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Nutrient and antinutrient composition of *Amaranthus muricatus*

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Abstract. The chemical and nutritional composition of the aerial parts of *Amaranthus muricatus* was assessed. This is a wild plant growing well in arid and poor soil, which are not suitable for cereals. The material under study was boiled, dried and ground and then evaluated for chemical, nutritional and antinutritional properties. Protein concentration of the amaranth flour was 15.74 g/100 g and total dietary fiber content was high (53.81 g/100 g), with 79% coming from insoluble dietary fiber. The calcium content was 1533 mg/100 g and the Ca/P ratio (5.94) was markedly higher than the value suggested in the 1989 Recommended Dietary Allowances. Fifty-nine percent of the fatty acids present were unsaturated with linoleic acid accounting for 40% of the total fatty acid content. Nitrate, hemagglutinin, trypsin inhibitor and saponin contents were within the non-critical values. Net protein utilization (NPU), true digestibility (tD) and biological value (BV) measurements indicated a nitrogen gain of 74% that of casein. The results of this study indicate a nutritive potential for the *A. muricatus* leaves, therefore, domestication of this plant is suggested along with assessment of its chemical and nutritional properties.

Key words: *Amaranthus muricatus*, Antinutrients, Chemical Composition, Nutritive Value, Plant leaves

Introduction

Amaranthaceae, which are closely related to *Chenopodiaceae*, are widely distributed in the tropical and subtropical regions of the world. Some of them, however, also grow well in warm climates. In ancient times, man started to cultivate vegetables to be used as food or for medicinal purposes. Probably, *Amaranthaceae* seeds and leaves were among these. The consumption of some wild species continues, but there is a growing tendency to replace them with leaves of cultivated species [1].

The nutritional value of *Amaranthaceae* is equivalent to that of spinach, and its protein, iron and calcium contents are higher than those of cabbage and lettuce. It has been reported that *Amaranthaceae* grains and leaves ex-

hibit high biological value. *Amaranthaceae* grains can be used in the making of bread, cookies and jam and leaves can be consumed raw or cooked in salad [2]. Leaves have a major nutritional potential, considering the lack of economically accessible protein sources to people of poor resources [3].

Amaranthus muricatus is a perennial plant, an unusual characteristic since most *Amaranthaceae* are annuals. It is characterized by decumbent stems with spiny formations around a pulpy root, lanceolate alternated leaves measuring 3–8 cm in length and 3–10 mm wide, unisexual flowers in terminal spikes and small black seeds 1.5 mm in diameter. It grows in the spring and blossoms in the summer and is widespread in the Argentinean provinces of Buenos Aires, Santa Fé and Córdoba, as well as in Uruguay [4].

Many Latin American government and non-government organizations are currently working on the design and implementation of actions to mitigate hunger and malnutrition that affects vulnerable groups. In this context, the study of the nutritional value of *Amaranthaceae* might contribute information about potential food sources to be incorporated in regional diets.

The aim of the present work was to assess the chemical and nutritional properties of a wild *Amaranthus muricatus* species, by determination of the general chemical composition of flour from leaves and stems, identification of some antinutrients, minor components of nutritional interest, and evaluation of the protein biological quality.

Materials and methods

Sample acquisition and preparation

The aerial parts of mature *A. muricatus* plants before blossoming were employed. The material was collected in early summer (December) in 'La Piedra Blanca,' a farm located in Lujan, province of San Luis, Argentina, where it grows wild. Ten kilograms of plant material were collected in a single day.

Moisture content was determined in the fresh material immediately after collection. The material was then boiled in drinking water (1:2 sample: solvent ratio) for 10 min in a 10-liter stainless steel container, manually pressed to eliminate excess water and dried in an air current oven (EHR/F/I Dalvo, Argentina) at 45 °C for 48 h. The dried product was ground in an electric coffee grinder (CG-8 Stylo, 220V-50 Hz 90 W, China) and sieved through a 200 um nylon sieve. The prepared amaranth flour was stored in 1500 mL polyethylene (HDPE) containers with screw-top lids at –20 °C (Kohinoor Freezer, Argentina) for 30 days until analyzed.

In previous assays (unpublished data), feeding Wistar rats flour prepared from raw *A. muricatus* material led to loss of animal weight and 20% mortal-

ity. Such effects might have been due to the presence of a soluble thermolabile factor. Therefore, flour obtained from the cooked material was used in this study.

Physicochemical methods

Moisture content, ether extract and ash were determined using the AOAC methods [5]. The Zn, Fe, Cu, Na, K and Mn contents were determined by atomic absorption spectrophotometry (Instrumentation Laboratory AA/AE Spectrophotometer 751, Wilmington, Massachusetts, USA). Phosphorus was determined by a colorimetric method [6]. Calcium was colorimetrically assayed using the chloroanylic acid technique [7]. Crude protein, $N \times 6.25$, was determined using the Kjeldahl method as modified by Winkler [8]. Soluble and insoluble fiber contents were determined according to Prosky [9].

Fatty acids were extracted according to Stanbie [10] and determined as methyl esters by gas chromatography, on a 3 M packed column chromatograph (Varian 3300, Berkeley, Carolina, USA), 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 flux N_2:20 mL/min. In the derivation process, diazomethane in methyl ether [11] was used as the methylating agent. A residue was obtained by evaporating the solution containing the derivated products under a nitrogen stream with N_2 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 Chemical Company, St. Louis, MO, USA) was run in parallel to identify fatty acids. The relative percentages were calculated from the peak areas.

Antinutrient evaluation

Nitrates were determined using the method of Cataldo [12]. Hemoagglutinating activity with previous saline extraction was done according to the method of Do Prado [13] with quantification following the method proposed by Das Gupta [14]. Trypsin inhibitors were determined using the method of Kakade [15].

Saponins were determined by measuring hemolytic activity [16] and foam index [17]. Hemolytic activity was evaluated using goat blood cells, which were observed for a period ranging from 30 min to 12 h. A numerical score was used: 0 (no hemolysis within 12 h), 1 (10% hemolysis within 12 h), 2 (20–40% hemolysis within 12 h), 3 (50–90% hemolysis within 12 h), 4 (100% hemolysis within 12 h), 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 minutes. 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 seconds, 2 frequencies per second. The filtrate solution was allowed to stand for 15 minutes and the height of the foam was measured. The foaming index was calculated as 1000/a, where a was the volume in mL 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.

Biological assay

Protein quality of the amaranth flour was measured by three different indices: net protein utilization (NPU), true digestibility (tD) and biological value (BV) noted by Miller and Bender [18]. Four groups (four animals per group) of 30-day-old Wistar rats weighing 45–50 g ($\pm$ 0.5 g weight difference) were used. One group received a protein free diet, the second received a control diet (casein), and the remaining two groups received a diet with protein contributed by the material under study. The animals were kept in individual, suspended cages with screen bottoms. All animals received water and food *ad libitum* for 10 days. Ingestion was recorded on days 3, 6 and 10 and weight gain was recorded at the end of the experiment. All diets were prepared according to the method of Sambucetti et al. [19] and contained 10% protein. In the protein-free diet, dextrin was used as a substitute. Salts and hydrosoluble and lyposoluble vitamins were added as recommended by Harper [20] in all diets.

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

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

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:

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

Table 1. Proximate chemical composition of flour from *Amaranthus muricatus* leaves and stems

Determination	(g/100 g)
Moisture (MF)[a]	78.50
Residual moisture	6.53
Protein (N × 6.25)	15.74
Ash	13.77
Ether extract (petroleum ether)	1.20
Total carbohydrates [b]	62.76
Soluble dietary fiber	11.38
Insoluble dietary fiber	42.43
Total dietary fiber	53.81

[a] Fresh basis.
[b] Calculated as 100–(% residual moisture + % protein + % ether extract + % ash).

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

$$Y = \frac{N_{(\text{in grams})}}{H_2O_{(\text{in grams})}} \times 100 \tag{2}$$

By equating equations (1) y (2), N is calculated as:

$$N_{(\text{in grams})} = \frac{H_2O(2.92 + 0.02X)}{100}$$

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:

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

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

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

Statistical analysis was done by Student's 't' test. Significance was accepted at $p \leqslant 0.05$.

Table 2. Mineral content in flour from *Amaranthus muricatus* leaves and stems

Elements	(mg/100 g)
Zinc	0.21
Iron	1.40
Copper	ND[a]
Calcium	1533.00
Total phosphorus	257.74
Sodium	450.00
Potassium	200.00
Manganese	ND[a]

[a] Not detected.

Results and discussion

The results of chemical determinations, shown in Table 1, are similar to those obtained for flour from leaves and stems of six non-conventional plants reported by Arellano et al. [21]. Fiber content was lower, though one must consider that only crude fiber determinations were done by those investigators while in the present study both soluble and insoluble fractions were assessed.

Table 2 data show the results of the mineral analyses. The Ca/P ratio (6:1) was much higher than the value suggested in the Recommended Dietary Allowances (1:1). The Ca content was triple the value reported by Sánchez Marroquin [22] for *Amaranthus hypocondriacus* (360.4 to 375.3 mg%).

The major saturated fatty acid (Table 3) was palmitic acid (61%). Unsaturated fatty acids represented 59% of the total, with the most prevalent being linolenic (40%) and oleic (35%) acids. These results are in agreement with the fact that these two unsaturated acids are predominant in lipids of vegetable origin [23]. The unsaturated/saturated fatty acid ratio was 1.4, lower than the value found for *Amaranthus hypocondriacus* [24].

Table 4 information shows the values for the antinutrient factors. A nitrate concentration of 720 mg/100 g was found in the sample submitted to boiling, while in raw samples, the concentration was 1600 mg/100 g. The nitrate content in the cooked sample was lower than that found in the aerial parts of *Amaranthus retroflexus* (760 to 3170 mg/100 g dry weight), while the value in the raw sample was within this range. The oxalate concentration was within the range recorded for the *A. retroflexus* species (2.66–5.36%) [25].

The results of the protein quality evaluations are shown in Table 5. Nitrogen gain for rats consuming the amaranth flour diet was 74% that of rats

Table 3. Fatty acid content in flour from *Amaranthus muricatus* leaves and stems

Carbon atoms	Acid (comon name)	Percentage
14:0	Myristic	2.58
16:0	Palmitic	25.00
!8:0	Stearic	12.60
22:0	Behemic	0.95
16:1	Palmitoleic	6.37
18:1	Oleic	20.59
18:2	Linoleic	23.75
18:3	Linolenic	8.16
20:4	Arachidonic	ND[a]

[a] Not detected.

Table 4. Antinutrient factors in flour from *Amaranthus muricatus* leaves and stems

Antinutrient factors	
Nitrates (NO_3^-) (mg/100 g)	720.00
Hemoagglutinant activity	ND[a]
Hemolytic activity (hemolysis degree)	ND
Foam index[b]	200.00
Antitrypsin activity (TIU/mg sample)[c]	ND
Oxalic acid (g/100 g)	4.99

[a] Not detected.
[b] 1000/a; a = mL of filtrate in the tube that reached 1 cm of foam. When no tube exhibited 1 cm of foam, foam index <100.
[c] Trypsin inhibited units per mg of flour.

eating the casein diet ($p < 0.005$). Digestibility was low, being 63% of the value obtained for the group consuming the casein diet ($p < 0.001$). Higher food intake and weight gain were recorded in animals fed the experimental diet. The low nitrogen gain in contrast to the high food intake value could be due to the presence of some unanalyzed antrinutrient factors or low digestibility, which decrease protein absorption. The high fiber content of the amaranth flour may have also influenced the protein quality results since fiber decreases the utilization of nutrients such as protein [26].

Several organizations in Latin America are designing policies to foster the incorporation of foods rich in macro and micro nutrients into the diet, with

Table 5. Biological quality of flour from *Amaranthus muricatus* leaves and stems

	Casein	*A. muricatus*
Net protein utilization (NPU)	72 ± 6.5[a]	53 ± 7.0 **
True digestibility (tD)	95 ± 11.0	59 ± 4.3 ***
Biological value (BV)[b]	76	98 *
Average food intake in g, by rat in 10 days (I)	3.0 ± 0.4	7.3 ± 0.8 ***
Weight gain in g by rat in 10 days (Δp)	8.5 ± 1.1	12.0 ± 1.4 **

[a] $\bar{X} \pm SD$.
* $p < 0.05$ versus control by Studentïs 't' test.
** $p < 0.005$ versus control by Studentïs 't' test.
*** $p < 0.001$ versus control by Studentïs 't' test.
[b] BV = NPU / tD.

special emphasis on the use of regional or autochthonous plants. The results of this study indicate that the nutritional value of *A. muricatus* might make it a candidate to be domesticated and gradually incorporated into traditional diets.

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