

## Leguminous Flour from the Native Argentinian Forest, Their Contribution to Antioxidant Defense

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### ABSTRACT

The oxygen-reactive species may generate oxidative stress, which could result in degenerative diseases. Antioxidant mechanisms usually act in a coordinated way, and they are grouped in two defense systems: enzymatic and non-enzymatic system. The metallo-dependent enzymes of the organism and the natural substances present in legumes have the capacity to delay, decrease or inhibit the oxidative processes.

This work aimed to evaluate the bioaccessibility of zinc and bioactive compounds of flour obtained from Argentinian native forest fruits corresponding to the family of leguminous plants: white carob (*Prosopis alba*) and Chañar (*Geoffroea decorticans*), in order to use them in human food.

The study was done on carob flour (CF) and chañar flour (CHF). The minerals were quantified by atomic absorption spectrometry. Bioaccessibility (D%) was estimated by dialysate percentage after *in vitro* digestion. The potential contribution (PC) was calculated. Their phenolic concentrations were obtained using the Folin Ciocalteu method and their antioxidant activity was evaluated *in vitro* using the radical DPPH (1,1-diphenil-2-picrihydracil) and expressed as the percentage of the trapping capacity against DPPH.

CF and CHF presented contents of Zn of 1.20 and 1.48 mg / 100g; D% 24 and 28; PC 0.29 and 0.41 respectively.

CHF contains  $112 \pm 18.3$  mg EAG/100g of total phenols and AF  $156 \pm 16.9$  mg EAG/100g.

The results showed that the antioxidant potential was higher in CHF 39.78% than CF: 16.4%, in preparations with 100 mg/l. The values for the discoloring percentage of DPPH radical were corrected considering the standard quercetine with a value of 100% antioxidant capacity. The samples under study are good sources of Zn, cofactor of the enzyme superoxide dismutase and of antioxidants, mainly of phenolic compounds. Therefore, these flours could be suitable for functional foods formulation.

### Keywords

Antioxidants, Carob flour, Glucose, Beans, Forest.

### Introduction

Antioxidants can be synthesized by the human organism, or otherwise be digested through the foods, in which phenolic compounds, flavonoids and non-flavonoids are found. Chemically, they are a group of natural compounds, secondary metabolites

produced by plants to protect themselves from other organisms. Phenolic compounds are the largest group of non-energetic substances present in foods of vegetal origin and, as natural antioxidants, they can effectively detain free radicals, absorb the ultraviolet light and chelate transition metals, thus they stop progressive autoxidative damage. In addition to this property, they provide foods with aroma, colour and astringency [1]. Over the last years, it has been demonstrated that a diet rich in vegetable

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polyphenols can improve health and reduce the incidence of cardiovascular diseases [2,3].

The Zn is part of the active nucleus of the enzymes with antioxidant activity; it keeps hepatic, cardiac and reproductive functions in good conditions and it is considered to be a protector against cancer as well. Between 3 and 38% of the zinc of the diet is absorbed in the proximal gastrointestinal tract. This absorption seems to be regulated by the synthesis of the intestinal protein called metallothionein (low molecular weight protein rich in cysteine) which has the capacity to bind different divalent metals as Zn, Cu and Cd. The absorption also depends on the quantities of Zn in the diet and the presence of substances that interfere with it, such as:

- The fibre and phytates which form compounds and decrease their absorption.
- Ca, Cu and Cd compete and can replace the Zn in the transporting protein, consequently they inhibit its absorption.
- Glucose, lactose and determined proteins favor the Zn absorption.

The recommended Zn intake for an adult range between 8mg per day for women and 11mg per day for men [4].

Studies carried out with lab rats have demonstrated that Zn stimulates the production of cytokines, inhibits the release of histamine and is found in the superoxide dismutase. Indigenous communities associate the *Prosopis* fruit/carob and its products to their origins and identity. Carob (*Prosopis* sp.) pods and seeds are flour sources which small farmers in the North West of Argentina (NOA) use for the manufacture of traditional regional foods.

The flours obtained after the drying process of the pods are used as substitute products of cocoa and coffee since they do not contain stimulant substances such as caffeine and theobromine. Furthermore, they are a proper ingredient in the preparation of sweet products such as cakes, muffins and cookies because of their high sugar content as well as good aroma and taste. The study of the nutritional properties of the carob pod flour revalues the potential use of this fruit in the food industry of functional foods.

Another leguminous plant from the woods which has grown in the wild is the chañar (*Geoffroea decorticans*). Regarding its harvest, it is done manually since when the fruits attain a degree of ripeness their detachment is spontaneous. In the same way as the carob, the chañar fruit has a high nutritional value. Maestri [5], also studied the centesimal composition of chañar seeds, opening up the possibilities for the use of these for the generation of oils and as a possible proteinic source. Currently, they continue to be part of the diet of some indigenous communities as Wichis, Tobas, Chorotes, etc. [6-8].

Beans (*Vicia faba*) are grains which also grow in the Argentinian northwest, and its consumption is popular in South America. Due to their hardness and great resistance to low temperatures, they are the best adapted leguminous plants to the regions Quebrada and Puna in Jujuy, Argentina and they represent a source of energy,

protein and micronutrients such as folic acid, niacin, vitamin C, magnesium, and potassium for the inhabitants of these regions. The high lysine content of its protein is appropriate as a complement of the cereal protein.

In addition to its intrinsic importance, the native forest represents a raw material reservoir for the rural populations which allows them to meet daily needs, as the treatment of health conditions, nutrition, fodder provision, energy, among others.

The objective of this work is to evaluate the Zn bioactive compounds and bioaccessibility of the flours obtained from the fruits of the native forest in Argentina corresponding to the leguminous family: white carob tree (*Prosopis alba*), Chañar (*Geoffroea decorticans*) and beans (*Vicia faba*) in order to use them in human consumption.

In addition, a survey was carried out so as to elucidate consumption trends, potential consumers and the effective behavior of consumers with new foods, in which a non-traditional raw material is incorporated as well as the values that intervene in their purchasing decisions.

## Materials and Methods

### Flour obtention

Work was done on obtaining carob flour (CF), chañar flour (CHF) and beans flour (BF). To do it, carob pods were bought from Cooperativa Agro Naciente in Colonia El Simbolar, in Departamento Robles, Santiago del Estero province, Argentina. These pods were dried in a mechanical convection stove (forced air circulation) (DALVO, DHR/F/I) at  $48 \pm 2^\circ\text{C}$  and at an air speed of 2m/s over a 96h period. Subsequently, the fruits were ground during 90s at 2900rpm in a knife-cutting mechanical action device CROYDON. The result of the milling process was sieved in a shaker-vibrator sieve ZONYTEST to obtain the fraction sought (a sieve non-passing fraction at a mesh width of 80) with a particle size ranging from 177 to 500  $\mu$ .

To obtain chañar flour, complete, selected and clean *Geoffroea decorticans* fruits were used, which were provided by collectors from the town Guanaco Sombriana, Departamento Atamisqui, Santiago del Estero, Argentina. After being dried, they were ground by a mechanical action equipment Croydon, Mod: MC560494, 50Hz and 2890 rpm, fitted with 8 stainless steel cutters mounted on a vertical axis, for a 1.5 m; the woody part was separated through a sieve ASTM N°8 (2380  $\mu$ m) and the pericarp was sieved to select granulometrically the flours comprised between 35 ASTM (500 $\mu$ m) and 80 ASTM (177 $\mu$ m) sieves.

Bean flour was provided by Cooperativa CAUQUEVA in Jujuy province, Argentina.

Zn was quantified by atomic absorption spectrometry. The bioaccessibility (D%) was estimated in CF and CHF through the dialized percentage post digestion in vitro. The potential contribution (PC) was calculated as it is indicated in the following formula:  $PC = D\% \times \text{mineral content} / 100$ .

The Zn dializability (D) was calculated as the amount of dialized mineral expressed as the total mineral content percentage in the sample. The Zn content was measured by atomic absorption spectrometry, using a Perkin Elmer equipment connected to a graphite furnace. To validate the Zn determinations by atomic absorption spectrometry, SRM Infant Formula 1846 (NIST) was used as an external reference standard.

### Statistical analysis

The results were analyzed using ANOVA and LSD (least significant differences) to determine the significant differences between the samples, at a 95% confidence level. applying software Statgraphics plus 3.0.

### Phenols

The phenols concentration was determined with the Folin Ciocalteu method which is based on the phenols capacity to react with oxidizing agents. Folin Ciocalteu reagent contains molybdate and sodic tungstate which react with any kind of phenol, forming phosphomolybdic-phosphotungstic complexes. The electron transfer to a basic pH reduces phosphomolybdic-phosphotungstic complexes to oxides, intense blue colour tungsten (W8O23) and molybdenum (Mo8O23) chromogens, this color being proportionate to the number of hydroxyl groups of the molecule. The modified micromethod [9], was carried out for this test. A gallic acid calibration curve was built using a stock solution of 500 mg/L (concentration).

The measures in the spectra (HITACHI U1900) at 765 nm. The phenols concentration in the samples was calculated on the basis of the calibration curve and was expressed as mg equivalent to Gallic Acid/100 sample grams.

For the search of free radicals' scavengers, a bioassay "in vitro" of the radical DPPH (1,1 -diphenyl-2-picrylhydrazyl), in accordance with what was reported by Tapia et al. [10], was used. This assay is based on the fact that the radical DPPH (Aldrich), intense violet in color, when it is trapped loses its characteristic color, expressed as % of trapping capacity with DPPH.

$$\% \text{ decoloration} = (1 - \text{Ac}/\text{Ab}) * 100 \%$$

Where: Ac is the compound absorbance and Ab is the absorbance of the blank of assay (control).

A value equal to 100 (hundred) corresponds to the maximum trapping capacity of free radicals, while a value close to 0 (zero) indicates a reduced or null capacity.

In a complementary way, a survey was conducted, considering surveys as "Information-gathering techniques which consist of the formulation of questions to people who should respond them on the basis of a questionnaire" [11].

In a first step, people were interrogated regarding knowledge about and consumption habits of the flours under study and food products with influence on health. The following step was to know the

reason for which they consume or do not such products and if they would consume new foods, manufactured with these leguminous flours. Finally, people were asked about the interpretation of advertisements on healthy properties on foods labels. The aim of this survey was to try to find out people's knowledge level of an important characteristic in these products.

### Target population

Men and women older than 20 years old who were interested in responding the questionnaire.

Data collection period: the questionnaire was carried out from October to November, 2016.

### Sample

220 structured questionnaires were applied.

### Interviewee selection

People from the city of Santiago del Estero who wished to participate in the survey.

### Results

CF, CHF, BF presented Zn 1.20, 1.48 and 3.20 mg/100g content respectively.

Given the high dietary fibre content (data not shown) of the CF, CHF and, since zinc is considered to be an essential element as a cofactor of enzymes which prevent oxidative stress it is of interest to know its potential availability (Table 1).

| Flours | Zn    |              |
|--------|-------|--------------|
|        | D (%) | PC (mg/100g) |
| Chañar | 28    | 0.41         |
| Carob  | 24    | 0.29         |

**Table 1:** Total Zn content, Bioaccessibility and potential contribution in the flour.

The cinch content of the beans flour is 3.2mg/100; this value is greater than that of the wheat flour fortified by law 256+30 (1.76mg/100g). If CHF and CF dializability values are compared with those obtained by other researchers [12], they are in the same order of magnitude.

The high fibre content of these flours which ranges between 12.75 and 15.46 g% [13], in addition to the presence of anti-nutritional factors specific to the leguminous plant's seeds [14], hinder the action of the digestive enzymes what explains the D% values obtained.

Polyphenols are powerful antioxidants. In Table 2 polyphenols contents found in the different flours are shown. According to the results shown, no significant differences were observed in total polyphenols contents between CF and BF, being both contents significantly higher than that of CHF. However, the antioxidant capacity (Table 2) of the latter, turned out to be considerably greater than that of the other flours, suggesting that in chañar flour

there are other components with antioxidant activity, in addition to the polyphenols.

| Flour        | X (mg EAG/100g) | SD   |
|--------------|-----------------|------|
| Chañar Flour | 112             | 18.3 |
| Carob Flour  | 156             | 16.9 |
| Beans Flour  | 158             | 31.8 |

**Table 2:** Total polyphenol content.

The total polyphenols content found is similar to the results reported by other researchers in different fractions of *Prosopis alba*, expressed as g of Polyphenols/100 g on a dried base, Pods  $0.235 \pm 0.009$ , Pulp  $0.27 \pm 0.02$  Seed  $0.27 \pm 0.01$  [15].

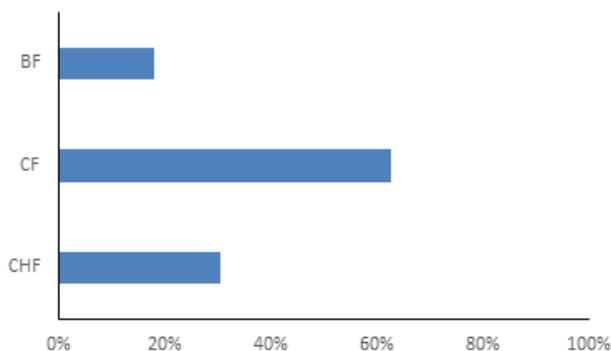
| Flour        | Antioxidant Activity % of the trapping capacity by DPPH* |         |         |
|--------------|--|---------|---------|
|              | 100 mg/L   | 50 mg/L | 10 mg/L |
| Chañar Flour | 39.78  | 29.43   | 14.28   |
| Carob Flour  | 16.40  | 8.06    | 4.00    |
| Beans Flour  | 20.00  | 12.00   | 6.20    |

**Table 3:** Trapping capacity by DPPH%.

The results showed that the antioxidant potential was greater in CHF 39.78 % than in BF and CF: 16.4%, in 100mg/l preparations, following the same order for 50 and 10 mg/l extracts. The radical DPPH discoloration percentage values were collected considering the quercetin standard with a 100% value of antioxidant capacity.

The antioxidant activity of the phenolic compounds found is interesting from the technological and nutritional point of view [16]. Thus, these compounds intervene as foods natural antioxidants, consequently the foods preparation with raw materials with a high phenolic compound content implies the reduction of the use of additives and also facilitates the production of healthier foods, which may even constitute a functional food.

Graphic 1 shows the summary of the results of the surveys carried out to establish the level of knowledge of flours under study expressed in %.



**Figure 1:** Knowledge level of leguminous flours.

Regarding consumption frequency, it was found that 5% of the surveyed population consumes CF frequently, 37% consumed it once and 57% never consumed it; unlike CF, 11.9 % and 3.5

% of the surveyed population only consumed CHF and BF once respectively, nobody consumes them frequently and 88.1 and 96.5 % have never consumed them. Therefore, it is important to disseminate their properties and characteristics in order to promote their use. In the case of the beans flour, its lack of consumption may be due to the fact it is not a characteristic resource from Santiago del Estero.

When the population was asked to mark with a cross if they would consume new baked products or/and pasta manufactured with these leguminous flours, from the total surveyed 80% would incorporate them in the form of pastas and 78.20 % as baked products.

100% of the surveyed population would like to consume foods which influence on health care in a positive way; likewise, 100% buy foods which include these characteristics in their labels. However, only 61 % consumes them daily, 30% sometimes does it and 9% never uses them. Even though the consumer buys products which claim to promote health, 89 % purchases them because they consider they have a scientific base, although they do not understand the statements in the labels. 30% of the population surveyed knows the word antioxidant; nevertheless, they do not know its meaning exactly, but they believe it is good for health.

In the fieldwork carried out on the consumption of foods promoted as functional, it was found out that a new aspect in the choice of food products is that they must help in health care. Consumers tastes are slowly being modified and oriented to the consumption of food that follow the principles of an optimal diet.

## Conclusion

The samples studied are good Zn sources, cofactor of the superoxide dismutase and of phenolic compounds with antioxidant activity.

CHF presents a greater antioxidant capacity than the other flours under study what encourages to delve into the study of other compounds with this activity.

There is an intention to consume these flours in the form of baked products or pastas in 78.20% and 80% of the population surveyed respectively.

For all the reasons above, these flours could be appropriate for the formulation of functional products.

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