomentosa* fruits was 47 %, and for each 5.00 g of fruit pulp was found 2.35 ± 0.62 g of water. The analysis of the moisture content was carried out with the aim of using the same amount of moisture free pulp of the fruits of *B. tomentosa in natura* and lyophilized in the extraction process to compare the ascorbic acid contents. Thus, the water content was considered for calculations of the ascorbic acid determination in the *in natura* sample of *B. tomentosa.*

The levels of ascorbic acid found in the lyophilized and *in natura* pulps of the *B. tomentosa* fruits submitted to the different extractive solutions are shown in Figure 1.

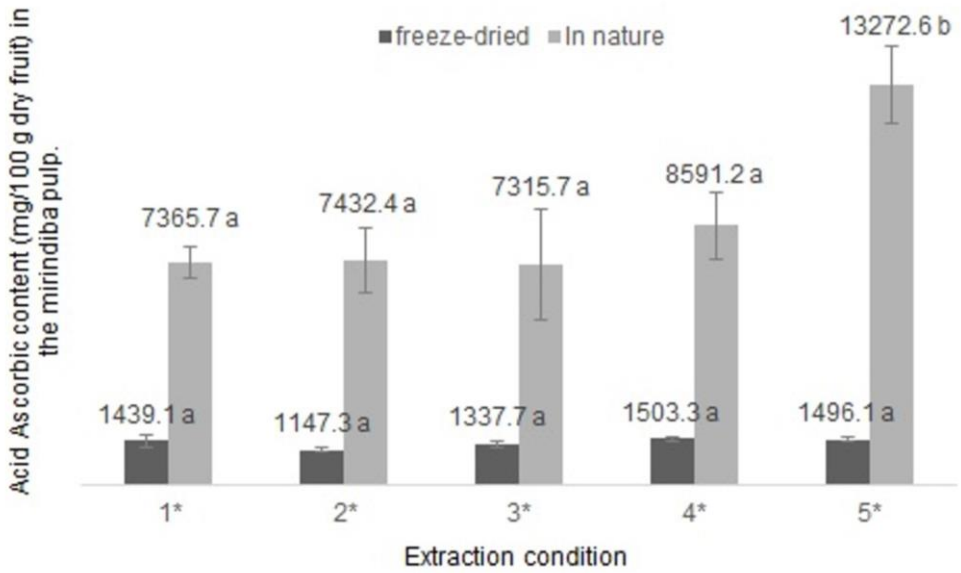

Figure 1. Ascorbic acid contents in freeze-dried and *in natura* pulps of *B. tomentosa* fruits, submitted to different extractive solutions

1*= Metaphosphoric acid 1 %; 2*= Metaphosphoric acid 3 %; 3*= Oxalix acid 0.5 %; 4*= Perchloric acid 10 % and metaphosphoric acid 1 %; 5*= Acetic acid 8 %, EDTA 2 mmol L^{-1}, H_2SO_4 0.15 mol L^{-1} and metaphosphoric acid 3 %. Average values followed by the same letter in the columns do not differ significantly of the Tukey test (p = 0.05).

According to Figure 1, it is possible to observe the high values of ascorbic acid found in the freeze-dried and *in natura* samples of *B. tomentosa*. The highest average ascorbic acid content was 13272.6 mg 100 g^{-1} for the *in natura* sample and the lowest was 7315.7 mg 100 g^{-1}. As for the lyophilized sample, there was not a great variation between the observed levels, being these between 1147.3 mg 100 g^{-1} and 1503.3 mg 100 g^{-1}. Although differences in ascorbic acid levels were observed in the *in natura* and lyophilized pulps, the two presented high contents of this compound.

The ascorbic acid sources are classified in different levels, according to the present levels in the foods, being: high source

(contains of 100-300 mg 100 g^{-1} of fruit); (contains 50-100 mg 100 g^{-1} fruit) and low source (contains 25-50 mg 100 g^{-1} fruit).[30] Note that *B. tomentosa* fruits can be classified as a source high ascorbic acid.

The *B. tomentosa* fruits studies are still recent and therefore there are no data in the literature on this fruit parameters. When comparing the *B. tomentosa* fruits ascorbic acid contents with the contents of some exotic tropical fruits, it is possible to observe that the contents of *B. tomentosa* fruits are much higher than those presented on bacuri fruits (2.4 mg 100 g^{-1}); cajá (26.5 mg 100 g^{-1}); carnauba (78.1 mg 100 g^{-1}); gurguri (27.5 mg 100 g^{-1}); jambolão (112.0 mg 100 g^{-1}); jussara (186.0 mg 100 g^{-1}); murta (181.0 mg 100 g^{-1}) and cupuaçu (3.3 mg 100 g^{-1}).[31,32]

The ascorbic acid levels found for the fruits of *B. tomentosa* were also higher than those presented by fruits such as acerola and cashew, fruits recognized for having high contents of this compound. Studies evaluating the ascorbic acid content of these fruits found average values ranging from 441 to 494 mg 100 g^{-1} in acerola pulp, and 109 and 115 mg 100 g^{-1} for cashew pulp.[33] Other studies have also shown lower ascorbic acid levels for acerola, compared to the values obtained for *B. tomentosa*, ranging from 1114.07 mg 100 g^{-1} to 1456.22 mg 100 g^{-1}.[34] This shows the potential of *B. tomentosa* fruits as a source of ascorbic acid.

Another fruit with a high content of ascorbic acid is camu-camu (Myrciaria dubia), a native Amazon Region fruit, the ascorbic acid content determined in the pulp of this fruit was 1882 mg 100 g^{-1}.[31] Other contents of this compound found in camu-camu were 840 mg 100 g^{-1} for the lyophilized extract.[35] In another study, camu-camu presented a value of 2061.04 mg 100 g^{-1} in predominantly red fruits.[36] Although the ascorbic acid content in the *B. tomentosa* fruits lyophilized pulp were lower than those found in *in natura* pulp, these levels are still very high when compared to the values obtained for other fruits, which are considered by the literature as rich in ascorbic acid, including acerola and camu-camu fruits.

According to statistical analysis, Figure 1 shows that for the lyophilized pulp there was no significant variation in the ascorbic acid content in relation to the extraction condition, demonstrating that the extraction conditions do not influence the ascorbic acid content obtained after the freeze-drying process of the fruit pulp of *B. tomentosa*. As for for the pulp of the fruit *in natura*, it is possible to observe that when using the solution of acetic acid 8 %, EDTA 1 mmol L^{-1}, sulfuric acid 0.15 mol L^{-1} and metaphosphoric acid 3 %, the content ascorbic acid was significantly higher than those found with the use of other extractive solutions (13272.6 mg 100 g^{-1}). Thus, the influence of the extraction conditions on the ascorbic acid content in the *in natura* pulp is observed. During the extraction process, ascorbic acid degradation can occur and the presence of metaphosphoric, acetic and sulfuric acids will aid in the inhibition of its oxidation, while EDTA will complex the metal ions present, in order to obtain a higher acid content end of extraction.[20]

The higher ascorbic acid content found in the pulp *in natura* in relation to those presented by the lyophilized pulp may be justified in view of a possible decrease in the oxidative ascorbic acid processes, as well as their less adherence to the fibers and tissues when the pulps are *in natura.* Other justifications for the found differences between the ascorbic acid contents can also be related to the sensitivity to heat, moisture content, oxygen, pH, temperature, light, and the presence of metallic ions.[37-39] The loss in the acid content of the lyophilized pulp is considered high because it is a lyophilization process, which is considered as the best fruit drying method when compared to the others. It is important to note that, due to the porous structure of lyophilized products, inadequate storage can lead to oxidative reactions that will influence the ascorbic acid content in the final product.[40]

The ascorbic acid content determination of in foods is important both for its nutritional value and for the fact that it is used by the food industry as an antioxidant additive.[41] The importance of this nutrient rich ingredients is even more relevant, considering that this compound is not synthesized by the human organism, being indispensable its intake in the diet. Studies show that the reduction in the risk of developing degenerative diseases is due to the combination of micronutrients, antioxidants, phytochemical substances and fibers present in foods of plant origin. In view of this, fruits with good sources of ascorbic acid represent an alternative to the insertion in the diet.[42]

3.2. Phenolic compounds and flavonoids content determination

The lyophilized pulp extract from the *B. tomentosa* fruits exhibited high amounts of phenolic compounds of 16,158.00 ± 24.69, in which the results were expressed as mg of gallic acid 100 g^{-1} of dehydrated pulp. Phenolic compounds have received attention from researchers because of their nutritional and medicinal effects, with emphasis on protection against free radicals. These compounds have been shown to be the main agents of the antioxidant activity presented by fruits and vegetables, responsible for preventive actions of cardiovascular diseases, cancer, among other degenerative diseases related to aging. The antioxidant activity of phenolic compounds occurs through distinct mechanisms, but the most important is the ability to reduce free radicals.[43]

Denardin *et al.* evaluated the total phenolic contents of different fruits, such as araca, butia, pitangas (red e purple), and blackberries (Xavante and Cherokee). Observed the highest levels of 799.80 ± 54.7 and 816.50 ± 63.6 (mg of gallic acid 100 g^{-1} fresh weigh) to fruits purple pitanga and blackberries cultivar Xavante, respectively. These contents are lower than that found for the lyophilized pulp of *B. tomentosa* fruits of 16,158.00 ± 24.69 mg of gallic acid 100 g^{-1}.[43]

Studies realised by Velderrain-Rodrígues *et al.* with pineapple, mango and papaya fruits showed total phenolic compounds expressed as mg of gallic acid per 100 g of fruit, 107.63 ± 1.01, 274.30 ± 9.32, 212.17 ± 2.40 mg 100 g^{-1}, respectively.[44] These levels were also lower than found in *B. tomentosa* extract. The *B. tomentosa* phenolic compounds content was also superior to that found in lyophilized pulps of Brazilian tropical fruits such as acerola (12,466 ± 1256 mg of gallic acid 100 g^{-1}) and guava (1152 ± 52 mg of gallic acid 100 g^{-1}).[45] This shows the *B. tomentosa* fruit potentiality as a source of phenolic compounds in relation to other fruits.

The *B. tomentosa* fruits also exhibited considerable amounts of total flavonoids of 593.0 ± 0.40 mg quercetin 100 g^{-1} of dehydrated pulp, when compared to lyophilized pulps of other Brazilian tropical fruits. Studies conducted by Paz *et al*. show levels of total flavonoid compounds (expressed mg epicatechin per g of fruit) of 672 ± 40 (açaí), 158 ± 23 (acerola), 184 ± 31 (caja), 217 ± 27 (guava), 70 ± 1 g (mango), 46 ± 6 (pineapple), 252 ± 21 (soursop) and 178 ± 32 (tamarind), demonstrating the *B. tomentosa* fruit pulp potential as a source of flavonoid compounds when compared to other Brazilian fruits.[45]

This research revealed the presence of considerable amounts in the analysed fruit of flavonoids and total phenolic compounds, as well as a high content of ascorbic acid, underlining the benefits of this fruit as a highly nutritious food.

3.3. Antioxidant capacity determination

The *B. tomentosa* fruits lyophilized pulps had presented relevant antioxidant activities through the FRAP (3,303.81 ± 50.47) and DPPH (50.29 ± 0.61) assays, in which the results were expressed as mM of ferrous sulphate g^{-1} dehydrated pulp and g of dehydrated pulp g^{-1} DPPH, respectively. The DPPH and FRAP assays are simple and can be used to analyze different antioxidant mechanisms presented

by the compounds, since these are based on different concepts.[43] The analyzing method of the antioxidant potential through the free radical sequestration capacity DPPH analyzes the radical's reactivity to hydrogen-donating molecules, whereas the FRAP method is based on the ability of the antioxidant compounds to reduce the ferric complex of [2,4,6-tri(2-pyridyl)-1,3,5-triazine] (TPTZ) $[(Fe^{+3}–(TPTZ)_2]^{+3}$.[46]

The antioxidant activity against the FRAP method presented by the *B. tomentosa* fruits was higher than those presented by other fruits such as butia (9.32), araça (89.09), blackberries *Xavante* (52.51) and *Cherokee* (66.60), and purple pitanga (81.62), all results being expressed in µM ferrous sulfate g^{-1} sample.[43] Rufino *et al*. evaluated the antioxidant activity of other Brazilian fruits under the FRAP method, such as acerola (*Malpighia emarginata*), açaí (*Euterpe oleracea*), camu-camu (*Myrciaria dubia*) and puçá-preto (*Mouriri pusa*), also found for these fruits less ability to reduce the ferric complex in relation to *B. tomentosa* (3,303.81 ± 50.47 µM ferrous sulfate g^{-1} sparing), with activities ranging from 32.1 ± 6.5 to 279.0 ± 1.5 µM ferrous sulfate g^{-1} extract, being the smallest and highest activity hit by the fruits açaí and camu-camu, respectively.[31]

By means of the DPPH free radical scavenging assay, it was observed that 50.29 ± 0.61 grams of the obtained extract from the lyophilized *B. tomentosa* fruits pulp were able to neutralize 1 gram of DPPH. Considering the results reported in the literature by Rufino *et al*. for other fruits recognized as having high antioxidant activity, *B. tomentosa* fruits showed high antioxidant potential, since their activity was higher (lower g value of extract g^{-1} of DPPH) than those presented by fruits such as acerola (670 ± 64.5), açaí (4264 ± 1381), camu-camu (478 ± 1.2) and puçá-preto (414 ± 14.4), also determined in g of sample g^{-1} of DPPH.[31] Another study carried out with fruits from the Brazilian Cerrado region also showed a lower capacity to neutralize the DPPH radical than that obtained for *B. tomentosa* fruits. The value found by the authors was 721.85 ± 0.95 for the araçá, 951.52 ± 0.42 for the buriti, 337.88 ± 0.59 for the cagaita, 1310.23 ± 0.42 for the yellow mombin, 2681.91 ± 1.27 for the mangaba and 148.59 ± 9.02 for the marolo.[1]

Although the fruit pulp extract of *B. tomentosa* presented high antioxidant activity against the FRAP and DPPH methods, it did not exhibit relevant activities in the protection of the oxidation of the β-carotene/linoleic acid system, as presented in Table 1. In the whitening of the β-carotene/linoleic acid system the antioxidant capacity is determined by measuring the products of the degradation of linoleic acid by oxidation.[46]

According to the Table 1 presented results in, it was observed that at concentrations below 0.5 g L^{-1} the extract exhibited pro-oxidant activity, and antioxidant activity was observed only at concentrations of 0.5 and 0.75 g L^{-1}. The presence of pro-oxidant activity in the β-carotene/linoleic acid bleaching assay is usually presented by fruits with a high ascorbic acid content, since ascorbyl radical formation may occur during the oxidation of the system.[47,48] Pro-oxidant activity against the β-carotene/linoleic acid protection assay was also observed for the acerola, fruit which also presents high levels of ascorbic acid.[49]

Table 1. Antioxidant analysis of *B. tomentosa* fruit extract by the β-carotene/linoleic acid method

Concentrations ($g\ L^{-1}$)	Antioxidant activity (% de inhibition)
0.1	-23.76 ± 9.78
0.2	-22.14 ± 8.83
0.5	9.69 ± 6.49
0.75	25.49 ± 4.71

The high antioxidants potential presented by the *B. tomentosa* fruits extract in the DPPH and FRAP assays may be associated to the high levels of ascorbic acid and phenolic compounds presented by this fruit. Alves *et al*. evaluated the antioxidant capacity of the cashew, gabiroba, and cagaita fruits, by different methods, including the DPPH and FRAP assays, and found a significant correlation between the antioxidant capacity and the ascorbic acid content.[50]

In another study, using the FRAP method to evaluated the antioxidant capacity of Brazilian tropical fruits, the authors reported a positive correlation between ascorbic acid content and antioxidant capacity.[31] A strong correlation between the content of phenolic compounds and the antioxidant capacity, through the DPPH and FRAP assays, has also been reported in studies with Brazilian fruits, including caju-do-cerrado, gabiroba, cagaita and camu-camu fruits.[31,50] The results obtained by these authors suggest that ascorbic acid and phenolic compounds may be the most important contributors to the antioxidant capacity presented by the *B. tomentosa* fruit extract studied in this study.

4. Conclusion

According to the obtained results concluded that the *B. tomentosa* fruits pulps present high levels of ascorbic acid. However, the *in natura* pulp presents higher contents of this compound when compared to the lyophilized pulp under the same extraction methods. Despite this difference, it can be stated that these fruits present high levels of ascorbic acid when compared with other fruits recognized for having high amounts of this compound. In relation to the different extraction conditions, no influence was observed on the ascorbic acid contents presented in the lyophilized pulp. For the *in natura* pulp, the extractive solution composed of 8 % acetic acid, EDTA 2 mmol L^{-1}, sulfuric acid 0.15 mol L^{-1} and 3 % metaphosphoric acid showed to be more efficient for the extraction of ascorbic acid in relation to the others.

The *B. tomentosa* fruits represent a promising source for the development of foods with functional, once this research also revealed the presence of considerable amounts of phenolic compounds and flavonoids when compared with other fruits. Moreover, was possible to observe that this fruit exhibits health-protection potential, with a pronounced antioxidant capacity towards the assay of the DPPH radical and reduction of iron ion (FRAP). However, considering its high ascorbic acid content, it acts as a pro-oxidant in the assay that evaluates the protection capacity of the β-carotene/linoleic acid system. The results indicate that this fruit is an excellent source of phenolics compounds and ascorbic acid, and have a great antioxidant potential, suggesting their use as a food functional. However, others studies are required to identify and quantify the phenolic compounds present, as well as determine the contribution of the major constituents to the antioxidant activity presented by the fruit.

Acknowledgments

The authors wish to acknowledge the "Conselho Nacional de Desenvolvimento Científico e Tecnológico" (CNPq), the Fundação de Amparo à Pesquisa do Estado de Minas Gerais" (FAPEMIG) and the "Coordenação de Aperfeiçoamento de Pessoal de Nível Superior" (CAPES) for financial support.

References

[1] Schiassi, M. C. E. V.; Souza, V. R. de; Lago, A. M. T.; Campos, L. G.; Queiroz, F. Fruits from the Brazilian Cerrado region: Physico-chemical characterization, bioactive compounds, antioxidant activities, and sensory evaluation. *Food Chemistry* **2018,** *245*, 305. [CrossRef]

[2] Marquete, N. F. S.; Valente, M. C. Flora da Reserva Ducke, Amazonas, Brasil, Combretaceae. *Rodriguésia* **2005**, *56*, 131. [CrossRef]

[3] Lorenzi, H.; *Árvores Brasileiras: manual de identificação e cultivo de plantas arbóreas do Brasil*, 5a. ed., Instituto Plantarum: Nova Odessa, 2008.

[4] Silva, M. A. B.; Melo, L. V. L.; Ribeiro, R. V.; Souza, J. P. M.; Lima, J. C. S.; Martins, D. T. O.; Silva, R. M. Levantamento etnobotânico de plantas utilizadas como anti-hiperlipidêmicas e anorexígenas pela população de Nova Xavantina-MT, Brasil. *Revista Brasileira de Farmacognosia* **2010**, *20*, 549. [CrossRef]

[5] Eloff, J. N.; Katerere, D. R.; McGaw, L. J. The biological activity and chemistry of the southern African Combretaceae. *Journal of Ethnopharmacology* **2008**, *119*, 686. [CrossRef] [PubMed]

[6] Mendonça, R. C.; Felfili, J. M.; Walter, B. M. T.; Silva Jr., M. C.; Rezende, A. V.; Filgueiras, T. S.; Nogueira, P. E.; Fagg, C. W. Em *Cerrado: ecologia e flora*; Sano, S. M. A.; Ribeiro, J. F., eds.: Embrapa Cerrados: Planaltina, 2008, cap. 15. [Link]

[7] Oliveira, R. G.; Godoy, H. T.; Prado, M. A. Quantificação dos isômeros ácido L-ascórbico e ácido D-iso- ascórbico em geleias de frutas por cromatografia líquida de alta eficiência. *Química Nova* **2012**, *35*, 1020. [CrossRef]

[8] Beultler, J. A.; Cardellina, J. H.; McMahon, J. B.; Boyd, M. R. Anti-HIV and cytotoxic alkaloids from *Buchenavia capitata*. *Journal of Natural Products* **1992**, *55*, 207. [CrossRef]

[9] Li, X. C.; Joshi, A. S.; Elsholy, H, N.; Khan, S. I.; Jacob, M. R.; Zhang, Z.; Khan, I. A.; Ferreira, D.; Walker, L. A.; Broedel, S. E.; Raulli, R. E.; Cihlar, R. L. Fatty acid synthase Inhibitors from Plants: Isolation, Structure Elucidation, and Sar studies. *Journal Natural Products* **2002***, 65*, 1909. [CrossRef] [PubMed]

[10] Batista, A. L.; *Tese de Doutorado*, Universidade Federal do Mato Grosso do Sul, Brasil, 2012. [Link]

[11] Glória, E. C.; *Dissertação de Mestrado*, Universidade Federal de Tocantins, Brasil, 2014. [Link]

[12] Fang, T.; Zhen, Q.; Liao, L.; Owiti, A.; Zhao, L.; Korban, S. S.; Han, Y. Variation of ascorbic acid concentration in fruits of cultivated and wild apples. *Food Chemistry* **2017**, *225*, 132. [CrossRef]

[13] Baynes, J. W.; Dominiczak, M. H.; *Bioquímica médica*, 4a. ed., Elsevier: Rio de Janeiro, 2015.

[14] Freire, J. M.; Abreu, C. M. P.; Rocha, D. A.; Corrêa, A. D.; Marques, N. R.; Quantificação de compostos fenólicos e ácido ascórbico em frutos e polpas congeladas de acerola, cajú, goiaba e morango. *Ciência Rural* **2013**, *43*, 2291. [CrossRef]

[15] Righetto, A. M.; *Tese de Doutorado*, Universidade Estadual de Campinas, Brasil, 2003. [Link]

[16] AOCS Official Method Da2b-42. *Official methods and recommended practices of the American Oil Chemists Society.* 4a. ed., American Oil Chemists Society: Champaign, 1994.

[17] Pimentel, F. A.; Cardoso, M. G.; Salgado, A. P. S. P.; Aguiar, P. M.; Silva, V. F.; Morais, A. R.;

Nelson, D. L. A convenient method for the determination of moisture in aromatic plants. *Química Nova* **2006**, *29*, 373. [CrossRef]

[18] Couto, M. A. L.; Canniatti-Brazaca, S. G.; Quantificação de vitamina C e capacidade antioxidante de variedades cítricas. *Ciência e Tecnologia de Alimentos* **2010**, *30*, 15. [CrossRef]

[19] Valente, A.; Sanches-Silva, A.; Albuquerque, T. G.; Costa, H. S. Development of an orange juice in-house reference material and its application to guarantee the quality of vitamin C determination in fruits, juices and fruit pulps. *Food Chemistry* **2014**, *154*, 71. [CrossRef]

[20] Franke, A. A.; Custer, L. J.; Arakaki, C.; Murphy, S. P. Vitamin C and flavonoid levels of fruits and vegetables consumed in Hawaii. *Journal of Food Composition and Analysis* **2004**, *17*, 1. [CrossRef]

[21] Cabral, T. A.; Cardoso, L. de M.; Pinheiro-Sant'ana, H. M. Chemical composition, vitamins and minerals of a new cultivar of lychee (*Litchi chinensis* cv. Tailandes*)* grown in Brazil. *Fruits* **2014**, *69*, 425. [CrossRef]

[22] Ferreira, D. F. Sisvar: a computer statistical analysis system. *Ciência e Agrotecnologia* **2011**, *35*, 1039. [CrossRef]

[23] Pérez- Jiménez, J.; Saura-Calixto, F. Literature data may underestimate the actual antioxidant capacity of cereals. *Journal of Agricultural and Food Chemistry* **2005**, *53*, 5038. [CrossRef]

[24] Zhou, S. H.; Fang, Z. X.; Lu, Y.; Chen, J. C.; Liu, D. H.; Ye, X. G. Phenolics and antioxidante properties of bayberry (*Myrica rubra* Sieb. Et Zucc.) pomace. *Food Chemistry* **2009**, *112*, 395. [CrossRef]

[25] Bao, J.; Cai, Y.; Sun, M.; Wang, G.; Corke, H. Anthocyanins, flavonols, and free radical scavenging activity of chinese bayberry (*Myrica rubra*) extracts and their color properties and stability. *Journal Agriculture Food Chemistry* **2005**, *53*, 2327. [CrossRef]

[26] Yepez, B.; Espinosa, M.; Lopez, S.; Bolanos, G. Producing antioxidant fractions from herbaceous matrices by supercritical fluid extraction. *Fluid Phase Equilibria* **2002**, *194*, 881. [CrossRef]

[27] Pulido, R.; Bravo, L.; Saura-Calixto, F. Antioxidant activity of dietary as determined by a modified ferric reducing/antioxidant power assay. *Journal Agriculture and Food Chemistry* **2000**, *48*, 3398. [CrossRef]

[28] Rufino, M. S. M.; Alves, R. E.; Brito, E. S.; Mancini Filho, J.; Moreira, A. V. B., *Metodologia Científica: Determinação da Atividade Antioxidante Total em Frutas no sistema β-caroteno/ácido linoleico*. Embrapa: Fortaleza, 2006. [Link]

[29] Phillips, K. M.; Tarrago-Trani, M. T.; Gebhardt, S. E.; Exler, J.; Patterson, K. Y.; Haytowitz, D. B., Stability of vitamin C in frozen raw fruit and vegetable homogenates. *Journal of Food Composition and Analysis* **2010**, *23*, 253. [CrossRef]

[30] Andrade, R. S. G. Diniz, M. C. T.; Neves, E. A.; Nóbrega, J. A. Determination and distribution of ascorbic acid in three tropical fruits. *Ecletíca Química* **2002**, *27*, 393. [CrossRef]

[31] Rufino, M. S. M.; Alves, R. E.; Brito, E. S.; Pérez-Jiménez, J.; Saura-Calixto, F.; Mancini-Filho, J. Bioactive compounds and antioxidant capacities of 18 non-traditional tropical fruits from Brazil. *Food Chemistry* **2010**,*121*, 996. [CrossRef]

[32] Canuto, G. A. B.; Xavier, A. A. O.; Neves, L. C. Caracterização físico-química de polpas de frutos da Amazônia e sua correlação com a atividade anti-radical livre. *Revista Brasileira de Fruticultura* **2010**, *32*, 4. [CrossRef]

[33] Scherer, R., Rybka, A. C. P., Godoy, H. T. Determinação simultânea dos ácidos orgânicos tartárico, málico, ascórbico, e cítrico em polpas de acerola, açaí e caju e avaliação da estabilidade em sucos de caju. *Química Nova* **2008**, *31*, 1137. [CrossRef]

[34] Lima P. C. C.; Souza, B. S.; Borges, S. S.; Assis, M. D. O. Caracterização e avaliação de frutos de aceroleira. *Revista Brasileira de Fruticultura* **2014**, *36*, 550. [CrossRef]

[35] Sandoval, M.; Melchor, V.; Okuhama, N. Antioxidante and biological properties of *Myrciaria dubia*: role in cytoprotection. *Free*

Radical Biology and Medicine **2001**, *31*, S37. [Link]

[36] Alves R. E.; Filgueiras, H. A. C.; Moura, C. F. H., *Caracterização de frutas nativas da América Latina*, FUNEP: Jaboticabal, 2000.

[37] Yamashita, F.; Benassi, M. T.; Tonzar, A. C.; Moriya, S.; Fernandes, J. G. Produtos de acerola: estudos de estabilidade de vitamina C. *Ciência e Tecnologia de Alimentos* **2003**, *23*, 92. [CrossRef]

[38] Uddin, M. S.; Hawlander, M. N. A.; Ding, L.; Mujumdar, A. S. Degradation of ascorbic acid in dried guava during storage. *Journal of Food Engineering* **2002**, *51*, 21. [CrossRef]

[39] Fennema, O. R.; *Química de los alimentos*, Editorial Acribia: Zaragoza, 1993.

[40] Marques, L. G.; *Tese de Doutorado*, Universidade Federal de São Carlos, Brasil, 2008. [Link]

[41] Bianchi, M. L. P.; Antunes, L. M. G. Radicais livres e os principais antioxidantes da dieta. *Journal of Nutrition* **2009**, *12*, 123. [CrossRef]

[42] Aguiar, L. P.; *Dissertação de Mestrado*, Universidade Federal do Ceará, Brasil, 2001. [Link]

[43] Denardin, C. C.; Hirsch, G. E.; Da Rocha, R. F.; Vizzotto, M.; Henriques, A. T.; Moreira, J. C. F.; Guma, F. T. C. R.; Emanuelli, T. Antioxidant capacity and bioactive compounds of four Brazilian native fruits. *Journal Food Drug Analysis* **2015**, *23*, 387. [CrossRef]

[44] Velderrain-Rodrígues, G.; Quirós-Sauceda, A.; Mercado-Mercado, G.; Ayala-Zavala, J. F.; Astiazarán-García, H.; Robles-Sánchez, R. M.; Wall-Medrano, A.; Sayago-Ayerdi, S.; González-Aguiar, G. A. Effect of dietary fiber on the bioaccessibility of phenolic compounds of mango, papaya and pineapple fruits by an *in vitro* digestion model. *Food Science and Technology* **2016**, *36*, 188. [CrossRef]

[45] Paz, M.; Gullon, P.; Barroso, M. F.; Carvalho, A.; Domingues, V. F.; Gomes, A. M.; Becker, H.; Longhinotti, E.; Delerue-Matos, C. Brazilian fruit pulps as functional foods and additives: evaluation of bioactive compounds. *Food Chemistry* **2015**, *172*, 465. [CrossRef]

[46] Aktumsek, A.; Zengin, G.; Guler, G. O.; Cakmak, Y. S.; Duran, A. Antioxidant potentials and anticholinesterase activities of methanolic and aqueous extracts of three endemic *Centaurea* L. species. *Food and Chemical Toxicology* **2013**, *55*, 291. [CrossRef]

[47] Hassimotto, N. M. A.; Genovese, I. S.; Lajolo, F. M. Antioxidant activity of dietary fruits, vegetables, and commercial frozen fruit pulps. *Journal of Agricultural and Food Chemistry* **2005**, *53*, 2932. [CrossRef]

[48] Kalt, W.; Forney, C. F.; Martin, A.; Prior, R. L. Antioxidant capacity, vitamin C, phenolics, and anthocyanins after fresh storage of small fruits. *Journal of Agricultural Food Chemistry* **1999**, *47*, 4641. [CrossRef]

[49] Duarte-Almeida, J. M.; Santos, R. J.; Genovese, M. I.; Lajolo, F. M. Evaluation of the antioxidant activity using the β-carotene/linoleic acid system and the DPPH scavenging method. *Ciência e Tecnologia de Alimentos* **2006**, *26*, 448. [CrossRef]

[50] Alves, A. M.; Dias, T.; Hassimotto, N. M. A.; Naves, M. M. V. Ascorbic acid and phenolic contents, antioxidant capacity and flavonoids composition of Brazilian Savannah native fruits. *Food Science and Technology* **2017**, *37*, 564. [CrossRef]