Fractionation procedures, electrophoretic characterization, and amino acid composition of Amaranth seed proteins

ARTICLE *in* JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY · JUNE 1992

Impact Factor: 2.91 · DOI: 10.1021/jf00018a002

Fractionation Procedures, Electrophoretic Characterization, and Amino Acid Composition of Amaranth Seed Proteins

Ana P. Barba de la Rosa,† Jacques Gueguen,‡ Octavio Paredes-López,*,† and Gerard Viroben‡

Laboratorio Biotecnologia de Alimentos, Centro de Investigacion y de Estudios Avanzados–IPN, Unidad Irapuato, Apdo Postal 629, 36500 Irapuato, Gto, Mexico, and Laboratoire de Biochimie et Technologie des Proteines, Institute National de la Recherche Agronomique, Rue de la Geraudiere, B.P. 527, 44026 Nantes Cedex 03, France

Proteins from *Amaranthus hypochondriacus* were extracted according to the Osborne classification. Various extracting agents were tested for protein solubilization. It was found that the best agent for extraction of albumins and globulins was Na_2HPO_4, pH 7, and for glutelins was $Na_2B_4O_7$ + 1% (w/v) SDS + 0.6% (v/v) 2-mercaptoethanol, pH 10. The major and minor seed protein fractions were albumins and prolamins, respectively. Defatting with hexane remarkably affected the distribution of protein solubility classes. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis patterns of nonreduced and reduced albumins showed a major band at 36–34 kDa; the globulin fraction showed several major bands between 38 and 36, 33 and 31, and 27 and 21 kDa plus some others within this range and over. Reduced globulin, prolamin, and glutelin fractions shared some common electrophoretic bands (67, 38, 35, 26, and 24 kDa). Albumins and globulins were rich in lysine and valine. Prolamins had high contents of sulfur amino acids and phenylalanine, and glutelins were rich in leucine, threonine, and histidine.

INTRODUCTION

Amaranth is a dicotyledonous and fast growing plant, tolerant to drought, high temperatures, and pests. In this environment the plant produces higher yields of seeds than other conventional cereals. Seeds contain 15–18% protein with acceptable levels of lysine, tryptophan, and sulfur amino acids (Dowton, 1973; Hauptli et al., 1979).

Protein fractionation according to an Osborne-modified procedure for whole and defatted amaranth flour has been reported (Konishi et al., 1985; Paredes-López et al., 1988). However, there is a lack of information on the optimal conditions for protein fractionation and on the biochemical and functional properties of these proteins.

The objectives of this study were to devise fractionation procedures of the main protein fractions from amaranth proteins and to characterize them with regard to their molecular weight and amino acid composition.

MATERIALS AND METHODS

Materials. Mature seeds of *Amaranthus hypochondriacus* (Mercado cultivar, waxy type) were harvested at the Experimental Station of the Instituto Nacional de Investigaciones Forestales y Agropecuarias (INIFAP), Chapingo, Mexico. Flour was obtained by grinding whole seeds in a ball mill (Prolabo) and attained an average particle size of about 100 μm. It was defatted with hexane in a 10% (w/v) suspension under continuous stirring during 24 h and then air-dried at room temperature. Whole and defatted flours were stored at 4 °C until used.

Protein Fractionation Procedures. *Albumin + Globulin + NPN Fraction.* Extraction of the albumin + globulin + NPN (non-protein nitrogen) fraction was carried out on both whole and defatted flour. To compare their efficiencies as protein solvent, the following extracting agents were assayed: (a) 0.1 M Na_2HPO_4, pH 7; (b) 0.1 M Na_2HPO_4 + 5% (w/v) K_2SO_4, pH 7; (c) 0.8 M NaCl aqueous solution; and (d) 0.1 M Na_2HPO_4 + 5% (w/v) K_2SO_4 + 0.8 M NaCl, pH 7.

Suspensions of flour/extracting agents (1:10 w/v) were stirred for 1 h at room temperature and centrifuged at 9000*g* for 20 min. Then a second extraction was done with the same agent, and finally the extraction residue was washed with deionized water, centrifuging in both cases at the same previous speed. Supernatants were collected and dialyzed (MW cutoff at 6000, Spectrapor spectrum, Medical Industries, Inc., Los Angeles, CA) at 4 °C against deionized water for 5 days; dialysate was changed every day. The content of dialysis tubes was centrifuged at 9000*g* for 20 min. Supernatant (albumin fraction), pellet (globulin fraction), and extracted residue were freeze-dried. Supernatant without dialysis was termed total extract.

Prolamin Extraction. A subsample of the freeze-dried residue resulting from albumin and globulin extraction from whole and defatted flour was finely ground and separately extracted under magnetic stirring for 1 h with 70% aqueous 2-propanol (2-PrOH) and centrifuged at 9000*g* for 20 min. Pellet was washed with water and centrifuged as described. Supernatants were pooled and dialyzed against acetic acid (1% v/v) for 5 days, changing dialysate every day, and then freeze-dried. After albumins and globulins were extracted from whole flour, another subsample of the corresponding freeze-dried residue was defatted as described previously and subjected to extraction of prolamins with this same procedure.

Glutelin Extraction. After prolamins were extracted, subsamples of freeze-dried residue from both flours, defatted or non, were removed for glutelin extraction. Five extracting agents were tested: (a) 0.1 M NaOH; (b) 0.1 M $Na_2B_4O_7$, pH 10; (c) 0.1 M $Na_2B_4O_7$ + 1% (w/v) SDS, pH 10; (d) 0.1 M $Na_2B_4O_7$ + 0.6% (v/v) 2-mercaptoethanol (2-ME), pH 10; and (e) 0.1 M $Na_2B_4O_7$ + 1% (w/v) SDS + 0.6 (v/v) 2-ME, pH 10. Samples of freeze-dried residue were suspended in the agents by magnetic stirring during 1 h and centrifuged at 9000*g* for 20 min. Supernatants were pooled and dialyzed like the prolamin fraction.

A micro-Kjeldahl method (Nkong and Ballance, 1982) was used to determine protein content. The protein/nitrogen coefficient used was 5.85 (Paredes-López et al., 1990). Solubility experiments and protein content were determined in duplicate.

Electrophoresis. *One-Step, One-Dimension Electrophoresis.* All gels were run using minislabs (Touzart et Matignon Model HE-6410). Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) was carried out according to the method of Laemmli (1970) with and without reduction of the protein by 2-ME in 15% (w/v) polyacrylamide gels. Protein samples (1

* Author to whom correspondence should be addressed.
† Unidad Irapuato.
‡ Institute National de la Recherche Agronomique.

0021-8561/92/1440-0931$03.00/0

Table I. Protein Fractionation of Whole and Defatted Amaranth Flour[a] (Grams of Protein/100 g of Protein Dry Basis)

fraction	extracting agent	amaranth flour: whole	amaranth flour: defatted	amaranth flour: whole defatted[b]
albumins + globulins + NPN	0.1 M Na_2HPO_4 (pH 7)	66.1[a] ± 0.7	51.6[c] ± 0.9	66.1
	0.1 M Na_2HPO_4 + 5% (w/v) K_2SO_4 (pH 7)	64.1[a] ± 1.9	55.0[b] ± 1.9	
	0.8 M NaCl	58.3[b] ± 2.6	54.4[b] ± 2.1	
	0.1 M Na_2HPO_4 + 5% (w/v) K_2SO_4 + 0.8 M NaCl (pH 7)	65.8[a] ± 2.9	50.8[c] ± 1.7	
prolamins[c]	2-ProH (70% v/v)	0.7 ± 0.1	1.2 ± 0.2	1.3 ± 0.2
glutelins[d]	(A) 0.1 M NaOH	23.8[fg] ± 1.4	40.6[ab] ± 0.9	20.9[g] ● 0.7
	(B) 0.1 M $Na_2B_4O_7$ (pH 10)	22.5[fg] ± 0.3	37.5[c] ± 3.0	22.7[fg] ± 1.0
	(C) 0.1 M $Na_2B_4O_7$ + 1% (w/v) SDS (pH 10)	26.2[e] ± 0.9	41.6[a] ± 1.2	23.6[fg] ± 1.1
	(D) 0.1 M $Na_2B_4O_7$ + 0.6% (v/v) 2-ME (pH 10)	23.3[fg] ± 0.9	38.8[bc] ± 0.4	23.0[fg] ± 1.4
	(E) 0.1 M $Na_2B_4O_7$ + 1% (w/v) SDS + 0.6% (v/v) 2-ME (pH 10)	28.5[d] ± 1.1	42.2[a] ± 0.9	21.7[fg] ± 0.7
residue	(A) 0.1 M NaOH	4.6	4.5	6.7
	(B) 0.1 M $Na_2B_4O_7$ (pH 10)	5.9	6.2	4.9
	(C) 0.1 M $Na_2B_4O_7$ + 1% (w/v) SDS (pH 10)	3.2	3.4	3.7
	(D) 0.1 M $Na_2B_4O_7$ + 0.6% (v/v) 2-ME (pH 10)	5.7	5.0	4.6
	(E) 0.1 M $Na_2B_4O_7$ + 1% (w/v) SDS + 0.6% (v/v) 2-ME (pH 10)	2.5	2.8	6.9
N recovery, %	(A) 0.1 M NaOH	95.2	97.9	95.0
	(B) 0.1 M $Na_2B_4O_7$ (pH 10)	95.2	96.5	95.0
	(C) 0.1 M $Na_2B_4O_7$ + 1% (w/v) SDS (pH 10)	96.2	97.8	94.7
	(D) 0.1 M $Na_2B_4O_7$ + 0.6% (v/v) 2-ME (pH 10)	95.8	96.6	95.0
	(E) 0.1 M $Na_2B_4O_7$ + 1% (w/v) SDS + 0.6% (v/v) 2-ME (pH 10)	97.8	97.8	96.0

[a] Means ± standard deviation not sharing a common superscript letter are significantly different at $p = 0.05$ by Duncan's multiple range test. [b] Albumins + globulins + NPN fraction was extracted from whole amaranth flour, after which defatting was performed for prolamin and glutelin extraction. [c] Extracted from the residue recovered after fractionation with 0.1 M Na_2HPO_4, pH 7. [d] They were extracted separately by A–E agents. NPN, Non-protein nitrogen; ME, mercaptoethanol; SDS, sodium dodecyl sulfate.

mg/mL) were dissolved in 0.1 M Tris-HCl, pH 6.8, and 2% (w/v) SDS. Reduction of disulfide bridges was performed by 2-ME (5% v/v) at 100 °C for 1 min. Electrophoresis was conducted at a constant current of 20 mA for gel for 2–3 h. After electrophoresis, the gel was fixed with trichloroacetic acid (12.5% w/v) for 30 min and stained overnight by addition of Coomassie Brilliant Blue G250 in a final concentration of 0.25%. Destaining was achieved by washing the gel during 2 h with acetic acid/ethanol/water (4.5/4.5/1 v/v/v) and then overnight with a solution of acetic acid (5% v/v).

Molecular weights of protein subunits were calculated using the following standard proteins: phosphorylase B (94 000), bovine serum albumin (67 000), ovalbumin (43 000), trypsin inhibitor (20 100), and lactalbumin (14 400).

Two-Step, One-Dimension Electrophoresis. To separate the high molecular weight subunits of prolamin and glutelin fractions a two-step, one-dimensional electrophoresis was performed. After the first electrophoresis in nonreductive conditions, bands 1 or 2 mm wide, which contained high molecular weight proteins that did not enter the gel, were cut off and transferred to an Eppendorf vial containing the extraction solution (0.07 M Tris-HCl + 3.4% w/v SDS + 12.7% w/v glycerol + 7.5% v/v 2-ME, pH 6.8). A small amount of bromophenol blue was also added as tracking dye. After incubation for 3 h at 60 °C, extraction and reduction were completed by heating the sample at 100 °C for 5 min. Reduced extracts of subunits from prolamins and glutelins were then separated by a second one-dimension SDS–PAGE electrophoresis (Khelifi and Branlard, 1991).

Amino Acid Analysis. Amino acid analysis was performed by reversed-phase high-performance liquid chromatography (RP-HPLC) of the phenylthiocarbamyl amino acid derivatives (Mora-Escobedo et al., 1990). Protein samples of 10 mg were hydrolyzed with 6 N HCl (Suprapur, Merck) in a Pico-Tag workstation (Waters Chromatography Division, Millipore Corp.) at 110 °C for 24 h. For determination of methionine and cysteine the protein samples (10 mg) were oxidized with performic acid at 0 °C for 16 h and hydrolyzed (Spindler et al., 1984). After dilution with an internal standard and filtration, 10 μL was used for derivatization with phenyl isothyiocyanate. The derivatized samples were analyzed on a C-18 Pico-Tag column. Data were integrated using a Model 19-740 integrator (Waters Chromatography). Duplicate runs were carried out, and mean values are reported.

RESULTS

Fractionation of the Proteins into Solubility Classes. Table I shows that extractability of albumins + globulins + NPN from whole flour by Na_2HPO_4 was at the same range as those obtained by this agent plus K_2SO_4 and plus NaCl (64–66%); the extraction with NaCl alone was lower (58%). Other workers (Gueguen and Barbot, 1988) have observed that addition of K_2SO_4 to extracting agents improved the protein solubility from peas. In all cases defatting tended to decrease the protein solubilization, which ranged from 51 to 55% (Table I), possibly due to protein denaturation by the solvent as well as by the extraction of nitrogenous compounds by hexane as reported by Paredes-López et al. (1988).

The yield of protein extraction with Na_2HPO_4 was in good agreement with the value reported by Konishi et al. (1985) for a waxy flour at 100% recovery. Protein extraction yields with NaCl were higher than those reported by Paredes-López et al. (1988), who found 45.7 and 48.6% on whole and defatted flours, respectively, under higher ionic strength conditions.

In view of previous results, the albumin + globulin + NPN residue from 0.1 M Na_2HPO_4, pH 7, was used as the first extraction step before prolamin and glutelin extraction. The amounts of prolamins extracted by aqueous 2-PrOH, from the corresponding residue, was 0.7 and 1.2% for whole and defatted flours, respectively (Table I).

Similar yields of extraction were obtained for glutelins from whole flour by using either NaOH or $Na_2B_4O_7$ as extracting agent (Table I). The addition of SDS slightly increased the extraction yield. On the other hand, the presence of a disulfide cleaving agent did not improve the solubilization of these proteins, but the combination of SDS and 2-ME in the $Na_2B_4O_7$ increased moderately the protein extraction. In general, glutelins from defatted flour followed a similar trend. This may indicate that disulfide bridges are not much involved in the solubility of the glutelin fraction. The greatest difference concerning solubility of this fraction was between whole and defatted materials used for the extraction. The former and latter

Table II. Albumin/Globulin Ratios Estimated with the Extracts from Whole and Defatted Flours by Different Extracting Agents[a]

extracting agent	albumins/globulins	
	whole flour	defatted flour
0.1 M Na_2HPO_4 (pH 7)	2.5	1.9
0.1 M Na_2HPO_4 + 5% (w/v) K_2SO_4 (pH 7)	2.0	2.0
0.8 M NaCl	2.2	2.1
0.1 M Na_2HPO_4 + 5% (w/v) K_2SO_4 + 0.8 M NaCl (pH 7)	2.4	2.3

[a] Mean values of duplicates.

samples gave glutelin solubility ranges of 22–28 and 38–42%, respectively. It clearly appears that hexane induced the insolubilization of some proteins normally soluble in Na_2HPO_4 (e.g., globulins) but improved the extractability of the glutelin fraction. The influence of defatting on protein extractability from cereals, following Osborne procedures, has been noticed by other authors (Byers et al., 1983).

On the basis of previous results, a third extraction procedure was tested to avoid the effect of hexane on all Osborne protein classes. In this procedure, as was mentioned previously, the defatted step was applied on the residue of whole amaranth flour resulting from the extraction of albumin + globulin + NPN fractions with Na_2HPO_4. Results are presented in Table I under whole defatted amaranth flour. Glutelin-type protein yields ranged from 21 to 24% instead of from 38 to 42% when defatted flour was used. In these conditions, it is expected that the presence of globulin-type proteins, in the glutelin fraction has been reduced or eliminated. Comparison of these proteins with those from whole and defatted flours is also required. Interestingly, nitrogen recovery in all cases was very much alike.

Table II shows the relationship between albumin and globulin values. This relationship was found to be around 2.0–2.5 and 1.9–2.3 for whole and defatted flours, respectively. These flours gave similar ratios for each of the extracting agents, except for Na_2HPO_4 which gave ratios of 2.5 and 1.9, respectively. This means that the effect of defatting was different for this particular agent.

Electrophoretic Characterization of Protein Fractions. There were no differences in electrophoretic patterns of albumins and globulins extracted from whole and defatted samples. However, a better definition of subunits was observed for defatted samples. Therefore, these samples were used for all electrophoretic studies. Figure 1 shows that there were outstanding similarities between the electrophoretic patterns under nonreductive conditions of albumins extracted with Na_2HPO_4 (lane 1a), Na_2HPO_4 + K_2SO_4 (lane 2a), and NaCl (lane 3a) but not with that with Na_2HPO_4 + K_2SO_4 + NaCl (lane 4a). Lanes 1a/b–4a/b belong to the sample of albumins + globulins before dialysis. The three former samples (1a, 2a, and 3a) showed a major band at 34 kDa. Various minor to major bands were observed in the molecular weight range 28 000–14 000 in NaCl and in Na_2HPO_4 + K_2SO_4 + NaCl albumins, which appeared absent in Na_2HPO_4 albumins. The solubility of these components may depend on the ionic strength of the extracting agents, as observed by Gueguen et al. (1988) for pea proteins. On the other hand, globulins extracted with Na_2HPO_4 and with Na_2HPO_4 + K_2SO_4 agents were very much alike, but striking differences were shown by those samples from NaCl and from Na_2HPO_4 + K_2SO_4 + NaCl (Figure 1). All globulins showed a major band at 38 kDa, which is not in agreement with the results of Gorinstein et al. (1991b), who reported a major globulin band at 18–14 kDa. This 18–14 kDa was only present in some of the samples.

The four albumin fractions exhibited a strong resemblance under reductive conditions (Figure 2); only minor differences may be found in some minor bands. All subunits migrated between 94 and 11 kDa, with a clear-cut major band at 36 kDa which is about in the same region as that observed in albumins without 2-ME (Figure 1). In other words, it remained after reduction. This subunit was also reported by Mora-Escobedo et al. (1990) for another type of *A. hypochondriacus*. Reduced globulins migrated as well in the same 94–11-kDa range (Figure 2). Various well-defined components were detected in the relatively high to moderate molecular weight region 92 000–50 000. This type of globulin polypeptide has been reported by other workers for pea convicilin (Croy et al., 1980) and for soybean conglycinin (Utsumi and Kinsella, 1985). All four globulins showed major bands at 54 kDa, a doublet-like at 38–36 kDa, which persisted after 2-ME reduction, a band at 34 kDa, three bands at 27–21 kDa, and two bands at 15–14 kDa. Mostly minor subunits appeared at 33–31 and 20–18 kDa, which might be related to the polypeptide chains constitutive of the 12.7S-type globulin from amaranth seeds as found by Konishi et al. (1985).

SDS–PAGE of amaranth prolamins in the absence of 2-ME showed components α of high molecular weight which did not enter the gel, β at the top of the gel ($\gg$94 kDa), and γ represented by a doublet with major bands at 67–60 kDa plus another major band at 94 kDa (Figure 3a). Various minor components appeared along the prolamin pattern. On the other hand, differences between glutelin patterns, without 2-ME, were most noticeable in comparisons of this protein class extracted in NaOH, $Na_2B_4O_7$, and $Na_2B_4O_7$ + SDS (Figure 3b,c,d, respectively); better clear-cut subunits appeared in the latter pattern. $Na_2B_4O_7$ + 2-ME glutelins exhibited a subunit distribution somewhat different from that of previous samples, which did not change by SDS addition to the extracting agent. As for prolamins, glutelins had protein residues that did not enter the gel.

Remarkable changes were noticed after reduction of prolamins with 2-ME (Figure 4a); five dense and well-defined bands were obtained at about 67, 38, 35, 26, and 24 kDa. These same bands, in various degrees of concentration, plus some others in the 94–11-kDa range, were shown by all reduced glutelins (Figure 4b–f). Interestingly, in spite of the reduction effect there appeared to remain residual protein which was unable to enter the gel.

Components α, β, and γ of prolamin and glutelin fractions shown in Figure 3 were studied by a two-step, one-dimension electrophoresis. All of these components, either from prolamins or glutelins (Figure 5a–e), exhibited in common at least two major to minor doublets at 54–52 and 34–33 kDa, plus some bands at the 27–22-kDa region. The doublet at 54–52 kDa in Figure 5c (prolamin γ) and the bands at 27–22 kDa in Figure 5d (glutelin γ) were rather slight. Different minor bands appeared at about 94–67 kDa. Gorinstein et al. (1991a) have recently reported that alcohol-soluble proteins from amaranth are mostly composed by 14–10-kDa subunits, which is not in agreement with our findings. Results in Figures 3a and 5a–c show major subunits from amaranth prolamins of relatively high, medium, and low molecular weight.

Figure 6 summarizes and compares the four solubility classes from amaranth seeds under nonreductive and reductive electrophoretic conditions. In the absence of 2-ME all fractions, but mostly globulins/prolamins/glu-

Table III. Amino Acid Composition of Amaranth Protein Fractions[a] (Grams of Amino Acid/100 g of Crude Protein)

amino acid	albumins	globulins	prolamins	glutelins	FAO/WHO/UNU ref pattern	
					preschool child	adult
isoleucine	3.7 ± 0.1	4.2 ± 0.2	6.2 ± 0.2	5.8 ± 0.1	2.8	1.3
leucine	5.7 ± 0.0	5.7 ± 0.2	5.7 ± 0.2	10.5 ± 0.2	6.6	1.9
lysine	7.6 ± 0.3	6.7 ± 0.2	4.2 ± 0.2	4.6 ± 0.0	5.8	1.6
methionine	4.1 ± 0.2	3.4 ± 0.1	7.4 ± 0.2	3.1 ± 0.1	2.5[b]	1.7[b]
cystine	5.9 ± 0.2	3.9 ± 0.2	6.5 ± 0.2	6.2 ± 0.2		
phenylalanine	5.1 ± 0.2	5.0 ± 0.1	9.0 ± 0.2	6.8 ± 0.2	6.3[c]	1.9[c]
tyrosine	3.3 ± 0.2	4.3 ± 0.1	4.0 ± 0.0	3.8 ± 0.3		
threonine	3.9 ± 0.2	4.1 ± 0.2	3.2 ± 0.1	8.6 ± 0.2	3.4	0.9
tryptophan	nd[d]	nd	nd	nd	1.1	0.5
valine	4.5 ± 0.1	4.7 ± 0.2	2.7 ± 0.1	3.8 ± 0.1	3.5	1.3
histidine	2.5 ± 0.2	1.1 ± 0.2	1.1 ± 0.2	4.7 ± 0.1	1.9	1.6
alanine	5.1 ± 0.3	4.0 ± 0.2	4.7 ± 0.1	3.6 ± 0.1		
arginine	8.1 ± 0.2	9.5 ± 0.2	9.4 ± 0.2	2.7 ± 0.1		
aspartic acid	6.2 ± 0.3	8.7 ± 0.2	6.2 ± 0.2	6.1 ± 0.1		
glutamic acid	17.5 ± 0.2	17.3 ± 0.2	13.4 ± 0.3	13.2 ± 0.3		
glycine	6.2 ± 0.2	6.6 ± 0.1	4.4 ± 0.1	4.9 ± 0.1		
proline	3.7 ± 0.2	3.9 ± 0.1	4.7 ± 0.2	4.6 ± 0.2		
serine	4.8 ± 0.2	4.9 ± 0.2	5.1 ± 0.2	5.3 ± 0.1		

[a] Fractions isolated from defatted flour using 0.1 M Na_2HPO_4, pH 7, as described under Materials and Methods. [b] Requirements for methionine + cystine. [c] Requirements for phenylalanine + tyrosine. Mean values of duplicates ± standard deviation. [d] nd, not determined.

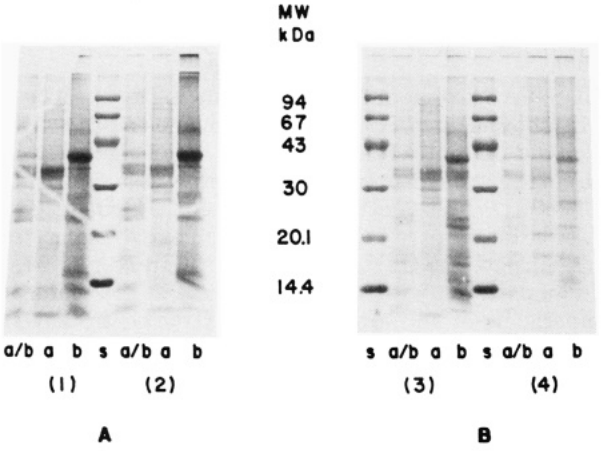

Figure 1. (A, B) Electrophoretic patterns of nonreduced protein fractions extracted from defatted amaranth flour by different extracting agents. a/b, albumins + globulins; a, albumins; b, globulins; s, standard reference proteins. 1, 0.1 M Na_2HPO_4, pH 7; 2, 0.1 M Na_2HPO_4 + 5% (w/v) K_2SO_4, pH 7; 3, 0.8 M NaCl; 4, 0.1 M Na_2HPO_4 + 5% (w/v) K_2SO_4 + 0.8 M NaCl, pH 7.

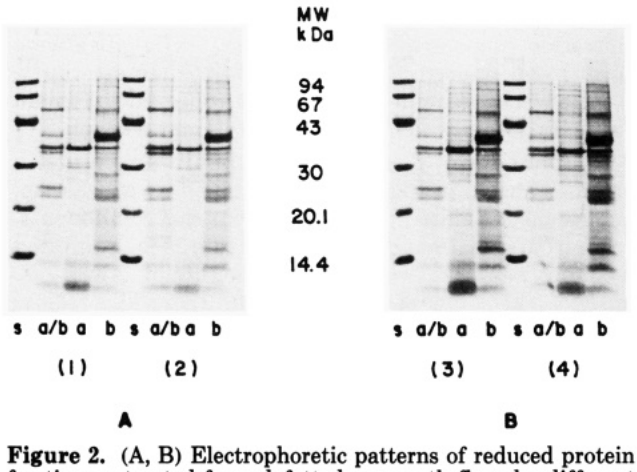

Figure 2. (A, B) Electrophoretic patterns of reduced protein fractions extracted from defatted amaranth flour by different extracting agents. a/b, albumins + globulins; a, albumins; b, globulins; s, standard reference proteins. 1, 0.1 M Na_2HPO_4, pH 7; 2, 0.1 M Na_2HPO_4 + 5% (w/v) K_2SO_4, pH 7; 3, 0.8 M NaCl; 4, 0.1 M Na_2HPO_4 + 5% (w/v) K_2SO_4 + 0.8 M NaCl, pH 7.

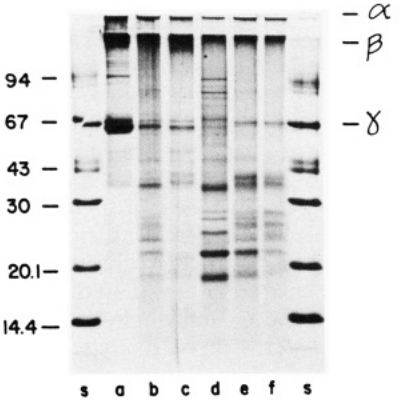

Figure 3. Electrophoretic patterns of unreduced amaranth prolamins, extracted with 70% 2-PrOH, and glutelins, extracted with different extracting agents. a, prolamins. Glutelins: b, 0.1 M NaOH; c, $Na_2B_4O_7$, pH 10; d, $Na_2B_4O_7$ + 1% (w/v) sodium dodecyl sulfate (SDS), pH 10; e, $Na_2B_4O_7$ + 0.6% (v/v) 2-mercaptoethanol (ME), pH 10; f, $Na_2B_4O_7$ + 1% (w/v) SDS + 0.6% (v/v) 2-ME, pH 10. For components α, β, and γ, see text.

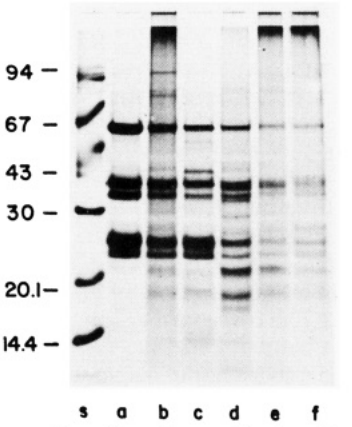

Figure 4. Electrophoretic patterns of reduced amaranth prolamins, extracted with 70% 2-PrOH, and glutelins, extracted with different chemical agents. a, prolamins. Glutelins: b, 0.1 M NaOH; C, $Na_2B_4O_7$, pH 10; d, $Na_2B_4O_7$ + 1% (v/v) sodium dodecyl sulfate (SDS), pH 10; e, $Na_2B_4O_7$ + 0.6% (v/v) 2-mercaptoethanol (ME); f, $Na_2B_4O_7$ + 1% (w/v) SDS + 0.6% (v/v) 2-ME, pH 10.

telins, shared some common electrophoretic bands (e.g., at 67 kDa). Common subunits, especially for globulins/prolamins/glutelins, were more noticeable in the presence of 2-ME (e.g., at 67, 38, 35, 26, and 24 kDa); these common components might come from the high molecular weight proteins that did not migrate into the gel. Electrophoretic

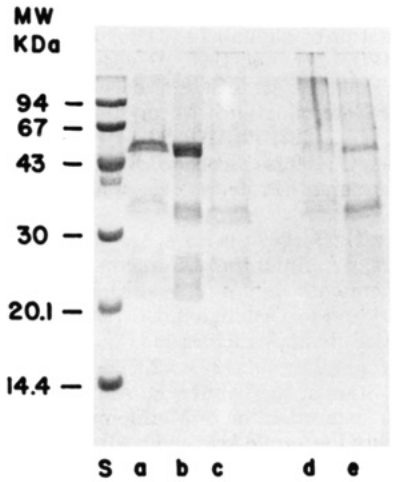

Figure 5. Two-step one-dimension electrophoresis of prolamin (a–c) and glutelin (d, e) fractions. Components α (a, d), β (b, e), and γ (c) are shown. α, β, and γ for prolamins were isolated from samples shown in Figure 3a and α and β for glutelins from Figure 3d. s, standard reference proteins.

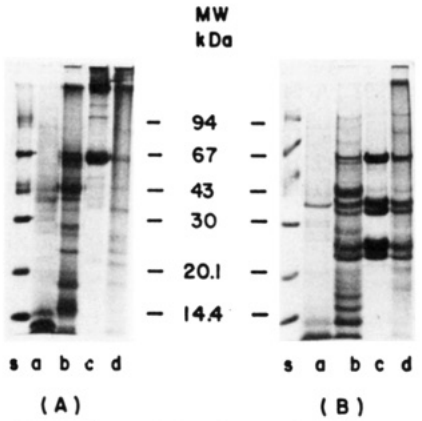

Figure 6. Comparison of the electrophoretic patterns of the four amaranth protein fractions. a, albumins; b, globulins; c, prolamins; d, glutelins; s, standard reference proteins. A, without 2-mercaptoethanol (ME); B, with 2-ME.

similarity among protein fractions was also reported by Wen and Luthe (1985) for rice proteins.

Amino Acid Composition. Amino acid compositions of different amaranth fractions are given in Table III. In terms of essential amino acids, albumins and globulins were relatively rich in lysine and valine, in agreement with previous results (Mora-Escobedo et al., 1990). Prolamins had large amounts of sulfur amino acids and phenylalanine, and glutelins showed high levels of leucine, threonine, and histidine. It should be noted that of all fractions glutelins exhibited the highest proportion of essential amino acids. In general, the four fractions contained adequate amounts of several essential amino acids for preschool children and all of those essential for adults, except for histidine in globulins and prolamins (FAO/WHO/UNU, 1985). Of nonessential amino acids, albumins and globulins were high in glutamic acid and glycine but low in proline, which is an opposite feature to that exhibited by both prolamins and glutelins. In amino acid compositions, amaranth proteins show some remarkable differences from those of both cereals and legumes (Peterson, 1978; Duarte-Correa et al., 1986).

DISCUSSION

The various extraction procedures used in the present study showed that defatting had a great influence on the distribution of protein solubility classes from amaranth seeds. Protein insolubilization after whole flour was defatted with hexane may lead to an overestimation of the glutelin fraction. To avoid this artifact, it would be preferable to carry out a quantitative evaluation of protein solubility classes on the whole flour without defatting. On the other hand, for qualitative studies of albumins and globulins it would be advisable to include in the procedure the defatting step. It significantly improved the technical quality of the electrophoretic patterns. For qualitative studies of prolamin and glutelin classes, the defatting step could be done after the extraction of albumins and globulins from the nondefatted flour. This suggestion is based on the consideration that starting from a defatted flour might lead to an incomplete extraction of globulins and consequently to a qualitative modification of the glutelin fraction. However, this aspect requires further investigation. As can be seen from the solubilization data, amaranth proteins have a unique distribution of fractions, as reported by various workers (Konishi et al., 1985; Paredes-López et al., 1988; Gorinstein et al., 1991b). The ratio albumins/globulins for whole and defatted flours was found to be between 1.9 and 2.5, which is in agreement with values previously found by Paredes-López et al. (1988). For globulins we found electrophoretic bands in reductive conditions in the 38–36-, 32–31-, and 24–22-kDa regions, as reported by Konishi et al. (1985). These polypeptides might be the components of the 12.7S-type protein, an 11S-like protein, found by the previous workers and by Barba de la Rosa et al. (1992) in amaranth globulins.

A great similarity was observed between electrophoretic patterns of reduced prolamins and glutelins. These fractions shared major polypeptides of 67, 38, 35, 26, and 24 kDa plus some others. Components of prolamins and glutelins which did not enter the gel in nonreductive conditions gave, after reduction, common polypeptides at 54–52, 34–33, and 27–22 kDa. This implies that these amaranth fractions have high molecular weight polypeptides linked by disulfide bridges. Thus, differences in the solubility properties exhibited by these two protein families might be ascribed to variations in polarity of similar subunits and to the amount of disulfide links.

In conclusion, this study confirms the unique distribution and composition of amaranth seed proteins. The conformation of these proteins should be studied in more detail.

ACKNOWLEDGMENT

We gratefully acknowledge financial support from the European Economic Community and from Consejo Nacional de Ciencia y Tecnología—México.

LITERATURE CITED

Barba de la Rosa, A. P.; Paredes-López, O.; Gueguen, J. Characterization of Amaranth Globulins by Ultracentrifugation and Chromatographic Techniques. *J. Agric. Food Chem.* **1992**, following paper in this issue.

Byers, M.; Miflin, B .J.; Smith, S. S. A Quantitative Comparison of the Extraction of Protein Fractions from Wheat Grain by Different Solvents, and of the Polypeptide and Amino Acid Composition of the Alcohol-Soluble Proteins. *J. Sci. Food Agric.* **1983**, *34*, 447–462.

Croy, R. R. D.; Gatehouse, J. A.; Tyler, M.; Boulter, D. The Purification and Characterization of a Third Storage Protein (Convicilin) from the Seeds of Pea (*Pisum sativum L*). *Biochem. J.* **1980**, *1191*, 509–516.

Dowton, W. J. S. Amaranthus Edulis: A High Lysine Grain Amaranth. *World Crops* **1973**, *35*, 30–26.

Duarte-Correa, A.; Jokl, L.; Carlsson, P. Amino Acid Composition of Some *Amaranthus p.* Grain Proteins and of its Fractions. *Arch. Latinoam. Nutr.* **1986**, *36*, 466–476.

FAO/WHO/UNU. *Energy and Protein Requirements*; Technical Report Series 724; WHO: Geneva, 1985.

Gorinstein, S.; Arnold, N. J.; 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.; Mashe, 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.

Gueguen, J.; Barbot, J. Quantitative and Qualitative Variability of Pea (*Pisum sativum L.*) Protein Composition. *J. Sci. Food Agric.* **1988**, *42*, 209–224.

Gueguen, J.; Chevalier, M.; Barbot, J.; Schaeffer, F. Dissociation and Aggregation of Pea Legumin Induced by pH and Ionic Strength. *J. Sci. Food Agric.* **1988**, *44*, 167–182.

Hauptli, H.; Zutz, R. L.; Jain, S. K. Germoplasm Exploitation in Central and South America. In *Proceedings of the Second Amaranth Conference*; Rodale Press: Emaus, PA, 1979; pp 117–120.

Khelifi, D.; Branlard, G. A New Two-Step Electrophoresis Method for Analysing Gliadin Polypeptides and High and Low Molecular Weight Subunits of Glutenin of Wheat. *J. Cereal Sci.* **1991**, *13*, 41–48.

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.

Laemmli, U. K. Cleavage of Structural Proteins During the Assembly of the Head of Bacteriophage T4. *Nature* **1970**, *227*, 680–685.

Mora-Escobedo, R.; Paredes-López, O.; Ordorica-Falomir, C. Characterization of Albumins and Globulins from Amaranth. *Lebensm. Wiss. Technol.* **1990**, *23*, 484–487.

Nkonge, Ch.; Ballance, M. A Sensitive Colorimetric Procedure for Nitrogen Determination in Micor-Kjeldahl Digests. *J. Agric. Food Chem.* **1982**, *30*, 416–420.

Paredes-López, O.; Mora-Escobedo, R.; Ordorica-Falomir, C. Isolation of Amaranth Proteins. *Lebensm. Wiss. Technol.* **1988**, *21*, 59–61.

Paredes-López, O.; Barba de la Rosa, A. P.; Hernádez-López, D.; Carabez-Trejo, A. *Amaranto—Caracteristicas Alimentarias y Aprovechamiento Agroindustrial*; Organización de los Estados Americanos: Washington, DC, 1990.

Peterson, D. M. Subunit Structure and Composition of Oat Seed Globulin. *Plant Physiol.* **1978**, *62*, 506–509.

Spindler, M.; Stadler, R.; Tanner, H. Amino Acid Analysis of Feedstuffs: Determination of Methionine and Cystine after Oxidation with Performic Acid and Hydrolysis. *J. Agric. Food Chem.* **1984**, *32*, 1366–1371.

Utsumi, S.; Kinsella, S. E. Structure-Function Relationship in Food Proteins Subunit Interactions in Heat-Induced Gelation of 7S, 11S and Soy Isolate Proteins. *J. Agric. Food Chem.* **1985**, *33*, 297–303.

Wen, T. N.; Luthe, D. S. Biochemical Characterization of Rice Glutelin. *Plant Physiol.* **1985**, *78*, 172–177.

Received for review August 22, 1991. Revised manuscript received December 9, 1991. Accepted March 11, 1992.

Registry No. Lys, 56-87-1; Val, 72-18-4; Phe, 63-91-2; Leu, 61-90-5; Thr, 72-19-5; His, 71-00-1; SDS, 151-21-3; Na_2HPO_4, 7558-79-4; $Na_2B_4O_7$, 1330-43-4; 2-mercaptoethanol, 60-24-2.