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Chemical and nutritional properties of different fractions of *Prosopis alba* pods and seeds

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Abstract The objective of this work was to study chemical and nutritional aspects of different fractions of *Prosopis alba*. Flours from whole pod, pericarp (pulp) and seeds were obtained. Polyphenols were mainly located in pulp but antioxidant activity was higher in whole pod flour and seeds. In seeds, the fraction with the highest polyphenols and antioxidant activity was the seed coat or testa. Protein content was higher in whole pod flour (5.81 %) than in pulp flour (3.52 %), presenting the seed an appreciable amount 33.6 %. These proteins were composed by monomer subunits of 85, 67, 38, 16 and 14 kDa and no prolamins and anti-tryptic activity were detected. *P. alba* flours presented high content of soluble sugars, mainly composed by sucrose, and also high amount of insoluble dietary fiber. The major mineral was potassium. The whole pod, due to the contribution of seeds, contained high amount of calcium, magnesium, iron and zinc, all indispensable minerals for human nutrition. Therefore, *P. alba* flours, mainly containing the seeds, constitute nutritional ingredients for bakery and gluten free products.

Keywords *Prosopis alba* pods · *Prosopis alba* seeds · Protein profile · Mineral composition · Nutritional value

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Introduction

American carob belongs to *Prosopis sp*. with almost 45 species distributed along America, Africa and Asia. Argentina constitutes the country with the greatest diversity (nearly 27 species) all over the world [1]. Flours obtained from roasted pods of both *Ceratonia siliqua* and *Prosopis sp.* are used as cacao and coffee substitutes with the advantage that do not contain the excitant substance caffeine [2]. *Prosopis sp.* flours can be used, due to its high sugar content, excellent taste and aroma, for flavoring applications and as ingredients in pastries, muffins and biscuits. Despite this wide possibility of uses, this flour is not generally employed in any commercial application at a significant scale.

The fruit of *Prosopis sp.* is a pod formed by pericarp and seeds. The pericarp is constituted by a thin epicarp, a cork like mesocarp and a woody endocarp which surrounds the seeds. The seed is formed by episperm, endosperm and cotyledons [3, 4]. Different parts of *Prosopis pallida* and *Prosopis juliflora* pods were analyzed by Grados and Cruz [3]. These researchers studied different parts of pericarp (exocarp, mesocarp or pulp, endocarp) and seeds (episperm, endosperm and cotyledon). The main sugar was sucrose (46 g sucrose/100 g pulp) and the polysaccharide of the endosperm was a galactomannan. In the pulp, vitamin C, nicotinic acid, and calcium pantothenate were also found. A significant content of vitamin C and E was found in the seed. The dietary fiber of the pulp and endocarp hulls was basically insoluble dietary fiber. Meyer et al. [5] studied the chemical composition of different fractions of pods from *Prosopis velutina*. Exo and mesocarp powders contained most of the pod sugar and flavor components while endocarp hulls were comprised mainly of fiber. From the seed a gum fraction with a mannose to galactose ratio

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of 1.55 was obtained. Galactomanans derived from *Prosopis sp.*, named mesquite gums, were also widely studied due to its application as food additives [6] in dietetic foods and foods for diabetic people; also as substitute of Arabic, guar and LBG in gum candies.

One of the most important food components are polyphenols due to their proved antioxidant capacity. Tissue damage due to free radicals conduct to oxidative stress that affect different structures and cellular components, promoting degenerative conditions for different diseases such as cancer, diabetes or cardiovascular disorders. In such way, molecules with antioxidant activity such as polyphenols contribute to decreasing risk of development such chronic diseases [7]. C-glycosyl flavonoids and O-flavonol glycosides were found to be the main constituents identified in phenolic-enriched extracts of algarrobo pods [8]. Eight and fourteen flavonoid glycosides were identified in *P. alba* and *P. nigra* pods extract, respectively. These molecules exhibited antioxidant and anti-inflammatory activities in vitro. Although in vitro studies can afford valuable biochemical information, extrapolation of the obtained data must be careful. Few in vitro studies utilize concentrations sufficiently low to have any relevance to potential bioactivities in vivo [9]. Kubatka et al. [10] revealed that high-dose of polyphenol sample, in a breast cancer model with female rats, inhibited tumor frequency by 58 % and tumor incidence by 24 %, and lengthened latency by 8 days in comparison with healthy animals. Histopathological analysis of tumors showed significant decrease in the ratio of high/low grade carcinomas.

A single polyphenol can generate several metabolites during absorption, thus, the compounds that reach cells and tissues are chemically, biologically and functionally different from those of the dietary form. Most dietary polyphenols are converted in glucuronide, methyl and sulfate metabolites in the small intestine, while in the large intestine are breakdown to phenolic acid and nonphenolic catabolites. Though, the protective effect of dietary phenolics was thought to be due to their antioxidant properties (decrease of free radicals), there is now evidence that the metabolites of dietary phenolics, which appear in the circulatory system in very low concentrations, exert modulatory effects in cells through selective actions on different components of the intracellular signalling cascades, vital for cellular functions [9].

Some studies have been performed on nutritional composition and bioactivity of flours and seeds of *Prosopis alba* [11, 12]. However, an analysis about the distribution of components, particularly protein and minerals, in the different parts of pod and seed has not been yet reported. This information would be useful to establish nutritional quality of flours and the contribution of seed to this nutritional profile. Therefore, the objectives of the present work were to distinguish protein and non-protein nitrogen contents, to determine protein content and analyze the protein profile, to evaluate polyphenols and antioxidant activity, carbohydrates and fiber amounts and mineral composition (major and trace elements) in different parts of *P. alba* seeds and pods.

Materials and methods

Separation of different fractions from *Prosopis alba* pods and seeds

Prosopis alba fruits utilized were collected in Ingeniero Juárez (Formosa, Argentina) (harvested on December 2010), and provided by the germplasm bank of Faculty of Natural Sources of the National University of Formosa (UNaF). A first step of hydration of pods was performed for improving seeds separation. The remainder fraction after seeds separation constitutes the pulp. Pod samples, with and without seeds, were then dried in forced-convection oven (Sanjor SL60SF, Buenos Aires, Argentina) at 80 °C during 4 h. Average size and weight of whole pods and seeds were determined.

After drying process, samples were ground in a domestic mill (1.3 HP power, Moulinex, Alençon, France). Ground samples were passed through sieves of 1000 μm (#18) and 500 μm (#35) (ALEIN, Munro, Argentina) according to ASTM specifications [13]. Coarse, medium and fine fractions were obtained; the fine fraction, with a milling yield of 60 %, was utilized for the assays. Whole pod and pulp flours were stored at 4 °C until analysis.

Seeds were separated into three fractions: testa, endosperm and germen. Seeds were immersed in boiled water for hydration up to reaching 20 °C and then were rested overnight at 4 °C. After hydration, the three parts were manually separated and freeze-dried.

Moisture of all flours was determined by vacuum drying (50 mm Hg) at 70 °C during 2 h [14].

Composition of different fractions from *Prosopis alba* pods and seeds

Nitrogen content and protein analysis

Total nitrogen content was determined by Kjeldahl method [14]. Non protein nitrogen was determined by Kjeldahl method in the soluble fraction after precipitation of proteins with 24 % trichloroacetic acid (TCA) and ulterior centrifugation at $6300 \times g$ during 15 min at 20 °C. Protein content was calculated considering protein nitrogen as the difference between total and non-protein nitrogen, and multiplying this value by the 6.25 factor.

Proteins, obtained by extraction from samples in different media, were analyzed by electrophoresis (SDS-PAGE) under different conditions. With the aim of removing lipids and polyphenols, samples were washed with petroleum ether at 20 °C during 40 min, and then centrifuged at 9360×*g*, 15 min at 4 °C. The precipitate was washed with the water:acetone (1:1) solvent at 20 °C during 40 min. After that, proteins were extracted for 1 h at 40 °C with a 0.086 M Tris-base, 0.045 M mM glycine, 2 mM EDTA, pH 8.3 buffer (B), the same buffer containing 2 % sodium dodecyl sulfate (SDS) (B_{SDS}) and the pH 8.3 buffer containing 2 % SDS plus 0.5 % DTT ($B_{SDS+DTT}$). Dispersions were centrifuged at 9360×*g* for 10 min at 15 °C. The supernatant was analyzed by SDS-PAGE. A 12 % continuous running gel with a 4 % stacking gel was prepared. A dissociating buffer system containing 1.5 M Tris-base, 0.5 % SDS, pH 8.8 for the separating gel and 0.125 M Tris-base, 0.96 M glycine, and 0.5 % SDS, pH 8.3, for the running buffer, was utilized. Electrophoresis was performed in a Mini Protean III at a constant voltage of 60 V (stacking gel) and 120 V (continuous gel) with a Power-Pack 300 (Bio-Rad, Richmond, CA, USA). Low MW markers (Amersham calibration kit, GE, USA) used included Phosphorylase b (97 kDa), Albumin (66 kDa), Ovalbumin (45 kDa), Carbonic Anhydrase (30 kDa), Trypsin Inhibitor (20.1 kDa) and α-Lactalbumin (14.4 kDa).

Absence of prolamins was checked by the competitive ELISA method with polyclonal antibodies developed by Chirdo, Añón [15]. Briefly, sequential competitive ELISA was based in the use of rabbit polyclonal anti-gliadin antibodies. A pre-incubation step was carried out by incubating during 2 h at 37 °C, the appropriated dilution of polyclonal antibody and the ethanolic extracts (70 % ethanol) from samples or standard for the calibration curve. Next, each pre-incubated sample was added to gliadin coated wells and incubated during 30 min at 37 °C. Goat anti-rabbit IgG horseradish peroxidase conjugate (Bio-Rad) was incubated for 1 h at 37 °C. The color reaction was developed using *o*-phenylenediamine. The enzymatic reaction was stopped after 20 min with 2 M-sulphuric acid. The optical density was determined at 490 nm.

Soluble sugars and dietary fiber

Soluble sugars were identified and quantified by HPLC method described by Eliasson [16]. Samples (1 g) were defatted with hexane at 40 °C by stirring 1 h at 650 rpm and centrifuged 10 min at 2655×*g*. Supernatant was discarded and 13 mL of MiliQ water was added. One mL of potassium ferrocyanide (15 % w/v) and 1 mL of zinc acetate (30 % w/v) solutions were incorporated. Dispersion was stirred during 30 min at 70 °C. After reaching ambient temperature, 10 mL of acetonitrile was added. Samples were centrifuged 10 min at 2655×*g*. Supernatants were filtered through a filter of 0.45 μm pore diameter.

Extracts were analyzed using an HPLC Waters 1525 (Millipore Corp., Milford, MA, USA). A Hypersil Gold Amino 250 column (i. d. 4.6 mm) of 25 cm large (particle size: 5 μm) maintained at 35 °C, and a refraction index detector was used. A system of acetonitrile:water 80:20 was used as mobile phase at a flow rate of 1.2 mL/min. Standards of glucose, fructose and sucrose (20 mg/mL, Sigma Corp.) were used for calibration curve. Curve areas were analyzed by PeakFit v4.12 software (Systat Software, California, USA).

Total dietary fiber and insoluble dietary fiber were determined according to AOAC methods [14] by enzymatic hydrolysis using the Megazyme (K-TDRF) kit (Megazyme, Wicklow, Ireland).

Lipid and moisture content

Flours were first dried at 100 °C during 2 hs and then lipids were extracted during 2 h with petroleum ether (boiling range 35–60 °C) in a Soxhlet extractor and weighed [14]. For moisture determination, samples were dried in a convection air oven at 135 °C during 2 h [14].

Nutritional characterization of different fractions from *Prosopis alba* pods and seeds

Mineral profile

Ash was analyzed by incineration in a muffle furnace at 550 °C [14]. Minerals were determined from ash samples in a Shimadzu AA-6650 (Kyoto, Japan). Ashes were dissolved in nitric acid solution (1:1). For calcium determination, samples were diluted with lanthanum oxide at final concentration of 0.5 % w/v. Iron, calcium, manganese, zinc and magnesium were determined by atomic absorption spectroscopy, using a lamp with an empty cathode of the same metal. Potassium levels were determined by atomic emission spectroscopy. Wavelengths were 248.3, 422.7, 285.2, 766.9, 279.5 and 213.9 for Fe, Ca, Mg, K, Mn and Zn, respectively. Acetylene was used as combustible and air as oxidant. Assays were performed by duplicate.

Polyphenols and antioxidant activity

Polyphenols were extracted from samples with 50 % acetone–water solution (3:1 solvent:sample) with stirring in a Eppendorf Thermomixer Comfort (Eppendorf, Hamburg, Germany) at 650 rpm during 40 min at 4 °C [17]. Extracts were centrifuged in a microfuge at 2655×*g* during 10 min at 20 °C. Supernatant was utilized for determining polyphenol content and antioxidant activity. Total polyphenols was

determined by Folin–Ciocalteau method and expressed as gallic acid equivalents [18].

Antioxidant activity was assayed using the method of the [2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)] radical ($ABTS^{\cdot+}$) [19]. A stock solution 7 mM of $ABTS^{\cdot+}$ ammoniac salt containing 2.45 mM of potassium persulfate was prepared and stored overnight in the dark at 20 °C. This solution of $ABTS^{\cdot+}$ was diluted with ethanol up to obtaining an absorbance value of 0.700 ± 0.03 at 734 nm. One mL of the $ABTS^{\cdot+}$ ethanolic solution was added to 10 µL of the acetone sample extract, this solution was mixed during 20 min and the absorbance was measured at 734 nm. Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) was used as standard and results were expressed as Trolox equivalent antioxidant capacity in µmol Trolox equivalents/100 g of dried sample.

Antitryptic activity

The presence of protease inhibitors (anti-nutritional factors) was analyzed using the method of Sobral and Wagner [20]. Porcine pancreatic trypsin (90 U/mg) and bovine hemoglobin were used as enzyme and substrate. Peptides released due to enzymatic hydrolysis were quantified by Folin–Ciocalteau method. Absorbance was measured at 650 nm. Soybean protein isolate, containing trypsin inhibitor, was used as control sample.

Statistical analysis

All measurements were performed by duplicated. Statistical analysis was performed using the Origin 8 Pro software (Origin Lab Corporation, Northampton, USA). Parameters were subjected to one-way ANOVA according to the general linear model procedure with least-square mean effects. Significantly different means were determined according to Fisher's least significant differences (LSD) test. Mean and standard deviation were calculated for each parameter.

Results and discussion

Morphological characterization of *Prosopis alba* pods and seeds

Prosopis alba pod (Fig. 1) present a medium size of 17.0 ± 3.3 cm large and weighs 5.7 ± 1.5 g; and contains 26 ± 4 seeds which correspond to 10 % of the total weight of the pod (Fig. 1a). Each seed has a medium size of 5.61 ± 0.56 mm large and 3.27 ± 0.34 mm width and weigh 27.0 ± 4.4 mg (Fig. 1b-A). Seed was formed by three fractions: coat or testa (Fig. 1b-B), endosperm (Figura 1b-C), and germ that contains cotyledons (Fig. 1b-D); in proportion

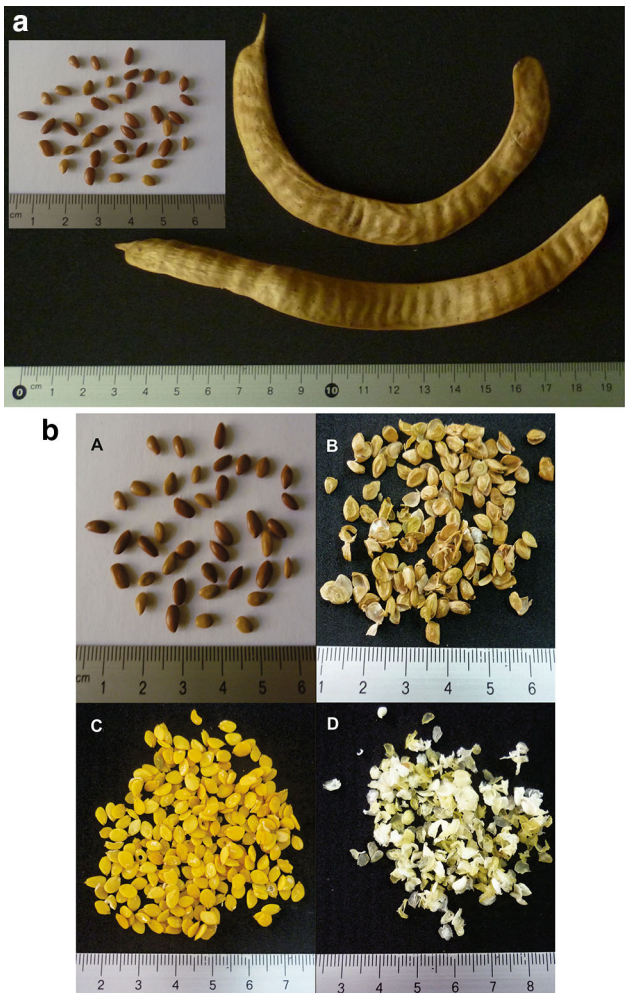

Fig. 1 Pods and seeds of *Prosopis alba* (**a**). Different parts of seeds (**b**): (*A*) whole seed, (*B*) coat seed or testa, (*C*) endosperm, (*D*) germen

of 1:1:2 with values in percentage of 24.3, 22.8 and 52.9 %, respectively. *P. alba* presented some differences respect to other species of *Prosopis sp*. Bravo, Grados [6] reported almost equivalent pod size (19 × 2 cm) and a weight of 12 g for *P. pallida*, with the same number of seeds in a pod. Zolfaghari, Harden [21] reported a proportion of seeds in pods of 15 % (w/w) for *Prosopis glandulosa*, while Becker and Grosjean [22] found a relation seed:pericarp 25:75 for *P. velutina*. *C. siliqua* L. seed was formed by a coat (30 %) rich in antioxidants, endosperm (45 %) and germ (25 %) [23]. *P. alba* presented, as in the case of *C. siliqua* L., extremely hard seeds but with high content of germ fraction.

Polyphenol content and antioxidant activity of different fractions of *Prosopis alba* pods and seeds

Polyphenols are important components in a food ingredient, due to their proved antioxidant capacity. Polyphenols

content and antioxidant activity of different fractions of *P. alba* pods are shown in Fig. 2. No significant differences in polyphenol content between flours from whole pod (pod with seeds) and pulp (pod without seeds) were observed (Fig. 2a). Results suggest that due to the proportion of seeds in pods (10 % of pod weight) polyphenols are mainly present in the exterior part of the fruit. Nevertheless, antioxidant activity of seeds (2865 µmol Trolox/100 g d.w.) was significantly and approximately three times higher than that detected in flours (801 or 984 µmol Trolox/100 g d.w.), suggesting that in the seed, besides polyphenols, there are other components responsible of this activity, like unsaturated lipids. Pod flour presented a higher content of lipids (1.51 ± 0.28 g/100 g d.w.) than pulp flour (1.11 ± 0.07 g/100 g d.w.), suggesting there is a contribution of seeds to the lipid amount of pod. Lipid content of seeds can be approximated considering the difference in lipid content of pod and pulp flours, jointly with the average weight of a pod, a seed and the number of seeds in a pod. Calculation conducted us to a lipid content value of 10.37 g lipids/100 g seeds. This value is a bit lower than that obtained experimentally by Lamarque et al. [24] (12.7 % w/w of lipids) for *P. alba* seeds. Seed components with antioxidant activity would probably be unsaturated lipids, these authors [24] also reported a composition (based on total lipid content) of 27.6 and 52.5 % of oleic and linoleic acids, respectively. Comparing to *P. alba*, Escobar, Estévez A [25] found a low value of lipids (10.2 %) in seeds of *Prosopis chilensis*.

Analysis of different part of *P. alba* seed (Fig. 2b) shows that the great part of polyphenols are concentrated in the seed coat or testa (2.02 ± 0.05 g GAE/100 g d.w.). Very low and non-significantly different values between germen (0.143 ± 0.006 g GAE/100 g d.w.) and endosperm

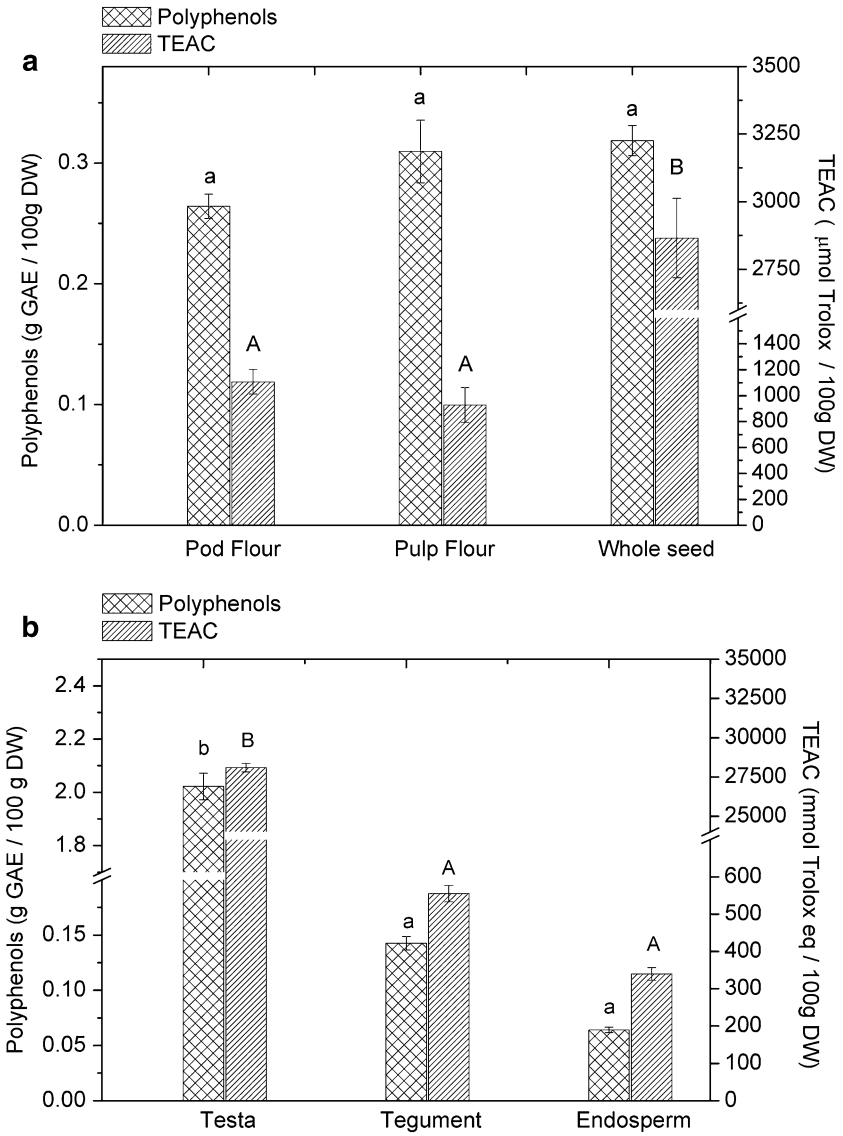

Fig. 2 Polyphenol content and antioxidant activity of different fractions of *Prosopis alba*. Different fractions of pods (**a**) and seeds (**b**)

(0.064 ± 0.002 g GAE/100 g d.w.) were detected. Antioxidant activity also resulted considerably higher in testa (28094 ± 269 μmol Trolox eq/100 g d.w.) respect to germen and endosperm. Results suggest that polyphenols and other components of seeds with antioxidant capacity are mainly located in testa.

Nitrogen and protein content of different fractions of *Prosopis alba* pods and seeds

One of the main and most important food components from the nutritional point of view is protein. The level of total nitrogen of whole pod flour was significantly superior (48 %) to that obtained for pulp flour, suggesting that seed makes an important contribution to this parameter. Zolfaghari, Harden [21] found for *P. glandulosa* pod a high content of non-protein nitrogen in pericarp and protein nitrogen in seeds. Therefore, non-protein nitrogen was studied in *P. alba* fractions. This parameter resulted to be 26 and 36 % of the total nitrogen for pod and pulp flour, respectively (Table 1). No significant differences between non-protein nitrogen of both flours were observed; although these values were different from that obtained for seeds. Seeds presented a low value of non-protein nitrogen content and represented 10 % of total nitrogen of this pod component. Calculating protein content with the value of protein nitrogen and using the 6.25 factor considered previously by Cattaneo et al.[11] for these proteins, pod flour presented considerable higher protein content than pulp flour, suggesting an important contribution of seeds to the protein content of the whole pod. The whole seed presented a protein content of 32.3 %, whereas the value for germen was 63.7 %, coincident with the proportion of germen in seed. Similar results were found by Escobar, Estévez A [25] for germen of *Prosopis chilensis* with a protein value of 63.6 %.

Not only is important the quantity, also the quality of protein and the proportion of different amino acids, mainly the essential ones and their relationship with human nutrition requirements. Cattaneo et al. [11] found that amino acids present in high proportion were glutamine, arginine and asparagine, followed by proline and serine. Felker and Bandurski [26] previously found for *P. chilensis* and *juliflora* a similar amino acid composition than that obtained for *P. alba*. Meyer et al. [5] reported that limiting amino acids for *P. velutina* were tyrosine and methionine + cysteine. The high content of glutamine and arginine of germen converts this fraction in a potentially valuable food ingredient for sportsmen due to the contribution to the increase of muscular mass, collagen synthesis and glycogen production.

González Galán etal. [27] reported values of protein between 8.8 and 11.0 % (d.w.) for different *Prosopis* species, belonging the last value to *P. alba*. Discrepancies in protein content value could be due to the fact that non-protein nitrogen was not subtracted from total nitrogen content. Our results agree with those obtained by Zolfaghari et al. [21]. These authors reported contents of total nitrogen of 1.70 and 0.84, and values of non-protein nitrogen of 0.41 and 0.31 for pods and pulp, respectively. They also studied nitrogen content in seeds and they proposed that most of the non-protein nitrogen of the pod belongs to pulp, while most of protein nitrogen is contributed by the seed. The non-protein nitrogen of the pulp is mainly inorganic nitrogen in the form of NO_3^- and NH_4^+ [22].

It is well known that the nature of proteins (globular or fibrillar) as well as their structure (secondary and tertiary) are important factors to be considered when analyzing their functional properties in foods. The type of proteins present in *P. alba* seeds were studied by electrophoresis, whose profiles are shown in Fig. 3.

The pH 8.3 buffer (B), a medium of low ionic strength, was not able to extract proteins from seeds, suggesting that proteins are stabilized with non covalent (hydrophobic and hydrogen bonds) and covalent (disulphide) bonds. The incorporation of SDS to the buffer (B_{SDS}) favored protein solubilization; more quantity of bands could be observed. High molecular mass aggregates, and also proteins of 38, 16 and 14 kDa were extracted. In less proportion, series of proteins with molecular mass between 40 and 85 kDa were observed. This dissociating agent allowed rupturing hydrogen and mainly hydrophobic bonds from insoluble protein aggregates. When the disulphide reducing agent dithiothreitol (DTT) was incorporated to buffer B, it led to dissociation of insoluble and soluble protein aggregates. Thus, defined bands of proteins of 85, 67 and 38 kDa were obtained indicating that these proteins were linked with disulphide bonds forming the aggregates. Besides, proteins of 16 and 14 kDa, as in the case of B_{SDS}, were also

Table 1 Nitrogen and protein content of different fractions of *Prosopis alba* pods

g/100 g sample (d.w.)	Pod flour	Pulp flour	Whole seed
Total Nitrogen	1.26 ± 0.02a	0.88 ± 0.01b	5.753 ± 0.072c
Non Protein Nitrogen	0.326 ± 0.001a	0.316 ± 0.04a	0.585 ± 0.001b
Protein (%Npx6.25)	5.81	3.53	32.3

Different letters in the same row indicate significant differences ($p < 0.05$) among samples

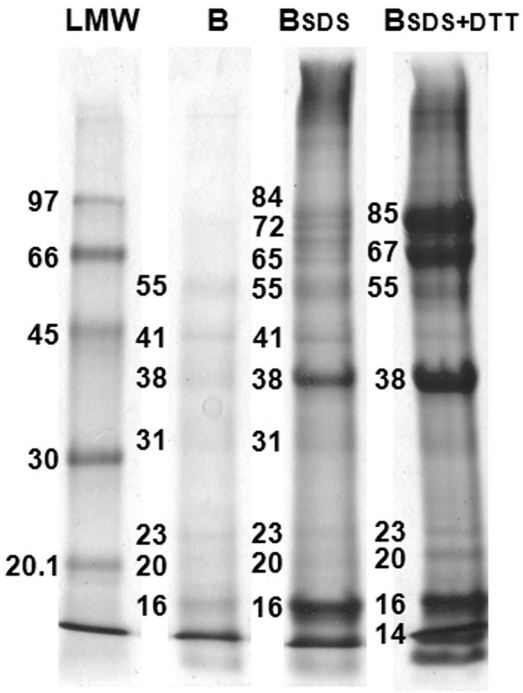

Fig. 3 SDS-PAGE of *Prosopis alba* seed proteins. Proteins extracted under: non dissociating (*B*), dissociating (B_{SDS}) and reducing ($B_{SDS+DTT}$) conditions. *LMW* low molecular weight markers

observed. The 38 kDa protein was probably linked by disulphide bonds but also by non covalent bonds, as it can be deduced from its presence in the B_{SDS} profile. Proteins of 85 and 67 kDa, that were absent in B_{SDS} extract, would be considered as monomers, due to the fact that were only extracted with the $B_{SDS+DTT}$ buffer. *P. alba* proteins were quite different from those present in European Carob *C. siliqua* or soybean (*Glycine max*.). Bengoechea et al. [28] also extracted from carob, with SDS, soluble aggregates of high molecular mass and a protein of 70 kDa, that after the action of β-mercaptoethanol were dissociated in two monomers of 48 and 24 kDa. Soybean seed present in major proportion two types of globulins [29]: the 7S and 11S globulins. The 7S globulin that is a trimeric glycoprotein (150 kDa) composed of three subunits: α', α and β of 85, 80 and 50 kDa, respectively, associated by hydrophobic bonds; while the 11S globulin consists of two apposed hexagonal rings each containing three hydrophobically associated subunits consisting of pairs of 55 kDa of disulfide-linked acidic (35 kDa) and basic (20 kDa) polypeptides. We can deduce from Fig. 3 that *P. alba* do not present the 11S neither the 7S globulins observed in soybean. *C. siliqua* and *P. alba* seeds, despite they belong to Fabaceae family like soybean, presented very different protein composition.

The type of proteins present in seed flours will influence the functional properties and determine the kind of food product that is able to be obtained. These proteins did not present antitryptic activity, possibly due to the heat treatment performed on pods before milling. In addition, the absence of prolamins (limit content: 0.1 mg/100 g flour) in pulp and pod flours was confirmed with the competitive enzyme-immune assay employing polyclonal antibodies performed according to Chirdo et al. [15] suggesting that *P. alba* flours constitute suitable ingredients for gluten free products.

Sugar and fiber of different fractions of *Prosopis alba*

Table 2 shows that the major sugar present in *P. alba* fruit was sucrose, while the minor component was glucose. High content of total sugars (sucrose, glucose plus fructose) was found in pulp flour (70.7 % w/w, d.w.) in comparison to whole pod flour (62.7 % w/w, d.w.), suggesting that these components would be absent in seeds. Da Silva et al. [30] reported for flour of whole pod of *P. juliflora* from Brazilian northeast, less content of total soluble sugars (56.5 %).

Total dietary fiber content was higher, although insoluble dietary fiber was lower in pod flour, comparing to pulp flour (Table 2). These results suggest that seeds contribute to fiber, mainly with soluble fiber, like galactomannans (mesquite gum) that are included in endosperm fraction. This value of total dietary fiber of *P. alba* was lower than that observed in *P. pallida* (31 %) but higher than the value detected in *C. siliqua* (14 %) [6]. Choge et al. [31] found for whole pod of *Prosopis julliflora* of Kenya a content of total soluble sugars significantly lower (13 % d.w.) than the value obtained for *P. alba* (62.7 % d.w.), with less amount of glucose (0.8 % d.w.) and sucrose (7.5 % d.w.). Nevertheless, levels of total dietary fiber were approximately double (48 % d.w.). In our case, fiber could have been retained in the coarse fraction after sieving process. Bravo et al. [6] found for *P. pallida* and also for *C. siliqua* pulps a total soluble sugar content of about 50 %, being sucrose the main constituent of this fraction (95 % of total sugars). Glucose, fructose, galactose and arabinose were the minor constituents. In our case, sucrose represented the 60 % of total sugars.

The high fiber content of *Prosopis sp*., from nutritional point of view, converts these flours in potential ingredients for formulations of enriched-fiber bakery products. There are no reports up to the moment regarding to nutritional value of *Prosopis sp.* evaluated through assays in humans; however, Mediterranean carob has been more studied. Zunft et al. [32] found that carob pulp enriched in insoluble fiber lowers total and LDL cholesterol in hypercholesterolemic patients. Gruendel et al. [33] showed that the consumption of a carob pulp beverage from *C. siliqua*,

Table 2 Sugar and fiber content of different flours of *Prosopis alba* pods

g/100 g (d.w)	Sucrose	Glucose	Fructose	FDT	FDI
Pod flour	41.4 ± 0.63a	9.60 ± 0.13a	11.7 ± 0.11a	25.1 ± 0.33a	20.9 ± 0.64a
Pulp flour	44.1 ± 0.32a	12.5 ± 0.32b	14.1 ± 0.42a	22.6 ± 0.54b	22.1 ± 0.45a

FDT total dietary fiber, *FDI* insoluble dietary fiber

Different letters in the same column indicate significant differences ($p < 0.05$) among samples

enriched in insoluble dietary fiber and polyphenols, decreases LDL cholesterol and lowers triglycerides mainly in females; these beneficial effects on human blood lipid profile may be effective in prevention and treatment of hypercholesterolemia disease.

Mineral content of different fractions of *Prosopis alba*

The ash content of pod and pulp flours were 3.373 ± 0.001 and 3.263 ± 0.081 (g/100 g d.w.), respectively. Ashes contain minerals that are essential in human nutrition. Among the major minerals studied, potassium was the one present in great proportion, followed by calcium and magnesium (Fig. 4a). In addition, pod flour presented the highest amount of potassium, suggesting that the major quantity of this mineral is provided by the pulp. The content of calcium of this fruit was significantly lower than the potassium one (Fig. 4a); although it was high (ten times) respect to other flours such as cereal flours [34]. Seeds contributed considerable to calcium content of pod. It can also be observed that almost all magnesium was provided by seeds; this is an expected fact because this mineral is essential for the formation of chlorophyll molecule that participates in photosynthesis process originated in cotyledons.

Calcium is an important macronutrient because an adequate life-long intake of this mineral can reduce osteoporosis risk in elders. The recommended daily intake (RDI) of calcium, based in a diet of 2000 calories for females between 30 and 50 years old, is 1000 mg (USDA, 2012). In Argentina calcium result a deficient nutrient presenting 94 % of women between 10 and 49 years old an intake below the RDI [35]. Calcium content obtained for pulp flour of *P. alba* was double of that obtained by Martinez Meyer et al. [36] for *Phaseolus vulgaris* L. flour; and was close to the content present in lupine seeds (*Lupinus albus*) (176 mg Ca/100 g sample) [34]. Therefore, the high quantity of this mineral makes *P. alba* flours from whole pod or from seeds, a potential ingredient for calcium supplementation in diet. Magnesium content of these carob flours (69.2 and 49.6 mg/100 g pod and pulp flour, respectively) (Fig. 4a) was similar to that found in *C. siliqua* (54 mg/100 g flour); and higher and lower than values detected for wheat (25 mg/100 g flour) and lupine (198 mg/100 g flour) samples, respectively.

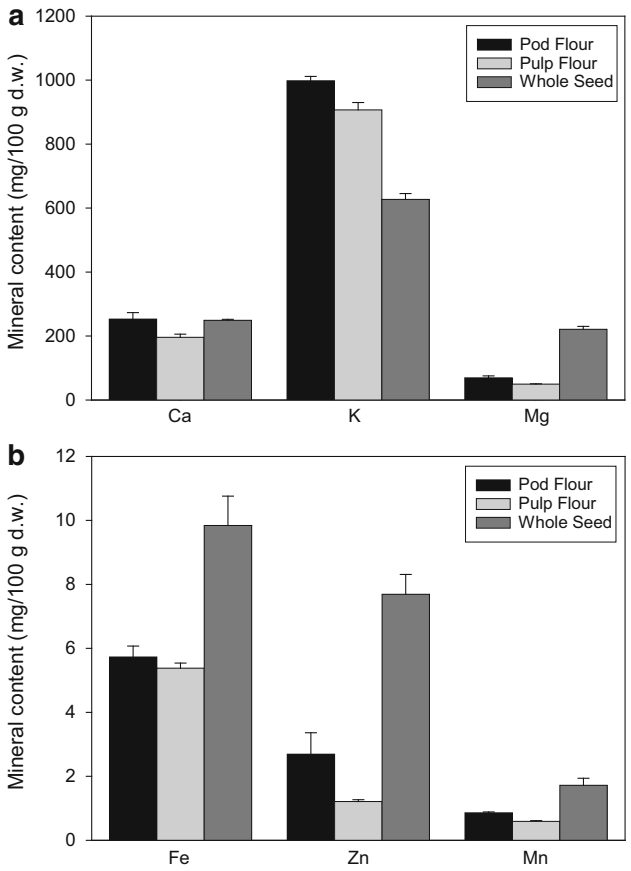

Fig. 4 Mineral content of pod and pulp flour and whole seeds of *Prosopis alba*. **a** Major minerals. **b** Trace minerals

Among the trace minerals assays (iron, zinc, manganese), the mineral found in major proportion was iron; the seed presented a great amount of this element, but also the external part of the fruit (Fig. 4b). Other important trace element found mainly in seeds was zinc, fact that can be deduced from the high values of zinc in seeds and from the difference in the amount of this mineral between pod and pulp flours. Manganese was found in minor proportion than the other minerals, with a contribution similar of seeds and external teguments.

Iron and zinc are important micronutrients in human nutrition [36]. Iron deficiency in children can affect neurotransmitter systems influencing brain functioning with the consequent risk of development of mental diseases.

Iron contents of pod and pulp flours of *P. alba* were 57.3 ± 3.4 and 53.8 ± 1.6 ppm, respectively; values higher than those reported for *C. siliqua* L. flour 29.4 ppm, [34] and for Argentinean iron-enriched white wheat flour (30 ppm). Comparing with other leguminous sources, values of iron obtained for *P. alba* flours resulted slightly lower than those reported for *P. vulgaris* L. (6.4–8.1 mg/100 g) [36] but higher than iron contents of *L. albus* (4.4 mg/100 g) [34].

A deficiency of zinc affects immune system function leading to growth retardation. Zinc content of pod flour was almost twice higher than values observed in carob (*C. siliqua* L.) and wheat flours, similar to that exhibit by bean (2.1-2.5 mg/100 g) and lower than that presented by lupine (4.75 mg/100 g).

Barminas et al. [37] found for *Prosopis africana* seeds similar contents of calcium, but lower levels of potassium, iron and zinc than those obtained in this work for *P. alba* seeds.

Conclusions

Prosopis alba flours presented interesting chemical and nutritional properties: high contents of soluble sugar, dietary fiber, polyphenols with antioxidant activity, minerals, protein of good quality and absence of prolamines and antitryptic activity. Due to these characteristics, *P. alba* flours are suitable as high-quality nutritional ingredients for bakery products.

The whole pod, due to the contribution of seed, contains high amount of calcium, iron and zinc, indispensable minerals for human nutrition. Although protein content of this pod flour is low, protein is mainly located in seeds. Subunit composition of proteins is different from carob (*C. siliqua* L.) and from other leguminous such as soybean.

Results suggest that from a nutritional point of view, it is convenient the use in food formulation, the whole pod flour; due to the nutrients provided by seeds present in this ingredient. For that purpose, it is appropriate to have an adequate milling process so as impeding loosing the seed fraction during sieving and thus obtaining whole meal flour. In the case that seeds were separated with the purpose of isolating gum and protein for being used as food ingredients, pulp flour (without seeds) obtained as subproduct also constitutes a high quality material, of higher nutritional quality than wheat flour, suitable for gluten-free products.

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