Protein Fractions in Amaranth Grain and Their Chemical Characterization

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Protein distribution in each of two selections of *Amaranthus caudatus*, *Amaranthus hypochondriacus*, and *Amaranthus cruentus* was established by the Landry and Moreaux fractionation scheme. Some of these fractions were analyzed for their amino acid content. Two extraction sequences were used, which differed in the first solvent, water in one and 0.5 M NaCl in the other. No differences were found in the protein fractions distribution between species or cultivars of the same species, independent of the fractionation sequence used. The solvent sequence resulted in differences in fraction I (albumin), II (globulin), III (prolamine), V (glutelin-like), and some in fraction VI (glutelins). With water as first solvent, albumins averaged 20.7, globulins 19.2, prolamines 2.2, glutelins 44.4, with 13.4% in the residue of the total protein. Albumins were rich in tryptophan, threonine, and lysine; globulins in sulfur amino acids and lysine; prolamines in threonine and leucine; and glutelins in tryptophan and leucine.

Amaranth grain has been identified as a very promising food crop because of its exceptional nutritive value as judged by its protein and lipid content, as well as for its essential amino acid composition that has a relatively high lysine content (Teotonico and Knorr, 1985). It is of significance that cereals are deficient in lysine and tryptophan in corn, lysine and threonine in rice (Deshpande et al., 1955; Rosenberg et al., 1960), and lysine in wheat (Howe et al., 1965), with adequate amounts of other essential amino acids. Amaranth protein amino acid content closely approximates the amino acid pattern of the FAO/WHO standard protein with the exception of leucine (United Nations University, 1980). Information on amino acid content of individual proteins is very limited (Duarte-Correa et al., 1986). Thus, the purposes of this study were to determine the amounts of albumins, globulins, prolamines, and glutelins present in the protein of three species of amaranth grain, represented by two varieties each, and to determine their amino acid composition.

MATERIALS AND METHODS

Materials. Six amaranth samples shown in Table I were used. All of them originally came from Rodale Center in the United States but were grown, in 1984, at INCAP's experimental farm in Guatemala. Seeds were milled in a Cyclone mill to pass a 40-mesh screen and defatted by exhaustive extraction with hexane, prior to protein extraction and fractionation.

Methods. *Protein Fractionation.* Proteins were extracted stepwise following the method of Landry and Moureaux (1980), with some modifications. Thus, 5 g of each sample was mixed with 50 mL of extractant under magnetic stirring. Two fractionation sequences were performed, which were essentially the same, except that in the first sodium chloride was the first solvent followed by water, while in the second water was the first solvent followed by sodium chloride. The solvent sequence following the above change was the same. A second difference was that in the first fractionation scheme nitrogen in the extracts was determined by the Kjeldahl method, while in the second the method of Bradford (1976) was used. This method determines only protein nitrogen. The time (min) and number of extractions were as follows: fraction I (H_2O) 60, 30; fraction II (0.5 M NaCl) 60, 30; fraction III (55% isopropyl alcohol) 60, 30, 15; fraction IV (55% isopropyl alcohol with 0.6% 2-mercaptoethanol) 30, 30; fraction V (borate buffer (pH 10) with 0.6% 2-mercaptoethanol and 0.5 M NaCl) 60, 30, 15; fraction VI (borate buffer (pH 10) with 0.6% 2-mercaptoethanol and 0.5% sodium dodecyl sulfate). Fractions I and II were extracted at 4 °C, while the rest were extracted at room temperature. After extraction, the mixtures were centrifuged for 15 min at 30000*g* and protein was determined in the combined supernatants for each solvent by the micro-Kjeldahl method when sodium chloride was the first solvent and by the method of Bradford (1976) when water was the first solvent. Protein was then precipitated by dropping the pH of the solution to 1 with 0.1 N HCl and by heating to 75 °C. The precipitate was centrifuged and freeze-dried prior to nitrogen determination by the standard micro-Kjeldahl procedure and amino acid analysis. The residue of extractions was analyzed for nitrogen, and percent protein was calculated as % N × 6.25.

Amino Acid Analysis. Amino acid determinations were made with an LKB 4151 Alpha Plus amino acid analyzer on protein hydrolysates obtained by hydrolyzing the sample with 6 N HCl for 4 h at 100 °C in a sealed tube under vacuum. No corrections were made for damage to sulfur or other amino acids during hydrolysis.

Tryptophan was determined colorimetrically by the method modified by Villegas et al. (1982).

Table I. Protein Content (% N × 6.25) of Six Amaranth Samples

sample	% protein
A. caudatus (var. 2) (A-982)	14.5 ± 0.4
A. caudatus (var. 3) (A-1113)	14.6 ± 0.1
A. hypochondriacus (var. 4) (A-718)	14.7 ● 0.4
A. hypochondriacus (var. 5) (A-720)	15.9 ± 0.5
A. cruentus (var. 7) (82S-1011)	15.3 ± 0.4
A. cruentus (var. 8) (82S-434)	16.1 ± 0.4

RESULTS AND DISCUSSION

Table I presents protein content of the materials used in this study. It is similar to values previously reported (Bressani et al., 1987). For protein extraction two solvent sequences were tested in which the first solvent used was either sodium chloride in one case and water in the other. Other solvents were used in the same sequence. Proteins that were extracted in each step as described before (Guiragossian et al., 1978; Misra et al., 1975; Nwasike et al., 1979) were as follows: fraction I, albumins; frac-

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Table II. Protein Fractions as Percentage of Total Protein

amaranth species	fraction[a] II	I	III	IV	V	VI	residue
caudatus (var. 2)	41.9 ± 2.5	6.7 ± 2.0	8.5 ± 1.9	1.6	8.4 ± 0.1	21.1 ± 1.0	14.3 ± 0.7
caudatus (var. 3)	39.3 ± 0.5	8.7 ± 1.3	6.9 ± 0.9	1.5 ± 0.1	9.1 ± 1.4	26.8 ± 4.4	16.4 ± 0.8
hypochondriacus (var. 4)	33.3 ± 1.4	8.2 ± 1.9	7.1 ± 3.4	1.6 ± 0.3	8.6 ± 1.5	22.1 ± 5.2	23.6 ± 0.6
hypochondriacus (var. 5)	43.7 ± 1.5	8.9 ± 1.3	7.5 ± 3.7	2.0 ± 1.2	6.0 ± 0.1	21.5 ± 0	24.0 ± 4.0
cruentus (var. 7)	47.5 ± 8.1	9.7 ± 5.2	9.2 ± 5.7	2.4 ± 0.9	6.9 ± 2.3	26.6 ± 0	16.7 ± 0.5
cruentus (var. 8)	40.9 ± 2.6	8.1 ± 1.2	8.9 ± 3.6	2.1 ± 0.1	8.4 ± 0.7	20.5 ± 5.5	14.7 ± 1.6
$\bar{x}$ ± SD	41.1 ± 4.3	8.4 ± 1.0	8.0 ± 1.0	1.9 ± 0.3	7.9 ± 1.1	23.1	18.3 ± 4.0

[a] Solvent sequence: II, 0.5 M NaCl; I, water; III, isopropyl alcohol (55%); IV, isopropyl alcohol + 2-MeEtOH; V, borate buffer (pH 10) + 2-MeEtOH + 0.5 M NaCl; VI, borate buffer (pH 10) + 2-MeEtOH + sodium dodecyl sulfate. Nitrogen analysis: micro-Kjeldahl.

Table III. Protein Fractions as Percentage of Total Protein

amaranth species	fraction[a] I	II	III	IV	V	VI	residue
caudatus (var. 2)	20.4 ± 2.4	20.5 ± 5.6	0.5 ± 0.2	1.0 ± 0.2	15.3 ± 2.1	32.5 ± 12.2	10.0 ± 1.6
caudatus (var. 3)	25.4 ± 2.5	20.4 ± 3.8	0.5 ± 0.2	1.4 ± 0.2	15.7 ± 2.0	25.1 ± 6.1	11.6 ± 1.7
hypochondriacus (var. 4)	17.0 ± 1.1	15.7 ± 2.9	0.7 ± 0.2	0.5 ± 0.2	13.8 ± 3.9	34.7 ± 2.4	17.6 ± 2.5
hypochondriacus (var. 5)	21.4 ± 0.2	20.5 ± 2.0	0.9 ± 0.1	2.0 ± 0.5	2.9 ± 2.6	33.4 ± 0.7	18.8 ± 2.5
cruentus (var. 7)	21.1 ± 4.8	19.3 ± 0.3	0.8 ± 0.0	2.8 ± 0.2	14.8 ± 1.0	31.0 ± 5.6	10.3 ± 0.8
cruentus (var. 8)	18.9 ± 3.8	18.8 ± 0.1	1.0 ± 0.7	0.8 ± 0.4	16.1 ± 4.0	30.9 ± 5.8	12.3 ± 0.2
$\bar{x}$ ± SD	20.7 ± 2.6	19.2 ± 1.7	0.7 ± 0.2	1.0 ± 0.8	13.1 ± 4.6	31.3 ± 3.1	13.4 ± 3.8

[a] Solvent sequence: I, water; II, 0.5 M NaCl; III, isopropyl alcohol (55%); IV, isopropyl alcohol + 2-MeEtOH; V, borate buffer (pH 10) + 2-MeEtOH + 0.5 M NaCl; VI, borate buffer (pH 10) + 2-MeEtOH + sodium dodecyl sulfate. Nitrogen analysis: Bradford.

tion II, globulins; fraction III, prolamines; fraction IV, prolamine-like or proteins that were soluble in alcohol after the disulfide bonds in the protein had been reduced with 2-mercaptoethanol; fraction V, proteins that are alkali soluble after the disulfide bonds are broken and have some of the characteristics of glutelin (glutelin-like); fraction VI, true glutelin, which is a complex high molecular weight protein mixture that could be solubilized only by treating with a reducing agent and a detergent, at alkali pH. Results obtained from the protein fraction when NaCl was the first solvent are shown in Table II. Some variability was observed between varieties of the same species in all fractions, with some exceptions such as fraction IV. However, average fraction distribution between species was similar. For all amaranth selections, fraction II was the highest, accounting for 41.1% of the total protein, followed by fraction VI. Fraction IV, the prolamine-like, gave the lowest values. Residual levels of protein were highest for *Amaranthus hypochondriacus*, which could be responsible for low values for fractions II and VI. The values presented in the table are different from those reported by Konishi et al. (1985). However similar amounts for *A. hypochondriacus* were found in fractions V and VI. Fractions III and IV were higher and Fractions I and II together were smaller in this study. The same was true for *Amaranthus caudatus* and *Amaranthus cruentus* except that fraction VI in this study was higher than the values reported by Konishi et al. (1985). No explanation can be offered for the differences between results of the present study and those of Konishi et al. (1985). One possibility may be the nitrogen content of the samples, which is lower in the samples used in this study. It is possible for higher protein-containing samples to have more nonprotein nitrogen than those of lower content.

Table III shows the results of the study in which water was the first solvent. In this case nitrogen extracted was evaluated by the method of Bradford (1976), which only measured protein nitrogen. There were only small differences between varieties of the same species and between species as already indicated. Fraction V, glutelin-like, is similar in all samples studied, with the exception of *A. hypochondriacus* variety 5, which gave a value of 2.9% of total proteins in comparison to an average of 15.1 ± 0.8% for the other five samples. This sample also differed in the amount of nonextracted nitrogen, that is, protein in the residues of extraction. The samples of both, *A. caudatus* and *A. cruentus*, showed an average of 11.1 ± 1.1% of total proteins in the residues, whereas the sample of *A. hypochondriacus* had an average of 18.2 ± 0.9%.

Table IV. Comparison of the Protein Distribution from the Two Extraction Sequences Studied

fraction	*A. caudatus* A[a]	B	*A. hypochondriacus* A	B	*A. cruentus* A	B
I	7.7	22.9	8.5	19.2	8.9	20.0
II	40.6	20.5	38.5	18.1	44.2	19.1
I + II + NPN	48.3	43.4	47.0	37.3	53.1	39.1
III	7.7	0.5	7.3	0.8	8.4	0.9
IV	1.5	1.2	1.8	1.2	2.0	1.9
V	8.7	15.5	7.3	8.3	8.1	15.4
VI	23.9	28.8	21.8	34.0	21.8	30.9
nitrogen	2.33		2.45		2.51	

[a] Key: A, 0.5 M NaCl first solvent; B, H_2O first solvent.

Average results from the two solvent sequences are summarized in Table IV. The sequences starting with NaCl (A) gave a higher value for the sum of albumin, globulin, and NPN than B (water). The last source of nitrogen could be assumed to be present since the method to evaluate extraction of the N was the Kjeldahl method for sequence A but not for B, which used the Bradford method. One could then assume the difference to be NPN, which represented 4.9, 9.7, and 14.0% of the total nitrogen in the samples of *A. caudatus*, *hypochondriacus*, and *cruentus*. It is of interest to indicate that these amounts correlated positively with the nitrogen content of the samples, which were 2.33, 2.45, and 2.51. This, then, may partially explain the differences between the results presented here and those from Konishi et al. (1985). From data under B, the globulin/albumin ratios for *A. caudatus*, *A. hypochondriacus*, and *A. cruentus* were 0.89, 0.94, and 0.95, which differs from that reported by Abdi and Sahib (1976) (G/A 0.28) and by Konishi et al. (1985) (G/A 2.1). These results do not agree with those reported by

Table V. Protein Composition of Different Cereals (Percent of Extracted Protein)

material	albumins + globulins	prolamines	glutelins	source
triticale				
germ	59	10	31	Lupano and Anon, 1985
endosperm	32	29	38	
corn				
common	6	55	40	Chung and Pomeranz, 1985
opaque-2	49	19	32	Landry and Moureaux, 1980
wheat	15	69	16	Chung and Pomeranz, 1980
oats	79–81	10–16	5	Chung and Pomeranz, 1980
sorghum	16	52	32	Chung and Pomeranz, 1980
amaranth	40[a]	2	44	this study
amaranth	55.9–62.2[b]	1.0–3.1	21.5–29.4	Konishi et al., 1985
amaranth	(65, 17)	11	7	Duarte-Correa et al., 1986

[a] Values calculated from Table II. [b] Albumins + globulins + NPN.

Table VI. Amino Acid Composition of Amaranth Grain Samples (Grams/16 g of N)

	A. caudatus		*A. hypochondriacus*		*A. cruentus*		
amino acid	var. 2 A-982	var. 3 A-1113	var. 4 A-718	var. 5 A-720	var. 7 82S-1011	var. 8 82S-434	FAO/WHO pattern
tryptophan	1.3			1.4	1.4		1.0
aspartic acid	10.2	11.9	11.3	11.7	12.6	11.4	–
threonine	3.6	3.7	3.4	3.5	3.9	3.8	4.0
serine	5.2	4.9	4.7	5.2	5.9	4.9	–
glutamic acid	21.9	21.5	17.9	15.6	21.3	22.0	–
glycine	10.3	9.6	10.3	9.0	9.4	10.4	–
alanine	5.4	7.7	6.9	9.5	8.0	8.9	–
valine	5.8	5.7	5.6	6.6	5.7	5.1	5.0
cystine	1.0	–	–	0.9	–	–	3.5[a]
methionine	2.7	2.8	2.9	3.0	3.0	3.0	
isoleucine	3.2	3.1	3.1	3.0	3.5	2.7	4.0
leucine	5.7	5.5	6.0	6.0	5.7	6.2	7.0
tyrosine	5.3	5.5	4.5	5.2	4.6	5.2	6.0[b]
phenylalanine	5.2	4.5	4.2	4.9	5.2	5.3	
histidine	3.7	4.3	4.1	4.3	5.0	4.3	–
lysine	6.4	7.0	10.1	7.7	7.8	6.9	5.4
arginine	10.7	13.4	10.2	12.7	9.6	11.3	–
proline	5.5	5.6	6.6	8.0	6.6	6.7	–
total EAA[c]	40.2	–	–	42.2	40.8	–	35.9

[a] Total sulfur amino acids (methionine + cystine). [b] Total aromatic amino acids (phenylalanine + tyrosine). [c] All amino acids listed under FAO/WHO pattern.

Duarte-Correa et al. (1986), who used a complex fractionation technique. They reported a distribution of 65, 17, 11, and 7% albumin, globulin, prolamine, and glutelin, respectively. Correlation coefficients between total protein and the different fractions were for albumins r = −0.189 (NS), for globulins r = 0.106 (NS), for total prolamines r = 0.453 (NS) and for total glutelins r = −0.327 (NS).

The correlation between total proteins and prolamine-like was 0.254 (NS), with glutelin 0.194 (NS), and with glutelin-like −0.440 (NS). The correlation coefficient of prolamine (without prolamine-like) is 0.953 (significant, p <0.01). Thus, a higher protein content in the grain could be attributed to prolamine content.

Table V shows protein distribution of different cereals as percentages of extracted protein, adapted from different sources for comparison purposes. From these data, it is obvious that amaranth protein contains low amounts of prolamines with high levels of glutelins and also relatively high levels of albumins plus globulins.

The essential amino acid pattern for total proteins of the amaranth samples is presented in Table VI. There were no large differences between species, except for lysine which was quite high in the *A. hypochondriacus* variety 4 sample. As has been indicated by other investigators (Teutonico and Knorr, 1985), and on the basis of the FAO/WHO essential amino acid reference pattern (United Nations University, 1980), the limiting amino acid appears to be leucine, although threonine and isoleucine are also low. Amaranth grain contains a relatively good essential amino acid content, particularly in lysine, which is deficient in cereal grain protein.

Table VII. Amino Acid Composition of Amaranth Albumins (Grams/16 g of N)

	A. caudatus		*A. hypochondriacus*		*A. cruentus*		
amino acid	var. 2	var. 3	var. 4	var. 5	var. 7	var. 8	$\bar{x}$
tryptophan	1.6	1.6	1.6	1.3	1.2	1.4	1.4
aspartic acid	9.5	9.4	11.4	10.2	8.7	9.2	9.7
threonine	4.5	4.4	5.3	4.3	4.3	4.5	4.5
serine	4.5	4.7	5.4	4.8	5.3	4.9	4.9
glutamic acid	19.7	18.8	20.7	20.9	19.3	18.8	19.7
glycine	4.5	4.1	5.2	4.6	5.0	4.8	4.7
alanine	4.2	3.7	4.8	4.4	3.7	3.9	4.1
valine	4.8	5.4	5.4	4.9	4.9	5.2	5.1
cystine[a]	nd	nd	1.1	1.0	nd	nd	1.0
methionine[a]	2.3	2.0	2.5	2.5	2.8	2.6	2.4
isoleucine	4.2	3.8	4.7	4.3	4.1	4.0	4.2
leucine	6.1	5.9	7.6	6.5	7.5	7.1	6.8
tyrosine	3.9	4.2	2.6	3.9	4.0	3.6	3.7
phenylalanine	5.3	4.5	5.1	4.8	4.8	4.5	4.8
histidine	2.2	2.0	2.3	2.2	2.3	2.3	2.2
lysine	7.2	7.3	7.1	6.6	7.4	7.9	7.2
arginine	7.5	7.1	7.6	7.3	7.4	6.8	7.3
total EAA	39.9	39.1	43.0	40.1	41.0	40.8	41.1

[a] No corrections were made for amino acid loss during hydrolysis. nd = nondetectable.

Tables VII–XI show the amino acid compositions for the different extracted fractions of all the samples (Tables VII, VIII, and XI) and of the fractions of three samples (Tables IX and X). Proline content was not measured in any of the samples. Variation of amino acid content

Table VIII. Amino Acid Composition of Amaranth Globulins

amino acid	*A. caudatus* var. 2	*A. caudatus* var. 3	*A. hypochondriacus* var. 4	*A. hypochondriacus* var. 5	*A. cruentus* var. 7	*A. cruentus* var. 8	$\bar{x}$
tryptophan	0.8	1.0	0.8	0.7	0.5	0.6	0.7
aspartic acid	6.1	9.8	10.4	9.4	10.0	7.1	8.8
threonine	2.8	2.7	3.0	2.5	2.9	2.9	2.8
serine	3.5	3.4	3.8	3.6	4.0	3.3	3.6
glutamic acid	30.2	28.9	31.6	33.3	30.4	24.4	29.8
glycine	4.8	4.9	5.0	5.0	5.2	4.4	4.9
alanine	3.1	3.4	3.5	3.0	3.2	2.5	3.1
valine	3.3	3.2	3.4	3.2	3.3	3.3	3.3
cystine[a]	5.6	4.0	4.3	4.6	4.4	3.7	4.4
methionine[a]	3.8	3.6	4.3	3.3	3.2	3.5	3.6
isoleucine	3.4	2.9	2.9	2.9	2.9	2.9	3.0
leucine	3.8	4.4	4.6	4.3	4.0	4.2	4.2
tyrosine	4.2	3.7	3.9	3.8	4.0	3.9	3.9
phenylalanine	5.5	5.6	5.3	5.0	5.2	5.4	5.3
histidine	2.8	2.2	2.2	2.3	2.3	2.0	2.3
lysine	6.4	6.2	7.5	6.4	6.4	5.0	6.3
arginine	11.9	12.0	11.6	13.2	12.3	11.4	12.1
total EAA	39.6	39.8	12.8	39.1	39.5	35.4	37.5

[a] No corrections were made for amino acid loss during hydrolysis.

Table IX. Amino Acid Composition of Amaranth Prolamines (Grams/16 g of N)

amino acid	*A. caudatus* (var. 2)	*A. hypochondriacus* (var. 5)	*A. cruentus* (var. 7)	$\bar{x}$
tryptophan	nd	0.7	1.0	0.8
aspartic acid	8.7	6.7	7.0	7.5
threonine	5.1	4.3	4.0	4.5
serine	6.2	5.2	5.5	5.6
glutamic acid	15.6	14.2	15.1	15.0
glycine	7.2	4.9	5.2	5.8
alanine	4.6	4.7	4.0	4.4
valine	5.5	4.6	4.2	4.8
cystine[a]	nd	nd	nd	nd
methionine[a]	0.6	1.1	nd	0.8
isoleucine	4.3	4.6	5.0	4.6
leucine	9.0	6.9	6.9	7.6
tyrosine	2.7	2.6	2.0	2.4
phenylalanine	4.6	4.6	4.7	4.6
histidine	2.5	1.9	2.0	2.1
lysine	6.0	5.1	4.8	5.3
arginine	6.6	5.7	5.8	6.0
total EAA	37.8	34.5	32.6	35.4

[a] No corrections were made for amino acid loss during hydrolysis. nd = nondetectable.

Table X. Amino Acid Composition of Amaranth Glutelin-like Proteins (Grams/16 g of N)

amino acid	*A. caudatus* (var. 2)	*A. hypochondriacus* (var. 5)	*A. cruentus* (var. 7)	$\bar{x}$
tryptophan	0.1	nd	0.6	0.4
aspartic acid	4.8	5.0	5.4	5.1
threonine	2.2	2.3	2.2	2.2
serine	2.7	3.5	3.5	3.2
glutamic acid	8.6	14.8	19.1	14.1
glycine	2.8	4.4	5.5	4.2
alanine	2.2	2.1	2.1	2.1
valine	2.6	1.9	1.8	2.1
cystine[a]	nd	nd	nd	nd
methionine[a]	1.1	nd	nd	1.1
isoleucine	2.2	1.9	1.9	2.0
leucine	3.5	3.0	2.7	3.1
tyrosine	1.9	1.8	2.0	1.9
phenylalanine	2.7	3.4	2.8	3.0
histidine	1.4	2.4	3.0	2.3
lysine	2.3	2.5	3.9	2.9
arginine	3.2	6.2	7.9	5.8
total EAA	18.6	16.8	17.9	18.7

[a] No corrections were made for amino acid loss during hydrolysis. nd = nondetectable.

Table XI. Amino Acid Composition of Amaranth Glutelin (Grams/16 g of N)

amino acid	*A. caudatus* var. 2	*A. caudatus* var. 3	*A. hypochondriacus* var. 4	*A. cruentus* var. 7	*A. cruentus* var. 8	$\bar{x}$
tryptophan	1.7	2.1	1.6	1.7	1.6	1.7
aspartic acid	7.9	7.4	8.4	9.3	7.3	8.1
threonine	3.4	4.1	2.8	3.7	3.4	3.5
serine	4.6	5.0	3.9	4.7	4.3	4.5
glutamic acid	13.6	14.8	16.9	17.7	16.7	15.9
glycine	4.7	5.2	4.5	4.9	4.7	4.8
alanine	3.5	3.6	3.6	4.7	4.1	3.9
valine	3.1	3.5	3.8	5.7	4.4	4.1
cystine[a]	nd	nd	nd	nd	nd	nd
methionine[a]	1.2	1.5	nd	0.2	nd	1.3
isoleucine	2.9	3.2	3.3	4.9	3.8	3.6
leucine	5.4	6.0	6.3	8.9	7.1	8.7
tyrosine	2.8	3.0	1.6	3.1	2.3	2.6
phenylalanine	4.1	4.9	3.9	5.5	4.5	4.6
histidine	2.3	2.9	4.1	2.3		2.9
lysine	3.9	4.7	5.0	5.0		4.6
arginine	5.5	6.9	7.7	7.3		6.8
total EAA	28.5	33.0	28.3	38.2		34.7

[a] No corrections were made for amino acid loss during hydrolysis. nd = nondetectable.

between varieties for each protein fraction was not very large, with a few exceptions.

Albumin composition (Table VII) shows *A. hypochondriacus* variety 4 to be slightly higher in threonine content and slightly lower in tyrosine than the other samples. In general, of all fractions, albumins have the highest lysine content, which confirms data of Duarte-Correa et al. (1986).

Globulin composition is shown in Table VIII. Of all fractions they are the highest in glutamic acid, methionine, and cystine content. However, they have slightly lower tryptophan, threonine, valine, leucine, isoleucine, and lysine contents than albumins. Both albumins and globulins have high essential amino acid contents, as compared to the rest of the fractions and of those of total protein.

The amino acid pattern of prolamines (Table IX) show unexpected values. First of all, they are relatively high in both lysine and tryptophan content which is very unusual, and they also have a threonine content as high as albumins. Their valine values are comparable to those of the albumin fractions, a fact that was not expected either. However, they are the highest leucine-containing fractions and are very low in sulfur amino acids. Their aromatic amino acid content is slightly lower than in either albumins or globulins. Their essential amino acid content is higher than expected although leucine and threonine contribute greatly.

Glutelin-like proteins, fraction V (Table X), have the lowest lysine values of all the fractions and also low valine, isoleucine, leucine, tyrosine, and phenylalanine contents. Hence, their essential amino acid content is very low.

Finally, with respect to fraction VI (Table XI), the most outstanding factor is that glutelins have the highest tryptophan values. Also, lysine content is lower than in albumins, globulins, and prolamines.

Correlation coefficients between amino acids of the whole grain and their contribution in the different fractions were all nonsignificant. The correlations between total content of each amino acid and percentage of each fractions were nonsignificant with two exceptions. True prolamines with total lysine and true prolamines with total threonine showed correlation coefficients of -0.836 ($p < 0.05$) and -0.889 ($p < 0.05$), respectively. Correlation coefficients between each of the essential amino acids were

statistically significant in four cases: namely, total lysine and total threonine ($r = 0.853$, $p < 0.05$), total leucine and total isoleucine ($r = 0.988$ $p < 0.001$), total leucine and total valine ($r = 0.883$, $p < 0.05$). This is very interesting because threonine has turned out in various studies (Bressani et al., 1987) to be limiting in amaranth protein. According to the findings of this study, a sample with more total lysine would also contain more total threonine; hence, when searching for high lysine varieties, high threonine contents should also be found.

In summary, results obtained give chemical evidence of the high nutritional quality of amaranth protein as judged by protein fractionation and by the amino acid patterns of each fraction. Unexpectedly, relatively high values were found for lysine in prolamines, as well as low values for the same amino acid in the glutelin-like fraction and in glutelins.

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