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Effects of Various Reagents on Textural Properties of Soy Protein Gel

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대두단백겔의 물성에 미치는 분자결합력 저해 시약의 영향

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Abstract

The changes in gel characteristics of soy protein as a result of various reagents that alter specific interactions which affect the formation and textural properties of gels, were studied. The reagents were added to 15% soy protein solutions prior to heat treatment. The gels were not formed with urea, indicating that hydrogen bonds significantly contributed to the formation and hardness of soy protein gel. Hydrophobic interactions and disulfide bonds compensated for hydrogen bonds and the contributions of electrostatic interactions to gel hardness are relatively insignificant. The force primarily responsible for gel cohesiveness appeared to be disulfide bonds, because a significant decrease in cohesiveness was found only with the presence of N-ethylmaleimide. Adhesiveness decreased only with the addition of urea, and thus the contribution of hydrogen bonding to adhesiveness of gel could be concluded to be present. However, adhesiveness was suggested to be interpreted not only with molecular forces involved in gel formation but also with hydration properties of protein.

Key words : soy protein, textural properties, protein gelation, molecular forces

Introduction

Soybean proteins are useful functional ingredients in the manufacture of processed foods and traditional Oriental foods. Heat-induced protein gels are of importance for the structure and properties of many food products. The formation of the protein network is considered to be a result from a balance between protein-protein and protein-solvent interactions, and between attractive and repulsion forces between adjacent polypeptide chains(1). Hydrophobic interactions, electrostatic interactions, hydrogen bonding, and disulfide crosslinks are known to provide the attractive forces. Their relative contribution may vary with the nature of the protein, the environmental conditions, and various steps in the gelation process(2). The effects of salts, reducing agents, denaturants and water-miscible solvents on the heat-induced gelation of soybean proteins have been studied to determine the mechanism and the molecular forces involved in the gelation process(3~9). The results also led to the suggestion that stabilization of the three-dimensional network gel structures may involve hydrogen bonding, hydrophobic associations, ionic interaction, and disulfide linkage. Several variables can affect the gelation process of soy proteins.

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According to Kwon and Snyder(10), the presence of reducing agents can possibly inhibit gelation at low concentrations if disulfide cross link between protein molecules is important in the gelation process. It was reported by Schmidt et al.(11) that NaCl(up to 0.5 M) and $CaCl_2$(up to 30 mM) increased gel strength of whey protein and blended systems. Gel strength of whey protein increased with moderate cysteine addition and was maximum at a level of 25 mM cysteine. Mulvihill and Kinsella(12) supported these observations. According to their report, β-lactoglobulin is the principal protein component of whey. Hence, its properties play a major role in determining the gelling properties of whey protein(13, 14). They also reported in another report(15) that the formation and maintenance of β-lactoglobulin gel structure were mainly affected by the contribution of hydrogen bonding and hydrophobic interactions, and disulfide bonds contributed to cohesiveness and elasticity/springiness of β-lactoglobulin gel. The effects of heat treatments on the hydrophobicity of coconut proteins were studied using a fluorescent probe method by Bae et al.(16). 8-anilino-1-naphthalene sulfonic acid and all trans-retinol were used as hydrophobic probes to estimate the aromatic and aliphatic hydrophobicities of coconut proteins. The increases in aromatic and aliphatic hydrophobicities with heat treatments showed similar trends with the increase in protein precipitation, thus verifying that the decreased solubility of coconut protein is partially caused by the increased hydrophobicity, and thus it was suggested that hydrophobic interaction played a important role in the formation of coconut protein gel. They also suggested in another report(17) that electrostatic interactions and hydrophobic interactions contributed to coconut tofu formation and hydrogen bonding contributed to the maintenance of coconut tofu structure.

The objectives of this study were to determine changes in the characteristics of soy protein gel as the results of various reagents (i.e., NaCl, NaSCN, propylene glycol, N-ethylmaleimide(NEM) and urea) that altered specific interactions, affecting the textural properties of soy protein gel.

Materials and Methods

Materials

Soybeans used in this study were purchased locally on an as-needed basis. All chemicals used in this study were of reagent grade.

Soy protein concentrate preparation

The experiments were conducted with soy protein concentrates(SPC) prepared according to the method of Campbell et al.(18). The process started with clean, dehulled and cracked soybeans, which were then flaked and extracted with hexane to remove soybean oil. The defatted soy flakes were then processed through an isoelectric leaching process to produce the SPC product. Ten-fold distilled water was added to the defatted soy flakes and then extraction of soy proteins was carried out using a Waring Blender (Model No. 34BL22) with a slow speed at 35℃ for at least 4 min. The pH of extraction water was adjusted to 9 by addition of 0.5N NaOH solution. After squeezing the soybean milk through cheese cloth with a press, it was centrifuged at 500Xg for 5min to remove more residues. The pH of the supernatant solution was controlled at 4.5 with 0.5N HCl to precipitate soy proteins. Then the precipitates were collected, neutralized and freeze-dried to be utilized in further studies. The protein content of produced SPC was determined by Kjeldahl method (19).

Gel preparation

Gels were prepared by the modified method of Utsumi et al.(20). An aliquot (5㎖) of 15% soy protein solution(w/v) in 30mM Tris-HCl buffer (pH 8.0) was transferred to syringes (inside diameter, 15㎜) which had been sealed at one end with polyvinylidene chloride film. After heated at 95℃ for 30min, the syringes containing 15% soy protein solution were cooled immediately by immersing in cold water, and the gels in the syringes were kept at 4℃ for 20hr with a pressure of 500g/㎠ to ensure complete gelation. The reagents were added to the soy protein solution prior to heat treatment.

Texture profile analysis of gels

The gels formed in the syringes were carefully removed and cut into 10㎜ length, and subjected to a double-compression test for textural properties, after equilibrating in an air-tight container at room temperature for 1hr. The textural properties of gels were measured with the Yamaden Rheometer Model RE-3305 using a pin punch of 0.3㎜ diameter. The moving speed of the pin punch was 5㎜/sec with a chart speed of 50 ㎜/min. The samples were compressed to 75% of their original height. The full-scale load of 5kg was applied.

Results and Discussion

Textural alterations in soybean gels by various reagents that are protein structure destabilizers and stabilizers, and affect gel formation and textural properties should be related to the magnitude of contribution of each molecular force in the texture of the protein gel. Thus, the effects of various reagents on the changes in textural properties of soy protein gels should allow interpretation of the molecular forces contributing to the textural properties of the protein network structure. The protein content of SPC used in this study was 91%.

Effects of NaCl and NaSCN on textural properties of soy protein gel

The effects of NaCl and NaSCN on the texture of soy protein gels are summarized in Figs. 1 and 2. Upon heating at pH 8.0 without addition of NaCl, 15% soy protein solution formed a soft and nonelastic gel, whose hardness was not affected by NaCl concentration up to 0.1M but then progressively decreased up to 0.5M(Fig. 1). In the presence of NaSCN, the gel hardness decreased at the low concentration(0.05M~0.2M) of NaSCN but the value remained relatively unchanged at NaSCN concentrations higher than 0.2M (Fig. 2). Therefore, the effects of NaCl and NaSCN on hardness of gel are attributed to charge neutralization effects; the contribution of electrostatic interactions to the formation and hardness of soy protein gel is present but relatively low. The results were partly in accordance with the thermodynamic finding of Babajimopoulos et al.(1) who concluded that electrostatic interactions were not so important in gelation of 7S globulin. Higher concentrations of NaSCN(≤1M) exert a destabilizing effects on hydrophobic interactions in addition to its charge effect.

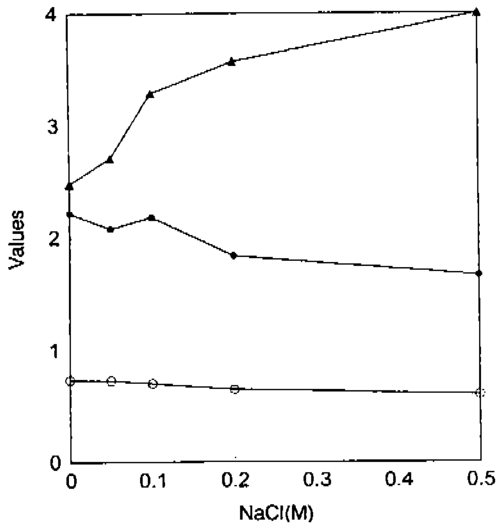

Fig. 1. Effect of NaCl on textural properties of 15% soy protein gel.
- Hardness(10^7dyne/cm^2)
- Cohesiveness
- Adhesiveness(10^6dyne/cm^2)

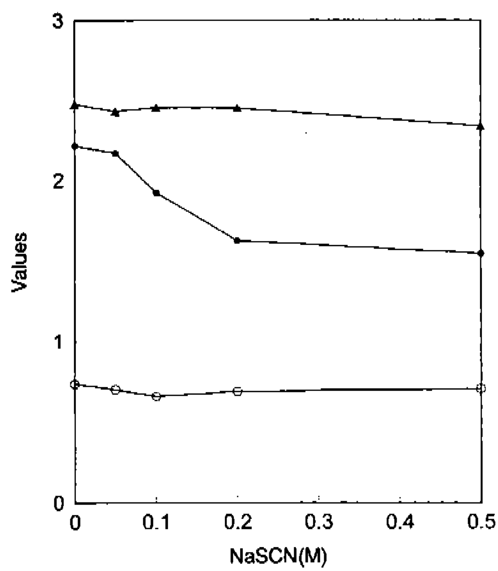

Fig. 2. Effect of NaSCN on textural properties of 15% soy protein gel.
- Hardness(10^7dyne/cm^2)
- Cohesiveness
- Adhesiveness(10^6dyne/cm^2)

The suppressive effects of NaSCN at low concentration were greater than those of NaCl. This suggested that hydrophobic interactions may also be slightly perturbed at this level. NaCl has a stabilizing effect on protein structure(21) and a salting out effect on the basic subunits(22). Utsumi and Kinsella(9) also observed similar effects of NaCl and NaSCN on the hardness of soy isolate gels, except for higher suppressive effect of NaCl than NaSCN. Gel recovery following compression as measured by cohesiveness were not influenced significantly by the addition of either NaCl or NaSCN, suggesting that the contribution of electrostatic interactions to the cohesiveness of soy protein gel was insignificant (Figs. 1 and 2). Gel adhesiveness was not affected by the addition of NaSCN. However, in the presence of NaCl, the gel adhesiveness increased sharply at the low concentration of NaCl (≤0.1M) and the value increased gradually at NaCl concentrations higher than 0.1M. These phenomena also implied that the contribution of electrostatic interactions to the adhesiveness of soy protein gel was insignificant. The increased adhesiveness by the addition of NaCl might be caused by an increase in solubility of SPC and thus increased viscosity of gel. This result was in accordance with the report of Samson et al.(23). The ions of salt might react with the charged groups of proteins and decrease the electrostatic attraction between opposite charges of neighboring molecules. Moreover, the solvate connected with these ions serves to increase the solvation of the proteins and thereby increases their solubility.

Effects of propylene glycol on textural properties of soy protein gel

The effects of propylene glycol on the texture of soy protein gels were similar with those of NaCl and summarized in Fig. 3. The hardness of soy protein gel decreased gradually with the increase in propylene glycol concentration. This result was in accordance with our previous report(17) dealing with coconut tofu, but different from the report of Utsumi and Kinsella(9). Propylene glycol diminishes the hydrophobic contributions, but it enhances the contribution of hydrogen bonds and electrostatic interactions by lowering the dielectric constant. The results in Fig. 3 indicated that the gel became softer with decreased involvement of hydrophobic interaction although the contribution of electrostatic interaction and hydrogen bond were enhanced(4, 24). This means that hydrophobic interaction contributes to the hardness of soy protein gel. However, the addition of propylene glycol had very little effects on the gel adhesiveness and cohesiveness. The small increase in cohesiveness might be resulted from enhanced electrostatic interactions, which was related with the ignorable decrease in cohesiveness by the addition of NaCl in the previous NaCl test (Fig. 1), indicating that very little of these were involved in cohesiveness of the protein network.

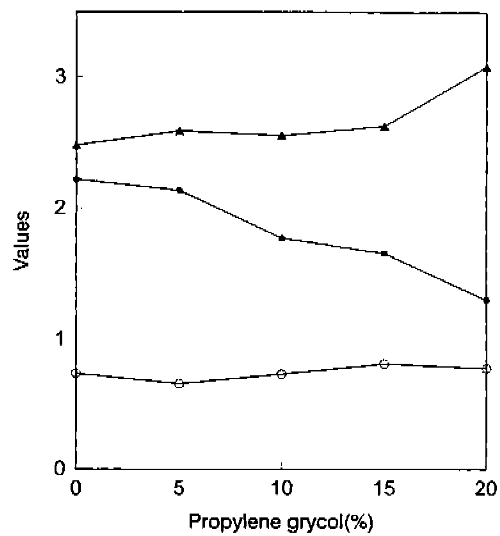

Fig. 3. Effect propylene glycol on textural properties of 15% soy protein gel.
-●- Hardness(10^7dyne/cm^2)
-○- Cohesiveness
-▲- Adhesiveness(10^6dyne/cm^2)

Effects of N-ethylmaleimide on textural properties of soy protein gel

The effects of N-ethylmaleimide(NEM) on the texture of soy protein gels were similar with those of NaCl and summarized in Fig. 4. In the presence of NEM, soy protein formed fragile gels whose hardness decreased until NEM concentration reached to 0.01M, but then increased above 0.02M. Catsimpoolas and Meyer(3) observed similar effects and attributed these effects to cleavage of intermolecular and intramolecular disulfide bonds at low and high concentrations, respectively. The cleavage of intermolecular disulfide bonds resulted in inhibition of gel formation and thus, decreased gel hardness. The cleavage of intramolecular disulfide bonds may facilitate the exposure of a large number of functional groups from the interior of the protein to the aqueous environment. This may facilitate more protein-protein interactions and cause the increase in hardness of gel at high NEM concentration, again. Thus, disulfide bonds influence the hardness of soy protein gel. The cohesiveness decreased at the low concentration($\leq$0.02M) of NEM and remained relatively unchanged at the higher concentration than 0.02M. This phenomenon suggested that intermolecular disulfide bonds were important in imparting cohesiveness of gels. However, the adhesiveness increased sharply at the low concentration($\leq$0.02M) of NEM and increased gradually at the concentrations higher than 0.02M. This phenomenon was also caused by the increased solubility of soy proteins by diminished intermolecular disulfide bonds. Thus, it can be supposed that adhesiveness of soy protein gel is related to disulfide bonds in inverse proportion.

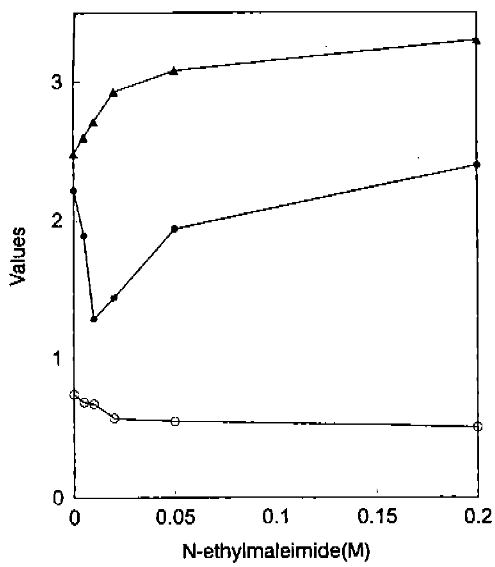

Fig. 4. Effect of N-ethylmaleimide on textural properties of 15% soy protein gel.
-●- Hardness(10^7dyne/cm^2)
-○- Cohesiveness
-▲- Adhesiveness(10^6dyne/cm^2)

Effects of urea on textural properties of soy protein gel

The effects of urea on the texture of soy protein gels were described in Fig. 5. In the presence of urea lower concentration than 2M, the translucent and elastic gels were formed. However, highly viscous sols rather than gels were formed, and thus, textural analysis was almost impossible when the concentration levels of urea were higher than 2M. The hardness and adhesiveness sharply decreased, while cohesiveness increased when the urea concentrations were between 1 and 2M. As urea is an effective denaturant, probably as its concentration increased more extensive unfolding of soy protein occurred upon heating, thus facilitating more extensive disulfide bonding, while progressively decreasing hydrogen bonding and hydrophobic interactions(15). Therefore, it was suggested that the increased cohesiveness of soy protein gel was due to the enhanced disulfide bonding by the addition of urea as discussed previously in NEM test and this result is in accordance with the conclusion of Mulvihill et al.(15). Hardness was decreased more with the addition of urea than that with the addition of propylene glycol, suggesting that hydrogen bonding played important role on formation and hardness of soy protein gel. Adhesiveness was decreased only with an addition of urea, and thus the contribution of hydrogen bonding to adhesiveness of gel was significant. However, adhesiveness was considered to be influenced by protein hydration as well as hydrogen bonds, according to the results of NaCl and propylene glycol tests (Figs. 1 and 3). Cheftel et al.(2) also mentioned that adhesiveness is related to water absorption, swelling and wettability of proteins.

In conclusion, these results suggest that hydrogen bonds are important in the formation and hardness of soy protein gel, hydrophobic interactions and disulfide bonds compensated for hydrogen bonds, and the contributions of electrostatic interactions are relatively insignificant. However, it is unclear whether the contribution of hydrogen bonds alone is sufficient to form the network structure of gel or not. The force primarily responsible for gel cohesiveness appeared to be disulfide bonds, because a significant decrease in cohesiveness was found only with the presence of

NEM. Adhesiveness was decreased only with the addition of urea, and thus the contribution of hydrogen bonding to adhesiveness of gel could be concluded important. However, adhesiveness of gel should most likely be interpreted not only with molecular forces involved in gel formation but also with hydration properties of protein.

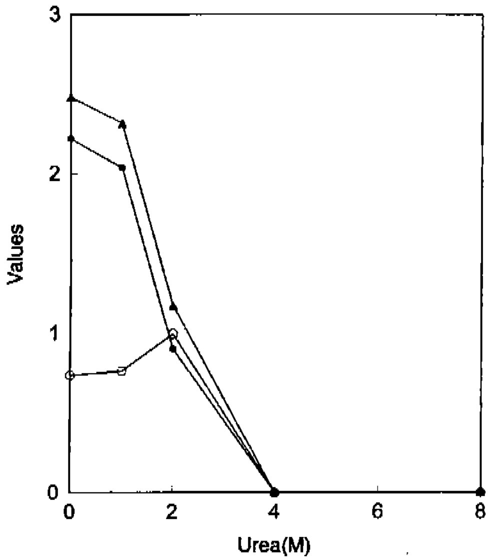

Fig. 5. Effect of urea on textural properties of 15% soy protein gel.
-●- Hardness(10^7dyne/cm^2)
-○- Cohesiveness
-▲- Adhesiveness(10^6dyne/cm^2)

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요 약

분자 결합력에 영향을 미치는 시약들에 의한 대두단백겔의 텍스처 변화를 고찰하였다. 각 시약(NaCl, NaSCN, propylene glycol, NEM, urea)을 15% 대두단백질 용액에 농도별로 첨가하여 열처리로 겔을 형성시킨 후, 텍스처 변화를 고찰하였다. Urea의 존재 하에 겔은 형성되지 않음으로서, 대두단백겔의 형성과 경도에 수소결합이 가장 큰 영향을 미침을 나타내었다. 또한, propylene glycol과 NEM이 대두단백겔의 텍스처에 미치는 영향을 고찰함으로서 소수결합과 황화결합이 대두단백겔의 형성과 경도에 부수적으로 관여함이 밝혀졌으며, 전자결합의 공헌도는 상대적으로 미미하였다. 같은 방법으로 대두단백겔의 응집성과 점착성에 미치는 분자 결합력을 고찰한 결과, NEM을 첨가하였을 때에만 대두단백겔의 응집성이 감소하였으므로 응집성에는 황화결합이 주로 영향을 미치는 것으로 보인다. 또한, urea를 첨가하였을 때에만 대두단백겔의 점착성이 감소한 것으로 보아, 점착성에는 수소결합이 주로 영향을 미치는 것으로 보이나, NaCl 혹은 NaSCN을 첨가하여 단백질간의 전자결합 혹은 소수결합을 저해하였을 때, 오히려 겔의 점착성이 증가한 것으로 보아, 점착성에는 수소결합뿐만이 아니라 단백질의 용해도와 같은 다른 물성도 큰 영향을 미치는 것으로 짐작된다.

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