Influence of the composition of unripe genipap (*Genipa americana* L.) fruit on the formation of blue pigment

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Abstract The physical and chemical characteristics of unripe genipap fruits and the proximate and amino acid compositions of the endocarp and mesocarp of the unripe fruits were determined, placing special emphasis on the possible role of the protein-amino acid fraction on the formation of the typical blue pigment of the matrix. The two parts of the fruit analyzed have low energy (49.88 kcal/100 g for mesocarp and 43.48 kcal/100 g for endocarp) and high fiber content (7.88 % for mesocarp and 16.76 % for endocarp). The endocarp showed protein content (3.19 %) five times higher than the mesocarp (0.62 %), which may explain in part the greater amounts of blue pigment formed in the endocarp when compared to the mesocarp. Furthermore, the pH found in mesocarp (4.49) and endocarp (5.21) is within the optimum range for the formation of the blue pigment. A significant color change ($\Delta E^* = 26.45$) was observed in endocarp during its exposure to the air for 2 h. Free aspartic and glutamic acids and cystine were the predominant amino acids in the

mesocarp, while glutamic and aspartic acids and leucine were predominant in the endocarp. According to the results, the formation of blue pigment does not cause any change in the amino acid composition.

Keywords *Genipa americana* L. · Proximate composition · Amino acid profile · Blue pigment · Food composition · Maturation stage

Introduction

The *Genipa americana* L. species belongs to *Rubiaceae* family and is originally from the Amazon region, where it grows especially in lowland areas. This plant is widely distributed throughout tropical and subtropical areas in Latin America (UNCTAD 2005). The ripe genipap fruits are used in the manufacture of liquor, jams, juices, wines, candies and syrups (Silva and Tassara 2005).

When the unripe fruit is cut open and its interior is exposed to the air, the pulp becomes gradually dark, acquiring an intense blue color, which has been widely used by Brazilian Indians in body painting and ceramics (Cavalcante 1991). The blue pigment is formed from the reaction between genipin (Fig. 1a), a colorless iridoid present in genipap, and primary amine sources such as amino acids and proteins (Touyama et al. 1994).

The detailed mechanism of the blue pigment formation is not clear, but it has been proposed to be the result of an oxygen radical-induced polymerization and dehydrogenation of several intermediary pigments, producing water-soluble polymers of high molecular weight (Mi et al. 2000; Park et al. 2002).

The blue pigment has peculiar features as natural pigment, it exhibits high stability to heat, light and pH changes, being much more stable than phycocyanin which is a blue pigment

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Fig. 1 Chemical structures of genipin (a) and geniposide (b)

extracted from blue-green algae (Fujikawa et al. 1987; Paik et al. 2001). Food Industry in East Asia countries such as Japan and Korea have used the blue pigment obtained from geniposide (Fig. 1b) extracted from fruits of *Gardenia jasminoides* which also belongs to the *Rubiaceae* family. The geniposide is a glycosylated form of genipin, and needs to be treated with the enzyme β -glucosidase to release the aglycone (Cho et al. 2006).

Genipap fruits are an underexploited source of blue pigment and few studies have been conducted concerning this property. The characteristics of genipap blue pigment should be intrinsically related to the nutrient composition of the unripe genipap, but no information is found in the literature to this respect. Therefore, the present study was designed to determine the physical characteristics, proximate composition and amino acid content of unripe genipap fruits and relate them to the formation of blue pigment in this fruit.

Materials and methods

Raw material

The unripe genipap fruits were obtained from “Ilha das Onças” (latitude 1°27'00”, longitude 48°33'00” and altitude of 11 m), an island in Amazon (Brazil) where the plant grows naturally. Fifty fresh fruits were characterized by the following physical parameters: weight, 140.10 ± 20.58 g, and 63.22 ± 3.17 mm and 73.10 ± 4.41 mm for transverse and longitudinal diameters, respectively.

After physical measurements fruits were peeled, mesocarp (pulp) and endocarp (inner part of the fruit including connective tissue and seeds) were separated and each part was homogenized and stored at -18°C until analysis of proximal composition. Ten fruits were used for the analysis of amino acids. They were peeled, separated into endocarp and mesocarp and each part was homogenized separately. Half of each sample was analyzed still fresh and the other half was cut open and subjected to oxidation for synthesis of blue pigment by exposing the material to the air for 2 h.

Proximate composition

All analyses were performed for the mesocarp and endocarp. Total solids, ash, protein, pH, total and reducing sugars; total and insoluble dietary fibers were determined according to AOAC (2005) standard procedures, methods n° 920.151, 940.26, 920.152, 943.02, 925.35; 925.36; 985.29, and 991.42, respectively. Soluble fibers were estimated by the difference between total and insoluble fibers. Lipid content was determined according to Bligh and Dyer (1959). The energy value was estimated according to FAO (2003), considering the following specific factors for fruits: total energy (kcal/100 g) = (percent of protein $\times$ 3.36 kcal/g) + (percent of lipids $\times$ 8.37 kcal/g) + (percent of carbohydrate $\times$ 3.60 kcal/g). Water activity was determined by a digital thermo hygrometer with internal temperature control set at 25°C (DECAGON, Aqualab TE 8063, Pullman, USA). All analyses were performed in triplicate, except the dietary fiber analysis, which was performed in quadruplicate.

Color analysis

The blue pigment formation was monitored by measuring the color of mesocarp and endocarp through CIELAB parameters (L^* , a^* and b^*) obtained by a colorimeter (Konica Minolta CR-400, Osaka, Japan), equipped with the light source D65, and observation angle of 2° . The fruits were peeled for reading mesocarp color and cut in half for reading the endocarp color. Measurements were taken in triplicate at four equidistant points around the circumference of the fruit for both mesocarp and endocarp. The color was measured at 15 min intervals for 2 h. Furthermore, the total color difference (ΔE^*) was calculated using Eq. (1).

$$\Delta E^* = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \quad (1)$$

Amino acid profiles

The amino acid profile was determined in an HPLC TSP (Thermo Separation Products), equipped with quaternary pumps (model 600), on-line degasser, and UV detector (Spectro System UV2000 - WATERS). The UV spectrum was obtained at 254 nm. Total and free amino acids were determined according to the method of White et al. (1986), as modified by Hagen et al. (1989).

Total amino acids analysis was performed in samples that underwent hydrolysis with 6 N HCl for 24 h at 100°C . Following reaction of the released amino acids with phenylisothiocyanate (PITC), the derivatives were separated on a RP C18 LUNA column (catalog number 00G-4252-EQ; $100\text{ \AA}$; particle size $5\text{ }\mu\text{m}$, $250 \times 4.6\text{ mm}$ i.d.; Phenomenex, Torrance, CA, USA) at 50°C and a binary eluant system

Table 1 Physicochemical characteristics of the mesocarp and endocarp from unripe genipap^a

Characteristic ^{b, c}	Mesocarp	Endocarp
Moisture (%)	80.87±0.42 ^a	68.01±1.38 ^b
Ash (%)	0.95±0.04 ^a	0.88±0.07 ^a
Protein (%)	0.62±0.02 ^a	3.19±0.08 ^b
Lipid (%)	0.29±0.03 ^a	0.54±0.06 ^b
Reducing sugars (%)	3.20±0.08 ^a	2.87±0.07 ^b
Totals sugars (%)	10.69±0.20 ^a	9.63±0.08 ^b
Insoluble fiber (%)	7.10±0.26 ^a	14.73±1.31 ^b
Total fiber (%)	7.88±0.39 ^a	16.76±0.65 ^b
Total energy (kcal/100 g)	49.88±0.92 ^a	43.48±1.27 ^b
Water activity	0.99±0.00 ^a	0.98±0.00 ^a
pH	4.49±0.02 ^a	5.21±0.01 ^b

^a Results expressed as mean±standard deviation^b Different letters in same line indicate a significant difference between samples ($p < 0.05$)^c On a wet basis

consisting of (A) 60 mM sodium acetate buffer in triethylamine and (B) acetonitrile in water with EDTA. Quantification was carried out by comparison with a standard mixture (Thermo Scientific, Rockford IL, USA) and DL-2-aminobutyric acid was used as an internal standard from Sigma (St. Louis, MO). The determination of free amino acids was carried out in 0.5 g samples in 80 % methanol/0.1 M HCl v/v, and 1 mL of α -aminobutyric acid, added as internal standard, in a 10 mL volumetric flask and centrifuged at $8500 \times g$ for 15 min. The supernatant was filtered through a 0.22 μ m membrane and a 40 μ L aliquot was derivatized as described above, for the injection of 20 μ L into the HPLC. The data were processed in a PC 1000 software.

Statistical analysis

The data on the major nutrients (proximate composition) and amino acid profiles were subjected to analysis of variance (ANOVA) establishing comparisons between the two parts

Fig. 2 Changes in color parameters of mesocarp and endocarp along time of exposure to the air

of the fruit (mesocarp and endocarp). For the amino acid profile, ANOVA was followed by the Tukey test when significant differences were detected. Statistical analyses were processed into a range of 95 % ($p < 0.05$) and using the software Statistica for Windows.

Results and discussion

Proximate composition

The average fruit weight was 140.10 g, this value represents approximately half of weight reported in the literature for ripe genipap (Hansen et al. 2007; Santos et al. 2007) and the average transverse and longitudinal diameters were 63.22 and 73.10 mm, respectively, which are smaller than those reported for ripe genipap (Hansen et al. 2007; Silva et al. 1998).

The mesocarp water content was 12.86 % higher than the endocarp (Table 1). The moisture of the mesocarp was close to that found for ripe genipaps (de Souza et al. 2012). Despite the difference in moisture content, the two portions of the fruit did not differ significantly regarding water activity, with values 0.99 and 0.98, for mesocarp and endocarp, respectively, thus suggesting high susceptibility to chemical and enzymatic reactions, as well as microbial development (Jay et al. 2005). There was no significant difference between the ash content of the two tissues of the fruit; although they appear to

be slightly lower (1.22 %) than reported for ripe genipap (Santos et al. 2007).

The protein content exhibited by the endocarp was five times higher than mesocarp, which may explain in part the greater amounts of blue pigment formed in the endocarp when compared to the mesocarp. As in most fruits, the lipid content of genipap was low, but the concentration in the endocarp was 86 % higher than in the mesocarp. The large difference between endocarp and mesocarp occurs because the endocarp is the place of nutrient reserve deposition for the embryo (Damodaran et al. 2007).

There were also significant differences in sugar content between the two parts of the fruit. The mesocarp had significantly higher levels of total and reducing sugars, than the endocarp. The levels of reducing and total sugars in this study with unripe fruits were much lower than those reported in the literature for ripe genipaps (Hansen et al. 2008; Santos et al. 2007).

The content of total and insoluble fiber present in the endocarp was twice the content found in the mesocarp for these two components. The insoluble fiber accounted for most of the dietary fiber in the two tissues (90 % in the mesocarp and 87 % in the endocarp). High fiber contents in unripe fruits are common, but along the maturation process, degradation of hemicellulose and protopectin occurs decreasing the concentration of fibers; especially in the mesocarp, rather than the endocarp, where seeds are formed mainly from the insoluble fibers cellulose and lignin (Damodaran et al. 2007).

The total energy of the endocarp was lower than the mesocarp, but both can be considered low in energy and are

Table 2 Total amino acid profile in mesocarp and endocarp (fresh and oxidized) of unripe genipap^a

Amino acid (mg/100 g) ^b	Mesocarp		Endocarp	
	Fresh	Oxidized	Fresh	Oxidized
Aspartic acid	93.32±31.65 ^a	95.76±28.06 ^a	209.15±1.63 ^b	218.48±4.41 ^b
Glutamic acid	77.72±6.33 ^a	75.63±7.74 ^a	304.23±8.44 ^b	319.48±25.07 ^b
Serine	9.29±8.08 ^a	11.97±7.12 ^a	95.98±2.86 ^b	105.02±9.45 ^b
Glycin	26.92±3.93 ^a	27.34±4.76 ^a	99.17±7.82 ^b	102.77±11.14 ^b
Histidine	19.75±0.49 ^a	17.60±0.99 ^a	51.30±4.39 ^b	50.67±3.86 ^b
Arginine	17.54±5.99 ^a	12.31±0.98 ^a	99.18±5.90 ^b	100.33±4.70 ^b
Threonine	26.49±1.99 ^a	28.99±7.09 ^a	78.68±3.37 ^b	84.56±0.63 ^b
Alanine	42.88±2.01 ^a	39.53±0.04 ^a	116.38±5.97 ^b	120.35±7.00 ^b
Proline	32.56±6.42 ^a	32.39±5.81 ^a	93.33±6.32 ^b	100.11±9.04 ^b
Tyrosine	22.16±0.48 ^a	23.55±1.63 ^a	75.77±5.19 ^b	84.66±6.59 ^b
Valine	30.69±2.41 ^a	31.22±4.07 ^a	104.25±4.03 ^b	111.34±8.01 ^b
Methionine	2.48±1.01 ^a	1.68±2.38 ^a	7.11±0.55 ^b	7.17±0.24 ^b
Cystine	74.58±10.30 ^a	74.16±4.01 ^a	58.44±15.22 ^a	44.56±0.34 ^a
Isoleucine	25.28±2.43 ^a	25.63±2.93 ^a	88.86±5.43 ^b	95.07±7.82 ^b
Leucine	52.78±5.96 ^a	57.36±6.85 ^a	176.13±14.53 ^b	185.08±17.71 ^b
Phenylalanine	28.76±6.14 ^a	29.659±0.65 ^a	94.53±1.46 ^b	103.06±5.01 ^b
Lysine	41.44±0.90 ^a	37.796±1.28 ^a	121.93±4.71 ^b	117.89±1.25 ^b

^a Results expressed as mean ± standard deviation

^b Different letters in same line indicate a significant difference between samples ($p < 0.05$)

comparable to savanna fruits like gabiroba (47.4 kcal/100 g) and murici (46.4 kcal/100 g), and Amazonian fruits like cubiu (43.8 kcal/100 g) (Silva et al. 2008; Yuyama et al. 2007).

The pH of the mesocarp was significantly lower than in endocarp, but both results were higher than reported values for ripe genipaps (de Souza et al. 2012; Hansen et al. 2008; Santos et al. 2007). The reduction of pH during ripening of fruits is not very common, but occurs in fruits such as banana and acerola due to accumulation of organic acids (Carvalho et al. 2011; Ferreira et al. 2009).

The values of pH of both parts of the fruit are within the optimal range for the formation of blue pigment (4.5 to 6.0) according to Cho et al. (2006). On the other hand, ripe genipap has a acid pH around 3.0 hampering the reaction because the amine group is protonated, resulting in substitution of the ester group of genipin and leading to the formation of a secondary amide, which results in low molecular weight polymers with low absorption in the visible range (Mi et al. 2005). For the blue pigment occur, the higher the molecular weight, the greater its tinctorial strength (Park et al. 2002).

Color analysis

The blue pigment formation occurred mainly in the endocarp with decreased yellowness (a^*) and redness (b^*) (Fig. 2), tending to the blue-green region of the color space. Simultaneously, the fruit darkening occurred, as featured by the reduction of the L^* parameter. The endocarp total color change (ΔE^*) was 26.45 in 2 h of exposure to the air. ΔE^* values larger than 12 units represents a large color difference, which can be easily perceived by human eyes (Guan and Luo 1999).

Amino acid profiles

Attempts to determine the free amino acid profile were unsuccessful because even in the freshly opened fruit, the concentrations were below the quantification limits in addition to the presence of unknown chromatographic peaks. This suggested that reaction of trace-level amino acids may have occurred already with some constituent of the matrix analyzed, most probably, genipin, and that in order to determine if amino acids of the fruit do participate in the transformation of genipin, an oxygen-free atmosphere would have to be used in the analysis.

The predominant amino acids in the mesocarp were the glutamic and aspartic acids, followed by cystine, and in the endocarp the predominant amino acids were glutamic acid, aspartic acid and leucine (Table 2). The endocarp, both fresh and oxidized, exhibited significantly higher levels than the mesocarp in most of the amino acids, except for cystine, whose levels did not differ between the analyzed portions of the fruit.

No significant differences between either fresh and oxidized mesocarp, or fresh and oxidized endocarp for all amino acids were observed. The data suggest that the formation of blue pigment do not affect the amino acids structure, since they are all recovered after acid hydrolysis.

Conclusions

The data presented here show that mesocarp and endocarp portions of the unripe genipap were characterized as having low total energy and high fiber contents. The endocarp was found to contain a high concentration of protein, but development of the typical blue pigment of the matrix (endocarp and mesocarp) appeared to have no effect on the total amino acid contents, once they are all recovered after acid hydrolysis. However, the pH of immature genipap seemed to be quite favorable for the development of the typical blue pigment either in the endocarp as in the mesocarp.

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