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Characterization of Sunflower Protein

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Sunflower protein isolates were prepared based on their solubility in alkali, water, and sodium chloride. The protein isolates were analyzed for proximate composition and amino acid content, and their solubility was determined over the pH range 1–11. The proteins were fractionated and characterized by gel filtration chromatography, ultracentrifugation, and gel electrophoresis and found to contain six to seven protein fractions with molecular weights ranging from 450×10^3 to 10×10^3. The major fraction had a molecular weight of 125×10^3, and the average molecular weight was calculated to be 180×10^3.

In recent years sunflower production and utilization has increased in many countries (Bureau Agricultural Economics, 1979) and considerable work has been done on processing, nutritional, and functional properties and food uses of sunflower oilseed meal protein (Robertson, 1975; Dorrell, 1978; Sosulski, 1979). Studies on the characterization of sunflower protein are either incomplete (Sabir et al., 1973; Baudet and Moss, 1977) or were carried out with protein that had been modified by chemical treatment (Joubert, 1955; Rahma and Rao, 1979). In this paper we have fractionated and characterized sunflower protein obtained by isolating the albuminous fraction in water, the globular fraction in salt, and the total protein in alkali solutions by gel filtration, chromatography, ultracentrifugation, and gel electrophoresis.

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MATERIALS AND METHODS

Materials. Proteins were isolated by suspending the dehulled and defatted (petroleum ether, 40–60 °C) sunflower oilseed meal (10%) in water (pH 6.5), 1 M NaCl (pH 7.0), and 1 M NaOH (pH 11.0), separately, to isolate albuminous, globular, and total sunflower protein, respectively. The suspension was stirred for 1 h at room temperature and then centrifuged. The supernatant was adjusted to pH 4.5 with 1 M HCl and centrifuged. The protein precipitate was dialyzed against distilled water and freeze-dried. The water-isolated albuminous fraction does not necessarily represent the albumins only; salts present in the meal might have solubilized some globular proteins, and similarly, the salt-isolated globular proteins might

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contain some albuminous proteins.

Analysis. Moisture, ash, and protein contents of the protein isolates were determined according to the methods of AOAC (1980). Chlorogenic acid was measured following the method of Dorrell (1976). The amino acids were determined by hydrolyzing the protein with 6 M HCl at 110 °C for 24 h, filtering, evaporating to dryness, and analyzing the residue, redissolved in citrate butter (pH 2.2), on an amino acid analyzer (Spinco 120C, Beckman).

Protein Solubility. A suspension (1%) of the protein in water was adjusted to various pHs with 1 M NaOH or 1 M HCl. The suspension was occasionally shaken for 1 h at 20 °C and then centrifuged at 12000 rpm for 15 min at 10 °C. The supernatant was analyzed for nitrogen content by the AOAC (1980) method.

Gel Filtration. Protein (20 mg/mL) dissolved in Tris-HCl buffer (pH 8.3) was applied (1 mL) onto a column (2.5 × 90 cm) packed with Sephadex G-200 or Sephacryl S-200 (Pharmacia Fine Chemicals) equilibrated with the same buffer. The void volume of the column was determined with blue dextran 2000 (Pharmacia Fine Chemicals). The protein was eluted with the same buffer at 5 mL/h at 20 °C in 3-mL fractions. The elution pattern of the protein was determined by measuring the absorbance at 280 nm in a spectrophotometer. To determine the molecular weight of the protein fractions, the column was calibrated with proteins of known molecular weight (ferritin, 450000; katalase, 240000; aldolase, 158000; albumin, 68000; albumin, 45000; chymotrypsinogen, 25000; cytochrome *c*, 12000; all obtained from Boehringer Mannheim).

Ultracentrifugation. The sedimentation velocity ($s_{20,w}$) was determined by using a protein solution (0.5–1.0 g/100 mL) in 0.1 M phosphate buffer (pH 7.5) at 20 °C in an analytical ultracentrifuge (Beckman Model E) equipped with schlieren optics, at 59840 rpm. $s_{20,w}$ was calculated by the method of Schachman (1959).

Electrophoresis. Electrophoretic mobility of the proteins was determined on a discontinuous polyacrylamide gel (2.5–27.5%) (Gradipore Gradient Laboratories) following the method of Lambin (1976) with and without sodium dodecyl sulfate ($NaDodSO_4$). Protein (0.5–1 mg/mL) dissolved in 0.1 M Tris–borate buffer (pH 8.1) was applied (10–15 μL) on the gel slab. Electrophoresis was performed in an apparatus (GE-2/4, Pharmacia Fine Chemicals) at 400 V for 1 h. The protein was fixed with 5% sulfosalicylic acid plus 10% w/v trichloroacetic acid and stained by Coomassie blue (0.1%) and destained with methanol–acetic acid–water (3:1:6). The protein bands were read in a densitometer (ATAGO). To determine the molecular weight of the bands, proteins with known molecular weight (standard protein kit, Pharmacia Fine Chemicals) were electrophoresed along with sunflower protein. For electrophoresis with $NaDodSO_4$, the protein, dissolved in the above buffer with 0.1% $NaDodSO_4$, was incubated for 3 h at 37 °C and then dialyzed with the same buffer for 16 h. Electrophoresis was done in the same manner with the same buffer having 0.1% $NaDodSO_4$.

RESULTS AND DISCUSSION

Isolation of Protein. Isolation of sunflower protein in water, NaCl, and NaOH gave yields that were 22.0, 57.4, and 95% of the total sunflower protein, respectively. The higher solubility of sunflower protein in salt solution than in water indicates that globular protein was present to a greater extent than albuminous protein (Gheyasuddin et al., 1970).

Proximate Composition. No appreciable difference was observed in the proximate composition of the three isolates. The average moisture, protein, ash, and chlorogenic acid content was 5.5, 90.0, 3.8, and 2.0 g/100 g of samples, respectively, on a dry weight basis.

Table I. Amino Acid Content of Sunflower Protein Isolates

amino acid[a]	amount present, g/100 g of protein: alkali isolate	water isolate	salt isolates	ideal (FAO, 1957)
lysine	3.2	3.5	3.1	4.2
threonine	3.2	3.7	3.1	2.8
half-cystine	1.2	2.3	2.0	
methionine	1.9	2.9	2.0	
total S amino acids	3.1	5.2	4.0	4.2
valine	4.9	5.0	4.3	4.2
isoleucine	4.2	4.1	4.0	4.2
leucine	5.9	6.2	5.7	4.8
phenylalanine	5.2	4.1	4.4	5.6

[a] Tryptophan was not analyzed.

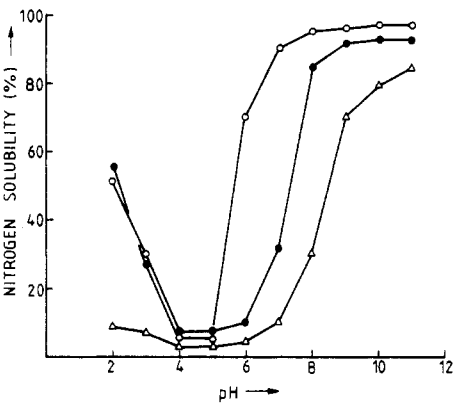

Figure 1. Protein solubility of sunflower protein isolate at various pH levels: (●) alkali isolate; (○) water-isolate; (△) salt isolate.

Amino Acid Content. Water-isolated protein contained relatively more lysine, threonine, and S amino acids and less phenylalanine compared to alkali- and salt-isolated protein (Table I). Salt-isolated protein contained more half-cystine and less valine than alkali-isolated protein. All the isolates contained less lysine than the FAO pattern of essential amino acid requirements while other amino acid levels were comparable (FAO, 1957).

Protein Solubility. Water-isolated protein was more soluble and salt-isolated protein was less soluble around the neutral pH than alkali-isolated protein (Figure 1). Water-isolated protein showed a small range of minimum solubility at pH 4–5 while salt- and alkali-isolated proteins showed a relatively wide range of minimum solubility at pH 4–6.

Gel Filtration. Gel filtration with Sephadex G-200 separated the alkali-, water-, and salt-isolated protein into six to seven fractions (Figure 2). Chlorogenic acid was distributed in all fractions but the low molecular weight fractions had relatively higher amounts. The protein–phenolic complexes present in the isolates (Sabir et al., 1974) are capable of strong hydrogen bonding with Sephadex G-200 gel, and such bonding would retard the elution of the protein fractions and thus may need higher elution volumes, resulting in an apparent decrease in molecular weight (Sabir et al., 1973; Brook and Mundy, 1970).

Gel filtration with Sephacryl S-200 was, therefore, conducted and showed much lower amounts of low molecular weight protein than with Sephadex G-200. Gel filtration with Sephacryl S-200 is shown in Figure 3 and quantified

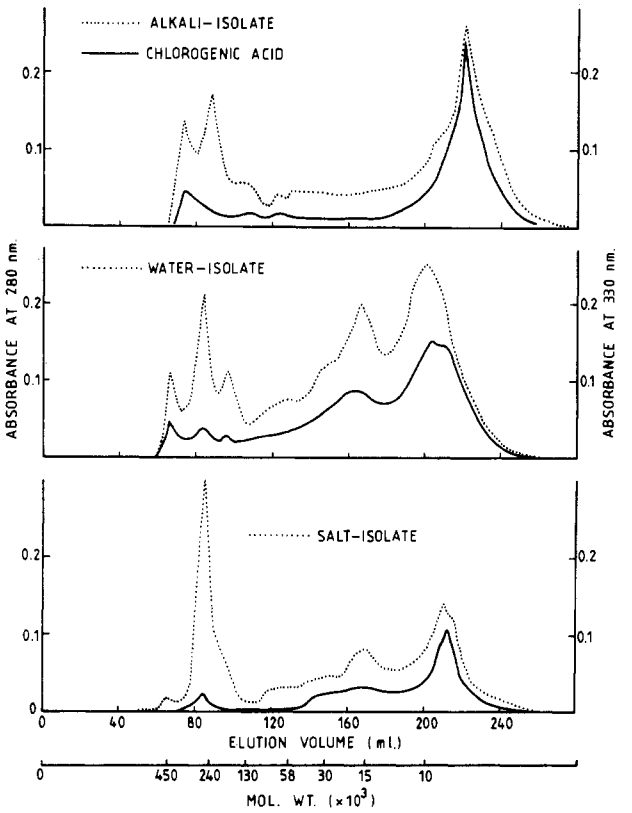

Figure 2. Gel filtration of sunflower protein with Sephadex G-200.

Table II. Fractionation of Sunflower Proteins by Gel Filtration with Sephacryl S-200

fraction no.	elution volume, mL	M_r (×10³)	amount of protein in the isolates, %		
			alkali	water	salt
1	70-72	≃400	32	15	24
2	101	150		33	
3	106-108	125-120	37		22
4	126	70		25	
5	154-156	≃25	7		26
6	166	20			11
7	182	10			9
8	196	<10	2		
9	204	<10		18	
10	208-210	<10	4		8
11	228	<10	17	8	

in Table II. Alkali-isolated proteins showed six protein fractions with three major fractions having molecular weights of 400 × 10³, 125 × 10³, and 10 × 10³ and their relative amounts being about 32, 37, and 17%, respectively. Water-isolated and salt-isolated proteins had five and six fractions with four and three major fractions, respectively. Salt-induced proteins had less low molecular weight fractions than the other fractions. The average molecular weight of the protein in alkali, water, and salt isolates were calculated to be about 180 × 10³, 130 × 10³, and 135 × 10³, respectively. Chlorogenic acid was distributed as with Sephadex G-200. A similar distribution of chlorogenic acid was reported by Rahma and Rao (1979) in the fractions of acid–alcohol-treated and alkali-isolated protein. Sabir et al. (1973) separated salt-soluble protein into five fractions on Sephadex G-200 and reported that only the low molecular weight fractions were bound to chlorogenic acid.

Ultracentrifugation. Ultracentrifugation separated the alkali-, water-, and salt-isolated proteins into three fractions with $s_{20,w}$ values of 12.4, 7.6, and 3.1, 12.1, 7.1, and 1.7, and 16.5, 10.4, and 1.2, respectively. The $s_{20,w}$ values

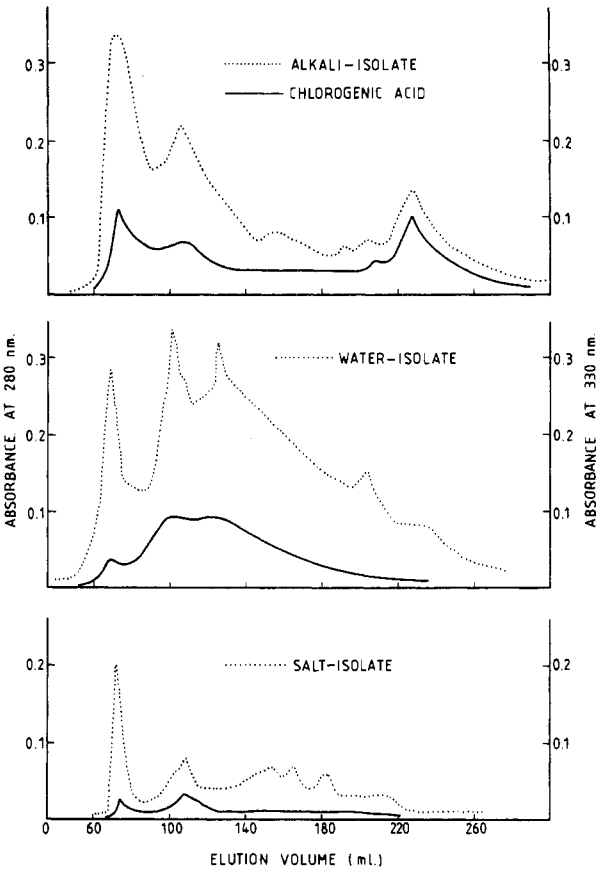

Figure 3. Gel filtration of sunflower protein with Sephacryl S-200.

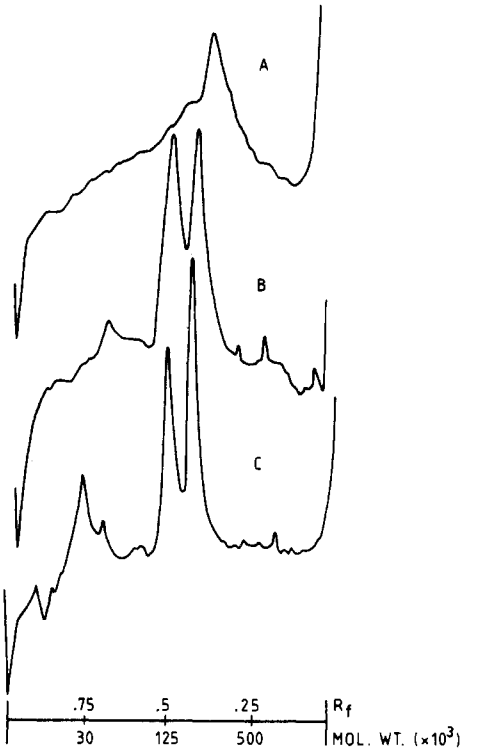

Figure 4. Electrophoretic mobility of sunflower protein without $NaDodSO_4$: (A) alkali isolate; (B) water isolate; (C) salt isolate.

obtained here were similar to those reported by Rahma and Rao (1979), Joubert (1955), and Sabir et al. (1973) and confirms that sunflower protein is made of two major components with $s_{20,w}$ values of about 7–8 and 11–12 S. The components having $s_{20,w}$ values less than 7 S may be degradation products and values higher than 12 S may be

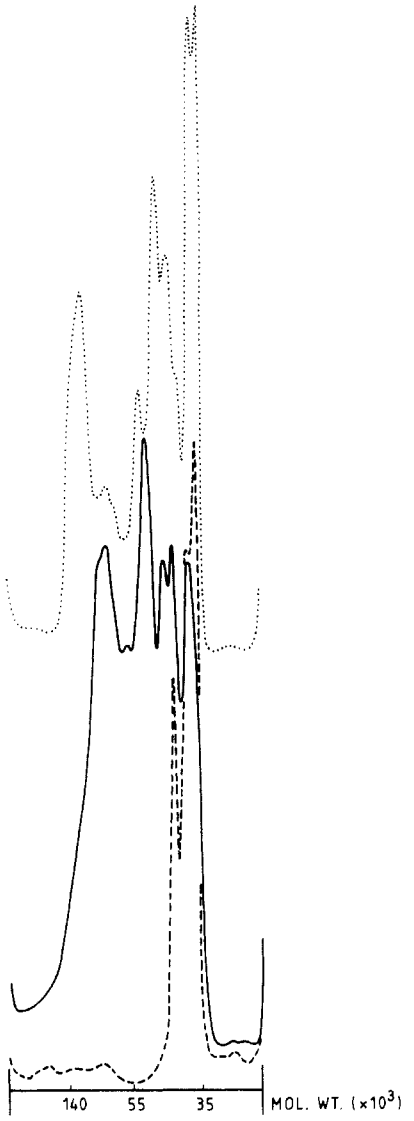

Figure 5. Electrophoretic mobility of sunflower protein with 0.1% $NaDodSO_4$: (—) alkali isolate; (- - -) water isolate; (···) salt isolate.

the aggregated products of 7 S and 11 S components (Rahma and Rao, 1979).

Electrophoresis. Electrophoretic mobility of alkali-isolated protein without $NaDodSO_4$ showed a single band with molecular weight of about 270×10^3 (Figure 4). Water- and salt-isolated protein had two and three major bands with molecular weights of 180×10^3 and 105×10^3 and 125×10^3, 80×10^3, and 15×10^3, respectively. Figure 5 shows the electrophoretic mobility of sunflower protein with $NaDodSO_4$. Alkali-isolated protein showed six fractions with molecular weights ranging from 85×10^3 to 25×10^3 while water- and salt-isolated protein contained six and seven fractions with molecular weights ranging from 240 to 15×10^3 and 130 to 25×10^3, respectively.

The overall data obtained in the analysis of sunflower protein isolates by various techniques showed that albuminous water isolate and globular salt isolate were composed of five to six and six to seven fractions, respectively. Alkali-isolate representing the total sunflower protein was composed of six to seven fractions with molecular weights ranging from $\simeq 400 \times 10^3$ to 10×10^3. The major protein in the alkali isolate had a molecular weight of 125×10^3 and above, and the average molecular weight of protein in the alkali isolate was calculated to be about 180×10^3.

The results obtained with water-isolated albuminous and salt-isolated globular protein cannot be quantitatively compared with the total sunflower protein isolated by alkali because, during the alkali isolation, the protein might have undergone some changes (Provansal et al., 1975) and salts present in the flour might have solubilized some globular protein, thereby inflating the results of water-isolated albuminous fractions (Gheyasuddin et al., 1970). However, the different nature of the protein in the isolates as extracted show that the functional properties and therefore the food application of the isolates would also differ (Kabirullah and Wills, 1981).

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