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Centesimal composition, bioactive compounds, physicochemical parameters in *in natura* Genipapo (or Huito) (*Genipa americana L.*)

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Abstract

Introduction: Cerrado is a diversified biome which has many types of fruit species, among which the Genipapo (*Genipa americana* L.) stands out. *Objective*: To analyze the centesimal composition, bioactive compounds and physicochemical parameters in *in natura* (fresh) Genipapo. *Methodology*: Genipapo was bought in shops located in the Brazilian states of Minas Gerais and Goiás. The analyses were performed in triplicate and the results were expressed as mean and standard deviation. Moisture, proteins, lipids, ashes, dietary fiber, vitamin C, titratable acidity, soluble solids and pH were determined following the methods of the Association of Official Analytical Chemists and Adolfo Lutz Institute. In addition, analyses of "available" carbohydrates were performed by the phenol-sulfuric method and by difference; the levels of beta-Carotene were analyzed by spectrophotometry. Antioxidant activity was performed by the DPPH assay. *Results*: The Genipapo pulp showed 70.0% moisture; 0.5% protein; 0% lipids; 1.1% ashes; 6.3% dietary fiber; 20.1% "available" carbohydrates; 22.1% "available" carbohydrates (by difference); 26.0 mg vitamin C/100 g; 0 mg beta-Carotene/100 g; 176.3 mg of phenolic compounds/100 g; pH 3.85; 15.2 ºBrix total soluble solids (TSS); 0.40% titratable acidity and 70.2% antioxidant capacity. *Conclusion*: The *in natura* Genipapo showed considerable values for moisture, total carbohydrates (mainly dietary fiber), vitamin C, phenolic compounds, acidity, soluble solids and high antioxidant activity. Thus, its inclusion in the diet could improve quality, contributing to food and nutritional security of the Cerrado population.

Keywords: Cerrado Fruits. Jenipapo. Centesimal Composition. Bioactive Compounds. Physicochemical Parameters. Antioxidant Activity.

Introduction

Genipapo (*Genipa americana* L.) is a fruit of the Cerrado (vast tropical savanna ecoregion of Brazil, particularly in the states of Goiás and Minas Gerais) belonging to the *Rubiaceae*[1] family and stands out for its wide geographic distribution within the American continent, and can be found in Brazil and other countries (from Mexico to northern Argentina).[2] It has a thin bark, globular shaped berries and brownish pulp (Figure 1).

Figure 1. Picture of whole Genipapo (*Genipa americana L.*) (left) and cut in half (right).

By presenting characteristic and sharp flavor and odor, Genipapo can be used in various ways in a diet: *in natura* (fresh), in preparations (jams, candied sweets and ice cream) and beverages (especially liqueurs, juice, wine and aguardientes (generic term for alcoholic beverages that contain between 29% and 60% alcohol by volume)).[3] The Cerrado has a large variety of native fruit species, but this biome has been threatened by intense degradation and exploitation due to agricultural expansion.[4,5]

The high content of soluble solids present in Genipapo provides better taste and higher yield during processing, increasing its acceptance as food and use as raw material by the food and cosmetics industry. On the other hand, the Genipapo high acidity is considered an intrinsic and characteristic feature, since most fruits present low acidity when ripe. Thus, the acceptance of Genipapo *in natura* may be decreased.[3,6] An interesting fact is that the high acidity of Genipapo promotes its conservation and prevents the proliferation of some microorganisms.[7,8] Genipapo

also has a high water content and significant carbohydrate content (especially dietary fiber) and bioactive compounds (especially phenolic compounds) [9] and, on the other hand, has a low content of proteins, lipids and ashes.[3]

Given the above, the understanding of the components present in the fruits of the Cerrado, in particular Genipapo, and its health benefits, is essential, since the determination and the knowledge of the chemical composition of the foods will provide the correct decision-making in dietetics, besides collaborating with the selection of appropriate equipment for production processes.[10] Thus, the analysis of the nutritional and physicochemical composition of the fruits of the Cerrado, as developed by Hamecek et al.[3] and by Da-Paz and colleagues,[11] has been extremely important also due to the scarcity of research developed mainly on the fruits of the Cerrado *in natura*, particularly in relation to Genipapo.

This study aims to analyze the centesimal composition (moisture, proteins, lipids, ashes, "available" carbohydrates and dietary fiber), bioactive compounds (beta-Carotene, vitamin C and phenolic compounds) and determine some physicochemical parameters (pH, acidity, soluble solids and antioxidant activity) in Genipapo (*Genipa americana L.*) *in natura*, in order to increase new data of nutritional and physicochemical composition, particularly with respect to "available" carbohydrates (by a quantitative method, and not only by difference), to the phenolic compounds and antioxidant activity.[12]

Methodology

Genipapo samples (about two kilograms) *in natura*, whole, ripe and with bark were acquired by consumers and randomly distributed. The acquisition of the Genipapo fruit was held in the trading in the Brazilian cities of Uberlândia, MG, between the months of December 2012 and February 2013, and Goiandira, GO, in January 2014. The sample acquired in Goiandira, GO, was used only to quantify the content of phenolic compounds, due to seasonality and scarcity of fruits in the region of Uberlândia.

The fruits were transported in cool boxes containing reusable ice, and sent to the Laboratório de Bromatologia e Microbiologia de Alimentos da Faculdade de Medicina da Universidade Federal de Uberlândia (Laboratory of Nutritional Science and Food Microbiology of the Medical School of the Federal University of Uberlândia). Before the time of the analyses, the Genipapo pulp was extracted manually and/or with the aid of utensils. All analyses were performed in a completely randomized design, consisting of: taking a test, a sample unit (about two kilograms), a type of fruit (Genipapo) and three replications (triplicate), in a factorial arrangement: 1 x 1 x 1 x 3.[11,13-17]

Moisture analysis was carried out by the method of drying in an oven at 65 °C for 72 hours. For determination of protein content, the methodology of micro-Kjeldahl (960.52) was used,[18] whose conversion factor was 5.75.[19] Lipid content was determined by the Goldfish method [10] using diethyl ether as an extraction solvent. For analysis of the ash content, methodology 018/IV of Instituto Adolfo Lutz (an analytical laboratory, based in São Paulo) was followed,[20] which consisted of burning in an oven at 550 °C for six hours. The contents of total dietary fiber were determined by the enzymatic-gravimetric method (985.29) [18] with the use of enzymes α-amylase, amyloglucosidase and protease.

For "available" carbohydrate analysis, two methods were used: the phenol-sulfuric 12 method and by difference.[19] The "available" carbohydrate analysis by the phenol-sulfuric method [12] took place by dehydration with sulfuric acid, followed by the formation of colored compounds from reactions with phenol and determined in absorbance at 492 nm. In addition, "available" carbohydrates by difference were calculated by the following formula: % "available" CHO (a carbohydrate is a biological molecule consisting of carbon (C), hydrogen (H) and oxygen (O) atoms) = 100% – (% moisture + % proteins +% lipids + % ashes + % dietary fiber).

The levels of vitamin C were determined by oxidation of ascorbic acid with potassium iodate, a recommended method 364/IV of Instituto Adolfo Lutz.[20] The determination of beta-Carotene content was performed by the spectrophotometric method of Masaijasu et al.[21] The phenolic compounds were analyzed by the method of Folin-Cicolteau, described by Singleton & Rossi,[22] using gallic acid as a standard. The values were expressed in milligrams (mg) of gallic acid equivalents (GAE) per 100 g.

The titratable acidity (TA) was determined according to method 016/IV of Instituto Adolfo Lutz,[20] which was based on a titration in a sodium hydroxide solution 0.1 N until reaching the pink color. The total soluble solids were analyzed by direct reading in a digital refractometer according to method 932.12 recommended by AOAC (Association of Official Agricultural Chemists).[18] The pH was determined by direct reading in a potentiometer, according to method 017/IV proposed by Instituto Adolfo Lutz.[20]

The antioxidant activity was analyzed by the method described by Brand-Williams, Cuvelier & Berset,[23] which is the determination of the percentage (%) of free radical oxidation inhibiting 2,2-diphenyl-1-picryl-hydrazyl (DPPH) using the ethanolic extract.

It is important to note that initially the determination of the moisture content of the samples of Genipapo was performed and the remaining dry matter was used for the analyses of the centesimal composition of the fruit. The levels of vitamin C, beta-Carotene, phenolic compounds and the physicochemical parameters were analyzed in the fruit *in natura*. The energy value in

kilocalories (kcal) was estimated using the calculation: multiplication of the contents of "available" carbohydrates (by difference), proteins and lipids by their conversion values 4, 4 and 9 kcal/g. As for the energy value in kilojoules (kJ), it was determined by multiplying the energetic value in kcal by the 4.2 conversion factor.

The values obtained in the analyses of the nutritional composition and physicochemical parameters were expressed on a wet basis and on a dry basis. The statistical analyses consisted in determining the mean and the standard deviation, using software *Microsoft Office Excel* (2010).[24]

Results

The data on the nutritional composition of Genipapo *in natura* analyzed in this study are shown in Table 1.

Table 1. Nutritional composition and energy value of Genipapo (*Genipa americana L.*) *in natura*, on a wet basis and on a dry basis. Uberlândia, MG, 2013.

Nutritional parameters	Wet base Average values ±DP	Dry basis Average values ±DP
Moisture (%)	70.0 ± 0.07	-
Proteins (%)	0.5 ± 0.04	1.7 ± 0.13
Lipids (%)	0.0 ± 0.00	0.0 ± 0.00
Ashes (%)	1.1 ± 0.08	3.6 ± 0.27
"Available" CHO [1] (%)	22.1 ± 0.49	73.7 ± 1.65
"Available" CHO [2] (%)	20.1 ± 0.68	67.0 ± 2.26
TDF (%)	6.3 ± 0.41	20.9 ± 1.38
beta-Carotene (mg/100g)	0.0 ± 0.00	0.0 ± 0.00
Vitamin C (mg/100g)	22.5 ± 6.93	75.0 ± 23.12
Phenolic compounds[3]-(mg EAG/100 g)	176.3 ± 3.84	587.7 ± 12.80
TEV[4] (kcal/100 g)	90.7 ± 1.79	302.2 ± 5.98
TEV[5] (kJ/100 g)	385.1 ± 0.59	1283.8 ± 1.98

Average values ± standard deviation (SD); n = 3 (triplicate of analysis); TDF = total dietary fiber; CHO[1] = "available" carbohydrates by difference; CHO[2] = "available" carbohydrates by the phenol-sulphuric method; Phenolic compounds = total phenolic[3]; TEV[4] = total energy value in kcal/100 g; TEV[5] = total energy value in kJ/100 g;

Table 2 shows the results of the physicochemical parameters of fresh Genipapo.

Table 2. Physicochemical parameters of Genipapo (*Genipa americana* L.) *in natura*, on a wet basis. Uberlândia, MG, 2013.

Physicochemical parameters	Average values ±DP
pH	3.9 ± 0.00
Total Soluble Solids (ºBrix)	15.2 ± 1.56
Titratable Acidity (% citric acid)	0.4 ± 0.03
Antioxidant Activity (%)[1]	70.2 ± 1.27

Average value ± standard deviation (SD); n = 3 (triplicate of analysis). [1]Percentage (%) of inhibition of oxidation of the free radical 2,2-diphenyl-1-picryl-hydrazyl (DPPH).

Discussion

According to the data found in this study, Genipapo presented high moisture content (70%). This value was similar to the one from another study, which found 74.0% moisture, [25] and also similar to the one for some fruits of the Cerrado, such as buriti (*Mauritia flexuosa*) and araticum (*Annonaceae* family), which had 68.9% and 78.8% moisture, respectively.[26] Nonetheless, certain studies have found higher moisture values in Genipapo: 80.4%[3] and 81.3%.[27] It should be highlighted that moisture is a parameter that should be considered because it facilitates the deterioration of fruits and microbiological contamination. Thus, the conservation of Genipapo *in natura* may be hampered and hence its life reduced by high water activity.[3]

The protein content (0.5%) found in this study was higher than the 0.04% reported by Torres,[27] and the value of lipids (0,0%) has not corroborated the result by Andrade et al.[28] who have found 1.5%, but it was close to that found by Brasil[29] (0.3%). This way, Genipapo was not considered a source of lipids.

The value for ashes (1.1%) described in this research was higher than the average of 0.71% found by Souza.[25] However, the value was similar to 1.2%, described by Santos.[30] The ashes indirectly indicate the mineral content of a food; therefore, Genipapo could present some minerals in its nutritional composition. It should be pointed out that the value for ashes found in Genipapo was within the acceptable range for fruit (0.3 to 2.1%).[10,25]

"Available" carbohydrate content by difference on a dry basis (73.7%) found in this study was close to 74.4% described by Hamacek et al.[3] also on a dry basis. This parameter was compared on a dry basis, because the moisture content found in another study[3] has shown difference compared to the present research.

It is important to highlight that so far it has not been possible to find other studies which would determine the "available" carbohydrates content in Genipapo the by phenol-sulfuric method. When confronting the "available" carbohydrate values of this research by both methods (by difference and phenol-sulfuric), it was found that the results were close to each other (22.1% and 20.1%, respectively). The total dietary fiber content (6.3%) observed here was lower than the 9.4% reported by Brasil.[29]

The energy value (90.7 kcal/100 g) of the present study was higher than the 85.1 kcal/100 g found by Torres[27] and lower than the 113.0 kcal/100 g described by Brasil.[29] An adult individual who consumes 100 g of Genipapo pulp will get: 4.5% of the daily energy recommended; between 5.9% and 8.0% of the daily recommendation of CHO; between 0.7% and 1.1% of the daily recommendation for protein; 0% of the daily recommendation of lipids; and 25.2% of the daily recommendation of dietary fiber, according to recommendations in the *Guia Alimentar da População Brasileira (Food Guide of the Brazilian Population)*, of the Ministério da Saúde (Ministry of Health).[31]

The relevance of consuming Genipapo regarding food quality occurs primarily by the high amount of total carbohydrates, especially with respect to dietary fiber. The consumption of dietary fiber by the Brazilian population has dramatically decreased over the past few decades, possibly due to a dietary transition of the population (drastic changes in eating habits), socioeconomic changes and lifestyle in Brazil.[32,33] Resume and enhance the consumption of foods rich in dietary fiber, mainly from foods that are part of the local food culture (Cerrado), is extremely important and has even been recommended by the Brazilian government agencies.[34,31]

Dietary fiber has many benefits for the body, such as a reduced risk of several chronic diseases (obesity, colon cancer, cardiovascular disease, diabetes mellitus, among others); and reduction in bowel transit time and are thus very useful for the prevention of constipation.[35,36]

The variations in the results of the centesimal composition (moisture, proteins, ashes, lipids, "available" carbohydrates and dietary fiber) of Genipapo found in this study, compared to other studies, can be justified for several reasons such as: maturation stage of the fruit; geographic distribution of the Genipapo tree, which features soils with distinct mineral wealth and different climates; and diversified Genipapo phenotypes. Moreover, the use of different methodologies can generate results that corroborate or not the present research.[25,37]

With respect to the bioactive compounds (beta-Carotene, vitamin C and phenolic compounds) laid down in Genipapo, it was found that the fruit had not beta-Carotene in its nutritional composition, since it was brownish. Beta-Carotene is found mainly in yellow-orange color food.[38] Although Genipapo did not present the typical color of a food source of beta-Carotene, an analysis of this bioactive component was performed just in order to make sure that the Genipapo did not present it in its composition. It should be noted that, to date, there are no other studies to report on beta-Carotene content in Genipapo.

Vitamin C content found in this study (26.0 mg/100 g) was similar to the 21.3 mg/100 g noticed by Muniz, Júnior-Silva, Santos.[2] It should be highlighted that the presence of bioactive substances (such as vitamin C) has a protective effect in relation to noncommunicable diseases (NCDs; also known as chronic diseases).[39] Vitamin C stands out due to its antioxidant property (reducing agent and electron donor), protecting the body against the actions of free radicals; its participation in numerous biochemical reactions, mainly acting as an enzyme cofactor; and its ability to enhance iron bioavailability, due to the reduction of ferric iron (Fe^{3+}) and ferrous iron (Fe^{2+}).[40,41]

The content of phenolic compounds observed in this study was 176.3 mg EAG/100 g, a data that did not confirm the content of 338.9 mg EAG/100 g described by Rezende et al.[42] Among the various classes of natural antioxidants, phenolic compounds have been highlighted due to their properties to capture free radicals and therefore the ability to protect the human organism.[43] It should be reiterated that the analysis of the phenolic compounds can be influenced by the nature of the compound, method used, solvent used, sample size, time and storage conditions, pattern used and interfering products (waxes and fats).[44]

The bioactive compounds determined in Genipapo were high, especially in relation to vitamin C and phenolic compounds contents, and these data have probably affected the antioxidant activity of the fruit (70.2%).[9] The antioxidants are able to stabilize or neutralize the free radicals, which can attack various biological components (cell membrane, membrane lipids, etc.) and cause them damage.[45] In addition, clinical and/or epidemiological studies have shown that populations that have a high consumption of foods with high antioxidant activity show a significant reduction in the risk of developing several noncommunicable diseases (NCDs; also known as chronic diseases) such as cancer, obesity and cardiovascular diseases.[39]

In relation to the physicochemical characteristics, the pulp of Genipapo presented acid pH, high levels of TSS and a high antioxidant activity. The value of pH (3.86) found in this study has corroborated what was described by Hamacek et al.,[3] who have found 3.87. The low pH content in food is a quality attribute because it facilitates the preservation of fruit, and other products,

and prevents the growth of microorganisms. In addition, acidic foods are appreciated and widely used by the food industry. On the other hand, fresh food with high acidity generally provides low sensory acceptance to consumers.[7,25,37]

The total soluble solids (TSS) found in this study (15.2 ºBrix (sugar content of an aqueous solution)) were numerically lower than the 18.3 ºBrix described by Souza.[25] The value of TSS of the Genipapo was high compared to tropical fruits (pineapple, malpighia glabra, papaya) and from Amazon (cupuaçu, mangaba, araza).[25] TSS content indicates the amount of total sugars and other compounds, which give sensory characteristics of plants (fruit and/or vegetables) – color, taste, smell, texture – and degree of maturity of fruit.[25] Studies indicate there could be an increase in the total soluble solids content in Genipapo stored at room temperature for up to 28 days.[46] This way, high TSS indices are related to better taste and higher yield of the fruit in preparations.[25,47]

Titratable acidity (TA), described in the present study (0.40%) was lower than the 0,71% found by Souza,[25] according to which the TA is used for fruit production for flavor, and its high content can reduce the acceptance of Genipapo in its fresh form. Titratable acidity may present progressive increase in Genipapo stored at room temperature for 28 days.[46] TA higher in the ripe Genipapo can be considered a characteristic of the Genipapo, since in most fruits TA decreases with the progression of ripening.[47]

Like in the centesimal composition (moisture, proteins, ashes, lipids, "available" carbohydrates and dietary fiber), the bioactive compounds (vitamin C, phenolic compounds and carotenoids) and the physicochemical parameters (pH, acidity, soluble solids and antioxidant capacity) have also admitted changes when compared with other studies. Basically such variations were probably due to changes in Genipapo harvest time, rainy season, soil quality and composition, seasonality, edaphological conditions and different analyses methodologies.[25,37,40]

In addition to the nutritional aspects (highlighted in relation to dietary fiber, vitamin C, phenolic compounds and antioxidant activity), encouraging the consumption of Genipapo could improve food and nutrition security of the Cerrado population, since there would be a resumption of food practices promoting health and respecting the food culture of the local population. Encouraging local food culture, in this case by means of the consumption of Genipapo by the Cerrado population, meets the recommendations of the Brazilian government agencies, especially the Ministry of Health.[31]

Conclusion

Regarding the centesimal composition, Genipapo *in natura* has presented high moisture content and total carbohydrates (about 27% on a wet basis), with emphasis on dietary fiber. As for the bioactive compounds, the highlight was for vitamin C and phenolic compounds.

Regarding the physicochemical values, Genipapo showed high acidity and low pH, appreciable amounts of soluble solids and high antioxidant activity. As a conclusion, the inclusion of Genipapo in the meals of the population of the Cerrado could improve diet quality, thus contributing to food and nutrition security.

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