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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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ABSTRACT

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.

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° = arctang (b/a) where 0° = red-purple, 90° = yellow, 180° = bluish-green and 270° = blue, CIRG index = 180-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.

2.4. Functional phytochemicals

2.4.1. Total phenolic compounds and total flavonoids extraction and quantification

The different samples were extracted three times with 95°ethanol (1 g of powder per 5 mL of ethanol) under stirring (40 cycles/min) in an ultrasonic bath for 30 min at room temperature. Then, the samples were centrifuged at 12,000×*g* during 10 min. The combined organic phase was taken to dryness under reduced pressure to afford the phenolic enriched extract (PEE). Total phenolic content was determined in the PEE according to Singleton, Orthofer, and Lamuela-Raventos (1999). The results were expressed as mg gallic acid equivalents (GAE)/100 g powder of whole fruit or each fruit part. The flavonoid content was measured following the report by Zhishen, Mengcheng, and Jianming (1999) using $AlCl_3$ and $NaNO_2$. The results were expressed as mg quercetin equivalents (QE)/100 g powder of whole fruit or each fruit part.

2.4.1.1. Recovery efficiency. The recovery efficiency of phenolic compounds was assessed using chlorogenic acid and rosmarinic acid as internal standard during the extraction procedure. Briefly, two sets of each sample were prepared with triplicate for each powder. In order to carry out the assay, 0.5 g of powder of seeds, skin and pulp of chilto were extracted three times with 95°ethanol in the same condition as described in Section 2.4.1. Set 1, the samples were obtained by spiking chlorogenic acid and rosmarinic acid (100 μg of each one) before extraction. Set 2, the samples were obtained without internal standard and then, were extracted. The extractions were concentrated by evaporation and analyzed directly by HPLC-DAD. HPLC system consisting of a Waters 1525 Binary HPLC Pumps system with a 1500 Series Column Heater, and a Waters 2998 photodiode array detector (PDA), an XBridgeTM C18 column (4.6 mm × 100 mm, 5 μm; Waters corporation, Milford, MA) with binary gradient solvent system was used. The system was composed of solvent A (0.1% acetic acid in water) and solvent B (0.1% acetic acid in methanol) (conditions: 10–57% B from 0 to 45 min and 57–100% B from 45 to 65 min) were used. The flow rate was set at 0.5 mL/min. The recovery efficiency was estimated as the ratio of the mean peak area of an internal standard spiked prior to extraction (set 1) to the peak area of the same amount of the internal standard spiked in the mobile phase. A calibration curve was prepared using commercial standards of chlorogenic acid and rosmarinic acid to determine the relationship between the peak area and concentration. The concentration was expressed as mg/100 g dry weight. Three replicates were used for all samples.

2.4.2. Tannins extraction and quantification

Each powder sample (1 g) was extracted with 12.5 mL acetone: water (70:30, v:v) according to Costamagna et al. (2013). The total condensed tannins content in each sample was determined according to Prior et al. (2010). Results were expressed as mg procyanidin B2 equivalent (PB2E)/100 g powder of whole fruit or each fruit part.

The acetone was evaporated and the aqueous fraction was separated in two fractions. One of them was submitted to acid hydrolysis. The total gallic acid content was measured in the hydrolyzed fraction with the rhodanine method (Inoue & Hagerman, 1988). Free gallic acid was determined in the non hydrolyzed fraction. The content of hydrolyzed tannin was determined by difference between them and the results were expressed as mg gallic acid equivalents (GAE)/100 g powder of whole fruit or each fruit part.

2.4.3. Pigments and ascorbic acid

The anthocyanin, carotenoids and ascorbic acid were extracted from each sample according to Costamagna et al. (2013) and the total content of each metabolite was determined according to Lee, Durst, and Wrolstad (2005), Rodríguez-Amaya (1999) and Barros, Heleno, Carvalho, and Ferreira (2010), respectively. Ascorbic acid was also determined by HPLC-DAD according to Phillips, Council-Troche, McGinty, Rasor, and Tarrago-Trani (2016).

2.4.4. Crude fiber

Fiber content (962.09) was determined according to AOAC (2005).

2.5. Simulated gastroduodenal digestion

The inhibitory effect on enzymes related to metabolic syndrome was determined using polyphenolic extracts before and after simulated gastroduodenal digestion (GD). The polyphenolic extracts were submitted to simulated GD according to Tenore, Campiglia, Giannetti, and Novellino (2015) with minor modifications. Briefly, for the salivary digestion, the extract (4 mg GAE) was mixed with 6 mL of artificial saliva at pH 6.8 (KCl [89.6 g/L], KSCN [20 g/L], NaH_2PO_4 [88.8 g/L], Na_2SO_4 [57.0 g/L], NaCl [175.3 g/L], $NaHCO_3$ [84.7 g/L], urea [25.0 g/L] and α-amylase [48.3 mg/mL]). The mixture was incubated for 3 min at 37 °C. Then, for the gastric digestion, pepsin (14,800 U) dissolved in HCl 0.1 M was added. The pH was adjusted to 2 and the mixture was incubated at 37 °C during 2 h. For pancreatic (duodenal) digestion, the pH was adjusted to 6.5 with $NaHCO_3$ (0.5 M). Then, pancreatin (8 mg/mL) and bile salts (50 mg/mL) (1:1, v/v), dissolved in water (20 mL), were added and the mixture was incubated at 37 °C for 2 h. After artificial digestions, polyphenols were extracted with ethyl acetate and the organic phase was taken to dryness and resuspended in dimethylsulfoxide (DMSO) (1 mg GAE/mL).

2.6. Enzyme inhibition and antioxidant assays

2.6.1. Inhibitory activity of enzymes related to metabolic syndrome

2.6.1.1. α-Glucosidase inhibition. The enzyme inhibition was performed according to Costamagna et al. (2016) with some modifications using *p*-nitrophenyl α-D-glucopyranoside as substrate. The pre-incubation of the α-glucosidase enzyme and PEE of seeds, skin, and pulp powder (2.5–20 μg/mL) (before and after simulated GD digestion) was carried out at 4 °C during 10 min. The IC_{50} values were calculated by interpolation of doses response curves. Orlistat (tetrahydrolipstatin, ATC code: A08AB01, Elea Laboratory, Ciudad Autónoma de Buenos Aires, Argentina) was used as positive control.

2.6.1.2. α-Amylase inhibition. The α-amylase inhibitory activity using starch as substrate was assayed using Amilokit® (Wiener Lab Group, Rosario, Argentina, Cat N° 1021001) according to the fabricant instructions as reported by Costamagna et al. (2016). The PEE from seeds, skin and pulp powder (5–40 μg GAE/mL) were assessed (before and after simulated GD digestion) and the results are reported as IC_{50} values. IC_{50} values denote the μg GAE/mL of PEE required to inhibit the enzyme by 50%.

2.6.1.3. Lipase inhibition. Lipase activity was assayed by measuring the enzymatic hydrolysis of *p*-nitrophenyl palmitate to *p*-nitrophenol in a microplate reader (BiotekELx808) at 400 nm according to Costamagna et al. (2016) in presence (final concentration between 2.5 and 20 μg/mL) and in absence of PEE from seeds, skin and pulp powder (before and after simulated GD digestion). After pre-incubation (Enzyme-PEE) at 4 °C for 10 min the enzyme reaction was carried out during 20 min. IC_{50} values were determined

as μg GAE/mL of PEE required to inhibit the enzyme activity by 50%.

2.6.2. Antioxidant activity

2.6.2.1. Total antioxidant capacity assay. The antioxidant capacity assay of PEE was carried out by the improved ABTS radical cation ($ABTS^{\cdot+}$) method as described by Re et al. (1999). Results were expressed as the concentration of PEE (before and after simulated GD digestion) necessary to scavenge 50% of ABTS radical cation (SC_{50}). BHT was used as reference compound.

2.6.2.2. Protection of oxidative hemolysis. The protection of oxidative hemolysis of red blood cells (RBC) by PEE (0.1–2.5 μg GAE/mL) (before and after simulated GD digestion) was determined according to Mendes, de Freitas, Baptista, and Carvalho (2011), using azo compound solution of AAPH. The extent of hemolysis was determined spectrophotometrically. The reaction mixture was incubated during 1 h at 37 °C and then was centrifuged (4000×*g*) for 3 min. The absorption of the supernatant was read at 545 nm. The percent hemolysis was calculated and the IC_{50} values were determined as the concentration of extracts (before and after simulated GD digestion) necessary to protect the RBC from oxidative hemolysis by 50%. BHT was used as reference compound.

2.7. Identification of phenolics by HPLC–ESI-MS/MS

The PEEs of tree tomato pulp, skin and seeds were analyzed by HPLC–ESI-MS/MS to compare the samples and to identify the extract constituents. Mass spectra were recorded using an Agilent 1100 (Agilent Technologies Inc., CA, USA) liquid chromatography system connected through a split to an Esquire 4000 Ion Trap LC/MS(n) system (Bruker Daltoniks, Germany). Ionization was performed at 3000 V assisted by nitrogen as nebulizing gas at 50 psi and as drying gas at 365 °C and a flow rate of 10 L/min. Negative ions were detected using full scan (*m*/*z* 20–2200) and normal resolution (scan speed 10,300 *m*/*z*/s; peak with 0.6 FWHM/*m*/*z*). The trap parameters were set in ion charge control (ICC) using manufacturer default parameters, and maximum accumulation time of 200 ms. The mass spectrometric conditions for analysis were: electrospray needle, 4000 V; end plate offset, −500 V; skimmer 1, 56.0 V; skimmer 2, 6.0 V; capillary exit offset, 84.6 V; capillary exit, 140.6 V. Collision induced dissociation (CID) spectra were obtained with a fragmentation amplitude of 1.00 V (MS/MS) using helium as the collision gas and was automatically controlled through SmartFrag option. The mixture was analyzed using a MultoHigh 100 RP 18-5μ (250 × 4.6 mm) column (CS-Chromatographie Service GmbH, Langerwehe, Germany) maintained at 25 °C. The HPLC–MS analyses were performed using a linear gradient solvent system consisting of 1% formic acid in water (A) and acetonitrile as follow: 90–85% A over 15 min, maintained to 20 min and changing to 82% A from 20 to 25 min, 82 to 70% A from 25 to 50 min, maintained to 70% A from 50 to 80 min, and returning to 90% A from 80.01 to 90 min. The flow rate was 0.5 mL/min and the volume injected was 20 μL. The compounds were monitored at 280 nm.

2.7.1. Quantification of rosmarinic acid and 3-caffeoylquinic acid

The main phenolics in the PEE were quantified using external calibration curves prepared with pure standards at λ 330 nm. The reference compounds rosmarinic acid (98%, Sigma Aldrich, St. Louis, MO, USA) and 3-caffeoylquinic acid (98%, Phytolab GmbH, Verstenbergsgreuth, Germany) were used in concentrations ranging from 5 to 500 mg/L (r = 0.9998) and 10 to 1000 mg/L (r = 0.9999), respectively. The analytical parameters were calculated in agreement to the ICH reference (ICH, 2005), with LOD of 7.03 and 5.91 mg/L and LOQ of 21.32 and 17.91 mg/L for rosmarinic and 3-caffeoylquinic acid, respectively. Results are expressed as mg of the corresponding compound/100 g extract.

2.8. Toxicity

2.8.1. Acute toxicity test

The acute toxicity level of the extracts was studied using *Artemia salina* as test organism (Svensson, Mathiasson, Martensson, & Bergatröm, 2005). The *Artemia salina* cysts were hatched in artificial seawater. After 24 h of incubation at 25 °C, the hatched larvae were transferred to microplates containing seawater and 2.5–200 μg GAE/mL of each extract. Solvent control (DMSO) without extract and positive control of potassium dichromate (10–40 μg/mL) were included in the experiment. All the plates were incubated during 24 h at 25 °C. The total number of dead larvae was counted for each concentration.

2.8.2. Mutagenicity

The mutagenic effect of chilto powder extracts was evaluated on two *Salmonella typhimurium* strains (TA98 and TA100). The plate incorporation assay was performed according to Maron and Ames (1983), by adding 0.1 mL of the overnight bacterial culture, 0.05 mL of PEE at different concentrations (175–500 μg GAE/plate) and 2 mL of top agar on minimal agar. The plates were then incubated at 37 °C for 48 h. After incubation, his+ revertant colonies were counted and compared to the number of revertant colonies in the controls. The positive control used was 4-nitro-*O*-phenylenediamine (4-NPD; Aldrich Chemical Co.), 10 μg/plate. Solvent control was carried out adding 0.05 mL DMSO/plate. An extract was considered mutagenic when the mean number of revertants was double or greater than two times that of the negative control. Three plates per experiment were assayed and two separate experiments were carried out for each concentration tested and for positive and negative controls.

2.9. Statistical analysis

All measurements were replicated three times and data were analyzed by Anova. Correlations coefficients (*r*) were also calculated.

3. Result and discussion

3.1. Determination of maturity index

Fruit acceptability by consumers depends basically on a combination of several quality attributes, which include color, sugar content, and acidity, between others. The fruit chromatic characteristics were determined to select to those in the same ripening grade ($L^* = 62.33 \pm 1.89$, $a^* = 15.72 \pm 1.81$, $b^* = 39.92 \pm 2.32$, chroma = 42.90, hue angle = 68.50 and CIRG index = 1.06). The SSC of the yellow-orange cultivars varied with an average value of 9.01 ± 0.5, lower than that found in fruits from Colombia, Ecuador and Spain (Acosta-Quezada et al., 2015; Schotsmans, East, & Woolf, 2011). Total acidity (TA values) were higher (TA = 1.9% of citric acid) than those of the red chilto (Acosta-Quezada et al., 2015; Schotsmans et al., 2011), values between 0.76 and 1.71.

During industrial processing of chilto fruit, the skin and the seeds are removed and considered waste material. The pulp, seed and skin represent 51.5%, 39.4% and 9.1%, respectively, of the total fresh fruit weight. Therefore, about half of the fruit weight (49%) is considered waste and would be discarded. Nevertheless, this material may be useful as a source of natural functional food ingredients. For this reason, the powder of ripe fruit pulp, skin and seeds was obtained by lyophilization of fresh material. The yields were as follows: 24.04 g powder/100 g fresh skin; 13.28 g powder/100 g fresh seed and 12.02 g powder/100 g fresh pulp. The

powders were characterized from a nutritional and functional point of view.

3.2. Nutritional composition of powder obtained from ripe fruit, pulp, skin and seeds

The carbohydrate content in yellow-orange chilto whole fruit (1.17 g/100 g fresh weight and 9.11 g/100 g dry weight) from Argentinean Yungas was similar to the carbohydrate content of different varieties of commercial tomato (*Solanum esculentum*) (1.01–2 g/100 g fresh weight) and higher than orange tree tomato (Acosta-Quezada et al., 2015). The storage efficiency of sucrose in yellow-orange fruits was higher than the glucose and fructose storage (Table 1). This result is similar to that reported in orange-colored fruits of other places such as Valencia, Spain (Acosta-Quezada et al., 2015; Schotsmans et al., 2011; Vasco, Avila, Ruales, Svanberg, & Kamal-Eldin, 2009). However, Ordóñez et al. (2005) reported that in mature red fruits from Argentina, a high acid invertase activity could release high concentrations of hexose by hydrolysis of sucrose during ripening of fruits. In yellow-orange chilto, the level of glucose and fructose was similar to that found in orange fruits from Ecuador but higher than hexose level in fruits from Spain (Vasco et al., 2009). The pulp, seeds and skin powder contained 7.62, 11.91 and 2.23% of total sugar and storage more sucrose than glucose and fructose (Table 1). The total protein level (2.6 g/100 g fresh weight or 18.95 g/100 g dry weight) similar to that reported in chilto fruits from Spain and Ecuador (Schotsmans et al., 2011; Vasco et al., 2009) and higher than in *Solanum esculentum* fruit (0.55–1.05 g/100 g fresh weight) (Guil-Guerrero & Rebolloso-Fuentes, 2009). The powder of yellow-orange chilto whole fruits, pulp, seed and skin contain 18.95%; 12.95%; 20.95% and 8.84% of total protein, respectively. The fat content in fresh fruits and in powder of each fruit parts was low (Table 1). The fiber level of chilto whole fruit (2.08 g/100 g fresh weight and 16 g/100 g dry weight) was higher than in *Solanum esculentum* fruit (0.74–1.60 g/100 g fresh weight). The fiber content in the powder of each fruit part was high, with values of 23.46, 19.04 and 28.42 g/100 g for skin, pulp and seeds, respectively (Table 1). For these reason, the chilto powder could have beneficial health effects promoting improved functioning of the digestive system (González-Castejón & Rodríguez-Casado, 2011).

The microelement content of chilto seeds, skin and pulp powder is high in potassium and low in sodium. Also, high calcium content in the skin and high magnesium content in seeds was observed (Table 1). The content of K, Ca and Mg in "chilto" whole fruit was higher than in commercial *Solanum esculentum* fruit (Guil-Guerrero & Rebolloso-Fuentes, 2009).

3.3. Phytochemical composition of chilto powder

3.3.1. Ascorbic acid

The percent ascorbic acid content (in mg) of 100 g pulp, seed and skin powder was 84.1, 56.8 and 51.1 mg, respectively (Table 1). Hence, the consumption of 100 g of chilto powder may be enough to cover the daily requirements in ascorbic acid, recommended daily allowance of 60–90 mg per person (men and women) (Levine, Wang, Padayatty, & Morrow, 2001). The ascorbic acid content in whole fruit was lower (15.21 mg/100 g fresh fruit or 117 mg/100 g dry weight) than the reported in *S. esculentum* red fruit (100–200 mg/100 g fresh weight) (Guil-Guerrero & Rebolloso-Fuentes, 2009).

3.3.2. Polyphenolic compounds

The total polyphenols content was 415.2, 523.8 and 179.4 mg GAE/100 g of pulp, skin and seed powder, respectively (Table 1). The pulp and skin powder were higher in phenolic and contained higher levels of total soluble phenolic compounds than the seeds without jelly. In fruits from Ecuador, the level of phenolics was higher in skin or peel (Vasco, Ruales, & Kamal-Eldin, 2008; Vasco et al., 2009). The flavonoid content of chilto skin powder was higher (265.70 mg/100 g powder) than pulp (223.80 mg/100 g powder) and seeds (175.62 mg/100 g powder), respectively. Since the average value of flavonoids mean uptake is in the range from 23 to 35 mg per day (Manach, Scalbert, Morand, Rémésy, & Jiménez, 2004), 8 g of skin powder are sufficient to meet the requirements of flavonoids. At the moment, recommended daily allowance (RDA) of total flavonoids could be between 250 and 400 mg/day, respecting the seasonality of food sources (Peluso &

Table 1
Nutritional and phytochemical composition of Argentinean *Solanum betaceum* seeds, pulp and skin powder.

Phytochemical and minerals content	Whole fruits	Skin	Pulp	Seeds
Dry extract (g/100 g fresh fruit)		0.41	1.30	2.34
Yield (g skin, pulp and seed powder/100 g fresh fruits)	–	2.18	6.19	5.2
Total phenolic (mg GAE/100 g of powder)	684.5 ± 22.0^{a}	523.8 ± 3.0^{b}	415.2 ± 4.0^{c}	179.4 ± 3.0^{d}
Flavone and flavonol (mg QE/100 g of powder)	157.69 ± 9.0^{d}	265.70 ± 3.60^{a}	223.80 ± 2.90^{b}	175.62 ± 3.10^{c}
Anthocyanins (mg C-3GE/100 g of powder)	1.78 ± 0.50^{a}	1.01 ± 0.01^{a}	ND	ND
Ascorbic acid (mg AAE/100 g of powder)	117.0 ± 10.2^{a}	51.1 ± 2.1^{c}	84.1 ± 2.0^{b}	56.8 ± 2.3^{c}
Carotenoids (g βCE/100 g of powder)	1.41 ± 0.09^{a}	1.37 ± 0.09^{a}	0.93 ± 0.05^{b}	0.53 ± 0.03^{c}
Total sugar (g GE/100 g of powder)	9.11 ± 1.50^{ab}	2.23 ± 0.50^{c}	7.62 ± 1.90^{b}	11.91 ± 2.00^{a}
Glucose (g/100 g of powder)	0.99 ± 0.08^{a}	0.92 ± 0.02^{a}	0.40 ± 0.01^{c}	0.79 ± 0.03^{b}
Fructose (g /100 g of powder)	2.49 ± 0.03^{a}	2.72 ± 0.01^{a}	1.04 ± 0.01^{c}	1.91 ± 0.03^{b}
Sucrose (g/100 g of powder)	8.10 ± 0.03^{a}	7.85 ± 0.02^{a}	3.23 ± 0.05^{c}	6.82 ± 0.01^{b}
Soluble Protein (g/100 g of powder)	0.16 ± 0.02^{a}	0.13 ± 0.01^{ab}	0.11 ± 0.01^{b}	0.15 ± 0.01^{a}
Total Protein (g /100 g)	18.95 ± 1.50^{b}	8.84 ± 1.00^{d}	12.95 ± 1.00^{c}	20.95 ± 1.80^{a}
Fat (g/100 g of powder)	0.20 ± 0.02^{bc}	0.21 ± 0.01^{b}	0.15 ± 0.01^{c}	0.31 ± 0.03^{a}
Fiber(g/100 g of powder)	16.00 ± 1.00^{c}	23.46 ± 2.00^{ab}	19.04 ± 1.90^{bc}	28.42 ± 3.20^{a}
Yield (%, g powder/100 g fresh fruits or skin, pulp, seed)	13.00 ± 1.00^{b}	24.04 ± 3.20^{a}	12.02 ± 2.50^{b}	13.28 ± 1.80^{b}
Minerals (mg/100 g of powder)				
Na	–	15.40 ± 1.40^{ab}	16.30 ± 1.00^{a}	13.20 ± 0.70^{b}
Mg	–	136.60 ± 8.30^{c}	170.60 ± 6.00^{b}	243.60 ± 10.50^{a}
K	–	3892.30 ± 190.70^{b}	4975.70 ± 213.00^{a}	4073.20 ± 214.70^{b}
Ca	–	247.90 ± 21.80^{a}	133.03 ± 3.60^{b}	111.20 ± 8.20^{b}
Fe	–	2.70 ± 0.10^{b}	2.10 ± 0.70^{b}	4.40 ± 0.20^{a}
Cu	–	0.20 ± 0.01^{c}	0.50 ± 0.02^{b}	0.70 ± 0.02^{a}
Zn	–	0.80 ± 0.04^{b}	1.00 ± 0.06^{ab}	1.90 ± 0.03^{a}

The protein, sugars, fat and all phytochemical concentrations are expressed in 100 g of powder obtained from whole fruits, skin, pulp or seeds according to the fruit part analyzed. ND: no detected. Different letters (a, b, c, d) in the same line in phytochemical analysis and (a, b, c) in the mineral analysis show significant differences in the content among each part of fruits according to Tukey's test ($p \leqslant 0.05$).

Palmery, 2015). The content of phenolic compounds and flavonoid are higher in chilto fresh fruit (88.92 mg GAE/100 g fresh weight and 20 mg QE/100 g fresh weight, respectively) than in *Solanum lycopersicum* fruit (10.51 mg GAE/100 g fresh weight and 9.45 QE/100 g fresh weight, respectively) (Liu et al., 2012). However, the composition and identity of the flavonoids in diet is relevant for the biological effects. Condensed and hydrolyzed tannins were not detected in the pulp, skin and seed powder.

3.3.3. Pigments

To determine the pigment content, differential extractions of the chilto fruit powder seeds, skin and pulp were carried out. Carotenoids are major compounds in the whole fruit powder (1.41 ± 0.09 g β-CE/100 g). The carotenoid content in the skin powder was higher than in pulp and seed powder (Table 1). Total carotenoid content was higher in chilto fruits from Tucuman (Argentina) than in fruits from Brazil and Ecuador (Vasco et al., 2009) and in several varieties of *S. lycopersicum* (Guil-Guerrero & Rebolloso-Fuentes, 2009). The carotenoids from the yellow colored tree tomato fruit were identified by HPLC–PDA-MS (Mertz, Brat, Caris-Veyrat, & Gunata, 2010). The authors identified 26 compounds from the crude extracts and after saponification.

The anthocyanin level was very low (1.78 mg C-3GE/100 g powder) and was detected only in skin. This result is coincident with the reported for fruits from Colombia where the anthocyanin level in red fruits (7818 mg/100 g) was higher than in the orange fruits (Osorio et al., 2012). The identified pigments in red fruits were delphinidin 3-O-(6-O-α-rhamnopyranosyl-β-glucopyranosyl)-3′-O-β-glucopyranoside, delphinidin 3-O-(6-O-α-rhamnopyranosyl)-β-glucopyranoside, cyanidin 3′-O-(6-O-α-rhamnopyranosyl)-β-glucopyranoside and pelargonidin 3-O-(6-O-α-rhamnopyranosyl)-β-glucopyranoside (Hurtado, Morales, González-Miret, Escudero-Gilete, & Heredia, 2009), delphinidin 3-O-α-L-rhamnopyranosyl-(1,6)-β-D-glucopyranoside-3-O-β-D-glucopyranoside, cyanidin-3-O-rutinoside, pelargonidin-3-O-rutinoside and delphinidin-3-O-rutinoside (Hurtado et al., 2009; Osorio et al., 2012). De Rosso and Mercadante (2007) described the anthocyanin and carotenoid content of *C. betaceae* fruits from Brazil. The main carotenes were β-cryptoxanthin and β-carotene while the three anthocyanins identified were delphinidin 3-rutinoside, cyanidin 3-rutinoside and pelargonidin 3-glucoside-5-rhamnoside.

3.4. Functional properties

3.4.1. Effect of PEEs on key enzymes involved in the development of metabolic syndrome

The metabolic syndrome, characterized by glycemic index imbalance, glucose intolerance, hypertension, dyslipidemia and/or obesity, is an early sign of potential future development of type II diabetes characterized by postprandial hyperglycemia. The effect of PEE from seed, pulp and skin powder before and after simulated GD digestion was assessed towards enzymes associated with metabolic syndrome, such as α-glucosidase, α-amylase and pancreatic lipase. The effect of chilto PEE on metabolic enzymes was summarized in Table 2.

3.4.1.1. α-Glucosidase and α-amylase inhibition. The increase of blood post-prandial glucose can be reduced through inhibition of enzymes involved in the release of glucose from food such as α-glucosidase, a membrane-bound enzyme located in the epithelium of the small intestine. Management of hyperglycemia in diabetes mellitus by oral α-glucosidase inhibitors is currently limited to acarbose, voglibose and miglitol aimed at delaying digestion of dietary carbohydrates to maintain postprandial blood glucose at normal levels (Ghani, 2015). The continuous administration may cause several adverse effects, such as abdominal discomfort, diarrhea, flatulence, and hepatotoxicity (Ghani, 2015). Therefore, novel inhibitors of α-glucosidase are necessary given the therapeutic challenge of type II diabetes mellitus. Much research has focused on glycosidase inhibitors to control hyperglycemia, but many forms of starch are also digested as rapidly as glucose absorption. Slowing the digestion and breakdown of starch may have beneficial effects on insulin resistance and glycemic index control in diabetics. The PEE from chilto seed, pulp and skin powder were active towards α-amylase and α-glucosidase with IC_{50} values of 5.1, 11.0 and 10.5 μg GAE/mL for α-glucosidase and 19.4, 15.0 and 13.5 μg GAE/mL for α-amylase, respectively. The IC_{50} value of the reference compound acarbose was 25.0 μg/mL for α-glucosidase and 1.25 μg/mL for α-amylase, respectively (Table 2). The PEEs were more active towards α-glucosidase than the reference compound acarbose but presented lower effect on α-amylase. The results suggest that PEE of chilto fruits powder might be able to reduce glucose uptake/absorption. In this sense, chilto fruits powder may be a dietary complement to control hyperglycemia in diabetic patients. However, further evaluation of the *in vivo* hypoglycaemic activity is necessary to verify potential beneficial effects.

3.4.1.2. Pancreatic lipase. Pancreatic lipase is an enzyme that has an impact on metabolic syndrome (Grove, Sae-tan, Kennett, & Lambert, 2012). Lipase, primarily produced in the pancreas, hydrolyses lipids to form fatty acids in a way that they can be absorbed in the human digestive system. The inhibition of pancreatic lipase is the main prescribed treatment for weight management and obesity (Birari & Bhutani, 2007). Orlistat, a commercial

Table 2
Effect of polyphenols enriched extract of seeds, pulp and skin powder from Argentinean *Solanum betaceum* before and after simulated gastroduodenal digestion (GD) and reference compounds on enzymes related to carbohydrate metabolism, fat metabolism and oxidative processes.

S. betaceum flour		IC_{50} (μg GAE/mL)			SC_{50} (μg GAE/mL)	IC_{50} (μg GAE/mL)
		Enzyme of metabolic syndrome			ABTS	AAPH
		α-Amylase	α-Glucosidase	Lipase		
Seeds	Before GD	19.4 ± 2.0^{b}	5.1 ± 0.5^{a}	5.3 ± 0.5^{a}	1.38 ± 0.05^{c}	0.91 ± 0.05^{b}
	After GD	7.0 ± 1.5^{A}	13.5 ± 0.4^{A}	4.6 ± 0.3^{A}	5.80 ± 0.03^{A}	1.21 ± 0.02^{a}
Pulp	Before GD	15.0 ± 1.0^{a}	11.0 ± 1.1^{b}	14.0 ± 1.4^{c}	1.09 ± 0.10^{b}	0.40 ± 0.03^{a}
	After GD	22.5 ± 1.0^{B}	17.4 ± 0.9^{B}	15.2 ± 1.6^{B}	1.20 ± 0.07^{B}	0.52 ± 0.06^{b}
Skin	Before GD	13.5 ± 1.2^{a}	10.5 ± 1.0^{b}	11.7 ± 1.1^{b}	0.8 ± 0.1^{a}	0.50 ± 0.0^{a}
	After GD	18.1 ± 1.3^{C}	17.5 ± 0.8^{B}	7.9 ± 0.9^{C}	0.70 ± 0.03^{C}	0.87 ± 0.01^{c}
Reference compound		Acarbose	Acarbose	Orlistat	BHT	BHT
IC_{50} (μg/mL)		1.25 ± 0.10	25.00 ± 1.00	0.08 ± 0.01	55.00 ± 0.10	0.65 ± 0.01

SC_{50}: Concentration of polyphenolic extract necessary to scavenge 50% of ABTS.
IC_{50}: Concentration of polyphenolic extract necessary to inhibit 50% of oxidative hemolysis or enzyme activity. Different letters (a, b, c or A, B, C) in the same column in each assay show significant differences among effect of polyphenols on enzyme activity according to Tukey's test ($p \leqslant 0.05$).

drug for obesity treatment, is an inhibitor of pancreatic lipase that produces several secondary effects (Mohamed, Ibrahim, Elkhayat, & El Dine, 2014). In order to find alternative natural sources for obesity prevention and treatment, we evaluated PEE of chilto fruit seeds, pulp and skin on lipase activity. The results are showed in Table 2. The inhibitory activity of seeds phenolic extract on lipase (IC_{50}: 5.3 μg GAE/mL) was higher than the pulp extract (IC_{50}: 14.0 μg GAE/mL) and skin (IC_{50}: 11.7 μg GAE/mL) and better than the inhibition reported for white and green tea polyphenols (Gondoin, Grussu, Stewart, & McDougall, 2010).

3.4.1.3. Effect of polyphenolic extracts after simulated GD digestion. After simulated GD digestion, the polyphenolic seeds extract was more active on α-amylase and less active on α-glucosidase than the untreated sample. The pulp and skin extracts showed similar activity before and after artificial GD digestion (Table 2).

3.4.2. Antioxidant activity

The role of oxidative stress in the initiation and progression of metabolic syndrome (diabetes and obesity) leads to the hypothesis that antioxidants can be used as therapeutic agents for its treatment or prevention. For this reason and considering that chilto extract possess hypoglycaemic and anti-obesity capacity, the antioxidant activity of the powder, before and after simulated GD digestion was analyzed. All the PEE showed a dose-response effect on the ABTS cation radical reducing capacity with SC_{50} value between 0.8 and 1.38 μg GAE/mL. Overall, the scavenging activity was higher than commercial natural and synthetic antioxidants used in food industry such as BHT (SC_{50} = 55 μg/mL). All fractions showed protection capacity of RBC on the oxidative hemolysis (IC_{50} values between 0.4 and 0.9 μg GAE/mL). The antioxidant power of the polyphenolic extracts before and after simulated GD was similar except for seeds extract, whose antioxidant capacity decreased after treatment with digestive enzymes.

3.5. Toxicity and mutagenicity assays

The toxicity of extracts against *Artemia salina* was studied. The extracts were no toxic at the assayed concentration range on this test organism. The mutagenicity was evaluated by the Ames assay. In a series of experiments preceding the mutagenicity studies, it was ascertained that the amounts of phenolic compounds added to the *S. typhimurium* culture do not influence their viability (data not shown). None of the preparations were mutagenic in strains TA98 or TA100 under the conditions used in this assay which indicates the inexistence of mutagens that cause base substitution (detected in TA100) and frameshift (detected in TA98) mutations. The absence of mutagenicity for the chilto preparations studied in the *Salmonella* tested strains indicates that DNA does not seem to be a relevant target for phenolic components of chilto.

3.6. Identification of polyphenolics

The HPLC–ESI-MS/MS analysis of the samples (Fig. 1, Table 3) allowed the tentative identification of 31 constituents, including 12 caffeic acid derivatives and related phenolics (compounds **1**–**5**, **8**–**11**, **13**, **14** and **16**), 10 rosmarinic acid (RA) derivatives (compounds **15**, **18**, **21**–**24**, **28**-**31**), and 7 flavonoids (compounds **6**, **12**, **19**–**20**, **25**–**27**). Selected ion chromatograms were used to identify the main constituents and related compounds in the extracts. The ions at *m*/*z* 179, 301, 353 and 359 amu were used for the caffeoyl, quercetin, caffeoylquinic acid and rosmarinic acid derivatives, respectively. Identification of the constituents was supported by literature (Espin et al., 2016; Wu, Meyer, Whitaker, Litt, & Kennelly, 2013) as well as by comparison with standards when available.

The mass spectra of compounds **1**, **3**, **10** and **14** with a $[M-H]^-$ ion at *m*/*z* 341 shows neutral loss of 162 atomic mass units (amu), leading to the base peak at *m*/*z* 179, in agreement with caffeoyl hexosides. The compounds **4**, **9**, **11** and **13** presented a $[M-H]^-$ ion at *m*/*z* 353 amu and the loss of hexose, with a base peak at

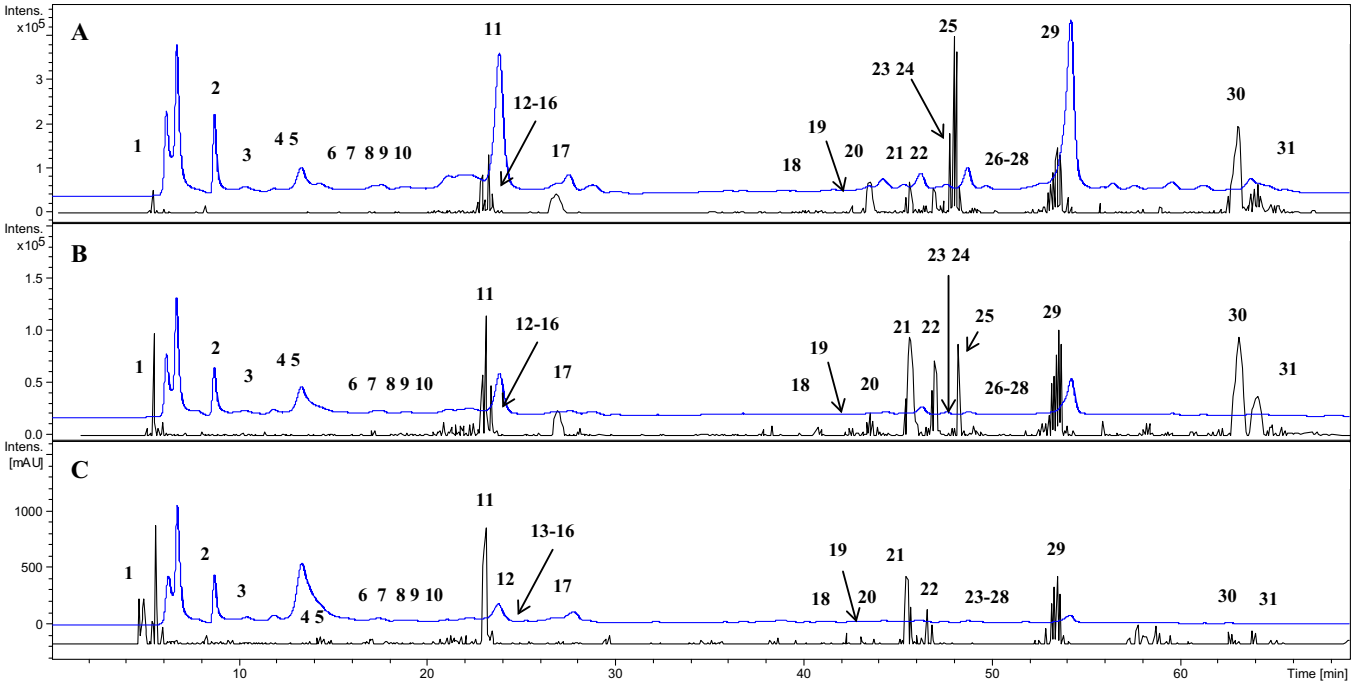

Fig. 1. HPLC chromatograms of the phenolic-enriched extracts of Argentinean *Solanum betaceum* skin (A), pulp (B), and seed (C) powder. Detection: UV, 280 nm (in blue) and total ionic current-all MS^n (in black). Peak identification is given in Table 3. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 3
Tentative identification of phenolics in Argentinean *Solanum betaceum* fruit pulp, skin and seed powder extracts.

Peak	Rt (min)	$[M-H]^-$	MS^2	Tentative identification
1	5.5	341	179 (100), 161 (27)	Caffeoyl hexoside
2	8.0	689	515 (100), 353 (25), 173 (100)	Dicaffeoylquinic acid derivative
3	9.9	341	179 (100)	Caffeoyl hexoside
4	17.5	353	191 (100), 179 (42)	Caffeoylquinic acid
5	17.6–20.1	515	455 (40), 425 (36), 353 (100), 191 (21)	Caffeoylquinic acid hexoside
6	18.3	419	387 (80), 287 (100), 255 (60)	Dihydrokaempferol pentoside
7	18.4	583	385 (100)	Unknown
8	19.5	677	353 (100), 191 (5)	Caffeoylquinic acid dihexoside
9	20.3	353	191 (100), 179 (19), 173 (3)	Caffeoylquinic acid
10	20.6	341	179 (100), 135 (17)	Caffeoyl hexoside
11	21.0–23.3	353	353 (100), 191 (92)	3-Caffeoylquinic acid[a]
12	23.4	401	269 (100), 160 (44)	Apigenin pentoside
13	23.6	353	191 (100)	Caffeoylquinic acid
14	24.2	341	179 (100)	Caffeoyl hexoside
15	24.2	375	179 (100)	Hydroxyrosmarinic acid
16	25.8	401	353 (100)	Caffeoylquinic acid derivative
17	26.8	385	247 (56), 223 (100), 205 (21)	Synapoyl hexoside
18	44.6	683	521 (100), 359 (100)	Rosmarinic acid dihexoside
19	44.8–44.9	447	301 (100)	Quercetin rhamnoside
20	45.6–46.0	609	301 (100)	Rutin (Quercetin rutinoside)[a]
21	45.8–46.5	521	359 (100)	Rosmarinic acid hexoside 1
22	47.2–47.5	521	359 (100)	Rosmarinic acid hexoside 2
23	47.3–47.4	505	343 (100)	Isorinic acid hexoside
24	47.3–47.4	637	521 (100), 359 (11)	Malonylrosmarinic acid hexoside
25	48.2	463	301 (100)	Quercetin hexoside
26	48.4	593	285 (100)	Kaempferol rutinoside
27	48.6	477	301 (100)	Quercetin glucuronide
28	49.0	521	359 (100)	Rosmarinic acid hexoside
29	55.1–56.0	719	359 (100), 197 (18), 179 (27), 161 (100)	2M-H, Rosmarinic acid[a]
30	63.3	727	565 (100), 359 (26)	Rosmarinic acid hexoside derivative
31	64.0	343	179 (100)	Isorinic acid

[a] Identified by comparison with standards.

m/*z* 191 and were identified as isomeric caffeoylquinic acids. Compounds **5** and **8** with *m*/*z* of 515 and 677 amu, respectively, losses a neutral fragment of *m*/*z* 162 (compound **5**) or two hexose units (compound **8**) leading to a base peak at 353 amu. The compounds were identified as caffeoylquinic acid hexoside **5** and caffeoylquinic acid dihexoside **8**, respectively. Compound **2** with *m*/*z* of 689 shows MS^2 ions at *m*/*z* 515, 353 and 173 and was assigned as a dicaffeoylquinic acid derivative. Compound **7** with a $[M-H]^-$ ion at *m*/*z* 583 shows a neutral loss of 198 leading to a base peak at 385 amu. While a compound with deprotonated ion $[M-H]^-$ at *m*/*z* 385 amu was described for the Ecuador sample of tree tomato and identified as dehydrodiferulic acid, the compound from the Argentinean fruit should be isolated to establish unambiguously the identity by spectroscopic means. The compound **16** with a $[M-H]^-$ ion at *m*/*z* 401 shows a neutral loss of 48 amu leading to a base peak at 353 amu and was tentatively assigned as a caffeoylquinic acid derivative. Compound **17** was assigned as sinapoyl hexoside based on the neutral loss of 162 amu, leading to the base peak at *m*/*z* 223. The mass spectra of compounds **18**, **21**, **22**, **24**, **28** and **29** shows a base peak at *m*/*z* 359, in agreement with rosmarinic acid, previously identified in tree tomato by Espin et al. (2016). Compounds **21**, **22** and **28** (521 amu) shows the neutral loss of 162 amu and were identified as rosmarinic acid hexosides while compound **24** ($[M-H]^-$ ion at *m*/*z* 637) show the loss of a malonyl and a hexose and was identified as rosmarinic acid malonyl hexoside. The compound **18** shows the loss of two hexose units and was identified as rosmarinic acid dihexoside. The mass spectrum of compound **15** show a $[M-H]^-$ ion at *m*/*z* 375, differing in 16 atomic mass units (amu) from rosmarinic acid and show a base peak at 179 amu, being assigned as hydroxyrosmarinic acid. Compound **29** is in agreement with rosmarinic acid while **31** differs in 16 amu, in agreement with isorinic acid. The compound **23** with a $[M-H]^-$ ion at *m*/*z* 505 show neutral loss of a hexose leading to a base peak at *m*/*z* 343 and was tentatively assigned as isorinic acid hexoside. The compound **30** ($[M-H]^-$ ion at *m*/*z* 727) presented the loss of a hexose and further fragmented to a *m*/*z* 359 ion, being assigned as rosmarinic acid hexoside derivative.

The mass spectra of the compounds **19**, **20**, **25** and **27** shows neutral loss of 146 (rhamnose), 308 (rutinose), 162 (hexose) and 176 atomic mass units (glucuronic acid), respectively, leading to a base peak at *m*/*z* 301, in agreement with quercetin. The compounds were assigned as quercetin rhamnoside, rutinoside, hexoside and glucuronide, respectively. The compounds **6** and **26**, with a $[M-H]^-$ ion at *m*/*z* 419 and 593 shows a neutral loss of 132 and 308 amu, respectively, leading to a base peak at *m*/*z* 287 and 285, in agreement with dihydrokaempferol and kaempferol, respectively. The compounds were tentatively identified as dihydrokaempferol pentoside and kaempferol rutinoside, respectively. The compound **12** showed the loss of pentose unit, leading to the *m*/*z* 269 ion, being assigned as apigenin pentoside. The tentative identification of compounds **1–31** is summarized in Table 3. The main compounds detected in the samples were caffeoylquinic acid and rosmarinic acid.

Selected ion chromatogram analysis was carried out for the identification of the main phenolics of *S. betaceum* PEE, according to the two major groups of constituents reported for *S. betaceum* fruit, namely caffeoylquinic acid derivatives and rosmarinic acid (RA)-related compounds (Espin et al., 2016). In our study, additional compounds and some flavonoid glycosides were identified. The chromatograms of the pulp, skin and seeds extract (Fig. 1) showed the same main compounds as identified by Espin et al. (2016) but also constituents that are reported for the first time for this fruit.

The compound **23** was tentatively identified as isorinic acid hexoside. Isorinic acid differs from rosmarinic acid in one hydroxyl function and has been reported from several plant species

(Dictionary of Natural Products on DVD, 2016). Caffeoylquinic acids, rosmarinic acid and its derivates, are characterized as natural antioxidants and potential natural anti-diabetic and anti-obesity compounds, because are inhibitors of α-glucosidase and amylase (Chen et al., 2014; Ghani, 2015; McCue & Shetty, 2004;) and inhibitors of lipase (Mohamed, 2014). Therefore, it is also feasible that the effect of *chilto* extracts on enzyme related to metabolic syndrome could be attributed to caffeoylquinic and rosmarinic acids (CQA and RA) and its derivatives present in the extracts. In previous papers, it was demonstrated that phenolic substances that are able to form quinones (*i.e.* caffeic acid, chlorogenic acid, gallic acid, etc) are more reactive than those phenolics that cannot form quinones, and Rohn, Rawel, and Kroll (2002) suggested that semi-quinones formed may react with amino acid side chains and free thiol groups on the enzyme. In the *chilto* extract, the antioxidant flavonoid rutin, with suitable pharmacokinetic properties was detected. Rousselot (2004) stated that improved antioxidant status may be one mechanism by which dietary antioxidant treatment contributes to the prevention and reduction of diabetic complications. In other Solanaceae fruits including *Solanum esculentum*, CQA and RA were not found. However, phenolic acids like gallic acid, chlorogenic acid and its derivatives, caffeic acid, syringic acid, *p*-coumaric acid and its derivatives have been reported (Liu et al., 2012; Navarro-González, García-Valverde, García-Alonso, & Periago, 2011). The flavonoids rutin, myricetin, quercetin, kaempferol and enantiomers of naringenin were identified in tomato seed (Peng, Zhang, & Ye, 2008; Torres, Davies, Yañez, & Andrews, 2005) while rutin, naringenin, and rutin derivatives were described in tomato peel (Navarro-González et al., 2011). The dihydrochalcone phloretin 3′,5′-di-*C*-*β*-glucopyranoside and the flavonol quercetin 3-*O*-(2″-*O*-*β*-apiofuranosyl-6″-*O*-rhamnopyr anosyl-glucopyranoside were reported in whole tomato fruits (Slimestad, Fossen, & Verheul, 2008). All tomatoes fruit fractions showed antioxidant activity (Liu et al., 2012; Navarro-González et al., 2011; Toor & Savage, 2005).

Under our experimental conditions, the percent recovery of the reference compounds chlorogenic acid and rosmarinic acid were in the range 96–100% and 100–105% for chlorogenic acid and rosmarinic acid, respectively. The content of the main phenolics, expressed as mg/100 g extract were as follows. For the 3-caffeoylquinic acid (compound **11**, Fig. 1 and Table 3): 1724.1 ± 80.7, 1324.8 ± 49.6 and 1663.4 ± 4.5 in the skin, pulp and seed extract, respectively. The content of rosmarinic acid (compound **29**) was 871.8 ± 3.5, 497.8 ± 6.8 and 345.1 ± 10.6 for skin, pulp and seed extract, respectively. Other derivatives identified by HPLC–MS were present in amounts below the LOQ and thus were not included in the quantification (ICH, 2005, accessed 11.06.2016). For the samples from Ecuador, Espin et al. (2016) reported values ranging from 25.04 to 163.62 mg/100 g dry weight for 3-O-caffeoylquinic acid (CQA) and 12.22 to 121.89 mg/100 g dry weight for rosmarinic acid (RA), respectively.

4. Conclusions

The dry powder of Argentinean chilto is a potential source of the biologically active phenolic CQA and RA. When referred to dry fruit part and percent of the total fruit, the skin, pulp and seed can be considered a good alternative to obtain a dietary supplement or functional food with nutraceutial potential, a potential source of dietary fiber and bioactive compounds. Additional studies are necessary to disclose the potential of the waste material from chilto processing to improve metabolic syndrome, including clinical trials. Following the guidelines of FAO and Biodiversity International (formerly the International Plant Genetic Resources Institute), this study will contribute to bring new insight in the research field of Argentina biodiversity principally underutilized genetic resources that could be used as non conventional food.

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