Effect of Milk Concentration on Heat-Induced, pH-Dependent Dissociation of Casein from Micelles in Reconstituted Skim Milk at Temperatures between 20 and 120 °C

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The effect of pH, milk concentration, heating temperature, and heating time on the dissociation of casein from the micelles in reconstituted skim milk was investigated. For the 10% total solids milk samples, low levels of casein were rendered soluble at pH below 6.7 regardless of heating temperature, whereas increasing levels of casein were solubilized as the pH was increased from 6.7 to 7.1. The quantity of soluble casein increased with temperature to a maximum dissociation at about 60−80 °C, decreased as the temperature was raised to 100 °C, and then increased as the temperature was raised further. The dissociation behavior of α_s-casein and β-casein showed similar dependence on temperature to that of the total casein. In contrast, the dissociation of κ-casein increased with increasing temperature over the entire temperature range studied. Most casein dissociation occurred during the first minutes of heating, with little further change on prolonged heating. As the milk was concentrated, a higher level of dissociation of casein, particularly κ-casein, was observed at lower pH and at higher temperatures, particularly above 80 °C.

Keywords: *Casein micelles; dissociation; heat treatment; reconstituted skim milk; concentrated skim milk*

INTRODUCTION

The heat treatment of milk at temperatures ≥90 °C results in the pH-dependent dissociation of casein from the casein micelles (Kudo, 1980; Singh and Fox, 1985; Nieuwenhuijse et al., 1991; Singh and Creamer, 1991a; Anema et al., 1993). At pH values below 6.7, the denatured whey proteins associate with the casein micelles and little casein is rendered soluble. At higher pH values, increasing levels of casein, predominantly κ-casein complexed with the denatured whey proteins, are dissociated from the casein micelles. The dissociation of κ-casein from the casein micelles is also dependent on the concentration of the milk. As the milk solids concentration is increased, the proportion of κ-casein dissociating at any particular pH is increased, and the pH at which this dissociation first occurs is progressively shifted to lower values (Nieuwenhuijse et al., 1991; Singh and Creamer, 1991a; Anema et al., 1993).

Recently, Anema and Klostermeyer (1997a) showed that a pH-dependent dissociation of the casein micelles could be induced on heating reconstituted skim milk at temperatures below 100 °C. Low levels of casein were rendered soluble at pH below 6.7 regardless of heating temperature, whereas increasing levels of casein were solubilized as the pH was increased from 6.7 to 7.1. This pH-dependent dissociation of the casein micelles showed an unusual dependence on temperature. Low levels of casein were dissociated at 20 °C. The quantity of casein solubilized increased with temperature to a maximum dissociation at about 70 °C, and then decreased at higher temperatures. The dissociation behavior of α_s-casein and β-casein at pH ≥6.7 showed similar dependence on temperature to that of the total casein, i.e., increasing dissociation with increasing temperature to a maximum dissociation at 70 °C and decreased dissociation at higher temperatures. In contrast, the dissociation of κ-casein increased essentially linearly with increasing temperature over the entire temperature range studied.

The present study expands the study of Anema and Klostermeyer (1997a) by examining the effect of milk concentration, heating temperature, and heating time on the pH-dependent dissociation of protein from the casein micelles in reconstituted skim milk over a 20−120 °C temperature range, with particular emphasis on the effect of heating at temperatures below 100 °C.

EXPERIMENTAL PROCEDURES

Milk Supply. Low heat skim milk powders (whey protein nitrogen index above 6; Sanderson, 1970) were obtained from Kiwi Cooperative Dairies, Pahiatua, New Zealand. Experimental skim milk samples were prepared by reconstituting the skim milk powder to 10, 17.5, or 25% (w/w) total solids (TS) in water purified by deionization followed by filtration through a Milli-Q apparatus (Millipore Corp., Bedford, MA). The reconstituted skim milk samples were allowed to equilibrate at ambient temperature (about 20 °C) with gentle stirring for 2 h before further treatment.

Adjustment of pH and Heat Treatments. Reconstituted skim milk samples were adjusted to pH values in the range from 6.3 to 7.1 by the slow addition of 1 M HCl or 1 M NaOH to well-stirred solutions. The pH-adjusted solutions were allowed to equilibrate for 3 h. Subsamples of milk (6 mL) at each pH were transferred to glass vials and heated, with continuous rocking, for the required times in a thermostatically controlled oil bath preset to temperatures in the range from 20 to 120 °C. After heat treatment, the milk samples were cooled to room temperature by immersion of the glass vials in cold running water.

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Turbidity Measurements. The turbidity of the heated skim milk samples was measured at 900 nm using a Shimadzu UV260 spectrophotometer and a 3 mm quartz cell. The average of five turbidity measurements was recorded.

Ultracentrifugation. Soluble caseins were defined as those that did not sediment from the milk during ultracentrifugation at 30 000 rpm (63 000*g* average) at 20 °C in a Beckman L8-80M ultracentrifuge and the associated Beckman Ti-80 rotor (Beckman Instruments Inc., Palo Alto, CA). To partially compensate for the differences in viscosity and concentration of the milk samples, the duration of the centrifuge run was 60, 90, and 120 min for the 10, 17.5, and 25% TS milks, respectively. Weighed aliquots of each milk sample (approximately 15 mL) were transferred to centrifuge tubes and ultracentrifuged. The clear supernatant was carefully removed from the pellets, and the protein content was determined by gel electrophoresis and laser densitometry.

Gel Electrophoresis and Laser Densitometry. Sodium dodecyl sulfate−polyacrylamide gel electrophoresis (SDS−PAGE) was performed using a Bio-Rad mini-gel slab electrophoresis unit (Bio-Rad Laboratories, Richmond, CA) as described previously (Anema and Klostermeyer, 1997a). SDS−PAGE gels were scanned using a Molecular Dynamics Model P.D. computing densitometer (Molecular Dynamics Inc., Sunnyvale, CA). The integrated intensities of the major casein bands were determined using the Imagequant software associated with the densitometer. The quantity of each protein in the ultracentrifugal supernatants was determined as a percentage of that in the original milk sample.

Statistical Analysis. Repeat experiments with milk samples from the same source produced essentially identical results to those presented here. Where appropriate, the results in graphs show the means and an error bar representing the maximum standard deviation observed between the duplicate measurements. In addition, an experiment on four separate reconstituted skim milk samples from different milk powder sources was conducted and subjected to a full statistical analysis. In this latter experiment, the pH was adjusted to 6.5 and 7.1, and the samples were heated at 20, 60, 90, and 120 °C only. The results were analyzed using the generalized linear model procedure (PROC GLM) in SAS (SAS Institute Inc., Cary, NC). Significance was determined at the 0.05 level.

RESULTS AND DISCUSSION

The effect of pH on the turbidity (relative to the sample at 20 °C) for the reconstituted skim milk samples of 10, 17.5, and 25% TS heated at temperatures in the range from 20 to 90 °C for 10 min is shown in Figure 1. The results for the 10% TS milk samples are in good agreement with that reported previously (Anema and Klostermeyer, 1997a). In general, a similar behavior was noted for all three milk concentrations; however, at temperatures above 70 °C, aggregation of the micelles in some of the milk samples introduced variable results. At pH 6.3, the turbidity of the milk increased with increasing heating temperature, which was probably due to the partial aggregation of the casein micelles at this low pH. At pH 6.5, there was only a small change in the turbidity of the milk with temperature up to 60 °C, and a marked, essentially linear increase in turbidity with increasing temperatures above 60 °C. For the milk samples at pH 6.7 or above, the turbidity decreased with increasing temperature to a minimum at about 60 °C and then increased again at higher temperatures. As the pH was increased from 6.7 to 7.1, the magnitude of the minimum in turbidity at 60 °C was greater, and the increase in turbidity at the higher temperatures was less pronounced.

The differences in turbidity behavior with pH and temperature for the three milk concentrations were generally small. The milk samples at higher concentrations showed signs of aggregation of the casein micelles at pH 6.9 and 7.1 and the higher temperatures, as noted by the marked increase in turbidity. The 25% TS samples at pH 6.3 and 6.5 showed evidence of a more marked turbidity decrease at temperatures between 20 and 70 °C than samples at lower milk solids concentrations. The changes in turbidity at higher milk solids and at pH 6.7 or above were less marked for the milks at higher solids concentration.

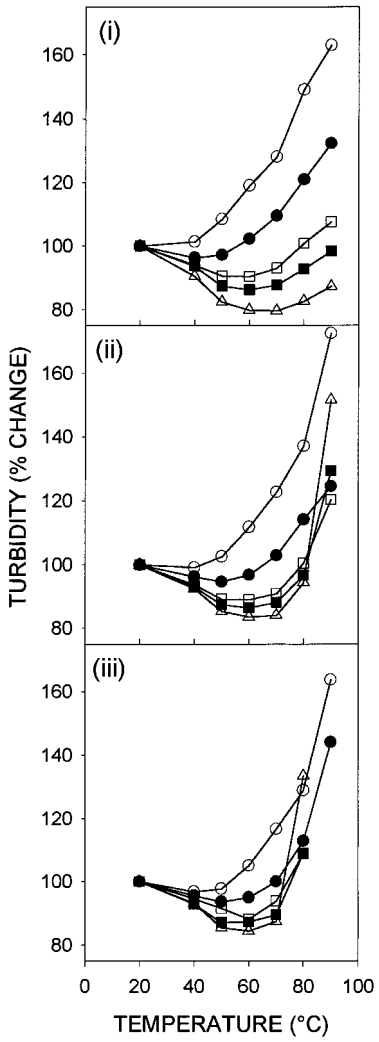

Figure 1. Effect of temperature and pH on the turbidity of reconstituted skim milk samples after heating for 10 min. Results represent the percentage change in turbidity relative to the 20 °C samples. (i) 10% TS milk; (ii) 17.5% TS milk; (iii) 25% TS milk. ○, pH 6.3; ●, pH 6.5; □, pH 6.7; ■, pH 6.9; △, pH 7.1.

Figure 2 shows the changes in turbidity with heating time at pH 6.55 or 6.9 for the milk samples at the three concentrations. For the 10% TS sample at pH 6.55, the turbidity was relatively unchanged on heating at 20, 40, or 60 °C, whereas at higher milk concentrations a small decrease in turbidity was observed with increasing temperature. At 80 and 100 °C, the turbidity increased with increased heating times for all milk concentrations, and this increase was more pronounced at higher temperatures. At pH 6.9, the turbidity progressively decreased with heating to 60 °C, with most of the decrease occurring during the initial heating period. The behavior was similar for all milk concentrations. At 80 °C, the turbidity initially decreased on heating and then increased as the heating time was prolonged, with the

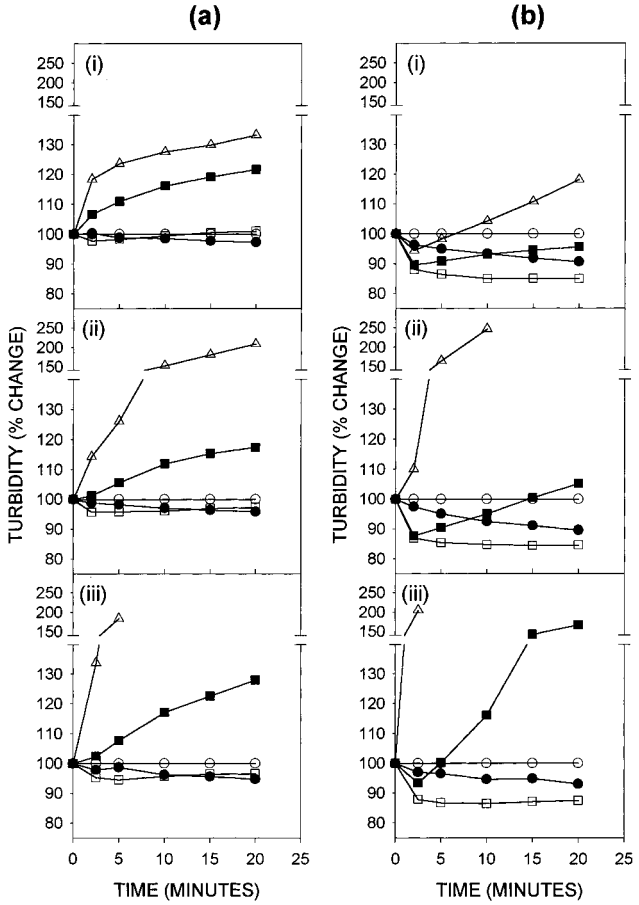

Figure 2. Effect of heating time and temperature on the turbidity of reconstituted skim milk samples. Results represent the percentage change in turbidity relative to the 20 °C samples. (a) pH 6.55. (b) pH 6.9. (i) 10% TS milk; (ii) 17.5% TS milk; (iii) 25% TS milk. ○, 20 °C; ●, 40 °C; □, 60 °C; ■, 80 °C; △, 100 °C.

increase being more pronounced at higher milk solids concentrations. With the exception of the 10% TS sample, which showed a slight initial decrease, the turbidity of the samples heated at 100 °C increased from the onset of heating. The 17.5 and 25% TS samples showed very dramatic increases in turbidity, which indicated the onset of micellar aggregation.

The unusual behavior of the turbidity of heated 10% TS skim milk samples has been reported to be due to a combination of a temperature-dependent dissociation of protein from the casein micelles as the pH of the system is increased and the association of denatured whey proteins with the casein micelles at temperatures above 70 °C (Jeurnink, 1992; Anema and Klostermeyer, 1997a). The turbidity results shown in Figures 1 and 2 suggest that a similar pH-dependent dissociation of casein micelles was occurring in the concentrated milk samples. This supposition was confirmed by an SDS−PAGE analysis of ultracentrifugal supernatants obtained from the heated skim milk samples of 10, 17.5, and 25% TS at a range of pH values (Figure 3). A densitometric evaluation of the casein protein bands [total casein, α_s-casein (α_{s1}-casein and α_{s2}-casein combined), β-casein, and κ-casein] yielded the results shown in Figure 4. The dissociation of these proteins from the milk samples showed similar trends for all three milk solids concentrations, although differences in the quantity of soluble casein were observed. For all three milk concentrations and at 20 °C, the level of total casein in

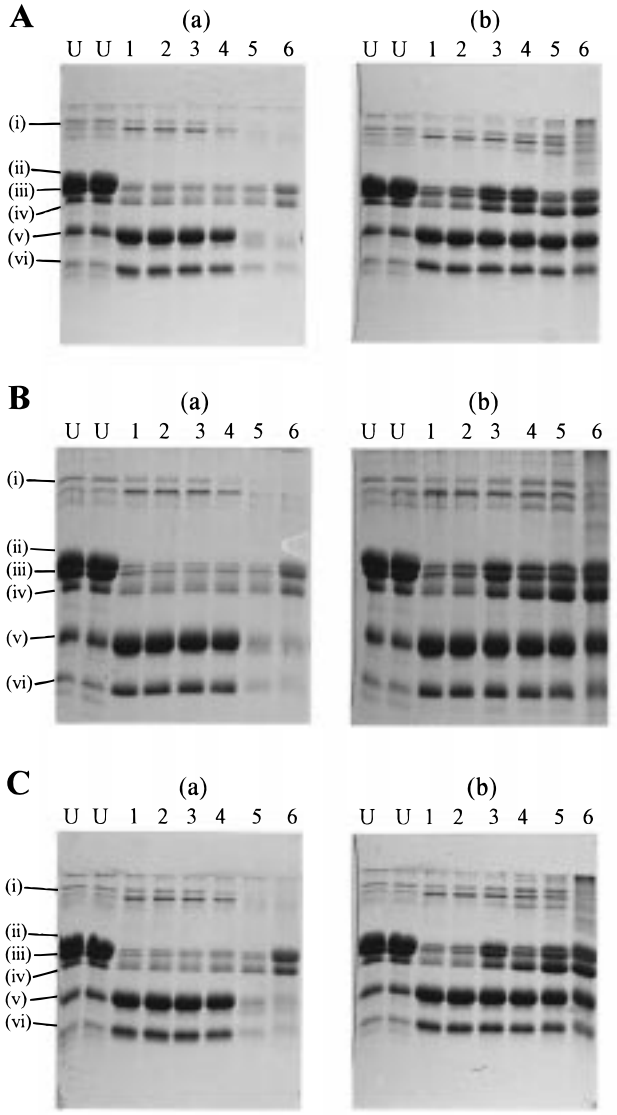

Figure 3. Selected SDS−PAGE patterns of milk samples and supernatants from heated skim milk samples. A, 10% TS milk; B, 17.5% TS milk; C, 25% TS milk. (a) pH 6.3; (b) pH 7.1. Lanes U, unheated milk. Lanes 1−6, supernatants from milk heated at 20, 40, 60, 80, 100, and 120 °C, respectively. (i) Immunoglobulin G, lactoferrin, and bovine serum albumin; (ii) α_s-casein; (iii) β-casein; (iv) κ-casein; (v) β-lactoglobulin; (vi) α-lactalbumin.

the supernatants was low (Figure 4a). At pH 7.1, the proportion of the casein liberated into the serum increased with increasing temperature up to a maximum dissociation at about 60−80 °C, decreased as the temperature was increased to 80−100 °C, and then increased again as the temperature was raised further. A similar temperature dependence of micellar dissociation was observed at pH 6.9 and 6.7; however, lower levels of casein were liberated at each heating temperature as the pH was decreased. Low levels of casein were found in the supernatants from the samples at pH 6.3 and 6.5, although this level increased for the 17.5 and 25% TS samples when heated at 120 °C.

The dependence of α_s-casein and β-casein dissociation on pH and temperature showed similar trends to that observed for the total casein (Figures 4b and 4c, respectively). In contrast, the effect of temperature and pH on the dissociation of κ-casein was different from that of the total casein, α_s-casein, or β-casein (Figure

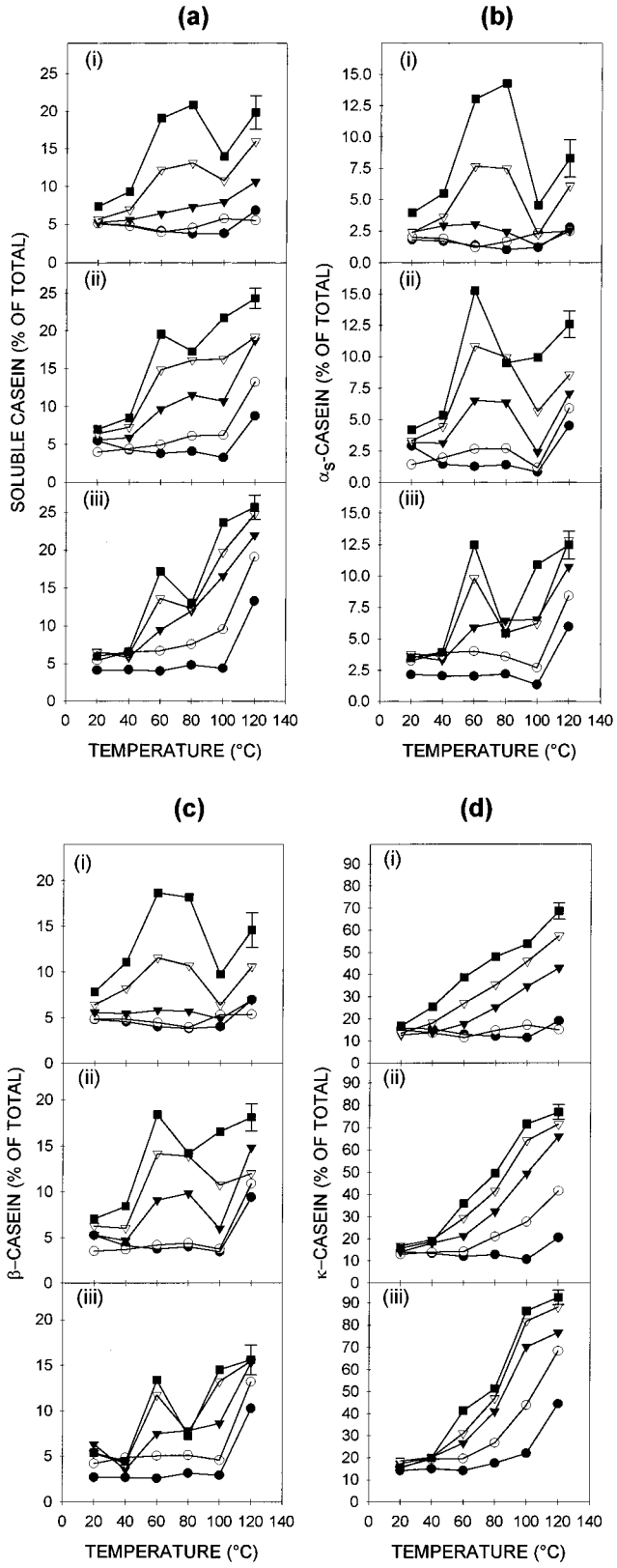

Figure 4. Effect of temperature and pH on the level of protein in the supernatants obtained from (i) 10% TS, (ii) 17.5% TS, and (iii) 25% TS reconstituted skim milk samples. (a) Total casein; (b) α_s-casein; (c) β-casein; (d) κ-casein. ○, pH 6.3; ●, pH 6.5; □, pH 6.7; ■, pH 6.9; △, pH 7.1. Error bars, included on the pH 7.1/120 °C samples, represent the maximum standard deviation observed between duplicate measurements for all results.

4d). For the 10% TS samples at pH 6.3 and 6.5 or the 17.5% TS sample at pH 6.3, low levels of κ-casein were dissociated from the micelles at all heating temperatures. For the 10 and 17.5% TS samples at higher pH and the 25% TS samples at all pHs, the level of κ-casein dissociating from the micelles increased with increasing pH and with increasing temperature throughout the range investigated. For the 25% TS samples, there appeared to be a more marked increase in dissociated κ-casein at temperatures above 80 °C. At any particular temperature or pH, a higher level of κ-casein than β-casein, and a higher level of β-casein than α_s-casein, was dissociated from the micelles, in accord with previous investigations (Singh and Fox, 1985; Singh and Creamer, 1991a; Anema et al., 1993; Anema and Klostermeyer, 1997a).

Four separate reconstituted skim milk samples of 10 and 25% TS, adjusted to pH 6.5 and 7.1, were treated at 20, 60, 90, and 120 °C, and the levels of casein present in the ultracentrifugal supernatants were determined by SDS−PAGE and laser densitometry. At each milk solids concentration, the four reconstituted skim milk samples behaved similarly, although there was some variation in the level of dissociated casein between samples (Figure 5). For both the 10 and 25% TS milk samples, the dissociation behavior of the casein proteins was similar to that observed in Figure 4, indicating that this type of dissociation may occur in all reconstituted skim milk samples and that the effect of pH, temperature, and milk solids concentration was similar in all milk samples.

A statistical analysis of the results in Figure 5 was conducted. For both milk concentrations and for each protein investigated, a similar dissociation level was observed at 20 °C at both pHs. At higher temperatures, a significantly ($p < 0.0001$) higher dissociation level was observed at pH 7.1 than at pH 6.5. For the 10% TS milk at pH 6.5 and for each protein investigated, no significant difference in dissociation level was observed at all temperatures, whereas at pH 7.1 the dissociation levels at adjacent temperatures were significantly different ($p < 0.01$). Similarly for the 25% TS milk samples at pH 6.5 and for total casein, α_s-casein, and β-casein, no significant difference in dissociation levels was observed with temperatures up to 90 °C, although a significantly ($p < 0.01$) higher level was observed at 120 °C. For κ-casein at pH 6.5, a significant ($p < 0.01$) monotonic increase in dissociation level was observed with increasing temperature. For the 25% TS milk samples at pH 7.1 and for the total casein, β-casein, and κ-casein, a significant ($p < 0.007$) increase in dissociation was observed with increasing temperatures, although the differences between 90 and 120 °C were not significant for the total casein and κ-casein. For the α_s-casein at pH 7.1, a significant ($p < 0.0001$) increase in soluble protein is observed between 20 and 60 °C, whereas the 60, 90, and 120 °C responses were consistent with each other.

Some significant differences in the dissociation behavior between the 10% TS and the 25% TS milk samples were observed, which were dependent on the particular protein, the temperature, and the pH. At 120 °C and pH 6.5, a significantly ($p < 0.0001$) higher level of dissociation was observed for the 25% TS milk samples than the 10% TS milk samples, whereas a statistically similar dissociation level was observed at all other temperatures. A significantly ($p < 0.0001$) higher level of κ-casein dissociation was observed for the 25% TS milk samples at 90 and 120 °C at both pHs when compared with the 10% TS milk samples, whereas

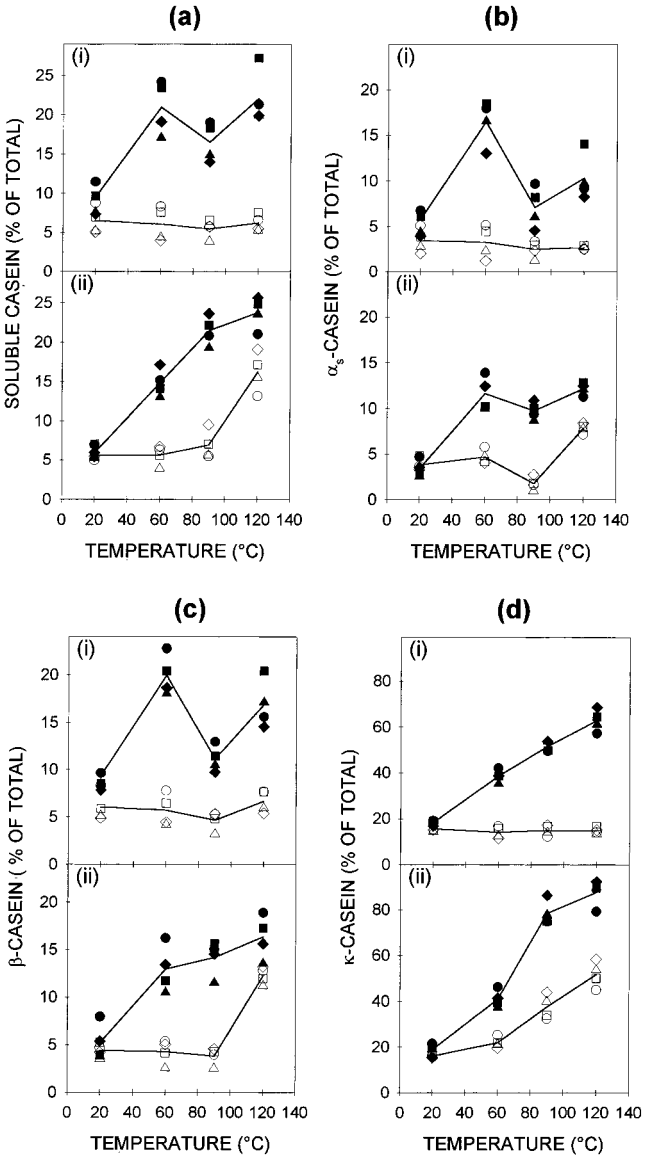

Figure 5. Comparison of the dissociation behavior with pH and temperature for four reconstituted skim milk samples. (i) 10% TS milk; (ii) 25% TS milk. (a) Total casein; (b) α_s-casein; (c) β-casein; (d) κ-casein. (○, ●) milk 1; (□, ■) milk 2; (△, ▲) milk 3; (▽, ▼) milk 4. Open symbols, pH 6.5; closed symbols, pH 7.1.

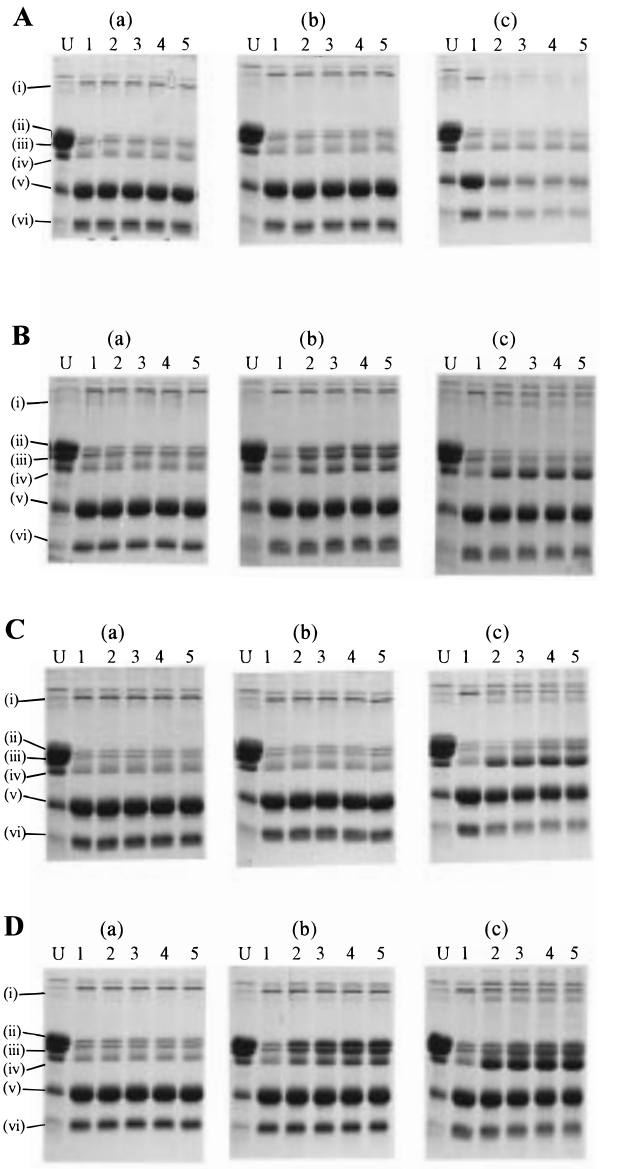

Figure 6. Selected SDS−PAGE patterns of milk samples and supernatants from heated skim milk samples heated for various times. (A) 10% TS milk, pH 6.55; (B) 10% TS milk, pH 6.9; (C) 25% TS milk, pH 6.55; (D) 25% TS milk, pH 6.9. (a) 20 °C; (b) 60 °C; (c) 100 °C. Lanes U, unheated milk. Lanes 1−6, supernatants from milk heated for 0, 5, 10, 15, and 20 min, respectively. (i) Immunoglobulin G, lactoferrin, and bovine serum albumin; (ii) α_s-casein; (iii) β-casein; (iv) κ-casein; (v) β-lactoglobulin; (vi) α-lactalbumin.

the dissociation levels were comparable at lower temperatures. For the total casein, α_s-casein, and β-casein at pH 7.1, a significantly ($p < 0.0001$) higher dissociation level was observed at 60 °C, and a significantly ($p < 0.03$) lower dissociation level was observed at 90 °C for the 10% TS milk when compared with the 25% TS milk, whereas at 20 and 120 °C the dissociation levels were similar between the two milk samples.

Overall, the statistical analysis of the results in Figure 5 indicated that, for each of the proteins of interest, there was a statistically significant difference in dissociation behavior at the two pH values. For the milk samples at pH 7.1, the dissociation levels at adjacent heating temperatures were significantly different, indicating that the shapes of the dissociation profiles with temperature were real. In addition, some interesting differences in dissociation behavior between the 10% and 25% TS milk samples were observed, with the differences being dependent on the temperature, pH, and protein of interest.

The effect of heating time at various temperatures on the protein composition of ultracentrifugal supernatants obtained from the 10% TS milk samples at pH 6.55, 6.9, and 7.1, or the 25% TS milk samples at pH 6.55 and 6.9, is shown in Figure 6, and the densitometric analysis of the major casein protein bands is shown in Figure 7. For the 10% TS milk sample at pH 6.55, low levels of casein were soluble at all heating temperatures and times (Figure 7a). In contrast, at pH 6.9 and pH 7.1, a marked dissociation of the casein micelles occurred. The level of dissociation was dependent on the type of casein protein and the temperature of heat treatment, with a lesser dependence on the duration of the heat treatment (Figure 7b,c). The dissociation behavior of the different casein proteins (total casein, α_s-casein, β-casein, and κ-casein) at any particular heating time was similar to that observed in Figure 4. For the total casein, α_s-

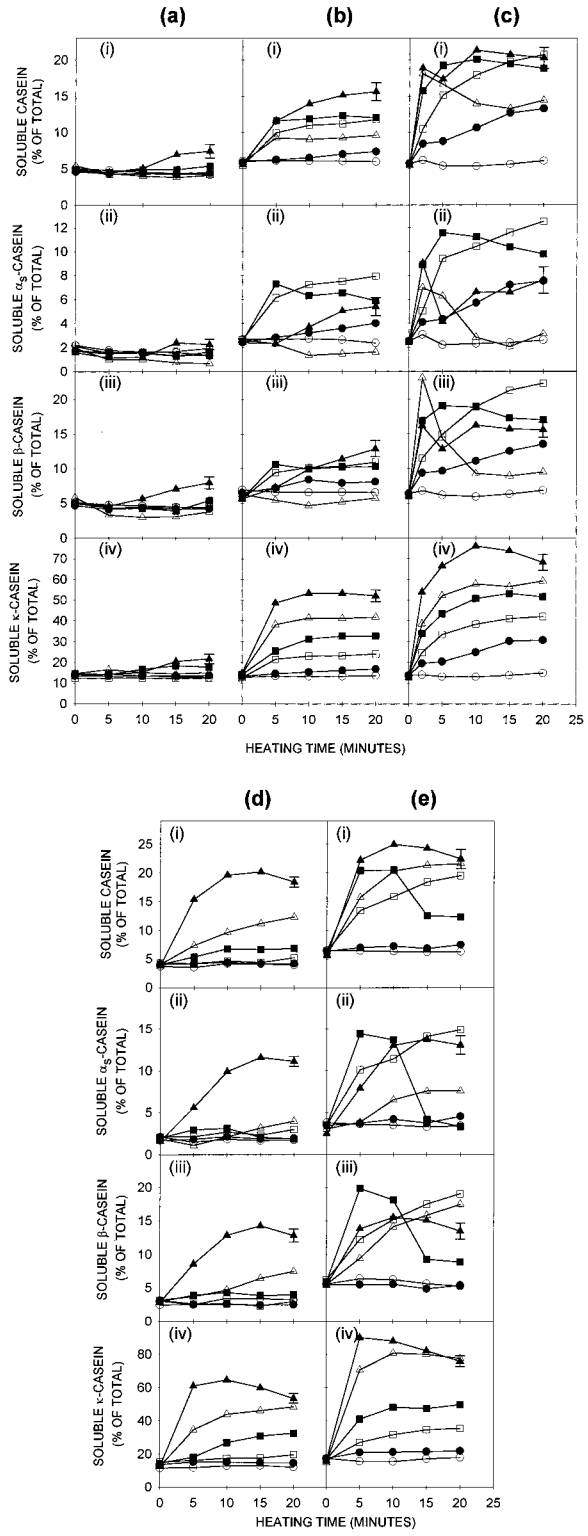

Figure 7. Effect of heating time, temperature, and pH on the level of protein in the supernatants obtained from (a) 10% TS milk, pH 6.55, (b) 10% TS milk, pH 6.9, (c) 10% TS milk, pH 7.1, (d) 25% TS milk, pH 6.55, and (e) 25% TS milk, pH 6.9. (i) Total casein; (ii) α_s-casein; (iii) β-casein; (iv) κ-casein. ○, 20 °C; ●, 40 °C; □, 60 °C; ■, 80 °C; △,100 °C; ▲, 120 °C. Error bars, included on the 20 min/120 °C samples, represent the maximum standard deviation observed between duplicate measurements for all results.

casein, and β-casein, the dissociation increased with temperature to a maximum dissociation at about 60−80 °C, decreased when the temperature was raised to 80−100 °C, and then increased again at 120 °C. In contrast, κ-casein dissociation at any particular heating time increased progressively and essentially linearly with increasing heating temperature throughout the temperature range. In general, the samples at pH 6.9 and 7.1 showed a similar dependence on temperature and heating time, although higher levels of dissociation were observed at the higher pH.

For the 25% TS milk samples at pH 6.55, little casein was dissociated at temperatures up to 60 °C, whereas at higher temperatures the level of dissociated casein increased with increasing temperature. The levels of soluble α_s-casein and β-casein were low at temperatures up to 100 °C, whereas these levels increased with heating time at 120 °C. The level of soluble κ-casein increased with increasing temperature, although the levels of dissociation at temperatures up to 60 °C were only slightly higher than for the samples at 20 °C. At pH 6.9, the dissociation of total casein, α_s-casein, and β-casein followed similar trends, and showed a complex dependence on temperature. At any particular heating time, the dissociation level increased with temperature to a maximum dissociation at about 60−80 °C, decreased between 80 and 100 °C, and then increased again at 120 °C. Only the sample at 80 °C showed a major dependence on heating time, with a marked increase in dissociation at the early stages of heating and the levels decreasing markedly on prolonged heating. At any particular heating time, the level of κ-casein dissociation increased with heating temperature, with a marked increase in dissociation above 80 °C. At both pH values, the dissociation of κ-casein was relatively unaffected by the heating time; however, at 120 °C, the level of dissociation appeared to decrease slightly on prolonged heating. This was probably due to changes in the staining intensity of κ-casein on the SDS−PAGE system as a consequence of heat-induced protein modifications, as has been reported previously (Anema and Klostermeyer, 1997b).

Although there are numerous reports on the pH-dependent dissociation of micellar casein when milk samples of various concentrations are heated (Kudo, 1980; Singh and Fox, 1985; Nieuwenhuijse et al., 1991; Singh and Creamer, 1991a,b; Anema et al., 1993), comparisons with the present study are limited as these earlier studies examined the dissociation behavior at a single heating temperature, usually 120 °C. The dissociation of casein micelles over the entire 20−120 °C temperature range is similar to that reported to occur at high temperatures in that κ-casein is the major protein dissociated; however, a substantial proportion of the α_s-casein and β-casein is dissociated, particularly at temperatures below 100 °C. The observed effect of milk concentration on the dissociation behavior of κ-casein, especially at temperatures above 80 °C, where increased levels of κ-casein are rendered soluble at higher milk concentrations, is in agreement with earlier observations (Singh and Creamer, 1991a; Nieuwenhuijse et al., 1991; Anema et al., 1993; Anema and Klostermeyer, 1997b).

The effect of the heating time on the levels of soluble casein indicates that the dissociation process occurs rapidly, with the majority occurring during the first minutes of heating. There is little change in the dissociation level on prolonged heating. These results are in accord with the reports of Anema and Klostermeyer (1997b) and Nieuwenhuijse et al. (1991) on the effect of heating time on κ-casein dissociation. Singh

and Creamer (1991b) reported that the level of dissociation of α_s-, β-, and κ-casein from the casein micelles in 25% TS milk samples at pH 6.55 and 6.85 increased progressively with heating time at 120 °C and that the maximum dissociation was similar at both pH values; however, a longer heating time was required to attain maximum dissociation at the lower pH values. In the current investigation, the levels of soluble α_s-casein and β-casein after heating at 120 °C were similar at the two pH values; however, the level of κ-casein was markedly higher at pH 6.9 than at pH 6.55 at all heating times.

The temperature-dependent change in dissociation behavior at about 60−80 °C corresponds to the temperature at which the whey proteins begin to denature (Dannenberg and Kessler, 1988). The change in dissociation behavior at this temperature may be a consequence of the interaction of the denatured whey proteins with κ-casein. The complex formed between κ-casein and denatured β-lactoglobulin is less effective at stabilizing α_s-casein and β-casein in the presence of calcium ions than uncomplexed κ-casein (Zittle et al., 1962). It is possible that, at temperatures below 70 °C, the dissociated α_s-casein and β-casein are stabilized by the dissociated κ-casein and remain in the serum as small soluble aggregates. The stabilizing action of κ-casein is reduced on complexing with β-lactoglobulin; therefore, either α_s-casein and β-casein do not dissociate from the micelles or the dissociated casein precipitates in the presence of calcium on cooling and deposits with the casein micelles on ultracentrifugation.

The mechanism by which the casein is dissociated from the micelles remains unknown. Casein micelle structure is maintained by CCP, hydrophobic interactions, and, presumably, other forms of electrostatic interactions. It is reasonable to assume that a temperature- and pH-induced modification to one or more of these effects is responsible for micellar disaggregation. Hydrophobic interactions reportedly increase with temperature to a maximum at about 80 °C and have little dependence on pH (Tanford, 1980). Therefore, changes to hydrophobic interactions are unlikely to explain the observed effects of temperature and pH on micelle dissociation, although they may play a role in the change in dissociation behavior at temperatures above 60 °C. The dependence of the dissociation on pH suggests that electrostatic interactions may play a critical role. Anema and Klostermeyer (1997a) suggested that the dissociation of casein micelles may occur through changes in the nature of the CCP at elevated pH and temperatures to a form less capable of maintaining micellar structure, particularly at higher pH where the charge on the proteins is greater. However, the experimental evidence required to support this hypothesis, such as detailed investigations on changes to the composition and structure of the CCP in milk at various temperature and pH combinations, is lacking.

The results of this study have demonstrated that there is a considerable pH- and temperature-dependent dissociation of casein protein from the micelles over the entire 20−120 °C temperature range, and that a similar dissociation behavior is observed at all milk solids concentrations. The dissociation is a rapid phenomenon, occurring during the early stages of heating. The dissociation level is primarily dependent on the temperature of the heat treatment and, in general, only slightly dependent on the duration of the heat treatment. Further research on this dissociation behavior is currently in progress, particularly the role of whey proteins and mineral components, and a comparison of the present results with those from fresh skim milk counterparts.

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