Conformational Changes and Some Functional Characteristics of Gelatin Esterified with Fatty Acid

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This study investigated the effects of attachment of fatty acid chains to gelatin molecules on their conformation and some functional properties in order to determine the effectiveness of this procedure for improving the emulsifying properties of gelatin. The esterification conferred upon the gelatin molecules a folded configuration resulting in a λ_{max} of fluorescence emission shift to lower wavelengths and decreases in solubility, intrinsic viscosity, and gelling ability. In contrast, surface hydrophobicity increased with extent of esterification, and the esterified derivatives exhibited higher emulsion stabilities. These results indicated that the oligomeric structure and functional attributes of the gelatin were altered with the modification.

Keywords: *Nitrogen solubility; specific viscosity; gelling ability; fluorescence emission spectra; surface hydrophobicity; emulsion stability*

INTRODUCTION

Some foods exist naturally as emulsions; some prepared foods depend strictly on the creation and maintenance of an emulsion for their consistency or structure and flavor. Therefore, emulsion stabilization is a major concern of the food industry and constitutes one of the most important aspects of food science and technology.

To form an emulsion, proteins acting as emulsifying agents spread around the surface of oil droplets as a rigid interfacial film (*1*, *2*). Due to their amphiphilic nature, proteins are absorbed at the interface between oil and water, causing a pronounced reduction of interfacial tension. The ability of proteins to bind lipids is important for such applications as emulsifiers, surfactants, meat replacers, and extenders. The capacity of a protein to stabilize emulsions is intimately related to the interfacial area that can be coated by the protein, which is ultimately dependent on the shape of the protein and a suitable balance between hydrophilic and hydrophobic moieties (*3*, *4*). From this observation, it appears that the attachment of lipophilic functional groups in a reasonable proportion to protein molecules could be an effective tool for improving the capacity of proteins to stabilize emulsions or protein emulsifying functions.

Despite the fact that chemical modification procedures have been intensively investigated by many scientists as a means to modify the conformation, the structure and, consequently, the functional attributes of proteins (*5*−*12*), none of the current techniques (acylation, guanidination, and benzonylation, for example) has been reported to improve effectively their emulsifying properties. However, recent studies conducted by Bucevschi and co-workers (*13*) have demonstrated the possibility of also modifying collagen hydrolysate functionalities through esterification with methanoic, ethanoic, and propionic acids, respectively.

On the basis of their results, one may tentatively believe that the attachment of long carbon chains of fatty acids to proteins could also be achieved through esterification procedures. Therefore, we initiated studies on the attachment to protein molecules of carbon chains of fatty acids through esterification procedures in order to make clear the features of protein esterification and elucidate the changes in the physicochemical properties of the protein following the modification. The kind of protein investigated was gelatin, an animal protein of significant interest in colloid chemistry. Results of our preliminary investigations have indicated that the reaction condition applied was characterized by the fact that, besides the esterification reaction, there was no N−O acyl group formation between amino groups of the gelatin and carboxylic groups of the fatty acid. Factors affecting the esterification reaction were water content, pH value, temperature, reactants ratio, and reaction time. In this paper, we report the conformational and functional behavior of pigskin gelatin esterified with stearic acid under the optimal condition established by the preliminary studies.

MATERIALS AND METHODS

Materials. Pharmaceutical and food grade gelatins manufactured from pigskin according to an alkaline method were purchased from Shanghai Gelatin Factory, Shanghai, China. After their molecular weight distributions have been evaluated by means of gel permeation chromatography, the sample with apparently high purity (narrow molecular weight distribution) and average molecular weight between 80000 and 90000 Da, a range of interest for food and pharmaceutical applications, was selected for our investigations. The column used in the chromatographic study was a thermostat type (1.6 × 100 cm) produced by Shanghai Jinhua Separation Equipment factory. Sephadex G-100, blue dextran (100000 Da), and standard proteins (albumin, 68000 Da; ovalbumin, 43000 Da; chymotrypsinogen, 25000 Da; and cytochrome *c* 12500 Da) were products of Sigma Chemical Co. Stearic acid, of analytical

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grade, produced by the Sea-salt Domestic Chemical Reagent Factory (Zhejiang, China) was provided by the Chemical Deparment of Wuxi University of Light Industry.

Methods. *Modification Procedure.* To prepare the esterified derivatives, powders of gelatin were heat-dispersed in distilled water to a concentration of 5−30% (w/v), and the pH of the sol was adjusted with 6 N HCl to a value of 4.5. After the addition of fatty acid at levels of 0, 0.05, 0.10, 0.20, and 0.40 g/g of protein, the reaction mass was brought to a temperature of 50−90 °C and stirred for 2−8 h. At the end of the reaction, excess fatty acid was removed by recrystallization at a temperature below its melting point. Gelatin sols at high concentrations can easily form gels, so the reaction mass was diluted with distilled water to a concentration of 1−2% soluble matter and the temperature was maintained at 30 °C so that gelation did not occur; however, the non-reacted fatty acid solidifies and separates out (from the medium). Then the recrystallized fatty acid was removed using centrifugation and vacuum filtration consecutively. The filtrate held at 40 °C was then subjected to ultrafiltration on a hollow fiber membrane (5000 Da) to achieve concentration of the product and removal of residual soluble fatty acid. Finally, the product was vacuum-dried, ground, and packed.

The extent of esterification was followed by the titration method reported by Ning (*14*): An aliquot of 1 g was collected from the reaction medium into a 50 mL conical flask for free fatty acid determination. The conical flask containing the aliquot was kept in a water bath at 30 °C to prevent gelation. Then 10 mL of a solution prepared with absolute alcohol and ethyl ether in the proportion 1:2 was added. The mixture was titrated under constant stirring with 0.1 mol KOH solution in the presence of phenolphthalein. The volume of KOH solution read at the end point of the titration was used to calculate free fatty acid content and determine the amount of fatty acid esterified per gram of gelatin expressed as percent of the theoretical esterification capacity of the gelatin. A sample treated in the same manner but without addition of fatty acid to the reaction medium was used as blank.

Nitrogen Solubility. Protein solubility was estimated according to the method of Paulson and Tung (*15*) with slight modification. Native gelatin solution (5%w/v) was prepared by dissolving at 50 °C a 5 g sample in 95 mL of deionized water; the pH must be adjusted to 6.5 with 2 N NaOH or 2 N HCl without altering the protein concentration. The solution was centrifuged at 12000*g* for 30 min, and the supernatant was analyzed for nitrogen content by using the micro-Kjeldahl method (*16*). Similarly, the esterified gelatin derivatives were analyzed for nitrogen content, and their solubilities were expressed as the percent nitrogen solubility of the native gelatin.

Viscosity Measurement. The specific viscosities of the gelatin and its derivatives (0.2% w/v protein in 0.01 M phosphate buffer, pH 7.0) were determined using an Oswald-type viscometer maintained at 40 °C in a water bath. The time for the sample to flow through the capillary tube under gravity was used to calculate specific viscosity (S_v) using the formula given by Kim and Kinsella (*17*): $S_v = t_s/t_0 - 1$, where t_s and t_0 are the flow times for the sample and the buffer solution, respectively.

Gel Strength. A 5% w/v solution was prepared by dissolving at 60 °C a 2.5 g protein sample in 47.5 mL of 0.1 phosphate buffer, pH 6.0. The solution, contained in a 50 mL beaker (4 × 5.5 cm) and covered with laboratory film (Parafilm M, American National Can, Greenwich, CT) to prevent dehydration and formation of a thicker layer at the surface, was cooled to room temperature and stored at 7 °C for 12 h. After equilibration to room temperature for 1 h, gel strengths were measured with type L1000S Lloyd material testing instrument equipped with an iron bar of 13 mm diameter operated at a dropping velocity of 50 mm/min.

Intrinsic Fluorescence Spectra. The intrinsic emission fluorescence spectra of native and esterified gelatins were determined in a Hitachi 650-60 recording fluorescence spectrophotometer. Protein concentration was 0.01% (w/v) in 0.01 M phosphate buffer, pH 7.0. Protein solutions were excited at 280 nm, and the emission intensities were recorded from 285 to 380 nm at a constant slit of 5 nm for both excitation and emission.

Measurement of Surface Hydrophobicity. Protein surface hydrophobicities were determined using the fluorescence probe 1-anilino-8-naphthalene sulfonate (ANS**)** (*15*). Fluorescence intensity (FI) was monitored with the model 650-60 Hitachi fluorescence spectrophotometer operated at a constant excitation and emission slit of 5 nm. Freshly prepared ANS (10 μL of 8.0 mM in 0.01 M phosphate buffer, pH 7.0) was added to 2 mL of buffer solutions containing proteins at concentrations between 0.0005 and 0.02% (w/v); the mixtures were excited at 390 nm, and their emissions were measured at 470 nm. Net fluorescence intensity (NFI) for each dilution was obtained by subtracting the FI without probe from that with probe. The slope of NFI versus percent protein were calculated by the least linear regression and specified as surface hydrophobicity (S_0).

Heat Stability of Emulsion and Emulsifying Stability Index (ESI). These parameters were estimated following the method of Pearce and Kinsella (*18*). Emulsions were prepared by dissolving a 0.3 g sample at 60 °C in 30 mL of distilled water and cooling to room temperature (25 °C). Ten milliliters of pure soybean salad oil (Fortune, produced by East-Ocean Oils and Grains Industries Co. Ltd., Zhangjiagang, Jiangsu, China) was added, and the mixture was homogenized using the Ultra-Turrax T25 type Janke and Kunkel homogenizer operated at 13500 rpm for 2 min. The emulsions were held at 80 °C with gentle stirring. Aliquots (1 mL) of each emulsion were taken periodically for serial dilution with water and SDS solution to give final dilutions of 1000-fold and SDS concentration of 0.1%. The turbidity was monitored at 500 nm by reading the absorbance in a 1 cm path length cuvette on a type 722 spectrophotometer. The plot of absorbance versus heating time characterized the heat stability of emulsions. The ESI was calculated as follows: $\mathrm{ESI} = T(\Delta t/\Delta T)$; $T = 2.303(A/l)$, where A represents the observed absorbance, l the path length of the cuvette, T the turbidity occurring during the time interval Δt, and ΔT the change in turbidity.

Statistical Analysis. All data reported are mean values of at least duplicate analyses on each sample except in the case of fluorescence spectra. Analysis of SD was carried out on the DE data using an fx-3600P Casio scientific calculator produced by Casio Computer Co. Ltd., and significance differences among treatments were determined by the single-factor ANOVA program on Microsoft Windows 95 at the level of 5%.

RESULTS AND DISCUSSION

Conformational and functional changes of gelatin accompanying esterification were estimated by changes in solubility, specific viscosity, gel strength, fluorescence spectra, surface hydrophobicity, and emulsifying properties, specifically heat stability of emulsion and emulsifying stability index.

Solubility characteristics of proteins are among the most important functional properties because many functional performances of proteins strictly depend on their capacity to go into solution initially (*19***)**. Therefore, the elucidation of solubility behavior is of extreme necessity during protein studies for new functional properties or improvement of specific functional attributes. The solubility profiles of native and esterified gelatins as shown in Table 1 indicate that esterification affected the solubility of gelatin, which decreased in a linear manner with extent of esterification. The differences in solubility following esterification were significant ($P < 0.05$), especially after a 31% degree of esterification had been reached. The decrease in nitrogen solubility, on the one hand, could be due to the blocking of some solubility enhancing factors (hydroxyl group, −OH) and, on the other hand, was expected as the result of the introduction of fatty acid chains into

Table 1. Changes in Solubility, Viscosity, Gelling Capacity, and Surface Hydrophobicity of Gelatin with Esterification

extent of esterification (%)	nitrogen solubility index (%)	specific viscosity[a]	gel strength[b] ($N\ cm^{-2}$)	surface hydrophobicity (FI/% protein)
0	100	0.351 ± 0.025	2.07 ± 0.14	71.41 ± 1.32
17.5	95.4 ± 0.41	0.347 ± 0.063	1.78 ± 0.17	78.38 ± 0.55
31.6	91.66 ± 0.79	0.34 ± 0.009	1.71 ± 0.73	80.34 ± 0.95
34.4	90.93 ± 0.37	0.331 ± 0.053	1.54 ± 0.10	82.13 ± 1.25
41.8	89.0 ± 0.32	0.32 ± 0.025	1.49 ± 0.20	83.98 ± 0.79

[a] Specific viscosity of 0.2% w/v protein in 0.01 M phosphate buffer, pH 7.0. [b] Gel strength of 5% w/v protein in 0.01 M phosphate buffer, pH 6.0.

the protein molecules. Alterations in solubility of proteins have been widely discussed in many papers. Paulson and Tung (*15*) found protein solubility to be significantly affected by the linear and quadratic effects of succinylation and pH as well as interaction effects between these variables. Such effects have been observed with succinylated fish, leaf, soy proteins, canola protein isolates, etc. In all cases of improved protein solubility, conformational alteration was associated with the replacement of short-range attractive forces with short-range repulsive forces, which promotes unfolding of protein molecules. In the present study, hydroxyl groups were replaced with aliphatic nonpolar carbon chains that cannot promote protein−water interaction. The decrease observed in solubility following esterification of gelatin should therefore be expected.

The specific viscosity of gelatin decreased slowly with increasing degree of esterification (Table 1). This could be related to a decrease in hydrodynamic volume as a result of the association of fatty acid chains to the protein molecules through reaction with hydroxyl functional groups, which may have altered the net charge and provoked changes in the oligomeric structure of the gelatin. Specific viscosity has also been used to estimate conformational changes of soy glycinin accompanying succinylation. Contrary to the conformational alterations of the estrified gelatin, Kim and Kinsella (*17*) found succinylation to increase hydrodynamic volume and consequently the viscosity of soy glycinin. This does not contradict our observations because succinylation confers upon the protein hydrophilic functional groups, whereas in the present work the functional groups incorporated with the protein were hydrophobic moieties.

The gel strength of gelatin decreased also progressively with increasing degrees of esterification (Table 1). This also reflected decreased hydrodynamic potentials probably due to the progressive substitution of hydroxyl groups of the gelatin molecules by the hydrophobic carbon chains of fatty acid. From the number of suggestions regarding the mechanism of protein gel formation, it is accepted that hydrogen bonding plays an important role in the formation of the highly ramified three-dimensional network (crystallite) that supports the gel (*20*). A decrease in hydroxyl groups may adversely affect the formation of the gel-supporting crystallite and consequently provoke a decrease in gel strength as was observed with the progressive esterification of gelatin. It must be pointed out that the general effect of the esterification on gel strength was significant ($P < 0.05$); however, no significant variation in gelling ability was noted beyond a 33% degree of esterification. This demonstrated that the implication of hydroxyl groups in gel formation is of limited value

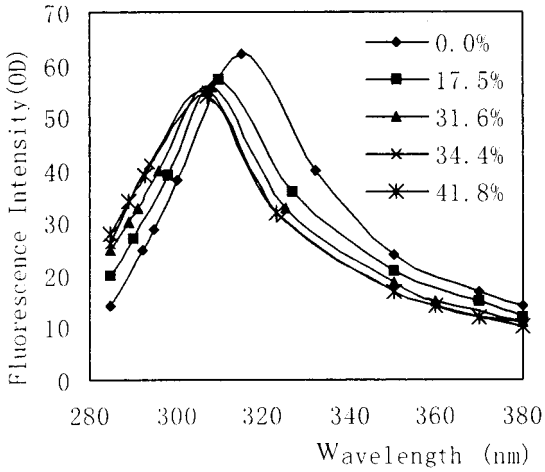

Figure 1. Intrinsic fluorescence spectra of 0.1% native and esterified gelatins in 0.01 M phosphate buffer, pH 7.0.

and revealed the importance of other forces such as hydrophobic and electrostatic interactive forces and/or disulfide cross-links in the building up of the architecture of gels.

The use of fluorescence spectra to evaluate changes in the conformation of proteins has been the central part of many investigations (*17*, *21*, *22*). The intrinsic fluorescence spectra of native and esterified gelatins are shown in Figure 1. It appears that the λ_{max} moved to lower wavelengths with increased extent of esterification. The λ_{max} shifted progressively from 315 to 307 nm, showing a maximum shift of 8 nm. This shift of λ_{max} toward the ultraviolet field indicated that alteration in the oligomeric structure of the gelatin following esterification with fatty acid was associated with the orientation of some interactive domains hiding from the polar environment toward the internal part of the gelatin molecules, conferring upon them folded configurations. Rao and Rao (*21*) observed a λ_{max} fluorescence emission shift of ∼15 nm to longer wavelengths following succinylation of glycinin. Damodaran and Kinsella (*22*) reported a similar shift after succinylation of soy globulins, whereas Kim and Kinsella (*17*) observed a maximum red shift of 5 nm for succinylated soy glycinin. From these observations, it would seem to be consistent that fluorescence emission shifts vary with the conformational features of proteins, which are dependent upon their nature and their net charges.

Surface hydrophobicity has been mentioned as one of the fundamental properties for understanding the basis of protein functionality (*23*), and a number of investigations have established a perfect correlation between this parameter and other functional attributes such as emulsifying properties (*24*−*26*), foaming characteristics (*27*), and ζ potential (*15*). Thus, the treatment effects on surface hydrophobicity were investigated as an important index for evaluating the functional and conformational characteristics of the esterified gelatin. The results are reported in Table 1. Surface hydrophobicity increased only slightly with increased degree of esterification. The attachment of aliphatic carbon chains to gelatin molecules up to 0.350 mmol/g of protein increased only slighly the surface hydrophobicity. Therefore, particular attention has to be paid to the probe used in determining the surface hydrophobicity. It has been reported that protein hydrophobicity may be classified into two types of hydrophobicity, aromatic and aliphatic, as influenced by aromatic and aliphatic

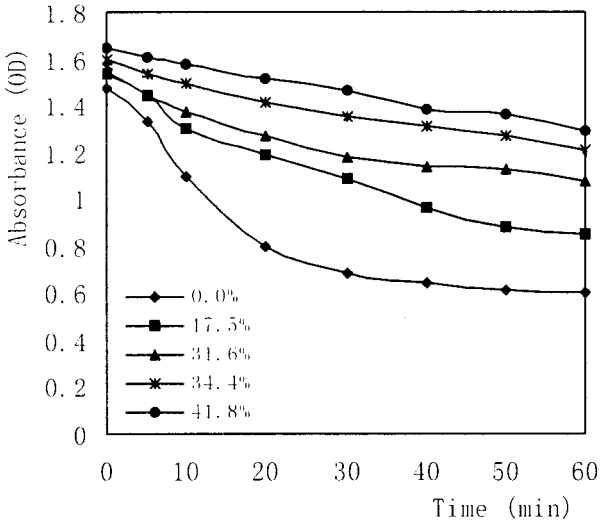

Figure 2. Heat stability of emulsions prepared with native and esterified gelatins.

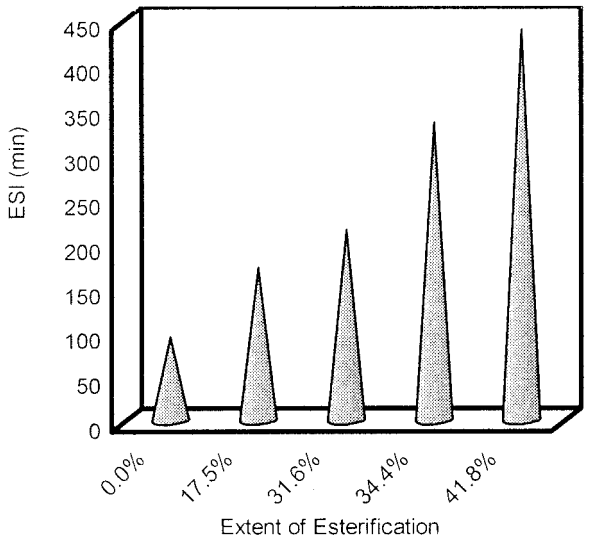

Figure 3. Emulsion stability index of native and modified gelatins.

residues, respectively. ANS, the probe used in this study, is defined as an indicator of aromatic hydrophobicity. The increase in ANS hydrophobicity following esterification could support the results of Hayakawa and Nakai (*28*), who found no difference between the two types of hydrophobicity. In this case, the rate of increase in surface hydrophobicity could be related to changes in gelatin configuration following esterification. In aqueous solution, the charged groups of protein molecules are oriented to the aqueous phase, whereas hydrophobic domains are displayed toward the center of the molecules. Due to the balance between hydrophobic and hydrophilic residues in combination with constraints imposed by amino acid sequence, it is not possible for all of the hydrophobic groups to be buried in the interior of the molecules. Probably, the amount of hydrophobic groups forced to remain on the surface of the gelatin molecules following the esterification increased progressively and contributed to surface hydrophobicity.

Emulsifying properties, commonly discussed in terms of emulsifying capacity, emulsifying stability, and emulsifying activity, are important in many food and pharmaceutical applications of ingredient proteins. Various methods have been made available to measure these indices. The method of centrifuging heated emulsion and expressing the emulsifying stability in terms of the height of the cream layer as a percentage of the initial height of the emulsion is widely used (*19*, *29*). However, the turbidimetric method of Pearce and Kinsella (*18*) was preferred in this investigation because these authors assumed that the emulsifying stability measured with the method of Yasumatsu et al. (*30*) may not be a valid indication of emulsifying stability. The ESI of esterified gelatin was significantly different from that of unmodified gelatin (Figure 3). It increased progressively from <100 min for native gelatin to >400 min for 41% esterified derivatives as a result of emulsion stability enhancement promoted by the hydrophobic carbon chains. The stability effect of the esterification was also materialized by the heat stability profile given in Figure 2. Generally, emulsions are thermodynamically unstable. At the operational level, a stable emulsion is one that under any condition undergoes very slowly the processes leading to separation of oil and water phases. It appeared from our investigation that esterification enhanced the heat stability of emulsions, the turbidity of which exhibited little fluctuation with increasing extent of incorporation of fatty acid chains into the protein molecules.

LITERATURE CITED

(1) Sester, C. S. Water and Food Dispersions. In *Food Theory and Application*; Bowers, J., Ed.; Macmillan Publishing: Riverside, NJ, 1992; pp 7−68.
(2) Friberg, S. *Food Emulsions*; Dekker: New York, 1976.
(3) Pearce, K. N.; Kinsella, J. E. Emulsification Properties of Proteins: Evaluation of Turbidimetric Technique. *J. Agric. Food Chem.* **1978**, *26*, 716−723.
(4) Aoki, E.; Taneyama, O.; Inami, M. Emulsification Properties of Soy Protein: Characteristics of 7S and 11S Proteins. *J. Food Sci.* **1980**, *45*, 534−538.
(5) Beuchat, L. R. Functional and Electrophoretic Characteristics of Succinylated Peanut Flour Protein. *J. Agric. Food Chem.* **1977**, *25*, 258−261.
(6) Choi, Y. R.; Kusas, E. W.; Rhee, K. C. Succinylation of Cottonseed Flour: Effects on the Functional Properties of Protein isolates Prepared from Modified Flour. *J. Food Sci.* **1981**, *46*, 954−955.
(7) Choi, Y. R.; Kusas, E. W.; Rhee, K. C. Effect of Acylation of Defatted Cottonseed Flour with various acid Anhydrides on Protein Isolates. *J. Food Sci.* **1982**, *47*, 1713−1716.
(8) Choi, Y. R.; Kusas, E. W.; Rhee, K. C. Molecular Structure and Functionalities of Protein isolates Prepared from Defatted Cottonseed Flour Succinylated at Various Levels. *J. Food Sci.* **1983**, *48*, 1275−1278.
(9) Shukla, T. P. Chemical Modification of Food Proteins. In *Food Protein Deterioration: Mechanism and Functionality*; Cherry, J. P., Ed.; American Chemical Society: Washington, DC, 1982; p 275.
(10) Johnson, E. A.; Brekke, C. J. Functional Properties of Acylated Pea Protein Isolates. *J. Food Sci.* **1983**, *48*, 722−725.
(11) Kim, S. H.; Kinsella, J. E. Surface Active Properties: Effects of Progressive Succinylation on Film Properties and Foam Stability of Glycinin. *J. Food Sci.* **1987**, *52*, 1341−1343.
(12) Feeney, R. E.; Yamasaki, R. B.; Geoghegan, K. F. Chemical Modification of Food Proteins: An Overview. In *Modification of Proteins; Food, Nutrition and Pharmacological Aspects*; Feeney, R. E., Whitaker, J. R., Eds.; American Chemical Society: Washington, DC, 1982; pp 3−19.

(13) Bucevschi, M. D.; Chirita, G.; Colt, M.; Chirita, M. Chemical Modification of Collagen Hydrolyzates. *J. Am. Leather Chem. Assoc.* **1999**, *94*, 89−95.
(14) Ning, Z.-X. *Handbook of Food Components Analysis*; Light Industry Publishing Society: Beijing, China, 1978; p 184.
(15) Paulson, A. T.; Tung, M. A. Solubility, Hydrophobicity and Net Charge of Succinylated Canola Protein Isolate. *J. Food Sci.* **1987**, *52*, 1557−1561.
(16) Huang, W.-S. General Methods of Food Components Analysis. In *Food Control and Analysis*; Huang, W.-S., Ed.; China Light Industry Publishing Society: Beijing, China, 1997.
(17) Kim, S. H.; Kinsella, J. E. Effects of Progressive Succinylation on Some Molecular Properties Of Soy Glycinin. *Cereal Chem.* **1986**, *63*, 342−345.
(18) Pearce, K. N.; Kinsella, J. E. Emulsifying Properties of Proteins: Evaluation of a Turbidimetric Technique. *J. Agric. Food Chem.* **1978**, *26*, 716−723.
(19) Kim, S. Y.; Park, P. S.-W.; Rhee, K. C. Functional Properties of Proteolytic Enzyme Modified Soy Protein Isolate. *J. Agric. Food Chem.* **1990**, *38*, 651−656.
(20) Ferry, J. D. Protein Gels: Interpretation of gelation as network formation. *Adv. Protein Chem.* **1948**, *4*, 40−47.
(21) Rao, A. G. A.; Rao, M. S. N. Effect of Succinylation on the Oligomeric Structure of Glycinin. *Int. J. Pept. Protein Res.* **1979**, 14307.
(22) Damodaran, S.; Kinsella, J. E. Interaction of Carbonyls with Soy protein: Conformational Effects. *J. Agric. Food Chem.* **1981**, *29*, 1253.
(23) Wu, W. U.; Hettiarachchy, N. S.; Qi, M. Hydrophobicity, Solubility, and Emulsifying Properties of Soy Protein Peptides Prepared by Papain Modification and Ultrafiltration. *J. Am. Oil Chem. Soc.* **1998**, *75*, 845−850.
(24) Nakai, S. Structure Function Relationship of Food Protein with Emphasis on the Importance of protein Hydrophobicity. *J. Agric. Food Chem.* **1983**, *31*, 672−678.
(25) Voutsinas, L. P.; Voutsinas, P.; Cheung, E.; Nakai, S. Relationship of Hydrophobicity to Emulsifying Properties of Heat Denatured proteins. *J. Food Sci.* **1983**, *48*, 26−32.
(26) Petruccelli, S.; Anon, M. C. Relationship Between the Method of Obtention and Structural and Functional Properties of soy Protein isolates. 2. Surface Properties. *J. Agric. Food Chem.* **1994**, *42*, 2170−2176.
(27) Townsend, A.-A.; Nakai, S. Relationships Between Hydrophobicity and Foaming Characteristics of Food Proteins. *J. Food Sci.* **1983**, *48*, 588−594.
(28) Hayakawa, S.; Nakai, S. Relationship of hydrophobicity and Net Charge to the Solubility of Milk and Soy Proteins. *J. Food Sci.* **1985**, *50*, 486-491.
(29) Chobert, J.-M.; Bertrand-Harb, C.; Nicolas, M.-G. Solubility and Emulsifying Properties of Caseins and Whey Proteins Modified enzymatically by Trypsin. *J. Agric. Food Chem.* **1988**, *36*, 883−892.
(30) Yasumatsu, K.; Sawada, K.; Moritaka, S.; Misaki, M.; Toda, J.; Wada, T.; Ishii, K. Whipping and Emulsifying Properties of Soybean Products. *Agric. Biol. Chem.* **1972**, *36*, 719−727.

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