Development of new food protein through chemical modification of rice bran proteins

Dongho Bae* and In Sook Jang

Department of Applied Biology and Chemistry, Konkuk University, Seoul 143-701, Korea

Received October 1, 1999

Protein concentrate was produced and succinylated from rice bran to assess and improve its functional properties for the purpose of expanding the uses of rice bran proteins. The most effective solvent for the extraction of rice bran proteins was 20% aqueous ethanol at pH 9. The protein content of rice bran protein concentrate produced was 70.0% and the total protein yield was 64.3%. The extent of succinylation of free amino groups in the modified products was 72.8%. Though the modified protein products showed good functional properties including solubility, emulsion properties, and oil absorption capacity, it did not form gel. Succinylation improved solubility and emulsion and gelling properties. These improvements in functionality will enhance the value of rice bran proteins, thus enabling them to be more competitive with other food proteins.

Key words: *rice bran, protein extraction, succinylation, functional properties.*

At present, large parts of rice brans are being used as animal feeds, although some 800 million people world wide are currently consuming diets inadequate in calories and protein. Considering the large quantities and excellent nutritional qualities which rice bran can provide to human, the present use appears wasteful. A number of papers have been published on the extraction of proteins from both full-fat and defatted rice bran[1-3] and the functional properties of rice bran protein concentrates.[4] However, their direct uses in the food industry have been limited due to poor functional properties on which the transformation of new protein sources into attractive foods depends. It would be beneficial to develop rice bran protein products with good functional properties, which could more easily be incorporated as food supplements. Among various physical and chemical methods used to improve protein functional properties, acylation, including succinylation, is one of the most effective and has been applied to many plant proteins including glandless cottonseed,[5] soybean,[6] leaf,[7] rapeseed,[8] oat,[9] and canola.[10] Succinylation can increase the net negative charges of protein, introduce bulky side groups and change the protein conformation. Hence, it can affect not only protein functionalities but also protein-mineral-phytate interactions.[8] The ε-amino group of lysine is the most readily succinylated group due to its relatively high reactivity and steric availability for reaction.[11] Since reactivity changes with the changes in amino acid profiles, the functional properties of proteins can be improved or impaired in different ways by chemical modifications, depending on the modification methods and natures of proteins used. The purposes of this study were to succinylate the rice bran and to determine the functional properties of the modified protein.

*Corresponding author
Phone: 82-2-450-3756; Fax: 82-2-456-7011
E-mail: donghoya@kkucc.konkuk.ac.kr

Abbreviations: EA, emulsion activity; ES, emulsion stability; FC, foaming capacity; FS, foaming stability; RBPC, rice bran protein concentrate; OA, oil absorption; WA, water adsorption.

Materials and Methods

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

Extractability of rice bran protein. The effects of sodium chloride, ethanol, and sulfuric acid concentrations of 0~1 M, 0~60%, and 0~1%, respectively, in the extraction solvent [rice bran to water ratio of 1:10 (w/v)] on the extractability of rice bran protein were studied to maximize the recovery of rice bran protein into aqueous solution. Extraction of the protein from rice bran was carried out using a Waring Blender (Model 34BL22) with a slow speed at 50°C for at least 4 min. The acidity of extraction was adjusted to 9 (except on the study for H_2SO_4 effects) by addition of 2 N NaOH solution to improve the protein extraction. After squeezing the protein solution through cheese cloth with a press and centrifugation at 4,300 g (Sorvall RC2-B Refrigerated Centrifuge) for 20 min, the supernatant extracts were filtered through filter paper (Whatman No. 1) to remove flocculent materials. The protein content of each supernatant was analyzed, and protein yield of each sample was calculated as follows;

$$Protein\ yield\ (\%) = \frac{\%protein\ in\ solution \times weight\ of\ solution}{\%protein\ in\ rice\ bran \times weight\ of\ rice\ bran}$$

Preparation of succinylated rice bran protein concentrate. Modified rice bran protein was obtained by succinylating rice bran protein concentrate. Modified methods of Bera and Mukherjee[4] were used in which raw rice bran obtained from a commercial rice mill was mixed with a 20% ethanol solution at pH 9.0 and 50 for 30 min. The extracted mass was centrifuged at 1,000 g for 30 min. The protein in the supernatant was then precipitated by adjusting the pH to 4.0 with 2 N HCl and again centrifuged at 1,000 g for 30 min. The solids were recovered and resuspended. Succinic anhydride was added to the protein solution at levels of 0.25 g per g protein, and the pH was maintained at 8.0 by adding 2 N NaOH. The solution was then dialyzed overnight at 4°C against distilled water and freeze-dried.

Chemical analysis. The extent of succinylation was quantified from the free lysine content of the succinylated and unmodified protein samples by the modified ninhydrin assay.[7,12-14] Ninhydrin solution (1m*l*) was added to a 1% protein solution (1m*l*), and the mixture was heated in a boiling water bath for 5 min and cooled immediately to 25°C. Distilled water (5m*l*) was added, and the absorbance was determined at 580 nm against a distilled water-ninhydrin solution blank. The absorbance indicates the number of free amino acid groups available for reaction with ninhydrin reagent, and the difference in absorbances between unmodified and succinylated proteins reflects the extent of succinylation.

$$\text{S uccinylation}(\%) = \frac{No.\ of\ amino\ groups_{unmodified} - No.\ of\ amino\ groups_{modified}}{No.\ of\ amino\ groups_{unmodified}} \times 100$$

Protein contents were determined according to the method of Lowry *et al.*[15] or AOAC[16] using a nitrogen to protein conversion factor of 6.25.

Evaluation of functional properties. The functional properties of succinylated rice bran proteins were compared with those of natural rice bran proteins. Nitrogen solubility was determined according to the method of Chobert *et al.*[17] Protein samples were dispersed in distilled water (0.1% protein, w/v) by mixing with a Vortex. The acidity was adjusted from 1.0 to 12.0 with HCl or NaOH of high normality to limit dilution and was stirred with a magnetic stirrer for 1 h. Samples were centrifuged at 20,000 g for 30 min, and the protein content of the supernatant was determined. The amount of soluble protein was expressed as % of total protein.

Emulsion activities (EA) and emulsion stabilities (ES) of the samples were determined according to the method of Yatsumatsu *et al.*[18] Each isolate (0.7 g) was suspended in distilled water (20 m*l*), and soybean oil (20 m*l*) was added. This mixture was homogenized at a high speed (10,000 g) for 1 min. The emulsion was centrifuged at 2,000 g for 5 min. The emulsifying activity was calculated as the height of emulsified layer divided by the height of total contents in the tube.

$$Emulsifying\ activity\ (\%) = \frac{height\ of\ emulsified\ layer}{height\ of\ whole\ layer\ in\ centrifuge\ tube} \times 100$$

The ES of each sample was measured by recentrifugation after heating at 80°C for 30 min.

Foaming capacity (FC) and stability (FS) were also assessed by the procedure of Yatsumatsu *et al.*[18] One percent protein solution of each sample was prepared and whipped at 12,000 g for 10 min. The height of the foam was recorded using 100 m*l* graduated cylinder as an index of the FC of the protein

$$\text{Foaming capacity} = \frac{\text{ml of volume}}{\text{g of protein}} \times 100$$

The height of the foam recorded after 30 min of standing at 24 indicated FS.

Water adsorption (WA) and oil absorption (OA) were determined according to the methods of Quinn and Paton[19] and Thompson *et al.*,[20] respectively, and expressed as ml of water and oil absorbed/g of protein. For the WA determinations, 0.1 g of the protein concentrate was added to 5 m*l* buffer solution (pH 7). The solution was agitated on a vortex mixer and centrifuged for 15 min at 500 g. After the clear supernatant was discarded, the pellet was weighed. Oil absorption (OA) of protein was measured in the same manner as WA except 5 m*l* of soybean oil was substituted for the 5 m*l* of buffer solution.

Gel forming properties were determined according to the modified method of Utsumi *et al.*[13] An aliquot (5 m*l*) of 10% protein solution (w/v) in buffer (pH adjusted from 1.0 to 12.0 with HCl or NaOH) was transferred to syringes (inside diameter, 15 mm) which had been sealed at one end with polyvinylidene chloride film. After heating at 95 for 30 min, the syringes containing 10% protein solution were cooled immediately by immersing in cold water, and the gels in the syringes were kept at 4°C for 20 h with a pressure of 500 g/cm^2 to ensure complete gelation. Calcium sulfate (0.2 N) was added to the protein solution to enhance the gel formation prior to heat treatment. The curds formed in the syringes were carefully removed and cut into 8 mm length. They were then subjected to a double-compression test for hardness, cohesiveness, and adhesiveness with a 50-kg

reversible load cell, after equilibrating in an air-tight container at room temperature for 1 h. Curd texture was measured with the Instron Universal Test Instrument Model 1011 using a pin punch of 1.2 mm diameter. The moving speed of the pin punch was 20 mm/min with a chart speed of 50 mm/min. The samples were compressed to 75% of their original height. The full-scale load of 5 kg was used.

Statistical analysis. All data were analyzed with the Statistical Analysis System.[21] Analysis of variance using Duncan's multiple range test was conducted to determine the significant differences among samples.

Results and Discussion

Protein extraction and succinylation. The protein content of starting material, rice bran for protein extraction, was 11.2% (wet basis). Efficiency of the extraction was the main yield-determining factor for utilization in food ingredients. Effects of solvent type and concentration were studied to maximize the protein recovery in aqueous form. Sixty-six percent of total protein, a reasonable recovery since according to Betschart *et al.*,[3] about 75% of rice bran proteins were albumin and glutelin, the water soluble and alkali soluble proteins, respectively, was extracted from rice brans into distilled water adjusted to pH 9 without any addition of salt (Fig. 1). The presence of NaCl in the solvent could not improve the extraction of rice bran proteins, although the content of globulin, a salt-soluble protein, was about 20%. Effects of H_2SO_4 concentration in the solvent on protein extraction were studied to increase the protein solubility by cleavage of disulfide bonds in rice bran protein. However, the extractability significantly decreased with the addition of 0.2% H_2SO_4 due to decreased pH caused by the addition of H_2SO_4 (Fig. 1), but remained relatively unchanged with further addition of H_2SO_4. The most efficient solvent for the extraction of proteins from rice bran was 2030% aqeous ethanol. The protein recovery was further improved by the addition of ethanol to the extraction solvent due to solubilized prolamine, an alcohol-soluble protein (Fig. 2). The aqueous ethanol extracted about 98% of the protein from rice bran at pH 9. The extracted protein was precipitated by adjusting the pH to 4.0 with 2 N HCl and centrifuged at 1,000 g for 30 min to recover the rice bran protein. The recovered solids were then resuspended, neutralized, and freeze-dried to produce rice bran protein concentrate (RBPC). The protein content of RBPC was 70.0%, and the total protein yield was 64.3%. About 35% of

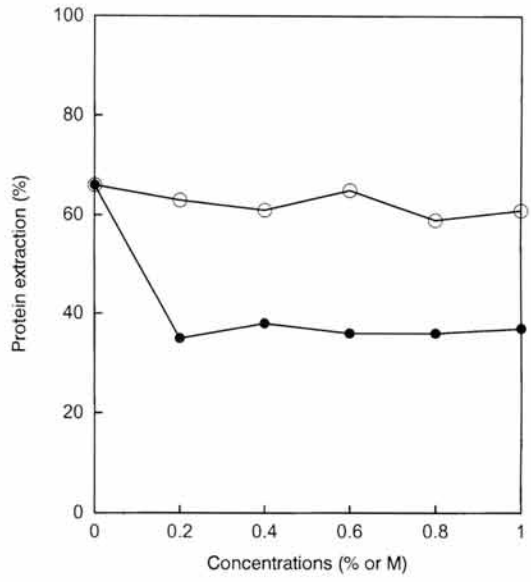

Fig. 2. Effects of H_2SO_4 (●) and NaCl (○) concentrations on extraction of protein from rice bran.

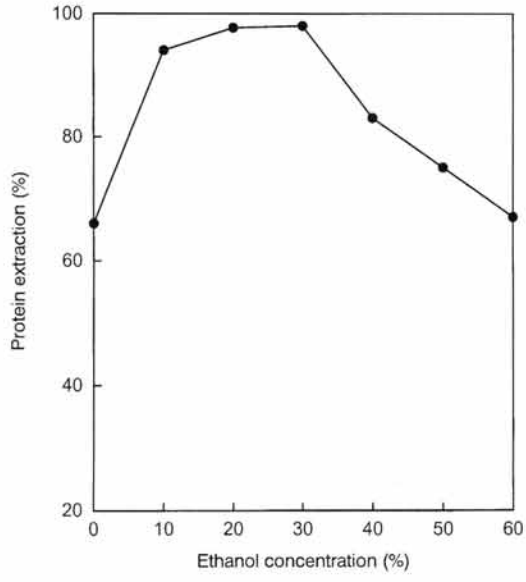

Fig. 1. Effect of ethanol concentration on extraction of protein from rice bran.

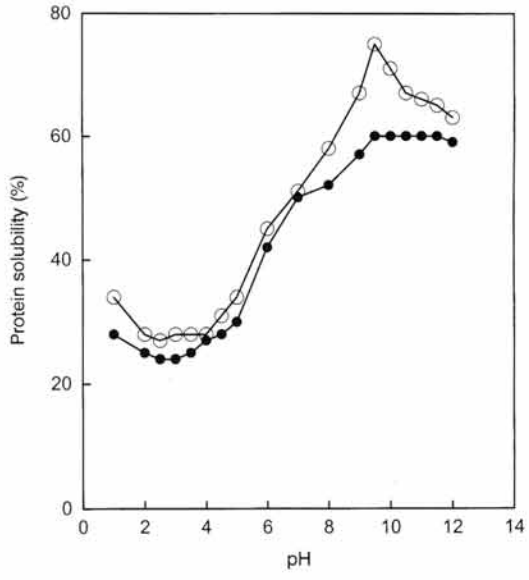

Fig. 3. Nitrogen solubility profiles of unmodified (●) and succinylated (○) rice bran protein concentrates.

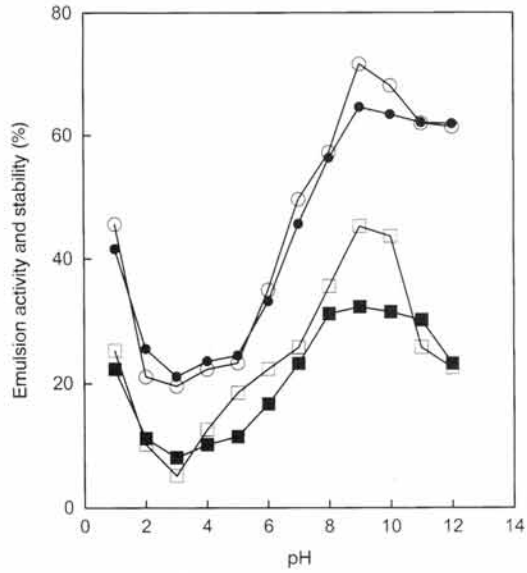

Fig. 4. The pH-emulsion ability and stability profiles of unmodified (emulsion ability, ●; emulsion stability, ■) and succinylated (emulsion ability, ○; emulsion stability, □) rice bran protein concentrates.

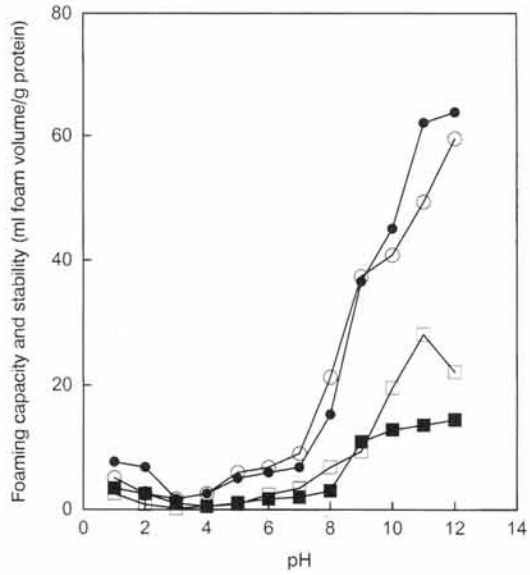

Fig. 5. The pH-foaming capacity and stability profiles of unmodified (foaming capacity, ●; foaming stability, ■) and succinylated (foaming capacity, ○; foaming stability, □) rice bran protein concentrates.

proteins were lost in the rice bran residues during the extraction and the solubles during the precipitation at pH 4.0. The protein concentrate was dissolved in distilled water to produce 5% protein solution, 0.1% (w/w of protein isolate) of succinic anhydride was added, and the pH of the solution was adjusted to 10. After stirring for 3 h for sufficient reaction and dialysis for 24 h, the protein solution was refreeze-dried to produce succinylated rice bran protein concentrate (S-RBPC). The extent of modification of free amino groups was 72.8% for the S-RBPC.

Solubilities. The protein solubility profiles (Fig. 3) of RBPC and S-RBPC showed good water solubilities in the alkaline pH region and minimum solubilities at pHs 3 and 2.5, respectively. In general, at between range of isoelectric point, proteins have a partial negative or positive charge and can thus may enhance solubilities, and succinylation shifted the isoelectric point of proteins approximately 0.5 pH unit due to increasing net negative charges. The profile of RBPC confirmed that protein solubility was maximum at pH>9, which is in accordance with the findings of Betschart *et al.*[3] Succinylation markedly enhanced the solubility of rice bran especially at alkaline pHs and solubilized 75.0% of rice bran protein at pH 9.5. Increased solubility is often observed in succinylated proteins,[6, 10] with greater nitrogen solubility due to increased protein-water interactions as the net negative charges increased upon succinylation.[6]

Emulsion properties. The EA and ES indices of RBPC and S-RBPC are presented in Fig. 4. Succinylation of RBPC increased the EA at pH 8-9, indicating that, while the improvement in EA by succinylation of rice bran proteins may not be significant at acidic and neutral pHs, the increase could be substantial at slightly alkaline pHs. The pH-ES profiles revealed the highest emulsion stabilities for all the protein samples at slightly alkaline pHs (Fig. 4). The increases in emulsion stabilities at neutral and slightly alkaline pHs might have resulted from swelling of protein molecules and consequent increases in viscosities of solutions. At extremely high pHs, protein hydrolysis caused decreases in emulsion stabilities. Nakai[22] has reported that emulsifying property was dependent on not only solubility but also hydrophile-lipophile balance of the particular protein. Thus, acylation of the proteins which increased net negative charges might be expected to unfold the protein structure and expose more hydrophobic groups which then could change the hydrophile-lipophile balance towards a more favorable level. However, Fig. 4 shows that the pH-EA and pH-ES curves resemble the pH-solubility curves (Fig. 1), with minimum EA and ES at pH 3, indicating that the emulsion properties were mainly influenced by protein solubility in this study. The good EAs and ESs of RBPC and S-RBPC suggest possible applications in emulsified foods at alkaline pHs.

Foaming properties. The pH-FC and pH-FS profiles are shown in Fig. 5. The pH-FCs of rice bran samples increased with increases of pHs, which was caused by protein swelling and unfolding. S-RBPC showed slightly lower FC than RBPC at alkaline pHs. Although phosphorylation inceased the solubility of rice bran protein and soluble protein contributes to foaming, an excessive increase in charge may reduce the cohesive interactions between succinylated rice bran proteins and prevent the formation of rigid and elastic film at high pHs. However, succinylation improved the FS of rice bran protein at neutral and alkaline pHs. Once rigid foam formed from S-RBPC,

Table 1. Water and oil absorption capacities of unmodified (RBPC) and succinylated (S-RBPC) rice bran protein concentrates and textural properties of heat-induced gels prepared from unmodified and succinylated rice bran protein concentrates with or without 0.2 N $CaSO_4$ addition.[1)]

Protein isolates	Water absorption (/g)	Oil absorption (/g)	Textural properties			
			Hardness (N)	Adhesiveness (Nm)	Cohesiveness (Nm)	Springiness (mm)
RBPC	1.60[a]	6.56[a]	3)			
S-RBPC	1.57[a]	6.65[a]	1.19(1.61)[2)]	- 0.08(- 0.07)	0.25(0.27)	0.25(0.30)

[1)]Values are means of three determinations.

[2)]Values in parentheses are textural properties of gel prepared with the addition of $CaSO_4$.

[3)]not detected.

[a]The same letters in the same column indicate no significant difference at $p<0.05$ level by Duncan's multiple range test.

further increased viscosity resulting from succinylation decreased the drainage of lamella liquid, hence increased FS.

Water and oil absorption. WA and OA values of RBPC and S-RBPC are compared in Table 1. WA capacity was negligibly decreased by succinylation, which could be attributed to the increases in protein solubilities. It had been reported that highly soluble protein exhibits poor WA.[23)] The WA of oat protein was decreased by acetylation and succinylation.[9)] The OA value of both isolates were much higher than WA. It is mainly attributed to the physical entrapment of oil and the number of nonpolar side chains on proteins that bind hydrocarbon chains on the fatty acids.[24)] The result of this study suggested that rice bran proteins had high surface hydrophobicity, high content of hydrophobic amino acids in rice bran proteins[25)] supporting the suggestion. The effect of succinylation on the OA of RBPC was insignificant.

Gelling properties. Ten percent protein solution prepared with RBPC failed to produce heat-induced gel and remained in sol (Table 1) and weak gels were produced from S-RBPC only at pH 4 both regardless of the addition of $CaSO_4$. Although the increased electrostatic repulsions by succinylation interfered with the gel formation of proteins, the increased solubility of rice bran proteins by succinylation contributed to gelation at their isoelectric pH, at which the electrostatic repulsions between the protein are minimized. The role of pH in protein gelation is extremely complex. Alkali treatment has been suggested as a method to improve gelation by unfolding the soy proteins.[26)] However, since the proteins in the S-RBPC differ considerably in that they are partially denaturated during milling process and/or unfolded by succinylation, they would respond differently to alkali treatment. It is conceivable that the combination of alkali and heating in gelation causes partial hydrolysis and other rearrangements detrimental to gelation. Alkali treatment has also been shown to affect the disulfide linkages of proteins,[27)] which would probably affect the gelation of rice bran protein. To assess textural properties of protein gel (Table 1), the gel prepared from S-RBPC was subjected to texture profile analysis (TPA). The importance of calcium bridging to the gelation of milk protein has been suggested.[28)] Addition of $CaSO_4$ improved the measured gel hardness moderately, which was in accordance with the report of Schmidt *et al.*[29)] on peanut protein gelation.

In conclusion, the present data show that rice brans are effective starting materials for protein concentrate production. Succinylation of amino acids has three major effects on the physical characteristics of proteins; it increases net negative charge, changes conformation, and increases the propensity of proteins to dissociate into subunits. The changes in the physicochemical properties of rice bran protein concentrate by succinylation improve some functional properties, including solubility and emulsion and gelling properties. This improvement in functionality will enhance the value of rice bran proteins enabling them to be more competitive with other widely used food proteins. Moreover, considering their present low end uses, the economic merits of these processes are favorable and attractive.

Acknowledgments. The authors wish to acknowledge the financial support of the Korea Research Foundation made in the program year of 1998.

References

1. Chen, L. and Houston, D. F. (1970) Solubilization and recovery of protein from defatted rice bran. *Cereal Chem.* **47**, 72-79.
2. Connor, M. A., Saunders, R. M. and Kohler, G. O. (1976) Rice bran protein concentrates obtained by wet alkaline extraction. *Cereal Chem.* **53**, 488-496.
3. Bestschart, A. A., Fong, R. Y. and Saunders, R. M. (1977) Rice by-products: Comparative extraction and precipitation of nitrogen from U. S. and Spanish bran and germ. *J. Food Sci.* **42**, 1088-1093.
4. Bera, M. B. and Mukherjee, R. K. (1989) Solubility, Emulsifying and foaming properties of rice bran protein concentrates. *J. Food Sci.* **54**, 142-145.
5. Childs, E. A. and Park, K. (1976) Functional properties of acylated glandless cottonseed flour. *J. Food Sci.* **41**, 713-714.
6. Franzen, K. L. and Kinsella, J. E. (1976) Functional properties of succinylated and acetylated soy protein concentrate. *J. Agric. Food Chem.* **24**, 788-795.

7. Franzen, K. L. and Kinsella, J. E. (1976) Functional properties of succinylated and acetylated leaf protein. *J. Agric. Food Chem.* **24**, 914-919.
8. 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.
9. Ma, C. Y. (1984) Functional properties of Acylated oat protein. *J. Food Sci.* **49**, 1128-1131.
10. Paulson, A. T. and Tung, M. A. (1987) Solubility, hydrophobicity and net charge of succinylated canola protein isolate. *J. Food Sci.* **52**, 1557-1569.
11. Schwenke, K. D. (1997) Enzyme and chemical modification of protein. In *Food Proteins and Their Applications*, Damodaran, S. and Paraf, A. (eds.) pp. 393-423, Marcel Dekker, Inc., New York, U.S.A.
12. Wanasundara, P. K. and Shahidi, F. (1997) Functional properties of acylated flax protein isolates. *J. Agric. Food Chem.* **45**, 2431-2441.
13. 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.
14. Paulson, A. T. and Tung, M. A. (1988) Rheology and microstructure of succinylated canola protein isolate. *J. Food Sci.* **53**, 821-825.
15. Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. (1951) Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193**, 265-275.
16. AOAC. (1984) In *Official Methods of Analysis* (14th ed.) Association of Official Analytical Chemists, Washington, DC.
17. Chobert, J. M., Bertrand-Herb, C., and Nicolas, M. G. (1988) Solubility and emulsifying properties of caseins and whey proteins modified enzymatically by trypsin. *J. Agric. Food Chem.* **36**, 883-892.
18. Yatsumatsu, K., Sawada, K., Moritaka, S., Toda, J. and Ishii, K. (1972) Whipping and emulsifying properties of soybean products. *Agr. Biol. Chem.* **36**, 719-727.
19. Quinn, J. R. and Paton, D. (1979) A practical measurement of water hydration capacity of protein materials. *Cereal Chem.* **56**, 38-40.
20. Thompson, L. U., Liu, R. F. K. and Jones, J. D. (1982) Functional properties and food applications of rapeseed protein concentrate. *J. Food Sci.* **47**, 1175-1180.
21. SAS Institute, Inc. (1985) In *SAS Users Guide Statistics*. SAS Institute, Inc., Cary, NC.
22. Nakai, S., Ho, L., Helbig, N., Kato, A. and Tung, M. A. (1980) Relationship between hydrophobicity and emulsifying properties of some plant proteins. *Can. Inst. Food Sci. Technol. J.* **13**, 23-31.
23. Hermansson, A. M. (1973) Determination of functional properties of protein foods. In *Proteins in Human Nutrition*, Porter, J. W. G. and Rolls, B. A. (eds.) pp. 407, Academic Press, London.
24. Al-Kahtani, H. A. and Abou-Arab, A. A. (1993) Comparison of physical, chemical, and functional properties of *Moringa peregrina* (AL-Yassar or AL-Ban) and soybean proteins. *Cereal Chem.* **70**, 619-626.
25. Houston, D. F., Allis, M. E. and Kohler, G. O. (1969) Amino acid composition of rice and by-products. *Cereal Chem.* **46**, 527-537.
26. Kelley, J. J. and Pressey, R. (1966) Studies with soybean protein and fiber formation. *Cereal Chem.* **43**, 195-206.
27. Nashef, A. S., Osuga, D. T., Lee, H. S., Ahmed, A. I., Whitaker, J. R. and Feeney, R. E. (1977) Effects of alkali on proteins: Disulfides and their products. *J. Agric. Food Chem.* **23**, 245-251.
28. Kalab, M. and Emmons, D. B. (1972) Heat-induced milk gels. 5. Some chemical factors influencing the firmness. *J. Dairy Sci.* **55**, 1225-1231.
29. Schmidt, R. H., Illingworth, B. L. and Ahmed, E. M. (1978) Heat-induced gelation of peanut/whey protein blends. *J. Food Sci.* **43**, 613-615.