Characterization of Soluble Amaranth and Soybean Proteins Based on Fluorescence, Hydrophobicity, Electrophoresis, Amino Acid Analysis, Circular Dichroism, and Differential Scanning Calorimetry Measurements

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Intrinsic fluorescence (IF), surface hydrophobicity (S_o), electrophoresis, amino acid analysis, circular dichroism (CD), and differential scanning calorimetry (DSC) were used to study folded and unfolded soluble proteins from *Amaranthus hypochondriacus* (A. h.) and soybean (S). Globulin (Glo) and albumin subfractions (Alb-1 and Alb-2) were extracted from A. h. and S and denatured with urea. Electrophoretic and functional properties indicated a significant correlation between soluble protein fractions from soybean and amaranth. The protein fractions shared some common electrophoretic bands as well as a similar amino acid composition. The larger percent of denaturation in protein fractions, which is associated with enthalpy and the number of ruptured hydrogen bonds, corresponds to disappearance of α-helix. The obtained results provided evidence of differences in their secondary and tertiary structures. The most stable was Glo followed by the Alb-2 fraction. Predicted functional changes in model protein systems such as pseudocereals and legumes in response to processing conditions may be encountered in pharmaceutical and food industries. These plants can be a substitute for some cereals.

Keywords: *Amaranth; soybean; amino acids; proteins; electrophoresis; amino acids; fluorescence; calorimetry; denaturation; spectroscopy*

INTRODUCTION

Amaranth belongs to pseudocereals and contains about 13.2−18.2% protein. Soybean represents legumes with a high percentage of proteins of three times in comparison with amaranth. A better balanced amino acid composition (lysine from 3.2 to 6.4%) was found in both plants in comparison with the major cereals (2.2 to 4.5%). This makes amaranth a promising plant as a food or source of dietary proteins and as potential matrixes for drug release even in comparison with the soybean proteins (*1*−*3*). The most important task in the introduction of this plant as a new protein source is the prevention of the destruction of lysine. Some studies have shown that the popping process causes minimal destruction to the quality of amaranth protein. However, other studies reveal significant destruction of lysine, thus decreasing the protein quality of the grain and its dietary supply of lysine (*3*). Nowadays, a ton of amaranth is produced annually in Mexico for special food purposes such as candies, flakes, and flours distributed by stores specialized in nutraceutical foods (*3*). According to our investigations and literature data, amaranth and soybean have albumins and globulins as storage proteins (*4*−*6*). Our recent papers were focused on the structure and functional properties of plant globulins (*2*, *7*−*9*). To our knowledge, there are no references in the recent literature about the differences and identity of electrophoretic patterns in isolated seed protein fractions such as albumins and its subfractions, and globulins of amaranth and soybean.

This paper reports on the characterization of albumin and globulin fractions from *Amaranthus hypochondriacus* and soybean (*Glycine max*) by electrophoresis, amino acid analysis, fluorescence, hydrophobicity, circular dichroism, and differential scanning calorimetry. The identity and differences between pseudocereal and legume were based on amino acid analysis, electrophoretic patterns, and functional properties of protein fractions.

MATERIALS AND METHODS

Sample Preparation. Whole mature seeds from *A. hypochondriacus* (Mexico) and soybean (*G. max)* with low oil content (Brazil) were ground on a mill through a 60-mesh screen. The meal was defatted in a Soxhlet extractor with *n*-hexane for 10 h and then was stored at 5 °C after removal of hexane.

Protein Extraction. Proteins were extracted stepwise according to the following methods (*5*, *10*, *11*). The meal (1 g) was extracted with a solvent: sample ratio of 10:1 (v/w) and vigorously shaken. The extracts were separated by centrifuging at 10000*g* for 10 min. Each step was repeated twice. The

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sequence of the used solvents was the following: (a) water-albumins (Alb-1.Os), 0.5 M NaCl (Glo.Os), then water (Alb-2.Os), and (b) 0.5 M NaCl, then separated by dialysis against water [albumins-1 (Alb-1.K) and globulins (Glo.K)]. Another fraction (Alb-2.K) was extracted with water after removing Alb-1.K and Glo.K. Then all fractions Glo, Alb-1, and Alb-2 were dialyzed and freeze-dried. The nitrogen content in each fraction was determined by the micro-Kjeldahl method combined with a colorimetric determination (*12*).

Amino Acid Analysis. Samples of extracted proteins were hydrolyzed with 6 M HCl and 3% phenol solution in an MLS-MEGA-Microwave system for 20 min at 160 °C. The energy was 1000 W the first 5 min and then 500 W for 15 min. The samples were then dissolved in 100 μL of HCl (20 mM) and filtered through a 0.45 μm filter. Derivatization was done with 6-aminoquinolyl-*N*-hydroxysuccinimidyl carbamate (*13*). The sample was injected into a Multi-Pump Gradient Water HPLC system with a vertex knauer column B1184742 (Knauer, Berlin), length XID 150 × 4, 6 mm ID, spherimage-80 = DS2-5 μm. The Millenium chromatography manager system from Waters (Waters, Milford, MA) was used to evaluate the amino acids. Scanning fluorescence detector was used at an excitation of 250 nm and emission of 395 nm. Gradient program consisted of 40% acetate phosphate buffer and 60% acetonitrile.

The values of leucine and lysine were added and presented as a total of both. TYR + CYS eluted together and are presented as TYR. The results are given as g/100 g of protein.

Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS−PAGE). SDS−PAGE was performed according to Laemmli (*14*) using gels of $T = 10\%$ and $C = 2.7\%$ with some modifications (*15*). The gels were 1.5-mm thick and consisted of a 2-cm stacking gel and a 8-cm running gel. A total of 25 μg of protein was applied to sample slots. Electrophoresis was carried out at 75 mA for 95 min. Gels were stained with 0.25% Coomassie Brilliant Blue R in methanol/water/acetic acid (5:5:1 v/v) and destained in the same solvent. MW standards IV (Merck, Darmstadt, Germany) were used to estimate protein subunit molecular masses in kDa: carbonic anhydrase (30); ovalbumin (42.7); bovine serum albumin (66.25); ovotransferin (78). Amaranth albumin 1 (A.Alb-1), amaranth albumin 2 (A.Alb-2), amaranth globulin (A.Glo), soybean albumin 1 (S.Alb-1), soybean albumin 2 (S.Alb-2), and soybean globulin (S.Glo) were dissolved in sample buffer that contained 10% glycerol, 5% 2-ME, and 2% SDS in 0.0625 M (Tris-HCl), pH 6.8. Then the extracts were boiled for 5 min before being loaded. Similar bands of Alb-2 from amaranth and soybean at 34.2 and 36.4 kDa, respectively, were cut from the electrophoresis gels. Dialysis against GLY after electrophoretic separation to avoid the artifacts from the glycine buffer and mercaptoethanol was done. Hydrolyzed gel was analyzed as a blank for comparison. The entire protein bands were hydrolyzed with 6 M HCl containing 3% phenol solution in an MLS-MEGA II microwave (MLS GmbH, Leutkirch, Germany) for 20 min at 160 °C. The energy supply was 1000 W for the first 5 min and then 500 W for 15 min (*16*). The amino acid concentration was determined as described by Cohen and Michaud (*13*).

Fluorescence Spectra. Fluorescence measurements were done using a model FP-770 Jasco spectrofluorometer. The temperature of the samples was maintained at 30 °C with a thermostatically controlled circulating water bath. Fluorescence emission spectra measurements for all native and denatured Glo, Alb-1, and Alb-2 samples were taken at excitation wavelengths (nm) of 274 and 295 and recorded over the frequency range from the excitation wavelength to a wavelength of 500 nm. Fluorescence intensity (FI) was measured according to Arntfield et al. (*4*) and Zemser et al. (*17*).

Hydrophobicity (S_o). S_o was determined by 1-anilino-8-naphthalenesulfonate (ANS)-fluorescent probe measurements with 0.01 M phosphate buffer, pH 7.0, from 0.001 to 0.02% protein concentration at $\lambda_{ex} = 357$ nm. The fluorescence intensity was measured at 513 nm (*18*). Index of protein hydrophobicity was calculated as the initial slope of fluorescence intensity versus protein concentration (%) plot.

Differential Scanning Calorimetry (DSC). The thermal denaturation of proteins was assessed with a Perkin-Elmer DSC system 4. Lyophilized samples of about 1 mg were sealed in aluminum pans (*19*). Denatured samples were prepared by homogeneous mixture of native protein and denaturant in the dry state: protein sample + urea (1:1). Then the mixed sample of 1 mg was sealed in an aluminum pan in the same way as the native one. As a reference, an empty pan was used. The scanning temperature was 30−120 °C at a heating rate of 10 °C/min. Indium standards were for temperature and energy calibrations. T_d and ΔH were calculated from the thermograms (*20*).

Circular Dichroism (CD) Spectra. CD spectra were measured with a Jasco J-600 spectropolarimeter (Japan Spectroscopic Co., Ltd., Japan) at room temperature under constant nitrogen purge. Solutions (0.03 mg/mL) of proteins were prepared by dissolving the lyophilized powder in 0.01 M phosphate buffer, pH 7.2. The absorbancies of all solutions were kept below 1.0 (*17*, *21*). Denaturation of proteins was performed with 8 M urea. CD spectra represent an average of eight scans collected in 0.2-nm steps at average rate of 20 nm/min over the wavelength range 180−250 nm of far-UV (FUV). CD spectra were baseline-corrected. The data are presented as the mean residue ellipticities (θ). Secondary structure content was calculated using nonlinear least-squares curve-fitting program and the results of CD measurements (*22*), allowing the comparison of secondary structures from different plants.

Statistics. To verify the statistical significance of studied parameters means (M), their 95% confidence intervals of 3 times analyzed samples ± SD were defined. The p values of < 0.05 were considered significant.

RESULTS AND DISCUSSION

Protein Extraction. The seed proteins were fractionated as albumins and globulins. The amount of total protein in *A. hypochondriacus* was 15% and in soybean was 40%.

Using two different extractions, the following yields were obtained: Alb-1.Os/Glo.Os/Alb-2.Os = 40:21:3.6 and Alb-1.K/Glo.K/Alb-2.K = 39:19:4.0. For soybean, these proportions were the following: 46:23:5. The protein extracts contained pure subfractions of albumins and globulins. This was demonstrated by electrophoresis as well as compared with our previous results and literature (*1*−*8*). Our results are in agreement with others (*5*, *6*, *23*) differing only on the extraction procedure and in the sequence of the solvents used.

Amino Acids of Total Proteins. Amaranth proteins show a significantly higher ($p < 0.05$) concentration of GLU, GLY, and MET than soybean, while TYR + CYS and the essential amino acids such as ILE, LEU, and PHE are significantly higher ($p < 0.05$) in soybean than in amaranth (Table 1). The sum of essential amino acids was 40.7 and 54.4 g/100 g of protein in amaranth and soybean, respectively. The results of some amino acids in soybean (ASP, GLU, ARG, PRO, and LYS) were lower than in the literature (*24*, *25*). It can be explained by the biological variation of low-fat soybean as well as by fluorescence measurements used for amino acid determinations (*13*). Consequently, the essential amino acid concentration was higher in soybean than in amaranth. Both amaranth and soybean can cover preschool child and adult requirements of ILE, LEU, PHE, VAL, and HIS, while MET and THR requirements are met from amaranth and soybean, respectively. Requirements of LYS for adults (not for preschool children) can be covered with amaranth and soybean proteins (*3*, *6*, *24*). The protein composition in these plants is relatively high and digestible (*24*).

Amino Acids of Albumins. S.Alb-1.K has a higher concentration of essential amino acids than A.Alb-1.K. In general, both S.Alb-1.K and A.Alb-1.K have concen-

Table 1. Total Amino Acid Concentration of Amaranth and Soybean Flour [g/100 g of Protein]

amino acids	amaranth	soybean
ASP	3.5 ± 0.31[a]	2.9 ± 0.02
SER	5.2 ± 0.11	4.4 ± 0.19
GLU	8.2 ± 0.30	5.9 ± 0.10
GLY	16.0 ± 0.50	8.5 ± 0.27
HIS	2.6 ± 0.08	3.3 ± 0.22
THR	3.0 ± 0.09	3.6 ± 0.17
ALA	6.8 ± 0.11	6.7 ± 0.51
ARG	4.3 ± 0.04	2.6 ± 0.48
PRO	1.5 ± 0.08	1.7 ± 0.01
TYR*	1.2 ± 0.06	3.3 ± 0.24
VAL	6.3 ± 1.77	7.7 ± 0.39
MET	3.4 ± 0.00	0.7 ± 0.00
LYS	2.3 ± 0.17	1.7 ± 0.92
ILE	6.0 ± 0.18	7.7 ± 0.15
LEU	10.6 ± 0.08	14.0 ± 0.13
PHE	9.1 ± 0.81	19.1 ± 0.90
essential amino acids	40.7	54.4

[a] Standard error.

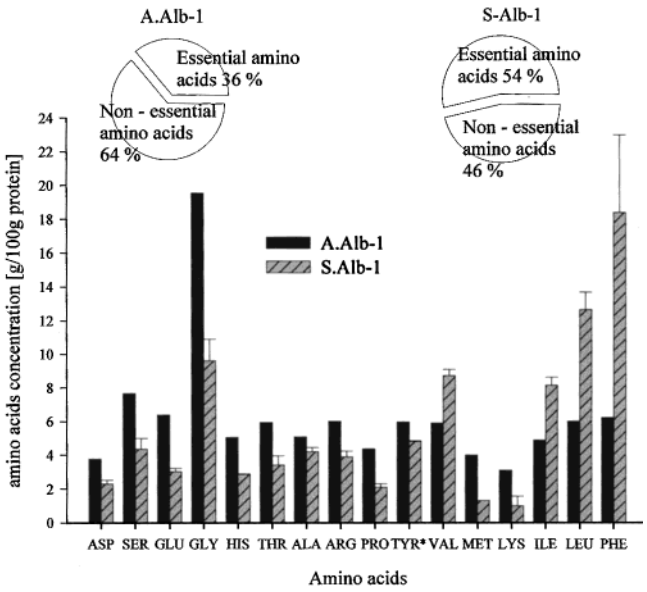

Figure 1. Amino acid concentration and total essential and nonessential amino acids of A.Alb-1.K and S.Alb-1.K. Bars indicate standard error. * = TYR + CYS.

trations of essential amino acids almost as high as amaranth and soybean flour (Figure 1 and Table 1), making this fraction important for further investigations.

The essential amino acid composition of S.Alb-2.Os is 37% and higher than in amaranth (Figure 2A). A.Alb-2.Os has more ($p < 0.05$) GLY than S.Alb-2.Os, but less ($p < 0.05$) TYR + CYS, VAL, ILE, LEU, and PHE. A.Alb-2.Os has 35.5 g/100 g of protein of essential amino acids, which corresponds to 12% less essential amino acids than in total proteins (Figure 2A). On the other hand, albumin 2 of soybean contains 56.6 g/100 g of protein of essential amino acids, which is comparable to the total amount of essential amino acids in total proteins. The high concentration of essential amino acids in albumin 2 indicates that this protein fraction is similar to storage proteins in pseudocereals (about 6.5−6.7% of total protein). For this reason, it is recommended to continue studies on this fraction.

Amino Acids of Globulins. The globulin fraction of amaranth has significantly higher ($p < 0.05$) concentrations of ASP, SER, GLU, GLY, ARG, and LYS than soybean globulins, while soybean has higher concentrations of ($p < 0.05$) VAL, ILE, and LEU than amaranth

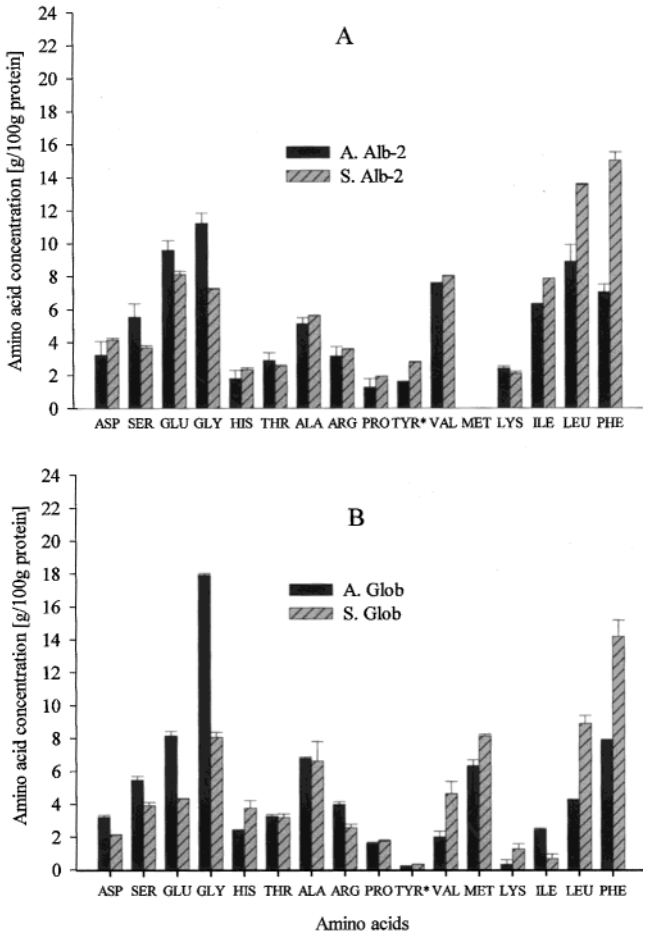

Figure 2. Amino acid concentration of (A) amaranth (A.Alb-2.Os) and soybean albumins-2: (S.Alb-2.Os) and (B) amaranth (A.Glo.Os) and soybean (S.Glo.Os) globulins. Bars indicate standard error. * = TYR + CYS.

globulins (Figure 2B). The essential amino acid concentration in soybean globulins is 62% higher than amaranth globulins.

Comparison between Albumins-2 and Globulins. The albumin-2 fractions of both amaranth and soybean have high concentrations of the essential amino acids VAL, ILE, and LEU (Figure 2A and 2B). Other authors have also found high amounts of VAL in amaranth albumin (*6*). Globulins of amaranth and soybean have high concentrations of LEU and PHE and differ from albumin-2 by high MET concentrations (Figure 2A and 2B).

Comparison of amino acid concentrations of A.Alb-1.Os, A.Alb-1.K, A.Alb-2.Os, and A.Alb-2.K fractions extracted by two different methods was done. The fraction of A.Alb-1.Os (data not shown) had a similar amino acid concentration as A.Alb-1.K (Figure 1) extracted according to Konishi et al. (*5*). A.Alb-1.K had about 52% more GLY than A.Alb-1.Os, while the total of essential amino acids in A.Alb-1.Os was 14% higher than in A.Alb-1.K. The higher amount of VAL (73%) in A.Alb-1.Os than in A.Alb-1.K partly explains the higher amount of essential amino acids in A.Alb-1.Os.

A.Alb-2.Os (data not shown) had 53% more GLY, but 15% less of total essential amino acids than A.Alb-2.K (Figure 1A). Both methods, Osborne (*10*) and Konishi et al. (*5*), show similar concentrations for other analyzed amino acids.

In terms of essential amino acids, soybean fractions and A.Alb-1.Os were relatively rich in lysine, valine, and

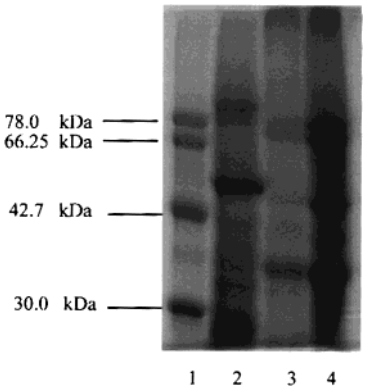

Figure 3. SDS−PAGE of soybean proteins; 1 = standard, 2 = S.Alb-1.Os, 3 = S.Alb-2.Os, 4 = S.Glo.Os.

leucine than A.Alb-2.Os and A.Alb-1.K. Threonine was the highest for Alb-1.Os, but all the fractions had very small variations in their amount. Alb-2.Os were relatively low in the amounts of valine (2.32 times), leucine, and lysine (1.7 times) than Alb-1.Os. The amounts of histidine, threonine, and phenylalanine differ from Alb-2.Os. Glycine amounts were twice as high in Alb-2 in comparison with Alb-1. Alb-1.Os had lower amounts of valine, leucine, and lysine than Alb-1.K, but the other essential amino acids were similar in their values. All amaranth fractions and subfractions were higher than the corresponding fraction of soybean in histidine, threonine, and phenylalanine.

SDS−PAGE. Electrophoresis showed that Glo.Os and Alb-2.Os contained similar major bands in the range of 36−38 kDa. Albumins and globulins of soybean showed some patterns with two major bands at 20 and 38 kDa and another two at 50 and 70 kDa with less material. Alb-2.Os of soybean differed from Alb-1.Os and contained less protein in the three subunits of 20, 38, and 50 kDa than Alb-1.Os. The Alb-2.Os fraction, which was extracted from amaranth, had less protein than in the soybean fraction. The main group of albumins has a molecular mass below 18 kDa, and the second has a mass between 45 and 50 kDa. Electrophoretic patterns of albumin-2 fractions showed that the main protein subunits were concentrated in (kDa): 34.2 for amaranth and 36.4 for soybean. The electrophoretic separation of these plants showed similarity. Variations in protein separation of amaranth and soybean Alb-2.Os were not found in major fractions, only in minor bands; therefore, the amaranth bands are not shown.

S.Alb-1.Os shows a main band with low molecular mass under 30 kDa as well as a band with a molecular mass above 42 kDa and a third band with a high molecular mass above 78 kDa (Figure 3). The SDS−PAGE of S.Alb-2 is more similar to S.Glo.Os than to S.Alb-1.Os. S.Alb-1.Os shows a main band of 36.35 kDa, which is also present in S.Glo.Os but in a more intensive way (Figure 3). S.Glo.Os also shows two other main bands with a molecular mass of 42 and above 66 kDa.

Amino Acids of SDS−PAGE Band of Albumin 2. The analyzed electrophoresis bands of amaranth and soybean, with a molecular mass of 34.2 and 36.4 kDa, respectively, is in reach of GLY and the sulfur-containing amino acid CYS (Figure 4). The sum of the essential amino acids in this band is 38 and 17 g/100 g of proteins in amaranth and soybean, respectively. The analyzed band of soybean has a lower nutritional value than of the total essential amino acid concentration (Figures 1 and 2). The albumin-2 band of soybean with a molecular

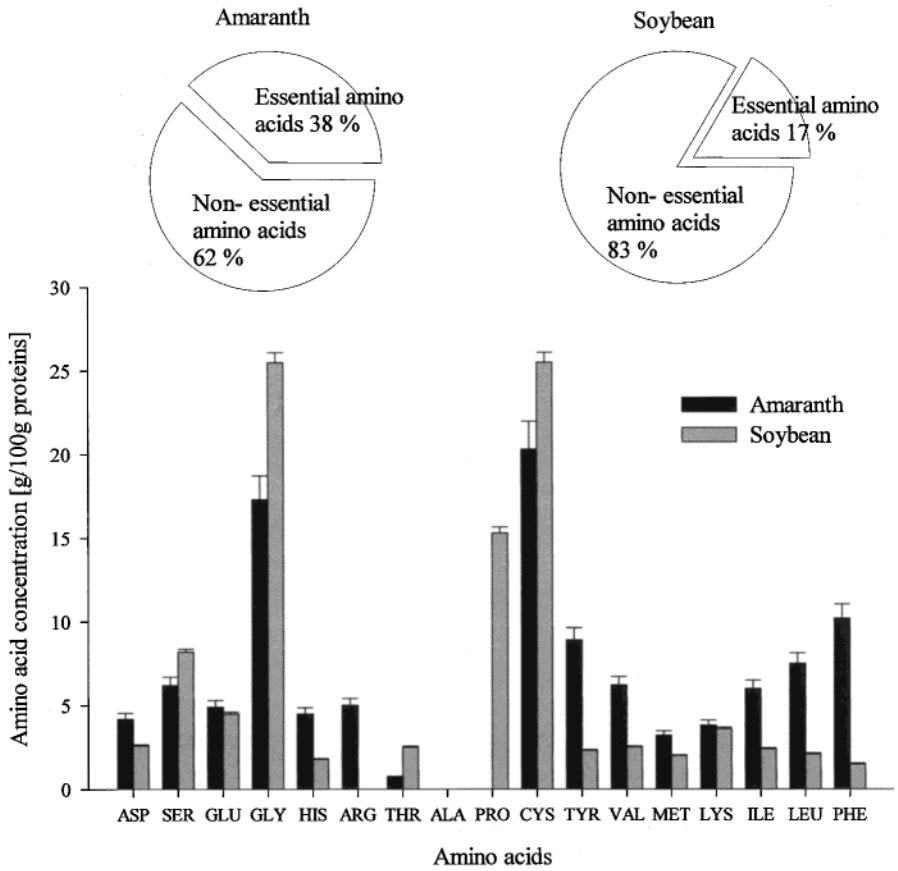

Figure 4. Amino acid concentration and total essential and nonessential amino acids of electrophoresis band at 34.2 and 36.4 kDa from A.Alb-2.Os and S.Alb-2.Os., respectively. Bars indicate standard error.

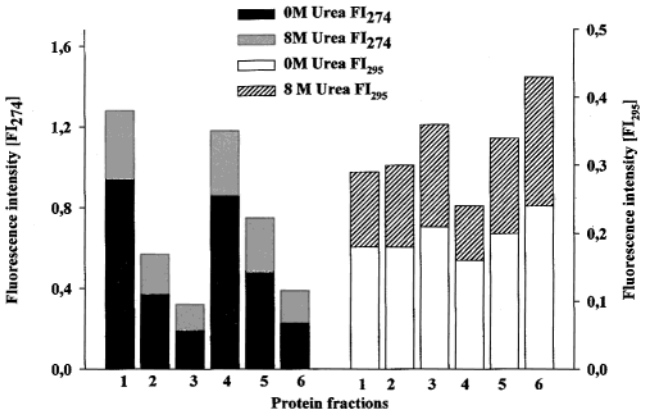

Figure 5. Fluorescence properties at different wavelengths of native and denatured (8 M urea) of amaranth (A) and soybean (S) proteins. 1 = A.Alb-1.Os; 2 = A.Alb-2.Os; 3 = A.Glo.Os; 4 = S.Alb-1.Os; 5 = S.Alb-2.Os; 6 = S.Glo.Os.

mass of about 36 kDa does not seem to be responsible for the high nutritional value of soybean. Albumin-2 can be important due to its high content of CYS in amaranth and soybean and its high PHE concentration in amaranth in the analyzed bands.

Fluorescence Spectra. Fluorescence spectra of Glo.Os, Alb-1.Os, and Alb-2.Os demonstrated peaks (nm) at 338, 346, and 351, respectively. It means that tryptophan residues are situated closer to the surface of the molecule in the case of albumins (Alb-1 and Alb-2) and consistent with the less compact and more hydrophobic structure in comparison with Glo. At λ excitation = 274 nm, very slight shoulders were seen only in Glo (λ emission = 308 nm; $I = 0.19$) and in Alb-2 (λ emission = 308.5 nm, $I = 0.37$) which was evidence of tyrosine. At λ excitation = 295 nm, tyrosine was not shown. Fluorescence measurements showed decreasing fluorescence intensity and a shift in the maximum of emission (*6*) reflecting unfolding of these proteins with urea (Figure 5). Fluorescence intensity at 295 nm for A.Glo+8M urea was 0.15 in comparison with 0.12 for A.Alb-2+ 8M urea. On the basis of measured and calculated data of all fractions and subfractions, the percentage of denaturation for Glo, Alb-1, and Alb-2 proteins with 8 M urea was 28.6, 52.2, and 33.3%, respectively. When the concentration of urea increased, the fluorescence intensity decreased gradually. Probably the distance between the tryptophan and the tyrosine residues was increased. The difference in the extent of denaturation between the protein fractions may be explained by the differences in the amounts of amino acids and by the sulfur bridges existing in such proteins.

Urea induces transition by interfering with the hydrophobic regions in the interior as well with the hydrogen-bonding pattern involved in the polar regions of the peptide chain in the protein molecule (*4*, *26*, *27*).

Surface Hydrophobicity. Hydrophobicity properties of native and denatured amaranth and soybean fractions Glo.Os, Alb-1.Os, and Alb-2.Os are shown in Figure 6. Surface hydrophobicity was the highest for Alb-1. Alb-2 had a higher value than Glo. Glo has more compact tertiary structure and less hydrophobic surface than albumins. The increase in surface hydrophobicity was correlated with the increase in the extent of protein denaturation (55.2 and 50% for amaranth and soybean, respectively). It can be explained by the altered (Figure 6), partially unfolded globulin and albumin fractions.

CD Spectra. Unfolding of albumins and globulin is a result of promoted interactions between exposed

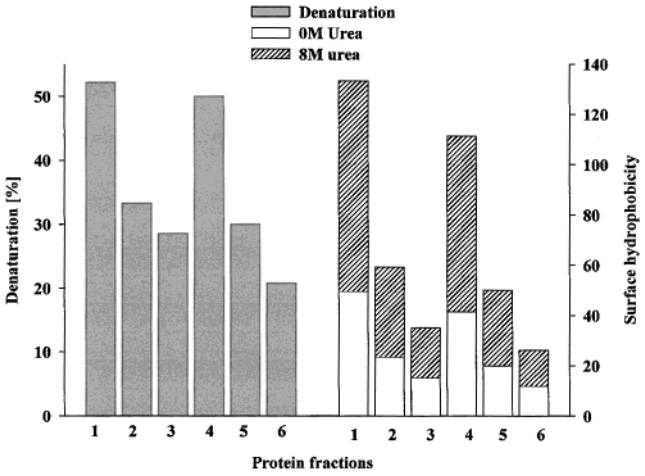

Figure 6. Dependence of surface hydrophobicity of amaranth (A) and soybean (S) proteins on the percent of their denaturation. 1 = A.Alb-1.Os; 2 = A.Alb-2.Os; 3 = A.Glo.Os; 4 = S.Alb-1.Os; 5 = S.Alb-2.Os; 6 = S.Glo.Os.

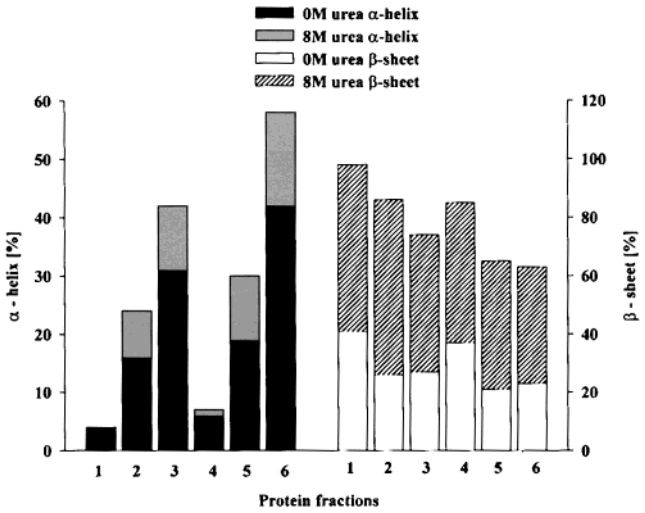

Figure 7. Effect of urea on the secondary structure composition of amaranth (A) and soybean (S) proteins. 1 = A.Alb-1.Os; 2 = A.Alb-2.Os; 3 = A.Glo.Os; 4 = S.Alb-1.Os; 5 = S.Alb-2.Os; 6 = S.Glo.Os.

functional groups, which involve transconformations of α-helix, β-sheet, and aperiodic structure. Denaturation increased β-sheet content and sacrificed α-helix content (Figure 7).

For soybean proteins, similar data were obtained, especially in denatured form of the protein fractions: β-sheet content and in the sacrifice of α-helix. The CD profiles of proteins also typified that of a protein having α-helix with minimum near 206 nm with nonstable structure due to the nonpresence of the disulfide cross-links.

As can be seen from the data observed for all protein samples, the denaturation was evident at 8 M urea. Our results are in agreement with others, which are matched in the gelling and functional behavior of the plant protein (*28*, *29*). These observations are in agreement with Marcone and Yada (*1*) and our previous results (*7*), which have explained this by existing polymeric species and partly as a result of sulfhydryl disulfide interchange reaction.

DSC Measurements of Albumins and Globulins. The native structure of proteins from amaranth and soybean was stable up to a critical temperature and then disrupted with intense heat absorption (Table 2).

Table 2. Thermodynamic Properties[a] of Native and Denatured Amaranth (A) and Soybean (S) Proteins: A.Glo.Os, A.Alb-1.Os, and A.Alb-2.Os and S.Glo.Os, S.Alb-1.Os, and S.Alb-2.Os

proteins	T_d[b] (°C)	ΔH[c] (kcal/mol)	ΔS[d] (kcal/mol K)	n[e]	%D[f]
A.Glo.Os	79 ± 6.8	172.7 ± 16.1	0.490	41	0
A.Glo.Os + U[g]	70 ± 6.2	118.7 ± 10.3	0.346	29	29.3
A.Alb-1.Os	59 ± 5.1	77.6 ± 7.2	0.234	20	0
A.Alb-1.Os + U	48 ± 4.4	32.2 ± 2.9	0.105	9	55.0
A.Alb-2.Os	77 ± 6.7	159.8 ± 14.7	0.457	38	0
A.Alb-2.Os +U	67 ± 5.9	106.4 ± 9.0	0.313	26	31.6
S.Glo.Os	83 ± 7.8	193.3 ± 18.1	0.542	45	0
S.Glo.Os + U	74 ± 6.6	146.4 ± 13.2	0.422	35	22.2
S.Alb-1.Os	64 ± 5.5	83.1 ± 7.7	0.247	21	0
S.Alb-1.Os +U	55 ± 4.8	38.7 ± 3.1	0.118	10	52.4
S.Alb-2.Os	80 ± 6.9	183.0 ± 17.1	0.518	43	0
S.Alb-2.Os +U	71 ± 6.0	124.2 ± 11.7	0.361	30	30.2

[a] Mean values of triplicates ± standard deviation. [b] Temperature of denaturation. [c] Enthalpy. [d] Entropy. [e] Number of broken hydrogen bonds. [f] Denaturation. [g] Urea (1:1).

Disordering of the system takes place upon heating. Comparison of the thermograms of native fractions from amaranth and soybean showed not only the difference in the temperature of denaturation and the enthalpy but also the broadening of the peak. Broadening of the peak with slight shift in T_d to a lower value as well as a decrease in ΔH indicates denaturation and less stable structure. A considerable number of molecules shift to a state that contributes much less to the unfolding transition, thus causing a significant decrease in the calorimetric enthalpy. The enthalpy changes of the initial and remaining DSC endotherm were measured and used for calculation of percent of denatured proteins (Table 2). The entropy (S) values, which are associated with state transition and affirmed disordering of protein structure, were also calculated (Table 2). Our recent (*6*) and present results (Table 2) agreed with others in that broadening of peaks indicates the existence of intermediate forms different from the native one (*28*, *29*). Thermal protein denaturation involves the rupture of disulfide bonds (which contributes a $\Delta H = 25$ kcal/mol and a negligible ΔS) and of n hydrogen bonds ($\Delta H = 4$ kcal/mol and $\Delta S = 0.012$ kcal mol^{-1} (protein molecule)$^{-1}$). Other interactions such as electrostatic and hydrophobic have not been calculated. Thus, the number of broken hydrogen bonds can be calculated as $n = \Delta S/0.012$ and $n = (\Delta H - 25)/4$, where $n =$ number of broken hydrogen bonds; ΔS is the entropy and ΔH is enthalpy of denaturation (*30*). Our calculations have shown that denaturation of native amaranth globulins involves the rupture of 41 hydrogen bonds in comparison with 45 for soybean, 38 for A.Alb-2, and 43 for S.Alb-2 (Table 2). We assume that probably during thermal denaturation only the rupture of hydrogen bonds is involved, since the presence of reducing agents such as 2-ME did not affect DSC characteristics, suggesting that disulfide bonds present in globulin polypeptides do not contribute to the thermal response of the protein. These results are in agreement with others based on the influence of hydrogen bonds disruption and enthalpy changes in DSC (*30*). Disulfide bonds and hydrophobic interactions contribute mainly to the stability of globulin gels. These results are in agreement with our data about the soybean globulins, which have shown the intermediate stability between those of rice and sesame globulins (*31*). DSC measurements of mixed globulins from soybean indicated one transition between 74 and 95 °C, with a maximum at 86.2 °C as reported by Arntfield and Murray (*4*), Bora et al. (*32*), and Gorinstein et al. (*7*). Gluten proteins showed a lower temperature of denaturation than amaranth (88.4 °C). For S.Glo.Os (Table 2), the maximum of denaturation was at 83 °C. Our results were close to those who stated that soybean globulins 7 S and 11 S showed 76 and 97 °C denaturation, respectively. Soybean 11 S globulins have a higher temperature of denaturation than amaranth globulins (*29*). The thermal stabilities of soybean and amaranth globulins and albumins-2 can be explained by the hydrophobic type of interaction between the subunits (*29*). The strength of such hydrophobic-type forces increases with the temperature (*33*). The low denaturation temperatures of our samples (Table 2) probably characterize the behavior of extracted proteins using a different sequence of solvents. Globulins have a more stable structure than albumins as demonstrated by their higher temperature of denaturation. Our results are in agreement with the authors of ref *28* who showed that oat albumin has an 87 °C denaturation andglobulin has a 110 °C denaturation. The percent of thermal denaturation in albumins and globulins showed similar changes in protein denaturation, which was characterized by fluorescence (Figure 5 and Table 2). The number of hydrogen bonds (n) ruptured during the process was used for the determination of the degree of denaturation (%D).

With previous urea-induced denaturation, the number of hydrogen bonds was reduced to about 30−50%. This trend is associated with the disruption of hydrogen bonds during heat denaturation and reflects a decrease in the α-helix content of denatured protein (*34*), which was shown in CD measurements (Figure 7). Hence, hydrogen bonding is the main stabilizing force in protein stability. Addition of protein denaturants such as urea led to a decrease in enthalpy and T_d, indicating protein denaturation and loss of cooperativity.

In this paper, systematic information on the changes in conformation, which takes place in protein solutions and dry state upon denaturation with urea was obtained for soluble protein fractions of amaranth and soybean. A new application of DSC to determine the structural stability in dry-heated solids of plant proteins is proposed.

Progressive unfolding in proteins was measured as a function of fluorescent light intensity, peak response, and shift in the maximum of emission. Amino acid analysis of the extracted fractions and their comparison has shown that the investigated plants have a high nutritional value. Amaranth and soybean were analyzed for protein content. Albumin subfractions and globulin were isolated and separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS−PAGE). The comparison was done by the obtained electrophoretic patterns and their amino acid analysis. Electrophoretic patterns of extracted proteins showed that the main protein subunits were concentrated between 10 and 45 kDa. A similarity was found between amaranth and soybean protein fractions, but the amount of essential amino acid concentration of total proteins was higher in soybean than in amaranth. The amino acid composition of pseudocereals showed that these plants can be used as a substitute for some cereals.

The thermodynamic data associated with transition were calculated and the number of bonds broken during denaturation was determined. Amaranth proteins may be an important option to proteins from soybean in view that the former macromolecules have various nutritional and functional properties as good as, or better than, the latter ones. In summary, thermal denatur-

ation parameters, S_o, and the content of secondary structure have shown that Glo.Os and Alb-2.Os showed similar conformational changes and degree of denaturation suggesting that Alb-2.Os is also a storage protein as Glo.Os.

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