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Polyphenolic compounds and anthocyanin content of *Prosopis nigra* and *Prosopis alba* pods flour and their antioxidant and anti-inflammatory capacity

María Jorgelina Pérez[1], Ana Soledad Cuello[1], Iris Catiana Zampini[1], Roxana Mabel Ordoñez[1], Maria Rosa Alberto[1], Cristina Quispe[2], Guillermo Schmeda-Hirschmann[2&], María Inés Isla[1*&]

[1]Laboratorio de Investigación de Productos Naturales (LIPRON), Instituto de Química del NOA (INQUINOA.CONICET). Facultad de Ciencias Naturales e IML. Universidad Nacional de Tucumán, San Miguel de Tucumán, Argentina.

[2]Laboratorio de Química de Productos Naturales, Instituto de Química de Recursos Naturales, Universidad de Talca, Casilla 747, Talca, Chile.

[&] Both authors had the same participation

*Corresponding author: Dra. María Inés Isla

INQUINOA- CONICET

Universidad Nacional de Tucumán.

San Lorenzo 1469

4000 - San Miguel de Tucumán. ARGENTINA

E-mail: misla@tucbbs.com.ar

Abstract

The aim of this study was to determine the content of total free and bound phenolics, free and bound flavonoids, anthocyanins, alkaloids and the profile of polyphenols of edible ripe pods of *Prosopis alba* and *Prosopis nigra.*

P. alba flour showed significantly higher total (sum of Free- and Bound) phenolic content and total flavonoid compounds than *P. nigra* ($p < 0.05$) while *P. nigra* had higher concentrations of anthocyanins than *P. alba* ($p < 0.05$). The *P. nigra* flour shows a pattern characterized by the occurrence of anthocyanins as well as 14 flavonoid glycosides, with higher chemical diversity than *P. alba*, which shows 8 flavonoid glycosides as relevant constituents. The main compounds were quercetin *O*-glycosides and apigenin-based *C*-glycosides. The phenolic composition of two South American algarrobo pod flour is presented for the first time. *P. nigra* pods having higher content of anthocyanins are darker (purple) than those of *P. alba* (light brown). Furthermore, the sugar-free polyphenolic extracts of *P. nigra* and *P. alba* as well as anthocyanins enriched extracts from *P. nigra* showed antioxidant activity. *P. nigra* and *P. alba* polyphenolic extracts showed activity against a pro-inflammatory enzyme. In conclusion, algarrobo pods meal contained biologically active polyphenols, with a positive impact on human health.

Keywords: *Prosopis*; mesocarp flour, phenolic compounds; anthocyanins, antioxidant capacity, antiinflammatory capacity.

1. Introduction

Genus *Prosopis* belongs to the *Fabaceae* family, subfamily *Mimosoideae* and comprises 44 species distributed mainly in arid and semiarid, tropical and subtropical countries (Burkart, 1976). In Argentina there are 29 *Prosopis* species with 14 endemic taxa, being the main diversity centre of America (Rzedowski, 1988). *Prosopis* shows a clear preference for arid climates, thrives in a large area that extends from the south-western part of the United States to the Argentinean Patagonia, being characteristic of the Monte and Chaco desert region in Argentina from Salta to Chubut provinces (Cardozo, Ordóñez, Zampini, Cuello, Dibenedetto & Isla, 2010). Several uses have been reported for *Prosopis* species. *Prosopis* pods constitute a food source for human and animals of Monte desert (Arenas, 2003; D'Antoni & Solbrig, 1977; Felger, 1977; Fagg & Stewart, 1994; Felker, Grados, Cruz & Prokopiuk, 2003; Felker, Takeoka & Dao, 2013). They are relevant food sources for Amerindians in the Paraguayan Chaco (Schmeda-Hirschmann, 1994, 1998) as well as, in Chile (Astudillo, Schmeda-Hirschmann, Herrera & Cortés, 2000; Schmeda-Hirschmann, Razmilic, Gutierrez & Loyola, 1999). Different food products are made from *Prosopis* species: drinks (añapa, aloja and chicha), syrup, flour, sweets (arrope, patay, jam), etc (Escobar, Estévez, Fuentes & Venegas, 2009). In Argentinean traditional medicine, the fruit of *Prosopis* is used as sedative, and pathologies associated with inflammatory processes like asthma and bronchitis (Carrizo, Palacio & Roic, 2002). Gum exuded from bark wounds is comparable to commercial Arabic gum (Anderson & Farquhar, 1982). According to the Argentinian Food Code, "algarrobo flour" is produced by grinding whole mature pod of *P. alba* and *P. nigra*. In the traditional grinding process most of the seeds are discarded due

to the hardness of the endocarp. In a previous paper we determined the nutritional and functional properties of flour obtained from the mesocarp fraction of *P. alba* and *P. nigra* from Northwestern Argentina (Cardozo, Ordoñez, Zampini, Cuello, Dibenedetto, & Isla, 2010). The highest free phenolics, flavonoids and condensed tannin content in both flours were found in aqueous extractions after boiling. Results indicate that the food processing procedures, such as heating, can lead to a disassociation of some phenolic compounds from cellular structures (bound-phenolic) (Cardozo, Ordoñez, Zampini, Cuello, Dibenedetto, & Isla, 2010). Some of these bound phenolics could be hydrolyzed from their attached macromolecule by intestinal enzymes, and reach the colon intact where they are released by the colon microflora (Andreasen, Kroon, Williamson & García-Conesa, 2001). The free phenolic compounds in the digestive tract can exert activity either on site and/or at remote sites after absorption, reducing the incidence of colon cancer and other chronic diseases (Andreasen, Kroon, Williamson & García-Conesa, 2001). Furthermore, the purple colour of *Prosopis* pods and the different colour that showed the pods flour from different collections of *Prosopis nigra* draw our attention (Figure 1 and 2). The colour of foods and beverages is one of the most important properties to obtain their acceptability. Normally, food colour is due to naturally occurring pigments or synthetic colorants added to confer the desired colour to the final product. Although synthetic colours had been favored over the past 100 years, their use has been reduced in the past four decades by its carcinogenic properties. For this reason, there is renewed interest in finding foods with natural colorants.

Anthocyanins are phenolic pigments occurring in a wide variety of sources such as fruits (grapes, red raspberry, cranberries) (Bassa & Francis, 1987; Rommel,

Heatherbell & Wrolstad., 1990), vegetables (red cabbage, red radish, sweet potato) (McDougall, Fyffe, Dobson & Stewart, 2007), cereals (purple corn) and petals of some flowers (Escribano-Bailón, Santos Buelga & Rivas Gonzalo, 2004).

Anthocyanins are considered to be potential replacements for synthetic colorants. However, anthocyanin containing products are susceptible to color deterioration during processing and storage. Nevertheless, their bright colors, water solubility that allows their incorporation into aqueous food systems and a number of health benefits, such as, improved visual acuity, anticancer and antiviral activities makes them attractive objects for study.

A better characterization of the bioactive metabolites occurring in South American phytogenetic resources is relevant for a sustainable management and for developing local species into potential new crops. The aims of this study were to determine the content of free phenolic, bound phenolic, anthocyanin, antioxidant capacity and phenolic fingerprint analysis of flour obtained from different accessions or morphotypes of *Prosopis alba* and *Prosopis nigra.*

2. Materials and Methods

2.1. Sample preparations and processing

Prosopis nigra (Griseb.) Hieron. and *P. alba* (Griseb) ripe pods were collected in Amaicha del Valle, Tucumán, Argentina in March 2012 and 2013. The cultivars were identified by Dra. Soledad Cuello and voucher specimens were deposited in the INQUINOA herbarium. Pods were classified on the basis of their colour as follows: 1 light brown, 2 light brown with purple spots, 3 brown with purple spots, and 4 purple.

The pods were brushed to remove foreign material and dried at 50ºC until reaching constant weight. Dried pods were ground to obtain the fine flour (149 μm) and fiber.

2.2. *Extraction and determination of free and bound polyphenolic compounds content*

Prosopis flour (180 g) was extracted three times with methanol: water (MeOH:H_2O), 70:30 (1:10 w/v) with an ultrasonic bath for 1 h at 25°C. The extracts were filtered, and two fractions were obtained (soluble and residue). The soluble fractions were combined, and the MeOH evaporated "*in vacuo*" (40°C). Residual water was freeze - dried by lyophilization. The resulting dried extract was suspended by using an ultrasound bath in 50 mL distilled water and then was mixed with Amberlite XAD7 (500 g). The resin was rinsed with 1L distilled water and eluted with 1L HPLC grade MeOH.

The eluate was concentrated under reduced pressure to give 1.03 g of a crude MeOH extract and then stored at -20°C until use.

The extraction of bound phenolics was performed by using the previously described method with a slight modification (Adom & Liu, 2002). The residue from the free phenolic extraction was mixed with 20 mL of 2 N NaOH with shaking at 60°C for 15 min. After adjusting the pH to 2 with 6N HCl, the mixture was filtered and extracted six times with ethyl acetate. The pooled ethyl acetate fraction was dried under reduced pressure by using the cold trap, and then reconstituted with 1 mL of MeOH for a cell-wall bound (Bound) fraction.

All reconstituted solutions were stored at -20 °C until analyzed. Phenolic content was determined by Folin-Ciocalteu reagent, according to Singleton, Orthofer & Lamuela-

Raventos (1999). Results were expressed as g gallic acid equivalents/100 g dry weight (g GAE/ 100 g flour). The total flavonoid content in all preparations was determined with aluminum chloride ($AlCl_3$) according to Min, McClung & Chen, (2011). Total flavonoid contents were expressed as g quercetin equivalents/100 g dry weight (g QE/100 g flour).

Free phenolic analysis by HPLC-DAD

Extracts were analyzed by HPLC coupled to a diode array detector to set the conditions for HPLC-DAD-MS/MS. The HPLC system used for DAD analysis was a Shimadzu (Shimadzu Corporation, Kyoto, Japan) equipment consisting of a LC-20AT pump, a SPD-M20A UV diode array detector, CTO-20AC column oven and a LabSolution software. A MultoHigh 100 RP 18-5µ (250x4.6 mm) column (CS-Chromatographie Service GmbH- Germany) maintained at 25 °C was used.

The HPLC analysis was performed using a linear gradient solvent system consisting of 1% formic acid in water (A), acetonitrile (B), and water (C) as follows: 70% A, 12% B and 18% C to 50% A, 20% B and 30% C over 15 min, followed by 50% A, 20% B and 30% C over 5 additional minutes (t = 20min), to 50% A, 20% B and 30% C from 20 min to 70% A, 12% B and 18% C to 35 min. Flow rate: 1 mL/min. Volume injected: 20 µL. Detection: 254 nm, DAD from 200 to 600 nm.

Identification of free phenolics by HPLC-ESI-MS/MS

Data were recorded on a LC-ESI-MS/MS system which consisted of the HPLC HP1100 (Agilent Technologies Inc, CA-USA) connected through a split to the mass spectrometer Esquire 4000 Ion Trap LC/MS(n) system (Bruker Daltonik GmbH,

Germany). Ionization was performed at 3000 V assisted by nitrogen as a nebulizing gas at 24 psi and as a drying gas at 365 ºC and a flow rate of 6 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). Trap parameters were set in ion charge control (ICC) using manufacturer default parameters, and maximum accumulation time of 200 ms. Collision induced dissociation (CID) was performed by collisions with helium background gas present in the trap and automatically controlled through SmartFrag option.

The identification of *Prosopis* pods constituents was carried out by comparison of the spectral properties (UV and ESI-MS and MS/MS) of the compounds with those of reference samples when available or literature data.

NMR analysis

The NMR spectra of isolated compounds and fractions were recorded on a Bruker Avance 400 (Bruker, Rheinstetten, Germany) spectrometer at 400 MHz for ^{1}H and 100 MHz for ^{13}C in $CDCl_3$ or MeOH-d_4. Chemical shifts are given in ppm with residual chloroform or methanol as the internal standard.

2.3. Extraction and determination of anthocyanins content

Prosopis nigra flour (P8) was mixed with different extracting media at a solid–liquid ratio of 1:5 and maintained overnight at 5 °C. The extracting media used included water, acidified water with 1% HCl, methanol, acidified methanol with 1% HCl, ethanol, acidified ethanol with 1% HCl, water: ethanol (1:1, v:v), and mixture of ethanol and acidified water with 1% HCl. The anthocyanin extract obtained in each

extraction was filtered to remove fibrous particles. The filtrates obtained for different extracting media were centrifuged separately at 10,000 x *g* for about 10 min to remove the suspended particles. The anthocyanin content in each of these extracts was measured by using the pH differential method, the results being compared (Abdel-Aal, Young & Rabalski, 2006). The better extraction medium was selected and used for the other flour samples.

Total anthocyanins were evaluated by the pH differential method (Lee, Durst, & Wrolstad, 2005). Acetonic extract in 25 mM potassium chloride solution (pH 1.0) and 400 mM sodium acetate buffer (pH 4.5) were measured simultaneously at 520 nm and 700 nm, respectively. The content of total anthocyanins was expressed as mg cyanidin-3-glucoside equivalents per 100 g of dry weight (mg C3GE/100 g DW).

$$\textit{Anthocyanin pigment (mg/L)} = \frac{A \times Mw \times DF \times 10^3}{\varepsilon \times L}$$

Where A= Absorbance (pH 1.0) - Absorbance (pH 4.5), Mw is the molecular weight of anthocyanin (433.2 g/mol). DF is the dilution factor, ε is the extinction coefficient (31,600 L/cm.mol) and L is the path length (1 cm).

2.4. Extraction and determination of alkaloid content

Total alkaloids were measured by using bromothymol blue (BTB) as the colouring agent (Önal, Kepekçi & Öztunç, 2005). One mL of water, standard or extract was added to 2 mL of BTB reagent (4.78×10^{-4}M in phosphate buffer pH 5). After mixing, the ion-pair complex formed was extracted with 5 mL chloroform by shaking vigorously for exactly 1 min. Absorbance was measured at 414 nm against the blank

prepared with water. Total alkaloids were calculated as µg of apomorfine chlorhydrate equivalents (µg ACE) per 100 g of flour.

2.5. Measurement of antioxidant capacity

2.5.1. ABTS free radical scavenging activity

The antioxidant capacity assay was carried out by the improved $ABTS^{\bullet+}$ method as described by Re, Pellegrini, Proteggente, Pannala, Yang & Rice-Evans (1999). The $ABTS^{\bullet+}$ was generated by reacting 7 mM ABTS and 2.45 mM potassium persulfate at room temperature (23ºC) after incubation, in the dark for 16 h. The $ABTS^{\bullet+}$ solution was obtained by dilution in methanol of the stock solution to an absorbance of 0.70 at 750 nm. $ABTS^{\bullet+}$ solution (100 µL) was added to samples and mixed thoroughly. Absorbance was recorded at 750 nm using a spectrophotometer Thermo Scientific (Model Multiscan Go) 1 min after initial mixing and up to 6 min. Results were expressed in terms of percentage of scavenging activity.

2.5.2. DPPH scavenging activity

In this method, a solution of 2,2-diphenyl-1-picrylhydrazyl (DPPH) in 96º ethanol (300 µM) was prepared, and 1.5 mL of this solution was added to 0.5 mL of the samples at varying concentrations. Twenty minutes later, the absorbance was measured at 515 nm.

The percentage of radical scavenging activity (RSA%) was calculated by using the following equation:

$$RSA\ \% = [(A_0 - A_s)/A_0] \times 100.$$

Where A_0 is the absorbance of the control and A_s is the absorbance of the samples at 515 nm. SC_{50} values denote the µg GAE/mL required to scavenge 50% DPPH free radicals.

2.5.3. β-Carotene- linoleic acid bleaching assay

In this assay, the antioxidant capacity is determined by the formation of conjugated diene hydroperoxides arising from linoleic acid oxidation, which results in the discolouration of β-carotene. The reaction is carried out according to Veliogu, Mazza, Gao & Oomah, (1998) with some modifications. β-Carotene (1 mg) was added to 20 µL of linoleic acid, 200 µL of the Tween 40 and 10 mL of distilled water. Then, the mixture was submitted to sonication during 10 min until total homogeneization. Then, 40 mL of 50 mM H_2O_2 was added. Next, 1mL of this mixture was transferred into test tubes containing different concentrations of the sample or positive controls. As soon as the emulsion was added to each tube, the zero time point absorbance was measured at 470 nm using a spectrophotometer. The emulsion was incubated for 2 h at 50 ºC. A blank, devoid of β-carotene, was prepared for background subtraction.

2.6. Measurement of anti-inflammatory capacity

2.6.1. Cyclooxygenase inhibition studies

The ability of the extracts to inhibit the conversion of arachidonic acid (AA) to prostaglandin H_2 (PGH_2) by human recombinant cyclooxygenase 2 (COX-2) was determined by using a COX inhibitor screening assay kit (No. 560131; Cayman Chemical, Ann Arbor, MI, USA). Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) produced from PGH_2 by reduction with stannous chloride was measured by enzyme immunoassay (EIA) in a

microplate reader (Biotek ELx 808). The assay to obtain the 100% of COX activity was performed with and without dimethyl sulfoxide (DMSO) as solvent control. The inhibitory assays were performed in the presence of different *Prosopis* extracts concentrations (until 3 μg GAE/mL) before and after Amberlite XAD-7 and dissolved in DMSO. The commercial anti-inflammatory drug nimesulide was used as reference. Pre-incubation time between COX-2 enzyme and inhibitor was 10 min with 2 min incubation in the presence of AA at 37 ºC. Enzyme control was performed with enzyme that had been inactivated by placing them in boiling water for 3 min. Detection limit was 29 pg of PG/mL. Extract anti-inflammatory effect was evaluated by calculating percentage inhibition of PGE_2 production.

2.6.2. Lipoxygenase inhibition studies

The UV absorbance-based assay of Taraporewala & Kauffman (1990) was used. Lipoxygenase (LOX) activity was determined by using a continuous spectrophotometric method, based on the enzymatic oxidation of linoleic acid to the corresponding hydroperoxide. The absorption at 234 nm was recorded as a function of time. The reaction mixture contained 50 mM linoleic acid in 0.2 M sodium borate buffer pH 9, 500 U LOX-1 (Sigma-Aldrich) and different concentrations of *Prosopis* extracts up to 30 μg GAE/mL. The assay to obtain the 100% of LOX activity was performed with the sample vehicle. The anti-inflammatory effect was evaluated by calculating percentage of hydroperoxide production inhibition each 30 sec for 5 min by absorbance at 234 nm (Spectrophotometer Thermo Scientific, Model Multiscan Go). The anti-inflammatory drug naproxen was employed as reference compound.

2.7. Statistical analysis

Sampling and analyses were performed in triplicate, and the data are presented as mean ± standard deviation. The correlation between two variants was analyzed by Pearson test and by Spearman test. Wilcoxon analysis and principal component analysis (PCA) were also performed with the Infostat software.

3. Results and Discussion

Prosopis nigra (Griseb.) Hieron. and *P. alba* (Griseb) ripe pods were collected in Amaicha del Valle, Tucuman, Argentine at 2,000-2,100 meters above sea level. The colour of *P. alba* pods was light brown in all samples (colour 1) while *P. nigra* pods were light brown with purple spots (colour 2, 3 or 4). The pods were dried at 50ºC and grounded to obtain the fine flour.

3.1. Phytochemical composition

Free- and bound-PCs content of *Prosopis* pods flour are shown in Table 1. Free-PC in *P. nigra* and *P. alba* flour ranged from 0.21 to 0.67 g GAE/100 g flour. The bound-PCs were the major contributors of total-PCs (sum of Free- and Bound-PC) in *P. alba* flour (57–86% of the total), whereas the bound-PC accounted for 17-36% of the total-PC in *P. nigra* flour. The results found in *P. alba* flour are consistent with Adom & Liu (2002), whose study indicated that bound-PCs of corn, wheat, oat and rice, dominantly contributed to the total-FC.

The total free PCs were higher than the total free phenolics reported in white wheat flour of 0.0044–0.014 g GAE/100 g and similar to wheat bran of 0.126–0.316 g GAE/100 g (Vaher, Matso, Levandi, Helmja & Kaljurand,2010).

The bound flavonoids were the major contributors of total flavonoids (89 to 92% of total flavonoids) in *P. alba* flour while in *P. nigra*, the bound- and free- flavonoids were variable.

The anthocyanin content of *P. nigra* and *P. alba* pods flour was also evaluated (Table 1). In general, the total anthocyanin concentration was higher in *P. nigra* flour (7.25 to 65.27 g/100 g flour) than in *P. alba* flour (0 to 11.34 g/100 g). Data suggest that the total anthocyanin concentrations vary not only between species but also between varieties.

The total alkaloid content showed values between 0.053 and 0.168 µg ACE/100 g flour of *P. nigra* and 0.065 to 0.147 µg ACE/100 g flour of *P. alba*.

Principal component analysis (PCA) showed a clear separation in two groups: *P. alba* and *P. nigra* flour (Figure 1) according to phytochemical composition. The scattered plot clearly showed discrimination between *P. nigra* and *P. alba* by principal component PC1, while PC2 mainly indicates the variation of metabolites within the species.

The colour of the algarrobo pods is directly related to the content of anthocyanins according to Spearman correlation analysis (ρ=0.74; p=0.0026<0.01) (Figure 1, Table 1). *P. nigra* pods having higher content of anthocyanins are darker in colour: G-2 (light brown with purple spots), G-3 (brown with purple spots), and G-4 (purple) than those of *P. alba* (light brown).

3.2. HPLC-ESI-MS/MS analysis

Anthocyanins

Flour obtained from *P. alba* and *P. nigra* pods shows different colors, ranging from off white to grayish purple, with darker colours for *P. nigra* flour. Extraction of the *P. nigra* pods flour with methanol afforded violet red colored extracts. Mass fragmentation patterns and tentative identification of the anthocyanins from *P. nigra* pods flour is presented in Table 2. The HPLC chromatogram showing the flour anthocyanins profile is shown in Figure 2.

HPLC analysis of the phenolic-enriched extract showed a main compound (peak 2) at retention time (Rt) of 11.7 min, with a maximum absorption of 516 nm and a $[M]^+$ ion at *m/z* 449. MS/MS analysis indicated the neutral loss of 162 amu, in agreement with a hexose. Compound 2 was assigned as cyanidin-3-hexoside, in agreement with literature (Ha, Lee, Park, Pae, Shim, Ko et al., 2005). Seven minor compounds were also detected and tentatively assigned as anthocyanidins, namely peaks 1, 3-8. Peaks 1, 2 and 5 shared the same anthocyanidin (cyanidin, *m/z* 287), 4, 7 and 8 presented malvidin (*m/z* 331) peak 3 contained peonidin (*m/z* 301) and 6 petunidin (*m/z* 317) as the anthocyanidin moiety, respectively (Wu & Prior, 2005). The MS/MS spectrum of compound 1 showed fragment ions at *m/z* 449 and *m/z* 287, in agreement with the consecutive loss of a rhamnose and a hexose, being tentatively identified as cyanidin rhamnosyl hexoside. The compound 5, with *m/z* 535 and MS/MS ions at *m/z* 449 and *m/z* 287, lose a malonoyl (86 amu) and a hexose (162 amu), in agreement with cyanidin malonoyl hexoside. The malvidin derivatives 4 and 7 presents a $[M]^+$ ion at 655 and 639 amu, which in MS/MS losses two hexoses and a rhamnose and a hexose, respectively, leading to the same anthocyanidin. Diglycosides were identified as malvidin dihexoside and malvidin-rhamnosyl-hexoside, respectively. The closely related compound 8 losses a hexose from the $[M]^+$ ion at *m/z* 493 and was identified

as malvidin-3-hexoside. Monoglycosides 3 and 6 losses a hexose from their $[M]^+$ ions and were assigned as peonidin-3-hexoside and petunidin-3-hexoside, respectively. The anthocyanins are reported for the first time for *Prosopis* species flour.

Alkaloids

The alkaloid tryptamine, occurring in low amounts, was isolated from the pods flour and identified by NMR analysis and mass spectrometry. Tryptamine was reported as a constituent of *Prosopis* pods by Tapia, Feresin, Bustos, Astudillo, Theoduloz & Schmeda-Hirschmann (2000) as well as by Astudillo, Schmeda-Hirschmann, Herrera & Cortés, (2000). Other nitrogen-containing compounds isolated and identified from Argentinean and Chilean *Prosopis* species include phenethylamines, piperidine alkaloids, (Tapia et al., 2000; Astudillo, Jürgens, Schmeda-Hirschmann, Griffith, Holt & Jenkins, 1999; Astudillo, Schmeda-Hirschmann, Herrera & Cortés, 2000) and maltoxazine from *P. tamarugo* (Schmeda-Hirschmann & Jakupovic, 2000). However, they were not detected in our samples.

Flavonoids O- and C-glycosides

The main constituents identified in the phenolic-enriched extracts of algarrobo pods were C-glycosyl flavonoids and *O*-flavonol glycosides. HPLC chromatograms of methanol extract from *P. alba* and *P. nigra* flour are shown in Figure 3. The structure of some constituents identified or tentatively identified in the flour is presented in Fig. 4. The HPLC-DAD-MS data of the phenolic identified in methanol extracts of *P. alba* and *P. nigra* pods are presented in Table 3.

Eight *O*- and *C*-flavonoid glycosides were identified in *P. alba* while fourteen were now described from *P. nigra* pods extract. Common in both samples were the quercetin glycosides **10**, **12** and **21**, the kaempferol hexoside **22** and the *C*-glycoside isovitexin **20**.

Compounds **10**, **12**, **16**, **18** and **21** were assigned as quercetin glycosides based on the MS/MS experiments. While the compound **10** shows consecutive losses of two hexoses and a rhamnose, **12** losses two hexoses and **21** one hexose, leading to an aglycone of *m/z* 301, in agreement with quercetin. Compounds **16** and **18** differ in sugar attachment, as **16** shows first the loss of a rhamnose and **18** an hexose unit. Flavonoid **12**, eluting at Rt 18.7 min shows an UV spectrum compatible with a 3-*O*-substituted flavonol bearing two or more OH functions in the B-ring. MS/MS spectrum showed loss of two hexoses leading to the aglycone of *m/z* 301, in agreement with a di *O*-hexoside of quercetin. The assignation is in agreement with the data reported by Ferreres, Llorach & Gil-Izquierdo (2004) for glycosyl flavonoids. The quercetin methyl ether derivatives (**13** and **24)** were detected as minor constituents and differ in sugar moieties, the placement of the OCH_3 function in the aglycone cannot be given based only on the MS analysis. Compounds **19** and **22** were tentatively identified as an hexoside-rhamnoside and as a hexoside of kaempferol, based on the sugar loss and the base peak of *m/z* 285, in agreement with the aglycone kaempferol. The derivative **25** differs from **19** in 14 amu, supporting a methyl ether of kaempferol.

Several *C*-glycosyl flavonoids (compounds **9**, **11**, **14**, **15** and **20**) occur as main constituents in the meal extract. Most of them are based on the aglycone apigenin. The differentiation of vitexin **15** and isovitexin **20** was based on the elution sequence

as well as on the relative abundance of the $[M-90]^-$ ion at *m/z* 341. In the compound eluting at Rt 22.8 min, the intensity of the $[M-90]^-$ ion at *m/z* 341 ion was 9% while for the isomer eluting at Rt 24.1 min, the abundance was 31%. This finding is in agreement with the report of Ferreres, Silva, Andrade, Seabra & Ferreira (2003) on the *C*-glycosyl flavones from quince (*Cydonia oblonga*) seeds. The elution sequence and relative ion abundance of compounds **11** and **14** are in agreement with isoschaftoside and schaftoside, reported from *Prosopis* pods syrups by Quispe, Petroll, Theoduloz & Schmeda-Hirschmann (2014).

3.3. Comparative phenolic composition of P. alba and P. nigra pods flour

HPLC-ESI-MS/MS analysis of the methanol extracts of *P. alba* and *P. nigra* pods meal allowed the identification or tentative identification of 25 compounds, including 8 anthocyanins and 17 flavonoids (Figures 2 and 3, Tables 2 and 3). *P. nigra* meal shows a typical pattern characterized by the occurrence of anthocyanins as well as 14 glycosides, with higher chemical diversity than *P. alba*, which shows 8 flavonoid glycosides as relevant constituents. The main compounds were quercetin *O*-glycosides and apigenin-based *C*-glycosides. Both groups of compounds are naturally occurring antioxidants. Flavonoid *C*-glycosides and *O*-glycosides have shown to present antioxidant, anti-inflammatory, and antiplatelet activity, among others (Barreca, Belloco, Leuzzi & Gattuso, 2014; Zucolotto, Goulart, Montanher, Reginatto, Schenkel & Frode, 2009; Velozo, Ferreira, Santos, Moreira, Guimaraes, Emerenciano et al.,2009; Piccinelli, Garcia Mesa, Armenteros, Alfonso, Arevalo, Campone et al., 2008).

3.4. *Antioxidant capacity of P. nigra and P. alba flour enriched with phenolic compounds*

Scavenging activity of stable free radical:

DPPH and ABTS assays are able to evaluate the ability of antioxidants to donate hydrogen to free radicals leading to non toxic species and therefore inhibiting the oxidation of biomolecules. The free phenolic extracts *P. nigra* enriched with free-flavonoids (40-60% of total flavonoids) from eight flour samples showed ABTS scavenging activity with SC_{50} values between 7 and 29 µg/mL while only three samples reached scavenging activity of 50% DPPH radicals with values between 19 and 22 µg/mL. Similar results were obtained for *P. alba* (6 to 17 µg/mL for ABTS and 11 to 15 µg/mL for DPPH, Table 4). Furthermore, *P. nigra* extracts enriched with bound phenolics showed SC_{50} values between 4 and 12.5 µg/mL while *P. alba* showed values between 2.5 and 7 µg/mL for ABTS (Table 4). The same behavior was shown by bound phenolics on DPPH scavenging activity (Table 4). According with the statistical analyses, the bound phenolic compounds were more active than free phenolic compounds. These results are in agreement with that reported from others foods of bound phenolics released upon alkaline, acid or enzyme hydrolysis (Liyana-Pathirana & Shahidi, 2006; Kim, Tsao, Yang & Cui, 2006). A significant correlation ($p < 0.05$) was observed between antioxidant potential determined by ABTS and DPPH with *P. alba* free phenolic compounds (R^2= 0.6800 and 0.7149, respectively) and *P. alba* bound-phenolic compounds (R^2= 0.8852 and 0.7856, respectively). In contrast, no significant correlation ($p \geq 0.1$) was found between stable free radical scavenger activity and phenolic content (bound or free) in *P. nigra* extracts.

Bound phenolics were considered to have health benefits because they may escape from upper gastrointestinal digestion conditions along with cell wall materials and are absorbed into blood plasma during digestion of intestinal microflora (Andreasen, Kroon, Williamson & García-Conesa, 2001).

Protection against lipoperoxidation

Membrane lipids are rich in unsaturated fatty acids that are very susceptible to oxidative processes. The inhibitory capacity of phenolic extracts against the coupled oxidation of β-carotene and linoleic acid, was tested. Both free and bound phenolic compounds of *P. alba* and *P. nigra* showed protective effect on lipids peroxidation with IC_{50} values between 1.5 and 14.5 µg/mL (Table 4). Free phenolic compounds of *P. nigra* were more active than bound phenolics of both *Prosopis* and free phenolic compounds of *P. alba*. There was a significant correlation ($p< 0.05$) between lipoperoxidation inhibition and bound or free phenolic content in *P. alba* extracts ($R^2= 0.8001$ and 0.9174, respectively). However, in *P. nigra* only a significant correlation with bound phenolic content was observed ($R^2= 0.6485$).

3.5 *Stability and antioxidant capacity of pigment (anthocyanin) of Prosopis nigra in different extractive solutions*

Anthocyanins, a group of hydrophilic flavonoids, are natural colorants, which are responsible for the red and purple colors of pigmented cereal grains, fruits and petals (Abdel-Aal , Young, & Rabalski, 2006). Color is one of the most important properties of foods and beverages and is the basis for their acceptability. The selection of a non-

toxic extraction solution for natural food pigments is an important factor in the food industry. Thus, ethanol and water were used to extract the pigments.

The anthocyanin content of *P. nigra* was higher than *P. alba* pods flour (Table 1). For this reason *P. nigra* flour was selected in order to find the suitable extracting medium for the maximum extraction of anthocyanin. Different experiments were carried out with three extraction media, pure water, water with HCl (1%), water with alcohol (96°) and their combination. In general, the extraction of anthocyanin was better with acidified water as compared to pure water. This can be mainly attributed to the presence of hydrochloric acid, which stabilizes the pigments and decreases the pH to a level where the absorbance of the anthocyanins is maximum (Table 5). Ethanol alone or acidified was not suitable as an extracting solvent, because a minimum quantity of water (with and without acid) is required for the extraction of hydrophilic anthocyanin. The stability of anthocyanin in the extraction media was also evaluated during 3 months (Table 5). In all conditions a decrease in the anthocyanin content was observed after the storage for three months but the anthocyanins were more stable in acidified water.

Regarding the $ABTS^{\bullet+}$ scavenging capacity, the activity was determined at pH 5 and 7. At pH 1.0, anthocyanins were predominantly in the flavylium cation form, whereas the proportion of this form significantly decreased at pH 3.0 and almost disappeared at pH 5.0. In fact, at pH 5.0 the absence of absorption bands in the visible spectrum indicates that the anthocyanins present in the functional extract were mostly in the colourless forms of hemiacetals and/or chalcones. The extract obtained with acidified water (SC_{50}= 1.2 µg C3GE/mL) was more active as antioxidant than ethanol/water/H^+ and ethanol/H^+ (SC_{50}= 7.4 and 8.3 µg C3GE /mL, respectively)

(Figure 5, Table 4). The antioxidant activity of aqueous extract/H^+ was similar to that of methanolic extract/H^+ (Table 4).

Considering the easy extraction of pigments from *P. nigra* flour and its stability in aqueous system during storage as well as its biological properties, the extract enriched in anthocyanins could be added to food systems.

3.6. *Anti-inflammatory activity of Prosopis methanolic extract*

Compounds that have radical scavenging activities may thus be expected to have therapeutic potentials for several inflammatory processes. In the present study, the anti-inflammatory activities of *P. nigra* and *P. alba* polyphenolic extracts were measured against isolated pro-inflammatory enzymes (COX and LOX). Products of these enzymes (prostaglandins, leukotrienes, hydroperoxide) are important mediators of inflammation.

In the inhibitory COX-2 enzyme assays, the concentrations able to inhibit 50% enzyme activity (IC_{50}) for *P. nigra* crude extracts were 0.66 ± 0.03 µg GAE/mL. And the IC_{50} value for the same extract after passing through Amberlite XAD-7 was 1.00 ± 0.09 µg GAE/mL. The same effect was observed in *P. alba* with IC_{50} values of 0.70 ± 0.04 and 1.11 ± 0.09 µg GAE/mL crude extract and after passing through Amberlite. Reference drug, nimesulide showed an IC_{50} of 0.39 µg/mL. However, no inhibition was observed in LOX enzyme for extracts of *P. nigra* and *P. alba* up to a concentration of 30 µg GAE/mL. So the extracts were selective for COX. The IC_{50} value of naproxen for LOX enzyme was 14 µg/mL.

These findings together with the antioxidant activity of *Prosopis* species suggest that *P. nigra* and *P. alba* pods flour consumption may contribute to the reduction of inflammation and could be preventive against related diseases.

Conclusions

P. alba and *P. nigra* flour showed different phytochemical composition. The dark colour of the algarrobo pods of *P. nigra* is directly related to the anthocyanins content. Both species contained biologically active polyphenols with a possible positive impact on human health and could be used as functional food.

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Figures

Figure 1. Score plot (PC1 vs. PC2) of principal component analysis (PCA) obtained from phytochemical analysis of different *P. alba* and *P. nigra* samples

Figure 2. HPLC chromatogram of the methanol extract of *Prosopis nigra* pods at 500 nm. **1**: Cyanidin rhamnosyl hexoside; **2**: Cyanidin-3-hexoside; **3**: Peonidin-3-hexoside; **4**: Malvidin dihexoside; **5**: Cyanidin malonoyl hexoside; **6**: Petunidin-3-hexoside; **7**: Malvidin rhamnosyl hexoside; **8**: Malvidin-3-hexoside.

Figure 3. HPLC chromatograms of methanol extract from *P. alba* and *P. nigra* flour. Ultraviolet (UV) detection at 254 nm. Compounds: **9**: Apigenin-di-C-hexoside (Vicenin II); **10**: Q-dihexoside rhamnoside; **11**: Apigenin-C-hexoside-C-pentoside (Isoschaftoside); **12**: Q-dihexoside; **13**: Q-methylether dihexoside; **14**: Apigenin-C-hexoside-C-pentoside (Schaftoside); **15**: Vitexin; **16**: Q-rhamnoside-hexoside; **17**: Unknown; **18**: Q-hexoside- rhamnose; **19**: K-hexoside-rhamnoside; **20**: Isovitexin; **21**: Q-hexoside; **22**: K-hexoside; **23**: Apigenin hexoside-rhamnoside; **24**: Q methyl ether-hexoside-rhamnoside; **25**: K-methyl ether hexoside-rhamnoside. Q: quercetin; K: kaempferol. The structure of compounds **2**, **9**, **11**, **14**, **15**, **20** and tryptamine is shown in Fig. 6.

Figure 4. Structure of selected compounds tentatively identified in methanolic extracts of *Prosopis* pods flour.

Figure 5. $ABTS^{\bullet+}$ scavenging activity of anthocyanin-rich extracts obtained from *Prosopis nigra* flour. Extraction solvents: (-♦-) acidic water (H_2O/H^+); (-■-) acidic ethanol; (-▲-) acidic water plus ethanol (H_2O/H^+/EtOH) or (-x-) acidic methanol ($MeOH/H^+$).

Figure 1.

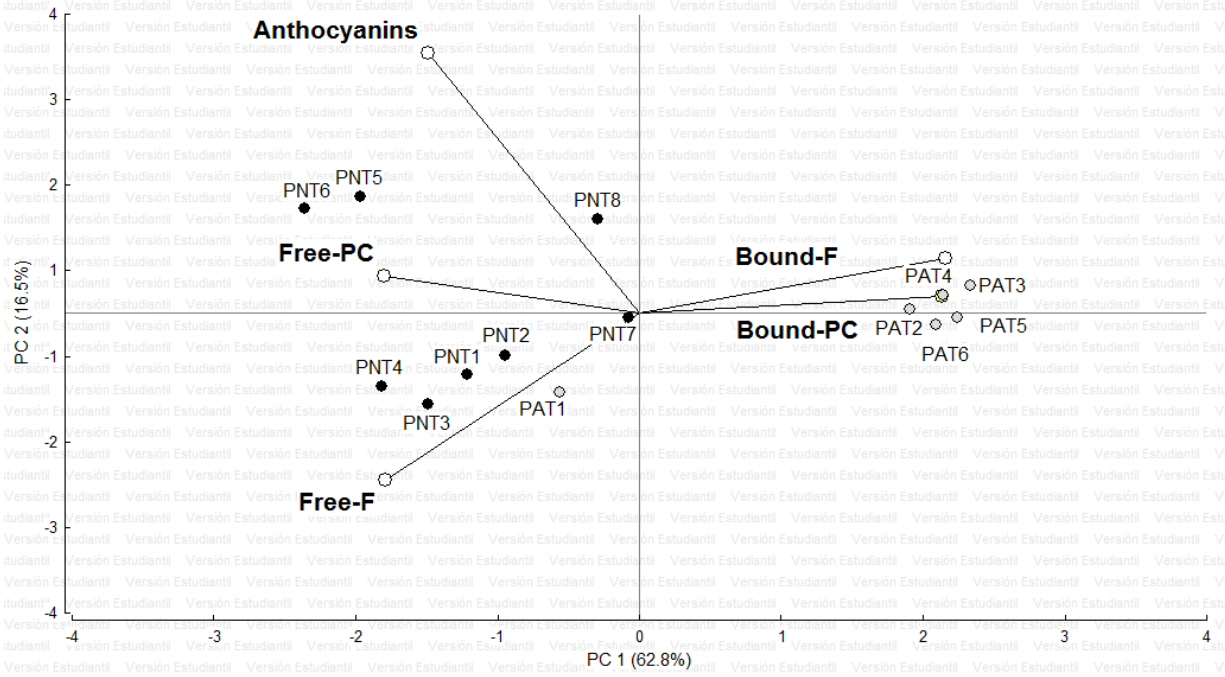


Figure 2.

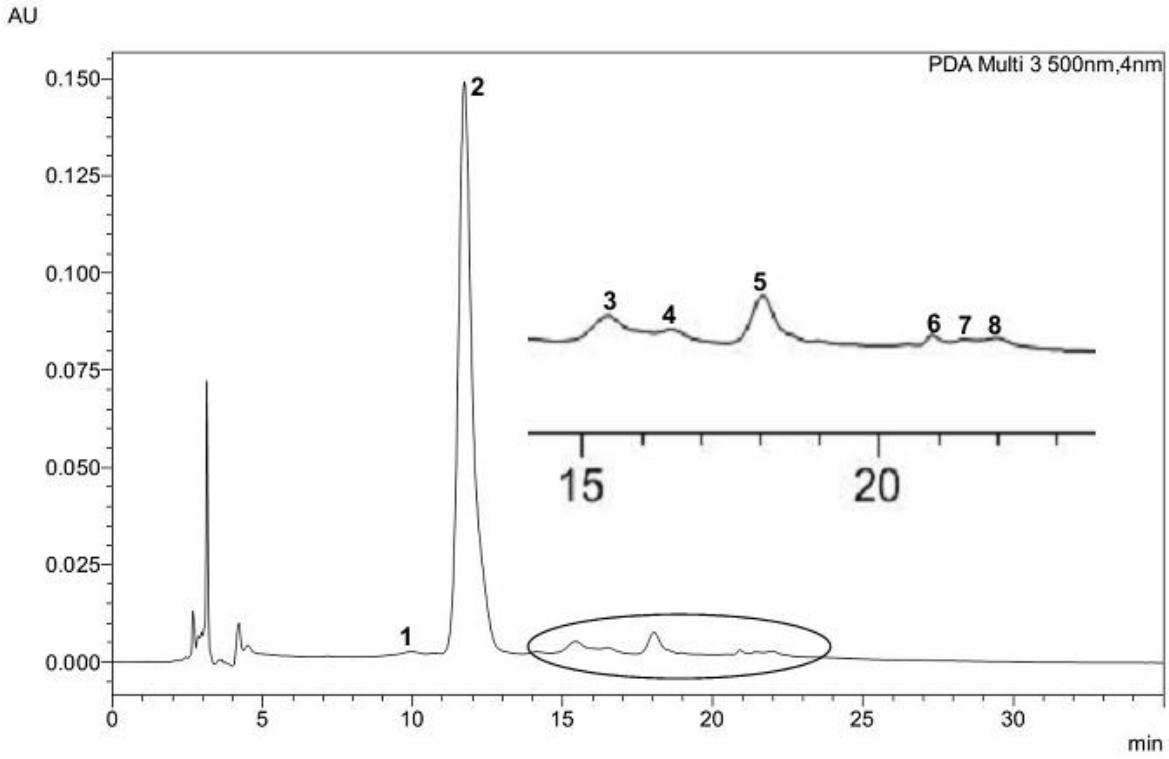


Figure 3.

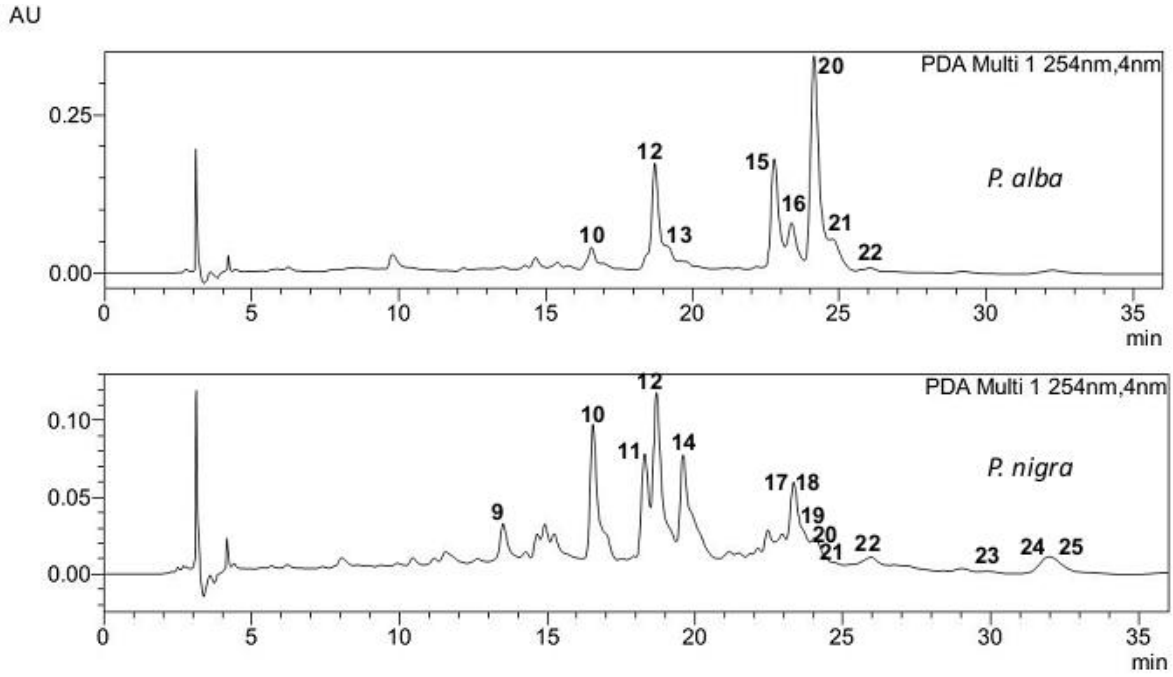
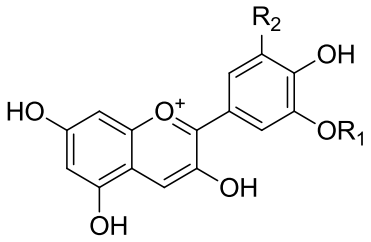
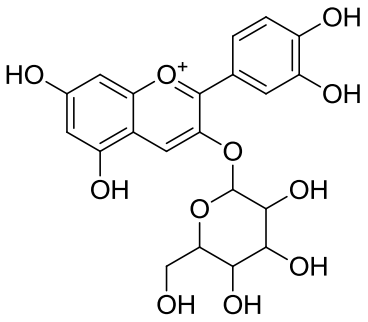


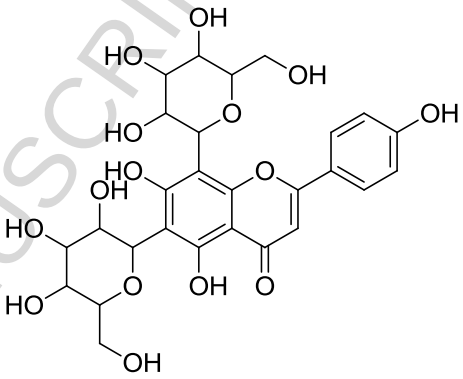
Figure 4.



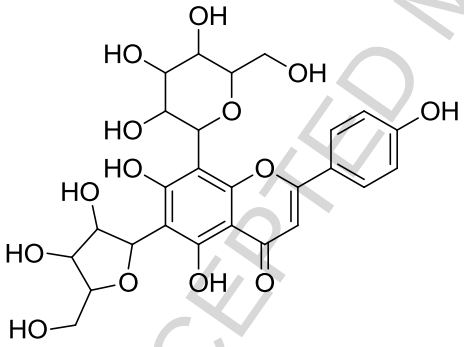
	R_1	R_2
Cyanidin	H	H
Peonidin	CH_3	H
Petunidin	CH_3	OH
Malvidin	CH_3	OCH_3



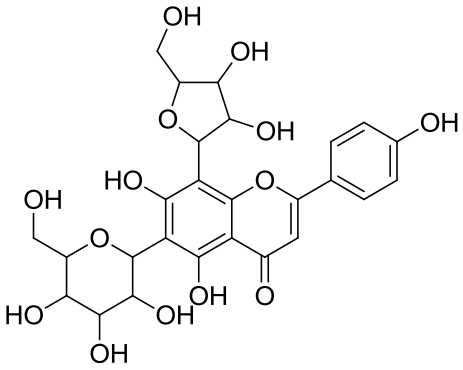
2 Cyanidin-3-hexoside



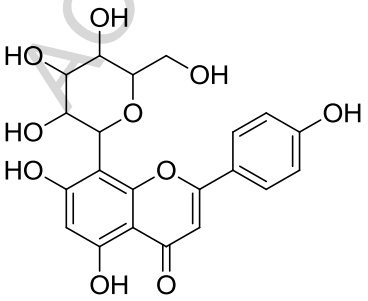
9 Vicenin II



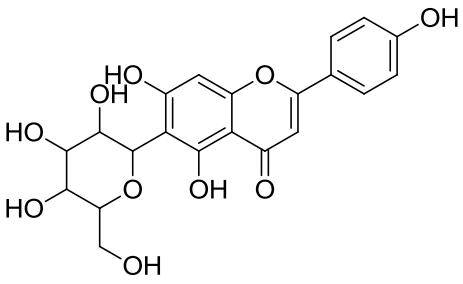
11 Isoschaftoside



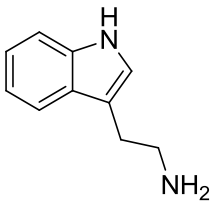
14 Schaftoside



15 Vitexin



20 Isovitexin



Tryptamin

Figure 5.

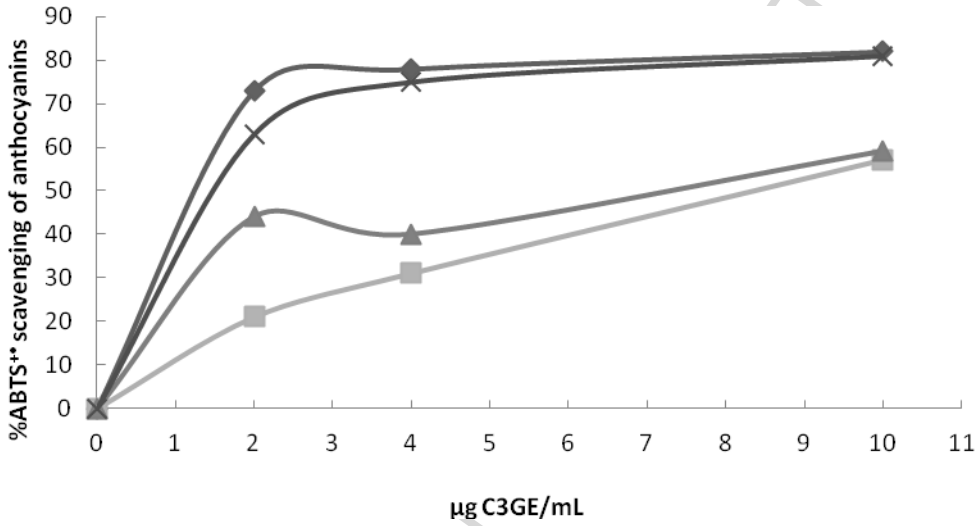


Table 1. Free and bound phenolic compounds (F-PC and B-PC), free and bound flavonoid (F-F and B-F), anthocyanin and alkaloid content in *Prosopis nigra* (PN) and *Prosopis alba* flour (PA) from Tucuman (T), Argentina.

Samples	F-PC	B-FC	F-PC + B-PC	F- F	B-F	F- F+B-F	Anthocyanins	Alkaloids
				Prosopis nigra				
PNT1	0.52±0.05	0.14 ±0.05	0.66	9.34 ±0.90	6.94 ±0.10	16.28	7.25±0.50	0.086±0.02
PNT2	0.46±0.05	0.25 ±0.03	0.71	10.23 ±0.60	16.67 ±0.10	26.90	16.20±1.00	0.053±0.02
PNT3	0.42±0.05	0.08±0.01	0.50	11.96±0.50	0.82±0.05	12.78	11.63±1.00	0.077±0.08
PNT4	0.38±0.10	0.08±0.01	0.46	13.88±0.60	1.09 ±0.05	14.97	25.95±1.00	0.105±0.06
PNT5	0.48±0.05	0.27 ±0.02	0.74	8.71±0.09	4.43 ±0.09	13.14	65.27±1.50	0.094±0.05
PNT6	0.66±0.10	0.14 ±0.02	0.80	8.88 ±0.10	11.78 ±0.50	20.66	56.22±1.50	0.121±0.01
PNT7	0.33±0.05	0.16 ±0.01	0.47	5.12±0.05	8.11±0.50	13.23	13.77±1.00	0.168±0.02
PNT8	0.35±0.10	0.15 ±0.01	0.49	3.47±0.05	11.02 ±1.00	14.49	38.78±1.00	0.115±0.02
				Prosopis alba				
PAT1	0.46±0.05	0.63 ±0.05	1.09	8.75±0.07	4.90 ±0.07	13.60	0.00	0.094±0.08
PAT2	0.22±0.05	1.13 ±0.10	1.35	4.11±0.05	37.25 ±0.09	41.35	6.96±1.00	0.069±0.02
PAT3	0.33±0.02	2.12 ±0.10	2.45	5.21±0.04	51.91 ±0.05	57.12	11.35±1.00	0.123±0.00
PAT4	0.21±0.03	1.36 ±0.05	1.56	3.30±0.08	35.84 ±0.05	39.14	8.45±1.00	0.065±0.03
PAT5	0.42±0.06	2.37 ±0.05	2.79	6.56±0.09	55.52 ±0.05	62.08	2.56±0.05	0.147±0.05
PAT6	0.26±0.02	1.68 ±0.08	1.94	4.53±0.09	35.40 ±0.04	39.93	1.77±0.05	0.079±0.05

F-PC and B-PC expressed as g GAE/100 g flour; F-F and B-F expressed as g QE/100 g flour; anthocyanin expressed as g C3GE /100 g flour; alkaloids expressed as µg apomorfine chlorhydrate equivalents (ACE)/100 g flour

Table 2. HPLC-DAD-MS data of anthocyanins in phenolic-enriched methanol extract of *Prosopis nigra* pods meal

Peak	R_t (min)	Vis λ_{max} (nm)	$[M]^+$ (*m/z*)	MS/MS (*m/z*)	Tentative identificaction
1	9.2	-	595	449, 287	Cyanidin rhamnosyl hexoside
2	11.7	516, 280	449	287	Cyanidin-3-hexoside
3	15.4	517, 274	463	301	Peonidin-3-hexoside
4	16.5	515, 292	655	493, 331	Malvidin dihexoside
5	18.0	518, 284	535	449, 287	Cyanidin malonoyl hexoside
6	20.8	522, 269	479	317	Petunidin-3-hexoside
7	21.4	-	639	493, 331	Malvidin rhamnosyl hexoside
8	22.0	520, 272	493	331	Malvidin-3-hexoside

Table 3. Identification of phenolic compounds in *Prosopis alba* (PA) and *P. nigra* (PN) pods meal by HPLC-DAD-ESI-MS data. Q: quercetin; K: kaempferol.

Peak	R_t (min)	UV λ_{max} (nm)	$[M-H]^-$	Fragmentation pattern (relative abundance)	Tentative identificaction	Species
9	13.5	332, 271	593	575 (5), 503 (30), 473 (100), 383 (18), 353(28)	Apigenin-di-C-hexoside (Vicenin II)	PN
10	16.50	351, 295 sh, 265 sh, 257	771	609 (40), 591 (80), 505 (38), 343 (24), 301 (100), 300 (90)	Q-dihexoside rhamnoside	PA, PN
11	18.3	336, 271	563	503 (41), 473 (100), 443 (64), 383 (24), 353 (33)	Apigenin-C-hexoside-C-pentoside (Isoschaftoside)	PN
12	18.7	351, 295 sh, 265 sh, 257	625	505 (33), 463 (42), 445 (46), 343 (12), 301 (100), 300 (87)	Q-dihexoside	PA, PN
13	19.4	-	639	477 (100), 315 (1)	Q-methylether dihexoside	PA
14	19.6	336, 271	563	545 (16), 503 (10), 473 (100), 443 (50), 383 (30), 353(37)	Apigenin-C-hexoside-C-pentoside (Schaftoside)	PN
15	22.8	338, 295 sh, 268	431	341 (9), 311 (100)	Vitexin	PA
16	23.2	353, 290 sh, 266, 256	609	301 (100)	Q-rhamnoside-hexoside	PA
17	ca. 23.5	-	603	555 (5), 483 (36), 443 (100), 323 (5)	Unknown	PN
18	23.9	345, 275 sh, 267	609	301 (100)	Q-hexoside-rhamnose	PN
19	24.0	-	593	285 (100)	K-hexoside-rhamnoside	PN
20	24.1	338, 296 sh, 270	431	341 (31), 311 (100)	Isovitexin	PA, PN
21	24.9	-	463	301 (100)	Q-hexoside	PA, PN
22	25.0	-	447	285 (100)	K-hexoside	PA, PN

23	28.5-29.5	-	577	269 (100)	Apigenin hexoside-rhamnoside	PN
24	31.9	-	623	477 (1), 300 (33), 315 (100)	Q methyl ether-hexoside-rhamnoside	PN
25	32.0	347, 298 sh, 265	607	299 (100), 284 (9)	K-methyl ether hexoside-rhamnoside	PN

Table 4. Radical cation ABTS scavenging activity of *P. nigra* (PNT) and *P. alba* (PAT) pods flour extracts obtained by differential methods: anthocyanin, free phenolic compounds and bound-phenolic compounds.

		P. alba				*P. nigra*			
	Extraction method	Sample	$ABTS^{+\bullet}$	$DPPH^{\bullet}$	AA	Sample	$ABTS^{+\bullet}$	$DPPH^{\bullet}$	AA
			SC_{50}	SC_{50}	IC_{50}		SC_{50}	SC_{50}	IC_{50}
Anthocyanin	H_2O/H^+					PNT5	1.2 ± 0.5^{a}*		
	$EtOH/H^+$					PNT5	$8.3\pm0.5^{c,d,e,f}$*		
	$H_2O/H^+/EtOH$					PNT5	$7.4\pm0.5^{b,c,d,e}$*		
	$MeOH/H^+$					PNT5	1.5 ± 0.5^{a}*		
Free phenolic compounds	$MeOH/H_2O$	PAT1	13.0 ± 1.1^{b}	15 ± 0.5^{c}	14.5 ± 0.5^{b}	PNT1	22.0 ± 1.0^{i}	ND	$7.5\pm0.5^{c,d,e}$
		PAT2	13.0 ± 1.1^{b}	$13\pm0.5^{b,c}$	$11.0\pm0.5^{a,b}$	PNT2	23.0 ± 1.0^{i}	ND	$3.0\pm0.5^{a,b}$
		PAT3	6.0 ± 1.0^{a}	$14\pm1.0^{b,c}$	10.0 ± 1.0^{a}	PNT3	29.0 ± 1.0^{j}	20 ± 1.0^{a}	$9.0\pm1.0^{d,e,f}$
		PAT4	7.0 ± 1.0^{a}	ND	$13.0\pm1.0^{a,b}$	PNT4	22.0 ± 0.5^{i}	19 ± 1.0^{a}	$7.5\pm0.5^{c,d,e}$
		PAT5	16.0 ± 0.5^{b}	ND	$13.0\pm1.0^{a,b}$	PNT5	15.0 ± 0.5^{h}	ND	$7.0\pm1.0^{c,d,e}$
		PAT6	17.0 ± 0.5^{b}	11 ± 1.0^{b}	$12.5\pm0.5^{a,b}$	PNT6	$7.0\pm0.5^{b,c,d,e}$	ND	1.5 ± 0.5^{a}
						PNT7	$10.0\pm1.0^{e,f,g}$	22 ± 0.5^{a}	$4.0\pm0.5^{a,b,c}$
						PNT8	$12.0\pm1.0^{f,g,h}$	ND	$9.5\pm1.0^{d,e,f}$
Bound-phenolic compounds		PAT1	4.5 ± 1.0^{a}	ND	10.0 ± 1.0^{a}	PNT1	$6.0\pm.0.5^{b,c,d}$	30 ± 0.5^{b}	$5.0\pm0.5^{a,b,c}$
		PAT2	3.5 ± 1.1^{a}	1.5 ± 0.5^{a}	$13.0\pm0.5^{a,b}$	PNT2	$12.5\pm0.5^{g,h}$	17 ± 1.0^{a}	$9.0\pm0.5^{d,e,f}$
		PAT3	2.5 ± 1.0^{a}	1.5 ± 0.5^{a}	$12.5\pm0.5^{a,b}$	PNT3	$4.0\pm0.5^{a,b}$	17 ± 1.0^{a}	$6.5\pm0.5^{b,c,d}$
		PAT4	3.0 ± 0.5^{a}	1.5 ± 0.5^{a}	$10.0\pm1.0^{a,b}$	PNT4	$9.5\pm0.5^{d,e,f,g}$	ND	$11.5\pm0.5^{f,g}$
		PAT5	7.0 ± 0.5^{a}	1.5 ± 0.5^{a}	$11.5\pm0.5^{a,b}$	PNT5	$4.5\pm0.5^{a,b,c}$	18 ± 1.0^{a}	$6.5\pm1.0^{b,c,d}$
		PAT6	3.0 ± 0.5^{a}	1.7 ± 0.5^{a}	$11.5\pm0.5^{a,b}$	PNT6	ND	ND	14.0 ± 0.5^{g}
						PNT7	ND	17 ± 1.0^{a}	$10.5\pm0.5^{e,f,g}$
						PNT8	ND	17 ± 1.0^{a}	$10.0\pm0.5^{d,e,f}$

Values are reported as mean±standard deviation of triplicates. Different letters inthe same column show significant differences among each treated group, according to the Tukey test ($p \leq 0.05$).

SC_{50} : Scavenging activity of 50% $ABTS^{+\bullet}$ or $DPPH^{\bullet}$ .The values are expressed in µg GAE/mL in free phenolic compounds or bound phenolic compounds

*SC_{50} values expressed in µg *C3* GE/mL.

IC_{50} : inhibitory concentration of 50% lipoperoxidation. The values are expressed in µg GAE/mL. ND: not determined because the maximal inhibition was around 30%

Table 5. Anthocyanin content in *Prosopis nigra* pods flour. The concentration of anthocyanin was determined immediately after flour extraction (T_0) and after three months of extracts storage at 4 °C (T_3). The total anthocyanins content was determined under different extracting conditions.

Extraction solvent	Anthocyanins (g/100 g pods flour)	
	T_0	T_3
H_2O	12.44±0.20[a]	0.91±0.10[a]
H_2O/H^+	13.98±0.20[b]	12.41±0.30[a]
EtOH	0	0
$EtOH/H^+$	0	0
H_2O/EtOH	18.13±0.30[c]	8.00±0.10[a]
H_2O/H^+/EtOH	12.08±0.20[a]	10.74±0.20[a]
$MeOH/H^+$	12.08±0.30[a]	8.00±0.10[a]

Values are reported as mean±standard deviation of triplicates. Different letters inthe same column show significant differences among each treated group, according to the Tukey test ($p \leq 0.05$).

Highlights

The polyphenolic composition of *Prosopis* flour was analyzed by **HPLC-ESI-MS/MS.**

The main phenolics were quercetin *O*-glycosides and apigenin-based *C*-glycosides.

The *P. nigra* flour showed the occurrence of anthocyanins profile.

P. nigra and P. alba flour contained bioactive polyphenols with impact in health

This is the first report on phenolic composition of *P. alba* and *P. nigra* flour