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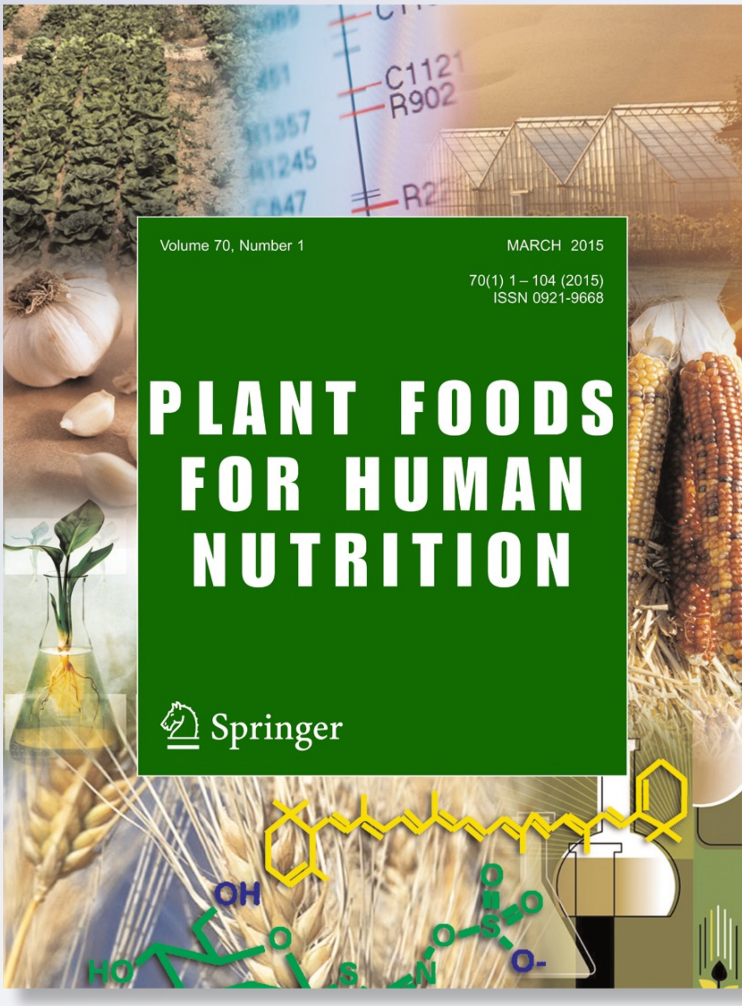

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ORIGINAL PAPER

Evaluation of the Nutritional Quality of the Grain Protein of New Amaranths Varieties

Elba Graciela Aguilar · Graciela de Jesús Albarracín · María Angelina Uñates · Hugo Daniel Piola · José Manuel Camiña · Nora Lilian Escudero

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Abstract The efforts for promoting the consumption of food of plant origin are increasingly growing. The amaranth grain is an important vegetable protein source, superior in content and quality to traditional cereals. In the central-west region of Argentina, new amaranth varieties have been obtained to optimize its agronomic traits and promote its use. In this work, the analysis of the wholemeal flour protein from seeds of two new varieties of *Amaranthus cruentus* var. *Candil* (CC) and *Amaranthus hypochondriacus* var. *Dorado* (HD), as well as from advanced lines of *Amaranthus hypochondriacus* x *Amaranthus cruentus* H17a (H17) and *Amaranthus cruentus* G6/17a (CG6), was carried out in order to elucidate their nutritional contribution to human diet. The amino acids profile and the chemical score (CS) were determined, and the protein quality was evaluated *in-vivo* through the following indexes: net protein utilization (NPU), true digestibility (tD), biological value (BV) and protein digestibility corrected amino acid score (PDCAAS). In general, the amino acids values of the different varieties exceeded the requirements established by the WHO/FAO/UNU; however, valine was the limiting amino acid in all cases. The values obtained (%) were within the following ranges: NPU, 33.56–46.04 %; tD, 68.80–75.40 %; BV, 44.53–64.28%; and PDCAAS, 23.69–36.19 %. These results suggest that the new amaranth flours varieties can be adequate for human consumption and as complementary protein source.

E. G. Aguilar · G. d. Albarracín · M. A. Uñates · H. D. Piola · N. L. Escudero (✉)
Faculty of Chemistry, Biochemistry and Pharmacy, National University of San Luis, Chacabuco 917. 5700, San Luis, Argentina
e-mail: nlesc@unsl.edu.ar

J. M. Camiña
Faculty of Exact and Natural Sciences, National University of La Pampa, and Institute of Environmental and Earth Sciences (INCITAP-CONICET), Avenida Uruguay 151, 6300 Santa Rosa, Argentina

Keywords Amaranths · Chemical Score · Net Protein Utilization · True Digestibility · Biological Value

Abbreviations

AAS	Amino acid score
AIN-93G	American Institute of Nutrition 1993 for growth
AOAC	Association of Official Analytical Chemists
BV	Biological value
CC	*Amaranthus cruentus* var. *Candil*
CG6	*Amaranthus cruentus* G6/17a
CS	Chemical score
H17	*Amaranthus hypochondriacus* x *Amaranthus cruentus* H17a
HD	*Amaranthus hypochondriacus* var. *Dorado*
NPU	Net protein utilization
PDCAAS	Protein digestibility corrected amino acid score
tD	True digestibility
WHO/FAO/UNU	World Health Organization/ Food and Agriculture Organization of the United Nations/ United Nations University

Introduction

The Amaranthus genus (Amaranthaceae Family) is constituted by both cultivated as well as wild plants of cosmopolitan distribution. In America, its cultivation was already known by the oldest civilizations; being this a practice that was replaced by the introduction of new crops from the old world, and a situation that is currently being sought to reverse. The amaranth is an annual crop, highly efficient and of rapid growth, which can grow up to 3,300 m above sea level and prosper under adverse agronomic conditions (saline, acid or alkaline soils; drought; low and high temperatures), easily adapting to different environments [1].

The amaranth seed protein contribution is around 16 %, which is higher than wheat (12–14 %), rice (7–10 %), corn (9–10 %) and other cereals of regular consumption. This protein is exceptional regarding its quality due to its high lysine content, and therefore, an optimum nutritional supplement for conventional cereals that are poor in this amino acid [2]. Since the amaranth seeds are gluten-free, they are also considered as an important food source for the population affected by the celiac disease [3–5].

The protein value of a food depends on both, the amount as well as the quality of the protein, being the quality the attribute that contributes to the growth and the health maintenance. The protein quality, or nutritional value, is the protein capacity to replace the nitrogen that the organism inevitably loses as a consequence of the metabolism in the biological processes. This depends on the composition in essential amino acids and their proportions, as well as its digestibility.

This means that the proteins must be assessed not only in terms of amount and amino acid profile in a food, but also in terms of their availability [6]. Based on these aspects, the determination of some parameters is performed, such as: NPU (net protein utilization), tD (true digestibility) and BV (biological value). On the other hand, considering the great amount of species and varieties of this genus, there is not enough scientific information available on the biological quality of their proteins.

Based on the above, in this study, the proteins quality was evaluated by chemical methods and biological tests of the flour obtained from the seeds of two new varieties and two advanced lines of *Amaranthus* from the central region of Argentina, in order to evidence their nutritional contribution to the human diet.

Materials and Methods

Sample

Seeds of new varieties of *Amaranthus cruentus* var. *Candil* (CC) and *Amaranthus hypochondriacus* var. *Dorado* (HD), as well as advanced lines of *Amaranthus hypochondriacus* x *Amaranthus cruentus* H17a (H17) and *Amaranthus cruentus* G6/17a (CG6), were supplied by the Faculty of Agronomy and Veterinary, National University of Río Cuarto, Córdoba, Argentina (experimental crops from 2012 vintage).

Sample Pretreatment

Dried seeds were ground in a grain mill and sieved through a 200 μm diameter mesh. The flours obtained were kept in closed containers protected from light, in a cool and dry environment until analysis.

Chemical Analysis

The protein analysis was carried out by the Kjeldhal method of the Association of Official Analytical Chemists (official method 984.13; AOAC, 2006) [7]. The determination of the amino acid composition was performed following the technique established by the AOAC Official Method (official method 982.30; AOAC, 2006) [7]. Samples were defatted for 6 h with hot petroleum ether and then hydrolyzed with 6 mol L^{-1} HCl solution under vacuum for 24 h. The determination of methionine and cysteine and/or cystine was performed by performic acid oxidation, followed by acid hydrolysis. Tryptophan was determined after alkaline hydrolysis with 4.2 mol L^{-1} NaOH solution and subsequent neutralization with 6 mol L^{-1} HCl solution. The quantification of three hydrolysates was performed with a Beckman amino acid analyzer. Amino acids standard solutions were provided by Sigma (St. Louis, MO). In all cases, analyses were performed by triplicate.

The amino acid score (AAS) was calculated for essential amino acids using the World Health Organization/ Food and Agriculture Organization of the United Nations/ United Nations University 2007 (WHO/FAO/UNU 2007) protein as pattern, through the following equation [8],

$$AAS = \frac{\text{mg of amino acid in 1g test protein}}{\text{mg of amino acid in requirement pattern}}$$

The chemical score (CS) is given by the limiting amino acid, which is found in greater deficit in relation to the reference protein [9].

Biological Assay

The protein quality of the amaranths flour was evaluated using three different indices: NPU, tD, and BV [10, 11]. Six groups (six animals per group) of 30-day-old *Wistar* rats weighing 30–40 g, were used for the biological assay. The first group received a protein-free diet, the second received a control diet (casein), and the remaining groups received a diet with protein provided by the flours under study. The composition of the supplied isocaloric diets is described in Table 1. The animals were kept in individual suspended cages with screen bottoms. Temperature and relative humidity were held at 21±1 °C and 60 %, respectively. Lighting was controlled by alternating 12-h periods of light and darkness. All animals received potable

Table 1 Diets composition

Nutrients (g/kg)	Protein-free	Casein	CC	HD	H17	CG6
Protein	0	111	760.5[a]	618.4[a]	765.1[a]	664.4[a]
Soybean oil	70	70	17.5[b]	33.8[b]	-[b]	8.3[b]
Mineral Mix	35	35	35	35	35	35
Vitamin Mix	10	10	10	10	10	10
Choline Bitartrate	2.5	2.5	2.5	2.5	2.5	2.5
Cystine	0	3	0	0	0	0
Dextrin	882.5	768.5	174.5	300.3	187.4	279.8

[a] The amount of flour incorporated in the diets was defined taking into account the protein content of the sources CC: 13.2 %, HD: 16.2 %, H17:13.07 % and CG6:15.05 %

[b] Amount of oil added to achieve the 7 % of lipids, taking into account the fat content obtained for the sources CC: 6.9 %, HD: 5.9 %, H17: 9.3 % and CG6: 9.3 %

CC Amaranthus cruentus var. *Candil, HD Amaranthus hypochondriacus* var. *Dorado, H17 Amaranthus hypochondriacus* x *Amaranthus cruentus* H17a, *CG6 Amaranthus cruentus* G6/17a

water and food *ad libitum* for 14 days. Ingestion was recorded on days 3, 6, 10 and 14; weight gain was recorded at the end of the experiment. Feces were collected and weighed. After the experiment, the euthanasia of the animals was performed through a carbon dioxide chamber. Subsequently, a thoracic and abdominal incision was performed, and the rats were weighed and placed in a forced air oven at 100–105 ° C for 48 h to determine the body water by weight difference. All diets were prepared according to the recommendations of the American Institute of Nutrition, 1993 (AIN-93G) [12] and the NPU prepared at 10 % of protein and 7 % of lipids [10].

NPU is defined as the portion of nitrogen intake that is retained, expressed as:

$$NPU = B-(BK-IK)\backslash\mathrm{divI}x100 \quad (I)$$

where B is the corporal nitrogen of the experimental group; BK is the corporal nitrogen of the group on the protein-free diet; IK 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 rats age in days, and Y is calculated as

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

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

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

tD was determined along with NPU, and it was considered as the absorbed nitrogen with respect to the N intake. Unabsorbed nitrogen was calculated by quantification of the fecal nitrogen in the group fed with the protein-free diet. tD is expressed as:

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

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

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

The official preferred method for evaluation of protein quality in the United States is the protein digestibility corrected amino acid score (PDCAAS) for all foods for human ages of 1 year and older. PDCAAS calculation needs an amino acid reference and the essential amino acid content of the samples [13, 14].

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

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

Statistical Analysis

The results obtained were analyzed using one-way variance analysis (ANOVA), comparing means by the Tukey-Kramer test (5 % confidence level) [15].

Results and Discussion

Considering that the present study was performed with whole meal flours of new varieties of amaranths, it is necessary to delve into the knowledge of the protein composition and its nutritional value. The protein content, in g/100 g dry sample,

Table 2 Amino acid score (AAS) for essential amino acids from seed flour of the new varieties: *Amaranthus cruentus* var. *candil* (CC) and *Amaranthus hypochondriacus* var. *dorado* (HD), and of the advanced lines: *Amaranthus hypochondriacus* x *Amaranthus cruentus* H17a (H17) and *Amaranthus cruentus* G6/17a (CG6)

Amino acids	FAO[a]	CC	AAS	HD	AAS	H17	AAS	CG6	AAS
Histidine	18	40	>100	42	>100	49	>100	40	>100
Isoleucine	31	41	>100	28	90	29	94	32	>100
Leucine	63	71	>100	67	>100	69	>100	69	>100
Lysine	52	47	90	60	>100	69	>100	65	>100
Sulfur AA	26	52	>100	55	>100	74	>100	37	>100
Aromatic AA	46	79	>100	66	>100	59	>100	55	>100
Threonine	27	49	>100	38	>100	44	>100	40	>100
Tryptophan	7.4	10	>100	14	>100	10	>100	12	>100
Valine	42	20	48	19	45	16	38	14	33
CS		48		45		38		33	

[a] Requirements in essential amino acids (mg g^{-1} protein) proposed by the WHO/FAO/UNU (2007) for preschoolers of 1 to 3 years old

Results are expressed in mg de AA g^{-1} protein

Sulfur AA: Methionine+Cysteine

Aromatic AA: Phenylalanine+Tyrosine

CS Chemical Score

of these varieties was around 13.07 for H17 and 16.2 for HD (see legend in Table 1). These results are similar to the reported by other authors [16–18]. Table 2 shows the amino acid profile and its comparison with the protein used as reference according to the WHO/FAO/UNU [8], and also the CS for each of the varieties. In all cases, valine was the limiting amino acid, obtaining a greater CS for CC (48), while the CG6 presented a lower value (33). However, it is noteworthy that in all cases, lysine presented values similar to other species of amaranth, but greater than cereals that are deficient in this amino acid. Moreover, the content of lysine was superior to that of the reference protein in all cases, except for CC which has an AAS equal to 90. In general, the essential amino acids of these varieties exceed the requirements established by the WHO/FAO/UNU [8], so that the balance of essential amino acids results better than that of many vegetable proteins.

Shown in Table 3 are the different parameters of the biological studies that allowed the evaluation of the protein quality of the studied samples. The intake and weight gain of the animals fed with amaranths flour did not present significant differences with respect to the foods with casein-based diet, except for CC, where the weight gain was significantly lower in comparison to casein, HD and H17. However, the weight evolution was in progressive increase in the four experimental groups, which indicates that the protein was adequate to maintain the animal growth. The weight of the feces did not show significant differences between the different varieties, but was significantly greater than that corresponding to the animals fed with a casein-based diet. This is

Table 3 Biological quality from seed flour of the new varieties: *Amaranthus cruentus* var. *candil* (CC), and *Amaranthus hypochondriacus* var. *dorado* (HD), and of the advanced lines: *Amaranthus hypochondriacus* x *Amaranthus cruentus* H17a (H17) and *Amaranthus cruentus* G6/17a (CG6)

	Casein	CC	HD	H17	CG6
Intake in 14 days (g)	82.34±6.85^{a}	73.96±4.97^{a}	72.10±4.37^{a}	81.56±8.49^{a}	77.74±7.88^{a}
Weight gain in 14 days (g)	15.09±5.76^{a}	3.85±2.36^{b}	13.77±6.93^{a}	15.90±2.36^{a}	10.40±6.40^{ab}
Feces weight in 14 days (g)	2.40±0.52^{a}	7.27±0.82^{b}	8.42±3.45^{b}	9.58±2.29^{b}	9.49±0.83^{b}
NPU	86.00±6.35^{a}	33.56±11.52^{b}	46.04±5.74^{b}	43.54±6.32^{b}	34.50±9.53^{b}
tD	96.00±7.25^{a}	75.40±7.53^{b}	70.50±6.44^{b}	68.80±8.04^{b}	71.80±7.71^{b}
BV	89.00±6.84^{a}	44.53±15.29^{b}	63.24±8.91^{ab}	64.28±7.86^{ab}	49.15±11.37^{b}
PDCAAS %	100^{a}	36.19±1.32^{b}	31.72±1.27^{b}	26.14±1.19^{c}	23.69±1.05^{c}

The values within the same row with different superscripts are significantly different at $p<0.05$.

NPU Net protein utilization, *tD* true digestibility, *BV* Biological value, *PDCAAS* Protein digestibility corrected amino acid score. The results are expressed as mean±standard deviation expected considering the high fiber content of the experimental diets, which according to previous studies their values are around 15.91/100 g for HD and 17.80/100 g for H17 [19]. On the other hand, the results of the biological tests for the studied diets showed significantly lower values in comparison to the casein-based diet, which is expected since casein is a source of pure protein with a balanced amino acid profile.

The NPU, defined as the fraction of nitrogen intake that is retained, allows evaluating the effectiveness of the protein for the normal growth and development. The studied samples did not present significant differences in the NPU values (33.56–46.04 %), but were significantly lower than the obtained for casein, which is in agreement with previous reports for other amaranth species such as *A. quitensis* (42.50 %) [20] and *A. standleyanus* (44.00 %) [21], and lower than the means of the ranges reported in literature reviews performed by Paredes López [22] and Bressani [16], which are around 76.10 and 74.8 %, respectively. On the other hand, the obtained NPU results were similar to the corresponding to conventional cereal grains, such as corn (51.1 %) and wheat (40.3 %) [23].

The digestibility *in vitro* is also an important parameter for the evaluation of a protein nutritional potential, but can overestimate the true nutritional value since it does not consider the biologically unavailable amino acids. Consequently, the tD is an important variable in the consideration of the nutritional adequacy of a protein source, since it represents the portion of nitrogen of the diet that is available for maintenance and growth functions. The tD values obtained in this work are around 68.80 % for H17 and 75.40 % for CC; it is considered that they have a good digestibility due to their vegetable protein condition, although they were lower than casein and other proteins of animal origin; however, they were similar to the obtained for other amaranths species [24].

The biological value of a protein represents the fraction of absorbed nitrogen that is retained by the organisms to maintain the integrity of the tissue, the development and the growth. In this study, the amaranth varieties CC and CG6 showed a BV of approximately 50 % of the obtained for casein ($p<0.01$). However, for the HD and H17 varieties, the BVs were 63 and 64 %, respectively, without presenting significant differences with respect to casein. These last values were similar to the ones published by Escudero et al., 2004, who determined a BV of 64 % for the *Amaranthus cruentus* flour [25]. The values found for the experimental proteins were lower that the means reported by Paredes López of 80.50 % [22], and of 75 % by other authors [26].

The PDCAAS is the index recommended by the FAO/OMS to evaluate the nutritional quality of the human consumption proteins. According to the established by Kannan et al. [27], the CS corrected by the true digestibility (tD) takes into account three critical parameters in the evaluation of the protein quality: essential amino acids profile, digestibility and ability to supply the essential amino acids according to the requirements for humans. To fulfil the most rigorous protein requirements, the PDCAAS compares the AA profile of a protein under study with the needs of preschoolers of 1 to 3 years old, which represent the most demanding requirements, except for the infants that are comparable to human milk. The PDCAAS values calculated for the mentioned amaranth varieties were low (23.69–36.19 %) compared to the casein diet, as well as with respect to the cereals (58.5 %) and the soybeans (78 %), but similar to reported for dried fruits [28]. The low PDCAAS values found in the experimental diets were due to valine, which was the limiting amino acid for the four investigated varieties.

As conclusion, the introduction of these new species is interesting because they have favorable characteristics, such as a greater performance potential, greater adaptability, easier harvesting and resistance to parasites, among others. This work intended to stimulate the introduction of new protein sources, as well as their nutritional benefits, in the feeding of the human population. However, according to the evaluation of new amaranth varieties performed here, it is reasonable to recommend complementing the protein values of these amaranths with cereals, legumes and foods of animal origin or with ingredients rich in valine.

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Conflict of interest All authors confirmed that they have no conflict of interest.

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