SOYBEAN PROTEIN PRODUCTS are available in a variety of forms suitable for use in the fabrication of foods. These food ingredients differ in composition, particle size, texture, solubility, and many other properties, but the common denominator of all these products is protein.

Until recently, soy proteins were selected primarily for their functional effects in imparting desirable properties to fabricated foods. Now, however, they are being incorporated for their nutritional value. Nevertheless, functional properties of proteins are still a significant factor, since texture, mouthfeel, water absorption, and the like are often critical in determining success or failure in the marketplace.

An understanding of the physical and chemical characteristics of soy proteins and their relationship to functional properties is therefore essential, if these proteins are to be used advantageously. This article intends to present this information.

CELLULAR STRUCTURE

Soybeans are unique in composition compared to wheat and corn. They contain little or no starch but consist of about 20% oil and 40% protein. The oil is contained in numerous inclusions called spherosomes (0.2–0.3 μ in diameter). The bulk of the protein is contained in storage sites called aleurone grains or protein bodies (2–20 μ in diameter). The protein bodies are surrounded by a membrane, thought to consist of phospholipids, which is stable to diethyl ether and hexane.

The protein bodies contain the major proteins found in soybeans (Wolf, 1970a) and are unusually high in protein content. Tombs (1967) obtained preparations containing 98% protein by sucrose density gradient centrifugation. The spherosomes have not yet been isolated and characterized, but methods used with other seeds appear applicable for this purpose.

COMMON FORMS

Soybeans are usually processed into a variety of forms in which the ordered structure of the intact seed is destroyed. Figure 1 compares the compositions and yields of various forms.

The simplest processing consists of steaming the beans and removing the hulls, which are more than 85% carbohydrate (cellulose and other polysaccharides). Grinding the dehulled material yields full-fat flours, the crudest form of soy protein. (The term flour, when used with soy, refers merely to particle size. Soy meals ground to pass through a 100 mesh or finer
screen are called flours, but no other similarity to wheat flour is intended.

Disruption of the spherosomes and extraction of the oil with hexane results in defatted flakes and flours with protein contents of 50% or more.

Increases in protein to 70% are achieved by removal of about half of the carbohydrates of defatted flakes and flours by extraction with such solvents as aqueous alcohols or dilute acid. Such extraction eliminates the sugars and some minor constituents and produces soy protein concentrates.

The purest soy protein forms are the soy protein isolates, from which the bulk of the oil and carbohydrates has been separated, leaving less than 10% non-protein material (ash and minor constituents).

As the protein content increases, processing becomes more complex because multi-step procedures are necessary to remove the non-protein constituents selectively. Also, loss of protein occurs when isolates are prepared because of incomplete extraction and precipitation of protein.

AMINO ACID COMPOSITION

The chemical composition of soy proteins is important because of its relationship to nutrition and reactions of the proteins during processing. The latter is as yet poorly understood, but it is known that solubility of isolates is altered when intermolecular disulfide bonds are formed by cysteine residues in the 2 major proteins.

The essential amino acid compositions for the 3 major forms of the proteins are listed in Table 1. The differences are caused by fractionation during processing.

Soy proteins are a good source of all the essential amino acids except methionine and tryptophan. The high lysine content makes soy protein a useful complement to cereal proteins that are deficient in lysine. A mixture of soy protein concentrate and wheat flour is nutritionally superior to either protein source alone; a soy:wheat protein ratio of 3:1 gives an optimum protein efficiency ratio (Wilding et al., 1968).

PROTEIN CLASSIFICATION

Soybeans contain neither an alcohol-soluble protein similar to gliadin nor a glutenin-like protein, both of which are unique to wheat gluten. Consequently, soy proteins lack the dough-forming properties of wheat flour. Indeed, adding soy proteins to wheat flour in baked goods dilutes the gluten and starch. Consequently, soy flours and other forms cannot be used simply to replace wheat flour.

The majority of soy proteins are classified as globulins. Such proteins are insoluble in water at their isoelectric points but dissolve in water or dilute salt solutions at pH values above or below their isoelectric points. The high water extractability of the proteins from defatted meal indicates that the phospholipid membrane surrounding the protein bodies is easily broken.

SOLUBILITY OF PROTEINS

The dependence of soy protein solubility on pH is clearly shown in Figure 2. When defatted meal is dispersed in distilled water at pH 6.5, nearly maximum

![Fig. 1—APPROXIMATE COMPOSITIONS (dry basis) and yields of protein forms obtained by processing soybeans](image-url)
protein solubility occurs. Raising the pH with alkali increases the solubility slightly, but adding acid reduces the solubility abruptly to a minimum in the range of pH 4–5, which is the isoelectric region. At lower pH values, the proteins become positively charged and redissolve.

The solubility curve in Figure 2 is typical only of a meal or flour that has received a minimum of heat treatment during extraction of the oil. Soy proteins are sensitive to moist heat and are rapidly insolubilized by steaming.

These solubility properties are important factors governing food applications of soy proteins, and solubility is often used as a quality control test. If a given product is slightly acid (pH 3.5–6.5), problems with solubility can be anticipated.

At present there is interest in using proteins in acidic beverages, but soy proteins as normally prepared do not qualify for this use. Soybeans contain phytate, which complexes with proteins when they are precipitated with acid. If the phytate is removed, the isoelectric point shifts to more alkaline pH values, and the proteins become soluble at low pH (Smith and Rackis, 1957).

Solubility of the proteins is often required to obtain desired functional properties, and soluble ingredients are always easier to formulate into foods. Consequently, almost all concentrates and isolates are neutralized and sold as proteinates, with sodium proteinate the major form available.

**SOLUBILITY OF ISOLATES**

Commercial preparation of soy protein isolates is based on solubility of the proteins. Undenatured flakes or flours are extracted with dilute alkali at pH 8–9, and the clarified extract is acidified to pH 4.5 to precipitate globulins, which are then washed, neutralized, and spray-dried.

Isolates are only partially soluble in pH 7.6, 0.5 ionic strength buffer (Nash and Wolf, 1967). Laboratory-prepared isolates vary considerably in solubility (37–73%), but solubility increases and is less divergent (66–78%) when 0.01 M mercaptoethanol is added to the buffer.

Portions of 2 of the major proteins occur in soybean meal as polymers linked by disulfide bonds. The polymers are extracted by water in the initial isolation step, but they are polymerized further during isoelectric precipitation and become insoluble.

Addition of mercaptoethanol to the buffer breaks the disulfide cross-links, and the proteins are resolubilized. A portion of the isolates, however, remains insoluble even with added mercaptoethanol.

Other reactions causing protein insolubility occur during isoelectric precipitation. These reactions depend upon time and pH of acid treatment (Nash et al., 1971).

Commercial isolates vary appreciably in solubility. While several manufacturers supply isolates similar in chemical composition, these products may differ in physical properties because of processing variations. All sources of supply should therefore be evaluated for a given food application.

Table 2—**ULTRACENTRIFUGE FRACTIONS** of soybean proteins

<table>
<thead>
<tr>
<th>Protein fraction</th>
<th>Percentage of total¹</th>
<th>Components</th>
<th>Molecular weight</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>2S</td>
<td>22</td>
<td>Trypsin inhibitors</td>
<td>8,000</td>
<td>Millar et al. (1969)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cytochrome c</td>
<td>12,000</td>
<td>Wu and Scheraga (1962)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.3S globulin</td>
<td>18,200</td>
<td>Fridman et al. (1968)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.8S globulin</td>
<td>32,000</td>
<td>Vaintraub and Shutov (1969)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Allantoinase</td>
<td>50,000</td>
<td>Vaintraub and Shutov (1969)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Yuan (1969)</td>
</tr>
<tr>
<td>7S</td>
<td>37</td>
<td>Beta-amyase</td>
<td>61,700</td>
<td>Gertler and Birk (1965)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Hemagglutinins</td>
<td>110,000</td>
<td>Lis et al. (1966)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Lipoxigenases</td>
<td>108,000</td>
<td>Stevens et al. (1970)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>7S globulin</td>
<td>186,000–210,000</td>
<td>Koshiyama (1968a)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11S</td>
<td>31</td>
<td>11S globulin</td>
<td>350,000</td>
<td>Wolf and Briggs (1959)</td>
</tr>
<tr>
<td>15S</td>
<td>11</td>
<td></td>
<td>600,000</td>
<td>—</td>
</tr>
</tbody>
</table>

¹ Source: Wolf et al. (1962)
What is soy protein?

MOLECULAR SIZE DISTRIBUTION

Soy proteins represent a mixture of molecules that differ in size, charge, and structure. The differences in size are shown best by ultracentrifugation (Fig. 3) or by gel filtration (Hasegawa et al., 1963). An ultracentrifuge separates water-extractable proteins of defatted meal into 4 fractions, with approximate sedimentation rates of 2, 7, 11, and 15S. Relative amounts and molecular weights for the components of these 4 fractions are given in Table 2. About 80% of the proteins have molecular weights of 100,000 or higher.

Fractionation has yielded a number of different proteins from the 2S and 7S fractions. The 2S fraction contains several trypsin inhibitors, cytochrome c, alantoinase, and 2 globulins with no known biological activity.

The 7S fraction makes up more than one-third of the total protein but contains at least 4 different proteins: beta-amylase, hemagglutinin, lipoxygenase, and 7S globulin. Multiple forms of hemagglutinin and lipoxygenase exist. Although devoid of known biological activity, the 7S globulin shares with hemagglutinin the distinction of being a glycoprotein (Koshiyama, 1969).

Another one-third of the total protein is made up of the 11S globulin (also called glycinin), which may account for the bulk of the 11S fraction. The 15S fraction has not yet been isolated and characterized, but based on its sedimentation rate it has a molecular weight of half a million or higher.

Table 2 indicates that soy proteins are more of a mixture than is suggested by Figure 3, and additional components will most likely be obtained upon further fractionation.

GEL ELECTROPHORETIC BEHAVIOR

Under various conditions, gel electrophoresis confirms that soybean proteins are a complex mixture. For example, starch gel electrophoresis of acid-precipitated globulins in 5 M urea with alkaline buffer reveals 14 protein bands, whereas acidic conditions reveal 15 bands (Puski and Melnychyn, 1968). The 11S globulin exhibits 18 bands in alkaline gels and 10 bands in acid gels. These studies also indicate that some globulin constituents are basic, since several bands migrate toward the cathode at pH 8.7.

Agar gel electrophoresis of water-extractable proteins without urea gives a continuous smear of components, but when these proteins are diffused against a mixture of their antibodies, at least 12 precipitin arcs form (Catsimpoolas et al., 1967). The larger number of protein bands found with urea-containing gels can be ascribed to a breakdown of larger units into sub-units.

ASSOCIATION-DISSOCIATION REACTIONS

A characteristic property of the 7S and 11S globulins is their ability to undergo rapid and reversible association-dissociation reactions under mild changes in ionic environment. For example, the 7S globulin at pH 7.6, 0.5 ionic strength exists as a monomer with molecular weight of 180,000–210,000, but at 0.1 ionic strength, it has a molecular weight of 370,000 as a result of dimerization (Koshiyama, 1968a). Likewise, the 11S globulin forms a faster-sedimenting form when ionic strength is lowered from 0.5 to 0.1, but the extent of association is low (Naismith, 1955).

SUB-UNIT STRUCTURE

The 7S and 11S globulins possess an additional feature that adds to their complexity—both proteins are built up of sub-units. Interactions between sub-units are very specific, and there appear to be no sub-units common to the 2 proteins.

- The 11S Globulin has a quaternary structure consisting of 12 sub-units, based on the following amino-terminal residues: 8 glycines, 2 phenylalaines, and either 2 leucines or 2 isoleucines (Catsimpoolas et al., 1967).

Upon isoelectric focusing in urea-mercaptoethanol, 6 sub-units separate. These results suggest that the 11S molecule (molecular weight of 350,000) is actually a dimer of 2 identical monomers, each consisting of 6 sub-units (Catsimpoolas, 1969). A surprising result is that 3 of these sub-units are acidic, with isoelectric points at pH 4.75, 5.15, and 5.40, and 3 are basic, with isoelectric points at pH 8.00, 8.25, and 8.50. The acidic and basic sub-units have molecular weights of 37,200 and 22,300, respectively (Catsimpoolas et al., 1969).
What is soy protein?

1971). Interactions between the acidic and basic sub-units may help stabilize the 11S molecule.

Two structures have been proposed for the 11S molecule on the basis of electron microscopic studies. The first consists of 2 doughnut-like structures stacked one on top of the other (Catsimpoolas, 1969), and the other consists of 2 split-rings facing each other (Saio et al., 1970). A feature common to both models, however, is a hole in the center of the structure.

The quaternary structure of the 11S molecule is disrupted by a variety of conditions, including ionic strength, high and low pH, high concentrations of urea, detergents, and temperatures above 80°C (Wolf, 1970b).

- The 7S Globulin contains 9 amino-terminal residues and, presumably, 9 sub-units of single polypeptide chains (Koshiyama, 1968a). A single glycopeptide has been isolated; it contains the same sugars as found in the intact 7S molecule (38 mannose and 12 glucosamine residues). Consequently, the carbohydrate appears to be a single polysaccharide unit attached to one of the protein sub-units (Koshiyama, 1969).

The 7S structure is disrupted by the usual reagents capable of dissociating proteins into sub-units. In 8 M urea or 4 M guanidine hydrochloride, the molecular weight drops from 180,000 for the native form to 22,500–24,000—or about one-ninth of the original size (Koshiyama, 1971).

In sodium dodecyl sulfate solution, the molecular weight of 7S globulin is 34,000. Binding of about 40 molecules of detergent per sub-unit would account for the discrepancy in molecular weight under the different conditions of protein dissociation (detergent vs urea or guanidine hydrochloride).

In 0.01 N sodium hydroxide, the 7S globulin dissociates into a unit with a sedimentation coefficient of only 0.4S. Two species sedimenting with coefficients of 2S and 5S are formed in acid solutions at low salt concentrations. Salts inhibit the conversion of 7S globulin into the 2S and 5S forms, and the conversion is reversed by dialyzing the protein to pH 7.6, 0.5 ionic strength (Koshiyama, 1968b). The 7S globulin is less sensitive to irreversible changes in structure than the 11S globulin, but the reason for this difference is unknown.

Spinning of protein isolate into fibers for use in meat analogs is an example of a food system in which the quaternary structures of the 7S and 11S globulins are disrupted. Breakdown into sub-units occurs when the isolate is dissolved in alkali to prepare the spinning dope (Kelley and Pressey, 1966).

**EFFECTS OF HEAT**

Foods are almost universally heated during processing or in preparation for eating. Consequently, soy proteins used as food ingredients are exposed to varying degrees of heat treatment. Little is known about the physical and chemical changes that take place at the molecular level when soy proteins are heated, but the most obvious change noted is loss of solubility.

For example, as shown by Figure 4, if defatted or full-fat flakes are steamed at atmospheric pressure, protein extractability decreases from an initial value of 80% to about 20% in 10 min. Neither presence nor absence of oil affects the insolubilization of the proteins.

Most commercial soy flours are steamed to place them along the lower parts of the solubility curves shown in Figure 4. Flakes or flours used for isolates or as a source of enzymes for bleaching wheat flour pigments receive a minimum of heat treatment and therefore have solubilities corresponding to the upper parts of the curves.

**DENATURATION STUDIES**

Several studies have been made to try to determine why soy proteins are insolubilized by heating.

- **Solubilization.** Shibasaki et al. (1969) employed a series of solvents to selectively solubilize heat-denatured

<p>| Table 3—SELECTIVE SOLUBILIZATION of soy proteins* |</p>
<table>
<thead>
<tr>
<th>Solvent</th>
<th>Percentage of meal protein solubilized</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>4</td>
</tr>
<tr>
<td>Buffer pH 8.6</td>
<td>16</td>
</tr>
<tr>
<td>Buffer pH 8.6 + 0.1 M mercaptoethanol</td>
<td>24</td>
</tr>
<tr>
<td>Buffer pH 8.6 + 0.1 M mercaptoethanol + 8 M urea</td>
<td>76</td>
</tr>
</tbody>
</table>

* Source: Shibasaki et al. (1969)
soy proteins. Results for a defatted meal steamed for 3 hr are shown in Table 3. Water and buffer dissolved little protein, as is typical for many denatured proteins. Addition of mercaptoethanol to the buffer increased solubility slightly, but the combination of buffer + mercaptoethanol + urea solubilized three-fourths of the protein.

Since high concentrations of urea are believed to break hydrogen and hydrophobic bonds, these appear to be the major bonds responsible for insolubilization of soy proteins in heated meals. The role of intermolecular disulfide bonds appears minor. Differences in sensitivity to steaming were noted for several protein components, as detected by starch gel electrophoresis.

- **High Concentrations.** Although soy proteins are readily insolubilized when meal is steamed, concentrated solutions of the proteins form gels when heated. Heating commercial isolates in concentrations above 7% increases viscosity and causes gelation (Ciccarelli et al., 1984). Gels form within 10-30 min at 70-100°C.

  Cysteine and sodium sulfite, disulfide bond-cleaving agents, help solubilize the isolates. Lower viscosities of unheated and heated dispersions of isolates, and inhibit gelation. Disulfide bonds apparently contribute to the gel structure.

- **Gelation Scheme.** In further studies of gelation of soy protein, Catsimpoolas and Meyer (1970) proposed the following scheme:

  ![Diagram](https://example.com/gelation_scheme.png)

  The protein dispersion (sol) is irreversibly converted to a high viscosity progel, which upon cooling becomes more viscous and gels. The progel-gel transition, however, appears reversible. When the protein system is heated excessively (125°C), a metasol forms that is incapable of gelling upon cooling. Cleavage of disulfide bonds likewise results in a metasol.

- **Lipids.** Gelation of soy proteins in systems containing lipids reveals a complex behavior (Catsimpoolas and Meyer, 1971). Apparent viscosities of progel and gel are increased either by shortening the fatty acid chain length of the glyceride or by decreasing the extent of esterification of the hydroxy groups of glycerol. Gel viscosities are higher with saturated fats than with unsaturated fats. Gelation of soy proteins is also enhanced by addition of either phosphatides or cholesterol.

- **Low Concentrations.** Reactions occurring during heating have also been studied in solutions at concentrations below those required for gelation. Ultracentrifugation and gel filtration studies show that heating solutions of water-extractable soybean proteins causes aggregates of 11S and 15S proteins, plus part of the 7S fraction (Watanabe and Nakayama, 1962; Saio et al., 1968).

  - **Purified 11S.** Because of difficulties in interpreting results with the unfractionated soy protein mixture, Wolf and Tamura (1969) studied the effects of heating purified 11S protein, which is a simpler system. When 0.5% solutions (pH 7.6, 0.5 ionic strength) of the proteins are heated at 100°C, turbidity increases rapidly, followed by precipitation of protein.

  Ultracentrifugation studies revealed an interesting series of changes during heating, as shown in Figure 5. The 11S protein disappeared and a fast-sedimenting aggregate (90-100S) formed in less than 5 min. Upon continued heating, the soluble aggregate became larger in size and precipitated. In 7 min, the soluble aggregate disappeared completely, and precipitation ceased.

  As the 11S component disappeared, a slow-sedimenting fraction of 3-4S (labeled 4S in Fig. 5) appeared, as well as a transient 7S intermediate designated 7S. The 4S fraction reached a maximum concentration of 40%, in 3-7 min and appeared stable to heating for up to 30 min.

  Addition of 0.1-0.5 M mercaptoethanol hastened the precipitation reaction, and no soluble aggregate was detected. When the protein was heated with a sulfhydryl-blocking agent (N-ethylmaleimide), the 3-4S fraction and the soluble aggregate formed, but the latter did not precipitate upon prolonged heating.

  These observations indicate the following sequence of events:
...What is soy protein?

11S → A sub-units + [B sub-units]

Soluble aggregates

Insoluble aggregates

Heat (reaction a) disrupts the quaternary structure of the 11S protein and releases 2 types of sub-units: A sub-units (3-4S fraction) that remain soluble upon heating, and B sub-units that consist of the portion of the 11S molecule which aggregates. Aggregation of B sub-units by reaction b is apparently very rapid because they are not detected in a slow-sedimenting form. The soluble aggregates are then precipitated through reaction c, which may be catalyzed by sulfhydryl compounds but is blocked by N-ethylmaleimide.

● Turbidity. Catsimpoolas et al. (1970) studied heat denaturation of 11S protein in dilute solutions (0.014-0.059%) by measuring turbidity. Aggregation occurred at temperatures above 70°C and was favored by low ionic strength and by mercaptoethanol. Aggregation was maximal between pH 4.0 and 6.0. A combination of ionic and hydrophobic bonding was proposed as the basis for aggregation.

FURTHER RESEARCH NEEDED

Further details on properties and food uses of soy proteins can be found elsewhere (Wolf, 1970b; Wolf and Cowan, 1971), but hopefully this review of some physical and chemical properties of soy proteins will serve as a guide in their use in food systems.

Some properties, such as solubility as a function of pH, are obviously important. Other properties, such as the structures of the major proteins, cannot be correlated with the behavior of the proteins in crude systems, e.g., when soy flour is added to a bread dough.

However, there can be little doubt that these proteins are sensitive to treatments, including heat, changes in pH and ionic strength, and addition of lipids and other materials. A variety of reactions is likely to take place, but many of them will only be discovered and understood through further research.

REFERENCES
