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NUTRITIONAL VALUE ANALYSIS OF ATRIPLEX LAMPA PROTEIN CONCENTRATES OBTAINED BY THERMOCOAGULATION AND ULTRAFILTRATION

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A B S T R A C T

The present work is intended to evaluate the quality of the protein extracted from *Atriplex lampa*, using membrane technology. This shrub is highly available in the area studied. Ultrafiltration followed by discontinuous diafiltration was conducted, and a protein concentrate by ultrafiltration (PCU) was obtained. Then, PCU was contrasted with a protein concentrate obtained by acid thermocoagulation (PCT) through the modified Ostrowski method, suitable for forage. Results indicated that PCT was limited in its Biological Value by the sulfur-containing aminoacids with a chemical score (CS) of 79.99 for methionine + cysteine. Lysine content was remarkable, with an availability of 51%. PCU showed a CS of 82.85.A high content of the remaining essential amino acids was found, particularly that of lysine with an enhanced availability (58%). In general, PCU, when compared to PCT, had a higher protein content (850g/kg), greater salt reduction (25 g/kg), and higher availability of lysine, threonine and tryptophan. By improving the taste, its use in formulations for animal supplements is feasible. The preparation of a protein concentrate starting from leaves of *Atriplex lampa* is considered a potential source of protein production for animal feeding whenever the high content of salt affecting the palatability of the product can be decreased.

INTRODUCTION

Protein is required for most normal functions of goats, including maintenance, growth, reproduction, lactation and hair production.Pregnant and lactating goats prefer forage species with high protein contents (Allegretti, *et al.*, 2012). Roig (1981) has suggested that as *Atriplex halimus* is used in Israel, *Atriplex lampa* may be used as forage due to its protein content. Several species studied were found to have protein values ranging from 160 to 203 g/kg (De Kock, 1980). In addition, Betschart and Kinsella (1974) stated that leaf proteins are important sources of lysine, methionine and tryptophan, making them an adequate complement to cereal grain proteins which are deficient inthese amino acids.

In Argentina, the species of the genus *Atriplex* show an enormous potential in the recovery of degraded arid soils and the production offorage and fuel from firewood.The plant has the ability to thrive in fragile and deteriorated environments such as the phytogeographic districts of the Chaco regions: Arid, Semiarid and Monte, where goat livestock is extensively bred.Since *Atriplex* is available throughout the year (Allegretti, *et al.*, 2012), it constitutes an important protein supplement for cattle, and anenergy contribution during periods in which the forage availability of the natural grassland is quantitatively and qualitatively reduced (Food and Agriculture Organization FAO, 1997).

Argentina has an area known as Monte Phytogeographic Province extending from Salta (23º S) in the North, to the province of Chubut (46º S) in the South. The Province of San Luis (33º 20’ S) is located within this biogeographic unit in which approximately 70 % of its territory is arid and semiarid with annual precipitations of 100 mm. As a result, there is a growing need of reinforcing and intensifying studies related to existing wild plant species in order to be used as protein source.

Several studies regarding the methods for protein extraction from fresh leaves have been reported (Sinclair, 2009). Traditional methods for obtaining protein isolates show disadvantages that limit the nutritional and functional value of proteins (Ostrowski *et al.*, 1978). Research conducted during the last decades has shown that aqueous extraction techniques and separation by ultrafiltration produce protein concentrates of high quality (Villeneuve and Mondor, 2014).

Ultrafiltration (UF) is a technique that replaces or complements traditional methods with the following advantages: short processing times and an increase of protein recovery with higher purity and lower energy consumption, among others. Consequently, obtaining products with highly desirable functional and nutritional properties is feasible (Scott, 1995). In general, the separation through membranes is economically more convenient than traditional techniques

involving extraction and precipitation. Nichols and Cheryan (1981) proved that the application of UF followed by diafiltration results in protein concentrates of high purity. Discontinuous diafiltration (DD) is the process by which the UF can rapidly and efficiently remove salts or low molecular weight species. It consists in the dilution of retentate with treated water, and subsequent ultrafiltration to continue the selective extraction of salts. It has become evident that the worldwide production of proteins needs to be increased. Hence, the separation techniques using membranes may constitute a major contribution to this area.

The aim of the present study was to compare the nutrition value of concentrates obtained by two methodologies: the Ostrowski method by acid thermocoagulation and UF concentration followed by purification through discontinuous diafiltration (UF-DD).This work proposes the exploitation of a natural resource (*A. lampa*) available in the region as protein concentrate by reducing its salt content, and resulting in better palatability.

MATERIALS AND METHODS

Preparation of plant material

A. lampa leaves were obtained from Monte, in Beazley, near San Luis city (Argentina).This species flowers from October to November and bears fruits from November to January (Moquin Dietrich, 1981). The material was harvested before flowering, when the plant had new leaves andthe protein content was higher.Twenty kilograms were harvested in a working day and immediately transported to the laboratory, where the stems were manually removed to prevent excessive humidity loss. Finally, the separated leaves were stored in polyethylene bags and kept frozen at -30ºC until they were used.

Protein concentrate obtained from A. lampa

Based on bibliographical information (De Fremery *et al.*, 1972), the method for foliar protein extraction was modified. Once the leaves reached room temperature, they were humidified using 2% sodium metasulfide. This prevented protein concentrate (PC) darkening, resulting from the formation of pigments in the plant extracts, caused by the binding of quinones to the protein sulfhydryl groups (Plaz*aet al.*, 2003). The juice from fresh leaves was extracted using a mechanical disintegrator (5 mmhole diameter).Protein extraction conditions from *A. lampa* leaves stemmed from the pH, using a ratio 1:5 (g of leaves/ml deionized water). Laboratory assays included dispersions of 100 g of grinded leaves in 500 ml of deionized water, agitation for 2h at pH 10 (IKA RW 16 Basic, Buenos Aires, Argentina), and a continuous flow of water at 5ºC passing through the tank to keep the temperature low during the extraction, thus minimizing proteolysis. Throughout the maceration process, organic acids were released because of vacuole breakage causing a pH decrease. By the addition of 5 N NaOH, the pH was kept constant. The insoluble material was separated from the aqueous extract by centrifugation (Sorvall Superspeed Angle Rotors GSA, Neutown, CT, USA) for 15 minutes at 1000 x G and 5°C (Fernández *et al.*, 1999). The leaf paste was pressed with a hydraulic press (7881-L05 Sepco, Atlanta, USA), and 5kP a of pressure was applied after each extraction to remove all the extractable juice from the green pulp.

In order to achieve additional extractability, the insoluble fibrous residue was re-suspended in half the initial quantity of deionized water. Temperature and pH were kept unchanged but, to ensure the removal of all soluble components, the extraction was applied for 60 further minutes. A third extraction was conducted to obtain a larger quantity of released nitrogen from the macerated fiber through a treatment with non-ionic detergent (10 g/l Tween 20) and agitation for 30 minutes. As a result, chloroplasts broke and the proteins were released from chlorophyll and lipids complexes. A greater quantity of nitrogen may be extracted when detergents are used over the residues after the initial alkaline extraction in relation to when they are added to the leaves in conjunction with the extractant. Once the extraction liquids were collected, 2% sodium metasulfide was added again to prevent protein loss. Laboratory protocols were applied to determine the nutritional value use of silicotungstic (STA) and trichloroacetic acids (TCA) in combination with detergents to segregate the nitrogen and protein content from the plant material in categories differing in digestibility (Licitra *et al.*, 1996). The extract was used in the two methods applied in this study. A schematic representation of the protein extraction process is shown in Figure 1.

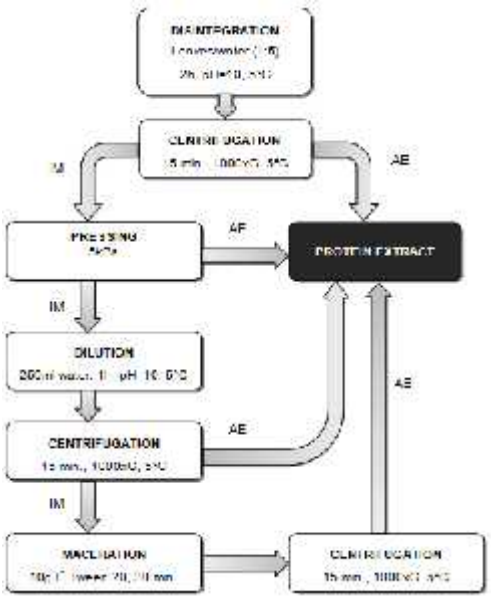

Fig. 1 General scheme of the process for obtaining protein extract from *A. lampa* leaves. The leaves were subject to different processes; the insoluble material (IM) and aqueous extract (AE) resulting in each step take different paths to obtain the final protein extract.

To obtain the protein concentrate suitable for forage by thermocoagulation (PCT), the method proposed by Ostrowski *et al.* (1978) was used as the base, and some modifications were incorporated. Liquids obtained were collected adjusting the pH to 3.5 using 5N HCl, and the protein clot was precipitated by passing through a current of water vapor up to 85ºC. Subsequent cooling to room temperature and centrifugation at 3000 rpm for 30 minutes were applied. The concentrate obtained was double-washed with 0.05N HCl, then with *deionized* water and finally with 95° ethylic alcohol,in all caseskeeping the ratio clot/solvent at 1:4.Alcohol wash decreased the content of chlorophyll and saponins, both soluble in ethanol.The yellowish-brown PCT obtained was dried in a vacuum furnace at 35 °C and ≤ 50 mmHg.

To obtain the protein concentrate by ultrafiltration (PCU), assays using membrane techniques through UF-DD were conducted. This desalinization process enhanced the palatability of the concentrate. In order to separate the solids that were not removed by centrifugation within the alkaline aqueous extract, microfiltration using a frontal flow module with a <1 µm Hytrex membrane (Osmonic, USA) was used. The UF studies were carried out using a Pellicon Cassette (Millipore® PLGC-type, USA) system, having a molecular weight cut-off (MWCO) of 10 kDa, and a membrane area of 0.465 m^2 (Fernández *et al.*, 2007). The operating conditions were the following: transmembrane pressure (TMP) of 1.4 bar, 18.6 l of sample at 30°C and a recirculation rate (*Q*) of 3.5 l/min.The UF was reached with a volume concentration ratio (VCR) of 12, and a final concentrate volume of 1.55 l.In the DD process, the permeable solutes were eliminated from the concentrateby volume reduction followed by the addition of the same quantity of water previously treated, and the subsequent re-ultrafiltration to VCR=12. The PCU was dried just as PCT.

Chemical composition

The total protein content was obtained by Kjeldahl method (Digestion block and Steam distillation unit Kjeldahl Semi-Automatic "Pro-Nitro S", Selecta, Spain).The conversion factor used for expression of the results was 6.25 (AOAC, 2005). Moisture was determined by drying in a vacuum oven (HER/F/I Dalvo, Buenos Aires, Argentina) at temperatures below 70ºC and pressure ≤ 50 mm Hg until constant weightwas reached (AOAC, 2005).Ash contentwas determined by incineration in a muffle furnace(HI Dalvo, Santa Fe,Argentina) at 525ºC(AOAC, 2005). Sodium and potassium content was quantified by atomic absorption spectrophotometry (Instrumentation Laboratory Spectrophotometer 751, Wilmington, Massachusetts, USA) (Ureand Mitchell, 1975). Total dietary fiber was determined by the enzymatic-gravimetric method (Prosky *et al.*, 1988).

Determination of the amino acid composition

After treatment with hydrochloric acid, hydrolyzed amino acids from the samples were derivatized with phenylisothiocyanate, and the resulting phenylthiocarbonyl derivatives were separated and quantified by the reserve phase HPLC using Merck-Hitachi equipment, consisting of an L-6200 pump, an L-4000 UV detector, and a D-2500 chromato-integrator. ASuperspher 60 RP-Select B column of 250 mm x 4 mm (Merck KGaA, Darmstadt, Germany) and α-aminobutyric acid as internal standard were used. Data were corrected by a normalized factor of 95% hydrolysis.The method described by Hurrell *et al.* (1979) was used to determine the available lysine.

Biological assays

Saltbush-quality concentrates (*A. lampa)* were analyzed by three different indexes: net protein utilization (NPU), based on the body nitrogen yield and defined as the portion of nitrogen intake that is retained, true digestibility (TD) and biological value (BV) as noted by Miller and Bender (1955). Four sets of 30 day-old Wistar rats weighing 50 g (± 0.5) were used (eight animals per set). The animals were housed and cared for at the Animal Resource Facilities, Facultad de Química, Bioquímica y Farmacia, Universidad Nacional de San Luis (Argentina), in accordance with the National Institutes of Health guide for the care and use of Laboratory animals.The first set received a control diet (casein), the second received dried leaf flour (DLF), and the remaining two sets were fed with PCT and PCU. The animals were kept in individual suspended cages with screen bottoms. Temperature and relative humidity were kept at 25 ± 2 ºC and 50%, respectively. Lighting was controlled by alternating 12 h periods of light and darkness. All the animals received potable water and food *ad libitum* for 10 days. Weight gain was recorded at the end of the experiment. Feces were collected and weighed. All diets were prepared according to the method of Sambucetti *et al.*, (1973) as follows: 10% proteins, 14.5% corn oil, 5% salt mixture, 0.25% hydrosoluble vitamins, 0.5% lyposoluble vitamins, 0.15% choline (as citrate) and 69.6% of dextrin. In the protein-free diet, dextrin was used as substitute. Salts and hydrosoluble and lyposoluble vitamins were added to all diets as Harper (1959) suggested.

Statistical analysis

Results are expressed as the mean ± SEM. The significance of differences between groups was evaluated by one-way analysis of variance (ANOVA), followed by the Tukey-Kramer multiple comparison test using 5 % of significance level. A *P*value less than 0.05 was considered statistically significant. The relative standard deviations for the determination of the amino acid composition analyses were approximately ±10%.

RESULTS

In order to evaluate the nutritional value of the *A. lampa* protein by biological methods, three different indexes were determined, namely,NPU, TD and BV. When a diet prepared with DLF was provided, the recordedintake during the ten days of experience was quite low, resulting in poor NPU values (Table 1). The PCT was biologically evaluated, and enhanced values for NPU were obtained in relation toDLF.The intake levels and weight gain of PCT diminished in contrast with the casein reference diet.The data obtained from the evaluation of the PCU biological quality are summarized in Table 1.The biological indexes for PCU were higher than PCT and the intake was comparable with the casein diet. Regarding the final performance of PCU, the percentage of nitrogen obtained in the protein concentrate with respect to the initial quantity present in the fresh leaves was 30.00 ± 1.8 %, in contrast to the PCT, which was 18 ± 1.2 %.

Table 1 *Biological quality of the DLF, PCT and PCU obtained from A. Lampa contrasted to Casein and PC obtained from M. sativa*

	Casein	DLF	PCT	PCU	PC Alfalfa
		A. lampa	*A. lampa*	*A. lampa*	*M. sativa*
NPU	89.00 ± 8.98^a	21.00 ± 2.6^b	50.65 ± 3.21^c	63.00 ± 4.00^b	62.4 ± 6.3^b
TD	97.20 ± 9.89^a	-	69.42 ± 6.96^c	79.00 ± 5.21^b	82.8 ± 3.1^b
BV	91.60	-	72.90	79.80	75.4
Intake	85.00 ± 8.98^a	30.70 ± 3.2^b	78.49 ± 3.26^c	86.40 ± 10.10^a	
WG	30.00 ± 3.27^a	-	18.66 ± 1.30^c	23.10 ± 1.29^b	

DLF = dried leaf flour; PCT = protein concentrate by acid thermocoagulation; PCU = protein concentrate by ultrafiltration; PC = protein concentrate; NPU = net protein utilization; TD = true digestibility; BV = biological value; Intake = Average food intake (g/rat per 10 days); WG = Weight gain (g/ rat per 10 days). Results expressed as mean (g/Kg, dry-weight basis) ± SD. Values on the same row with different superscript letters differ significantly ($P < 0.05$).

The chemical composition of PCT was determined, and results showed a decrease in salt content and an increase in

protein concentration in relation to DLF (Table 2). Data regarding the chemical composition of PCU are also included. From the data analysis, it can be inferred that PCU had a higher protein content and lower salt content than PCT. It is important to note that the values determined for sodium and potassiumfor PCU corresponded to the minimum requirements for ruminants (Na = 0.6 g/Kg and K = 5-8 g/Kg), according to the National Research Council (1981).

Table 2 *Chemical composition of DLF contrasted to PCT and PCU obtained from A. Lampa*

	DLF	PCT	PCU
Humidity	62.7 ± 1.98	76.3 ± 3.11	76.0 ± 1.97
Protein	269.3 ± 7.99	593.7 ± 4.66	850.0 ± 9.89
Total Dietary Fiber	152.4 ± 2.95	10.2 ± 0.56	10.0 ± 0.42
Total Ashes	378.0 ± 4.24	147.0 ± 1.13	25.0 ± 3.39
Potassium	49.5 ± 4.10	23.5 ± 1.15	5.0 ± 0.35
Sodium	60.5 ± 3.68	35.0 ± 4.63	0.8 ± 0.19

DLF = dried leaf flour; PCT = protein concentrate by acid thermocoagulation;
PCU = protein concentrate by ultrafiltration.
Results expressed as mean (g/Kg, dry-weight basis)

Table 3 summarizes the amino acid content and the evaluation of the plant protein quality throughthe chemical score (CS), whichwas calculated using anamino acid pattern (FAO/OMS, 1981). The values obtained indicate that the protein ofDLF was limited in its BV by the sulfur-containing amino acids (methionine + cysteine). The content ofthe remaining essential amino acids was also important, particularly that of lysine.The evaluation of the PCT protein quality through CS indicated that the protein was limited in its BV by the same sulfur-containing amino acids. Moreover, the lysine content wasnotable, withan availability of 51%. The resultingvalues indicated that the protein of PCU was still limited in its BV by the sulfur-containing amino acids, improving the lysine availability (58%).

The results on nitrogen obtained in the protein concentrate are in accordance with previous studies conducted by Ostrowski (1979) regarding the quality and quantity of the protein extracted from different herbs and grasses through acid thermocoagulation, in comparison with the UF process. Besides, the acid thermocoagulation method decreased the availability of some essential amino acids. The loss of lysine availability of PCT was attributed to the process applied (Bhatty and Finlayson, 1973). This might result from the formation of cross-linking among different amino acids of the proteinor from a severe heating during the thermocoagulation process, in which the highly reactive lysine ε-amino groups interact with the sugars present in the hot plant liquor (Maillard reaction). This reaction was not observed when the protein concentration by UF wasperformed at low temperatures (approximately between 25ºC and 30ºC). Among the essential amino acids, lysine and threonine are the most heat-labile (Lund, 1973). However, Meredith *et al*., (1974) have shown that threonine and valine are subject to a higher degree of degradation throughout the heating process, in comparison with methionine and lysine. Additionally, the PCT process causes the denaturation of the proteins, making them insoluble in water and not favorable from the nutritional perspective. According to Lewis (2001), the most limiting amino acids for the pig diet are lysine and threonine. Moreover, threonine content is comparable to the protein concentrate obtained from alfalfa leaves (*Medicago sativa)*, known as Pro-Xan. The latter is obtained by membrane techniques, and it is used as chicken feed (Enochian *et al*., 1980).

Table 3 *Amino acid content and chemical score in DLF contrasted to PCT and PCU obtained from A. Lampa*

Amino Acids	**FAO[1]**	**DLF**		**PCT**		**PCU**	
(g/16g N)		AA	CS	AA	CS	AA	CS
Lysine	5.5	8.70	>100	8.77	>100	9.60	>100
Histidine	-	0.20	-	0.30	-	0.20	-
Arginine	-	5.90	-	5.97	-	6.42	-
Aspartic Acid	-	6.80	-	7.90	-	7.58	-
Threonine	4.0	4.90	>100	4.74	>100	5.08	>100
Serine	-	3.80	-	3.90	-	4.10	-
Glutamic Acid	-	9.00	-	9.50	-	9.70	-
Proline	-	4.00	-	4.95	-	4.70	-
Glycine	-	6.30	-	5.50	-	6.50	-
Alanine	-	5.70	-	6.25	-	6.50	-
Valine	5.0	5.60	>100	5.32	>100	6.20	>100
Tryptophan	-	1.60	-	1.75	-	1.90	-
Cysteine + Methionine	3.5	3.19	91.13	2.80	79.99	2.90	82.85
Isoleucine	4.0	4.80	>100	5.55	>100	5.30	>100
Phenylalanine + Tyrosine	6.0	10.00	>100	10.91	>100	10.51	>100
Leucine	7.0	8.60	>100	9.84	>100	9.95	>100

DLF = dried leaf flour; PCT = protein concentrate by acid thermocoagulation; PCU = protein concentrate by ultrafiltration; AA = amino acid; CS = chemical score.
1FAO/OMS (1981).
Values correspond to the average of two determinations.

DISCUSSION

In this study the low intake in the rats feeding with DLF was shown to be attributed to the low palatability of the diet by the high salt content. Results suggested that salts act as a non-nutritive diluent. Given this evidence, the PCT was obtained through a modification of the Ostrowski *et al.*, (1978) method, suitable for forage. Although the NPU of PCT increased in comparison with DLF, the value was still low. Furthermore, an slight improvement in leaf palatability was observed as a result of the salt content decrease.

Regarding the PCU digestibility, previous studies have reported values of soy flour ranging from 85 to 90% (Carlsson and Hanczakowski, 1985). Consequently, the PCU from *A. lampa* is, at least, 11% less digestible than soy flour. These data, in comparison with the results obtained by using an alfalfa protein concentrate (*M. sativa)*, suggest that the behavior of both concentrates through *in vivo* assays are some what similar (Hernández *et al.*, 1997). Additionally, if BV is compared to other samples such as cow milk casein (79.7) or soy flour (76.6), the values resemble thoseof our concentrate which

were higher than the protein isolated from soy (66.4) (FAO, 1970).

The NPU was higher in PCU than PCT, confirming our previous assumption regarding the influence of the concentration procedure. The weight gain observed with the PCT diet was small as a result of its low palatability, because the salt content was still present. This fact constitutes an advantage for the PCU. The digestibility value of the PCTwas lower than PCU. The heat contributed to the formation of disulfide bonds in the amino acids with the formation of irreversible steric perturbations that prevent the action of the digestive enzymes (Serna-Saldivar *et al.*, 1988).The PCU exhibited outstanding lysine content. Likewise, the PCU is comparable to various flours used as balanced supplements for lysine- and threonine-deficient animal foods, such as cotton-seed, flax-seed, corn gluten and soy flour. By feeding animals, it has been experimentally determined that the limiting amino acids in the protein of corn are, in order of importance, the following: lysine, tryptophan and threonine (Benton *et al.*, 1956). In general, PCU has a higher content of lysine, threonine and tryptophan than PCT.

CONCLUSIONS

Through aqueous extraction and UF-DD concentration techniques, it has been demonstrated that a concentrate from foliar proteins may be obtained. Regarding the final performance, PCU is better than PCT for obtaining higher protein content from the same quantity of plant material. Besides, the CS is improved for methionine + cysteine to 82.85 with a high availability of essential amino acids like lysine, threonine and tryptophan. The salt content decreases to 25g/Kg as well as the values for sodium and potassium, reduced to 0.8g/Kg and 5 g/Kg, respectively, which are values close to the requirements for ruminants. By improving the taste, its use in formulations for animal supplements becomes feasible.

This work is currently at alaboratory technical feasibility stage, and the preliminary economic analysis of the process has not yet been conducted. However, approximations are favorable because of the great raw material availability.*A. lampa* is a shrub widely distributed and abundant in the semiarid region of San Luis,Argentina, it grows wild and might be used as a sustainable protein source.Taking into account that plant protein is the most inexpensive primary source available within the agro-ecosystem, the optimization of the overall technological process involving its production could have a major impact. The application of the concentrate as a dietary supplement for the rural population is also considered. Consequently, the appropriate and responsible use of this resource could also benefit the local communities.

References

Allegretti, L., Sartor, C., Paez Lama, S., Egea, V., Fucili, M. and Passera, C. 2012. Effect of the physiological state of Criollo goats on the botanical composition of their diet in NE Mendoza, Argentina. Small Rumin. Res. 103, 152-157.

Association of Official Analytical Chemists (AOAC). 2005. Official methods of analysis, 18th edition. AOAC, Arlington, VA, USA.

Benton, D.A., Harper, A.E., Spivey, H.E. andElvehjem, C.A.1956. Leucine, isoleucine and valine relationships in the rat. Arch. Biochem. Biophys. 60, 147-155.

Betschart, A.A. andKinsella, J.E.1974.Influence of storage composition, amino acid content, and solubility of soybean leaf protein concentrate. *J.Agric.Food Chem*. 22, 116-123.

Bhatty, R.S. andFinlayson, A.J.1973. Extraction of nonprotein nitrogen from oilseeds meals with different solvents. Cereal Chem.50, 329-335.

Carlsson, R. andHanczakowski, P.1985. The nutritive value of mixtures of white leaf protein and food proteins. *J. Sci. Food Agric.*36, 946-950.

De Fremery, D., Bickoff, E.M. and Kohler, G.O. 1972. PRO-XAN process. Stability of proteins and carotenoid pigments in freshly expressed alfalfa juice. *J. Agric. Food Chem*. 20, 1155-1158.

De Kock, G.C.1980. Drought resistant fodder shrub crops in South Africa. Le Houérou HN (ed.), Browse in Africa: The current state of knowledge. International Livestock Centre for Africa, Addis-Ababa399-410.

Enochian, R.V., Kohler, G.O., Edwards, R.H., Kuzmicky, D.D. andVosloh, C.J. 1980. Producing PRO-XAN (leaf protein concentrate) from alfalfa: economics of an emerging technology. United States Department of Agriculture, Agricultural Economic Report Nº 445.

FAO.1970. Amino acid content of foods and biological data on proteins. FAO, Rome, Italy.

FAO. 1997. Especies arbóreas y arbustivas para las zonas áridas y semiáridas de América Latina. Serie: zonas áridas y semiáridas Nº 12. Programa conjunto FAO/PNUMA de control de la desertificación en América Latina y el Caribe. Santiago de Chile.

FAO/OMS.1981. Feed information summaries and nutritive values, of FAO Animal Production and Health Series12. FAO, Rome, Italy.

Fernández, S.S., Mucciarelli, S. and Pérez Padilla, A. 1999. Protein extraction from *Atriplex lampa* leaves: Potential use as forage for animals used for human diet. Plant Foods Hum Nutr 54, 251-259.

Fernández, S.S., Menéndez C., Mucciarelli, S. and Pérez Padilla, A.2007. Saltbush (*Atriplex lampa*) leaf protein concentrate by ultrafiltration for use in balanced animal feed formulations. *J. Sci. Food Agric.*87, 1850-1857.

Harper, A.E. 1959. Aminoacid balance and imbalance. I. Dietary level of proteinand aminoacid imbalance.*J.Nutr*. 68, 405-418.

Hernández, T., Martínez, C., Hernández, A. and Urbano, G.1997. Protein quality of alfalfa protein concentrates obtained by freezing. *J. Agric. Food Chem*.45, 797-802.

Hurrell, R.F., Lerman, P. and Carpenter, K.J. 1979. Reactive lysine in foodstuffs as measured by a rapid dye-binding procedure. *J. Food Sci*. 44, 1221-1227.

Lewis, A.J.2001. Amino acids in swine nutrition. In Lewis AJ, Southern LL (eds.) Swine Nutrition, 2nd edn. CRC Press, Boca Raton Florida, US 131-150.

Licitra, G., Hernandez, T.M. and Van Soest, P.J. 1996. Standardization of procedures for nitrogen fractionation of ruminant feeds. Anim. Feed Sci. Technol. 57, 347-358.

Lund, D.B. 1973. Effects of heat processing. Food Technol27, 16-18.

Meredith, F.I., Gaskins, M.H. and Dull, G.G. 1974. Amino acid losses in turnip greens (*Brassica rapa L.*) during handling and processing. *J. Food Sci*. 39, 689-691.

Miller, D.S. and Bender, A.E.1955. The determination of the net utilization of proteins bya shortened method. Br. *J.Nutr*.9, 382-388.

Moquin Dietrich.1981. *Atriplex lampaDarwiniana* 23, 126-127.

National Research Council (NRC).1981. Nutrient requirements of goats: angora, dairy, and meat goats in temperate and tropical counties. Nutrient requirements of domestic animals 15. National Academy Press, Washington, DC, USA.

Nichols, D.J. andCheryan, M.1981. Production of soy isolates by ultrafiltration: factors affecting yield and composition. *J. Food Sci*. 46, 367-372.

Ostrowski, H.T., Carlsson, R. andTragardh, C. 1978. Isolation and purification of proteins from green vegetation for direct human consumption. In: Linko P, Mälkii Y, Olkku J, Larinkari G (eds) Food Process Engineering 1. Food processing systems. London: Applied Science Publishers Ltd, 864–870.

Ostrowski, H.T. 1979. The isolation of protein concentrates from pasture herbage and their fractionation into feed- and food-grade products. *Journal of Food Processing and Preservation* 3, 105-124.

Plaza, L., Muñoz, M., De Ancos, B. and Cano, M.P. 2003. Effect of combined treatments of high-pressure, citric acid and sodium chloride on quality parameters of tomato puree. European Food Research and Technology 216, 514-519.

Prosky, L., Asp, N.G., Schweizer, T.F., De Vries, J.W. andFurdaI.1988. Determination of insoluble, soluble, and total dietary fiber in foods and food products: interlaboratory study. *Journal Association off Official Analytical Chemists* 71, 1017-1023.

Roig, F.A.1981. Flora de la ReservaEcológica de Ñacuñan. Editorial Zeta, Mendoza, Argentina. Cuaderno Técnico (IADIZA) 3, 5-176.

Sambucetti, M.E., Gallegos, G. andSanahuja, J.C.1973. Isolated protein from linseed meal I. Nutritive value and toxicological tests. ArchivosLatinoamericanos deNutrición 23, 79-94.

Scott, K.1995. Handbook of industrial membranes,1st ed., Elsevier Advanced Technology, Oxford, UK.

Serna-Saldivar, S.O., Tellez-Giron, A. and Rooney, L.W. 1988. Production of tortilla chip from sorghum and maize. *Journal of Cereal Science* 8, 275-284.

Sinclair, S. 2009. Protein extraction from pasture: The plant fractionation bioprocess and adaptability to farming systems. Ministry of Agriculture and Forestry, New Zealand, SFF No. C08/001.

Ure, A.M. and Mitchell, R.L.1975. Lithium, sodium, potassium rubidium and cesium. In Dean J.A. andRains TC, eds. Flame emission and atomic absorption spectrometry. Dekker, New York, USA.

Villeneuve, S. and Mondor, M. 2014. Processing and bread-making potential of proteins isolated from malted and no malted pea seeds by ultrafiltration/ diafiltration. Food Bioscience 8, 33-36.

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