International Journal of Advanced Research in Botany (IJARB)
Volume 1, Issue 1, Jul-Sep 2015, PP 23-33
www.arcjournals.org

Chemical and Nutritional Characterization of Fruits from *Geoffroea Decorticans* Tree (Chañar) and their Parts, from Argentine Subtropical Forest

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Abstract: *Geoffroea decorticans, chañar, is an abundant tree in the north of Argentina and limiting countries. Its fruits are rich in minerals, carbohydrate, protein and fibers. A novel approach was developed, where after coarse milling and sieving of the fruits, exocarp and mesocarp is separated from the stones (endocarp and seed). Subsequent fine grinding gave flours named A and B, respectively. But, in another original process, the stones can be coarse milled and two new fractions can be obtained, C and D. Flour A representing 61% of the fruit was low in crude fiber (CF, 5.13%) and fat (1.89%), but high in carbohydrate (81.95%) and protein (8.90%). Flour B was higher in fat and fibers (6.66% and 46.8%), but lower in protein (5.02%). The carbohydrate content was almost the same in both. Flour C had almost nothing of fat and minor quantities of protein but was rich in fibers (47.0%). Flour D contained most of the stone protein and the remaining oil in a 23 % concentration. The main minerals present in the different fractions were Ca, Mg, and Fe, while heavy metals were not significant. Samples showed all essential amino acids in free state except the sulfur ones, but the proteins presented all of them, with some fractions exceeding the chemical score of 100. These results enable to suggest some of these flour fractions as raw material for food or feed.*

Keywords: *Chañar, fruit, Proximate analysis, Flours, Aminoacids, Electrophoresis.*

1. INTRODUCTION

The chañar tree is a common inhabitant of the natural forest of Argentina and neighboring countries. It is commonly found forming small woods, due its clonal reproduction capability. Currently, its fruits are underexploited resources, in spite of being consumed by native people since long time ago. Alternatives for sustainable exploitation would be welcome, generating a fairly wide and innovative workspace as harvesting and seed storage and product manufacturing ranging from the food itself (sweet, oil), to fuels (bio-oil). Its potential as raw material is justified keeping in mind the sustainability and renewability of the exploitation, proposing the collection and use for the fruit, keeping the tree in the forest.

Leguminous trees are important sources of food in many countries (*Prosopis: juliflora, alba Griseb, nigra, ruscifolia Griseb., Geofroea decorticans, Styphonolobium burseroides; Acacia: bilimekii, aroma, caven; Cercidium praecox, Tamarindus indica)* but surprisingly, little or no information about sustainable uses for chañar tree is known. Studies made by Becker (1983), show that fruit from chañar are good source of sugar or as a fermentation substrate. The same author also highlights the important oil content in chañar seed. According to Silva *et al.* (1999), the exocarp and mesocarp of chañar constitute important sources of carbohydrates, fiber and flavonoids. The pericarp of *Geoffroea decorticans* has high glucose and laevulose contents, and protein level similar to the chicken egg (10-15%), so could be a good dietary supplement for cereals. Freyre *et al.* (2003) and Lamarque *et al.* (2000) showed that chañar fruits from the semiarid region of the province of Cordoba present a 21% of proteins and a 45% oil in the seeds, with an oleic/linoleic relationship of 1.75. Zamora Rueda, *et al.* (2008) compared the functional properties of fruits from mistol (Zizyphus mistol, Rhamnacease) and chañar, and concluded that the fiber extracted from the latter one has functional properties acceptable for its use in human feeding. Although Charpentier (1998) highlighted the nutritional quality of the whole flour from these fruits, he emphasized that the grinding does not remove the woody portion, thus reducing the nutritional quality of the products.

The aim of this study was to show the possibility of this tree as supplier of raw material for the food industry. Quantitative data on the composition of the flours obtained and the proximal and electrophoretic study of the proteins present in the seed are provided to show the nutritional value and potential of this fruit.

2. MATERIALS AND METHODS

Ripe fruits from Geoffroea decorticans were collected in the months of November and December 2009, from selected trees in the central-west region of the province of Formosa, Argentina. The trees were chosen in the knowledge they had a very low risk to be cut or damage by fire, and their location was recorded with a GPS (Garmin Nuvi, ± 50 m). Fruits were collected from the trees and the soil under their crowns, in samples of 2 to 4 kg. The fruits were later chosen to remove defectives and / or green ones, plus other impurities presents.

Grinding and Sieving Devices:

- Hammer mill: (1.5 HP, 2850 rpm) with a 0.50 mm mesh
- Disc mill: (2 HP, 1500 rpm).
- Blender, equipped with 5 stainless steel blades, driven by a 1HP motor at 1450 rpm (220 V, 50 Hz).
- Vibrating sieve with stainless steel meshes.

Flours A, B, C and D were obtained following the steps traced in figure 1.

Seed Separation by Hand: the mesocarp was removed with the aid of a knife, and the hard endocarp tissue broken, to free the seeds.

Water Washing of Flour A: mixtures 1:4 (w/v) of flour A and warm water (60ºC) were shaken for 10 minutes and then filtered through a canvas and centrifugated at 87g (GVequipamientos). The solid residue so obtained was hammer milled with 0,5 mm mesh.

Physical and Chemical Analysis: moisture, protein, oil and ash were determined by the standard method and in triplicate according to AOAC (1999). Crude fiber (CF) and acid detergent fiber (ADF) was performed according to Osborne and Voogt (1986). The carbohydrate content was calculated from the remaining difference (Wattanapat *et al.*, 1994). Minerals were measured with an atomic absortion spectrophotometer Perkin Elmer Analyst 600 (Maldonado and Guzman, 1998). The acidity and saponification indexes were determined by standard method from the AOAC (1999). The refraction index was taken with an Abbe refractometer and the density, according to the pycnometer method.

Aminoacids Quantification: flours A and that from seeds were milled and defatted with hexane (10% w/v) by continuous stirring for 24 hs at 5ºC. Part of flour A was previously washed with hot water to remove sugar and soluble compounds.

The free aminoacids present in the original samples, were previously extracted with dilute HCl, according to the official method of analysis of AOAC International, 994.1210, modified by Mufari (2010). To obtain the total amino acid profile, the flours were hydrolyzed in 6 M HCl for 24 hours to free them. In both cases, the respective chromatograms were obtained in a Perkin Elmer 200 HPLC, with UV-visible detector and a Zorbax Eclipse Plus C18 (4.6 x 150 mm, 5 um) column from Agilent Technologies.

The chemical score (CS) was calculated using the following formula: CS = (a / b) * 100, where: a (grams of an aminoacid in sample protein), b (grams of same aminoacid in pattern protein).

The amino acid requirements for preschool children (2-5 years), schoolchildren and adults were employed as a reference, (Tapia *et al.*, 1985).

Protein Characterization: The electrophoresis of proteins was performed according to the method of Laemmli, 1970. Flours were defatted in hexane (10% W / V) with stirring for 24 hours at 5 ° C. Defatted meals were suspended in buffer at pHs between 3 and 11 (1:10 w/v, ionic strength of 0.5), stirred for 30 minutes and then centrifuged at 10,000 g for 15 minutes. The supernatant was recovered and diluted 1:2 v/v with sample buffer to be employed in electrophoresis runs. For assays with denatured proteins, the sample is preheated for 4 minutes at 95 ° C. Native-PAGE: 0.5 M Tris-HCl pH

6.8, glycerol, 0.5% w/v bromophenol blue. In the case of SDS-PAGE, 2% w/v SDS was added and, for the reducing conditions, 2-mercaptoethanol 5% v/v, was employed.

All electrophoresis were performed on a Minilab (Bio-Rad Mini Protean Tetra System). Protein extracts were prepared and used immediately. 5 µL were placed in each well. Native-PAGE: the resolving gel containing 6% w/v acrylamide in buffer 1.5 M Tris-HCl pH 8.8. SDS-PAGE: The stacking gel containing 6% acrylamide in buffer 0.5 M Tris-HCl pH 6.8. The resolving gel containing 12% w/v acrylamide in buffer 1.5 M Tris-HCl pH 8.8. 10% SDS was added in both cases. The run electrode buffer containing Tris-glycine pH 8.3, 15 mA constant current for 2 to 3 hours was employed. Protein bands were stained with Coomassie Brilliant Blue R250. A Molecular weight marker (Promega;10, 15, 25, 35, 50, 75, 100, 150 and 225 kDa bands provided) was used for molecular weight determinations.

3. RESULTS AND DISCUSSION

Wild plants are considered under-exploited food sources; however, natives and rural population used to consume them as food and for domestic animal feeding (Ozkan, *et al.*, 2011; Jezierny, *et al.*, 2010). An important advantage of most wild or semi-wild plants is their tolerance to extreme natural conditions. Also, some edible wild legume species, could be employed as animal feed (Carvalho, *et al.*, 2011), this would leave more traditional grains available for food and, at the same time, enhance the production of animal feed (Carvalho, *et al.*, 2011). As showed in Table 1, the carbohydrate content positioned the fruit as highly energetic. Ash is also important and in agreement with the level of minerals found in chañar fruit, where highlight potassium and calcium, while the toxic metals are negligible (table 2). Charpentier (1998) observed lesser concentrations of potassium and calcium, but higher in magnesium, for the same species. Potassium shows low affinity for organic chelate, that explains its presence in high amounts in plant tissues (Valillo, *et al.*, 2006). The presence of crude protein in the whole fruit is superior to those reported by Charpentier (1998), and near to *Prosopis ruscifolia* and *P. glandulosa* (12.7% and 11.6%, respectively in dry base; Freyre *et al.*, 2003). The *Prosopis* belongs to the same family and shares the same ecological environment of *Geoffroea decorticans* and its fruits are consumed by the same cultures in the same season. The part of the fruit with higher protein content is the seed (24.3%) with a value similar to Asian legumes, such as: *Vigna mungo* (21.9%) and *Cicer arietinum* (25.5%); but more than others such as: *Hymeneae caurbaril* (10.6%), *Cymbopetalum penduliflorum* (11.3%), *Hymeneae stigonocarpa* (9.0%) (Matuda and Netto, 2005).

Table1. *Proximate composition of the whole fruits and milling fractions of chañar (dry basis)*

	Whole fruits	Seed	Fraction A	Fraction B	Fraction C	Fraction D
Fraction yield (%)	100	5.4 ± 1.5	61.0 ± 1.5	39,0 ± 1.0	27.2 ± 1.5	11.7 ± 1.5
Ash (%)	3.69 ± 0.2	3.92 ± 0.02	4.79 ± 0.01	8.30 ± 0.04	0.74 ± 0.01	24.1 ± 0.3
Protein (%)	10.0 ± 0.2	24.26 ±0.5	8.90 ± 0.24	5.02 ± 0.6	2.42 ± 0.3	131.4 ± 5.4
Lipids (%)	4.36 ± 0.5	50.22 ± 0.6	1.89 ± 0.14	6.66 ± 0.2	<dl	231 ± 9.8
Carbohydrate (%)	81.9 ± 1	21.6 ± 1.2	84.4 ± 1	80.02 ±0.8	96.84 ± 0.34	613 ± 16
CF (%)*	25.25 ± 0.2	nd	5.13 ± 0.07	46.8 ± 1	47.0 ± 1	39.05 ±0.64
ADF (%)**	36.23 ± 0.5	nd	30.33 ± 0.05	60.59 ± 0.75	61.57 ± 0.75	46.3 ± 1.3

** Crude fiber; **acid detergent fiber; nd: not determinated; <dl: below the detection limit*

Table 1 also shows a relatively low lipid content in these fruits. Further, most of this oil is in the seed, representing less than 12% of the fruit but bearing near 50% of its weight in chañar oil. This high oil content of seeds is compared with some others, such as groundnut (38-58%), colza (40-60%) and sunflower (32-40%) (Grosso *et al.*, 1994). Studies by Lamarque, *et al.*, (2000) and Maestri, *et al.*, (2001) on chañar seeds, of specimens coming from others regions of Argentina, showed results very similar to those found in this work. Data obtained from other seed wild legume Prosopis, showed a fat content between 4 and 6% (Freyre *et al.*, 2000 and 2003, Del Valle *et al.*, 1983), well below the result presented here. Some quality parameters of the oil are showed in table 2. As can be seen, the free acid content (1.25%) is lower than those from sunflower oil, peanut (2.5%) and olive (2%), but little higher than soybean oil (1.13%; Chasquibol Silva, 1997). Besides possessing most of the oil, chañar seeds also show high presence of proteins, Ca, Zn and Fe, as can be seen in tables 1 and 3, respectively. Nevertheless, separation of seeds from the stone is quite difficult and a mechanical method which allows a clean separation had not been developed until now.

The acid detergent fiber (ADF) allows the measure of lignin and cellulose but not hemicellulose (Seguras *et al.*, 2007). The crude fiber (CF) mainly expresses lignin, which surrounds the cellulose and hemicellulose, thereby restricting the access to these carbohydrates and making them almost completely indigestible either for monogastrics or polygastrics. Therefore, it has been used as an indicator to predict the digestibility of dry matter and non-used energy from food. It can be seen from table 1 that lignin is the major fiber constituent of chañar fruits, reducing its availability for animal or human intake.

Table2. *Mineral composition of chañar fruits and their milling fraction.*

Mineral	Whole fruits (mg / kg)*	Seed (mg / kg)*	Fraction A (mg / kg)*	Fraction B (mg / kg)*
Na	170.40 ± 1.32	nd	nd	61.6 ± 1.9
K	13511.70 ± 2.01	nd	nd	2891.1 ± 9.4
Cu	13.52 ± 0.02	nd	nd	4.85 ± 0.02
Mg	515.6 ± 3.5	4674.5 ± 14	564.4 ± 1.8	298.6 ± 32.5
Zn	9.70 ± 0.05	34.8 ± 0.2	11.07 ± 0.04	4.04 ± 0.65
Ca	657.6 ± 1.7	848.7 ± 0.9	763.5 ± 0.4	382.3 ± 3.9
Fe	21.2 ± 0.6	46.8 ± 0.4	17.93 ± 0.14	12.3 ± 1
Pb	0.54 ± 0.003	<dl	nd	0.11 ± 0.01
Cd	<dl	<dl	<dl	<dl
Cr	1.50 ± 0.001	<dl	<dl	0.65 ± 0.01
Mo	0.51 ± 0.001	nd	nd	0.30 ± 0.01
Mn	4.04 ± 0.01	nd	nd	1.87 ± 0.04

**dry basis; nd: not determinated; <dl: below the detection limit*

Manual separation of mesocarp and endocarp is a task that requires time and labor intensive. The procedure showed in figure 1 allows mechanical separation of the pulp (exo and mesocarp), called flour A, from endocarp (stone), named flour B. Flour called A possessing most of the pulp presented a significant reduction in the fiber content, mainly crude fiber (table 1). On the opposite, flour B, from the stone milling, showed most of the CF and, as said before, it is not recommended for food formulations. In flour A, CF was almost nine times lesser than in B and very near to those observed in the specie P. ruscifolia (6.55%, dry basis; Freyre *et al.* 2000) and also in P. alba (4.82%), both considered good sources of dietary fiber by Gonzalez *et al.*, (2008).

The presence of Magnesium, calcium, zinc and iron in fraction A was notably higher than B (figure 1), in spite of its relative importance (39% of the fruit) and the higher fiber content. This result could not only be due to the higher cellulose content in flour A, but also to the fact that pulp holds most of the fruit water. The proteins content of flour A and its high carbohydrate level (table 1) resemble those reported for other species of the same family (Freyre *et al.*, 2003; Gonzalez *et al.*, 2008). These characteristics joined with the low CF, make this flour proper for food and livestock.

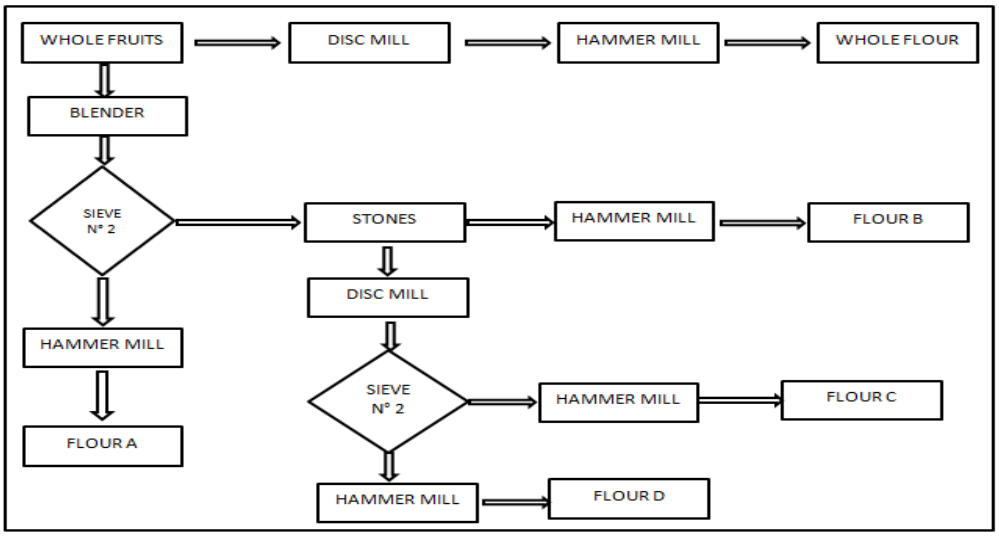

Figure1. *Diagram of mechanical grinding of fruits from Geoffroea decorticans*

As showed in figure 1, after disc milling of the stones and proper sieving, two new flours are obtained, named C and D. This procedure enabled to concentrate most of the oil in flour D. While

lipids represented just 4,4% of the whole fruit, in flour D it raised to 23% (table 1), similar to other vegetal materials as soybean, commonly employed as an oil source (Gunstone, *et al.*, 2007).

The removal of oil from D would even raise the protein content in this fraction and the so obtained skimmed flour could be devoted to animal feeding. But if the high level of lignin were a problem, both flours C and D could still be destined to energy production. Recently, bio-oils were obtained from flours C and D with good yields, Bertero *et al.*, (2013).

Table3. *Parameters of Geoffroea decorticans crude oil.*

Parameter	**Value**
Acidity Index (%)	2.49 ± 0.01
Acidity (% oleic)	1.25 ± 0.01
Saponification Index (mg KOH/g)	164.0 ± 1.4
Ester Index (Is-Ia)	161.5± 1.4
Peroxides Index ((meq 02/kg))	4.34 ± 0.35
Density (g / ml)	0.910 ± 0.002
Refractive Index (25º C)	1.467 ± 0.001
Seed fat yield (soxhlet extraction, dry basis) (%)	50.2 ± 0.6
Fat yield from flour D. Cold pressed (dry basis). (%)	10.1 ±1.9
Fat yield from flour D by Soxhlet (dry basis). (%)	23.1± 1.0

3.1 Protein Quality in Chanar Fruit

FAO has stated that a protein is biologically complete when it contains all essential amino acids in an amount equal to or greater than that established for each amino acid in a reference protein or pattern (FAO/WHO/UN, 1985). Therefore, proteins that possess one or more limiting amino acids are considered biologically incompletes; because they limit protein synthesis, cannot be fully utilized by the body. A better way to consider protein quality is the so called amino acid count; it means the ratio between the content of the same amino acid in the sample protein and the reference (Tapia *et al.*, 2000).

Table4. *Content of free and totals amino acids in FA, FAW and FS expressed in grams of amino acid per 100 g of protein plus averages and standard deviation.*

gAA/100g(CP)	Content of free amino acids			Total amino acid content (acid hydrolysis)			References		
	FA	FAW	FS	FA	FAW	FS	SBM	QM	FM
Asp	2.94±0.02	0.60±0.02	0.06±0.001	10.35±0.06	10.71±0.5	17.90±0.41	13.4	7.79	14.24
Glu	2.18±0.01	0.36±0.01	0.19±0.01	8.72±0.3	10.09±0.2	33.10±0.36	17.66	13.57	13.11
Ser	5.53±0.01	0.82±0.01	0.09±0.003	0.47±0.05	0.49±0.04	3.60±0.07	5.96	3.93	3.63
His*	4.16±0.04	0.93±0.02	0.08±0.001	7.95±0,22	9.53±0.16	4.15±0.12	4.68	2.86	2.46
Gly	0.60±0.02	0.13±0.06	0.03±0.002	4.28±0.5	5.47±0.4	2.59±0.40	4.47	5.57	4.66
Thr*	0.94±0.04	0.40±0.02	0.02±0.0002	3.54±0.04	3.6±0.2	1.57±0.01	4.47	3.07	3.3
Arg*	3.85±0.03	0.47±0.02	0.38±0.002	8.25±0.3	8.71±0.5	6.01±0.01	nd	8.21	nd
Ala	2.73±0.08	0.38±0.01	0.04±0.003	5.07±0.4	5.64±0.4	2.28±0.02	5.11	4.14	5.96
Pro	4.83±0.05	0.21±0.002	0.44±0.04	10.38±0.6	6.27±0.02	6.69±0.05	5.74	2.50	4.4
Tyr	0.93±0.01	0.27±0.01	0.06±0.001	2.49±0.3	2.55±0.3	1.52±0.05	3.62	2.29	4.15
Val*	3.82±0.003	0.99±0.03	0.01±0.003	5.13±0.5	5.62±0.2	1.95±0.45	5.53	5.07	5.57
Met*	< dl	< dl	< dl	1.56±0.2	1.66±0.2	2.60±0.07	0.96	0.93	2.98
Cys	< dl	< dl	< dl	0.64±0.1	0.72±0.01	0.82±0.02	0.35	0.44	Nd
Ileu*	1.59±0.004	0.12±0.001	0.01±0.0001	3.96±0.3	4.25±0.3	0.48±0.01	5.32	3.79	5.17
Leu*	1.30±0.006	0.39±0.003	0.02±0.0003	5.69±0.2	6.74±0.4	1.91±0.03	6.81	6.29	8.29
Phe*	1.17±0.03	0.33±0.002	0.03±0.0002	3.55±0.4	3.91±0.3	3.57±0.10	5.11	3.71	4.53
Lys*	0.33±0.01	0.26±0.01	0.06±0.001	2.57±0.1	3.83±0.1	2.70±0.04	6.60	4.29	9.59

*CP: crude protein (dry basis); nd: not determinated; dl: detection limit; *essential amino acids; SBM: soybeans meal; FM: fish meal (Ozkan, et al., 2011); QM: Quinoa meal (Mufari, 2010)*

In general, grain legumes are considered sources of protein and a wealth of data on their composition are available. Protein content is quite variable depending on genotypes and sometimes, into them (Wiseman and Cole, 1988; Gatel, 1992). As Showed table 1, proteins can be found in the four fractions from chañar fruits.

Considering the mesocarp (flour A) and seed in chañar, both have higher protein content than wild legumes such as *S. burseroides* and *A. bilimekii* (4.3% and 3.5% and 35.5% and 8.6%, respectively;

Sotelo *et al.*, 1999). Chañar mesocarp showed an important presence of soluble materials, principally low molecular weight sugars and amino acids. When these solubles are removed, the protein content is drastically raised. So, the presence of sugar is now 15,4% while it was just 8,9% in the non-treated flour. Nevertheless, it is highly probable that some protein losses happened during this process, but it is observed from the results that they never exceeded those from carbohydrates. In the case of seeds, proteins are the second most important component. After fat removal, polypeptides represented 48,7% of the residue.

The free and total amino acid composition for flour A, prewashed flour A and seed flours are shown in Table 4. The three samples presented all the amino acids in the free state (except sulfur), excelling in flour A Ser, His and Pro. The washing notably reduced the free amino acids presence in flour A, as can be seen in table 4, but the lower contents are in seed flour, with values did not exceed 0,1% in most cases. Flour A constitutes an excellent raw material for products that require high content in free aminoacids, sport drinks being an example (Ovalles *et al.*, 2002).

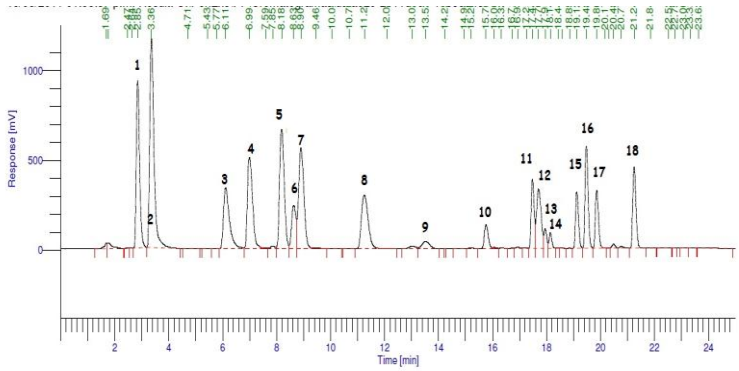

Figure2. *Amino acid profile of the acidic hydrolysis product from the fraction FS.*

Figure 2: HPLC Perkin Elmer. Conditions: Zorbax Eclipse Plus C18 (5µm ; 4,6 x 150 mm) Agilent Technologies column. Detection: UV-V (280 nm). Mobile phase: buffer 25 mM sodium acetate (pH = 6) and (B) acetonitrile with a flow of 0.9 mL/min at room temperature. Flow rate: 0.5 ml/min. Injection volume: 20 µl. Identification: 1: Asp; 2: Glu 3: Ser; 4: His; 5: Gly; 6: Thr ; 7: Arg; 8: Ala; 9: Pro; 10: Tyr; 11: NH4Cl; 12: Val; 13: Met; 14: Cys; 15: Ileu; 16: Leu; 17: Phe; 18: Lys

The most relevant amino acids found in proteins from flour A are Arg, Val, Leu, Ileu and Phe (table 4). In FAW the main proteins were the same, but their presences were drastically reduced. Sulphured amino acids are relevant and in higher levels than those found in some Prosopis (Nieblas, *et al.*, 1996) and also, in wheat flour (Suarez and Lopez, 2009). The essential amino acids content in chañar seed approaches that from soybeans, an important plant protein source. In fact, chañar seed protein showed higher concentration in sulfur amino acids, but lower in the other essential amino acids (Table 4). Seed protein is rich in aminoacids such as Pro, Glu and Asp and with a presence of Phenylalanine (3.6%) similar to that of fish meal (4.5%). Except for Histidine, all the essential aminoacids were in lower concentration than egg white pattern, a characteristic observed in most wild legume seeds (Carvalho, *et al.*, 2011).

Table5. *Chemical score in FA, FAW and FS*

Essential amino acids	2-5 years old children (*)				Adults (*)		
	FA	FAW	FS		FA	FAW	FS
His	418	396	218		418	396	218
Thr	126	102	56		104	102	46
Val	88	109	34		147	109	56
Met-Cys	88	108	137		88	108	137
Ileu	63	107	8		141	107	17
Leu	167	118	56		86	118	29
Phe	173	107	145		96	107	81
Lys	234	149	245		44	149	47

** FAO/WHO patterns (1985)*

According to FAO (1985), chemical score values over 80 are considered desirable. As showed in table 5, chañar seeds (FS) are not deficient in sulfur amino acids, both for children and adults, with a

chemical score of 137 (Met-Cys). Nevertheless, they contain many limiting amino acids for children (Ileu; Val, Thr and Leu), and adults (Ileu, Leu, Thr, Lys and Val). On the contrary, fraction A presented just one limiting amino acid for children: isoleucine and Lysine for adults.

A further limitation in legumes as food sources, are the secondary plant metabolites, such as condensed tannins, protease inhibitors, alkaloids and α-galactosides. Thermal procedures have been shown to be adequate to reduce the content or activity of several metabolites (Alonso *et al.*, 2000), particularly those the termolabile group (protease inhibitors, lectins), and tannins. Furthermore, heat treatment technologies are known to induce conformational changes in storage proteins, which may render them more accessible to digestive enzymes, and thus increase amino acids digestibility (Jezierny, *et al.*, 2010).

Figure3. *Native-PAGE protein extract, pH=11.*

Protein Characterization in Chañar Seed:

Native-PAGE electrophoresis of chañar extract showed four bands (Rm: 0.20, 0.34, 0.46 and 0.60, figure 3), representing four different protein families. The biggest band presented the lowest movility (0,20). In the SDS-PAGE runs, all the extracts showed similar protein profiles, just varying in their intensities according to the pH rise, the higher observed at pH over 6 (Figure 4). More weakly colored bands were observed at pH 3 to 4. Color intensity is commonly related with protein concentration in the band, those in figure 4 seem to be more strongly colored at pH 7 - 8 than others higher; nevertheless, protein solubility is assumed to increase with pH. It is well known that in Bradford method for protein determinations, the color intensity depends on pH (Oseas da Silva & Zezzi Arruda, 2006); the dye used is closely related with the coomassie blue employed here to stain electrophoresis bands. Then, using the intensity of the bands stained for inferring protein concentrations therein at different pHs, does not seem to be appropriate. The profiles showed a broad range of molecular weights, ranging from 11 to 164 kDa, highlighting the bands at 68, 53, 32, 25 and 12 kDa (Figure 5). When the electrophoresis was run in reducing conditions, numerous new bands arose (Figure 6). Prominent protein bands were observed at 95, 76, 39, 24 and 12 kDa. Again, the profile resulted invariant with the pH but, surprisingly the first two, high-molecular weight strongly-stained bands were not observed in non-reducing conditions. Nevertheless, weakly-stained high-molecular weight bands at non reducing conditions, can be observed in figure 5; the disulfur bonds reduction with β-mercaptoethanol surely have freed protein subunits, able to form stronger associations with the dye (Figure 7). In spite of the weak staining, the high molecular weight proteins are present in solution and, in this way, available for the human gut.

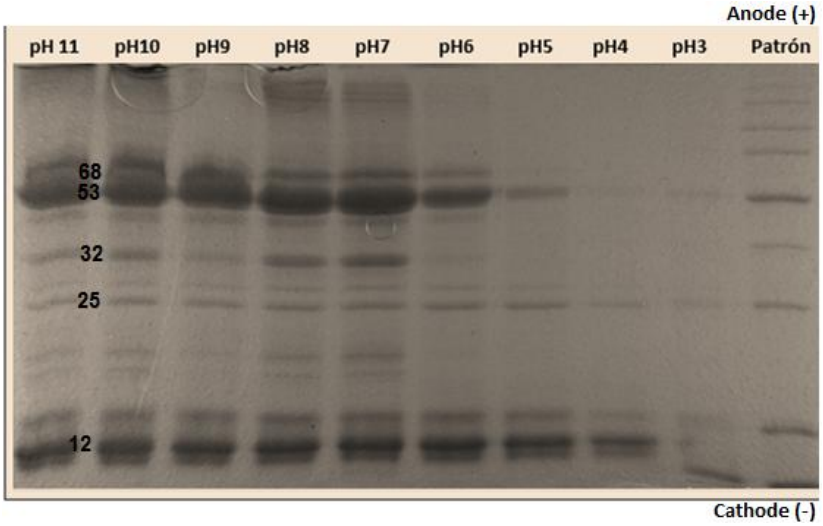

Figure4. *SDS-PAGE protein extracts of pH 3 to 11. Molecular weight (kDa)*

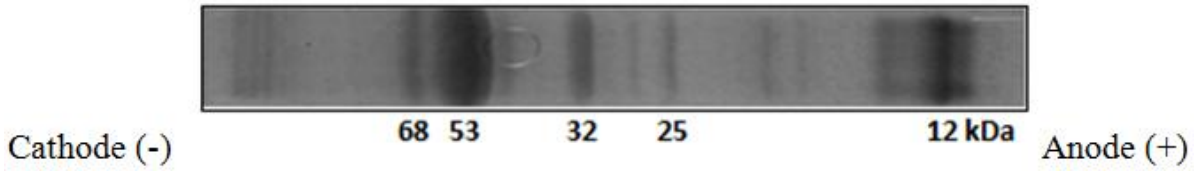

Figure5. *SDS-PAGE proteic extrac a pH 11.*

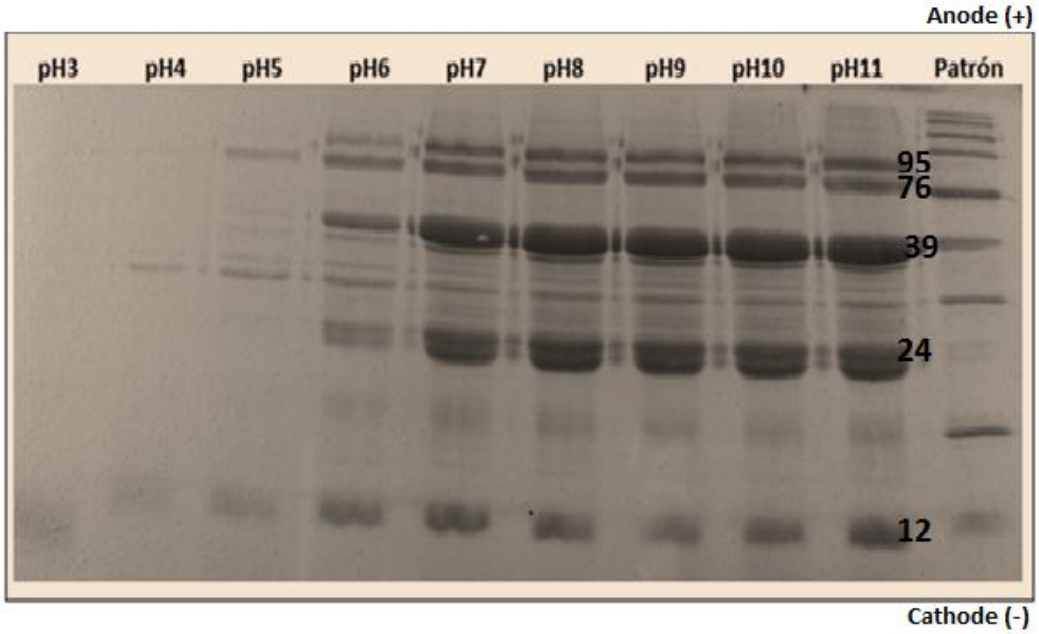

Figure6. *SDS-PAGE with a reducing agent of protein extracts pH 3 to 11. Molecular weight (kDa)*

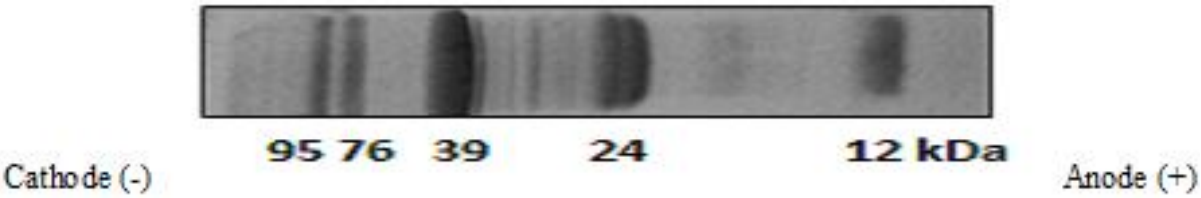

Figure7. *SDS-PAGE protein extract with reducing agent to pH 11.*

4. CONCLUSIONS

The process presented here, allowed the mechanical separation of the woody part of the fruit, improving its nutritional quality. Also, the possibility of a better exploitation of this resource seems possible, not damaging the tree, being an environmentally friendly way to use the resources. The procedures described in this paper are simple and escalable up to an industrial level.

The results showed the chañar fruits as an attractive and alternative source of nutrients for humans or their livestock, through the application of simple technologies. This enabled to obtain a low-lignin-high-protein flour, that could be suitable for humans or animals, and also a low-protein-high-lignin flour that could be devoted to charcoal production or energy generation. In addition, the latter can be divided into two new flours, one bearing most of the raw fiber and the other with the lipids in levels high enough to make the extraction economical.

The digestibility of chañar fruits and the presence or not of anti-nutrients should also be investigated, as it is important in order to establish its quality.

ACKNOWLEDGEMENTS

To the Fondo Nacional de Ciencia y Técnica (FONCyT) in partnership with the Universidad Nacional de Formosa (UNAF) and the Ministry of Culture and Education of the Province of Formosa for funding.

Lic. Vicente Gianna, for his suggestions and comments. Mr. Marcelo Sanchez and Ms. Marina Clavero for your collaboration.

REFERENCES

[1] AOAC International. (1999). Official Methods of Analysis of the Association of Official Analysis Chemist. 16th Edition, 5th Revision, Gaithersburg, USA.

[2] Alonso, R., Aguirre, A., Marzo, F. (2000). Effects of extrusion and traditional processing methods on antinutrients and in vitro digestibility of protein and starch in faba and kidney beans. *Food Chem*. **68**, 159-165.

[3] Becker, R. (1983). Nutritional quality of the fruit from de chañar tree (*Geoffroea decorticans*). *Ecology of Food and Nutrition* **13** (2), 91-97

[4] Bertero, M., Gorostegui, H. A., Orrabalis, C. J., Guzmán, C. A., Calandri, E. L., Sedran U. 2014. Characterization of the liquid products in the pyrolysis of residual chañar and palm fruit biomasses. *FUEL*, **116**, 409-414

[5] Carvalho, A.; Farias, D.; Rocha-Bezerra, L.; Sousa, N.; Cavalheiro, M.; Fernandes, G.; Facanha, L.; Maia, A.; Sousa, D.; Vasconcelos, I.; Gouveia, S.; Machado, O. (2011). Preliminary assessment of the nutritional composition of underexploited wild legumes from semi-arid Caatinga and moist forest environments of northeastern Brazil. *Journal of Food Composition and Analysis* **24**, 487-493.

[6] Charpentier, M. (1998). Valores Nutricionales de las Plantas Alimenticias Silvestres del Norte Argentino. Instituto de Cultura Popular-Comisión Europea, Reconquista, Argentina. pp. 91-93.

[7] Chasquibol Silva, N. (1997). Characterization of crude, neutral and blenched oil from pulp and peel of jungle fruit *Paraqueiba sericea tulasne* "Umari". *Grasas y Aceites* **48** (1), 11-16

[8] Del Valle F. R.; Escobedo, M.; Muñoz, M. J.; Ortega, R.; Bourges, H. (1983). Chemical and nutritional studies on Mesquite beans (*Prosopis juliflora*). *Journal Food Science* **48**, 791-797.

[9] FAO/ OMS/ ONU. (1985). Necesidades de energía y de proteínas. Informe de una reunión consultiva conjunta de expertos. Serie de informes técnicos N° 724. Roma.

[10] Freyre, M. R.; Rozycki, V. R.; Bernardi, C. M.; Baigorria, C. M.; Martínez-Navarrete, N.; Camacho, M. M. (2000). Composición y propiedades de semilla de vinal (*Prosopis ruscifolia).* Series de Ciencias e Ingeniería de Alimentos **2**, 229-239.

[11] Freyre, M. R.; Astrada, E.; Blasco, C.; Baigorria, C. M.; Rozycki, V. R.; Bernardi, C. (2003). Valores Nutricionales de Frutos de Vinal (*Prosopis ruscifolia)*: Consumo Humano y Animal. *Sociedad Mexicana de Nutrición y Tecnología de Alimentos* **4** (1), 41-46.

[12] Gatel, F. (1992). In: First European Conf. on Grain Legumes. Angers, Francia. pp: 461.

[13] Grosso, N. R; Lamarque, A. L.; Maestri, D. M.; Zygadlo, J. A; Guzmán, C. A. (1994). Fatty ácid variatión of Runner peanut (*Arachis Hypogaea* L.) among geographic localities from Córdoba (Argentina). *J. Am. Oil Chem. Soc.* **71** (5), 541-542.

[14] González, G. A.; Correa, A. D.; Patto de Abreu, C. M.; Piccolo Barcelos, M. (2008). Chemical Characterization of integral flour from the *Prosopis spp*. of Bolivia and Brazil. *Archivos Latinoamericanos de Nutrición* **58** (3), 309-314.

[15] Jezierny, D.; Mosenthin, R.; Bauer, E. (2010). The use of grain legumes as a protein source in pig nutrition: A review. *Animal Feed Science and Technology* **157**, 111-128

[16] Gunstone, F. D.; Harwood, J. L.; Dijkstra, A. J. (2007). The Lipid Handbook. 3º ed. C.R.C. Press. E.E.U.U. p. 51-54.

[17] Lamarque, A. L.; Maestri, D. M.; Zygadlo, J. A; Guzmán, C. A. (2000). Chemical evaluation of *Geoffroea decorticans* seeds as source of oil and protein. *Grasas y Aceites* **51** (4), 241 – 243.

[18] Maestri, D. M; R. H. Fortunato; J. A. Greppi; A. L. Lamarque. (2001). Estudios de composición de semillas y frutos de dos variedades de Geoffroea decorticans. *Journal of Food Composition and Analysis* **14** (6), 585-590.

[19] Maldonado, E. M.; Guzmán, C. A. (1998). Contenido de algunos elementos y cenizas totales en semillas de nueve cultivares de maní de la provincia de Córdoba (Argentina). *Fyton* **42** (2), 185-189.

[20] Matuda, T.; Netto, M. (2005). Caracterização química parcial da semente de Jatobá-do-cerrado (*Hymeneae stigonocarpa* Mart.*)*. Ciencia y Tecnol. Alimentos. Campiñas, **25** (2), 353-357.

[21] Mufari J. R. (2010). Tesis: Determinación y cuantificación de proteínas solubles y de aminoácidos totales en harina de quinoa. Facultad de Ciencias Químicas. Universidad, Argentina.

[22] Nieblas Ortega, Moreno, M.; Burgueño, M. (1996). Protein Quality and Antinutritional Factors of Wild Legume Seeds from the Sonoran Desert. *J. Agric. Food Chem.* **44**, 3130-3132.

[23] Osborne, D. R.; Voogt, P. (1986). The Analysis of Nutrients in Foods. Editorial: Academic Press Inc. (London) Ltd. Section 4: Carbohydrates pp. 158 - 161, 248 -219.

[24] Oseas Da Silva, M. A.; Zezzi Arruda, M. A. (2006). Mechanization of the Bradford Method for the spectrophotometric determination of total proteins. *Analytical Biochemistry*, **351**, 155-157

[25] Ovalles, J.; León, L.; Vielma, R.; Medina, A. (2002). Determinación del contenido de aminoácidos libres del agua de coco tierno por HPLC y revisión electrónica sobre la nueva tecnología para el envasado del agua de coco. Revista de la facultad de farmacia **44**, 70-78.

[26] Ozkan, C.; Atalay, A.; Guven, I.; Kaya, E.; Sagocak, A. (2011). Crude protein and amino acid composition of some protein sources used livestock production in south of Turkey. *Asian journal of animal and veterinary advances.* **6** (7): 750 – 753.

[27] Seguras, S. F.; Echeverri, F. R.; Patiño, L. A.; Mejía, G. A. (2007). Descripción y discusión acerca de los métodos de análisis de fibra y del valor nutricional de forrajes y alimentos para animales. VITAE. Revista de la facultad de química y farmacéutica, **14** (1), 72-81.

[28] Silva, R.; Ruiz, R. L.; Ruiz, S. O. (1999). Estudio Fitoquímico de Frutos de *Geoffroea decorticans* (Gill. Ex Hook. et Arn.) Burk. *Leguminosae* (Fabaceae). Acta Farm. Bonaerense **18** (3), 217-219.

[29] Sotelo, A.; Migliaro, P.; Toledo, A.; Contreras, J. (1999). Chemical composition, digestibility and antinutritional factors content of two wild legumes: Styphonolobium burseroides and Acacia bilimekii. *Plant Foods for Human Nutrition* **54**, 59–65.

[30] Suarez, M.; Lopez, L. (2009). Alimentación saludable. Editorial: Hipocrático S.A (Buenos Aires). Sección 11: Evaluación de la Calidad Proteica. pp. 48-49.

[31] Tapia, M.; Moron, C.; Ayala, G.; Fries, A. (2000). Valor Nutritivo y Patrones de consumo. En: Cultivos Andinos subexplotados y su aporte a la alimentación, 2° Edición, Organización de las Naciones Unidas para la Agricultura y la Alimentación. Oficina Regional de la FAO para América Latina y el Caribe.

[32] Vallilo, M.; Lamardo, L.; Gaberlotti, M.; Oliveira, E. Moreno, P. (2006). Composição química dos frutos de *Campomanesia adamantium* (Cambessédes) O.BERG. Ciencia y Tecnol. Alimentos. Campiñas, **26** (4), 805-810.

[33] Wattanapat, R.; Nakyama, T.; Beuchat, L. R; Phillips, R. D. (1994). Kinetic of acid hydrolysis of defatted peanut flour. J. *Food Science,* **59** (3), 621 – 625.

[34] Wiseman, J. y Cole, D.J.A. (1988). En: Recents Advances Animal Nutrition. Butterworths. pp: 13.

[35] Zamora Rueda, G.; Gutiérrez, C.; Campero, V.; Barrionuevo, M. J. (2008). Comparación de las propiedades funcionales entre frutos y fibras de mistol y chañar. II Jornadas de Jóvenes Investigadores. Universidad Nacional de Tucumán. ISBN: 978-987-1366-20-0. Tucumán.

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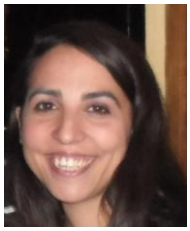

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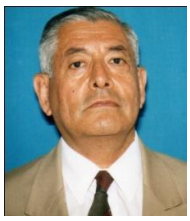

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