Evaluation of Four *Amaranthus* Species through Protein Electrophoretic Patterns and Their Amino Acid Composition

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Amaranth seed proteins of four species, *Amaranthus cruentus*, *A. flavus*, *A. caudatus*, and *A. hypochondriacus*, were fractionated as albumins and globulins, alcohol-soluble proteins A1 and A2, and glutelins G2 and G3. Their average values were 61.3, 1.4, and 24.1, respectively. The main protein subunits have molecular masses of between 10 and 45 kDa. Variations, found for some minor bands, were also detected by amino acid analysis. Albumins, globulins, and glutelin G3 have much higher lysine contents than the alcohol-soluble and glutelin G2 protein fractions. Globulins were only intermediate in comparative contents of methionine and cystine.

INTRODUCTION

The seed varieties of the Amaranthaceae family originated from Central and South America and the vegetable ones from southeast Asia (Saunders and Becker, 1984; Stone and Lorenz, 1984). The main species are *Amaranthus cruentus* and *A. hypochondriacus* (Konishi et al., 1985b; Teutonico and Knorr, 1985). Amaranth had been cultivated as minor group crops in Latin America: *A. caudatus* in Argentine, Peru, and Brazil; *A. hypochondriacus* in Mexico (Bressani et al., 1987; Kauffman and Haas, 1983; Teutonico and Knorr, 1985).

The amaranth seed containing starch, proteins, amino acids, lipids, minerals, and vitamins has potential in the future for food and feed resources (Konishi et al., 1985a,b; Sugimoto et al., 1981; Teutonico and Knorr, 1985). There is a great deal of work on its chemical composition, protein and amino acid content, and nutritional value, including feeding tests (Afolabi and Oke, 1981; Becker et al., 1981; Connor et al., 1980; Correa et al., 1986; Pandey and Pal, 1985; Pant, 1985). In addition, Konishi et al. (1985a) and Abdi and Sahib (1976) have characterized globulin isolated from seed of *A. hypochondriacus*. Correa et al. (1986) showed some results on amino acids composition of proteins in *Amaranthus* grain.

To our knowledge there are no data available in the literature about the features of diverse protein fractions isolated from main species of Amaranth seeds. This paper deals with the relative amounts, molecular polymorphism, and amino acid composition of the protein fractions present in Amaranth seeds.

MATERIALS AND METHODS

Sample Preparation. Whole mature seeds of *A. cruentus* (purple), *A. cruentus* (yellow), *A. flavus* (black), *A. caudatus* (yellow-brown), and *A. hypochondriacus* (yellow) were used in this study.

Seeds of *Amaranthus* were ground on a mill through a 32-mesh screen. The flour was defatted with cold acetone (10 mL/g) for 48 h at −20 °C and then air-dried. The defatted flour was milled through a 60-mesh screen and stored at 4 °C until use.

Amaranth protein fractions were isolated with the same sequence of solvents developed by Landry and Moureaux (1970). Some modifications in duration of extraction were applied. The meal (1 g) was extracted with a solvent/sample ratio of 10/1 (v/w) and vigorously shaken. The extracts were isolated by centrifuging at 10000*g* for 10 min. Each step was repeated twice.

The solvent sequence and the isolated proteins were the following: 0.5 M NaCl, water [albumins (Alb) and globulins (Glo)]; 70% (v/v) 2-propanol [alcohol-soluble proteins A1 (ASP A1)]; 70% 2-propanol containing 0.6% (v/v) 2-mercaptoethanol (2-ME) [alcohol-soluble proteins A2 (ASP A2)]; 0.01 M sodium carbonate buffer (pH 10) containing 0.6% (v/v) 2-ME [glutelin G2 (Glu G2)] and then the same solvent plus 0.5% (w/v) sodium dodecyl sulfate (SDS) [glutelin G3 (Glu G3)].

Globulin was separated from albumin by dialysis against water at 4 °C for 72 h.

The nitrogen content in each fraction was determined by micro-Kjeldahl method, combined with a colorimetric determination (Nkonge and Ballance, 1982). Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) was performed according to the procedure of Laemmli (1970), using both homogeneous (15% w/v) and gradient (10–20% w/v) gels. The amount of protein applied to sample slots was 50 μg. Gels were stained with 0.25% Coomassie Brilliant Blue R250 in 2-propanol/water/acetic acid (1/8/1 v/v/v) and destained in the same solvent. Standards obtained from Sigma Chemical Co. of 14, 20, 24, 29, 36, 45, and 60 kDa were used for the molecular weight estimation of protein subunits.

Freeze-dried samples were hydrolyzed with 6 N HCl in sealed tubes for 20 h at 110 °C with and without previous oxidation with performic acid. The vacuum-dried hydrolysate was analyzed and applied on a Beckman 120 C automatic amino acid analyzer (Spackman et al., 1958). For tryptophan determination samples were hydrolyzed with 4 N LiOH for 24 h at 110 °C followed by treatment with 6 N HCl for 22 h at the same temperature. Tryptophan levels were also obtained by Spies W method (Gorinstein et al., 1988; Spies, 1967, 1968). Mean values of eight determinations are reported in this study. All statistical data were determined by Duncan's (1955) multiple range test.

0021-8561/91/1439-0851$02.50/0

Table I. Nitrogen Content and Protein Distribution for Seeds of Four Amaranth Species

species	total pro	protein fractions[a,b]						
		Alb + Glo + P	ASP A1	ASP A2	Glu G2	Glu G3	residue	recovery, %
A. cruentus (purple)	14.8 ± 0.8	64.5 ± 1.2	0.5 ± 0.1	0.4 ± 0.1	5.0 ± 0.3	20.2 ± 0.4	8.6 ± 0.3	99.2
A. cruentus (yellow)	15.3 ± 0.9	58.8 ± 1.3	0.6 ± 0.3	0.4 ± 0.2	4.5 ± 0.5	18.7 ± 0.4	13.1 ± 0.4	96.1
A. flavus	15.1 ± 0.3	59.3 ± 2.2	2.1 ± 0.2	0.4 ± 0.1	7.5 ± 0.5	20.0 ± 0.3	8.1 ± 0.2	97.4
A. caudatus	16.6 ± 0.8	66.5 ± 3.1	0.7 ± 0.1	0.3 ± 0.1	4.8 ± 0.3	14.2 ± 0.4	13.7 ± 0.3	100.1
A. hypochondriacus	15.0 ± 0.7	57.3 ± 1.8	0.9 ± 0.2	0.5 ± 0.1	7.1 ± 0.3	18.7 ± 0.3	12.1 ± 0.3	96.6
A. hypochondriacus[c]		59.4	0.6	0.4	6.9	22.5	12.6	102.4

[a] Expressed as percentage of nitrogen contained in the fraction to nitrogen contained in seed. [b] Pro, protein; Alb, albumins; Glo, globulins; P, nonprotein nitrogen; ASP, alcohol-soluble proteins; Glu, glutelins. [c] From Konishi et al. (1985a).

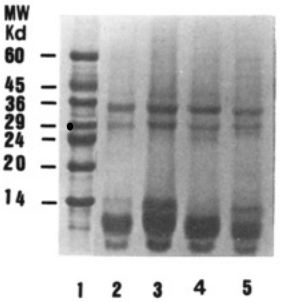

Figure 1. SDS–PAGE of albumins from Amaranth seeds in 10–20% gradient PAAG. (1) Standard; (2, 3, 4, 5) *A. cruentus* (purple), *A. flavus*, *A. caudatus*, *A. cruentus* (yellow), respectively.

RESULTS AND DISCUSSION

Table I shows the average proportions of albumin, globulin, alcohol-soluble proteins A1 and A2, and glutelins G2 and G3 expressed as percentages of total nitrogen present in seed. Protein content of defatted Amaranth seeds of four species was 14.9–16.6% on a dry weight basis. The major fractions representing 84% of the bulk nitrogen were globulins, albumins, and glutelins; ASP A1 and A2 showed only 1.4%. As can be seen from these data, Amaranth proteins have a unique distribution of protein fractions. Some differences between species for the homologous fractions were reported.

The percentages of the solubilized fractions ranged from 87 to about 92% and were different from values reported by Correa et al. (1986), who used Padhye and Salunkhe's (1977) method for extraction. These authors found higher levels for alcohol-soluble proteins (11%) and albumins and a very low level for glutelins. Our prolamin fraction is much lower. The protein distribution in Amaranth seed as found in this study was similar to one reported for rice (Padhye and Salunkhe, 1979). The ratio of globulins to albumins in Amaranth was 2.1, similar to that given by Konishi et al. (1985a) but different from those reported by Abdi and Sahib (1976) and Correa et al. (1986).

In this study electrophoretic patterns of albumins, globulins, and glutelins are presented in Figures 1–3. SDS–PAGE of albumins, globulins, and glutelins showed similar patterns for homologous protein fractions isolated from different species. The main subunits of albumins, irrespective of species, had molecular masses in the range of 10, 29, 30, and 37 kDa. Some differences were shown in the minor fractions in the region 48–58 kDa. *A. cruentus* (purple) and *A. caudatus* were similar and differed from *A. flavus* and *A. cruentus* (yellow) mainly in the region of 14 kDa, where especially *A. flavus* showed a very intensive band (Figure 1). Globulins from four varieties had a main subunit of 14–18 kDa.

The patterns of *A. cruentus* (purple) and *A. flavus* were similar as well as for *A. hypochondriacus* and *A. cruentus* (yellow). *A. caudatus* differed from other species by the presence of subunits of about 22 kDa (Figure 2).

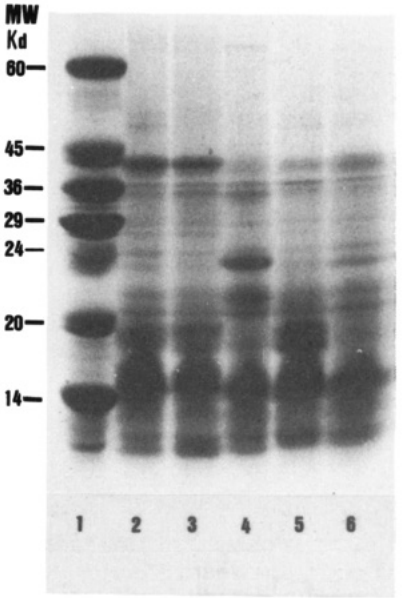

Figure 2. SDS–PAGE of globulins from Amaranth seeds in 15% PAAG. (1) Standard; (2, 3, 4, 5, 6) *A. cruentus* (purple), *A. flavus*, *A. caudatus*, *A. hypochondriacus*, *A. cruentus* (yellow), respectively.

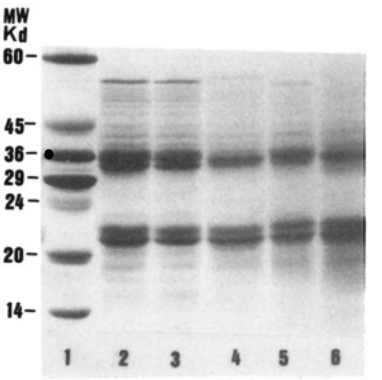

Figure 3. SDS–PAGE of glutelins G2 from Amaranth seeds in 15% PAAG. (1) Standard; (2, 3, 4, 5, 6) *A. cruentus* (purple), *A. flavus*, *A. caudatus*, *A. hypochondriacus*, *A. cruentus* (yellow), respectively.

Subunits of glutelins G3 of 21 and 22 kDa and between 30 and 37 kDa were present in four varieties. Some differences were found in minor bands from 48 to 58 kDa (Figure 3). The subunits of any major protein fraction were concentrated between 10 and 45 kDa. Probably only by electrophoretic patterns it is difficult to make a conclusion about the different compositions of specific varieties. Amino acid analysis revealed this difficulty.

Table II shows the amino acid composition of all protein fractions of *A. hypochondriacus*, as one of the species, which is used for food purposes. In composition of essential amino acids albumin had the highest lysine content

Table II. Amino Acid Composition of Protein Fractions Isolated from *A. hypochondriacus* (Mole Percent)

	protein fractions						
	whole meal	Alb	Glo	ASP A1	ASP A2	Glu G2	Glu G3
lysine	5.5	6.7	5.7	2.5	3.8	2.9	5.9
histidine	2.7	2.1	3.8	1.7	2.3	3.4	2.7
arginine	7.5	0.7	8.2	6.5	6.6	10.1	6.5
tryptophan	0.9	0.6	0.6	0.9	10.8	0.1	0.3
aspartic acid	8.5	8.0	7.5	7.4	9.4	9.1	10.3
threonine	4.5	5.2	4.7	6.7	4.5	3.7	5.5
serine	7.1	12.7	5.6	6.7	9.6	7.1	6.9
glutamic acid	14.5	16.4	13.7	11.4	15.7	16.6	11.7
proline	4.6	2.9	4.6	5.8	5.8	6.8	5.0
glycine	12.8	14.2	8.9	14.6	13.3	10.3	9.4
alanine	6.3	8.1	5.8	9.4	6.9	5.4	7.5
$^1/_2$ cystine	1.6	2.2	4.4	1.9	1.2	1.6	1.3
valine	4.9	4.4	6.0	6.6	4.2	4.1	5.5
methionine	2.2	2.3	2.8	4.5	0.9	1.2	1.4
isoleucine	3.5	2.9	4.3	2.7	3.3	4.1	4.1
leucine	6.0	5.4	6.1	6.7	6.0	6.2	8.3
tyrosine	3.7	2.7	2.9	1.6	2.4	3.1	3.4
phenylalanine	3.3	2.5	4.5	2.5	3.5	4.1	4.4

Table III. Amino Acid Composition of Globulin Fraction Isolated from Diverse Species (Mole Percent)

	species of amaranth						
	cruentus						
	purple	yellow	*flavus*	*caudatus*	*hypochondriacus*	*hypochondriacus*[a]	*hypochondriacus*[b]
lysine	5.8	5.0	4.9	4.5	5.7	3.7	6.0
histidine	2.5	3.2	2.0	2.3	3.8	2.8	2.8
arginine	9.8	8.5	11.6	10.0	8.2	7.9	11.3
tryptophan	0.9	0.8	0.4	1.4	0.6		
aspartic acid	8.0	7.7	7.6	8.8	7.5	10.1	9.1
threonine	3.85	4.3	2.8	2.9	4.7	4.1	3.0
serine	5.6	6.2	4.6	6.0	5.6	8.6	4.4
glutamic acid	16.6	14.9	21.7	19.3	13.7	16.6	21.6
proline	3.9	5.0	3.8	4.6	4.6	5.4	4.2
glycine	10.0	9.2	11.4	12.1	8.9	9.2	4.4
alanine	6.0	6.0	5.0	6.4	5.8	6.2	3.0
$^1/_2$ cystine	4.1	3.7	5.3	2.6	4.4	0.9	
valine	4.2	4.8	3.0	2.6	6.0	6.1	4.7
methionine	3.2	3.9	3.7	3.7	2.8	1.2	5.3
isoleucine	3.1	3.6	1.8	2.1	4.3	5.2	3.9
leucine	5.6	5.6	4.0	4.4	6.1	7.5	6.2
tyrosine	3.1	3.5	3.2	2.4	2.9	2.7	4.2
phenylalanine	4.0	4.2	3.2	3.7	4.5	5.3	6.0

[a] Konishi et al. (1985a). [b] Correa et al. (1986).

(6.74%); globulin was rich in methionine and cystine (7.13%), isoleucine (4.34%), and valine (6.02%).

Glutelin was rich in phenylalanine and tyrosine (7.44%) and leucine (7.2%). Prolamin showed high levels of tryptophan (0.82%) and threonine (5.57%). This fraction contained also 3.16% lysine and 5.79% proline (nearly the same amount as glutelins and globulins). Glutamic acid (13.2%) in the prolamin fraction was lower than in other protein fractions. The amount of leucine (6.36%) was close to that in the globulin fraction.

This agrees with the data of Konishi et al. (1985a) that prolamin in Amaranth is not a storage protein. In some references (Gorinstein et al., 1986; Konishi et al., 1985a) it was shown that other cereals such as normal corn prolamins contain about 42% total nitrogen.

Prolamin, the major storage protein in corn seeds, is characterized by the absence of lysine and tryptophan and enrichment in proline, glutamic acid, and leucine (Landry and Moureaux, 1970).

Therefore, according to this determination prolamins of Amaranth do not have true properties of this fraction of proteins and have to be called an alcohol-soluble fraction. Our data are not in agreement with those of Correa et al. (1986). If prolamins have high threonine and leucine contents as for the other fractions, this fraction cannot be classified as a prolamin.

In whole meal of *A. hypochondriacus* the amounts of essential amino acids were in agreement with nutritional requirements (FAO, 1973; Oke, 1980).

Table III shows the amino acid composition of the most abandant fraction—globulin—of different Amaranth species. All Amaranth species were rich in glutamic acid, glycine, arginine, and aspartic acid. Amounts of alanine, lysine, serine, and leucine were respectively about 5–6%.

Our results were compared with the only available literature for *A. hypochondriacus* of Correa et al. (1986) and Konishi et al. (1985a), which were similar to Konishi et al. (1985a), who showed also enrichment of the globulin fraction in glutamic and aspartic acids. The data of amino acid composition differed from those Correa et al. (1986) in methionine and phenylalanine contents. The protein fractions and amino acid composition of Amaranth were closer to the amino acid composition of soybean than to the amino acid composition of cereals (Konishi et al., 1985a).

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Received for review April 12, 1990. Revised manuscript received November 20, 1990. Accepted December 6, 1990.