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Physicochemical characterization of the structural stability of some plant globulins

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The fluorescence properties of amaranth, soybean, rice, sorghum and maize globulins, and cassava globulin-like proteins were measured as a function of fluorescent light intensity, peak response and shift in the maximum of emission using the fluorescence of tryptophan at 295 nm. Application of differential scanning calorimetry (DSC) of these globulins gave a quantitative estimation of their thermal stabilities in solid state. The thermodynamic data associated with transition and the number of ruptured hydrogen bonds were calculated. Differences in secondary structure and α-helical content were observed. Relative structural stabilities of native plant globulins were also estimated by X-ray diffractometry and Fourier transform infrared (FT–IR) measurements. Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

The nutritional quality of plant seed storage proteins depends mainly on the amount of essential amino acids and on their digestibility (Gorinstein *et al.*, 1991; Paredes-López *et al.*, 1994; Segura-Nieto *et al.*, 1994). Globulins represent the main storage proteins in legumes and oats (Peterson, 1978; Borroto & Dure, 1987; Wright, 1987), tubers (Gorinstein *et al.*, 1988) and amaranth (Gorinstein *et al.*, 1991; Paredes-López *et al.*, 1994; Segura-Nieto *et al.*, 1994). Glutelins are normally the most abundant storage proteins in rice, as prolamins are in sorghum and maize (Rodrigues, 1991; Wu, 1994). The use of these proteins is based on their functional properties such as emulsification, solubility and foaming abilities (Kinsella & Phillips, 1989). It was shown by Konishi & Yoshimoto (1989) and Marcone & Yada (1992) that amaranth globulins, as well as some other proteins (Voutsina *et al.*, 1983; Utsumi *et al.*, 1984; Wang & Damodaran, 1991), have the properties of heat stable emulsifiers. Corn–soybean meal diet with optimum dietary proportions of tryptophan and lysine is widely used for animal feed, based mostly on the globulin fractions of these plants (Yu *et al.*, 1993). Content of globulins show wide variations in plants; amaranth contains 14%, soybean 18%, rice 2%, sorghum 5%, maize 10% and cassava 0.2% of globulins (Landry & Moureaux, 1970; Gorinstein *et al.*, 1988, 1991; El-Khalifa & El-Tinay, 1994; Paredes-López *et al.*, 1994; Segura-Nieto *et al.*, 1994; Yuno-Ohta *et al.*, 1994). It is difficult to follow the conformational changes in food systems of such large proteins as globulins (Arntfield *et al.*, 1987). Conformational changes in proteins widely used in food systems and pharmacy have been characterized for fababean, amaranth and quinoa globulins, and vicilin and ovalbumin by differential scanning calorimetry (DSC), circular dichroism (CD) and intrinsic fluorescence (IF) (Arntfield & Murray, 1981; Arntfield *et al.*, 1987; Zemser *et al.*, 1994; Gruen *et al.*, 1987; Gorinstein *et al.*, 1995). There are investigations showing comparison between soybean, rice, oats, sorghum and amaranth in relation to purification, nutritional, structural and functional properties (Krishnan *et al.*, 1992; Gorinstein, 1993; El-Khalifa & El-Tinay, 1994; Marcone *et al.*, 1994; Segura-Nieto *et al.*, 1994). However, there is a lack of information on the structural stability of such proteins. Our recent study (Gorinstein *et al.*, 1996) was focused on the

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denaturant-induced secondary and tertiary structural changes of amaranth globulins (A–G) as followed by measurements of fluorescence intensity and wavelength of the peak output response and circular dichroism.

This work reports a new application of DSC to determine thermal stability of dry-heated solid plant globulins. X-ray diffraction, Fourier transform infrared (FT–IR) and circular dichroism spectroscopy (CD) were also applied in this study.

MATERIALS AND METHODS

Sample preparation

Whole mature seeds of amaranth [*Amaranthus hypochondriacus* (*A. hyp.*)], soybean, cultivated and wild rices (*Oryza sativa* and *Oryza rufipogon*), sorghum with low tannin content, maize pipoca-flint and roots of wild cassava (*Manioc, manihot esculenta*), respectively, were investigated (Hiromoto & Vello, 1986; Rodrigues, 1991). Seeds of all cereals and plants were ground on a mill with a 60-mesh screen and defatted in a Soxhlet extractor with *n*-hexane for 10 h. Roots of cassava were cut, lyophilized and also milled. The meal was stored at 4°C after removal of *n*-hexane. Plant globulin fractions were isolated with the same sequence of solvents as used in Landry & Moureaux (1970). One gramme of the meal was extracted with a solvent/sample ratio of 10/1(/v/w) and vigorously shaken. The extracts were centrifuged at 10 000*g* for 10 min. Each step was repeated twice. Albumins (Alb) and globulins (G) were extracted with 0.5 M NaCl. Then globulins were separated from albumins using dialysis against H_2O at 4°C for 72 h and then freeze-dried. All protein solutions were prepared from freeze-dried samples in 0.01 M phosphate buffer, 0.4 M NaCl and 0.02% sodium azide, pH 7.2. Protein assays were performed by the Lowry method (Lowry *et al.*, 1951).

Amino acid analysis

Analysis of globulins was done by the procedure of Spackman *et al.* (1958). Freeze-dried samples were hydrolysed with 6 N HCl in sealed tubes for 66 h at 110°C with and without previous oxidation with performic acid. The vacuum-dried hydrolysate was analysed and applied on a Beckman 120C automatic amino acid analyser. For tryptophan determination, samples were hydrolysed with 4 N LiOH for different periods such as 20, 24, 28, 36 and 40 h at 110°C followed by treatment with 6 N HCl for 22 h at the same temperature (Lucas & Sotelo, 1980).

Fluorescence

Fluorescence measurements were carried out using a Model FP-770 Jasco-Spectrofluorometer. The sample temperature was 30°C and the protein concentration was 0.015%, which corresponded to the absorbance less than 0.1 in a 1 cm path length to receive a linear increase in a relative fluorescence intensity. Absorbance values were determined using a Uvikon 930 spectrophotometer. Fluorescence emission spectra of all proteins were measured at excitation wavelengths (nm) of 274 and 295 and recorded over the frequency range from the excitation wavelength to a wavelength of 450 nm. All data were determined in triplicate for each experimental condition (Zemser *et al.*, 1994).

Circular dichroism spectra (CD)

CD spectra were measured with a Jasco J-600 spectropolarimeter (Japan Spectroscopic Co. Ltd, Japan) using a 1 cm quartz cell at room temperature under constant nitrogen purge. Solutions (0.03 mg/ml) of globulins were prepared by dissolving the lyophilized powder in 0.01 M phosphate buffer, 0.4 M NaCl and 0.02% sodium azide, pH 7.2. The absorbances of all solutions were kept below 1.0 (Matsuura & Manning, 1994; Zemser *et al.*, 1994). CD spectra represent an average of eight scans collected in 0.2 nm steps at a rate of 20 nm/min over the wavelength range 180–250 nm of far-UV (FUV). CD spectra were baseline-corrected, and the data are presented as the mean residue ellipticities (Θ). Helical content of globular globulins was estimated with the following two equations:

$$\% \ \alpha\text{-helix} = \frac{(-[\Theta]_{222\,\mathrm{nm}} - 2340)}{(30300 - 2340)} \times 100 \qquad (1)$$

$$\% \ \alpha\text{-helix} = \frac{(-[\Theta]_{208\,\mathrm{nm}} - 4000)}{(33000 - 4000)} \times 100 \qquad (2)$$

where $\Theta_{222\,nm}$ and $\Theta_{208\,nm}$ are residue ellipticities at 222 and 208 nm, respectively (Chen *et al.*, 1972; Yang *et al.*, 1986).

Differential scanning calorimetry (DSC)

The thermal denaturation of globulins was studied with a Perkin-Elmer DSC System 4. Lyophilized samples (about 1 mg) were sealed in aluminium pans. As a reference an empty pan was used. The scanning temperature was 30–120°C at a heating rate of 10°/min. Indium standards were used for temperature and energy calibrations. T_d and ΔH were calculated from the thermograms (Gorinstein *et al.*, 1995).

Fourier transform infrared (FT–IR) measurements

A Perkin-Elmer 2000 FTIR spectrometer was used to record IR spectra. The samples for measurements were prepared from granulated protein, and the pellets were pressed applying 10 000 kg/cm^2 for 15 s.

X-ray diffraction

X-ray diffractograms of native plant proteins were recorded by a Rigaku (MAX-IIIA, Rigaku Keisoku

Co,) powder X-ray diffractometer, using the method of Hizukuri (1978). The X-ray, CuKα irradiation was performed with a monochrometer. The operating conditions were as follow: voltage 35kV, current 25mA, angles 2Θ–3° to 2Θ–40°, scanning speed 1°/min, chart speed 5 mm/min, time constant 1 s and count rate 2 kpcs. Samples were densely packed between a two glass plates using an aluminium frame. Values of intensities were read from the curves over the angular range 4–30° which includes most of the crystalline peaks. Per cent of crystallinity was determined by an integral method. d spacings were computed by Bragg's law, using $\lambda = 2d \sin \Theta$ (where λ is the wavelength of the X-ray beam = 1.5405 Å, d is the spacing between unit cell edges of the specific crystal to be studied and Θ is the angle of diffraction). The quantitative measurement of crystallinity was undertaken according to Nara *et al.* (1978). Each point of minimum intensity on the X-ray diffractograms of proteins was joined by a smooth curve. The upper region under the most prominent peaks in proteins was the area of 100% crystalline fraction.

RESULTS AND DISCUSSION

Amino acid composition of globulins

Table 1 contains results of amino acid analysis. Special treatment was used to prevent the destruction of tryptophan (Lucas & Sotelo, 1980). The data of other amino acids were omitted because the fluorescence measurements were based only on tryptophan, tyrosine and phenylalanine content. The amount of tryptophan in investigated samples varied from 0.3 to 1.3. In all globulins tyrosine was higher than the amount of tryptophan and phenylalanine, except in the amaranth sample.

Intrinsic fluorescence properties of globulins

According to the content of tryptophan, tyrosine and phenylalanine found in globulins (Table 1), it can be expected that at least two emission peaks are detected. Khan *et al.* (1980) showed that tyrosine has a lower extinction coefficient than tryptophan, and also that the energy transfer shows the dominance of the tryptophan fluorescence response. The presence of even one tryptophan residue in globulins gives a spectrum with a single peak of tryptophan emission in the interval 331–342 nm at both excitation wavelengths of 274 and 295 nm (Arntfield *et al.*, 1987). It means that excitation at 274 or 295 nm results in the emission spectra of native globulins with a single emission peak. Replacement of the excitation wavelength of 274 nm by 295 nm resulted in a decrease of relative fluorescence intensity that coincided with a decrease in tryptophan emission and almost complete absence of tyrosine fluorescence (Khan *et al.*, 1980). Tryptophan appears to be the only aromatic amino acid to absorb light at this radiation wavelength. At the excitation wavelength of 295 nm (Fig. 1B) the spectra were typical for tryptophan content, but the peak of amaranth globulins was displaced to a longer wavelength (341.5 nm) and demonstrated a more polar environment for the tryptophan residues. The fluorescence intensity of amaranth (Fig. 1A,a) is much higher than for sorghum and cassava (Fig. 1A,d and Fig. 1A,e) and similar to that for rice. The spectra of rice, soybean, sorghum and cassava showed an emission peak maximum for the range 333.5–335 nm. The fluorescence intensity at 274 nm of soybean, sorghum, rice, amaranth ad cassava globulins was 0.68, 0.35, 0.84, 0.90 and 0.31, respectively, showing the highest intensity for amaranth (Fig. 1A). But the fluorescence intensity at 295 nm was different compared with the previous one, showing, for soybean, sorghum, rice, amaranth and cassava, values of 0.54, 0.18, 0.72, 0.36 and 0.23, respectively, with the highest value for rice. Amaranth proteins may show remarkably high contents of phenylalanine plus tyrosine (Fig. 1A), whereas rice proteins appear to contain high levels of tryptophan (Fig. 1B,b). At an excitation wavelength of 274 nm, shoulders at approximately 309 nm were seen in globulins from sorghum (Fig. 1A,d) and cassava (Fig. 1A,e) which is evidence of tyrosine. At λ excitation of 295 nm, tyrosine is not shown.

Figure 2 shows the fluorescence spectra of *Oryza sativa* and *Oryza rufipogon*. At an excitation wavelength of 274 nm, peaks for *Oryza sativa* and *Oryza rufipogon* (Fig. 2A,a and Fig. 2A,b) had the same position but a difference in tyrosine intensity. The sample of cultivated rice had more tyrosine than the wild sample. At 295 nm, peaks were typical for tryptophan (Fig. 2B,a and Fig. 2B,b) with more tryptophan in *Oryza sativa.* For the wild rice a shoulder appeared at 400 nm, probably corresponding to the tyrosine residues in this sample.

CD studies

The CD spectra of plant globulins in the far ultraviolet (FUV) region are shown in Fig. 3. It has been shown that polypeptide chains can assume only a limited number of stable structures. The α-helix, the interchain hydrogen bonded β-structure and a fully extended parallel or antiparallel arrangement of peptide chains were reported (Chen *et al.*, 1972; Matsuura & Manning,

Table 1. Amino acid composition of plant (g amino acid/100 g protein)

Amino acid	Amaranth	Soybean	Rice	Sorghum	Cassava
Tryptophan	0.6	1.0	1.3	0.8	0.3
Tyrosine	2.9	3.1	5.0	2.1	4.0
Phenylalanine	4.5	1.9	3.3	1.7	1.1

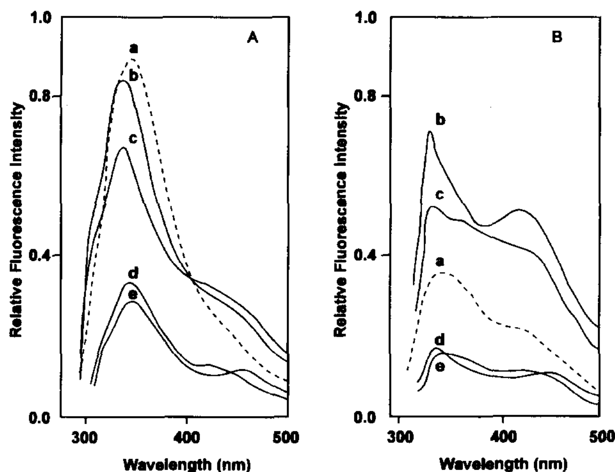

Fig. 1. Fluorescence emission spectra of globulins from a = amaranth, b = rice, c = soybean, d = sorghum and e = cassava. Excitations at (A) 274 and (B) 295 nm, respectively.

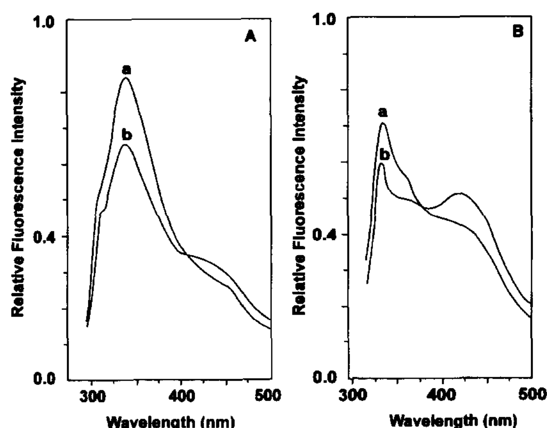

Fig. 2. Fluorescence emission spectra of globulins from a = *Oryza sativa* and b = *Oryza rufipogon*. Excitations at (A) 274 and (B) 295 nm, respectively.

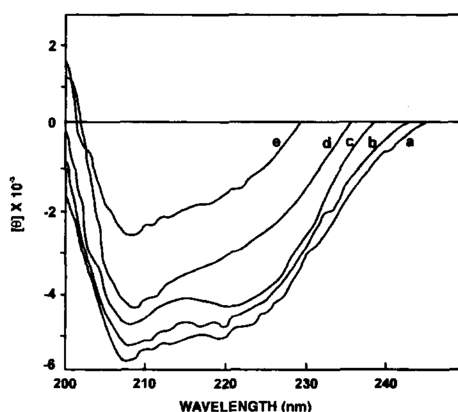

Fig. 3. Circular dichroism spectra of globulins. Far-UV from a = cultivated rice, b = maize, c = amaranth, d = soybean and e = sorghum.

1994). Optical activity of α-helix in FUV permits the use of CD studies for the investigation of conformational changes in protein solutions, and the CD band position for various structures has been reported (Sarkar & Doty, 1966; Chen *et al.*, 1972). Globulins from amaranth, soybean, sorghum, maize and rice (Table 2 and Fig. 3) showed a band with minimum at 208 nm, and that for cassava at 216 nm (Table 2), which represents the β-structure of the protein. The corresponding mean residue ellipticity (Θ) was $-1285\,\text{deg}\,\text{cm}^2\,\text{dmol}^{-1}$ (Table 2). If it is assumed that the molecular ellipticity for 100% β-structure is $-23\,000\,\text{deg}\,\text{cm}^2\,\text{dmol}^{-1}$ at 216 nm (Sarkar & Doty, 1966), the proportion of this structure in amaranth globulins is estimated to be 6% as was shown in our previous work (Gorinstein *et al.*, 1996). These results are in correspondence with those of Lilley (1986) who showed that conglutin $\delta 1$ and $\delta 2$ of lupins are rich in α-helix, consisting of about 38%, and 31.4% in amaranth. Crude globulins, which are a mixture of 7S and 11S globulins, probably represent the total amount of α-helix and β-sheet. Soybean globulins also showed a slightly higher amount of α-helix than amaranth. It can also be explained by the purity of soybean sample, which contains the 7S and 11S oligomers. Sorghum, maize and rice showed a low percentage of the α-helical structure (Table 2). The cultivated and wild rices showed a typical α-helix pattern, but the CD spectrum for the wild rice was not as smooth as for the cultivated one, corresponding to different residue ellipticities (not shown). The CD profile for amaranth globulins was typical of a protein having considerable α-helix, with a minimum near 208 nm, having a stable structure owing to the presence of the disulphide crosslinks, which is in agreement with Lilley (1986) and Marcone & Yada (1992). The difference in CD spectra of these globulins in comparison with results of Marcone & Yada (1992) can be explained by the purity of the sample (crude globulins) as well as by the difference in pH of the buffers used for CD measurements.

DSC measurements of globulins

The DSC method has been extensively used to study unfolding in the liquid state, being highly sensitive to conformational changes. The DSC scans for plant globulins are shown in Fig. 4. The native structure of globulins from amaranth, rice, soybean, sorghum and cassava was stable up to a critical temperature and then disrupted with intense heat absorption (Fig. 4 and Table 3). Disordering of the system takes place upon heating. A considerable number of globulin molecules shifts 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 per cent of denatured globulin. The entropy (S) values which are associated with state transition and affirmed disordering of protein structure were also calculated (Table 3). Comparison of the thermograms of native globulins from amaranth, rice, soybean, sorghum and cassava (Fig. 4) showed not only the difference in the temperature of denaturation and the enthalpy, but also broadening of the peak. Broadening

Table 2. Secondary structure composition (H-, β- and -R) of globulins estimated from CD

Proteins	Form	λ (nm)	[Θ] (deg cm^2 dmol^{-1})	Percentage
Amaranth	H	208	−13 110	31.4
Soybean	H	208	−15 546	39.8
Sorghum	H	208	−5343	4.6
Maize	H	208	−5947	6.7
Rice	H	208	−4087	3.0
Cassava	ß	216	−1285	5.6

H = α-helix; β = β-sheet; R = disordered structure; λ,wavelength; [Θ], residue ellipticity.

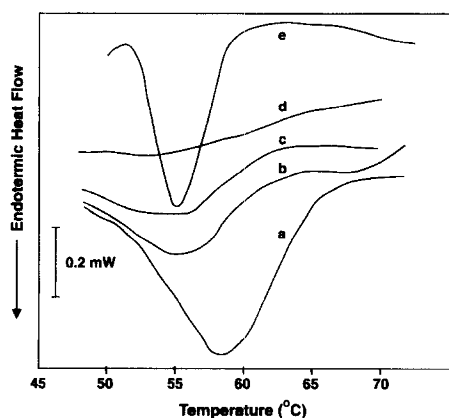

Fig. 4. DSC thermograms of salt-soluble proteins from a = cultivated rice, b = sorghum, c = amaranth, d = cassava and

of the peak with slight shift in T_d to a lower value, as well as a decrease in ΔH, indicates denaturation and a less stable structure (Fig. 4,c and Fig. 4,d). It has been well documented (Wang & Damodaran, 1991; Nagano *et al.*, 1994) that broadening of peaks indicates the existence of intermediate forms different from the native one. This means that the conformation of the protein molecule has shifted towards the unfolded state, which is associated with the disruption of hydrogen bonds during heat denaturation, and reflects a decrease in α-helix content of denatured protein. Kato *et al.* (1987) showed the dependence of intensity ratio of the amide II band to the amide I band on the α-helix content. The influence of hydrogen bond disruption on enthalpy changes in DSC was also reported by Wagner & Añon (1985). According to them thermal protein denaturation involves the rupture of one disulphide bond (which contributes a ΔH of 25 kcal/mol and a negligible ΔS) and of n hydrogen bonds ($\Delta H = 4$ kcal/mol and $\Delta S = 0.012$ kcal/mol per protein molecule). Thus, the number of broken hydrogen bonds can be calculated as: $n = \Delta S/0.012$ and $n = (\Delta H - 25)/4$, where n is the number of broken hydrogen bonds; ΔS is entropy and ΔH is enthalpy of denaturation. Our calculations have shown that denaturation of native amaranth globulins involves the rupture of 19 hydrogen bonds in comparison with 80 for rice, 50 for soybean, 29 for sorghum and 14 for cassava. We assume that, 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 disulphide bonds present in globulin polypeptides do not contribute to thermal response of the protein. It was shown (Yuno-Ohta *et al.*, 1994) that rice globulin gels were intermediate between those of gels of soybean and sesame globulins. Disulphide bonds and hydrophobic interactions contributed mainly to the stability of rice globulin gels; the contribution of disulphide bonds to both the formation and stability were greater than for sesame globulin gels (Yuno-Ohta *et al.*, 1994). These results are in agreement with our data. 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 & Murray (1981) and Bora *et al.* (1994). Wheat gluten proteins showed a lower temperature of denaturation than amaranth (88.4°C). They found that soybean globulins 7S and 11S showed denaturation at 76 and 97°C, respectively; soybean 11S globulins, have a higher temperature of denaturation than amaranth globulins (Nagano *et al.*, 1994). The thermal stabilities of globulins, especially of soybean and amaranth, can be explained by the hydrophobic type of interaction between the subunits

Table 3. Thermodynamic properties of native and denatured globulins

Proteins	T_d (°C)	ΔH (kcal/mol)	ΔS (kcal/mol K)	n
Rice	60.1	320.51	0.962	80
Soybean	56.0	197.90	0.602	50
Sorghum	56.7	115.08	0.349	29
Amaranth	59.0	75.30	0.227	19
Cassava	50.6	54.85	0.169	14

T_d = temperature of denaturation; ΔH = enthalpy; ΔS = entropy; n = number of broken hydrogen bonds.

(Nagano *et al.*, 1994). The strength of such hydrophobic type forces increases with the temperature (Konishi & Yoshimoto, 1989). The low denaturation temperatures of our samples (Fig. 4 and Table 3) probably characterize the behaviour of crude salt-soluble proteins, which were a mixture of albumins and globulins. Globulins have a more stable structure than albumins as demonstrated by their higher temperature of denaturation; oat albumin has 87°C as its temperature of denaturation and globulin 110°C (Wang & Damodaran, 1991).

Our data show that DSC can be used to study the effect of medium composition and heating on the tertiary and quaternary structures. Some of these treatments (heating, pH adjustment, salt addition) are required in food processing where proteins are present as major ingredients. Since the functional properties of proteins are greatly influenced by their conformation, DSC is a valuable tool in assessing the potential of globulins as functional ingredients in different food systems (Bora *et al.*, 1994).

FT–IR spectrometry

Kaiden *et al.* (1987) applied infrared (IR) spectroscopy for the study of the secondary structure of a living protein. On the basis of this investigation, IR spectra of globulins were obtained to analyse the differences in these proteins. These changes arise from the nature of proteins from cereals and other plants were observed by the changes in the amide I, II and III bands (Ker *et al.*, 1993). We assigned the broad band in the 1300–1250 cm^{-1} region to α-helix, the relatively sharp band in 1240–1230 cm^{-1} region to β-sheet and a broad, medium intensity band in the 1270–1240 cm^{-1} region to a disordered structure. All plant globulins showed similar bands at 3300 cm^{-1} (amino acid peak) and at 2900 cm^{-1} (-CH_2 stretching). Amide I, II and II bands in the range of 1630–1660, 1510–1540 and 1310–1235 cm^{-1}, respectively, differ for various plant globulins. Displacement of bands position and absence of amide III bands can be seen in most of the investigated samples, but cassava did have an amide III band. Globulin spectra of cassava showed alterations in the 1313–1235 cm^{-1} region (i.e. the amide III band). A clear sharp band at 1235 cm^{-1}, corresponding to the β-sheet, became broader and smaller in the cassava sample, which can be attributed to the fact that the β-sheet, is the main ordered structure of this globulin. The intensities of the amide I and II bands were very similar in sorghum, maize and rice samples, indicating that the α-helix content of these globulins is lower in comparison with amaranth and soybean, where the intensities of the amide I and II bands were as much as double. In spectra of these globulins the band at 1515 cm^{-1} which is associated with the β-sheet or random structure is not shown. The absence of the amide III band in amaranth, soybean, sorghum, rice and maize showed that these globulins are mostly composed of the α-helix as the main ordered structure (Kato *et al.*, 1987). These results are in agreement with our recent data from circular dichroism (Gorinstein *et al.*, 1996).

X-ray diffractometry

Soybean, amaranth and sorghum native globulins showed two peaks at 3.12 and 3.23 Å (Table 4). The intensities of these peaks were different (Fig. 5). The relative crystallinity was determined taking into consideration these two peaks. The crystallinities of samples a, b and c (Table 4) were, respectively, 100, 94 and 84%. From the results shown in Table 4 and Fig. 5, it may be concluded that the crystallinity of globulins is connected with the degree of denaturation. The larger enthalpy of denaturation, determined by DSC, corresponds to the larger crystallinity of the sample.

In summary, soybean (legume), amaranth (pseudocereal), rice, maize and sorghum (cereals), and cassava (tuber) were screened for variability of crude globulins. Amino acid composition of maize globulins in tryptophan, tyrosine and phenylalanine, as well as intrinsic

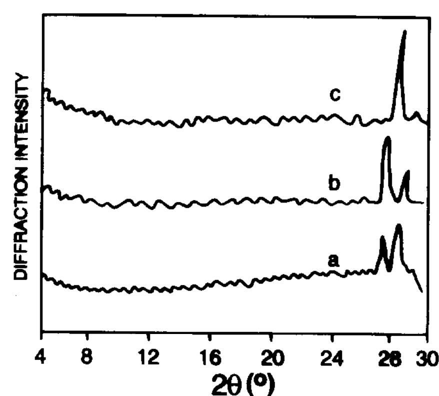

Fig. 5. X-ray diffraction patterns of plant globulins: a = soybean, b = amaranth and c = sorghum.

Table 4. X-ray diffraction spacings in plant globulins

Number	Proteins	Interplanar spacings ***d*** (Å): very strong (vs), strong(s); medium (m) and weak (w) intensities	Relative crystallinity(%)
a	Soybean	3.23(m); 3.12(vs)	100
b	Amaranth	3.23(vs); 3.12(m)	94
c	Sorghum	3.23(vs); 3.12(w)	84

fluorescence properties, thermal stability and X-ray patterns were similar to sorghum; therefore these data were omitted. Globulins from amaranth and soybean contained more α-helix and less random coil structures than other investigated samples. In both intrinsic fluorescence and CD spectra, differences in the amount of amino acid and α-helical content for cultivated and wild rices were detected.

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