# Soybean Proteins: Their Functional, Chemical, and Physical Properties

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Soybean proteins are available to the food industry in the form of flours and grits, concentrates, and isolates with respective protein contents of 40–50%, 70%, and 90% or more. These proteins, when added to a variety of foods, supply desirable functional properties, such as emulsification, fat absorption, moisture holding, thickening, and foaming. Soybean proteins are mainly globulins with minimum solubilities near pH 4.5, and consist of a mixture of components with molecular weights ranging from 8000 to 600,000. Two of the major components, the 7S and 11S globulins, form insoluble disulfide polymers, undergo association-dissociation reactions, and have quaternary structures. The quaternary structures are disrupted by acids, alkalis, urea, detergents, and heat. Concentrated solutions of protein isolates increase in viscosity and gel when heated. Heating dilute solutions of 11S globulin causes about one-half of the protein to precipitate, while the other half is converted into a 3–4S form that remains soluble.

Soybeans have a long history as a protein foodstuff in the Orient. Over the centuries Oriental people developed a variety of soybean foods, including tofu, shoyu (soy sauce), miso, and tempeh. Processes used include cooking, grinding, extracting, fermenting, and sprouting. In contrast to these well-established food patterns in the Orient, the use of soybean protein food in the United States is still in its infancy. Soybeans have a short history of use in this country, since soybean processing was not a full-fledged industry until about the mid 1930's. In the past 35 years, however, soybeans have become our major source of edible oil, and the meal provides an important source of protein for animal feeds. Food use of soybean protein in the form of defatted flours also began in the 1930's, but this market has developed slowly; present usage is primarily as supplements or additives to provide desirable functional properties in standard food products. Availability of a greater variety and of more refined forms of soy proteins in recent years (Eley, 1968; Horan, 1966; Meyer, 1966; Ziemba, 1966) plus rising prices of animal proteins have generated considerable interest in soy proteins as substitutes for milk and meat proteins. For example, sodium caseinate is meeting increased competition from sodium soy proteinate in a number of formulated foods because the soy product is lower in cost.

At present the various forms of soy proteins are still utilized primarily for their functional effects rather than for their nutritional properties. However, a number of companies are now working on foods in which soy proteins are the major source of protein and are exploiting the functional properties of the proteins in the successful development of these products. The different forms of soy proteins now available and some of their functional properties are briefly reviewed here and elsewhere (Wolf, 1969). Chemical and physical properties of soy proteins important in food uses are also covered.

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## FORMS OF SOYBEAN PROTEINS

**Flours and Grits.** Present forms of soybean proteins used as raw materials by the food industry are conveniently classified into three major groups based on protein content. Typical analyses for these products are shown in Table I. Least refined forms are flours and grits, which have varying fat contents, particle sizes, textures, and degrees of heat treatment (Horan, 1966). Flours are prepared by grinding soybean flakes to 100 mesh or finer, whereas grits are coarser than 100 mesh. Minimum protein contents of these materials range from 40 to 50%, depending on the fat content. Defatted flours have approximate compositions as shown in Table II.

Proteins, carbohydrates, and ash are the major constituents of defatted flour; the remainder consists of residual lipids and a number of such minor components as saponins, isoflavones, and compounds responsible for the typical flavors of raw soybean flour and grits. About one-half of the flour carbohydrates are the oligosaccharides—sucrose, stachyose, and raffinose—while the other half is made up of polysaccharides, which are insoluble in water or alcohols (Aspinall *et al.*, 1967).

**Concentrates.** Soybean protein concentrates are more refined than flours and grits and contain 70% or more protein on a dry basis. Protein concentrates are prepared from defatted flakes or flour by removing the oligosaccharides, part of the ash, and some of the minor components in one of three ways (Figure 1). The first method involves washing defatted flakes or flour with 60–80% aqueous alcohol (O'Hara and Schoepfer, 1965; Mustakas *et al.*, 1962). The proteins and polysaccharides are insoluble in alcohol, while the sugars and other compounds dissolve and are removed. The concentrate is then dried at its natural pH which is near neutrality. A second procedure uses an acid leach at about pH 4.5 to remove the sugars. At this pH the major globulins are at their isoelectric point; both the proteins and polysaccharides are insoluble under these conditions. The leaching step may be carried out at or above room temperatures. The wet protein concentrate is then neutralized and dried (Moshy, 1964; Sair, 1959). The third procedure

**Table I. Commercial Forms of Soybean Proteins[a]**

| Form | Protein, % | Fat, % | Moisture, % |
|---|---|---|---|
| Flours and grits | | | |
| Full-fat | 41.0[b] | 20.5[b] | |
| High-fat | 46.0 | 14.5 | 6.0 |
| Low-fat | 52.5 | 4.0 | 6.0 |
| Defatted | 53.0 | 0.6 | 6.0 |
| Lecithinated | 51.0 | 6.5 | 7.0 |
| Concentrates | 66.2 | 0.3 | 6.7 |
| Isolates | 92.8 | <0.1[c] | 4.7 |

[a] Analytical data from Horan (1966); Meyer (1966); Tremple and Meador (1958). Table taken from Wolf (1969). [b] As is basis; normal moisture 5–10%. [c] Taken from Circle and Johnson (1958).

**Table II. Composition of Defatted Soy Flour[a]**

| Constituent | Percent |
|---|---|
| Protein (N × 6.25) | 50.5 |
| Carbohydrates | 34.2 |
| Fiber | 3.2 |
| Ash | 5.8 |
| Fat (ether extract) | 1.5 |

[a] As is basis; normal moisture 5–10%. Taken from Horan (1966).

uses moist heat to denature and insolubilize the proteins in the flakes or flour followed by a water wash to remove the sugars and other minor components (McAnelly, 1964). Although concentrates prepared by any of these methods contain 70% or more protein, their physical properties will differ with the method of preparation (Meyer, 1966). For example, concentrates prepared by acid leaching plus neutralization in the absence of heat treatment will have a higher content of soluble protein than concentrates obtained by heat and alcohol treatment. Concentrates have a reduced flavor level as compared to flours and grits because some of the flavor constituents are removed by the concentration processes.

**Isolates.** The most refined forms of soybean proteins are the isolates, which contain 90% or more protein (Meyer, 1966). They are prepared (Figure 2) by removing the water-insoluble polysaccharides, as well as the oligosaccharides and other low-molecular weight components that are separated in making protein concentrates. Defatted flakes or flours, which have received a minimum of moist heat treatment, are extracted with water plus alkali at a pH of 7 to 8.5. The insoluble

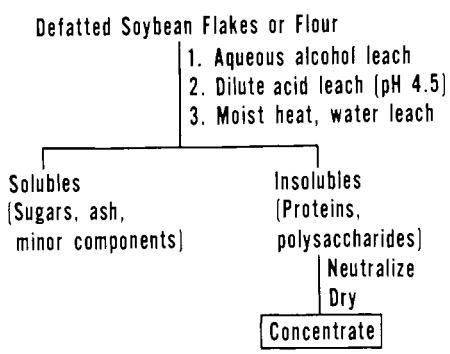


**Figure 1. Flowsheet for three methods of commercial preparation of soybean protein concentrates**

The neutralization step applies only to the acid-leach procedure

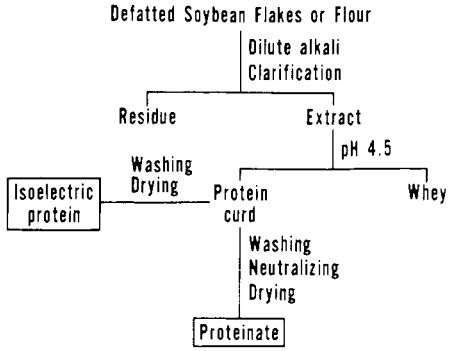


**Figure 2. Flowsheet for commercial isolation of soybean protein**

Taken from Wolf (1969)

residue, which contains the water-insoluble polysaccharides, plus residual protein is then separated. In the next step the clarified extract containing the bulk of the proteins plus the sugars is adjusted to about pH 4.5. This treatment precipitates the proteins, which are then removed by centrifugation or filtration. The supernatant or filtrate (whey in Figure 2) contains the sugars, ash, and minor constituents. The precipitated proteins are then washed and dried to give the isoelectric protein. More commonly, the protein is neutralized before drying. This procedure yields the proteinate form, which has the advantage of being water dispersible, while the isoelectric protein is insoluble in water. Isolates may analyze more than 95% protein (Kjeldahl nitrogen X 6.25), dry basis, but contain 2–4% ash (Meyer, 1966) and 3–4% of minor constituents. The latter are extractable with aqueous alcohols and consist of saponins, phospholipids, sterol glycosides, isoflavones, and unidentified compounds (Nash *et al.*, 1967).

**Prices and Production.** Comparative selling prices and recent production estimates of flours and grits, concentrates, and isolates are given in Table III. Flours and grits, the least refined of the proteins, also cost the least and are consumed in the largest amounts (Eley, 1968; Ziemba, 1966). Production of flours and grits for food use in 1967 was somewhat more than 200 million pounds if one includes the amounts used to make the corn-soy-milk blend, or CSM, which was purchased by the U.S. Government for distribution in developing countries. Concentrates sell for about three times as much as flours and grits, whereas isolates are about six times as expensive. Total production of these different protein forms is equivalent to 7–8 million bushels of soybeans, or less than 1% of the total U.S. soybean production in 1968. This percentage emphasizes that there is a large

**Table III. Prices and Production Estimates for Soybean Proteins[a]**

| Protein Form | Protein Content, % | Price per Pound, Cents | Price per Pound of Protein, Cents | Annual Production,[b] Million Pounds |
|---|---|---|---|---|
| Flours and grits (defatted) | 50 | 6½–7 | 13–14 | 105–110[c] |
| Concentrates | 70 | 18–28 | 26–40 | 17–30 |
| Isolates | 90+ | 35–39 | 39–43 | 22–35 |

[a] Taken from Meyer (1966) and Eley (1968). [b] Data for 1967. [c] An additional 100 million pounds was used in a corn-soy-milk (CSM) product.

Table IV. Functional Properties of Soybean Properties in Food Systems

| Functional Property | Protein Form Used[a] | Food System | References |
|---|---|---|---|
| Emulsification | | | |
| Formation | F, C, I | Frankfurters, bologna, sausages | Rock *et al.* (1966); Pearson *et al.* (1965); Inklaar and Fortuin (1969) |
| | | Breads, cakes, soups | Wood (1967); Tremple and Meador (1958) |
| | | Whipped toppings, frozen desserts | Circle and Johnson (1958) |
| Stabilization | F, C, I | Frankfurters, bologna, sausages | Rock *et al.* (1966); Pearson *et al.* (1965); Inklaar and Fortuin (1969) |
| | | Soups | Wood (1967) |
| Fat absorption | | | |
| Promotion | F, C, I | Frankfurters, bologna, sausages, meat patties | Rock *et al.* (1966); Wood (1967) |
| Prevention | F, I | Doughnuts, pancakes | Ziemba (1966); Eley (1968); Johnson (1970) |
| Water absorption | | | |
| Uptake | F, C | Breads, cakes | Wood (1967); Tremple and Meador (1958); Turro and Sipos (1968) |
| | | Macaroni | Paulsen (1961) |
| | | Confections | Ziemba (1966) |
| Retention | F, C | Breads, cakes | Wood (1967); Tremple and Meador (1958) |
| Texture | | | |
| Viscosity | F, C, I | Soups, gravies, chili | Wood (1967); Ziemba (1966) |
| Gelation | I | Simulated ground meats | Anson and Pader (1958); Circle *et al.* (1964); Frank and Circle (1959) |
| Chip and chunk formation | F | Simulated meats | Ziemba (1966, 1969) |
| Shred formation | F, I | Simulated meats | Rusoff *et al.* (1962) |
| Fiber formation | I | Simulated meats | Ziemba (1966, 1969); Thulin and Kuramoto (1967) |
| Dough formation | F, C, I | Baked goods | Circle and Johnson (1958) |
| Film formation | I | Frankfurters, bologna | Circle and Johnson (1958); Ziemba (1966) |
| Adhesion | C, I | Sausages, lunch meats, meat patties, meat loaves and rolls, boned hams | Rock *et al.* (1966); Ziemba (1966) |
| | | Dehydrated meats | Coleman and Creswick (1966) |
| Cohesion | F, I | Baked goods | Circle and Johnson (1958) |
| | | Macaroni | Paulsen (1961) |
| | | Simulated meats | Rusoff *et al.* (1962) |
| Elasticity | I | Baked goods | Circle and Johnson (1958) |
| | | Simulated meats | Rusoff *et al.* (1962) |
| Color control | | | |
| Bleaching | F | Breads | Wood (1967) |
| Browning | F | Breads, pancakes, waffles | Wood (1967); Eley (1968) |
| Aeration | I | Whipped toppings, chiffon mixes, confections | Ziemba (1966); Eldridge *et al.* (1963); Circle and Johnson (1958) |

[a] F, C, and I represent flours, concentrates, and isolates, respectively.

potential source of these low-cost food proteins available in this country.

## FUNCTIONAL PROPERTIES

Soybean proteins in their various forms have functional properties that make them useful in food systems (Table IV). These functional properties are generally attributed to the proteins. When the cruder proteins are used, however, the other constituents may also contribute to the overall effect observed. For example, in flours, grits, and concentrates, not only the proteins, but also the polysaccharides, will absorb water. Thus, these products will take up more water than an equivalent amount of protein as an isolate.

Soybean proteins aid in formation of emulsions and help to stabilize the emulsions during subsequent processing, as in the production of ground meat products. Fat absorption can be promoted or controlled with soy proteins. In ground meats, soy proteins bind fat, whereas in doughnuts and pancakes, soy flour helps to prevent excessive fat absorption during frying. Soy proteins are hydrophilic and therefore absorb and retain water. For example, soy flours enable bakers to add more water to doughs to improve handling characteristics. In contrast, addition of soy flour to macaroni decreases water absorption during cooking so that a more cohesive product results. Soy proteins in baked goods and confections not only increase water absorption, but also enhance moisture retention in the finished product and thereby help to maintain freshness.

Texture of foods can be varied several ways by using soybean proteins. A simple example is the use of flours and concentrates to thicken soups and gravies. In finely ground meats, soy proteins aid in the formation of a gel, which acts as a matrix for holding moisture and fat and gives desirable chewiness. Textured products are being prepared commercially by extrusion of soy flour under pressure and heat to form chips, chunks, flakes, and a variety of other shapes. These products are flavored to resemble such meats as hamburger, stew meats, and chili beef (Ziemba, 1969). Soy flours or isolates can also be converted into meatlike products by agitation in aqueous slurries at high

temperatures. In this treatment the proteins are oriented and coagulated to form shredded masses that, again, have meatlike textures (Rusoff *et al.*, 1962).

A third process for simulating the texture of meats is more complex than extrusion or agitation-coagulation. A concentrated alkaline solution of protein isolate is forced through a spinneret into an acid-salt bath to form a protein fiber. The spun fibers are then treated with binders, flavors, fats, and colors and are manipulated to form meatlike products like ham, bacon, beef, and chicken.

Soy proteins can be converted into doughlike materials under certain conditions, but they lack the dough-forming properties unique to wheat proteins. Addition of soy proteins to bread dilutes the wheat gluten and starch, depresses loaf volume, and affects crumb texture; thus, soy proteins cannot be used simply to replace wheat flour (Pomeranz *et al.*, 1969).

The film-forming properties of soy proteins are useful in meat products, such as frankfurters and other ground meats, while the adhesive qualities of soy proteins are used to bind meat particles together in sausages, meat patties, meat loaves, and chicken rolls. Cohesive and elastic properties are imparted to baked goods and simulated meats by addition of soy products.

The function of soy flour in controlling color in foods is at least twofold: the flour may decrease color or enhance it. A decrease in color is exemplified by the use of undenatured soy flour as a bleaching agent in bread. Such flours contain the enzyme lipoxygenase (E.C. 1.13.1.13), which oxidizes polyunsaturated fats. The oxidized fats in turn are believed to bleach the wheat carotenoids, which are yellow; the bleaching effect produces a bread with a whiter crumb. Recent work, however, shows that carotene is not bleached by the classical lipoxygenase but by another enzyme which is heat sensitive (Kies *et al.*, 1969). Soy flour in bread also gives an enhanced and brighter color to the crust, presumably as a result of reactions between the soy proteins and the wheat flour carbohydrates. In breading mixes, pancakes, and waffles, soy flour improves browning as well as minimizing fat absorption during frying.

Soybean proteins are used as aerating agents in whipped toppings and frozen desserts. Pepsin hydrolyzates of soy proteins are also used as whipping agents for confections and chiffon mixes. Further details of functional properties of soy proteins can be found elsewhere (Horan, 1966; Johnson, 1970; Meyer, 1966).

## CHEMICAL AND PHYSICAL PROPERTIES

Although soybean protein products impart functional properties to foods, the physical and chemical aspects involved in functionality are still hazy. At present there are no physical or chemical tests that will predict how soy proteins will behave in a given food system before actual testing of the proteins in the food product. Johnson (1970) has recently emphasized that the only reliable way to determine such behavior is to incorporate the ingredient into the formulation and produce the finished product. Nevertheless, an understanding of the chemical and physical properties of soybean proteins is helpful in the successful use of the proteins in foods.

**Amino Acid Composition.** An important chemical property of soy proteins is their amino acid composition, which determines the nutritional value of the proteins. Table V lists the essential amino acid compositions for three major forms of the proteins. Fractionation of protein, which occurs when meals and flours are processed into concentrates and isolates, probably accounts for the differences in the amino acid contents noted between the different protein forms. Of primary interest are the lysine and methionine contents. The proteins are high in lysine and thus are useful supplements for cereals, which tend to be low in this amino acid. On the other hand, methionine is the first limiting amino acid in soy proteins and this limitation must be considered when the proteins are added for nutritional purposes rather than simply for functionality.

**Table V. Essential Amino Acid Content of Soybean Proteins**

| Amino Acid | Grams Amino Acid per 16 Grams Nitrogen | | |
|---|---|---|---|
| | Meal[a] | Concentrate[b] | Isolate[a] |
| Lysine | 6.9 | 6.6 | 5.7 |
| Methionine | 1.6 | 1.3 | 1.3 |
| Cystine | 1.6 | 1.6 | 1.0 |
| Tryptophan | 1.3 | 1.4 | 1.0 |
| Threonine | 4.3 | 4.3 | 3.8 |
| Isoleucine | 5.1 | 4.9 | 5.0 |
| Leucine | 7.7 | 8.0 | 7.9 |
| Phenylalanine | 5.0 | 5.3 | 5.9 |
| Valine | 5.4 | 5.0 | 5.2 |

[a] Values from Rackis *et al.* (1961). [b] Values from Meyer (1966). Table taken from Wolf (1969).

**Protein Solubility.** An important physical property of soy proteins is solubility. The major soybean proteins are globulins, which are insoluble at their isoelectric points. These proteins are soluble, however, in water or dilute salt solutions at pH values above or below the isoelectric point. The relationship between pH and extractability of proteins from defatted soybean meal is shown in Figure 3, taken from Smith and Circle (1938). In these experiments defatted meal was dispersed in water and the pH was adjusted with hydrochloric acid or sodium hydroxide. When meal is dispersed in water, a pH of about 6.5 is reached and nearly maximum solubility of the proteins occurs. In contrast, there is a marked minimum in solubility at pH 4 to 5, the isoelectric region. This pronounced effect of pH on solubility explains the apparent inconsistency in the use of water

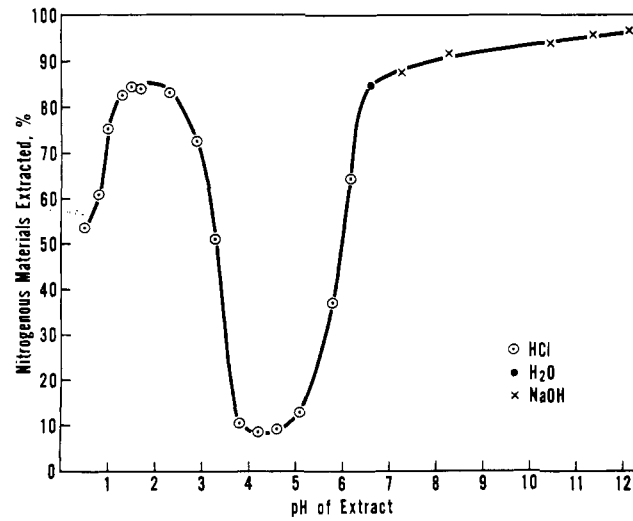


**Figure 3. Extractability of proteins in defatted soybean meal as a function of pH**

Taken from Smith and Circle (1938)

Table VI. Uses of Soy Flours of Different Nitrogen Solubility Index (NSI) Values[a]

| NSI | Uses |
|---|---|
| >85 | Enzyme bleaching of bread |
| 50–60 | Breads, cakes, sweet doughs, cookies, macaroni, doughnuts |
| 25–35 | Beverages, pancakes, waffles, gravies, soups, sausage products, dietary foods, infant foods |
| 10–20 | Crackers, beverages, cookies, cereals, infant foods |

[a] Compiled in part from Johnson (1970).

to extract soybean proteins and their classification as globulins. Globulins are a class of proteins normally considered to be insoluble in water at their isoelectric points but soluble in salt solutions at the same pH. A soybean meal dispersion in water, however, is not at the isoelectric point but is about 2 pH units higher. This solubility behavior (Figure 3) is also the basis for the isolation of soy proteins as described earlier (Figure 2). The proteins are extracted near or above neutrality and separated from the extract by adjusting the pH to 4.5.

Solubility of the proteins is required if the desired functional properties are to be achieved. A soluble product is also easier to formulate into certain foods. For these reasons almost all concentrates and isolates are neutralized and sold as the proteinates. The major form is sodium proteinate, but potassium and calcium proteinates are also available (Meyer, 1966).

Insolubility in the isoelectric pH region can be eliminated by hydrolyzing the proteins with pepsin to a much lower molecular weight than that of the original proteins. Such hydrolyzates are used primarily as foaming agents in candies, chiffon mixes, and cake mixes.

Another important factor influencing solubility of soy proteins is heat treatment. The extractability curve shown in Figure 3 is characteristic of a meal or flour that has received a minimum of heat treatment during removal of solvent from the flakes following solvent extraction to remove the oil. It is well known that if meal or flour is treated with moist heat, the proteins become insoluble. For example, 10 min of atmospheric steaming will reduce the protein extractability of meal or flour from 80 to 20% (Belter and Smith, 1952).

Raw soy flours and grits have a characteristic flavor unacceptable in many foods. One method for reducing the intensity of the raw flavor is to treat the flours or grits with steam. Since heat treatment also insolubilizes the proteins, functional properties are often impaired. As a result, a compromise is made between reduction of flavor level and loss of functionality by controlling the extent of heat treatment. To meet the need for products with varying flavor levels and functionality, the soy industry provides a series of flours and grits heated to different degrees. The amount of heat treatment is measured by determining the extent of protein denaturation. Several methods have been devised for assessing heat denaturation by measuring solubility of the protein. In one method the amount of Kjeldahl nitrogen that will disperse in water under specified conditions is determined, and the result is expressed as the percent of the total Kjeldahl nitrogen in the sample. This percentage is the nitrogen solubility index or NSI, as it is commonly referred to (AOCS, 1965). It should be emphasized that this method is empirical and that food processors must determine the NSI range of the flour or grits which gives optimum results for a given product. Soy flours of varying NSI values are used in different foods as shown in Table VI.

At the top of the list is soy flour, which has received a minimum of heat treatment during removal of the solvent from the flakes following solvent extraction to remove the oil. The NSI values for such flours will vary with the source. Some manufacturers can supply flours with NSI values in the 90's, whereas flours provided by other processors will be somewhat lower. Flours of this type are used as a source of enzyme for bleaching bread. They are used at a level of 0.5 part per 100 parts of wheat flour, which is a level low enough so that the raw soy flavor is not detected in the baked bread.

When larger amounts of soy flours are added to breads and other baked goods, flours are used that have received more heat treatment (NSI 50–60). Flours with NSI values as low as 10–20 are suitable in other foods (Table VI). Certain food types are listed for more than one NSI range, a situation which reflects differences in specific food items within a general class. An example is cookies, which are listed for NSI values of 50–60 and 10–20.

**Solubility of Isolates.** As described earlier, isolates are prepared by extracting undenatured flakes or flour with water near neutrality and then precipitating the proteins by adjusting the extract to the isoelectric point of the proteins. Studies at the Northern Laboratory have shown that after isoelectric precipitation, isolates are no longer completely soluble in pH 7.6, 0.5 ionic strength buffer (Wolf *et al.*, 1964; Nash and Wolf, 1967). We found considerable variation in solubilities of laboratory preparations but that all isolates increased in solubility when the buffer contained 0.01*M* mercaptoethanol. Two of the major fractions of soy protein account for this behavior. A portion of these proteins exists in soybean meal as aggregates held together by disulfide bonds. These aggregates are extracted by water in the initial isolation step, but further aggregation occurs during isoelectric precipitation, and the proteins are insolubilized. These insoluble forms are then resolubilized by adding mercaptoethanol to the buffer, which breaks the disulfide crosslinks. Other reactions causing protein insolubility also occur during isoelectric precipitation, since a portion of the isolate remains insoluble even when mercaptoethanol is present in the buffer.

We also measured solubilities of commercial isolates and found an even greater variation than noted with the laboratory preparations. Although isolates from various manufacturers will be similar in chemical composition, their physical properties may be quite different because of variations in processing. It is therefore advisable to consider all sources of supply when soy proteins are evaluated for a given food product.

**Complexity of Soybean Proteins.** Soybean proteins consist of discrete groups of proteins which cover a broad range of molecular sizes. This range is shown most simply by use of the ultracentrifuge (Wolf and Briggs, 1959) or by gel filtration (Hasegawa *et al.*, 1963). A typical ultracentrifuge pattern for water-extractable proteins of defatted meal shows four major fractions designated 2, 7, 11, and 15S on the basis of their sedimentation rates (Figure 4). Approximate amounts of each sedimenting fraction are given in Table VII. Fractionation studies show that the proteins are mixtures more complex than indicated by Figure 4. Components isolated from the different fractions are also listed in Table VII. Several trypsin inhibitors and cytochrome c exist in the 2S fraction; other minor proteins may also be present.

Table VII. Approximate Amounts and Components of Ultracentrifuge Fractions of Water-Extractable Soybean Proteins

| Fraction | Percent of Total[a] | Components | Molecular Weight | Reference |
|---|---|---|---|---|
| 2S | 22 | Trypsin inhibitors | 8000, 21,500 | Steiner and Frattali (1969) |
| | | Cytochrome c | 12,000 | Fridman *et al.* (1968) |
| 7S | 37 | Hemagglutinin | 110,000 | Lis *et al.* (1966) |
| | | Lipoxygenase | 102,000 | Mitsuda *et al.* (1967) |
| | | β-Amylase | 61,700 | Gertler and Birk (1965) |
| | | 7S Globulin | 180,000–210,000 | Koshiyama (1968a) |
| 11S | 31 | 11S Globulin | 350,000 | Wolf and Briggs (1959) |
| 15S | 11 | ... | ~600,000 | ... |

[a] Taken from Wolf *et al.* (1962).

The 7S fraction makes up about one-third of the total soybean protein and consists of at least four different proteins: hemagglutinin, lipoxygenase, β-amylase, and a component designated 7S globulin. The 7S globulin represents more than one-half of the total 7S fraction (Wolf and Sly, 1967). The 11S fraction makes up another one-third of the soybean protein; a single protein called 11S globulin accounts for most of this fraction. The remaining one-tenth of the protein constitutes the 15S fraction, which has not been isolated and characterized but which, on the basis of its sedimentation rate, has a molecular weight of half a million or more. Table VII emphasizes that soybean proteins comprise a mixture of components and that about 80% of the proteins have molecular weights of 100,000 or greater.

Complexity of soybean proteins is further demonstrated by hydroxylapatite chromatography (Wolf and Sly, 1965; Vaintraub, 1965), gel filtration (Hasegawa *et al.*, 1963), starch gel electrophoresis (Shibasaki and Okubo, 1966; Puski and Melnychyn, 1968), and immunoelectrophoresis (Catsimpoolas *et al.*, 1968).

**Properties of 7S and 11S Globulins.** The 7S and 11S globulins, which represent the two major proteins of soybeans, have been purified and characterized. Several interesting properties they have, however, make them difficult to study. A property common to both proteins is the ability to form disulfide-linked polymers, which contribute to insolubility of soy protein isolates as described earlier (Nash and Wolf, 1967). The disulfide polymers also cause turbidity (Briggs and Wolf, 1957) and increase viscosity (Circle *et al.*, 1964) of soybean protein solutions. Depolymerization occurs readily when the proteins are treated with mercaptoethanol, sodium sulfite, or cysteine.

A second property shared by both proteins is sensitivity to their ionic environment. They undergo association–dissociation reactions with changes in ionic strength. The most clear-cut demonstration of these reactions is observed with the 7S globulin. At pH 7.6 and 0.5 ionic strength, the 7S protein exists as a monomer with a molecular weight of 180,000–210,000, whereas at 0.1 ionic strength the protein sediments with an $s_{20,w}$ of about 9S has a molecular weight of 370,000, and is obviously a dimer of the unit observed at the higher ionic strength (Koshiyama, 1968a). The 11S protein is also converted into a faster sedimenting form when ionic strength is lowered from 0.5 to 0.1, but the extent of association is low (Naismith, 1955). Both proteins undergo these reactions reversibly.

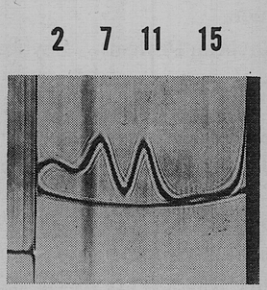


**Figure 4. Ultracentrifuge pattern for water-extractable soybean proteins**

Solvent is pH 7.6, 0.5 ionic strength buffer containing 0.01*M* mercaptoethanol (Wolf and Briggs, 1959). Numbers across top of pattern are sedimentation coefficients in Svedberg units. Taken from Wolf (1969)

A third property characteristic of the 7S and 11S globulins is their quaternary structure. The 11S protein contains eight glycine, two phenylalanine, and two leucine (isoleucine) amino terminal residues per mole (Catsimpoolas *et al.*, 1967). Although these results suggest a minimum of 12 polypeptide chains and 12 subunits per molecule (assuming absence of disulfide crosslinkages between polypeptide chains), only six different subunits separated on isoelectric focusing in urea-mercaptoethanol (Catsimpoolas, 1969). A dimer structure of two identical monomers each containing six subunits was proposed for the 11S molecule. The quaternary structure of the 11S molecule is disrupted by high and low pH, by high concentrations of urea, detergents, phenol-acetic acid-mercaptoethanol-urea mixtures, and by temperatures above 80° C (Wolf and Briggs, 1958; Wolf *et al.*, 1958; Catsimpoolas *et al.*, 1969; Wolf and Tamura, 1969).

The 7S globulin contains nine amino-terminal residues and, presumably, nine subunits which undergo a number of reactions (Koshiyama, 1968b). In acid solutions at low salt concentrations, the 7S protein forms two species which sediment with coefficients of 2S and 5S. Conversion into the 2S and 5S species in acid is inhibited by salts, and is reversed by dialyzing the protein to pH 7.6, 0.5 ionic strength. In 0.01*N* sodium hydroxide, the 7S globulin is converted to a form with a sedimentation coefficient of only 0.4S. Conversion of the 7S protein into the slow-sedimenting forms suggests disruption of a subunit structure.

An example of a food system in which the quaternary structures of the 7S and 11S globulins are disrupted is in the spinning of protein isolates into fibers used for textured food products. Separation of subunits occurs when the proteins are dissolved in alkali to prepare the spinning dope (Kelley and Pressey, 1966).

**Effect of Heat on Soybean Proteins.** Heat is a physical treatment given to many foods, and is a common process applied to soy flours as described earlier. Although it is well known that moist heat readily insolubilizes the proteins, little is understood about the physical and chemical changes that

take place at the molecular level as a result of heating. When solutions of soy proteinates in concentrations above 7% are heated, the solutions become more viscous and then gel (Circle *et al.*, 1964). Gels form within 10–30 min at 70° to 100° C. Disulfide cleaving agents, such as cysteine and sodium sulfite, which act as solubilizing agents for isolates lower viscosities of unheated and heated dispersions of the proteinates and inhibit gelation. Aoki and Sakurai (1968) report that soybean protein can be heated in dilute solution, concentrated, and then reheated to form gels. They prepared isolates by a modification of the procedure outlined in Figure 2; the meal extracts were heated to 90° C with steam before precipitating the protein by adding hydrochloric acid or calcium chloride. Gels resulted when the precipitated protein was converted to the proteinate form, diluted to 20% dispersions, and heated at 90° or 95° C. Sodium bisulfite and mercaptoethanol inhibited gelation in agreement with the results of Circle *et al.* (1964) concerning the effects of disulfide-cleaving agents on gelation. Disulfide bonds obviously play a part in gelation. Sulfhydryl-disulfide interchange during heating may result in intermolecular crosslinkages, which stabilize the gel network, or intramolecular disulfides may help to maintain conformations of individual molecules, which favor other interactions necessary for gelation.

When dilute solutions of the water-extractable proteins are heated, the 11S and 15S fractions plus a part of the 7S fraction aggregate as indicated by ultracentrifugation and gel filtration (Watanabe and Nakayama, 1962; Saio *et al.*, 1968). If solutions of the 11S protein are heated above 70° C, they become turbid, and protein precipitates at 90° C. Disc electrophoresis of heated solutions shows dissociation into subunits, but heating at 90° for 1 hr does not destroy the ability of the 11S protein to react with its antibody (Catsimpoolas *et al.*, 1969).

We have recently carried out more detailed studies on the effects of heating the 11S protein (Wolf and Tamura, 1969). When 0.5% solutions of 11S protein are heated at 100° C (pH 7.6, 0.5 ionic strength), the solutions quickly become turbid and precipitation of protein follows. Changes in the solution as a function of time of heating as detected by ultracentrifugation, are shown in Figure 5. The 11S component disappeared in less than 5 min and a soluble aggregate of 80–100S appeared. On continued heating the soluble aggregate continued to grow in size and then precipitated completely in about 7 min. Disappearance of the 11S component was accompanied by formation of a slow-sedimenting fraction with an $s_{20,w}$ value of about 3–4S (designated as 4S in Figure 5) and transient appearance of a 7S intermediate designated $\overline{7S}$. Concentration of the 4S fraction reached a maximum in 5 to 7 min and appeared stable to continued heating for more than 30 min. When the protein solutions were heated with 0.1 to 0.5*M* mercaptoethanol, precipitation occurred more rapidly than in the absence of the reducing agent, and formation of the 80–100S intermediate was not detected. Heating of 11S protein with the sulfhydryl-blocking agent, *N*-ethylmaleimide, formed a soluble aggregate but no precipitate. Obviously, heating disrupts the subunit structure of the 11S molecule and the following scheme is proposed to account for the observations noted:

$$11S \xrightarrow{(a)} \text{A-subunits} + [\text{B-subunits}]$$

$$[\text{B-subunits}] \xrightarrow{(b)} \text{Soluble aggregates} \xrightarrow{(c)} \text{Insoluble aggregates}$$

In this scheme, A-subunits represent the protein that remains soluble (3-4S fraction), while B-subunits represent that portion of the 11S molecule which is converted into aggregates by reaction (b), which apparently is rapid as compared to reaction (a). Reaction (c) appears to be catalyzed by mercaptoethanol but is blocked by *N*-ethylmaleimide.

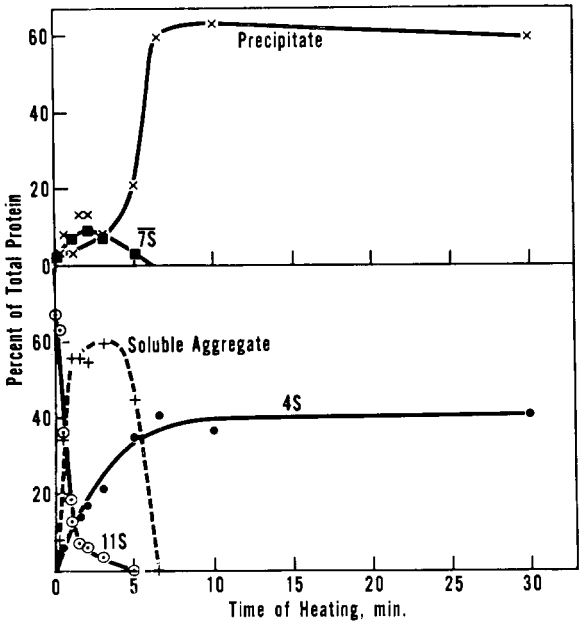


**Figure 5. Changes in ultracentrifugal composition of 11S globulin solution as a function of time at 100° C**

Taken from Wolf and Tamura (1969)

## CONCLUSIONS

This brief summary of some of the physical and chemical properties of soybean proteins emphasizes the complexity of these materials and suggests that they may undergo a wide range of reactions in food systems. Although progress is being made in determining the chemistry of soybean proteins, we are still unable to explain their functional properties on a physical or chemical basis. Further studies are needed on the individual proteins and on their reactions, not only with one another, but with constituents like water, lipids, and starch, before we can attempt to explain such properties as water and fat absorption and gelation in foods.

Current trends in food processing are toward fabrication of foods from basic ingredients—fats, proteins, and carbohydrates—with greater emphasis on nutritional quality. As a result of improvements in flavor and functional properties of soybean proteins, these materials are now moving from a role of merely providing functional properties to serving both as functional additives and as a source of nutritive protein.

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