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Comparison of the Chemical Composition and Nutritional Value of *Amaranthus cruentus* Flour and Its Protein Concentrate

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Abstract. Plants constitute an alternative source of proteins in the human diet, with advantages over animal proteins because of their low content of saturated fats and absence of cholesterol. Within the framework of a wider research project on the role of *Amaranthus cruentus* (Ac) in lipid metabolism, in this work the chemical composition and biological value of the Ac flour and its protein concentrate were compared. Proximate chemical composition, amino acid and fatty acid profiles, some antinutrient factors, and biological values were determined for Ac seed flour and its protein concentrate obtained by extraction at pH 11 and precipitation at pH 4.5. The flour protein content was 16.6 g% while that of the concentrate was 52.56 g%. The content of the soluble dietary fiber with a hypolipemic function was notably higher in the protein concentrate (12.90 g%) than in the seed flour (4.29 g%). The protein concentrate also exhibited a higher content of insoluble dietary fiber. The Ac flour and the concentrate contain 75.44 and 56.95% unsaturated fatty acids, respectively. Squalene, which affects the biosynthesis of cholesterol, was detected both in the flour and the concentrate oils, with a higher content in the concentrate (9.53%) as compared to the flour (6.23%). Comparison of the amino acid composition with the FAO pattern protein indicated that the concentrate does not have limiting amino acids, while the flour has leucine, threonine, and valine. The content of lysine was high in both the flour and the concentrate, making these products particularly useful as a complement for cereal flour, which is deficient in this amino acid. The biological quality analysis demonstrated an improvement in the quality of the concentrate. The presence of saponins, phytic acid, and trypsin inhibitors in the concentrate, which favor the metabolism of lipids, suggests that consumption of the concentrate might reduce the risk of heart disease.

Key words: *Amaranthus cruentus*, Chemical composition, Nutritive value, Protein concentrate

Introduction

The cultivation of amaranth dates back 5000–7000 years. The Mayas were probably the first to use amaranth as a productive culture, and the Incas and Aztecs adopted it later. With the coming of the Spanish colonists, amaranth consumption and cultivation was banned for being a ritual food and its cultivation was only continued in a small scale.

In more recent times, the cultivation of amaranth reappeared not only in Mexico and Central America, but also in Andean territories of South America as well as in some Asian, European, and African countries. In the last two decades, the study of this plant has attracted a great deal of interest.

Amaranth, a dicotyledon, is considered a pseudocereal and belongs to the group of photosynthetic plants with metabolism C_4, which allows it to grow in areas unsuitable for other crops, and has a greater photosynthetic yield [1, 2].

Amaranth grains contain about 15% of protein and 60% of starch. Its amino acid profile makes it an attractive protein source and, because of its high lysine content, it tends to be viewed as a protein that, if consumed along with other cereals, can provide a "balanced" protein source [3, 4]. As a consequence there has been an increasing interest in using amaranth flour in blends with wheat or maize. The amaranth species are receiving a great deal of attention in developing countries as a means to fight protein malnutrition.

The amaranth seeds can be subjected to several treatments such as popping, toasting, or grinding to be consumed as suspensions with water or milk or to be included in bread, tortillas, cookies, or other preparations [5].

Tocols have been characterized in amaranth seeds and include gamma and delta tocotrienols, the unsaturated forms of vitamin E—all with antioxidant activity—and are under scrutiny as hypocholesterolemic and antitumor agents [6].

Nutraceutical effects for this crop are probably associated with fiber [7], squalene, tocols, or the lectin biomarker called amaranthin [8].

Techniques have been developed for obtaining protein concentrates of high nutritional value for use in several food preparations. Furthermore, the functional properties, composition, and structural characterization of the protein concentrates have been widely studied [9–11].

Studies carried out in our laboratory have shown the hypocholesterolemic effect in male Wistar rats under diets using flour and protein concentrate of *Amaranthus cruentus* (Ac) seeds as protein source.

The purpose of this work was to compare the chemical composition and nutritional value of the Ac flour and protein concentrate, focusing on components that affect lipid metabolism, such as dietary fiber, phytic acid, saponins, lectins, trypsin inhibitors, and squalene, in order to assess their relative potential as nutraceuticals. The results obtained may permit to propose the protein concentrate and the flour as nutraceuticals.

Materials and Methods

Flour Preparation

A. cruentus seeds were provided by the Agronomy Department of Universidad Nacional de La Pampa, Argentina, obtained from a 1999 harvest of an experimental cultivation.

Raw seeds were dried in an air-current oven (EHR/F/I Dalvo, Argentina) at 45 °C for 48 hours. The dried product was ground in an electrical coffee grinder (CG-8 Style, 220 V, 50 Hz, 90 W, China) and sieved through a 200-μm nylon sieve. A light brown and slightly yellowish flour was obtained.

Preparation of the Protein Concentrate

The protein concentrate was obtained following the technique used by Lúquez [12]. The flour aqueous dispersion was subjected to the study of the pH influence on the solubility of the Ac flour, in a pH interval between 1 and 11.5, with 11 and 4.5 as the maximum- and minimum-solubility pH values, respectively. The flour/solvent ratio used was 1:7 so as to achieve optimum extraction conditions. The process was followed by determining nitrogen in aliquots of the different solutions. Once the optimum extraction conditions were set, the protein concentrate was obtained as follows. In 3.5 l of deionized water, 500 g of flour was dispersed and treated with 5 *N* HONa to achieve a pH of 11. The solution was mechanically shaken for 2 hours at room temperature. The solution was centrifuged at 4000 rpm for 20 min, discarding the insoluble material, and then acidified with 5 *N* HCL to pH 4.5. The solution was let to stand overnight under refrigeration till complete sedimentation. The precipitate was separated by centrifugation at 4000 rpm for 20 min and washed three times with deionized water. The obtained precipitate was dried in stove at 45 °C under forced air current for 48 hours. A light-colored protein concentrate was obtained, which was ground in an electrical coffee grinder (CG-8 Style, 220 V, 50 Hz, 90 W, China) and sieved through a 200-μm nylon sieve. From 100 g of flour, 16 g of protein concentrate were obtained. The procedure is shown in Figure 1.

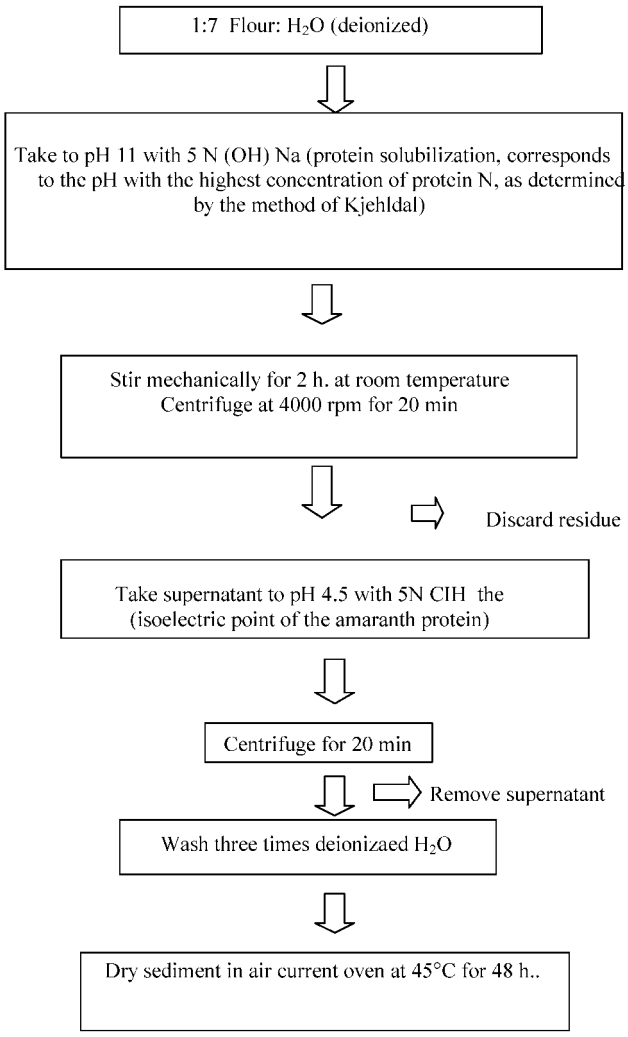

Figure 1. Preparation of the protein concentrate.

Chemical Determinations

Analyses were performed in triplicate, with the exception of amino acids and fatty acids, which were done in duplicate, and the mean value was expressed for dry matter.

The following was determined in each sample: moisture; ash content by the AOAC method [13]; crude protein N $\times$ 5.85 by the method of Kjeldahl modified by Winkler [14]; total soluble and insoluble fiber by the gravimetric enzymatic method proposed by Prosky [15]; fatty acids were extracted according to the method of Stanbie [16] and determined as methyl esters by gas chromatography using a 3300 Varian chromatograph (Berkeley, Carolina) equipped with a 10% SP-2330 packed column. The experiments were performed under the following operation conditions: injector temperature, 270 °C; FID detector temperature, 270 °C; initial temperature, 180,°C for 2 min; final temperature, 210 °C for 12 min; ΔT, 5 °C/min; and nitrogen flow rate, 20 ml/min. In the derivation process, diazomethane in methyl ether [17] was used as the methylating agent. A residue was obtained by evaporating the solution containing the derivated products under a nitrogen stream, with the current starting from the solution containing the derivated products. The residue was dissolved in 1–2 ml of acetone and injected into the chromatograph. A standard solution (Sigma, St. Louis, MO) was run in parallel to identify fatty acids. The relative percentages were calculated from the peak areas. For the determination of squalene,

lipid extraction was performed by refluxing the samples in hexane in a Soxhlet apparatus for 36 hours. The hexane was evaporated and the total lipid weight was determined. Lipids were then saponified with 75% ethanol/KOH for 90 min at 60 °C, and the solvent was removed by rotary evaporation under vacuum. The residue was redissolved in water, extracting three times with ethyl ether, thus separating the unsaponifiable matter (ether extract) from the saponifiable matter. Subsequently, the ether extract was washed three times with water, and squalene content was determined by reversed-phase liquid chromatography. The determinations were performed with a Beckman (model 332) liquid chromatograph equipped with a variable-wavelength detector (model 164) operated at 205 nm. The retention times were measured with a Varian 4290 integrator and a Spherisorb 5-μm C8 column (150 × 4.6 mm) was used in all experiments. The flow rate was 1.2 ml/min, and the mobile phase consisted of acetonitrile/water (90:10). Squalene (Sigma) was used as standard for the quantitative determinations. For the determination of the amino acid composition, the samples were defatted for 6 hours with hot petroleum ether and then hydrolyzed with 6 *N* HCl under vacuum for 24 hours. Final quantification was performed on a Beckman 119 CL amino acid analyzer. Tryptophan was determined according to the technique of Lombard and Lange [18]. The amino acid score (AAS) was calculated for essential amino acids, using the FAO/WHO/UNU 1985 protein as pattern [19].

Antinutrient Evaluation. The following studies were performed by triplicate and mean values are expressed in dry matter. Nitrates were determined using the method of Cataldo [20]. Hemoagglutinating activity (lectins) was determined by previous saline extraction according to the method of Do Prado [21], with quantification following the method proposed by Das Gupta [22]. Trypsin inhibitors were determined using the method of Kakade [23]. Saponins were determined by measuring the hemolytic activity [24] and foam index [25]. Hemolytic activity was evaluated using goat blood cells which were observed for a period ranging from 30 min to 12 hours. A numerical score was used: 0 (no hemolysis within 12 hours), 1 (10% hemolysis within 12 hours), 2 (20–40% hemolysis within 12 hours), 3 (50–90% hemolysis within 12 hours), 4 (100% hemolysis within 12 hours), 5 (100% hemolysis within 30 min). Values 0–2 were considered to indicate low hemolytic activity and values 3–5 were considered indicative of high activity. The foaming index was determined by the following procedure: about 1 g of the plant material was reduced to a coarse powder (sieve size no. 1250), weighed accurately and transferred to a 500-ml conical flask containing 100 ml of boiling water. Moderate boiling was maintained for 30 min. The solution was cooled and filtered into a 100-ml volumetric flask and sufficient water was added through the filter to dilute the volume to 100-ml. The above decoction was placed into 10 stoppered test tubes (height 16 cm, diameter 16 mm) in a series of successive portions of 1, 2, 3, up to 10 ml and the volume of the liquid in each tube was adjusted with water to 10 ml. The tubes were stoppered and shaken in a lengthwise motion for 15 sec, two frequencies per second. The filtrate solution was allowed to stand for 15 min and the height of the foam was measured. The foaming index was calculated as $1000/a$, where a is the volume in milliliters of filtrate used for solutions in which the foam reached 1 cm. If the foam did not reach 1 cm, the index was reported as <100. Phytic acid was determined by the colorimetric quantitative method of Rucci and Bertoni. Briefly, the phytate is extracted with trichloroacetic acid and precipitated as the ferric salt, the iron content of the precipitate is determined colorimetrically and the phytate phosphorus content calculated from this value assuming a constant 4Fe:6P molecular ratio in the precipitate [26].

Biological Assay. Protein quality of the amaranth flour and of its concentrate was measured by three different indices: net protein utilization (NPU), true digestibility (tD), and biological value (BV) as noted by Miller and Bender [27]. Six groups of 30-day-old Wistar rats weighing 45–50 g (±0.5 g weight difference) were used (four animals per group). One group received a protein free diet, the second received a control diet (casein), and the remaining groups received a diet with protein contributed by the material under study: amaranth flour (two groups) and concentrate (two groups). The animals were kept in individual, suspended cages with screen bottoms. Temperature and relative humidity were held at 25 ± 2 °C and 50%, respectively. Lighting was controlled by alternating 12-h periods of light and darkness. All animals received potable water and food *ad libitum* for 10 days. Ingestion (I) was recorded on days 3, 6, and 10, weight gain (ΔP) was recorded at the end of the experiment. Feces were collected and weighed. All diets were prepared according to the method of Sambucetti [28] and contained 10% protein; corn oil, 14.5%; salt mixture, 5%; hydrosoluble vitamins, 0.25%; lyposoluble vitamins, 0.5%; choline (as citrate), 0.15%; and dextrin, 100 g. In the protein-free diet, dextrin was used as a substitute. Salts and hydrosoluble and lyposoluble vitamins were added as recommended by Harper [29] in all diets.

Net Protein Utilization (NPU) is defined as the portion of N intake that is retained. The formula used was

$$\text{NPU} = \frac{B - (B_K - I_K)}{I} \times 100$$

where B is the corporal nitrogen of the experimental group; B_K is the corporal nitrogen of the group on the protein-free diet; I_K is the nitrogen intake of the group on the protein free diet; and I is the nitrogen intake in the experimental

group. Corporal nitrogen (N) was calculated by using the following equation:

$$Y = 2.92 + 0.02X \quad (1)$$

where X is the age in days of rats, and Y is calculated as

$$Y = \frac{\mathrm{N}_{(\text{in g})}}{\mathrm{H_2O}_{(\text{in g})}} \times 100 \quad (2)$$

By equating Eqs. (1) and (2), N is calculated as

$$\mathrm{N\,(in\,grams)} = \frac{\mathrm{H_2O}\,(2.92 + 0.02X)}{100} \quad (3)$$

True digestibility (tD) was determined together with NPU, and was considered as the absorbed nitrogen with respect to the N intake. Unabsorbed nitrogen was calculated by quantification of the fecal nitrogen in the lot fed the protein-free diet. The formula used was

$$\mathrm{tD} = \frac{I - (F - F_K)}{I} \times 100$$

where I is the ingested nitrogen; F is the fecal nitrogen in the group that received the experimental diet; and F_K is the fecal nitrogen of the group eating the protein-free diet.

The biological value (BV) was calculated as the NPU/tD quotient.

The official preferred method for evaluation of protein quality in the United States is PDCAAS for all foods for humans ages 1 year and older. PDCAAS calculation needs an amino acid reference and essential amino acid content of samples. For a given essential amino acid, amino acid score (AAS) was calculated by dividing the content of this amino acid in a sample by the content of this amino acid in the reference:

$$\mathrm{AAS} = \frac{\text{amino acid composition}}{\text{amino acid reference}}$$

PDCAAS was the product of the lowest AAS ($\mathrm{AAS_1}$) in a food by tD of the food [30]

$$\mathrm{PDCAAS} = \mathrm{AAS_1} \times \mathrm{tD}$$

The results obtained were subjected to one-way variance analysis (ANOVA), with a 5% significance level, supplemented by the multiple comparison of means test of Tukey–Kramer.

Results and Discussion

Table 1 shows the proximate composition of the material under study. Protein content of the flour was 16.60 g% while that of the concentrate was 52.56 g%. The protein, ash, and moisture values are similar to those obtained by Becker [31].

Table 1. Proximate chemical composition of *Amaranthus cruentus* flour and protein concentrate

Determinations	Flour (g/100 g)	Protein concentrate (g/100 g)
Protein ($N \times 5.85$) (dw)	16.60 ± 0.18	52.56 ± 0.45
Moisture	10.30 ± 0.09	3.10 ± 0.04
Ash (dw)	3.35 ± 0.05	3.70 ± 0.05
Soluble dietary fiber (dw)	4.29 ± 0.12	12.90 ± 0.32
Insoluble dietary fiber (dw)	5.54 ± 0.14	20.69 ± 0.57
Total lipids (dw)	8.77 ± 0.95	5.90 ± 0.75

Note. Results are expressed as mean ± standard deviation of triplicate determinations. dw: dry weight basis.

The protein concentrate had a higher proportion of soluble and insoluble dietary fiber, a tendency that has been reported by other authors [32]. Soluble dietary fiber has a hypolipemic action because it elevates the viscosity in the small intestine, decreasing lipid absorption, and bonds to biliary acids, thus increasing cholesterol catabolism by transforming it into biliary acids in the liver. Besides, soluble dietary fiber is fermented by the microflora in the large intestine, and propionate, one of the fermentation products, can inhibit cholesterol synthesis in the liver. Soluble dietary fiber can also affect the lipid metabolism in the liver and peripheral tissues by modification of the secretion of the gastrointestinal hormones insulin and glucagon [33]. Insoluble dietary fiber stimulates the motor activity of the digestive tract, reduces transit time, and increases the volume of the feces.

The fatty acid composition of Ac flour (Table 2) was similar to that reported by Becker [34], containing about 75.44% of unsaturated fatty acids and rich in linoleic acid. In the concentrate, the percentage of unsatured fatty acid decreased to 56.95%, which was probably due to the treatment

Table 2. Fatty acid and squalene composition of *Amaranthus cruentus* flour and protein concentrate

Fatty acid	Flour oil (%)	Protein concentrate oil (%)
Palmitic 16:0	20.75	34.59
Palmitoleic 16:1	16.57	7.15
Stearic 18:0	3.79	8.44
Oleic 18:1	23.57	42.96
Linoleic 18:2	35.31	5.81
Linolenic 18:3	ND[a]	0.56
Arachidic 20:0	ND	ND
Arachidonic 20:4	ND	0.48
Saturated fatty acid	24.54	43.03
Insaturated fatty acid	75.45	56.96
Squalene	6.23	9.53

Note. Values are mean of duplicate determinations.
[a]Not detected.

Table 3. Amino acid composition of *Amaranthus cruentus* flour and protein concentrate

Amino acid	FAO (mg AA/g protein)[a]	Flour (Mg AA/g protein)	AAS[b]	Protein concentrate (mg AA/g protein)	AAS[b]
Isoleucine	28	30	>100	29	>100
Leucine	66	58	87	83	>100
Lisine	58	82	>100	72	>100
Methionine + cystine	25	36	>100	26	>100
Phenylalanine + tyrosine	63	63	100	63	100
Threonine	34	27	78	37	>100
Tryptophan	11	18	>100	14	>100
Valine	35	32	90	46	>100
Histidine	19	25	>100	23	>100
Arginine	—	83	—	85	—
Alanine	—	34	—	38	—
Aspartic acid	—	71	—	67	—
Glutamic acid	—	143	—	132	—
Glycine	—	67	—	75	—
Proline	—	37	—	45	—
Serine	—	54	—	70	—

Note. Values are mean of duplicate determinations.
[a]Based on FAO/OMS/UNU (1985).
[b]AAS: amino acid score.

to which it was subjected. Araquidonic acid and linolenic acid were detected in the protein concentrate but not in the flour. The presence of the linolenic acid in the concentrate is of importance since the incidence of ω-3 in reducing risks of heart disease and cancer is well known [35, 36]. Squalene was present in both the flour and the concentrate, with a higher content in the concentrate. The Ac flour oil contains 6.23%, a value similar to results reported by Becker, while the Ac concentrate oil contains 9.53%. Squalene affects the biosynthesis of cholesterol, since it is an inhibitor by feedback of the activity of HMG-CoA reductase (hidroxy-methyl-glutaryl CoA reductase), an enzyme limiting cholesterol synthesis [37].

Table 3 gives the amino acid profile. Overall, the amino acid compositions of the flour and the concentrate do not differ significantly, but an increase in the values of leucine and threonine were detected in the concentrate. Both exhibit an elevated lysine content, which makes them suitable complements of cereal flours, which are deficient in this amino acid. Comparison with the FAO pattern protein showed that the concentrate does not exhibit limiting amino acids, while the flour has threonine, leucine, and valine, with chemical scores of 78, 87, and 90, respectively

Table 4 shows the results of the antinutrient analysis. Nitrates are present in amounts that do not pose a health hazard. With regard to hemoagglutinating activity (lectins), a 50% reduction of the hemoagglutination value was observed in the concentrate, which becomes important to assess the concentrate since lectins are responsible for the decrease of the nutritional value. According to Liener, the partial activity of lectins can be reduced or completely eliminated by means of humid heat, for example, by pasteurization in a subsequent industrialization process [38]. With regard to saponins, a higher proportion was detected in the concentrate. Anderson et al. have suggested that saponins should not be considered as antinutrient factors since they have hypocholesterolemic and anticarcinogenic effects. Saponins reduce blood cholesterol through the increase of biliary excretion. The concentration of phytic acid was similar to that in soy, and, like in soy, its concentration was same in the concentrate. Phytic acid interferes with Zn adsorption, regulates the Zn/Cu ratio in blood, and probably plays an important role in cholesterol regulation since high Zn/Cu ratios are associated with high cholesterol in humans [39]. Furthermore, phytates form complexes with the basic protein residues, thus leading to a paralysis of many enzymatic digestive reactions. This

Table 4. Antinutrient factors of *Amaranthus cruentus* flour and protein concentrate

	Flour	Protein concentrate
Hemoagglutinant activity	1/64	1/32
Hemolytic activity (hemolysis degree)	1	2
Foam index[a]	<100	<100
Antitrypsin activity (TIU/mg sample)[b]	2.62 ± 0.05	1.99 ± 0.03
(TIU/mg protein)[c]	18.14 ± 0.12	4.10 ± 0.04
Nitrates (NO_3^-) (mg/100 g)	58.20 ± 3.20	21.45 ± 1.75
Fitic acid (as P) (mg/100 g)	1.90 ± 0.02	2.00 ± 0.02

Note. The results are expressed as mean ± standard deviation of triplicate determinations.
[a]$1000/a$; a = amount of filtrate (in ml) in the tube that reached 1 cm of foam. Since no tube exhibited 1 cm of foam, foam index <100.
[b]TIU/mg flour = trypsin inhibited units per milligram of flour.
[c]TIU/mg protein = trypsin inhibited units per milligram of protein.

Table 5. Biological quality of *Amaranthus cruentus* flour and protein concentrate

	Casein	Flour	Protein concentrate
Net protein utilization (NPU)	72.25 ± 4.90a	51.50 ± 3.30b	57.45 ± 4.59b
True digestibility (tD)	95.00 ± 8.98a	81.00 ± 1.83b	82.00 ± 5.94b
Biological value (BV)	76	64	70
Average food intake in g by rat in 10 days (I)	85.00 ± 8.98a	63.00 ± 3.55b	83.00 ± 2.45a
Weight gain in g by rat in 10 days (Δp)	30.00 ± 3.27a	13.00 ± 2.58c	19.50 ± 3.14b
PDCAAS[a]	103[b]	63	83

Note. The results are expressed as mean ± standard deviation. Means on the same row with different superscripts differ significantly ($P < 0.05$).
[a]PDCAAS = amino acid value corrected for protein digestibility.
[b]Expressed as sodium caseinate (Pascale R et al., 1997).

might be one of the factors causing a decrease of PDCCAS in the flour and the protein concentrate with respect to animal proteins. However, the true role of phytic acid, either as an antinutrient or as having beneficial activity, remains to be discussed [40]. Trypsin inhibitors were found both in the flour and the concentrate. Their presence would stimulate gastrointestinal secretion, including that of cholecystokinin, which causes a contraction of the biliary vesicle muscles, leading to biliary secretion in the intestinal tract [41].

From Table 5 it can be seen that the NPU value of the flour is 71% that of casein, which is an acceptable level. For the concentrate, the NPU value was 79.5% that of casein, indicating an improvement of the biological quality as compared to the flour. Intake was higher in the rats fed with the concentrate than in those on the flour diet, which was accompanied by increased weight. The values obtained by PDCAAS were 63 and 83 for the flour and the concentrate, respectively. The PDCAAS value of sodium caseinate, which was taken as a standard reference, is 103.00, according to reports by Pascale R. [30], while the value informed by the same author for soy is 78.2. The values here obtained for Ac indicate that the nutritional quality of the concentrate is enhanced with respect to the flour, and is higher than that reported for soy flour.

Conclusion

From the results obtained it can be concluded that processing of the *Amaranthus cruentus* flour to obtain a protein concentrate leads to a marked improvement of its nutritional quality and increases the content of factors that directly or indirectly influence lipid metabolism. These results match results obtained in previous works as regards the effect of the Ac flour and protein concentrate on the lipid metabolism of rats. Consumption of the concentrate may decrease the risk of cardiovascular disease.

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