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Chemical and functional characterization of skin, pulp and seed powder from the Argentine native fruit mistol (*Ziziphus mistol*). Effects of phenolic fractions on key enzymes involved in metabolic syndrome and oxidative stress

María Eugenia Orqueda [a], Iris Catiana Zampini [a], Sebastian Torres [a,1], María Rosa Alberto [a], Liudis Leidy Pino Ramos [b], Guillermo Schmeda-Hirschmann [b,1], María Inés Isla [a,*,1]

[a] Laboratorio de Investigación de Productos Naturales (LIPRON), Instituto de Bioprospección y Fisiología Vegetal (INBIOFIV-CONICET), Facultad de Ciencias Naturales e Instituto Miguel Lillo, Universidad Nacional de Tucumán, San Miguel de Tucumán, Tucumán, Argentina
[b] Laboratorio de Química de Productos Naturales, Instituto de Química de Recursos Naturales, Universidad de Talca, Casilla 747, 3460000 Talca, Chile

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ABSTRACT

The mistol (*Ziziphus mistol*) is a native food tree occurring in dry areas of Northwestern and Central Argentina. Studies on its functional properties and bioactive compounds are scarce so far. In this work we assess the nutritional and functional components of lyophilized powder of mistol skin, pulp and seed. The powder from the different fruit parts have moderate carbohydrate content and are an important source of flavonoids, fiber, potassium, magnesium and calcium. The HPLC–ESI-MS/MS analysis of the polyphenols enriched extracts (PEE) of the samples allowed the identification of 17 compounds including 16 flavonoids and a procyanidin. The PEE showed antioxidant capacity and were able to inhibit α-glucosidase, α-amylase and pancreatic lipase, enzymes related to metabolic syndrome. The results suggest potential of the lyophilized skin, pulp and seed powder from mistol as functional food or dietary supplement in the prevention or treatment of diseases associated with metabolic syndrome.

1. Introduction

The *Ziziphus* genus (Rhamnaceae) comprises approximately 170 pantropical species, 25 of which are native to America and the Caribbean (Islam & Simmons, 2006). They are mainly trees and shrubs adapted to arid environment due to their drought stress capacity (Arndt, Clifford, & Popp, 2001). *Ziziphus* species are important fruit trees with food and medicinal value (Mizrahi, Nerd, & Sitrit, 2002).

The *Ziziphus mistol* Grisebach tree, is distributed in semiarid areas of Brazil, Paraguay and Argentina (Tortosa, 1995). It is an important genetic resource of saline environments of the Chaco forests (Ragonese, 1967). Its fruits, known as mistol, have been used as food, forage for cattle and in traditional medicine (Scarpa, 2004). At present, the mistol fruits commercialized in local markets are gathered from wild growing trees, as there are no commercial orchards of the species. Cardozo, Ordoñez, Alberto, Zampini, and Isla (2011) reported the nutritional composition and functional properties (antioxidant and anti-inflammatory activities) of aqueous and alcoholic extracts from ripe *Ziziphus mistol* fruits. The mistol seed oil contain 22% of its fatty acids as 18:3, n-3 (alpha-linolenic acid) and 12% as linoleic acid. Eynard, Muñoz, Lamarque, Silva, and Guzmán (1992) have shown that the mistol fruit oil is a good source of edible fat for growing and reproducing mice. The modulating effect of dietary *Z. mistol* seed oil on two murine mammary gland adenocarcinomas having low and high metastatic abilities was reported. From these results, it is suggested that *Z. mistol* seed oil may be of potential value in nutritional oncology (Muñoz, Piegari, Guzman, & Reynard, 1999;

Abbreviations: GE, glucose equivalent; βC E, β-carotene equivalents; C3-G E, cyanidin-3-glucoside equivalents; PB2 E, procyanidin B2 equivalents; QE, quercetin equivalents; GAE, gallic acid equivalents; PEE, phenolic enriched extract; ABTS, 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid); BHT, butylated hydroxytoluene; RBC, red blood cells; AAPH, 2,2′-azo-bis(2-amidinopropane) dihydrochloride.

* Corresponding author at: INBIOFIV-CONICET, Universidad Nacional de Tucumán, San Lorenzo 1469, 4000 San Miguel de Tucumán, Argentina.
E-mail addresses: eorqueda@yahoo.com.ar (M.E. Orqueda), zampini@csnat.unt.edu.ar (I.C. Zampini), sebatk@hotmail.com (S. Torres), mralberto@csnat.unt.edu.ar (M.R. Alberto), lipino@utalca.cl (L.L. Pino Ramos), schmeda@utalca.cl (G. Schmeda-Hirschmann), misla@tucbbs.com.ar (M.I. Isla).
[1] The three authors contributed equally to the manuscript.

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Muñoz, Silva, Lamarque, Guzman, & Eynard, 1995). The aqueous extracts of *Z. mistol* fruits have potential application in reducing the severity of the chronic evolution of hemolytic uremic syndrome produced by *Escherichia coli* (Albrecht et al., 2011). This pathology has been associated with the oxidative stress of diverse tissues, and mainly with oxidative damage of the blood components and kidney (Albrecht et al., 2011).

No information is available on the properties and composition of the powder obtained from mistol fruit seed, pulp, and skin. The aim of the present study was to assess the composition and enzyme inhibitory activity of powder obtained from different parts of ripe fruit of mistol harvested in Northern Argentina. The phytochemical composition and the activity of its polyphenolic components against enzymes relevant in hyperglycemia, dyslipidemia, and oxidative stress related with metabolic syndrome were investigated.

2. Material and methods

2.1. Standards and reagents

Ethanol (95%) was purchased from Reagents S.A. (Santa Fe, Argentina), acetone (≥99.5%) was provided by Research A.G. (Buenos Aires, Argentina) and DMSO (≥99.9%) was acquired from Biopack (Buenos Aires, Argentina). The enzymes α-amylase, α-glucosidase, lipase, pepsin, pancreatin, and other reagents such as *p*-nitrophenyl α-D-glucopyranoside, *p*-nitrophenyl palmitate,2,2 -azino-bis (3-ethylbenzo thiazoline-6-sulphonic acid) (ABTS), 2,2-azo-bis(2-amidinopropane) dihydrochloride (AAPH), 4-aminoantipyrine, horse radish peroxidase (HRP), phenazinmethosulfate (PMS), β-nicotinamide adenine dinucleotide (NADH), nitroblue tetrazolium (NBT), Griess reagent, BHT, quercetin and ascorbic acid were purchased from Sigma-Aldrich (St. Louis, MO, USA).

2.2. Plant material

Ripe fruits of *Ziziphus mistol* Griseb. were collected from different trees in February to March 2015 from wild growing populations in Fernandez, Robles Department, Santiago del Estero, Argentina (27°55′25.1″S; 63°53′36.7″W) (Fig. 1). The fruits were transported refrigerated to the laboratory, washed with tap water and the different fruit parts were separated by hand to obtain the skin (outer epidermis), pulp, and seeds. The pulp portion remaining on the seed surface was removed washing with distilled water and the washing liquid was put together with the pulp fraction. Then, each fruit part was frozen (−80 °C), lyophilized and powdered to obtain the starting material for the study.

The lyophilized powders were vacuum packed into oxygen barrier bags (Multivac, DZ-400, China) and stored at −20 °C until its analysis.

2.3. Nutritional analysis

All assays were carried out in triplicate using standard methods for plant food matrixes (AOAC, 2005).

2.3.1. Sugar, protein, and fat

The extraction and analysis of sugars and soluble proteins were carried out as previously described by Costamagna, Ordoñez, Zampini, Sayago, and Isla (2013). Total neutral and reducing sugars were measured using the phenol-sulphuric acid and Somogyi-Nelson method, respectively. The total nitrogen (N) content in each fraction was determined by the Kjeldahl method. Crude protein content was calculated by multiplying Kjeldahl N by 6.25. Total lipid content was gravimetrically measured after extraction of crude fat with petroleum ether at 40–60 °C in a Soxhlet apparatus during 4 h.

2.3.2. Moisture, ash, and minerals

The moisture content was determined by evaluating the difference in weight between the fresh sample and the sample dried at 40 °C until constant weight. The dried sample was placed in a muffle (500 °C) until the ashes were obtained. 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, according to Orqueda et al. (2017). 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 polyphenols and total flavonoids

One g of skin, pulp, or seeds powder were extracted with 5 ml of 95° ethanol under stirring (40 cycles/min) in an ultrasonic bath for 30 min at room temperature and then centrifuged at 12,000*g* during 10 min. The extraction procedure was repeated three times for each sample. The organic phase was taken to dryness under reduced pressure to obtain 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 in the PEE was determined as described by Zhishen, Mengcheng, and Jianming (1999) using $AlCl_3$ and $NaNO_2$ reagents. The results were expressed as mg quercetin equivalents (QE)/100 g powder of each fruit part.

2.4.2. Tannins

The tannins extracts were obtained according to Costamagna et al. (2013). One g of each powder sample were extracted with 12.5 mL acetone:water (70:30, v:v). The total condensed tannins content in each fruit part was quantified as described by Prior et al. (2010). Results were expressed as mg procyanidin B equivalent (PB_2E)/100 g powder of each fruit part. Hydrolysable tannins were measured by the rhodanine method (Inoue & Hagerman, 1988). Free acetone aqueous fraction was submitted to acid hydrolysis and then the gallic acid content was measured in this hydrolyzed fraction (total gallic acid) and in the non-hydrolyzed fraction (free gallic acid). 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 each fruit part.

2.4.3. Pigments and ascorbic acid

The pigments (anthocyanin and carotenoids) and ascorbic acid were extracted from each sample according to Costamagna et al. (2013). The total content of each metabolite was measured as described by Lee, Durst, and Wrolstad (2005), Rodríguez-Amaya (1999) and Barros, Heleno, Carvalho, and Ferreira (2010), respectively.

2.4.4. Crude fiber

The 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 PEE before and after simulated gastroduodenal digestion (GD). The PEE was submitted to GD according to

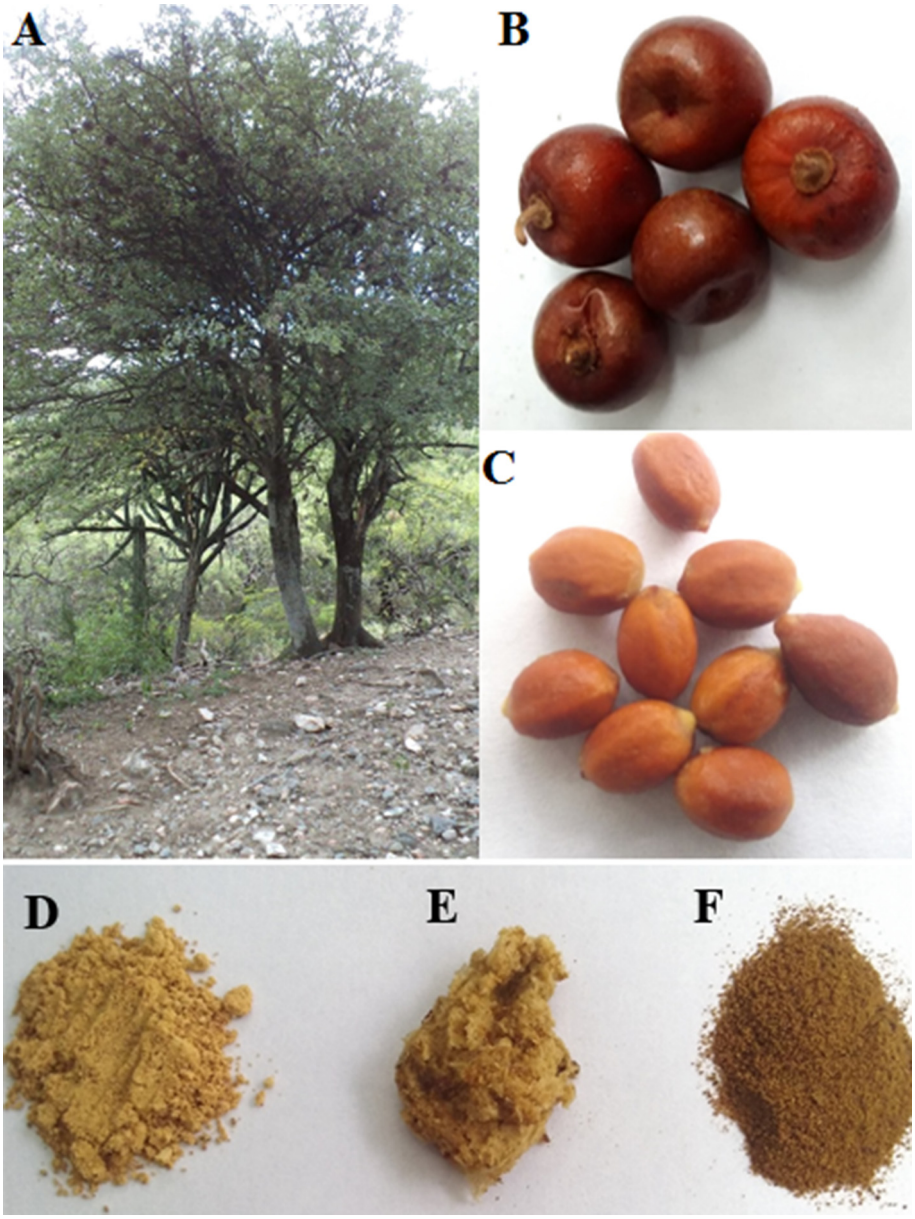

Fig. 1. *Ziziphus mistol* tree (A). Detail of ripe fruits (B) and seeds (C) of *Z. mistol*. Powders of skin (D), pulp (E), and seeds (F) of *Z. mistol* fruits.

Costamagna et al. (2016). Briefly, PEE (4 mg GAE) were mixed with 6 mL of artificial saliva (salts, urea [25.0 g/L] and α-amylase [48.3 mg/mL]; pH 6.8) during 3 min at 37 °C. Then, the pH was adjusted to 2 and pepsin (14,800 U), dissolved in HCl 0.1 M, was added and the mixture was incubated at 37 °C during 2 h (gastric digestion). For duodenal digestion, the pH was adjusted to 6.5 with $NaHCO_3$ (0.5 M) and 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 GD, phenolic compounds were extracted with ethyl acetate, and the organic phase was taken to dryness and resuspended in DMSO (1 mg GAE/mL).

2.6. Inhibitory activity of enzymes related to metabolic syndrome

2.6.1. α-Glucosidase inhibition

The α-glucosidase inhibitory activity was performed according to Costamagna et al. (2016) with some modifications. *p*-Nitrophenyl α-D-glucopyranoside was used as substrate for enzyme assay. α-Glucosidase enzyme was pre-incubated with PEE of seed, skin, and pulp powder (2.5–20 μg/mL) at 4 °C during 10 min before enzyme reaction. The IC_{50} values were calculated by interpolation of doses response curves. Acarbose (ATC code: A10BF01, Sigma-Aldrich) was used as positive control.

2.6.2. α-Amylase inhibition

The α-amylase inhibitory activity using starch as substrate was assayed using Amilokit® (Wiener Lab Group, Rosario, Argentina, Kit No. 1504163370) as reported by Costamagna et al. (2016). Enzyme was mixed with the PEE from seed, skin and pulp powder (2.5–40 μg GAE/mL) during 5 min at 4 °C before starting the enzymatic reaction. IC_{50} values were determined. IC_{50} values denote the μg GAE/mL of PEE required to inhibit the enzyme by 50%. Acarbose (ATC code: A10BF01, Sigma-Aldrich) was used as positive control.

2.6.3. 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 GAE/mL) and in absence of PEE from seed, skin, and pulp powder (before and after simulated GD). IC_{50} values were determined as μg GAE/mL of PEE required to inhibit the enzyme activity by 50%. Orlistat (tetrahydrolipstatin, ATC code: A08AB01, Elea Laboratory) was used as positive control.

2.7. Antioxidant activity

2.7.1. Total antioxidant capacity assay

The total antioxidant activity of PEE was measured by the improved ABTS radical cation ($ABTS^{\cdot+}$) method as described by Re et al. (1999). The results were expressed as the concentration of PEE necessary to scavenge 50% of ABTS (SC_{50}). BHT and quercetin were used as reference compounds.

2.7.2. Protection of oxidative hemolysis assay

The protection of oxidative hemolysis of red blood cells (RBC) by PEE (0.1–2.5 μg GAE/mL) was assayed as reported by Mendes, de Freitas, Baptista, and Carvalho (2011), using AAPH reagent. The grade of hemolysis was measured spectrophotometrically. The reaction mixture was incubated during 1 h at 37 °C and then was centrifuged, (4000*g* for 3 min). Then, the absorbance (545 nm) of the supernatant was read and the percent of hemolysis was calculated. The IC_{50} values were determined as the concentration necessary to protect the RBC from oxidative hemolysis in 50%. Quercetin was used as reference compound.

2.7.3. H_2O_2 scavenging assay

The capacity of PEE to scavenging hydrogen peroxide was measure according to Fernando and Soysa (2015) with some modifications. The mixture containing PEE (2.5–40 μg GAE/mL) and H_2O_2 was pre-incubated during 3 min at 37 °C. Then, a solution of phenol (12 mM) and 4-aminoantipyrine was added to the mixture. The amount of hydrogen peroxide was spectrophotometrically determined catalyzing its conversion by a horseradish peroxidase (HRP), in a colored quinone that was read at 504 nm. The SC_{50} values were determined as the concentration (μg GAE/mL) necessary to scavenge 50% of H_2O_2. Quercetin and ascorbic acid were used as controls.

2.7.4. Superoxide radical scavenging assay

Superoxide radical scavenging activity of PEE was measured with the phenazinmethosulfate (PMS) method of Valentão et al. (2001). The superoxide radicals were generated by oxidation of NADH (β-nicotinamide adenine dinucleotide) in a system with PMS, and manifested with the reduction of nitroblue tetrazolium (NBT). The reaction mixture consisted of 40 μL of sodium phosphate buffer 20 mM, 40 μL of PMS 60 μM, 30 μl of NBT 1 mM, 40 μL of NADH 9.8 mM and the PEE at different concentrations (10–100 μg GAE/mL). After 20 min of incubation at room temperature, the absorbance was read at 560 nm. The SC_{50} values were calculated as μg GAE/mL necessary to inhibit the 50% of superoxide radicals. Quercetin was used as reference compound.

2.7.5. Hydroxyl radical scavenging assay

The experiment was performed based on the deoxyribose degradation assay developed by Chobot (2010). The reaction mixture contained PEE (0.5–10 μg GAE/mL) in KH_2PO_4/KOH buffer (pH 7.4), 50 μL of 10.4 mM 2-deoxy-D-ribose, 50 μL of 50 μM $FeCl_3$, 50 μL of 52 μM EDTA. To start the Fenton reaction, 50 μL of 10 mM H_2O_2 and 50 μL of 1.0 mM ascorbic acid were added. The reaction mixture was incubated 1 h at 37 °C. Then, 500 μL of 2-thiobarbituric acid (1%, w/v) dissolved in trichloroacetic acid (3%, w/v) was added. After 20 min at 100 °C, the absorbance was read at 532 nm. The hydroxyl radical scavenging activity was expressed as SC_{50} values (μg GAE/mL necessary to inhibit by 50% the degradation of 2-deoxy-D-riboseby the hydroxyl radicals).

2.7.6. Nitric oxide scavenging assay

The capacity of PEE to scavenging nitric oxide was performed based on sodium nitroprusside method described by Govindarajan et al. (2003). The reaction mixture containing different concentrations of PEE (10–60 μg GAE/mL), sodium nitroprusside (100 mM) and sodium phosphate buffer (0.2 M; pH 7.4). The mixture was incubated during 60 min at 37 °C. Then, Griess reagent was added and incubated for 15 min in the dark. The antioxidant capacity was measured spectrophotometrically at 550 nm. SC_{50} was defined as the phenolic compound concentration (μg GAE/mL) necessary to scavenge 50% of nitric oxide. Ascorbic acid was used as positive control.

2.8. Identification of phenolic compounds

The PEEs of mistol pulp, skin, and seed powder 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 and drying gas (50 psi at 365 °C) at 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% to 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, 70% to 65% A from 50 to 55 min, 65% to 10% A from 55 to 75 min, and returning to 90% A from 75 to 80 min and kept to 90% for 80 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. Quantification of the main compounds was performed using a Shimadzu equipment (Shimadzu Corporation, Kyoto, Japan) with a LC-20AT pump, SPD-M20A UV diode array detector and CTO-20AC column oven, and a Labsolution software. The analyses were performed with a MultoHigh 100 RP-18 column, 5 μm, 250 × 4.6 mm, (CS-Chromatographie Service GmbH, Langerwehe, Germany) maintained at 30 °C, using a linear gradient solvent system consisting of H_2O-formic acid (99:1, v/v, solvent A) and acetonitrile (ACN) (solvent B) as follows. 90% to 82% A from 0 to 25 min, 82% to 60% A from 25 to 50 min, 60% to 55% A from 50 to 65 min, 55% to 10% A from 65 to 70 min, 10% to 90% A from 70 to 80 min, isocratic from 80 to 90 min. The compounds were monitored at 350 nm. Spectra from 200 to 600 nm were recorded for peak characterization. Calibration curve was prepared with pure standards of rutin (PhytoLab, Vestenbergsgreuth, Germany, code 89270) as analyte (0.5–80 ppm) and quercetin-3-O-glucuronide (PhytoLab 80349) as internal standard (50 ppm), r = 0.997 at 350 nm. Pulp and skin samples were injected at 19 mg/mL and seed was injected at 9.5 mg/mL. The peaks areas were determined at 350 nm.

2.9. Statistical analysis

All measurements were replicated three times. Statistical analysis was performed by one-way ANOVA followed by Tukey's multiple comparison test ($p < 0.05$).

3. Result and discussion

Traditional communities of Northwestern Argentina use *Ziziphus mistol* fruits in hot water infusion (mistol tea), as a substitute of coffee and to prepare beverages used in folk medicine (Cardozo et al., 2011). The very pleasant taste of the fruit is appreciated in a traditional food preparation known as "bolanchao", a snack prepared from a paste made from this fruit (Boelcke, 1989). In a previous work we reported the chemical composition of some of these home-made preparations using mistol fruits (Cardozo et al., 2011). The multiple health-promoting properties present in mistol, make this fruit an excellent source of natural functional food ingredients. For this reason, the powder of ripe fruit pulp, skin, and seed, obtained by lyophilization of fresh material, was chemically and functionally characterized. The yields of flour obtained expressed as g powder by 100 g fruit weight were as follows: skin 36.5%, pulp 30.5%, and seed 42.5%, respectively (Table 1).

Table 1
Macronutrient and micronutrient contents of Argentinean *Ziziphus mistol* skin, pulp, and seed powder.

Nutrients and mineral content	Skin powder	Pulp powder	Seeds powder
Total soluble sugar (g/100 g powder)	31.2 ± 1.0[b]	42.1 ± 1.2[c]	2.1 ± 0.1[a]
Glucose (g/100 g powder)	6.9 ± 0.4	9.5 ± 0.4	5.5 ± 0.2
Fructose (g/100 g powder)	18.6 ± 1.2	22.2 ± 1.0	10.5 ± 0.5
Sucrose (g/100 g powder)	4.2 ± 0.1	5.4 ± 0.2	2.5 ± 0.5
Protein (g/100 g powder)	8.1 ± 0.5[a]	15.5 ± 1.2[b]	2.5 ± 0.2[c]
Fat (g/100 g powder)	9.8 ± 1.0[a]	12.3 ± 1.2[a]	18.5 ± 1.5[b]
Fiber (g/100 g powder)	23.2 ± 2.0[c]	13.8 ± 1.6[a]	18.8 ± 1.6[b]
Yield (%, g powder/100 g skin, pulp, seed)	36.5 ± 1.0[b]	30.5 ± 1.2[a]	42.5 ± 2.1[c]
Ash	7.6 ± 0.3[c]	4.6 ± 0.5[a]	6.4 ± 0.4[b]
Moisture	13.9 ± 0.8[a]	40.3 ± 1.3[c]	18.5 ± 0.9[b]
Minerals (mg)			
Na	25.0 ± 1.6[a]	216.8 ± 7.1[b]	14.6 ± 0.1[a]
Mg	155.7 ± 5.8[b]	204.7 ± 5.8[c]	32.4 ± 0.3[a]
K	1369.2 ± 63.7[b]	1599.9 ± 32.5[c]	272.4 ± 6.8[a]
Ca	297.7 ± 16.5[a]	565.4 ± 23.7[c]	374.0 ± 3.2[b]
Fe	6.0 ± 0.1[b]	4.8 ± 0.1[a]	6.4 ± 0.2[c]
Cu	0.4 ± 0.0[b]	1.4 ± 0.04[c]	0.3 ± 0.0[a]
Zn	0.7 ± 0.0[a]	22.7 ± 0.4[b]	1.0 ± 0.1[a]

Different letters in the same line show significant differences in the nutrients and mineral content among each part of the fruit, according to Tukey's multiple comparison test ($p \leq 0.05$).

3.1. Nutritional and phytochemical composition of mistol pulp, skin, and seeds powder

3.1.1. Macronutrient composition

The total soluble sugar content in *Z. mistol* fruits powder was higher in pulp (42.1%) and skin (31.2%) than in seed (2.1%) (Table 1). The carbohydrate content in pulp and skin powder was similar to those previously reported in aqueous (47.5 g/100 g dry weight) and alcoholic (34.2 g/100 g dry weight) beverages made from pulp and skin of mistol fruits (Cardozo et al., 2011). This result is in accordance with data informed for the fruits of *Ziziphus jujuba* from Asia (between 48.3 and 73.2 g/100 g dry weight) (Guo et al., 2015). The main sugar in all mistol powders was fructose (10.5–22.2%). The crude protein values in pulp powder of mistol (15.5%) was higher than in skin and seed powder (8.1% and 2.5%, respectively) (Table 1). The protein content in mistol pulp is high compared to fleshy fruits and moderate with respect to some nuts. Protein values from mistol pulp powder were similar or higher than those observed in nuts, drupes or legumes such as chickpeas (18.5%), walnuts (15.2%), hazelnuts (14.9%), pecans (9.2%), macadamia nuts (7.9%) or jujube (4.6–6.8%) (Bullo, Juanola-Falgarona, Hernandez-Alonso, & Salas-Salvadó, 2015; Choi et al., 2012; Cosiansi, Milanesi, Da Riva, & Hayipanteli, 2002). The fat content in the powder of each fruit parts was moderate (Table 1). The fat concentration was higher in seed powder (18.5 g/100 g flour) than in pulp and skin powder. The fiber content of each fruit part powder was 23.2, 13.8 and 18.8% for skin, pulp and seed, respectively (Table 1). The high fiber values make these powders ideal supplements to improve the health of the digestive system (González-Castejón & Rodríguez-Casado, 2011).

3.1.2. Micronutrient composition

The *Z. mistol* fruit, in particular the pulp, is rich in beneficial minerals such as potassium, magnesium, calcium and zinc (Table 1). The pulp powder also provides moderates amounts of sodium (216.8 mg/100 g powder), while skin and seeds showed low sodium content. The high content of K and moderate or low content of Na may be of interest for electrolytesreplacement in oral rehydration therapies. The results suggested that due to its high protein, fiber and carbohydrate and moderate fat content, the mistol pulp powder could be considered for the formulation of therapeutic foods or food supplements for the treatment of malnutrition.

3.1.3. Phytochemical composition

3.1.3.1. Polyphenolic compounds. Mistol fruit is an excellent source of phenolics (Cardozo et al., 2011). The content of total polyphenols was higher in seeds (425.2 mg GAE/100 g) and pulp (356.0 mg GAE/100 g) than in skin powder (188.8 mg GAE/100 g) (Table 2). The levels of phenolics in *Z. mistol* fruit flour were lower than those previously described in mistol beverages (797.2–834.8 mg GAE/100 g) or in *Z. jujuba* fruits (706.9–1320.0 mg GAE/100 g) from different regions of China (Cardozo et al., 2011; Sun, Liang, Shan, Viernstein, & Unger, 2011). However, phenolics values in seed and pulp powder were similar or higher than those from Iranian *Z. jujuba* (149 mg GAE/100 g) or Indian *Ziziphus mauritiana* (145–216 mg GAE/100 g), or even than those reported for other fruits known for their high phenolics content or antioxidant properties, such as grapes (425 mg GAE/100 g), figs (195 mg GAE/100 g) or plums (565 mg GAE/100 g) (Miletic, Popovic, Mitrovic, Kandic, & Leposavic, 2014; Najafabadi, Sahari, Barzegar, & Esfahani, 2017; Sun et al., 2011). The main phenolic compounds in mistol powder were flavonoids. Hydrolyzed tannins were not detected in the pulp, skin and seed powder, which is consistent with the previous reported for *Z. mistol* fruits (Cardozo et al., 2011). Condensed tannins were only present in seed powder (153.4 mg procyanidin-B2/100 g powder). Anthocyanins were only detected in mistol skin powder (63.5 mg C-3GE/100 g), in values similar to those reported for Chinese and Iranian *Z. jujuba* fruits (31–79 mg C-3GE/100 g) (Najafabadi et al., 2017; Sun et al., 2011). The flavonoid content of mistol seed powder was higher (444.0 mg QE/100 g powder) than pulp (130.0 mg QE/100 g powder) and seed (144.0 mg QE/100 g powder), respectively. It is noteworthy that between 60 and 90 g of mistol seed powder are required to reach the recommended daily allowance (RDA) of total flavonoids (250–400 mg per day) (Peluso & Palmery, 2015).

3.1.3.2. Ascorbic acid and carotenoids. In concordance with *Z. mistol* fruits from other region of Argentina (Cardozo et al., 2011), carotenoids were not detected in the mistol powders (Table 2). The ascor-

Table 2
Functional compounds content of Argentinean *Ziziphus mistol* skin, pulp, and seed powder (mg per 100 g of each powder).

Phytochemical content	Skin powder	Pulp powder	Seeds powder
Total phenolic (mg GAE)	188.8 ± 4.5[a]	356.0 ± 5.1[b]	425.2 ± 6.0[c]
Flavone and flavonols (mg QE)	144.0 ± 3.0[b]	130.0 ± 3.3[a]	444.0 ± 4.2[c]
Condensed tannins (mg procyanidin-B2)	nd	nd	153.4 ± 6.5
Anthocyanins (mg C-3GE)	63.5 ± 2.1	nd	nd
Ascorbic acid (mg AA)	42.5 ± 2.1[c]	30.5 ± 1.2[a]	36.5 ± 1.0[b]

Different letters in the same line show significant differences in the functional compounds content among each part of the fruit, according to Tukey's multiple comparison test ($p \leq 0.05$).
nd: not detected (below detection limit).

bic acid content recorded in skin, pulp and seed powder was 42.5, 30.5 and 36.5 mg AA/100 g powder, respectively (Table 2). The ascorbic acid content in mistol is lower than in other *Ziziphus* fruits, such as jujube (110–757 mg AA/100 g) (Gao, Wu, & Wang, 2013; Najafabadi et al., 2017; Sun et al., 2011). However, 100 g of powder are sufficient to cover about 50% of the recommended daily allowance of 60–90 mg of ascorbic acid per person (Levine, Wang, Padayatty, & Morrow, 2001).

3.2. Functional properties

3.2.1. Effect on oxidative stress

During aerobic metabolism, reactive oxygen species (ROS), such as hydroxyl radicals ($HO^{\cdot}$), superoxide radical ($O_2^{\cdot-}$) and hydrogen peroxide (H_2O_2) are produced as by-products. Also, during certain pathology conditions, an overproduction of cell signaling molecule $NO^{\cdot}$ can occur. Simultaneous production of $O_2^{\cdot-}$ and $NO^{\cdot}$ leads to the formation of reactive nitrogen species (RNS). Both, ROS and RNS are involved in the development of several ageing-related or chronic degenerative disorders, such as diabetes, metabolic syndrome, cardiovascular diseases, chronic inflammatory diseases or cancer (Pacher, Beckman, & Liaudet, 2007). It is well known that the antioxidant properties of fruits are correlated to the presence of efficient ROS/RNS scavengers, active natural antioxidants such as flavonoids, polyphenols, anthocyanins or vitamin C. The regular intake of these natural antioxidants can decrease the risk of ageing-related diseases by reducing oxidative stress (Sun et al., 2011). In order to evaluate the potential of mistol powder as therapeutic alternative to prevent the initiation or progression of these diseases, the antioxidant activity of the skin, pulp, and seed powder, was analyzed before and after simulated GD. All mistol powder exhibited $ABTS^{\cdot+}$ scavenging activities with SC_{50} values between 0.50 and 1.10 μg GAE/mL before simulated GD (Table 3). In concordance with the content of total phenolics, the $ABTS^{\cdot+}$ reducing capacity was higher in seed and pulp powder (0.5 μg GAE/mL) than in skin powder. Powder digestion did not considerably affect antioxidant activities, with a decrease between 1.1 and 1.6 times after GD,in pulp and seeds powder, respectively. The potency of seed and pulp powder was higher than that of cotyledon flour from *Prosopis alba* (7.1 μg GAE/mL), and similar to those of pulp and skin flour from chilto (*Solanum betaceum*) (0.8–1.1 μg GAE/mL), two native food plants that grows in Northern Argentina (Cattaneo et al., 2016; Orqueda et al., 2017). Also, the ABTS cation scavenging activities of PEE from mistol seed and pulp powder were 2.8 times higher than the natural antioxidant quercetin used in food industry. All powders displayed protection capacity against red blood cells oxidative hemolysis (IC_{50} values between 0.18 and 0.36 μg GAE/mL), higher than that recorded for chilto powder (0.40 and 0.91 μg GAE/mL) (Orqueda et al., 2017). Simulated GD of PEE from mistol powders decreased protection capacity of red blood cells oxidative hemolysis only between 1.3 and 1.5 times.

The PEE from mistol powders exhibited effect as $HO^{\cdot}$, $O_2^{\cdot-}$, H_2O_2, and $NO^{\cdot}$ scavenger (Table 3), with higher antioxidant potency as $HO^{\cdot}$ scavenger, the most extreme reactive and oxidative molecule. The best performance as ROS/RNS scavenger was displayed by skin powder, showing higher antioxidant capacity than quercetin and ascorbic acid. Overall, there was no considerably decrease in ROS/RNS scavenger activity after simulated GD. According to our results, the powders have higher antioxidant activity in one assay system than in the others, pointing out that there is no universal method to measure the antioxidant capacity of all samples consistently and with precision.

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

The metabolic syndrome is a pathology associated with hyperglycemia, glucose intolerance, hypertension, dyslipidemia and/or abdominal obesity. This condition is an early sign of eventual development of more serious diseases, such as type II diabetes or cardiovascular disease. The effect of PEE from mistol pulp, skin, and seed powder (before and after simulated GD) was assessed towards α-amylase, α-glucosidase and pancreatic lipase, enzymes closely related with metabolic syndrome.

3.2.2.1. α-Glucosidase and α-amylase inhibition. If the release of glucose from food is blocked, the glucose uptake in gastrointestinal tract decreases, contributing to a decrease in blood post-prandial glucose. This can be achieved through the inhibition of the enzymes α-glucosidase and α-amylase, in charge of the digestion of dietary carbohydrates. Currently, glucose uptake is restricted through the use of commercial drugs (acarbose, voglibose or miglitol) that target these enzymes, but with the cost of causing several adverse gastrointestinal effects (Orqueda et al., 2017). The PEE from mistol skin, pulp, and seed powder were able to inhibit both α-amylase and α-glucosidase (Table 3). The inhibitory effect was higher towards α-glucosidase, with IC_{50} values between 3.5 and 5.0 μg GAE/mL, stronger than that observed for the reference compound acarbose (25.0 μg GAE/mL). In comparison to other fruit flours, the inhibition of α-glucosidase by mistol was similar to that of chilto powder (5.1–11.0 μg GAE/mL), but lower than that of chañar flour (0.68 μg GAE/mL) (Costamagna et al., 2016; Orqueda et al., 2017). Regarding α-amylase, the IC_{50} values of PEE of mistol powder (5.9–23.5 μg GAE/mL) were lower than that of reference compound acarbose (1.25 μg GAE/mL). A higher inhibitory effect on one of this enzyme is the preferred effect. In that way, the digestion of carbohydrates is not totally inhibited, preventing fermentation of high amounts of sugars in the colon, responsible for gastrointestinal discomforts (Costamagna et al., 2016). In this sense, mistol pulp or seed powder (strong inhibitory effect on α-glucosidase with moderate effect on α-amylase) could represent the better dietary complements to control glucose uptake.

3.2.2.2. Lipase inhibition. Orlistat is one of the most prescript commercial drugs for obesity and weight management treatment (Mohamed, Ibrahim, Elkhayat, & El Dine, 2014). This drug is a very strong inhibitor of pancreatic lipase that practically suppresses lipids hydrolysis and therefore acylglycerol and fatty acids absorption. It results, in addition to its pharmacological effect, in several secondary effects (Mohamed et al., 2014). Inhibitors from natural sources able to moderately inhibit pancreatic lipase could result in better choices to metabolic syndrome prevention or treatment. Indeed, the PEE of mistol skin, pulp and seeds powder were able to moderately inhibit pancreatic lipase (IC_{50}: 5.9–9.0 μg GAE/mL) in comparison to Orlistat (IC_{50}: 0.08 μg GAE/mL) (Table 3). Similar inhibitory activity towards pancreatic lipase was previously

Table 3
Effect of polyphenols enriched extract of skin, pulp and seed powder from Argentinean *Ziziphus mistol* before and after simulated gastroduodenal digestion (GD) and reference compounds on enzymes related to carbohydrate metabolism, fat metabolism and oxidative processes. Results are presented as SC_{50} or IC_{50} in μg GAE/mL.

Z. mistol powder	Enzyme inhibition (IC_{50} values in μg GAE/mL)			SC_{50} (μg GAE/mL)	IC_{50} (μg GAE/mL)	Antioxidant/free radical scavenging assays SC_{50} (μg GAE/mL)			
	α-Amylase	α-Glucosidase	Lipase	ABTS	AAPH	O_2.−	HO.	H_2O_2	NO.
Seeds									
Before GD	23.5 ± 2.1^{d}	5.0 ± 0.5^{ab}	9.0 ± 1.0^{b}	0.50 ± 0.05^{a}	0.20 ± 0.01^{ab}	27.20 ± 2.10^{ab}	4.20 ± 0.08^{b}	23.30 ± 0.01^{c}	29.00 ± 3.00^{a}
After GD	26.8 ± 3.2^{d}	6.2 ± 0.9^{b}	15.3 ± 1.5^{c}	0.81 ± 0.02^{b}	0.31 ± 0.02 c^{d}	25.30 ± 1.00^{a}	4.00 ± 0.02^{b}	25.23 ± 0.23^{d}	35.23 ± 1.25^{a}
Pulp									
Before GD	16.0 ± 1.1^{c}	4.8 ± 0.2^{ab}	8.0 ± 0.9^{ab}	0.51 ± 0.04^{a}	0.18 ± 0.01^{a}	75.01 ± 3.00^{c}	8.70 ± 0.60^{c}	28.10 ± 0.08^{e}	55.00 ± 6.10^{b}
After GD	10.5 ± 2.1b^{c}	5.0 ± 0.6^{ab}	10.5 ± 1.0^{b}	0.56 ± 0.05^{a}	0.26 ± 0.02^{bc}	80.00 ± 1.52^{d}	1.30 ± 0.85^{a}	31.00 ± 0.52^{f}	56.42 ± 7.5^{b}
Skin									
Before GD	5.9 ± 0.7^{ab}	3.5 ± 0.6^{a}	5.9 ± 0.5^{a}	1.10 ± 0.09^{c}	0.28 ± 0.02^{c}	25.01 ± 0.90^{a}	2.40 ± 0.60^{a}	10.00 ± 0.01^{a}	24.00 ± 1.00^{a}
After GD	4.3 ± 1.1^{a}	5.6 ± 0.5^{b}	8.2 ± 0.6^{ab}	2.30 ± 0.02^{d}	0.36 ± 0.06^{d}	30.01 ± 0.23^{b}	4.12 ± 0.20^{b}	11.23 ± 0.50^{b}	28.23 ± 0.96^{a}
Reference	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

Different letters in the same column show significant differences in the functional activities among each part of the fruit, according to Tukey's multiple comparison test ($p \leq 0.05$).

recorded for chilto (IC_{50}: 5.3–14 μg GAE/mL) (Orqueda et al., 2017) and chañar flour (IC_{50}: 4 μg GAE/mL) (Costamagna et al., 2016). According to these results, we suggest that mistol powder could be useful as an alternative for treatment or prevention of metabolic syndrome. However, further evaluation of the *in vivo* hypoglycemic and lipid lowering activities is necessary to verify these potential beneficial effects.

3.3. Identification of phenolic compounds in Z. mistol fruit powders by HPLC-ESI-MS/MS

The HPLC-ESI-MS/MS analysis of the samples (Fig. 2, Table 4) allowed the tentative identification of 17 compounds including 16 flavonoids and a procyanidin. Selected ion chromatograms were used to identify the main constituents and related compounds in the extracts. The ions at *m/z* 285, 301 and 315 amu were used to detect kaempferol, quercetin and rhamnetin/isorhamnetin derivatives, respectively. The ion at *m/z* 289 allowed to detect the possible occurrence of catechin and procyanidin derivatives. Compound **1** presented a $[M-H]^-$ ion at *m/z* 577 amu and the neutral loss of 126, 152 and 288 amu, in agreement with a fragmentation mechanism involving heterocyclic ring fission (HRF), retro Diels-Alder (RDA) and methidequinone (QM), respectively. The compound was assigned as (epi) catechin-(epi) catechin dimer (Procyanidin B2), in agreement with literature (Li & Deinzer, 2007). Procyanidin B2 was previously isolated from the Rhamnaceae *Z. jujuba* (Choi, Ahn, Kozukue, Levin, & Friedman, 2011).

Compounds **2**, **3** and **6** with *m/z* of 595, 593 and 597 amu, respectively, show neutral losses of 90 and 120 amu from the pseudomolecular ion and from the base peak, typical for flavone di-C-

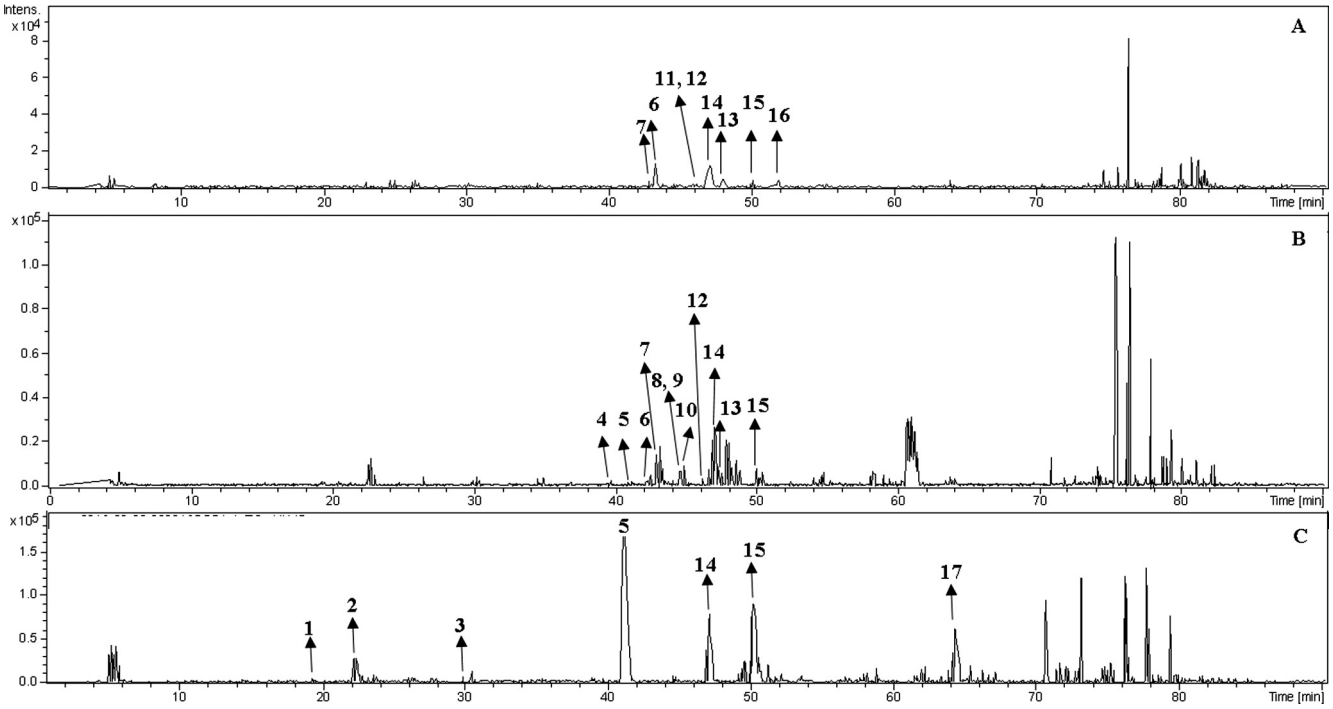

Fig. 2. HPLC chromatogram of polyphenols from *Ziziphus mistol* fruit pulp (A), skin (B), and seeds (C). Detection: TIC-all MS^n. Compounds: (1) Procyanidin B2; (2) Chalcone naringenin-di-C-hexoside; (3) Apigenin di-C-hexoside; (4) Kaempferolhexoside glucuronide; (5) Spinosin (Vitexin/isovitexin hexoside methyl ether); (6) di-C-hexosylphloretin; (7) Quercetin-3-O-rutinoside; (8) Quercetin-dihydrocaffeoyl; (9) Quercetin hexoside; (10) Rh/IRh dihexoside rhamnoside; (11) Kaempferol glucuronide; (12) Kaempferol rutinoside; (13) Rh/IRutinoside; (14) Quercetin rhamnoside hexoside; (15) Quercitrin; (16) Kaempferol hexoside rhamnoside; (17) Quercetin.

Table 4
Tentative identification of phenolics in Argentinean *Ziziphus mistol* fruit skin, pulp, and seed powder extracts.

Peak	Rt (min)	$[M-H]^-$	MS/MS	Tentative identification
1	19.4	577	559 (46), 451 (76), 425 (100), 407 (52), 289 (52)	Procyanidin B2
2	22.3	595	577 (43), 505 (39), 475 (100), 415 (67), 385 (18), 355 (47)	Chalconaringenin-di-C-hexoside
3	30.0	593	503 (29), 473 (100), 353 (47), 289 (13)	Apigenin di-C-hexoside
4	39.8	623	605 (70), 385 (66), 285 (100)	Kaempferol hexoside glucuronide
5	41.0–41.6	607	487 (11), 427 (100)	Spinosin (Vitexin/isovitexin hexoside methyl ether)
6	42.1–43.3	597	505 (15), 477 (100), 387 (20), 357 (51)	di-C-hexosylphloretin
7	42.7–42.9	609	301 (100)	Quercetin-3-O-rutinoside
8	44.6	465	445 (8), 301 (100)	Quercetin-dihydrocaffeoyl
9	44.9	463	301 (100)	Quercetin hexoside
10	44.8–45.0	785	623 (9), 315 (100)	Rh/IRh dihexoside rhamnoside
11	45.9	463	285 (100)	Kaempferol glucuronide
12	46.0	593	285 (62)	Kaempferol lrutinoside
13	46.8–47.9	623	315 (100)	Rh/IRh rutinoside
14	47.1–47.4	609	447 (27), 429 (33), 301 (100)	Quercetin rhamnoside hexoside
15	50.2–50.5	447	301 (100)	Quercitrin
16	52	593	431 (33), 413 (60), 285 (100)	Kaempferol hexoside rhamnoside
17	64.4	301	179 (100), 151 (35), 257 (13)	Quercetin

glycosides. The aglycones of compounds **2**, **3** and **6** were assigned as chalconaringenin, apigenin and phloretin, respectively. Apigenin glycosides have been previously isolated from *Ziziphus* species (Asgarpanah & Haghighat, 2012; Cheng et al., 2000; San & Yildirim, 2010). The mass spectrum of compound **6** was consistent with 3′,5′-di-C-β-D-glucosylphloretin, previously isolated from *Ziziphus spina-christi* (Pawlowska, Camangi, Bader, & Braca, 2009) and also detected in *Z. jujuba* (Zhang et al., 2014). However, the identity of the sugar and the exact placement cannot be determined. Compound **2**, with a pseudomolecular ion differing in two atomic mass units from compound **6** and showing a similar fragmentation pattern, was tentatively assigned as the chalcone formed by the deprotonation of carbons C-2 and C-3 of phloretin leading to chalconaringenin. The mass spectrum of compound **5** showed the neutral loss of 162 and 120 amu, suggesting the presence of C- and O-glycosides in the molecule. By comparison with literature, the spectrometric data of **5** was consistent with spinosin, previously isolated from *Z. jujuba* Mill var. spinosa (Cheng et al., 2000).

Four kaempferol (K) derivatives were tentatively identified in the samples (compounds **4**, **11**, **12** and **16**). The compounds differ in the identified and number of the sugar units and present neutral loss of 176 and 162 amu (glucuronic acid and hexose, compound **4**), 176 (glucuronic acid, compound **11**), 308 (rutinose, compound **12**), 162 and 146 (hexose and rhamnose, compound **16**). The glycosides were assigned as K-hexoside glucuronide (**4**), K-glucuronide (**11**), K-rutinoside (**12**) and K-hexoside-rhamnoside (**17**), respectively. Kaempferol rutinoside has been identified in *Z. jujube* and *Z. spina-christi* (Pawlowska et al., 2009).

The mass spectra of the compounds **7**, **8**, **9**, **14** and **15** shows neutral loss of 308 (rutinose), 164 (dihydrocaffeic acid), 162 (hexose), 162 and 146 (hexose and rhamnose) and 146 (rhamnose) atomic mass units, respectively, leading to a base peak at *m*/*z* 301, in agreement with quercetin (Q). The compounds were assigned as Q rutinoside (**7**), Q dihydrocaffeoyl (**8**), Q hexoside (**9**), Q rhamnosidehexoside (**14**) and Q rhamnoside (**15**), respectively. Peak **17** was identified as the aglycone quercetin based on comparison with a standard sample as well as by the fragmentation pattern, in agreement with Fabre, Rustan, de Hoffmann, and Quetin-Leclercq (2001). Quercetin rutinoside (rutin) is widespread in plants and has been described as a constituent of *Z. jujuba*, *Z. jujuba* var. spinosa and *Z. mauritiana* (Choi et al., 2011; Guo et al., 2011; Memon, Memon, Luthria, Pitafi, & Bhanger, 2012). The compound **14** is related to Q-3-O-rhamnoside-galactoside reported from *Z. mistol* (Pelotto & del Pero Martínez, 1993). Quercitrin**15** was isolated from *Z. spina-christi* (Nawwar, Ishak, Michael, & Buddrus, 1984). Quercetin 3-O-(2,6-di-O-rhamnosyl-glucoside) 7-O-glucuronide has been reported in *Z. spina-christi* (Karar et al., 2016). The mass spectra of compounds **10** and **13** shows a base peak at *m*/*z* 315 and neutral losses of 162 and/or 308 amu, suggesting the occurrence of rhamnetin/isorhamnetin (Rh/Irh) glycosides. Compound **10** first loss 162 amu (hexose) and then a 308 amu fragment and was tentatively identified as Rh/Irh-dihexoside-rhamnoside. Compound **13** loss 308 amu and was assigned to Rh/Irhrutinoside.

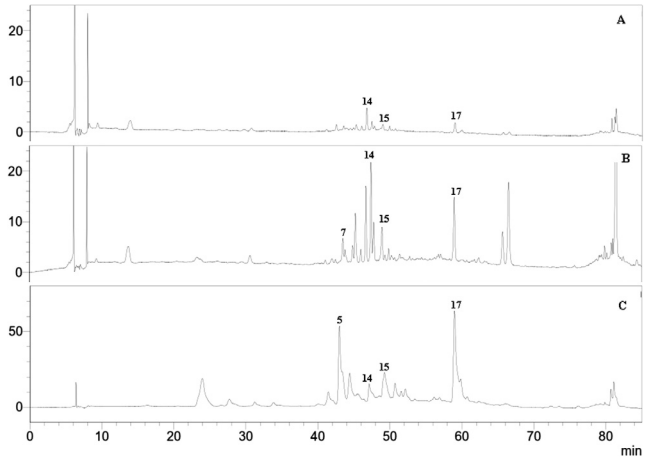

Fig. 3. HPLC chromatogram of main polyphenols from *Ziziphus mistol* fruit pulp (A), skin (B), and seeds (C). Detection: UV, 350 nm. Compounds: (5) Spinosin; (7) Quercetin-3-O-rutinoside; (14) Quercetin rhamnoside hexoside; (15) Quercitrin; (17) Quercetin.

Quantification of the main identified compounds occurring in mistol pulp, seed and skin extract was carried out by HPLC-DAD using the internal standard method. The chromatograms are shown in Fig. 3. Results are expressed as μg equivalents of rutin per gram of extract. The compound **5** content, occurring only in the seeds was 1.27 ± 0.02 μg equivalents of rutin per gram of extract while **7**, identified in the skin accounted for 0.0176 ± 0.0003 μg equivalents of rutin per gram of extract. The flavonoid **14** was found in skin (0.0800 ± 0.0001 μg equivalents of rutin per gram of extract), pulp (0.0242 ± 0.0004) and seeds (0.967 ± 0.007). Compound **15** could be quantified in pulp (0.032 ± 0.001 μg equivalents of rutin per gram of extract), seed (0.97 ± 0.02) and skin (0.041 ± 0.001). The quercetin (compound **17**) content of skin, pulp and seeds was 0.0892 ± 0.0001,

0.034 ± 0.001 and 2.06 ± 0.04 μg equivalents of rutin per gram of extract, respectively.

4. Conclusions

Until the first half of the twentieth century, consumption of mistol fruit as fresh fruit, infusions and "bolanchao" by the inhabitants of Northwestern and Central Argentina was frequent. Changes in food habits led to less consumption of the native foods and increased intake of refined carbohydrate-rich diet. The multiple and beneficial functional properties of mistol fruit can boost its consumption as a non-conventional food or dietary supplement for the treatment or prevention of metabolic syndrome and related pathologies. The powder of mistol fruit can be considered a potential source of dietary fiber and biologically active compounds, such as flavonoids and its derivatives as well as procyanidin derivatives. Further studies including *in vivo* studies and clinical trials are necessary to confirm the potential medicinal and dietary values of mistol fruit and its flour for humans.

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References

Albrecht, C., Pellarin, M., Baronetti, J., Rojas, M., Albesa, I., & Eraso, A. (2011). Chemiluminescence determination of antioxidant property of Zizyphus mistol and Prosopis alba during oxidative stress generated in blood by hemolytic uremic syndrome-producing *Escherichia coli*. *Luminescence, 26*, 424–428.

AOAC (2005). *Official methods of analysis* (18th ed.). Arlington, VA, USA: Association of Official Analytical Chemists.

Arndt, S. K., Clifford, S. C., & Popp, M. (2001). Ziziphus – a multipurpose fruit tree for arid regions. In S. W. Breckle, M. Veste, & W. Wucherer (Eds.), *Sustainable land use in desert* (pp. 388–399). Berlin, Heidelberg: Springer-Verlag.

Asgarpanah, J., & Haghighat, E. (2012). Phytochemistry and pharmacologic properties of Ziziphus spina-christi (L.) Willd. *African Journal of Pharmacy and Pharmacology, 6*(31), 2332–2339.

Barros, L., Heleno, S., Carvalho, A., & Ferreira, I. (2010). Lamiaceae often used in Portuguese folk medicine as a source of powerful antioxidants: Vitamins and phenolics. *Food Science and Technology, 43*, 544–550.

Boelcke, O. (1989). *Plantas vasculares de la Argentina* (second ed.). Buenos Aires: Editorial Hemisferio Sur.

Bullo, M., Juanola-Falgarona, M., Hernandez-Alonso, P., & Salas-Salvadó, J. (2015). Nutrition attributes and health effects of pistachio nuts. *British Journal of Nutrition, 113*, 79–93.

Cardozo, M. L., Ordoñez, R. M., Alberto, M. R., Zampini, I. C., & Isla, M. I. (2011). Antioxidant and anti-inflammatory activity characterization and genotoxicity evaluation of *Ziziphus mistol* ripe berries, exotic Argentinean fruit. *Food Research International, 44*, 2063–2071.

Cattaneo, F., Costamagna, M., Zampini, I. C., Sayago, J., Alberto, M., Chamorro, V., ... Isla, M. I. (2016). Flour from Prosopis alba cotyledons: A natural source of nutrient and bioactive phytochemicals. *Food Chemistry, 208*, 89–96.

Cheng, G., Bai, Y., Zhao, Y., Liao, N., Tao, J., Liu, Y., ... Xu, X. (2000). Flavonoids from *Ziziphus jujuba* Mill var. spinosa. *Tetrahedron, 56*, 8915–8920.

Chobot, V. (2010). Simultaneous detection of pro- and antioxidative effects in the variants of the deoxyribose degradation assay. *Journal of Agricultural and Food Chemistry, 58*, 2088–2094.

Choi, S. H., Ahn, J. B., Kim, H. J., Im, N. K., Kozukue, N., Levin, C. E., & Friedman, M. (2012). Changes in free amino acid, protein, and flavonoid content in Jujube (*Ziziphus jujube*) fruit during eight stages of growth and antioxidative and cancer cell inhibitory effects by extracts. *Journal of Agricultural and Food Chemistry, 60*, 10245–10255.

Choi, S. H., Ahn, J. B., Kozukue, N., Levin, C. E., & Friedman, M. (2011). Distribution of free amino acids, flavonoids, total phenolics, and antioxidative activities of jujube (*Ziziphus jujuba*) fruits and seeds harvested from plants grown in Korea. *Journal of Agricultural and Food Chemistry, 59*(12), 6594–6604.

Cosiansi, J. F., Milanesi, E., Da Riva, D., & Hayipanteli, S. (2002). La flexión en el proceso de extracción de semillas de *Prosopis flexuosa* en relación a las características anatómicas delfruto. *Agriscientia, 19*, 55–62.

Costamagna, M. S., Ordoñez, R. M., Zampini, I. C., Sayago, J. E., & Isla, M. I. (2013). Nutritional and antioxidant properties and toxicity of *Geoffroea decorticans*, an Argentinean fruit and products derived from them (flour, arrope, decoction and hydroalcoholic beverage). *Food Research International, 54*, 160–168.

Costamagna, M. S., Zampini, I. C., Alberto, M. R., Cuello, A. S., Torres, S., Pérez, J., ... Isla, M. I. (2016). Polyphenols rich fraction from *Geoffroea decorticans* fruits flour affects key enzymes involved in metabolic syndrome, oxidative stress and inflammatory process. *Food Chemistry, 190*, 392–402.

Eynard, A. R., Muñoz, S., Lamarque, A., Silva, R., & Guzmán, C. A. (1992). Lipidic composition and nutritional evaluation of the (Rhamnacea) *Zizyphus mistol* oil seed as the sole source of fat for mice. *Communicative and Integrative Biology, 10*, 213–224.

Fabre, N., Rustan, I., de Hoffmann, E., & Quetin-Leclercq, J. (2001). Determination of flavone, flavonol, and flavanone aglycones by negative ion liquid chromatography electrospray ion trap mass spectrometry. *Journal of the American Society for Mass Spectrometry, 12*(6), 707–715.

Fernando, C. D., & Soysa, P. (2015). Optimized enzymatic colorimetric assay for determination of hydrogen peroxide (H_2O_2) scavenging activity of plant extracts. *Methods X, 2*, 283–291.

Gao, Q., Wu, S., & Wang, M. (2013). The Jujube (Ziziphus jujuba Mill.) fruit: A review of current knowledge of fruit composition and health benefits. *Journal of Agricultural and Food Chemistry, 61*, 3351–3363.

González-Castejón, M., & Rodríguez-Casado, A. (2011). Dietary phytochemicals and their potential effects on obesity: A review. *Pharmacological Research, 64*, 438–455.

Govindarajan, R., Rastogi, S., Vuayakumae, M., Shirwalkar, A., Kumar, S., Rawat, A., ... Pushpangadan, P. (2003). Studies on the antioxidant activities of *Desmodium gangeticum*. *Biological and Pharmaceutical Bulletin, 26*, 1424–1427.

Guo, S., Duan, J. A., Tang, Y., Qian, Y., Zhao, J., Qian, D., & Shang, E. (2011). Simultaneous qualitative and quantitative analysis of triterpenic acids, saponins and flavonoids in the leaves of two *Ziziphus* species by HPLC–PDA–MS/ELSD. *Journal of Pharmaceutical and Biomedical Analysis, 56*(2), 264–270.

Inoue, K. H., & Hagerman, A. E. (1988). Determination of gallotannins with rhodanine. *Analytical Biochemistry, 169*, 363–369.

Islam, M. B., & Simmons, M. P. (2006). A thorny dilemma: Testing alternative intrageneric classifications within Ziziphus (Rhamnaceae). *Systematic Botany, 31*, 826–842.

Karar, M. G. E., Quiet, L., Rezk, A., Jaiswal, R., Rehders, M., Ullrich, M. S., & Kuhnert, N. (2016). Phenolic profile and in vitro assessment of cytotoxicity and antibacterial activity of *Ziziphus spina-christi* leaf extracts. *Medicinal Chemistry, 6*(3), 143–156.

Lee, J., Durst, R. W., & Wrolstad, R. E. (2005). Determination of total monomeric anthocyanin pigment content of fruits juices, beverages, natural colorants, and wines by the pH differential method: Collaborative study. *Journal of AOAC International, 88*, 1269–1278.

Levine, M., Wang, Y., Padayatty, S., & Morrow, J. (2001). A new recommended dietary allowance of vitamin C for healthy young women. *Proceeding of the National Academy of Sciences of the United States of America, 98*(17), 9842–9846.

Li, H. J., & Deinzer, M. L. (2007). Tandem mass spectrometry for sequencing proanthocyanidins. *Analytical Chemistry, 79*(4), 1739–1748.

Memon, A., Memon, N., Luthria, D., Pitafi, A., & Bhanger, M. (2012). Phenolic compounds and seed oil composition of *Ziziphus mauritiana* L. fruit. *Polish Journal of Food and Nutrition Sciences, 62*(1), 15–21.

Mendes, L., de Freitas, V., Baptista, P., & Carvalho, M. (2011). Comparative antihemolytic and radical scavenging activities of strawberry tree (*Arbutus unedo* L.) leaf and fruit. *Food and Chemical Toxicology, 49*, 2285–2291.

Miletić, N., Popović, B., Mitrović, O., Kandić, M., & Leposavić, A. (2014). Phenolic compounds and antioxidant capacity of dried and candied fruits commonly consumed in Serbia. *Czech Journal of Food Science, 32*(4), 360–368.

Mizrahi, Y., Nerd, A., & Sitrit, Y. (2002). New fruits for arid climates. In J. Janick & A. Whipkey (Eds.), *Trends in new crops and new uses* (pp. 378–384). Alexandria: ASHS Press.

Mohamed, G. A., Ibrahim, S. R. M., Elkhayat, E. S., & El Dine, R. S. (2014). Natural antiobesity agents. *Bulletin of Faculty of Pharmacy, Cairo University, 52*, 269–284.

Muñoz, S., Piegari, M., Guzman, C., & Reynard, A. (1999). Differential effects of dietary Oenothera, *Zizyphus mistol*, and corn oils, and essential fatty acid deficiency on the progression of a murine mammary gland adenocarcinoma. *Nutrition, 15*, 208–212.

Muñoz, S. E., Silva, R. A., Lamarque, A., Guzman, C. A., & Eynard, A. R. (1995). Protective capability of dietary *Zizyphus mistol* seed oil, rich in 18:3, n-3, on the development of two murine mammary gland adenocarcinomas with high or low metastatic potential. *Prostaglandins, Leukotrienes and Essential Fatty Acids, 53*, 135–138.

Najafabadi, N., Sahari, M., Barzegar, M., & Esfahani, Z. (2017). Effect of gamma irradiation on some physicochemical properties and bioactive compounds of jujube (*Ziziphus jujube* var vulgaris) fruit. *Radiation Physics and Chemistry, 130*, 62–68.

Nawwar, M. A. M., Ishak, M. S., Michael, H. N., & Buddrus, J. (1984). Leaf flavonoids of *Ziziphus spina-christi*. *Phytochemistry, 23*(9), 2110–2111.

Orqueda, M. E., Rivas, M., Zampini, I. C., Alberto, M. R., Torres, S., Cuello, S., ... Isla, M. I. (2017). 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. *Food Chemistry, 216*, 70–79.

Pacher, P., Beckman, J., & Liaudet, L. (2007). Nitric oxide and peroxynitrite in health and disease. *Physiology Reviews, 87*(1), 315–424.

Pawlowska, A. M., Camangi, F., Bader, A., & Braca, A. (2009). Flavonoids of Zizyphus jujuba L. and *Zizyphus spina-christi* (L.) Willd (Rhamnaceae) fruits. *Food Chemistry, 112*, 858–862.

Pelotto, J. P., & del Pero Martínez, M. A. (1993). Flavonoid variation with the plant age in *Zizyphus mistol* leaves. *Biochemical Systematics and Ecology, 21*, 645–646.

Peluso, I., & Palmery, M. (2015). Flavonoids at the pharma-nutritioninterface: Is a therapeutic index in demand? *Biomedicine & Pharmacotherapy, 71*, 102–107.

Prior, R. L., Fan, E., Ji, H., Howell, A., Nico, C., Payne, M. J., & Reed, J. (2010). Multilaboratory validation of a standard method for quantifying proanthocyanidins in cranberry powders. *Journal of the Science of Food and Agriculture, 90*, 1473–1478.

Ragonese, A. E. (1967). *Vegetación y ganadería en la República Argentina*. Buenos Aires, Argentina: Colección Científica del Instituto Nacional de Tecnología Agropecuaria, I.S.A.G..

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., & Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decoloration assay. *Free Radical Biology & Medicine, 26*, 1231–1237.

Rodríguez-Amaya, D. B. (1999). *A guide to carotenoid analysis in foods*. Washington DC: ILDI Press.

San, B., & Yildirim, A. N. (2010). Phenolic, alpha-tocopherol, beta-carotene and fatty acid composition of four promising jujube (*Ziziphus jujuba* Miller) selections. *Journal of Food Composition and Analysis, 23*, 706–710.

Scarpa, G. F. (2004). Medicinal plants used by the Criollos of Northwestern Argentine Chaco. *Journal of Ethnopharmacology, 91*, 115–135.

Singleton, V. L., Orthofer, R., & Lamuela-Raventos, R. M. (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. *Methods in Enzymology, 299*, 152–178.

Sun, Y., Liang, Z., Shan, C., Viernstein, H., & Unger, F. (2011). Comprehensive evaluation of natural antioxidants and antioxidant potentials in Ziziphus jujube Mill. var. spinosa (Bunge) Hu ex H.F. Chou fruits based on geographical origin by TOPSIS method. *Food Chemistry, 124*, 1612–1619.

Tortosa, R. D. (1995). Rhamnaceae. In A. T. Hunziker (Ed.), *Flora Fanerogámica Argentina* (9, pp. 1–18). Córdoba, Argentina: PROFLORA-CONICET.

Valentão, P., Fernandes, E., Carvalho, F., Andrade, P. B., Seabra, R. M., & Bastos, M. L. (2001). Antioxidant activity of Centaurium erythraea infusion evidenced by its superoxide radical scavenging and xanthine oxidase inhibitory activity. *Journal of Agricultural and Food Chemistry, 49*(7), 3476–3479.

Zhang, R., Chen, J., Shi, Q., Li, Z., Peng, Z., Zheng, L., & Wang, X. (2014). Phytochemical analysis of Chinese commercial *Ziziphus jujube* leaf tea using high performance liquid chromatography–electrospray ionization-time of flight mass spectrometry. *Food Research International, 56*, 47–54.

Zhishen, J., Mengcheng, T., & Jianming, W. (1999). The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. *Food Chemistry, 64*, 555–559.