Effects of Specific Interaction Altering Reagents on Hardnesses of Succinylated Soy Protein Gel

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The changes in gel characteristics of soy protein and succinylated soy protein due to various specific interaction-altering reagents which affect the formation and textural properties of gels, were studied. The reagents were added to 15% soy protein solutions prior to heat treatment. Succinylated soy protein formed harder gel without the addition of reagents. Hardly no gels were formed with urea, indicating that hydrogen bonds significantly contributed to the formation and hardness of the gel and the effects of urea on the hardness of succinylated soy protein gel were more significant. Disulfide bonds were important in the formation of hard gels whether they were succinylated or not, but the contributions of hydrophobic interactions to gel hardness were relatively insignificant. The hardness reducing effects of NaCl and NaSCN were more significant in succinylated soy protein gel. As such, electrostatic interactions were important for succinylated soy protein to form hard gel but not for unmodified soy protein.

Key words : *soy protein, succinylation, protein gelation, molecular forces.*

Heat-induced protein gels are of importance to the structure and properties of many food products. Though traditional animal proteins have better gel forming ability than plant-based proteins, they cannot continue to adequately meet the demands for food protein sources due to their high cost and limited supply. On the other hand, the relatively abundant protein sources derived from plants have poor functional and nutritional qualities. Thus, chemical acylation of amino acid residues with compounds containing functional groups such as succinic anhydride has been suggested as one means of improving functional qualities of plant-based proteins. This chemical modification has been applied to food proteins such as oat concentrate, cottonseed flour, rapeseed, canola, and soybean proteins.[1-5] Succinylated soy protein has a greater emulsion capacity, foaming ability, viscosity, and a lower isoelectric point compared with unmodified protein.[6] However, gelling properties of succinylated soy protein has not yet been studied.

The formation of the protein network is considered to be a result of a balance between protein-protein and protein-solvent interactions and attractive and repulsion forces between adjacent polypeptide chains.[7] 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.[8] 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.[9-15] 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. According to Kwon and Snyder,[16] the presence of reducing agents can inhibit gelation at low concentrations if disulfide cross link between protein molecules is important in the gelation process. Gel strength of whey protein increased with moderate cysteine addition and was maximum at the level of 25 mM cysteine. Mulvihill and Kinsella[17] reported 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 the 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.*[18] 8-Anilino-1-naphthalene sulfonic acid and all trans-retinols 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

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Abbreviation: NEM, N-ethylmaleimide.

the decreased solubility of coconut protein is partially caused by the increased hydrophobicity. It was therefore suggested that hydrophobic interaction played an important role in the formation of coconut protein gel. They also suggested in another report[19] that electrostatic and hydrophobic interactions contributed to coconut tofu formation and hydrogen bonding to the maintenance of coconut tofu structure.

The objectives of this study were to investigate the effects of succinylation on the hardness of soy protein gel and to determine changes in the characteristics of succinylated soy protein gel as a result of the addition of various reagents (i.e., NaCl, NaSCN, propylene glycol, N-ethylmaleimide (NEM) and urea) that alter specific interactions, affecting the textural properties of succinylated 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.*[20] 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 processed through an isoelectric leaching process to produce SPC product. Tenfold distilled water was added to the defatted soy flakes, and the extraction of soy proteins was carried out using a Waring blender (Model No. 34BL22) in slow speed at 35°C for at least 4 min. The pH of extraction water was adjusted to 9 by addition of 0.5 N NaOH solution. After squeezing the soybean milk through cheese cloth with a press, it was centrifuged at 500 × g for 5 min to remove more residues. The pH of the supernatant solution was controlled at 4.5 with 0.5 N HCl to precipitate soy proteins. The precipitates were then collected, neutralized, and freeze-dried to be utilized in further studies. The protein content of produced SPC was determined by Kjeldahl method.[21]

Succinylation of soy protein and determination of succinylation extent. Succinylation was performed by the modified method of Franzen & Kinsella.[22] Previously prepared soy protein isolate (2 g), consisting of 81% protein, was dispersed in 250 m*l* of distilled water, and 0.5 g increments of succinic anhydride were added for a total of 2 g with stirring for 75 min. During succinylation, the pH was maintained at 7~8 with 3.5 M NaOH. After the pH stabilized, the solution was dialyzed with a seamless cellulose dialyzer tubing (a pore diameter of 4.8 mm) against distilled water at 4°C for 44 hrs and freeze-dried for further experiments.

In order to quantify the extent of chemical modification of soy protein, the modified ninhydrin assay[22,23] was used. Ninhydrin solution (1 m*l*) was added to a 1% aqueous protein solution (1 m*l*), and the mixture was heated at 100°C in a boiling water bath for 5 min and cooled immediately to 25°C. Distilled water (5 m*l*) was added, and the absorbance was determined at 580 nm against a distilled water - ninhydrin solution blank. The absorbance indicated the number of free amino acid groups available for reaction with ninhydrin reagent, and the difference in absorbances between unmodified and succinylated proteins reflected the extent of succinylation.

$$Succinylation(\%) = \frac{\text{No.of amino groups}_{unmodified}\text{-No. of amino groups}_{modified}}{\text{No. of amino groups}_{unmodified}} \times 100$$

Gel preparation. Gels were prepared by the modified method of Utsumi *et al.*[24] An aliquot (5 m*l*) of 15% soy protein solution (w/v) in 30 mM Tris-HCl buffer (pH 8.0) was transferred to syringes (inside diameter 15 mm) sealed at one end with polyvinylidene chloride film. After heat treatment at 95°C for 30 min, 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°C for 20 hr with a pressure of 500 g · cm^{-2} to ensure complete gelation. The reagents were added to the soy protein solution prior to heat treatment.

Hardness analysis of gels. The gels formed in the syringes were carefully removed and cut into 10 mm length. They were then subjected to a compression test for hardness, after equilibrating in an air-tight container at room temperature for 1 hr. The hardness 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 mm · sec^{-1} with a chart speed of 50 mm · min^{-1}. The samples were compressed to 75% of their original height. The full-scale load of 5 kg was applied.

Results and Discussion

Hardness alterations in soybean and succinylated soy protein gels by various reagents, which 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 and succinylation extent were 81% and 86.41%, respectively.

Effects of NaCl and NaSCN on hardness of gels. The effects of NaCl and NaSCN on the hardness of soy protein and succinylated soy protein gels are summarized in Figs. 1 and 2. Upon heating at pH 8.0 without addition of NaCl, both soy protein solutions formed a soft and nonelastic gel, whose hardness was progressively decreased with the

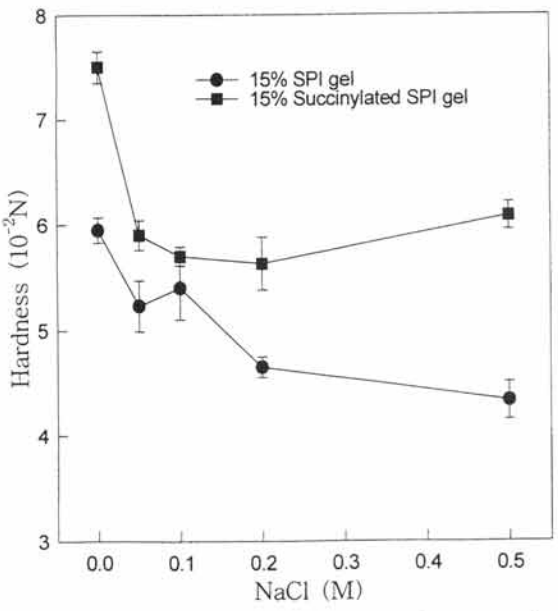

Fig. 1. Effects of NaCl on the hardnesses of soy protein and succinylated soy protein gels. The ions of salt might react with the charged groups of proteins and decrease the electrostatic attraction between opposite charges of neighboring molecules.

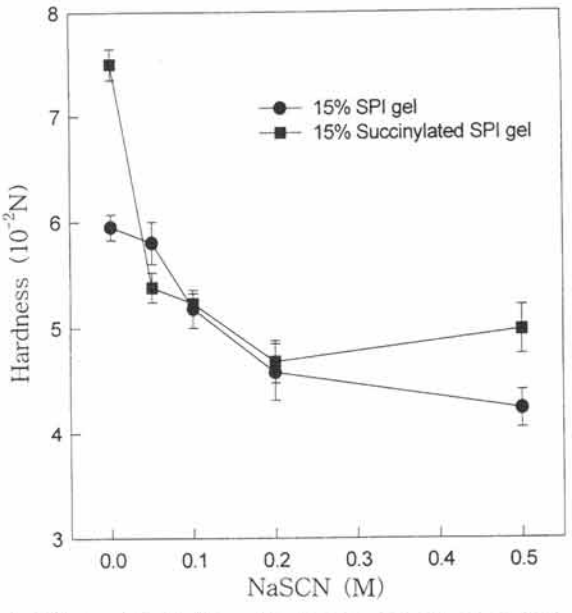

Fig. 2. Effects of NaSCN on the hardnesses of soy protein and succinylated soy protein gels. NaSCN has charge neutralization effect of protein.

presence of 0.05 M NaCl and remained relatively unchanged at higher concentrations of NaCl (Fig. 1). 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, thereby increasing their solubility. At all concentrations of NaCl, succinylated soy protein gels showed higher hardnesses than unmodified ones. The higher hardness of succinylated soy protein gel might be caused by an increase in solubility resulting from succinylation and thus increased viscosity of gel.[6] In the presence of NaSCN, the soy protein gel hardness decreased progressively at low concentrations (0.05 M~0.2 M) of NaSCN. The value remained relatively unchanged at NaSCN concentrations higher than 0.2 M (Fig. 2), while the succinylated soy protein gel hardness decreased remarkably at the NaSCN concentrations between 0 to 0.05 M. 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 lower than that of succinylated soy protein gel. This result, in accordance with the findings of Paulson and Tung,[25] was caused by the large numbers of negative charges supplied by succinylation. The results of unmodified soy protein gel were partly in accordance with the thermodynamic finding of Babajimopoulos *et al.*[14] who concluded that electrostatic interactions were not so important in gelation of 7S globulin. Higher concentrations of NaSCN ($\leq$ 1 M) exert a destabilizing effect on hydrophobic interactions in addition to its charge effect. The suppressive effects of NaSCN on the hardness of succinylated soy protein gel 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[26] and a salting out effect on the basic subunits.[27] On the other hand, the hardness of unmodified soy protein gel decreased gradually at all tested NaCl concentration ranges bewteen 0 to 0.5 M, suggesting that the contribution of hydrophobic interactions to gel formation was more important in soy protein gel than in succinylated soy protein gel.

Effects of propylene glycol on hardness of gels. The effects of propylene glycol on the hardness of gels are summarized in Fig. 3. The hardness of both gels decreased gradually inversely to propylene glycol concentration, and succinylated soy protein gels showed higher hardnesses than unmodified ones at all concentrations of propylene glycol. These results were in accordance with our previous report[19] on coconut tofu, but different from the report of Utsumi and Kinsella.[15] Propylene glycol diminishes the hydrophobic contributions, while it enhances the contribution of hydrogen bonds and electrostatic interactions by lowering the dielectric constant. The results in Fig. 3 indicate that the gel became softer with decreased involvement of hydrophobic interaction although the contribution of electrostatic interaction and hydrogen bond were enhanced,[10,28] implying that hydrophobic interaction contributes to the hardness of soy protein gels regardless whether they were succinylated or not.

Effects of NEM on hardness of gels. The effects of NEM on the hardness of both protein gels were similar with those of NaCl and are summarized in Fig. 4. In the presence of

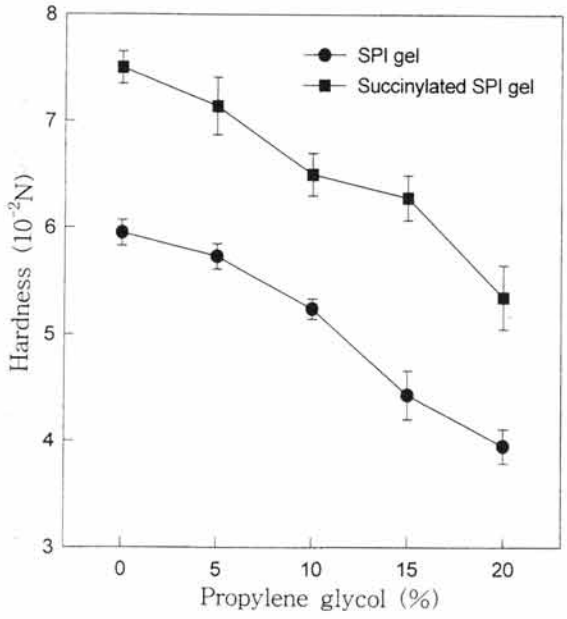

Fig. 3. Effects of propylene glycol on the hardnesses of soy protein and succinylated soy protein gels. Propylene glycol diminishes the hydrophobic contributions, while it enhances the contribution of hydrogen bonds and electrostatic interactions by lowering the dielectric constant.

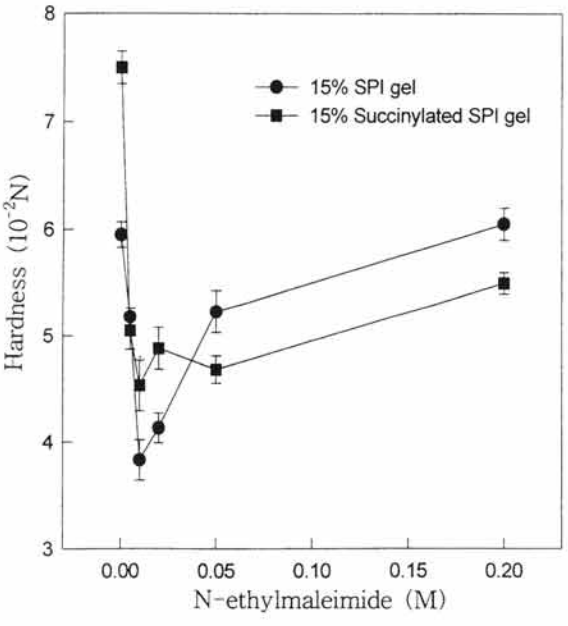

Fig. 4. Effects of N-ethylmaleimide on the hardnesses of soy protein and succinylated soy protein gels. N-ethylmaleimide has cleavage effect of intermolecular and intramolecular disulfide bonds in proteins.

NEM, both proteins formed fragile gels whose hardness decreased until NEM concentration reached 0.01 M then increased above 0.02 M. Thus, disulfide bonds influence the hardness of soy protein gel. Catsimpoolas and Meyer[9] observed similar effects and attributed these effects to cleavage of intermolecular and intramolecular disulfide bonds at low and high concentrations, respectively. The

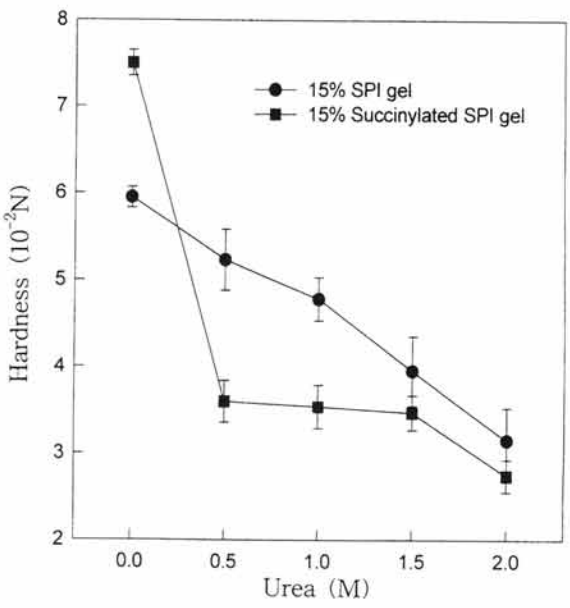

Fig. 5. Effects of urea on the hardnesses of soy protein and succinylated soy protein gels. Urea facilitates disulfide bonding, while progressively decreases hydrogen bonding and hydrophobic interactions.

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 promote more protein-protein interactions and again cause increase in hardness of the gel at high NEM concentration. The recovery of hardness in succinylated soy protein gel was not significant. The hardnesses were lower than those of unmodified ones at higher NEM concentrations than 0.05 M, suggesting that protein-protein interactions following the cleavage of intramolecular disulfide bonds would not occur in succinylated soy protein gel.

Effects of urea on hardness of gels. The effects of urea on the hardness of gels are described in Fig. 5. In the presence of urea, lower concentration than 2 M, 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 2 M. The hardness of succinylated soy protein gel sharply decreased in the presence of 0.5 M urea, while that of unmodified one gradually decreased at all ranges of urea concentration. As urea is an effective denaturant, with the increase in its concentration, more extensive unfolding of soy protein occurred upon heating, thus facilitating disulfide bonding, while progressively decreasing hydrogen bonding and hydrophobic interactions.[17] Hardness decreased more with the addition of urea than that of propylene glycol, suggesting that hydrogen bonding plays an important role on the formation and hardness of soy protein gel and succinylated soy protein gel.

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, while the contributions of electrostatic interactions are relatively insignificant. Hydrogen bonds and electrostatic interactions were of much greater importance in the formation of succinylated soy protein gel because the inhibition of hydrogen bonding and electrostatic interactions resulted in sharply decreased hardnesses of succinylated soy protein gels. However, the higher hardness of succinylated soy protein gel might be accomplished by the contributions of disulfide bond and hydrgen bond, because inhibitions of disulfide bond and hydrgen bond led to the formation of weaker succinylated soy protein gels than unmodified ones.

References

1. Ma, C. Y. (1984) Functional properties of acylated oat protein. *J. Food Sci.* **49**, 1128-1131.
2. Child, E. A. and Park, K. K. (1976) Functional properties of acylated grandless cottonseed flour. *J. Food Sci.* **41**, 713-714.
3. Thompson, L. U. and Cho, Y. S. (1984) Chemical composition and functional properties of acylated low phytate rapeseed protein isolate. *J. Food Sci.* **49**, 1584-1587.
4. Paulson, A. T. and Tung, M. A. (1987) Solubility, hydrophobicity and net charge of succinylated canola protein isolate. *J. Food Sci.* **52**, 1557-1569.
5. Franzen, K. L. and Kinsella, J. E. (1976) Functional properties of succinylated and acetylated soy protein. *J. Agric. Food Chem.* **24**, 788-795.
6. Kinsella, J. E. (1979) Functional Properties of Soy Protein. *J. Am. Oil Chem. Soc.* **56**, 242-258.
7. Hermansson, A. M. (1986) Soy protein gelation. *J. Am. Oil Chem. Soc.* **63**, 658-666.
8. Cheftel, J. C., Cuq, J. L., Lorient D. and Fennema, O. R. (1985) Amino Acids, Peptides and Proteins. In *Food Chemistry*, Fennema, O. R. (2nd ed.) p. 290, Marcel Dekker Inc., New York.
9. Catsimpoolas, N. and Meyer, E. W. (1970) Gelation phenomena of soybean globulins. I. Protein-protein interactions. *Cereal Chem.* **47**, 559-568.
10. Catsimpoolas, N. and Meyer, E. W. (1971) Gelation phenomena of soybean globulins. II. Protein-water-miscible solvent interactions. *Cereal Chem.* **48**, 150-159.
11. Circle, S. J., Meyer, E. W. and Whitney, R. W. (1964) Rheology of soy protein dispersions. Effect of heat and other factors on gelation. *Cereal Chem.* **41**, 157-165.
12. Furukawa, T., Ohta, S. and Yamamoto, A. (1979) Texture-structure relationships in heat-induced soy protein gels. *J. Texture Stud.* **10**, 333-340.
13. Shimada, K. and Matsushita, S. (1980) Gel formation of soybean 7S and 11S proteins. *Agric. Biol. Chem.* **44**, 637-644.
14. Babajimopoulos, M., Damodaran, S., Rizvi, S. S. H. and Kinsella, J. E. (1983) Effects of various anions on the rheological and gelling behavior of soy proteins: Thermodynamic observations. *J. Agric. Food Chem.* **31**, 1270-1276.
15. Utsumi, S. and Kinsella, J. E. (1985) Forces involved in soy protein gelation: Effects of various reagents on the formation, hardness and solubility of heat-induced gels made from 7S, 11S, and soy isolate. *J. Food Sci.* **50**, 1278-1282.
16. Kwon, T. W. and Snyder, H. E. (1987) In *Soybean Utilization*, p. 132, Van Nostrand Reinhold Co. Publ., New York.
17. Mulvihill, D. M., Rector, D. and Kinsella, J. E. (1991) Mercaptoethanol, N-ethylmaleimide, propylene glycol and urea effects on rheological properties of thermally induced β-lactoglobulin gels at alkaline pH. *J. Food Sci.* **56**, 1328-1341.
18. Bae, D. H., Lee, W. Y. and Choi, Y. H. (1995) Estimation of hydrophobicity changes of coconut proteins upon heat treatment. *Foods Biotechnol.* **4**, 189-193.
19. Bae, D. H., Yoon, K. S. and Choi, Y. H. (1997) Effects of various reagents on hardness and formation of coconut tofu. *Foods Biotechnol.* **6**, 34-38.
20. Campbell, M. F., Kraut, C. W. and Yackel, W. C. and Yang, H. S. (1985) Soy protein concentrate. In *New Protein Foods : Seed Storage Proteins*, Altschul, A. A. and Wilcke, H. L. (eds.) p. 38, Academic Press, USA.
21. AOAC (1990) In *Official Methods of Analysis*, (15th ed.) Assoc. Off. Anal. Chem. Washington DC.
22. Franzen, K. L. and Kinsella, J. E. (1976) Functional properties of succinylated and acetylated leaf protein. *J. Agric. Food Chem.* **24**, 914-919.
23. Wanasundara, P. K. and Shahidi, F. (1997) Functional properties of acylated flax protein isolates. *J. Agric. Food Chem.* **45**, 2431-2441.
24. Utsumi, S., Nakamura, T. and Mori, T. (1982) A micro-method for the measurement of gel properties of soybean 11S globulin. *Agric. Biol. Chem.* **46**, 1923-1930.
25. Paulson, A. T. and Tung, M. A. (1988) Rheology and microstructure of succinylated canola protein isolate. *J. Food Sci.* **53**, 821-825.
26. Damodaran, S. and Kinsella, J. E. (1981) The effects of neutral salts on the stability of macromolecules. *J. Biol. Chem.* **256**, 3394-3401.
27. German, B., Damodaran, S. and Kinsella, J. E. (1982) Thermal dissociation and association behavior of soy proteins. *J. Agric. Food Chem.* **30**, 807-813.
28. Tanford, C. (1962) Contribution of hydrophibic interactions to the stability of the globular conformation. *J. Am. Chem. Soc.* **84**, 4240-4247.