Peanut Protein and Product Functionality[1]

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Interest in the potential of peanut seed as a source of edible vegetable protein products has been stimulated by an increase in our understanding of protein physicochemical properties, improved protein extraction, fractionation and characterization techniques, advanced technologies to produce high quality and diverse protein ingredients and understanding the interrelationship between physicochemical, functional and nutritional characteristics of protein products. Further expansion in the processing and utilization of peanut products may be constrained by economic conditions rather than by limitations in functionality, nutritional quality, or consumer acceptability. Should changes occur to improve the competitive position of peanuts, the potential contributions of their protein products may be more fully realized.

Worldwide production of peanut (*Arachis hypogaea* Linn) seed is estimated to be approximately 20.4 million metric tons or, an equivalence of 4.8 million metric tons of potentially edible 44% protein meal (1). The United States is a major producer of peanut seed, contributing approximately 10% of world production, and processes most of this commodity to edible foods—namely, peanut butter, roasted and boiled snacks and confections (2). On a worldwide scale, however, peanut seed is grown primarily for the oil, which is favored for cooking and as a salad oil. Oil extraction produces a protein-rich co-product which may be used for human consumption, if processed from edible-grade peanut seed by commercially accepted food processes. This material is available as flakes, grits or flours and may be further processed to high protein concentrates and isolates. Guidelines for all of the processing steps are available for preparing food-grade peanut flour, including raw material selection, processing, packaging and handling (3). New developments in practical manufacturing conditions are continually occurring to improve these specifications. It is clear that peanut protein products can contribute to the physicochemical, functional and nutritional characteristics of foods in which they are incorporated.

PROTEIN PROPERTIES

Protein of peanut seed has been the subject of numerous investigations for over 100 years (4–7). Depending on the type and the amount of extraction medium used, different quantities of protein with varying solubility properties can be prepared from peanut meal or flour (8,9; Figs. 1 and 2). Seed extracts contain two types of globulins; arachin (stored in aleurone grains or protein bodies) and conarachin (present in cellular cytoplasm) and a number of other protein components distinguishable by chromatographic and standard polyacrylamide gel electrophoretic techniques (8; Fig. 1). Arachin can be precipitated from a 10% sodium chloride extract by diluting with water, then either making the salt extract 20% saturated with ammonium sulfate or saturating it with carbon dioxide. Conarachin precipitates by either further dialyzing the filtrate against water after removal of arachin or by saturating it with ammonium sulfate. The method of cold temperature precipitation (cryoprecipitation) can also be used to fractionate arachin and conarachin (10,11).

Classical studies showed that arachin and conarachin can be purified to α-arachin and α-conarachin on the basis of their behavior with selected extraction procedures and electrophoresis in agar and polyacrylamide gels (12,13; Fig. 3). α-Arachin was specifically shown to contain four different kinds of polypeptide chains (α,β,γ, and δ), Singh and Dieckert (14,15) demonstrated with sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis that an arachin-P6 isolate from a 10% NaCl extract contained nine polypeptide subunits. Basha and Cherry (9) showed that water, sodium phosphate buffer (I = 0.03; pH 7.9) and 10% NaCl neither completely extracted nor separated arachin, conarachin and non-arachin proteins. Fractionation of peanut seed proteins with 1 M NaCl-20 mM sodium phosphate buffer yielded α-arachin and α-conarachin fractions which were distinguished on standard polyacrylamide gels (Fig. 4). On SDS gels, α-arachin had one minor and four major components of molecular weights between 20,000 and 80,000, and α-conarachin had two major and three minor components with molecular weights of 23,000 to 84,000.

Yamada *et al.* (16) demonstrated that there were two molecular species of arachin (I and II). Arachin I existed as a monomer (molecular weight 180,000) in 0.01 M sodium phosphate buffer, pH 7.9, but it associated reversibly to a dimer (350,000) in 0.3 M sodium phosphate buffer, pH 7.9. Arachin II existed as a dimer (350,000) under both conditions. Tombs (12,13) showed earlier that the molecular weight of α-arachin was approximately 320,000. In a follow-up study, Yamada *et al.* (17) showed by isoelectric focusing in a sucrose gradient that the 180,000 molecular weight component of arachin consisted of six different subunits (molecular weights between 19,500 and 40,500; Fig. 5). Arachin and conarachin extracted in 10% NaCl, 0.01 M sodium phosphate buffer, pH 7.9, and separated by sucrose density gradient centrifugation, both form a dimer (350,000) and a monomer (180,000) at high ionic strength, suggesting that these globulins have similar size polypeptides. Four and five components of arachin and conarachin, respectively, identified on SDS electrophoretic gels by Basha and Cherry (9) had comparable molecular weights. More recently, Krishna *et al.* (18) showed yet another form of variation, namely, varietal or genetic classes of arachin polypeptides distinguishable on SDS polyacrylamide gels as follows: (i) classes A and B, both containing three acidic subunits of arachin and a basic subunit, but varying in molecular weight; (ii) class C, an additive pattern of A and B;

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Mention of specific products/companies is for convenience, and does not constitute an endorsement by the U.S. Department of Agriculture, over other products/companies not mentioned.

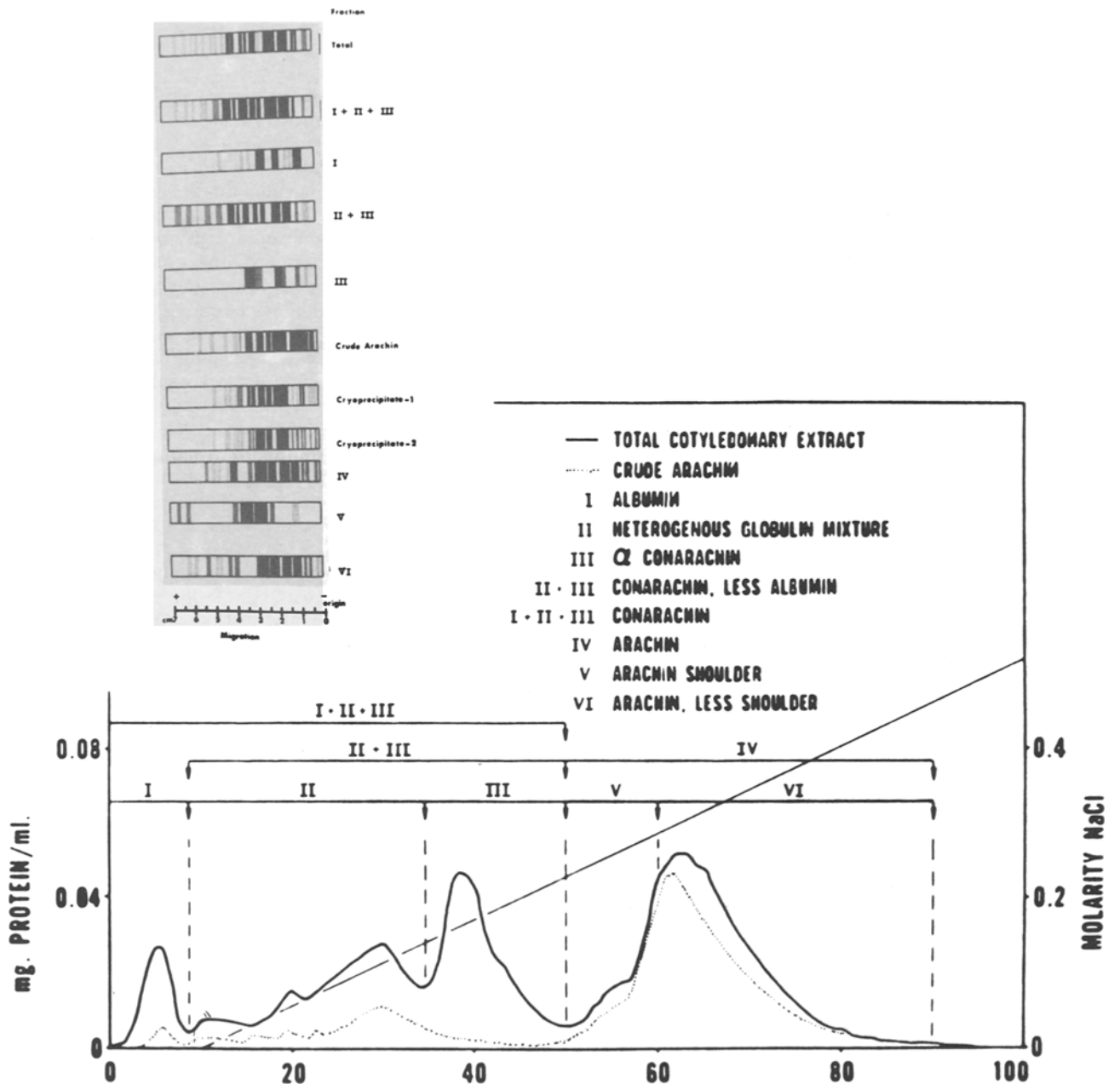

FIG. 1. DEAE-cellulose chromatographic elution patterns and standard polyacrylamide electrophoretic gels of peanut seed proteins (8).

and (iii) class D, two acidic polypeptides and one basic subunit (Fig. 6). Tombs (12,13) showed the presence, in certain seed, of a variant form B of α-arachin which contained only β, γ and δ chains. Cherry (19) demonstrated the existence of variable patterns in the arachin region of standard polyacrylamide gels of extracts from wild *Arachis* species.

Because current methods may not be sophisticated enough to completely isolate and dissociate α-arachin and α-conarachin to their smallest subunits, or purifying conditions may alter molecular bonds in their structures to forms that resist polypeptide dissociation, research needs to continue on the basic physical and chemical properties of peanut proteins to clarify these issues. Moreover, studies are needed to characterize the genetic variability that exists among polypeptides of the storage globulins. As one approach, peanut seed arachin and non-arachin proteins can be fractionated by ion-exchange and gel filtration chromatography and polypeptides. Of these separated components examined by two-dimensional polyacrylamide gel electrophoresis employing isoelectric focusing in the first dimension and SDS electrophoresis in the second (20–23; Fig. 7). The composite patterns show as many as 74

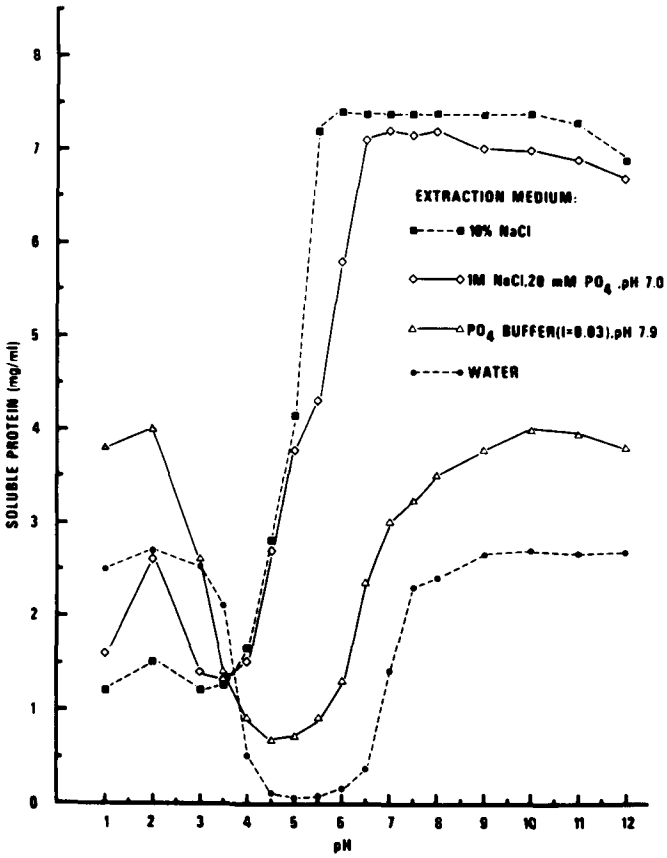

FIG. 2. Protein solubility profiles of peanut proteins (9).

major and 125 minor components with molecular weights ranging between 15,000 and 175,000. Peanut seed protein were recently resolved by high-performance liquid chromatography into four major and six minor fractions with molecular weights ranging from 80,000 to 480,000 (24).

Procedures used to prepare peanut seed proteins usually begin by grinding full-fat or fat-free meals with water, buffer, alkaline pH or salt solutions. The solubilized proteins are then fractionated according to one or more of their physicochemical properties relative to ammonium sulfate saturation, pH solubility, chromatographic conditions and/or temperature (heat, cryoprecipitation). Gel electrophoretic techniques used to characterize the physicochemical properties of proteins have shown them to be heterogeneous, varying in numbers and types of polypeptide subunits depending on their purity and degree of dissociation. However, with regard to use as food ingredients, these differences are probably minor since isolates of arachin and conarachin prepared by various techniques have similar amino acid compositions (4,5). Therefore, emphasis should also be placed on developing techniques for preparing partially purified protein products, namely, concentrates and isolates, from peanut seed that have unique physicochemical, functional and nutritional properties for use in selected foods.

PROTEIN PRODUCT PROCESSES

Technology is available to produce edible protein products from peanut seeds, which are generally classified as flours, concentrates and isolates that contain up to

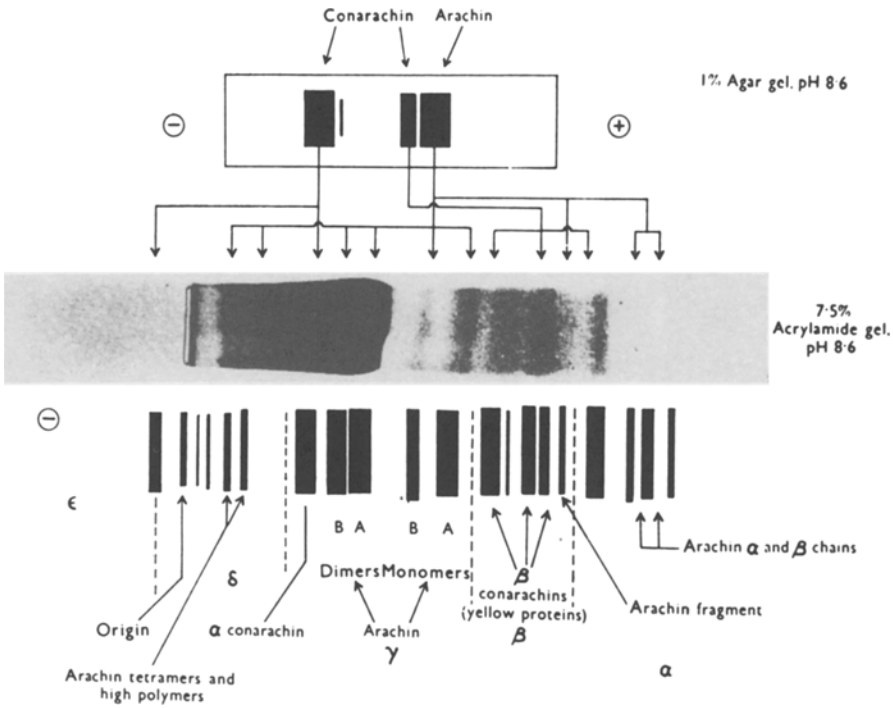

FIG. 3. Electrophoretic classification of peanut proteins (12,13). The result of agar-gel electrophoresis is shown at the top; these zones are connected to those corresponding to α-arachin and α-conarachin in the acrylamide gel shown below. Below this is a diagram of the gel, for clarity, with identification of some of the components. The gel has been split into five areas; α, β, γ, δ and ε.

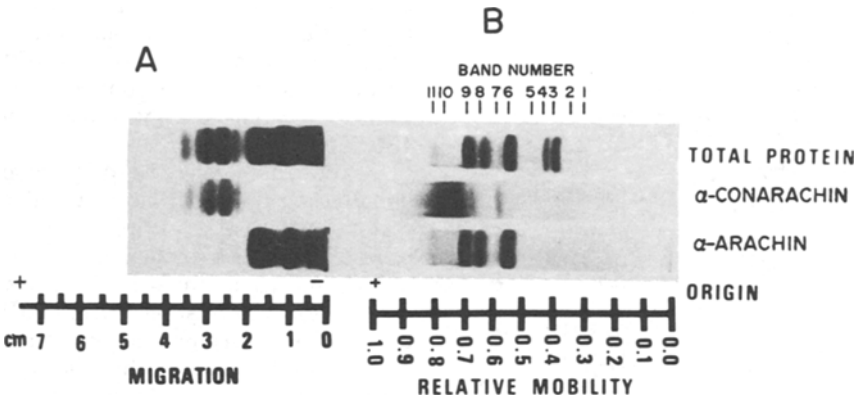

FIG. 4. Standard- (A) and SDS- (B) polyacrylamide gel electrophoretic patterns of the pH 7.0, 1 M sodium chloride-20 mM sodium phosphate buffer extracted peanut proteins (9).

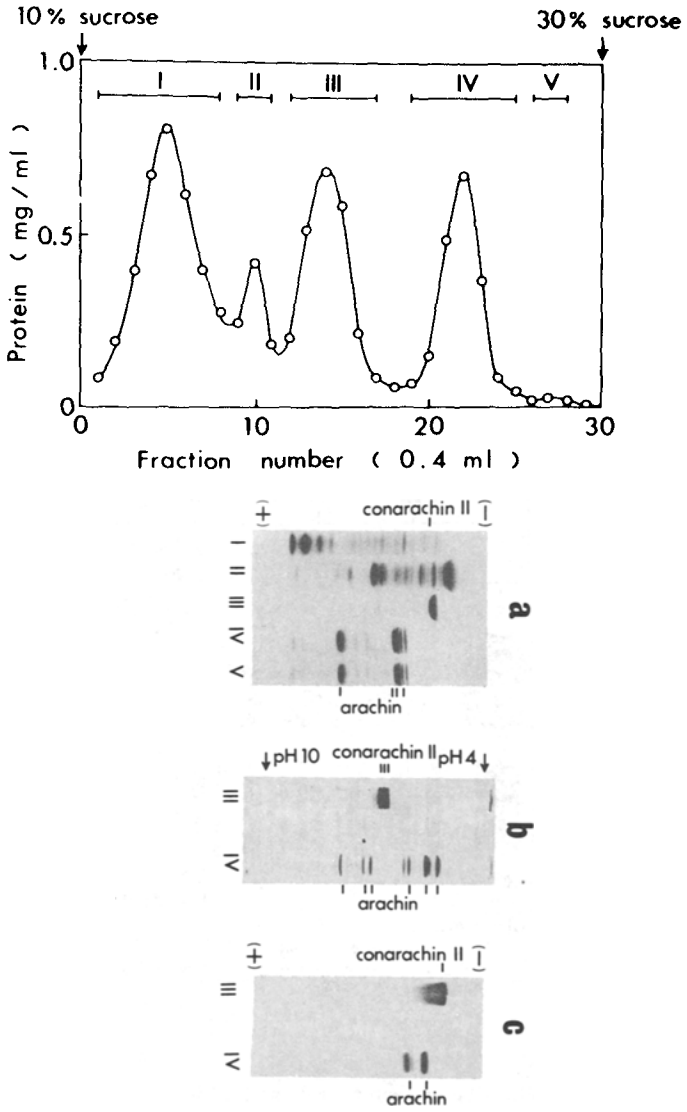

FIG. 5. A typical sedimentation pattern of soluble protein on sucrose density gradient centrifugation (SDGC) (16,17). Gel electrophoresis of the protein components fractionated by SDGC; (a) SDS-gel electrophoresis; (b) gel isoelectric focusing in urea; and (c) standard gel electrophoresis.

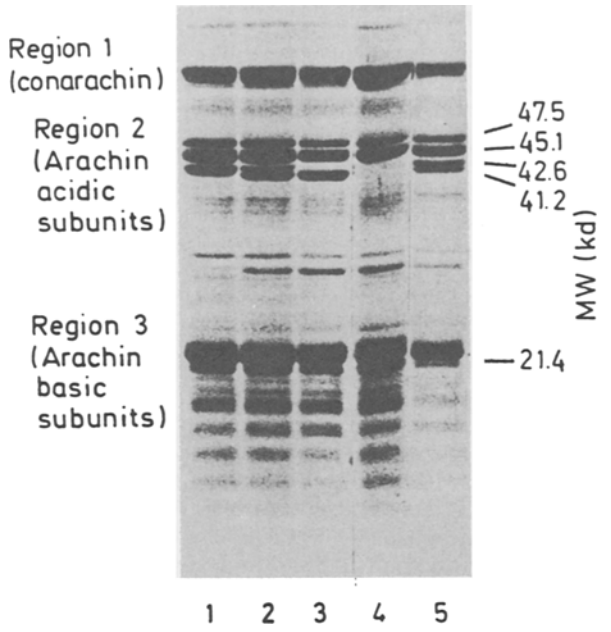

FIG. 6. SDS polyacrylamide gel electrophoretic patterns of peanut protein from different genotypes (18). track 1 is variety SP; 2, TG-8; 3, TG-1; 4, TG-18; and 5, a mixture of SP and TG-1. The numbers in the margin are molecular weights of the arachin polypeptides.

69%, 70–85% and 86–100% protein, respectively (4–7). A group of peanut products with protein content ranging from 25% (full-fat products) to 95% (isolates) has been developed experimentally, some of which are commercially available (5; Fig. 8). Processes for preparing these peanut ingredients are similar, in principle, to those for corresponding soybean products.

The literature contains information on oil mill principles and operations in selected countries, industrial production of peanut products (2–6, 25,26), and pilot plant investigations (27). Hydraulic pressing, screw pressing, solvent extraction and pre-pressing followed by solvent extraction are the basis of these processes to remove oil and prepare defatted meals; the solvent generally used is hexane. Ayres and coworkers (28,29), and Steele (30) presented an improved modified prepress solvent extraction technique for processing of edible flour and grits from peanut seed. A process for making pre-cooked full-fat, partially and defatted flakes from peanut seed was developed (31–33); full-fat peanut flakes are being marketed. These products are said to be essentially free of flavor and white in color, which are important characteristics for their use by the food industry as vegetable protein ingredients.

Process variations, including leaching at the isoelectric point, aqueous alcohol leaching, air-classification, liquid cyclone fractionation, moist-heat denaturation followed by water leaching and aqueous extraction have been developed at the experimental level to produce concentrates with 60–85% protein content (4–7,26,34–39; Fig. 8). Bitter-tasting and odoriferous, or off-flavored compounds, including peanut flavor, trypsin inhibitor, aflatoxins and flatulent-causing sugars are usually removed in the preparation of protein concentrates from peanut products.

Processing of peanut isolates containing 90% or more protein involves extracting the water-soluble polysaccharides, water-soluble sugars and other minor constituents, in addition to those constituents that are necessary to make concentrates. The basic methods involve the extraction of proteins from defatted peanuts with aqueous alkaline solution followed by removal of insoluble material by centrifugation and/or filtration methods, precipitation of the proteins at their isoelectric pH, collection of the precipitated protein curds by centrifugation or filtration methods, and drying of the isolates by spray-drying or lyophilization procedures (4–7,25,26,40–50; Fig. 8).

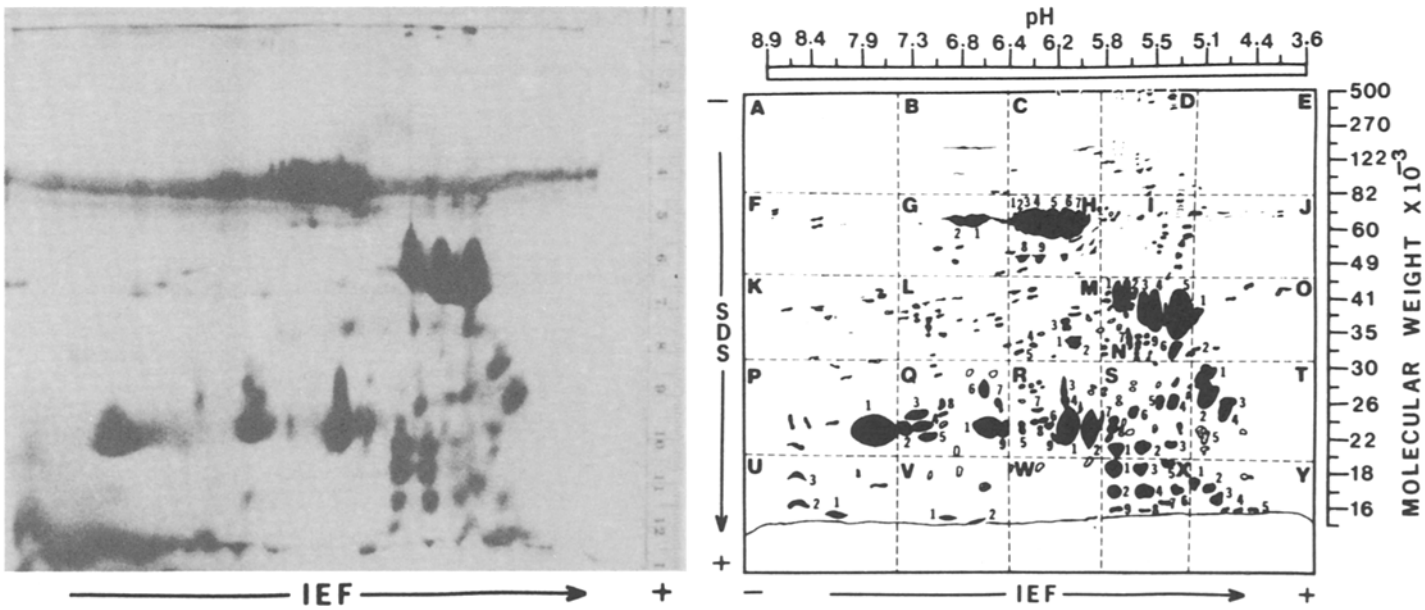

FIG. 7. Actual and diagrammatic composite, two-dimensional gel electrophoretic patterns of the polypeptides in peanut seeds (20–23). Varieties studied include Florigiant, Florunner, 439-16-10-3, 439-16-10-1-1, Early Bunch and Jenkins Jumbo Runner.

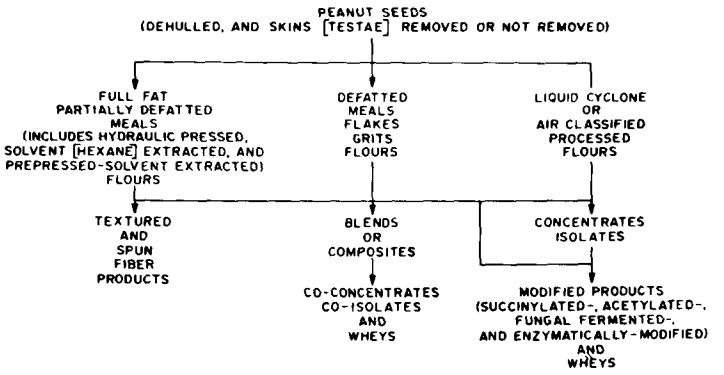

FIG. 8. Peanut protein products (5).

Variables involved in protein isolation include particle size of the flour, flour-to-water ratio, pH, time and temperature of extraction and speed and length of centrifugation. These variables effect yield and extractability of protein. Aqueous alkaline extraction is conducted at pH values below 9.0 to minimize protein denaturation. An added note is that the aqueous alkaline process for isolates includes a pH 8.0 extraction followed by a filtration to separate the proteins from the fiber fraction, steps that are not included in making concentrates (44-47). The protein isolates can be neutralized before drying to make them water dispersible.

Research has been reported showing that large-pore industrial hollow-fiber ultrafiltration membranes can be used in conjunction with a special difiltration technique to produce flavor-free, light-colored protein isolates and concentrates (49). Flavor- and color-causing constituents are simultaneously removed from the protein products. The yield and quality of products from these new processes suggest a viable potential for commercialization.

PROTEIN PRODUCTS

Selected peanut protein products including flakes, flours, concentrates and isolates processed by the methods discussed above were obtained from various laboratories in the United States for comparative studies of proximate composition, amino acids and selected func-

TABLE 1

Selected Peanut Protein Products and Sources Available for Compositional, Functionality and Nutritional Studies (5)

Clemson Univ., full-fat, partially-defatted, and defatted flakes (31-33)
Texas A&M Univ., aqueous processed full- and low [or partially-defatted]-fat concentrates and isolate (38,44-47,50)
Gold Kist extensive and mild heat processed flours (28-30)
Georgia Experiment Station flour (51)
Pert blanched, defatted, Crown Test No. 1, and AVOCA flours (38,41,44-47,50)
Swift direct solvent extraction processed flour (52,53)
Southern Regional Research Center (SRRC) hexane defatted and liquid cyclone processed (LCP) flours (40,41)
Univ. Florida, freeze-dried and spray-dried concentrates (54,55)
Georgia Institute of Technology isolate (50)

TABLE 2

Proximate Composition of Selected Peanut Protein Products (5)

	Proximate composition (%)					
Product[a]	Moisture	Protein (N × 6.25)	Fat	Fiber	Ash	N-Free extract (carbohydrate)
Clemson defatted flakes	5.27	58.88	0.77	0.67	4.51	29.90
Gold Kist mildly heated flour	10.33	59.88	2.31	3.75	4.65	19.08
Georgia Experiment Station flour	9.91	57.25	1.15	3.25	4.09	24.35
Pert AVOCA flour	3.75	59.63	0.23	3.40	4.69	28.30
Swift flour	3.34	60.63	1.12	3.65	4.72	26.54
SRRC flour	2.12	63.13	2.08	3.15	4.44	25.08
Texas A&M partially-defatted concentrate	4.81	44.13	29.02	4.40	1.46	16.18
Florida spray-dried concentrate	8.94	88.50	1.50	1.55	2.97	0
Georgia Institute of Technology isolate	7.40	92.25	0.34	2.40	1.42	0

[a]From Table 1.

tional properties (5; Table 1). Protein compositions of the products ranged between 44.13% and 92.25%, based on the calculation of % nitrogen multiplied by 6.25 (Table 2)—these percentages depended on the degree to which fat, fiber, ash and nitrogen-free material were removed during processing of the various products. Typical polyacrylamide gel electrophoretic patterns of peanut proteins are those of Gold Kist mildly heated flour, Georgia Experiment Station flour, Swift flour and SRRC flour (Fig. 9). Adjusting the pH of aqueous suspensions of the Texas A&M University partially defatted concentrate and Georgia Institute of Technology isolate to 8.2 increased protein solubility and improved the gel patterns. These gel patterns are typical of changes that occur in proteins when various heat and pH conditions are used during processing of peanut products (4,5).

Table 3 presents the amino acid composition of the selected peanut products. Most of the products had similar compositions varying in the intermediate range only between 0.1% (histidine, methionine) and 3.3% (glutamic acid). In fact, most of the amino acids within the intermediate range differed by less than 0.7%.

Functionality, or functional property, is defined as "any property of a substance, besides nutritional, that affects its utilization" (56). Functionality, and

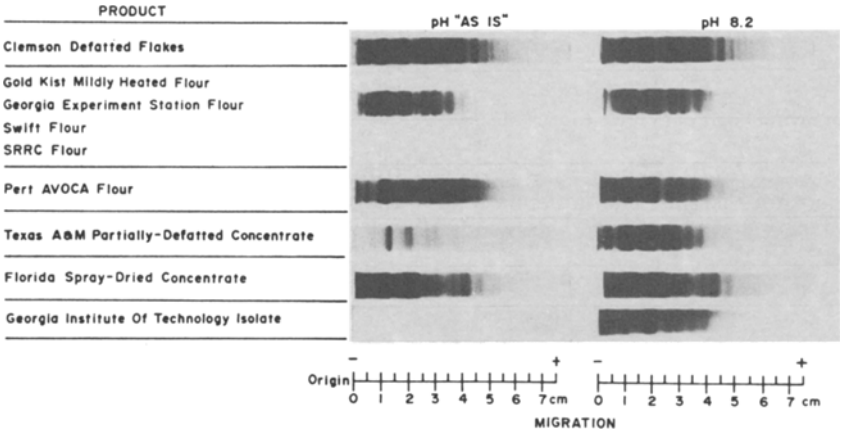

FIG. 9. Standard polyacrylamide gel electrophoretic patterns of proteins from peanut products (5).

TABLE 3

Low, Intermediate Range and High Amino Acid Compositions of Proteins in Peanut Products (5)

	Composition (g/16 g nitrogen)		
Amino acid	Low[a]	Intermediate[b] range	High[a]
Lysine[c]	3.0 (GIT)	3.1- 3.4	3.6 (CD,GES)
Histidine[c]	2.1 (PA,SRRC)	2.2- 2.3	2.4 (GKM)
Arginine[c]	11.2 (SRRC)	12.4-13.0	13.1 (GIT)
Aspartic acid	11.0 (SRRC)	11.2-13.4	13.9 (FSD)
Threonine[c]	2.1 (GKM)	2.3- 2.8	2.9 (FSD)
Serine	4.2 (SRRC)	4.3- 5.0	5.2 (FSD)
Glutamic acid	18.7 (SRRC)	19.2-22.5	23.5 (FSD)
Proline	4.0 (SRRC)	4.1- 4.8	5.7 (FSD)
Glycine	4.8 (FSD)	5.2- 6.4	6.6 (CD)
Alanine	3.6 (SRRC)	3.9- 4.2	4.5 (GKM)
Half-cystine	0.7 (GKM)	0.8- 1.0	1.1 (GIT)
Valine[c]	3.4 (GKM)	4.0- 4.5	5.2 (FSD)
Methionine[c]	0.8 (GES,FSD)	0.9- 1.0	1.1 (PA)
Isoleucine[c]	3.2 (SRRC)	3.3- 3.6	4.2 (FSD)
Leucine[c]	5.9 (SRRC)	6.3- 7.0	7.9 (FSD)
Tyrosine	3.9 (SRRC)	4.0- 4.6	4.9 (GIT,FSD)
Phenylalanine[c]	4.8 (SRRC)	4.9- 5.5	6.2 (FSD)

[a]CD = Clemson defatted flakes; GKM = Gold Kist mildly heated flour; GES = Georgia Experiment Station flour; PA = Pert AVOCA flour; SRRC flour; GIT -Georgia Institute of Technology Isolate; and FSD = Florida spray-dried concentrate.
[b]Values of other products listed in Table 1 are within this range.
[c]Essential amino acid.

TABLE 4

Protein Solubility, Emulsifying and Foaming Properties of Selected Peanut Product-Water Suspensions at "As Is" and Adjusted pH Values (5)

Product[a]	pH ("As Is"; adjusted)	Total protein soluble (%)	Emulsifying properties			Foaming properties	
			Capacity (ml oil)	Apparent viscosity (cps)	Type	Increase (%)	Type
Clemson defatted flakes	6.78	24.6	69.9	26,240	Salad dressing-like	55.0	Very thin
	to 4.0 to 8.2	28.8	*b*	—	—	—	—
Gold Kist mildly heated flour	6.66	40.0	65.6	2,720	Pourable suspension	55.0	Thin
	to 4.0 to 8.2	53.8	85.4	83,040	Mayonnaise-like	70.0	Thin
Georgia Experiment Station flour	6.74	38.0	78.0	5,760	Pourable suspension	100.0	Thick egg-like
	to 4.0 to 8.2	53.3	89.7	70,880	Mayonnaise-like	120.0	Thick egg-like
Pert AVOCA flour	6.48	12.6	85.6	72,320	Mayonnaise-like	100.0	Medium
	to 4.9 to 8.2	37.2	88.6	126,240	Mayonnaise-like	90.0	Very thick
Swift flour	6.62	51.0	75.4	12,960	Salad dressing-like	75.0	Thin
	to 4.0 to 8.2	80.3	80.6	64,164	Mayonnaise-like	110.0	Medium
SRRC flour	6.75	49.4	73.8	31,040	Thick salad dressing-like	60.0	Thin
	to 4.0 to 8.2	52.6	76.9	54,880	Mayonnaise-like	55.0	Thin
Texas A&M partially defatted concentrate	4.96	0	*c*	—	—	10.0	Very thin
	to 4.0 to 8.2	42.3	68.0	82,080	Mayonnaise-like	40.0	Very thin
Florida spray-dried concentrate	8.32	60.5	97.1	160,000+	Mayonnaise-like	105.0	Medium
	to 4.0 to 8.2	62.1	77.0	160,000+	Mayonnaise-like	100.0	Thick egg-like
Georgia Institute of Technology isolate	4.69	3.8	*c*	—	—	32.5	Very thin
	to 4.0 to 8.2	34.6	102.9	160,000+	Mayonnaise-like	400.0	Thick egg-like

[a]From Table 1.
[b]Insufficient sample.
[c]None formed.

particularly the role of protein, is a high priority area of research. The functional properties that govern the role of proteins in food applications are color, flavor, texturization, solubility, viscosity, adhesion or cohesion, gelation, coagulation, aeration or foamability, water and oil absorption and emulsification. Of these, solubility, foamability and emulsification are popular to study because they form the basis of many food systems. The inherent properties of proteins or their molecular conformation, denaturation, aggregation, pH solubility and susceptibility to divalent cations affect their performance in model and commercial functional systems. This is especially noted in foamability and emulsification profiles of proteins that closely resemble protein solubility curves and thus the factors that influence solubility properties, including protein composition and structure, methods and conditions of extraction, processing and storage, or treatments used to modify protein character, also influence these functional properties.

Products having high protein solubility after adjusting the pH from "native pH" to 4.0 to 8.2 had good emulsifying properties (Table 4); i.e., these products had high oil capacities and viscosities, and produced mayonnaise-like emulsions. These properties are considered important qualities in the formation of quality meat systems. McWatters and Cherry (57) showed that adjusting the pH of peanut meal suspensions from 6.7 ("native pH") to 4.0 to 8.2 dramatically improved their functional behavior in emulsion formation. The best emulsions were formed with concentrates and isolates, even though some of these products had lower protein solubilities at pH 8.2 than certain flours. This is probably due to the high concentration of protein, especially the major globulin, arachin, in the concentrates and isolates, which is thought to contribute greatly to the functionality of peanut products (4,5). Peanut products, especially those having the highest percentage of soluble protein, were also shown to have excellent foaming properties (Table 4). These observations show that peanut proteins readily unfold at interfaces and expose their hydrophobic regions facilitating deformation of the liquid and enhancing aeration of the solution. As discussed above, this is affected by pH, ionic strength, temperature and protein content.

Interest in the potential of peanut seed as sources of edible vegetable protein ingredients has been stimulated by an increased understanding of protein physicochemical and functional properties. Further expansion in the processing of peanut seed to vegetable protein products could be realized if present constraining economic conditions are eased.

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