Effects of Maturation and Storage on Solubility, Emulsion Stability and Gelation Properties of Isolated Soy Proteins

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Solubility, emulsion stability and gelation of isolated soy proteins and their 7S and 11S fractions from three stages of seed maturity were studied. The pH-solubility profile was similar irrespective of maturation and six-month storage. The 11S protein was more soluble in the acidic pH range than the 7S protein. Oil-in-water emulsion stability of isolated soy protein from mature soybeans was higher than that from the immature ones. This is due to the fact that there was more 7S fraction in the mature soybeans, and 7S protein was found to form a more stable emulsion than that formed from 11S protein. The result suggests that isolated soy protein from mature soybeans would serve as a better emulsifying agent. Heat-induced soy protein gels became weaker as the soybeans became more mature. This can be attributed to the higher content in the immature soybeans of 11S fraction which gives a stronger gel than 7S fraction in the protein from immature soybeans.

KEY WORDS: **Disulfide content, effect of pH and storage, effect of salt, emulsion stability, gel strength, heat-induced gel, isolated soy proteins, NSI, 7S/11S ratio, soybean maturation.**

Functional properties such as solubility (1-4), emulsion stability (5-7) and gelation (8-10) of isolated soy protein have been extensively studied. Soy proteins are used in numerous food products for their specific functionality (11). Solubility as a function of pH would allow selective solubilization of protein, and information obtained can be applied to products such as acidic beverages. It has been reported that storage proteins, 7S and 11S proteins, are the major fractions of isolated soy protein. The 11S protein was found to be quite soluble in the pH range of acidic beverages and could be a rich source of acid-soluble proteins useful in beverages, mayonnaise and salad dressings (12).

Isolated soy protein acts as a surfactant to stabilize an emulsified structure to extend shelf-life in products such as soups, sausages and mayonnaise. Further fractionation of soy protein indicated that an emulsion of the 7S protein-rich fraction was more stable than those of the 11S protein-rich fraction in the pH range 2-10 (13).

In protein gelation, isolated soy protein contributes to the gel matrix to hold water and other ingredients in such products as tofu and yogurt. It was found that the 7S and 11S fractions differ in gelation behavior (14). The heat-induced 11S protein gel exhibited stronger gel strength than the heat-induced 7S protein gel.

Previous studies (15,16) indicate that quantities of major soy protein fractions, 7S and 11S, varied with maturity. Water-imbibing capacity and rheological properties of isolated soy protein changed with maturity, and these changes correlated with the change in the ratio of 7S to 11S protein (17). The functional properties of isolated soy protein represent the composite properties of all component proteins (12). Thus, a variation in functional properties of isolated soy protein at different maturation stages would be expected.

The objectives of this study were to i) investigate the effect of soybean maturity and storage time on solubility, emulsion stability and gelation of isolated soy protein; ii) study these functional properties of 7S and 11S protein fractions; and iii) examine the effect of salt on these functional properties.

MATERIALS AND METHODS

Sample preparation. Soybeans of the Williams variety were planted on the South Farms of the University of Illinois at Urbana-Champaign. Three maturation stages of the soybeans used throughout this study were harvested and stored for six months according to the procedures described by Yao *et al.* (15).

Isolation of soy protein was prepared by the method of Horan (18). For fractionation of 7S and 11S proteins, the method developed by Thanh and Shibasaki (19) was applied. Adjusting the pH of freeze-dried isolated soy protein to pH 6.4 with 2 N HCl causes precipitation of the 11S fraction. After centrifugation, the supernatant was adjusted to pH 4.8 and the 7S fraction was separated from the whey proteins.

Protein content. Protein content was calculated as 6.25 times micro-Kjeldahl nitrogen content (20).

Disulfide content. The procedure for measuring disulfide content in heat-induced soy protein gel developed by Beveridge *et al.* (21) was used.

Emulsion stability. Emulsion stability was determined according to the method of Beuchat (22) except an Omni mixer (Omni Corporation International, Inc., Waterbury, CT) was used for blending. One gram of soy protein was suspended into 50 mL of distilled water at 25°C for 15 min. Soybean oil (50 mL) was added and blended for 30 sec. About 20 g of emulsified mixture was transferred to a graduated centrifuge tube and centrifuged at 380 × *g* for 10 min. Emulsion stability was read as mL of liquid phase separated per 20 g emulsion upon centrifugation.

Solubility. Solubility of soy protein samples was determined by the AOCS procedure (23) for Nitrogen Solubility Index (NSI). NSI was calculated by dividing the protein content of the supernatant by the total protein content in the sample expressed as percent.

Gel strength. For determining gel strength, soy protein dispersion (15% wet basis, pH 7.0) contained in an aluminum dish (5.0 cm in diameter and 2.2 cm in height) was heated at 94 ± 1°C for 30 min. After heating, the aluminum dishes were immediately placed in an ice bath to cool. Gel strengths of the heat-induced gels were measured by using an Instron Universal Testing Instrument, Table Model TM-M equipped with a CTM tension cel (Instron Corporation, Canton, MA) with cross head speed 2 cm/min, chart speed 5 cm/min, a full-scale load range of 1 kg and plunger of 1 cm diameter. The gel strength was determined from the highest point on the chart.

Statistical analyses. Data were analyzed using analysis of

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variance (24). The F-test was used to test significant differences at the 5% level. If the F-test proved significant, the least-significant-difference procedure was applied to determine significant differences among treatment means (25).

RESULTS AND DISCUSSION

Solubility. Solubility of isolated soy protein is expressed as NSI over the pH range 3.5-8.0 (Fig. 1 and Table 1). Within this range, isolated soy proteins from all maturation stages gave similar pH-NSI profiles (Fig. 1). Solubilities were minimal at pH 4.5-4.8 and rose when the pH was either increased or decreased. Solubility of isolated soy proteins from all maturation stages remained similar after storage for six months (Table 1). Different solubility profiles of similar shape were obtained for 7S and 11S proteins with the solubility of the 11S protein shifting towards a higher pH by about 0.5 units (Fig. 2). It was previously shown (15) that proteins isolated from Williams soybeans increased in the 7S/11S ratio during maturation from 0.34 in stage one to 0.64 in stage three. According to the increase in the 7S/11S ratio, the points in Figure 1 might be expected to have different solubility profiles rather than a common profile. However, the increase in the 7S/11S ratio during maturation was not sufficient to result in a significant change in solubility profile (Fig. 1).

In order to determine whether the same quantity of extractable protein is present in soybeans irrespective of maturity, soybeans at three stages of maturity were ground and dispersed in distilled water adjusted to pH 3.5, 4.5 and 8.0. Mixtures of equal volumes of soybean suspensions and 20% ice-cold trichloroacetic acid solution were made and the extractable proteins were determined from the supernatants after centrifugation at 165 × *g* for 5 min. Approximately the same amount of extractable protein was obtained at each stage of maturity. This finding suggested that similar solubility profiles of the isolates from three maturation stages

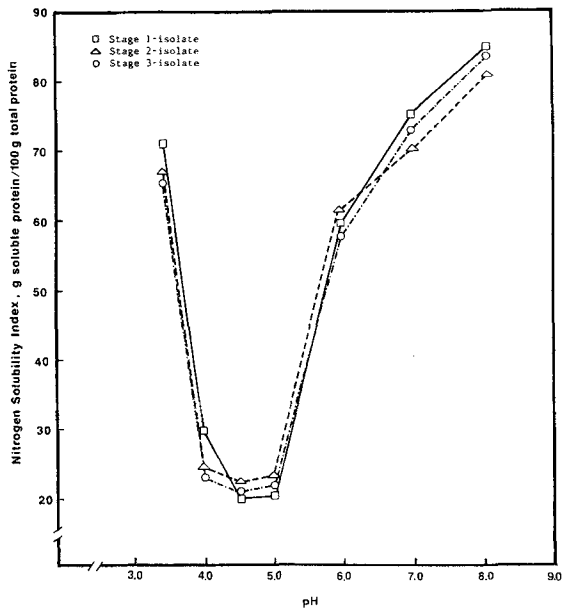

FIG. 1. Effect of pH on nitrogen solubility index of isolated soy proteins from seeds at three stages of maturity.

TABLE 1

Effect of Soybean Maturity and Storage time on Nitrogen Solubility Index (NSI) of Isolated Soy Proteins at Various pHs

Maturation stage	pH	NSI (g soluble protein/100 g total protein) 0 months storage	3 months storage	6 months storage
1 (immature)	3.5	65.07 ± 1.07	64.10 ± 2.26	64.18 ± 5.39
	4.0	22.11 ± 0.94	15.45 ± 1.48	21.20 ± 0.29
	4.5	20.44 ± 1.30	21.75 ± 1.77	22.73 ± 2.13
	5.0	22.04 ± 0.21	25.85 ± 1.20	23.95 ± 0.71
	6.0	56.85 ± 1.03	51.80 ± 1.13	50.39 ± 1.81
	7.0	69.86 ± 3.53	70.90 ± 2.26	66.47 ± 2.12
	8.0	82.67 ± 3.02	78.85 ± 2.05	80.66 ± 2.32
2	3.5	66.98 ± 0.13	63.75 ± 1.78	65.71 ± 1.39
	4.0	23.36 ± 2.07	18.50 ± 2.12	22.93 ± 0.05
	4.5	22.09 ± 0.76	21.65 ± 0.92	21.06 ± 0.69
	5.0	22.94 ± 0.39	26.85 ± 2.62	22.06 ± 0.42
	6.0	60.44 ± 2.59	56.70 ± 0.99	58.47 ± 0.42
	7.0	68.59 ± 3.71	71.90 ± 2.40	69.39 ± 1.53
	8.0	76.81 ± 0.27	78.65 ± 0.49	70.83 ± 0.52
3 (mature)	3.5	71.84 ± 0.24	71.50 ± 2.12	68.63 ± 0.08
	4.0	30.11 ± 0.43	24.11 ± 1.27	24.47 ± 1.57
	4.5	18.64 ± 0.13	18.87 ± 0.52	18.57 ± 0.56
	5.0	18.41 ± 0.16	17.85 ± 0.21	18.54 ± 2.54
	6.0	65.66 ± 1.92	64.17 ± 1.33	67.14 ± 2.50
	7.0	73.10 ± 1.82	75.17 ± 1.35	74.17 ± 2.95
	8.0	83.72 ± 3.08	80.99 ± 0.77	83.73 ± 2.26

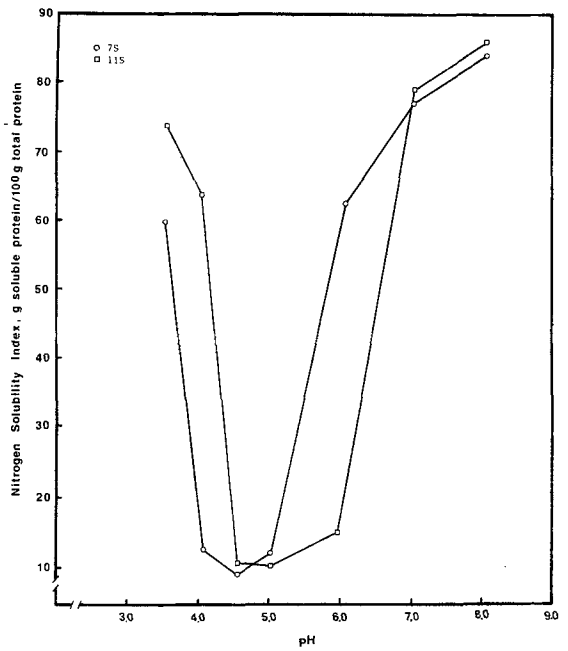

FIG. 2. Effect of PH on nitrogen solubility index of 7S and 11S soy proteins.

were mainly due to insufficient increase in the 7S/11S ratio during maturation (Fig. 1).

Effect of salt on solubility. Solubility of soy protein in aqueous systems can vary considerably with many factors. Besides pH (Fig. 1 and 2), ionic strength affects protein solubility significantly. In this study, 2% sodium chloride was added to protein dispersions and their NSIs were measured and plotted in Figure 3. In the presence of 2% sodium chloride, the pH-NSI profile shifted away from the control position in

such a way that solubility increased in the vicinity of the isoelectric point (4.0-5.5) and decreased outside of this pH range. Similar results were also observed for 7S and 11S proteins (Fig. 4 and 5). These results can be interpreted as salting-in and salting-out. Salting-in effect is the phenomenon in which neutral salts in low concentration increase protein solubility. This is because the number of charges on protein molecules is increased and the repulsive forces between molecules are enhanced. Thus, the dissociable groups of protein molecules tend to ionize and become easier to suspend in solution, eventually resulting in higher solubility. Since there is no net charge on protein molecules near the isoelectric point, the added sodium chloride increased the number of charges on protein molecules and caused salting-in effects. These results agree with those of others that at pH 4 to 5, sodium chloride (0.20-0.75 M) progressively increased solubility of soy protein while between pH 7 and 10, sodium chloride reduced solubility with increasing ion concentration (26,27).

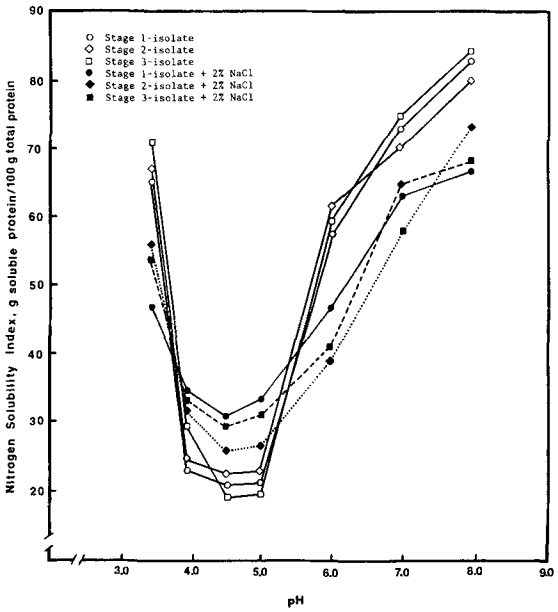

FIG. 3. Effect of pH and sodium chloride on nitrogen solubility index of isolated soy proteins from seeds at three stages of maturity.

Emulsion stability. Emulsions are colloidal systems in which dispersed immiscible droplets within a liquid phase are stabilized by some interphasic compounds (28-30). Emulsion capacity and emulsion stability of soy proteins are important due to extensive uses of the proteins as binders in meat products. The emulsion capacity is measured as the amount of oil which can be emulsified by a given amount of protein before collapse of the emulsion system. Since the determination of emulsion capacity of protein is more or less subjective, emulsion stability was chosen for this study. In this study, stabilities of oil-in-water emulsions stabilized by isolated soy protein at three stages of maturity were determined by measuring amount of water separation upon centrifugation.

The results shown in Table 2 indicate that protein isolate from the mature bean provided better emulsion stability than that from the immature one. Comparing the emulsion

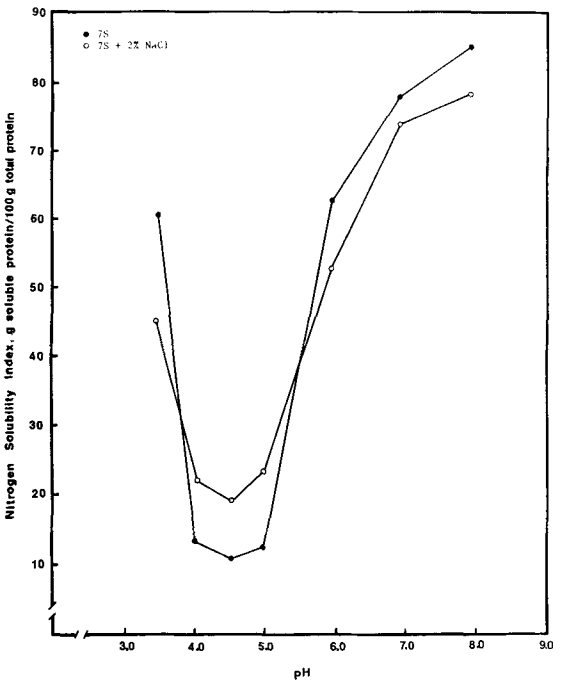

FIG. 4. Effect of PH and sodium chloride on nitrogen solubility index of 7S soy proteins.

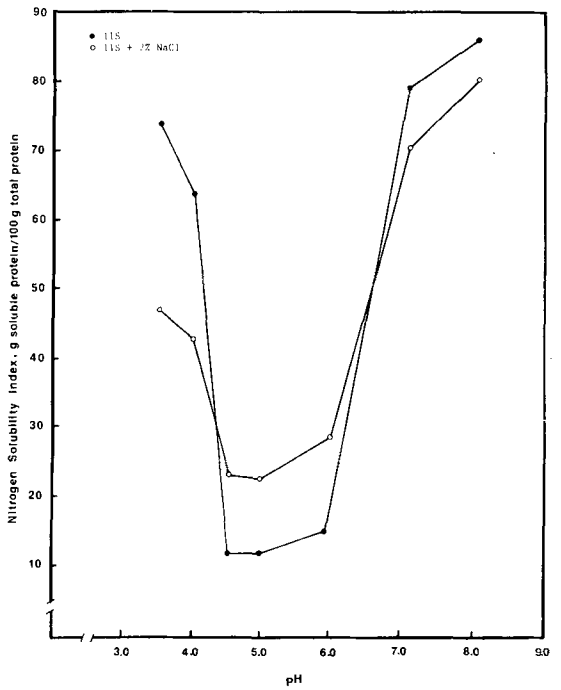

FIG. 5. Effect of pH and sodium chloride on nitrogen solubility index of 11S soy proteins.

stabilities of isolates with their 7S/11S ratio in Figure 6, it is clear that emulsion stability increased with increasing 7S fraction in the mixture. The linear correlation coefficient for the relationship between emulsion stability and 7S/11S ratio was 0.8458. Therefore, the quantities of 7S and 11S protein present in isolated soy protein appear to be the major factor influencing the stability of isolated soy protein

stabilized oil-in-water emulsions. This result is in agreement with observations of Aoki *et al.* (13); at neutral pH, 7S protein provided better emulsion stability as compared to 11S protein.

TABLE 2

Effect of Soybean Maturity and Storage Time on Emulsion Stability of Isolated Soy Proteins, 7S and 11S Proteins

Maturation stage	Protein sample	Emulsion stability (mL of water separation/20 g emulsion[1]) 0 months storage	3 months storage	6 months storage
1 (immature)	isolate	8.0 ± 0.2[a]	8.4 ± 0.6[a]	8.1 ± 0.1[a]
2	isolate	6.6 ± 0.4[b]	7.2 ± 0.4[b]	7.4 ± 0.1[b]
3 (mature)	isolate	5.6 ± 0.3[c]	6.2 ± 0.2[c]	6.4 ± 0.2[c]
Mean	7S	5.3 ± 0.1[c]	6.0 ± 0.2[c]	5.2 ± 0.5[d]
Mean	11S	8.3 ± 0.5[a]	8.8 ± 0.7[a]	8.6 ± 0.4[a]

[1]Means with a common underline in the same horizontal row do not differ significantly at the 5% level. Means in the same vertical column bearing different superscripts differ significantly at the 5% level.

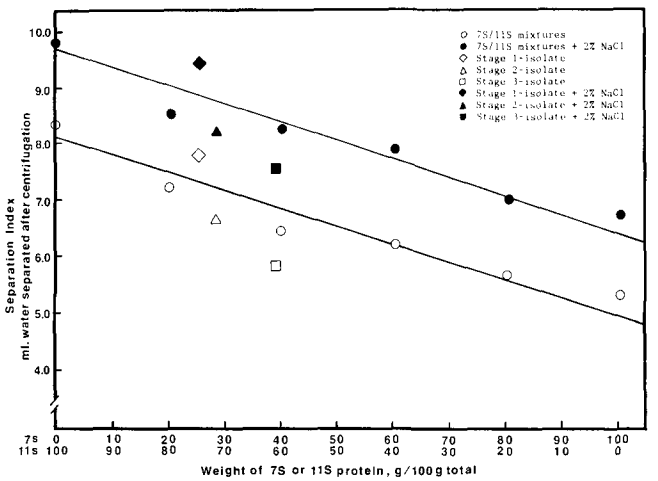

FIG. 6. Stability of oil-in-water emulsions stabilized by isolated soy protein from seed at three stages of maturity compared to that of physical mixture of 7S and 11S fractions with and without 2% sodium chloride added.

Heat-treated peanuts exhibit emulsion capacities similar to or better than the non-heated control (31). It was desirable to determine the effect of heat treatment of a soy protein on its emulsion stability. Upon heating prior to emulsification, emulsion stability was increased. These results may be due to partial denaturation of proteins upon heating. Heating causes unfolding of protein molecules and exposing of hydrophobic or apolar groups (12). The exposed protein conformation would facilitate interaction between protein and oil during emulsification. Therefore, more stable oil-in-water emulsions would be formed by heated proteins.

Emulsion stability of isolated soy proteins from all maturation stages remained approximately the same after storage (Table 2). It has been shown that the presence of an emulsifier (such as protein), which lowers interfacial tension and forms a strong protective membrane around the fat globules, is the most important factor in preparing a stable emulsion (32). Since storage time did not affect the solubility of isolated soy proteins (Table 1), it should not affect emulsion stability as well.

Effect of salt on emulsion stability. The effect of salt on stabilities of oil-in-water emulsions stabilized by soy protein was also investigated at neutral pH. Two-percent sodium chloride was added to mixtures of 7S and 11S fractions in various ratios as mentioned before. The emulsion stability was measured and the results are shown in Figure 6. A linear relation between emulsion stability and various 7S/11S ratios was found in the presence of 2% sodium chloride; separation decreased with increasing amounts of 7S protein in the mixture. The linear correlation coefficient for the curve with 2% sodium chloride was 0.9181. Emulsion stabilities of oil-in-water emulsions stabilized by isolated soy proteins during maturation also followed the curve with 2% sodium chloride added.

As observed above (Fig. 3), the solubility of isolated soy protein decreased independent of maturity at neutral pH in the presence of 2% sodium chloride. As mentioned earlier, the decrease in protein solubility would reduce the emulsion stability of a protein stabilized oil-in-water emulsion. In addition, 2% sodium chloride also impairs the stability of both 7S and 11S protein stabilized oil-in-water emulsions. This is expected since they are the major fractions in isolated soy proteins and can be attributed to the decrease in solubility at neutral pH in the presence of 2% sodium chloride (Fig. 4 and 5).

At pH 4.5, the emulsion stability of 7S protein stabilized oil-in-water emulsions with and without 2% sodium chloride added showed 5.5 mL and 4.4 mL of water separated per 20 g emulsions after centrifugation, respectively. The increase in emulsion stability can be explained by the increase in solubility caused by salting-in effects at the isoelectric point. The solubility of 7S protein at pH 4.5 with 2% sodium chloride added was higher (18.5 g soluble protein/100 g total protein) than that of 7S protein without 2% sodium chloride (12.3 g soluble protein/100 g total protein) at the same pH. Therefore, emulsion stability appears to be related to protein solubility in the system. Generally, the oil-in-water emulsion stabilized by mature isolated soy protein remained relatively more stable in the presence of 2% sodium chloride than that stabilized by the immature isolated soy protein.

Gelation. Formation of protein gel usually requires heating causing unfolding of polypeptides of the protein subunits (8,9). Upon subsequent cooling, unfolded polypeptides reassociate to form the gel (10). Protein gels are composed of three-dimensional networks cross-linked by a combination of forces, i.e., hydrogen bonds, ionic interactions, hydrophobic associations, disulfide linkages (9). The ability of protein to form gels is valuable in food applications because they hold water, flavors, sugars and other ingredients in the gel matrix (12) and contribute to texture. Shemer (33) indicated that heating to 60-70°C is required to induce gelation for a 10% soy protein dispersion. However, when the soy protein dispersion was heated to above 100°C, it lost its gel-forming ability due to destruction of the secondary and tertiary structures.

In this study, gelation was induced by heating isolated soy

proteins at neutral pH from three maturation stages. The results of gel strength and disulfide content of heat-induced soy protein gels during maturation are shown in Table 3. Maturation resulted in reduced gel strength and disulfide content of isolated soy protein gel. It is interesting to note that the fraction giving the stronger gel, 11S, also contained significantly more disulfide (Table 3). Kinsella (12) and Circle *et al.* (8) stated that these disulfide bonds play an important role in heat-induced gel formation. A correlation between the 7S/11S ratio in the mixture and the gel strength was established in Figure 7. The gel strength decreased linearly (r = 0.9679) from 120 to 60 g as the 7S/11S ratio increased from 0 to 100%. The gel strength of soy proteins isolated during maturation also followed this trend (Fig. 7). It can be concluded that 7S/11S ratio in isolated soy protein determines gel strength of heat-induced soy protein gels. Hashizume *et al.* (34) also reported that gels formed from 11S proteins were firmer than those formed from 7S.

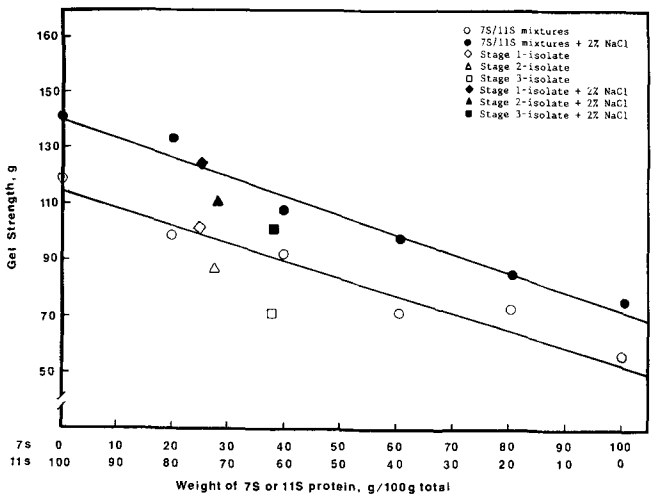

FIG. 7. Strength of heat-induced isolated soy protein gels from seed at three stages of maturity compared to that of physical mixtures of 7S and 11S fractions with and without 2% sodium chloride added.

In addition to being independent of maturation stage, gelation of soy proteins was not affected by storage (Table 3). The gel strength of protein gels from immature isolated soy protein remained stronger than those from mature isolated soy protein during storage. The 11S soy protein gel also turned out to be firmer than the 7S protein gel after six months storage of the beans (Table 3).

Effect of salt on gelation. Isolated soy protein-based gels usually contain a variety of food ingredients. Sodium chloride is frequently used to enhance flavor. In this study, 2% sodium chloride was incorporated into synthetic mixtures of 7S and 11S fractions in various ratios, and heat-induced gels were made as described earlier. Gel strength results are shown in Figure 7.

A linear relation (r = 0.8757) between the ratio of 7S/11S and gel strength was also obtained at 2% sodium chloride. Gel strength increased when 2% sodium chloride was added. The proteins isolated at the three stages of maturity fell on the same curve. A similar result was observed by Circle *et al.* (8). Although salt stabilizes the quaternary structure of soy proteins, heating at temperatures as high as 95°C would be sufficient to dissociate protein quaternary structure into its subunits (34). Protein gelation was considered to be an irreversible process of unfolding, rearrangement and recombination of polypeptide (9). During this process, various interactions between protein molecules and between protein and other ingredient molecules can take place. Increased salt concentration or ionic strength can enhance hydrophilic or ionic interactions among polar groups of the involved molecules, and this should lead to the formation of a stronger gel. This hypothesis was tested by increasing salt concentration to 10% with all five samples listed in Figure 7; none of them showed any gel formation. Extremely high ionic strength exerts a salting-out effect on proteins and causes failure of gel formation. Additionally, soy proteins associate and dissociate differently in various ionic environments (35,36). Changes in solvation states caused by high ionic strength affect the stability of protein conformation (37). Conformational changes lead to unfolding of internal or hydrophobic groups, resulting in hydrophobic interactions between protein molecules. Furthermore, when excess sodium chloride

TABLE 3

Effect of Soybean Maturity and Storage time on Disulfide Content and Gel Strength of Heat-Induced Gels of Isolated Soy Proteins, 7S and 11S Proteins

Maturation stage	Protein sample	Disulfide content (μM/g sample[1])	Gel strength (g[1]) 0 months storage	3 months storage	6 months storage
1 (immature)	isolate	14.32 ± 1.47[b]	101.1 ± 3.5[b]	104.0 ± 5.5[b]	102.4 ± 3.9[b]
2	isolate	9.89 ± 1.36[c]	83.3 ± 4.2[c]	78.9 ± 2.9[c]	82.1 ± 5.0[c]
3 (mature)	isolate	4.19 ± 0.47[d]	61.1 ± 5.7[b]	58.8 ± 3.5[d]	56.4 ± 3.8[d]
Mean	7S	2.84 ± 0.79[d]	55.6 ± 4.1[d]	54.3 ± 3.7[d]	53.5 ± 2.8[d]
Mean	11S	26.62 ± 0.16[a]	116.7 ± 4.3[a]	120.4 ± 6.0[a]	116.1 ± 5.4[a]

[1]Means with a common underline in the same horizontal row do not differ significantly at the 5% level. Means in the same vertical column bearing different superscripts differ significantly at the 5% level.

is added to a protein suspension, the Na^+ and Cl^- ions tend to screen the charged amino acid residues, preventing protein and water interactions (38). As a result, it is not possible for protein to interact with water to form a three-dimensional gel matrix in a high salt environment.

Effect of modification of disulfide groups on emulsion stability and gelation of soy protein. Many physical properties of foods are imparted by proteins; thus, altering protein structure through physical or chemical means would provide direction for designing functional properties and fabricating new foods. For example, in the baking industry, some oxidizing agents are used to enhance dough strength, and reducing agents are used to reduce elastic properties of doughs. It is generally believed that the function of the reducing agent is to control the extent of disulfide linkages (39,40).

For this study, sodium sulfite, a reducing agent for disulfide bonds of proteins, was applied to reduce disulfide linkages in isolated soy protein. The emulsion stability and gel strength of isolated soy proteins from the immature stage (36% solids content) were determined before and after treatment with sodium sulfite. The results are shown in Table 4. Reducing disulfide content resulted in a very slight increase in emulsion stability but a very large decrease in gel strength.

TABLE 4

Effect of Disulfide Content on Emulsion Stability and Gel Strength of Soy Protein

Treatment	Disulfide content (μM/g)	Emulsion stability (mL water separated per 20 g emulsion)	Gel strength (g)
Control	15.43 ± 1.31	8.2 ± 0.2	100.6 ± 2.0
Sodium sulfite	3.88 ± 0.22	7.6 ± 0.3	36.3 ± 3.2

The emulsion stability of isolated soy protein treated with sodium sulfite was 7.6 mL water separated per 20 g emulsion while that of untreated sample was 8.2 mL water separated (Table 4). Thus, the emulsion stability of the treated sample was slightly higher than that of the untreated sample. The increase in emulsion stability can be attributed to the reduction of disulfide linkages which leads to partial unfolding of polypeptide chains. Thus, more hydrophobic surface area is exposed to the environment. Such conformation may facilitate the interaction between oil globules and protein molecules during emulsification. A more stable oil-in-water emulsion would be formed.

Gel strength of the heat-induced soy protein gel treated with sodium sulfite was 36.3 g and that of the untreated sample was 100.6 g (Table 4). Therefore, gel strength of the treated sample was weaker than that of the untreated sample. This result was expected since disulfide linkage has been known to play an important role during gel formation. Circle *et al.* (8) and Shemer (33) indicated that the thinning action of sodium sulfite on both sol and gel forms of 10% sodium soy proteinate dispersions is probably exerted by cleavage of these disulfide bonds. This conclusion is further supported by the ineffectiveness of two other reducing agents, sodium hypophosphite and sodium nitrite, which presumably have no action on the disulfide linkage (8).

REFERENCES

1. Smith, A.K., and S.J. Circle, *Soybean: Chemistry and Technology,* AVI Publishing Company, Westport, CT, 1978.
2. Johnson, D.W., *J. Am. Oil Chem. Soc. 45*:402 (1970).
3. Wolf, W.J., and T. Tamura, *Cereal Chem. 46*:331 (1969).
4. Wolf, W.J., *J. Agric. Food Chem. 18*:969 (1970).
5. Inklaar, P., and J. Fortuin, *Food Technol. 23*:103 (1969).
6. Pearson, A.M., M. Spooner, G. Hegerty and L.J. Bratzler, *Ibid. 19*:1841 (1965).
7. Hutton, C., and A.M. Campbell, *J. Food Sci. 42*:454 (1977).
8. Circle, S.J., E.W. Meyer and R.W. Whitney, *Cereal Chem. 41*:157 (1964).
9. Catsimpoolas, N., and E.W. Meyer, *Ibid. 47*:559 (1970).
10. Saio, K., M. Terashima and T. Watanabe, *J. Food Sci. 40*:541 (1975).
11. Wolf, W.J., and J.C. Cowan, *Soybeans as a Food Source,* CRC Press, Inc., Cleveland, OH, 1975.
12. Kinsella, J.E., *J. Am. Oil Chem. Soc. 56*:242 (1979).
13. Aoki, H., O. Taneyama and M. Inami, *J. Food Sci. 45*:534 (1980).
14. Saio, K., I. Sato and T. Watanabe, *Ibid. 39*:777 (1974).
15. Yao, J., L.S. Wei and M.P. Steinberg, *J. Am. Oil Chem. Soc. 60*:1245 (1983).
16. Tanteeratarm, K., L.S. Wei and M.P. Steinberg, *J. Food Sci. 54*:593 (1989).
17. Yao, J., L.S. Wei and M.P. Steinberg, *Ibid. 53*:464 (1988).
18. Horan, F.E., *J. Am. Oil Chem. Soc. 51*:67A (1974).
19. Thanh, V.H., and K. Shibasaki, *J. Agric. Food Chem. 24*:1117 (1976).
20. *Official Methods of Analysis,* 13th edn., AOAC, Washington, DC, 1984.
21. Beveridge, T., S.J. Toma and S. Nakai, *J. Food Sci. 39*:49 (1974).
22. Beuchat, L.R., *J. Agric. Food Chem. 25*:285 (1977).
23. *Official and Tentative Methods of the American Oil Chemists' Society,* 3rd edn., AOCS, Champaign, IL, 1984.
24. Hunsberger, D.V., and P. Billingsley, *Elements of Statistical Inference,* Allyn and Bacon, Inc., Boston, MA, 1978.
25. Duncan, D.B., *Biometrics 11*:1 (1955).
26. Anderson, R.L., W.J. Wolf and D. Glover, *J. Agric. Food Chem. 21*:251 (1973).
27. Van Megen, W.H., *Ibid. 22*:126 (1974).
28. Acton, J.C., and R.L. Saffle, *J. Food Sci. 35*:852 (1970).
29. Tornberg, E., and G. Lundh, *Ibid. 43*:1553 (1978).
30. Yasumatsu, K., K. Sawada, S. Moritaka, M. Misaki, J. Toda, T. Wada and K. Ishii, *Agric. Biol. Chem. 36*:719 (1972).
31. Cherry, J.P., K.H. McWatters and L.R. Beuchat, in *Functionality and Protein Structure,* edited by P.E. Akiva, American Chemical Society, Washington, DC, 1979, pp. 1-26.
32. Hansen, L.J., *Food Technol. 14*:565 (1960).
33. Shemer, M., *Studies on the Effect of Thermal Processing on the Chemical, Nutritional and Functional Properties of Soybean Protein,* Ph.D. Thesis, University of Illinois, Urbana, IL, 1973.
34. Hashizume, K., N. Nakamura and T. Watanabe, *Agric. Biol. Chem. 39*:1339 (1975).
35. Wolf, W.J., in *Soybeans: Chemistry and Technology,* edited by A.K. Smith, and S.J. Circle, AVI Publishing Co., Westport, CT, 1978, pp. 93-143.
36. Hermansson, A.M., *J. Texture Studies 9*:33 (1978).
37. Franks, F., and D. Eagland, *CRC Critical Reviews in Biochemistry 3*:165 (1975).
38. Bello, J., D. Haas and H.R. Bello, *Biochem. 5*:2539 (1966).
39. Ewart, J.A.D., *J. Sci. Food Agric. 23*:567 (1972).
40. Ewart, J.A.D., *Ibid. 23*:687 (1972).

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