Composition, Solubility, and Gel Electrophoretic Properties of Proteins Isolated from Florunner (*Arachis hypogaea* L.) Peanut Seeds

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Defatted peanut (*Arachis hypogaea* L.) meal was homogenized with various extraction media to determine optimum conditions for qualitative and quantitative recovery of proteins. Maximum recovery of both arachin and nonarachin proteins was accomplished with 1 M NaCl–20 mM sodium phosphate buffer (pH 7.0). Other extraction media compared and found to be less efficient in recovering soluble seed proteins were 10% NaCl, sodium phosphate buffer (pH 7.9, $I = 0.03$), and water. A method was developed to prepare relatively pure isolates of arachin and nonarachin proteins from the total soluble fraction extracted with 1 M NaCl–20 mM sodium phosphate buffer using a series of simple steps involving differential solubility, cryoprecipitation, and dialysis; the degree of purity of these isolates was determined by "standard" polyacrylamide disc gel electrophoretic techniques. Gel electrophoresis of sodium dodecyl sulfate dissociated proteins showed these isolates each contained five different components having molecular weights between 20000 and 84000. In addition, the arachin and nonarachin components were found to be glycoproteins containing both neutral and amino sugars. These isolates differed in amino acid composition, e.g., nonarachin proteins contained approximately twice as much serine (7.5 g/100 g of protein), half-cystine (2.7), methionine (2.0), and lysine (5.6) compared to arachin.

Among the various sources of plant proteins considered possible as food ingredients, peanut seeds have excellent potential because of their high protein content (Dechary and Altschul, 1966; Johnson and Lay, 1974). In earlier studies, peanut proteins were fractionated and classified as albumins, arachin, and nonarachin or conarachin (Johns and Jones, 1916; Jones and Horn, 1930; Johnson and Shooter, 1950; Johnson et al., 1950; Evans et al., 1962; Johnson and Naismith, 1963; Daussant et al., 1969a,b; Dawson, 1968, 1971; Cherry et al., 1973). The latter two fractions were composed mainly of complex large molecular weight globulins known as α-arachin and α-conarachin, respectively, plus several related polypeptides or subunit components not completely separated by the usual techniques (Neucere and Ory, 1970; Cherry et al., 1973). Subunit components could be dissociated from these large molecular weight globulins depending on pH, ionic strength, protein concentration, and time lapsed after preparation of the isolate (Johnson and Shooter, 1950; Johnson et al., 1950; Dechary et al., 1961; Johnson and Naismith, 1963; Cherry et al., 1973). The existence of genetically related variant forms of arachin was also shown (Tombs, 1963; Cherry et al., 1971; Cherry, 1974). More recent development of improved extraction procedures and use of sodium dodecyl sulfate (SDS)–polyacrylamide gel electrophoresis showed that arachin could be prepared in a relatively pure form (Singh and Dieckert, 1973a,b; Shetty and Rao, 1974). This storage globulin was very complex containing as many as nine polypeptide subunits ranging in molecular weight from 10000 to 71000. Other studies suggested that this globulin consisted of as few as four or as many as seven subunits (Shetty and Rao, 1974). These data indicated that arachin may be differentially susceptible to various procedures used to isolate and dissociate it in the presence of detergent and a reducing reagent.

Research with soybeans (Wolf, 1970) produced technology to use these materials as commercially available food-grade vegetable protein ingredients. Presently, methods are being developed to prepare flours, meals, concentrates, and isolates from peanut seeds for the same purpose (Smith, 1971; Mattil, 1973). For example, a wet processing method was developed to obtain from peanuts maximum amounts of oil and proteins (Rhee et al., 1973a,b). These products compare favorably with those of other legume seeds as ingredients in foods such as cereals and synthetic meat products. However, more research on peanut proteins is needed to improve understanding of their physicochemical, functional, and/or nutritional properties.

This paper examines various extraction media to prepare arachin and nonarachin proteins from peanuts. The physicochemical and "standard" and SDS–gel electrophoretic properties as well as the amino acid and carbohydrate compositions of these proteins are analyzed and compared.

MATERIALS AND METHODS

Protein Extraction Methods. Florunner (*Arachis hypogaea* L.) peanut seeds (testa free) were ground in a Wiley mill (using a 60-mesh screen) and the resulting material defatted repeatedly with hexane until essentially fat free (step 1, Figure 1). The defatted meal was air-dried and stored in a closed container at 0 °C until used. The fat free meal was homogenized in a mortar with a pestle in either deionized water, sodium phosphate buffer (pH 7.9, $I = 0.03$), 1 M NaCl–20 mM sodium phosphate buffer (pH 7.0), or 10% NaCl at different meal to extraction medium ratios between 1:4 and 1:60 (w/v). The homogenates were centrifuged at 20000*g* for 20 min at 22 °C. The resulting supernatants were analyzed for protein content by the method of Lowry et al. (1951).

Protein Fractionation Methods. The fat free meal (step 1, Figure 1) was homogenized either in deionized water, sodium phosphate buffer ($I = 0.03$; pH 7.9), 1 M NaCl–20 mM sodium phosphate buffer (pH 7.0), or 10% NaCl at a meal to medium ratio of 1:18 (w/v) with a mortar and pestle and centrifuged at 20000*g* for 20 min at 22 °C (step 2, Figure 1). The supernatant or total soluble protein fractions were dialyzed for 48 h at 4 °C against deionized water (step 3, Figure 1). The dialysates were centrifuged

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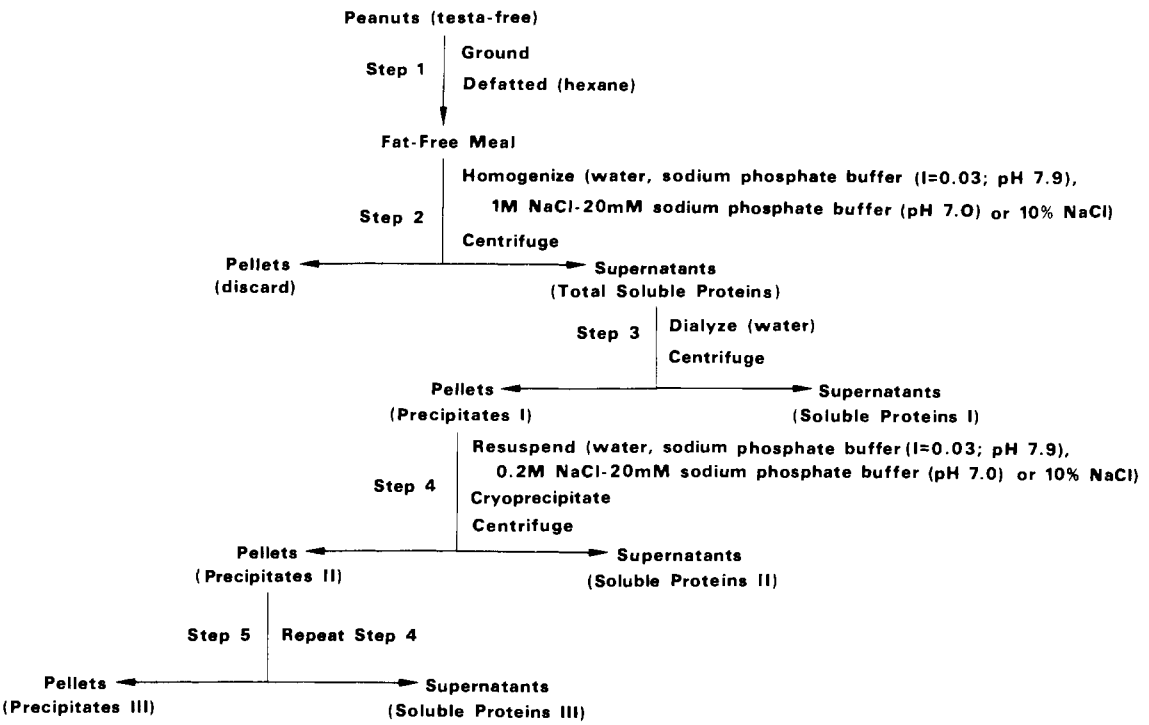

Figure 1. Isolation and fractionation of peanut seed proteins. For further discussion of procedures in the scheme, see Materials and Methods.

at 20000*g* for 20 min at 4 °C and, except in the case where 1 M NaCl was replaced with 0.2 M NaCl–20 mM sodium phosphate buffer (pH 7.0), the resulting pellets resuspended in their respective extraction medium, precipitated at 4 °C for 24 h (cryoprecipitation), and then centrifuged (step 4, Figure 1). The latter step was repeated (step 5, Figure 1). The resulting supernatants (solubles I, II, and III; steps 3, 4, and 5) and final precipitates were dialyzed against deionized water for 24 h at 4 °C and lyophilized.

Protein Solubility Relative to pH. Total soluble protein extracts prepared with the four extraction media in step 2, Figure 1, were first dialyzed against water to lower the level of salts and then made up to equal volumes (250 ml) and finally 20-ml aliquots were adjusted to pH levels between 1 and 12 with either 1 N NaOH or 1 N HCl. The protein mixtures were allowed to equilibrate for 3 h at 4 °C and then centrifuged at 30000*g* for 20 min. The amount of protein soluble at each pH was determined by the method of Lowry et al. (1951).

Polyacrylamide Gel Electrophoresis. All protein preparations characterized by standard gel electrophoresis were dialyzed against water to lower the level of salts and then electrophoresed in 10% polyacrylamide gels following the procedures of Canalco ("Disc Electrophoresis", 1973) and Cherry et al. (1970).

Protein Subunits and Molecular Weights. Proteins were characterized for subunit composition and molecular weights following the method of Palmiter et al. (1971). Briefly, protein solutions were mixed with 10% trichloroacetic acid (1:1, v/v) and the precipitated material was redissolved (2 mg/ml) in a dissociation buffer of 1.5% dithiothreitol (DTT), 1.0% sodium dodecyl sulfate (SDS), 1.2% Tris, and 20% glycerol while being heated in a boiling water bath for 5 min.

SDS–polyacrylamide gel electrophoresis was performed on 5% gels as similarly described by Weber and Osborn (1969); sample and spacer gels were excluded. Preparations of dissociated protein (100–400 μg/gel) were applied on gels, electrophoresed at 2 mA/gel for the first 20 min, and then at 10 mA/gel for 140 min at room temperature. The electrophoretic reservoir buffer (pH 7.1) contained 1.93% disodium phosphate–7% H_2O, 0.39% monosodium phosphate–H_2O, and 0.1% SDS. After electrophoresis, the proteins were fixed by incubating the gels in 20% sulfosalicylic acid for 16–18 h at 37 °C and stained with coomassie blue R-250 (0.25% in water); the background stain was removed with 7.5% acetic acid. β-Galactosidase (mol wt 130000), lactic acid dehydrogenase (150000), bovine serum albumin (68000), catalase (66000), trypsin (23800), pepsin (35500), ribonuclease (13700), and α-chymotrypsin (24000) were used as molecular weight markers on SDS gels.

Sugar Estimation. Lyophilized protein preparations (100 mg) were hydrolyzed with 10 ml of 1 N HCl for 1 h in an autoclave at 124 °C, cooled, filtered, and dried. The dried hydrolysates were redissolved in 2 ml of 0.3 N HCl and applied to a Dowex XW (100–200 mesh) cation exchange column (8 × 70 mm) following the procedures of Gardell (1953). The column was eluted in 2-ml portions with 0.3 N HCl until a total volume of 26 ml (13 fractions) was used. Neutral sugars were determined in the first three fractions (Yemm and Willis, 1954) and amino sugars were examined in the remaining ten eluates (Rondole and Morgan, 1955).

Total Amino Acid Analysis. Lyophilized protein extracts (50 mg) were hydrolyzed in 20 ml of 6 N HCl at 110 °C for 15 h. After hydrolysis, the pH of the hydrolysates was adjusted to 2.2 with 12 N NaOH and made up to 50 ml with water. Samples were analyzed for total amino acids in a Durrum D-500 amino acid analyzer (Young et al., 1974).

RESULTS

Protein Solubility. Ratios of peanut meal to extraction medium used to determine optimum solubility of proteins in water, sodium phosphate buffer, 1 M NaCl–20 mM sodium phosphate buffer, or 10% NaCl are shown in Figure 2. The results show that different amounts of protein can be recovered from defatted peanut meal depending on the type and amount of extraction medium

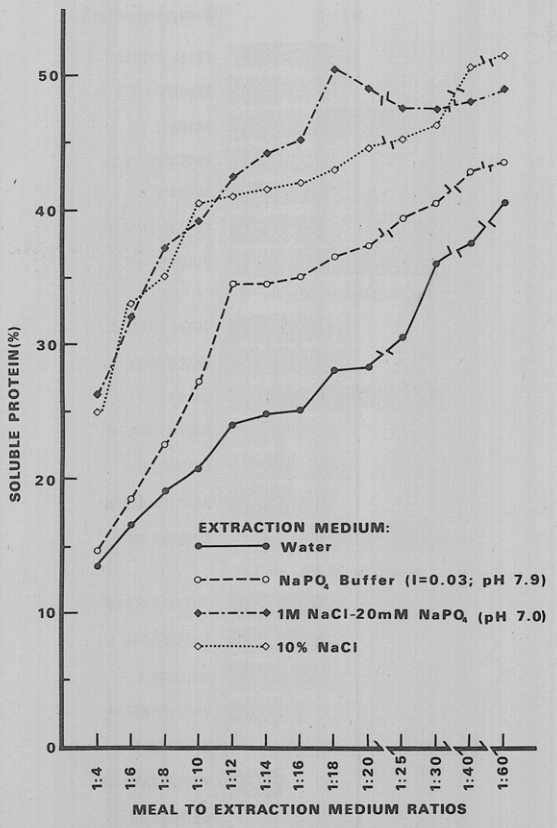

Figure 2. Percentage protein of fat-free meal solubilized relative to various ratios of meal and different extraction media (1:4–1:60, w/v).

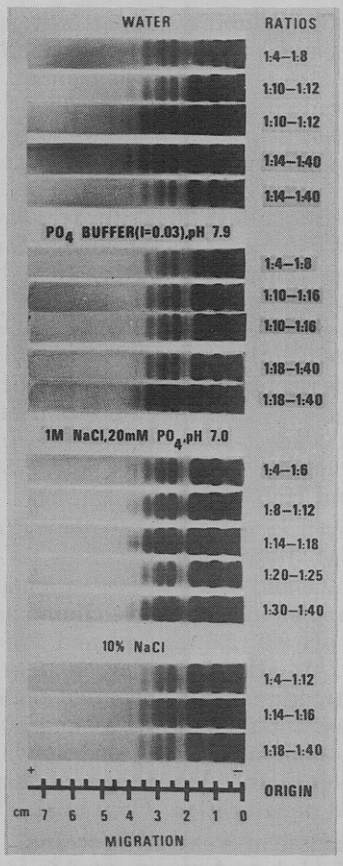

Figure 3. Polyacrylamide disc gel electrophoretic patterns of proteins solubilized in extracts of various ratios of fat-free meal and different extraction media. Some gel patterns containing two levels of protein from various extraction ratios are shown for clarity of all possible bands.

used. Of the four extraction media compared, protein recovery was lower in water and sodium phosphate buffer than where extracts contained sodium chloride (cf. meal to medium ratios 1:4 to 1:60). In addition, the ratio of meal to medium was important in determining the amount of soluble protein recovered. For example, as the ratio of meal to water, sodium phosphate buffer, or 10% NaCl was increased from 1:12 to 1:18, amounts of soluble protein increased slightly (remaining at approximately 25, 35, and 42%, respectively). On the other hand, protein levels in 1 M NaCl–20 mM sodium phosphate buffer extracts increased from 42 to 51% as the ratio was increased. Protein recovery improved with increasing volumes (to 1:60) of water, sodium phosphate buffer, or 10% NaCl but maximum solubilization was attained with 1 M NaCl–20 mM sodium phosphate buffer at a ratio of 1:18. This latter extraction ratio accounted for more than 95% of the protein in peanut seeds.

Since protein levels improved with increasing volumes of extraction media, it was of further interest to determine the qualitative aspect of these solubilized proteins using "standard" polyacrylamide gel electrophoresis. These data (Figure 3) showed that at lower ratios (1:4 to 1:8), water and sodium phosphate buffer extracts contained small amounts of protein migrating in region 1.5–2.5 cm on gels; arachin is normally located in this area of the patterns (Cherry et al., 1973). Moreover, gel patterns showed that arachin content in 10% NaCl extracts did not increase as much as those of the other three media between ratios of 1:4 to 1:40; the gel pattern for the ratio of 1:60 in all comparisons was similar to that of 1:40. A minor band in region 2.0–2.5 cm was not prominent in gels of water extracts when compared to those of other extraction media. Thus, gel electrophoresis showed that 1 M NaCl–20 mM sodium phosphate buffer uniformly solubilized peanut proteins both quantitatively and qualitatively regardless

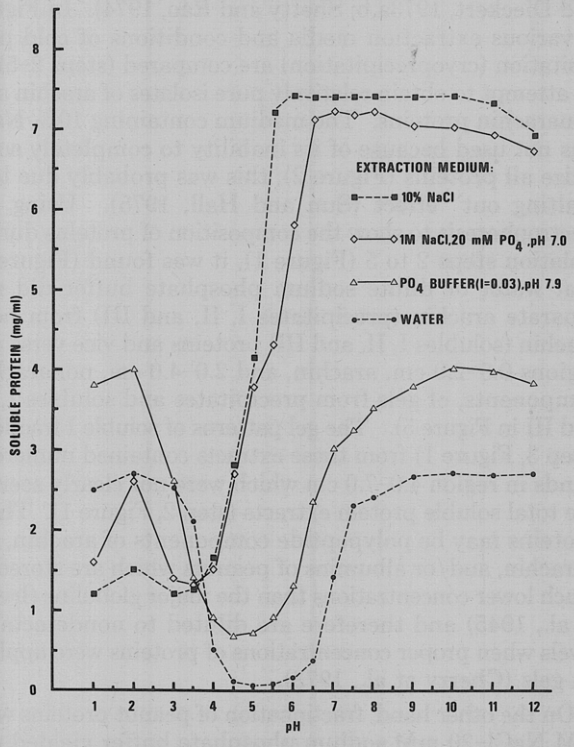

Figure 4. The influence of pH and extraction medium on the solubility of peanut seed proteins.

of the meal to extraction medium ratio used; maximum solubilization was attained at a meal to medium ratio of 1:18 (w/v).

pH Effects. Solubility properties relative to pH of proteins prepared with different extraction media are presented in Figure 4. Proteins from extraction media containing sodium chloride (1 M, 10%) had isoelectric points between pH 3.0 and 3.5, while those of sodium phosphate buffer and water were approximately pH 4.5 and 5.0, respectively. Moreover, protein levels at these isoelectric points differed (cf. approximate values of 1.3, 1.2, 0.6, and <0.1 mg/ml for media containing 1 M NaCl–sodium phosphate buffer, 10% NaCl, sodium phosphate buffer, and water, respectively). The high protein levels at the isoelectric points of extracts containing either sodium chloride (1 M, 10%) or sodium phosphate buffer may be related to the activity coefficient of protein charged groups or a salting-in effect (Betschart, 1974). Further, salt concentration decreases the influence of pH on protein solubility (Smith and Circle, 1938; Van Megen, 1974; Sun and Hall, 1975).

Protein levels in extraction media containing sodium chloride (1 M, 10%) increased from 1.3 to 7.5 mg/ml between pH 3.5 and 6.5. The largest amounts of soluble proteins extracted in water and sodium phosphate buffer were above pH 9.0 (2.6 mg/ml) and 10.0 (4.0 mg/ml), respectively. At pH values above 10.0, protein levels in different extraction media decreased slightly as a result of alkaline denaturation. At pH 2.0 and 1.0, protein levels in all extraction media increased above those at the isoelectric points, and then decreased, respectively, as a result of acid denaturation (Nash et al., 1971).

Fractionation of Peanut Proteins. Although numerous methods have been reported for fractionation of peanut proteins, most of them are either complex or inefficient in separating arachin and nonarachin components into highly purified extracts (Johns and Jones, 1916; Jones and Horn, 1930; Dechary et al., 1961; Tombs, 1965; Neucere, 1969; Dawson, 1971; Cherry et al., 1973; Singh and Dieckert, 1973a,b; Shetty and Rao, 1974). In Figure 1, various extraction media and conditions of cold precipitation (cryoprecipitation) are compared (steps 2–5) in an attempt to obtain relatively pure isolates of arachin and nonarachin proteins. The medium containing 10% NaCl was not used because of its inability to completely solubilize all proteins (Figure 2); this was probably due to a "salting out" effect (Sun and Hall, 1975). Using gel electrophoresis to show the composition of proteins during isolation steps 2 to 5 (Figure 1), it was found (Figure 5) that water or dilute sodium phosphate buffer did not separate arachin (precipitates I, II, and III) from nonarachin (solubles I, II, and III) proteins and vice versa (cf. regions 0.5–2.0 cm, arachin, and 2.0–4.0 cm, nonarachin components, of gels from precipitates and solubles I, II, and III in Figure 5). The gel patterns of soluble I fractions (step 3, Figure 1) from these extracts contained numerous bands in region 4.0–7.0 cm which were not clearly seen in the total soluble protein extracts (step 2, Figure 1). These proteins may be polypeptide components of arachin, conarachin, and/or albumins of peanuts which are stored in much lower concentrations than the major globulins (Irving et al., 1945) and therefore are diluted to nondetectable levels when proper concentrations of proteins were applied on gels (Cherry et al., 1973).

On the other hand, fractionation of peanut proteins with 1 M NaCl–20 mM sodium phosphate buffer yielded relatively pure arachin (region 0.5–2.0 cm) and nonarachin (region 2.0–4.5 cm) fractions (cf. separation of these two fractions in soluble and precipitates I, II, and III in Figure 5). In contrast to fractions of water and sodium phosphate buffer, this medium selectively removed nonarachin components from arachin and vice versa (solubles and precipitates II and III, Figure 5). The electrophoretic patterns of final arachin and nonarachin proteins (soluble III, BCS_3; precipitate III, BCP_3) which were prepared with 1 M NaCl–20 mM sodium phosphate buffer, dialyzed against water, and lyophilized to a creamy white crystalline-like powder are shown in Figure 6. The arachin isolate contained mainly the components of region 1.0 and 1.5 cm of the total protein fraction (step 2; Figure 1) while the nonarachin fraction showed the bands located in region 2.0–4.0 cm of the original extract ("standard" electrophoretic gel patterns; Figure 6A).

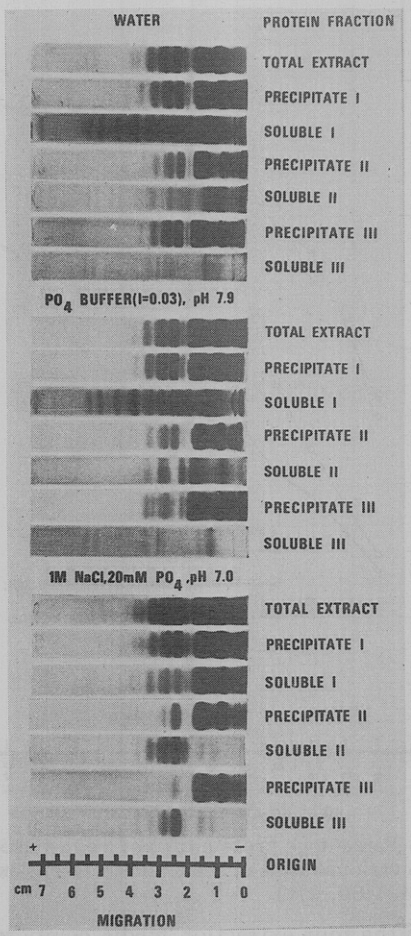

Figure 5. Polyacrylamide disc gel electrophoretic patterns of proteins fractionated using different extraction media. For details on preparation of the various fractions shown, see Figure 1.

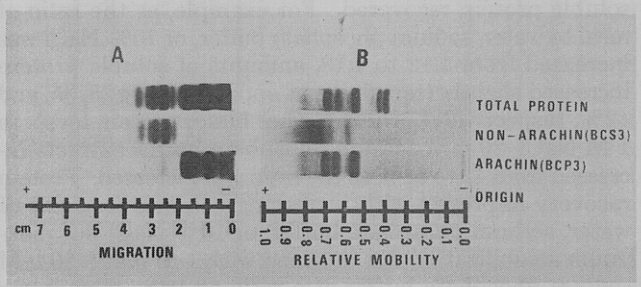

Figure 6. Standard (A) and SDS- (B) polyacrylamide disc gel electrophoretic patterns of the total proteins extracted with 1 M NaCl–20 mM sodium phosphate buffer (pH 7.0) at a meal to salt-buffer ratio of 1:18 (w/v) and the final purified fractions of nonarachin and arachin proteins made from this preparation. The proteins, labeled as BCS_3 and BCP_3, respectively, are lyophilized samples resulting from step 5 in Figure 1.

Table I. Molecular Weights of the Protein Fractions Obtained during Protein Fractionation[a]

Protein fractions[b]	1	2	3	4	5	6	7	8	9	10	11
Total	130 000	115 000	84 000	78 000	66 000	50 500	45 000	37 000	31 500		21 000
Soluble I	130 000	115 000	84 000	78 000	66 000	50 500		37 000	31 500		21 000
Soluble II			84 000						31 000	26 000	21 500
Soluble III			84 000				46 000	34 000	31 000		23 000
Arachin			81 000			50 500		37 000	32 000		20 000

[a] Protein fractions were dissociated with SDS and DTT and subjected to SDS gel electrophoresis. Molecular weights (±2000) were determined using protein standards as markers. [b] See Figure 1 for description of fractions.

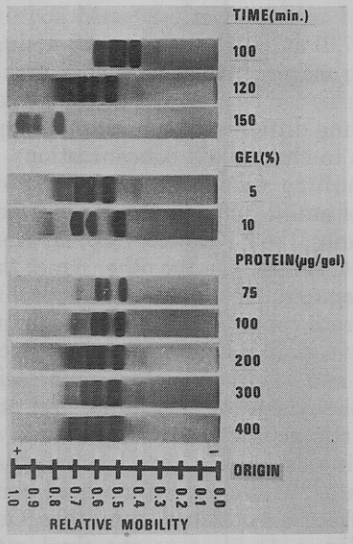

Figure 7. SDS–polyacrylamide disc gel electrophoretic patterns of arachin proteins (BCP_3 from Figure 6). Gel electrophoresis was performed using various protein levels of 75 to 400 μg/gel on 5 or 10% polyacrylamide gels for time intervals between 100 and 150 min.

Proteins of the total soluble extract of 1 M NaCl–20 mM sodium phosphate buffer, arachin (precipitate III, BCP_3), and nonarachin fractions (soluble III, BCS_3) were dissociated with SDS and DTT. The components resulting from this procedure were distinguished according to their molecular weights by SDS gel electrophoresis (Figure 6B; Table I). Proteins in the total soluble fraction showed five major (regions 0.4, 0.43, 0.55, 0.65, and 0.7) and five minor (0.3, 0.35, 0.38, 0.58, and 0.82) components on SDS gels. On the other hand, arachin had four major (0.55, 0.65, 0.7, and 0.82) and one minor (0.4) band(s). Nonarachin proteins produced two major (0.73 and 0.78) and three minor (0.58, 0.68, and 0.80) components. Although arachin and nonarachin components differed slightly in their mobilities, the possibility of improving techniques to characterize common subunits in these two isolates cannot be precluded at this time. Although the component composition of the total soluble fraction resembled that of arachin, nonarachin proteins cannot be clearly distinguished because they constitute a small portion of the total protein (Johns and Jones, 1916; Jones and Horn, 1930; Cherry et al., 1973).

The number of components observed on SDS gels is related to the duration of electrophoresis, polyacrylamide concentration, and protein levels applied to the gels (Weber and Osborn, 1969). Therefore, a study was conducted to examine the effects of these factors on SDS gel patterns of arachin. These data (Figure 7) showed that increasing the duration of electrophoresis from 100 to 150 min increased the component separation and gradually made them difficult to discern in the gels. Better separation of arachin components occurred on 10% rather than on 5% gels. It was also noted that these bands become diffuse as protein levels in the gels were increased above 100 μg/gel. On the other hand, minor components (region 0.3–0.4 and 0.6–0.8) could not be distinguished clearly in the gels until protein levels of 200 μg/gel were applied. These data show the importance of determining optimum conditions for SDS gel electrophoresis before true compositions and molecular weights of protein can be made using these procedures.

Table II. Carbohydrate Content (%) of Protein Fractions Prepared with 1 M NaCl–20 mM Sodium Phosphate Buffer (pH 7.0)

	Neutral sugars	Amino sugars
Total extract[a]	0.4762	0.1549
Arachin[b]	0.1294	0.0293
Nonarachin[b]	0.1338	0.1221

[a] Fractions of step 2, Figure 1. [b] Fractions of step 5, Figure 1.

Glycoproteins. The carbohydrate content of total soluble protein, arachin, and nonarachin fractions was analyzed to determine the possible glycopeptide nature of these peanut seed components. The data (Table II) show that all of these protein fractions of peanuts contained both neutral and amino sugars. The proteins in the total soluble fraction contained approximately 0.48% neutral and 0.15% amino sugars. The nonarachin proteins accounted for most of the amino sugars (0.12%). Arachin and nonarachin proteins both contained similar amounts of neutral sugars (approximately 0.13%). These data suggest that many of the peanut proteins are, in effect, glycoproteins. Other legume seed proteins from beans (Pusztai, 1966), soybeans (Koshiyama, 1966), mung beans (Ericson and Chrispeels, 1973), and peas (Basha and Beevers, 1976) have also been found to be glycoproteins.

Total Amino Acids. The total amino acid composition of the various protein fractions prepared from peanuts with 1 M NaCl–20 mM sodium phosphate is shown in Table III. Proteins in the total soluble fraction (step 2, Figure 1) reflect the typical amino acid composition of peanuts, i.e., low in half-cystine, methionine, lysine, and tyrosine and high in aspartic acid, glutamic acid, and arginine. On the other hand, significant differences were observed in the amino acid composition of the total soluble fraction, solubles I, II, and III (BCS_3), and arachin (BCP_3; precipitate III). In general, levels of serine, half-cystine, glycine, methionine, and lysine were higher in the nonarachin proteins than in the total soluble fraction and arachin. The glycine content of soluble III was four and six times higher than the latter two preparations, respectively. However, aspartic acid, threonine, proline, alanine, valine, isoleucine, phenylalanine, and histidine were lower in solubles I, II, and III and arachin than the original salt–buffer extract. Moreover, during the stepwise purification (solubles I to II to III), levels of certain amino acids in nonarachin proteins either decreased (aspartic acid, threonine, proline, alanine, valine, isoleucine, and phenylalanine), did not change (glutamic acid, leucine, and

Table III. Amino Acid Composition (% of Protein) of Different Protein Fractions Resulting during Fractionation in 1 M NaCl–20 mM Sodium Phosphate Buffer

AA	T. prot.[a]	Sol. I[b]	Sol. II[b]	Sol. III (BCS_3)[b]	Precip. III (BCP_3)[c]
Asp	11.890	11.570	10.330	7.917	11.060
Thr	2.670	2.710	1.140	1.039	2.238
Ser	5.110	5.088	6.768	7.519	4.947
Glu	19.830	18.670	19.960	15.160	18.400
Pro	4.440	4.304	3.051	1.693	4.522
Cys	0.335	1.607	3.293	2.673	1.421
Gly	6.590	3.174	10.490	24.600	4.224
Ala	4.120	3.657	2.008	1.738	3.524
Val	3.880	3.954	1.574	1.587	3.406
Met	0.510	1.013	1.286	1.982	0.660
Ile	3.400	3.052	1.553	1.426	2.807
Leu	6.535	5.859	5.275	4.130	5.678
Tyr	4.090	4.252	5.136	5.722	5.069
Phe	6.385	7.266	3.765	3.061	8.020
His	3.170	3.515	2.718	2.796	3.780
Lys	3.875	3.810	4.287	5.621	3.097
NH_4	1.540	1.891	2.185	1.649	1.987
Arg	11.585	13.980	15.650	11.770	14.130

[a] Total soluble protein, step 2, Figure 1. [b] Solubles I, II, III, steps 3, 4, and 5, Figure 1. [c] Precipitate III, step 5, Figure 1.

arginine), or increased (serine, half-cystine, glycine, methionine, tyrosine, and lysine). These data suggest that there is a possibility of preparing proteins with different nutritional value and functional properties from peanuts using appropriate fractionation procedures.

DISCUSSION

In most cases, procedures used to prepare peanut seed protein begin by grinding full-fat or fat-free meal with either water, buffer or salt solutions (Irving et al., 1945; Dechary et al., 1961; Tombs, 1965; Neucere, 1969; Dawson, 1971; Cherry et al., 1973; Singh and Dieckert, 1973a,b). Fractionation of solubilized proteins then follows taking advantage of one or more of their physicochemical properties relative to ammonium sulfate saturation, pH solubility, chromatographic properties, and/or temperature (heat, cryoprecipitation). The proteins in each fraction are then characterized as to their purity and physicochemical properties. Improving techniques such as those used for gel electrophoresis and amino acid analysis to better define the composition of these protein fractions have shown them to be heterogeneous. Interestingly, many of the protein isolates prepared by different procedures are in fact similar based on criteria such as gel electrophoretic patterns and amino acid composition. Differences among isolates were related to the degree of purity of the major α-arachin and α-conarachin components. This in turn has been related to the degree of dissociation of these major globulins during extraction procedures or variations in chromatographic, salt saturation, or heat coagulation procedures (Johnson and Shooter, 1950; Johnson and Naismith, 1963; Tombs and Lowe, 1967; Tombs et al. 1974). However, with regard to food uses, these differences are probably minor since arachin prepared by either calcium chloride, ammonium sulfate, or cryoprecipitation shows similar amino acid composition (Dawson, 1971). Conarachin fractions prepared by ammonium sulfate saturation, acetic acid precipitation, or dialysis were also similar in amino acid composition; these preparations differed only in the content of certain amino acids. This is also true for a highly purified arachin fraction (P6) recently made by Singh and Dieckert (1973a,b). The amino acid composition of P6 compares closely with those of arachin fractions prepared by Tombs (1965), Dawson (1971), and Neucere (1969); P6 has higher amounts of serine, glycine, alanine, and valine than the other arachin preparations. Thus, use of various fractions of peanut proteins as food ingredients may not require extensive purification of the individual components. Instead, techniques for proper preparation of partially purified protein extracts from peanut seeds with unique physicochemical, functional, and nutritional properties for use as specific food ingredients may be more important.

The present work has shown that good total protein extracts containing many of the arachin and conarachin components in peanuts can be made from fat-free meal homogenized in 1 M NaCl–20 mM sodium phosphate buffer (pH 7.0) at a 1:18 (w/v) ratio. Using this extract as a starting material, relatively pure arachin and nonarachin proteins can be prepared by using a series of simple steps involving differential solubility, cryoprecipitation, and dialysis to change salt concentrations. The arachin product resulting from this process contains similar quantities of amino acids as those reported by previous workers (Tombs, 1965; Neucere, 1969; Dawson, 1971; Singh and Dieckert, 1973a). On the other hand, the nonarachin fraction prepared by our isolation technique differs somewhat from conarachin preparations of previous investigations; e.g., it is higher in serine, glycine, tyrosine, methionine, and lysine. The other amino acids are similar (cysteine, phenylalanine, histidine, and arginine) to or lower (aspartic acid, threonine, glutamic acid, proline, alanine, valine, isoleucine, and leucine) than previously reported data. However, amino acid compositions of conarachin fractions obtained from steps 3 and 4 (solubles I and II, Figure 1) prior to the final purified isolate compared well with published data.

Gel electrophoresis of arachin and nonarachin proteins showed that they were relatively pure. Dissociation of these fractions with SDS and DTT showed that each of them contained at least five subunit components ranging in molecular weight from 20000 to 81000. The subunit components of the two isolates, although slight in some cases, differed in molecular weight. However, the possibility still exists that common subunit components are present in both isolates. Other investigations have shown that the number of subunit components of arachin can range from four to seven having molecular weights between 10000 and 71000 (Singh and Dieckert, 1973b; Shetty and Rao, 1974). Quite possibly, current methods may not be efficient in dissociating arachin to a basic subunit having a molecular weight of 10000. This may also be related to the isolation conditions for arachin which may alter molecular bonds in the basic structure to forms which resist SDS-induced dissociation.

In conclusion, current trends in food processing are toward fabrication of foods from basic ingredients such as proteins with greater emphasis on functional and nutritional properties rather than purity. Ample data are now available on isolation techniques and physicochemical properties of various protein components in peanuts. Understanding the molecular properties of proteins may produce new ingredients as unique functional and nutritive additives in foods.

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Protein and Amino Acid Composition of Three Varieties of Iraqi Dates at Different Stages of Development

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Protein contents and amino acid composition of date fruits were determined in three Iraqi varieties (Khastawi, Khadhrawi, and Zahdi) at different stages of development. Protein concentration for the three varieties was highest at the green stage. Seventeen total amino acids were detected and determined; their concentrations (dry basis) varied. Khastawi and Khadhrawi showed a higher concentration than the Zahdi variety. At the green stage, concentrations of glutamic acid, aspartic acid, lysine, leucine, alanine, and serine were highest. At the yellow and ripe stages, glutamic acid, aspartic acid, lysine, leucine, proline, and glycine were present at a high concentration. For most amino acids, the concentration of amino acids is higher at the yellow stage than at the ripe stage.

Iraq annually produces a considerable amount of dates, two-thirds of which is exported and the remainder of which is utilized locally for consumption or production of vinegar, syrup, and some alcoholic beverages. Several important varities grow in different parts of Iraq. These varieties differ in their color, taste, texture, sugar, protein, and amino acid contents. Date fruits are edible only when they are in the yellow or ripened stage and quite undesirable by man when they are at the green stage. Dates and date syrup are consumed by a large number of Iraqi's and in some low income families they are considered as an important source of food mainly because of their carbohydrate contents.

Qualitative and quantitative studies on the constituents of dates, such as carbohydrates, fats, proteins, and amino acids, at the ripe stage were reported: Ashmawi et al. (1956), Ragab et al. (1956), and Auda et al. (1974).

Amino acid and oligopeptide contents of California dates at different stages of development have been reported by Globbelaar et al. (1955) and Rinderknecht (1959). The amino acid composition of three varities of Iraqi dates at the ripe stage has also been reported by Al-Rawi et al. (1967) and Al-Aswad (1971).

There is very little information on the changes in proteins and amino acids that occur during development and ripening of some varieties of dates. Therefore, we measured changes in proteins and amino acids as a part of a study on the preservation and extension of the market life beyond the harvest season for these varieties which are grown in the Republic of Iraq.

MATERIALS AND METHODS

Sampling. Samples of three common varieties (Khastawi, Khadhrawi, and Zahdi) were obtained from Za'afarania Horticultural Experiment Station. For each

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