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Physicochemical characterization of jicaro seeds (*Crescentia alata* H.B. K.): A novel protein and oleaginous seed

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ABSTRACT

Jicaro (*Crescentia alata*) is a tropical tree native to Mesoamerica well adapted to severe drought conditions. The seeds of the fruits were analyzed for protein, fatty acid, dietary fiber, phytate, polyphenol, tannin, tocopherol, sugar, mineral, amino acid and trypsin inhibitor contents. The jicaro cotyledons contained 43.6 ± 1.15 g protein/100 g and 38.0 ± 0.20 g fat/100 g (d.w.), which is comparable to most protein-rich and oleaginous seeds. Among the lipids, 77.6% were unsaturated fatty acids, particularly oleic acid, and essential amino acids represented 16.0 ± 0.9% (d.w.) of the protein fraction, which is similar to soybean amino acid contents. A proteomic analysis and SDS-PAGE electrophoresis revealed that the proteins are mostly of low molecular weight (~10 kDa), and the storage protein 2S albumin dominated. Jicaro seed trypsin inhibitory activity was low (0.1 trypsin inhibitor units TIU/mg), which enhances the digestibility of its proteins. The jicaro seed cotyledon represents an autochthonous and high-quality food source.

1. Introduction

Jicaro (*Crescentia alata*), which belongs to the family Bignoniaceae, is an ancient tree of American tropics (Vázquez-Yanes et al., 1999). Jicaro fruit has a calabash shape, and it was widely used during pre-Columbian era not only as a staple food but also as food utensils and containers. Jicaro fruit has a lignified pericarp that contains a sweet pulp surrounding many seeds. Jicaro fruit seeds, which represent 5% of the whole fruit, are dark brown, flat and heart shaped with a pleasant characteristic odor. Little research concerning jicaro exists, and few studies have attempted to characterize the composition of jicaro seed. Several decades ago, oil and fatty acid composition of jicaro seed was partially determined with respect to its main fatty acids (61.8% oleic, 15.0% linoleic) (Lewy, 1960). A more recent work performed on Guatemalan fruits showed that the seeds contained 38% fat and 26% protein (Figueroa and Bressani, 2000). These preliminary data were nonetheless of very high importance, as even in silvopastoral systems, with an average of 350 trees per hectare, each tree produces 750 fruits per year corresponding to 52 kg of fresh seeds (Carballo et al., 2005), which represents a high potential for commercial production. Additionally, the tree is well adapted to Pacific region of Central America, where severe droughts can occur, and thanks to deep root system, jicaro can absorb nutrients and water from savanna soils (Bucheli et al., 2013). Jicaro is one of the only trees to remain green year-round in the dry regions of Central America.

Jicaro seed was an important staple food in some pre-Columbian communities (Staller, 2010), and it is still widely consumed in Mesoamerica, especially in El Salvador and Nicaragua, as a very popular beverage called “horchata”. Nonetheless, because of real value of this seed remains unknown, consumption is decreasing and preservation of jicaro trees in silvopastoral systems must be strengthened. In the context of adaptation to climate change, a renewed interest in sustainable and biodiverse food sources with high nutritional and functional potential, has led to the rise of new market opportunities. For this

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reason, the objective of the present study was to describe the physicochemical characteristics of jicaro seeds and their main nutritional properties.

2. Materials and methods

2.1. Raw materials

Jicaro seeds (*Crescentia alata*) were obtained from the village Caserio Los Zarzarles (latitude: 12.66°N, longitude: −86.44°W, altitude: 115.7 m) in the municipality of León during two harvesting season, November 2013 and 2014. The seeds were prepared following a traditional process. Mature fruits were halved, and the seed-containing pulp was placed in polystyrene bags to ferment for three days. The seeds were then separated and washed before being sun dried. A total of 100 kg of dried seeds were collected from different jicaro fields, gathered and mixed at the Universidad Nacional Autónoma de Nicaragua (UNAN) of the city of Leon in the Pacific coast. Foreign material was removed. The seeds were washed again and sun dried to obtain a unique homogenized batch, representative of the overall production. All analyses were performed on samples of this representative batch.

Cotyledons and seed coats were separated manually from jicaro seeds. Each seed part was ground separately into a powder by using a PREP'LINE knife blender (SEB, Ecully, France) before analysis. Commercial yellow soybean (Markal, Saint-Marcel-lès-Valence, France) was bought in a supermarket in Montpellier (France) and was used as control.

2.2. Chemical analysis

Jicaro seed, cotyledon and seed coat samples were analyzed in triplicate. Dry matter content of 5 g sample was determined according to PR NF ISO 6496 standard using differential weighing after oven drying at 103 °C to reach a constant weight.

Micro-Kjeldahl method was used according to the Official Method 950.48 (AOAC, 1995) to determine total protein content (Nx6.25) with an automatic Foss analytical AB Kjeltec™ 8400 apparatus (Foss, Höganäs, Sweden). Each sample was previously mineralized with concentrated sulfuric acid and a mixture of 0.15 g of $CuSO_4 \cdot 5H_2O$, 5.0 g K_2SO_4, and 0.15 g titanium dioxide (Thompson Capper Ltd., Hardwick, UK) (Venkatachalam and Sathe, 2006).

Fat content was determined by using an ASE™ 350 accelerated solvent extractor (DIONEX Corp., Sunnyvale, CA, USA). A 1 g sample was placed in the stainless steel extraction cell in the presence of 0.5 g sand. The extraction solvent was pure petroleum ether at 60 °C and 100 bar. Flush was set to 100%, number of cycles to 5 with a static time of 7 min, and purge duration was 90 s. The extraction solvent containing lipids was collected in a 50 mL flask that had been previously dried and weighed. Flasks were weighed again after total evaporation of the solvent. Lipid content was expressed as g fat per 100 g sample (dry weight basis).

2.3. Soluble sugars

Sugar analysis was performed on the lipid-free meal remaining in the extraction cell. A 80% ethanol solution (v/v) was used as the solvent at 60 °C and 100 bar. Extracts of recovered sugars were diluted 25-fold with deionized water and filtered to 0.45 μm before injection of 10 μL into the chromatographe. Soluble sugars were separated using high performance ionic chromatography (HPIC) with a DX600 system equipped with a Carbopac MA-1 column (250 × 4 mm), a Carbopac MA-1 guard column (25 × 4 mm) and a Dionex ED50 pulsed amperometric detector (PAD) (DIONEX Corp., Sunnyvale, CA, USA). Chromatographic conditions were described by Valente et al. (2013).

The determination of α-galactosides (raffinose, stachyose and verbascose) was performed according to a procedure described by Muzquiz et al. (1999). A sample containing phenyl α-D-glucoside (100 μg) as an internal standard and 48% aqueous ethanol was added. Extraction was performed using a sonication for 60 min, followed by centrifugation at 700 × *g* for 10 min. Combined supernatants were heated at 85 °C under reflux for 30 min, cooled and centrifuged at 700 × *g* for 5 min. The supernatant was evaporated to dryness. The residue was dissolved in water, and an aliquot was transferred into a glass-stoppered test tube. Acetonitrile (1.0 mL) was then added with shaking, and the mixture was stored overnight at 4 °C. The sample was injected into an HPLC DX600 system (DIONEX Corp., Sunnyvale, CA, USA).

2.4. Fiber content

Neutral detergent fiber was prepared from 1 g sample according to the method of Van Soest and McQueen (1973), which sequentially yields neutral detergent fibers (NDF), acid detergent fibers (ADF) and acid detergent lignins (ADL). For NDF, a neutral detergent solution (sodium lauryl sulfate, USP-grade and ethylenediaminetetraacetic acid, EDTA; pH 7) at boiling temperatures with a 0.2 mL of heat-stable α-amylase Termamyl® (Laboratoires HUMEAU, La Chapelle-sur-Erdre, France) was used to dissolve the easily digested pectins and cell contents (proteins, starch sugars, and lipids), leaving a fibrous residue NDF (cellulose, hemicellulose and lignin). For ADF, 100 mL an acid detergent solution (20 g cetyl trimethylammonium bromide and 0.5 M H_2SO_4) were used to dissolve hemicellulose and minerals. Finally, for ADL, a 3 h digestion was performed with 72% H_2SO_4 in a crucible. Waste mineralization was performed at 550 °C for 4 h. The results are reported on a dry matter basis, as cellulose (ADF-ADL), hemicellulose (NDF-ADF) and lignin (ADL).

2.5. Mineral composition

Mineralization (500 °C) of the sample in an ash furnace (Thermo Scientific™ Thermolyne™ 6000 series 408, Waltham, Massachusetts, USA) was performed prior to the analysis of P, K, Na, Ca, and Mg and similarly for trace elements (Fe, Mn, Cu and Zn) until the ashes were cleared. Ash was then digested with hot concentrate hydrochloric acid until the destruction of organic matter, as described by Pinta (1973) methodology. Mineral contents were performed by inductively coupled plasma atomic emission spectroscopy ICP-AES) (Varian Vista-Pro, Palo Alto, CA, USA) and quantified against standard solutions of know concentrations. Total mineral content was defined as the sum of all the minerals analyzed.

2.6. Phytate determination

Phytate content was measured as described by Sekiguchi et al. (2000). Jicaro seeds (0.1 g) were placed in 2 mL 0.5 M HCl overnight at room temperature with constant stirring. After centrifugation (10 min, 10,000 × *g*, 10 °C), the supernatant was recovered and then diluted in 0.2 M borax buffer (pH 8.0). Phytate content (inositol hexakisphosphate, IP6) was measured using HPIC with a DX600 system, equipped with an ATC-1 trap column, an AG11 guard column and an AS11 column, detection was performed with a conductivity cell connected to an ED50 detector after removing anions on an ASRS 300 (DIONEX Corp., Sunnyvale, CA, USA). The injected volume was 10 μL. Phytate elution was performed using a basic gradient of 200 mM NaOH. The gradient was linear from 30 to 80 mM NaOH for 8 min, before returning to and holding initial conditions for 8 min to re-stabilize the system. Data were analyzed