



Evaluation of antioxidant capacity, genotoxicity and polyphenol content of non conventional foods: Prosopis flour

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ABSTRACT

Fruits such as *Prosopis* pod have been food sources (patay, arropo, chicha or aloja) of inhabitants of arid and semi-arid lands in South America. The aims of the present study were determine some nutritional and functional properties as well as genotoxicity of flour obtained from *Prosopis* ripe pods that were submitted to different processing. Sucrose constituted the main sugar for flours obtained from *Prosopis alba* and *Prosopis nigra*. Decoctions and macerations showed around 2.9% and 1.4% of soluble proteins, respectively. The highest free phenolics, flavonoids and condensed tannins contents were observed in aqueous extractions with heating. None of the samples presented phytic acid levels high enough to constitute a nutritional problem. Antioxidant activity (AA) was evaluated by DPPH, ABTS and β -carotene bleaching assays. Results showed that the antioxidant potential was significantly higher in flour obtained from *P. nigra* pods than in that from *P. alba* pods, and it was also higher in aqueous extracts than in alcoholic ones. Data obtained suggests that compounds responsible for AA are thermostable; therefore, *Prosopis* flour might be capable of retaining a significant amount of antioxidant capacity after heating. *Prosopis* extracts did not show any mutagenic effect with and without metabolic activation. *Prosopis* flour proved to be a non conventional, novel and rich source of antioxidant compounds that could help to prevent pathologies associated with oxidative stress.

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1. Introduction

Antioxidants retard the development of the unpleasant flavour brought about by the oxidation of unsaturated fatty acids, usually present as triacylglycerides and/or polar lipids. Nowadays there is a general trend towards replacing synthetic antioxidants in food processing with natural oxidation inhibitors or with ingredients that naturally possess antioxidant activity. Naturally occurring nutritive and non-nutritive antioxidants have recently become a major area of scientific research, but it should be kept in mind, however, that they are not necessarily less toxic than synthetic compounds.

Leguminous plants are cultivated throughout the world and consumed in various dishes. Their seeds are a source of many substances with antioxidant properties, including phenolic compounds. A recent epidemiological study showed that bean and

lentil consumption is related to a lower incidence of breast cancer (Adebamowo et al., 2005). Soybean and lupin seed have attracted a lot of attention for their protein content (Sugano, 2006) and antioxidant activity (flavonoids, tocopherols, phospholipids, amino acids and peptides).

The genus *Prosopis* belongs to the Fabaceae family, subfamily Mimosoideae and comprises 45 species distributed mainly in arid and semi-arid, tropical and subtropical countries. *Prosopis* genera have been widely studied all over the world for their ecological value since they are extremely resistant to heat, drought, alkalinity and salinity. They also contribute to soil stabilization and improvement through nitrogen fixation (Bernardi, 2000). 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).

Several uses have been reported for these species. The exuded gum produced from the wounds in the bark is comparable to commercial gum Arabic (Anderson & Farquhar, 1982).

Ripe *Prosopis* pods and leaves are of economic significance for ruminant breeding (Abdullah & Abdel hafes, 2004; Obeidat,

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Abdullah, & Al-Lataifeh, 2008) and the 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 (Pasiiecznik, Harris, & Smith, 2003; PFMN Database, 2003). *Prosopis nigra* fruit is commonly used for sore eyes, as a sedative, and diuretic as well as against venereal diseases, asthma and dyspepsia (Carrizo, Palacio, & Roic, 2002).

Prosopis species fruits constitute a food source for human and animal of Monte desert (Arenas, 2003; D'Antoni & Solbrig, 1977; Fagg & Stewart, 1994; Felger, 1977). Different food products are made from *Prosopis* species: drinks (añapa, aloja and chicha), syrup, flour, sweets (arroppe, patay, jam), etc. (Escobar, Estévez, Fuentes, & Venegas, 2009; Odibo, Ezeaku, & Ogbo, 2008; Roig, 1993). Although *Prosopis* pod flour (80–100 mesh) and wheat flour have approximately equal energy and protein contents, wheat flour is neutral in taste, and it is used for its textural properties in stimulating volume increase, while *Prosopis* flour does not have gluten (Felker, Grados, Cruz, & Prokopiuk, 2003; Saunders et al., 1986) and does not stimulate volume increase, but has a rather sweetish taste. *Prosopis* flour has a coffee or cocoa like taste and aroma (Felker et al., 2003).

In this work, we analyzed the soluble phytochemical components as well as the antioxidant capacity and genotoxicity of flour from *Prosopis* pods, collected in Northwestern Argentina.

Since literature has shown the influence of biochemical transformations on the concentrations of different metabolites during food processing (Odibo et al., 2008) different extractions were performed.

2. Materials and methods

2.1. Sample preparations and processing

P. alba (Griseb.) and *P. nigra* (Griseb.) Hieron. ripe pods were collected in La Unión, Departamento Rivadavia, Salta, Argentina in December 2007. The fruits were brushed to remove foreign material and dried at 50 °C until reaching constant weight. Dried pods were ground to pass through a 80 µm sieve for the analysis. Samples were extracted with water and ethanol.

2.2. Ethanol extraction

10 g of *Prosopis* flour were mixed with 100 ml of 96° ethanol. The mixture was homogenized for 7 days at room temperature and centrifuged for 20 min at 10,000g. The supernatant was filtered through Whatman No. 4 filter paper. The supernatant was dried under reduced pressure at 40°C and weighed.

2.3. Aqueous extraction with boiling

Five gram of ground samples were decocted in 100 ml of distilled water for 20 min. The decoction was left to cool at room temperature, centrifuged for 20 min at 10,000g and filtered through Whatman No. 4 filter paper. Supernatants were freeze-dried.

2.4. Sugar determination

The phenol–sulphuric acid method (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956) was used for determination of total neutral sugars. Results were expressed as g of glucose/100 g dry weight.

Reducing sugars were measured using the Somogyi–Nelson method (Nelson, 1944; Somogyi, 1945). Results were expressed as g of glucose/100 g dry weight.

Glucose was determined by the glucose-oxidase method (Jorgensen & Andersen, 1973). Sucrose was estimated by the resorcinol method (Cardini, Leloir, & Chiriboga, 1955). Fructose was measured according to Roe (1934). Results were expressed as g of glucose, sucrose and fructose/100 g dry weight, respectively.

2.5. Protein determination

Soluble protein concentration was determined by the method of Lowry, Rosebrough, Farr, and Randall (1951) using bovine serum albumin (BSA) as standard. Results were expressed as g of BSA/100 g dry weight. Protein total ($N \times 6.25$) was determined according to the AOAC (1998) methods.

2.6. Free phenolics content

A derived method of Folin–Ciocalteu, according to Singleton, Orthofer, and Lamuela-Raventos (1999) was used: the reaction mixture contained 50 µl of each preparation, 2 ml of distilled water, 200 µl of Folin–Ciocalteu reagent and 800 µl of sodium carbonate (15.9% w/v). The reaction mixture was heated at 50 °C for 5 min in a water bath. Absorbance was measured at 765 nm. Results were expressed as g gallic acid equivalents/100 g dry weight (g GAE/100 g DW).

2.7. Total flavonoid content determination

Flavonoid content was determined by the aluminium chloride colorimetric method (Woisky & Salatino, 1998), with minor modifications. A 0.5 ml of 2% aluminium chloride ethanolic solution was added to 0.5 ml of sample diluted in ethanol. After 1 h at room temperature, the absorbance was measured at 420 nm. Total flavonoid contents were expressed as g quercetin equivalents/100 g dry weight (g QE/100 g DW).

2.8. Proanthocyanidin content

About 1.2 ml of *n*-butanol–HCl solution (95:5, v/v) and 40 µl of iron reagent (2% ferric ammonium sulphate in 2 N HCl) were added to 200–400 µl of extract. The test tubes were covered with glass marbles and heated at 100 °C for 50 min. Absorbance was measured at 550 nm. Proanthocyanidin content was expressed as g of quebracho tannin equivalent/100 g dry weight (g QTE/100 g DW), (Porter, Hrstich, & Chan, 1986).

2.9. Phytic acid determination

Phytic acid levels were determined in flour by the method of Vaintraub and Lapteva (1988) with some modifications. Ground samples (0.5 g) were mixed with 10 ml of 3.5% HCl for 1 h. Then, the sample was shaken and centrifuged at 3000g for 10 min. One hundred and fifty microliter of the supernatants were diluted with distilled water and adjusted to a final volume of 1.5 ml. Five hundred microliter of Wade reagent (0.03% solution of FeCl₃·6H₂O containing 0.3% sulphosalicylic acid) was added and mixed for 30 s. Absorbance was measured at 500 nm. Phytic acid was used as the standard and results were expressed as g phytic acid/100 g dry weight.

2.10. Measurement of antioxidant capacity

2.10.1. ABTS free radical scavenging activity

The antioxidant capacity assay was carried out by the improved ABTS⁺ method as described by Re et al. (1999). The ABTS⁺ was generated by reacting 7 mM ABTS and 2.45 mM potassium persulfate after incubation at room temperature (23 °C) in the dark for 16 h. The ABTS⁺ solution was obtained by dilution in ethanol (for

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