Solvation of Rennet Curd from High-Heat-Treated Milk Influenced by Acid Type and pH

Gilbert I. Imafidon[†] and Nana Y. Farkye*

Dairy Products Technology Center, Dairy Science Department, California Polytechnic State University, San Luis Obispo, California 93407

Mean solvation of caseins in unheated milk was 2.29 g of H_2O/g of dry matter. Mean solvation of casein−whey protein complex in high-heat-treated (85 °C for 10 min) milk was 3.44 g of H_2O/g of dry matter. Rennet treatment of heated or unheated milk reduced curd solvation, respectively, to 2.64 and 0.78 g of H_2O/g of dry matter. Curd solvation decreased with milk pH and was dependent on the acid type used in acidification of the milk. Mean curd solvation, in decreasing order, resulting from acid type used was acetic = hydrochloric > phosphoric > lactic acids.

Keywords: *Solvation; acids; curd*

INTRODUCTION

Rennet coagulation of milk results in the entrapment of water, protein (mainly caseins), fat, and calcium to form cheese curd. The amount of water incorporated influences cheese texture and firmness (de Jong, 1978) and is used to distinguish soft cheeses from hard cheeses. Also, water activity is important in controlling enzymatic and microbial reactions in cheese. One of the methods of increasing moisture content of cheese is to subject milk to high-heat treatment. When milk is heated at high temperatures (>70 °C) for cheese manufacture, the whey proteins that are normally lost become trapped in the curd (Melachouris and Tuckey, 1966) due to their interaction with the caseins. The disadvantage of heating milk for cheese-making at high temperatures is the adverse effect on rennet coagulation and the high moisture content of resultant cheese. Poor rennet coagulation of highly heated milk can be reversed by acidification of milk before rennet addition (Banks, 1988; Marshall, 1986; van Hooydonk et al., 1986; Imafidon and Farkye, 1993).

Rennet coagulability of highly heated milk is greatly enhanced when the pH of milk is cycled (i.e., acidification to pH 5.5 to disintegrate the casein micelles and subsequent neutralization to pH 6.2−6.4) before rennet addition (Pyne and McGann, 1960; van Hooydonk et al., 1977; Singh et al., 1988; Reddy and Kinsella, 1990; Imafidon and Farkye, 1993). Recently, Farkye et al. (1995) reported that acid-curd cheese made with citric, lactic, or acetic acid retained different levels of moisture and had different textural properties. This suggests that properties of rennet curd obtained from acidified milk may be influenced by the type of acid used for pH adjustments. Therefore, the objective of this study was to determine the solvation of milk proteins and rennet curd made from highly heated milk acidified with different acids.

MATERIALS AND METHODS

Heat Treatment and pH Adjustment of Milk. Bovine whole milk was obtained from California Polytechnic State University creamery. The milk was adjusted to pH 7.5 with 5 M NaOH, heated in a water bath to 85 °C for 10 min, and then cooled in an ice bath at 4 °C. The cold milk was acidified with 2 M HCl to pH 5.5 and held overnight at 4 °C. Then, predetermined amounts of 2 M HCl or 5 M NaOH were added to samples of the acidified milk to give incremental pH values from 5.2 to 6.8. This mode of acidification and alkalinization is called pH cycling. Control experiments were conducted using heated or unheated milks with no pH adjustments.

Measurement of Solvation. Milk samples (∼25 mL) were accurately weighed into a centrifuge tube and centrifuged (94800*g* for 1.5 h) in a Model L7-35 ultracentrifuge (Beckman Instruments, Inc., Fullerton, CA) equilibrated at 29 °C. The supernatant was decanted, and the wet pellet was accurately weighed, freeze-dried for 36 h, and reweighed. The difference in weight between the wet and dry pellet was calculated as solvation, grams of H_2O per gram of dry sample (Thompson et al., 1969). Moisture content of the samples was determined according to the microwave oven method (AOAC, 1990).

Influence of Rennet on Solvation of Heated or Unheated Milk. Samples (200 mL) of the milk prepared as described above and having a final pH of 5.2−6.8 were weighed into 250 mL centrifuge tubes and equilibrated at 30 °C for 1 h. One milliliter of a 1:20 dilution of single-strength calf rennet (Chr. Hansen's, Lab., Inc., Milwaukee, WI) was stirred into the milk. The rennet-treated milk was left undisturbed for 1 h. The coagulum was broken to facilitate whey expulsion, and the tube and its contents were centrifuged at 10000*g* for 30 min at 29 °C. The whey was discarded and the wet curd weighed, freeze-dried, and reweighed for calculation of solvation.

Influence of Acid Type on Solvation of Rennet Curd. To study the effects of acid type, 2 M H_3PO_4, acetic, citric, lactic, or malic acid was used to replace 2 M HCl during acidification of the milk. The procedures for rennet treatment, centrifugation, moisture determination, and solvation calculation were followed as described above.

Statistical Analysis. Statistical analysis was done using the general linear models procedure on the SAS software package (SAS Institute Inc., Cary, NC). The model was

$$Y_{ijk} = \mu + \alpha_i + \beta_j + \alpha\beta_{ij} + \epsilon_{ijk}$$

where μ is the overall mean, α_i is the acid *i* effect, β_j is the pH *j* effect, $\alpha\beta_{ij}$ is the interaction of acid × pH, and ϵ_{ijk} = error.

Least-squares means comparisons and probability of differences were computed for pH and acid type effects.

RESULTS AND DISCUSSION

Table 1 shows the solvation of pellet obtained after ultracentrifugation of heated or unheated milk (pH 6.7).

* Author to whom correspondence should be addressed.

[†] Present address: Bioproducts, Inc., 1048 S. Hieland Rd., St. Anne, IL 60964.

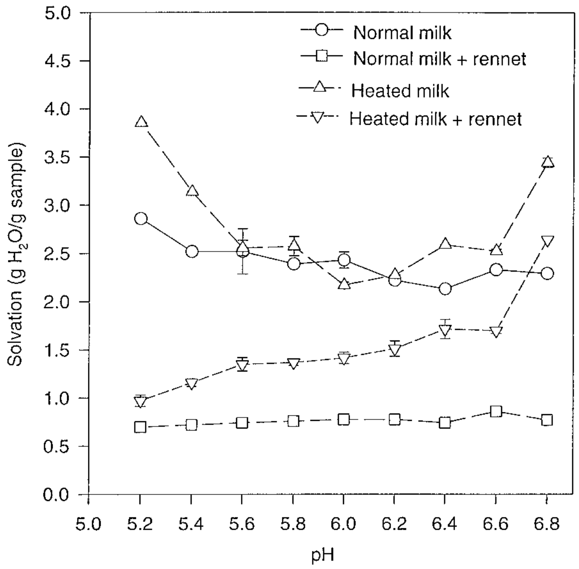

Figure 1. Effect of rennet treatment on the solvation of protein in heated (85 °C for 30 min) or unheated milk.

Table 1. Solvation of Curd from Rennet-Treated Heated or Unheated Milk

treatment	sample type	solvation (g of H_2O/g of dry matter)
unheated milk	pellet	2.29 ± 0.02
unheated milk + rennet	curd	0.78 ± 0.01
heated milk	pellet	3.44 ± 0.01
heated milk + rennet	curd	2.64 ± 0.01

Mean solvation of casein micelles from unheated milk was 2.29 g of H_2O/g of dry matter. This agrees with the results of Thompson et al. (1969) and Richardson et al. (1974), who found that solvation of casein micelles in skim milk is in the range 1.6−2.3 g of H_2O/g of dry matter. Mean solvation of protein material sedimented from the high-heat-treated milk was 3.44 g of H_2O/g of dry matter. Tarodo de la Fuente and Alias (1975) also reported increased solvation of proteins in milk heated to 90 °C. The increased solvation of protein material from the high-heat-treated milk is due to sedimentation of some whey proteins along with the caseins. In unheated milk, whey proteins remain soluble in the milk serum and do not sediment during ultracentrifugation. When milk is heated to >70 °C, denaturation of whey proteins occurs (Sawyer, 1969; Hill, 1989). The heat-denatured whey proteins interact with caseins and sediment when milk is subjected to ultracentrifugation.

The mean solvation of rennet curd was, respectively, 0.78 or 2.64 g of H_2O/g of dry matter for the unheated and highly heated milks (Table 1). Rennet treatment of normal milk reduces solvation of casein micelles (Creamer, 1985; Konstance et al., 1995). Similarly, rennet curd from highly heated milk was less solvated than the protein material sedimented from the highly heated milk containing no rennet. The reduced solvation of curd from rennet-treated milk is due to partial loss of κ-casein. In milk, rennet hydrolyzes κ-casein to *p*-κ-casein (f1−105) and (glyco)macropeptide peptide, GMP (f106−169). *p*-κ-Casein remains attached to the casein particles while the macropeptide is lost in the whey. Anema and Creamer (1993) found that casein micelle solvation correlated strongly ($r = 0.87$) with the level of κ-casein in milk, suggesting that partial loss of κ-casein from the micelle may reduce solvation. The high solvation of curd from the highly heated milk reflects the reduction in the amount of GMP released from κ-casein by rennet (Reddy and Kinsella, 1990; Imafidon and Farkye, 1994).

The relationships between pH values of milk after pH cycling on pellet or curd solvation from heated and unheated milks are shown in Figure 1. Mean solvation of pellet from pH-cycled unheated milk ranged from 2.1 to 2.9 g of H_2O/g of dry pellet and was independent of the final pH of milk. The pellet from the highly heated milk was more solvated than that from the unheated milk. In the highly heated milk, pellet solvation decreased with pH between 6.8 and 6.0 and then increased between pH 6.0 and 5.2. Snoeren et al. (1984) found that, in the pH range from 5.4 to 6.6, the solvation of casein micelles was lowest at pH ≈6.0.

Mean curd solvation for rennet-treated unheated milk was 0.76 g of H_2O/g of dry curd regardless of milk pH. Curd from rennet-treated highly heated milk was more solvated than that from unheated milk. In addition, curd solvation decreased with the pH of milk at rennet addition. This suggests that acidification of high-heat treated milk for cheese-making may be beneficial in controlling the moisture content of resultant cheese.

The solvation of rennet-treated highly heated milk acidified with various acids is shown in Table 2. Solvation of curd from rennet-treated highly heated milk was significantly ($P < 0.001$) influenced by acid type or milk pH. In addition, the interaction term, acid type × pH, was statistically significant ($P < 0.001$). Overall means for curd solvation were 1.40, 1.40, 1.24, and 1.35 g of H_2O/g of dry matter, respectively, for milk acidified with acetic, HCl, or lactic acid or H_3PO_4. Least-squares mean comparisons of curd solvation for the four acids show the order acetic = HCl > H_3PO_4 > lactic acid.

Creamer (1985) suggested that the gradual decrease in solvation as milk pH decreases is due to neutralization of negative charges on the caseins. In the high-heat-treated milk, the net negative charge on the casein−whey protein complex is expected to be large.

Table 2. Solvation[a] of Rennet Curd Prepared from Highly Heated Milk Acidified to Various pH Values Using Different Acids

curd solvation (g of H_2O/g of dry matter) milk pH at renneting	acetic	hydrochloric	lactic	phosphoric	malic	citric
6.6	1.59 ± 0.02	1.70 ± 0.01	1.40 ± 0.01	1.74 ± 0.00	n/a[b]	n/a
6.4	1.64 ± 0.01	1.72 ± 0.02	1.50 ± 0.00	1.57 ± 0.08	n/a	n/a
6.2	1.52 ± 0.01	1.51 ± 0.01	1.46 ± 0.01	1.30 ± 0.00	n/a	n/a
6.0	1.55 ± 0.00	1.42 ± 0.02	1.39 ± 0.08	1.43 ± 0.00	n/a	n/a
5.8	1.34 ± 0.13	1.37 ± 0.01	1.31 ± 0.00	1.43 ± 0.11	1.71 ± 0.02	n/a
5.6	1.31 ± 0.03	1.35 ± 0.00	1.08 ± 0.01	1.24 ± 0.02	1.39 ± 0.01	1.86 ± 0.01
5.4	1.19 ± 0.01	1.16 ± 0.06	0.96 ± 0.01	1.12 ± 0.00	1.15 ± 0.04	1.16 ± 0.04
5.2	1.06 ± 0.02	0.97 ± 0.04	0.81 ± 0.01	0.98 ± 0.01	1.00 ± 0.02	0.97± 0.03
5.0	1.10 ± 0.01	0.92 ± 0.11	0.82 ± 0.00	0.92 ± 0.00	0.93 ± 0.01	0.98 ± 0.01

[a] Means ± SE of duplicate determinations on three replicates. [b] n/a, not available. Milk did not clot in 3 h at 30 °C.

Thus, the percent decrease in solvation of highly heated milk as pH is reduced is lower than that of unheated milk. Pearce (1976) reported that rennet-treated micelles carry a net negative charge. However, the net negative charge on rennet-treated micelles is expected to be small (Creamer, 1985) because of the exterior of the micelles contains positively charged *p*-κ-casein. This may be the reason for the reduced solvation of curd from rennet-treated acidified milk.

Rennet-treated highly heated pH-cycled milk using citric acid for pH adjustments did not coagulate (within 3 h at 30 °C) at pH ≥5.8. Similarly, highly heated milk acidified with malic acid before rennet addition did not coagulate at pH ≥6.0. When coagulation occurred at milk pH of 5.6 or 5.6, curds produced from highly heated milk acidified with citric or malic acid were most solvated. Citric acid is known to partially disintegrate casein micelles (Heertje et al., 1985) by sequestering Ca^{2+} ions. Protein solvation is inversely related to Ca^{2+} concentration (Konstance et al., 1995). Therefore, the higher levels of curd solvation for milk acidified to pH 5.6−6.0 with citric and malic acids may reflect the low levels of ionic calcium in those samples.

CONCLUSIONS

Acid type has been shown to affect the structure of acid gels from highly heated milk (Harwalkar and Kalab, 1981). This study shows that rennet curd from highly heated milk is influenced by acid type. Acidification of highly heated milk to improve the rennet coagulability resulted in curd with different levels of water binding, as measured by solvation. Use of acetic or hydrochloric acid resulted in curds that were most solvated. Lactic acid produced the least solvated curds. Acidification of highly heated milk to pH ≥5.8 or ≥6.0 with citric or malic acids, respectively, prevented rennet coagulation. Curd solvation increased with milk pH at rennet addition.

LITERATURE CITED

Anema, S. G.; Creamer, L. K. Effect of the A and B variants of both α_{s1}- and κ-casein on bovine casein micelle solvation and κ-casein content. *J. Dairy Res.* **1993**, *60*, 505−516.

AOAC. *Official Methods of Analysis*, 15th ed.; Association of Official Analytical Chemists: Washington, DC, 1990.

Banks, J. M. Elimination of the development of bitter flavour in Cheddar cheese made from milk containing heat-denatured whey proteins. *J. Soc. Dairy Technol.* **1988**, *41*, 37−41.

Creamer, L. K. Water absorption by renneted casein micelles. *Milchwissenschaft* **1985**, *40*, 589−591.

de Jong, L. The influence of moisture content on the consistency and protein breakdown in cheese. *Neth. Milk Dairy J.* **1978**, *32*, 1−14.

Farkye, N. Y.; Prasad, B. B.; Rossi, R.; Noyes, O. R. Sensory and textural properties of Queso Blanco-type cheese influenced by acid type. *J. Dairy Sci.* **1995**, *78*, 1649−1656.

Harwalkar, V. R.; Kalab, M. Effect of acidulants and temperature on microstructure, firmness and susceptibility to syneresis of skim milk gels. *Scanning Electron Microsc.* **1981**, *3*, 503−513.

Heertje, I.; Visser, J.; Smit, P. Structure formation in acid milk gels. *Food Microstruct.* **1985**, *4*, 267−277.

Hill, A. R. The β-lactoglobulin-κ-casein complex. *Can. Inst. Food Sci. Technol. J.* **1989**, *22*, 120−123.

Imafidon, G. I.; Farkye, N. Y. Rennet coagulability of high-heat treated milk influenced by time of pH adjustment. *J. Food Sci.* **1993**, *58*, 1300−1302.

Imafidon, G. I.; Farkye, N. Y. Influence of pH on chymosin action in solutions of different κ-casein variants. *J. Agric. Food Chem.* **1994**, *42*, 1598−1601.

Konstance, R. P.; Holsinger, V. H.; Thompson, M. P. Instrumental texture parameters and solvation characteristics of mixed casein gels. *J. Dairy Sci.* **1995**, *78*, 784−793.

Marshall, R. J. Increasing cheese yields by high heat treatment of milk. *J. Dairy Res.* **1986**, *53*, 313−322.

Melachouris, N. P.; Tuckey, S. I. Changes of the proteins in Cheddar cheese made from milk heated at different temperatures. *J. Dairy Sci.* **1966**, *50*, 800−805.

Pearce, K. N. Moving boundary electrophoresis of native and rennet-treated casein micelles. *J. Dairy Res.* **1976**, *43*, 27−36.

Pyne, G. T.; McGann, T. C. A. 1960. The colloidal phosphate of milk. III. Nature of its association with casein. *J. Dairy Res.* **1960**, *27*, 403−417.

Reddy, I. M.; Kinsella, J. E. Interaction of β-lactoglobulin with κ-casein in micelles as assessed by chymosin hydrolysis: Effect of temperature, heating time, β-lactoglobulin concentration, and pH. *J. Agric. Food Chem.* **1990**, *38*, 50−58.

Richardson, B. C.; Creamer, L. K.; Pearce, K. N.; Munford, R. E. Casein micelle structure. II. Structure and composition of casein micelles in ovine and caprine milk as compared with those of bovine milk. *J. Dairy Res.* **1974**, *41*, 239−247.

Sawyer, W. H. Complex between β-lactoglobulin and κ-casein. A review. *J. Dairy Sci.* **1969**, *52*, 1347−1355.

Singh, H.; Shalabi, S. I.; Fox, P. F.; Flynn, A.; Barry, A. Rennet coagulation of heated milk: influence of pH adjustment before or after heating. *J. Dairy Res.* **1988**, *55*, 205−215.

Snoeren, T. H. M.; Klok, H. J.; van Hooydonk, A. C. M.; Damman, A. J. The voluminosity of casein. *Milchwissenschaft* **1984**, *8*, 461−463.

Thompson, M. P.; Boswell, R. T.; Martin, V.; Jenness, R.; Kiddy, C. A. Casein pellet solvation and heat stability of individual cow milks. *J. Dairy Sci.* **1969**, *52*, 796−798.

Torado de La Fuente, B.; Alais, C. Solvation of casein in bovine milk. *J. Dairy Sci.* **1975**, *58*, 293−300.

van Hooydonk, A. C. M.; Boerrigter, I. J.; Hagedoorn, H. G. pH-induced physico-chemical changes of casein micelles in milk and their effect on renneting. 2. Effect of pH on renneting of milk. *Neth. Milk Dairy J.* **1986**, *40*, 297−313.

van Hooydonk, A. C. M.; de Koster, P. G.; Boerrigter, I. J. The renneting properties of heated milk. *Neth. Milk Dairy J.* **1987**, *41*, 3−18.

Received for review August 14, 1995. Accepted June 11, 1996.[⊗] Funds for this study were provided by Dairy Management, Inc.

JF950555R

[⊗] Abstract published in *Advance ACS Abstracts*, August 1, 1996.