Heat stability of yak micellar casein as affected by heat treatment temperature and duration

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ORIGINAL PAPER

Heat stability of yak micellar casein as affected by heat treatment temperature and duration

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Abstract Heat treatment is one of the essential operations widely used in most dairy processes, and heat stability is one of the essential properties of milk. Casein micelles are the major component in milk responsible for the heat stability of milk during processing. This study assessed the effects of heat treatment temperature and duration on the average size, turbidity, polydispersity index and heat stability of casein micelles in yak skim milk and distilled water. The results showed that whey protein had an important role in influencing the heat stability of casein micelles. The average size, polydispersity index and turbidity of micelles in skim milk were higher than those of micelles in distilled water in all cases while the heat stability of casein micelles in skim milk was lower than those in distilled water. As a result of the heat treatment, the size of micelles in skim milk increased due to complex of casein/whey protein formed via covalent bonds, whereas it decreased in distilled water attributed to the change of hydrophobicity in micelles. The size distribution of particles broadened with increasing heating temperature, resulting in the increase in turbidity and polydispersity index of casein micelles both in skim milk and distilled water. The micelles in skim milk combined with whey protein during heating. These findings will help processors design appropriate heating conditions for yak milk and yak casein products and help identify new opportunities for product development.

Keywords Yak milk · Casein micelles · Size of micelles · Turbidity · Polydispersity index · Heat stability

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1 Introduction

Heat treatment is one of the essential operations widely used in most dairy processes for either improving the technological–functional properties of dairy products or ensuring their safety and shelf life (Oldfield et al. 2000; Law and Leaver 1997). The heat stability of milk is an essential property for the production of a wide range of heat-treated products. Casein is the main protein in milk and found as self-associating colloids called casein micelles, which are mainly composed of α_{s1}-, α_{s2}-, β- and κ-casein (McMahon and Oommen 2008). Casein micelles are at the core of the dairy industry, and their physico-chemical properties make them valuable in a wide number of applications (de Kort et al. 2012; de Kruif et al. 2012; de Kruif 1999). Casein micelles are stable but not fixed structures, and they are capable of withstanding the severe treatments commonly associated with dairy processing. However, certain conditions can dissociate or aggregate micelles, resulting in structural or property changes (Sauer and Moraru 2012; Liu and Guo 2008; Chakraborty and Basak 2007; Vasbinder and de Kruif 2003; Horne 2002; Anema and Li 2000). The heat stability of casein micelles and changes in their structure, size, degree of aggregation or dissociation and functional properties with large-scale changes in temperature and duration of heat treatment have mostly been studied in cow milk (Anema et al. 2004; Vasbinder and de Kruif 2003; Corredig and Dalgleish 1999; Anema 1998; Anema and Klostermeyer 1997). It was concluded that the extent of dissociation or aggregation of casein protein from the micelles depended on the composition of the solution as well as the temperature and duration of the heat treatment.

Whey protein plays an important role in the heat stability of casein micelles in milk. There have been a large number of studies on heat-induced casein/whey protein interactions and the functional properties of casein after heat treatment in cow milk (Sauer and Moraru 2012; Kehoe and Foegeding 2011; Kethireddipalli et al. 2011; Morand et al. 2011; Kücükcetin 2008; Vasbinder et al. 2003; Vasbinder and de Kruif 2003; Corredig and Dalgleish 1999; Law and Leaver 1997). It was proved that denatured whey proteins binding covalently to κ-casein on the micelle surface above 70 °C, resulting in change of casein micelles' characteristic.

Yak milk is a special material in Qinghai-Tibet Plateau. In recent years, yak milk production has been increasing to 40 million tons each year. However, industrially processed yak milk dairy products are no more than 25% (Li et al. 2011). The remainder of yak milk is produced and consumed by traditional means, such as butter, fermented skimmed and whole milk and Qula in China (Liu et al. 2013; Li et al. 2011). Considering protein fractions, yak milk contains more protein and caseins than cow milk (Li et al. 2010). Compared with cow caseins, yak caseins contain more α_{s2}- and β-casein and less α_{s1}- and κ-casein (Wang et al. 2013). It was also reported that the amino acid sequence of yak casein is different from that of other caseins (Cui et al. 2012; Bai and Yin 2011; Zhang et al. 2010). Concerning minerals, yak milk contains approximately 1,545 and 922 mg/kg of calcium and phosphate, respectively, which are greater than the values for cow milk (Li et al. 2011). As a consequence of these compositional differences, several properties of the micellar systems of these milk types differ noticeably from those of cow milk, notably micelle composition, size, mineralization and hydration (Wang et al. 2013; Li et al. 2006). These are some of the reasons why results obtained with cow milk cannot be easily extrapolated to other types of milk.

Do the differences in protein composition influence the structure, the composition and the size of yak casein micelles, which could further affect heat stability? The objective of this research was to investigate the influence of heat treatment at different temperatures and durations on the size, scatter factor, turbidity, topography and heat stability of yak casein micelles in milk and distilled water. A good understanding of particle size, structure and heat stability of yak casein micelles is important in the technological design of processing for yak milk and casein products.

2 Materials and methods

2.1 Materials

The yak milk used in this study was collected from Tianzhu grassland, on the Qinghai-Tibetan Plateau, in northwest China. After milking, 0.02% (*w*/*v*) sodium azide was added to inhibit bacterial growth. The samples were then put in sterile plastic bottles and stored in a box filled with ice. The samples were transported to the laboratory within 6 h. The yak milk was defatted twice by centrifugation (TDD5M, Changsha Pingfan Instrument Co. Ltd., Changsha, China) at 4,000×*g* for 10 min at 20 °C.

The skim milk was centrifuged using a Beckman Optima XL-100 K refrigerating ultracentrifuge (Beckman Coulter, USA) at 120,000×*g* for 40 min at 20 °C (Françoise et al. 2009). The supernatant was removed, and the firm pellet at the bottom was the casein micelles. The casein micelles were dispersed in a volume of distilled water equal to the volume of skim milk before centrifugation, which resulted in a solution of pure casein micelles at the same concentration found in yak milk.

2.2 Heat treatment of casein micelles

Subsamples of skimmed milk and casein micelles' solution (20 mL) were transferred to glass vials and heated, with continuous rocking, for predetermined times in a thermostatically controlled water bath (SHA-B, the Jingda instrument factory of Jintan city, Jiangsu, China) preset to temperatures ranging from 30 to 90 °C. After heat treatment, the milk samples were cooled to room temperature (20 °C) by immersion of the glass vials in cold running water within 3 min then equilibrated within 2 h at room temperature. The size, turbidity and heat stability were measured immediately afterwards.

2.3 The size and polydispersity index of casein micelles

The average size and polydispersity index of casein micelles were determined by laser light scattering (Delsa TM Nano, Beckman Coulter Inc., CA, USA) within 5 min. Before measurement, the milk samples were diluted 100 times with a simulated milk ultrafiltrate, which was made according to Beliciu and Moraru (2009).The experimental temperature was 25 °C, the refractive index of the solvent was 1.333, and viscosity was 1.002 mPa.s. The wavelength was 632.8 nm. The particles were characterised by mean diameter D and polydispersity index. The polydispersity index was obtained from the equipment directly, and D was calculated by the following equation (Wang et al. 2013):

$$D = \frac{\sum_j V_j D_j^3}{\sum_j V_j D_j^2} \tag{1}$$

where Vj is the relative volume fraction of all casein micelles with diameter Dj. Each experiment was repeated three times to acquire the average diameters of yak casein particles.

2.4 Turbidity measurements

The turbidity of skim yak milk and casein micelle samples was measured according to the method of Partschefeld et al. (2007), with slight modification. A total of 200 μL of samples was diluted with distilled water to 10 mL. The absorbancy of the solution above 633 nm was measured using a UV-2100 spectrophotometer (Beijing Beifen-Ruili Analytical Instrument Co., Ltd., Beijing, China) and a 1-cm quartz cell. The turbidity of the solution was indicated by the absorbancy.

2.5 Scanning electron microscopy (SEM)

Casein samples of 1 mL were fixed overnight in 10 mL of 2.5% glutaraldehyde in phosphate buffer (0.1 M) at pH 7.0. The samples were mounted on copper stubs and dehydrated in a graded ethanol series (10, 30, 50, 70, 90 and 100%*v*/*v*) every 30 min then coated with gold. At least three images of typical structures were recorded at a magnification of×30,000 and×50,000 using a JSM-6701 F (JEOL Ltd. Tokyo, Japan) microscope operating at 5 kV.

2.6 Heat stability

The heat stability of samples was expressed as the rate of deposition. After heat treatment, 20-mL samples were centrifuged at 40,000×*g* in a 50-mL tube for 10 min at 20 °C. The supernatant was removed, and the firm pellet at the bottom was weighed. The rate of deposition was calculated using the following formula:

$$\text{The rate of deposition\%} = (\text{the weight of precipitation}/\text{the weight of solution before centrifugation}) \times 100\% \tag{2}$$

2.7 Statistical analysis

All data were expressed as mean ± SD (standard deviation) from at least three independent trials. The differences between the mean values of multiple groups were analysed by one-way analysis of variance (ANOVA) with Duncan's multiple range tests. ANOVA data with $P<0.05$ were identified as statistically significant. PASW Statistics 18.0 software (SPSS Inc., Chicago, IL, USA) and Origin 8.0 (OriginLab Corporation, Northampton, MA, USA) were used to analyse and report the data.

3 Results and discussion

3.1 The size of casein micelles

After heat treatment at different temperatures for different durations, the average size of casein micelles decreased in distilled water, whereas it increased in skim yak milk (Fig. 1). The average diameter of native yak casein micelles was 211.3 nm, which increased to 223.1 nm after 30 °C treatment with 5 min, and then increased to 272.1 nm within 25 min at same temperature. After heat treatment at 90 °C within 5 min, the average diameter of casein micelles in skim milk was 369.0 nm, it increased to 372.6 nm within 25 min. The average diameter of casein micelles in distilled water was 198.2 nm, which increased to 205.6 nm after 30 °C treatment within 5 min, and then increased to 236.9 nm within 25 min at same temperature. After heat treatment at 70 °C within 5 min, the average diameter of casein micelles in distilled water was 171.8 nm, which decreased to 138.8 nm within 25 min. After heat treatment at 90 °C within 5 min, the average diameter of casein micelles in distilled water was 192.2 nm, which increased to 208.0 nm within 25 min. The particle size of yak casein micelles in skim milk was similar to that reported by Wang et al. (2013) and larger than cow casein micelles reported to be approximately 200 nm (Beliciu and Moraru 2009).

At treatment temperatures >70 °C, the particle size of casein micelles in skim milk evens out, reaching a plateau as β-lactoglobulin molecules denature and bind

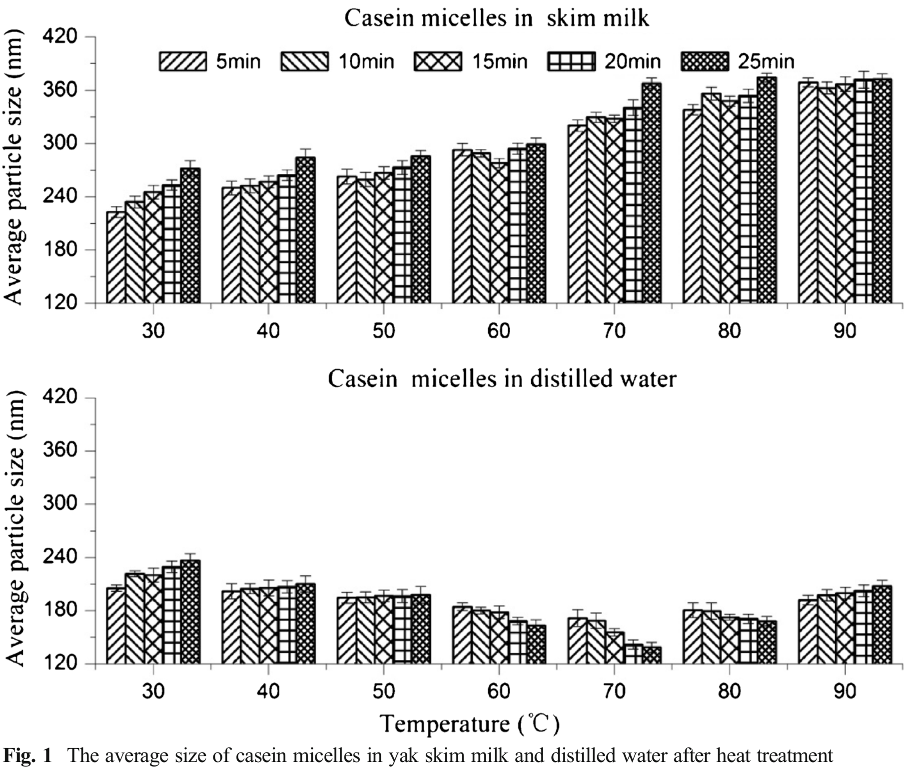


Fig. 1 The average size of casein micelles in yak skim milk and distilled water after heat treatment

with κ-casein via disulphide bonds (Kethireddipalli et al. 2011; Corredig and Dalgleish 1996). Relatively small particle size changes at low temperature treatments (40–60 °C) may be due to small variations in the degree of hydration. The size of casein micelles in skim milk markedly changed depending on the duration of heat treatment. At the same temperature, the size of micelles increased with a longer duration. In all cases, the maximum size of casein micelles was achieved after heat treatment for 25 min. The size of the micelles also increased, due to the formation of whey protein–casein micelle complex. As a consequence, it modified casein micelle hydration and reactivity. Below 70 °C, the size of micelles with 25-min heat treatment was not significantly different ($P>0.05$)

In comparison with casein micelles in skim milk, the size of casein micelles in distilled water decreased with increasing temperature. Below 50 °C, the size of casein micelles decreased with increasing heat treatment duration, the same with 90 °C and the opposite within 60–80 °C. It was reported that at low temperatures, the hydrophobic bonds become weaker, which causes the micelle structure to become looser and more porous and the micelle diameter to become larger. Additionally, longer heat treatment duration led to a partial dissolution of micellar calcium phosphate, which also causes the voluminosity of the casein micelles to increase (Gaucheron 2005). The intensity of hydrophobic bonds decreased when samples were treated at 80–90 °C and particle size reverted to similar values as observed at 40–50 °C.

The polydispersity indexes are presented in Fig. 2. The polydispersity indexes, i.e. scatter factors, were interpreted as follows: $0\leq P\leq 0.02$, which is characteristic of monodisperse or nearly monodisperse systems; $0.02<P\leq 0.08$, which is a characteristic of narrow particle size distributions; and $P>0.08$, which indicates broader size distributions (Chappellaz et al. 2010; Beliciu and Moraru 2009). According to the polydispersity data in Fig. 2, the yak casein micelles had narrow particle size distributions ($P<0.08$) and broader size distributions after heat treatment above 70 °C ($P>0.08$). In all cases, the polydispersity of casein micelles depended on the heat treatment duration, i.e. it increased with increasing duration. At 60 °C with 15-min heat treatment, the polydispersity of particles in skim milk exceeded 0.08. The same polydispersity was achieved with 25-min treatment of micelles in distilled water. Compared to casein micelles in distilled water, the polydispersity index of micelles in skim milk were larger than those of micelles in distilled water after heat treatment above 70 °C, which attributed to the formation of the whey protein–casein micelle complex. After heat treatment below 70 °C, the dissociation of minerals from micelles induced a little dissociation of casein monomers from micelles, so the number of soluble proteins increased, the polydispersity index of micelles increased. With 80–90 °C heat treatment, the size of the particles in distilled water increased; hence, the polydispersity index increased.

3.2 Turbidity

It is well known that the turbidity of aggregated proteins depends on the number and size of aggregates along with the scatter factor of their particles (Kehoe and Foegeding 2011; Liu and Guo 2008). The large size and high scatter factor of particles usually lead to the high turbidity of the solution. Therefore, solution turbidity provides a macroscopic overview of the effect of heat treatment on the aggregation of casein micelles of yak milk. The turbidity of both the casein micelles in skim milk and distilled water

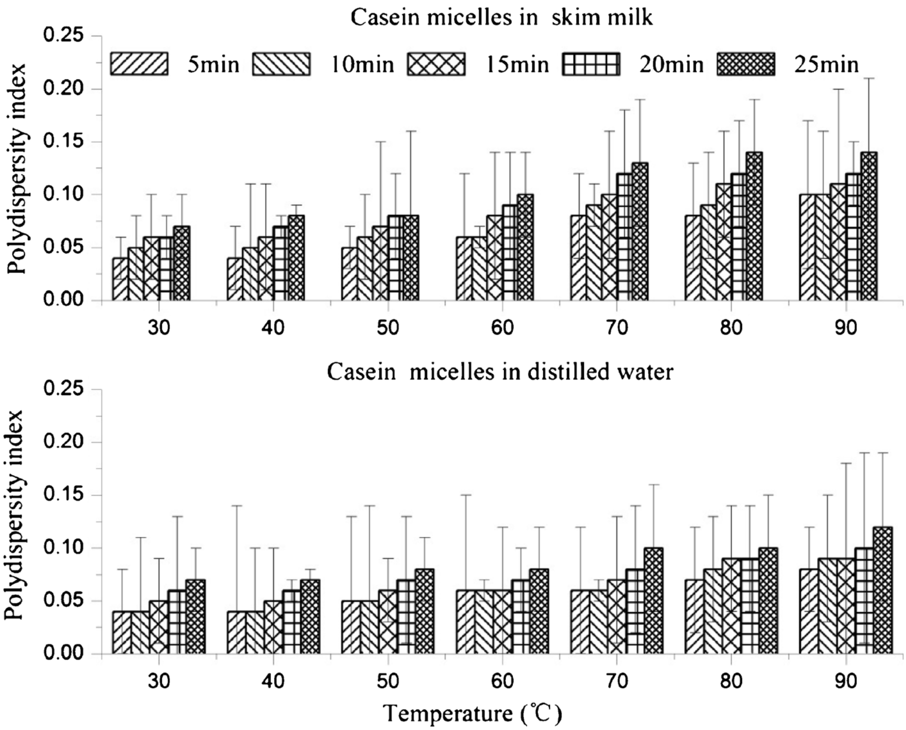


Fig. 2 The polydispersity index of casein micelles in yak skim milk and distilled water after heat treatment

increased with increasing temperature (Fig. 3). The turbidity of skim milk was higher than that of distilled water due to the higher protein content (casein and whey protein).

The turbidity of skim milk increased gradually below 70 °C heat treatment and then increased sharply. This was attributed to the formation of the whey protein–casein micelle complex, which led to the larger size and higher polydispersity index of casein micelles. The turbidity of skim milk depended on the treatment duration. A longer treatment duration resulted in a larger number of whey protein–casein micelle complex and a higher turbidity value for each treatment. For treatments above 70 °C, there was a formation of the whey protein–casein micelle complex, the size of particles increased rapidly, and turbidity increased sharply.

The turbidity of casein micelles in whey protein-free solution increased with increasing heat treatment temperature. This may have resulted from an increase in the number of particles due to any dissociation of casein monomers and minerals that may occur. Because of the dissociation of minerals from casein micelles, the stability of micelles decreased, and a little of casein monomers separated from the micelles, the number of particles increased and consequently the scatter factor of particles increased, which increased the turbidity.

3.3 Scanning electron microscopy (SEM)

The topography of casein micelles in distilled water and skim milk after heat treatment at different temperatures for 15 min obtained by SEM are shown in Fig. 4. The

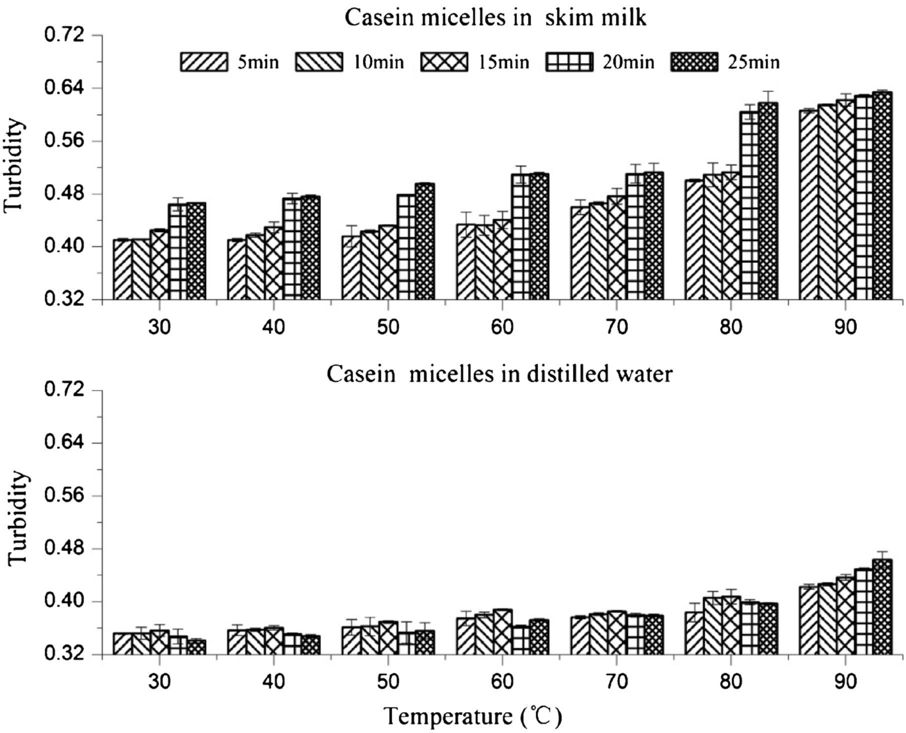


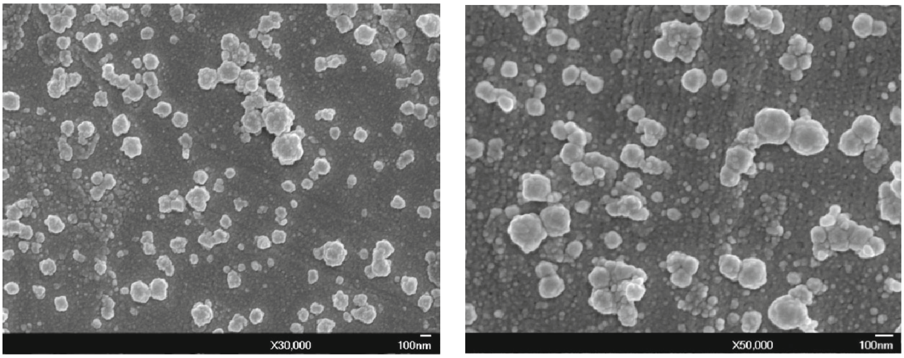
Fig. 3 Turbidity of casein micelles in yak skim milk and distilled water after heat treatment

topography of casein micelles in skim yak milk was similar to that of casein micelles in distilled water. The size of yak casein micelles was 100–300 nm, which was in agreement with the dynamic light scattering results. The size of casein micelles in skim milk increased with increasing heat treatment temperature because of the formation of the whey protein–casein micelle complex. After heat treatment below 70 °C for 15 min, hydrophobic bonds became more intense, resulting in a shrinking of the micelle in distilled water, which caused a decrease in particle size.

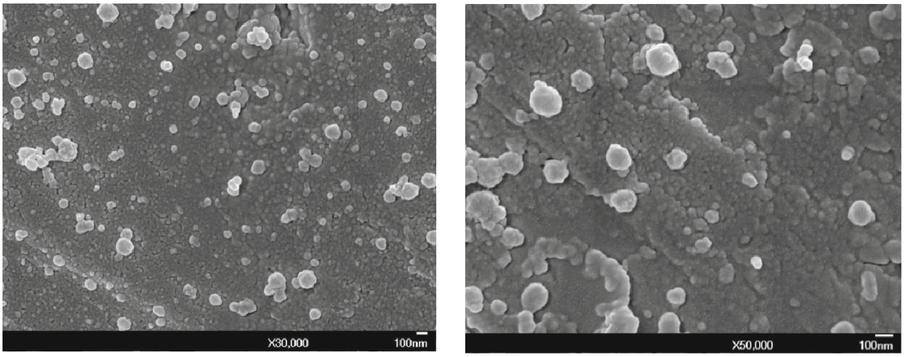
The collapse of the casein micelles at 90 °C was observed for both skim milk and distilled water. But this collapse seems to begin before in skim milk, at 70 °C. Moreover, it seems to have less rough parts on the casein micelles in distilled water at 90 °C, perhaps due to the absence of whey protein complexes attached to the surface of casein micelles. The particles of casein micelles in distilled water after 90 °C treatment were larger than those after 70 °C treatment, but were smaller and more homogeneous than those after 30 °C treatment. After 90 °C treatment, the size distribution became uniform, but the larger particle size increased the turbidity.

3.4 Heat stability

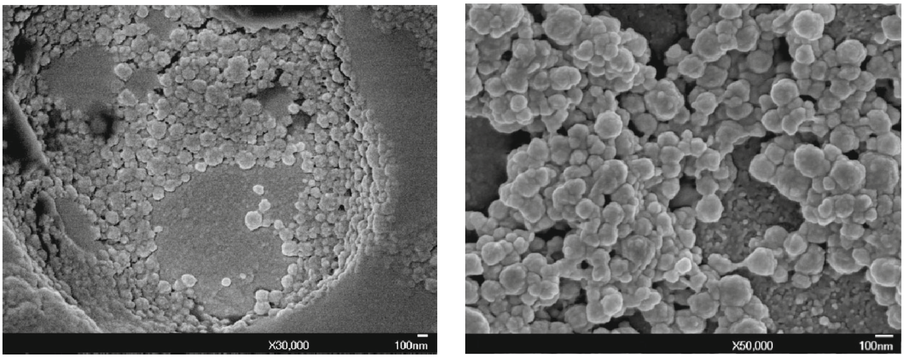
Heat stability of casein micelles in skim milk and distilled water, as indicated by the rate of deposition, is shown in Fig. 5. Heat stability of casein micelles decreased with increasing treatment temperature and duration in all cases. Heat stability of



a. Casein micelles in distilled water with 15 min heat treatment at 30 °C



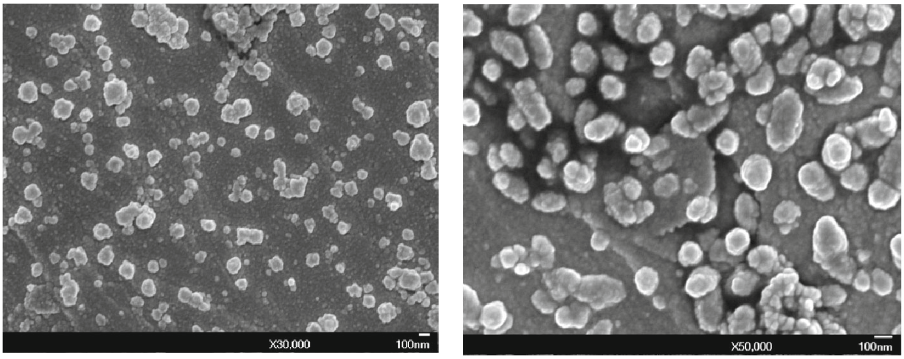
b. Casein micelles in distilled water with 15 min heat treatment at 70 °C



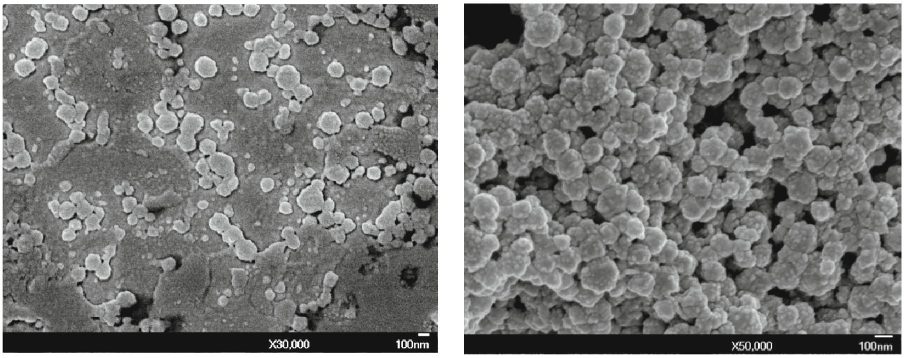
c. Casein micelles in distilled water with 15 min heat treatment at 90 °C

Fig. 4 The topography of casein micelles in yak skim milk and distilled water after heat treatment at different temperatures for 15 min (SEM, ×30,000, ×50,000). **a** Casein micelles in distilled water with 15 min heat treatment at 30 °C. **b** Casein micelles in distilled water with 15-min heat treatment at 70 °C. **c** Casein micelles in distilled water with 15-min heat treatment at 90 °C. **d** Casein micelles in skim milk with 15-min heat treatment at 30 °C. **e** Casein micelles in skim milk with 15-min heat treatment at 70 °C. **f** Casein micelles in skim milk with 15-min heat treatment at 90 °C

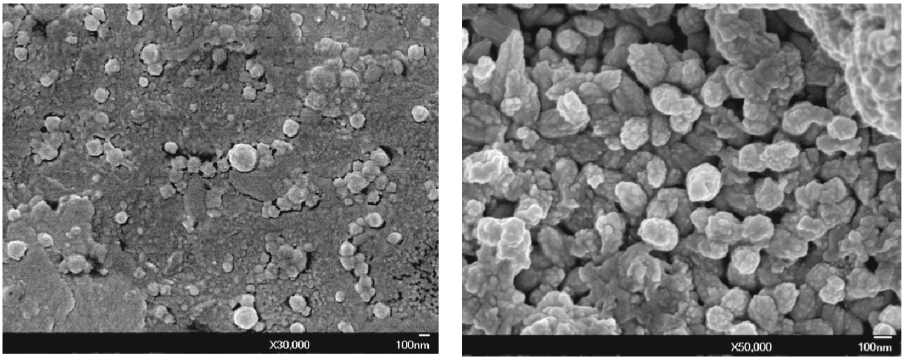
casein micelles in skim milk was lower than that of casein micelles in distilled water, which was attributed to the formation of the whey protein–casein micelle complex that consequently increased the particle size and made casein micelles easier to separate by centrifugation. There was more complex of whey–casein with



d. Casein micelles in skim milk with 15 min heat treatment at 30 °C



e. Casein micelles in skim milk with 15 min heat treatment at 70 °C



f. Casein micelles in skim milk with 15 min heat treatment at 90 °C

Fig. 4 (continued)

longer heating durations, so the rate of deposition increased. Heat stability of micelles with heating below 70 °C changed little at the same treatment duration. Above 70 °C, the rate of deposition of micelles in skim milk increased significantly because of the presence of whey proteins in the skim milk, which most likely denature and form complexes with the casein micelles when heated to >70 °C. The complexes are most likely centrifuged into a pellet and used to determine an artificially higher rate of deposition in the skim milk.

Compared to casein micelles in skim milk, although the rate of deposition of micelles in distilled water was lower, it increased sharply with increasing heating duration. Anema and Li (2000) reported that the dissociation of minerals from micelles with heat treatment decreased micelle stability. With increasing heating duration, the thermal motion was accelerated, more minerals and casein monomers may have dissociated from micelles, and heat stability decreased.

4 Conclusion

The main conclusion of this study was that the size of casein micelles changed during heat treatments in the range of 30–90 °C, and this instability increased with treatment temperature and duration. The whey protein had an important role in affecting the heat stability of casein micelles. The average size, polydispersity index and turbidity of micelles in skim milk was higher than that in distilled water in all cases while the heat stability of micelles in skim milk was lower than that in distilled water. During heating, the casein micelles in skim milk became bigger due to the formation of whey–casein complex, but they shrinked in distilled water resulting in more intensity of hydrophobic bonds. Therefore, the size of particles in skim milk increased, whereas it decreased in distilled water. The change of particle size caused the size distribution of casein micelles to broaden, so the

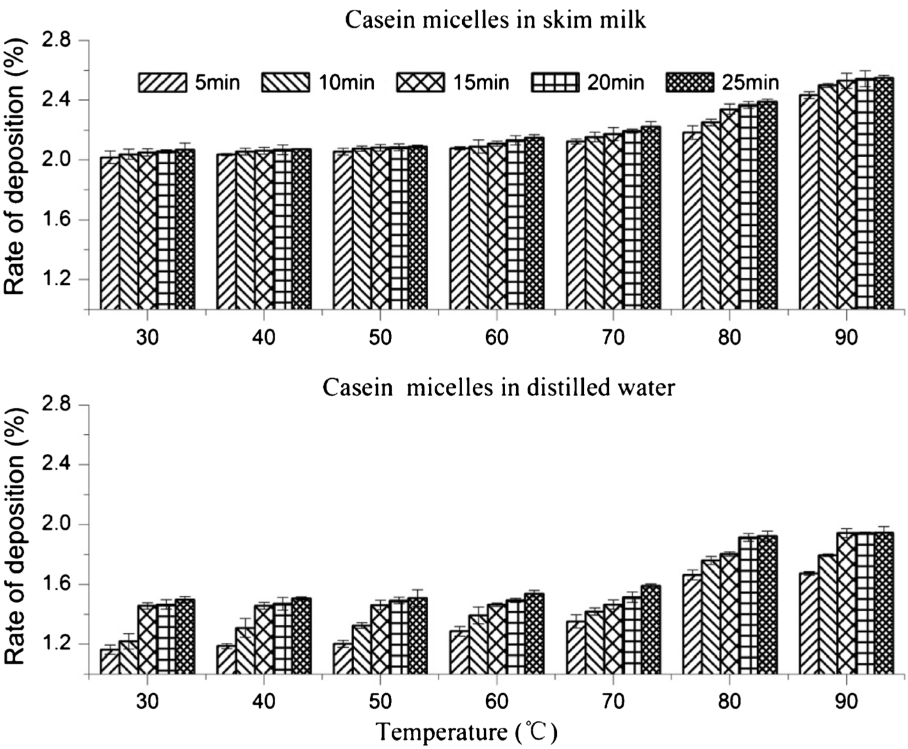


Fig. 5 The heat stability of casein micelles in yak skim milk and distilled water after heat treatment

turbidity and polydispersity index increased with increasing heating temperature. At higher temperatures, the reaction between casein and whey protein was severe, resulting in a sharp increase in size, turbidity and polydispersity and a decrease in heat stability of casein micelles in skim milk. During heating, the minerals may have dissociated from micelles, which decreased the heat stability of micelles in distilled water. These findings will help processors design appropriate heating conditions for yak milk and yak casein products and help identify new opportunities for product development.

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