are glutamic acid, aspartic acid, lysine, leucine, and valine.

The selective fractionation method reported in this paper is simple and thus appears to be suitable for a large-scale plasma fractionation.

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# Effect of Methods To Remove Polyphenols from Sunflower Meal on the Physicochemical Properties of the Proteins

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Sunflower meal was extracted four times with 40% aqueous acetone, 40% aqueous ethanol, or 40% aqueous methanol to remove polyphenols. The flaked kernels were also extracted five times with water (pH 6.0) at 70 °C. With organic solvents the efficiency of polyphenol removal was 95–98% whereas with water it was only 83%. Extraction with water and organic solvents increased the protein content of the meal and decreased the total sugar content. The amino acid composition of the protein did not change. Nitrogen solubility of the extracted meals was lower than that of the unextracted meal in water in the range pH 2–8. However, there was no change in the gel filtration or sedimentation velocity pattern. In polyacrylamide gel electrophoresis, methanol-extracted meal proteins had higher mobility. Between 300 and 400 nm the fluorescence emission intensity of extracted meal proteins was higher than that of the unextracted meal proteins. Extracted meal proteins were hydrolyzed to a greater extent by trypsin/$\alpha$-chymotrypsin/papain than the unextracted meal proteins.

Sunflower (*Helianthus annuus*) is an important source of vegetable oil, and the meal after extraction of oil is a valuable source of proteins (Sosulski and Bakal, 1969). Sunflower proteins have unique organoleptic and functional properties that would make them useful in processed foods (Sosulski, 1979). There are no known toxic constituents and antinutritional factors in sunflower meal (Clandinin, 1958). The major difficulty in the utilization of sunflower meal in human diets is the presence of hulls and polyphenols in the seed. The hulls contribute to high crude fiber content of the meal. The presence of polyphenols causes the meal and the proteins to become colored during alkaline extraction of the proteins (Smith and Johnson, 1948): the polyphenols are colorless at low and neutral pH values and assume greenish yellow to brown color in the range pH 8.0–11.0. They also lower the nutritive value (Jung and Fahey, 1981), since they interact with essential amino acids such as lysine and methionine

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(Pierpoint, 1970), and inhibit proteases (Sastry, 1984). Other pigments in sunflower also contribute to the color (Pomenta and Burns, 1971). The problem of high crude fiber content of the meal can be overcome by dehulling the seed before oil extraction.

The major polyphenols present in sunflower seed are chlorogenic acid (CGA), caffeic acid (CA), and quinic acid (QA). The content of CGA, which forms nearly 70% of the total polyphenols, ranges from 1.42 to 4.0% (Dorrel, 1976). Many methods have been described for the removal of polyphenols from sunflower seed/meal, and all of them involve extraction with the solvents hot 70% ethanol or near-absolute methanol (Smith and Johnson, 1948), 50% ethanol (Joubert, 1955), 80% methanol (Mikalojcak et al., 1970), 80% ethanol (Pomenta and Burns, 1971): diffusion extraction with water and organic solvents (Sosulski et al., 1972); continuous extraction with 0.001 N HCl at 80 °C (Sosulski et al., 1973); countercurrent extraction with water or aqueous ethanol (Fan et al., 1976), butanol–0.005 N HCl (Sodini and Canella, 1977), 4% NaCl solution (Sastry, 1978), or alkali solution containing 0.1% $Na_2SO_3$ (Taha and Nockrashy, 1981). Methods for obtaining light-colored sunflower protein isolate have also been reported: extraction of the protein with 0.25% $Na_2SO_3$ solution followed by precipitation at pH 5.0 (Gheyasuddin et al., 1970), washing of sunflower meal five times with water of pH 4.5 followed by alkali peptization and isoelectric precipitation (O'Connor, 1971), extraction of the broken kernels with 0.2% $Na_2SO_3$ at pH 10.0 followed by acid precipitation (Hagenmaier, 1974), and extraction with water at pH 9.0 followed by isoelectric precipitation (Lawhon et al., 1982).

Sripad et al. (1982) made a systematic study of the extractability of polyphenols in various aqueous–organic solvents as a function of solvent composition, pH, and temperature. In addition to water, methanol, ethanol, 2-propanol, and acetone were used by these investigators. The choice of solvent system was guided by the solubility of the polyphenols in various solvents as reported in the literature. They observed that water adjusted to pH 8 extracted nearly 70% of CGA in a single extraction. Methanol, ethanol, 2-propanol, and acetone at 20–60% concentration in water caused maximum extraction of CGA (~70%) in a single extraction.

There are a number of reports on the effect of polyphenol removal from the sunflower meal on its functional properties (Kilara et al., 1972; Fleming et al., 1974; Lin et al., 1974; Sosulski and Fleming, 1977; Canella, 1978; Rahma and Narasinga Rao, 1981). However, there are no reports on the effect of polyphenol removal on the physicochemical properties of the proteins. We have in this investigation determined the proximate composition of the meal, and the amino acid composition, solubility, gel filtration, gel electrophoresis, sedimentation velocity, fluorescence spectral characteristics, and hydrolysis by proteases of the proteins from the extracted meal. The solvents used for polyphenol removal were 40% aqueous acetone, 40% aqueous ethanol, 40% aqueous methanol, and water (Sripad et al., 1982).

## EXPERIMENTAL SECTION

**Materials.** Sunflower seed, EC 68415, a Russian variety, grown in the State of Karnataka during the season 1979–1980, was obtained from Agro Seed Corp., Mysore, India. It was stored in the cold (~4 °C). The sources of the chemicals used were as follows: CGA, CA, QA, trypsin, α-chymotrypsin, tris(hydroxymethyl)aminomethane (Tris), blue dextran, Sepharose 6B-100, Sigma Chemical Co.; papain, CSIR Centre for Biochemicals; sodium nitrite, ammonium persulfate, amido black, alkali-soluble casein, acid-soluble casein, fluorodinitrobenzene, methyl chloroformate, E. Merck; acrylamide, bisacrylamide, Koch-Light Laboratories; *N,N,N'N'*-tetramethylethylenediamine (TEMED), β-mercaptoethanol, Fluka, A.G. The organic solvents were distilled once before use. All other chemicals and reagents were of analytical reagent grade.

**Methods.** *Preparation of Polyphenol-Free Meals.* Sunflower seeds were dehulled in a centrifugal disk huller (Sastry, 1978), flaked, and extracted with hexane to obtain a defatted meal. It was mixed with 40% acetone, methanol, or ethanol in water at a meal to solvent ratio of 1:10 (w/v) and shaken for 2 h. The extraction, which was done at room temperature (~28 °C), was repeated until the supernatant gave a negative test for CGA. Generally the fourth extract did not give a positive test for CGA when tested by the method of Pomenta and Burns (1971). The slurry was filtered under suction, dried in a current of air at 35 °C, powdered, and passed through 60-mesh sieve (BSS).

Extraction with water (pH 6.0) was carried out on dehulled broken kernels of sunflower seed at 70 °C with a kernel to solvent ratio of 1:10 (w/v). The extraction was done five times, each of 1-h duration. The extract was removed by filtration and the residue dried in an air current at 40 °C, defatted with hexane, powdered, and passed through 60-mesh sieve.

*Protein Content of the Meal.* This was determined by the Kjeldahl nitrogen method. A factor of 6.25 was used to convert nitrogen to protein value.

*Estimation of "Available" Lysine.* This was estimated by the method of Carpenter and Ellinger (1955) using lysine as standard.

*Trypsin Inhibitory Activity.* This was determined by the method of Smith et al. (1980) using $N^{\alpha}$-benzoyl-L-arginine *p*-nitroanilide as substrate.

*Estimation of Total Sugars.* They were estimated by the phenol–sulfuric acid method (Dubois et al., 1956) and are expressed as glucose equivalent.

*Extraction of Total Proteins.* The meal was extracted in 1 M NaCl solution at meal to solvent ratio of 1:10 (w/v) with stirring for 1 h. The slurry was centrifuged at 6000 rpm for 30 min. The supernatant was dialyzed against 1 M NaCl solution or 0.025 M Tris–glycine buffer of pH 8.3 containing 0.5 M NaCl. For PAGE experiments NaCl was omitted from the buffer.

*Estimation of CGA, CA, and QA.* The method of Pomenta and Burns (1971) was used. Pure CGA, CA, and QA were employed as standards.

*Nitrogen Solubility.* A 2-g portion of meal was mixed with 20 mL of water and the pH adjusted to the desired value by the addition of 1 N HCl or 1 N NaOH. After being shaken for 1 h at room temperature, the slurry was centrifuged at 6000 rpm for 30 min and the pH of the supernatant noted. Aliquots of 5.0 mL of the supernatant were taken for nitrogen estimation. The extracted nitrogen was expressed as percentage of the total nitrogen of the meal.

*Amino Acid Analysis.* About 10 mg of the meal was taken in 5 mL of 6 NHCl (electronic grade) and kept in a freezing mixture for 6–8 h. The tube was sealed under vacuum and kept for hydrolysis for 24 h in an air oven maintained at 110 ± 1 °C. After hydrolysis the tube was broken at the tip and HCl removed completely in a flash evaporator around 60 °C by washing the residue five to six times with glass-distilled water. The dried residue was taken in 0.2 M citrate buffer of pH 2.2, and about 40 μL containing 20 μg of the hydrolysate was loaded automat-

**Table I. Protein, Total Sugar, and Total Polyphenol Contents of Sunflower Meals**

| meal | protein, % + MD[a] | total sugars, % | polyphenol, % + MD | % rem polyphenols |
|---|---|---|---|---|
| unextracted | 50.8 ± 0.5 | 16.2 | 3.20 ± 0.1 | |
| 40% aq acetone extr (~28 °C) | 61.5 ± 0.5 | 0.8 | 0.07 ± 0.1 | 98 |
| 40% aq ethanol extr (~28 °C) | 63.5 ± 0.5 | 0.7 | 0.07 ± 0.1 | 98 |
| 40% aq methanol extr (~28 °C) | 58.9 ± 0.5 | 0.9 | 0.17 ± 0.1 | 95 |
| water extr (70 °C) | 54.8 ± 0.5 | 4.2 | 0.54 ± 0.1 | 83 |

[a] MD = mean deviation.

ically. The amino acid composition was directly calculated from the integrator and expressed as a percentage. LKB α-amino acid analyzer equipped with a programmer and integrator was used. Determinations were made in triplicate and average values obtained.

*Gel Filtration.* Sepharose 6B-100 was packed into a column, 1.8 × 100 cm, and equilibrated with 0.025 M Tris–glycine buffer of pH 8.3 containing 0.5 M NaCl. About 5 mL (~125 mg) of protein was loaded on the column and eluted with the same buffer. The eluent (3-mL) fractions were collected in an automatic fraction collector and the absorbances measured at 280 nm since the proteins have maximum absorption at that wavelength.

*Polyacrylamide Gel Electrophoresis (PAGE).* Electrophoresis was carried at pH 8.3 in 0.025 M Tris–glycine buffer using 8% gels. A constant current of 3 mA/tube for 45 min and 60 μg of the protein were used. Bromophenol was used as indicator dye. The gels were stained with 0.5% amido black in 7% acetic acid for 30 min and destained by diffusion with 7% acetic acid for 3–4 days.

*Ultracentrifugation.* The experiments were performed with a Spinco Model E analytical ultracentrifuge equipped with rotor temperature indicator and control (RTIC) unit and phase plate Schlieren optics. The protein solution (1%) in 0.025 M Tris–glycine buffer of pH 8.3 containing 0.5 M NaCl was run at 60 000 rpm. Photographs were taken at different intervals of time and the $S_{20,w}$ values calculated (Schachman, 1959).

*Fluorescence Emission Spectrum.* This was measured on a Perkin-Elmer Model 203 spectrofluorimeter using 0.01% protein solution in buffer. The emission intensity was measured in the range 300–400 nm at room temperature (~28 °C) after excitation at 280 nm.

*Hydrolysis with Proteolytic Enzyme.* The following general procedure was used (Arnon, 1970): To 1 mL of 1% protein solution in the desired buffer was added 1 mL of the enzyme solution. Before addition both were preincubated at 37 °C. After mixing, the solution was incubated at 37 °C for different time intervals in the range 2–30 min, taking a separate aliquot for each time interval. At the end of the time interval, the reaction was stopped by adding 2 mL of 20% TCA solution. The solutions were left for 10 min and centrifuged, and the absorbance of the supernatant was measured at 280 nm. Measurements were also made with 1% casein solution as substrate. Corrections for the appropriate blanks were applied. For measurements with trypsin 0.1 M sodium phosphate buffer of pH 7.6 was used, with α-chymotrypsin 0.1 M borate buffer of pH 7.8 containing 0.005 M $CaCl_2$ and 2% NaCl, and with papain Tris-HCl buffer of pH 8.0 containing 0.005 M cysteine hydrochloride and 0.002 M EDTA.

## RESULTS AND DISCUSSION

The protein, total sugar, and total polyphenol contents of sunflower meal extracted with different solvents are given in Table I. The total polyphenol content of the unextracted meal was 3.2%, a value within the range of values reported for a number of varieties of sunflower (Dorrell, 1976). The efficiency of removal of polyphenols by acetone, ethanol, or methanol was 98% whereas it was only 83% by water extraction. The protein content of the extracted meals increased over that of the unextracted meal whereas the total sugar content decreased. Obviously leaching out of nonprotein constituents and water-soluble sugars increased the protein content. An increase in protein content of sunflower meal due to extraction with solvents has been reported (Sosulski et al., 1973; Lin et al., 1974; Fan et al., 1976; Sodini and Canella, 1977; Rahma and Narasinga Rao, 1981). The amino acid compositions of sunflower meals are given in Table II. The amino acid composition of the unextracted meal protein agrees with the reported values (Rooney et al., 1972; Sabir et al, 1973, 1974; Sosulski and Fleming, 1977; Sodini and Canella, 1977; Bau et al., 1983). There were no significant differences in the amino acid compositions of variously extracted meal proteins compared with that of unextracted meal protein. Sodini and Canella (1977) reported that the amino acid composition of the proteins of acidic butanol extracted sunflower meal was similar to that of the unextracted meal.

**Table II. Amino Acid Composition of Sunflower Meals (g AA/16 g N)**

| amino acid | unextr + MD[a] | extracted with acetone | ethanol | water | methanol |
|---|---|---|---|---|---|
| aspartic acid | 9.03 ± 0.40 | 8.95 | 8.89 | 9.13 | 8.88 |
| threonine | 3.42 ± 0.08 | 3.39 | 3.42 | 3.63 | 3.55 |
| serine | 4.64 ± 0.13 | 4.60 | 4.56 | 4.80 | 4.55 |
| glutamic acid | 20.62 ± 0.68 | 21.05 | 21.89 | 19.77 | 19.76 |
| proline | 3.78 ± 0.37 | 4.07 | 4.29 | 4.29 | 4.26 |
| glycine | 5.46 ± 0.13 | 6.05 | 5.81 | 5.50 | 5.22 |
| alanine | 4.15 ± 0.24 | 3.99 | 4.22 | 4.55 | 4.33 |
| cystine | 1.10 ± 0.10 | 0.97 | 1.09 | 0.94 | 1.00 |
| valine | 3.54 ± 0.41 | 3.51 | 3.53 | 3.63 | 3.77 |
| methionine | 1.71 ± 0.20 | 1.57 | 1.60 | 1.76 | 1.55 |
| isoleucine | 2.89 ± 0.36 | 2.78 | 2.96 | 2.93 | 3.00 |
| leucine | 5.73 ± 0.25 | 5.69 | 5.81 | 5.97 | 5.99 |
| tyrosine | 1.83 ± 0.20 | 2.30 | 2.51 | 2.46 | 2.44 |
| phenylalanine | 4.03 ± 0.35 | 4.24 | 4.33 | 4.45 | 4.32 |
| histidine | 2.20 ± 0.18 | 2.42 | 2.17 | 2.22 | 3.11 |
| lysine | 3.42 ± 0.15 | 3.39 | 3.19 | 3.51 | 3.55 |
| ammonia | 3.17 ± 0.30 | 3.03 | 2.39 | 3.04 | 3.44 |
| arginine | 9.00 ± 0.38 | 8.95 | 8.66 | 8.66 | 8.44 |

[a] MD = mean deviation.

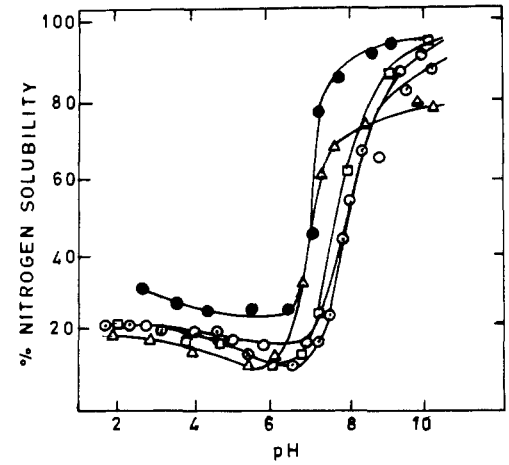


**Figure 1.** Nitrogen solubilities of sunflower meals in water at different pH values: ●, unextracted meal; ○, acetone-extracted meal; □, ethanol-extracted meal; ⊙, water-extracted meal; △, methanol-extracted meal.

**Nitrogen Solubility.** In Figure 1 the nitrogen solu-

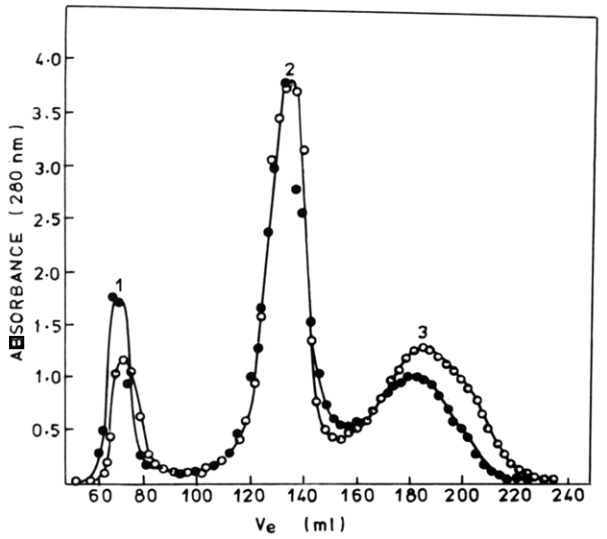


**Figure 2.** Gel filtration patterns of total proteins of sunflower meals (0.025 M Tris–glycine buffer, pH 8.3, containing 0.5 M NaCl): ○, unextracted meal; ●, methanol-extracted meal.

bilities of unextracted and extracted sunflower meals are given as a function of pH in water. The curve represents the average of two determinations. Minimum nitrogen solubility (20–30%) was observed between pH 4.0 and 6.5 and maximum solubility (80–95%) at pH 10. The data with the unextracted meal agree with the reported values (Smith and Johnson, 1948; Cater et al., 1972; Gheyasuddin et al., 1970; Canella, 1978; Rahma and Narasinga Rao, 1979; Bau et al., 1983). Except for the methanol extraction, extraction procedures reduced the nitrogen solubility in the entire pH range. In the range pH 9–10, the values were comparable to those of the unextracted meal. The profiles of ethanol-, acetone-, and water-extracted meals were nearly superimposable. However, that of methanol-extracted meal was different. It showed higher solubility than that of other extracted meals in the range pH 6.0–8.0 but lower solubility above pH 8.0. In the pH 7 range, it had the same solubility as the unextracted meal. The reduced nitrogen solubility of the variously extracted meals could be due to denaturation of the proteins caused by the solvents. It may be mentioned here that for water extraction a relatively high temperature of 70 °C was used to achieve efficient polyphenol removal (Sripad, 1982).

**Gel Filtration.** The gel filtration pattern of 1 M NaCl-soluble proteins of unextracted and methanol-extracted meals on Sepharose 6B-100 consisted of three peaks (Figure 2) with elution volumes of 70 ± 2, 134 ● 2, and 183 ± 2 mL. It has been reported that fraction II (134 ± 2 mL) corresponds to the 11S protein of sunflower and fraction III (183 ± 2 mL) to the 2S protein (Rahma and Narasinga Rao, 1981). Acetone-ethanol-, and water-extacted meal proteins also gave the same pattern (data not shown). There was no significant variation in either the elution volume or the proportion of the fractions. Peak I gave a UV absorption spectrum with a maximum in the range 250–260 nm. The ratio of absorbance at 280 nm to that at 260 nm was 0.62. This fraction appeared to be contaminated with nucleic acids (Layne, 1957). Also it became turbid 30 min after elution.

Sabir et al. (1973) reported that gel filtration of extracts of sunflower meal in 0.02 M phosphate buffer (pH 7.0) containing 2.5% NaCl on Sephadex G-200 gave five peaks. Rahma and Narasinga Rao (1979) reported that 1 M NaCl extract of sunflower meal gave three peaks in gel filtration on Sepharose 6B-100; our results are in agreement with this observation.

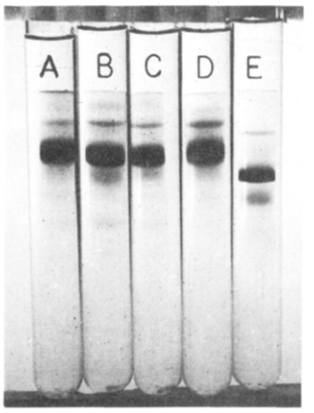


**Figure 3.** Polyacrylamide gel electrophoresis patterns of total proteins of sunflower meals (0.05 M Tris–glycine buffer, pH 8.3): A, unextracted meal; B, acetone-extracted meal; C, ethanol-extracted meal; D, water-extracted meal; E, methanol-extracted meal.

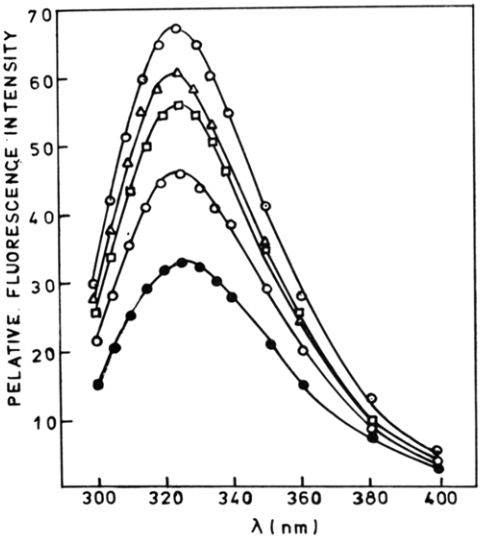


**Figure 4.** Fluorescence emission spectra of total proteins of sunflower meals (0.025 M Tris–glycine buffer, pH 8.3): ●, unextracted meal; ○, acetone-extracted meal; □, ethanol-extracted meal; ⊙, water-extracted meal; △, methanol-extracted meal.

**Polyacrylamide Gel Electrophoresis (PAGE).** The PAGE pattern of unextracted meal proteins consisted of one major band of low mobility, two to three faint bands of higher mobility, and one faint band at the top of the gel (Figure 3). The mobility of the major band and, in general, the PAGE patterns of the extracted meal proteins were the same as those of the unextracted meal proteins except in the case of methanol-extracted meal (Figure 3). In this case the major protein had a higher mobility and also the intensity of the fast-moving band was stronger (Figure 3E). The "available" lysine content of the methanol-extracted meal proteins was 2.60 ± 0.1 g/100 g of protein compared to 3.10 ± 0.1 in all other samples. This could be the reason for the higher mobility of methanol-extracted meal proteins.

**Ultracentrifugation.** The sedimentation velocity pattern of the total proteins of unextracted and extracted meals showed four peaks with $S_{20,w}$ values of 13.4–16.1, 10.5–10.9, 6.7–7.2, and 1.4–1.9. No changes in either the proportion of the fractions or their $S_{20,w}$ values were observed.

**Fluorescence Spectra.** The fluorescence emission intensity of the proteins was measured in the range 300–400 nm by exciting at 280 nm (Figure 4). The con-

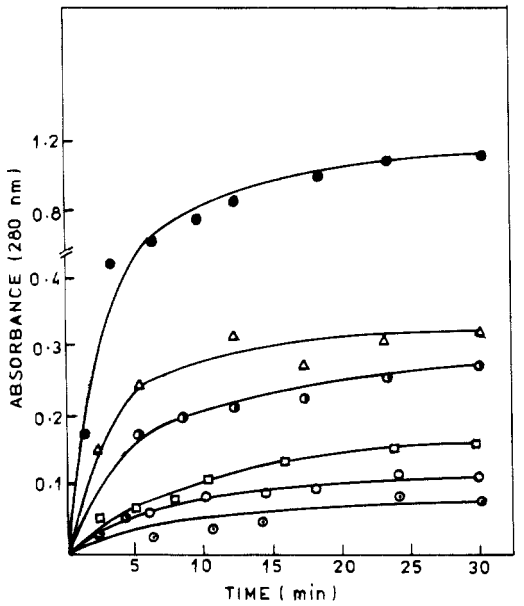


**Figure 5.** Hydrolyses of total proteins of sunflower meals by trypsin (0.1 M phosphate buffer, pH 7.6): ⊙, unextracted meal; ○, acetone-extracted meal; □, ethanol-extracted meal; ◑, water-extracted meal; △, methanol-extracted meal; ●, casein.

centration of the protein solution in all cases was the same, 0.01%. The fluorescence intensities of the extracted meal proteins between 300 and 400 nm were higher than those of the unextracted meal proteins at all wavelengths. However, there was no change in the emission maximum, and it was at 325 nm. The increase in fluorescence intensity could possibly be due to the removal of polyphenols; they cause quenching of fluorescence intensity since they have absorbance in the region 300–400 nm. If the presence of polyphenols was the sole reason for decreased fluorescence intensity, then it should increase with a decrease in CGA content. However, this was not so. For example, methanol-extracted meal had a CGA content of 0.17%; yet its fluorescence intensity was higher than that of ethanol-extracted meal whose CGA content was 0.07%. Also, acetone- and ethanol-extracted meal had the same CGA content, 0.07%; yet the former had higher fluorescence intensity. Thus, there did not appear to be a direct correlation between CGA content and fluorescence intensity. The fluorescence emission spectrum of a protein is dependent on its secondary structure also (Joly, 1965; Chen et al., 1969; Shiffrin et al., 1971). Possibly the secondary structures of variously extracted meal proteins differ.

**Hydrolysis by Proteolytic Enzymes.** The rate of hydrolysis of a protein with a proteolytic enzyme depends on the conformation of the protein (Linderstrøm-Lang, 1952; Harrington et al., 1959; Mihalyi and Godfrey, 1963). Therefore, a change in conformation may lead to an altered rate of hydrolysis by the same enzyme. In the present study hydrolysis was measured to determine whether CGA combined with the proteins would affect hydrolysis and the methods used for removal of polyphenols also affected hydrolysis. Two bond-specific enzymes, trypsin and α-chymotrypsin, and a nonspecific enzyme, papain, were used. The hydrolysis of casein was also determined for comparison.

The unextracted meal proteins were almost resistent to hydrolysis by trypsin (Figure 5). The rate and extent of hydrolysis of the extracted meal proteins increased 2–6-fold over those of the unextracted meal proteins. However, the rate and extent of hydrolysis of all the proteins were considerably lower than those of casein. There was no parallel between CGA content and hydrolysis. For example, methanol-extracted meal whose CGA content was 0.17% gave a rate at least 6 times higher than that of ethanol-extracted meal whose CGA content was 0.07%. It has been reported that CGA has an inhibitory effect on trypsin (Milic et al., 1968; Sastry, 1984). However, the inhibitory effect is not marked. Sastry (1984) has reported that CGA at $1 \times 10^{-3}$ M concentration inhibits trypsin activity only by ~20%. The concentration of CGA in the unextracted meal is $\sim 0.5 \times 10^{-3}$ M.

Hydrolysis of the unextracted and extracted meal proteins by α-chymotrypsin and papain followed the same order (data not shown). Thus, the three enzymes hydrolzyed the extracted meal proteins to a greater extent than the unextracted meal proteins. Among the extracted meals, methanol-extracted meal showed the highest susceptibility to hydrolysis.

Trypsin inhibition activity of unextracted meal was determined. It did not contain trypsin inhibitors. Also inhibition of trypsin by CGA was marginal. Thus, the increased hydrolysis of extracted meal proteins may not be due to the removal of trypsin inhibitors and CGA. Possibly exposure to organic solvents causes changes in the conformation of the proteins in such a way that more bonds are exposed and hydrolyzed. Organic solvents are known to bring about conformational changes in proteins (July, 1965). The effect was maximum with methanol.

**Registry No.** $N_2$, 7727-37-9; trypsin, 9002-07-7; chymotrypsin, 9004-07-3; papain, 9001-73-4; acetone, 67-64-1; ethanol, 64-17-5; water, 7732-18-5; methanol, 67-56-1.

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# Effects of pH and Salt on Yields, Trypsin Inhibitor Content, and Mineral Levels of Soybean Protein Isolates and Wheys

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Protein isolates were prepared from water extracts of defatted soybean flour or flakes by acid precipitation at pH's from 3.5 to 5.2 with or without added 0.1 N NaCl. The resulting wheys and precipitates were analyzed for trypsin inhibitor (TI) activity (TIA), phytic acid, and minerals. TIA varied from 70 mg of trypsin inhibited/g of protein in an isolate precipitated at pH 4.5 from an extract with no added NaCl to 8 mg/g at pH 5.2 with added NaCl. Protein isolate yields varied from 85% of extract proteins at pH 4.5 with no added salt to 59% at pH 5.2 with added salt. The TIA was concentrated to 450 mg/g of freeze-dried permeate by ultrafiltration and dialysis. Retention of the TIA was 50% or more by an ultrafiltration membrane with a 100-kDa cutoff. Since TI's have a molecular weight of 20K or less, their retention indicates they were aggregated to a higher molecular weight. The phytic acid level was highest and zinc level lowest in the pH 3.5 protein precipitate compared to levels in the pH 4.5 and 5.2 precipitates. Levels of Ca, Mg, K, Na, Fe, Mn, and Cu in isolates, wheys, and a TI concentrate were also determined. Extent of removal of minerals and phytic acid from an acid-soluble TI concentrate at pH 2.4 was in the order K > Mn > Zn > Mg > phytic acid > P > Ca > Fe > Cu.

Soybeans are an important source of dietary protein in many parts of the world. High levels of trypsin inhibitor activity (TIA), in raw soybeans, are of nutritional concern in spite of the fact that trypsin inhibitors (TI's) have higher levels of essential amino acids than some storage proteins. Although TI's are found in many food sources, soybeans, potatoes, and eggwhites have relatively high levels of TIA and have received special attention (Liener and Kakade, 1980; Rackis and Gumbmann, 1981; Rackis et al., 1986). Small changes in processing conditions (pH, salt concentration, etc.) have a relatively large influence on the levels of TI's and other proteins in soy protein isolates; processing also affects levels of phytate and minerals and causes changes in functional and nutritional properties (Anderson et al., 1973; de Rham and Jost, 1979). Short-term animal feeding studies have shown that raw soybean meal and purified soybean TI's inhibit growth and enlarge the pancreas in certain monogastric animals (Liener and Kakade, 1980; Rackis and Gumbmann, 1981; Struthers et al., 1982). Other studies showed that the effects of raw soy flour containing diets on the rat pancreas persist and become progressively more marked sometime between 30 and 60 weeks of feeding [see review by McGuiness et al. (1984)]. Therefore, 2-year rat feeding studies were undertaken to study the effects of raw and heated soybean protein products with varying levels of TIA (Rackis et al., 1985; Gumbmann et al., 1985; Liener et al., 1985; Baker and Rackis, 1985).

In support of the long-term feeding studies, we varied precipitation pH and added 0.1 N NaCl to the soybean meal extract to prepare a series of protein isolates with low TIA and corresponding wheys with high TIA. We also concentrated TIA further by combinations of ultrafiltration, dialysis, pH, and diafiltration with salt to separate TI's from other proteins, minerals, and carbohydrates. We determined the effects of acid pH on association of minerals with acid-soluble and acid-insoluble proteins and showed that precipitation pH affects phytate and zinc levels and protein isolate yields.

## MATERIALS AND METHODS

**Materials.** The starting materials for our preparations were a commercial, defatted soy flour, Nutrisoy 7B (Archer Daniels Midland, Decatur, IL), and dehulled, defatted soybean flakes prepared in the laboratory (Sessa et al.,

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