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Control of Heat-Induced Aggregation of Whey Proteins Using Casein

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The ability of α_{s1}/β-casein and micellar casein to protect whey proteins from heat-induced aggregation/precipitation reactions and therefore control their functional behavior was examined. Complete suppression (>99%) of heat-induced aggregation of 0.5% (w/w) whey protein isolate (pH 6.0, 85 °C, 10 min) was achieved at a ratio of 1:0.1 (w/w) of whey protein isolate (WPI) to α_{s1}/β-casein, giving an effective molar ratio of 1:0.15, at 50% whey protein denaturation. However, in the presence of 100 mM NaCl, heating of the WPI/α_{s1}/β-casein dispersions to 85 °C for 10 min resulted in precipitation between pH 6 and 5.35. WPI heated with micellar casein in simulated milk ultrafiltrate was stable to precipitation at pH >5.4. Protein particle size and turbidity significantly ($P \leq 0.05$) increased from an initial diameter of 165.5 nm in the unheated mixture to 272 nm following heating at 85 °C for 10 min at pH 6. Whey protein denaturation was significantly ($P \leq 0.05$) promoted when heated in the presence of micellar casein, but whey protein aggregation was controlled down to pH 5.4. The protective behavior of α_{s1}/β-casein and micellar casein differed in that the former inhibited denatured whey protein aggregation, whereas the latter system promoted denaturation but controlled aggregation.

KEYWORDS: Heat-induced aggregation; whey protein protection; α_{s1}/β-casein; whey protein isolate; micellar casein

INTRODUCTION

Recent studies have suggested that casein, which exists in nature as an unfolded random coil protein, has chaperone-like functions (*1*−*3*). Chaperone proteins in nature can prevent irreversible aggregation of proteins induced by thermal as well as nonthermal stress by providing hydrophobic surfaces to unfolding proteins. The important features deemed to be responsible for the chaperone activity of α_{s1}-casein are high hydrophobicity characterized by Bigelow's parameter of 1170 (*4*) along with high estimated net negative charge of ≈22 at pH 6.5 (*5*). The highly flexible nature due to the relatively high content of proline (8.5%) distributed uniformly along the chain together with the absence of a cystine group in the sequence also contributes to the chaperone-like activity.

Bhattacharyya and Das (*1*) identified α_{s1}-casein as having chaperone-like functions. It was shown that α_{s1}-casein could inhibit visual turbidity development of whey protein isolate (WPI) on heating at pH 6.6 in 10 mM phosphate buffer. Morgan et al. (*2*) showed that α_{s1}-casein could suppress the heat-induced aggregation of β-lactoglobulin at pH 7.0 in the presence of 0.2 M NaCl. Matsudomi et al. (*3*) have shown that α_{s1}-casein suppressed the heat-induced aggregation (80 °C) of ovotransferrin at pH 7.0. They suggested that α_{s1}-casein possibly interacted with the exposed hydrophobic surface of heat-denatured ovotransferrin and then the polyanion on the surface of the hydrophobically bonded casein−ovotransferrin complex prevented the coalescence of the complex by their repulsive electrostatic forces, thus preventing aggregation of the ovotransferrin. It has previously been shown that whole casein prevented gross heat-induced aggregation of whey proteins through nonspecific interaction, even in calcium-containing systems (*6*, *7*).

This study investigated the ability of α_{s1}/β-casein and micellar casein to modify the aggregation behavior of whey proteins during heat-induced denaturation under more extreme conditions of pH and ionic strength.

MATERIALS AND METHODS

Materials. WPI [93.3% (w/w) protein, 0.8% (w/w) fat, 1.7% (w/w) ash, and 4.3% (w/w) moisture] was obtained from Davisco International Inc. (Le Seuer, MN). Micellar casein [80.3% (w/w) protein, 7.9% (w/w) ash, 4.3% (w/w) fat, 1.9% (w/w) lactose, and 5.6% (w/w) moisture] was prepared in-house as described by Kelly et al. (*8*). α_{s1}/β-casein was prepared as described previously (*9*). All other chemicals were of analytical reagent grade and supplied by BDH (Poole, U.K.).

Preparation of Dispersions. WPI or α_{s1}/β-casein powder was dissolved in distilled/deionized water or 100 mM NaCl. The pH of solutions was adjusted using 1 N NaOH or 1 N HCl. Micellar casein/WPI mixtures were always dispersed in simulated milk ultrafiltrate (SMUF; *10*).

Heat Treatment and Measurement of Turbidity. All protein solutions were heated in tightly capped glass vials containing 1 mL of sample at various pH values in a water bath at 85 °C. At various time periods (from 1 to 10 min), vials were removed and immediately cooled

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in ice water to 4 °C. The turbidity was estimated through measurement of absorbance of the diluted sample at 600 nm in a Hitachi U-1100 spectrophotometer (Hitachi Ltd., Tokyo, Japan) and expressed as relative units (RU).

Measurement of Whey Protein Denaturation. Following the heating/cooling step, 0.5 mL of acetic acid/sodium acetate buffer (0.5 N, pH 4.7) was added to 0.5 mL of sample. The samples were centrifuged at 14000*g* for 10 min in an Eppendorf centrifuge 5417C (Unitech, Dublin, Ireland). The absorbance of the supernatant (appropriately diluted) was determined at 280 nm. Hellma quartz cuvettes (Hellma GmbH and Co., Mulheim, Germany) were used in a Hitachi U-1100 spectrophotometer (Hitachi Ltd.).

Measurement of Particle Size. Particle size analysis was performed at 22 °C using a Malvern Zetamaster (model 7EM; Malvern Instruments Ltd., Worcester, U.K.). The cumulative method was used to find the mean average (*z*-average) or the size of a particle that corresponds to the mean of the intensity distribution. Samples were diluted in water or SMUF at the appropriate pH value to come within the desired limits of the Zetamaster (60−90 kilocounts s^{-1}).

Statistical Analysis. The preparation of all dispersions and subsequent analyses on them were performed in triplicate. Analysis of variance (ANOVA) was carried out using SigmaStat (version 3.0; Jandel Scientific, Corte Madera, CA). Tukey's multiple-comparison test was used to determine differences between treatment means. Treatment means were considered to be significantly different at $P \leq 0.05$.

RESULTS AND DISCUSSION

Statistical Analysis. All dispersions were prepared in triplicate and subsequent analyses on them were performed in triplicate. Treatment means were considered to be significantly different at $P \leq 0.05$. Each curve shown in **Figures 1−8** represents the mean of triplicate trials, and the vertical bars shown in the figures represent the standard deviation between means.

Effects of α_{s1}/β-Casein on Heat-Induced Aggregation of WPI. Whereas most studies on the suppression of whey protein aggregation use α_{s1}-casein (*1*−*3*), a combination of α_{s1}- and β-casein was used in this study. This preparation was significantly depleted in both κ- and α_{s2}-casein as determined by HPLC. Both κ- and α_{s2}-casein contain disulfide bonds, which in theory could be susceptible to heat-induced thiol−disulfide interchange reactions with the free thiol group of unfolded β-lactogloblin (β-lg). In this part of the study, interaction with the casein, therefore, centered on the ability of casein to interact with unfolded whey protein molecules by physical or nonspecific interactions.

Figure 1 shows the effect of pH of heating on the turbidity development and particle size of insoluble protein aggregates formed from 0.5% (w/w) WPI heated at 85 °C for 10 min. Turbidity (0.0155 ± 0.001 RU) was evident on heating at pH 6.3, reaching a maximum at pH 6.0 (1.43 ± 0.13 RU). The mean protein particle size increased significantly ($P \leq 0.05$) from 89.6 ± 5.6 nm at pH 6.62 to 258.4 ± 18.8 nm at pH 6.0. Heating at pH values <6.0 resulted in precipitation. WPI did not exhibit a significant ($P \leq 0.05$) increase in turbidity when heated in the pH range of 6.35−7.5 under low ionic strength conditions. This pH-dependent aggregation behavior is typical for WPI heated under low ionic strength conditions, at which the denaturation rate is limited by its ability to aggregate at pH 7.0 and by its ability to unfold at pH values close to the isoelectric point (pH 5.2 for β-lg) (*11*, *12*). O'Kennedy et al. (*13*) found that the heat-induced denaturation level of β-lg was significantly higher at pH 5.0 (72% denaturation) compared to that at pH 7.0 (21% denaturation).

The heat-induced behavior of WPI solutions at pH 6.0 is thought to be intermediate between these extremes. These data

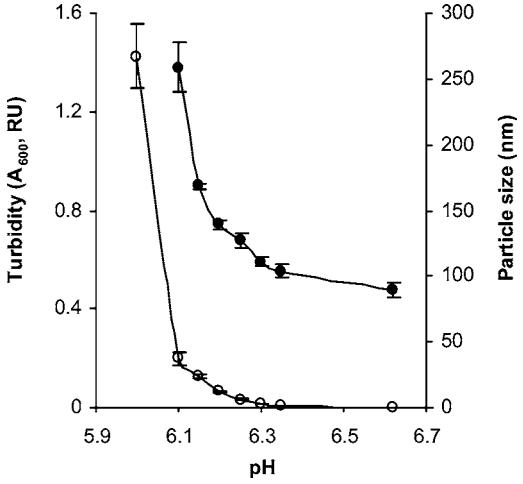

Figure 1. Effect of pH of heating (85 °C, 10 min) on turbidity development (○) and particle size (●) of 0.5% (w/w) WPI. Each curve represents the mean of triplicate trials. Vertical bars show standard deviation between means.

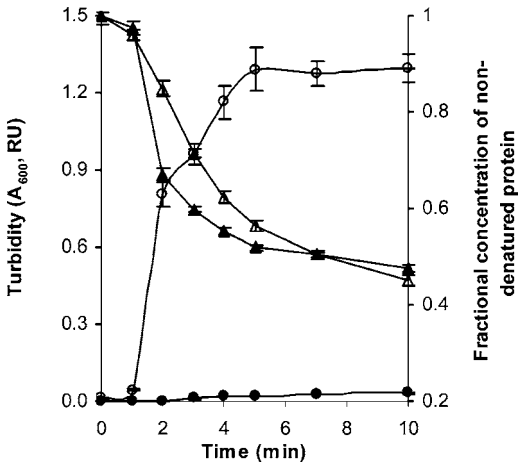

Figure 2. Effect of heating time (85 °C, pH 6.0) on turbidity development (○) of 0.5% (w/w) WPI and (●) 0.5% (w/w) WPI with 0.5% (w/w) α_{s1}/β-casein and fractional concentration of non-denatured protein (△) 0.5% (w/w) WPI and (▲) 0.5% (w/w) WPI with 0.5% (w/w) α_{s1}/β-casein. Each curve represents the mean of triplicate trials. Vertical bars show standard deviation between means.

suggested that suppression of heat-induced whey protein aggregation by casein at pH 6 should provide a challenging starting point for the protective behavior of the casein.

In the absence of casein, WPI (mainly β-lg) denatures and aggregates, when heated at pH 6, to form "stable" protein particles. The development of turbidity on heating of WPI solutions (0.5%, w/w) with and without α_{s1}/β-casein (0.5%, w/w) at pH 6.0 and 85 °C for various times is outlined in **Figure 2**. In the absence of casein, WPI showed significant ($P \leq 0.05$) increases in turbidity from 0.012 ± 0.001 to 1.16 ± 0.065 RU with increasing heating time from 0 to 4 min, after which the turbidity remained constant. A 1:1 (w/w) ratio of α_{s1}/β-caseinn/WPI resulted in a significant ($P \leq 0.05$) reduction in the extent of aggregation of the WPI (turbidity ≤ 0.03 RU). On the other hand, when the α_{s1}/β-casein solution was heated separately, the solution was still transparent at the concentrations used in this study. **Figure 2** also shows the levels of non-denatured protein on heating of WPI solutions (0.5%, w/w) or WPI solutions (0.5%, w/w) with α_{s1}/β-casein (0.5%, w/w) at pH 6.0 and 85 °C for various times. No significant difference ($P \leq 0.05$) in levels of denaturation was observed after heating at 85 °C for

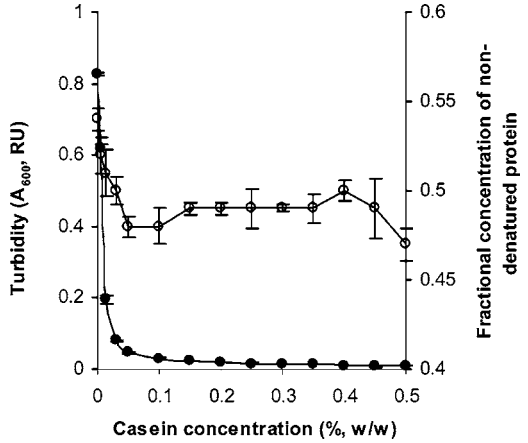

Figure 3. Effect of α_{s1}/β-casein concentration (0–0.5%, w/w) on heat-induced (85 °C, 10 min, pH 6.0) turbidity (●) and fractional concentration of non-denatured protein (○) of 0.5% (w/w) WPI solutions. Each curve represents the mean of triplicate trials. Vertical bars show standard deviation between means.

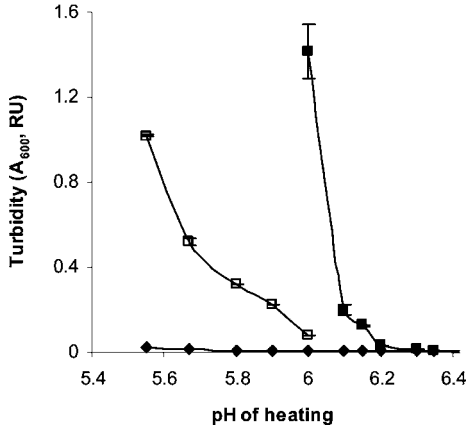

Figure 4. Effect of the pH of heating (85 °C, 10 min) on the turbidity of solutions of 0.5% (w/w) WPI (■) and 0.5% (w/w) WPI with 0.2% (w/w) α_{s1}/β-casein (□) or 0.2% (w/w) α_{s1}/β-casein (◆). Each curve represents the mean of triplicate trials. Vertical bars show standard deviation between means.

10 min between heated solutions of WPI (52.3 ± 0.64%) and the combination of WPI and α_{s1}/β-casein (54.8 ± 1.1%).

The effect of increasing concentrations of α_{s1}/β-casein (0−0.5%, w/w) on the development of turbidity and levels of denatured whey protein on heating WPI solutions (0.5%, w/w) at 85 °C and pH 6.0 for 10 min is outlined in **Figure 3**.

Heating WPI (0.5%, w/w) at 85 °C for 10 min at pH 6.0 resulted in a turbidity of 0.826 ± 0.011 RU. With the increased addition of α_{s1}/β-casein (0−0.5%, w/w), this turbidity was significantly ($P \leq 0.05$) reduced. Complete suppression of heat-induced aggregation was achieved at a 1:0.2 (w/w) ratio of WPI/casein, corresponding to a mole ratio of 1:0.15, if it is assumed that WPI is mainly composed of β-lg. However, a maximum of ≈50% denaturation of WPI was possible under these conditions of heating (pH 6.0, 85 °C, 10 min), giving an effective molar ratio of 1:0.3 (WPI/casein) in the soluble aggregates. However, the suppression of WPI aggregation was found to be >99% at a ratio of 1:0.1 (w/w), giving an effective molar ratio of 1:0.15.

Heating WPI (0.5%, w/w) at 85 °C for 10 min at pH 6.0 resulted in 46.0 ± 1.1% whey protein denaturation. However, heating the WPI in the presence of increasing levels of α_{s1}/β-casein (up to 0.5% w/w) resulted in whey protein denaturation levels ranging from 48 to 53%, which were not significantly ($P \leq 0.05$) different from the level of denaturation obtained form heating WPI in the absence of α_{s1}/β-casein.

The results indicate that ≈50% of the WPI was in an unfolded conformation but was not aggregated to a degree which could contribute to turbidity development. In agreement with previous authors (*1*−*3*) these results suggest that heat-induced whey protein aggregation was suppressed through an alternative interaction between casein and denatured whey protein in a concentration-dependent manner. The absence of thiol−disulfide interchange reactions between the casein and the denatured whey protein suggests the interactions were of a non-covalent nature and were therefore potentially reversible. Due to the extended structure of casein it is conceivable that it can provide the template to bind, either hydrophobically or electrostatically, 6−7 mol of β-lg for each mole of casein.

Although heating WPI at pH 6, under low ionic strength conditions, resulted in a turbid suspension of protein particles that were stable to spontaneous sedimentation, heating at pH values <6 resulted in gross precipitation. The effect of pH (<6) on the heat-induced aggregation of WPI (0.5%, w/w) in the presence (0.2%, w/w) or absence of casein is outlined in **Figure 4**. As the pH of heating was reduced to 5.5 the turbidity of the cooled mixtures increased. However, the turbidity or WPI aggregation with α_{s1}/β-casein was significantly ($P \leq 0.05$) suppressed compared to that of WPI solutions heated alone. Whereas the turbidity (0.025 ± 0.001 RU) of caseinate solutions was unchanged following the heating cycle at pH 5.5, it was observed that the casein was aggregated (turbid) at 85 °C but reversed on cooling (visually clarified). At pH 5.5 the charge on the casein has been severely reduced, and although the solution was clear at room temperature, its capacity to aggregate at elevated temperatures suggested that the balance of forces (electrostatic, hydrophobic) had been altered. Increasing the casein concentration to 0.3% (w/w) significantly ($P \leq 0.05$) decreased the aggregation of the protein mixture; however, further increases had no significant effect ($P \leq 0.05$) (results not shown). The denaturation/aggregation of WPI increased from 50% at pH 6.0 to 70% at pH 5.5. This is in agreement with O'Kennedy et al. (*13*), who found that the denaturation of β-lg at 78 °C was minimal at pH 6.0 but increased as the pH moved toward pH 5.0. It was concluded that the casein solution used in this study still exhibited significant protective properties in controlling the aggregation of WPI at increasingly acidic pH (5.5). It is also suggested that the efficiency of protective behavior was limited by the inherent inability of casein to hydrate fully at pH values <5.5 under low ionic strength conditions.

Effect of NaCl on WPI Aggregation in the Presence of α_{s1}/β-Casein. It was observed that the casein fraction used in this study could not be reliably dispersed at pH values <5.5, prior to mixing with the WPI solutions and heating. In the presence of 100 mM NaCl, dispersions could be achieved at (1%, w/w) concentrations down to pH 5.15. Heating the casein dispersions at these pH values resulted in clear solutions with minimal turbidity development (<0.02 RU). In contrast, heating the WPI/casein dispersions at pH 5.25 and 5.15 resulted in the formation of stable milky dispersions. However, subsequent heating of the WPI/casein dispersions to 85 °C for 10 min resulted in precipitation between pH 6 and 5.35. Matsudomi et al. (*3*) reported that the ability of α_{s1}-casein to suppress heat-induced ovotransferrin aggregation was weakened by the presence of NaCl. It was therefore suggested that the suppression of ovotransferrin aggregation by α_{s1}-casein was a result of ionic

interaction with phosphoserine residues. The results of the present work indicate that the interactions between casein and whey protein, under low ionic strength conditions, were also electrostatic in nature.

Complex coacervation is caused by the interaction between two oppositely charged colloids (*14*). De Kruif et al. (*15*) have suggested that soluble protein−polyelectrolyte complex formation can occur under low-salt conditions on the "wrong" side of the isoelectric point. This is ascribed to heterogeneity of the surface charge distribution on the protein surfaces. In the present work, it can be suggested that α_{s1}/β-casein, due to its innate lack of solubility at pH ~5.5 under low-salt conditions, was actually quite close to the effective isoelectric point, and thus complex coacervation with the whey protein was a possibility.

Overbeek and Bungenburg de Jong (*16*) ascribed salt effects in protein solutions to the ease with which ionizable groups can dissociate. They saw the effect as charge suppression between the various charge-carrying groups on the protein, and consequently the dissociation proceeded more easily at the same pH. More recently, de Kruif and Zhulina (*17*) used a salted brush theory to describe casein micelle behavior. The salted brush is characterized by the fact the charges along the chain are well screened by salt so that neighboring charges, on either the same or different chains, only weakly interact. Results showed that casein−casein interactions were inhibited in the presence of 100 mM NaCl, as evidenced by its increased solubility down to pH 5.15 (pH 5.5 in the absence of NaCl). However, the increased ionic strength may also have adversely inhibited its ability to control whey protein aggregation for similar reasons.

Conversely, under low ionic strength conditions casein was able to instantly interact with unfolded whey protein molecules, thus inhibiting their aggregation. It was interesting to observe that the only window of whey protein aggregation control in the presence of casein and 100 mM NaCl occurred at pH 5.15−5.25, at which the casein charge was severely reduced. In theory, therefore, any factor that reduced the effective charge on the casein and/or the whey protein should promote an association between the molecules. However, it is likely that the addition of salt after heating of dispersions of α_{s1}/β-casein and whey protein would have dissociated the preformed complexes resulting in precipitation of the denatured whey protein entity.

Effect of Micellar Casein on the Aggregation of WPI under High Ionic Strength Conditions. Although α_{s1}/β-casein has the capability to stabilize the aggregation of WPI under low ionic strength conditions, it loses this ability to protect in the presence of 100 mM NaCl. One method of suppressing the charge on casein is to preaggregate the protein in a controlled fashion. This could be as micelles as they occur in milk, where the charge has been reduced through calcium and calcium phosphate binding to negatively charged phosphoserine and carboxyl groups.

As a preliminary step to the introduction of micellar casein into WPI solutions, prior to heating, the effect of heating WPI in a SMUF solution was determined. This was necessary as the micellar casein has to be dispersed in SMUF to maintain its integrity. The effect of heating WPI (5 mg/mL) in SMUF is outlined in **Figure 5**. The pH was varied from 7.0 to 6.0, and the turbidity following heating/cooling was determined. Turbidity development following heating was pH-dependent, significantly ($P \leq 0.05$) increasing from 0.30 ± 0.012 RU at pH 7.0 to 8.71 ± 0.07 RU at pH 6.0. However, similar precipitation behavior was observed on heating at pH 6 as occurred when WPI was heated in the presence of 100 mM NaCl at pH 6. The denaturation level following heating WPI (5 mg/mL) in SMUF

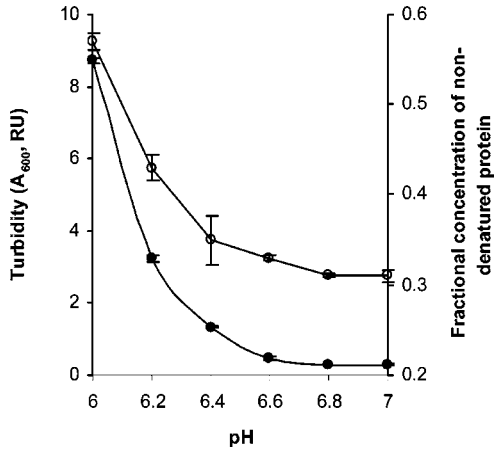

Figure 5. Effect of pH of heating (85 °C, 10 min) of 0.5% (w/w) WPI solutions in SMUF on turbidity development (●) and fractional concentration of non-denatured protein (○). Each curve represents the mean of triplicate trials. Vertical bars show standard deviation between means.

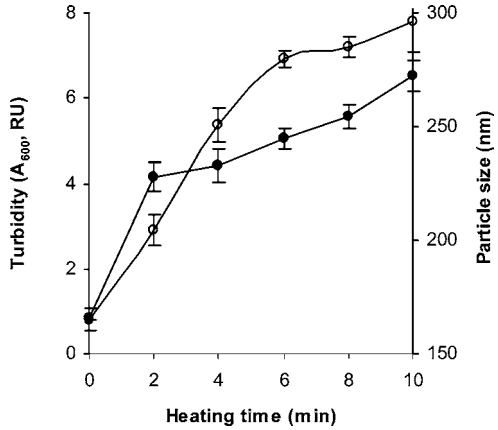

Figure 6. Effect of heating time (85 °C, pH 6.0) on turbidity development (○) and particle size (●) of mixtures of WPI (0.5%, w/w) and micellar casein (0.5%, w/w) in SMUF. Each curve represents the mean of triplicate trials. Vertical bars show standard deviation between means.

significantly ($P \leq 0.05$) increased from 43.2 ± 0.7% at pH 6.0 to 68.2 ± 0.44% at pH 7.0. This again suggested a minimum in denaturation on heating at pH 6.0 but with maximal development of turbidity and visual precipitation.

Development of turbidity following heating (85 °C for 0−10 min) of WPI (0.5%, w/w) in the presence of micellar casein (0.5%, w/w), in SMUF at pH 6, is outlined in **Figure 6**. The turbidity of the WPI/micellar casein mixtures significantly ($P \leq 0.05$) increased from 0.80 ± 0.012 to 7.82 ± 0.65 RU as the time of heating increased form 0 to 10 min. Micellar casein in SMUF, at the concentration used in this study, is quite stable to heating at 85 °C at this pH. However, no precipitation of the whey protein in the mixture was evident on heating at pH 6.0. It was observed that the protein particle size significantly ($P \leq 0.05$) increased from an initial diameter of 165.5 ± 4.8 nm in the unheated mixture to 272 ± 6.8 nm following heating for 10 min (**Figure 6**). The increase in turbidity was positively correlated to an increase in particle size; however, uncontrolled precipitation of the whey protein was prevented. It was concluded that micellar casein could control the aggregation of the whey protein during the heat-induced denaturation step, even though the casein micelles were dispersed in a relatively high ionic strength solvent.

The effect of decreasing micellar casein concentration (0.5−0.1%, w/w) on the heat-induced (85 °C, 10 min, pH 6) protein

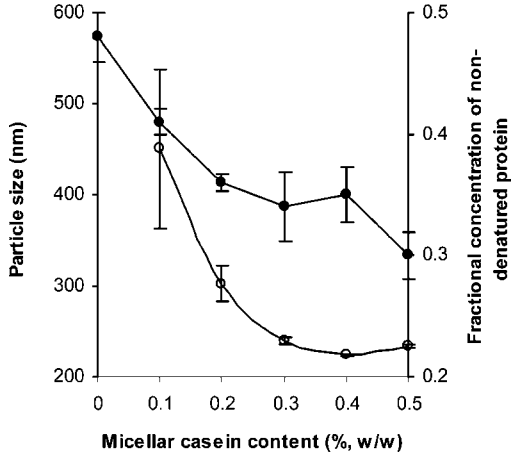

Figure 7. Effect of micellar casein concentration on the protein particle size (○) and fractional concentration of non-denatured protein (●) following heating of WPI 0.5% (w/w)–0.5% (w/w) casein mixtures (85 °C, 10 min, pH 6.0, SMUF). Each curve represents the mean of triplicate trials. Vertical bars show standard deviation between means.

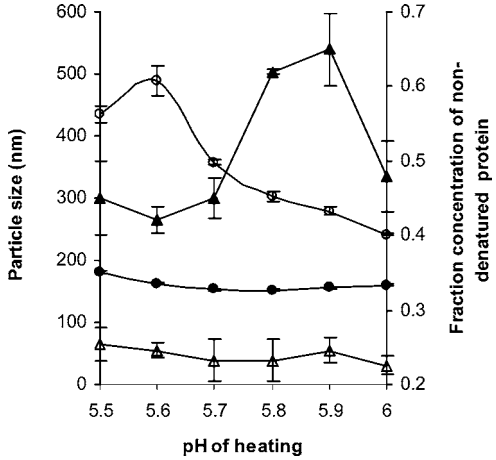

Figure 8. Effect of pH of heating (85 °C, 10 min, SMUF) on the particle size [○, mixture of 0.5% (w/w) WPI and 0.5% (w/w) micellar casein; ●, 0.5% (w/w) micellar casein] and fractional concentration of non-denatured protein [△, mixture of 0.5% (w/w) WPI and 0.5% (w/w) micellar casein; ▲, 0.5% (w/w) WPI]. Each curve represents the mean of triplicate trials. Vertical bars show standard deviation between means.

particle size and whey protein denaturation level is outlined in **Figure 7**. The mean protein particle size, in this experiment, significantly ($P \leq 0.05$) increased from 234 ± 2.2 nm when (0.5%, w/w) micellar casein was present in the mixture prior to heating to 450 ± 87 nm when the casein concentration was reduced to 0.1% (w/w). The level of whey protein denaturation was shown to significantly increase ($P \leq 0.05$) from 52.1 ± 1.6% when the WPI was heated in water to 70.8 ± 1.3% when the WPI was heated in 5 mg/mL micellar casein. Simple calculations suggested that 0.7 mg of denatured whey protein associated with 1 mg of casein in heated mixtures containing 0.5% (w/w) casein. When only 0.1% (w/w) casein was present during heating of the mixture, 2.95 mg of whey protein was associated with 1 mg of casein. The whey protein load on the casein micelle therefore dictated the protein particle size. Vasbinder and de Kruif (*18*) noted that all denatured whey protein was associated with the casein micelle following heating at pH 6.35. Whereas initial experimentation with α_{s1}/β-casein eliminated the possibility of thiol−disulfide interchange contributing to the interaction between casein and denatured whey proteins, heat-induced micellar casein-denatured whey protein interactions could involve thiol−disulfide interchange due to the presence of both κ- and α_{s2}-casein. Thiol−disulfide interchange reactions are less likely to occur at acidic pH (*17*); however, Vasbinder et al. (*20*) have shown that the formation of additional or secondary disulfide bond during acidification of preheated milk is not only possible but a requirement for optimal structure development in fermented milks.

There was no significant ($P \leq 0.05$) difference in the particle size of 0.5% (w/w) micellar casein dispersions when heated at pH values between 6.0 (160.2 ± 1.2 nm) and 5.6 (162.9 ± 1.6 nm; **Figure 8**). However, a significant ($P \leq 0.05$) increase in casein particle size to 180.6 ± 3.5 nm was observed on heating at pH 5.5. When WPI (0.5%, w/w)/phosphocasein (0.5%, w/w) mixtures were heated under gradually reduced pH conditions, a significant ($P \leq 0.05$) increase in particle size was observed (241 ± 1.06 nm at pH 6; 435 ± 13.9 nm at pH 5.5). The level of whey protein denaturation (≈75%) in WPI (0.5%, w/w)/phosphocasein (0.5%, w/w) mixtures showed little variation after heating at different pH values, although they were significantly ($P \leq 0.05$) higher than WPI solutions heated in the absence of casein. It seems to be reasonable to assume that the initial casein protein particle, as it exists between pH 5.5 and 6, acts as a template for the deposition of denatured whey protein. The presence of micellar casein during heating of the whey proteins promotes the aggregation step, thus resulting in an increase in total whey protein denaturation. As the pH of micellar casein is lowered the increased protonation of protein groups decreases the net negative charge of the micelle (*21*). Fay (*22*) showed that that the ζ potential of casein micelles decreased from −18 mV at pH 6.7 to −12 mV at pH 5.5. Solubilization of colloidal calcium phosphate (*23*, *24*) with acidification results in an incremental increase in Ca^{2+}, which will have significant effects on the integrity of the casein micelle. De Kruif (*25*) suggested that the casein micelle had a stabilizing brush layer of κ-casein on the particle surface, which, together with ionic strength, dictated the pH at which the casein particle destabilized. The protective behavior of α_{s1}/β-casein and micellar casein differ in that the former inhibits denatured whey protein aggregation, whereas the latter system promotes aggregation.

On the basis of these results it is suggested that casein has the ability to control the aggregation of heat-induced denatured whey proteins, even at acidic pH values, which can be used to predict and modify some of the functional behavior of milk proteins. These functional properties include water-binding, viscosity, gelation, or general structure/texture development (*20*, *26*) in milk protein-based products such as yogurt, sauces, or nutritional beverages.

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