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Rheological Properties of Concentrated Skim Milk: Influence of Heat Treatment and Genetic Variants on the Changes in Viscosity during Storage

ANNIE BIENVENUE, RAFAEL JIMÉNEZ-FLORES,* AND HARJINDER SINGH†

Dairy Products Technology Centre, California Polytechnic State University, San Luis Obispo, California 93407

Heat treatment during manufacturing of milk powder is one of the most important tools for manipulation of its functional properties, and it is the basis of the classification of these proteins into low-, medium-, and high-heat types. Slight differences in the sequences of the major proteins in milk (genetic variants) seem to have also a significant effect in milk powder processing (U.S. patent). Therefore, the effects of high-temperature storage and heat treatment on skim milk of defined genetic variants of β-lactoglobulin (β-LG) were measured. The samples had 45% total solids, the temperature of aging was 50 °C, and the heat treatment was 90 °C for 10 min prior to evaporation. Measurements on shear rate and on apparent viscosity were determined for each sample. During storage of the concentrated milk, the apparent viscosity and yield values increased markedly, and the age-dependent increase in viscosity in heat-treated concentrated skim milks was much more pronounced than in those prepared from unheated skim milks. The increase in apparent viscosity and yield value with storage time was notably different for milks containing different genetic variants. Unheated concentrated milks containing the B variant of β-LG showed the most rapid increase in apparent viscosity with storage time, whereas the viscosity increase was slowest in the concentrate containing the A variant. In contrast, heat-treated concentrated milks containing the A variant of β-LG showed the most rapid increase in viscosity with storage time, whereas the viscosity increase was slowest in the concentrate containing the AB variant. The changes in apparent viscosity of concentrated milk were largely reversible under high shear during the early stages of storage, but samples stored for a long time showed irreversible changes in apparent viscosity. Particle size analysis confirmed irreversible aggregation and fusion of casein particles during storage.

KEYWORDS: Concentrated milk; viscosity; yield stress; casein micelle aggregation; genetic variants

INTRODUCTION

The use of milk powders for recombination and manufacturing of various food products has increased over recent years, placing greater importance on the definition of the functional properties of powders. The functionality, particularly the reconstitution properties, of milk powders can be highly variable and occasionally unpredictable, and solutions to functionality problems tend to be largely empirical in nature. To produce milk powders that will function consistently, a greater background knowledge of what is causing functionality problems is needed.

The manufacturing process for skim milk powder consists of four distinct stages: (a) separation of raw milk and pasteurization (72 °C for 15 s) of the skim milk, (b) preheat treatment of skim milk, (c) concentration of preheated skim milk to ~45% total solids by evaporation of water under reduced pressure, and (d) spray-drying of the concentrate to remove most of the remaining water. Processes that can be manipulated prior to spray-drying include preheat treatment of skim milk prior to evaporation and the properties of the concentrate itself.

Preheat treatment of milk is one of the most important tools for manipulation of the functional properties of milk powders, and it forms the basis of classification of skim milk powders into low-, medium-, and high-heat types. When the preheat treatments are above 70 °C, denaturation of whey proteins commences, which is followed by their aggregation and association with casein micelles (*1*−*3*). These associations change the reactivity and interactions of casein micelles, which in turn influence the functionality of milk powders.

The viscosity of the concentrated milk prior to spray-drying has a major influence on the size of the droplets during atomization and hence the rate of drying and final powder particle size distribution. These factors in turn affect the physical and functional properties of the dried product (*4*−*6*). In addition,

* Corresponding author [telephone (805) 756-6103; fax (805) 756-2998; e-mail rjimenez@calpoly.edu].
† Present address: Riddet Centre, Massey University, Palmerston North, New Zealand.

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the viscosity of the concentrated skim milk restricts the extent of concentration that can be attained by evaporation without adversely affecting the properties of milk powder. The viscosity of the concentrate is affected by several factors, including temperature, protein content, total solids, preheat treatment of milk, and holding time of the concentrate (*7*−*11*). It has been established that the concentrated milks show non-Newtonian behavior, with the viscosity decreasing with increasing shear rate, and follow a power law behavior (*7*, *10*−*12*).

Heating of milk above 70 °C has a marked effect on its viscosity because of the denaturation of whey proteins. The denatured whey proteins associate with the casein micelles, resulting in an increased micelle size and change in micelle−micelle interactions, which increases the viscosity (*13*, *14*). Snoeren et al. (*15*) observed an increase in the viscosity of skim milk concentrates in proportion to the intensity of preheat treatment (95 °C for 5 min > 85 °C for 1 min > 70 °C for 10 s), which was considered to be due to increased protein voluminosity as a result of whey protein denaturation. Bloore and Boag (*7*) reported that a high-temperature−short-time (HTST) preheat treatment (113 °C for 10 s) gave a lower viscosity in concentrated skim milk than a low-temperature−long-time preheat treatment (80 °C for 120 s), although both of these treatments resulted in very similar whey protein denaturations.

No information is available on the combined effects of heat treatments and genetic variants of β-lactoglobulin (β-LG) on the rheological properties of concentrated skim milk. The objective of the work reported in this paper was to explore the physical and chemical changes that cause age thickening in skim milk concentrated to 45% total solids and to determine the effects of preheat treatment of skim milk and genetic variants of β-LG.

MATERIALS AND METHODS

Materials. All materials and reagents were purchased from Fisher Scientific (Pittsburgh, PA; www.fishersci.com) and were of analytical grade. Pasteurized skim milk was obtained on two occasions from the herd at the University of California, Davis, CA. Each genotype sample of milk was supplied by pooling the milk from seven to nine cows in different stages of lactation and given the same feed. The milk was selected to have only β-lactoglobulin variation and keep κ-casein and β-casein as constant genotypes (AB and A^2B, respectively).

Milk Composition. The total protein content was determined using the Kjeldahl method (*16*) using a conversion factor of 6.38. The non-casein nitrogen, not precipitated with 12% trichloroacetic acid, was also determined according to the Kjeldahl procedure. The total solids content was determined by oven-drying of preweighed samples at 105 °C for 24 h, cooling them in a desiccator for 2 h, and reweighing the samples. Ash content was determined by ignition of the above dried material at 550 °C in an electric muffle furnace.

Experimental Protocol. A diagram showing how the milk samples were processed is given in **Figure 1**. Each batch of skim milk was divided into two streams. The skim milk in the first stream was HTST pasteurized (72 °C for 15 s) and then evaporated to ∼45% total solids; the second stream involved heat treatment of skim milk at 90 °C for 10 min in a steam-jacketed vessel with a 5 min time for reaching 90 °C, followed by evaporation in a rising film single-effect evaporator at 55 °C with an evaporative capacity of 70 kg/h (Merriott Walker Corp., Birmingham, MI) to 45 ± 1% total solids. The total solids (TS) content was determined by CEM microwave oven and adjusted to 45% by addition of water, if needed. The concentrated skim milk prepared from pasteurized skim milk is referred to as an "unheated" sample, whereas the concentrated milk prepared from skim milk heated at 90 °C for 10 min is referred to as a "heat-treated" sample.

Samples of the concentrated milks were poured into 250 mL bottles, 0.02% sodium azide was added to prevent bacterial growth, and then the bottles were placed in a water bath thermostatically controlled at 50 °C. The pH of all samples was closely monitored, and no significant change from the initial pH was recorded in any sample. Samples were removed after 1, 2, 4, 6, 8, or 10 h for rheological measurements. Subsamples were diluted with Milli-Q water to give 9% TS for particle size and polyacrylamide gel electrophoresis analysis.

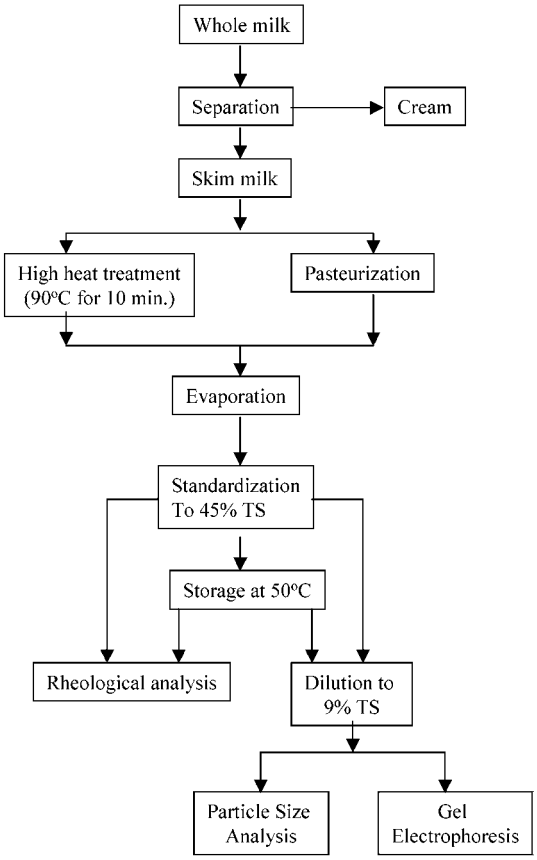

Figure 1. Experimental protocol for the manufacture and analysis of concentrated skim milks.

Measurement of Viscosity. Flow curves of milk evaporated to 45% TS were obtained on a controlled stress rheometer (Rheometric SR-5000, Rheometric Instruments, Piscataway, NJ) equipped with cup and bob geometry and temperature control. At least two concentrates were analyzed for each treatment. All measurements were performed in a steady stress sweep mode at 50 °C, after the samples had been stored in the water bath for different times. Samples were held for 5 min in the rheometer cup before the measurements were begun.

Measurement of Particle Size Distributions. Particle size distributions were determined using a laser diffraction particle size analyzer in the polarization intensity differential scattering (PIDS) optical mode (Beckman Coulter, model LS 230, Miami, FL). Diluted concentrated milks were added into the analysis chamber containing 1 L of milk serum (permeate from UF of milk using a spiral wound UF membrane 10 kDa MW cutoff, GEA Technology, Hudson, WI). Milk was added into the analyzer chamber until the required obscuration (∼50%) was achieved. The PIDS system uses three wavelengths of light (450, 600, and 900 nm) at two polarizations. Measurements are made at several scattering angles of the light polarized vertical to the scattering plane and the light polarized horizontal to the scattering plane. The difference in scattered intensity between the two polarizations is highly sensitive to particle size, wavelength, and angle of measurement. The PIDS system has the highest resolution when the particle size is smaller than the wavelength, that is, in the range between 100 and 400 nm.

Polyacrylamide Gel Electrophoresis (PAGE). The concentrated sample milks were diluted to 9% TS with Milli-Q water and examined using PAGE for protein analysis. Samples were dispersed in 0.5 M Tris-HCl buffer, containing 10% glycerol, 10% (w/v) SDS, and 0.05% bromophenol blue. For reducing conditions, 5% β-mercaptoethanol was

Table 1. Average Composition (Percent) of Skim Milk Containing Different β-Lactoglobulin Phenotypes

component	β-LG A	β-LG AB	β-LG B
protein	3.56	3.39	3.18
non-casein protein	1.08	1.02	0.77
ash	0.73	0.74	0.65
total solids	8.95	8.91	8.75

added to the samples followed by heating at 95 °C for 5 min in a boiling water bath. A 10 μL sample was then loaded onto the SDS gel, and the gel was run in a Mini-Protean system (Bio-Rad, Richmond, CA) at 200 V using a Bio-Rad power supply unit (model 1000/500, Bio-Rad). The SDS-PAGE systems have been described by Singh and Creamer (*17*); the protein bands were fixed and stained using a solution of Coomassie blue R-250.

RESULTS

Milk Composition. Table 1 shows the average composition of skim milks with different genetic variants used in this study. The average total protein concentration ranged from 3.2 to 3.6%, but the casein concentrations in different milks were fairy similar (in the range of 2.37−2.48%). The milks from cows with homozygous β-LG A variant had slightly higher total protein contents than did milks from cows having the B variant of β-LG, which was due to a higher whey protein content in the β-LG A variant milks (as indicated by the non-casein nitrogen contents). The ash content of milk with the homozygous β-LG variant B was slightly lower than that of the other milks. The pH of the samples was in the range of 6.65−6.72.

Apparent Viscosity. Figures 2 and **3** show the apparent viscosity of concentrated skim milk samples (45% TS) stored at 50 °C as a function of shear rate for each variant (**Figure 2a**) A, (**Figure 2b**) AB, and (**Figure 2c**) B. All milk samples exhibited shear-thinning behavior; that is, the apparent viscosity decreased rapidly with increasing shear rate.

At constant shear rate, the apparent viscosity increased with increasing storage time at 50 °C, the increase being more marked at low shear rates.

When the unheated samples were examined immediately after manufacture (i.e., 0 storage time), there were no differences in the apparent viscosities between the concentrated milk samples containing different genetic variants of β-LG (**Figure 2**). However, the rate of increase in apparent viscosity with storage time was notably different for samples containing different genetic variants; milk concentrate containing the B variant of β-LG (**Figure 2c**) showed the most rapid increase in viscosity with storage time, whereas the viscosity increase was slowest in the concentrate containing the A variant.

The effects of heat treatment of skim milk at 90 °C for 10 min prior to evaporation on the apparent viscosities of resulting 45% total solids concentrated milks were determined. Under these heating conditions, most of the whey proteins (>95%) were denatured and there were no differences in the extent of denaturation between the milk samples containing different β-LG variants. In the shear rate range of 1−1000 s^{-1}, the apparent viscosities of heated samples were considerably higher than those of the corresponding unheated samples (**Figure 2** versus **Figure 3**), although apparent viscosity still decreased considerably with increasing shear rate. Similar to the trends observed for unheated samples, storage of heat-treated samples at 50 °C resulted in a considerable increase in apparent viscosity. However, the rate of increase in viscosity with storage time was much higher in the heat-treated samples (**Figure 3**). In contrast to unheated systems, heat-treated concentrated milks

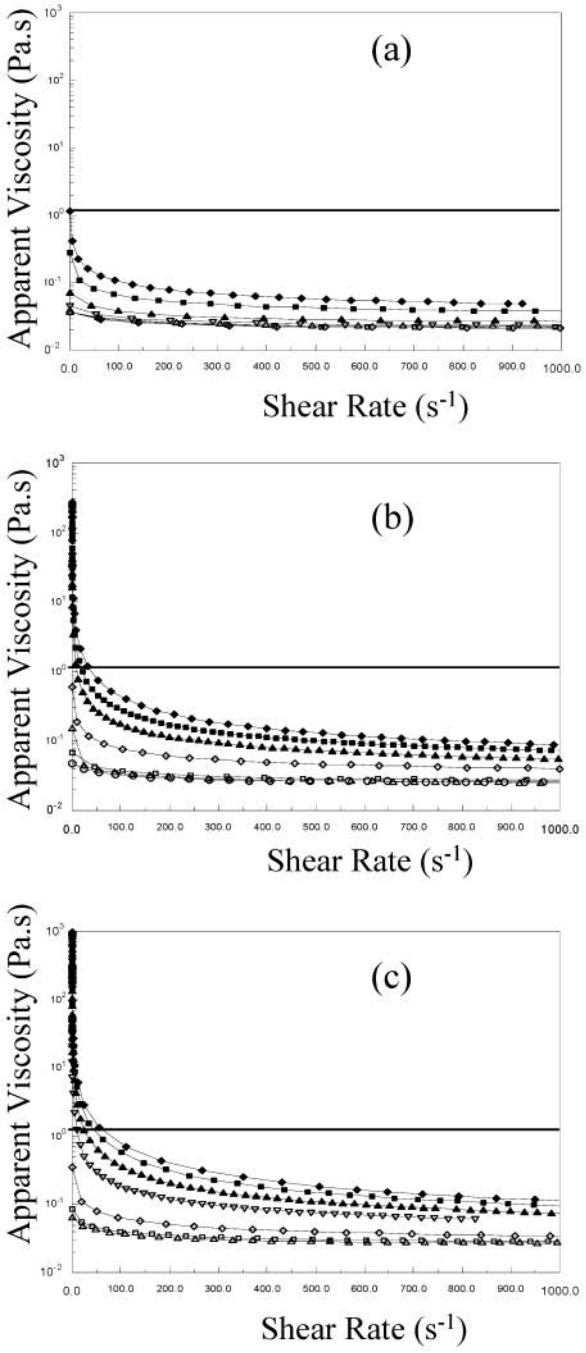

Figure 2. Apparent viscosity as a function of shear rate of concentrated skim milk (45% total solids), prepared from unheated (pasteurized) skim milk and stored at 50 °C for 0 (△), 1 (□), 2 (◇), 4 (▽), 6 (▲), 8 (■), or 10 (◆) h: concentrated skim milks containing β-LG A (**a**), AB (**b**), or B (**c**) variant. A horizontal line at 1 Pa s has been drawn for easy comparison of the changes among the different experiments.

containing the A variant of β-LG showed the most rapid increase in viscosity with storage time, whereas the viscosity increase was slowest in the concentrate containing the AB variant.

In all samples, it was clear that the apparent viscosity increased with storage time even at the highest shear rate used. In the unheated systems, concentrated milk with the B variant had a somewhat higher viscosity than the A variant, with the viscosity of AB milks being intermediate (**Figure 4**). This indicates an irreversible change in the structure of the material that could not be completely disrupted by shear forces. In the heat-treated concentrated milks, apparent viscosity at high shear

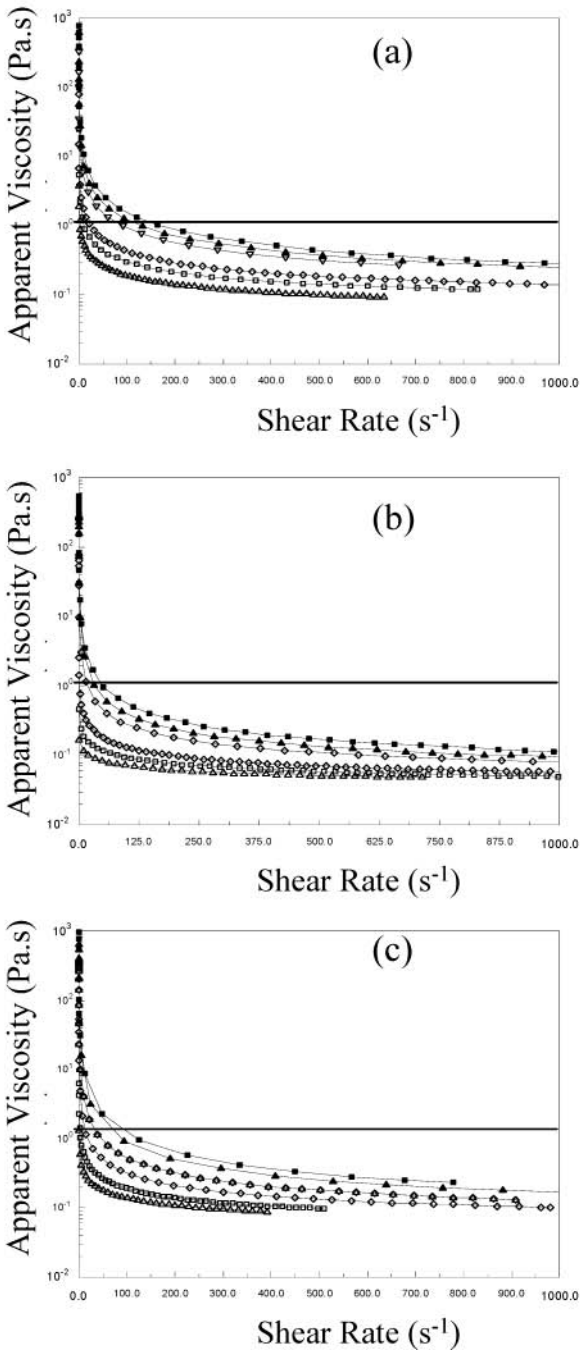

Figure 3. Apparent viscosity as a function of shear rate of concentrated skim milk (45% TS), prepared from skim milk heated at 90 °C for 10 min and stored at 50 °C for 0 (△), 1 (□), 2 (◇), 4 (▽), 6 (▲), or 8 (■) h: concentrated skim milks containing β-LG A (**a**), AB (**b**), or B (**c**) variant. A horizontal line at 1 Pa s has been drawn for easy comparison of the changes among the different experiments.

rates was considerably higher than that of the corresponding unheated concentrated milks at each storage time, with the A variant milk having the highest apparent viscosity followed by B and AB variants (**Figure 4**).

The non-Newtonian viscosity of concentrated milk was analyzed in terms of the Bingham equation over the linear high-shear region of τ versus γ curves, as described by Horne (*12*):

$$\tau = \tau_y + \eta_{PL}\,\gamma$$

η_{PL} is plastic viscosity (Pa s), and τ_y is Bingham yield stress

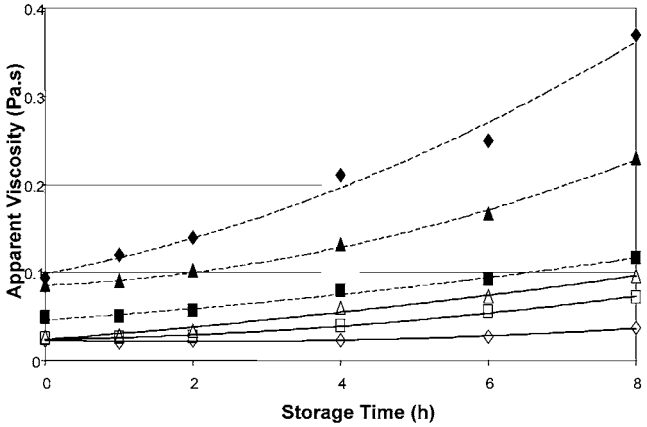

Figure 4. Apparent viscosity (at a shear rate of 1000 s^{-1}) of concentrated milks containing β-LG A (◇, ◆), AB (□, ■), or B variant (△, ▲). Open symbols represent concentrated milks prepared from unheated skim milk, whereas solid symbols represent concentrated milks prepared from skim milk that had been heated at 90 °C for 10 min prior to evaporation.

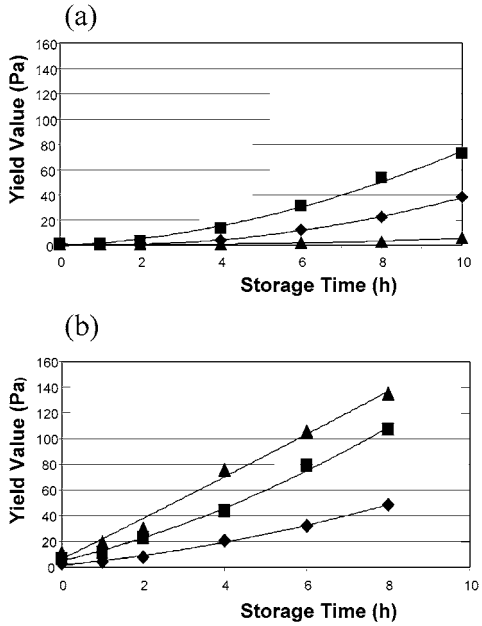

Figure 5. Bingham yield values, extrapolated from flow curves, of concentrated milks containing β-LG A (▲), AB (◆), or B variant (■): (**a**) concentrated milks prepared from unheated skim milk; (**b**) concentrated milks prepared from skim milk that had been heated at 90 °C for 10 min prior to evaporation.

(Pa). Yield stress were determined by extrapolating flow curves to $\gamma = 0$. It was found that this model fitted well (with correlation coefficients of >0.98) the experimental data at different storage times. The yield values obtained for concentrated milks containing different genetic variants are shown in **Figure 5**. The yield values increased with storage time, indicating that the structure of the material was changing with storage time. Unheated concentrated skim milks containing the B variant of β-LG showed the greatest increase in yield value with storage time, whereas the yield values in β-LG A containing milk remained the lowest throughout the storage time.

As expected, the yield values of heat-treated concentrate samples were markedly higher and increased more rapidly with storage time compared with the unheated samples (**Figure 5b**). Unlike the unheated samples, the yield values of β-LG A containing milk were highest throughout the storage time. Surprisingly, the concentrated milks containing the AB variant

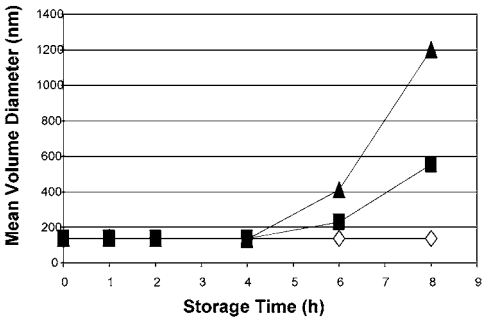

Figure 6. Changes in mean volume diameter, as a function of storage time at 50 °C, of concentrated milks (45% TS), prepared from unheated skim milk, containing β-LG A (◇), AB (■), or B variant (▲).

showed the slowest increase in apparent viscosity with storage time. It should be noted that the differences between the viscosities of unheated milks containing different genetic variants of β-LG were much smaller than those between the heat-treated samples.

Particle Size Distributions. The above concentrated milk samples after each storage time were diluted with water to ~9% TS and analyzed for particle size distributions; the changes in particle size reflect irreversible aggregation and fusion of casein particles. The mean particle sizes in unheated, rediluted concentrated milks as a function of storage time at 50 °C are shown in **Figure 6**. In accordance with the high-shear viscosity data (**Figure 4**), rediluted concentrated milks containing the B variant of β-LG showed the largest increase in mean particle size during storage for up to 8 h, whereas there was no significant change in the mean particle size of the A variant milk.

Heat treatment of skim milk prior to evaporation had little effect on the particle size distributions (**Figure 7**); however, there were considerable differences in the particle size distributions of the corresponding concentrated milks diluted with water to ~9% total solids immediately after evaporation. Unheated rediluted concentrated milk showed a single peak with particles ranging from 60 to 300 nm, typical of casein micelle size distribution in normal milk. However, in the heat-treated rediluted concentrated milk, the size distribution shifted toward larger particle sizes, with a large proportion of particles >200 nm. This indicates that in heat-treated samples irreversible interactions between casein and whey protein particles took place during evaporation itself. Consequently, the average particle size in heat-treated samples was much larger than in the unheated samples, and it continued to increase with storage time.

SDS-PAGE. To determine the type of interactions that may be involved in protein aggregation during storage of concentrated milk, all samples stored at 50 °C were subjected to gel electrophoresis in SDS-containing buffer systems. There were no obvious differences between the milks of different genetic variants. Typical gel patterns obtained for heat-treated concentrated milks are shown in **Figure 8**. Analysis of concentrated milk samples by SDS-PAGE under reducing conditions showed no apparent changes in the intensities of casein bands with storage time (**Figure 8b**), although the β-LG band gradually decreased in mobility and became blurred. Similar patterns were observed when SDS-PAGE was carried out under nonreducing conditions (**Figure 8a**), except that the intensity of κ-casein was much lower, which is to be expected as κ-casein exists as disulfide-linked polymers in the micelles and in heated systems β-LG and α-lactalbumin are linked with as κ-casein through disulfide bonds. These results indicate that the increase in particle size and probably high-shear viscosity during storage did not involve formation of additional covalent bonds but was probably the result of hydrophobic and ionic bonds. However, this does not rule out the possibility of formation of further disulfide linkages during storage between already aggregated whey proteins.

DISCUSSION

In addition to concentration of milk constituents, evaporation causes a number of other changes in milk (*18*). The pH of milk decreases while ionic strength increases during evaporation of milk. Both of these factors would reduce electrostatic repulsions between the micelles. The κ-casein hairy layer may collapse, resulting in a decrease in steric repulsion. Therefore, it would be expected that casein micelles in fresh skim concentrated milk would be weakly flocculated.

In the concentrated milk prepared from heat-treated skim milk, association of denatured or aggregated whey proteins at the micelle surface would lead to an increase in effective volume fraction (due mainly to the presence of a layer of aggregated

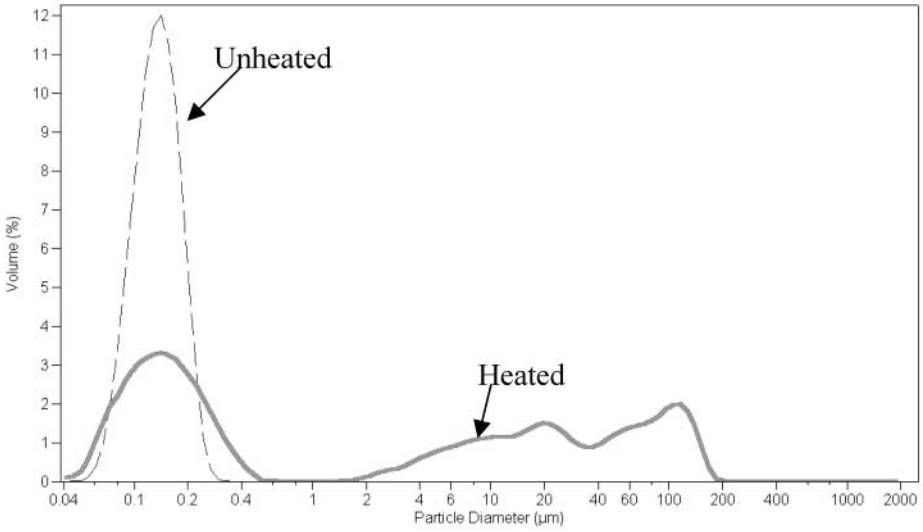

Figure 7. Particle size distributions of concentrated milks prepared from unheated skim milk and heat-treated skim milk. Concentrated milk samples (β-LG A variant milk) obtained immediately after evaporation were diluted with water to 9% total solids.

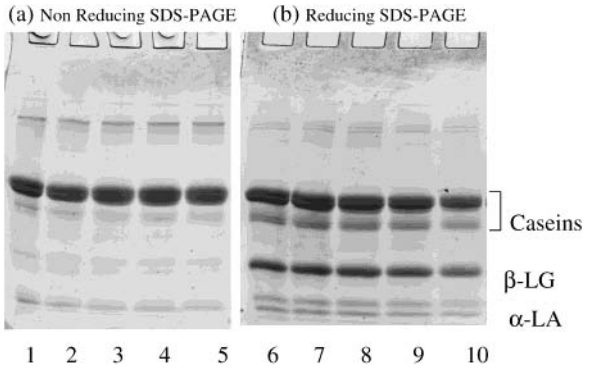

Figure 8. SDS-PAGE patterns, under nonreducing (**a**) and reducing conditions (**b**), of the concentrated milks (prepared from heat-treated skim milk) containing the β-LG A variant. Samples were stored at 50 °C for different times: (lane 2) 0 h; (lane 3) 4 h; (lane 4) 6 h; (lane 5) 8 h. The original skim milk is shown in lane 1.

whey proteins around the micelles and aggregated whey proteins in the serum) (*14*, *24*, *25*). In addition, because of changes in pH and ionic strength, there are likely to be further interactions between denatured whey proteins, involving both those associated with casein micelles and those in the serum. Therefore, the large increase in apparent viscosity and yield stress observed in concentrated skim milk that had been prepared from heat-treated skim milk could be attributed to combined effects of increased volume fraction and increased interactions between the casein micelles. In the heated systems, as the shear rate increases, the filament-like whey protein aggregates associated at the casein micelle surface would collapse, resulting in a decrease in apparent viscosity, and upon removal of shear, these aggregates would resume their original conformations and hence viscosity. However, the viscosity of heated systems, even at high shear rates, was considerably higher than that of the unheated concentrated systems.

The increase in apparent viscosity with storage time found in both unheated and heated concentrated skim milk systems indicates the occurrence of further interactions within the milk concentrate structure. Rearrangement of the three-dimensional structure during storage, resulting in greater degree of contacts between dispersed particles, may be responsible for increased apparent viscosity. Interestingly, the storage of the concentrates for longer times caused irreversible changes in the structure, as indicated by greatly enhanced apparent viscosity under high shear. This change probably arises from some kind of permanent clustering between the casein particles and the fact that these clusters are not broken down under high shear. The exact nature of chemical changes involved in this permanent clustering is uncertain. Particle size analysis indicated that fusion/aggregation of micelles does occur during storage of milk concentrates.

The age-dependent increase in viscosity in concentrated milks prepared from heat-treated skim milk was much more pronounced than those prepared from nonheated skim milk. During storage of concentrated milks prepared from heat-treated skim milk, the denatured whey proteins (both those associated with casein micelles and those in the serum) may further aggregate through sulfhydryl−disulfide and hydrophobic interactions, leading to increased cross-linking or bridging between the micelles. Particle size analysis confirmed that larger aggregates were formed during storage of heat-treated concentrated milks as compared to unheated concentrates.

The differences in the apparent viscosities of milks with different genetic variants reflect differences in milk compositions and reactivity of the proteins during heat treatment and evaporation. It is not obvious why the increase in apparent viscosity with storage time was more pronounced in unheated concentrates containing the B variant than that in the AB and A variant milks. As there were no significant differences in casein concentrations among these milks, it would be expected that the effective volume fractions of casein micelles, and hence the viscosities, would be similar. This indeed was the case when the samples were analyzed immediately after evaporation or after storage for up to 4 h. Differences in the rates of changes in viscosity during storage may be related to lower proportions of κ-casein in the β-LG B variant milks (*26*) and hence the greater sensitivity of casein micelles to aggregation due to the changes discussed above during storage. As discussed earlier, κ-casein is very important for the stability of casein micelles, and its fractional content is related to micelle size.

The behavior of heat-treated systems is largely determined by whey protein aggregation and association with casein micelles during heat treatment and interactions during evaporation and storage. Concentrates containing β-LG A had higher concentrations of whey proteins, and this could promote aggregation of whey proteins (*27*). It is possible that relatively more β-LG had associated with the casein micelles in the β-LG A containing milks after heat treatment as compared with the β-LG B variant milks, leading to greater interactions or cross-linking between the dispersed particles and resulting in much enhanced viscosity during storage. Another possibility is that association of β-LG A aggregates with the casein micelles gave rise to more elongated filament-like structures, whereas association of β-LG B formed a more compact layer at the micelle surface. The former structure would be expected to have a higher volume fraction and hence a higher apparent viscosity. Differences in the aggregation behaviors of β-LG A and B variants have been detected in heated cheese whey by Parris et al. (*28*), who reported that the A variant formed larger, more insoluble aggregates than the B variant. Further studies will be required to determine the nature of molecular interactions involved during the storage of concentrated skim milks and to further understand the contribution of differences in protein and mineral contents between different genetic variant milks to apparent viscosity.

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