Functionality of Dialyzed Soybean Extract

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ABSTRACT: The soybean extract that was dialyzed against distilled and deionized water and adjusted to pH 7.5 (dialyzed soybean extract; d-SE) gave a transparent liquid after heating. The heated d-SE had less beany flavor and formed a translucent gel by heating again with 0.2 M NaCl. Gelation occurred at a lower protein concentration with NaCl than that of the original soybean extract. The dynamic viscoelasticity showed that the translucent gel from the heated d-SE was melted and gelled by the repetition of heating and cooling, indicating reversible gel-sol transition. Cryoprecipitation could be avoided by the heating of d-SE. A gel was formed at 30°C by incubation of d-SE with NaCl. These properties of soybean extract may offer new applications as a food ingredient.

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KEY WORDS: Novel functionality of soybean extract, reversible gel, soybean, soybean extract.

Soybean protein is widely used as a food ingredient because of its versatile functional properties. The gel-forming properties of soybean protein have been extensively investigated, as have the emulsification, adhesiveness, and other properties (1). However, these properties and characteristics of soybean protein are not always desirable. For instance, heat-induced soybean protein gel is heat-irreversible and turbid. But if the gel from soybean protein were thermo-reversible and/or transparent in the manner of gelatin or agar gel, it could offer new applications as a food ingredient. As another undesirable example, the cryoprecipitation of soybean protein, in some cases, makes it difficult to store soybean protein at low temperature in soluble form.

Protein solution forms a turbid suspension or gel by heating. Some protein solutions, however, have been reported to form transparent solutions or sols after heating, even at high protein concentration, provided that low ionic strength and/or pH far from the isoelectric point of the protein are adopted (2). This means that the protein molecules heated under these conditions may form soluble aggregates. It is known that the soluble aggregates prepared by heating of protein solution create a gel network by addition of salt or adjustment of pH even at room temperature or lower (2). Such gels are elastic and less turbid. Soluble aggregate of protein has interesting characteristics as a potential food ingredient. These phenomena were observed not only in some albumin proteins (e.g., egg albumin and bovine serum albumin) but also in some globulins (e.g., β-lactoglobulin in milk whey proteins) (3).

The main fraction of soybean proteins is classified as globulin that can be solubilized in a salt solution. A buffer at neutral pH with high ionic strength or a salt solution has been used to solubilize the soybean protein from soybean flakes or powder. However, it is necessary to reconsider the definition of globulin, since some globulins are soluble in distilled water. The solubility strongly depends on the pH and other conditions. In this study, first, we attempted to determine whether precipitate forms when soybean extract (SE) is dialyzed against distilled and deionized water. Generally, the precipitation of soybean protein is promoted by low temperatures, that is, under cryoprecipitation. Second, if soybean protein can be solubilized in distilled water, it may be possible to prepare a soluble form of heat-denatured soybean protein by heating under a salt-free condition. This type of soybean protein may have novel applications as in the case of other proteins (2).

In this study, SE rather than soybean protein isolate was used, since the major component of SE is protein and the extract was more favorable for practical application. The objective of this study was to clarify whether SE-containing globulins are soluble in distilled water and whether the SE heated under the salt-free condition is soluble and whether it has different properties from those of SE.

MATERIALS AND METHODS

Preparation of dialyzed SE (d-SE) from defatted soybean flakes. Defatted soybean flakes, prepared by low-temperature extraction in order not to denature the soybean protein, were the kind gift of Fuji Oil Co., Ltd. (Osaka, Japan). To 20 g of defatted soybean flakes, 140 mL of distilled water was added. The suspension was kept for 1 h with stirring and centrifuged for 10 min at 2,500 × *g* to obtain the SE at room temperature. The pH of SE was adjusted to 7.5 by addition of 2 N NaOH. Then, the SE was dialyzed against distilled and deionized water that was changed every 3 h and three times; on each change, the pH of the dialysate was adjusted to 7.5, if necessary. The resulting dialysate was centrifuged at 10,000 × *g* for 10 min at room temperature. The pH of the dialysate was again confirmed to be 7.5, and pH was adjusted to 7.5 if it was shifted, and the dialysate was lyophilized and kept at –20°C

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until use. Distilled water was added to the lyophilized powder to dissolve it prior to use in the experiments. The obtained solution is the SE dialyzed against distilled and deionized water (d-SE). The pH of d-SE is 7.5.

Heat treatment of the d-SE and preparation of gel by the second heating with salt. The d-SE was heated at 80 or 90°C for 1 h. This heated d-SE was heated again at 75°C in the presence of 0.2 M NaCl, then cooled to obtain the gel. The protein concentration was measured based on the absorbance at 280 nm using $E^{1\%}_{1\,cm} = 10$.

Preparation of soybean protein isolate (SPI). The pH of the SE was adjusted to 4.5 by addition of HCl. The precipitate was collected by centrifugation, dissolved in distilled water, and neutralized with NaOH. This procedure was repeated three times to purify the SPI. The final solution was subjected to lyophilization.

Measurement of dynamic viscoelasticity. Viscoelastic properties were monitored at the frequency of 3 Hz by a dynamic rheometer (Rheolographsol, Toyoseiki, Inc., Tokyo, Japan). The sample solution (1.6 mL) was poured into a parallel plate cell and heated at a rate of 2°C/min from 25 to 75°C. An aliquot of liquid paraffin was poured on the sample to prevent evaporation. The storage modulus (G′) and loss modulus (G″) were measured. When heating and cooling had been repeated several times, temperature was increased to 75°C at a rate of 2°C/min and was kept at 75°C for 40 min, and then decreased to 25°C at a rate of 2°C/min. After 40 min at 25°C, the sample was heated to 75°C at a rate of 2°C/min. These steps were repeated successively.

Differential scanning calorimetry (DSC) analysis. Thermal denaturation of the SE was studied by DSC with a model DSC 100 calorimeter (Seiko Instruments Inc., Tokyo, Japan). The sample (50 µL, 75 mg/mL) was placed in a silver pan and heated to 120°C at a rate of 2°C/min.

RESULTS

Preparation of the d-SE. When SE was dialyzed against distilled and deionized water, its transparency could be maintained if its pH was held to 7.5 during dialysis. The beany flavor of SE was significantly reduced after dialysis, being particularly effective with an extraction using seven times weight of distilled water to a weight of flakes (4).

Effects of pH and NaCl concentration on turbidity. The pH and NaCl concentrations of the SPI solution were adjusted before the SPI solutions were heated. Figure 1 shows the change in turbidity for SPI sample by heating at various pH and NaCl concentrations. At pH values from 3 to 5, the samples were turbid with and without heating, because soybean proteins aggregated at the isoelectric points around pH 4.5. After being heated at 90°C for 1 h, protein aggregation was enhanced and the samples became turbid in a broader pH range, particularly with increased NaCl concentrations. For instance, at 100 mM or higher NaCl concentrations, the samples were turbid from pH 2 to 6. Similar findings have been reported for milk whey proteins and ovalbumin (3,5).

Since in the above experiments using SPI the protein concentration was not sufficient to produce a heat-induced gel, the d-SE at a concentration of 75 mg/mL and pH 7.5 was heated to 80 or 90°C. No samples were turbid after heating. To determine the effect of ionic strength on turbidity, d-SE was once heated at 90°C and pH 7.5 and heated again in the presence of 0.2 M NaCl at 75°C. Figure 2 shows the actual samples after the second heating, together with the d-SE heated with 0.2 M NaCl. The d-SE heated at 90°C in advance under low ionic strength and pH 7.5 showed less turbidity than the sample when heated with 0.2 M NaCl, although incorporation of bubbles in the sample made it difficult to find its clearness in Figure 2.

Reversibility of gel-sol transition. Agar and gelatin produce transparent and heat-reversible gels, whereas soybean protein yields a turbid and heat-irreversible gel. It was therefore, important to clarify whether the semitransparent gel prepared with NaCl by the second heating of the d-SE showed thermo-reversibility. First, the G′ and the G″ of the gel samples prepared from d-SE and heated d-SE were measured. The d-SE and the d-SE heated at 80 or 90°C for 1 h were heated again with 0.2 M NaCl. Changes in the G′ and G″ are shown in Figure 3. The temperature profile was as follows: the temperature was kept at 25°C for 10 min, increased from 25 to 75°C at 2°C/min, kept at 75°C for 10 min, and decreased from 75 to 25°C at 2°C/min. Neither the d-SE nor that heated at 80°C showed any increase in either G′ or G″, whereas the d-SE heated at 90°C showed an increase in both G′ and G″ during cooling. This profile is different from those for the usual heat-induced protein gelation. As shown in Figure 3, heating of d-SE at 80°C for 1 h was not adequate to induce enough gelation by the second heating with 0.2 M NaCl under this condition. Both G′ and G″ of the d-SE heated at 90°C with 0.2 M NaCl increased during cooling, suggesting a cold-set gel formation. To clarify the difference in effect between heating of d-SE at 80 and 90°C on the gelling behavior induced by the second heating with 0.2 M NaCl, DSC analysis of both samples after heating was carried out. Figure 4 shows the DSC thermograms of the d-SE, the d-SE heated at 80°C, and the d-SE heated at 90°C. The d-SE gave two endothermic peaks at about 70 and 90°C, corresponding to those of 7*S* globulin and 11*S* globulin, respectively (6). The d-SE heated at 80°C gave only one peak at 90°C, and the d-SE heated at 90°C gave no peak, indicating that soybean protein in the d-SE was not completely denatured by heating at 80°C but was denatured by heating at 90°C. This seems to be the reason why the d-SE heated at 80°C could not induce the gelation by the second heating in the presence of NaCl.

In the trial described in Figure 3, the temperature of the d-SE was maintained at 75°C for 10 min only and was then cooled. This experiment provides no information about the effect of prolonged heating on G′ and G″, nor about whether G′ and G″ decreased in the manner of gelatin or agar, typical cold-set gels, when the samples were heated again. Then, effect of prolonged heating and repetition of heating and cooling on G′ and G″ were examined. First, the d-SE heated at

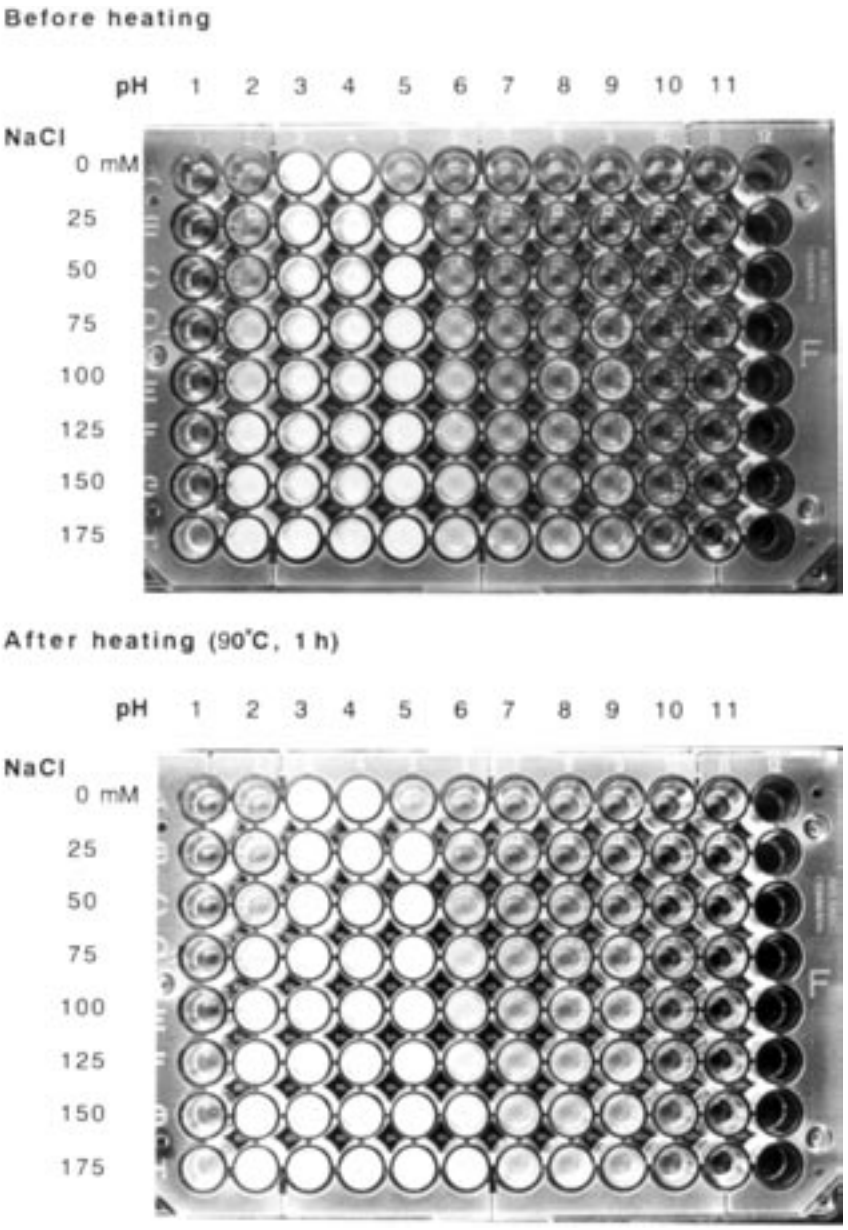

FIG. 1. Soybean protein isolate before and after heating at 90°C for 1 h. Each well contained 250 µL of soybean protein isolate solution (15 mg/mL). Soybean protein isolate was previously dialyzed against distilled water, and then pH, NaCl concentration, and protein concentration were adjusted as indicated.

90°C was kept at 25°C for 40 min with 0.2 M NaCl and then the temperature was increased from 25 to 75°C at 2°C/min. At 75°C the sample was kept for 40 min and was cooled to 25°C at a rate of 2°C/min. At 25°C it was again kept for 40 min and then heated. This step was repeated nine times. As shown in Figure 5, this procedure resulted in profiles similar to that shown in Figure 3. Both G′ and G″ decreased to their earlier low values by heating and increased again by cooling, indicating that the sample was gelled by cooling and then melted by the following heating. This transition was reversible. Both G′ and G″ values at 75°C gradually increased with the repetition of heating and cooling. This indicates that the gel cannot return to the state of the original sample by the repetition of heating and cooling, although the gel formed by cooling was melted by the following heating. Similarly both G′ and G″ values at 25°C also gradually increased with repetition of heating and cooling, meaning that the gel hardened.

As mentioned above, some proteins heated under salt-free and neutral condition form transparent and slightly viscous solutions or sols (7) having properties different from those of the native protein solution. One of the remarkable characteristics of these proteins is that their protein solutions are gelled only by addition of salt without heating, that is, gelled at around room temperature or lower. Here, we examined

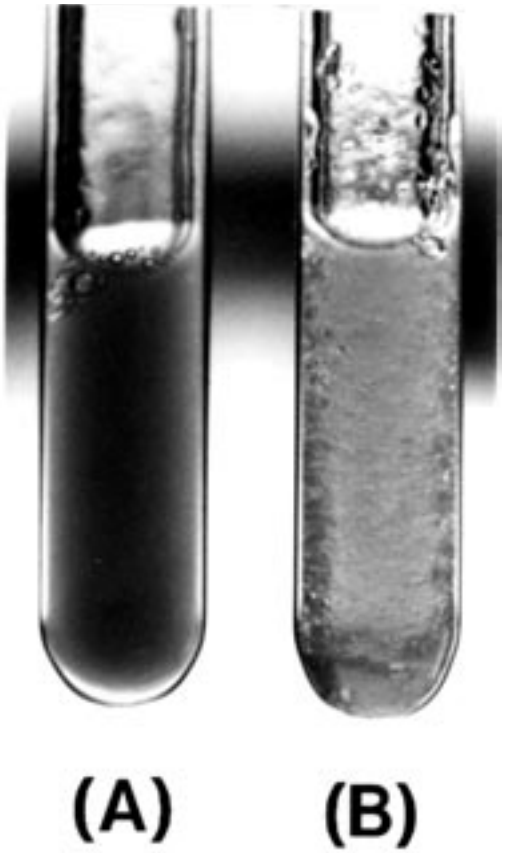

FIG. 2. Effects of the second heating in the presence of NaCl on the transparency of the soybean extract dialyzed against distilled water (A) and dialyzed and heated at 90°C under salt-free condition was heated again (B). Protein concentration, NaCl concentration, and pH were 75 mg/mL, 0.2 M, and 7.5, respectively.

whether the d-SE heated at 90°C also showed such properties. The d-SE (protein concentration, 80 mg/mL), heated at 90°C, was incubated at 30°C in the presence of 0.2 M NaCl. Figure 6 shows the changes in G′ and G″. Although the value of G″ was not changed, G′ gradually increased with the incubation, suggesting that gelation of the heated d-SE occurred only by addition of NaCl at 30°C.

DISCUSSION

The pH of SE tends to decrease during dialysis, which might be due to absorption of carbon dioxide from outside. Keeping pH to 7.5 was a critical condition to avoid the occurrence of precipitate and to obtain the clear liquid after heating. Therefore, pH of the dialysate was adjusted to 7.5 every 3 h, if necessary.

The beany flavor of soybean protein is not easily removed and reduces the applicability of soybean protein as a food material. Soybean protein is usually prepared by precipitation at the isoelectric point (pH 4.5). The SPI prepared by isoelectric precipitation maintains the beany flavor, suggesting that protein-bound flavor compounds cannot be removed by simple washing. As mentioned above, however, the beany flavor of the SE could be removed by dialysis against distilled water. As the amount of aldehydes in the SE remarkably decreased by dialysis (Kitabatake, N., and Y. Fujita, unpublished data), it seems that the beany flavor compounds do not interact strongly with the soybean protein in SE. On the other hand, the aldehydes bound to soybean proteins in SPI solution were not reduced by repetition of isoelectric precipitation (Kitabatake, N., and Y. Fujita, unpublished data), which was measured using

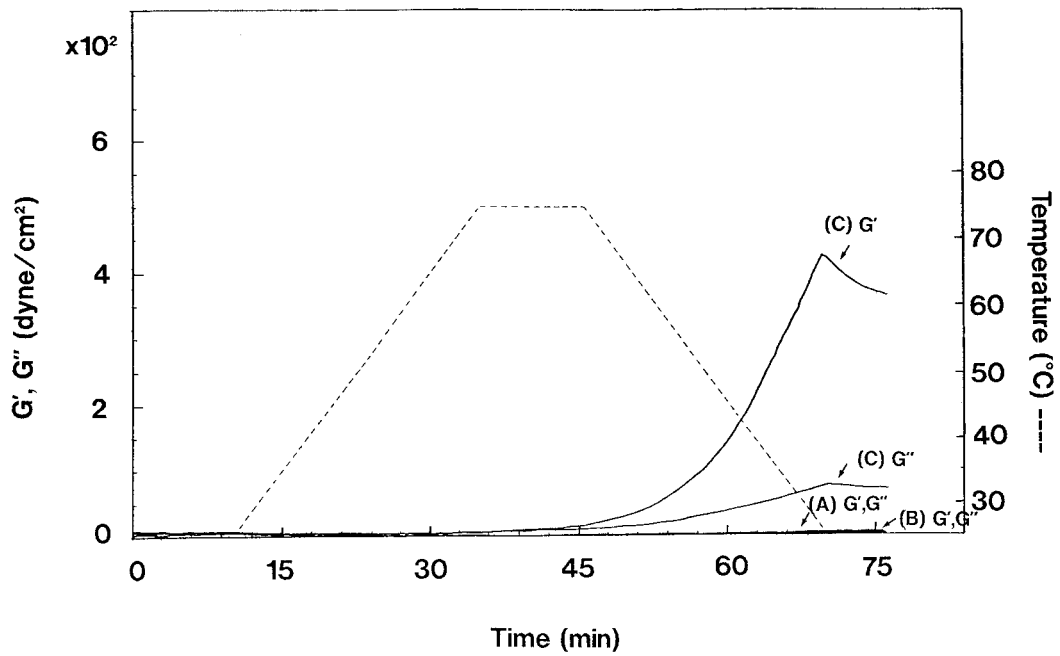

FIG. 3. Changes in storage modulus, G′, and loss modulus, G″, of the dialyzed soybean extract (A), dialyzed and heated at 80°C (B), and dialyzed and heated at 90°C (C). The protein concentration, NaCl concentration, and pH were 75 mg/mL, 0.2 M, and 7.5, respectively.

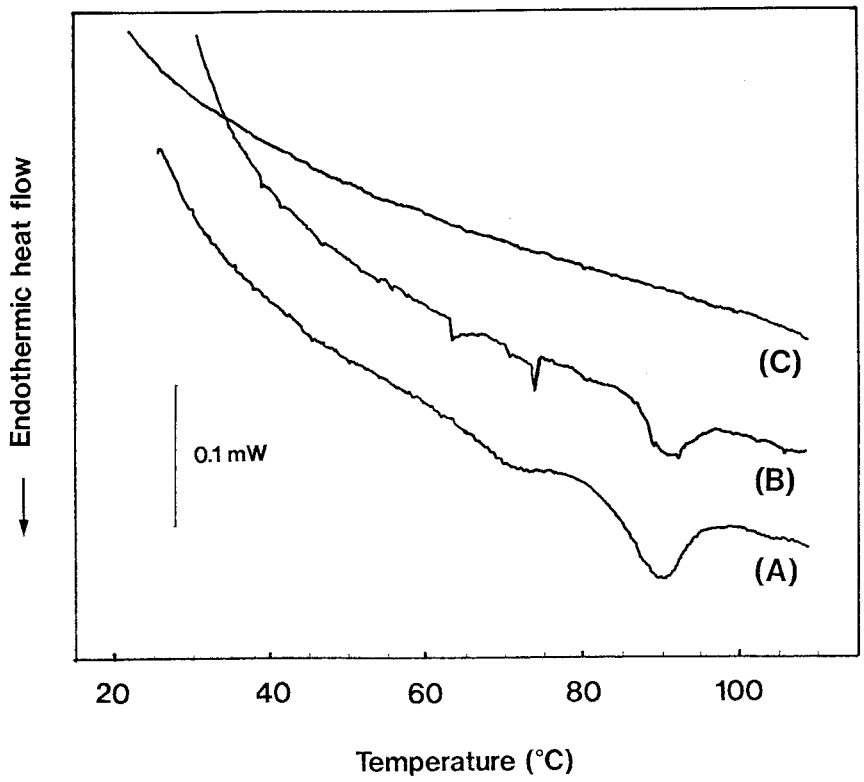

FIG. 4. Differential scanning calorimetry thermogram of the dialyzed soybean extract (A), dialyzed and heated at 80°C (B), and dialyzed and heated at 90°C (C). The protein concentration was 75 mg/mL. Details are shown in the text.

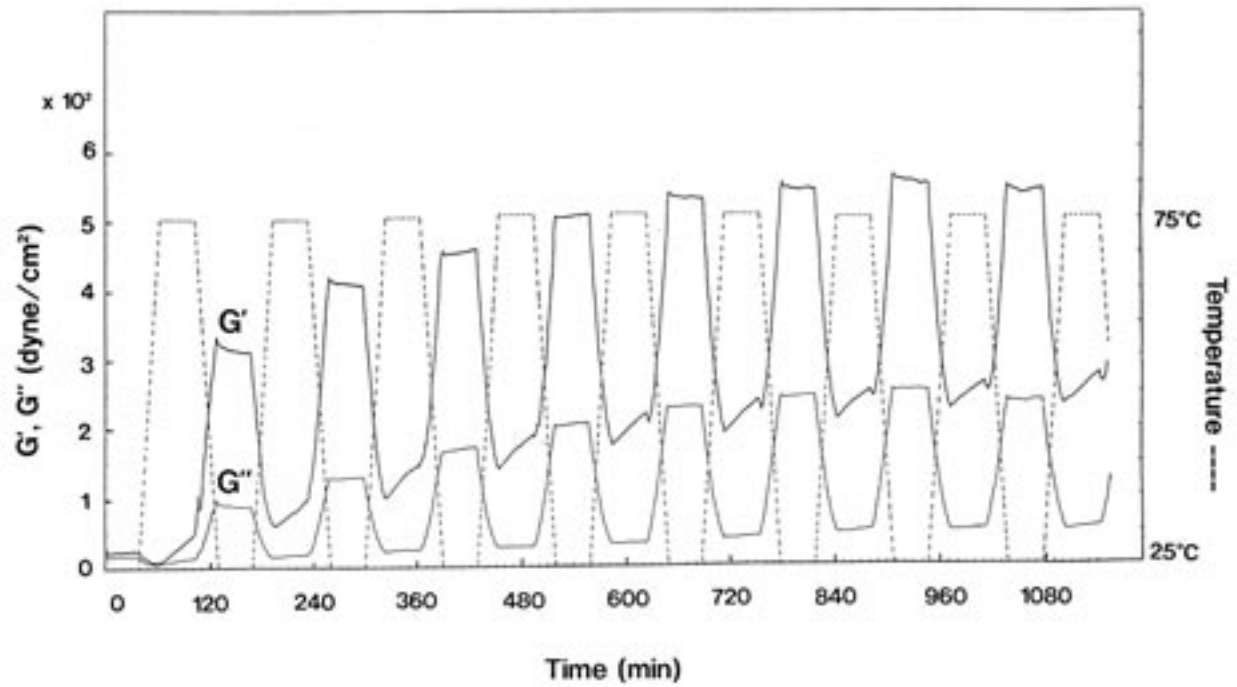

FIG. 5. Changes in storage modulus, G′, and loss modulus, G″, by the repetition of heating up to 75°C and cooling to 25°C, of the dialyzed soybean extract and heated at 90°C. The protein concentration, NaCl concentration, and pH were 75 mg/mL, 0.2 M, and 7.5, respectively. Dotted line is the temperature of the sample.

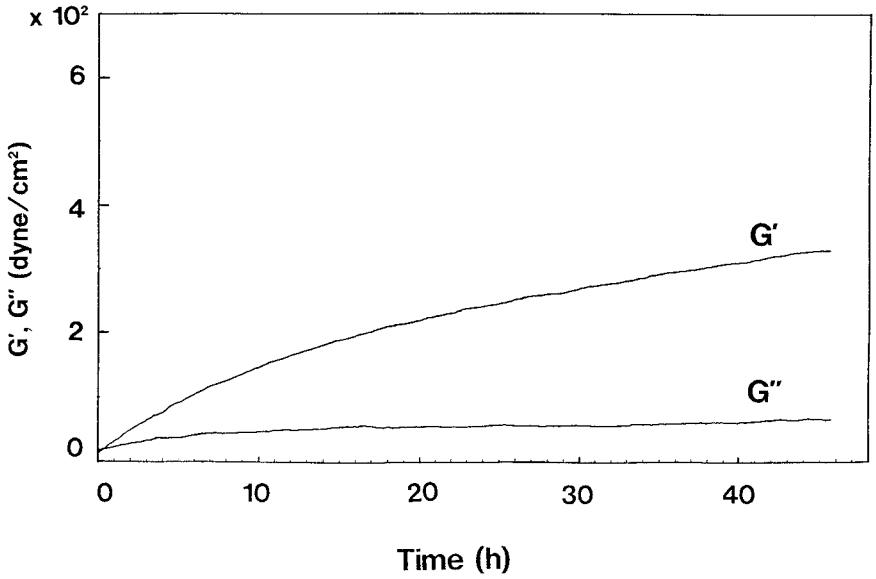

FIG. 6. Changes in storage modulus, G′, and loss modulus, G″, of dialyzed soybean extract and heated at 90°C. The samples were kept at 30°C in the presence of NaCl (0.2 M). The protein concentration and pH were 75 mg/mL and 7.5, respectively.

bovine liver aldehyde dehydrogenase (8). Therefore, it seems that beany flavor compounds in SE, which are primarily medium-chain aliphatic aldehydes, do not interact with soybean protein in SE and tightly adsorb or bind to soybean protein in the course of acid precipitation. And thus the beany flavor seems to be easily removed from SE by dialysis.

Under the conditions examined in this study, the reversibility of the gel to sol was apparent (Fig. 5), whereas at high protein concentration, the formed gel could not return to the sol state. In this case the formed gel was softened by the following heating and hardened by cooling. When the sample was heated a long time or by repeated cycles of heating and cooling, both the G′ and G″ values increased, which might mean that some covalent linkages between protein molecules, such as disulfide bonds, were formed and made the gel firm. Ovalbumin, which usually forms an irreversible gel by heating, shows heat reversibility (gel-sol transition) under specific conditions (9). Repetition of heating and cooling causes covalent linkages among ovalbumin molecules to increase and harden (2,9).

In conclusion, a new type of soybean extract sample has been prepared and might offer new applications of soybean flake extract as a food ingredient.

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