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Soy Protein Gelation

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Heat-induced protein gels are of importance for the structure and properties of many food products. Gel formation is a complex process which often involves several reactions such as denaturation, dissociation-association, and aggregation. The kinetics of the reactions involved will determine the type of structure formed. Protein gels can be divided into two types: gels formed by random aggregation and gels formed by association of molecules into strands in a more ordered way.

The two soy proteins glycinin and conglycinin both have the ability to form ordered structures consisting of strands 10–15 nm thick. The glycinin gel strands formed in distilled water are regular, and cross sections of strands showed a hollow cylindrical structure. In the presence of sodium chloride, glycinin forms an aggregated gel structure at 85 C, but at 95 C a regular structure similar to that found in distilled water was formed. The aggregated structure was interpreted as a transient state similar to the soluble aggregate formed on heating dilute solutions prior to dissociation into subunits.

Conglycinin gels are more irregular and more cross-linked than gels of glycinin. Also, the strands of conglycinin showed a complex mode of aggregation possibly in the form of double spirals. The addition of salt does not affect the microstructure of conglycinin gels as dramatically as in the case of glycinin gels.

Commercially produced soy protein isolates may behave quite differently from native soy proteins, due to processing conditions causing denaturation and various states of aggregation.

Heat-induced protein gels are important to the structure and properties of many food products. Heating of proteins may give rise to several reactions such as denaturation, association, dissociation and aggregation. Gel formation is a complex process involving several different reactions. The degree of random aggregation determines the type of gel structure formed. Association and dissociation reactions are of importance for the onset of gelation as well as for the orientation of molecules into strands. Denaturation is often required for gel formation to take place, but gels can form from already denatured proteins or spontaneously from native proteins under special conditions. An absolute prerequisite for gel formation is the interaction between protein molecules, strands or aggregates in such a way that some kind of a three-dimensional network is formed. As illustrated in Table 1, some of these reactions are induced by protein-water and some by protein-protein interactions (1).

The kinetics of the reactions involved in gel formation will determine the type of structure formed as well as the properties of the structure, e.g. water holding and rheological properties.

AGGREGATED AND ORDERED PROTEIN GELS

Protein gels can be divided roughly into two types, gels formed by "random" aggregation and gels formed by association of molecules into strands in a more ordered way. Due to small changes in the repulsive balance, gels of both types can be formed from one protein and the transfer from one type of gel structure to another can take

TABLE 1

Mechanisms and Conformations Involved in Structure Formation

Protein-solvent	Protein-protein
Mechanism(s)	Mechanism(s)
Dissociation	Association
Denaturation	Precipitation
Solubilization	Coagulation
Swelling	Flocculation
	Aggregation
Conformation(s)	Conformation(s)
Coil	Helix
	Native structure
	Three dimensional structure

place within 0.1 pH unit. Aggregated gels are non-transparent, whereas ordered gels from small globular proteins such as β-lactoglobulin or serum albumin may be completely transparent.

Aggregated gels can be described as being in various stages of phase separation. Generally speaking, the finest structure is obtained under conditions during which the tendency toward aggregation is just above that necessary for gels to form. An increased degree of random aggregation beyond this point will result in a coarser structure, as illustrated in Figure 1.

For example, heating above the gelation temperature will increase the tendency for random aggregation, and a coarser structure with larger aggregates will be formed. This is illustrated in Figures 2 and 3 showing 12% whey protein gels formed at 75 C and 95 C in distilled water. Similar results have been shown previously for blood plasma protein gels (2). The coarser the structure the poorer the water-holding properties of the gels. Consequently, the gels formed at 95 C have considerably poorer water holding properties than the gels formed at 75 C (3,4).

Information about molecular properties may be of limited value for the understanding of the structure and properties of aggregated, or phase separated, gels. Instead, it is important to obtain knowledge of the factors that promote and restrict aggregate growth, e.g. surface tension which may promote growth and the net surface charge of aggregates which may restrict growth and thereby determine the particle size distribution of an aggregated protein gel. For example, a structure almost identical with that shown in Figure 3 may be formed under certain conditions from myosin, which has completely different molecular properties from β-lactoglobulin, the main gel-forming protein in whey (5).

If the energy barrier against random aggregation is sufficient, the molecules can arrange themselves into strands and the second type of gel structure may form. It has been postulated previously that molecules of globular proteins expand due to unfolding during denaturation and then associate into strands in a linear way like a "string of beads" (6,7). As will be shown later, glycinin and conglycinin form ordered gels by a more complex mode of association. To understand the formation of ordered gel

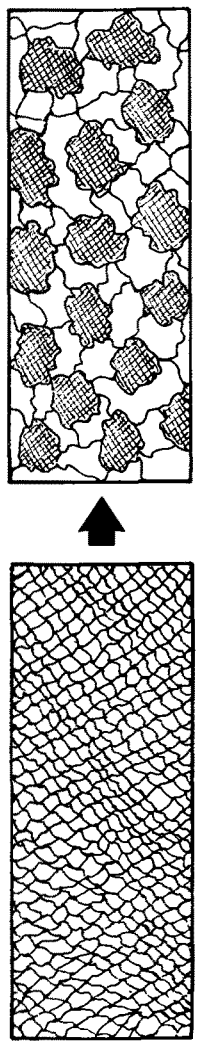

FIG. 1. Schematic illustration of a change in gel structure due to local aggregation phenomena.

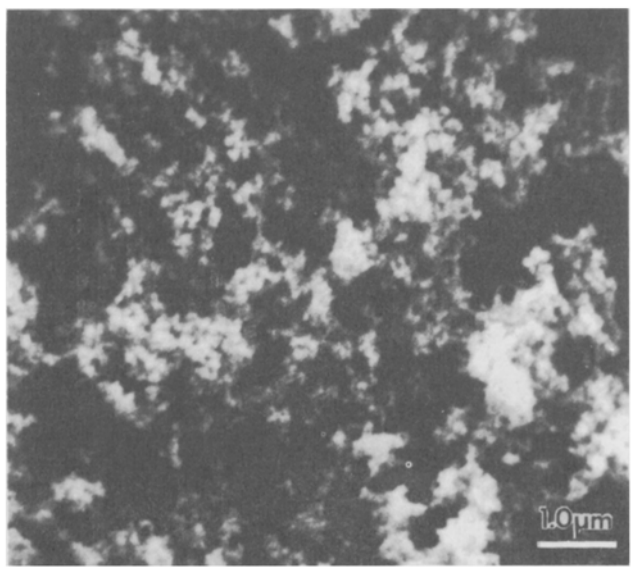

FIG. 2. Scanning electron microscopy (SEM) micrograph of a 12% whey protein gel heated to 75 C at pH 7.

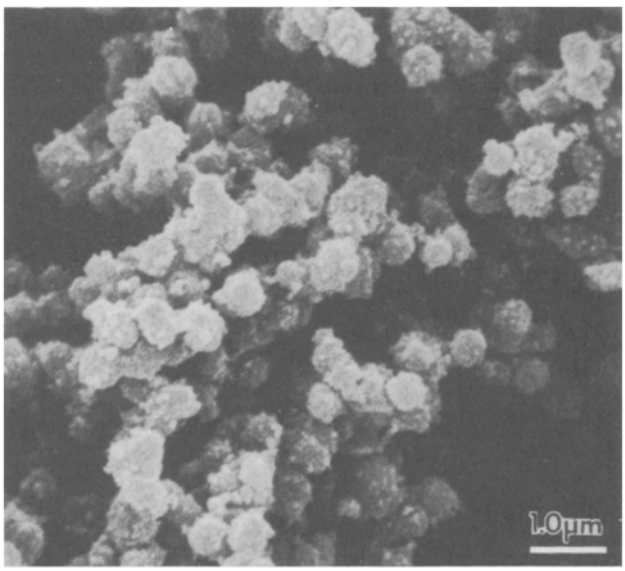

FIG. 3. SEM micrograph of a 12% whey protein gel heated to 95 C at pH 7.

structures, it is important to have knowledge of such reactions as denaturation, dissociation and association.

SOY PROTEINS

The major soy proteins glycinin and conglycinin both have the ability to form ordered gel structures. These proteins have complex quaternary structures that easily undergo association-dissociation reactions, depending on environmental conditions. Glycinin consists of alternately arranged acidic and basic subunits (8). The number of different acidic and basic subunits that have been identified varies from three to eight; the number varies for different soybean cultivars. The molecular weight of the subunits is 34800-45000 for the acidic and 19600-22000 for the basic subunits, and the isoelectric point 4.75-5.40 for the acidic and 8.05-8.5 for the basic subunits. Intermolecular -S-S-bond(s) exist between pairs of acidic and basic subunits (8-12). Acidic subunits not connected to a basic subunit by -S-S-bonds also have been reported (11,12).

β-Conglycinin makes up 90% of the conglycinin fraction and often exists in a dimer form, each dimer consisting of two cyclic trimers of subunits placed on top of each other. The molecular weight of the three subunits α, α' and β are 57000 for α and α' and 42000 for β; the isoelectric points are 4.9, 5.2 and 5.7-6.0 for α, α' and β subunits, respectively. β-Conglycinin has a very low sulphur content, and there are no intermolecular -S-S-bonds between subunits (13-16).

EFFECT OF IONIC STRENGTH ON DENATURATION AND DISSOCIATION

Denaturation of a soy protein isolate in the absence and presence of sodium chloride has been studied by differential scanning calorimetry (DSC). Figure 4 shows DSC thermograms of 10% soy protein dispersions in distilled water and in 0.2 M sodium chloride at pH 2-10 (1).

Two endothermic peaks can be observed in the pH range 4-9. Denaturation of conglycinin is responsible for

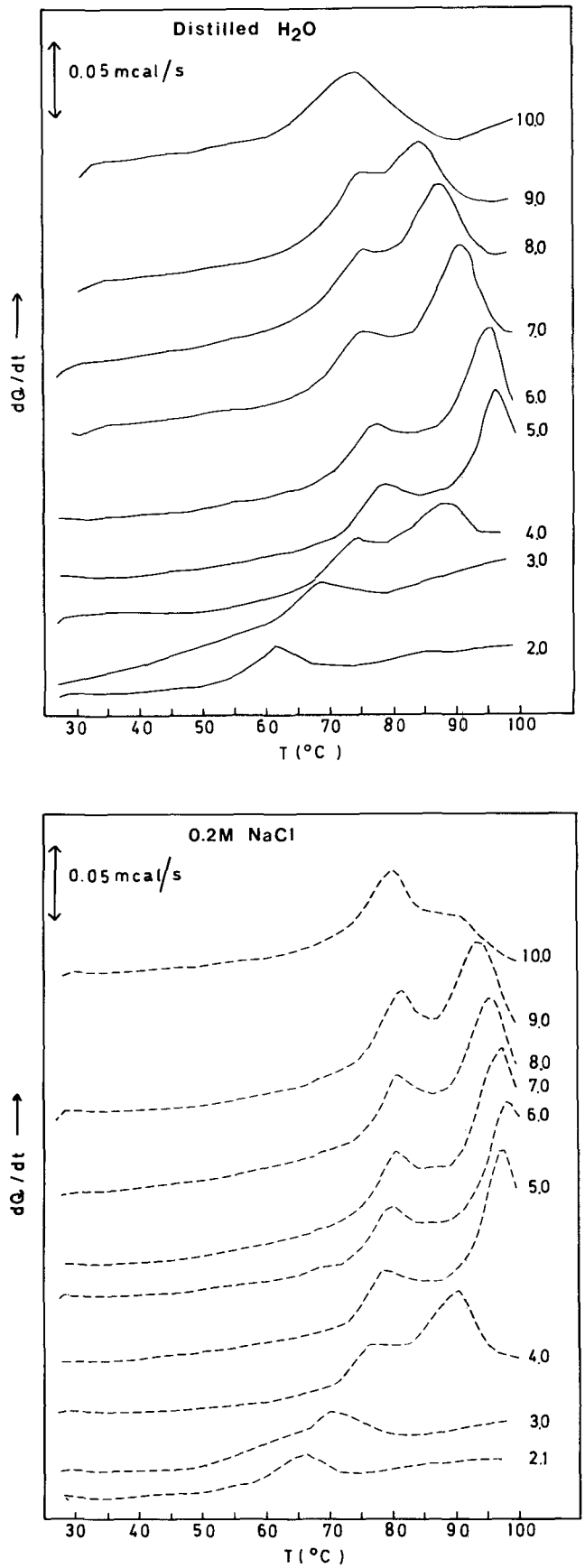

FIG. 4. DSC thermograms of 10% soy protein dispersions in distilled water (top) and 0.2 M NaCl (bottom) at pH 2.0 to 10.0 (1).

TABLE 2

Endothermic DSC Peak Characteristics as a Function of NaCl Concentration at pH 7

NaCl conc. (M)	Peak 1		Peak 2	
	T_d[a] (°C)	T_{max} (°C)	T_d (°C)	T_{max} (°C)
0	67.0	76.0	80.0	91.0
0.01	68.0	76.5	81.0	91.0
0.05	70.0	77.0	84.5	92.5
0.10	72.0	78.5	86.0	94.0
0.25	74.0	80.5	90.0	97.0
0.50	78.5	85.0	97.0	101.0
1.0	87.0	92.0	103.0	108.0
2.0	97.0	102.0	>103.0	>113.0

[a]T_d is the denaturation temperature and T_{max} the temperature at the peak maximum (1).

TABLE 3

Effect of Heat on the Ultracentrifugation Pattern of the 11S Globulin in 0.01 and 0.1 M NaCl (17)

Sample	NaCl (M)	11S (%)	4S (%)	28S (%)
Unheated	0.01	77	—	—
Heated to 80 C	0.01	—	52	48
Heated to 100 C	0.01	—	52	48
Unheated	0.1	77	—	—
Heated to 80 C	0.1	25	37	28
Heated to 100 C	0.1	—	45	55

the first and denaturation of glycinin for the second peak. The measurements made in distilled water are more pH dependent than those made in 0.2 M sodium chloride solutions. In distilled water the protein is most stable against denaturation at pH 5, close to the isoelectric region where the net charge is low. At extreme pH 2, 3 and 10, partial denaturation takes place even before heat treatment, both in the absence and in the presence of sodium chloride. The presence of salt stabilizes the quaternary structure and thereby the proteins against denaturation. This is clearly illustrated in Table 2, which shows the onset of denaturation of the two denaturation peaks (T_d) as well as the temperature of the peak maxima (T_{max}) at salt concentration in the range of 0.01–2.0 M.

It can be seen that the T_d of peak 1 increased by 19 C and the T_d of peak 2 by 22 C when the salt concentration was increased from 0.01 to 1.0 M.

No DSC data of isolated glycinin and conglycinin fractions have thus far been published, and it is possible that denaturation temperatures of pure fractions would deviate somewhat from the results of the soy protein isolate discussed above.

The presence of salt stabilizes the quaternary structure of glycinin against dissociation and thereby probably the protein against denaturation. Hashizume et al. (17) have investigated changes in the ultracentrifugation patterns of glycinin and conglycinin fractions when solutions were

TABLE 4

Effect of Heat on the Ultracentrifugation Pattern of the 7S Globulin in 0.01 and 0.1 M NaCl (17)

Sample Treatment	NaCl (M)	7S (%)	28S (%)
Unheated	0.01	91	—
Heated to 80 C	0.01	43	50
Unheated	0.1	91	—
Heated to 80 C	0.1	9	71

heated to 80 C and 100 C in the presence of 0.01 and 0.1 M sodium chloride. The results are shown in Tables 3 and 4.

As can be seen from Table 3, the amount of dissociation products (4S) was lower when the glycinin-rich fraction (11S) was heated in 0.1 than in 0.01 M sodium chloride solutions. Furthermore, 25% of the 11S fraction remained after heating to 80 C in 0.1 M NaCl, whereas no 11S fraction could be detected after heating in 0.01 M NaCl.

As can be seen from Table 4, no dissociation products were found after heating of the conglycinin-rich fraction (7S). Contrary to the results obtained with the 11S fraction, less of the 7S fraction remained after heating to 80 C in 0.1 M sodium chloride than after heating in 0.01 M NaCl. Aggregation was thus found to be favored by the higher salt concentrations.

The effect of salt on the denaturation temperature implies that the gelation temperature also will be affected by the salt concentration. The shear force increase as a function of the heating temperature was studied and used as a crude measurement of the gelation temperature (1). Figure 5 shows the shear force as a function of the heating temperature of soy protein dispersions at pH 7, when the salt concentration was increased from 0.2 to 2.0 M. It can be seen that the gelation temperature increased with increasing salt concentration, and at 2.0 M the gelation was completely suppressed at ≤100 C.

HEAT INDUCED ASSOCIATION-DISSOCIATION REACTIONS

Apart from the ultracentrifugation results obtained by Hashizume et al. (Table 4), no studies have been published on heat-induced association reactions of conglycinin-rich fractions. Glycinin has been far more extensively studied. Most of these studies have been made under the following experimental conditions: protein concentration 0.5%, pH 7.6, ionic strength 0.5, and heating temperature 100 C. As will be shown later, the results from these studies are of importance for the understanding of gel formation of glycinin in the presence of salt.

Wolf and Tamura (18) followed the changes in ultracentrifugation patterns during heating of 0.5% glycinin solutions at 100 C. Their results are summarized in Figure 6.

The 11S component completely disappeared after five min. A soluble aggregate was formed but disappeared within seven min of heating. With the decrease of the amount of soluble aggregate, a precipitate and a soluble 4S fraction were formed. A smaller amount of a transient

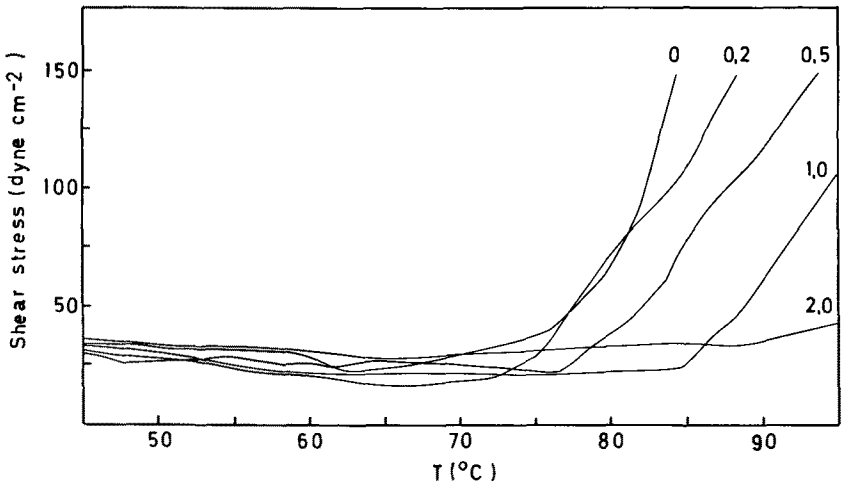

FIG. 5. Shear force at 300 s^{-1} as a function of temperature of 10% soy protein dispersions at pH 7 and NaCl concentrations of 0–2.0 M (1).

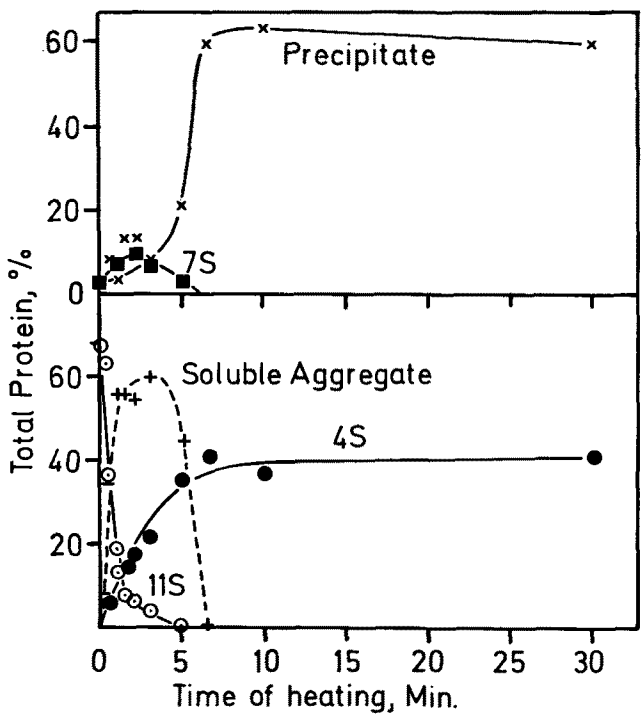

FIG. 6. Effect of heating time at 100 C on the ultracentrifugal composition of a 0.5% glycinin solution at an ionic strength of 0.5 and pH 7.6 (18).

7S component also was isolated. Yamagishi et al. (19) analyzed the composition of fractions corresponding to those described above. They found that the 4S component consisted of acidic subunits and that the transient 7S component probably was an oligomer of acidic subunits. The precipitate was found to consist of basic subunits. Similar results were obtained by Mori et al. (20). They concluded that heating of a 0.5% glycinin solution results in the formation of a transient soluble aggregate consisting of acidic and basic subunits with a ratio of acidic subunits slightly lower than that of the native molecule. On subsequent heating, this aggregate disappeared and a complete dissociation into acidic and basic subunits took place. The basic subunits precipitated due to their low solubility at pH 7.6, whereas the acidic subunits remained soluble.

In addition to the studies of 0.5% glycinin solutions, Mori et al. (20) studied the effect of heating of 5% glycinin solutions. At the higher protein concentration they found no separation into acidic and basic subunits of the soluble aggregate, but further aggregation and subsequent gel formation after five min of heating. They drew the conclusion that glycinin in concentrated solutions does not dissociate on heating and that the soluble aggregate was a precursor to the glycinin gel.

GLYCININ GELS

Hermansson (21) investigated the microstructure of glycinin and conglycinin-rich gels prepared in the presence and absence of 0.2 M sodium chloride and heated to various temperatures. Figure 7 shows the microstructure of a 12% glycinin gel made in distilled water and heated to 95 C for 30 min.

The gel consists of very regular strands. Cross-sections of strands show that they are built up like hollow tubes. Some cross-sections are encircled. The outer diameter of the strands is 12–15 nm. Micrographs at higher magnifications (400,000×) of cross-sections of strands indicated the strands are composed of subunits in some kind of spiral arrangement (21).

No major changes of the structure were observed when the heating temperature of gels made in distilled water was lowered from 95 C to 85 C. Quite different results were obtained in the presence of 0.2 M sodium chloride. As can be seen from Figures 8 and 9, an aggregated structure was formed at 85 C but when the heating temperature was raised to 95 C the structure changed from an aggregated structure to an ordered network of strands. The latter structure was somewhat denser than but similar to that formed in distilled water.

Contrary to the observations discussed earlier, of the effects of heating temperatures on aggregated gel

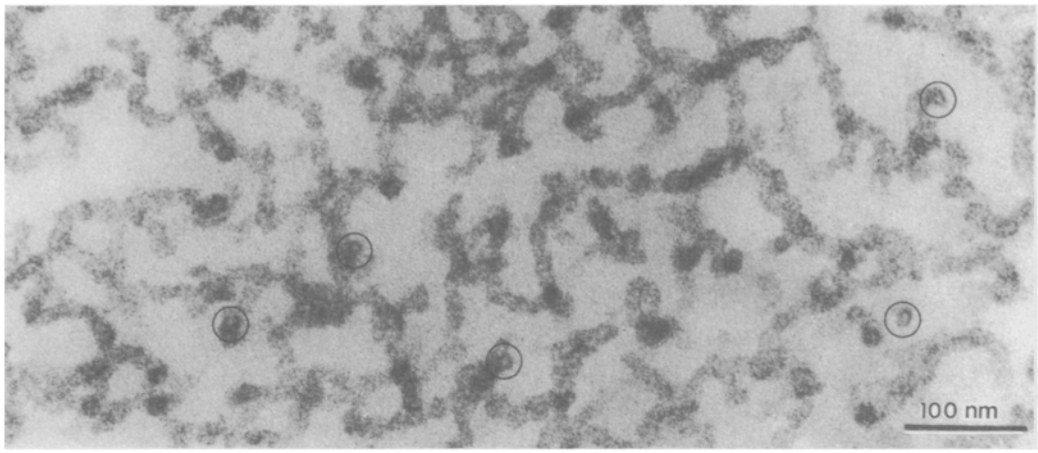

FIG. 7. Section of a 12% glycinin gel made in distilled water at 95 C at a magnification of 200000×. Examples of cross-sections of strands are encircled.

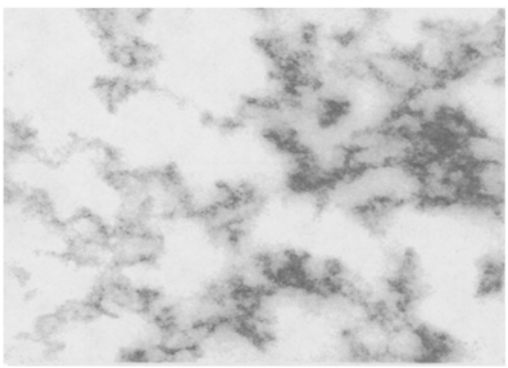

FIG. 8. Section of a 12% glycinin gel made in 0.2 M NaCl at 85 C at a magnification of 100000×.

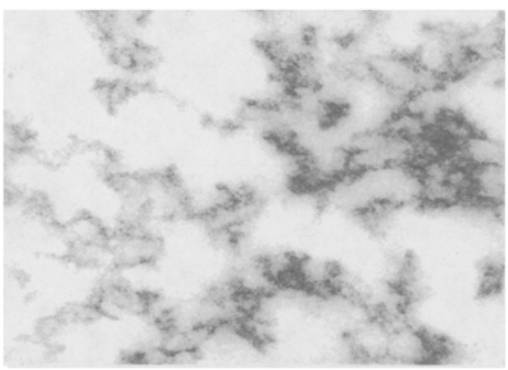

FIG. 9. Section of a 12% glycinin gel made in 0.2 M NaCl at 95 C at a magnification of 100000×.

systems, we here have a situation in which an increase in heating temperature causes a transition from an aggregated structure to a more ordered strand structure. The aggregated structure found at 85 C was unstable and could vary considerably with regard to the state of aggregation between different preparations, whereas the structure formed at 95 C in the presence of 0.2 M sodium chloride was very reproducible. The aggregated gel structure formed at 85 C in 0.2 M NaCl is believed to correspond to the transient soluble aggregate formed in dilute solutions and to the gel structure described by Mori et al. (20). There is no reason to believe that the behavior of glycinin should differ with regard to dissociation and denaturation between dilute and concentrated systems, but rather that different kinetics determine the dissociation and subsequent reassociation into regular strands in concentrated gel systems and the dissociation and separation into acidic and basic subunits in dilute systems.

It thus can be concluded that the regular strands of glycinin gels formed in distilled water at 85 C and after sufficient heating at 95 C in the presence of sodium chloride are caused by dissociation of the quaternary structure of glycinin and reassociation of the subunits into some kind of spiral arrangement. It is unlikely that there is complete separation into acidic and basic subunits fractions in the gel, because two types of strands and a more irregular structure would then be expected.

CONGLYCININ GELS

The microstructure of a 12% conglycinin-rich gel formed at 85 C in distilled water is shown in Figure 10.

The gel structure is denser and its network more strongly crosslinked than that of glycinin. The strands of the conglycinin-rich gel are more compact and less regular than those of glycinin. The strand thickness is generally in the range 10–14 nm, but small regions of strands are of finer dimensions. In the case of conglycinin, the strands are composed of some kind of circular arrangement and not of expanded subunits, according to the "strings of beads" mechanism. Examples of spiral structures are encircled.

Conglycinin-rich gels also were formed at 75 C in distilled water and at 75 C and 85 C in the presence of 0.2 M sodium chloride. Lower temperatures were chosen because conglycinin has a lower denaturation temperature than glycinin. There were no major changes between gels formed at 75 C and 85 C in distilled water or between gels formed at 75 C and 85 C in 0.2 M sodium chloride. The addition of salt produced a denser network structure.

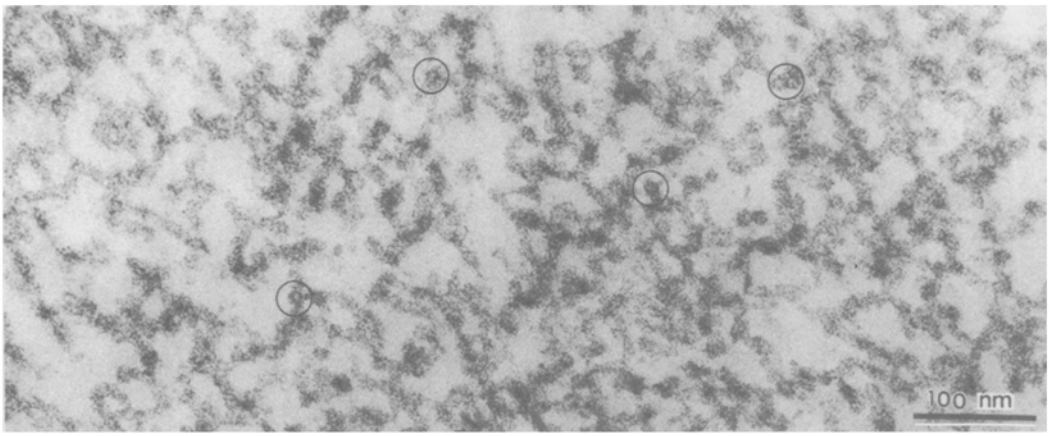

FIG. 10. Section of a 12% conglycinin gel made in distilled water at 85 C at a magnification of 200000×. Examples of circular arrangements are encircled.

GELS FROM SOY PROTEIN ISOLATES

The gel structure of a soy protein isolate (A) was investigated by electron microscopy by Hermansson and Buchheim (22). The isolate was a pilot plant preparation of an acid-precipitated soy protein isolate prepared under mild conditions (1). Twelve percent protein dispersions were heated to 85 C in distilled water and in 0.2 M sodium chloride at pH 7. As expected, a more aggregated structure was obtained in the presence of sodium chloride. This structure was more similar to that of glycinin than to that of conglycinin gels heated to 85 C. The magnification in this study was too low for any conclusions to be drawn about the composition of the strands.

Information about the gel structure of glycinin, conglycinin or acid precipitated soy protein isolates may be of limited value for the understanding of commercially available soy isolates, because processing during production may have induced completely different properties. Figure 11 shows DSC thermograms of the acid precipitated soy protein isolate (A) and the commercial isolates B, C and D, of which C and D are the most commonly used (23).

It can be seen that isolates C and D are completely denatured and the isolate B partially denatured. Surprisingly enough, isolate D showed high solubility outside the isoelectric regime, when the measurements were made in distilled water (Fig. 12). However, in the presence of 0.2 M sodium chloride the results turned out quite differently (Fig. 13). Now the solubility is low in the whole pH range 2–9. This finding is of practical importance because the soy protein isolate D will function quite differently, depending on how it is used. If a dispersion of isolate D is heated in distilled water, it will dissolve and form a relatively fine gel network in spite of the fact that it is completely denatured. The isolate also can form a gel in the presence of salt, but this gel is produced by swelling of spray dried particles as shown in Figure 14 and has no resemblance to protein gels (24).

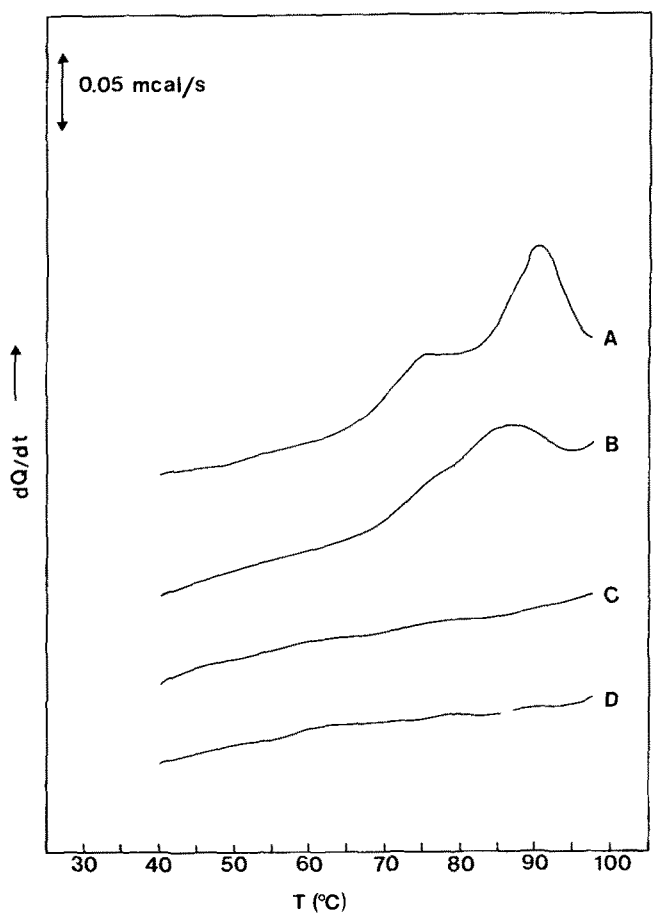

FIG. 11. Differential scanning calorimetry (DSC) thermograms of 10% soy protein isolates at pH 7.0 in distilled water. A: produced under mild conditions on pilot plant scale. Isolates B, C, and D: commercially available isolates (23).

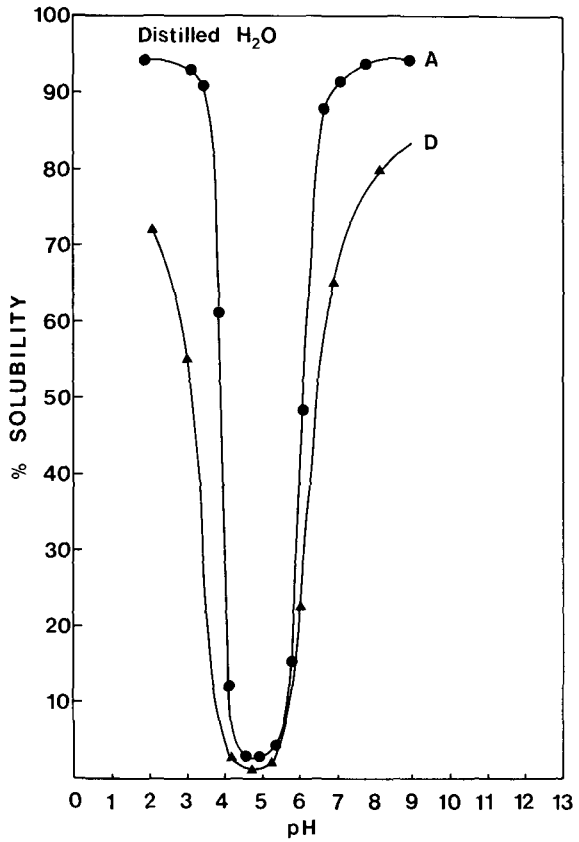

FIG. 12. Solubility as a function of pH in distilled water of 1% dispersions of the mildly produced soy protein isolate A and the commercial soy protein isolate D (23).

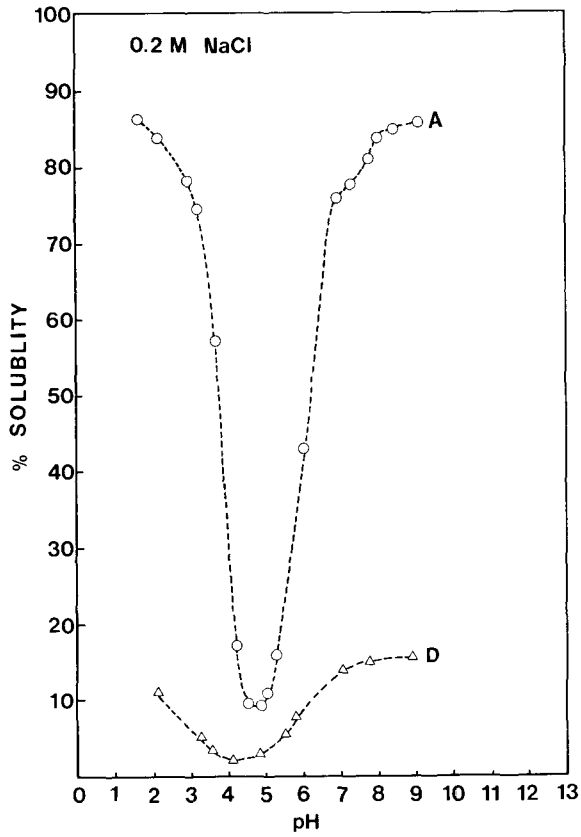

FIG. 13. Solubility as a function of pH in 0.2 M NaCl of 1% dispersions of the mildly produced soy protein isolate A and the commercial soy protein isolate D (23).

To illustrate the different forms of behavior of isolate D, depending on how it is added to a food formula, an experiment was made where 2% and 4% isolate D were added to a meat emulsion product in two different ways. It was either pre-swelled in the absence of salt and then added to the meat batter or added in its dry form after the salt had been added to the meat. Apart from the different ways of adding the soy protein isolate, the recipes and the processing conditions were the same.

The presence of swelled soy particles when added dry can easily be detected in the meat system both before and after heat treatment, as illustrated by Figure 15. Figure 16 shows that there was a marked difference in cooking loss, depending on the way in which the two isolate levels were added. When the isolate was added pre-swelled it could dissolve before the addition of salt and make a better contribution to the structure and the fat and water-holding properties of the product. When the isolate was added dry it stabilized the structure in the form of swelled particles and behaved more like a textured protein than a gelling ingredient.

The results show that protein can form gels of quite different dimensions, from ordered networks of strands to phase separated or aggregated structures, to the extreme where the protein is insoluble and a "gel" is formed from spray dried particles. Soy protein gels can be found in all these categories.

Soy glycinin and conglycinin have the ability to form ordered networks of strands. The strands of both proteins

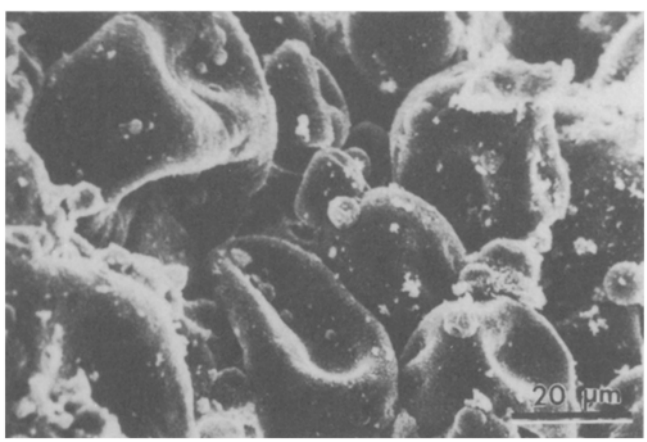

FIG. 14. SEM micrograph of a 12% soy protein "gel" of isolate D made in 0.2 M NaCl at 95 C at a magnification of 800×.

were composed of associated monomers arranged in a circular way. The glycinin strands were more regular and the degree of crosslinking lower than in the case of conglycinin-rich gels. The aggregated structure of glycinin formed at 85 C in the presence of salt was interpreted as a transient state corresponding to the soluble aggregate found in early stages of heating of dilute glycinin solutions.

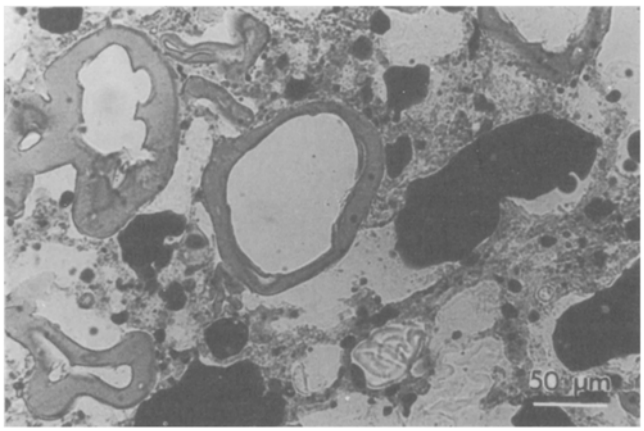

FIG. 15. Light micrograph of a cooked sausage with swelled particles of isolate D at magnification of 200×.

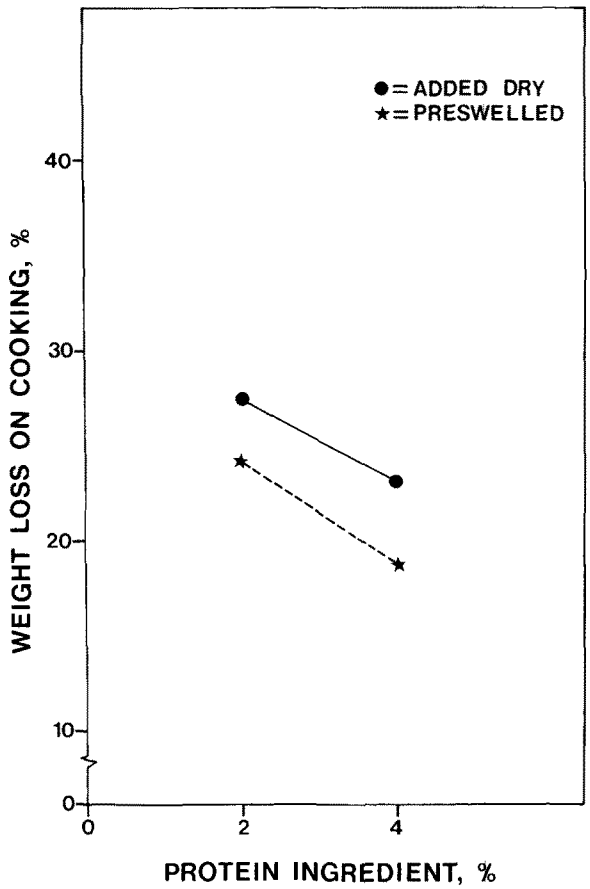

FIG. 16. Weight loss on cooking of sausage emulsions where isolate D has been added pre-swelled and in its dried form.

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