Sunflower Seed Total Proteins: Effect of Dry and Wet Heating

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Proteins in sunflower seeds, heat treated (dry and autoclaved), show changes in physicochemical properties. Changes include the ultraviolet absorption spectrum, specifically at 324 nm due to bound chlorogenic acid. Wet heating under steam pressure at 2 kg/cm² causes protein aggregation, as shown by polyacrylamide gel electrophoresis. Sedimentation velocity experiments show that dry heating at 150 °C and wet heating at 2 kg/cm² increase low molecular weight components (2S) while decreasing the percentage of high molecular weight proteins (11S). The gel filtration profiles of proteins from seeds treated at high temperatures (autoclaving at 2 kg/cm² and dry roasting at 150 °C) also show an increase in the percentage of low molecular weight proteins. Heat-treated total proteins are less susceptible to proteolysis by trypsin and chymotrypsin.

INTRODUCTION

Sunflower seed has been shown to be an excellent source of vegetable protein. During oil extraction, by screw press, the seeds are subjected to varying conditions of temperature and pressure to facilitate the release of oil (Altschul and Wilcke, 1985). It is well-known that temperature affects the physical, chemical, and functional properties of the proteins of the seed (Kinsella et al., 1985). The effect of heat, both wet and dry, results in changes in temperature and pressure of the system and can affect properties such as nitrogen solubility and extractability of different protein fractions and the interaction of polyphenols with the proteins (Venktesh and Prakash, 1993a-c).

Hence it was the aim of the present study to investigate the effect of temperature on the proteins and follow the changes that take place. The gel filtration pattern, amino acid composition, sedimentation velocity profile, polyacrylamide gel electrophoresis pattern, ultraviolet absorption spectra, fluorescence emission spectra, and rate of hydrolysis by proteolytic enzymes as a function of temperature both in the control protein and in the heat-treated protein are reported.

MATERIALS AND METHODS

Sunflower seeds of the variety *Helianthus annuus* L. were procured from National Seed Corp., Mysore, India. Chemicals used were from the following sources: acrylamide, bis(acrylamide) (*N,N*-methylenediacrylamide); TEMED (*N,N,N',N'*-tetramethylethylenediamine), trypsin, hemoglobin, α-chymotrypsin, Coomassie brilliant blue R-250, and chlorogenic acid, Sigma Chemical Co., St. Louis, MO; Sepharose 6B-100, Pharmacia Fine Chemicals, Uppsala, Sweden; boromophenol blue and sucrose, Koch Light Laboratories, England; ammonium persulfate, E. Merck, Darmstadt, Germany; calcium chloride, BDH, Bombay, India; sodium hydroxide, Astra IDL, Bangalore, India; dialysis tubings, Thomas Scientific Co., Swedesboro, NJ.

Methods. *Treatment of Seeds (Dry and Wet Heating).* Whole sunflower seeds were graded, cleaned, and divided into five batches. One batch, with no treatment, served as control. Two batches were roasted in a rotary roaster at 100 and 150 °C (from Ms. Bharat Co. India) and designated R₁ and R₂, respectively. The times taken for the seeds to reach the specified temperatures were 20 and 25 min, respectively. The seeds were held at the peak temperature for 5 min and then cooled by aeration. The remaining two batches were wet-heated by autoclaving at 1 and 2 kg/cm² at 121 and 135 °C, respectively,

for 25 min in a Conred Engelke, Hannover-Limmer, autoclave. They were designated A₁ and A₂, respectively. The seeds were cooled and dried at 50 °C for 3 h, in an Armstrong Smithe (India) cabinet dryer for further processing.

Preparation of Flour. All five batches of sunflower seeds were separately dehulled in a centrifugal sheller with aspirator. They were flaked in a rice flaker and solvent extracted with eight washings of *n*-hexane. The grits were then dried in a cabinet dryer at 45 °C for 4 h and powdered in a Brabender Quadramat-Senior automatic pilot mill to obtain flour of 150-μm size. The fresh flour thus prepared was used for all further analyses.

Proximate Analysis. a. Estimation. Moisture, fat, and nitrogen contents in the flour were determined according to the AOAC method (AOAC, 1984). Carbohydrate was estimated according to the method of Montogomery (1961) with glucose as standard. A conversion factor of 5.7 was used to calculate protein from nitrogen.

b. Chlorogenic Acid (CGA). The method of Pomenta and Burns (1971) was used. Chlorogenic acid was used as standard, in the concentration range 100-600 μg. The concentration of CGA in the sample was obtained from the standard graph and expressed as percent CGA. The content of CGA was also monitored by its absorbance at 324 nm.

c. Absorption Spectra. The absorption spectra of the proteins were recorded by a Beckman DU-8B recording spectrophotometer in the range 240-350 nm in a 1 cm path length quartz cell at 27 °C. Absorption coefficients of protein were determined by estimating nitrogen content (AOAC, 1984) and absorbance at 280 nm of a series of protein solutions of various concentrations. The absorption coefficient of a 1% protein solution was obtained from a plot of absorbance vs nitrogen content.

d. Gel Chromatography. Gel filtration of the total proteins was performed on Sepharose 6B-100 gel, packed into a 2 × 90 cm column. The packed column was equilibrated with phosphate buffer, pH 6.0, 0.02 M, containing 1 M NaCl with a flow rate of 20 mL/h. The absorbance was monitored both at 280 nm for proteins and at 324 nm for CGA in a Shimadzu UV-150-02 double-beam spectrophotometer.

e. Fluorescence Spectra. The fluorescence excitation and emission spectra of the proteins were recorded in a Shimadzu RF 5000 series spectrofluorometer at 27 °C. Protein solutions having an absorbance in the range 0.1-0.15 at 280 nm were used for the fluorescence measurements. The excitation spectra were recorded from 200 to 300 nm with emission at 330 nm, and the emission spectra were measured in the range 300-380 nm with excitation fixed at 280 nm. All measurements of fluorescence were monitored 10 s after excitation.

f. Polyacrylamide Gel Electrophoresis (PAGE). PAGE was performed with 10% acrylamide/0.4% methylene bis(acrylamide) as the cross-linking agent in 0.04 M phosphate buffer, pH 6.0. After a prerun of 30 min in the buffer, protein samples of 10-100 μg of protein concentration containing sucrose (15%) and

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Table I. Percent Composition of Moisture, Protein, Nonprotein Nitrogen (NPN), Residual Fat, Carbohydrates, and Polyphenols (Estimated as Free Chlorogenic Acid) for Various Samples of Sunflower Seed, Control and Heat Treated, Defatted Samples

sample[a]	moisture, %	protein, %	NPN, %	residual fat, %	carbohydrates, %	polyphenols, %
C	8 ± 0.5	49 ± 1	7.3 ± 0.2	3.4 ± 0.2	13.6 ± 0.9	3.80 ± 0.10
R_1	7 ± 0.3	49 ± 1	6.9 ± 0.1	2.2 ± 0.1	10.8 ± 0.8	3.80 ± 0.08
R_2	3 ± 0.1	48 ± 1	7.5 ± 0.2	1.2 ± 0.1	6.4 ± 0.3	1.95 ± 0.02
A_1	11 ± 0.5	46 ± 1	7.6 ± 0.2	1.8 ± 0.2	10.4 ± 0.5	3.10 ± 0.06
A_2	10 ± 0.4	47 ± 1	7.9 ± 0.3	3.2 ± 0.2	10.0 ± 0.6	1.95 ± 0.04

[a] C, control; R_1, roasted at 100 °C; R_2, roasted at 150 °C; A_1, autoclaved at 1 kg/cm2; A_2, autoclaved at 2 kg/cm^2.

bromophenol blue dye as indicator were loaded on the gel. The electrophoresis was performed at a constant current of 6 mA per tube until the dye reached the bottom of the gel. The proteins in the gel were fixed by immersion in 20% trichloroacetic acid for 1 h and stained in 0.1% Coomassie blue R-250. The gel was destained in 0.1% (v/v) acetic acid.

g. Sedimentation Velocity. Analytical ultracentrifugation measurements were performed with a Spinco Model E analytical ultracentrifuge equipped with rotor temperature indicator and control unit and phase plate schlieren optics. A standard 12-mm Kel F cell centerpiece was used. Protein solutions in the range 1–2% concentration were used for the experiments at 59 780 rpm and 27 °C. Photographs were taken at set time intervals. $S_{20,w}$ values were calculated according to the procedure described by Schachman (1959).

h. Amino Acid Analysis. The amino acid composition of the protein was determined in an LKB-ALpha-amino acid analyzer according to the procedure of Moore and Stein (1963). The amino acid composition was calculated from the areas of standards obtained from the integrator and expressed as percentage.

Hydrolysis with Proteolytic Enzymes. a. Trypsin. To 1 mL of a 1% protein solution in 0.1 M sodium phosphate buffer of pH 7.6 was added and mixed 1 mL of trypsin solution of predetermined concentration (100 µg/mL) in standardized 0.001 N HCl. Both solutions were preincubated at 37 °C. After mixing, the solution was incubated at 37 °C for different time intervals in the range 2–30 min. Separate aliquots were withdrawn for each time interval, and the reaction was stopped by adding 2 mL of a 20% TCA solution. The solutions were allowed to stand for 10 min and then centrifuged at 6000g, and the absorbance of the supernatant was measured at 280 nm. The blank was prepared by adding TCA solution in the beginning followed by 1 mL of trypsin solution and centrifugation at 6000g. The absorbance of the supernatant was recorded in a Shimadzu 150-UV-02 double-beam spectrophotometer.

b. α-Chymotrypsin. The hydrolysis with α-chymotrypsin was done in 0.1 M borate buffer of pH 7.8 containing 0.05 M CaCl₂. The experimental protocol followed was the same as for trypsin. The concentration of enzyme used was 100 µg/mL. A standard with hemoglobin was also done for both trypsin and α-chymotrypsin at predetermined concentrations (10 µg/mL) of enzyme.

RESULTS

The composition of the control and heat-treated defatted sunflower seed flours is given in Table I. R_2 has a low moisture level (3%) but in A_2 it is high (10%), probably because of high temperature and equilibrium moisture in these two treatments. The protein content of the control sample is 49 ± 1%. The value remains constant for all of the heat-treated samples, since it arises from the same source of control seeds being processed to different extents. The nonprotein nitrogen value is in the range 6.9–7.9%. The origin of nonprotein nitrogen in these oilseed proteins is generally free amino acids, peptides, and other nitrogen-containing components such as color (Prakash and Narasinga Rao, 1986). The residual fat is highest in the control samples. The lowest content of residual fat was observed in the 150 °C (R_2) treated sample (Table I). The high temperature during dry roasting conditions loosens the structure of the cells, leading to better accessibility of the solvent to the cell matrix, thus facilitating the extraction

Table II. Percent Nitrogen Solubility of Sunflower Seed, Control and Heat Treated, Defatted Flours in Water at pH 6 and in 1 M NaCl at pH 5.4[a]

sample	% nitrogen soluble in water (pH 6)	% nitrogen soluble in 1 M NaCl (pH 5.4)
control	15 ± 1.0	46 ± 2.0
roasted at 100 °C	12 ± 0.7	25 ± 1.8
roasted at 150 °C	11 ± 0.7	13 ± 1.9
autoclaved at 1 kg/cm²	10 ± 1.0	24 ± 2.0
autoclaved at 2 kg/cm²	10 ± 1.0	15 ± 1.0

[a] The error bars are the standard deviation of three samples.

of fat. On the other hand, in A_2, there is probably sufficient emulsification which may prevent the extraction of oil by the solvent. The percentage of carbohydrate has a very large variation from 13.6 to 6.4% in the control and the R_2 sample, respectively. This large change in carbohydrate content may reflect the effect of heat on the solubilization, gelatinization, protein carbohydrate interaction in sunflower seeds, and degradation of carbohydrate in the samples both in the presence and in the absence of moisture at high temperature (Bjarnason and Carpenter, 1970).

The free CGA content estimated as polyphenols in the control flour is 3.8 ± 0.1%. At elevated temperatures of 150 °C and autoclaved conditions of 2 kg/cm², the amount of polyphenols is nearly halved as compared to the control (1.95 ± 0.02%). Such decreases in free CGA content can be attributed to the interaction of the ligand with the various protein fractions at elevated temperatures. The R_1 and A_1 samples showed a decrease of about 25% free polyphenol (3 ± 0.08 and 3.1 ± 0.06%, respectively) as compared to that of the control.

The nitrogen solubility profiles of the control and treated samples in both water and 1 M NaCl at different pHs indicate a decrease in solubility as well as broadening of the solubility profile near the acidic pH region as the temperature and pressure are raised (Venktesh and Prakash, 1993a–c). The solubility values at ph 6 (Bio pH) for water and pH 5.4 for 1 M NaCl for all samples are shown in Table II. The data show that the proteins extracted in water do not vary significantly among the samples, whereas the salt-soluble proteins are affected by roasting at 150 °C and autoclaving at 2 kg/cm².

The amino acid composition of the five samples (control, R_1, R_2, A_1, and A_2) are shown in Table III. The control protein correlates with the amino acid composition of sunflower seed total proteins as reported (Schwenke et al., 1974; Youle and Huang, 1981). The protein is rich in glutamic and aspartic acids and some of the hydrophobic amino acids such as leucine, isoleucine, phenylalanine, and methionine. After different heat treatments, significant changes can be noted in the content of lysine with decreases in both samples R_2 and A_2. Also, there is a marginal decrease in the tyrosine and methionine content in these two samples as compared to C, R_1, or A_1. The ultraviolet absorption spectra of the sunflower seeds, control and heat processed, are shown in Figure 1. There are marked differences between the spectrum of the control

Table III. Amino Acid Analysis (Mole Percent) of Sunflower Seed Total Proteins of Control and Differently Treated Samples[a]

amino acid	C	R₁	R₂	A₁	A₂
lysine	2.93 ± 0.08	2.83	2.41	2.88	2.60
histidine	2.72 ± 0.10	2.38	2.24	3.07	2.99
arginine	7.80 ± 0.30	7.82	6.81	7.77	7.43
aspartic acid	10.30 ± 0.08	10.02	10.22	10.11	10.41
threonine	3.70 ± 0.06	3.65	3.29	3.25	3.62
serine	5.63 ± 0.09	5.24	5.85	5.79	6.57
glumatic acid	19.32 ± 0.10	18.96	17.63	19.50	19.40
proline	5.13 ± 0.18	5.55	4.23	5.22	3.96
glycine	8.10 ± 0.19	8.20	8.23	8.14	8.50
alanine	7.59 ± 0.20	7.80	7.17	8.06	7.42
valine	5.96 ± 0.21	5.80	5.74	5.78	4.46
methionine	2.14 ± 0.09	2.01	1.88	1.86	1.98
isoleucine	4.22 ± 0.20	4.00	3.14	4.13	4.67
leucine	6.02 ± 0.23	6.27	6.57	6.50	6.29
tyrosine	1.90 ± 0.18	1.65	1.44	1.42	1.17
phenylalanine	4.15 ± 0.14	3.93	3.64	3.59	4.05

[a] Average of three amino acid analyses. C, control; R₁, roasted at 100 °C; R₂, roasted at 150 °C; A₁, autoclaved at 1 kg/cm²; A₂, autoclaved at 2 kg/cm².
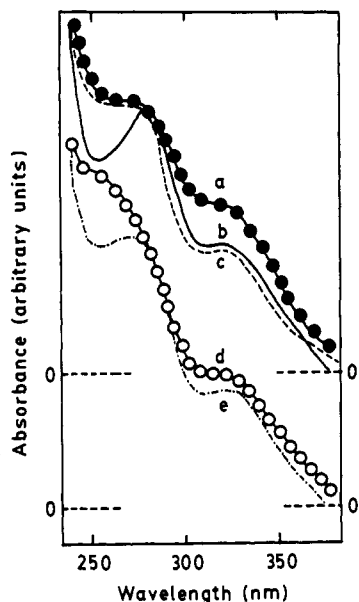

Figure 1. Ultraviolet spectrum of sunflower seed total proteins: (a) untreated, (b) roasted at 100 °C, (c) roasted at 150 °C, (d) autoclaved at 1 kg/cm², and (e) autoclaved at 2 kg/cm². All of the spectra were obtained at pH 6.0 phosphate buffer, 0.02 M, containing 1 M NaCl. The absorbances of the solutions were fixed to the same value at 280 nm for the purpose of comparison. Spectra d and e are arbitrarily shifted down on the Y axis for clarity.

and the spectra of various heat-processed samples. In Figure 2 is shown the fluorescence emission spectra of the control and the heat-treated samples. A₁ and R₁ samples show nearly 22 and 38% quenching, respectively, as compared to the control, whereas A₂ and R₂ samples show broader and nearly 73% quenching. This suggests that the exposure of the tryptophan residues takes place in the higher temperature treated samples as a result of denaturation. In Figure 3A is shown the polyacrylamide gel electrophoretic patterns of the control and heat-treated samples from where the proteins were extracted. The autoclaved samples, A₁ and A₂ shown in Figure 3Ad,e, indicate lesser numbers of protein bands as compared to C, R₁, or R₂ (Figure 3Aa–c). The absence of very fast moving components in these gels is indicative of the effect of heating in producing soluble aggregates either from the dissociated or from the denatured proteins.
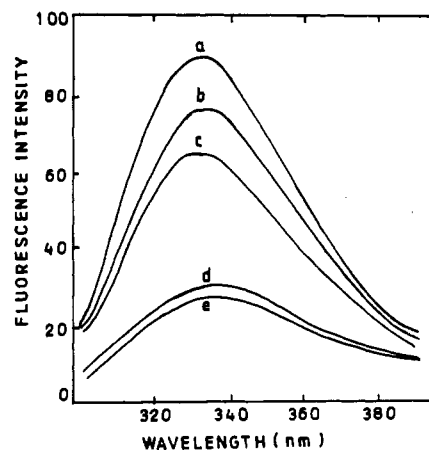

Figure 2. Fluorescence emission spectrum of total proteins of sunflower seed in pH 6.0 phosphate buffer, 0.02 M, containing 1 M NaCl. The excitation wavelength was 280 nm, and the protein concentration was maintained at 0.0075%. The fluorescence spectra were measured at least 10 s after excitation of the samples. The samples are (a) total proteins from the untreated seeds, (b) autoclaved at 1 kg/cm², (c) roasted at 100 °C, (d) autoclaved at 2 kg/cm², and (e) roasted at 150 °C.

These results indicate that there is dissociation and, to a certain extent, aggregation (as in the autoclaved samples) of the total proteins of sunflower seed heat-treated samples as compared to the control.

The sedimentation velocity pattern of control protein in pH 6.0 phosphate buffer, 0.02 M, in the presence of 1 M sodium chloride, has four components of sedimentation coefficients 17, 11, 7, and 2, the percentages of which are 2, 60, 5, and 30, respectively (Figure 3Ba, upper). Joubert (1955) reported that the total proteins of sunflower are 18.1S, 7.8S, and 1.7S. Schwenke et al. (1975) have reported that the 7S component could be a dissociation product of the 11S protein and the 17S protein an aggregate. In Figure 3B is shown the effect of roasting at 100 and 150 °C and autoclaving at 1 and 2 kg/cm². Roasting at 100 °C increases the 2S protein fraction from nearly 35 to 48% with a concomitant decrease in the 11S fraction (Figure 3Ba, lower). With further drastic roasting at 150 °C the 2S fraction increases to nearly 75% with only 15% of 11S being present in the system (Figure 3Bb). On the other hand, A₁ did not show any significant change either in sedimentation coefficient or in the percent fraction of the different components (Figure 3Bc, lower). In Figure 3Bc (upper) is shown the velocity sedimentation profile of the A₂ sample. The percent fraction of 2S indicates nearly twice the concentration compared to the control on the basis of total protein concentration in the sedimentation velocity run, with less than 25% of 11S being present in the system.

These results clearly indicate a certain hierarchy in the degree of effectiveness of dissociating the sunflower proteins, as measured by velocity sedimentation experiments by the various heat treatments. Empirically, it can be expressed as

$$R_2 > A_2 > R_1 > A_1 > C$$

The total proteins of the control were fractionated into four components, a, b, c, and d, by gel filtration (Figure 4), constituting 12, 44, 27, and 17%, respectively. The second and third components, b and c, are the major constituents. The fourth component had high absorbance at 324 nm.

In R₁ (Figure 4B) there is a marginal increase in the percentage of component b and a significant increase in component c, while in A₁ (Figure 4D) there is only a
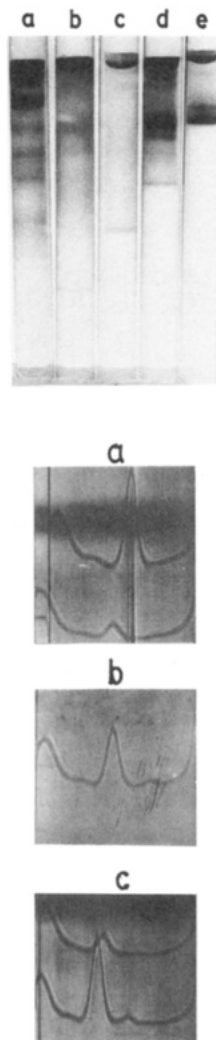
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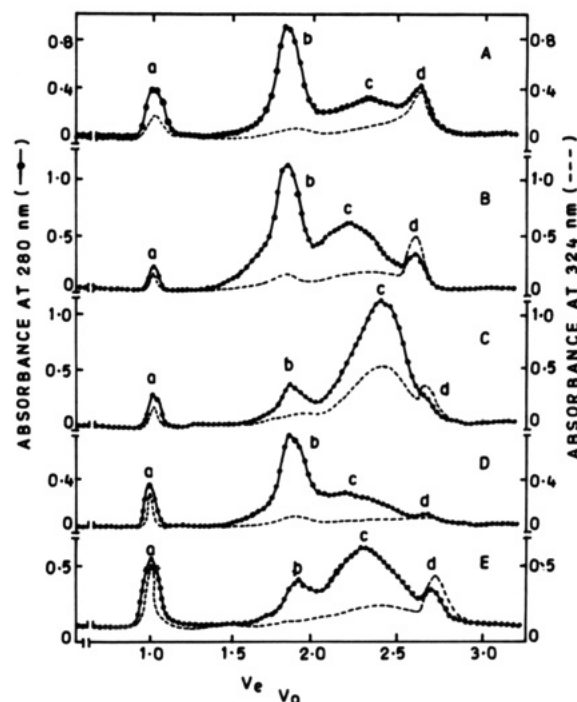
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Figure 4. Gel filtration pattern of sunflower seed total proteins, extracted in pH 6.0 phosphate buffer, 0.02 M, containing 1 M NaCl and dialyzed for 24 h in the same buffer, on a Sepharose 6-B 100 column. One milliliter of sample, containing 40 mg of proteins, was loaded at 27 °C. The absorbance was monitored at both 280 and 324 nm as shown. The data are normalized in terms of elution volume of individual peaks to that of the void volume of the column and are shown on the x-axis as V_e/V_0. The samples are (A) untreated total proteins, (B) roasted at 100 °C, (C) roasted at 150 °C, (D) autoclaved at 1 kg/cm², and (E) autoclaved at 2 kg/cm².

Figure 3. (A) Polyacrylamide gel electrophoresis pattern of sunflower seed total proteins in pH 6.0 phosphate buffer, 0.04 M, at 12% acrylamide concentration with 0.4% bis(acrylamide) as the cross-linking agent. The samples are (a) total proteins from untreated seeds (200 µg), (b) roasted at 100 °C (150 µg), (c) roasted at 150 °C (120 µg), (d) autoclaved at 1 kg/cm² (175 µg), and (e) autoclaved at 2 kg/cm² (120 µg). (B) Sedimentation velocity pattern of sunflower seed total proteins in pH 6.0 phosphate buffer, 0.02 M, containing 1 M NaCl. The sedimentation run was performed at 59 780 rpm at 27 °C, and the sedimentation proceeds from left to right. The time of photograph was 45 min after two-thirds of maximum speed was attained, unless otherwise stated. The bar angle was kept constant at 60°. A protein concentration of 14.5 mg/mL was used, unless stated otherwise. The samples are total proteins (a) upper, from untreated seeds; lower, from seeds roasted at 100 °C (40 min); (b) from seeds roasted at 150 °C; (c) upper, from seeds autoclaved at 2 kg/cm²; lower, from seeds autoclaved at 1 kg/cm² (35 min).
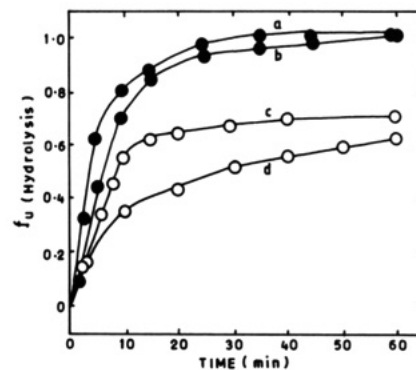
Figure 5. Proteolysis of denatured hemoglobin by (a) trypsin, (b) α-chymotrypsin (●), and sunflower seed total protein at 1% concentration by (c) trypsin and (d) α-chymotrypsin (○) as a function of time at 37 °C. f_u on the y axis is evaluated from the plateau readings of denatured hemoglobin evaluated as 1 and calculating all other values corresponding to it. $f_u = 0$ indicates no hydrolysis in the system.

marginal increase in these components as compared to the control pattern. High temperature, such as in R_2 (Figure 4C) and A_2 (Figure 4E), has a significant affect on the elution pattern of the total proteins. In both cases there is a reduction in the percentage of component b with a concomitant significant increase in the percentage of component c. This could suggest the dissociation of the proteins as a result of heating at elevated temperature. The absorbance at 324 nm also shows an increase along with component c with the heated samples, especially with R_1, R_2, and A_2 as shown in the figure.

The effect of hydrolysis by α-chymotrypsin on the treated and untreated protein samples shows that the extent of hydrolysis of the control proteins by α-chymo-

trypsin is much less than hemoglobin (Figure 5). In Figure 6 is shown the extent of hydrolysis (f_u) of different heat-treated samples, namely R_1, R_2, A_1, and A_2, by α-chymotrypsin (A) and trypsin (B). It can be seen that compared to the denatured hemoglobin and control proteins (Figure 5) the f_u of the heat-treated samples decreases and the greater the heat treatment, the less hydrolysis (Figure 5). The susceptibility to hydrolysis by α-chymotrypsin follows the pattern

$$C > A_1 > A_2 > R_1 > R_2$$

The extent of hydrolysis (f_u) by trypsin on the untreated protein (Figure 5c) is similar to that of α-chymotrypsin
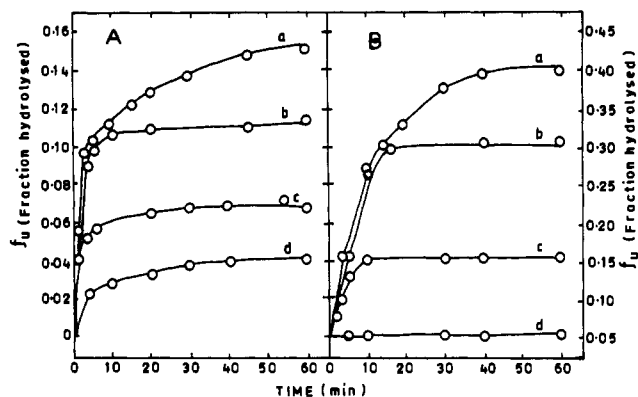
Figure 6. Proteolysis of heat-treated sunflower seed total proteins by (A) α-chymotrypsin and (B) trypsin as a function of time at 37 °C. f_u on the y axis is evaluated from the plateau readings of denatured hemoglobin evaluated as 1 and calculating all other values corresponding to it. $f_u = 0$ indicates no hydrolysis in the system. The samples are for (A) (a) autoclaved at 1 kg/cm², (b) autoclaved at 2 kg/cm², (c) roasted at 100 °C, and (d) roasted at 150 °C and for (B) (a) roasted at 100 °C, (b) roasted at 150 °C, (c) autoclaved at 1 kg/cm², and (d) autoclaved at ⁿ kg/cm². The protein concentration used was 1% in all expt. iments.

except that the plateau region is reached in about 15 min as compared to that of α-chymotrypsin (Figure 5d). Several seed proteins have also been shown to be resistant to trypsin as compared to standard proteins (Prakash and Narasinga Rao, 1986, 1988; Tasneem and Prakash, 1992).

In Figure 6 is shown the extent of hydrolysis (f_u) vs time plot for all proteins which are heat treated in the presence of α-chymotrypsin and trypsin. The proteins are hydrolyzed much more slowly by trypsin as compared to α-chymotrypsin, and f_u reaches a plateau region in about 15–20 min in all samples except in R₂, where it reaches it in 30–35 min. The susceptibility to hydrolysis by trypsin follows the pattern

$$C > R_1 > R_2 > A_1 > A_2$$

DISCUSSION

The effect of different heat treatments on the physicochemical properties of sunflower seed proteins indicates that both dry heating and wet heating alter the nitrogen solubility index, the ultraviolet absorption spectra, the fluorescence emission spectra, and the association–dissociation phenomena. These data show that heat treatment can bring about (a) different amounts of polyphenol interactions with the proteins, (b) changes in the physicochemical properties of the proteins on heating, and (c) varying degrees of association–dissociation and denaturation of proteins.

The absorbance maximum at 324 nm, which is also the absorption maximum of CGA, could be due to the binding of CGA or its oxidized products to the proteins (Prasad, 1988). It has been reported that in aqueous systems the hydrogen bonding between the hydroxyl groups of phenolic compounds and the peptide bond in proteins is strong and the equilibrium strongly favors the formation of complexes between polyphenols and proteins (Loomis and Battaile, 1966). The decrease in absorbance at 324 nm upon mild heating (R₁ and A₂) could be due to the inactivation of polyphenol oxidase. Besides the covalent binding of CGA to protein, through oxidation to o-quinones (chlorogenoquinone and related quinones), other polyphenolic compounds could also react noncovalently with

proteins through hydrogen, ionic, and hydrophobic interactions (Pierpoint, 1969a,b).

The lower percentage of various factions in the sedimentation velocity pattern of the heat-treated samples (A₂ and R₂) as compared to the control need not be only due to dissociation of the protein. It could be a result of (a) denaturation leading to loss of solubility of a particular protein fraction, (b) protein–protein association of the dissociated subunits leading to insoluble aggregates, and (c) chemical interactions at elevated temperatures possibly leading to aggregation of some of the low molecular weight components. Such changes in protein patterns can be differentiated as described by Prakash and Timasheff (1986). These treatments show a heirarchy of dissociation of sunflower seed proteins and agree very well with earlier results as monitored by PAGE, where similar results are shown with wet and dry heating. The gel filtration profiles of A₂ and R₂ indicate the dissociation of the high molecular weight proteins to the low molecular weight proteins which could, under specific conditions, aggregate. The hydrolysis of the total proteins, represented as f_u, where f_u is the fraction of the protein hydrolyzed (under similar concentration condition, taking denatured hemoglobin reaching the plateau region reading as 1), of untreated sunflower seed (control) proteins by α-chymotrypsin and trypsin as compared to that of denatured hemoglobin with the same enzyme indicates a decrease. α-Chymotrypsin does hydrolyze seed proteins to a lesser extent as compared to some of the standard proteins such as casein (Prakash and Narasinga Rao, 1986). Tasneem and Prakash (1992) have shown that the high molecular weight protein fraction of *Sesamum indicum*, α-globulin, is resistant to hydrolysis by proteolytic enzymes as a result of feedback inhibition by the products. In the case of sunflower seed proteins the results show (a) intrinsic resistance to hydrolysis by sunflower seed proteins to the proteolytic enzymes, (b) various heat treatments (both dry and wet) result in protein–protein association and structural changes along with interaction with ligand, and (c) varying degrees of extractability of different protein fractions of sunflower seed proteins.

The resistance to proteolysis of seed proteins can be attributed to several factors: (a) the presence of protease inhibitors, (b) the presence of ligands, (c) the presence of carbohydrate moieties as in glycoproteins, (d) specific structural regions of protein which have a special role to play in the stabilization of protein conformation, and (e) inhibition by hydrolysis products (Sharon and Lis, 1981; Prakash and Narasinga, Rao, 1986, 1988; Tasneem and Prakash, 1992). It is known that aspartic and glutamic acids, alanine, and lysine can partly inhibit the hydrolysis of the proteins (Hayase et al., 1973). The resistance to hydrolysis with proteases could also be due to the presence of trypsin inhibitors. However, the problem of trypsin inhibitors is of less importance in sunflower seeds as compared to soybeans and especially in the heat-treated samples.

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