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PROTEIN AND AMINO ACIDS OF NOPAL (*Opuntia ficus indica* (L.)[1]

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1. INTRODUCTION

It is not common to hear about the usage of cactus for food consumption, but according to VILLARREAL (*16*) there are regions in the world where the climate conditions are so extreme that for various years the rainfall is very reduced. The resultant low moisture extremely limits the development of other plants. In this case, the nopal - also called prickly pear - is almost vital to man and other animals for it provides water, a rare diet component during droughts made even more severe because of its need during the consequent high temperatures.

According to DIGUET (*3*), because of its extraordinary abundance, the nopal is an alimentary resource of first order for indigenous Mexicans, especially for those nomadic tribes of the desert and the sedentary remainings of the civilized centers of the pre-Colombian Anauac.

Discussing the edibility of some cacti, WEIMER (17) states that nearly all the flat lobed Opuntias (Nopales) are a source of food.

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The nutrient composition of cacti varies, depending on location, season, type of soil and age of the plant (*9*). This author also states that younger pads tend to have a higher carbohydrate content than older pads. These also contain less fiber than the older ones and more water, thus making them more palatable.

According to SPOEHR (*12*) the mucilaginous portion of the prickly pear pads was shown to have two main components. These were carbohydrate in the form of polysaccharides and complex organic acids (*13*). These results were confirmed by TELES *et alii* (*14*) when working on the circadian variation of those acids. A small amount of proteins and other mucilaginous substances, and inorganic salts were present. However, no data on the protein and free amino acids were presented for young pads and no effort was made to evaluate, at least chemically, its protein quality for human consumption. This work was carried out in order to add some information on the subject.

2. MATERIALS AND METHODS

2.1. *Crude Protein*

All equipment used was standard for determination of nitrogen by the macro Kjeldahl Method, as officially recommended (*1*) in a Kjeldahl battery designed for 24 samples at a time.

The reagents were prepared as officially indicated (*1*), substituting boric acid 2 percent for hydrochloric or sulfuric acid 0.5N when collecting the distilled ammonia.

A composite sample of four young pads of *Opuntia ficus indica* were collected from three specimens, immediately frozen, cut in $1cm^2$ squares, combined, lyophilized, ground to pass a 40 mesh sieve, and taken for analysis. Determination of the moisture was simultaneously carried out. The same procedure was repeated using pads from an entire 74 kilogram plant where 30 kilograms were dried in a vacuum oven at 60-70^0 C and 100 mm Hg for 48 hours. Forty-four kilograms of this sub-sample were frozen and lyophilized in batches of approximately 4 kilograms each. The two samples were ground, uniformed and the resulting powders analyzed separately.

The analyses were carried out according to recommendations of the A.O.A.C. (*1*). The procedure was slightly modified, as the NH_3 was received in boric acid 2% and then titrated with sulfuric acid 0.5 N. The crude protein value was obtained by multiplying the percent nitrogen by 6.25.

2.2. *Total Amino Acids*

The method of analysis, here used, was developed by REID (*11*) at the University of Arizona, and was used with slight modifications for the hydrolysis. The quantification was performed in a Beckman No. 121, automatic amino acid analyzer.

Lyophilized young pads containing 11.2% crude protein were taken as samples, kept in an air-tight container in a freezer at -20^0 C, were ground to pass a 60 mesh sieve and dried to a constant weight by vacuum oven. The temperature was kept below 50^0 C and the vacuum varied from 50 to 60 mm Hg. The dried material was then kept in a calcium chloride desiccator to allow the temperature to equilibrate with the environment.

As special reagents, sodium thioglycollate (ST) and thiodiglycol (TG) analytical grade were used. A small amount of each was added to buffers to minimize the conversion of methionine to the methionine sulfoxides during addition of the sample to the column and during the analysis (*8*). Sodium citrate buffer, pH 2.2: 19.6 g of sodium citrate were dissolved in approximately 400 ml of deionized water in a one-liter mixing cylinder; 16.5 ml of concentrated hydrochloric acid and 5.0 ml of thiodiglycol were added, diluted to a few ml of a liter with deionized water and mixed. The pH was adjusted to 2.2 using hydrochloric acid or sodium hydroxide as needed.

The analytical procedure used was developed by REID (*11*) and is here quoted *modo et forma* from his work:

1. Weigh quadruplicate 100 mg samples into 125 ml Erlenmeyer's flasks. Add 100 mg of sodium thioglycollate to two samples in each set of four.
2. Add approximately 20 ml of 6N HCl to each flask and cover by inverting a small beaker over each.
3. Autoclave overnight.
4. Quantitatively transfer each sample to 250 ml round-bottom flask using deionized water.
5. Evaporate the samples to dryness under vacuum.
6. Dissolve the samples with 20.0 ml sodium citrate buffer pH 2.2.
7. Filter the samples using Whatman #2 paper and collect the filtrate in vials. *Samples must be clear* .
8. Dilute the samples with buffer if necessary.
9. Adjust pH to 2.2 if necessary.
10. Allow the samples to sit overnight in the refrigerator before submitting

them for analysis. Refilter or draw off a clear sample from each vial to the analytical apparatus

2.3. *Free Amino Acids*

The method used was basically that recommended by NIEDERWEISER and PATAKI (*8*). The sample was submitted to a deproteinization and the free amino acids contained in the supernatant were analyzed in an amino acid analyzer.

Four young pads of nopal (*Opuntia ficus indica* L.) comprising 153 g were collected early in the morning, in the winter (temperature ambient being approximately 0^0 C) kept in crushed ice until analysis 30 minutes later.

Phosphotungstic acid solution. 20 g of $P_2 O_5$. $24WO_3$ were dissolved in 100 ml deionized water and used as precipitating agent (*1*).

The young pads were quickly chopped and liquefied in a blender for 5 minutes and the slurry was filtered through cheese-cloth. Seventy-five milliliters of phosphotungstic acid solution were added to one-hundred grams of slurry. After agitation with a glass rod to aid protein precipitation, the mixture was centrifuged in a International centrifuge, model HT (IEC 3401) with rotor for 400 ml, maximum volume, at 3,000 rpm for 5 minutes and let stand for 30 minutes. Fifty milliliters of the supernatant were filtered through a Whatman No. 2 filter paper and aliquots were taken for analysis.

3. RESULTS AND DISCUSSIONS

3.1. *Crude Protein*

The term crude protein has special significance. Protein is calculated from nitrogen analysis which includes nitrogen from nonprotein compounds as well as protein nitrogen. Consequently, protein values calculated from total nitrogen are quantitatively inaccurate and thus are "crude" estimations. From a practical standpoint, however, the error is relatively unimportant since, metabolically, protein metabolism is nitrogen metabolism. On the average, protein of an ordinary mixed dietary contains 16 percent nitrogen and therefore the factor 6.25 (100/16) was used for computing protein content (*10*).

The crude protein content of the nopal, as done by standard methods, averaged 11.03, standard deviation (SD) ± 0.021 for young pads, 4.82 (S.D.± 0.067) for whole plants when oven dried and 4.98 (S.D.± 0.119)

percent of dry weight. Those results are comparable to the ones found in the literature (Table 2): 0.50 and 3.33 (*2*); 1.10 and 10.0 (*4*); 0.4 and 6.70 (*5*); 0.66 and 3.88 (*6*); 0.93 and 7.94 (*15*).

In order to calculate the protein content on dry weigh basis, determinations of moisture content were carried out using Standard Methods (*1*) and listed in Table 1. The results were comparable to the ones found in the literature: 85.00(*2*), 88.90 (*4*), 94.00(*5*), 83.00 (*6*), and 88.28 (*15*).

TABLE 1. Percent moisture of young nopal pads (*Opuntia ficus indica* L.) determined by standard methods (*1*).

Sample Replications	A	B
A	95.28	95.25
B	95.29	95.29
C	95.33	95.26
D	95.19	95.20
Averages	95.27	95.25
Standard deviation (S.D.)	0.059	0.037

TABLE 2. Crude protein content of nopal (*Opuntia ficus indica* L.)

Replication	% Dry Weight			% Green Matter (Calculated)		
	Young Pads	Whole Plant		Young Pads	Whole Plant	
		Oven Dried	Lyophilized		Oven Dried	Lyophilized
A	11.03	4.90	4.85	0.53	0.88	0.82
B	11.00	4.85	4.90	0.53	0.87	0.83
C	11.04	4.75	5.05	0.53	0.86	0.86
D	11.05	4.78	5.10	0.53	0.86	0.87
Average	11.03	4.82	4.98	0.53	0.87	0.85
S.D.	0.021	0.067	0.119	0.00	0.095	0.023

3.2. *Amino Acids*

The component amino acids of the nopal proteins, plus the free amino acids were analyzed at the same time. The combined results are here called total amino acids, or simply amino acid content.

Information on the amino acid content of foods provides a useful indication of the nutritive value of protein when compared with protein of known high quality. The *protein score* proposed by the Food and Agriculture Organization of the United Nations and described in PIKE and BROWN (*10*), is based on comparison of amino acid content (as determined by chemical analysis) of food with that of whole egg or human milk, both of which are proteins of the highest quality and therefore considered to have a protein score of 100.

The amino acids as percent of protein, mg/g nitrogen, the chemical score, and protein score are presented in Tables 3 and 4. The protein scores for isoleucine and total sulfur amino acids were 87 and 48, respectively. All others were higher than 100. Taking the lower score for nopal proteins, it is 48 (total sulfur amino acids) when compared with whole egg protein.

According to MITCHELL (*7*), when x represents the chemical score and y the biologic value, the relation is: $y = 0.7966x + 26.92$. According to this equation and considering the chemical score for the 2nd limiting amino acid, the calculated biological value of the nopal proteins would be 72.60 when related to egg protein.

3.3. *Free Amino Acids*

The average free amino acid content of the deproteinized plant extracts were: lysine, 9; histidine, 4; arginine, 32; aspartic acid, 36; threonine, 7; serine, 39; glutamic acid, 20; proline, 8; glycine, 4; alanine, 15; cystein, 8; valine, 5; methionine, 1; isoleucine, 3; leucine, 4; tyrosine, 4; and phenylalanine, 9 mg per 100 grams of green material.

These amounts would not change much the general picture showed in Table 4, although the presence of cystein would have increased a minute amount the total sulfur containing amino acids.

4. SUMMARY

The moisture, crude protein, total amino acids, free amino acids, protein and chemical scores in pads of the *cactaceae,* commonly known as

TABLE 3. Amino acids as percent of sample, percent of protein and chemical score of nopal (*Opuntia ficus indica* L.).As % protein (g/16g N)

Amino acid	Whole egg	Nopal	Chemical score	
Lysine*	7.2	5.446	75.64	
Histidine*	2.3	2.205	95.87	
Ammonia		3.508		
Arginine*	7.2	4.493	62.40	3rd limiting amino acid
Aspartic acid		7.539		
Threonine		3.487		
Serine		3.472		
Glutamic acid		14.478		
Proline		n3.621		
Glycine		4.301		
Alanine		6.512		
Cystein*		trace	--	1st limiting amino acid
Valine*	7.5	4.972	66.29	
Methionine*	3.0	1.714	57.13	2nd limiting amino acid
Isoleucine*	6.0	3.755	62.58	
Leucine*	7.2	6.381	88.63	
Tyrosine	6.00**	2.917**		
Phenylalanine*		3.854**	64.23	
Tryptophan*		***		

* Considered essential amino acids.
** Aromatic combined value.
*** TRP is not determined quantitatively by this procedure.

TABLE 4. Protein score

Food	ISO	LEU	LYS	PHE	TYR	TOTAL SULFUR A.A.	THR	VAL	TOTAL ESSENTIAL
				Amino acid content in mg per g of nitrogen					
Nopal	235	399	340	241	182	107	218	311	2033
Whole egg	415	553	403	365	262	346	617	454	3415
				Percentage of total essential amino acids					
Nopal	11.6	19.6	16.7	11.9	9.0	5.3	10.6	15.3	100
Whole egg	13.3	17.8	12.9	11.7	8.4	11.1	10.2	14.6	100

nopal (*Opuntia ficus indica* L), were determined. The average results were: moisture, 95.3%; crude protein, 11.03% for young pads and 4.82 on whole plant (dry matter basis). The calculated biological value, based on the chemical score of the nopal proteins, was 72.60 when related to egg protein. Detailed description of the pertinent methodology was presented.

5. **RESUMO**

(PROTEÍNA BRUTA E AMINOÁCIDOS DO NOPAL (*Opuntia ficus indica* (L.))

Foram determinados a umidade, a proteína bruta, aminoácidos totais, aminoácidos livres, *chemical score* e *protein score* de raquetas jovens da cactácea conhecida como nopal (*Opuntia ficus indica* L.). Os resultados médios foram: umidade, 95,3%; proteína bruta, 11,03% para raquetas jovens e 4,82 na matéria seca da planta inteira. O valor biológico calculado com base no *chemical score* das proteínas do nopal foi de 72,60 quando relacionado com a proteína do ovo. Uma descrição detalhada da metodologia analítica empregada é apresentada.

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