Characterization of Amaranth (*Amaranthus hypochondriacus* L.) Seed Proteins

Magdalena Segura-Nieto,* Nélida Vázquez-Sánchez, Hilda Rubio-Velázquez, Laura E. Olguín-Martínez, Carlos E. Rodríguez-Nester, and Luis Herrera-Estrella

Departamento de Ingeniería Genética del Centro de Investigación y de Estudios Avanzados del IPN, Km 9.6, Libramiento norte carretera Irapuato-León, Apartado Postal 629, Irapuato, Gto CP 36500, Mexico

Amaranth seed has a high nutritional value due to the balanced amino acid composition of its proteins. These were isolated by sequential extractions. The percentage concentrations of albumins, globulins, prolamins, and glutelins were 51.0, 15.9, 2.0, and 31.1, respectively, of total protein extracted, the insoluble fraction being quantitatively negligible. Albumins are the richest in sulfur amino acids (4.4%), globulins in lysine (7.0%), and prolamins in leucine (10%) and threonine (7.2%). Glutelins are the poorest in lysine (4.2%). Upon electrophoresis, albumins were found to be the most polymorphous, while prolamins were made up of fewer and less abundant components. Two-dimensional gel electrophoresis analysis revealed the occurrence of some glutelins as the major proteins in the seed. In sucrose gradients, the albumins sediment in a major component ranging between 1.4S and 2.0S and a minor one of 4.6S. Globulins sediment in three components of 1.9S, 8S, and 13S.

INTRODUCTION

Amaranth is one of the few nongrass species with potential for becoming a cereal-like grain crop. One of the most important characteristics of the amaranth grain is that its storage protein content is higher and better balanced in essential amino acid than those of nearly all cereals (National Research Council, 1984; Teutonico and Knorr, 1985). Three species of the genus *Amaranthus* produce large seedheads loaded with edible seeds: *Amaranthus caudatus*, *Amaranthus cruentus* and *Amaranthus hypochondriacus*. The two last species are native to Mexico and Guatemala (Tucker, 1986). Amaranth is one of the 23 tropical plants recommended for studies aimed to enhance food quality in the tropics (Sauer, 1977; Duarte-Correa et al., 1986).

Amaranth seeds are small and lenticular in shape with an average diameter of 1.0–1.5 mm and an average weight of 0.6–1.2 mg (Jain and Hauptli, 1980; Teutonico and Knorr, 1985). They have a crude protein content ranging from 12.0 to 17.9% dry matter (Konishi et al., 1985; Singhal and Kulkarni, 1988). Proteins are relatively rich in lysine, tryptophan, and methionine and poor in leucine and isoleucine content with respect to the values reported by the Food and Nutrition Board (1980) (Flores and Teutonico, 1986; Duarte-Correa et al., 1986). This makes the amaranth grain an excellent complement to leucine-rich, lysine- and tryptophan-poor maize or sorghum (Becker et al., 1981) or to methionine- and cysteine-poor common beans or soybeans (Becker et al., 1981; Saunders and Becker, 1984).

Several studies on the amino acid composition of amaranth seed total proteins or on isolated groups of storage proteins have been carried out (Becker et al., 1981; National Research Council, 1984; Teutonico and Knorr, 1985; Duarte-Correa et al., 1986; Konishi et al., 1985; Mora-Escobedo et al., 1990; Bressani and García-Vela, 1990; Gorinstein et al., 1991a,b). However, no studies are available on the molecular characterization of the different classes of proteins contributing to the high level of some essential amino acids. Here we report a detailed characterization of the albumins, globulins, prolamins, and glutelin groups, of *A. hypochondriacus* seed proteins. This paper includes the following analyses: (a) determination of the relative abundance in the grain; (b) protein electrophoretic analysis in one and two dimensions; (c) analysis of the amino acid composition; and (d) determination of albumins and globulins sedimentation coefficients.

* Author to whom correspondence should be addressed.

MATERIALS AND METHODS

Seed Material and Protein Purification. Seeds of *A. hypochondriacus* L., azteca type, were used in this study. Seed meal was prepared by grinding frozen seeds in a Janke and Kukel mill at 4 °C. The meal was defatted by extraction with 3 × 5 mL of cold hexane for each gram of meal (v/w) (30 min, at 4 °C). The defatted meal was then separated by centrifugation and dried in a vacuum desiccator at room temperature.

Sequential extraction of amaranth protein groups was performed according to the method of Osborne (1924) with minor modifications. Each extraction step was performed twice at 4 °C (during 8 and 6 h, respectively) using the corresponding extraction solution. In the first extraction a rate of 10 mL of extractant/g of defatted meal was used, and 5 mL of extractant/g of meal was used for the second. In every step, extracts were isolated by centrifugation at 10000*g* during 20 min at 4 °C. Albumins were extracted with solution E-1 [0.1 M NaCl, 0.010 M K_2HPO_4, pH 7.5, 0.001 M ethylenediaminetetraacetic acid (EDTA), 0.1 mM phenylmethanesulfonyl fluoride (PMSF), 0.02% sodium azide (NaN_3)]. Globulins were extracted from the albumin-free pellet 1 with E-2 (1 M NaCl, 0.010 M K_2HPO_4, pH 7.5, 0.001 M EDTA, 0.1 mM PMSF, 0.02% NaN_3). Prolamins were then extracted using solution E-3 (60% 1-propanol, 0.010 M K_2HPO_4, pH 7.5) and finally glutelins with E-4 (0.010 M boric acid, pH 9.0, 1% SDS, 1% β-mercaptoethanol). After the extraction of the four protein groups, the residue did not contain significant amounts of proteins (2–4%) of the total proteins present in the original defatted meal.

Gel Electrophoresis and Amino Acid Composition. Protein was quantified using the bicinchoninic acid protein assay reagent (BCA, Pierce; Smith et al., 1985). Bovine serum albumin (BSA) was used as standard. Each sample was assayed in triplicate. The corresponding extractant for albumins and globulins was used as blank. Prior to protein determination, prolamins were first precipitated with acetone (5 mL for 1 mL of extract) at −70 °C for 1 h, and the pellet isolated after centrifugation (20 min at 10000*g*, 4 °C) was dried for 5–10 min in a speed vacuum centrifuge and then dissolved in 0.1 N NaOH.

0021-8561/92/1440-1553$03.00/0

Similarly, glutelins were precipitated during 20 min at 4 °C with trichloroacetic acid (TCA) at 10% final concentration; the pellet isolated by centrifugation was washed once with −20 °C cold 80% ethanol and twice with 96% ethanol. The dry pellet was dissolved in 0.1 N NaOH.

The electrophoresis in one and two dimensions (2D) was performed as described by Laemmli (1970) and O'Farrell (1975) and O'Farrell et al. (1977), respectively, with the following modifications in the first dimension: 3-[(3-Cholamidopropyl)-dimethylammonio]-1-propanesulfonate (Chaps) was added to 2% in the lysis buffer. Ampholines 3.5–10 and 5–7 in a rate of 4:1 were used for the isoelectric focusing (IE) (O'Farrell, 1975), and ampholines 3.5–10 at 3% were used for nonequilibrium pH gradient electrophoresis (NEPHGE) (O'Farrell et al., 1977). Gel dimensions for IE and NEPHGE were 0.3 cm diameter × 11.5 cm long and 15 × 15 × 0.15 cm for the sodium dodecyl sulfate–polyacrylamide slab gels (SDS–PAGE). The IE was first prerun as follows: 15, 30, and 30 min at 200, 300, and 500 V, respectively. Then the samples were applied and run for 16 h at 500 V. The NEPHGE was carried out for 5 h at 500 V with no prerun.

After electrophoresis, the 2D gels were fixed for 1 h in 25% TCA and for 30 min in solution D (10% acetic acid, 10% methanol in water) to remove ampholines and then stained for 30 min with 0.25% Coomassie brilliant blue R-250 in solution D and thoroughly destained with solution D. SDS–PAGE gels were stained without fixing.

Amino acid analysis of proteins was performed after hydrolysis of samples such as they were or oxidized (Hirs, 1967). The hydrolysis was done in 6 N HCl constant boiling point with 1% phenol at 110 °C during 24 h in vacuum. The amino acids from hydrolysates were coupled with phenyl isothiocyanate (PITC) and the phenylthiocarbamyl derivatives (PTC-aa) were analyzed using the Pico-Tag system of Waters Millipore as described by Bidlingmeyer et al. (1984).

Sedimentation Coefficient. The sedimentation coefficients of albumins and globulins were determined through ultracentrifugation on 5–20% linear sucrose gradients with a cushion of 0.5 mL of 60% sucrose in the solution used for the extraction of corresponding proteins. Calibration was performed with cytochrome *c* (1.71S), myoglobin (2.04S), chymotrypsinogen (2.54S), bovine serum albumin (4.6S), aldolase (7.35S), and catalase (11.20S). The sedimentation coefficient of amaranth albumins and globulins was calculated by extrapolation to the migration of the sedimentation markers in the sucrose gradients. Centrifugation was carried out at 35 000 rpm, in the Beckman SW40 rotor at 4 °C, during 72 h for albumins and 27 h for globulins.

RESULTS AND DISCUSSION

Electrophoretic Pattern of Amaranth Seeds of Different Color. Seeds of *A. hypochondriacus*, azteca type, display some heterogeneity in their color, varying from pale yellow to black. Over 95% of the seeds are pale yellow. To determine whether the protein electrophoretic pattern changes with grain color, total protein extracts from seeds of different color were analyzed on SDS–PAGE.

The electrophoretic pattern of total proteins originated from seeds of distinct color elicits several differences in the quantity and quality of the polypeptides, mainly in the regions of high and low molecular weight (Figure 1). Pale yellow seeds are rich in polypeptides with molecular weight below 12 000, while the darker ones are richer in polypeptides in the range 30 000–80 000. These differences should be borne in mind when comparative studies between different types of amaranth seeds are made.

Selective Extraction of Proteins. To characterize the proteins of amaranth grains, defatted meal of pale yellow seeds was differentially extracted to obtain albumins, globulins, prolamins, and glutelins protein groups. Each protein group was first analyzed by SDS–PAGE. As observed in Figure 2, the electrophoretic patterns of albumins and globulins are similar to those of *A. hypochondriacus* Nepal type already described by Mora-Escobedo et al. (1990). Albumins are a protein fraction with

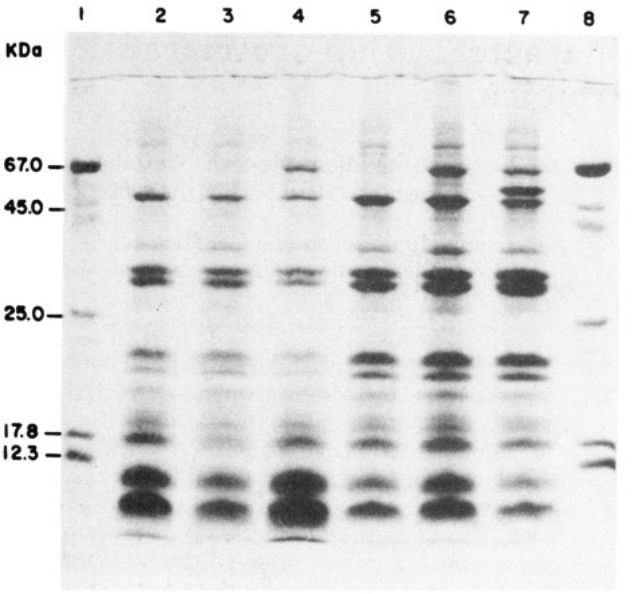


Figure 1. Electrophoretic pattern of distinct colored amaranth grains. Four seeds (approximately 1 mg per seed) of each color were homogenized in 200 μL of Laemmli sample buffer (Laemmli, 1970). Each lane from 2 to 7 was loaded with 80 μL of total extract. The colors of the seeds were pale yellow, yellow, strong yellow, light brown, dark brown, and black loaded from lane 2 to lane 7, respectively. Lanes 1 and 8 were loaded with protein molecular weight markers; the polypeptides of 45 and 25 kDa were partially degraded.

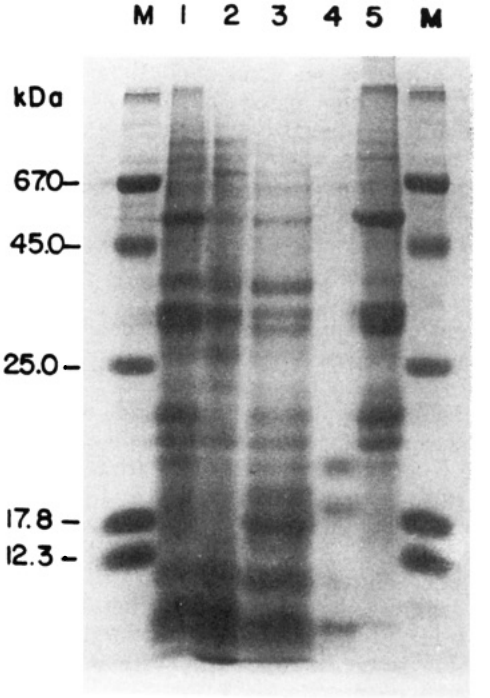


Figure 2. Electrophoretic pattern of protein groups extracted from amaranth defatted meal. (Lane M) Molecular weight markers; (lane 1) total proteins of amaranth defatted meal (200 μg); (lane 2) albumins (150 μg); (lane 3) globulins (200 μg); (lane 4) prolamins (30 μg); (lane 5) glutelins (80 μg).

polypeptides of very heterogeneous sizes, low molecular weight components being the most abundant (lane 2). Globulins have also heterogeneous polypeptide sizes (lane 3).

Prolamins have the lowest number of polypeptides, and the electrophoretic protein pattern is slightly different from the alcohol-soluble protein pattern shown by Gorinstein et al. (1991a). In most cases four main components

Table I. Protein Distribution in Amaranth Grain

A. hypochondriacus	albumins and globulins	prolamins	glutelins	ref[a]
azteca	**66.9**	**2.0**	**31.1**	**pd**
waxy	56.9	1.6	22.4	1
nonwaxy	59.4	1.0	29.4	1
HH5	82.0	11.0	7.0	2
78S-125 Nepal ND	47.5	1.4	29.3	3
A-718	40.0	2.0	44.0	4
yellow	59.0	1.7	26.3	5

[a] pd, present data; 1, Konishi et al. (1985); 2, Duarte-Correa et al. (1986); 3, Paredes-López et al. (1988); 4, Bressani and García-Vela (1990); 5, Gorinstein et al. (1991b).

were detected: two are approximately 18 and 22 kDa, respectively, and two are 10 and 12 kDa, respectively. The latter ones increased with their storage at −20 °C, suggesting they were degradation products of the higher molecular weight polypeptides. There are also some minor polypeptides in the region 30–40 kDa.

Finally, glutelins (lane 5) are made up of six main sets of polypeptides: one with a molecular weight around 60 000, three between 28 000 and 40 000, and two with average molecular weights of 20 000–23 000. A minor set is made up of five polypeptides in the range 67 000–93 000.

Protein Distribution. Albumins are the predominant group and represent 51% of the total proteins, followed by glutelins (31.1%), globulins (15.9%), and prolamins (2.0%).

Since the presence of some globulins in the albumin fraction cannot be excluded, the two groups of proteins will be considered together for the comparison of the present data with those reported in the literature (Table I).

The main differences observed pertain to the relative importance of albumins and globulins, and they could be attributed to the difference in experimental conditions used for extraction and quantitation.

In this study, albumins were directly isolated from the defatted meal using 0.1 M NaCl in the presence of EDTA buffered at 7.5 and longer extraction time. NaCl and EDTA at pH 7.5 were used because they extract more protein. In this case some of the globulins could be co-extracted, although they are usually removed with buffered 0.5 M NaCl (Gorinstein et al., 1991b; Bressani and García-Vela, 1990; Mora-Escobedo et al., 1990; Paredes-López et al., 1988).

Globulins were extracted after albumins in the presence of higher ionic strength, because this allows a more efficient protein extraction as has been previously observed (Paredes-López et al., 1988). In other studies globulins were directly extracted from defatted meal, for a shorter time, and at a lower ionic strength (0.5 M NaCl) (Gorinstein et al., 1991b; Bressani and García-Vela, 1990, in procedure A; Paredes-López et al., 1988; Konishi et al., 1985). These differences could affect the final recovery of albumins and globulins and would explain the high content of insoluble protein final residue (Konishi et al., 1985; Paredes-López et al., 1988).

Amino Acid Composition of Amaranth Grain Protein Groups Differentially Extracted. The amino acid composition of the four groups of proteins is shown in Table II. Albumins are the richest in sulfur amino acids (Met + Cys = 4.4%), globulins in lysine and valine (7.0 and 5.0%, respectively), and prolamins in leucine and threonine (10.0 and 7.2%, respectively); prolamins are high in lysine (6.7%) as well. Finally, glutelins have the highest content of isoleucine (5.0%), tyrosine (3.0%), and phenylalanine (4.3%). These values compared with published

Table II. Amino Acid Composition of Amaranth Seed Proteins[a]

amino acid	meal	albumins	globulins	prolamins	glutelins	+FNB
CA	1.3	1.9	1.5	1.0	0.6	
Asx	7.9	9.4	9.4	8.0	7.2	
Glx	14.9	20.0	17.5	10.0	11.1	
Ser	7.3	6.4	7.7	8.0	9.0	
Gly	10.7	10.5	13.9	10.7	10.3	
His*	3.0	2.3	2.3	1.8	2.4	
Arg	7.3	8.9	9.3	6.8	8.5	
Thr*	5.1	3.4	4.0	7.2	5.4	**4.1**
Ala	6.6	6.2	5.4	8.6	6.3	
Pro	5.7	5.0	4.0	4.5	5.9	
Mes*	3.3	2.5	1.6	1.7	2.6	
Tyr*	1.9	2.9	2.8	3.0	3.0	
Val*	5.9	4.0	5.0	4.5	5.0	**4.8**
Ile*	3.9	3.5	4.0	4.5	5.0	**4.2**
Leu*	6.2	5.5	6.0	10.0	8.0	**7.0**
Phe*	3.4	3.0	2.0	3.9	4.3	
Lys*	5.7	6.6	7.0	6.7	4.2	**5.1**
CA + Mes	**4.6**	**4.4**	**3.1**	**2.7**	**3.2**	**2.6**
Phe + Tyr	**5.4**	**5.9**	**4.8**	**6.9**	**7.3**	**7.3**

[a] The figures are expressed in percent in moles. An asterisk indicates an essential amino acid. CA, cysteic acid; MES, methionine sulfone; +FNB, Food Nutritional Board [NR, 1980, in Duarte-Correa et al. (1986)].

ones emphasize some differences. Mora-Escobedo et al. (1990) found a lysine contents of 10 and 4.6% for the albumins and globulins, respectively. Bressani and García-Vela (1990) isolated from varieties A-718 and A-720 of *A. hypochondriacus* albumins with 7.1 and 6.6% of lysine and globulins with 7.5 and 6.4% lysine, respectively. Duarte-Correa et al. (1986) reported 8.2 and 6% lysine for albumins and globulins, respectively, for type HH5. These differences might be attributed, partially, to the sample used in each study or to the extraction procedure of the proteins.

Two-Dimensional Electrophoresis of Amaranth Proteins. The relative content of each component present from each group of seed proteins in the grain can be estimated from the pattern of the whole defatted meal (Figure 3A).

Albumins have a complex polypeptide pattern revealing wide qualitative and quantitative variations. In addition, several sets of polypeptides with the same molecular weight but different isoelectric points were present (Figure 3B). The number of polypeptides and their relative abundance are difficult to assess. Many of them, however, can be detected among the total polypeptides from the defatted meal (panel 3A, Al, indicated with an arrow).

Globulins, being a mixture of very basic (half-arrow in Figure 3C) and acidic polypeptides, are better resolved by NEPHGE than by IEF (data not shown). The presence of acidic and basic globulin subunits has been previously described for several legumes (Boulter, 1981). Because amaranth globulins are not an abundant group of proteins in the grain, some of their polypeptides are not obvious among the total polypeptides from the whole meal (Gb, globulins in Figure 3A). In contrast, glutelins, although discrete in number (Figure 3D), are an abundant group of polypeptides in the grain, and most of them can be easily identified in the whole meal (Gt, labeled arrows in Figure 3A).

Sedimentation Pattern of Albumins and Globulins from Amaranth Grain. The sedimentation pattern of amaranth grain total albumins is shown in Figure 4. Two sets of components are evident: a major one, with coefficients between **1.40S** and **2.00S**, and a minor one, with a coefficient of **4.60S**. It is interesting to note that the 4.6S amaranth albumin comigrates with bovine serum

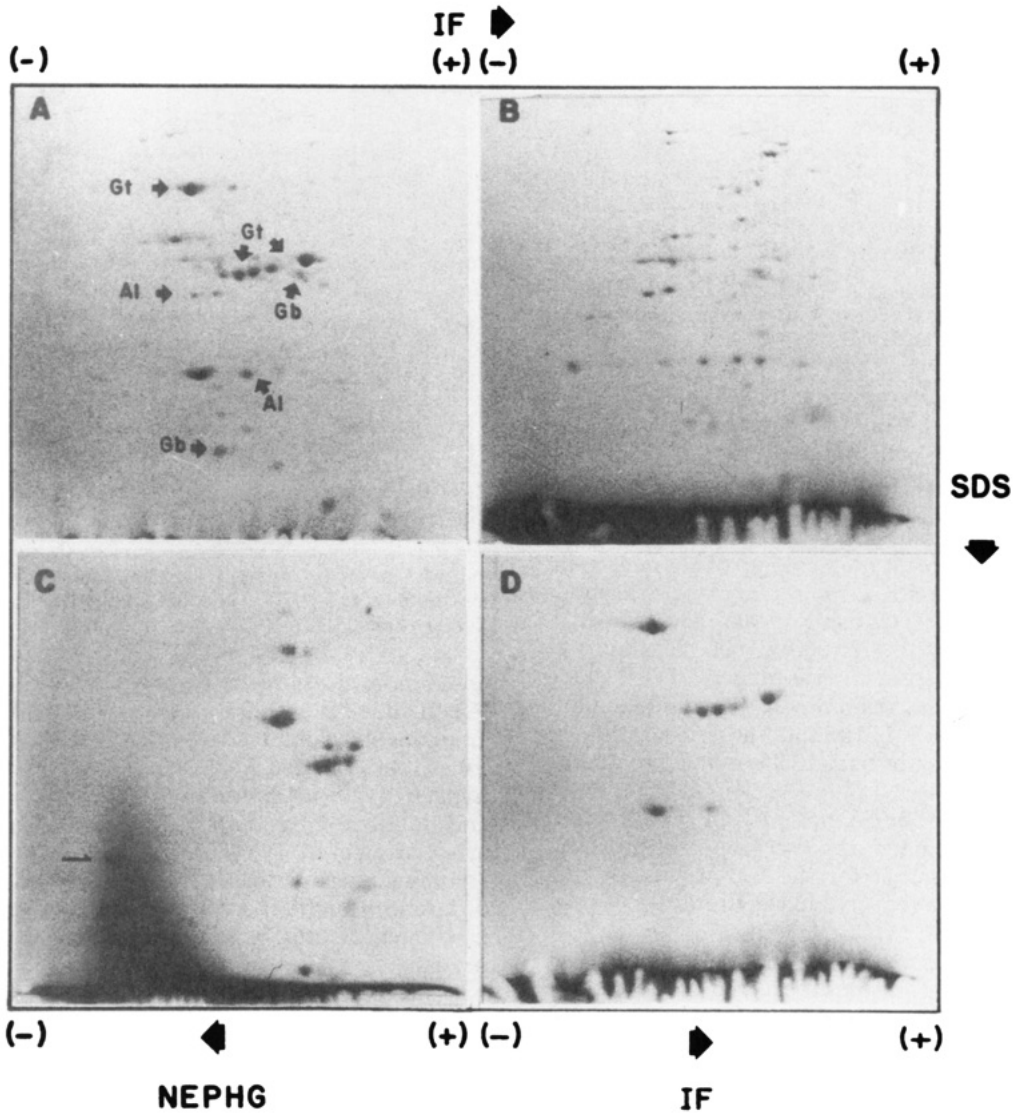


Figure 3. Two-dimensional electrophoretic pattern of amaranth polypeptides gradually extracted from defatted meal: (A) 250 μg of total extract of proteins from defatted meal; (B) 175 μg of albumins (Al); (C) 150 μg of globulins (Gb); (D) 80 μg of glutelins (Gt). IF, isoelectric focusing; NEPHG, Nonequilibrium pH gradient electrophoresis.

albumin (BSA) used as marker. There are also some aggregates that sediment to the bottom of the tube.

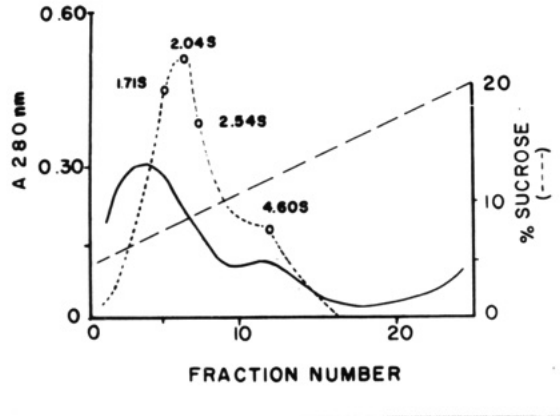
The electrophoretic pattern of the albumin fractions isolated by sucrose gradient is shown in the lower part of Figure 4. Some low molecular weight albumins sedimenting at the top of the gradient could be lost during the trichloroacetic acid precipitation used to prepare the samples to be loaded in the gel. This could explain the absence of polypeptides in the first fractions (lanes 1–3, Figure 4) and the very low concentration in lanes 4 and 5, even though these correspond to fractions from the gradient of higher protein content, as detected by absorbance at 280 nm (see Figure 4, upper part, continuous line). It is important to mention that the protein pattern in the gel does not represent the real concentration of these polypeptides in the total albumin group.

The main set of albumins sedimenting between 1.40S and 2.00S consists of polypeptides with molecular weights ranging from 4000 to 9000 (see fractions 4–7, Figure 5, lower part). These values are the same as those reported for many other 2S albumins (Youle and Huang, 1981; Crouch and Sussex, 1981; Altenbach et al., 1986; Krebbers et al., 1988). These 1.70S (1.40–2.0S) albumins are one of the major groups of polypeptides in the amaranth grain. The same holds for some oilseed albumins (Youle and Huang, 1981). The 2S proteins from some oilseeds are often rich in cysteine (Youle and Huang, 1981) and eventually in methionine (Ampe et al., 1986; Lilley et al., 1989; Altenbach and Simpson, 1990). Preliminary studies have shown the presence of methionine-rich polypeptides among the 2S amaranth albumins (Segura-Nieto et al., unpublished data).

In some legume seeds, globulins represent the major group of storage proteins (Derbyshire et al., 1976; Boulter and Derbyshire, 1978; Gatehouse et al., 1984; Kinsella et al., 1985). They can be categorized on the basis of sedimentation coefficients. The major groups are about 7S and 11S.

To further characterize amaranth globulins, their sedimentation behavior on linear sucrose gradients was determined. Amaranth globulins (continuous line) sedimented in three main sets (Figure 5) with coefficients of 1.9S, 8S, and 13S. The sedimentation coefficients of amaranth globulins suggest that they contain polypeptides of higher molecular weight than those present in 7S and 11S from pea globulins (half-arrows, Figure 5).

The electrophoretic analysis of these three sets reveals qualitative and quantitative differences. The 1.9–2.0S globulin component could interact with a fraction of albumins in such a way that the association is not broken



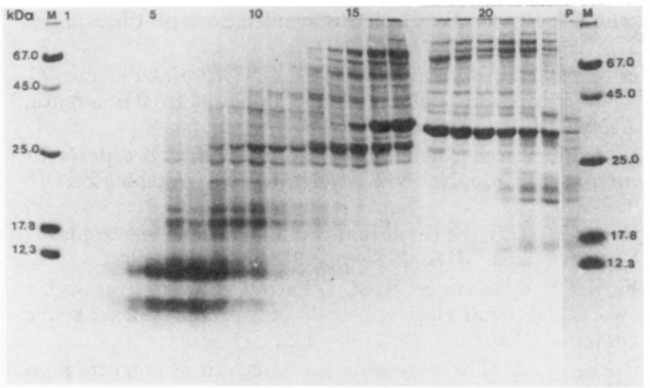
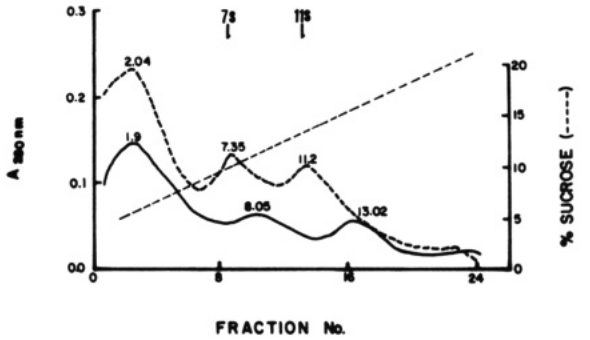


Figure 4. Analysis of the sedimentation pattern of amaranth albumins and proteins of known sedimentation coefficient. Approximately 3.0 OD of total albumins was loaded on a linear 5–20% sucrose gradient. The long dashed lines over the fractions indicate the range of the sucrose gradient. The continuous line indicates the absorbance of amaranth albumins. The absorbances of the protein markers of known sedimentation coefficient are indicated by broken lines; they were as follows: cytochrome *c*, 1.71S; myoglobin, 2.04S; chymotrypsinogen, 2.54S; bovine serum albumin, 4.6S. The lower part of the figure shows the electrophoretic pattern of all fractions from the gradient. Each well was loaded with approximately 40 μg of each fraction. Lane P was loaded with one-fifth of the dissolved pellet. Lanes M were loaded with the molecular weight markers.



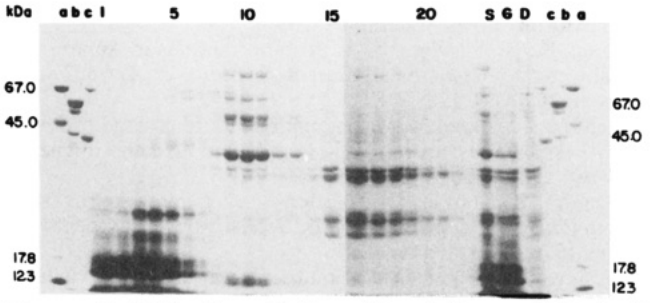


Figure 5. Estimation of the sedimentation coefficient of amaranth globulins and electrophoretic pattern of polypeptides from different fractions. The upper panel shows the sedimentation profile of proteins of known sedimentation coefficient together with amaranth globulins (broken lines). The protein markers and their corresponding sedimentation coefficient were as follows: cytochrome *c*, 1.70S; myoglobin, 2.04S; bovine serum albumin, 4.60S; aldolase, 7.35S; catalase, 11.20S. Amaranth globulins alone are indicated by a continuous line. The half-arrows point to the position where 7S and 11S pea globulins migrate. A preliminary estimation of the sedimentation coefficient of amaranth globulins is indicated on the peak of the continuous line. In the lower panel lanes a–c contain polypeptides of known molecular weight. Lanes 1–23 contain all of the fractions from the sucrose gradient. Lane S is amaranth defatted meal, and lane G is globulins before dialysis.

in the presence of 0.1 M NaCl. To test these assumptions, globulins were dialyzed against low ionic strength buffer (0.01 M K_2HPO_4, pH 7.5, 0.001 M EDTA, and 0.1 mM PMSF). Under these conditions true globulins were precipitated. One 2S fraction was found in the pellet and another in the dialyzate, indicating the presence of an albumin component in the globulin preparation. But electrophoretic analysis of the globulin fractions isolated by sedimentation is unable to assess the degree of similarity of the 2S fractions (2S gel albumins in Figure 4 vs 2S globulins in Figure 5). A more intensive analysis of these fractions is needed.

This study on the seed proteins from *A. hypochondriacus*, azteca type, grain showed that albumins and glutelins were the main groups. Certain individual glutelins were the most abundant of all seed proteins detected. With respect to the content of essential amino acids, albumins had the highest content in sulfur amino acids, globulins in lysine, and prolamins in leucine and threonine. On the other hand, the glutelin group appeared to have a better balanced concentration in essential amino acids, except for its lower lysine content. The sedimentation coefficients of the main albumin group were in the range 1.4–2.0S, like many other albumins from oilseeds (Youle and Huang, 1981), and in the case of globulins, three groups with the following sedimentation coefficients were found: 1.9S, 8S, and 13S, respectively, as has been described for several legumes (Derbyshire and Boulter, 1976; Gatehouse et al., 1984; Higgins, 1984). The storage proteins of amaranth have a different content of albumins, globulins, prolamins, and glutelins from those of cereals and legumes. These types of studies will establish the basis for more detailed analysis of the processes involved in the synthesis and deposition of amaranth storage proteins.

ACKNOWLEDGMENT

This research was supported by a grant from the Board on Science and Technology for International Development (BOSTID, New York) and from the Consejo Nacional de Ciencia y Tecnologia (CONACYT, Mexico) (Grant P220CCOR-892189). We thank QFB Aurora Verver y Vargas for technical assistance, Dr. Rafael Rivera for help with the computer, Dr. June Simpson for valuable criticism, and Mr. Antonio Cisneros for photographic work. We also thank the referees for their corrections and valuable comments.

LITERATURE CITED

Altenbach, S. B.; Simpson, R. Manipulation of methionine rich protein genes in plant seeds. *Trends Biotechnol.* **1990**, *8*, 156–160.

Altenbach, S. B.; Pearson, K. W.; Leung, F. W.; Sun, S. S. M. The stepwise processing of a sulfur-rich seed protein from Brazil nut (*Bertholletia excelsa*). In *Molecular Biology of Seed Storage Proteins and Lectins*; Shannon, L. M., Chrispeels, M. J., Eds.; American Society of Plant Physiologists: Rockville, MD, 1986; pp 137–146.

Ampe, C.; Van Damme, J.; de Castro, L.; Sampaio, M. J.; Van Montagu, M.; Vandekerckhove, J. The amino acid sequence of the 2S sulfur-rich proteins from seeds of Brazil nut (*Bertholletia excelsa* H.B.K.) *Eur. J. Biochem.* **1986**, *159*, 597–604.

Becker, R. E.; Wheeler, E. L.; Lorenz, K.; Stafford, A. E.; Grosjean, O. K.; Betschart, A. A.; Saunders, R. M. A compositional study of amaranth grain. *J. Food Sci.* **1981**, *46*, 1175–1180.

Bidlingmeyer, B. A.; Cohen, S. A.; Tarvin, T. L. Rapid analysis of amino acids using pre-column derivatization. *J. Chromatogr.* **1984**, *336*, 93–104.

Boulter, D. Biochemistry of storage protein synthesis and Deposition in the developing legume seeds. *Adv. Bot. Res.* **1981**, *9*, 1–31.

Boulter, D.; Derbyshire, E. General properties, classification and distribution of plant proteins. In *Plant proteins*; Norton, G., Ed.; Butterworths: London, 1978; pp 3–24.

Bressani, R.; García-Vela, L. A. Protein fractions in Amaranth grain and their chemical characterization. *J. Agric. Food Chem.* **1990**, *38*, 1205–1209.

Crouch, M. L.; Sussex, I. M. Development and storage-protein synthesis in *Brassica napus* L. embryos in vivo and in vitro. *Planta* **1981**, *153*, 64–74.

Derbyshire, E.; Boulter, D. Isolation of a legumin-like protein from *Phaseolus aureus* and *Phaseolus vulgaris*. *Phytochemistry* **1976**, *15*, 411–414.

Derbyshire, E.; Wright, D. J.; Boulter, D. Legumin and vicilin, storage proteins of legume seeds. *Phytochemistry* **1976**, *15*, 3–24.

Duarte-Correa, A.; Jolk, L.; Carlsson, R. Amino acid composition of some *Amaranthus sp.* grain proteins and of its fractions. *Arch. Latinoam. Nutr.* **1986**, *36*, 466–476.

Flores, H. E.; Teutonico, R. A. III.5 Amaranths (*Amaranthus spp.*): Potencial grain and vegetable crops. In *Biotechnology in Agriculture and Forestery*; Bajai, P. S., Ed.; Springer-Verlag: Berlin, 1986; Vol. 2, pp 568–578.

Food and Nutrition Board. *Recommended dietary allowances*, 9th ed.; National Academy of Sciences, National Research Council: Washington, DC, 1980.

Gatehouse, J. A.; Croy, R. E.; Boulter, D. The synthesis and structure of pea storage proteins. *CRC Crit. Rev. Plant Sci.* **1984**, *1*, 287–314.

George, A. A.; de Lumen, B. O. A novel methionine-rich protein in soybean seed: Identification, amino acid composition, and N-terminal sequence. *J. Agric. Food Chem.* **1991**, *39*, 224–227.

Gorinstein, S.; Arnao de Nue, I.; Arruda, P. Alcohol-soluble and total proteins from amaranth seeds and their comparison with other cereals. *J. Agric. Food Chem.* **1991a**, *39*, 848–850.

Gorinstein, S.; Moshe, R.; Greene, L. J.; Arruda, P. Evaluation of four *Amaranthus* species through protein electrophoretic patterns and their amino acid composition. *J. Agric. Food Chem.* **1991b**, *39*, 851–854.

Higgins, T. J. V. Synthesis and regulation of major proteins in seeds. *Annu. Rev. Plant Physiol.* **1984**, *35*, 191–221.

Hirs, C. H. W. Performic acid oxidation. *Methods Enzymol.* **1967**, *11*, 197–199.

Jain, S. K.; Huaptli, H. *Grain amaranth: A new crop for California*; Agronomy Progress Report 107; University of California, Cooperative Extension Service: Davis, 1980.

Kinsella, J. E.; Damodaran, S.; German, B. Physicochemical and functional properties of oil seed proteins with emphasis on soy proteins. In *New protein foods*; Academic Press: New York, 1985; Vol. 5, Chapter V, pp 107–175.

Konishi, Y.; Fumita, Y.; Ikeda, K.; Okuno, K.; Fuwa, H. Isolation and characterization of globulin from seeds of *Amaranthus hypochondriacus* L. *Agric. Biol. Chem.* **1985**, *49*, 1453–1459.

Krebbers, E.; Herdies, L.; De Clercq, A.; Seurinck, J.; Leemans, J.; Van Damme, J.; Segura, M.; Gheysen, G.; Van Montagu, M.; Vandekerckhove, J. Determination of the processing sites of an *Arabidopsis* 2S albumin and characterization of the complete gene family. *Plant Physiol.* **1988**, *87*, 859–866.

Laemmli, U. K. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature (London)* **1970**, *227*, 680–685.

Lilley, G. G.; Caldwell, J. B.; Kortt, A. A.; Higgins, T. J.; Spencer, D. Isolation and primary structure for a novel methionine-rich protein from sunflower seeds (*Helianthus annus* L.). In *Proceeding of the World Congress on Vegetable Protein Utilization in Human Foods and Animal Foodstuffs*; Applewhite, T. H., Ed.; American Oil Chemists Society: Champaign, IL, 1989; pp 497–502.

Mora-Escobedo, R.; Paredes-López, O.; Ordorica-Palomir, C. Characterization of albumins and globulins from amaranth. *Lebens. Wiss. Technol.* **1990**, *23*, 484–487.

National Research Council. *Amaranth: Modern prospects for an ancient Crop*; National Academy Press: Washington DC, 1984.

O'Farrell, P. H. High resolution two-dimensional electrophoresis of proteins. *J. Biol. Chem.* **1975**, *250*, 4007–4021.

O'Farrell, P. Z; Goodman, H. M.; O'Farrell, P. H. High resolution two dimensional electrophoresis of basic as well as acidic proteins. *Cell* **1977**, *12*, 1133–1142.

Osborne, T. B. The vegetable proteins. In *Monographs on Biochemistry*, 2nd ed.; Loongmans, Green: New York, 1924.

Paredes-López, O.; Mora-Escobedo, R.; Ordorica Palomir, C. Isolation of amaranth proteins. *Lebens. Wiss. Technol.* **1988**, *21*, 59–61.

Sauer, J. D. The history of grain *Amaranthus* and their use and cultivation around the world. In *Proceedings of the First Amaranth Seminar*; Rodale Press: Emmaus, PA, 1977; pp 9–13.

Saunders, R. M.; Becker, R. *Amaranthus*: A potential food and feed resource. *Adv. Cereal Sci. Technol.* **1984**, *6*, 357–396.

Singhal, R. S.; Kulkarni, P. R. Review: Amaranths, an underutilized resource. *Int. J. Food Sci. Technol.* **1988**, *23*, 125–139.

Smith, P. K.; Krohn, R. I.; Hermanson, G. T.; Mallia, A. K.; Gartner, F. H.; Provenzano, M. D.; Fujimoto, E. K.; Goeke, N. M.; Olson, B. J.; Klenk, D. C. Measurement of protein using bicinchoninic acid. *Anal. Biochem.* **1985**, *150*, 76–85.

Teutonico, R. A.; Knorr, D. Amaranth: Composition, properties, and applications of a rediscovered food crop. *Food Technol.* **1985**, *39* (4), 49–61.

Tucker, J. B. Amaranth: The once and future crop. *BioScience* **1986**, *36*, 9–13.

Youle, R. J.; Huang, H. C. Occurrence of low molecular weight and high cysteine containing albumins storage proteins in oil seeds of diverse species. *Am. J. Bot.* **1981**, *68*, 44–48.

Received for review April 13, 1992. Revised manuscript received June 3, 1992. Accepted June 19, 1992.