10

Legumes: Seed Composition and Structure, Processing into Protein Products and Prctein Properties

W. J. Wolf

The family Leguminosae includes more than 12,000 species in about 500 genera that are widely distributed from the tropics to the arctic regions. Although abundant in the temperate zones, legumes are especially numerous in the tropics. Man has cultivated some members of this family since remote antiquity and few families of dicotyledons are of greater importance than the legumes. Nonetheless, only a few legumes are of economic significance either as feeds or foods. Clovers, vetches, and alfalfa are among our most prominent forage crops, but of even more importance are peas, beans, lentils, peanuts, and soybeans that are grown as food plants.

Peas, beans, and lentils are consumed directly as foods in many parts of the world and are important sources of dietary protein in countries where animal proteins are scarce and expensive or are not consumed for religious or cultural reasons. Few of these legumes are processed industrially to concentrate the protein fraction. In Thailand, the mung bean (*Phaseolus aureus*) is processed for its starch for conversion into a spaghetti-like "bean thread" which is transparent rather than opaque when cooked. The protein-rich residue has been used to prepare a textured product. In England, the broad bean (*Vicia faba*) has been fractionated on a pilot scale into

protein isolates which in turn were spun into fibrous meat analogs. Field peas and broad beans are being developed as new sources of protein for food and feed used in Western Canada. Studies on flours, protein concentrates, and isolates from these two legumes have been reported (Fleming *et al.* 1975).

Large quantities of peanuts are consumed directly as well as processed into oil and meal. In the United States, for example, about one-half of the crop is used domestically as salted peanuts, peanut candy, and peanut butter; the latter accounts for about 50% of the edible uses. The remainder of the crop is either crushed for oil or exported. The high-protein meal obtained as a by-product in oil crushing goes largely into animal feeds. Edible grade defatted peanut flours and grits became available in the United States in the early 1970's.

Soybeans are rarely eaten in the United States, but have been processed into comparatively simple food products for many centuries in the Orient. Processing includes water extraction (soy milk) with coagulation by a calcium salt (tofu), roasting (kinako), and fermentation (miso, tempeh, and soy sauce). The bulk of the world soybean crop is processed into edible oil and soybean meal. As with peanut meal, the bulk of defatted soybean meal goes into animal feeds, but increasing amounts are being converted into edible flours and grits, protein concentrates, and protein isolates. Of all the legumes, soybean proteins have reached the highest degree of refinement and extent of development and are added to a wide variety of processed foods (Wolf and Cowan 1975).

Because of their growing importance as food ingredients, proteins from several legumes are receiving increased attention from biochemists and food scientists who are gradually unraveling their complex properties and trying to relate them to problems encountered in food applications. Only selected topics are reviewed here. Other sources should be consulted for more comprehensive information on production, composition, and processing (Deschamps 1958; Smith and Circle 1972; Wolf 1975); food uses, and nutritional properties (Bressani and Elias 1974); and biochemical, physical, and chemical properties of the proteins (Derbyshire *et al.* 1976; Millerd 1975).

SEED COMPOSITION

Proximate analyses for a selection of edible legumes are given in Table 10.1. A distinguishing feature of legume seeds is their high protein content; most legumes fall into the range of 20-30% protein. Another common feature of many legumes is a low fat (2-5%) and high carbohydrate (55-60%) content. Starch is often a major

TABLE 10.1
COMPOSITION OF SEVERAL LEGUMES[1]

Legume	Protein[2] (%)	Fat (%)	Ash (%)	Fiber (%)	Carbohydrate[3] (%)	Reference
Chick pea (*Cicer arietinum*)	20.6	5.4	2.8	10.3	61	Verma *et al.* (1964)
Lentil (*Lens esculenta*)	29.6	3.1	2.4	3.2	62	Singh *et al.* (1968)
Pea (*Pisum sativum*)	27.9	3.2	2.8	5.9	60	Singh *et al.* (1968)
Broad bean (*Vicia faba*)	31.8	0.9	3.6	8.5	55	Marquardt *et al.* (1975)
Peanut (*Arachis hypogaea*)	30.0	50.3	3.1	3.0	14	Hoffpauir (1953)
Soybean (*Glycine max*)	43.9	21.0	4.9	—[4]	30[4]	Kawamura (1967)

[1]Moisture-free basis.
[2]Kjeldahl N × 6.25.
[3]Measured by difference.
[4]Fiber is included in carbohydrate value.

constituent of the carbohydrate fraction. Broad beans, for example, may contain as high as 40% starch (Bhatty 1974).

When compared to other legumes, soybeans and peanuts are anomalous in their compositions. Soybeans have high protein and oil content while peanuts are very high in oil. Because of their high oil contents, soybeans and peanuts are classified as oilseeds for purposes of commerce. Their high oil contents are counterbalanced by low carbohydrate contents. Soybeans, for example, contain little or no starch; the carbohydrates are primarily cell wall polysaccharides plus the oligosaccharides sucrose, raffinose, and stachyose.

In addition to having a high protein content, most legumes have a reasonably good balance of essential amino acids (Table 10.2). They are generally a good source of lysine. Although peanuts are low in this amino acid, a more common deficiency is methionine which is the first limiting amino acid in many legumes, including soybeans.

SEED STRUCTURE

Legume seeds have a characteristic structure consisting of three major parts: seed coat (hull), cotyledons, and hypocotyl (including plumule). Table 10.3 shows the distribution of these parts for several selected legumes. Compositions of the three seed fractions for soybeans are given in Table 10.4. Similar studies on the distribution of nutrients in anatomical parts of other legumes are available (Lal *et al.* 1963; Singh *et al.* 1968). In many of the legumes, the cotyledons constitute about 90% of the seed. The seed coat is the next largest fraction, but because it is high in fiber, it contains an insignificant part of the total food value of the whole seed. In the preparation of

TABLE 10.2
ESSENTIAL AMINO ACID CONTENTS OF SEVERAL LEGUMES

	Chick Pea[1] (*Cicer arietinum*)	Lentil[2] (*Lens esculenta*)	Broad Bean[3] (*Vicia faba*)	Pea[4] (*Pisum sativum*)	Soybean[5] (*Glycine max*)	Peanut[6] (*Arachis hypogaea*)
Protein content, %	17.5	24.1	31.8	31.6	61.4	56.9
Amino acids			g/16 gN			
Arginine	7.98	8.45	10.6	9.2	8.42	—[7]
Histidine	2.57	3.81	2.8	2.5	2.55	—[7]
Isoleucine	4.53	6.30	4.5	4.4	5.10	3.2
Leucine	7.63	10.9	7.7	7.4	7.72	5.9
Lysine	7.72	7.96	7.0	7.7	6.86	3.1
Methionine	1.16	0.70	0.6	1.3	1.56	0.9
Phenylalanine	6.46	6.25	4.3	4.9	5.01	3.8
Threonine	3.86	4.47	3.7	3.8	4.31	2.3
Tryptophan	1.78	1.22	—[7]	1.3	1.28	—[7]
Valine	4.63	5.42	5.2	4.9	5.38	4.1

[1]Shehata and Fryer (1970).
[2]Khan and Baker (1957).
[3]Marquardt *et al.* (1975).
[4]Data for dehulled pea flour (Anon. 1974).
[5]Data for defatted soy flour (Rackis *et al.* 1961).
[6]Data for defatted peanut (Virginia variety) flour on as-is moisture basis (Conkerton and Ory 1976).
[7]Not reported.

TABLE 10.3
DISTRIBUTION OF SEED PARTS IN LEGUMES

Legume	Proportion of Whole Seed		
	Seed Coat (%)	Cotyledons (%)	Hypocotyl (%)
Pea (*Pisum sativum*)[1]	10.0	89.3	1.3
Lentil (*Lens esculenta*)[1]	8.1	90.0	2.0
French bean (*Phaseolus vulgaris*)[1]	8.6	90.4	1.0
Mung bean (*Phaseolus aureus*)[1]	12.1	85.6	2.3
Soybean (*Glycine max*)[2]	7.3	90.3	2.4

[1]Singh *et al.* (1968).
[2]Kawamura (1967).

TABLE 10.4
PROXIMATE COMPOSITIONS OF SOYBEANS AND SEED PARTS[1]

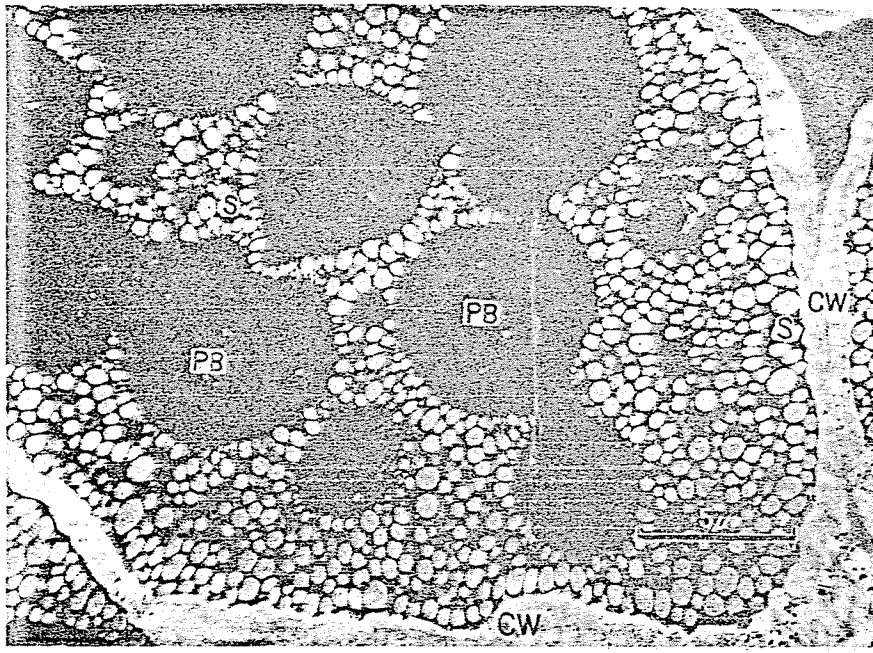
Fraction	Protein (N X 5.71) (%)	Fat (%)	Carbohydrate (%)	Ash (%)
Whole bean (100%)	40	21	34	5
Seed coat (8%)	9	1	86	4
Cotyledon (90%)	43	23	29	5
Hypocotyl (2%)	41	11	44	4

Source: Kawamura (1967).
[1]Moisture-free basis.

edible soy flours and grits, the hulls (seed coat) are removed and standards for defatted soy flour permit a maximum of 3.5% fiber. Undehulled, defatted soybean meal contains about 6% fiber.

Legume seeds are highly organized structures when examined at the subcellular level. The major constituents, proteins, lipids, and starch (when present) are neatly packaged as exemplified for the soybean (Fig. 10.1). Protein storage sites are called protein bodies and in soybeans vary from 2-20 μm in diameter, although many are about 5-8 μm. The lipid deposits are called spherosomes and in soybeans are about 0.2-0.5 μm in diameter. Peanuts contain protein bodies 2-10 μm in diameter along with occasional starch grains ranging from 5-10 μm in size (Bagley *et al.* 1963). Spherosomes in peanuts are about five times (1-2 μm) as large as those in soybeans (Jacks *et al.* 1967).

In the nonoil-bearing legumes, the prominent subcellar structures are protein bodies and starch granules. For example, in peas the protein bodies average 2 μm in diameter and the starch granules are egg shaped with lengths up to 15 μm (Varner and Schidlovsky 1963).



From Saio and Watanabe (1968)

FIG. 10.1. ELECTRON MICROGRAPH OF A SECTION OF A MATURE SOYBEAN COTYLEDON

Seed was soaked in water overnight, fixed with osmium tetroxide, and stained with uranyl acetate and lead citrate. Structures identified are protein bodies (PB), spherosomes (S), and cell wall (CW).

Starch granules are very prominent in broad beans (*Vicia faba*) and range from 10–40 μm in length to 10–25 μm in width; protein bodies in this legume are about 1–5 μm in diameter (McEwen *et al.* 1974). Cotyledon cells of lima beans (*Phaseolus lunatus*) likewise are very high in starch, and granules averaging about 25 μm in diameter are closely packed into the cells (Rockland and Jones 1974).

PROCESSING INTO PROTEIN PRODUCTS

Conversion of legumes into protein products suitable for incorporation into processed foods involves disruption of the highly ordered seed structure (Fig. 10.1) and fractionation by physical methods. These methods may be as simple as screening and aspiration to remove seedcoats or as complex as extraction of defatted flakes with aqueous solutions to dissolve proteins. In simple processing, some of original structures of the seed are detectable in the final products (Wolf and Baker 1975). Processing of soybeans has reached the highest degree of development, but the same basic principles have been applied to other legumes on laboratory and pilot-plant scales.

TABLE 10.5
PROXIMATE ANALYSES AND BIOCHEMICAL PARAMETERS OF SOY FLOURS, PROTEIN CONCENTRATES, AND PROTEIN ISOLATES

	Full-Fat Flour[1]	Toasted Defatted Flour[2]	Protein Concentrate[2,3]	Protein Isolate[2]
Moisture, %	3.4	6.5	8.0	4.8
Protein (N X 6.25), %	41.0	53.0	65.3	92.0
Crude fat, %	22.5	1.0	0.3	—
Crude fiber, %	1.7	3.0	2.9	0.25
Ash, %	5.1	6.0	4.7	4.0
PER[4]	2.15	2.3	2.3	1.1-1.6
Inactivation of trypsin inhibitors, %	89	—	—	—
Urease activity, pH change	0.1	—	—	—
Nitrogen solubility index	16	15-25	5	75

[1]Experimental sample (Mustakas *et al.* 1970).
[2]Tech. Serv. Manual, Central Soya Co., Chicago.
[3]Prepared by extraction with aqueous alcohol.
[4]Protein efficiency ratio corrected to casein = 2.50.

Full-Fat Soy Flours

These products are made on an industrial scale in the United States and England. Beans are cleaned, steamed, dried, cracked between corrugated rolls, dehulled by aspiration and screening, ground, and sieved (Pringle 1974). Alternatively, the beans may be cracked and dehulled prior to steaming. The steam treatment inactivates antinutritional factors plus enzymes such as lipoxygenases that can catalyze oxidation of lipids and thereby develop undesirable flavors (Mustakas *et al.* 1969).

An alternate process for preparing full-fat soy flours involves extrusion cooking. Beans are cracked, dehulled, heated, raised to 18% moisture, extruded (121-143°C), cooled, and ground (Mustakas *et al.* 1970).

Full-fat soy flours prepared by either method have a composition (Table 10.5) closely resembling that of whole soybeans because only the hulls (seedcoats) are removed during processing. Heat treatment during processing decreases solubility of the proteins as measured by the nitrogen solubility index and inactivates the bulk of the trypsin inhibitors. The protein efficiency ratio is about 90% of the value for the casein standard.

Full-Fat Peanut Flakes

Pilot-plant studies on preparation of full-fat peanut flakes were announced recently (Anon. 1976A). Developed at Clemson Uni-

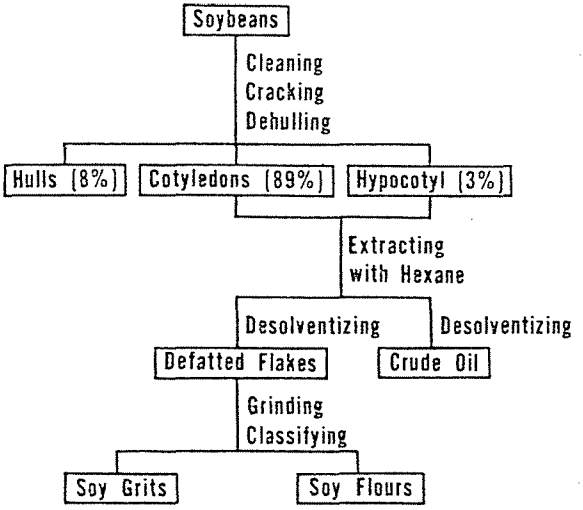


FIG. 10.2. OUTLINE OF COMMERCIAL PROCESSING OF SOYBEANS INTO OIL AND DEFATTED FLOURS AND GRITS

versity, the process has been licensed for commercialization. The peanuts are steamed, dried to 2-3% moisture, ground, precooked with water, and finally drum-dried to a bland, light-colored flake (Mitchell 1974).

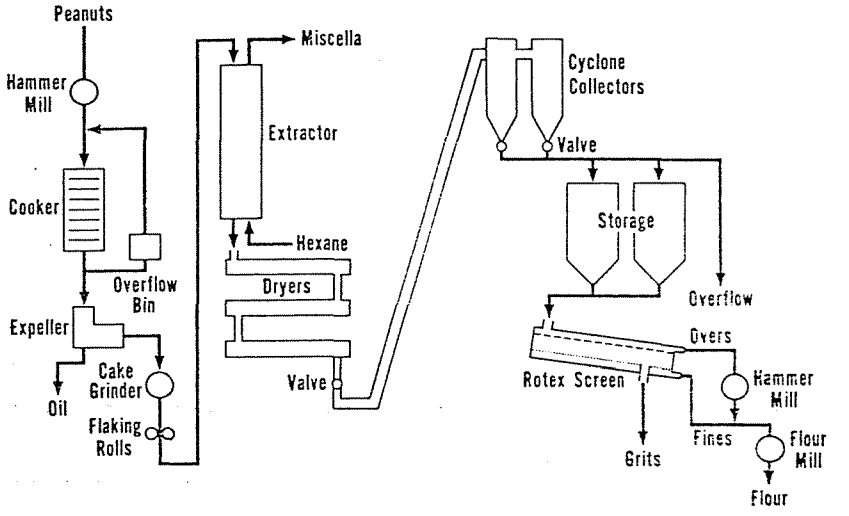
Defatted Soy Flours and Grits

These products are the major form of legume proteins used today as food ingredients. Commercial processing is outlined in Fig. 10.2. Soybeans are cleaned, cracked, and dehulled. The dehulled fraction is then conditioned by mild steaming, flaked, and extracted with hexane to remove the oil. Hexane is removed by desolventizing under conditions where the amount of moist heat treatment given to the flakes is controlled according to the desired end use (Becker 1971). The final step is grinding and classifying into grits (particles>100 mesh) and flours (particles≤100 mesh).

By definition defatted soy flours and grits have a minimum protein content of 50%, but often they will analyze higher (Table 10.5).

Defatted Peanut Flours and Grits

Edible peanut flours and grits are manufactured by one U.S. company by a combined prepress-solvent extraction method (Ayres *et al.* 1974). In the process (Fig. 10.3), peanuts are hammer milled, adjusted to 10% moisture, and cooked. The cooked peanuts are then fed to an expeller to press out the bulk of the oil and obtain a cake containing 8-12% oil. The press cake is ground, conditioned to 10% moisture, flaked, and extracted with hexane. After desolventizing,



From Ayres et al. (1974)

FIG. 10.3. OUTLINE OF COMMERCIAL PROCESSING OF PEANUTS INTO EDIBLE FLOURS AND GRITS

the flakes are screened to remove grits (16-60 mesh) and the fines are milled into a flour. The resulting flour has a crude protein content of 57%.

A pilot study has demonstrated that the prepressing step in conventional handling of peanuts (Fig. 10.3) can be eliminated by cutting the peanuts into thin slivers (Anon. 1976B). The thin slivers permit direct extraction of the oil as currently carried out on soybeans. Because the cooking and expelling step is avoided, defatted flakes or flours with high protein solubilities can be prepared.

A third alternative for making defatted peanut products consists of coarsely grinding skinned peanuts, mixing with water, homogenizing, and then drum-drying to form flakes. Extraction with a fat solvent yields defatted flakes (Mitchell 1976).

Pea Flours

Pilot studies on conversion of field peas into flours have been conducted by the Prairie Regional Laboratory in Saskatoon, Saskatchewan. These studies led to building of a pilot plant by Newfield Seeds Ltd. in Nipawin, Saskatchewan, and a commercial-scale plant is scheduled for completion in Saskatoon in the fall of 1976 (Anon. 1975). Whole or dehulled peas are pin-milled to yield a pale golden flour containing almost 32% protein on a moisture-free basis when dehulled peas are used. Product characteristics, composition, and functional and nutritional properties have been summarized (Anon. 1974; Youngs 1975). The flour has potential use as an ingredient for

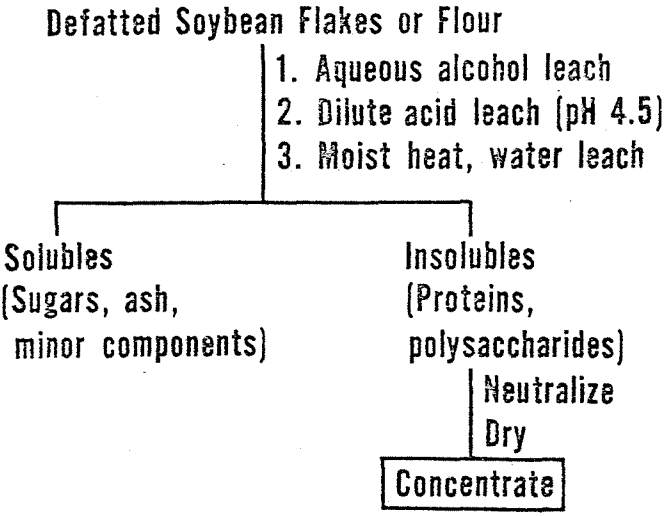


FIG. 10.4. OUTLINE OF PROCESSES FOR MANUFACTURING SOYBEAN PROTEIN CONCENTRATES
Initial extraction is made with one of the three solvent systems listed as described in the text.

snack foods, soup mixes and baby foods, but it will be primarily converted into protein concentrates as described below.

Soy Protein Concentrates

Removal of the soluble sugars (sucrose, raffinose, and stachyose) and other low-molecular-weight components from defatted soy flakes or flours raises the protein content to 70% or higher. The resulting products are called protein concentrates. Protein concentrates are made by three processes as outlined in Fig. 10.4. The first process employs aqueous alcohol to dissolve the sugars and nonprotein constituents, leaving the insoluble proteins and polysaccharides that constitute the protein concentrate after desolventizing (Mustakas *et al.* 1962). The second process uses a dilute acid leach at pH 4.5 to remove the soluble sugars. At this pH the globulins that make up the bulk of the proteins are at their isoelectric point and insoluble. After neutralizing and drying, the second type of concentrate is obtained (Sair 1959). In the third process, defatted flakes or flours are steamed to heat-denature and insolubilize the proteins. Washing with water then removes the sugars, and the denatured protein-polysaccharide residue is dried to yield the third type of protein concentrate (McAnelly 1964). A fourth process developed recently is a variation of the first. When the hexane-wet flakes leave the extractor in the usual hexane extraction (Fig. 10.2), they are mixed with alcohol. The hexane-alcohol mixture is then separated to remove residual lipids and flavor components not

removed by hexane alone. Residual hexane is then removed by selective desolventizing, and a final wash with aqueous alcohol yields a protein concentrate (Hayes and Simms 1973).

Concentrates made by the three processes have similar chemical compositions. Proximate analyses for a concentrate made by the aqueous alcohol extraction method are given in Table 10.5. The protein efficiency ratio of this concentrate is the same as that for flours, and protein solubility is low because of alcohol and heat denaturation resulting from processing.

Pea Protein Concentrates

The Prairie Regional Laboratory has extended its pilot studies on pea flours to the preparation of protein concentrates by two methods (Anon. 1974). In the first method, pin-milled pea flour (100 lb) is air classified to yield a starch fraction (65 lb) containing 2.5% protein and concentrate (35 lb) consisting of 56% protein. This method is being commercialized at present (Anon. 1975). In the second process, pea flour is slurried in lime water to give a pH of 9 and centrifuged. The resulting supernatant is spray or drum dried to yield one-third of the starting flour as a concentrate (60% protein). The starch fraction from the centrifuge is reslurried to wash it and again centrifuged. The starch solids (two-thirds of starting flour) containing 1.8% protein are then dried. Compositions, functional properties, nutritional properties, and product applications were evaluated (Anon. 1974; Bell and Youngs 1970; Youngs 1975).

Pea protein concentrate made by the aqueous process described above differs from soy protein concentrates in the nature of the carbohydrates present. In the pea concentrate, the insoluble polysaccharides (starch plus cell wall material) are removed and the soluble sugars retained, whereas in soy concentrates the insoluble polysaccharides are retained and the soluble sugars removed.

Peanut Protein Concentrates

An aqueous processing method developed on a pilot scale at Texas A&M University starts with blanched (skinned) peanuts which are ground and then dispersed in dilute acid to give a pH of 4.0. The acid-precipitated proteins plus insoluble polysaccharides are removed by screening and on drying yield a protein concentrate. The acid-soluble sugars (whey) and oil are separated by centrifuging (Rhee *et al.* 1973).

Soy Protein Isolates

These protein forms are made (Fig. 10.5) by extracting defatted flakes or flour having a high protein solubility [desolventized with a

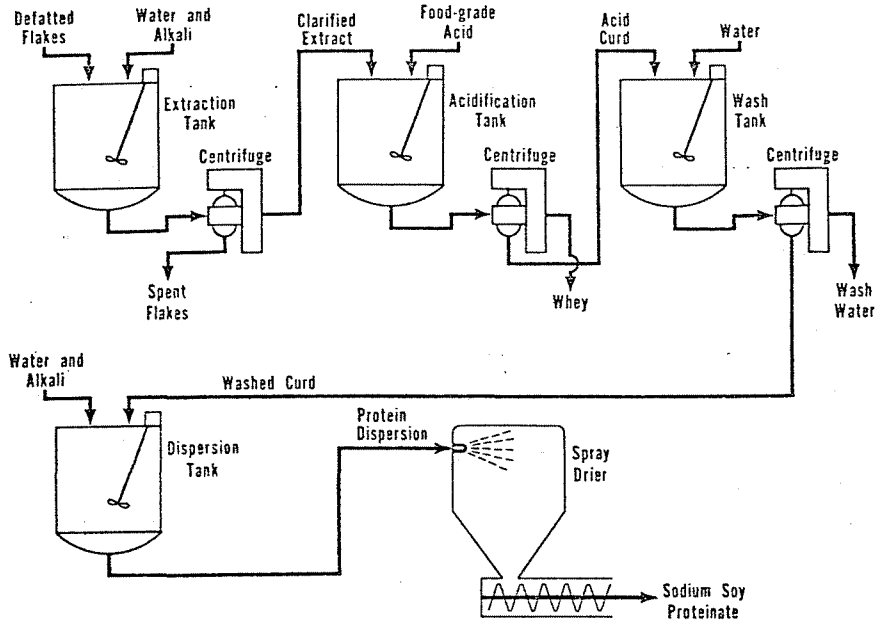


FIG. 10.5. OUTLINE OF PROCESS FOR COMMERCIAL PRODUCTION OF SOYBEAN PROTEIN ISOLATES

minimum of moist heat treatment as described by Becker (1971)] with dilute alkali (pH 7-9). The spent flakes (water-insoluble polysaccharides plus residual protein) are centrifuged off and resulting extract containing the soluble proteins and sugars is then acidified to pH 4.5. This is the isoelectric point for the bulk of the proteins and the pH of minimum solubility, hence the proteins precipitate. Centrifuging separates the whey (soluble sugars, some proteins, peptides, salts, and minor constituents) from the protein curd which is then reslurried with water to wash it. The washed curd can be concentrated and dried to yield the isoelectric (water insoluble) form of isolate. Usually the washed curd is resolubilized by neutralizing and then spray dried. This results in the proteinate form of isolate that is often preferred because of its water dispersibility. Proximate analyses for a commercial soy protein isolate are given in Table 10.5. Protein efficiency ratios for isolates are lower than for flours because of loss of essential amino acids in the proteins removed in the whey, residual antinutritional factors, and possibly low digestibility (Meyer 1971).

Broad Bean Protein Isolates

Pilot-scale preparations of isolates from broad beans (*Vicia faba*) are described in the literature (Flink and Christiansen 1973; Guinat

1969). Flink and Christiansen extracted bean flour at pH 8-10, centrifuged to remove starch and other insoluble materials, and then recovered the protein by precipitation at pH 3.5. Guinat extracted a flour with $NaHSO_3$ solution at pH 7.4 and screened off the insoluble residue. The resulting protein extract and suspended starch were then separated in a hydroclone. The clarified extract was acidified to pH 4.5 and centrifuged to isolate the protein.

Several years ago, isolates from *Vicia faba* were made in a commercial pilot plant by Rank, Hovis, McDougal, Ltd., in England. A secondary processor spun the isolates into a protein fiber that was converted into a meat analog. The process was technically feasible, but soy protein isolates are now used instead for the protein fibers because of uncertainty of an economical supply of broad bean (Smith 1976).

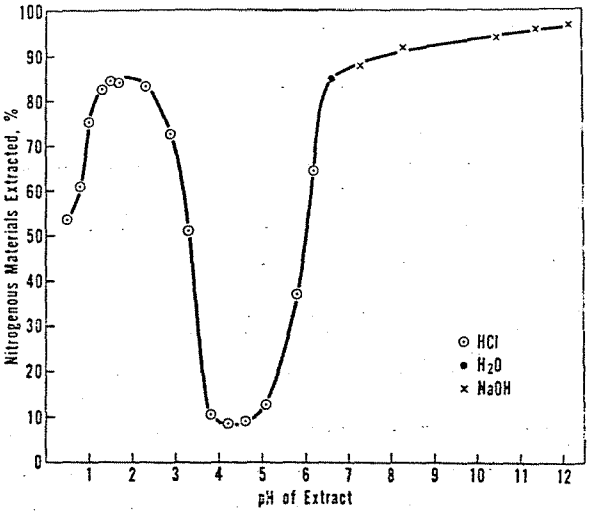
Peanut Protein Isolates

The aqueous method for processing peanuts developed at Texas A&M University can be conducted to yield isolates instead of protein concentrates as described earlier. Ground peanuts are dispersed in dilute alkali to give a final pH of 8.0. Screening removes the insoluble polysaccharide residue from the aqueous extract which is then acidified to precipitate the peanut globulins. Screening separates the protein curd, and centrifuging the aqueous phase separates the oil and whey fractions. Pilot-scale studies are available (Rhee *et al.* 1972).

PHYSICAL AND CHEMICAL PROPERTIES OF THE PROTEINS

Solubility

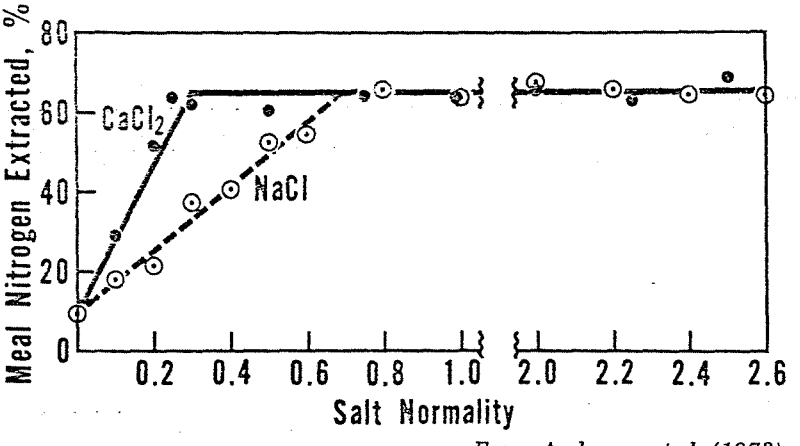
The bulk of the proteins in legume seeds are globulins which are characterized by being insoluble in water at their isoelectric points but are soluble in the presence of salts. The proteins may, however, be highly soluble in water if the pH is sufficiently removed from the isoelectric pH. This behavior is best illustrated by the classical studies of Smith and Circle (1938) on extractability of soybean meal proteins in water as a function of pH (Fig. 10.6). In water, the meal slurry gives a pH of 6.5-6.8 and over 80% of the proteins dissolve. If the pH is lowered progressively, less protein is dissolved and extractability reaches a minimum in the range pH 4-5, which is the isoelectric region for the bulk of the proteins. This is the basic principle employed in the preparation of soy protein isolates: extractions are made at pH 7-9 and the proteins are then recovered by acidifying the extracts to pH 4.5.



From Smith and Circle (1938)
FIG. 10.6. EXTRACTABILITY OF DEFATTED SOYBEAN MEAL PROTEINS AS A FUNCTION OF pH

Solubility-pH relationships similar to Fig. 10.6 have been reported for a large number of legumes (Ayres *et al.* 1974; Bhatty 1974; Fan and Sosulski 1974; Hang *et al.* 1970; Pusztai 1965).

The insolubility of soybean proteins at pH 4.5 can be overcome, at least partially, by adding sodium or calcium chloride to the extraction solvent (Fig. 10.7). Maximum solubility occurs with 0.3 *N* calcium chloride or 0.7 *N* sodium chloride. Higher concentrations of salts have no further effect and the remaining proteins apparently are denatured by the pH 4.5 treatment (Anderson *et al.* 1973).



From Anderson et al. (1973)
FIG. 10.7. EXTRACTABILITY OF DEFATTED SOYBEAN MEAL PROTEINS AT pH 4.5 AS A FUNCTION OF SALT CONCENTRATION

Molecular Size and Distribution of Proteins

Nearly 30 years ago, Danielsson (1949) examined the globulins of peas in the ultracentrifuge and found two major fractions that he designated vicilin and legumin. Vicilin sedimented as a 7S entity and legumin had a sedimentation coefficient of about 12S. He was able to separate the two by isoelectric precipitation at pH 4.5 in the presence of 0.2 *M* sodium chloride. Under these conditions, vicilin dissolved whereas legumin precipitated. Hence, it was simple to separate them on the basis of their differences in solubility at the isoelectric point. Molecular weights of the two proteins were estimated to be 186,000 (vicilin) and 331,000 (legumin).

In surveying the globulin fraction from 34 species of Leguminosae including peanuts (*Arachis hypogea*), soybeans (*Glycine max*), beans (*Phaseolus vulgaris*), and broad beans (*Vicia faba*), Danielsson found a remarkable similarity in the composition of the proteins. Practically all of the species contained counterparts of vicilin and legumin. These two fractions represent a large proportion of the total protein in a legume seed. For example, in *Vicia faba*, vicilin and legumin account for 90% of the protein found in the protein bodies (Bailey *et al.* 1970). A detailed review of the physical, chemical, and immunological properties of vicilin and legumin in various legumes appeared recently (Derbyshire *et al.* 1976). Further discussion of these protein fractions occurs below.

Protein Body Composition

Because of their high content in seeds and lack of demonstrated biological activity, vicilin and legumin are generally considered to be storage proteins and are probably packaged together in the protein bodies. In soybeans, the protein bodies consist almost exclusively of 7S and 11S fractions (Wolf 1970; Koshiyama 1972). Fig. 10.8A shows the ultracentrifuge pattern for total proteins extractable from defatted flour; 7S and 11S fractions make up about 70% of the total protein. The remainder of the proteins exists as 2S and 15S fractions. When the defatted flour is suspended in 20% sucrose at pH 5 and centrifuged in a sucrose density gradient (Tombs 1967), a homogenate fraction (proteins soluble at pH 5 and probably very similar to soybean whey proteins) and the protein bodies are obtained. The homogenate fraction (Fig. 10.8B) clearly consists of portions of the 2S and 7S fractions found in the total protein mixture. The protein bodies in turn contain a small amount of 2S protein, a high proportion of 7S protein, and all of the 11S protein found in soybean flour (Fig. 10.8C). Although the 7S protein found in unfractionated soybean protein is distributed between the

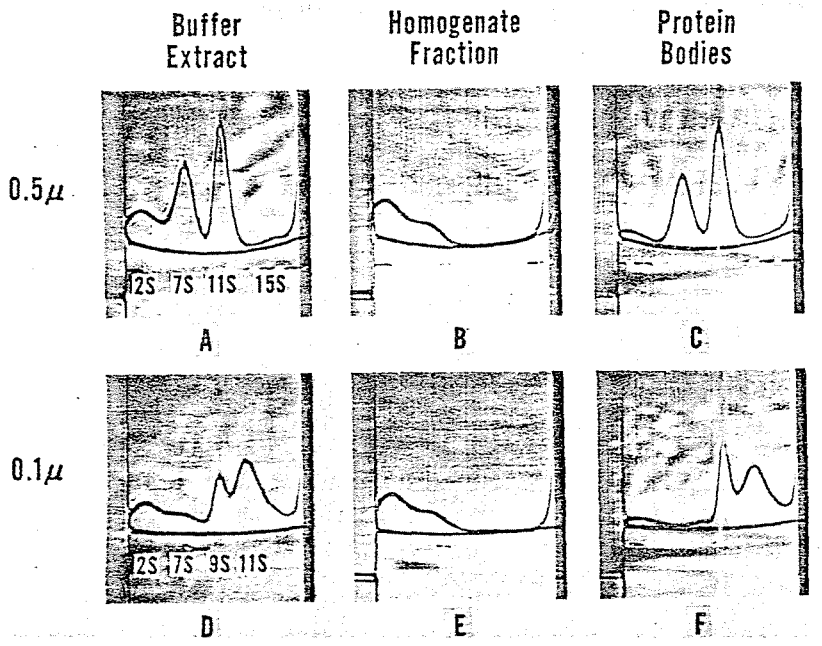


FIG. 10.8. ULTRACENTRIFUGE PATTERNS FOR UNFRACTIONATED SOYBEAN PROTEINS, HOMOGENATE FRACTION AND PROTEIN BODY PROTEINS

The unfractionated proteins were obtained by extracting undenatured, defatted flour with pH 7.6, 0.5 ionic strength buffer containing 0.01 *M* mercaptoethanol and dialyzing against the same solvent. Homogenate fraction and protein bodies were prepared by sucrose density gradient centrifugation (Tombs 1967). Analyses were conducted at pH 7.6, 0.5 ionic strength (A-C), and 0.1 ionic strength (D-F).

homogenate fraction and the protein bodies, a significant separation occurred. Koshiyama (1968A) isolated a 7S globulin that exists as a monomer (mol wt 180,000–210,000) at pH 7.6, 0.5 ionic strength, and forms a dimer ($S_{20,w} \sim 9S$, mol wt 370,000) at 0.1 ionic strength. This property is readily demonstrated even in the unfractionated mixture by analyzing the sample at 0.1 ionic strength (Fig. 10.8D); about two-thirds of the total 7S fraction dimerizes. Under the same conditions, the homogenate fraction shows the formation of only a barely detectable amount of dimer (Fig. 10.8E). In contrast, almost all of the 7S material in the protein bodies at 0.5 ionic strength (Fig. 10.8C) was converted into the dimer form at 0.1 ionic strength (Fig. 10.8F). Obviously the protein bodies contain predominantly the 7S fraction that is capable of forming a dimer at low ionic strength and the 11S protein.

7S Globulins

Although vicilin-like proteins occur in a large number of legumes (Derbyshire *et al.* 1976), comparatively few have been purified and

TABLE 10.6
7S GLOBULINS FROM LEGUMES

Legumes	Sedimentation Coefficient	Molecular Weight	References
Peanuts (*Arachis hypogaea*)	8.7 (S_{20})	190,000	Johnson and Naismith (1953)
Soybean (*Glycine max*)	7.9 ($S^{\circ}_{20,w}$)	193,000	Koshiyama (1968B)
Beans (*Phaseolus vulgaris*)	7.6 ($S^{\circ}_{20,w}$)	140,000	Pusztai and Watt (1970)
Broad bean (*Vicia faba*)	7.1 ($S_{20,w}$)	150,000	Wright and Boulter (1973)

characterized in detail. Table 10.6 lists four 7S globulins for which molecular weights are reported. Molecular weights for these four 7S globulins range from 140,000-190,000 and many 7S globulins fall into this range. The preparations from soybeans and beans appear to be glycoproteins. These proteins have subunit structures which can be disrupted by various means. For example, the 7S globulin from soybeans has nine N-terminal residues and in 8 *M* urea or 4 *M* guanidine hydrochloride dissociates into subunits with a molecular weight of 22,500-24,000 in accord with the presence of nine subunits (Koshiyama 1971). Not all of the 7S globulins possess the ability to associate at low ionic strength as observed for this protein from soybeans (Fig. 10.8D and 10.8F).

Recent work indicates the 7S globulin fraction in soybeans is much more complex than previously believed. Five components were isolated by chromatography on DEAE-Sephadex A-50 (Thanh *et al.* 1975). There are probably only subtle differences between them because all five components appear to be glycoproteins and dimerize at pH 7.6, 0.1 ionic strength. Likely, other legumes will exhibit similar heterogeneity of the 7S fraction when they are examined in sufficient detail.

11S Globulins

The legumin-like fraction of legumes appears less heterogeneous than the vicilin-like proteins and more representatives of this fraction have been isolated and characterized. Many of them have molecular weights of 300,000-400,000 (Table 10.7), and it has long

TABLE 10.7
11S GLOBULINS FROM LEGUMES

Legume	Sedimentation Coefficient	Molecular Weight	Reference
Peanuts (*Arachis hypogaea*)	13.2 ($S^{\circ}_{20,w}$)	340,000	Brand *et al.* (1955)
Soybeans (*Glycine max*)	12.3 ($S^{\circ}_{20,w}$)	320,000	Badley *et al.* (1975)
Beans (*Phaseolus vulgaris*)	11.6 ($S_{20,w}$)	340,000	Derbyshire *et al.* (1976)
Peas (*Pisum sativum*)	13.1 (S°_{20})	398,000	Johnson and Richards (1962)
Broad beans (*Vicia faba*)	11.4 ($S^{\circ}_{20,w}$)	328,000	Derbyshire *et al.* (1976)

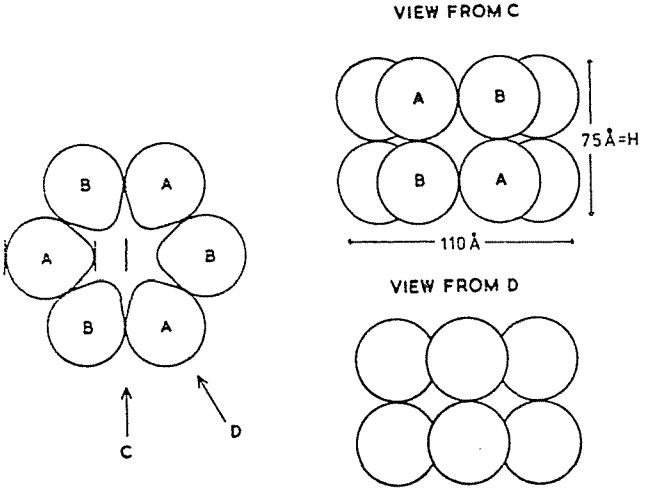
been recognized that these complex molecules are made up of subunits. A surprising feature observed in several 11S proteins to date is the presence of acidic and basic subunits. This structural detail has been found in the 11S or legumin fractions of *Vicia faba* (Wright and Boulter 1974), *Vicia sativa* (Vaintraub and Tuen 1971), and *Glycine max* (Catsimpoolas *et al.* 1971).

Glycinin, the 11S protein of soybeans, contains three acidic and three basic subunits with isoelectric points as follows (Catsimpoolas 1969):

Subunits	pI
Acidic	4.75, 5.15, 5.40
Basic	8.00, 8.25, 8.50

Each of these subunits apparently occurs twice in the 11S molecule because Badley *et al.* (1975) found 12 N-terminal residues per 320,000 g consisting of: 6 glycines, 2 leucines, 2 isoleucines, and 2 phenylalanines. The basic subunits terminate with glycine whereas the other N-terminal residues are found in the acidic subunits.

Electron microscopy using negative staining revealed that the 11S molecule consists of two hexagonal rings stacked on top of each other with six subunits in each ring (Fig. 10.9). When viewed from the top, there appears to be a hole in the center. Side views show six (view from D) or eight subunits (view from C) depending on the viewing position. Catsimpoolas (1969) and Badley *et al.* (1975) proposed that the acidic (A) and basic (B) subunits alternate in the



From Badley et al. (1975)

FIG. 10.9. MODEL OF SUBUNIT ARRANGEMENT IN SOYBEAN 11S PROTEIN
Left is top view and right shows side views from positions C and D as indicated by arrows on left.

hexagonal ring arrangement as well as in the two-layered structure (Fig. 10.9).

Small angle X-ray scattering measurements combined with electron microscopy indicate that the hollow oblate cylinder model for the glycinin molecule measures 110 × 110 × 75 Å. Pea legumin likewise appears to be a hollow cylinder; it is 85 Å in diameter and 85 Å high (Valentine 1959).

Although the quaternary structures of the 11S globulins of legumes are comparatively stable, it is possible to disrupt them stepwise:

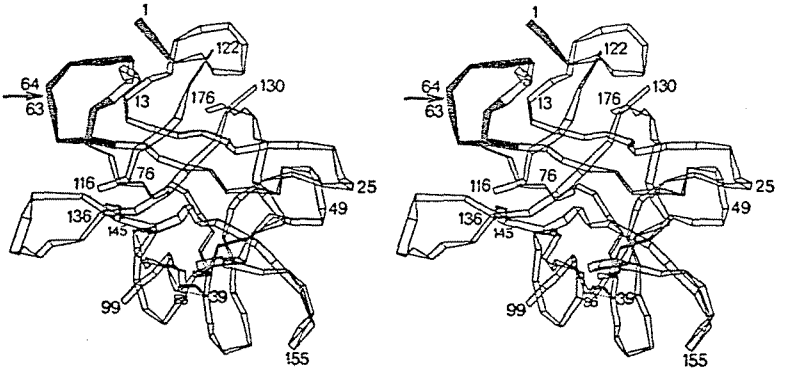
$$11S\ (A_6B_6) \rightarrow \sim 7S(2A_3B_3) \rightarrow \sim 3S(6AB) \rightarrow \sim 2S(6A + 6B)$$

Designations within parentheses indicate the extent of subunit interaction in terms of six acidic (A) and six basic (B) subunits as typified by glycinin. The conditions under which the various reactions occur vary widely depending on the particular protein. All of the interactions are noncovalent with the possible exception of the ~3S form where disulfide bonds have been implicated (Derbyshire *et al.* 1976; Badley *et al.* 1975).

Primary, Secondary and Tertiary Structures

Broad outlines of the quaternary structures of the major globulins of several legumes are emerging as just described. However, corresponding information about the primary, secondary, and tertiary structures of most legume proteins is very meager. In soybeans, for example, the primary structures are known for just two proteins: Bowman-Birk trypsin inhibitor (Odani and Ikenaka 1973) and Kunitz trypsin inhibitor (Koide and Ikenaka 1973). Although of physiological importance as antinutritional factors in their native state, both of these proteins are readily inactivated by moist heat and are present in small amounts as compared to the major globulins.

Significant details of the secondary and tertiary structures of soybean proteins are known only for the Kunitz trypsin inhibitor as a result of X-ray crystallographic studies of the crystalline complex between porcine trypsin and the inhibitor (Sweet *et al.* 1974). The X-ray studies confirmed previous optical rotatory dispersion and circular dichroism studies (Ikeda *et al.* 1968) that indicated the absence of α-helical structures. The molecule approximates a sphere of about 35 Å in diameter made up of crisscrossing loops wrapped around a core of hydrophobic side chains (Fig. 10.10). The polypeptide chain is folded in approximate β-sheet structures with little regular sheet formation.



From Sweet et al. (1974)

FIG. 10.10. STEREO PAIR OF MODEL OF POLYPEPTIDE CHAIN FOLDING IN KUNITZ SOYBEAN TRYPSIN INHIBITOR

A more complex protein, whose structure may be indicative of the major legume globulins having subunit structures, is concanavalin A, the agglutinin found in jack beans (*Canavalia ensiformis*). Below pH 6 concanavalin A exists as a dimer (2 identical subunits of 25,500 mol wt) and above pH 7 as a tetramer. X-ray crystallography and amino acid sequence determinations have yielded its structure at 2-Å resolution. The predominant structural elements are β-pleated sheets and random coils; no α-helices are present. The sites of interactions between subunits to form the dimers consist of β-pleated sheets, whereas side chain interactions contribute to the formation of the tetramers from two dimers (Edelman *et al.* 1972; Hardman and Ainsworth 1972).

Optical rotatory dispersion studies of soybean 11S globulin indicate an α-helical content of 5% and β-structure of 35% (Koshiyama 1972). Circular dichroism measurements suggest 9% α-helix, 33% β-pleated sheet, and 58% unordered structure for the 11S globulin; similar values were obtained for α-conarachin from peanuts (Jacks *et al.* 1973). Absence of appreciable amounts of α-helical structure in soybean and peanut globulins suggests that the gross structural features of Kunitz trypsin inhibitor and concanavalin A may be representative of the legume globulins.

CONCLUSIONS

Much has been learned about the properties of legume seed proteins, but clearly we have only scratched the surface. A few of the major globulins have been purified to a high degree and methods are being developed to separate the individual subunits. The next logical step is the determination of the amino acid sequences. Ultimately, the proteins must also be crystallized to permit an unequivocable

determination of their three-dimensional structures by X-ray crystallography. These are obviously formidable tasks. Nonetheless, such information should be invaluable to the food scientist in relating structure to functional properties and to the plant scientist confronted with the task of understanding the biosynthesis of specific seed proteins and their control mechanisms in order to improve the protein quality of legumes (Millerd 1975).

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