simpoolas et al., 1970), sesame α-globulin (Prakash and Nandi, 1977), mustard 12S protein (Kishore Kumar Murthy and Narasinga Rao, 1984), and arachin (Navin Kumar, 1982). Catsimpoolas et al. (1970) reported that reassociation of glycinin at low pH was due to hydrogen bonding between the uncharged carboxyl groups of unfolded polypeptide chains. Prakash and Nandi (1977) reported that reaggregation of sesame α-globulin was due to hydrophobic bonding since hydrogen bonding does not have significant stability in aqueous solution (Klotz and Franzen, 1962). Hjerten et al. (1974) reported that hydrophobic interaction increases with an increase in ionic strength of the medium while the opposite is true of hydrogen bonding based interactions. At low pH values ionic strength increases, and this could strengthen reaggregation based on hydrophobic bonding.

ABBREVIATIONS USED

Gdn·HCl, guanidinium hydrochloride; SDS, sodium dodecyl sulfate; CGA, chlorogenic acid; PAGE, polyacrylamide gel electrophoresis; CD, circular dichroism.

LITERATURE CITED

Catsimpoolas, N.; Wang, J.; Berg, T. *Int. J. Protein Res.* **1970**, *2*, 277.
Donovan, J. W. In *Physical Principles and Techniques of Protein Chemistry*; Leach, S. J., Ed.; Academic: New York, 1969; Part A, p 101.
Donovan, J. W. *Methods Enzymol.* **1973**, *XXVII*, 497.
Fasman, G. D.; Hoving, H.; Timasheff, S. N. *Biochemistry* **1970**, *9*, 3316.
Flanagan, M. T.; Hesketh, T. R. *Eur. J. Biochem.* **1974**, *44*, 251.
Herskovite, T. T. *Methods Enzymol.* **1967**, *XI*, 748.
Hjerten, S.; Rosengren, J.; Pahlnan, S. *J. Chromatogr.* **1974**, *101*, 281.
Imoto, T.; Andrews, L. J.; Banerjee, S. K.; Sharke, A.; Forester, L. S.; Rupley, J. A. *J. Biol. Chem.* **1975**, *250*, 8275.
Kishore Kumar Murthy, N. V.; Narasinga Rao, M. S. *Int. J. Peptide Protein Res.* **1984**, *23*, 94.
Klotz, I. M.; Franazen, J. S. *J. Am. Chem. Soc.* **1962**, *84*, 3461.
Koshiyama, I. *J. Sci. Food. Agric.* **1972**, *23*, 853.
Kronman, M. J.; Robbins, F. M. In *Fine Structure of Proteins and Nucleic Acids*; Fasman, G. D., Timasheff, S. N., Eds.; Marcel Dekker: New York, 1970; p 271.
Lapanje, S. *Physico-chemical Aspects of Protein Denaturation*; Wiley: New York, 1978.
Navin Kumar, K. D. Ph.D. Dissertation, University of Mysore at Mysore, 1982.
Nozaki, Y.; Tanford, C. *J. Biol. Chem.* **1967**, *242*, 4731.
Prakash, V.; Nandi, P. K. *Int. J. Peptide Protein Res.* **1977**, *9*, 319.
Prakash, V.; Narasinga Rao, M. S. *Proc.—Indian Acad. Sci., Chem. Sci.* **1984**, *93*, 1205.
Prakash, V.; Nandi, P. K.; Jirgensons, B. *Int. J. Peptide Protein Res.* **1980**, *15*, 305.
Rahma, E. H. Ph.D. Dissertation, University of Mysore at Mysore, 1979.
Rahma, E. H.; Narasinga Rao, M. S. *J. Agric. Food Chem.* **1981a**, *29*, 518.
Rahma, E. H.; Narasinga Rao, M. S. *J. Biosci.* **1981b**, *3*, 407.
Sastry, M. C. S. Ph.D. Dissertation, University of Mysore at Mysore, 1984.
Schachman, H. K. *Ultracentrifugation in Biochemistry*; Academic: New York, 1959.
Schultz, G. E.; Shrimer, R. H. In *Principles of Protein Structure*; Springer-Verlag: New York, 1979; p 27.
Schwenke, K. D.; Schultz, M.; Linew, K. J. *Die Nahrung* **1975a**, *19*, 425.
Schwenke, K. D.; Schultz, M.; Linew, K. J. *Die Nahrung* **1975b**, *19*, 817.
Schwenke, K. D.; Sologub, L. P.; Brando, E. E.; Tolstogrzov, W. B. *Die Nahrung* **1978**, *22*, 491.
Schwenke, K. D.; Pahtz, W.; Linew, K. J.; Raab, B.; Schultz, M. *Die Nahrung* **1979**, *23*, 241.
Shifrin, S.; Luborsky, S. W.; Grochowski, B. J. *J. Biol. Chem.* **1971**, *246*, 7708.
Steiner, R. F.; Edelhoch, H. *Nature* (London) **1962**, *193*, 375.
Strickland, E. H. In *CRC Handbook of Biochemistry and Molecular Biology—Proteins*; Fasman, G. D., Ed.; CRC: Boca Raton, FL, 1976; Vol. 3, p 141.
Tanford, C. *Physical Chemistry of Macromolecules*; Wiley: New York, 1961; p 390.
Tanford, C. *Adv. Protein Chem.* **1962**, *17*, 80.
Teale, F. W. J. *Biochem. J.* **1960**, *76*, 881.
Wang, K. P.; Hamlin, L. W. *Biochemistry* **1974**, *13*, 2578.
Wetlaufer, D. B. *Adv. Protein Chem.* **1962**, *17*, 303.
Yang, J. T.; Foster, J. F. *J. Am. Chem. Soc.* **1954**, *76*, 1580.
Yang, J. T.; Doty, P. *J. Am. Chem. Soc.* **1957**, *79*, 761.

Received for review September 15, 1986. Accepted April 13, 1987.

SDS-Catalyzed Deamidation of Oilseed Proteins

Frederick F. Shih* and Agnes D. Kalmar

The effects of sodium dodecyl sulfate (SDS) on the acid hydrolysis of cottonseed and other oilseed proteins have been investigated. Under relatively mild acid (0.2 N HCl) and temperature (70 °C) conditions, small amounts of SDS (0.02–0.06 M) catalyzed the hydrolysis of amide groups (deamidation) in cottonseed protein in preference to the hydrolysis of peptide bonds. High degrees of deamidation could be achieved with minimal peptide bond degradation, and functional properties of the protein such as solubility, water binding, emulsion capacity, and whippability were improved. The SDS-catalyzed deamidation of soybean and peanut proteins has also been investigated.

Oilseeds are a good source of low-cost protein, and there have been efforts to enhance the functional properties of oilseed proteins to make them more suitable for food uses. Enzyme treatments of oilseed proteins have been reported to improve the solubility but sometimes destroy the emulsifying capacity and foam stability (Sekul et al., 1978; Beuchat et al., 1975, Arzu et al., 1972). Chemical modifications normally improve functionality of the oilseed proteins using techniques that include sulfonation (Sair, 1961), succinylation (Franzen and Kinsella, 1976), phos-

Southern Regional Research Center, New Orleans, Louisiana 70179.

This article not subject to U.S. Copyright. Published 1987 by the American Chemical Society

phorylation (Sung et al., 1983), and deamidation (Matsudomi et al., 1981; McDonald and Pence, 1961; Finley, 1975; Matsudomi et al., 1985).

Of these methods, the deamidation approach is particularly attractive because oilseed proteins have a large number of amide groups. Even a 10% deamidation as achieved by treating soy protein with 0.05 N HCl at 100 °C for 30 min could result in significant changes in the functional properties (Matsudomi et al., 1985). However, to be effective for practical uses, deamidation has normally been conducted under rather severe conditions involving temperatures of 100 °C or higher and strong acids such as HCl at concentrations of 0.5 N or higher. Under these conditions, excessive degradation of the protein molecules often occurs, which could lead to undesirable results such as bitter taste and off-flavors. Ideally, mild and effective deamidating conditions should be obtainable by catalysis. One promising catalytic system consists of detergents such as sodium dodecyl sulfate (SDS). According to Steinhardt and Fugitt (1942), protein can be hydrolyzed at the amide and peptide bonds more effectively in the presence of anions with long carbon chains. Catalysis is most effective under conditions of relatively mild acid, low catalyst concentration, and low temperature, and as such, it enhances deamidation more than peptide bond hydrolysis. The SDS–acid combination has the potential of producing protein hydrolysates with optimum deamidation and minimal peptide bond hydrolysis.

This report concerns the SDS-catalyzed deamidation of oilseed protein, particularly the storage protein of cottonseed. We investigated (a) the catalytic effect of SDS and other detergents on the hydrolysis of amide and peptide bonds in cottonseed protein, (b) the changes in protein functionality as a result of deamidation, and (c) the effectiveness of SDS catalysis for the deamidation of different oilseed proteins.

MATERIALS AND METHODS

Materials. Defatted glandless cottonseed flour was obtained from the Food Protein Research and Development Center, Texas A&M University. The storage protein of cottonseed was prepared by 10% NaCl extraction of the flour after water extraction to remove nonstorage proteins, according to Zarins and Cherry (1982). Peanut protein was obtained from the pilot plant at the Southern Regional Research Center, and soy protein (Mira Pro 111), from A. E. Staley Mfg. Co., Galesburg, IL. Sodium dodecyl sulfate (SDS), sodium dodecyl sulfonate, sodium heptanesulfonate, and Triton X-100 were purchased from Sigma Chemical Co., St. Louis, Mo.

Deamidation Procedure. Hydrochloric acid (0.4 N, 200 mL) was added to a suspension of protein (4.0 g) in SDS solution (0.04–0.2 M, 200 mL). The mixture was stirred at 70 °C, and aliquots of supernatant solution were withdrawn at intervals for analysis. At the end of the reaction, the mixture was cooled and adjusted to pH 4.8 by dilute NaOH. The deamidated product was separated as an insoluble residue.

SDS Removal. After centrifugation, the separated insoluble residue was washed to remove SDS first with a mixture of acetone, acetic acid, triethylamine, and water (85:5:5:5) and then with acetone (Henderson et al., 1979). The product was dried under vacuum at room temperature. In our deamidation preparations, the deamidated protein normally consisted of 5–7% residual SDS without the extraction treatment. With the treatment, the residual SDS was found to be 0.02–0.05%, well below the 0.1% SDS allowed in egg white as emulsifier or the 0.5% in gelatin as wetting agent (Code of Federal Regulation, CFR 172.822, 1985). The residual SDS in the deamidated product was determined by the method of Haysushi (1975). The method takes advantage of water-insoluble salt formation between the detergent and methylene blue, which was extracted by chloroform. Absorbance of the chloroform extract was measured at 655 nm.

Determination of Percent Deamidation and Percent Peptide Hydrolysis. To 10.0 mL of supernatent solution from the hydrolysis mixture was added 5.0 mL of trichloroacetic acid (TCA) solution (50%). The precipitate formed was removed by centrifugation, and the solution was analyzed for ammonium ion and nitrogen. Ammonium ions in 10 mL of solution, 10^{-3}–10^{-5} M, were first converted quantitatively to ammonia by the addition of a strong base (1 mL, 10 N NaOH). The amount of ammonia was then determined by an ammonium electrode (HNU System, Inc., Newton Highlands, MA), against a calibration curve that correlates the millivolt readings of the electrode with the ammonia conentrations as generated from base-treated standard ammonium ion solutions. The ammonium nitrogen of the deamidated sample was calculated from the ammonia evolved in the deamidation, and the amide nitrogen refers to the ammonium nitrogen calculated from complete deamidation of the original protein sample. Complete deamdidation was achieved by hydrolysis with 2.0 N HCl at 100 °C for 4 h. Nitrogen in solid samples or in solution was analyzed by the Kjeldahl method. The dissolved nitrogen refers to nitrogen remaining in solution after TCA treatment. Subtraction of ammonium nitrogen from dissolved nitrogen gives non-ammonium nitrogen, which is a measure of peptide hydrolysis. Thus, percent deamidation = (ammonium nitrogen/amide nitrogen of protein) × 100, and percent peptide hydrolysis = (non-ammonium nitrogen/total nitrogen of protein minus amide nitrogen) × 100.

Functionality Analysis. At least duplicate determinations were run on all functionality tests. Water binding was measured at 84% relative humidity (over saturated KCl), according to Mellon et al. (1947). Solubility was measured with 2% (w/v) suspensions of protein in water. The suspensions were adjusted to pH 3.5, 4.8, and 6.5 by dilute HCl or NaOH, stirred for 0.5 h, and filtered (0.45 μm, Millipore). The filtrate was analyzed for nitrogen by the Kjeldahl method. In foam expansion measurement, samples of 1 g of protein in 15 mL of water were blended for 1 min in a Sorvall Omni-mixer operated at speed 5. The foam was transferred immediately to a graduated cylinder for measurement of foam expansion. After the foam was allowed to stand for 30 min, the residual foam volume was measured again for calculation of foam stability (percent foam remaining). Oil-in-water emulsions were studied according to McWatters and Cherry (1977). Peanut oil was delivered slowly from a buret into a suspension of protein in water during emulsion formation. Emulsion capacity was considered to be the point at which a sudden drop in viscosity occurred due to oil–water phase separation. The data were reported as milliliters of oil emulsified/10 mL of 7% protein–water suspension. The viscosity of emulsion was measured continuously with the addition of oil, and the value obtained at the oil volume 10 mL before phase separation was recorded as the emulsion viscosity.

RESULTS AND DISCUSSION

SDS-Catalyzed Deamidation of Cottonseed Protein. To investigate the effects of SDS on acid deamidation of cottonseed protein, the protein was treated with a combination of HCl and different concentrations of SDS. Figure 1 shows the degree of deamidation as a function of

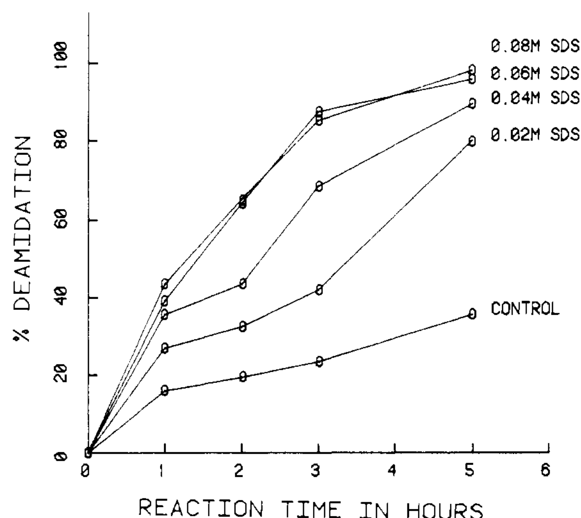

Figure 1. Effects of various concentrations of SDS on the deamidation of cottonseed protein. Samples consisting of 1% protein were treated with 0.2 N HCl and various amounts of SDS at 70 °C.

Table I. Deamidation of Cottonseed Protein under Various Conditions[a]

sample no.	acid (0.2 N)	catalyst (0.04 M)	deamidation, %
1	hydrochloric acid	sodium dodecyl sulfate	43.5
2	sulfuric acid	sodium dodecyl sulfate	45.0
3	phosphoric acid	sodium dodecyl sulfate	25.0
4	acetic acid	sodium dodecyl sulfate	2.8
5	hydrochloric acid	Triton X-100[b]	8.0
6	hydrochloric acid	sodium dodecyl sulfonate	39.0
7	hydrochloric acid	sodium heptanesulfonate	15.0

[a] Protein (1%) was heated at 70 °C for 2 h. [b] 1%.

reaction time at various concentrations of SDS. The degree of deamidation at a given reaction time increased with increased SDS concentration. However, this increase reached a maximum at about 0.06 M SDS; at SDS concentrations higher than 0.06 M, there was no further deamidation increase. Table I shows the effect of different acids and detergents on the deamidation of cottonseed protein. Of the common acids investigated, sulfuric acid is the most effective, followed by, in decreasing order, hydrochloric acid, phosphoric acid, and acetic acid. Detergents other than SDS have also been investigated in the HCl deamidation of cottonseed protein. Sodium dodecyl sulfonate was almost as effective as SDS, whereas sodium heptanesulfonate and Triton X-100 were relatively less effective.

According to Steinhardt and Fugitt (1942), the mechanism of catalysis was described as an increased basicity of the amide bonds as a result of a combination of these bonds with the large anion of the catalyst. The anions of dodecyl sulfate and dodecyl sulfonate, at 12 carbons, seem to be of the proper size and form to effect good catalysis. Our results agree with findings of Steinhardt and Fugitt (1942) who reported that alkyl sulfate and alkyl sulfonate anions of 12 carbons are effective catalysts for protein deamidation whereas those of 14 carbons and higher homologues tend to form aggregates and cause the lowering of hydrogen ions resulting in less effective deamidation.

Catalysis of SDS on Peptide Bond Hydrolysis. SDS catalyzed the hydrolysis of both the pendent amide groups and the peptide linkages. Figure 2 shows the plots of percent peptide bond hydrolysis vs. reaction time at various levels of SDS. More peptide bonds were hydrolyzed

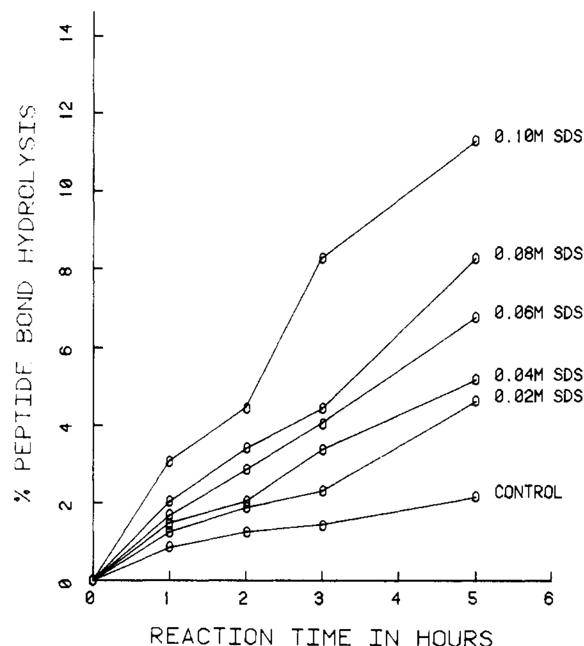

Figure 2. Effects of SDS on the peptide bond hydrolysis of cottonseed protein. Samples consisting of 1% protein were treated with 0.2 N HCl and various amounts of SDS at 70 °C.

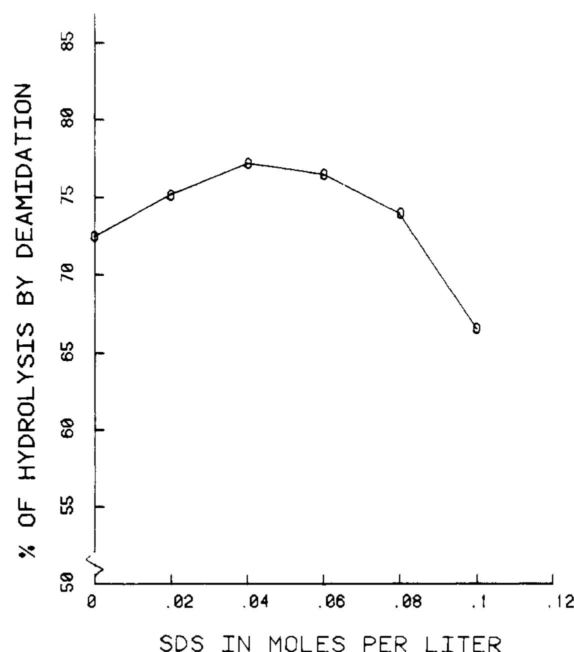

Figure 3. Effects of various concentrations of SDS on percent of hydrolysis by deamidation.

with increasing amounts of SDS in the reaction system, and unlike deamidation, there was no slow down of peptide bond hydrolysis at 0.06 M and higher SDS concentrations. From the data from Figures 1 and 2, the ratio of deamidation to total hydrolysis (deamidation plus peptide hydrolysis) was calculated and the resulting percent of total hydrolysis by deamidation was plotted as a function of SDS concentration (Figure 3). The plot indicates that the catalysis generally favored deamidation over peptide bond hydrolysis at SDS concentrations lower than 0.08 M, and the most desirable concentrations of the catalyst for deamidation ranged from 0.03 to 0.06 M SDS.

Functionality and Deamidation. Selected functional properties of deamidated cottonseed protein are listed in Table II. The peptide bond hydrolysis of the chosen samples does not exceed 1.9% for deamidations up to 42%.

Table II. Effect of Deamidation on Functional Properties of Cottonseed Protein[a]

functional property	deamidation, %			
	0	27.0	32.5	42.0
water-binding capacity, %	22.5	27.7	35.2	48.0
foam expansion, %	126	168	180	213
foam stability, %	95	97	98	98
emulsion capacity, mL of oil	56.0	88.0	97.0	109.0
emulsion viscosity, cps × 1000	40	75	89	122
solubility, %				
pH 3.0	25.0	35.5	39.0	42.5
pH 4.8	2.5	4.0	8.0	8.6
pH 6.5	42.5	48.9	50.8	60.5

[a] Deamidations of 0, 27.0, 32.5, and 42.0% were obtained by the reaction of cottonseed protein (1%) with 0.2 N HCl and 0.02 M SDS at 70 °C for 0, 1, 2, and 3 h, respectively.

Table III. Functional Properties of Soy and Peanut Proteins[a]

functional property	deamidation, %			
	soy		peanut	
	0	33.7	0	27.8
water-binding capacity, %	25.5	54.0	35.0	60.0
foam expansion, %	120	198	129	190
foam stability, %	92	98	91	98
emulsion capacity, mL of oil	60	150	70	160
emulsion viscosity, cps × 1000	47	98	54	95
solubility, %				
pH 3.0	25.0	30.3	40.4	51.1
pH 4.8	2.0	12.0	12.0	21.7
pH 6.5	30.9	45.5	58.9	88.0

[a] Protein (1%) was treated with 0.2 N HCl and 0.04 M SDS at 70 °C for 1.5 h. The controls (0% deamidation) were obtained at 0 h of the same treatment.

Thus, the changes in properties are mostly due to deamidation. As the protein was increasingly deamidated, it became more whippable and had greater capacity to emulsify oil. The increases in solubility were quite significant, considering that peptide bond hydrolysis was only 2% or less in the samples under investigation. One of the noticeable increases was in water-binding capacity. The water-binding capacity of oilseed proteins has been reported to correlate with the amount of hydrophilic groups minus amide groups during deamidation (Hagenmaier, 1972). The amide groups in protein, according to Bull and Breese (1968), not only do not bind water but actually inhibit water binding by the other polar groups. Deamidation converts an amide group to an acid group; by going from water antibinding to water binding, each conversion in the deamidation effects a double increase in the capactity to bind water.

SDS-Catalyzed Deamidation of Other Oilseed Proteins. Our investigations have been centered on cottonseed protein, but other oilseed proteins can also be deamidated in the presence of SDS. A list of functional properties is shown in Table III for deamidated samples of soy protein and peanut protein. The samples have been treated at 70 °C with 0.2 N HCl and 0.04 M SDS for 2 h. Under these conditions, deamidations of 27.8% for peanut protein and 33.7% for soy protein were achieved with very little hydrolysis (less than 1.1% in both cases) of the peptide bonds. General improvements in functional properties are demonstrated, and they can be attributed to the partial deamidation of the proteins as in the case of cottonseed protein (Table II). Greater deamidation of these proteins could result in further improvement in functional properties but could also cause more extensive hydrolysis of the peptide bonds.

ACKNOWLEDGMENT

We express appreciation to A. M. Hammond for nitrogen determinations.

Registry No. SDS, 151-21-3; H_2O, 7732-18-5.

LITERATURE CITED

Arzu, A.; Mayorga, H.; Gonzales, J.; Rolz, C. "Enzymatic Hydrolysis of Proteins". *J. Agric. Food Chem.* **1972**, *20*, 805–809.

Beuchat, L. R.; Cherry, J. P.; Quinn, M. R. "Physicochemical Properties of Peanut Flour as Affected by Proteolysis". *J. Agric. Food Chem.* **1975**, *23*, 616–620.

Bull, H. B.; Breese, K. "Protein Hydration". *Arch. Biochem. Biophys.* **1968**, *128*, 488–496.

Finley, J. W. "Deamidated Gluten: a Potential Fortifier for Fruit Juices". *J. Food Sci.* **1975**, *40*, 1283–1285.

Franzen, K. L.; Kinsella, J. E. "Functional Properties of Succinylated and Acetylated Soy Protein". *J. Agric. Food Chem.* **1976**, *24*, 788–795.

Hagenmaier, R. "Water Binding of Some Purified Oilseed Proteins". *J. Food Sci.* **1972**, *37*, 965–966.

Hayashi, K. "A Rapid Determination of Sodium Dodecyl Sulfate with Methylene Blue". *Anal. Biochem.* **1975**, *67*, 503–506.

Henderson, L. E.; Oroszlan, S.; Konigsberg, W. "A Micromethod for Complete Removal of Dodecylsulfate from Proteins by Ion-pair Extraction". *Anal. Biochem.* **1979**, *93*, 153–157.

McDonald, C. E.; Pence, J. W. "Wheat Gliadin in Foams for Food Products". *Food Technol.* **1961**, *15*, 141–144.

McWatters, K. H.; Cherry, J. P. "Emulsification, Foaming and Protein Solubility Properties of Defatted Soybean, Peanut, Field Pea and Pecan Flours". *J. Food Sci.* **1977**, *42*, 1444–1447.

Matsudomi, N.; Kaneko, S.; Kato, A.; Kobayashi, K. "Functional Properties of Deamidated Gluten". *Nippon Nogeikagakuk Kaishi* **1981**, *55*, 983–989.

Matsudomi, N.; Sasaki, T.; Kato, A.; Kobayashi, K. "Conformational Changes and Functional Properties of Acid-modified Soy Protein". *Agric. Biol. Chem.* **1985**, *49*, 1251–1256.

Mellon, E. F.; Korn, A. H.; Hoover, S. R. "Water Absorption. I. The Effect of Free Amino Groups in Casein". *J. Am. Oil Chem. Soc.* **1947**, 69, 827–831.

Saito, L. "Alkaline Glucinin for Meat Processing". U.S. Patent 3001875, 1961.

Sekul, A. A.; Vinnett, C. H.; Ory, R. L. "Some Functional Properties of Peanut Protein Partially Hydrolyzed with Papain". *J. Agric. Food Chem.* **1978**, *26*, 855–857.

Steinhardt, J.; Fugitt, C. "Catalyzed Hydrolysis of Amide And Peptide Bonds in Proteins". *J. Res. Natl. Bur. Stand. (U.S.)* **1942**, *29*, 315–327.

Sung, H. Y.; Chen, H. J.; Liu, T. Y.; Su, J. C. "Improvement of The Functionalities of Soy Protein Isolate through Chemical Phosphorylation". *J. Food Sci.* **1983**, *48*, 716–721.

Zarins, Z. M.; Cherry, J. P. "Storage Proteins of Glandless Cottonseed Flour". *J. Food Sci.* **1982**, *46*, 1855–1859, 1862.

Received for review December 22, 1986. Accepted May 11, 1987.