content and protein solubility can be observed in the low tannin variety. Finally, the complementary nature of the kafirin and glutelin distribution is shown in Figure 2. The excellent negative correlation between these two protein constituents in the case of BR64 strongly supports our contention that tannin–kafirin complexes behave as glutelins according to solubility characteristics. In preliminary studies we have compared the ability of the individual sorghum protein fractions to complex with purified tannins prepared from sorghum hulls by the method of Strumeyer and Malin (1975). Fractions II and III showed maximum complex formation leading to precipitation of the complexes from aqueous solution.

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Some Physicochemical Properties of Peanut Protein Isolates

N. J. Neucere* and E. J. Conkerton

Peanut proteins were separated by ion-exchange chromatography to yield five fractions. These were characterized by immunochemistry and electrophoresis. The bulk of the protein was concentrated in two fractions, representing the conarachin and arachin systems. One of the five protein fractions contained relatively high concentrations of methionine, lysine, and cystine. Studies of solubility in acidic sucrose buffers showed evidence that peanut protein isolates could possibly be utilized in high-protein citric acid-based beverages.

Isolation, characterization, nutritional quality, and functional properties of plant proteins have been the objects of intense studies in recent years. Protein isolates and concentrates from seeds play an important role in the fabrication of modern foods such as milk-like drinks and imitation meats. Soybean proteins, in particular, dominate the market for producing foods such as meat analogues and extenders and protein-rich beverages. Current literature on this subject, however, indicates that more of the other oilseeds and high-protein legumes will be used in diverse staple food products in the future.

In peanuts, the water- and salt-soluble proteins and enzymes have been characterized according to their elution patterns from chromatography on ion-exchange cellulose, and by other physicochemical methods (Cherry et al., 1973; Daussant et al., 1969; Dechary et al., 1961; Dieckert et al., 1962; Neucere, 1969; Thomas and Neucere, 1973). The nutritional quality of protein fractions isolated by density-gradient centrifugation from peanut cotyledons and in heated intact seed was reported by Jacks et al. (1972) and by Neucere et al. (1972). Peanut protein concentrates described by Rhee et al. (1973) have been reported to have potential in baking applications (Khan et al., 1975).

Fractionation of the peanut proteins by chromatography on DEAE–cellulose, reported by Dechary et al. (1961), was accomplished by elution with a linear sodium chloride gradient. The fractions were categorized into four groups: I, II, III, and IV. From the area under each chromatographic peak, it was estimated that the first fraction, I, which was not adsorbed onto the cellulose, comprised about 8.0% of the total proteins soluble in phosphate buffer, pH 7.9, ionic strength 0.03. Groups II and III (the conarachins) and group IV (arachin) comprised about 20, 29, and 43% of the total proteins, respectively. In the present investigation, the objective was to devise a serial elution chromatographic procedure for isolating protein fractions from peanuts that might be useful in large-scale preparations. The isolated fractions were partially characterized by electrophoresis and immunochemistry, tested for solubility in acidic sucrose solutions, and assessed for relative amino acid contents.

EXPERIMENTAL SECTION

Protein Extraction. Ten grams of cotyledons free of testae and axial tissues from Virginia 56R certified peanut seeds were homogenized in a Waring Blendor at medium speed with 30 mL of phosphate buffer, pH 7.9, ionic

Southern Regional Research Center, Agricultural Research Service, U.S. Department of Agriculture, Oilseed and Food Laboratory, New Orleans, Louisiana 70179.

strength 0.2, for 10 min (Dechary et al., 1961). The homogenate was centrifuged at 37 000*g* for 30 min and the supernatant was recentrifuged under the same conditions. The protein solution was separated from the fat pad and the pellet with a hypodermic needle and syringe. Finally, the protein extract was dialyzed against 1 L of phosphate buffer, pH 7.9, ionic strength 0.03, for 24 h at 5 °C before adsorption on ion-exchange cellulose. Protein concentration was 30 mg/mL.

Isolation of Protein by Chromatography. Fifty grams triethylaminoethyl-cellulose (TEAE), acquired from Brown Company Inc., were dispersed in water and packed in a 2.5 × 60 cm column at 25 °C. The cellulose was washed with 1.0 L of 0.01 M NaOH, followed with 3.0 L of phosphate buffer, pH 7.9, ionic strength 0.03; the flow rate was 100 mL/h. Two grams of protein in 1.0 L of the latter buffer was adsorbed on the cellulose. The breakthrough (B) was saved as the first isolate. The column was then washed with 1.0 L of the same buffer to give the second fraction (W). Further elution was accomplished successively (without a gradient) by use of three 1.0 L portions of low ionic strength (0.03) phosphate buffer containing 0.1, 0.2, and 0.3 M NaCl, respectively; these are designated as the 0.1, 0.2, and 0.3 fractions. These three fractions were each dialyzed against 3 L of deionized water for 48 h at 5 °C to remove NaCl. All fractions were freeze-dried and stored in sealed jars at 2 °C.

Analytical Methods. Protein contents were determined by the method of Lowry et al. (1951). Immune sera were prepared by the standard procedure of Antibodies Inc., Davis, Calif. Immunoelectrophoresis (IEA) was carried out according to Grabar and Williams (1953) in 1.5% Ionagar gel (Oxoid, Ltd., London). Each well was filled with 1.0 mg of protein before electrophoresis, which was conducted in 0.025 M veronal buffer, pH 8.2, at 4 V/cm for 2 h at room temperature. Antibody-in-gel electrophoresis was done according to Laurell (1966) with 2% antiarachin in 1.5% Ionagar, employing 100 V and 10 mA for 16 h at 25 °C. Each well contained 20 μg of protein. Disc electrophoresis was conducted by the method of Steward et al. (1965), using 10.0% polyacrylamide in the running gel and 3.0% in the stacking gel; electrophoresis was performed on 0.6 mg protein from each fraction at a constant current of 3 mA/tube for approximately 1 h at 5 °C. All protein zones on disc gels and immunoprecipitates were stained with 0.1% Amido Black in 7.0% acetic acid and destained with 7.0% acetic acid. One sample of each protein fraction was hydrolyzed, under nitrogen, with 6 N hydrochloric acid at 145 ± 2 °C for 4 h (Conkerton, 1973). Amino acid contents were determined by gas chromatography (Adams, 1974). Multiple runs on the gas chromatograph were made for each sample. Results were determined as relative mole percent:

$$\text{relative mole \%} = (A_a \times 1/R_a)/\Sigma(A_a \times 1/R_a) \times 100$$

where A_a = area of each amino acid peak on the chromatogram and R_a = amino acid molar response/serine molar response. The serine molar response is assigned a value of unity.

To determine relative protein solubilities in sucrose at acid pH, 5 mg of protein from each fraction was dissolved in 1.0 mL of citrate buffer, pH 3.0, containing 0.25 M or 0.5 M sucrose. Protein contents in each fraction were measured before and after removal of sucrose by dialysis. Residual sucrose in the samples after dialysis was determined by the phenol–sulfuric acid method of Dubois et al. (1956).

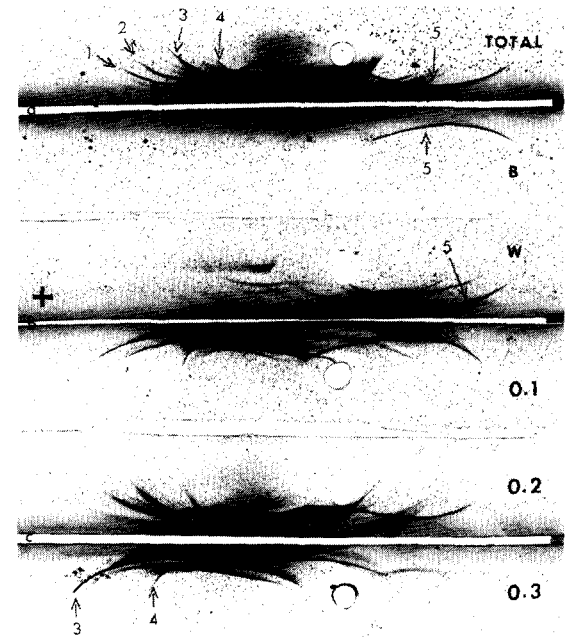

Figure 1. Qualitative immunoelectrophoresis of proteins in isolated fractions dissolved in phosphate buffer, pH 7.9, ionic strength 0.03. A total cotyledonary protein extract was placed in the upper well, and protein in samples of designated fractions was diffused in succeeding wells. Arrows correspond to 1, α_1-conarachin; 2, an arachin contaminant; 3, α-arachin; 4, another arachin contaminant; and 5, the major immunogenic protein in fractions B and W, respectively. Each sample contained 1.0 mg of protein and each trough (a, b, c) was filled with immune serum made against a total cotyledonary extract.

RESULTS AND DISCUSSION

Quantitation of Protein in Each Fraction. Gravimetric analysis showed that B and W, the two fractions eluted with phosphate buffer, represented 2.5 and 3.0% of the recovered protein. Subsequent serial elutions with sodium chloride solutions of increasing concentrations represented 6.9, 32.3, and 55.0% of the recovered protein (fractions 0.1, 0.2, and 0.3).

Characterization of Protein Fractions by Immunochemistry. In Figure 1, IEA shows representative data of the precipitin patterns of each fraction relative to the total proteins. From the previous study (Daussant et al., 1969), at least 14 precipitin lines were detected in a total protein extract from which arachin and conarachin were identified. The major precipitin line in the arachin fraction (arrow 3) was designated as α-arachin, and minor constituents present in that fraction (arrows 2 and 4) were designated as α-arachin contaminants. The major conarachin protein was designated as α_1-conarachin (arrow 1). Data in Figure 1 are intended primarily to show qualitative differences in the number and position of precipitin lines in each fraction. The breakthrough fraction, B, contained one major antigenic constituent that appeared in fraction W (arrows 5); trace quantities of this protein were present in the other fractions. Fractions W, 0.1, 0.2, and 0.3 obviously overlap in contents of specific proteins. For α-arachin and its so-called contaminants in the 0.3 fractions, definite anodic shifts in electrophoretic mobilities were observed (arrows 3 and 4). This observation was reported earlier (Neucere, 1969).

Analysis of Fractions by Disc Electrophoresis. Representative electrophoreograms of the proteins contained in each isolated fraction are shown in Figure 2. In each case, 0.6 mg of protein was layered on top of the stacking gel. Fractions B and W have similar patterns, but

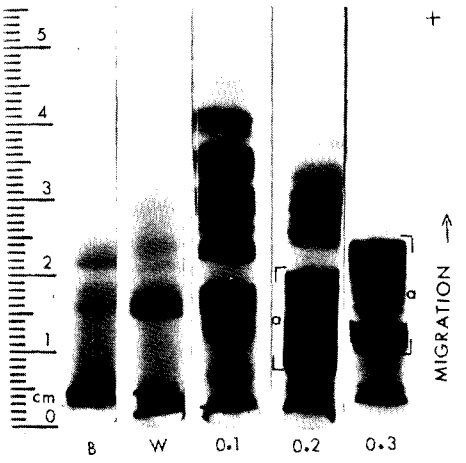

Figure 2. Disc electrophoresis of proteins in isolated fractions. Designated fractions at 0.6 mg of protein each were layered over the stacking gel before electrophoresis. Migration was from bottom to top. Bracketed areas (a) correspond to the position of polymeric forms of α-arachin in fractions 0.2 and 0.3; note the shift in migration of the major zones corresponding to arachin in fraction 0.3.

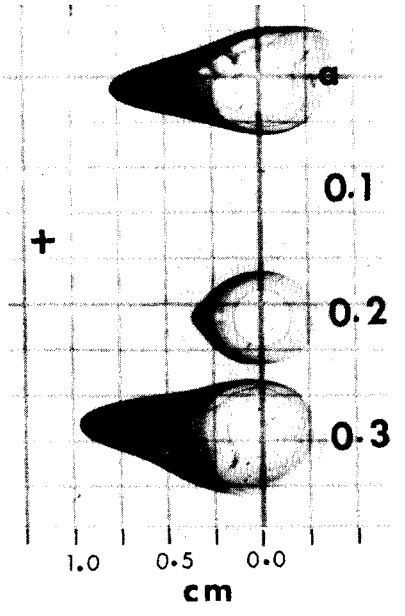

Figure 3. Semiquantitative analysis of α-arachin in fractions 0.1, 0.2, and 0.3 by antibody-in-gel electrophoresis. Standard α-arachin is designated by a. Each sample contained 20 μg of protein.

show differences in staining intensities of certain zones (e.g., zones at the origin and those 1.5 cm from the origin). Fractions 0.1 and 0.2 are the most complex, containing at least 12 protein zones, which are not completely resolved at this concentration. Both fractions 0.2 and 0.3 contained high concentrations of protein that migrated in areas between 0 and 2.5 cm from the origin. Polymeric forms of α-arachin have been reported to migrate in this region (Neucere, 1972); hence, some of the zones in the bracketed areas correspond to α-arachin and the so-called contaminants of α-arachin. The immunochemical data in Figure 1 corroborate these observations on disc gels.

Semiquantitative Analysis of α-Arachin in the 0.1, 0.2, and 0.3 Fractions. The data in Figure 3 are based on a technique described by Laurell (1966). In principle, the method involves the electrophoretic migration of a protein that forms a solid complex with its antibody embedded in agar gel. The length of the conical peak formed is directly proportional to the concentration of

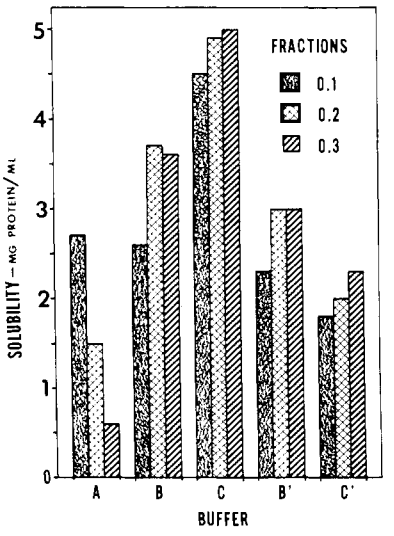

Figure 4. Solubility in acidic sucrose of proteins in fractions 0.1, 0.2, and 0.3 at pH 3.0, expressed as mg of protein solubilized/mL of buffer. A corresponds to 0.05 M citrate buffer, B to 0.05 M citrate–0.25 M sucrose, and C to 0.05 M citrate–0.5 M sucrose. B′ and C′ refer to samples in the sucrose buffers after removal of sugar by dialysis against buffer A.

protein. The results showed that most of the protein in fraction 0.3 (over 90%) consisted of polymeric forms of α-arachin. About 20% of polymeric forms of α-arachin was present in fraction 0.2, and fraction 0.1 contained no α-arachin.

Solubilities in Acidic Sucrose of Proteins from Major Fractions. The relative solubilities of protein in fractions 0.1, 0.2, and 0.3 in citrate–sucrose solutions are shown in Figure 4. Fraction 0.1 showed the highest solubility in citrate buffer (A) alone, but solubilities of the other two fractions increased after sucrose was added to the citrate buffer. Furthermore, solubility was higher in 0.5 M sucrose (C) than in 0.25 M sucrose (B) for all fractions. After removing the sucrose by dialyzing against 0.05 M citrate buffer, some precipitation occurred, but the solubilities of fractions 0.2 and 0.3 remained higher than was observed in the control samples (compare B′ and C′ with A). The reverse was observed for fraction 0.1. These differences in protein solubility may be due to irreversible modifications of tertiary and quaternary protein structures induced by sucrose at acid pH. Analysis for sugars in the dialyzed samples, using glucose as a standard, showed only traces of residual sugar.

Relative Mole Percent Amino Acid Composition of the Fractions. Relative amino acid composition data in Table I show that fractions B and W were similar. Except for the presence of cystine, these two fractions have an amino acid profile—high glycine and lysine contents—similar to the nonspecific peanut hemagglutinin reported by Dechary et al. (1970). Fraction 0.3, the α-arachin fraction, has a typical peanut protein amino acid profile, i.e., high concentrations of glutamic and aspartic acids and low concentrations of methionine, cystine, and lysine. Fraction 0.2 is similar to 0.3 but has slightly higher relative concentrations of methionine and lysine. Of the five, fraction 0.1 has the best amino acid profile for adequate human nutrition. Although this fraction represents only 6% of the original protein, the relatively high concentrations of methionine, lysine, and cystine make it more desirable as a protein supplement than the other four fractions.

From these data, it is evident that simple salt elution of a mixture of peanut proteins from ion-exchange media

Table I. Relative Mole Percent of Amino Acids in Peanut Protein Fractions[a]

Amino acid	B	W	0.1	0.2	0.3
Ala	9.4	6.6	6.8	6.2	7.5
Val	2.7	4.7	6.3	5.0	5.5
Gly	32.4	21.4	9.3	7.6	8.5
Iso	0.7	2.5	3.8	4.0	4.1
Leu	3.5	6.0	6.6	7.5	7.9
Pro	5.0	5.3	4.6	6.0	6.2
Thr	6.0	6.8	6.1	3.2	3.4
Ser	10.6	8.9	8.8	8.9	7.9
Asp	8.7	9.8	11.5	14.1	14.1
Met	1.5	1.9	4.6	2.4	0.8
Phe	1.4	2.4	3.7	4.7	4.4
Glu	11.1	10.8	16.3	20.0	18.0
Tyr	2.7	2.7	3.1	2.9	4.7
Lys	8.7	8.0	5.6	5.4	3.3
Arg			2.7	1.9	4.2
His	n.d.[b]	n.d.	n.d.	n.d.	n.d.
Cys	3.8	2.3	3.6	0.7	0.1

[a] RMR_{ser} assigned a value of unity. [b] n.d. = not determined.

does not completely separate individual proteins. However, proteins with similar ion-exchange capacities can be separated as groups by such a procedure. The bulk of the protein in peanuts, conarachin and arachin, as characterized in this study, is concentrated in two fractions, namely, the 0.2 and 0.3 fractions. From the amino acid profile in Table I, it is evident that these two fractions have relatively low concentrations of some essential amino acids. Thus their nutritional use (as opposed to functional use) would require appropriate amino acid supplementation along with other required nutrients in finished products.

Peanut protein isolates are well suited for fabricating diverse foods because of their bland taste and odor and desirable color. In baking, for example, bread supplemented with peanut protein concentrates was superior in flavor, taste, and crumb color to bread fortified with either whole peanut or soy flour (Khan et al., 1975).

Protein beverages based on plant proteins have been tested and found acceptable in several countries. These range from milk-type drinks (Mustakas, 1974) to protein-fortified fruit juices (Salunkhe and Bolin, 1972). Also, proteins isolated from cheese whey have been reported suitable for the fortification of carbonated beverages (Holsinger et al., 1973). In view of our data on the solubilities of peanut protein isolates in acidic sucrose, perhaps they would be suitable for use in citric acid based drinks. More information such as heat stability and change in turbidity on standing, however, must be obtained.

For use in food products other than beverages, e.g., dairy and meat analogues, other functional properties of these protein isolates (whippability, water retention, gelation, etc.) must be established because they are just as important as the nutritional aspects. Extensive research has been conducted in this area of food applications, especially on soy proteins (Circle and Smith, 1972; Wolf, 1970). Many unique food systems and concepts have been developed as a result of extended knowledge of these functional properties (Glicksman, 1976). The development and introduction of new foods based on plant proteins and their acceptance by consumers has been described by Rosenfield (1976).

The information in this report relates to a worldwide major problem of concern to food scientists, namely, the development of economic, nutritious diets through innovative and unconventional uses of isolated plant proteins. To accomplish this, however, it is essential to ascertain physicochemical properties of proteins and other natural ingredients that are relevent to processing methods and nutritional requirements. The full potential of protein isolates in diverse food applications can be realized once these parameters are established.

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