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Chemical and functional characterization of seed, pulp and skin powder from chilto (*Solanum betaceum*), an Argentine native fruit. Phenolic fractions affect key enzymes involved in metabolic syndrome and oxidative stress

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A B S T R A C T

The aim of this work was to assess the nutritional and functional components of powder obtained by lyophilization of whole fruits, seeds, pulp and skin from chilto (*Solanum betaceum* Cav) cultivated in the ecoregion of Yungas, Argentina. The powders have low carbohydrate and sodium content and are a source of vitamin C, carotenoid, phenolics, potassium and fiber. The HPLC–ESI-MS/MS analysis of the fractions enriched in phenolics allowed the identification of 12 caffeic acid derivatives and related phenolics, 10 rosmarinic acid derivatives and 7 flavonoids. The polyphenols enriched extracts before and after simulated gastroduodenal digestion inhibited enzymes associated with metabolic syndrome, including α-glucosidase, amylase and lipase and exhibited antioxidant activity by different mechanisms. None of the analyzed fruit powders showed acute toxicity or genotoxicity. The powders from the three parts of *S. betaceum* fruit may be a potential functional food and the polyphenol enriched extract of seed and skin may have nutraceutical properties.

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1. Introduction

The tree tomato or chilto (Solanaceae) (synonym: *Solanum betaceum* Cav. and *Cyphomandra betacea* (Cav.) Sendtn.) is a native Argentinean food plant. It is cultivated in the humid forest of the eastern Andean slopes of northwestern Argentina. In these ecosystems known as Yungas, agriculture expanded almost exclusively in the low land pre-montane sector in a process that began with the conversion of humid forest into sugar cane and citrus orchards; and then into soybean production (Grau & Brown, 2000). The destruction of the Yungas ecosystem has increased in the last decades, affecting the ecological conditions of the remaining forests with consequences over the occurrence and distribution of native plants and animals, energy and carbon fixation. *S. betaceum* fruit known as chilto is included in the Argentine Food Code as tree tomato. The ripe fruit is consumed mainly by the population from northwestern Argentina in salads, juice, jams and liquors, being commercialized mainly in local or regional markets. During processing, the skin and seeds are discarded. A better characterization of chilto fruits produced in Argentina as well as from their industrial by-products is relevant to know the potential nutraceutical and functional properties of this native food resource. Previously, the simultaneous accumulation of an antimicrobial protein and reducing sugars during chilto fruit ripening was reported (Ordóñez, Ordóñez, Nieva Moreno, Sayago, & Isla, 2006; Ordóñez, Vattuone, & Isla, 2005). The isolated protein showed inhibitory activity against hydrolytic enzymes produced by pathogenic

Abbreviations: GE, glucose equivalent; βCE, β-carotene equivalents; C3-GE, cyanidin-3-glucoside equivalents; PB2E, procyanidin B2 equivalents; QE, quercetin equivalents; GAE, gallic acid equivalents; PEE, phenolic enriched extract; ABTS, 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid); BHT, butylated hydroxytoluene; RBC, red blood cells; AAPH, 2,2′-azo-bis(2-amidinopropane) dihydrochloride.

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organisms and against growth of phytopathogenic bacteria and fungi. A possible participation of the protein in the plant defense mechanism and its use in agriculture as post-harvesting control agent has been proposed (Isla, Ordóñez, Nieva Moreno, Vattuone, & Sampietro, 2002).

The antioxidant activity of a maceration, decoction and juice of chilto fresh fruit in free cell and cell systems was demonstrated. This activity was related with the phenolic compounds content (Ordóñez, Cardozo, Zampini, & Isla, 2010). *Salmonella* microsome assays of decoction, maceration and juice showed no mutagenic effect (Ordóñez et al., 2010). The insoluble matter or waste material (seed and skin) obtained after juice preparation showed antioxidant activity by quenching free radicals (Ordóñez et al., 2010). However, no information is available on the properties and composition of the powder obtained from the waste material from industrial processing, including seeds, pulp and skin. At present, the Direction of Non-timber Forest Products from Argentina promotes the cultivation of chilto in its natural environment in the Yungas as a commercial crop for a sustainable management of the remaining montane forests. For this, the aim of the present study was to assess the chemical composition, biological activities and toxicity of powder obtained from different parts of ripe orange fruit of chilto (seeds, skin and pulp) collected in the Argentinean Yungas at Tucumán. The effect of polyphenolic components from the fruit extracts against enzymes relevant in hyperglycemia, dyslipidemia, and oxidative stress related with metabolic syndrome was studied. Knowledge of this natural resource of native forests of northwestern Argentina will promote consumption, cultivation and marketing of chilto fruit, generating a source of income to the inhabitants of the region.

2. Material and methods

2.1. Plant material

Fruits of *Solanum betaceum* Cav. (orange-yellow cultivar) were collected in Parque Sierra de San Javier, a protected area of the Universidad Nacional de Tucumán, Argentina at 600 m over sea level, during February and March 2014 and 2015. The taxonomic identity was confirmed at the Instituto Miguel Lillo, Tucumán, using the reference herbarium specimens. The fruits were harvested manually from different plants according to the ripening stage. The ripening stages for all samples were selected in agreement with those at which the fruits are usually consumed. After collecting, the fruits were packed in a portable refrigerator until they were transported to the laboratory (2–3 h). The fresh fruits were washed with tap water and skin, pulp and seeds were separated. The skin (outer epidermis) of the fruit was carefully separated from the flesh using a sharp knife. The seed fraction was seeds without the jelly portion. Pulp was the portion of tomato remaining after removal of the skin and seed fractions. Then, each fraction was frozen at −80 °C, lyophilized and grounded to obtain the powder. The powders were then placed in oxygen barrier bags, vacuum packed (Multivac, DZ-400, China) and stored frozen at −20 °C until its analysis.

2.2. Quality parameters of fresh chilto fruits

Approximately 100 g of fresh fruits were pressed to obtain the juice, that was used to determine total soluble solids using a digital refractometer (ATC Instruments, Chemillé, France) with automatic temperature compensation and the results were expressed in °Brix. The pH was measured directly in each sample with a pH meter (Adwa, Szeged, Hungary). Titratable acidity (TA), as the amount of alkali solution (0.1 M NaOH) required to neutralize the components of a given amount of sample (juice), was expressed in g citric acid/100 mL of product (AOAC, 2000). Chromatic parameters were measured with a colorimeter Chroma meter CR-400 (Konica Minolta, Tokyo, Japan) using the CIELab system. The color space was chosen to obtain the results expressed in the chromaticity coordinates L^*, a^* and b^* for the selected illuminant.

The coordinated L^* represents lightness (contribution of black or white varying between 0 and 100); a^* represents the contribution of green or red (negative or positive); and b^* represents the contribution of blue or yellow (negative or positive). The coordinated L^* is perpendicular to the plane containing the chromaticity coordinates a^* and b^*. Considering the coordinates L^*, a^* and b^*, the color is expressed through L^*, C^* and H, where: L^* is brightness; C^* is chroma or saturation; and H is tone (or hue angle, which indicates color variation in the plane formed by the coordinates a^* and b^*). These parameters were determined considering: $C^* = (a^2 + b^2)^{1/2}$, $H^\circ = \text{arctang}\ (b/a)$ where 0° = red-purple, 90° = yellow, 180° = bluish-green and 270° = blue, CIRG index = $180\text{-}h/(L^* + C^*)$ (Usenik, Štampar, & Veberic, 2009).

2.3. Nutritional components determinations

All analysis were carried out in triplicate using standard methods previously optimized and used for plant food matrixes according to Association of Official Analytical Chemists (AOAC, 2005) methods. Crude protein (920.87) content was calculated from the total nitrogen (N) content determined by Kjeldahl method using a conversion factor of 6.25. Total lipids (920.85) content was determined according to the Soxhlet extraction method with petroleum ether (40–60 °C) during 4 h.

2.3.1. Carbohydrate analysis

Sample powder (1 g) was extracted with 80% aqueous ethanol (4 mL) at 75 °C for 10 min and then centrifuged at 9000×*g* for 5 min. The remaining solids were extracted exhaustively with the same solvent system. All organic extracts were combined and then evaporated. Total neutral and reducing sugars were measured using the phenol-sulphuric acid and Somogyi-Nelson method, respectively (Costamagna, Ordoñez, Zampini, Sayago, & Isla, 2013). Glucose, fructose and sucrose were quantified by HPLC system coupled with a refractive index detector (Waters 410) according to Gancedo & Luh, 1986. A chromatographic separation of sugars involved acetonitrile: water (80:20, v/v) as the mobile phase at a flow rate of 1.5 mL/min and Agilent ZORBAX Carbohydrate column (4.5 × 250 mm) (GL Sciences Inc., Torrance, CA, USA). Eluted peaks were detected with a refractive index detector. A calibration curve was prepared using commercial standards of glucose, fructose and sucrose to determine the relationship between the peak area and concentration. The sugar concentrations were expressed as mg/100 g dry weight. Three replicates were used for all samples.

2.3.2. Mineral analysis

The mineral analysis was carried out by quadrupole inductively plasma mass spectrometry (Q-ICPMS) at the Instituto Superior de Investigación Desarrollo y Servicios en Alimentos, ISIDSA, Córdoba, Argentina. The mineral content and composition of ashes (993.14) was determined by atomic absorption spectroscopy according with the AOAC (2005) recommendations. The following ions were analyzed: sodium, magnesium, potassium, calcium, and iron. The results were expressed in mg/100 g of powder.

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