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Effects of β-Conglycinin and Glycinin on Thermal Gelation and Gel Properties of Soy Protein

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Abstract Dynamic shear moduli of isolated soy protein solutions upon heating were measured to monitor gelation. Onsets of gelation coincide with onset temperatures of denaturation in glycinin and β-conglycinin solutions, whereas in isolated soy proteins, onset of gelation was above denaturation temperature of β-conglycinin with storage modulus increasing in two steps. The first increase in storage modulus of isolated soy proteins occurred at about 78.5°C, while the second increase started at about 93°C. Gel properties of soy protein gels having different proportions of glycinin and β-conglycinin were measured by compression-decompression test. β-Conglycinin was responsible for gel elasticity. Glycinin significantly increased hardness, toughness, and fracturability of gels at high heating temperature near 100°C. Results reveal texture of soy protein gels can be controlled by regulating ratio of glycinin to β-conglycinin and heating temperature.

Keywords: β-Conglycinin, glycinin, soy protein, gel property, compression test

Introduction

Soy proteins play important roles in many foodstuffs because of their nutritional value and contribution to food texture (1). The gel-forming ability induced by the heating of soybean proteins is one of their most important functional properties (2, 3). For optimal use of soy proteins as functional ingredients, we, therefore, need better insight into the effects of processing conditions on gel properties. In particular, because the ability to form a gel contributes to the creation of texture in food systems, the effects of heat on protein solutions at high concentration should be studied in greater detail.

Soybeans contain glycinin and β-conglycinin as the major components of their storage proteins, which are generally known to contribute to the quality, particularly the physical properties, of foods made with soybeans (4). Studies revealed the proportion of glycinin and β-conglycinin in soybeans is responsible for the differences in the physical properties of tofu gel (5). In addition, the behavior and partial gelation mechanisms of glycinin, β-conglycinin, and isolated soy proteins have been elucidated (6). Furthermore, glycinin and β-conglycinin have been suggested to interact with each other in the isolated soy protein gel (7)

In a previous study, we investigated the mechanical properties and texture of soy protein gels made under various gelling conditions. Differences in heating temperature and protein concentration provided gels with different mechanical properties and texture (8). In addition, the texture of soy protein gels prepared under various gelling conditions was evaluated by means of three-dimensional representation of the gels through factor analysis of instrumental data (9). In the present work, to elucidate how the two major components of soybean storage proteins contribute to the heat-induced gelation, thermal gelation and gel properties of glycinin and β-conglycinin, and isolated soy proteins with different proportions of glycinin and β-conglycinin were investigated.

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Materials and Methods

Materials Defatted, low heat-treated soybean meal was donated by Ajinomoto Co. Inc. (Tokyo, Japan). Protein solubility (NSI), and concentrations of protein and fat in the defatted soybean meal were 85, 53, and 0.6%, respectively. Acid-precipitated soybean protein (APP-1), and glycinin-rich (Glycinin) and β-conglycinin-rich (β-conglycinin) fractions were prepared from the soybean meal according to the method of Thanh and Shibasaki (10). No further purification was attempted. The proteins were freeze-dried and stored in a refrigerator (4°C) until used. Glycinin fraction was added to the β-conglycinin fraction to reach the glycinin/β-conglycinin ratio of 1:1 (APP-2). Lyophilized proteins were suspended in 35 mM potassium phosphate buffer (pH 7.6) containing 0.4 M NaCl, and stirred. The suspensions were adjusted to pH 7.6 by adding 2 N NaOH dropwise. The protein concentration was 19% (w/w), unless otherwise stated.

Determination of 2S globulin, β-conglycinin and glycinin proportions The protein solutions (1%) were centrifuged at 20°C in 12 mL of 10-30% (w/v) linear sucrose gradient in 35 mM potassium phosphate buffer (pH 7.6, 0.4 M NaCl, 10 mM 2-mercaptoethanol, and 0.02 % NaN_3) at 248,850 g for 17.5 hr in a rotor (RPS 40T, Hitachi, Japan). After centrifugation, the gradient was divided into 0.4 mL fractions and measured at 280 nm simultaneously with a density gradient fractionator (Isco Inc., Lincolin, NB, USA). The protein content of each fraction was measured using the method of Lowry (11), and the proportion of 2S globulin, β-conglycinin, and glycinin were determined (Table 1). APP-1 and APP-2 gave glycinin/β-conglycinin ratios of 2.2 and 1.0, respectively.

Table 1. The 2S globulin, β-conglycinin and glycinin proportion of the soy protein preparations

Preparations	Protein components (%)[1)]		
	2S globulin	β-conglycinin	Glycinin
Glycinin	2.7	3.7	93.6
APP-1	19.4	25.1	55.5
APP-2	16.4	41.8	41.8
β-Conglycinin	25.0	65.0	10.0

[1)] Standard deviations for all values were <±1%.

Rheological measurement Complex shear modulus of the protein solutions was measured by a Rheolograph sol (Toyo Seiki Seisakusho, Tokyo, Japan) as a function of temperature. The sample solutions (1.6 mL) were put into parallel plate-type cells (internal dimensions, 2.6 mm × 15 mm × 45 mm). Subsequently, a blade (0.6 mm thick × 10 mm wide × 25 mm long) was inserted into the cell, and the surface of the sample was covered with silicone oil to avoid evaporation. The samples were heated at 1°C/min from 25 to 100°C, and were subjected to 3 Hz sinusoidal shear oscillations with an amplitude (displacement, ±100 μm), which was well within the linear region and did not disturb the gelation. The detected stress was divided into the component in phase with the applied strain (storage modulus of rigidity, G') and that ahead of the strain by π/2 (loss modulus of rigidity, G''). Values of the storage and loss moduli, which represent elastic and viscous elements, respectively, of a viscoelastic body, were recorded as functions of temperature. The gelation point was determined as the temperature at which storage modulus (G') starts growing.

Differential scanning calorimetry (DSC) Thermal behavior of the solution was studied by DSC with Seiko model SSC 5000-DSC100 (Seiko Electronic Co., Tokyo, Japan). The samples used for the gel preparation were accurately weighed and sealed in silver pans. An empty pan was used as the reference. The heating rate was 1°C/min from 25 to 120°C. A thermal analysis software (Com, Seiko, Tokyo, Japan) was used to acquire raw experimental data, and to save the onset (To) and peak (Tp) temperatures.

Preparation of gels Protein solutions were poured into the 2-mm gaps between two glass plates equipped with silicon spacers (2 mm thickness) for sealing, and heated at different temperatures (80 to 100°C) for 30 min. The gel sheets were removed and cut into 2-cm squares.

Measurement of mechanical properties of gels A uniaxial compression-decompression test was carried out using a compression tester (KES-FB 3, Kato Tech Co. Ltd., Tokyo, Japan) equipped with a cylindrical plunger having a cross-sectional area of 0.25 cm^2. The tests were performed five times at 20°C with the gel samples. The plunger descended at a rate of 1.2 mm/min. The soy protein gels were measured by compressing the samples till rupture. In the tests, the plunger returned automatically at the rupture point. Compression work (CW) and decompression work (DW) were determined as the area under the compression and decompression curves, respectively. Resiliency (RS) was calculated from the following equation:

$$RS\ (\%) = (DW/CW) \times 100 \quad (1)$$

Compressibility (CM) was measured as percent deformation using the following equation:

$$CM\ (\%) = [\text{deformation (mm)/sample thickness (mm)}] \times 100 \quad (2)$$

Statistical analysis All analyses were performed five times, and average values were used in all tables and figures. Statistical differences were determined using an analysis of variance in conjunction with Duncan's Multiple Range Test and Bonferroni's Multiple Comparison Test.

Results and Discussion

Gelation Because the gelation point is rheologically defined as the point at which storage modulus (G) starts growing, the temperature of gelation point was determined from the temperature versus dynamic shear modulus curve (Fig. 1). The dynamic shear modulus curve of β-conglycinin showed a steep rise in G' at around 76°C, which indicates the rapid formation of a viscoelastic gel matrix. Small increase in G' of β-conglycinin between 60 to 76°C may be due to contamination of 2S globulin. With increases in the ratio of glycinin, progressive delays were observed in the onset temperature of gelation. In the case of glycinin, G' increased markedly at about 93°C, which indicates the formation of gel structure at this temperature. In APP-1 and APP-2, G' showed a two-step increase during the gelation. The first increase occurred at about 79 and 78°C, respectively, while both showed second increase at about 93°C. Thus, the gelation behavior of soy protein can be divided into two gelation steps. Experiments with separate β-conglycinin and glycinin indicated that the first and second peaks originated from those of β-conglycinin and glycinin, respectively. DSC analysis was carried out to determine the thermal behavior of the protein solutions (Table 2). Beveridge *et al.* (12) compared DSC data to the

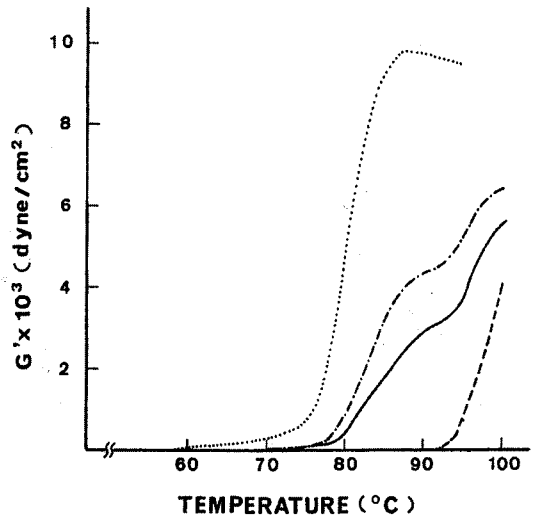

Fig. 1. Temperature versus dynamic shear modulus curves for gels. β-conglycinin (···), APP-2 (– · –), APP-1 (–), and glycinin (---).

Table 2. Values of onset (To) and peak (Tp) temperatures for β-conglycinin and glycinin in soy protein preparations

Preparations	β-conglycinin		Glycinin	
	To (°C)	Tp (°C)	To (°C)	Tp (°C)
Glycinin	-	-	92.8 ± 0.1	97.5 ± 0.1
APP-1	75.1 ± 0.2	79.8 ± 0.1	93.2 ± 0.1	97.8 ± 0.2
APP-2	75.3 ± 0.1	79.4 ± 0.2	93.8 ± 0.2	97.8 ± 0.1
β-Conglycinin	76.4 ± 0.2	80.0 ± 0.1	-	-

development of gel or network structure assessed as an increase in the storage modulus. They reported that the onset of structure development coincided with the peak temperature associated with maximum heat flow on the DSC curve (Tp) for most proteins in the mixture. However, Stading and Hermansson (13) used onset temperature of denaturation as denaturation temperature when comparing results of both DSC and rheological measurement. In this study, the onset of gelation coincided with the onset temperature of denaturation (To) for glycinin and β-conglycinin. For APP-1 and APP-2, the onset of gelation took place at about the peak temperature (Tp) of β-conglycinin, although small decreases in heat stability of β-conglycinin fractions were observed. The presence of undenatured free glycinin molecules is considered to disturb the formation of a viscoelastic gel matrix, and/or low contents of β-conglycinin delay gelation temperatures of APP-1 and APP-2 (Table 1).

Rupture force and compression work of the gels Self-supporting gels were formed in the 19% solutions of β-conglycinin, APP-1, and APP-2 from 80°C, whereas glycinin did not form the gels below 93°C. The effect of heating temperature on mechanical properties of soy protein gels is shown in Fig. 2 and 5, where the plots are based on the means, and 95% confidence intervals are not indicated due to small variation. Of the four mechanical properties, rupture force and compression work required to cause rupture are supposed to be related to the formation of substantial gel structure and its following stabilization by chemical bindings (14). From viewpoints of sensory properties, hardness and toughness appear to correspond to the high values of rupture force and compression work, respectively (15). Obviously, results on rupture force (Fig. 2) are very similar to those on compression work (Fig. 3). For example, glycinin gels showed marked increases of rupture force and compression work with elevation of heating temperature above 93°C, i.e., gelation point ($p< 0.05$), which suggests that the formation of new and substantial network structure occurred within the gel, and this substantial network structure stabilized by further formation of chemical bindings at these temperatures. The formation of substantial structure appears to be initiated by conformational changes of glycinin from its onset temperature of denaturation, 92.8°C (Table 2). These conformational changes are assumed to bring about the association of unfold molecules through hydrophobic interactions and intermolecular cross-linkages by disulfide binding, which leads to the formation of a rigid network structure. On the contrary, β-conglycinin gels showed a flat curve, with no marked changes over the temperature range of 80 to 100°C (Figs. 2 and 3; 0.05 level). This indicates that β-conglycinin starts to gelate at about 76°C and form substantial viscoelastic gel matrix through, perhaps, hydrophobic or hydrogen-bonding interactions (Fig. 1), but with no further formation of chemical bindings within the gel matrix at above 80°C. No increases of rupture force and compression work with the elevation of heating temperature may be related to no possibility of inter-molecular disulfide bonding, because β-conglycinin molecules have no cystein residues (16). APP-1 (glycinin/β-conglycinin=2.2) and APP-2 (glycinin/β-conglycinin=1.0) gels showed low values and nearly flat curves below 93°C, which, however, significantly increased above 93°C ($p<0.05$). Two-step gelation took place in APP-1 and APP-2 gels, that is, gelation of β-conglycinin below 93°C and that of glycinin above 93°C (Fig. 1). Soft and week gels of APP-1 and APP-2 below 93°C (Figs. 2 and 3) are supposed to sustain by network structure of β-conglycinin. Lower values of APP-1 and APP-2 gels than those of β-conglycinin gels over temperature

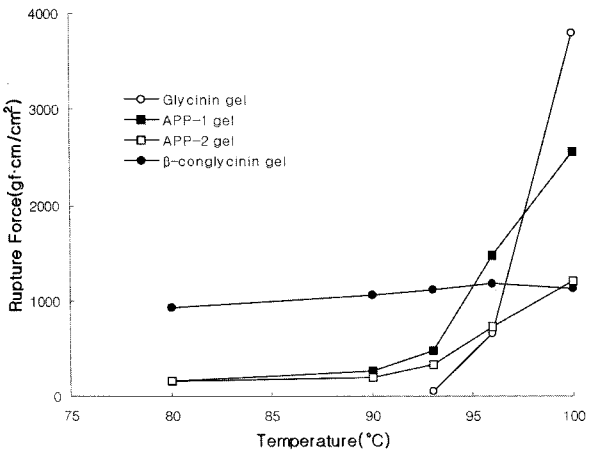

Fig. 2. Effect of heating temperature on the rupture force of soy protein gels. Symbols denotes ● β-conglycinin; □, APP-2; ■, APP-1; ○, glycinin, respectively. The gels were prepared from 19% protein solutions.

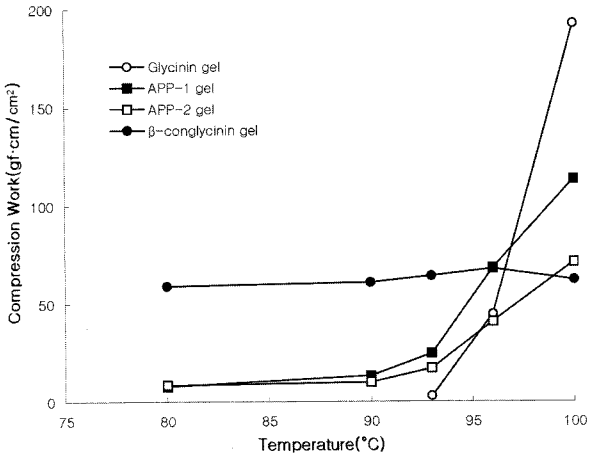

Fig. 3. Effect of heating temperature on the compression work of soy protein gels. Symbols denotes ● β-conglycinin; □, APP-2; ■, APP-1; ○, glycinin, respectively. The gels were prepared from 19% protein solutions.

ranges of 80 to 93°C may be due to the presence of free glycinin molecules that has no contribution on the network structure. Above 93°C, however, glycinin could play an important role in the formation of network structures of APP-1 and APP-2 gels. When heating temperature was very high, near 100°C, the values of rupture force and compression work were proportional to the contents of glycinin, which means that glycinin significantly contributes to the increases in hardness and toughness of soy protein gel.

Compressibility of the gels Changes in compressibility at rupture of the gels are shown in Fig. 4. Gel compressibility rose with increasing heating temperature. Marked increases in compressibility were observed when heating above 93 °C, except for β-conglycinin gel, which showed a constant curve over the temperature range of 80 to 100°C (0.05 level). In general, value of gel compressibility is proportional to glycinin content, particularly at high temperatures. Compressibilities of APP-1 and APP-2 gels were greater than those of β-conglycinin ones over the temperature range of 85-100, and 100°C, respectively. β-conglycinin and glycinin interact with each other during heating (17-19), which indicates that the complexes formed between the subunits of glycinin and β-conglycinin upon heating form a more unfracturable three-dimensional network than the β-conglycinin fraction alone when heated above 96°C.

Resiliency of the gels Resiliency decreased with increase of heating temperature (Fig. 5). The glycinin gels exhibited smaller resiliency than the β-conglycinin gels. This indicates that the β-conglycinin gels are more elastic than the glycinin gels. During thermal gelation, the glycinin undergoes sulfhydryl-disulfide interchange reaction, leading to the formation of very high molecular weight complexes (20). Though this disulfide-induced polymerization may lead to increase in gel hardness,it may also lower the elasticity of glycinin gels. Resiliencies of APP-2 gels were larger than the other gels when heated below 93°C. During heating both β-conglycinin and glycinin undergo dissociation and

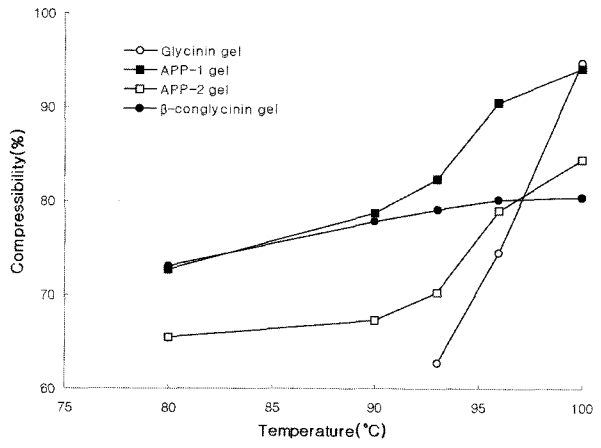

Fig. 4. Effect of heating temperature on the compressibility of soy protein gels. Symbols denotes ● β-conglycinin; □ , APP-2; ■, APP-1; ○ , glycinin, respectively. The gels were prepared from 19 % protein solutions.

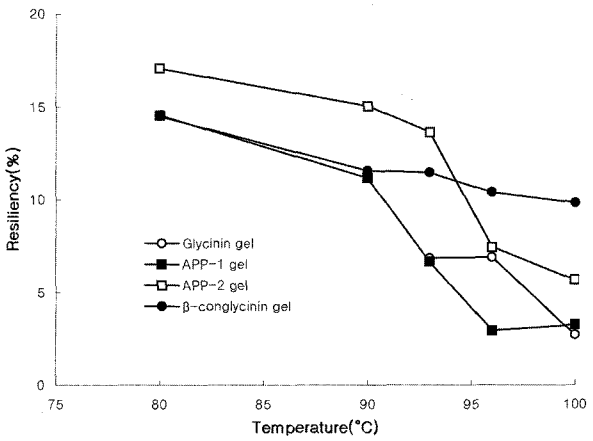

Fig. 5. Effect of heating temperature on the resiliency of soy protein gels. Symbols denotes ● β-conglycinin; □ , APP-2; ■, APP-1; ○ , glycinin, respectively. The gels were prepared from 19 % protein solutions.

denaturation, and the dissociated basic subunits of glycinin interact with the subunits of β-conglycinin, forming soluble complexes (18). This may be the reason for the observed higher gel elasticity of mixed soy protein solution.

In conclusion, main factors or conditions significantly affecting the mechanical properties of heat-induced gels of soy proteins were suggested based on the results of this study. In particular, glycinin contributes significantly to the increases in hardness and toughness of soy protein gel, and β-conglycinin has a large effect on elasticity of soy protein gel at heating temperature close to 100°C. Therefore, the texture of soy protein gels can be controlled by regulating the ratio of glycinin to β-conglycinin, as well as heating temperature.

Acknowledgments

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