



Research article

Integral use of Argentinean *Solanum betaceum* red fruits as functional food ingredient to prevent metabolic syndrome: effect of *in vitro* simulated gastroduodenal digestion



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ABSTRACT

The “red chilito” (*Solanum betaceum* Cav) is a native fruit from the Yungas forest of Argentina. Red chilito is a neglected and underutilized native species (NUS). The objective of this work was to evaluate the potentiality of pulp, seed and skin from “red chilito” as a functional food ingredient to add value to a native resource of Argentine Yungas to promote sustainable integral use of it. The powders have low carbohydrate and sodium content and are a source of vitamin C, phenolic acids (rosmarinic acid and caffeoylquinic acid), anthocyanins, condensed tannins, carotenoids, potassium, and fiber. The phenolics of chilito powders showed, before and after simulated gastroduodenal digestion, antioxidant activity (ABTS^{•+}; H₂O₂; O₂^{•-}; HO[•]) and were able to inhibit enzymes related to metabolic syndrome, such as α -glucosidase, α -amylase and lipase. Chilito powder showed hypoglycemic effect by increasing glucose adsorption, decreasing glucose diffusion rate and by promoting glucose transport across the cell membrane. These results suggest the potential of Argentinean “red chilito” fruits as functional food ingredients or dietary supplements to prevent metabolic syndrome principally by its antioxidant, hypoglycemic and hypolipemic effects.

1. Introduction

The study of native fruits as unconventional foods is an emerging area in Argentina (Cardozo et al., 2011; Costamagna et al., 2013; Orqueda et al., 2017). In Northwestern Argentina, many native fruits make up the food spectrum of rural communities. *Solanum betaceum* Cav., called “monte tomato” or “chilito” is one of them. *S. betaceum* is distributed in Northwest Argentina in the provinces of Catamarca, Salta, Jujuy and Tucumán in the “Selva Pedemontana” of the Yungas forest, between 400 and 700 m.a.s.l. It plays an outstanding ecological role in the context of the Yungas since it has a high biological diversity and serves as a refuge for species of other altitudinal levels of the mountain forest. The Selva Pedemontana has been strongly reduced in size by the transformation of its natural cover in areas of intensive agriculture, in particular, sugar cane.

S. betaceum produces edible fruits with an interesting growing market in its native Andean countries, as well as in other parts of the world (Prohens and Nuez, 2000). The main fruit types were conventionally recognized based on skin colour, namely dark purple, red and orange. At present, the sustainable crop of red and orange chilito is being carried out in Argentina Yungas in three provinces. The orange variety is being grown in Tucumán province and the red variety in Jujuy and Salta provinces. The orange variety of chilito was previously investigated for nutritional and phytochemical composition, antioxidant and inhibitory activity on key enzymes involved in metabolic syndrome (Orqueda et al., 2017). However, so far there have been no studies of the red variety grown in Argentina.

Although the native fruits can be incorporated in the diet as fresh ripe fruits, they have a limited shelf life, as physical, chemical and biological

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changes can occur after a few days of harvest. Under these circumstances, minimum processing is often necessary to achieve a longer shelf life, preserving their nutritional and functional values as well as their organoleptic properties. The objective of this work was to evaluate the potentiality of pulp, seed and skin from “red chilito” as a functional food ingredient to add value to a native resource of Argentine Yungas and promote sustainable integral use of it.

2. Materials and methods

2.1. Plant material

The fruits of *Solanum betaceum* (red variety) were harvested at the ripening stage in which they are consumed in Finca del Obispo, Villa Jardín de Reyes, Jujuy, Argentina, in December 2016. Skin, pulp and seeds were separated, freeze-dried and powdered. The powders were vacuum packed and stored at -20 °C. Scanning electron microscopy (SEM) of each powder was carried out. SEM of gold-coated (Fine Coat Ion Sputter JEOL JFC-1100) samples was performed by using a ZEISS SUPRA-55 VP field emission scanning electron microscope at Centro Integral de Microscopía Electrónica (CIME), CONICET-UNT. The micrographs were taken for each sample at a magnification of 10,000 and 50,000 (Figure 1).

2.2. Quality parameters of fresh fruits

The titratable acidity was analyzed for each sample (AOAC, 2000). To determine the different color parameters, a Chroma meter CR-400 colorimeter (Konica Minolta, Tokyo, Japan) was used through the CIE-Lab system. Chromaticity coordinates L*, a* and b* were obtained. The parameters were obtained from three independent measurements in the equatorial region of each whole fruit. Ten fruits were measured in total.

2.3. Determination of metabolic profile of fresh fruits by ¹H-NMR

To test the metabolic profile, whole fresh fruits were frozen in liquid nitrogen. Then, was homogenized with potassium phosphate buffer (1 M pH 7.4 in D₂O) and centrifuged at 4 °C for 30 min at 14000 rpm. The supernatant was recovered and the pH was adjusted to 7.4. The deuterated sodium salt of 3-trimethylsilyl-1-propane sulfonic acid (TSP) (except for the methyl groups bound to the silicon atom) was added as an internal standard. ¹H-NMR at 600.13 (MHz Bruker Avance II spectrometer) was used.

2.4. Calculation of sweetness index

The sweetness index for each powder was determined according to Keutgen and Pawelzik (2007). The sweetness index of each part of the chilito fruit, an estimate of total sweetness perception, was calculated based on the amount and sweetness properties of individual carbohydrates presents in the samples. The contribution of each sugar in the samples, taking into account that fructose and glucose are 1.7 and 0.75 times sweeter than sucrose, respectively was estimated. As a result, sweetness index = 0.75 [Glucose] + 1.0 [Sucrose] + 1.7 [Fructose].

2.5. Estimation of taste parameter of the fruit powder

The sugars were repeatedly extracted from each part of the fruit using 80% ethanol at 80 °C until their total depletion. Total soluble sugars were quantified by the Fenol-H₂SO₄ method proposed by Dubois et al. (1956). Reducing sugars were quantified using the Somogyi-Nelson method (Nelson, 1944; Somogyi, 1945). The glucose content was determined using the enzyme glycemia kit (Wiener lab. 1400101) which is based on

the glucose oxidase/peroxidase method. Fructose and sucrose were determined using the resorcinol-thiourea method proposed by Cardini et al. (1955) and Roe and Papadopoulos (1954).

2.6. Proximal composition of chilito powder

2.6.1. Protein, fat, fiber and minerals

To determine of crude protein, lipids and minerals content, the protocols previously described by Orqueda et al. (2017) was used. The total protein content was determined by measuring the total nitrogen (N) by the Kjeldahl method with a conversion factor of 6.25 (AOCS, 1989). For the determination of the total lipid content, the samples were extracted by Soxhlet with petroleum ether (40–60 °C) for 4 h and gravimetrically quantified.

The fiber content was determined according to AOAC (2005). Briefly, 1 g of fat-free powder was weighed, treated with 20 mL of 1.25% H₂SO₄ and incubated at 100 °C for 30 min. Then, 50 mL of 3.52% NaOH was added and incubated again at 100 °C for 30 min. Finally, the sample was dried at 110 °C until constant weight. It was then incinerated in muffle at 500 °C and the crude fiber content was determined by weight difference. The integrated total dietary fiber kit (WI 96461, Megazyme International Ireland, Ireland) was used to determine the dietary fiber content (McCleary, 2014). Mineral analysis was performed using quadrupole induction plasma mass spectrometry (Q-ICPMS) at the Instituto Superior de Investigación, Desarrollo y Servicios en Alimentos, ISIDSA, Córdoba, Argentina. The following ions were analyzed: sodium, magnesium, potassium, calcium, iron, copper and zinc.

2.6.2. Starch determination

The extraction and determination of the digestible and resistant starch content were carried out according to the method of Holm et al. (1986) with some modifications. The powders (0.1 g) were mixed with 10 mL of 0.1 M HCl and 0.1 g of pepsin at pH 1.5 and kept at 40 °C for 1 h. Then, 0.1 g of sodium azide was added and the mixture was hydrolyzed using 0.5 g of α-amylase (10 U/mg) at pH 6.9 for 16 h at 37 °C. Subsequently, the samples were centrifuged at 6,000 x g. The pellets were dispersed in 2 M KOH and maintained at room temperature for 30 min. Then, the digestible starch and the resistant starch were subjected to a digestion process using 50 μL of a solution of amyloglucosidase 10 mg/mL (14U/mg) pH 4.75 in a water bath for 30 min at 60 °C. The glucose content was determined by the glucose oxidase enzymatic assay (Wiener Lab., Argentina).

2.7. Phytochemical analysis

2.7.1. Extraction and determination of phenolic compounds

The phenolic compounds were obtained from chilito powders at 25 °C with 95° ethanol (1:5, W/V) until exhaustion with ultrasound during 30 min (Pothitirat et al., 2009). Then, the samples were filtered and dried under reduced pressure to obtain the phenolic enriched extract (PEE). In each PEE, total phenolic content and flavonoid content was determined according to Costamagna et al. (2013). Extraction and quantification of tannins and anthocyanins were carried out according to Costamagna et al. (2013).

2.7.2. Vitamin extraction and determination

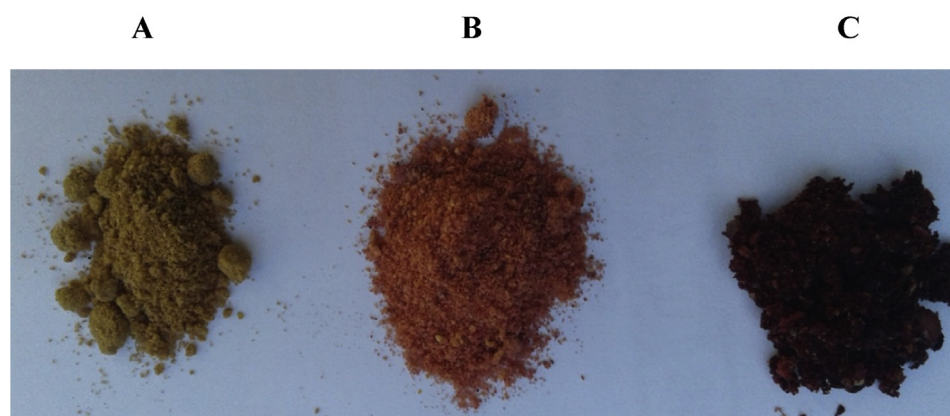
For vitamin C quantification, the powder was extracted with 2% H₃PO₄ ultrasound assisted during 10 min and the ascorbic acid content was determined according to Costamagna et al. (2013). Vitamin C was expressed as g L-ascorbic acid (g L-AA)/100 g powder.

For carotenoid quantification, the powder was extracted with petroleum ether:acetone (1:1, v/v) and total carotenoid was calculated

D)



II)



III)

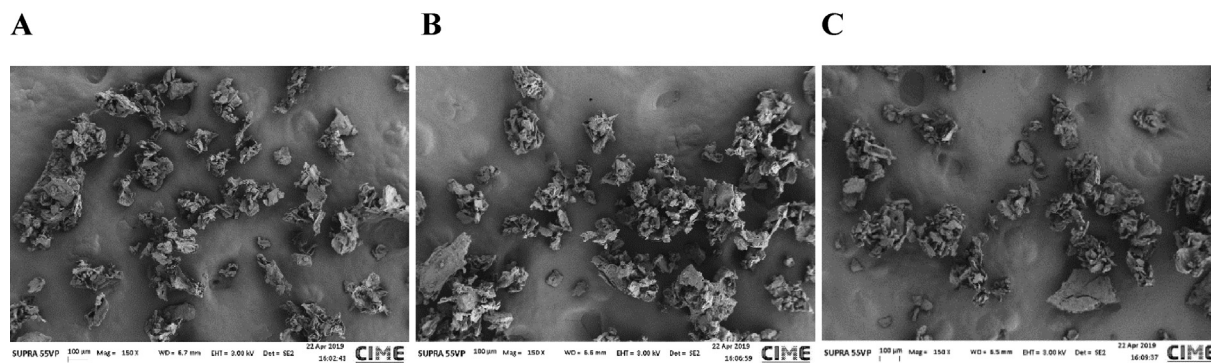


Figure 1. I) Red chilto fruit; II) (A)Seed; (B) pulp and (C) skin powder; III) Scanning electron micrographs of (A) seed powder; (B) pulp powder and (C) skin powder.

according to [Costamagna et al. \(2013\)](#). The results were expressed as g of β -carotene equivalents (g β -CE)/100 g of powder.

2.7.3. Phenolic profiling by HPLC-DAD

The extracts of the chilto samples (peel, pulp, and seeds) were analyzed by HPLC-DAD to obtain a phenolic fingerprint. The HPLC

system used was a Shimadzu equipment (Shimadzu Corporation, Kyoto, Japan) consisting of an LC-20AT pump, an SPD-M20A diode array detector, CTO-20AC column oven and a LabSolution software. An Inertsil® ODS-3 RP-C18 (5 μ , 250 \times 4.6 mm) column (GL-Sciences Inc, Tokio, Japan) maintained at 25 °C. The analyses were performed using a linear gradient solvent system consisting of 1% formic acid in water (A) and

acetonitrile (B) as follow: 90%–85% A over 15 min, maintained to 20 min, then 85% A for 20–25 min, 85% to 70% A for 25–50 min, maintained to 70% A for 50–80 min, and returning to 90% A for 80.01–90 min. The flow rate was 0.5 mL/min and the volume injected was 20 μ L. The compounds were monitored at 254, 280, 320 and 350 nm and spectra from 200 to 600 nm were recorded.

The main phenolics were quantified. The reference compounds rosmarinic acid (98%, Sigma Aldrich, St. Louis, MO, USA) and 3-caffeoyl-quinic acid (98%, Phytolab GmbH, Verstenbergsgreuth, Germany) were used in concentrations ranging from 5–500 mg/L. Results were expressed as mg of the corresponding compound/100g extract.

2.8. Simulated gastroduodenal digestion

The chilito powder was submitted to a simulated gastroduodenal digestion process (GD) according to Orqueda et al. (2017) using 0.5 g of each sample (skin, pulp and seed powder). Then, the powder samples were centrifuged at 10000 x g and the digested powders and without digestion were used to determine adsorption capacity and diffusion of sugars.

The polyphenolic enriched extracts (PEE) were also submitted to GD according to Orqueda et al. (2017). After GD, the polyphenols were extracted from the aqueous phase using ethyl acetate, dried and resuspended in dimethyl sulfoxide (DMSO) to enzymatic inhibition assays and in ethanol 95° to antioxidant assays. In all case, a negative control was performed using each of solvent.

2.9. Inhibition of enzyme involved in metabolic syndrome by chilito PEE

2.9.1. α -Glucosidase inhibition

The α -glucosidase inhibition activity of PEE was tested according to Costamagna et al. (2016). The α -glucosidase enzyme and PEE of each part of the fruit (2.5–20 μ g/mL) (before and after simulated GD digestion) were pre-incubated at 4 °C for 10 min. To start the reaction, the substrate *p*-Nitrophenyl- α -D-glucopyranoside was added. The results were expressed as IC₅₀ values (μ g GAE/mL of PEE necessary to inhibit the 50% of enzyme activity). Orlistat (tetrahydropipstatin, ATC code: A08AB01, Elea Laboratory, Ciudad Autónoma de Buenos Aires, Argentina) was used as a positive control.

2.9.2. α -Amylase inhibition

The inhibitory activity of α -amylase was measured using Amilokit (Wiener Lab Group, Rosario, Argentina, Cat. N 1021001) as reported by Costamagna et al. (2016) and following the manufacturer's instructions. Different concentrations of extract were used (5–40 μ g GAE/mL) (before and after the simulated GD) and the results were reported as IC₅₀ values. Acarbose was used as a positive control.

2.9.3. Lipase inhibition

The effect on lipase activity was tested by measuring of enzymatic hydrolysis of *p*-nitrophenyl palmitate according to Costamagna et al. (2016). The assays were carried out in presence (final concentration between 2.5 and 20 μ g/mL) and in absence of each PEE, before and after simulated GD digestion. After pre-incubation (enzyme-PEE) at 4 °C for 10 min, the enzyme reaction was carried out for 20 min. IC₅₀ values were calculated. Orlistat was used as a positive control.

2.10. Antihyperglycemic activity

2.10.1. Determination of glucose adsorption capacity of chilito powder

The glucose adsorption capacity of each powder (before and after GD digestion) was determined by Ahmed et al. (2011). The reaction mixture consisted of 10 mL of 20 mM glucose solution and 0.5 g of powder from each part of the fruit. The mixture was stirred well, incubated in a water bath with a stirrer at 37 °C for 4 h, centrifuged at 4,000 x g for 20 min and

the glucose content in the supernatant was determined. The glucose bound to the powder was calculated using the following formula:

$$\text{Glucose bound} = [(G1-G2)/(\text{Weight of the sample})] \times \text{volume of solution}$$

G1: glucose concentration in the original solution (20 mM).

G2: glucose concentration after 4 h.

2.10.2. Effect of chilito powder on glucose diffusion

The assay was performed according to Ahmed et al. (2011). To carry out the analysis, 5 mL of a 20 mM glucose solution and chilito powder samples (before and after GD digestion) (0.25, 0.5 and 1g) were dialyzed in dialysis bags against 40 mL of distilled water at 37 °C. The glucose content in the dialysate was measured at 30, 60, 120, 180 and 240 min using the enzymatic glycemia kit. A control was carried out without powder.

2.10.3. Effect of PEE on glucose intake by *Saccharomyces cerevisiae* cells

The assay was carried out according to Bhutkar et al. (2017). To prepare the suspension, commercial yeast was washed three times with distilled water and then centrifuged (3000 x g, 5 min) until the supernatant was clear. Then, a 10% (v/v) *S. cerevisiae* cells suspension was prepared in distilled water. One hundred microliter of this suspension was contacted with increasing concentrations of PEE (10–100 mg/mL) (before and after simulated GD digestion). Then, 1mL of 20 mM glucose solution was added to this mixture, incubated for 1 h at 37 °C and centrifuged at 2500 x g for 5 min. The glucose concentration in the supernatant was determined by enzymatic glycemia kit. The percentage of increase in glucose consumption by *S. cerevisiae* cells was calculated using the following formula:

$$\text{Increase in glucose uptake} = (\text{DO control}-\text{DO sample}) / (\text{DO control}) \times 100$$

For scanning electron microscopy, yeasts with and without treatment were coated with gold (Fine Coat Ion Sputter JEOL JFC-1100). ZEISS SUPRA-55 VP field emission scanning electron microscope was used. The micrographs were taken for each sample at a magnification of 10,000 and 50,000.

2.11. Antioxidant activity of PEE

Unless otherwise specified, antioxidant activity tests were carried out in 96 multiwell plates.

2.11.1. Total antioxidant capacity assay

The total antioxidant activity of PEE was tested using the ABTS radical-cation method (ABTS •⁺) (before and after simulated GD digestion) (Orqueda et al., 2017). Results were expressed as SC₅₀ (the concentration of PEE necessary to scavenge 50% of ABTS). BHT and quercetin were used as reference compounds.

2.11.2. Protection of oxidative hemolysis assay

The capacity of PEE to protect the red blood cell (RBC) membrane from oxidative hemolysis was carried out according to Orqueda et al. (2017), before and after the simulated GD digestion (0.1–2.5 mg GAE/mL). Antioxidant activity was measured using 2 mL cuvettes. The IC₅₀ values were determined as the concentration of PEE necessary to protect the RBC from oxidative hemolysis by 50%. Quercetin was used as a reference compound.

2.11.3. H₂O₂ scavenging assay

The hydrogen peroxide scavenging assay was assayed according to Fernando and Soysa (2015) method with a few modifications. PEE, before and after simulated GD digestion (2.5–40 mg GAE/mL) and H₂O₂ were pre-incubated for 3 min at 37 °C. Then, a solution of phenol (12 mM) and 4-aminoantipyrine was added to the mixture. The content of hydrogen peroxide was determined catalyzing its conversion by

horseradish peroxidase (HRP) and measured spectrophotometrically by the formation of a coloured quinone at 504 nm. The results were expressed as SC₅₀ values (mg GAE/mL), the concentration necessary to scavenge 50% of H₂O₂. Quercetin and ascorbic acid were used as controls.

2.11.4. Superoxide radical scavenging assay

The capacity of PEE to scavenging superoxide radicals was measured with the phenazine methosulfate (PMS) method (Cardozo et al., 2011). The superoxide radicals were generated by oxidation of NADH (β -nicotinamide adenine dinucleotide) in a system with PMS and were measured spectrophotometrically by the reduction of nitroblue tetrazolium (NBT). The reaction mixture contained extract, before and after simulated GD digestion at 10–100 mg GAE/mL. Then, the reaction was incubated 20 min at 25 °C and the optical density was read at 560 nm. The SC₅₀ values were measured as mg GAE/mL necessary to inhibit the 50% of superoxide radicals. Quercetin was used as an antioxidant reference compound.

2.11.5. Hydroxyl radical scavenging assay

The HO[•] scavenging capacity was measured as described by Cardozo et al. (2011). Antioxidant activity was measured using 2 mL cuvettes. The PEE (before and after simulated GD digestion) (0.5–10 mg GAE/mL) were added to a reaction mixture contained in KH₂PO₄/KOH buffer (pH 7.4), 50 mL of 10.4 mM 2-deoxy-D-ribose, 50 mL of 50 mM FeCl₃, 50 mL of 52 mM EDTA. To start the Fenton reaction, 50 μ L of 10 mM H₂O₂ and 50 μ L of 1.0 mM ascorbic acid were added and the reaction mixture was incubated 1 h at 37 °C. Then, 500 μ L of 2-thiobarbituric acid (1%, w/v) dissolved in trichloroacetic acid (3%, w/v) was added to the mixture and then, was incubated 20 min at 100 °C. The reaction absorbance was read at 532 nm. The antioxidant activity was expressed as SC₅₀ values (mg GAE/mL necessary to inhibit by 50% the degradation of 2-deoxy-D-ribose by the HO[•]).

2.11.6. Nitric oxide scavenging assay

The ability of the chilto extracts to scavenging nitric oxide was carried out according to Govindarajan et al. (2003). Different concentrations of PEE (before and after simulated GD digestion) (10–60 μ g GAE/mL), sodium nitroprusside (100 mM) and sodium phosphate buffer (0.2 M; pH 7.4) were incubated during 60 min at 37 °C. Then, the Griess reagent was added and incubated for 15 min in the dark. The antioxidant capacity was measured spectrophotometrically at 550 nm. SC₅₀ was defined as the phenolic concentration (mg GAE/mL) necessary to scavenge 50% of nitric oxide. Ascorbic acid was used as a positive control.

2.12. Toxicity assays

2.12.1. Acute toxicity

The acute toxic effect of the extracts was tested by the *Artemia salina* assay. After hatching, nauplius larvae with 24 h of incubation at 25 °C were used for the assay. The larvae (10 per well) were transferred into wells of a microplate containing 100 μ L of seawater. Then, increasing concentrations of extracts were added (2.5–250 μ g GAE/mL). Negative control with DMSO and positive control with potassium dichromate (10–40 μ g/mL) were assayed. After 24 h of exposition, the number of dead shrimps in each well was counted.

2.12.2. Salmonella mutagenicity assay

The mutagenic effect of red chilto skin, pulp and seed extracts were assayed on *Salmonella* Typhimurium strains TA98 and TA100 (Maron and Ames, 1983). Different concentrations of extracts (125–500 μ g GAE/plate) were used. His⁺ revertant colonies were counted and compared to the revertant colonies of the controls (100 μ L of DMSO). The reagent 4-nitro-o-phenylenediamine (4-NPD, 10 μ g/plate) was used as a positive control. Extracts are considered mutagenic when the average number of revertants colonies was double or higher than the revertants colonies of

controls. Three plates per experiment were assayed and two separate sets of experiments were performed in all cases.

2.13. Statistical analyses

Analyses were conducted at least three times with three different samples. Each experimental value is expressed as the mean \pm standard deviation (SD). The one-way ANOVA with Tukey posttest at a confidence level of 95% was used for the comparisons between groups. For correlation analyses between total phenolic content and bioactivities Pearson's correlation coefficient (r) at $p \leq 0.05$ was used. The analyses were developed using the statistic software InfoStat (Student Version, 2011).

3. Results and discussion

Chilto (orange and red) is a neglected and underutilized native species (NUS) in Argentina. The successful build-up as a commercially important crop requires the selection of cultivars with good fruit quality and the study of its potentialities as functional ingredient or food. For this reason, the study of red variety chilto grown in Argentinean Yungas and its comparison with the orange fruit is proposed.

3.1. Characterization of chilto fresh red fruits

3.1.1. Parameters of physical-chemical quality of fresh fruit

The quality of the fruits is the combination of characteristics, attributes and properties that give value to the food. The quality can also be defined as the set of parameters, such as soluble solids content (SSC), color and total acidity that determine that a certain product is the taste of a consumer or a group that you want to satisfy. The CIELAB parameters of colour were taken into account to ensure the homogeneity and quality of the sample (L*: 36.51 \pm 2.54; a*: 25.66 \pm 1.33; b*: 11.74 \pm 1.82; hue angle: 22.97; Chroma: 27.26 and CIRG index: 2.46) (Table 1). These values were different of the colorimetric parameters of orange chilto fruits of Northwestern Argentina (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) (Orqueda et al., 2017), showing differences in the shades of the epicarp among both varieties. The SSC value obtained for red fruits (9.20% \pm 0.2) was lower than those for red tree tomatoes fruits from Ecuador and Spain (11.00–12.00%) (Vasco et al., 2009; Acosta Quezada et al., 2015). The red fruit of *S. betaceum* from Argentina showed lower total acidity (0.9%) than the orange fruit from the same place (1.9%) (Orqueda et al., 2017). The difference in the acidity of the two varieties is clear in the taste, so the red chilto have a more pleasant and less acid taste than the orange fruit.

3.1.2. Metabolic profile of fresh red chilto

In fruits, fructose, glucose and sucrose are the main soluble sugars and differ in the level of sweetness. The sugars are important in the flavor of the fruit causing a strong impact on the organoleptic quality of the fruits (D'Angelo et al., 2018). In fresh fruits of red chilto the level of fructose was 9.91 mg/g FW, glucose 7.32 mg/g FW and sucrose 6.62 mg/g FW (Table 1) and accordingly, the sweetness index of red fruits was 28.97. The sweetness index of chilto was higher than pear, apple and blueberries juice index (Rizzolo and Cortellino, 2018).

Citric acid was the more abundant organic acid in the ripe red chilto (26.47 mg/g FW) followed by malic acid (4.86 mg/g FW). The level of GABA, glutamic and succinic acids were low, indicating the absence of GABA shunt pathway activity, and consequently, the lack of feeding to the tricarboxylic acid cycle, which diminishes the supply of NADH to the mitochondrial electron transport chain. Few free amino acids were detected, including L-asparagine and L-aspartic acid, allowing nitrogen assimilation and supplying oxaloacetate for the tricarboxylic acid cycle functioning, respectively. The level of ethanol was low indicating the absence of a fermentative process (Table 1).

Table 1. Metabolic profile of red *Solanum betaceum* fresh fruits by ¹H-NMR spectrometry and CIELAB parameters.

Metabolites	g/100 g fresh weight
D-fructose	0.99 ± 0.27
D-glucose	0.73 ± 0.14
Sucrose	0.66 ± 0.20
Gamma-aminobutyric acid	0.05 ± 0.02
L-alanine	0.01 ± 0.001
L-asparagine	0.17 ± 0.09
L-aspartic acid	0.14 ± 0.09
Citric acid	2.67 ± 0.54
L-malic acid	0.48 ± 0.13
Alpha-ketoglutaric acid	0.17 ± 0.12
Ethanol	0.05 ± 0.002
Methanol	0.10 ± 0.007
CIELAB parameters of colour	
L*	36.51 ± 2.54
a*	25.66 ± 1.33
b*	11.74 ± 1.82
hue angle	22.97
Chroma	27.26
CIRG index	2.46
Sweetness index	28.97
Total acidity	0.9
SSC	9.20% ± 0.2

In red chilito fruit, the methanol content was 1 mg/g fruit, similar to that reported for apple (Possner et al., 2014). Methanol is present in pectin, a component of the plant cell walls and the middle lamella.

Water-soluble pectin and methanol are released during processing of fruits and vegetables by enzymatic activities. To accurately measure the methanol contents of whole or processing fruits, it is important that pectin methyl esterase (PME) activity in the fruits is inactivated before analysis. In this way, when homogenizing the material, a rapid de-esterification of the pectin will occur altering the amounts of methyl esters and free methanol.

3.2. Chemical and functional characterization of chilito red fruit powder

Removal of skin and seeds of fruits is a common practice by the Argentine food industry, and the population consumes only the fruits pulp (crude or cooked).

For this reason, we analyzed the chilito skin, pulp and seed in order to give an added value to the waste material (skin and seed) in the industrial processing to obtain pulp, juice and other products.

3.2.1. Nutritional composition of the skin, pulp and seed powder

3.2.1.1. Macronutrients analysis. The soluble sugars content (8.04–20.5 g/100g DW) in the three fruit parts of the Argentinean red chilito was higher than in the orange chilito variety (2.23–11.91 g/100g DW) from the same ecoregion of Argentina (Orqueda et al., 2017). The carbohydrates content of red chilito fruits was lower than the ones reported for wild *S. cajanumense* of Ecuador (28.6 g/100 g DW) (Acosta-Quezada et al., 2015). The levels of glucose and fructose were higher in skin powder (1.02–3.23 g/100g) than in pulp or seeds powder (0.8–0.95 g/100g). These results were similar to those obtained for the orange chilito variety (0.90–2.72 g/100g) and much lower than for red tree tomatoes from Ecuador (9.0 g/100g) (Acosta-Quezada et al., 2015). The sucrose levels in ripe red chilito (4.23, 7.23 and 8.52 g/100g DW for skin, pulp and seeds, respectively) were higher than glucose (0.6–0.99 g/100g DW) and fructose (1.23–3.96 g/100g DW) (Table 2). According to these results, the sweetness index of the three fruit parts was 6.77, 10.71 and 15.99 for skin, pulp and seeds, respectively, higher than in the orange

chilito from the Argentine northwestern Yungas. Comparing with other flours or powders, the sweetness index of red chilito powder was lower than apple flours (59.85) (Pires et al., 2018). Digestible starch (DS) (0.02%, 0.01% and 0.006% for seeds, pulp and skin, respectively) and resistant starch (RS) (0.003% only in the pulp) were lower than for wheat flour (65% of total starch) (Swieca et al., 2017). In order to maintain normal postprandial glucose and fatty acid levels and avoid oxidative stress, a decrease in DS consumption must be achieved (Agama-Acevedo et al., 2018).

In the three fruit parts, the protein level was between 5.2–16.3 g/100 g powder, higher than the content found in New Zealand fruits, whose values were between 1.9–2.0 g/100g (Athar et al., 2003). Although the protein content of the chilito powder was lower compared to soybean flour (43.2 g/100g) (Van Ee, 2009), it is relatively high for an unconventional flour. This data together with the low fat content (0.42–0.9 g/100 g), makes these unconventional flours or powders an ingredient of interest in the food industry.

The total fiber level of skin, seed and pulp was high and similar to previously found in orange chilito (8.84–20.95 g/100g) (Orqueda et al., 2017). The dietary soluble fiber (DSF) content in red chilito skin, pulp and seeds was 9.72; 9.06 and 5.59%, respectively. The DSF content was higher than the reported in the flours of amaranth and quinoa (Kurek et al., 2018) and similar to wheat flour (Zhang et al., 2018). It is proven that dietary fibers produce certain healthy effects for consumers through increased fecal volume, stimulation of the fermentation of the colon, reduction of postprandial blood glucose and pre-prandial cholesterol level. These physiological effects are also well associated with reduced risk of cardiovascular diseases, cancer, diabetes, respiratory disease, infections, and others (Zhang et al., 2018). In conclusion, this study proved that chilito seeds and skin, two sub-products, as well as the pulp, could be exploited for their content in nutritional and functional components beneficial for human health.

3.2.1.2. Mineral analysis. The three chilito powders were high in potassium, with a content similar to banana (395 mg/100 g FW) (Livsmedelsverket, 2009) and low in sodium (below detection limits in pulp and seed powder) (Table 2), even below the sodium content of the orange variety of chilito (Orqueda et al., 2017). This potassium/sodium ratio in

Table 2. Macronutrient and micronutrient content of seed, pulp and skin powder of Argentinean red *Solanum betaceum*.

Nutrients and mineral content in 100 g powder	Skin	Pulp	Seeds
Soluble total sugar (g GE)	8.9 ± 0.4 ^a	10.5 ± 1.8 ^a	20.5 ± 1.2 ^b
Glucose (g GE)*	0.6 ± 0.01 ^a	0.91 ± 0.03 ^b	0.99 ± 0.06 ^b
Fructose (g GE)*	1.23 ± 0.5 ^a	1.65 ± 0.52 ^a	3.96 ± 0.2 ^b
Sucrose (g GE)*	4.23 ± 0.9 ^a	7.23 ± 0.5 ^b	8.52 ± 0.3 ^b
Digerible starch (mg)	6.53 ± 0.5 ^a	18.49 ± 0.2 ^b	22.52 ± 1.2 ^c
Resistant starch (mg)	ND	3.47 ± 0.60	ND
Total protein (g)	10.51 ± 0.6 ^a	11.2 ± 0.3 ^a	21.9 ± 0.2 ^b
Soluble protein (g AE)**	0.09 ± 0.01 ^b	0.05 ± 0.008 ^a	0.5 ± 0.01 ^c
Fat (g)	0.8 ± 0.1 ^c	0.2 ± 0.03 ^a	0.42 ± 0.03 ^b
Total fiber (g)	23.2 ± 2.0 ^a	20.8 ± 1.8 ^a	29.5 ± 1.2 ^b
Dietary soluble fiber (g)	9.72 ± 0.2 ^b	9.03 ± 0.2 ^b	5.59 ± 0.1 ^a
Minerals (mg/100 g)			
Na	1.2 ± 0.1	ND	ND
Mg	90.4 ± 2.0 ^b	76.4 ± 0.5 ^a	131.8 ± 3.5 ^c
K	2725.6 ± 69.0 ^b	1234.2 ± 26.7 ^a	1234.2 ± 26.7 ^a
Ca	212.9 ± 6.0 ^c	105.7 ± 1.9 ^b	56.5 ± 3.5 ^a
Fe	3.4 ± 0.05 ^b	ND	5.8 ± 0.06 ^a
Cu	3.74 ± 0.008 ^b	0.81 ± 0.02 ^a	0.90 ± 0.03 ^a
Zn	0.46 ± 0.04 ^a	1.20 ± 0.06 ^b	1.61 ± 0.04 ^b

bdl: below detection limit. Different letters (a, b, c, d) in the same line show significant differences in the macro and micronutrient content among each part of the fruit (skin, pulp and seeds), according to Tukey's multiple comparison ($p \leq 0.05$). *GE: Glucose equivalent; **AE: Albumin equivalent; ND: no detected.

red chilito powders is desirable in a healthy diet. The magnesium and calcium contents were higher than in tree tomatoes from Ecuador (Vasco et al., 2009), principally in seed powder. Compared with orange chilito, the magnesium, potassium and calcium content was lower in red chilito, although the iron content was higher in the seeds of red chilito (Orqueda et al., 2017). Since iron is an important mineral for the prevention of anemia due to iron deficiency, and the diverse clinical manifestations associated with this pathology, the content of this microelement converts chilito powder as an interesting functional ingredient (Abbaspour et al., 2014). However, other studies on the bioavailability of this mineral in chilito powder would be necessary.

3.2.2. Phytochemical composition of the skin, pulp and seed powder

3.2.2.1. Polyphenolic compounds content. Plant foods have large amounts of phytochemicals. There is evidence on the benefit that the consumption of foods containing phenolic compounds is associated with the prevention of chronic disorders such as cardiovascular diseases and type 2 diabetes (Costamagna et al., 2016). In recent times, the interest in these bioactive compounds has increased, as well as their impact on health. The content of phenolic compounds in red chilito was 408.9 ± 2.3, 334.0 ± 1.2 and 623.6 ± 1.6 mg GAE/100 g DW in the skin, pulp and seeds, respectively. The phenolic profile of pulp and skin was very similar. Two main compounds, rosmarinic acid and caffeoylquinic acid, were identified (Figure 2). These two hydroxycinnamoyl derivatives had been reported previously in the orange chilito from Argentina Yungas and in yellow and purple varieties from Ecuador (Espin et al., 2016; Orqueda et al., 2017) but in orange chilito the diversity of phenolic compounds was greater (12 caffeic acid derivatives and related phenolics, 10 rosmarinic acid derivatives and 7 flavonoids), (Orqueda et al., 2017). The total content of each phenolic acid (rosmarinic acid and caffeoylquinic acid) in red chilito skin powder was 0.291 ± 0.001 g/100 g powder and 0.354 ± 0.002 g/100 g powder, respectively. Several biological activities are attributed to these phenolic acids, such as antioxidant, anti-inflammatory, immunomodulatory, hypoglycemic, hypolipidemic, antimutagenic, antitumor, antimicrobial, hepatoprotective, neuroprotective, and nephroprotective properties (Spínola and Castilho, 2017; Costa et al., 2014).

The food preferences of the population are characteristic of each region and can define the intake of polyphenols in each of them. However, although the recommended intake depends on the region, preferences, age, among others, the presence of these bioactive compounds enhances the nutritional value of the fruits. Regarding diet contribution of phenolics, 100 g of fresh red chilito contributes 84.87 mg of phenolic compounds, including 43.96, 29.79 and 11.12 mg of these compounds in the seeds, pulp and skin, respectively.

Hydrolyzable tannins were not detected in any of the three powders. But, all red chilito powders contain condensed tannins (Table 3), mainly in the seed powder (265.0 mg/100 g DW). This is the first report on the content of condensed tannins for chilito. Condensed tannins are bio-functional compounds of interest for their multiple health attributes that include anti-inflammatory, antimicrobial, antiviral, antitumoral, hypocholesterolemic, and digestive enzymes (α -amylase, α -glucosidase and lipase) inhibitory activities and thus, their benefits in cardiovascular diseases, diabetes, and gastrointestinal disorders (da Silva et al., 2014).

Anthocyanins are a group of flavonoid compounds responsible of the colours of many plant structures (flowers and fruits) and are related with health-related properties such as effect reducing the risk of cardiovascular diseases (De Pascual-Teresa and Sanchez-Ballesta, 2008). In red chilito, the content of anthocyanins was 10.6, 62.5 and 65.23 mg EC/100 g DW for the pulp, skin and seeds, respectively. The total content of pigment (60.38 mg EC/100 g FW) in red chilito was much higher than the reported to orange chilito (1.78 mg/100 g, only in the skin) from Argentinean Yungas (Orqueda et al., 2017) and similar to that reported in blackberry (58.61 mg/100 g FW; Souza et al., 2014). These results provide evidence of difference in profile and quantities of phenolic compounds among the orange and red varieties. This information is essential for the development of selection and breeding programmes.

3.2.2.2. Vitamins. The ascorbic acid (vitamin C) content of the skin, pulp and seed powders is shown in Table 3. The results show that the powders are rich in vitamin C, a potent antioxidant compound with large biological activities. The vitamin C content of the red chilito powder was similar to orange chilito from Argentina and was higher than that of the edible tissues of oranges *Citrus sinensis* (Abeyasinghe et al., 2007).

Carotenoids were only detected in red chilito pulp (0.9 mg/100 g DW) while en orange chilito from Argentina was found in the three parts. These

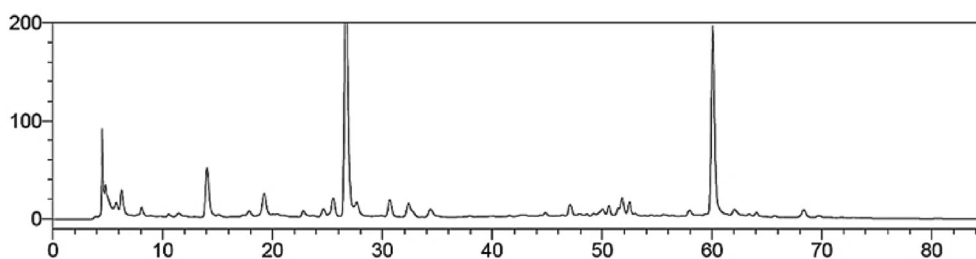


Figure 2. Phenolic compounds profile of red chilito skin polyphenolic extracts. Caffeoylquinic acid RT 27.1 min; Rosmarinic acid RT 60.1 min.

Table 3. Phytochemical content of seed, pulp and skin powder of *Solanum betaceum* fruits (red variety).

Phytochemical content	Skin	Pulp	Seeds
Total phenolic (mg GAE)	408.9 ± 2.3 ^b	334.0 ± 1.2 ^a	623.6 ± 1.6 ^c
Flavone and flavonol (mg QE)	195.3 ± 3.6 ^c	123.3 ± 0.9 ^a	180.5 ± 2.0 ^b
Condensed tannins (mg procyanidin B2)	75.7 ± 1.3 ^b	18.9 ± 1.2 ^a	265.0 ± 3.5 ^c
Hydrolyzable tannins (mg GAE)	ND	ND	ND
Anthocyanins (mg C-3GE)	62.5 ± 0.5 ^b	10.6 ± 0.6 ^a	65.23 ± 0.9 ^b
Carotenoids (g β carotene)	ND	0.9 ± 0.08	ND
Ascorbic acid (mg)	43.5 ± 2.5 ^a	75.9 ± 2.0 ^b	45.2 ± 0.6 ^a
%Yield (g powder/100 g fruit part)	26.0 ± 0.8 ^b	20.4 ± 0.5 ^a	25.9 ± 1.5 ^b

Different letters (a, b, c, d) in the same line show significant differences in the phytochemical content among each part of the fruit (skin, pulp and seeds), according to Tukey's multiple comparison ($p \leq 0.05$). ND: no detected. GAE: Gallic acid equivalent; QE: Quercetin equivalent; C-3GE: Cyanidin-3 glucoside equivalent.

pigments have multiple biological properties such as oxidative stress protection, anticarcinogenic capacity, macular degeneration prevention or bone health support (Hornero-Mendez et al., 2018).

3.2.3. Functional properties of seed, skin and pulp powders and PEE

The powders of pulp, skin and seed of chilito were submitted to simulated GD. Due to the extensive evidence that phenolic compounds are effective antioxidants and inhibitors of digestive enzymes, and the intention to also use the fruit powders as raw material for phenolic extracts preparation, the effect of gastroduodenal digestion was studied also on phenolics enriched extracts.

3.2.3.1. Hypoglycemic effect of chilito powder and PEE. Metabolic syndrome is a combination of at least three of the cardiovascular risk factors: obesity, dyslipidemia, hypertension and hyperglycaemia or Type 2 diabetes. All these risk factors are related to insulin resistance, oxidative stress and an inflammatory state. The level of postprandial hyperglycemia can be controlled by inhibiting the activities of α -amylase and α -glucosidase, slowing the diffusion and adsorption of glucose in the intestinal epithelium, stimulating insulin secretion by pancreatic cells and inhibiting the transporters of glucose at the cellular level (Costamagna et al., 2016). The commercial medicines used for treating diabetes may cause several side effects, including flatulence and diarrhea. Therefore, it is necessary to investigate natural sources of food for the prevention of postprandial hyperglycemia.

3.2.3.1.1. Effect of chilito powder on glucose adsorption, diffusion and transport.

Adsorption

The glucose adsorption capacity of red chilito powders before and after GD was assayed. The seed powder showed higher adsorption capacity than skin and pulp powders (Figure 3). No significant differences were observed between the glucose adsorption capacity of digested and undigested seed and pulp powders. The adsorption capacity of the digested chilito skin powder was greater than undigested. The results revealed that the adsorption capacity of chilito seeds powder was higher than acarbose, wheat bran and oats at a concentration of 10 mM of glucose (Ahmed et al., 2011) and similar to *Caesalpinia bonducella* and

Myristica fragrans seed (Bhutkar et al., 2018). These results indicated that the red chilito powders could decrease the amount of glucose available to be absorbed by the intestinal epithelium resulting in normal blood glucose values. Furthermore, condensed tannins, which are also present in red chilito powders, principally in seed powders could bind to the enzymes at non-specific sites, diminishing their activities and have been proposed as non-competitive inhibitors of these digestive enzymes (Barrett et al., 2018).

Diffusion

The effect of chilito powder before and after GD on retarding glucose diffusion across the dialysis membrane is represented in Figure 4. During the study, the movement of glucose across the dialysis membrane was monitored every 30 min for 240 min, and it was observed that the powder samples delayed the movement of glucose to the dialysate through the membrane compared to the control. The rate of glucose diffusion across the dialysis membrane was increased over time, but all the samples were able to inhibit glucose diffusion compared to the control (Figure 4). No significant differences were observed between the digested and undigested powders. These effects could be attributed to the network formed by the fibers where glucose is trapped (López et al.,

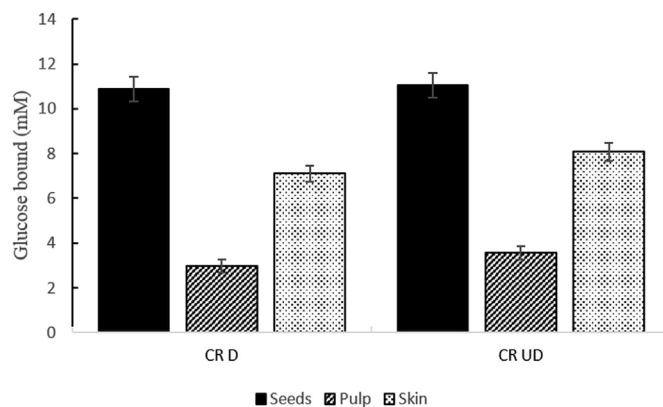


Figure 3. Glucose adsorption capacity of digested and undigested chilito powders. CR D: red chilito digested powders. CR UD: red chilito undigested powders.

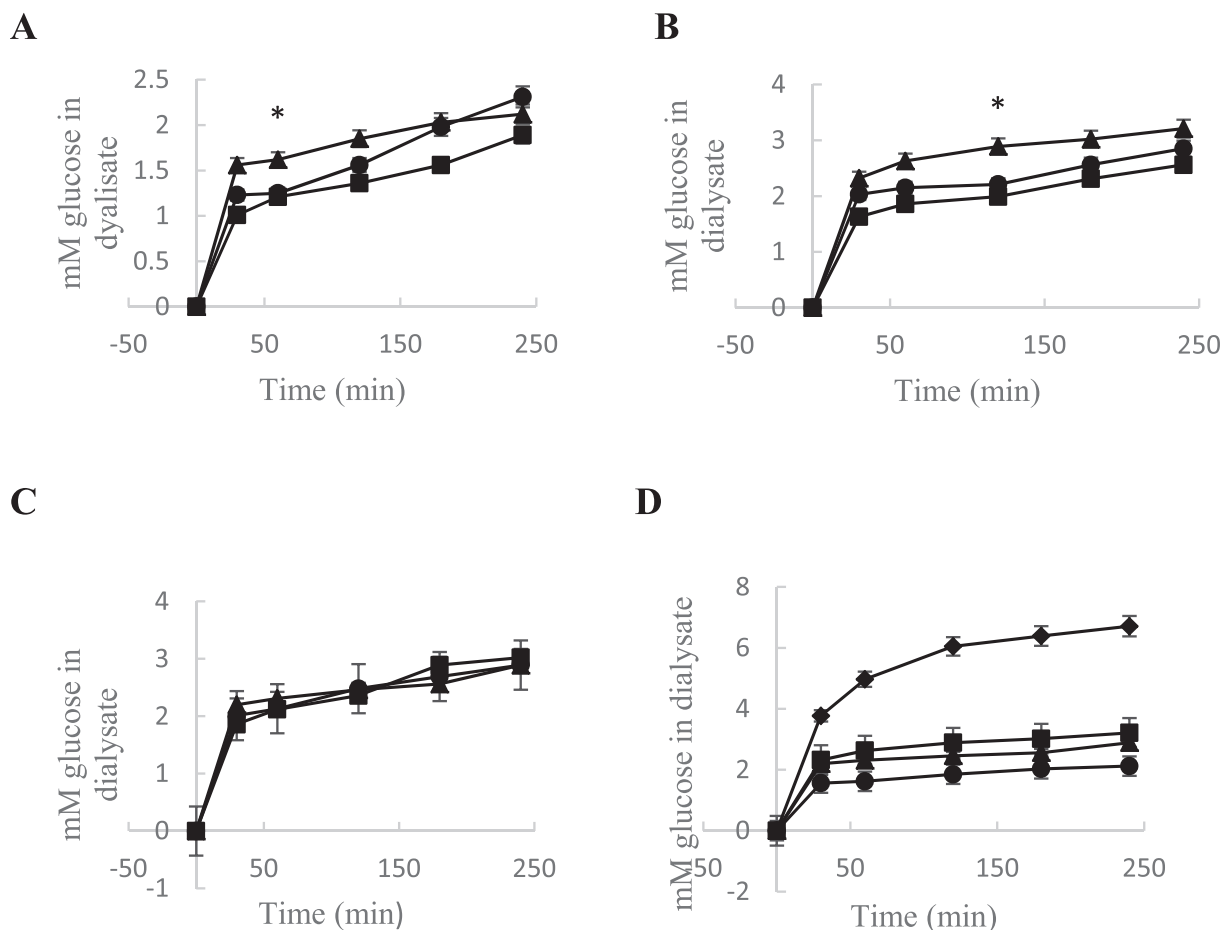


Figure 4. Effect of different amount of chilto powders on glucose diffusion: (A) Seed; (B) Pulp (B) and (C) Skin powders \blacktriangle –0.25 g; \bullet –0.5 g and \blacktriangle –1.0 g. (D) comparative effect on glucose diffusion of 0.25 g powders with control \blacklozenge –; seed \bullet –; pulp \blacksquare –; and skin \blacktriangle –. The symbol (*) indicates significant differences between seed and pulp extracts compared to skin extracts at a given time.

1996). The dietary fiber of the powders forms viscous gels in contact with water, which slows the access of glucose to the small intestine epithelium, decreasing its absorption.

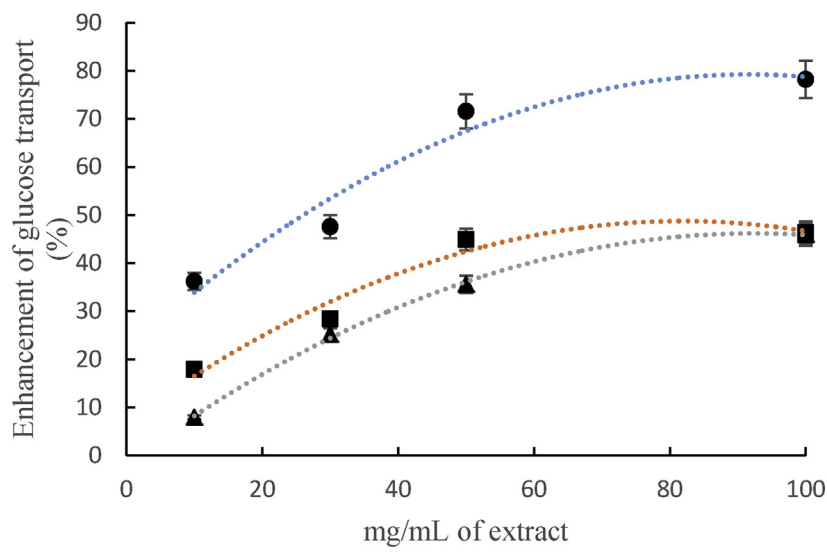
3.2.3.1.2. Effect of chilto PEE on glucose uptake by *S. cerevisiae* cells. The yeast model to evaluate the mechanism of glucose transport through the cell membrane is a test for the *in vitro* evaluation of the possible hypoglycemic effect. The results of the rate of glucose transport across the cell membrane in yeast cell system are represented in Figure 5 I. The presence of phytochemicals of chilto increase the uptake of glucose by the yeast cells, therefore the amount of glucose remaining in the medium is lowest after the incubation time. The area under the curves of glucose uptake obtained with polyphenols from seed powder (5901.52 ± 124.79) was higher than pulp (3504.01 ± 441.78), followed by skin (2789.50 ± 109.58). The chilto seeds polyphenolic extract was more effective to increase glucose transport up to 81% compared to the control (Figure 5I). Enhancement on glucose uptake by low concentration of seeds polyphenols was similar to chia and cinnamon extracts (Woldemariam and Van Winkle, 2015). Furthermore, SEM photographs showed that the cell surface structure of the yeasts was not affected in the presence of chilto extracts (Figure 5II). These results show that the chilto polyphenols could favor the entry of glucose into the cells, resulting in a decrease in blood glucose levels. Further *in vivo* experiments should be achieved in order to confirm that these natural extracts are possible antidiabetic nutraceuticals.

3.2.3.1.3. Inhibition of carbohydrate metabolism enzymes by PEE. Pancreatic α -amylase and α -glucosidase hydrolyse complex carbohydrates and disaccharides in absorbable monosaccharides in the intestinal epithelium (Costamagna et al., 2016). An objective in the treatment

of type 2 diabetes focuses on the inhibition of these enzymes as a way to lower blood glucose levels (Zhang et al., 2015). Anti-diabetic agents that are used in clinical practice, such as acarbose, voglibose, and miglitol are competitive inhibitors of these enzymes which delay the hydrolysis of carbohydrates and thus alleviate postprandial hyperglycemia (Scheen et al., 2015). However, the use of these drugs has some disadvantages including drug resistance, side effects, and even toxicity (Spínola et al., 2020). Due to their strong inhibitory activities, these enzyme inhibitors can produce several side effects, since dangerous ones like the excessive lowering of blood sugar levels when used in combination with other medications used to treat diabetes, to banaler such as abdominal pain, diarrhoea, and flatulence. In this sense, new natural inhibitors with moderate activities and good bioavailability can be useful to manage obesity, insulin resistance or type-2 diabetes. Bioavailability is dependent upon their release from the plant matrix (bioaccessibility), their stability during digestion process, and the efficiency of their transepithelial passage (Manach et al., 2004). So, the inhibitory capacity of amylase and glucosidase of red chilto polyphenolic extracts was determined before and after *in vitro* gastroduodenal digestion to determine the stability during digestion.

All extracts enriched in polyphenols of red chilto were active to inhibit both enzymes with IC_{50} values between 25.0–89.3 μ g of GAE/mL for α -glucosidase and 50.0–136.5 μ g of GAE/mL for α -amylase (Table 4). A strong correlation ($r^2 \geq 0.84$) between total phenolic contents and the inhibitory capacity of the enzymes (α -glucosidase and α -amylase) was observed with seeds, skin, and pulp extracts (Table 5). However, the correlations were only significant ($p < 0.05$) with seeds and pulp extracts against α -glucosidase. A very strong significant correlation between total

I.



II.

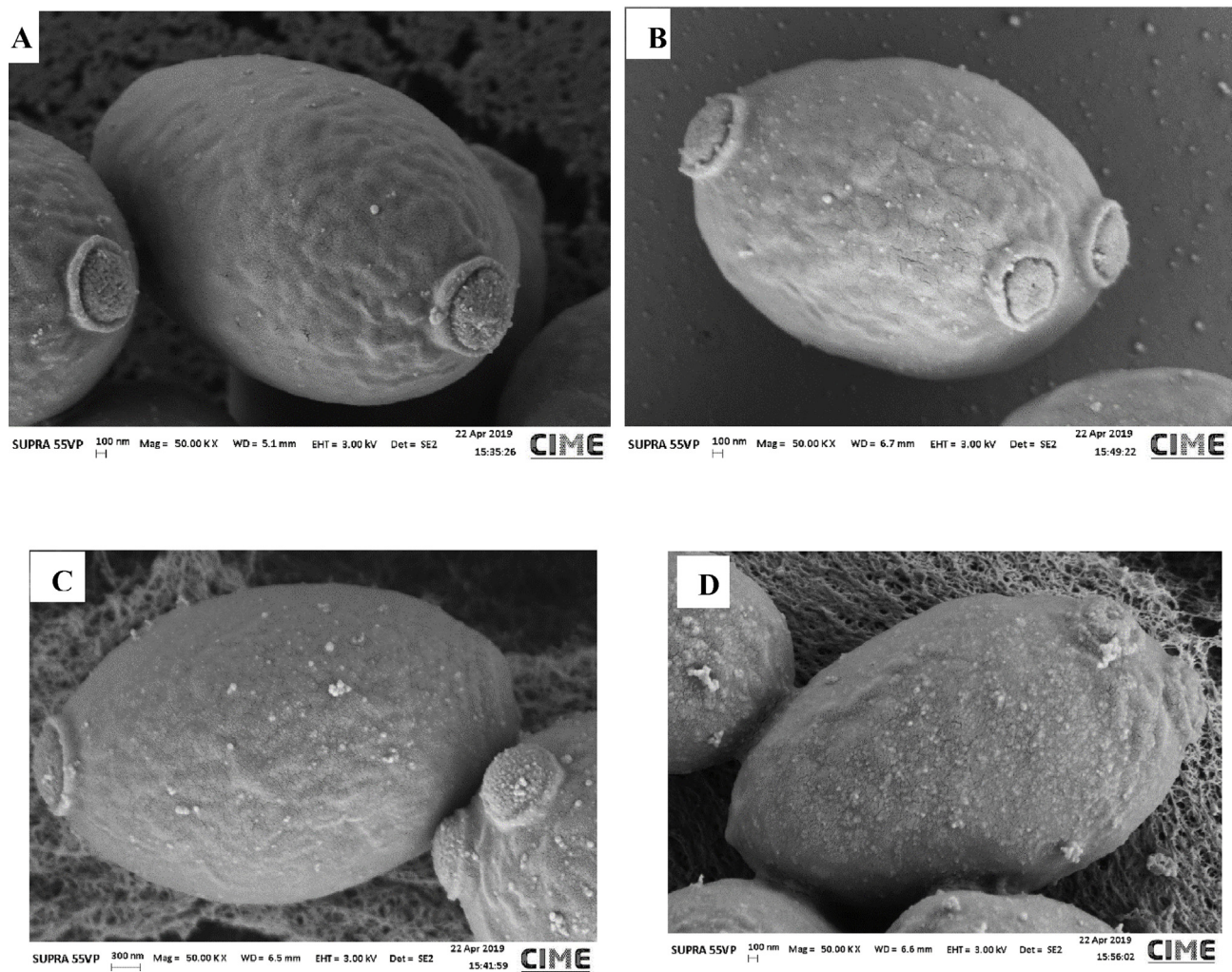


Figure 5. I. Effect of *S. betaceum* extracts on uptake of glucose by *S. cerevisiae* –●– seed; –■– pulp; –▲– skin. II. Scanning electron micrographs of (A) control yeast, (B) yeast treated with seed extract, (C) yeast with glucose, (D) yeast treated with polyphenolic enriched extract + glucose.

Table 4. Effect of phenolic-enriched extract (PEE) of Argentinean *Solanum betaceum* red fruit powder (seed, pulp and skin) before and after simulated gastroduodenal digestion (GD) on enzymes related to carbohydrate and fat metabolism and oxidative processes. Reference compounds are included for comparison.

<i>S. betaceum</i>	Enzyme IC ₅₀ (μg GAE/mL)			IC ₅₀ (μg GAE/mL)	SC ₅₀ (μg GAE/mL)				
	α amylase	α glucosidase	Lipase	AAPH	ABTS	O ₂ ⁻	HO [•]	H ₂ O ₂	NO
Seeds									
Before GD	50.0 ± 2.3 ^a	25.0 ± 1.2 ^a	4.5 ± 0.3 ^a	1.2 ± 0.05 ^a	4.5 ± 0.5 ^{ab}	22.1 ± 0.5 ^a	8.5 ± 0.06 ^c	5.8 ± 0.5 ^a	17.1 ± 0.6 ^a
After GD	35.6 ± 1.2 ^A	30.5 ± 1.1 ^A	6.5 ± 0.2 ^A	3.6 ± 0.02 ^C	5.8 ± 1.2 ^{AB}	26.6 ± 0.6 ^A	7.9 ± 0.2 ^B	7.8 ± 0.3 ^A	44.0 ± 1.3 ^B
Pulp									
Before GD	115.1 ± 2.1 ^b	42.3 ± 0.3 ^b	4.7 ± 0.9 ^a	1.0 ± 0.03 ^a	5.7 ± 0.6 ^b	74.0 ± 1.3 ^b	5.6 ± 0.1 ^b	14.6 ± 0.05 ^b	44.0 ± 3.2 ^b
After GD	120.0 ± 2.3 ^B	40.3 ± 0.6 ^B	5.9 ± 0.6 ^A	0.7 ± 0.06 ^A	8.2 ± 0.6 ^B	78.3 ± 2.0 ^C	10.2 ± 0.03 ^C	20.6 ± 0.6 ^C	49.8 ± 2.0 ^B
Skin									
Before GD	118.2 ± 2.6 ^b	76.0 ± 0.9 ^c	6.8 ± 0.3 ^b	2.6 ± 0.04 ^b	3.4 ± 0.3 ^a	66.0 ± 0.6 ^b	4.2 ± 0.09 ^a	20.6 ± 0.2 ^c	22.0 ± 2.1 ^a
After GD	136.5 ± 1.2 ^C	89.3 ± 2.6 ^C	8.5 ± 0.1 ^B	2.9 ± 0.01 ^B	5.6 ± 1.1 ^A	70.6 ± 0.9 ^B	6.3 ± 0.1 ^A	16.5 ± 0.5 ^B	30.6 ± 1.6 ^A
Reference compound	Acarbose	Acarbose	Orlistat	Quercetin	BHT	Quercetin	Quercetin	Quercetin	Ascorbic acid
IC ₅₀ (μg/mL)	1.25 ± 0.10	25.00 ± 1.00	0.08 ± 0.01	1.40 ± 0.03	0.65 ± 0.01	60.50 ± 4.70	30.00 ± 2.00	17.30 ± 0.50	37.19 ± 0.33

IC₅₀: Concentration of polyphenolic extract necessary to inhibit 50% of oxidative hemolysis or enzyme activity; SC₅₀: Concentration of PEE necessary to scavenge 50% of ABTS, O₂⁻, HO[•], H₂O₂, NO; GAE: Gallic acid equivalent; PEE: phenolic enriched extract. 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 or antioxidant activity according to Tukey's test ($p \leq 0.05$).

phenolic concentrations and α-glucosidase inhibition was observed with these two extracts ($r^2 \geq 0.96$, $p < 0.05$). The seeds powder was the most active extract, probably due to the higher contents of total phenolics and condensed tannins, the last ones recently proved to be effective inhibitors of α-glucosidase and α-amylase (Barrett et al., 2018). The PEE of red chilto were more active towards the α-glucosidase than the acarbose reference compound but had a lower effect on α-amylase. Also, it is noteworthy that the extracts obtained from the orange variety flours proved to be much more active than those of the red variety, probably due to its less phenolic components diversity than orange chilto (Orqueda et al., 2017).

Besides, there were no significant differences after subjecting the extracts to the digestive process, contrariwise to the PEE of seeds of orange variety (Table 4).

According to the literature, CQA and RA which are present in red chilto have a great capacity for inhibition of digestive enzymes (Lin et al., 2011; Spínola and Castilho, 2017). Previous reports showed that the concentration of rosmarinic acid is slightly reduced after gastric digestion and that was more stable in the basic pH of the pancreatic environment than in the acidic pH of the gastric juice (Costa et al., 2014; Porfirio et al., 2010). On the other hand, isomerization and hydrolysis of CQA and partial opening of lactones intramolecular ring were observed after incubation of CQA with artificial and human intestinal fluids (pH from 6.3 to 7.7), with slightly losses after 8-h incubation (Farah and Duarte, 2015). The results would indicate that the inhibitory effect could be attributed to

both phenolic acids and other minority compounds, non-detectable with the used methodologies.

3.2.3.2. Effect of chilto PEE on in vitro fat absorption. Obesity has become a serious health issue around the world due to the connection with a large number of pathological disorders, including metabolic syndrome, diabetes mellitus, hypertension, atherosclerosis and cancer. Inhibition of dietary fat absorption is one of the common approaches to decrease excessive energy intake. Pancreatic lipase hydrolyses lipids in absorbable fatty acids and monoglycerides and, therefore, their inhibition is a key target in the prevention and treatment of obesity. Orlistat is a commonly used antiobesity drug and the reference compound for the inhibition of pancreatic lipase, but it produces several adverse effects. Again, the potency of available commercial inhibitors is mostly responsible for these undesirable effects. Hence the importance of finding moderate lipase inhibitors and natural inhibitors from plant arise as an alternative. In this regard, the inhibitory activity of red chilto PEE was assessed on pancreatic lipase (Table 4) before and after GD digestion. The highest inhibitory activities were observed in the PEE from seeds and pulp before GD digestion (IC₅₀: 4.5 and 4.7 μg GAE/mL, respectively). These both extracts showed a very strong significant correlation between total phenolic contents and the ability to inhibit pancreatic lipase ($r^2 \geq 0.97$; $p < 0.05$) (Table 5). The inhibitory capacity of red chilto ethanolic extracts was similar to that of extracts of orange chilto and *Geoffroea decorticans* (IC₅₀: 4.0 μg GAE/mL) (Costamagna et al., 2016; Orqueda et al., 2017). The ability to inhibit lipase exhibited by the PEE from red chilto could be related to the presence of functional compounds such as caffeoylquinic

Table 5. Pearson's correlation coefficient (r) between total phenolic concentration and functional activities of seeds, pulp, and skin extracts. p-Value less than 0.05 was considered statistically significant.

Bioactivity	Seeds		Pulp		Skin	
	Pearson's coefficient	p-value	Pearson's coefficient	p-value	Pearson's coefficient	p-value
α-amylase inhibition	0.84	0.1586	0.85	0.1470	0.89	0.1078
α-glucosidase inhibition	0.96	0.0380	1.00	0.0026	0.92	0.0784
Lipase inhibition	0.98	0.0248	0.97	0.0273	0.90	0.0994
AAPH	0.95	0.0040	0.64	0.1748	0.75	0.0842
ABTS	0.99	0.0133	0.88	0.1166	1.00	0.0015
O ₂ ⁻	0.99	0.0096	0.96	0.0363	1.00	0.0038
H ₂ O ₂	0.99	0.0095	0.91	0.0863	0.98	0.0174
HO [•]	0.97	0.0260	1.00	0.0001	0.95	0.0455
NO [•]	0.96	0.0412	0.96	0.0445	0.91	0.0896

acids or rosmarinic acid which were previously described as pancreatic lipase inhibitors (Spínola and Castilho, 2017). However, after gastroduodenal digestion, the inhibitory activity of the red chilto PEE decreases by more than 20%, Table 4. The decrease in inhibitory activity after GD digestion may be related to changes in the identity and quantity of bioavailable polyphenols during the passage through the gastrointestinal tract (Costa et al., 2014; Porfirio et al., 2010; Farah and Duarte, 2015).

3.2.3.3. Antioxidant activity of chilto PEE. Several studies indicate that certain complications associated with the metabolic syndrome such as atherosclerosis, hypertension, visceral adiposity and insulin resistance may be associated to an increase in oxidative stress (Costamagna et al., 2016). Some researchers suggest that oxidative stress could be an early event in the pathology of these chronic diseases rather than merely a consequence or an innocent bystander. Considering this, the antioxidant activity of the three PEE was assessed before and after simulated GD (Table 4). Most of the extracts exhibited very strong significant correlation between total phenolic contents and antioxidant activities ($r^2 \geq 0.95$; $p < 0.05$) (Table 5). The extracts were able to scavenge ABTS radical with IC_{50} values between 3.4–8.2 $\mu\text{g GAE/mL}$. Also, the PEE were very efficient protectors of RBC membrane (0.7–3.6 $\mu\text{g GAE/mL}$) with major activity of pulps extracts (1 and 0.7 $\mu\text{g GAE/mL}$, before and after GD digestion). The activity was much higher than extracts of other fruits known for its capacity for the prevention of oxidation and cellular damage, such as quince (*Cydonia oblonga*) or sweet cherries (*Prunus avium*) (500 $\mu\text{g GAE/mL}$ and 73 $\mu\text{g GAE/mL}$, respectively) (Magalhães et al., 2009; Gonçalves et al., 2017); and similar to orange chilto extracts (0.40–1.21 $\mu\text{g GAE/mL}$, Orqueda et al., 2017). All the extracts were good scavengers of reactive oxygen and nitrogen species (ROS/RNS), with IC_{50} values between 4.2–78.3 $\mu\text{g GAE/mL}$. The PEE of seeds showed higher activity than pulp and skin PEE for the depuration of O_2^- , H_2O_2 and NO . However, the HO^\bullet radical is considered the most harmful ROS due to its extreme reactivity and oxidative potential, even reacting with compounds such as alkanes (Cardozo et al., 2011). The highest inhibitory potency recorded against this radical was observed in skin powder and was consistent with its higher contents of flavones and flavanols (Table 4). Simulated GD did not significantly affect the protective activity of biomolecules and ROS/RNS scavenging capacity of the PEE. Again, rosmarinic acid and caffeoylquinic acids could be contributing to the functional property of the PEE. Antioxidant capacities of these phenolic compounds were repeatedly demonstrated in both *in vitro* and *in vivo* experiments (Zhang et al., 2015; Marković and Tošović, 2016; Adomako-Bonsu et al., 2017).

3.2.4. Acute toxicity and genotoxicity tests

Toxicity tests are defined as the qualitative and quantitative studies of the deleterious effects caused by chemical or physical agents on the structure and function of living systems. These assays are used for the evaluation of safety and prevention of damage to man. In this sense, the toxicity of *S. betaceum* extracts was evaluated. None of the extracts of the three parts of the fruit produced significant changes in the number of *S. Typhimurium* colonies compared to the control at the concentration of 500 $\mu\text{g GAE/plate}$ (data not shown). This result indicates that none of the extracts has genotoxic effects in the cellular systems used, suggesting the absence of compounds that can cause mutations of the frameshift type (TA98) or substitution of bases (TA100). Also, the polyphenols enriched extracts of red chilto had not toxic effect on *Artemia salina* at the concentrations tested with respect to the negative control (DMSO). With the highest concentration tested (1000 $\mu\text{g GAE/mL}$) the viability of *A. salina* was not affected. Besides, no changes in larval movements were observed after the incubation period.

4. Conclusions

The red chilto fruits are a source of appreciated bioactive compounds, such as the rosmarinic acid, caffeoylquinic acids, condensed tannins, anthocyanins, ascorbic acid and soluble dietary fiber. These valuable compounds are also present in the skin, pulp and seed powders, turning

them into an excellent source of nutraceuticals and, thus, into dietary supplements or functional foods useful for the management of metabolic syndrome and its related pathologies. Red chilto fruits can alternatively be used as flours or powders to prepare diverse foods or as the raw material for the preparation of functional phenolic extracts. The (before and after GD digestion) seed powder showed high glucose adsorption capacity. The polyphenolic extracts obtained from seed flours were the most active for most of the antioxidant tests as well as to inhibit the enzymes related to the development of the metabolic syndrome and could favor the entry of glucose into the cells, resulting in a decrease in blood glucose levels. The high content of phenolic compounds with high antioxidant activity in seed extracts make them a good functional food candidate and could serve as an ingredient for the preparation of nutraceuticals for human health improvement. Skin powder was also active as an antioxidant supplement. These results provide added value to the different fruit parts that are normally discarded during industrial processes. Pulp powder is attractive because of the presence of bioactive compounds such as phenolic compounds, carotenoids and the high amount of vitamin C, which makes it an excellent dietary supplement with nutritional and phytochemical qualities. The results of the present work showed that both red chilto and the previously studied orange variety are potent inhibitors of digestive enzymes related to metabolic syndrome. The polyphenols of the orange variety displayed higher α -amylase and α -glucosidase inhibitory capacity, while the PEE of the red fruits showed higher inhibition of pancreatic lipase. These results highlight the use of both powder and polyphenolic extracts of red chilto in metabolic syndrome. Besides, natural products often have the advantage of having moderate activity, a wide spectrum of possible multiple/synergistic effects and have fewer side effects with low toxicity, which makes them of special interest for the development of alternative “biologically active products”. However, further research including *in vivo* studies and clinical trials are necessary to confirm the potential healthy-beneficial effects of red chilto fruits and its powders.

Declarations

Author contribution statement

Maria E. Orqueda: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Sebastian Torres, Iris C. Zampini, Estela M. Valle, Guillermo Schmeda-Hirschman, Maria I. Isla: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Florencia Cattaneo: Performed the experiments; Analyzed and interpreted the data.

Agustina Fernandez Di Pardo, Felipe Jiménez-Aspee: Performed the experiments.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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