Pazur, J. H.; Ando, T. *J. Biol. Chem.* **1959**, *234*, 1966.
Steel, R. G. D.; Torrie, J. H. "Principles and Procedures of Statistics"; McGraw-Hill: New York, 1960; p 72.
Thivend, P.; Mercier, C.; Guilbot, A. In "Methods in Carbohydrate Chemistry"; Whistler, R. L., Ed.; Academic Press: New York, 1972; Vol. VI, pp 100–105.
Topps, J. H.; Kay, R. N. B. *Proc. Nutr. Soc.* **1969**, *28*, 23A.

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Effects of Ultra-High-Temperature Pasteurization on Milk Proteins

Frederic W. Douglas, Jr.,* Rae Greenberg, Harold M. Farrell, Jr., and Locke F. Edmondson[1]

Ultra-high-temperature (UHT) pasteurization of skim milk (148 °C for 3 s) has been found to inactivate effectively foot and mouth disease virus. For determination of the effect of UHT pasteurization on milk proteins, the composition and properties of proteins from milk after this treatment were compared with those from conventional high-temperature–short-time pasteurized (HTST = 71.7 °C for 15 s) and raw skim milks. Vacuum-dried–acid-precipitated caseins and freeze-dried–dialyzed whey proteins were prepared from each product. Functional properties of casein such as solubility, viscosity, emulsifying capacity, and electrophoretic mobility were compared. For both casein and whey proteins, compositional comparisons were made among molar ratios of amino acids, total protein, and chemically available lysine. The solubility of milk caseins was reduced by UHT pasteurization. Whey protein nitrogen analyses show significant protein denaturation. No significant losses in nutritive value are indicated, and differences in viscosity and emulsification capacity are small.

Earlier studies (Burrows and Dawson, 1968; Hedger and Dawson, 1970; Sellers, 1969; Terbrüggen, 1932) indicated that the virus from foot and mouth disease (FMD) infected cows can survive in milk and derived milk products such as cheese (Blackwell, 1975) and casein (Cunliffe and Blackwell, 1977). FMD virus in whole milk, skim milk, and cream from experimentally infected dairy cows (Blackwell and Hyde, 1976) can survive conventional high-temperature–short-time (HTST = 71.7 °C for 15 s) pasteurization. Cunliffe et al. (1978, 1979) showed the persistence of infectious FMD virus up to 42 days in heat-dried caseins produced by acid precipitation of HTST-pasteurized skim milk from infected cows. Milk-borne FMD virus may pose a serious threat to FMD-free countries, and reliable procedures are needed to inactivate FMD virus in milk. Heat treatments considerably above pasteurization have been referred to as ultra-high-temperature (UHT) processes. According to the International Dairy Federation (1972), UHT processes refer to pasteurization techniques with temperatures of at least 130 °C in a continuous flow and holding times of ~1 s or more. Cunliffe et al. (1979) reported that UHT pasteurization effectively inactivates FMD virus in milk when carried out at 148 °C for 3 s or longer. It was of interest to determine the effect of this regimen on the properties of caseins and whey proteins prepared from UHT-pasteurized skim milk and to compare them with those prepared from HTST-pasteurized and raw skim milk. The results indicated that UHT pasteurization of skim milk resulted in interaction of casein and whey proteins, a reduction in solubility of casein at neutral pH or below, a decrease in chemically available lysine in whey protein, and a 56% denaturation of whey protein in the skim milk.

Eastern Regional Research Center, Agricultural Research, Science and Education Administration, U.S. Department of Agriculture, Philadelphia, Pennsylvania 19118.
[1]Retired.

EXPERIMENTAL SECTION

Sample Preparation. A schematic for the preparation of UHT-pasteurized, HTST-pasteurized, and raw caseins is shown in Figure 1. Fresh raw whole milk was obtained from a local dairy and separated cold. The raw skim milk, which contained 0.06% fat, was divided into three lots. Casein was prepared from 9.1 kg of lot no. 1 at 40 °C by precipitation at pH 4.6, with the addition of 1025 mL of 0.5 N HCl added slowly from a buret over a period of 15 min with stirring. After an additional 5 min of stirring, the casein settled and the whey was decanted and filtered. The casein was washed 4 times with water at pH 5.0. Water temperatures were 35, 45 (twice), and ~23 °C. Approximately 38.6 kg of lot no. 2 was pasteurized at 76.7 °C for 15 s in a triple tube heater, and the HTST-pasteurized casein was prepared as above from 9.1 kg of milk. Approximately 38.6 kg of lot no. 3 was sterilized in a tubular heat exchanger at 148.5 °C for 2.5 s, and 9.1 kg was used to prepare UHT casein as above except the skim milk was heated to 47.5 °C and 982 mL of 0.5 N HCl was added over a period of 7 min with stirring. Stirring was continued for an additional 10 min. The caseins from the raw, HTST-pasteurized, and UHT-pasteurized milks were dried in a shelf dryer under high vacuum.

Figure 2 shows the procedure for preparation of raw, HTST, and UHT freeze-dried whey proteins. Samples of all three wheys were dialyzed for 36 h in running chilled tap water and then for 12 h in distilled water. The dialyzed wheys were concentrated to approximately half of the

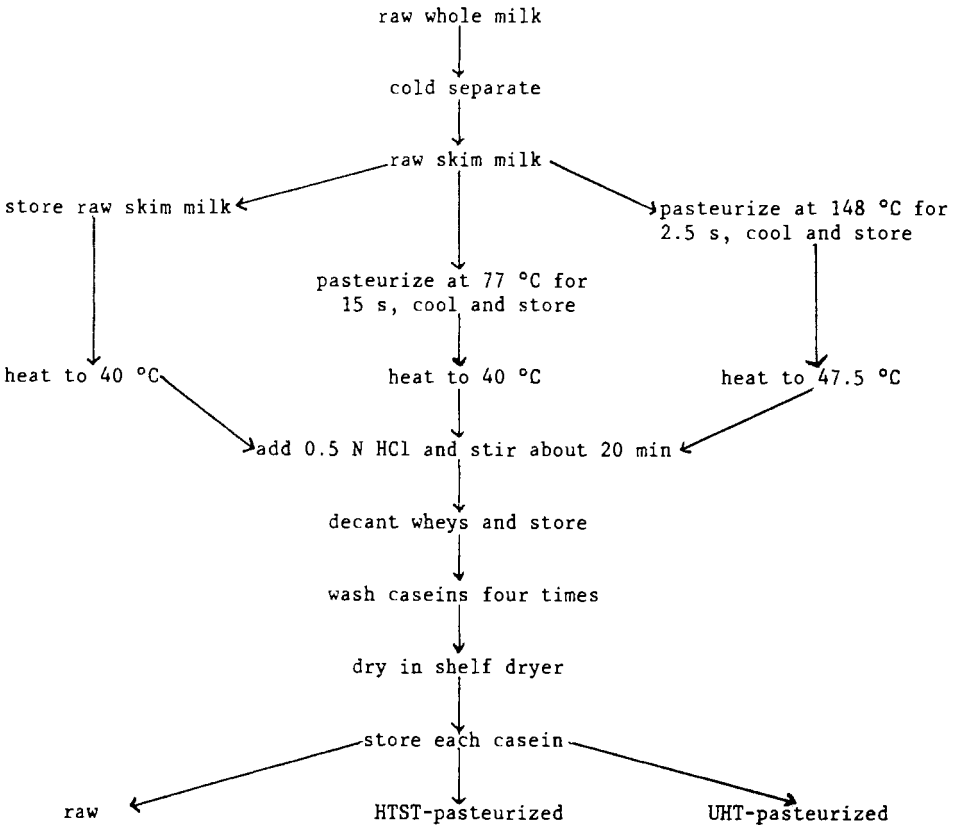

Figure 1. Schematic for the preparation of raw, HTST-pasteurized, and UHT-pasteurized caseins.

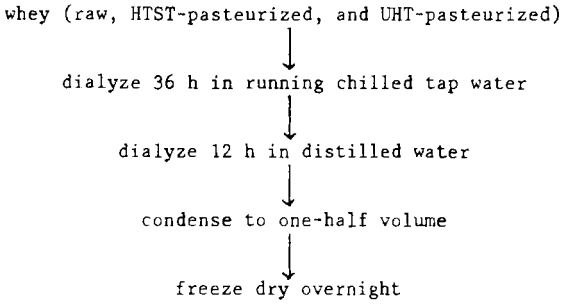

Figure 2. Schematic for the preparation of raw, HTST-pasteurized, and UHT-pasteurized whey proteins.

original volume and then freeze-dried overnight.

Reagents and Buffers. Potassium phosphate (monobasic)–sodium hydroxide buffers at pH 6.0, 6.8, and 8.0 were prepared by the procedure in Association of Official Analytical Chemists (1965a), sections 13.023 and 13.024. These buffers were made 0.15 M with respect to sodium chloride. Boric acid–potassium chloride buffer at pH 9.0 was prepared by the procedure listed in Association of Official Analytical Chemists (1965b), sections 13.023 (c, d), 13.024, and 33.047 (a). Corn oil was purchased from a local supermarket. All other reagents used were analytical grade.

Solubility Characteristics. The procedure of Lawhon and Cater (1971) was modified and used to determine solubility characteristics of the caseins. Essentially the method consisted of dispersing 1 g of each casein in 100.0 mL of each buffer and stirring for 5 min. The slurry was readjusted to the original buffer pH, stirred an additional 5 min, with the pH readjusted when necessary; stirring continued for an additional 5 min. The pH adjustments of the casein slurry were made with 0.01 N HCl and 0.015 N NaOH. Samples were centrifuged for 20 min at 2000 rpm and then filtered. Aliquots of 10.0 mL of supernatant were analyzed for nitrogen by the micro-Kjeldahl method (Association of Official Analytical Chemists, 1965c).

Viscosity Measurements. Casein solutions (2.5%) in buffers at pH 6.8, 8.0, and 9.0 were prepared by the modified technique of Lawhon and Cater (1971). The viscosity measurements of the casein solutions were determined in Ostwald-Cannon-Fenske viscometers. Samples were equilibrated 10 min in the viscometers at 30.4 °C prior to being measured.

Emulsifying Capacity. A modification of the procedure of Pearson et al. (1965) was used to determine the emulsifying capacity of the caseins. The vacuum-dried caseins were dispersed in buffer solutions at pH 6.8, 8.0, and 9.0. Samples were stirred for 15 min, and the pH was readjusted with 0.5 N NaOH. After dissolving, all samples were readjusted to pH 6.8. The emulsifying capacity was determined with corn oil with 25.0-mL aliquots of casein solutions containing from 120 to 250 mg of protein. The oil was added slowly from a buret to the sample in a beaker, with stirring at 1600 rpm. Just enough oil was added to break the emulsion as shown by a sudden drop in viscosity.

Gel Electrophoresis. Polyacrylamide gel electrophoresis of the casein and whey protein samples was carried out according to the method of Thompson et al. (1964) with 7% polyacrylamide in 4.5% M urea at pH 9.2, after the samples were reduced with 10% 2-mercaptoethanol. An eight-slot gel was run and samples were applied so that each slot received 83 μg of the sample on a dry weight basis.

Whey Protein Nitrogen. Whey protein nitrogen determinations of raw, HTST-pasteurized, and UHT-pasteurized skim milks were by the Methods of Laboratory Analysis for Dry Whole Milk, Nonfat Dry Milk, Dry Buttermilk and Whey (1961), with a modification of the Harland–Ashworth test used for whey protein nitrogen.

Available Lysine. Chemically available lysine was determined by the method of Kakade and Liener (1969) as modified by Greenberg et al. (1977).

Table I. Percentage of Soluble Nitrogen of Caseins

pH	% of soluble nitrogen[a] raw	HTST	UHT
6.0	78.78 ± 0.11	74.76 ± 0.29	50.98 ± 0.16
6.8	88.15 ± 0.41	84.11 ± 0.46	71.72 ± 0.32
8.0	94.38 ± 0.16	86.85 ± 0.21	78.05 ± 0.14

[a] $\bar{x} \pm \sigma$ for two determinations.

Table II. Relative Viscosity of 2.5% Casein Solutions

pH[b]	viscosity measurements[a] raw	HTST	UHT
6.8	1.53	1.45	1.29
8.0	1.66	1.57	1.38
9.0	1.60	1.52	1.55

[a] Viscosity relative to water. $\bar{x}$ for three determinations. Standard errors were ±0.01 or less. [b] Buffers at pH 6.8 and 8.0 were potassium phosphate–sodium hydroxide. At pH 9, a boric acid–potassium chloride buffer was used.

Amino Acid Composition. The procedure of Moore and Stein (1963) was used for automated amino acid analysis. Samples were hydrolyzed at 110 °C for 24 h with 5.7 N HCl containing phenol (10 μL/mL), in sealed evacuated tubes.

Radial Immunodiffusion. The distribution of β-lactoglobulin in the various fractions was determined by radial immunodiffusion. For this work it was necessary to obtain antibodies specific for β-lactoglobulin. The protein was purified by the method of Aschaffenberg and Drewry (1957). Antibodies to β-lactoglobulin were produced in rabbits by Cappel Laboratories, Cochraneville, PA. The antiserum produced was highly specific; it gave no cross-reactions to purified caseins or to α-lactalbumin by either Ouchterlony diffusion or immunoelectrophoresis (Crowle, 1973). Standard proteins and samples of each fraction were dissolved in 50 mM Tris, 4 mM citrate, 5 M urea, and 10 mM 2-mercaptoethanol. A 3-μL aliquot was withdrawn to assay for β-lactoglobulin content by the method of Mancini et at. (1965) with β-lactoglubulin in urea–2-mercaptoethanol used as the standard. A 100-μL aliquot was taken for estimating of the protein content of each fraction by the Coomassie dye binding method (Bradford, 1976), with either whole casein or β-lactoglobulin in the urea buffer used as the standard.

RESULTS AND DISCUSSION

Solubility Characteristics. The solubility of the caseins as shown in Table I provides evidence that there is a marked reduction in the solubility of casein from UHT-pasteurized milk compared with caseins from raw and HTST-pasteurized milks when determinations were made at neutral pH or below. The differences were less when the caseins were first dispersed in buffer at pH 8.0 and then titrated back to the appropriate pH. Low solubility of the UHT-treated sample is no doubt related to the heat-induced β-lactoglobulin–κ-casein interaction (Zittle et al., 1962). This complex formed by the sulfhydryl–disulfide interchange tends to dissociate at high pH, as was observed in this case. For some food uses, solubility may not be an important factor, but for applications where high solubility is needed, redispersion of UHT-pasteurized casein at an elevated pH followed by adjustment to the desired pH level is recommended.

Viscosity. Viscosity is the principal means for characterizing the flow of a fluid. The three caseins were dissolved in buffers at pH 6.8, 8.0, and 9.0 to make 2.5% solutions. Viscosity of the casein solutions was determined at 30.4 °C. Although there is a trend toward lower viscosity with increasing heat treatment (Table II), this is most likely contributed by protein interaction (Zittle et at., 1962) in the pasteurized samples causing low solubility at pH 6.8. As the pH increases, so does the viscosity for all of the samples. The viscosity for all three samples at all three pH values falls well within the range of skim milk (1.33 at 30 °C and 1.54 at 25 °C) (Whitaker et al., 1927). The differences in viscosity of the three samples probably would not affect most food uses of the caseins.

Table III. Emulsifying Capacity of Caseinates

pH	caseinate concn, mg/25 mL	mL of oil/mg of protein raw	HTST	UHT[a]
6.8	120	1.75	1.67	
	160	1.31	1.25	
	200	1.05	1.00	
	250	1.36	0.88	
8.0 → 6.8	120	1.75	1.75	1.67
	160	1.31	1.22	1.19
	200	1.05	0.95	1.05
	250	0.96	1.30	1.08
9.0 → 6.8	120	1.67	1.75	1.71
	250	1.28	1.24	1.28

[a] The UHT casein sample did not completely dissolve at pH 6.8; therefore, no emulsion was run.

Table IV. Total Protein and Chemically Available Lysine in Caseins and Whey Proteins

sample	protein,[a] g/100 g of sample	available lysine, g/100 g of protein[b]
casein		
raw	93.75	6.73 ± 0.16
HTST	95.24	6.95 ± 0.17
UHT	92.06	6.94 ± 0.15
whey protein		
raw	90.40	8.99 ± 0.20
HTST	84.86	8.75 ± 0.18
UHT	69.96	8.18 ± 0.17

[a] Moisture-free basis. [b] $\bar{x} \pm \sigma$ for duplicate samples of available lysine.

Emulsifying Capacity. In many food applications, emulsifying properties of ingredient proteins are important and are commonly discussed in terms of emulsifying capacity (EC). The EC denotes the maximum amount of oil that is emulsified under specified conditions by a standard amount of protein (Pearce and Kinsella, 1978). EC of the three vacuum-dried caseins was measured as a ratio of milliliters of oil per milligram of protein (Table III). At pH 6.8, the UHT-pasteurized casein sample did not dissolve completely; therefore, no emulsion test was run on this sample. Although the UHT casein is more difficult to dissolve, once solution is effected at pH 8.0 or 9.0 and then readjusted to pH 6.8, the emulsifying properties compare favorably with those of raw and HTST-pasteurized caseins at all concentrations.

Chemically Available Lysine and Total Protein. The results of determinations for total protein and chemically available lysine are listed in Table IV. The lyophilized whey protein samples have low total protein values, reflecting inefficient lactose removal by dialysis of the whey fraction. The available lysine levels when calculated on a gram per 100 g of protein basis are not significantly different for the raw, HTST-pasteurized, and UHT-pasteurized caseins. This indicates that as measured by this parameter there is no loss of nutritional value for the casein fractions. The UHT-pasteurized whey protein does show a small decrease (<10%) in available lysine as

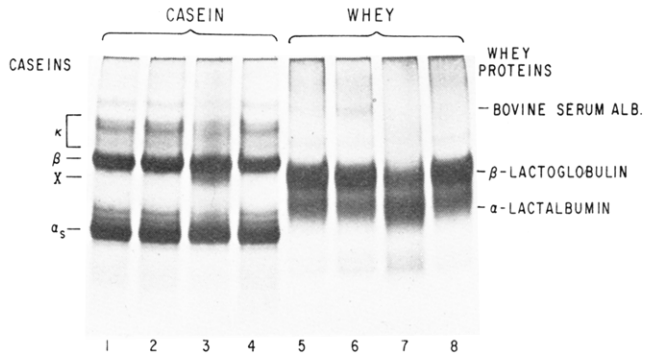

Figure 3. Gel electrophoresis of caseins and wheys. Slots 1 and 4 are raw casein, slot 2 is HTST-pasteurized casein, and slot 3 is UHT-pasteurized castein. Slots 5 and 8 are raw whey proteins, slot 6 is HTST-pasteurized whey protein, and slot 7 is UHT-pasteurized whey protein.

Table V. Radial Immunodiffusion Quantitation of the Percent β-Lactoglobulin in Casein and Whey Protein Fractions

sample	$\frac{\text{mg of β-lactoglobulin}}{\text{mg of total protein}} \times 100$[a]
casein	
raw	0.817 ± 0.072
HTST	1.56 ± 0.085
UHT	11.9 ± 1.4
whey protein	
raw	85.9 ± 7.1
HTST	67.6 ± 4.5
UHT	23.3 ± 0.9

[a] $\bar{x} \pm \sigma$ for four determinations.

compared to the raw whey sample, but this decrease is not enough to diminish significantly its nutritional value. A PER test on the whey and casein as well would provide confirmation of this but at this point there is no reason to expect otherwise.

Electrophoretic Mobility. Gel electrophoresis patterns of the raw, HTST-pasteurized, and UHT-pasteurized caseins and whey proteins are shown in Figure 3. The major casein fractions, α_s- and β-caseins are unaffected by the heat treatment in samples 1, 2, 3, and 4. Close examination of κ-casein bands in sample 3 shows a diminution in their intensity when compared with those of samples 2 and 4. In addition, sample 3 shows a band (X) below β-casein. Compared with samples 5–8, this band (X) represents β-lactoglobulin complexed with the casein as a result of the heat treatment (Zittle et al., 1962). Quantitation by single radial immunodiffusion carried out with antiserum specific for β-lactoglobulin gives further evidence that β-lactoglobulin is complexed with the casein in the UHT-pasteurized sample. Table V, comparing the levels of β-lactoglobulin in raw and UHT casein, shows that the raw casein contains only 0.817% β-lactoglobulin in contrast to the high level (11.9%) in the UHT-pasteurized sample. Also, raw whey protein contains a high level of β-lactoglobulin (85.9%) as compared to only 23.3% in the UHT-pasteurized sample. The complex precipitates with the casein, but the conditions under which the gel is run allows for the separation of the complex. Comparison of samples 7 and 8 of Figure 3 indicates that much of the β-lactoglobulin is carried out of the whey. The ratio of β-lactoglobulin to α-lactalbumin is diminished in sample 7, and on a dry weight basis (Table V) the whey protein was subsequently reduced from 85.9% β-lactoglobulin for raw dialyzed whey to 23.3% for UHT whey protein. The bovine serum albumin has been affected by the heat as well.

Table VI. Molar Ratios of Amino Acids in Raw, HTST-Pasteurized, and UHT-Pasteurized Caseins and Whey Proteins[a]

amino acids	molar ratios					
	caseins			wheys		
	raw	HTST	UHT	raw	HTST	UHT
Asp	1.72	1.74	1.88	3.13	3.03	3.62
Thr[b]	1.09	1.18	1.17	1.67	1.65	1.81
Ser[b]	1.60	1.60	1.63	1.70	1.73	2.03
Glu	5.04	4.73	4.83	4.49	4.62	4.98
Pro	3.34	3.05	3.00	1.66	1.75	2.06
Gly	0.80	0.81	0.83	1.03	1.04	1.01
Ala	1.07	1.10	1.23	1.99	1.99	1.48
½-Cys		0.02	0.15	0.77	0.72	0.69
Val	1.81	1.97	1.83	1.79	1.80	1.57
Met	0.59	0.59	0.45	0.62	0.39	0.35
Ile	1.30	1.39	1.40	1.66	1.87	2.00
Leu	2.33	2.47	2.57	3.45	3.61	3.29
Tyr	1.01	0.90	0.99	0.78	0.75	0.73
Phe	1.02	1.01	1.00	0.91	0.84	0.99
Lys	1.79	1.76	1.90	2.58	2.72	3.04
His	0.63	0.62	0.60	0.53	0.55	0.72
Arg	0.69	0.69	0.68	0.66	0.69	0.62

[a] Ratios were determined by averaging data using Lys, Arg, and Phe as divisors. [b] Uncorrected for losses.

Whey Protein Nitrogen. The principal interest in protein denaturation centers around the effects of heat treatment, which considerably affects the whey proteins. In the natural state, the milk whey proteins have a definite configuration which, when exposed to heat above a certain critical level, is disrupted and the characteristic properties of the protein are altered (Jenness and Patton, 1959). Analysis for whey protein nitrogen in the skim milks used to produce the three casein and whey protein samples showed a 56% denaturation in the UHT-pasteurized milk, a 0.4% denaturation in the HTST-pasteurized milk, and no denaturation in the raw milk.

Molar Ratios of Amino Acids. The amino acid composition as shown in Table VI provides further evidence of the interaction of whey proteins and casein during UHT pasteurization. The molar ratios of amino acids in UHT-pasteurized casein, compared with those in the raw and HTST-pasteurized samples, indicate elevated levels of aspartic acid, alanine, and cystine. Each of these is present in higher ratios in raw whey. Also, UHT-pasteurized casein contains depressed ratios of proline and histidine, with raw whey again reflecting lower levels of these residues. These comparisons suggest an admixture of whey proteins, thereby diluting the values for UHT-pasteurized casein compared with those of the unpasteurized sample. In the same fashion, UHT-pasteurized whey exhibits higher values of glutamic acid, proline, phenylalanine, and histidine, compared with those of raw whey and concomitant lower values for alanine and cystine. These results are consistent with the polyacrylamide gel patterns showing whey proteins in casein fractions but also indicate the presence of casein in the UHT-pasteurized whey fraction.

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LITERATURE CITED

Aschaffenberg, R.; Drewry, I. *Biochem. J.* **1957**, *65*, 273–277.
Association of Official Analytical Chemists "Methods of Analysis", 10th ed.; AOAC: Washington, DC, 1965 (a) pp 194–195; (b) pp 194–195, 640; (c) p 744.
Blackwell, J. H. *J. Dairy Sci.* **1975**, *59*, 1574–1579.

Blackwell, J. H.; Hyde, J. L. *J. Hyg.* **1976**, *77*, 77-83.
Bradford, M. M. *Anal. Biochem.* **1976**, *72*, 248-254
Burrows, R. S.; Dawson, P. S. *Vet. Rec.* **1968**, *82*, 387-388.
Crowle, A. J. In "Immunodiffusion", 2nd ed.; Academic Press: New York, 1973; pp 226-235.
Cunliffe, H. R.; Blackwell, J. H. *J. Food Prot.* **1977**, *40*, 389-392.
Cunliffe, H. R.; Blackwell, J. H.; Dors, R.; Walker, J. S. *J. Food Prot.* **1979**, *42*, 135-137.
Cunliffe, H. R.; Blackwell, J. H.; Walker, J. S. *J. Food Prot.* **1978**, *41*, 706-707.
Greenberg, R.; Dower, H. J.; Woychik, J. H. "Abstracts of Papers", 173rd National Meeting of the American Chemical Society, New Orleans, LA, March 1977; American Chemical Society: Washington, DC, 1977; AGFD 72.
Hedger, R. S.; Dawson, P. S. *Vet. Rec.* **1970**, *87*, 186-188.
International Dairy Federation, IDF Monograph on UHT Milk, IDF Bulletin, 1972.
Jenness, R.; Patton, S. In "Principles of Dairy Chemistry"; Wiley: New York, 1959; pp 147, 335.
Kakade, M. L.; Liener, I. E. *Anal. Biochem.* **1969**, *27*, 273-280.
Lawhon, J. T.; Cater, C. M. *J. Food Sci.* **1971**, *36*, 372-377.
Mancini, G.; Carbonara, A. O.; Heremans, J. F. *Immunochemistry* **1965**, *2*, 235-254.
Methods of Laboratory Analyses for Dry Whole Milk, Nonfat Dry Milk, Dry Buttermilk and Whey, USDA, AMS, Dairy Division and Grading Branch, Washington DC, 1961.
Moore, S.; Stein, W. H. *Methods Enzymol.* **1963**, *7*, 819-831.
Pearce, K. N.; Kinsella, J. E. *J. Agric. Food Chem.* **1978**, *26*, 716-723.
Pearson, A. M.; Spooner, M. E.; Hegarty, G. R.; Bratzler, L. J. *Food Technol. (Chicago)* **1965**, *19*, 103-107.
Sellers, R. F. *Br. Vet. J.* **1969**, *125*, 163-168.
Terbruggen, F. *Dtsch. Tieraerztl. Wochenschr.* **1932**, *40* (Part 1), 129-134.
Thompson, M. P.; Kiddy, C. A.; Johnston, J. O.; Weinberg, R. M. *J. Dairy Sci.* **1964**, *47*, 378-381.
Whitaker, R.; Sherman, J. M.; Sharp, P. F. *J. Dairy Sci.* **1927**, *10*, 361.
Zittle, C. A.; Thompson, M. P.; Cluster, J. H.; Cerbulis, J. *J. Dairy Sci.* **1962**, *45*, 807-810.

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Effects of Salts and Denaturants on Thermocoagulation of Proteins

Kazuko Shimada* and Setsuro Matsushita

Thermocoagulation of proteins containing different amounts of hydrophobic amino acids was investigated with regard to the effects of salts and denaturants. The formation of thermocoagulum of egg albumin was enhanced by the addition of salts, and the effect of salts followed the lyotropic series. For bovine serum albumin, salts of the higher order on the lyotropic series enhanced fromation of coagulum but those of the lower order inhibited it when the salt concentrations were increased. For soybean protein, an increase in turbidity at alkaline pH was observed when salts were added, while formation of thermocoagulum was inhibited. Guanidine hydrochloride enhanced the coagulum formation in a manner similar to that of salts. Sodium dodecyl sulfate and urea suppressed thermocoagulation. From the effects of salts and denaturants on thermocoagulation of these proteins, the mechanism of coagulum formation can be surmised from the standpoint of the hydrophobic amino acid content of proteins.

Thermocoagulation of proteins is widely utilized in food processing. However, there has been very little past research on this subject. Heat-treated solutions of serum albumin have been found to form an opaque coagulum, a clear gel, or an intermediate clot depending on the pH of the medium (Jensen et al., 1950). Seideman et al. (1963) reported that some factors (pH, addition of sucrose, etc.) affected the coagulation temperature of egg white. Differences in coagulation of egg white resulting from application of conventional heat and electronic exposure were compared by Baldwin et al. (1967). It has been observed that the microstructures of soybean protein curds and yeast protein curds which were examined by an optical microscope and a scanning electron microscope varied according to pH (Lee and Rha, 1978; Tsintsadze et al., 1978).

The three-dimensional network of the protein coagulum is believed to be formed by hydrophobic interactions, hydrogen bonds, and ionic attractions, but the mechanism of formation is still not well understood. Our previous paper showed that the amount of hydrophobic amino acids in proteins easily forming thermocoagulum differed from those in proteins forming thermoreversible gel (Shimada and Matsushita, 1980b). In this paper, we report the effects of salts and denaturants on thermocoagulation of proteins containing various amounts of hydrophobic amino acids. Egg albumin which coagulates on heating and bovine serum albumin and soybean protein which gel on heating were chosen as models in this study.

MATERIALS AND METHODS

Materials. Egg albumin was purchased from Nakarai Chemicals Ltd., Kyoto. Bovine serum albumin (BSA) (demineralized) was obtained from Povite Producten N.V. (Amsterdam, Holland). These proteins were defatted by acetone before use. Soybean protein solution, prepared from an aqueous extract of defatted soybean meal, was powdered by acetone treatment. Other chemicals were reagent grade.

Heat Treatment and Turbidimetry. Heat treatment was carried out with 5 mL of protein solution in a glass tube (105 × 15 mm). Protein solutions were heated in a

Research Institute for Food Science, Kyoto University, Kyoto, Japan.

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