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# Anti-inflammatory and antioxidant activities, functional properties and mutagenicity studies of protein and protein hydrolysate obtained from *Prosopis alba* seed flour

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ABSTRACT

*Prosopis* species are considered multipurpose trees and shrubs by FAO and their fruit constitute a food source for humans and animals. According to the "Código Alimentario Argentino", "algarrobo flour" is produced by grinding the whole mature pod, but in the traditional process most of the seeds are discarded. In this paper, the flour from seed was obtained. Then, the proteins were extracted and enzymatic hydrolysis was carried out. According to their amino acid profile and chemical score (>100%), the *Prosopis alba* proteins, are not deficient in essential amino acids considering the amount of amino acid necessary by adults. The protein isolate showed a good solubility (pH 7.4–9), emulsificant capacity, oil binding capacity and water adsorption capacity. The antioxidant ability of proteins was significantly increased with hydrolysis ($SC_{50}$ values: 50–5 μg/mL, respectively). Inhibitory activity of pro-inflammatory enzymes (lipoxygenase and phospholipase) was described. The mutagenicity/antimutagenicity of proteins and protein hydrolysates from seed flour were also analysed.

The results suggest that *P. alba* cotyledon flour could be a new alternative in the formulation of functional foods not only for its high protein content but also by the biological and functional properties of its proteins and protein hydrolysates.



## 1. Introduction

The genus *Prosopis* belongs to the Fabaceae family, subfamily Mimosoideae and involves about forty-four species distributed mainly in arid and semiarid tropical and subtropical regions of Southwest Asia, Africa and predominantly America. In America, *Prosopis* thrives in a large area that goes from the south-western part of the United States to the Argentinean Patagonia, being characteristic of the Monte desert in Argentina from Salta to Chubut provinces (Cabrera, 1994). *Prosopis* pods and leaves have economic significance for ruminant breeding (Abddel hafes & Abdullah, 2004; Obeidat, Abdullah, & Al-Lataifeh, 2008) and its wood is an important resource for construction, firewood and charcoal (Bogino & Villalba, 2008; Fagg & Stewart, 1994). *Prosopis alba* fruit is used to dissolve gallstones, and as an anti-bronchitic and laxative. Its flowers have diuretic properties; and the bark is used as an astringent and to heal eye infections (Pasiecznik, Harris, & Smith, 2004). *Prosopis* species fruits constitute a food source for humans and animals of Monte desert (Arenas, 2003; D'Antoni & Solbrig, 1977; Fagg & Stewart, 1994; Felger, 1977; Roic, Carrizo, & Palacio, 2002). Different food products are made from *P. alba* and *Prosopis nigra* pods: drinks (añapa, aloja and chicha), syrup, flour, sweets (arrope, patay, jam), etc. (Escobar, Estévez, Fuentes, & Venegas, 2009; Odibo, Ezeaku, & Ogbo, 2008; Roig, 1993). Felker, Takeoka, & Dao (2013) noted that the whole pods of different *Prosopis* species were ground for animal feeding, whereas the flour for human food is made only from the mesocarp fraction of washed and sorted pods. According to the "Código Alimentario Argentino", "algarrobo flour" is produced by grinding of whole mature pod of *P. alba* and *P. nigra*, but most of the seeds are discarded in the traditional process due to the hardness of the

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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* that grow in Northwestern of Argentina (Cardozo et al., 2010). The use of *P. alba* cotyledons is little known in Argentina, probably due to the difficulties in obtaining them, a situation that could change by implementing a simple technology. The cotyledons and their flour would represent a real alternative as a protein source in the formulation of animal feeds and human food. Estévez, Escobar, and Ugarte (2000) and Escobar et al. (2009) studied the utilisation of *Prosopis chilensis* cotyledons to obtain some products for human alimentation with high protein content (cereal bars and cookies).

Antioxidants play a vital role in both food systems as well as in the human body to reduce oxidative processes. In food systems, antioxidants are useful in retarding both lipid peroxidation and the formation of secondary lipid peroxidation products and thus help to maintain flavour, texture, and, in some cases, the colour of the food products during storage (Samaranayaka & Li-Chan, 2011). Proteins and protein hydrolysates derived from sources like milk, soy, egg and fish have also shown antioxidant activity (Elias, Kellerby, & Decker, 2008; Hagen & Sandnes, 2004; Peña-Ramos & Xiong, 2003). Food-derived peptides also have potential for controlling and modulating some inflammatory diseases like hepatitis, inflammatory bowel disease like Crohn's disease and ulcerative colitis and other chronic intestinal inflammations (Chattertona, Nguyen, Bering, & Sangild, 2013; Sato, 2012). Phospholipase $A_2$ ($PLA_2$) activity serves to release arachidonic acid from membrane phospholipids, which can then be processed by cyclooxygenase (COX) and lipoxygenase (LOX) enzymes to generate leukotrienes, prostaglandins and hydroperoxides which are potent mediators of inflammation. Thus, the inhibition of these enzymes is one of the important cellular mechanisms of anti-inflammation.

Since there has recently been an increased interest in non-conventional natural food from plant origin with beneficial properties for health, seed proteins from *P. alba* could be used as functional food or food additive. In this study, we examine the procedure to obtain *P. alba* cotyledon flour, the functional properties and biological properties (potential as antioxidant, inhibitor of pro-inflammatory enzymes as well as antimutagenic) of proteins obtained from them and its hydrolysates. The genotoxicity of proteins and its hydrolysates was also analysed.

## 2. Materials and methods

### 2.1. Plant material

Ripe *P. alba* (Griseb.) pods were collected in Amaicha del Valle (Tucumán, Argentina) in April 2010. They were brushed to remove foreign material and dried at 50 °C until reaching a constant weight.

Dried pods were ground to powder (Helix mill, Metvisa) and were passed through 2 mm and 4 mm mesh sieves. Two fractions were obtained: mesocarp flour and seeds with endocarp. Since seed-stone (endocarp) thickness and hardness affect the process of cotyledon extraction, a given weight of seeds was treated with sulphuric acid (seed-to-solvent ratio of 1:5 w/v) for 5 min to soften the endocarp. Then, the seeds were separated from the endocarp and rinsed. They were kept in water for 24 h to produce their imbibition. The seed cotyledons, mucilage (endosperm) and episperm were separated.

### 2.2. Preparation of Prosopis cotyledon flour

The cotyledons were dried at 50 °C until reaching a constant weight. Then the dried cotyledons were ground to obtain cotyledon flour. The flour was stored in plastic screw-capped bottles at −20 °C until further use.

### 2.3. Determination of total protein content

The total nitrogen content of cotyledon flour and protein isolate were determined by Kjeldahl digests. A factor of 6.25 was used to determine the percentage of total protein.

### 2.4. Soluble protein determination

Soluble protein content was determined by Bradford (1976) (BIO-RAD®) method using bovine serum albumin (BSA) as a standard. Results were expressed as mg of BSA/g dry weight of cotyledon flour.

### 2.5. Isoelectric point determination

To determine the isoelectric point (pI), the sample was diluted to a 5 mg soluble protein/mL and fractioned in several tubes. Glacial acetic acid was added to bring each tube to reach different pH values. The content of the tubes was mixed and left to rest for 20 min. Next, the tubes were centrifuged and the supernatant was separated to quantify soluble protein.

### 2.6. Preparation of protein isolates from cotyledon flour

*Prosopis* cotyledons flour was extracted with 0.5% (w/v) aqueous sodium hydroxide (NaOH) (pH 10; flour-to-solvent ratio of 1:4; w/v) for 20 min with stirring in a cool bath. The preparation was centrifuged at 9692*g* for 35 min at 4 °C (Sorvall RC-50) and the pellet was re-extracted two fold with the same solution and centrifuged. All supernatants were pooled and adjusted to their pI. The pellet obtained by centrifugation at 9692*g* for 30 min at 4 °C was lyophilised and stored at −20 °C.

### 2.7. Enzymatic hydrolysis of protein isolates

A 2% (w/v) protein isolate (PI) suspended in 50 mM citrate-phosphate buffer pH 2.5 was subjected to enzymatic hydrolysis. The enzyme–substrate ratio was 1:20 w/w. A pepsin solution (Sigma–Aldrich) was added to the mixture and was incubated with continuous stirring for 2 h at 37 °C. Then the pH was adjusted to 7 with 1 M NaOH, a pancreatin solution (Sigma–Aldrich) in 50 mM citrate-phosphate buffer pH 7 was added and the mixture was incubated for 2 h at 37 °C. The reaction was stopped by heating at 80 °C for 20 min and the resultant *Prosopis* protein hydrolysate was frozen and stored at −20 °C. The hydrolysis treatment was performed in triplicate.

### 2.8. Hydrolysate fractionation by ultrafiltration

The hydrolysate solution was filtered through a membrane (MILLIPORE®) with a 3 kDa obtaining two new fractions, *Prosopis* protein hydrolysate <3 kDa and >3 kDa.

### 2.9. Electrophoresis

#### 2.9.1. Tris-SDS–PAGE

SDS–PAGE (Laemmli, Amos, & Klug, 1976), was used to characterise the polypeptide profile of protein preparations. Typically, samples were electrophoresed on a 15% acrylamide separating gel with a 7% acrylamide stacking gel (1.0 cm × 10 cm × 1.5 mm). Protein samples were mixed with suitable volumes of SDS–PAGE sample buffer (0.05 M Tris–HCl, pH 6.8; 1% SDS; 0.01% bromophenol blue as the tracking dye; and 3% glycerol) containing 2% (v/v)