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Quantification of Bioactive Molecules, Minerals and Bromatological Analysis in Carao (*Cassia grandis*)

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Abstract

Medicinal plants have various beneficial conditions for humanity, one of them is its medicinal contribution due to the presence of phytochemicals and antioxidants, characterized by these bioactive compounds as the main source of nutraceuticals. The fruit of *Cassia grandis*, traditionally known as *carao*, is a plant that is attributed antimicrobial and medicinal properties. The objective of this work was to determine the bromatological, mineralogical composition and bioactive molecules of *carao* in the department of Choluteca (Honduras). Total phenolic compounds determined by the Folin-Ciocateau method resulted in higher concentrations in the seeds 11.1±0.3 mg EAG 100 g^{-1}. The antioxidant activity was also found to be higher in *carao* seeds, with concentrations of 7.31±0.11 µg g^{-1} of DPPH and total carotenoids showed higher concentration in the pulp with a concentration of 4.12±0.11 µg mL^{-1}. Among the macro minerals, high concentrations of magnesium and calcium stand out in the seed with a concentration of 18.27±0.14 mg 100 g^{-1} and 7.31±0.23 mg 100 g^{-1} respectively. Among the microminerals, iron stands out in higher concentrations than in the rest of the microminerals being higher in the shell with concentrations of 1.71±0.23 mg 100 g^{-1} followed by manganese in concentrations of 0.51±0.12 mg 100 g^{-1}.

Keywords: iron, carotenoids, biotechnology, DPPH

1. Introduction

The *carao*, whose scientific name is *Cassia grandis*, belongs to the legume family and is a sub family of the *Cesalpinaceae*, it is commonly known by different names such as *caña*, *fistula*, *cañandonga* and *carao* (Lagarto & Guerra, 2005; Carvalho, 2006; Lafourcade et al., 2014). There are approximately more than 500 *Cassia* species worldwide, represented as herbs, shrubs and trees (Sadiq et al., 2012; Korlam et al., 2016). This species is found in the wild mainly in India, China, East Africa, South Africa and in some countries of the American continent, such as Brazil, Colombia, Mexico, Cuba, El Salvador, Nicaragua, Costa Rica and Honduras (Lafourcade et al., 2014; Ramos et al., 2014; Marcía et al., 2017; Bomfim Gois et al., 2018). The *carao* fruits is rigid and can only be opened by applying mechanical forces, inside it many lobes are seen separated by very thin transverse partitions of yellowish color. A dark red flesh, thick consistency, strong odor, sweet taste and easily soluble in water (Jaime et al., 2012).

The *carao* is a tree that grows from 15 to 30 m high, with a width of 45 to 100 cm with a cylindrical shaft that branches from the middle, round crown with about 8 m in diameter, its bark is smooth, of brown gray color, 30 mm thick, the leaves are composed and alternate with 15 to 20 pairs of opposite leaflets, 2 to 5 cm long and 1 to 1.5 cm wide, rounded base and green , the inflorescences have 15 or more flowers, an intense pink color, the seeds are 2 to 4 cm long and 1.5 to 2.5 cm wide and the pod that contains it can reach up to 75 cm in length (Figure 1) (Ramos et al., 2014).

Figure 1. Carao (*Cassia grandis*)

Currently there are studies that validate the medicinal potential of *Cassia grandis*, due to its phytochemical composition, its antioxidant capacity and its high bioavailability. Romero et al. (2018) affirm that the seed of the fruit can be used as an anti-diabetic potential, due to its inhibitory effect of trypsin. Fruit pulp from in vivo models showed a reduction in blood glucose levels (Lafourcade et al., 2018; Lodha et al., 2019). Its nanodispersion exerts a hypoglycemic effect with a potent inhibition of alpha-glucosidase and pancreatic lipase (Lafourcade et al., 2019). In addition, the fruit of *Cassia grandis* has shown its anti-anemic potential from in vivo studies, due to its inorganic iron content and good bioavailability (Tillán et al., 2004; Lafourcade et al., 2014; Lafourcade et al., 2016). From studies of phytochemical characterization in carao fruit, it was determined that it contains good iron content, presence of saponins, porphyrins, flavonoids, tannins, phenols, essential oils and a high antioxidant capacity (Deshpande & Bhalsing, 2011; Jaime et al., 2012; Ramos et al., 2014; Kabila et al., 2017).

Other investigations on carao extracts (*Cassia grandis*) determined the presence of the galactomannan biopolymer and, due to its rheological characteristics, can be used as a substitute for gums in the food industry (Harsha & Kapoor, 2003). The objective of this work was to carry out a bromatological characterization of the different parts of the *carao* fruit, mineral analysis as well as composition of total phenolic compounds, antioxidant activity and carotenoids in the different parts of the fruit.

2. Material and Methods

2.1 Sample Collection and Preparation

The samples were collected in the department of Choluteca (Honduras) between February and March 2019, and were subsequently taken to the Biotechnology Laboratory of the National University of Agriculture, Catacamas (Honduras) where they were separated in different parts of the fruit and dried in an air circulation oven for 48 hours to maintain constant weight. Subsequently they were ground, sieved and stored in suitable containers until the time of the analysis.

2.2 Bromatological Analysis

The bromatological parameters analyzed to later determine the total energy value in the different parts of the fruit were the humidity, quantity of ashes carried out in a muffle at 600 °C and the percentage of ashes calculated by mass difference. Total proteins were determined by the Kjeldahl distillation method with previous sulfuric digestion. The percentage of total lipids was determined by Soxhlet type extractor with hexane and the percentage of carbohydrates was calculated by difference using Equation 1, according to the methodology described by IAL (2008).

$$\text{Energetic value (kcal 100 g}^{-1}\text{)} = (P*4) + (L*9) + (C*4) \quad (1)$$

Where, P = protein value (%), L = lipid value (%), C = carbohydrate value (%), 4 = kcal conversion factor determined in calorimetric pump for proteins and carbohydrates and 9 = kcal conversion factor determined in calorimetric pump for lipids.

2.3 Mineral Analysis

For the determination of minerals, the samples were first subjected to perchloric nitric digestion (3:1), the following elements being determined: Ca (λ = 422.70 nm), Mg (λ = 285.21 nm), Fe (λ = 248.33 nm), Zn (λ = 213.80 nm), Mn (λ = 279.48 nm), Cu (λ = 324.75 nm) by FAAS, Na and K by EAS and by molecular spectrophotometry Uv-visible phosphorus (λ = 660 nm) and sulfur (λ = 420 nm) according to the methodology described by EMBRAPA (2008).

2.4 Phenolic Compounds and Antioxidant Activity

Total phenolic compounds was determined using the Folin Ciocateau method with formation of a blue complex using gallic acid as a reference standard, the absorbance readings being performed on a Uv-Visible spectrophotometer at 765 nm according with the methodology described by Wolfre et al. (2013). To determine the antioxidant activity, the method of the radical 1,1-diphenyl-2-picrilhydrazil (DDPH) was used and the iron reduction method was used. In the first method, the absorbance reading at 515 nm was performed (Miranda & Fraga, 2006), the calibration being made from dilutions of a 60 mM DPPH solution in methanol. The second method of determining the antioxidant activity was the method based on the reduction of Fe^{3+} for Fe^{2+} according to the methodology proposed by Sanchez Moreno et al. (2008), with the readings in Uv-Visible molecular absorption spectrophotometry a 690 nm.

2.5 Total Carotenoids

For the quantification of total carotenoids, one g. of sample was extracted with 18 mL of acetone, the readings were made in UV-Visible molecular absorption spectrophotometer at 470 nm, 661 nm and 664 nm respectively, being calculated using Equations 2-4 described by Lichtenthaler and Buschmann (2001) (Equations 2-4),

$$\text{C carotenoids (mg mL}^{-1}) = (1000\ A_{470} - 1.90\ Ca - 63.14\ Cb)/214 \quad (2)$$

$$\text{Ca (mg mL}^{-1}) = 11.24\ A_{661} - 2.04\ A_{644} \quad (3)$$

$$\text{Cb (mg mL}^{-1}) = 20.13\ A_{664} - 4.19\ A_{661} \quad (4)$$

2.6 Statistical Analysis

The data was analyzed in the SPSS software, version 25.0, using, tukey test at ($P < 0.05$) to identify significant differences.

3. Results and Discussion

3.1 Bromatological Analysis

In Table 1, the values of the nutritional composition and total energy value for the different parts of the fruit studied are presented.

Table 1. Bormatological composition and total energy value in *carao*

Fruit parts (%)	**Humidity**	**Ashes**	**Lipids**	**Carbohydrates**	**Proteins**	**Energetic Value** (Kcal 100 g^{-1})
Pulps	26.72a	2.80b	0.21b	61.93c	8.34b	**282.97c**
Shell	8.19c	2.42c	0.14c	88.01a	1.24c	**358.26a**
Seeds	9.58b	3.74a	1.17a	75.40b	10.11a	**352.57b**
Whole fruits	17.31	3.14	0.74	71.4	7.41	**321.9**

Note. * Means with different letters in the same column indicate statistical differences ($P \leq 0.05$) with Tukey test.

The highest humidity values for the different parts of the fruit studied are in the pulp, with values of 26.72% and the part that presented less moisture was the crust with only 8.19%. The ash content in *carao* is one of the lowest bromatological parameters, with the seeds having the highest mineral value with 3.78%. Among the parameters that contribute to the energy value of the fruit are lipids, carbohydrates and proteins, the amount of lipids is very low, reaching the value at 1.17% for the seeds. Again, the amount of protein is higher for seeds with 10.11%.

Carbohydrates, including fibers, are the major constituents of *carao*, with the peel presenting the highest percentage of carbohydrates with 88.01%.

Among the parameters that contribute to the energy value of the fruit are lipids, carbohydrates and proteins. The amount of lipids is very low, reaching the value at 1.17% for the seeds. Again, the amount of protein is higher for seeds with 10.11%. Carbohydrates, including fibers, are the major constituents of *carao*, with the peel presenting the highest percentage of carbohydrates with 88.01%. Given the high percentage of carbohydrates found in legumes, and especially in *carao*, they can be used as an unconventional food source. The carbohydrate content in this legume is higher than that found in other tropical legumes such as Inga, whose percentage reaches 27.62% (Mendoza et al., 2016). As for the energy value, the part of the fruit that has an important contribution is the shell with 358.26 kcal 100 g^{-1}. The daily energy recommendations of legumes are around 2,000 kcal 100 g^{-1} in accordance with the specifications of the European Economic Community 90/496/EEC of September 24, 1990.

3.2 Mineralogical Analysis

Table 2 shows the values of the different minerals analyzed for the different parts of the fruit studied, as well as for the whole fruit of *carao*. Among the macro minerals, magnesium stands out as the majority, being its highest concentration for seeds with values of 18.27±0.14 mg 100 g^{-1}. This element is of great importance for the body as it is involved in numerous metabolic reactions (Wolfe & Cittadini, 2003). The recommendations of this element according to DRI (2011) are 420 mg day^{-1} for men and 320 mg day^{-1} for women. The next element in importance within the macro minerals is calcium which, like magnesium, this element is in higher concentrations in seeds with a concentration of 7.31±0.21 mg 100 g^{-1}. This element is essential for the mineralization of bones and teeth (França & Martini, 2014) being the recommendations of 1000 mg day^{-1} for both sexes, according to the recommendations of the DRI (2011). Sodium and potassium are also two important elements to maintain the electrolyte balance in the cell such as the sodium potassium pump (Cuppari & Bazanelli, 2010). In *carao* fruit, potassium is found in concentrations higher than sodium, reaching values of 8.23±0.18 mg 100 g^{-1} for seeds and instead sodium is found in higher concentrations in the pulp, in concentrations of 2.56±0.13 100 mg g^{-1}.

Table 2. Shows the macro and micromineral values in the different parts of the *carao*, as well as in the whole fruit

Concentration (mg 100g^{-1})	**Seeds**	**Pulps**	**Shells**	**Whole fruits**
Ca	7.31±0.21a	5.67±0.12b	4.67±0.17c	6.21±0.12
Mg	18.27±0.14a	14.31±0.12b	11.21±0.07c	15.46±0.07
K	8.23±0.18a	3.45±0.07b	2.43±0.14c	4.31±0.08
Na	0.85±0.07c	2.56±0.13a	1.31±0.07b	1.47±0.31
Fe	1.71±0.23a	1.54±0.12b	0.81±0.07c	1.14±0.21
Cu	0.21±0.08b	0.14±0.03c	0.71±0.12a	0.44±0.11
Zn	0.46±0.09a	0.34±0.11b	0.21±0.07c	0.27±0.13
Mn	0.51±0.12a	0.25±0.07b	0.21±0.07c	0.38±0.08
P	0.47±0.07a	0.21±0.02b	0.14±0.07c	0.26±0.08
S	0.08±0.01c	0.17±0.04a	0.11±0.03b	0.04±0.01

Note. * Means with different letters in the same line indicate statistical differences ($P \leq 0.05$) with Tukey test.

The daily recommendations for this element in adulthood are 8 mg day^{-1} for men and for women aged 19-50 years. The recommended concentrations are 18 mg day^{-1} and from 50 years of 8 mg day^{-1} according to the DRI (2011). Manganese is another of the micronutrients found in carao in significant concentrations, the highest concentration being for seeds with a concentration of 0.51±0.12 mg 100 g^{-1}. Manganese is the second micronutrient after iron of interest to plants (Malavolta, 2006), but at the same time it plays an antagonistic role with iron in the body, since in the diet, excess manganese can cause reduced absorption of iron causing anemia in addition to affecting the central nervous system (Roels et al., 1997). Zinc has different physiological functions in the cell, such as the hepatic mobilization of vitamin A, in sexual maturation, fertility and reproduction, phagocytic, cellular and humoral immune function (Manganaro, 2008) the concentration in *carao* seeds being 0.46±0.09 mg 100 g^{-1}. Copper is another essential nutrient not synthesized by the body, being found in fruits in concentrations between 0.02-0.66 mg 100 g^{-1} according to Amancio (2017).

In *carao*, the copper concentrations found are very low, being in greater concentration in the bark of this fruit in a concentration of 0.71±0.12 mg 100 g^{-1}. Two other elements analyzed in this fruit were phosphorus and sulfur. The highest phosphorus concentration was found in the seed with a concentration of 0.47±0.07 mg 100 g^{-1} acting in the energy metabolism of ATP, involved in carbohydrate metabolism and present at the same time in the synthesis of phosphated sugars, nucleic acids and coenzymes (Epstein & Bloom, 2006). Sulfur was found in low concentrations in *carao* with concentrations of 0.17±0.04 mg 100 g^{-1}, being an element that is also part of the structure of biomolecules such as proteins and found in the body in concentrations of up to 140 grams (Lisbon, 2015).

3.3 Phenolic Compounds, Antioxidant Activity and Total Carotenoids

Table 3 shows the values of phenolic compounds, antioxidant activity and total carotenoids in the different parts of the *carao* fruits.

Table 3. Phenolic compounds, antioxidant activity and total carotenoids in different parts of *carao*

Parts	**Total Phenolic Compounds** (mg EAG 100 g^{-1})	**Antioxidant Activity**		**Total carotenoids** ($\mu g\ mL^{-1}$)
		DPPH ($\mu g\ g^{-1}$)	**Iron reduction** (mg g^{-1})	
Pulp	5.6±0.2b	6.07±0.02b	0.21±0.01b	4.12±0.11a
Shells	2.3±0.1c	5.12±0.04c	0.18±0.02c	2.21±0.07c
Seeds	11.1±0.3a	7.31±0.11a	0.41±0.02a	3.76±0.03b
Fuit total	6.3±0.1	6.48±0.07	0.34±0.04	2.56±0.04

Note. * Means with different letters in the same column indicate statistical differences ($P \leq 0.05$) with Tukey test.

The total phenolic compounds determined in the different parts of the *carao* samples as well as in the whole fruit varied between 2.3±0.1 mg EAG 100 g^{-1} for the shell to concentrations of 11.1±0.3 mg EAG 100 g^{-1} for the seeds, this part being the one with the highest concentration of phenolic compounds. In comparison with other legumes such as *Vicia faba*, these values are within those determined by Valente et al. (2018), reaching values of 13±0.1 mg EAG 100 g^{-1}. The antioxidant activity, was carried out by two methods: by means of the DPPH technique and on the other hand by means of the iron reduction method, being again the *carao* seed who has the highest antioxidant activity with antioxidant activity values of 7.31±0.11 μg g^{-1} by the DPPH method and 0.34±0.04 mg g^{-1} by the iron reduction method. Godevac et al. (2008) study the antioxidant activity of nine species of *Fabaceae*, obtaining values higher than those obtained for *carao*. Other authors such as Pirela et al. (2011), study the antioxidant activity in the *Genisteae* also belonging to the *Fabaceae* family, obtaining values of 0.15 to 0.50 mg mL^{-1}, being lower than those determined in this work. The last group of molecules studied in this work are the carotenoids that give the compound a certain added biotechnological potential, since they are precursors of vitamin A, they have antioxidant, anti-inflammatory and anti-tumor properties (Rehman, 2020). The concentrations of this group of substances in the study species varied between 2.21±0.07 μg mL^{-1} for the cortex of the *carao*, reaching values of 4.12±0.11 μg mL^{-1} for the pulp, being in this part of the fruit where the highest amount of carotenoids is found.

4. Conclusions

Although *carao* has been traditionally used in countries of Central America as a nutritional alternative, specifically to meet the needs of iron in blood, there is not much data regarding its chemical composition, so this work serves to highlight the energy, mineralogical and Bioactive molecules that this fruit has to be used with biotechnological potential in neutraceutical foods.

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