AMINO ACID COMPOSITION OF SOME *Amaranthus sp.* GRAIN PROTEINS AND OF ITS FRACTIONS[1]

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SUMMARY

This study was carried out to determine the protein content of several *Amaranthus* sp. grains. Findings revealed this has a high lysine (5.3 to 6.3 of the protein) and sulphur amino acids content (3.4 - 4.0%), while leucine could well be limiting when those seeds are used as a sole protein source in food. Using the correction for *in vitro* protein digestibility, the chemical score varied from 50 to 67. The calculated protein efficiency ratios and biological values ranged from 1.39 to 1.80 and 53 to 68, respectively. Considering that amaranth grain is a good supplement to cereal grain, the protein of *A. hypochondriacus* HH5 (yellow seeds) and *A. anclancalius* (black seeds) was fractionated into albumin, globulin, prolamin and glutelin. The average proportions between those soluble proteins were 65:17:11:7, respectively. Albumin had the highest lysine content (7.3 - 8.2%), and globulin the highest methionine (4.1 - 5.3%) and phenylalanine (6.0 - 6.1%) content. Prolamin had the highest threonine (4.6 - 5.4%) and leucine (6.8 - 6.9%) content, while glutelin had a very low methionine content (0.6 - 1.0%).

Based on the above-mentioned findings, the authors conclude the variation in the amino acid composition of the protein fractions can be used for genetic protein improvement.

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INTRODUCTION

Cereals are the major food source for human diets. Nevertheless, their mean protein content is low (about 10%) and generally imbalanced. Lysine is particularly limiting, but other amino acids such as threonine (sorghum, wheat) and tryptophan (corn, millet) are limiting as well. On the other hand, there could be an excess of leucine which increases the need for niacin, being also partially responsible for pellagra (1).

The pseudo-cereal *Amaranthus* is one of 23 tropical plants, recommended for studies aimed to enhance food quality in the tropics (2). The present investigation on amaranth grain protein and its soluble-protein fractions constitutes part of such a study. Their amino acid composition was therefore determined, and some parameters for nutritive value estimated.

MATERIAL AND METHODS

Plant Material

A general description of the *Amaranthus* species studied and its cultivation conditions are given in Table 1. A more detailed description of those conditions was provided in a previous work (3). The seeds were dried at 60°C and ground to pass a 35 mesh sieve.

Analytical Methods

For their amino acid analysis, samples were hydrolyzed with 6*N* HCl in sealed tubes for 20 hr, at 110 ± 2°C, with and without previous oxidation with performic acid. The vacuum-dried hydrolysate was then dissolved in 0.2 M sodium citrate buffer, pH 2, filtered, and an aliquot applied on a Beckman 120 C automatic amino acid analyzer (4, 5). Tryptophan levels were obtained by a colorimetric method, after alkaline hydrolysis (6) and the nitrogen conversion factors were also determined (4, 5).

Nutritional Value

The nutritive value was estimated taking some parameters based on the amino acid composition of amaranth grains and of the ANRC casein (% protein: 7.2 lysine; 4.1 threonine; 0.9 cysteine; 2.2 methionine; 6.4 valine; 5.0 isoleucine; 9.8 leucine; 5.3 tyrosine; 5.4 phenylalanine, and 1.2 tryptophan). The following parameters were calculated: amino acid score - AAS (8), protein efficiency ratio - CPER (9) and biological value - CBV (10). When necessary, the *in vitro* protein digestibility values were taken from a previous experiment (3).

Protein Fractionation

NaCl and Na_2SO_4 in different concentrations (0.5, 1.0, 5 and 10%) were tested, so as to establish the best extraction conditions of the soluble

TABLE 1

AMARANTHUS SPECIES USED IN THIS EXPERIMENT AND CULTIVATION CONDITIONS

Fertilizer level (kg N/ha)	Latin name	Seed coat color	Other cultivation conditions[a]
0	*A. anclancalius*	Black	*Brazil (Belo Horizonte, Minas Gerais):* Dry, tropical climate, at high altitude (1000 m above sea level). No irrigation.
	A. gangeticus	Black	
	A. hypochondriacus HH5	Yellow	
	A. cruentus HH1	Yellow	
100	*A. anclancalius*	Black	*Puerto Rico (Mayaguez):* Hot, humid, rainy subtropical climate, at lowland area. No irrigation.
	A. hypochondriacus HH5	Yellow	
	A. mantegazzianus	Yellow	
200	*A. anclancalius*	Black	*California (Davis):* Hot, temperate climate, at dry lowland area. Irrigation (every 2 weeks).
	A. gangeticus	Black	
	A. hypochondriacus HH5	Yellow	
	A. mantegazzianus	Yellow	

[a] More details are presented in a previous paper (3).

proteins from *A. hypochondriacus* HH5 (yellow-coated seed) and *A. anclancalius* (black-coated seed). The fractionation into albumin, globulin, prolamin and glutelin was performed according to Padhye & Salunkhe (11). The amount of extracted protein from each fraction was also determined (12). The amino acids of the protein fractions were analyzed without performic acid oxidation, which means that the sulphur amino acids values are underestimated. A standard deviation of ± 8o/o was used for comparison purposes between the amino acid values determined.

RESULTS AND DISCUSSION

Whole Grain Protein

The amino acid composition of grains from different *Amaranthus* species and a reference protein (13) are presented in Table 2. Because no differences were detected in the seed amino acid compositions under different cultivation conditions, and considering a standard deviation of ± 8o/o, only the mean values for each *Amaranthus* species are given. Evidently, the fertilization level did not affect the particular amino acid values, without no differences observed between species. On the other hand, Carlsson (14) noted slightly higher values for some essential amino acids infertilized (200 kg N/ha) samples, in contrast with non-fertilized samples (*A. cruentus* HH3 and *A. hypochondriacus* HH 4/5).

TABLE 2

MEAN AMINO ACID COMPOSITION OF AMARANTH SEEDS (g/100 g PROTEIN)

Amino acids \ Samples[a]	Ahy	Aab	Agb	Amy	Acy	Reference protein[b]
Lysine	6.1	5.7	6.1	6.2	6.2	5.1
Threonine	4.6	4.4	4.3	4.3	4.5	4.1
1/2 cystine	2.1	2.0	2.1	2.0	2.0	
Methionine	1.7	1.7	1.8	1.8	1.7	
(Met + Cys)	3.9	3.7	3.9	3.8	3.7	2.6
Valine	4.4	4.6	4.5	4.6	4.8	4.8
Isoleucine	4.0	4.0	3.9	3.9	4.2	4.2
Leucine	6.2	6.0	6.0	6.3	6.1	7.0
Tyrosine	4.3	4.3	4.3	4.3	4.4	
Phenylalanine	4.8	4.7	4.6	4.8	4.7	
(Tyr + Phe)	9.1	9.0	8.9	9.1	9.1	7.3
Tryptophan	1.3	1.2	1.2	1.4	1.4	1.1
Histidine	2.7	2.8	3.2	3.0	2.7	
Arginine	8.1	8.1	8.5	9.4	7.9	
Aspartic acid	8.1	8.1	8.1	8.6	8.0	
Serine	8.0	8.7	8.6	7.1	7.9	
Glutamic acid	16.6	16.9	16.3	16.6	17.1	
Proline	4.6	4.5	4.4	4.5	4.5	
Glycine	8.4	8.6	8.8	7.4	8.3	
Alanine	3.9	3.8	3.7	3.9	3.6	
CF[c]	6.00	6.00	5.92	5.92	6.02	

[a] Ah (*A. hypochondriacus* HH5), AA (*A. anclancalius*), Ag (*A. gangeticus*), Am (*A. mantegazzianus*), Ac (*A. cruentus* HH1), y (yellow-coated seed), b (black-coated seed).

[b] FNB (13).

[c] Nitrogen to protein conversion factor.

The black-coated seeds (*A. anclancalius* and *A. gangeticus*) contained more serine and less tryptophan than the yellow-coated ones (Table 2). Higher values for serine, glycine and sulphur amino acids have been determined in black-coated seeds (14) when compared to "white" (yellow) seeds. Comparison of the present amino acid levels with those obtained by Carlsson (14) for *A. hypochondriacus* HH5 (white), *A. gangeticus* (black) and *A. cruentus* HH1 (white) as well as HH3 (brown), revealed some divergent results: slightly lower values for lysine (5.2 - 5.5o/o), leucine (5.6 - 5.7o/o), and phenylalanine (3.9 - 4.0o/o), while the methionine values were higher (2.3 - 2.6o/o). As to *A. cruentus*,

somewhat lower results were observed (15, 16) for tyrosine, threonine, and tryptophan, while the methionine and cystein values were higher in spite of the fact that one of the samples was a black-coated variety. In *A. hypochondriacus* much lower values were reported for lysine, phenylalanine and tyrosine (16, 17) and quite higher ones for proline and glycine (17). These divergencies could probably be attributed to differences in analytical methods and/or in seed varieties.

The average nitrogen-to-protein conversion factor of all samples analyzed was 5.97 (Table 2). Values comprised between 5.4 and 5.8 have been reported for several *Amaranthus* species (14) and 5.85 for *A. edulis (A. caudatus)* and *A. cruentus* (15).

Estimated Nutritive Value

When compared to a reference protein (13), the primary limiting amino acid of the *Amaranthus* studied, was leucine (Table 3), finding which is in accordance with the literature (14, 15, 18-22).

The amino acid score (AAS) of the *Amaranthus* species included in this work, varied from 0.81 to 0.90, i.e., values similar to those of animal proteins. Vegetable proteins present a delayed or incomplete digestion when compared to those of animal origin. Therefore, a digestibility factor should be considered to estimate protein utilization (1, 8). Using the *in vitro* protein digestibility values (Table 3), obtained in a preceding work (3), the corrected amino acid score (CAAS) ranged from 50 to 67 (Table 3). The black-coated seeds had a lower CAAS than the yellow ones.

Considering that the *in vivo* methods for nutritive value analysis of proteins are expensive and time consuming, a calculated protein efficiency ratio (CPER) and biological value (CBV) were used as a first approach (Table 3). Nevertheless, as happens with other *in vitro* methods, these may not give a completely true judgement of the proteins' nutritional value. The average CPER values were 1.45 for *A. anclancalius* and 1.39 for *A. gangeticus*, both black-coated seeds, while those values were 1.80 for *A. cruentus* HH1, 1.79 for *A. hypochondriacus* HH5, and 1.66 for *A. mantegazzianus*, all three of them with yellow-coated seeds. The digestibility factor is taken into account for calculation of the CPER. Therefore, those values could be compared to PER results from biological assays; *in vivo* PER values equal to 1.50 for *A. cruentus* and *A. hypochondriacus* have been reported (19, 20). Furthermore, Carlsson (14) showed that *A. hypochondriacus* HH5 with "white" seeds (yellow) gave higher daily weight gains (3.7 g/d) than *Amaranthus* sp. with black seeds (1.7 g/d ± 0.8).

The equation for calculated biological value (CBV) (10) gave values of 0.73 - 1.00 (Table 3). When these were corrected for the digestibility factor (CCBV) they now varied from 0.53 to 0.68. Again, black-coated seeds gave lower CCBV values than the yellow ones. *A. hypochondriacus* exhibited a biological value of 0.74 and a true digestibility of 0.76 (23).

The CAAS and CCBV values are similar if two samples are excluded (AhBy and AmCy, Table 3), for which very high and very low threonine values were recorded (4.8 and 4.2%, respectively). Since threonine plays a major role in the equation for CBV (10), a respective sub- and superestimation of the CCBV may result. The linear correlation coefficient

TABLE 3

ESTIMATION OF THE NUTRITIVE VALUE OF AMARANTH SEED PROTEIN

Parameters[b] \ Sample[a]	AhBy	AhPRy	AhCy	AaBb	AaPRb	AaCb	AgBb	AgCb	AmPRy	AmPRy	AcBy
LAA											
First	Leu	Leu	Leu	Leu	Leu	Leu	Leu	Leu	Leu	Leu	Leu
Second	Val	Val	Val	Ile	Ile	Ile	Ile	Ile	Ile	Ile	
Third	Ile	Ile	Ile	Val	Val	Val	Val	Val	Val	Val	
AAS	0.89	0.87	0.89	0.89	0.81	0.89	0.84	0.86	0.89	0.90	0.87
i. v. PD	0.76	0.76	0.70	0.64	0.61	0.64	0.62	0.62	0.67	0.68	0.70
CAAS	0.67	0.61	0.62	0.57	0.50	0.57	0.52	0.53	0.59	0.61	0.61
CPER	1.80	1.74	1.76	1.49	1.31	1.54	1.38	1.40	1.65	1.68	1.80
CBV	0.73	0.84	0.91	0.87	0.87	0.88	0.85	0.87	0.91	1.00	0.91
CCBV	0.55	0.59	0.64	0.56	0.53	0.56	0.53	0.54	0.61	0.68	0.64

[a] Ah *(A. hypochondriacus* HH5), Aa *(A. anclancalius)*, Ag *(A. gangeticus)*, Am *(A. mantegazzianus)*, Ac *(A. cruentus* HH1); B (Brazil), PR (Puerto Rico), C (California); y (yellow), b (black).

[b] LAA: limiting amino acids; AAS: amino acid scoring (8); i. v. PD: *in vitro* protein digestibility (3); CAAS: corrected AAS; CPER: calculated protein efficiency ratio (9); CBV: calculated biological value (10); CCBV: corrected CBV.

between CAAS and CCBV (8) was 0.91 (n = 9). The same statistical analysis for CAAS and CPER gave a correlation coefficient of 0.97 (n= 11).

In view of the fact that cereal proteins have low lysine and high leucine contents, and *Amaranthus* grain proteins exhibit high lysine and low leucine levels, a combination of those two protein sources would give a food with a relative good nutritional value. Rat assays showing this fact have been reported (16,23). The same was observed with cereal flours mixed with *Chenopodium quinoa* flour, another pseudo-cereal (Carlsson & Harczakowski, unpublished results).

Protein Fractionation

A 5%/o NaCl solution was chosen as extraction medium, as it gave the highest yields, 11.4%/o of the seed dry weight. A yield of 15.9%/o has been notified (25), but using another extraction method.

The protein fractions albumin, globulin, prolamin and glutelin from *A. hypochondriacus* HH5 (yellow seeds) and *A. anclancalius* (black seeds) presented the following average proportions of total solubilized protein, by dry weight, 65:17:11:7, respectively.

Seed proteins of species grown under three different cultivation conditions, were fractionated to examine environmental effects on the amino acid composition of the fractions. No major differences were observed. Average values are given in Table 4. As observed, in all fractions the glutamic acid was higher than in the whole seed protein. The amount of essential amino acids was higher in the globulin and prolamin fractions (about 40%/o of the protein), while it was lower for albumin and glutelin (34%/o, approximately).

Differences between fractions from yellow seeds (*A. hypochondriacus* HH5) and those from black seeds *(A. anclancalius)* were detected. As Table 4 depicts, therefore, the yellow seed fractions albumin, prolamine and glutelin were higher in their lysine, threonine, leucine and tyrosine content. If certain differences between yellow and black seed protein fractions are not taken into account, some differences between the fractions can be noted. Albumin, for example, had high contents of methionine, leucine, phenylalanine, and histidine, and a low level of glycine. Prolamin had high contents of threonine, valine, isoleucine, leucine and proline, and was low in arginine. Glutelin presented high levels of leucine and aspartic acid, but had lower levels of methionine and tyrosine. Thus, although differences in amino acid composition among species were difficult to notice, there do exist large differences between amino acids from different protein fractions, and in fractions between two species, selected for having different morphological seeds.

A high lysine content in the Opaque-2 variety of maize was also appreciated, this being related to an increase of the glutelin fraction and a reduction of prolamin (zein). More basic amino acids were present in the acid-soluble fraction than in that from the hybrid corn (25). By crossing Opaque-2 with other mutants with relatively higher contents of albumin, globulin and glutelin, all with relatively high lysine contents, the lysine levels could be increased further, as compared to hybrid maize (26,27). A similar relation was also observed for barley (28).

TABLE 4

MEAN AMINO ACID CONTENTS OF SEEDS AND PROTEIN ERACTIONS OF *A. HYPOCHONDRIACUS* HH5 AND *A. ANCLANCALIUS* (g/100 g PROTEIN)

Samples[a] / Amino acids	Seeds		Protein fractions							
			Albumin		Globulin		Prolamin		Glutelin	
	Ahy	Aab	Ahy	Aab	Ahy	Aab	Ahy	Aab	Ahy	Aab
Lysine	6.1	5.7	8.2	7.3	6.0	6.1	5.7	4.3	6.3	4.8
Threonine	4.6	4.4	3.5	2.8	3.0	3.1	5.4	4.6	4.5	4.0
Methionine	1.7	1.7	1.9	2.2	5.3	4.1	2.2	2.1	0.6	1.0
Valine	4.4	4.6	4.2	3.9	4.7	4.7	8.0	7.2	5.9	5.0
Isoleucine	4.0	4.0	3.8	4.0	3.9	4.1	4.8	5.0	4.0	4.0
Leucine	6.2	6.0	5.1	4.6	6.2	6.4	6.8	6.9	6.6	6.2
Tyrosine	4.3	4.3	3.8	3.8	4.2	4.4	4.9	3.9	3.2	2.9
Phenylalanine	4.8	4.7	3.6	3.6	6.0	6.1	3.9	4.7	4.4	4.7
Histidine	2.7	2.8	2.2	2.1	2.8	2.9	1.9	1.8	1.7	1.9
Arginine	8.1	8.1	11.3	14.0	11.3	11.2	5.8	7.7	7.3	10.0
Aspartic acid	8.1	8.1	8.3	6.6	9.1	9.0	8.2	7.1	11.4	8.8
Serine	8.0	8.7	4.7	3.9	4.4	4.6	4.5	3.6	5.1	4.8
Glutamic acid	16.6	16.9	24.3	27.6	21.6	21.2	16.2	20.8	19.8	24.6
Proline	4.6	4.5	3.7	3.6	4.2	4.2	6.1	6.0	4.6	4.7
Glycine	8.4	8.6	7.8	7.2	4.4	4.5	7.8	7.5	8.5	7.5
Alanine	3.9	3.8	3.5	2.6	3.0	3.4	7.7	6.8	6.1	5.0

a — Ahy: *A. hypochondriacus* HH5, yellow coated; Aab: *A. anclancalius*, black coated.

From the above-mentioned findings, the authors conclude that it seems possible to increase the level of the first limiting amino acid of the *Amaranthus* seed, leucine, by thorough genetic engineering or through selection means, by increasing the leucine-rich fractions.

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RESUMEN

COMPOSICION AMINOACIDICA DE LA PROTEINA DE SEMILLAS DE ALGUNAS VARIEDADES DE *Amaranthus* sp. Y DE SUS FRACCIONES

La proteína de diversas semillas de *Amaranthus* sp. tiene un elevado contenido de lisina (5.3 - 6.3°/o de la proteína) y amino ácidos azufrados (3.4 - 4.0°/o); la leucina, en cambio, puede ser limitante cuando esas semillas son utilizadas como fuente única de proteína en el alimento. Empleando la corrección para digestibilidad *in vitro* de la proteína, el puntaje químico varía de 50 a 67. Los valores calculados para el índice de eficiencia proteínica y del valor biológico variaron de 1.39 a 1.80 y de 53 a 68, respectivamente. Considerando que la semilla de amaranto es un buen suplemento para los cereales se acordó fraccionar proteínas de *A. hypochondriacus* HH5 (de semillas amarillas) y *A. anclancalius* (de semillas negras) en albúmina, globulina, prolamina y glutelina. Las proporciones promedio entre estas proteínas solubles demostraron ser de 65:17:11:7, respectivamente. La albúmina acusó el mayor contenido de lisina (7.3 - 8.2°/o); la globulina, en metionina (4.1 - 5.3°/o) y fenilalanina (6.0 - 6.1°/o) y la prolamina, en los aminoácidos treonina (4.6 - 5.4°/o) y leucina (6.8 - 6.9°/o). Según se constató, la glutelina tiene un nivel muy bajo de metionina (0.6 - 1.0°/o).

A partir de los hallazgos mencionados, los autores concluyen que la variación de las fracciones proteínicas puede ser utilizada con propósitos de mejoramiento genético de la proteína.

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