Papers from the symposium on

Functionality of Oilseed and Legume Protein

presented at the 78th AOCS annual meeting
held in New Orleans, LA, May 17-21, 1987

Current Status of Soy Protein Functionality in Food Systems[1]

C.V. Morr[2]
Department of Food Science, Clemson University, Clemson, SC 29634-0371

Much of the research discussed in this paper deals with the effects of processing treatments on the physicochemical and functional properties of total soy protein isolate, 7S and 11S components and/or their subunits. This is new and exciting information that should become significant for future developments within the soy protein ingredient manufacture and utilization field. However, these researchers generally failed to provide adequate experimental details concerning methodologies employed to prepare, characterize and utilize soy proteins, their components and subunits in functionality studies. There is also an obvious need to devise and implement the use of more uniform and/or standardized methodologies for this kind of work in the future.

The current scientific literature contains numerous references to soy protein functionality research that has been conducted and reported during the last five years. Much of this research has been conducted using sophisticated chemical and physicochemical techniques that detail the basic structural properties of the native soy proteins and determine the effects of processing and modification treatments on the soy proteins. Researchers are placing more emphasis on the basic properties of the major soy protein components (e.g., the 7S and 11S globulins and their subunits), and how they influence the functionality of the total soy protein system. A major portion of this research is being conducted in simple buffer and model systems that do not closely resemble food systems.

The objective of much of this research is to develop a systematic approach that would provide a better understanding of the physicochemical properties of the individual and total soy protein system. This information is essential for manufacturing and utilizing soy protein ingredients that will meet the food industry's functionality requirements. Although much of the soy protein functionality research has of necessity been conducted in rather simple model systems, additional emphasis is needed for research with more complex model food product systems. This latter research will become more meaningful as additional knowledge from the simple and model food systems research is applied to it.

One additional factor that indirectly relates to the subject of this paper is the rather dramatic increase in manufacture and sales of soy foods in the United States during the past several years, due to real and/or perceived nutritional and health advantages over their traditional food counterparts (1-4). These soy food products include aseptic and retort soy milk, tofu and spray dried tofu, soy-based yogurt and cheese, nondairy frozen desserts and toppings, spreads, etc. Such developments may alter the consumer's acceptance of soy proteins in conventional food products. However, long term improvement in consumer attitudes toward soy protein utilization in conventional food products will likely depend on the industry's ability to provide soy protein isolates and concentrates with consistently high quality and functionality.

The purpose of this paper is to highlight selected soy protein functionality research that has been published during the last five years. Special attention will be given to: (i) the need to develop and utilize newer technologies for manufacturing soy protein isolates and concentrates with improved sensory and functional properties; (ii) the application of new and more sophisticated research techniques to determine the basic structural properties of soy proteins and their subunits; (iii) consideration of the more recent efforts to determine the effects of heating and other processing treatments on the physicochemical and functional properties of soy proteins; and (iv) efforts to correlate the basic physicochemical properties of soy proteins and their subunits to the functionality of commercial isolates and concentrates in model and commercial food product systems.

FACTORS AFFECTING SOY PROTEIN FUNCTIONALITY

The major functional attributes of soy proteins continue to be hydration, rehydration and swelling; dispersibility; solubility; colloidal stability; acid coagulation and gelation; emulsification activity and stability; foam expansion and stability; and adhesion/cohesion. The successful application of soy protein ingredients in the food industry requires that they be capable of providing one or more key functional attributes at various critical stages in the fabrication, handling, storage, preparation and utilization of a given food product. Much of the research being reported in the scientific literature understandably deals with investigations of the functional properties of soy proteins in buffer and simple model systems that may or may not mimic the compositional and processing conditions of actual food systems.

As with other food proteins, soy proteins possess certain basic and endogenous physicochemical properties

[1]Presented at the 78th American Oil Chemists' Society Annual Meeting, May 17-21, 1987, New Orleans, LA.
[2]Present address: Dept. of Food Science and Technology, Ohio State University, OH 43210-1097.

TABLE 1

Physicochemical Parameters that Relate to Soy Protein Functionality

Purity, homogenity and subunit composition by SDS PAGE[a]
Amino acid composition and sequence of subunits
Subunit, isomer and polymer molecular weights as a function of pH, ionic composition and temperature
Association-dissociation of subunits and polymers
Total and available SH and SS groups
Isoelectric points of subunits and polymers
Thermal transition points and their reversibility by DSC[b]
Solubility as a function of pH, ionic composition and temperature
Surface properties
Optical rotation and dispersion properties
Polarization of fluorescence properties
Visible and UV spectroscopy properties

[a]Sodium dodecyl sulfate polyacrylamide gel electrophoresis.
[b]Differential scanning calorimetry.

(Table 1) that strongly control their functionality in both simple and complex food systems. These properties are more or less susceptible to alteration as a result of various processing treatments required to extract and remove the lipids during the preparation of defatted soy flakes, and for isolating, purifying and drying the soy protein isolate and concentrate. Their physicochemical and functional properties are also strongly subject to the compositional and processing conditions imposed on them during their utilization in the food product.

Nakai (5) investigated the structure-function relationships of food proteins. This work is important in understanding and controlling the functionality of soy and other food proteins. "Surface" and "effective" hydrophobicity correlated well with interfacial tension of proteins, which is important for emulsification, whereas total hydrophobicity was not well correlated with this functional attribute. The emulsifying properties of a protein molecule depend largely on a proper balance between hydrophile (hydrophilic) and lipophile (hydrophobic) groups. Partial protein denaturation tends to unfold the protein molecule to increase its effective hydrophobicity. Both hydrophobicity and solubility are involved in determining the emulsification properties of heat denatured proteins. Heat denaturation causes a lowering of protein solubility and this latter factor affects its hydrophobicity and emulsification properties. In order to function as a foaming agent, a protein molecule must also have a proper balance of hydrophilic and hydrophobic groups. In this case total hydrophobicity computed from amino acid composition correlated with foaming capacity better than did surface hydrophobicity. There is a strong negative correlation between foam stability and net charge density of the protein molecule. Hydrophobicity and viscosity may also play key roles in determining foam stability.

Heat denaturation causes an increase in surface hydrophobicity and a decrease in surface tension under conditions that denature the proteins (oval-albumin and lysozyme) and thus improve their emulsifying and foaming properties. Heat denaturation of proteins exposes their hydrophobic groups which subsequently interact and cause aggregation and loss of solubility. Disulfide interchange may also be involved in heat-induced aggregation of proteins. Exposed hydrophobicity values for unfolded proteins more closely correlate to thermal properties than does surface hydrophobicity. Significant correlations have also been reported for heat-induced gelation, exposed hydrophobicity and SH or SH plus reduced SS group concentrations of food proteins. Molecular flexibility is also an important factor for controlling surface properties such as emulsification and foaming. Hydrophobic, electrostatic (charge frequency) and steric parameters are important for describing the physicochemical properties of protein molecules. Since most proteins cannot be obtained in a crystalline state, it is necessary to use a computer program for predicting secondary structure, e.g., α-helix, β-sheet, β-turns and random structure. Additional application of the above considerations is needed to better understand and control the functionality of soy and other food proteins.

ALTERNATIVE PROCESSES FOR MANUFACTURING SOY PROTEIN ISOLATES AND CONCENTRATES

Although the industry persists in using several of the original processes for manufacturing soy protein isolates and concentrates, these processes need to be modified to provide ingredients with improved sensory and functional properties. Specific reasons for modifying soy protein manufacturing processes include: (i) to remove phenolic compounds that complex with the proteins during isolation to adversely affect their color, flavor, solubility, digestibility and functionality (6–9); (ii) to remove phytate that complexes with the proteins to alter solubility, trace mineral bioavailability, digestibility and functionality (6,10–12); (iii) to avoid drastic pH conditions that dissociate and denature the proteins to adversely affect their sensory and functional properties (13); and (iv) to avoid dehydration that causes irreversible protein structural changes and results in partial loss of solubility, flavor and functionality (9,14).

Newer and alternative process modifications for manufacturing soy protein ingredients with special sensory and functional quality include: (i) cation/anion processing for removing phytate and ionized phenolic compounds (10); (ii) activated carbon treatment to remove phenolics, pigments and flavor compounds (8); (iii) ultrafiltration and other membrane processing modifications (15); (iv) use of fresh soy protein ingredients that have not been subjected to drying; (v) use of sulfite and other chemical treatments to improve flavor and color of the soy protein ingredients (C. V. Morr, unpublished observations); (vi) supercritical fluid extraction of lipids and flavor compounds from soybeans (16); and (vii) fractionation and use of crude 11S glycinin (cold insoluble) and 7S conglycinin fractions with their unique functionality attributes. Since these process modifications would significantly increase the manufacturing costs, such special soy protein ingredients would probably be used primarily for those food applications that require the highest sensory and functional quality.

SOY PROTEIN NOMENCLATURE

The nomenclature and structural properties of the major soy proteins and their subunits have been reviewed

(17-19). Historically, there are four major soy protein fractions that have been categorized by ultracentrifugation in standard phosphate buffer into 2S, 7S, 11S and 15S components (20). The two major proteins, from a functionality standpoint, are the 7S β- and γ-conglycinins and 11S glycinin. These proteins are composed of subunits that are strongly subject to association-dissociation as a function of temperature, pH, phytate, ionic environment and heat processing. The molecular weights of these two major soy proteins are generally considered to be in the range of 320,000-350,000 (11S glycinin) and 150,000-175,000 (7S conglycinins). β-Conglycinin is composed of three different subunits (α, α′ and β) that range from about 44,000 to 59,000 daltons, and glycinin contains seven different acidic subunits (A_{1a}-A_6) and five basic subunits (B_{1a}-B_4) with molecular weights ranging from about 37,000 to 45,000 and 19,000 to 22,000, respectively (18). There are a number of key physicochemical differences in the 7S and 11S soy protein components, however. For example, 11S glycinin is insoluble at pH 6.4 and 2-5°C, whereas 7S conglycinin is soluble under these conditions, but insoluble at pH 4.8. The crude 7S and 11S fractions are usually purified by ion exchange, Sepharose and affinity chromatography on ConA Sepharose. The 7S β-conglycinins contain up to ≅3.9 mole % mannose and up to 1.25 mole % glucosamine. The literature contains numerous references that describe the physicochemical and functional properties of these major soy protein components and their subunits.

Although it is doubtrul that the industry would be able to provide highly purified 7S or 11S soy protein ingredients on a commercial scale, it should be possible to manufacture partially purified protein products that are enriched in one or the other of these proteins. Such developments would obviously not be advantageous to the industry until researchers have demonstrated a significant functionality advantage for such protein ingredients.

EFFECTS OF PHYTATE AND PHENOLICS REMOVAL

Phytate accounts for about 1.75-2% of soy protein isolates. A major protion of the phytate is in the 7S soy protein, and 11S protein contains only about 0.07% phytate (21,22). The difference in phytate content of 7S and 11S soy proteins may be responsible for their unique physicochemical and functional properties.

Removal of phytate by the ion exchange procedure causes little if any alteration of the molecular weight distribution and subunit composition of the 7S and 11S soy proteins. These results suggest that phytate is not required or involved in the assembly of the 7S and 11S soy protein subunits into their respective protein isomers. Research is needed to determine whether removal of phytate from soy protein extract will alter the fractionation characteristics of the 7S and 11S soy proteins. For example, would removal of phytate alter the relative solubility of 11S soy protein at pH 6.4 and 2-5°C or alter the ability of subsequent chromatographic procedures to fractionate these two proteins (23)?

Soy proteins also contain 1-1.5 mg phenolics/g (6), most of which can be readily removed by anion exchange and/or activated carbon treatments. Removal of phytate and phenolics increased the susceptibility of soy proteins to *in vitro* enzyme hydrolysis (9). The 11S soy protein that contained about 0.07% phytate was slightly more digestible by trypsin at pH 8, and pepsin at pH 2, than was 7S soy protein with its 1.41% phytate content. Kinetic studies indicated that phytate and phenolics inhibited the enzyme hydrolysis reaction by accumulation of hydrolytic end-products rather than by contributing to an adverse protein conformation or providing stearic hindrance to reaction sites on the protein molecules.

The major affect of phytate removal on soy protein isolate is improved solubility and foaming properties in the acid pH range (11). Phytate-reduced soy proteins had minimum solubility at pH 4.8-5.0, compared to pH 4.2-4.5 for control soy proteins. Phytate-reduced soy proteins provided a maximum foam expansion of about 1300% at pH 3, whereas control soy proteins provided a maximum foam expansion of about 1100% at pH 6.

The effect of partial (≅75%) phytate removal resulted in a 6-11% improvement in iron bioavailability on the basis of a standard chick hemoglobin repletion assay procedure (12). These beneficial results were interpreted as indicating a soy protein-Fe-phytate complex which must be digested to release endogenous and/or added iron for the needs of the growing chick. A number of different experimental treatments were compared for their ability to remove endogenous iron from laboratory soy protein isolates (24). The ion exchange procedure which removed ⩾95% of the phytate was only able to remove about 12% of the endogenous iron from soy protein isolate. None of the experimental treatments, including exhaustive dialysis after treatment with EDTA and various pH treatments, enzyme hydrolysis and others, were not able to release more than 30% of the endogenous iron. Treatment with ascorbic acid at pH 2 plus dialysis removed about 60% of the endogenous iron. This beneficial result of the latter treatment may have been due to reduction of the iron to its ferrous form, which is reportedly more soluble than the ferric form and may not have been as strongly bound to the protein-Fe-phytate complex. Failure of the ion exchange procedure to remove more than about 95% of the phytate from soy protein isolate may be due to its inability to release endogenous ferric ion from the protein-Fe-phytate complex.

Research by Schnepf and Satterlee (25) with added radiolabelled ^{59}Fe confirmed the ability of soy proteins and/or protein-phytate complex to bind iron. The release of exogenous iron was promoted by EDTA and ascorbic acid and by partial hydrolysis of the soy proteins. These workers proposed that soy protein aggregates strongly retained the added iron, whereas surface bound iron was more readily released.

THERMAL PROPERTIES OF SOY PROTEINS

Heating soy proteins at temperatures in excess of 70°C dissociates their quaternary structures, denatures their subunits, and promotes the formation of protein aggregates via electrostatic, hydrophobic and disulfide interchange bonding mechanisms. The current literature contains a number of references to the details of these treatments, both with total soy protein extracts and with mixtures of 11S and 7S soy protein fractions. These references document the influence that such heat-induced

protein alterations have upon the functional (e.g., mainly gelation emulsification and foaming) properties of soy proteins. The subject of heat effects upon soy protein systems has recently been reviewed by Kilara and Sharkasi (19). A few of the current references are discussed here to illustrate the nature of the studies and the results that they are generating.

It is generally accepted that hydrophobic and disulfide bonding is involved in and responsible for protein-protein aggregation caused by heating to temperatures above 70°C. However, the contribution of electrostatic and hydrogen bonding cannot be overlooked, especially for the gelation of heated soy protein systems that are subsequently cooled. Some of the apparent differences being reported by various researchers is undoubtedly due to variations in their protein systems and in the compositions of the respective buffer systems being used. For example, the concentration of NaCl, the presence of reducing compounds such as mercaptoethanol and pH all influence soy protein dissociation and aggregation reactions. Similarly, the data being generated from studies involving the total soy protein system, as in soy protein extract or acid precipitated soy proteins, will likely be quite different than those obtained by heating 7S and 11S soy protein solutions.

Acid precipitated soy protein isolate was heated in pH 7.6 phosphate buffer (26), and changes in SH and SS groups were determined in the soluble (16,000 $\times$ g supernatant) fraction. Available and masked SH groups decreased from about 1×10^{-8} to 0.3×10^{-8} moles/mL, and total SH decreased from about 2×10^{-8} to 0.4×10^{-8} moles/mL as a result of heating 90 min at 90°C. Simultaneously, the masked SS residues decreased from about 2.4×10^{-7} to 0.5×10^{-7} moles/mL, total SS residues decreased from about 2.7×10^{-7} to 2.1×10^{-7} moles/mL and available SS residues increased from about 0.4×10^{-7} to 1.6×10^{-7} moles/mL upon heating 90 min at 90°C. This heat treatment caused an increase in SS residue concentration in the 16,000 $\times$ g sediment (precipitate) fraction from about 0.35×10^{-7} to 0.6×10^{-7} moles/mL and a decrease in SH residue concentration from about 0.18×10^{-8} to zero. Data were also presented on difference-second derivative absorption spectra (250–300 nm) for heated acid precipitated soy protein in urea to that of the native protein in urea. The ratios express the quantities of aromatic amino acid residues buried in heat-treated protein. Results indicated that about 90% of each of phenylalanine, tyrosine and tryptophan residues buried in acid precipitated soy protein remained in the buried form after heating 30 min at 70°C. Even after heating 30 min at 90°C, 60% of the phenylalanine and tryptophan residues and 80% of the tyrosine residues remained buried. These changes indicate that such difference spectra may not be a reliable indication of the extent of conformation change due to loss of secondary structure produced by heating soy protein. For example, heating may cause at least three simultaneous or sequential reactions in soy proteins: (i) dissociation of 7S and 11S protein subunits; (ii) unfolding of the subunits' secondary structures; and (iii) reassociation of denatured subunits via disulfide, hydrophobic, electrostatic and other important bonding forces. Although the first two reactions should lower the percentage of buried aromatic groups, the third reaction would tend to counteract this change. Thus, in order to use such data as an indication of loss of secondary structure in the soy protein system it would likely be necessary to block the final reassociation reaction.

Heating soy proteins to 80°C causes 7S protein to gel, but it was necessary to heat 11S protein to >90°C to form a gel, indicating that 11S protein is less susceptible to gelation than is 7S protein. This relationship is also consistent with the observation that 11S protein has a higher thermal transition point (92°C) than 7S protein (77°C), as reported by German *et al.* (27). The minimum protein concentration for forming thermal gels is about 8% for total soy protein extracts, but 11S proteins form gels at concentrations of about 2.5% when heating is done in distilled water. Addition of NaCl tends to inhibit the formation of thermal soy protein gels. Typical food applications involve the heating of ⩾10% total soy protein extracts at temperatures of >95°C to form stable gels and/or texturized products.

Research on the role of 7S and 11S soy proteins and their subunits in gel formation was also reviewed by Kilara and Sharkasi (19). Heating β-conglycinin in distilled water caused its dissociation into subunits, but addition of NaCl promoted reassociation of the subunits. It has been reported that NaCl suppresses both soy protein denaturation and aggregation by stabilizing their quaternary structures. In order to obtain proper gel formation it is necessary to provide the necessary conditions to allow the several important steps—dissociation, denaturation and aggregation—to take place in such a manner as to provide a three-dimensional network that can adequately immobilize the bulk of the free solvent.

In contrast to much of the above information, German *et al.* (27) reported that heating soy protein solutions and mixtures of their 7S and 11S proteins for 30 min at 100°C did not cause protein-protein aggregation. These workers showed that heating purified 11S soy protein solutions at >90°C caused precipitation of only basic subunits via hydrophobic interactions. Apparently, they were able to rule out the role of disulfide interchange reactions due to the fact that heating was done under disulfide reducing conditions. They proposed that the 7S protein subunits interacted with and stabilized the 11S basic subunits to prevent protein aggregation when both 7S and 11S soy proteins were present during heating.

Heating mixtures of 7S and 11S soy proteins at 100°C in buffer containing 0.02 M N-ethylmaleimide resulted in protein-protein aggregation and precipitation (28). The largest sized aggregates that were recovered by centrifugation were enriched in 11S basic and 7S β subunits and the resulting supernatant fractions were enriched in 11S acidic and 7S $\alpha\alpha'$ subunits. Utsumi *et al.* (29) investigated the preferential association of 11S basic and 7S β subunits when 7S and 11S soy proteins were heated at 80°C in pH 8 Tris buffer containing 10 mM 2-mercaptoethanol. These treatments caused dissociation of the quaternary structure of both 7S and 11S globulins and formation of $>10^6$ dalton macro-complexes that were composed of both 7S and 11S subunits. It was determined by electrophoretic techniques that these macrocomplexes were preferentially composed of 11S basic and 7S β subunits and that the interaction mechanism was primarily electrostatic in nature. Disulfide bonds between 11S basic subunits are also involved in forming soluble macrocomplexes.

The early stages of the dissociation-association reaction of 11S soy globulin was investigated by sucrose density gradient centrifugation and SDS gel electrophoresis (30). Soluble aggregates of 8×10^6 molecular weight were formed when 0.5 and 5% 11S soy protein solutions were heated 1 min at 100°C in pH 7.6 phosphate buffer containing 0.4 M NaCl. Additional heating caused the disappearance of the soluble aggregates and eventually the complete dissociation of 11S protein into its acidic and basic subunits. An additional 5 min heat treatment caused formation of highly polymerized aggregates with the 5% protein solution. The soluble aggregates appeared to be functioning as intermediates that were involved in the gelation of 11S soy globulin.

Hermansson (31) reported that both protein-protein and protein-solvent interactions are involved in the gelation of soy proteins. The important properties of glycinin and conglycinins that affect their gelation properties were summarized as: (i) the high number of disulfide bonds that hold alternating acidic and basic subunits in the glycinin molecule; (ii) isoelectric points of the glycinin subunits range from pH 4.75-5.40 (acidic) to pH 8-8.5 (basic); (iii) the isoelectric points of conglycinin subunits range from 4.9 (α), 5.2 (α') and 5.7-6.0 (β); and (iv) conglycin has a low sulfur content and no intermolecular disulfide bonds. In terms of the mechanisms involved in forming gel structure, Hermansson (31) reported that: (i) gels consisting of ordered, regular strands resulted in transparent gels with good solvent-holding properties; (ii) random molecular aggregation results in non-transparent gels with poor solvent-holding properties; (iii) an energy barrier is needed to prevent random protein strand formation; and (iv) protein molecules undergo denaturation, unfolding, expansion and then associate into linear strands to form a transparent gel structure. Results of experiments with glycinin-enriched soy protein solutions were presented in terms of heating 12% protein solutions for 30 min with the following observations: (i) heating in water at 85 and 95°C produced gels with regular strands; (ii) heating in 0.2 M NaCl solution at 85°C produced aggregated structured gels; and (iii) heating in 0.2 M NaCl solution at 95°C produced gels with regular strands. Heating conglycinin-enriched solutions as above showed that heating in water at 75 and 85°C produced dense, crosslinked gel structures, and heating in 0.2 M NaCl at 75 and 85°C produced even denser gel structures than those obtained by heating in water. Heating a 12% dispersion of a laboratory soy protein isolate as above resulted in gels with structures resembling those of glycinin. The soy protein isolate used in this study was of a non-denatured state with good solubility away from its isoelectric point. However, working with commercial soy protein isolates in which the proteins were of a highly denatured state, resulted in poor solubility in 0.2 M NaCl solution and the formation of a gel by swelling of the spray dried particles rather than by dissolution, dissociation and association as with soluble soy protein products and fractions.

Beveridge *et al.* (32) compared the thermal gelation properties of a commercial soy protein isolate with those of whey protein concentrate and egg white using the dynamic shear storage modulus (G′) method. Whereas egg white rapidly produced a gel structure upon heating, whey protein concentrate produced a weaker gel structure at a slower rate. Whey protein concentrate gel exhibited a stronger reversible cooling "set" than did egg white protein. Gelaton of both proteins was by first order kinetics. In contrast, soy protein gelation did not follow first order kinetics, had considerable elasticity prior to heating, exhibited a rapid increase in G′ value upon heating and failed to exhibit a cooling set. Egg white protein and whey protein concentrate gels were shown by electron microscopy to consist of strands and networks of spherical particles, whereas commercial soy protein isolate gels consisted of very large particles embedded in a gel matrix.

The effects of anions of neutral salts (SO_4^{-2}, Cl^{-1}, Br^{-1}, and SCN^{-1} upon the gelation of laboratory soy protein isolate and 7S and 11S soy protein fractions was investigated by Babajimopoulos *et al.* (33) using viscosity, gel melting point and expansion-contraction parameters. The ΔH^0 of the gelation reaction was unaffected by the type of salt used. Based upon the results of this study it was concluded that hydrogen bonding and van der Waals interactions were the major forces involved in soy protein gelation and that hydrophobic and electrostatic interactions were negligible.

Mori *et al.* (34) investigated the mechanism of gelation in soy proteins by heating 0.5 and 5% glycinin solutions for 1 or 20 min at 100°C. Gels formed by heating 20 min were about four times as hard as those formed after heating 1 min. Electron microscopy indicated a similar microstructure for both gels. The dissociation effect of urea and 2-mercaptoethanol indicated that subunit junctions in the gels were due to disulfide and hydrophobic bonding.

Kitabatake *et al.* (35) investigated the continuous gelation properties of soy proteins by HTST extrusion cooking. Gels were formed by extrusion cooking of 18% protein isolate dispersions at residence times of 30-100 S in water at temperatures of 150-160°C. The influence of added NaCl, $CaCl_2$ and sucrose upon the gelation reaction was investigated. The gels were found to reversibly melt upon reheating to 120°C.

Nakamura *et al.* (36) investigated the gelation properties of 11S soy globulin from five different soybean cultivars. Heating was for 20 min at 100°C in 0.4 M NaCl solution. Protein complexes were fractionated by sucrose density gradient centrifugation and gels were characterized for turbidity, hardness and subunit composition by SDS polyacrylamide gel electrophoresis. Glycinin that contained acid subunit (AS) 4 that was not linked to a basic subunit via a disulfide bond had the most rapid gelation rate. The slowest gelation rate was for glycinin that lacked AS 4 subunit. The hardness of the gels depended on the content of subunit AS 3. Higher turbidity gels were formed from glycinins that contained the highest SH group content.

Utsumi and Kinsella (37) studied the forces involved in soy protein gelation and the effect of various reagents on the formation, hardness and solubility of heat-induced gels formed from 7S and 11S soy globulins and soy protein isolate. Gels were formed by heating in pH 8.0 Tris buffer with and without added reagents (NaCl, NaSCN, N-ethyl maleimide, ethanol, and propyleneglycol). Results indicated that electrostatic interactions and disulfide bonds were involved in gelation of 11S globulin, hydrogen bonding was largely responsible for gelation of 7S globulin and hydrogen bonding and hydrophobic

interactions were responsible for gelation of soy protein isolates. Results also demonstrated that 11S basic subunits interact with 7S subunits in soy protein isolate gels, but that certain acidic subunits play only a limited role in gelation of 11S soy globulin.

van Kleef (38) studied the gelation properties of 10–35 g/100 g soy protein isolate solutions and the resulting gels were characterized by electron microscopy, solubility, and swelling in several different solvents. Conclusions were that gel structure does not require extensive or continuous covalent cross-linking. Gel properties depend strongly upon pH, but are largely independent of ionic strength. Gelation at pH 10 provided strong, translucent gel structures with strong solvent-holding properties, but gelation at pH 5 produced weak gels with low solvent-holding properties. Soy protein gels were characterized as crosslinked protein aggregates.

Kamata *et al.* (39) investigated the relationship between conformational stability and surface hydrophobicity of limited tryptic digested native and heat denatured soybean glycinin. Digests of native glycinin (DNG) and the high molecular weight fraction of tryptic digest of heat-denatured glycinin (HMF) functioned best as emulsifiers. The low molecular weight fraction (LMF) and the fraction that precipitated during digestion of heat-denatured glycinin (PPT) functioned poorest as an emulsifier. The minimum concentration of detergent (SDS) required to provide complete dissociation of flocculated fat globules was termed "flocculation strength." These latter values were higher in the order of intact glycinin > DNG > HMF. There was a close relationship between flocculation strength and short term (30 min) emulsion stability. The percentage of protein adsorbed onto the emulsion globule surfaces was greater near the isoelectric point and varied according to the order of HMF > DNG > intact glycinin. These latter parameter values were closely related to long term (24 hr) emulsion stability. The conformational stability of emulsifier proteins was evaluated by the change in their intrinsic fluorescence at increasing concentrations of urea (0–8 M) and the surface hydrophobicity was estimated by the binding intensity of 1-anilino-8-napthalene sulfonate (ANS). The emulsion stability increased with decreasing conformational stability and increasing surface hydrophobicity of the emulsifier proteins.

Matsudomi *et al.* (40) investigated the effect of ionic strength (0.01–0.5) of pH 7.6 phosphate buffer during heat denaturation of 11S soybean glycinin on its surface hydrophobicity and surface functionality. Heating in low ionic strength buffer produced greater changes in surface hydrophobicity (binding of *cis*-parinaric acid) and the thermal transition points, determined from changes in surface hydrophobicity vs temperature plots, were lower than when heating was performed at high ionic strengths. These data indicate that glycinin is more sensitive to heat-induced denaturation at lower ionic strength than at high ionic strength. The stability of emulsions stabilized by heated glycinin increased greatly with heating temperature and were proportional to the increases in surface hydrophobicity. Emulsions stabilized by glycinin heated in low ionic strength buffer were more stable than those heated at high ionic strength and were again related to changes in surface hydrophobicity. Foaming properties correlated with surface hydrophobicity of heated soybean glycinin, but in this case the data suggested that this functional property is dependent on additional conformational factors as well.

Aoki *et al.* (41) reacted 4% solutions of acid precipitated soy proteins (APP) in pH 7 buffer with equal volumes of ethanol, n-propanol or isoproponal for 2 hr at 35°C to "lipophilize" them. The alcohol was removed by exhaustive dialysis and changes in HLB number, UV spectra difference, intrinsic viscosity and surface hydrophobicity were investigated. APP was chemically modified by acetylation and also partially hydrolyzed by reacting with dilute HCl. Alcohol-modified APP exhibited higher emulsion stability than unmodified proteins, especially in the isoelectric point range of pH 4.5. These modifications lowered the HLB number of the APP. The surface hydrophobicity, determined as ability to bind 1-anilino-8-sulphonate (ANS), and exposed tyrosine residues by UV difference spectra indicated that the treatments with alcohol had "lipophilized" the APP. The emulsion stability increased almost linearly with increase in surface hydrophobicity and intrinsic viscosity. It was concluded that alcohol modification of soy protein can be used to improve the emulsion stability in the slightly acidic range.

FUNCTIONALITY IN FOOD PRODUCTS

Mittal and Usborne (42) reviewed the factors that are important in evaluating soy protein ingredients in meat emulsion products. Soy proteins are incorporated into the product to enhance and stabilize the fat emulsion, improve viscosity, impart texture upon cooking and improve juice retention upon cooking. It is often necessary to add certain seasonings and spices to cover the undesirable flavor associated with soy protein ingredients. The high salt concentrations in certain meat emulsion systems tend to inhibit the viscosity forming and swelling properties of dispersed and texturized soy proteins. Isolated soy proteins have been advocated as a suitable replacement of the salt-soluble myofibrillar meat proteins in emulsified meat products. They reportedly provide cohesiveness, stabilize the emulsion, bind water and form a firm, resilient gel structure upon cooking.

Rivas and Sherman (43) determined the adsorption properties of 7S, 11S and acid precipitated soy protein (APSP), as well as meat protein fractions and their influence upon interfacial tension of an oil-water interface. The adsorption of these proteins at the interface is time-dependent and strongly influenced by their molecular configuration. Compact molecules adsorb more rapidly and cause a quicker decrease in interfacial tension than large, bulky protein molecules. Nonglobular, salt-soluble meat proteins exhibited the fastest rates of interfacial tension reduction and achieved the lowest equilibrium values. Water-soluble meat proteins functioned somewhat less effectively in this regard and soy proteins functioned poorly.

Lorchirachoonkul (44) investigated the factors that influence the colloidal stability of soy proteins in simulated milk beverages. The work was done with commercial soy protein isolate and UF membrane processed soy protein isolate. Calcium chloride decreased both stability and viscosity of the product. Tween 60 increased stability and carrageenan increased viscosity of the product. Addition of K citrate, K phosphate, Na tartrate and other calcium binding compounds improved the colloidal stability of the

soy proteins in the product. The UF membrane processed isolate functioned better than commercial soy protein isolate for this application.

REFERENCES

1. LaBell, F., *Food Proc. 48*:99 (1987).
2. Duxbury, D.D., *Ibid. 48*:100 (1987).
3. Lieb, M.E., *Dairy Foods 88*:32 (1987).
4. *Soya Newsletter 1*(2):1 (1987).
5. Nakai, S., *J. Agric. Food Chem. 31*:676 (1983).
6. Seo, A., and C.V. Morr, *Ibid. 32*:530 (1984).
7. Sosulski, F., *J. Am. Oil Chem. Soc. 56*:711 (1979).
8. How, J.S.L., and C.V. Morr, *J. Food Sci. 47*:933 (1982).
9. Ritter, M.A., C.V. Morr and R.L. Thomas, *J. Food Sci. 52*:325 (1987).
10. Brooks, J.R., and C.V. Morr, *Ibid. 47*:1280 (1982).
11. Chen, B.Y-H., and C.V. Morr, *Ibid. 50*:1139 (1985).
12. Rodriguez, C.J., C.V. Morr and M.E. Kunkel, *Ibid. 50*:1072 (1985).
13. Anderson, R.C., *Cereal Chem. 51*:707 (1974).
14. Greig, R.I.W., *Dairy Ind. Intern. 44*:5 (1979).
15. Lawhon, J.T., and E.W. Lusas, *Food Technol. 38*(12):97 (1984).
16. Friedrich, J.P., and A.C. Eldridge, U.S. Paten 4,493,854 (1985).
17. Peng, I.C., D.W. Quass, W.R. Dayton and C.E. Allen, *Cereal Chem. 61*:480 (1984).
18. Brooks, J.R., and C.V. Morr, *J. Am. Oil Chem. Soc. 62*:1347 (1985).
19. Kilara, A., and T.Y. Sharkasi, *Crit. Rev. Food Sci. Nutr. 23*:323 (1986).
20. Wolf, W.J., and J.C. Cowan, "Soybeans as a Food Source," CRC Press, Boca Raton, FL (1975).
21. Brooks, J.R., and C.V. Morr, *J. Agric. Food Chem. 32*:672 (1984).
22. Honig, D.H., W.J. Wolf and J.J. Rackis, *Cereal Chem. 61*:523 (1984).
23. Thanh, U.H. and K. Shibasaki, *Agric. Food Chem. 24*:1117 (1976).
24. Morr, C.V., and A. Seo, *J. Food Sci. 51*:994 (1986).
25. Schnepf, M.I., and L.D. Satterlee, *Nutr. Reports Intern. 31*:371 (1985).
26. Yamagishi, T., M. Takaya, F. Ebina and F. Yamauchi, *Agric. Biol. Chem. 48*:537 (1984).
27. German, B., S. Damodaran and J.E. Kinsella, *J. Agric. Food Chem. 30*:807 (1982).
28. Yamagishi, T., A. Miyakawa, N. Noda and F. Yamauchi, *Agric. Biol. Chem. 47*:1229 (1983).
29. Utsimi, S., S. Damodaran and J.E. Kinsella, *J. Agric. Food Chem. 32*:1405 (1984).
30. Mori, T., T. Nakamura and S. Utsumi, *J. Food Sci. 47*:26 (1981).
31. Hermansson, A.-M., *J. Am. Oil Chem. Soc. 63*:658 (1986).
32. Beveridge, T., L. Jones and M.A. Tung, *J. Agric. Food Chem. 32*:307 (1984).
33. Babajimopoulos, M., S. Damodaran, S.S.H. Rizvi and J.E. Kinsella, *Ibid. 31*:1270 (1983).
34. Mori, T., T. Nakamura and S. Utsumi, *Ibid. 34*;33 (1986).
35. Kitabatake, N., D. Megard and D. Cheftel, *J. Food Sci. 50*:1260 (1985).
36. Nakamura, T., S. Utsumi, K. Kitamura, K. Harada and T. Mori, *J. Agric. Food Chem. 32*:647 (1984).
37. Utsumi, S., and J.E. Kinsella, *J. Food Sci. 50*:1278 (1985).
38. van Kleef, F.S.M., *Biopolymers 25*:31 (1986).
39. Kamata, Y., K. Ochiai and F. Yamauchi, *Agric. Biol. Chem. 48*:1147 (1984).
40. Matsudomi, N., H. Mori, A. Kato and K. Kobayashi, *Ibid. 49*:915 (1985).
41. Aoki, H., O. Taneyama, N. Orimo and I., Kitagawa, *J. Food Sci. 46*:1192 (1981).
42. Mittal, G.S., and W.R. Usborne, *Food Technol. 39*:121 (1985).
43. Rivas, H.J., and P. Sherman, *J. Disp. Sci. Technol. 5*:143 (1984).
44. Lorchirachoonkul, S., *Diss. Abst. Intern. B45* (1):117 (1984).

[Received May 1, 1988; accepted January 17, 1990]
[J5915]