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Studies on the Interaction of Sunflower Albumins with Chlorogenic Acid

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Acidic butanol extraction removed 90% of the phenolics from sunflower meal. The phenol-free meal contained a higher albumin level than the defatted meal. Gel permeation chromatography showed four and two protein peaks for defatted and phenol-free albumins, respectively. Considerable variation in the electrophoretic mobilities in these two albumin preparations was observed. Amino acid analyses indicated low levels of phenylalanine, tyrosine, leucine, and valine in phenol-free albumin as compared to defatted albumin. Chlorogenic acid and phenol-free albumin interaction studies indicated aggregation of low molecular weight albumins in sunflower.

Sunflower (*Helianthus annuus* L.) is one of the larger sources of vegetable oil and protein of good nutritional quality. But the presence of phenolic compounds in the seeds contribute dark color to formulated foods and prevent the use of sunflower defatted meal (Cater et al., 1972; Gheyasuddin et al., 1970a; Lusas, 1985). Rahma and Rao (1981) have characterized the total proteins from defatted and chlorogenic acid free sunflower meal by disc gel electrophoresis, gel permeation chromatography, ion-exchange chromatography, and analytical centrifugation. Further, various studies have indicated that chlorogenic acid is associated with low molecular weight proteins (Prasad, 1987; Rahma and Rao, 1981; Sabir et al., 1974). Studies on interactions of chlorogenic acid with 11S sunflower protein have showed negative cooperativity in the presence of high salt concentration, high temperature, and 8 M urea (Sastry, 1984).

Attempts have been made to remove these polyphenolic substances to obtain colorless sunflower isolates from sunflower meal (Fan et al., 1976; Kilara et al., 1972; Lawhon et al., 1982; Sosulski et al., 1972). Lawhon et al. (1982) have developed a process for preparing white sunflower protein isolate, by using alkali extraction followed by acid precipitation under vacuum, and deoxygenated water was used in this procedure. However, these methods caused denaturation of the proteins and incomplete removal of color-forming phenols. Sodini and Canella (1977) described the use of acidic butanol reagent for removing phenolics from sunflower meal without causing detectable protein denaturation. The present paper compares albumins from phenol-free and defatted sunflower meal and in vitro binding studies of chlorogenic acid with phenol-free albumins.

MATERIALS AND METHODS

Dehulled seeds of the sunflower cultivar Morden were crushed and defatted with use of petroleum ether (40–60 °C) in a Soxhlet extractor. The defatted meal (DFM) was air-dried and ground to pass through a 100-mesh sieve. The phenol-free meal (PFM) was prepared by the procedure as described by Sodini and Canella (1977).

Solubility Fractionation. Proteins from sunflower meals were fractionated by the modified method of Gheyasuddin et al. (1970b) using a meal to solvent ratio of 1:10 (w/v): (a) distilled water to extract albumins; (b) 1 M sodium chloride to extract globulins; (c) 60% (v/v) aqueous 2-propanol to extract prolamines; (d) 0.4% sodium hydroxide to extract glutelins. The samples were extensively dialyzed against distilled water at room temperature and freeze-dried.

Nitrogen content in the samples and protein fractions was determined by the micro-Kjeldahl method and multiplied by 6.25 to obtain crude protein content (AOAC, 1980). Phenolic content was estimated by the method of Swain and Hillis (1959) using Folin–Denis reagent.

Amino Acid Composition. Amino acid analyses of defatted and phenol-free albumins (DFA and PFA) were performed according to Spackman et al. (1958) using a Hitachi Model KLA-3B amino acid analyzer.

Gel Permeation Chromatography. A 50-mg portion of albumin was dissolved in 4.0 mL of 0.02 M sodium phosphate buffer (pH 7.2) containing 0.01 mM β-mercaptoethanol, 0.02% sodium azide, and 2.5% sodium chloride. The solution was centrifuged at 5000 rpm for 10 min at room temperature, 3.5 mL of the supernatant was applied on a Sephadex G-200 column (3 × 40 cm) equilibrated with the same buffer, and 4.0-mL fractions were collected with use of an LKB 2112 redirac automatic fraction collector. Absorbances of the fractions were read at 280 and 328 nm for protein and phenolics, respectively.

Polyacrylamide Gel Electrophoresis. Portions of 100 μg of each protein were separated on 7.5% polyacrylamide gels with 0.02 M Tris–borate buffer (pH 8.8) and a current of 5 mA/gel for 1.5–2.0 h by the method of Davis (1964). The protein bands were visualized with Coomassie brilliant blue.

In Vitro Binding of Chlorogenic Acid to PFA. PFA and chlorogenic acid (CA) in the ratios 10:1, 10:1.5, and

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Table I. Protein[a] and Phenolic[b] Contents in Sunflower Meal and Solubility Fractions

		sunflower meal	solubility fraction			
			albumin	globulin	prolamine	glutelin
I.	protein content					
	(a) defatted	49.3	9.2	20.3	2.5	12.0
	(b) phenol-free	45.8	15.6	20.5	1.5	8.4
II.	phenolic content					
	(a) defatted	3.9	4.1	1.2	0.7	0.7
	(b) phenol-free	0.5	1.7	0.9	0.6	0.6
	% residual phenolic content in phenol-free	12.8	41.5	75.0	85.7	85.7

[a] Grams of protein/100 g of meal. [b] Grams of chlorogenic acid/100 g of meal or protein.

Table II. Amino Acid Composition of Albumins Extracted from Defatted and Phenol-Free Sunflower Meal[a]

	FAO/WHO,[b] 1973	DFA	PFA
lysine	4.2	3.4	3.6
methionine	2.2	3.0	3.3
cysteine	2.0	1.9	3.2
isoleucine	4.2	3.3	2.8
leucine	4.8	5.4	4.2
phenylalanine	2.8	3.5	1.7
tyrosine	2.8	2.7	1.6
threonine	2.8	3.5	2.9
valine	4.2	8.9	7.0
arginine		5.0	5.5
glycine		4.4	6.9
histidine		1.6	1.7
alanine		3.8	2.6
aspartic acid		8.6	5.9
glutamic acid		18.7	21.9
proline		4.6	4.7
serine		3.6	3.9
total		85.9	83.4

[a] Date reported as percent of protein. [b] FAO/WHO, 1973.

10:2 (w/w) were dissolved in 0.02 M sodium phosphate buffer (pH 7.2), and the resultant mixtures were stirred for 30 min at room temperature and dialyzed extensively against same buffer to remove excess or unbound CA. The CA-bound albumins were analyzed by polyacrylamide gel electrophoresis and gel permeation chromatography.

RESULTS AND DISCUSSION

The phenolic level and solubility of protein fractions from defatted and phenol-free sunflower meal are shown in Table I. Sunflower meal was found to be rich in salt-soluble proteins, and the protein solubility pattern was quite typical of that of other oilseeds (Kaushal et al., 1982; Madhusudhan and Singh, 1983; Prakash and Rao, 1986). The globulin contents of DFM and PFM were almost identical, whereas the PFA was significantly high as compared to DFA. DFM had higher prolamine and glutelin conents than PFM. Results also indicated a significant reduction (90% and 60%, respectively) in the phenolic content of PFM and PFA preparations. Differences between phenolic contents of defatted and polyphenol-free globulins, prolamines, and glutelins were rather low.

The amino acid analyses of DFA and PFA are shown in Table II. In general, these albumins are rich in glutamic acid, aspartic acid, valine, arginine, and histidine, characteristic of amino acid compositions of oilseed storage proteins (Kaushal et al., 1982; Madhusudhan and Singh, 1983; Prakash and Rao, 1986; Sabir et al., 1973, 1974). Data also indicated that sunflower albumins were rich in methionine, cysteine, leucine, phenylalanine, tyrosine, threonine, and valine as compared to the FAO provisional essential amino acid pattern (FAO/WHO, 1973). PFA contained low levels of phenylalanine, tyrosine, leucine, and valine as compared with the amino acid composition

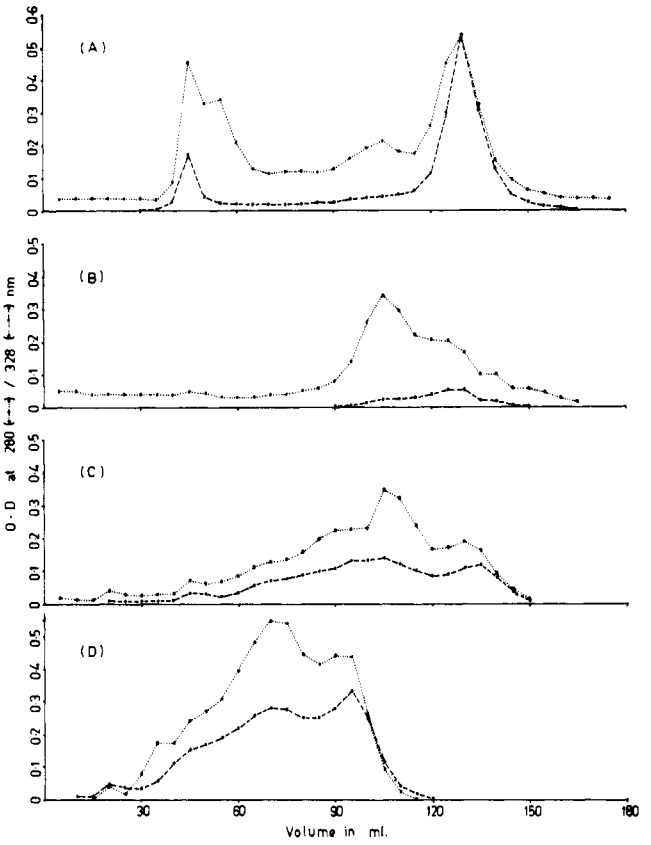

Figure 1. Sephadex G200 column fractionation of sunflower albumins: A, DFA; B, PFA; C, PFA with CA (10:1, w/w); D, PFA with CA (10:2, w/w).

of DFA. Probably, the small polypeptides, which are rich in phenylalanine, tyrosine, leucine, and valine, might have been extracted in the acidic butanol solvent with the phenolics.

Sephadex G-200 column chromatography of DFA and PFA yielded four and two major fractions (Figure 1). The distribution pattern of DFA was similar to the protein pattern obtained by Kabirullah and Wills (1983) on molecular sieve chromatography. But, the PFA exhibited only two peaks and the elution volume corresponded to that of DFA fractions III and IV. Absence of the first two fractions and increased OD at 280 nm for the fraction corresponding to the third fraction of DFA in PFA might indicate possible denaturation and dissociation of high molecular weight proteins during acidic butanol extraction. The chromatographic analyses of CA-bound PFA clearly indicate that low molecular weight proteins interact with CA in vitro, aggregate, and elute out as high molecular weight proteins (Figure 1C,D). Ribereau-Gayon (1972) has reported that protein–phenolic interaction results mainly from (a) hydrogen bonding between phenolic groups of phenols and receptor groups of proteins (NH, CO, OH) and (b) ionic bonding between anionic groups of phenols and

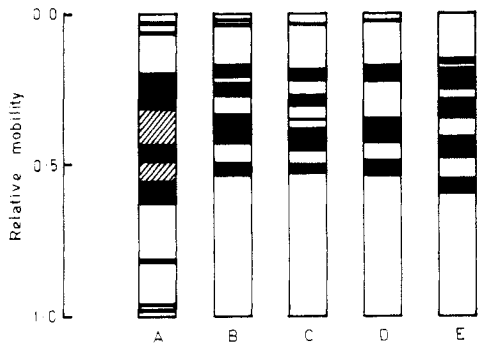

Figure 2. Polyacrylamide gel electrophoretic pattern of sunflower albumins: A, DFA; B, PFA with CA (10:1, w/w); C, PFA with CA (10:1.5, w/w); D, PFA with CA (10:2, w/w); E, PFA.

cationic groups of proteins. During the phenolic extraction using acidic butanol solvent, these bonds might have been disrupted, resulting in dissociation of the high molecular weight proteins.

A significant difference in the electrophoretic mobilities of DFA and PFA (Figure 2A,E) resulted in nine and five major bands, respectively. The reduction in number of bands in PFA might also be attributed to losses of proteins during acidic butanol extraction. The CA-bound PFA showed four to six major bands (Figure 2B–D). Further retardation in mobilities of the protein bands can also be observed in the CA-bound PFA. This might be due to the aggregation of sunflower albumins in the presence of phenolics, possibly making it more compact and basic in its conformation than PFA.

It has been reported earlier that the hydrogen bonding between hydroxyl groups of phenolic compounds and the peptide bond in proteins is known to be unusually strong and equilibrium in aqueous solutions strongly favors the formation of complexes between phenols and proteins (Loomis and Battaile, 1966; Pierpoint, 1970). Further, the ionic and salt linkages have been shown to involve the ionized carboxyl of hydrolyzable phenols and basic groups of proteins, in particular that of arginine and lysine. The covalent interaction of polyphenols involves reactions of *o*-quinones (oxidized products of the phenols) with proteins through amino, thiol, and active methylene groups (Pierpoint, 1969a,b). Although it can be reasonably concluded that a favorable combination of hydrogen bonding and covalent linkages of quinones is involved in the interaction of polyphenols with sunflower albumins, the exact nature of the protein–phenolic interaction is yet to be understood. Further studies are under way to establish the nature of binding of these phenolics with low molecular weight proteins and the physiological role of this class of proteins during sunflower seed development.

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