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Short communication

Yak milk whey protein denaturation and casein micelle disaggregation/aggregation at different pH and temperature

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ABSTRACT

At the natural pH of yak milk (pH 6.6), a low level (<30%) of κ-casein (κ-CN) was found in the serum phase after heating at 95 °C for 30 min, indicating that as much as 70% of the β-lactoglobulin (β-Lg) and κ-CN complexes is associated with the micelle colloidal particles. The β-Lg and κ-CN levels increased from 13.2% and 2.6% at pH 6.0 to 35.2% and 60.1% at pH 7.0, respectively, when yak milk was heated at 95 °C for 30 min. At pH 6.0–6.4, the denatured whey proteins were associated with the caseins in the colloidal phase, resulting in milk gelation upon heating. The distribution of β-Lg and κ-CN complexes increased in the serum phase, demonstrated by the increasing levels of both β-Lg and κ-CN with increasing pH; at high pH (6.6–7.0), large proportions of β-Lg and α-lactalbumin were lost, presumably forming complexes in the colloidal phase.

1. Introduction

Yak milk ranks the third in economic importance in China, after cow and buffalo milk (Zhong, Chen, Zhao, & Xiao, 2006). The protein and fat content of yak milk, 5.5–10% and 6–10%, respectively, are higher than those of cow milk (Neupaney, Kim, Ishioroshi, & Samejima, 1997). Therefore, yak milk could potentially be used for the development of functional foods. However, of this large volume, only a small quantity (approximately 25%) is processed industrially (Lu & He, 2009).

Temperature and pH are the most important factors that influence the physical functionality and stability of milk. When the temperature is above 65 °C, whey proteins unfold and start interacting with the caseins, which leads to aggregation. Properties of cow milk, including heat stability, show marked pH dependence. The dissociation of the casein micelles was pH-dependent when cow milk was heated at temperatures below 100 °C (Anema & Li, 2000; Guyomarc'h, Law, & Dalgleish, 2003). Low levels of the casein micelle dissociate at 20 °C. The content of soluble caseins in the serum increases with temperature to a maximum dissociation at about 70 °C, and then decreases as the temperature rises further (Anema, 1998). However, the casein micelle does not dissociate at pH < 6.7, regardless of the heating temperature, whereas dissociation is enhanced at pH 6.7–7.1 (Anema & Li, 2000).

There have only been a few reports on heat stability of milk from other animal species such as goat (Montilla & Calvo, 1997), buffalo (Mehriz & Ganguli, 1980), camel (Farah, 1986) and yak (Li et al., 2014). Although the mechanisms of heat-induced reactions are similar, the differences are due to different concentrations of milk proteins, casein micelle structure, partition of salts between colloidal and serum phases, and thus the extent of protein interactions (Huppertz, 2016; Singh, Creamer, & Fox, 2004).

So far most studies on yak milk have been focused on the chemical composition (He et al., 2011; Li et al., 2011; Zhong & Yu, 1996). A recent study concerning the heat stability of yak milk shows that the maximum heat stability of yak milk was at pH 6.8 (Li et al., 2014). This study investigated the effects of temperature and pH on the stability of yak milk protein at more commonly used processing temperatures ranging between 65 and 95 °C by assessing the distribution of whey protein/casein complexes in the serum or colloidal phase and its implication on the turbidity and viscosity of yak milk. Such knowledge provides a further understanding of yak milk heat stability influenced by the concentrations of specific milk proteins.

2. Materials and methods

2.1. Materials

Raw yak milk samples were obtained from local farm of Aba Country in Sichuan Province, China. The milk was skimmed by

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centrifugation at 4000×g for 30 min at 4 °C (Beckman L8-80M ultracentrifuge and Beckman 50.2 Ti rotor; Beckman Instruments Inc., Palo Alto, CA, USA) to remove the cream layer.

2.2. Adjustment of pH and heat treatment

Raw skim yak milk samples (10 mL) pH adjusted from 6.0 to 7.0 using 1 M NaOH or HCl were transferred into glass vials. The vials were immersed in an immersion circulator heater (Suzhou Bolinger Analytical Instruments Co., Ltd, Shanghai, China) and shaken in a horizontal plane with an oscillation of 5 cycle min^{-1}. The thermostatically controlled water bath was maintained at various temperatures of 65, 75, 85, 90 or 95 °C for 30 min. After heating, all samples were stored at 4 °C for 24 h.

2.3. High performance liquid chromatography analysis of milk proteins

The heated and unheated milk sample (10 mL) was transferred into 38.5 mL centrifuge tubes and centrifuged at 27,000×g for 1 h at 4 °C (Beckman L8-80M ultracentrifuge and 50.2 Ti rotor; Beckman Instruments Inc.) to separate the serum and colloidal phases. The protein components of serum phase and colloidal phase were determined using the reverse phase-high performance liquid chromatography (RP-HPLC) method described by Bonizzi, Buffoni, and Feligini (2009). The concentrations of whey proteins and caseins in the serum phase were calculated as a percentage of those in the original unheated yak milk.

2.4. Viscosity and turbidity analysis

Viscosity measurement was performed using a Ubbelohde capillary viscometer (Cannon Instrument Company, State College, PA, USA) according to the method described by Jeurnink and De Kruif (1993). Milk samples were transferred to a temperature-controlled room preset to 4 ± 0.4 °C and allowed to equilibrate for at least 4 h prior to viscosity measurement.

Milk turbidity measurement was carried out at 4 °C using a Shimadzu1601 PC UV spectrometer (Shimadzu, Kyoto, Japan) according to the method of Madadlou, Mousavi, Emam-Djomeh, Ehsani, and Sheehan (2009). The turbidity of the milk was monitored by transmittance at 450 nm.

2.5. Statistical analysis

All experiments were conducted in triplicate. Data were analysed using analysis of variance (ANOVA) and means were compared using Duncan's multiple range tests ($p < 0.05$).

3. Results and discussion

3.1. Protein composition in yak milk

The protein composition of the control yak milk is shown in Table 1. The summed concentration of the major proteins was 44.73 g L^{-1}, with 84.4% being caseins and 15.6% whey proteins. This casein-to-whey ratio is slightly higher than that in cow milk. Of the casein (CN) proteins, β-CN was at the highest concentration, followed by α_{S1}-CN, κ-CN and α_{S2}-CN in the ratio 4:2.4:1.2:1 (β:α_{S1}:κ:α_{S2}), which is different from that of cow milk (4:4:1:1) with both β-CN and κ-CN being greater in yak milk than cow milk. Among the whey proteins, β-lactoglobulin (β-Lg) was the predominant protein, followed by α-lactalbumin (α-La) and serum albumin (SA). However, the ratio and the amounts of the three major whey proteins in yak were different from those in cow milk.

Table 1
Concentration of major proteins in the raw skim yak milk.

Protein in yak milk	Content (g L^{-1})
β-Casein	17.29
α_{S1}-Casein	10.66
κ-Casein	5.31
α_{S2}-Casein	4.48
β-Lactoglobulin	5.87
α-Lactalbumin	0.65
Serum albumin	0.47
Total protein	44.73

β-Lg and SA concentrations in yak milk were relatively greater than those in cow milk, whereas α-La was lower, which gave a ratio of β-Lg-to-α-La 9:1 in yak milk compared with that of 4:1 in cow milk (Bonizzi et al., 2009).

3.2. Effects of pH and temperature on the contents of whey proteins and casein in the serum phase

The effects of pH and heating on whey proteins are shown as the percentages of remaining individual proteins in the serum phase, in Fig. 1A–C. In unheated samples nearly all (100%) whey proteins were present in the serum phase. At 65 °C at natural pH (6.6), a reduction in the concentration of individual whey proteins was observed, with SA being reduced by 8.7%, α-La by 9.3% and β-Lg by 4.1%. SA and α-La were reduced by another ~10%, whereas only a very small reduction was observed for β-Lg at 75 °C. With increasing the heating temperature to between 75 and 95 °C, the concentrations of these three whey proteins substantially decreased. The extent of denaturation for β-Lg was about 60% and α-La 50% at this pH. The result was similar to that of cow milk heated to 90–100 °C for 10–15 min (Anema & Li, 2000, 2015).

The differences observed could be due to the differences in the heating time, the concentrations of individual whey proteins and subsequent interactions between denatured whey proteins interacting with κ-CN (Anema, 1998, 2007; Donato & Guyomarc'h, 2009; Donato, Guyomarc'h, Amiot, & Dalgleish, 2007). The amounts of whey proteins decreased with increasing temperature and increased concomitantly with pH from 6.0 to 7.0 at any given temperatures. An obvious reduction in β-Lg occurred between 75 and 85 °C (38.9–54.6%), whereas the reduction in α-La began between 65 and 75 °C (12.7–14.2%). Changes in SA levels (35.7–39.2%) between different pH values, particularly at the higher temperatures between 85 and 95 °C, were higher than those for β-Lg (13.8–15.3%) and α-La (15.9–20.4%). The levels of β-Lg and α-La increased in the yak milk serum phase with increasing in pH between 75 and 95 °C, these levels were much lower than cow milk at the similar pH values between 6.7 and 7.1 (Anema & Klostermeyer, 1997; Anema & Li, 2015).

The levels of caseins in the serum phase after heat treatment at pH 6.0–7.0 are shown in Fig. 1D–F. The levels of caseins were low in all unheated samples. After heating at 65 °C at natural pH, about 21% κ-CN, 11% α_S-CN and 12% β-CN were found in the serum phase. On increasing the heating temperature, the level of κ-CN in the serum progressively increased to 31% at 95 °C. In contrast, the levels of α_S-CN and β-CN decreased to below 5% at 95 °C. The maximum levels of α_S-CN and β-CN in the serum were found upon heating at 65 °C (11–12%). The concentration at natural pH of κ-CN in serum phase at 65 °C (21%) was similar with cow milk (~15%), the changes in the levels of κ-CN with increasing in temperature was less steep in yak milk (~10%; Anema & Li, 2000, 2015). The reduction of α_S-CN and β-CN were in heat-induced protein complexes formed with whey protein at temperature from 85 to 90 °C (Pesic, Barac,

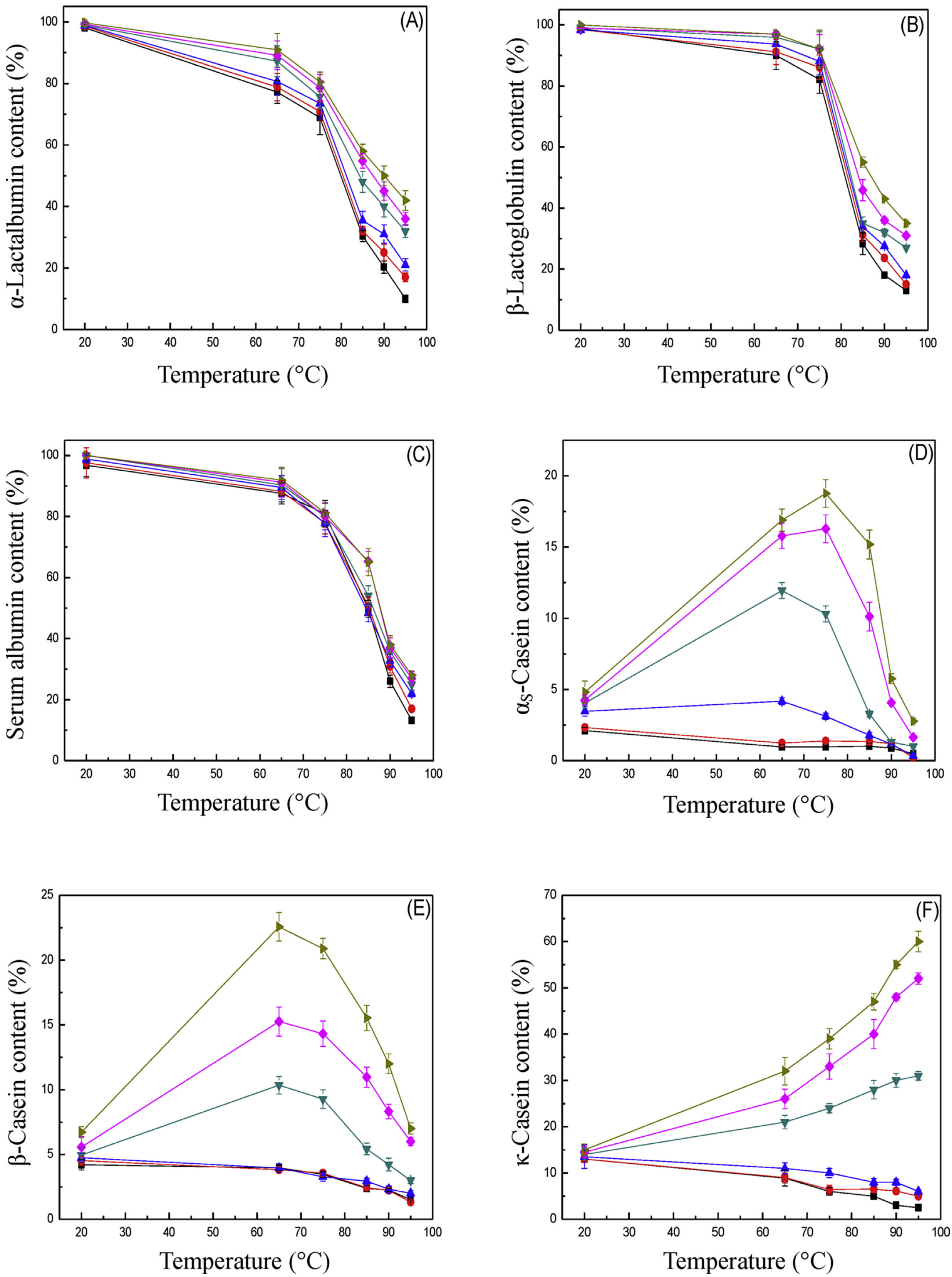

Fig. 1. Effect of temperature and pH on yak whey protein and casein in serum phase: A, α-lactalbumin; B, β-lactoglobulin; C, serum albumin; D, α-casein; E, β-casein; F, κ-casein after heating for 30 min at: ■, pH 6.0; ●, pH 6.2; ▲, pH 6.4; ▼, pH 6.6; ◆, 6.8; ▶, 7.0.

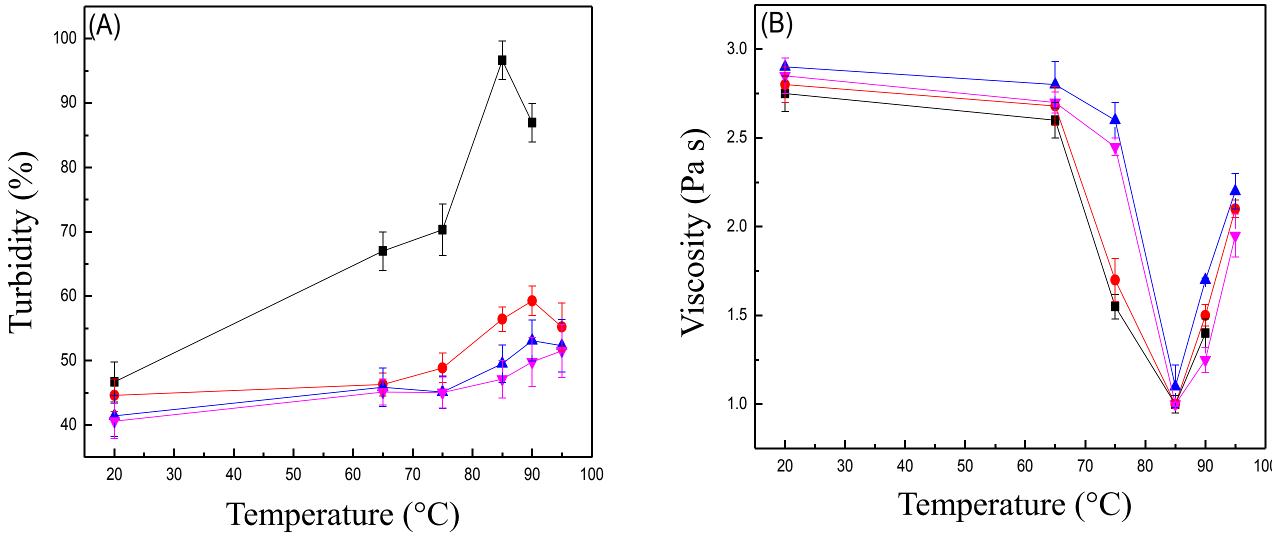

Fig. 2. Effect of pH on the yak milk turbidity (A) and viscosity (B) at different temperatures: ■, pH 6.4; ◆, pH 6.6; ▲, pH 6.8; ▼, pH 7.0.

Stanojevic, & Vrvic, 2014). The relatively lower levels of both κ-CN and β-Lg in the serum phase of yak milk upon heating to 95 °C suggest that as much as 70% of these proteins are associated with the micelle colloidal particles.

The levels of α_S-CN and β-CN in the serum phase decreased with increasing temperature from 75 °C onwards, at every pH value tested. At pH 6.0–6.4, the content of κ-CN in the serum declined following the increase in heating temperature. In contrast, at pH 6.6–7.0 the amount of κ-CN was increased concomitantly with temperature. At pH below 6.4, the dissociation of the casein micelle was low; together with the steep reduction in the levels of whey proteins in the serum phase upon heating, this indicates that most, if not all, of the denatured whey proteins were associated with casein micelles. With increasing pH from 6.6 to 7.0, the levels of caseins increased in the serum phase at a given temperature. The levels of α_S-CN and β-CN decreased, whereas κ-CN increased with increasing in heating temperatures.

3.3. *Effects of pH and temperature on the turbidity and viscosity of yak milk*

The turbidity and viscosity of milk samples heated at various temperatures in the range pH 6.4–7.0 are shown in Fig. 2A and B. Milk samples at pH 6.0–6.4 formed gels at 85–95 °C. The denatured whey proteins attached to casein micelles make the system susceptible to heat-induced aggregation in this pH range. The trend of turbidity and viscosity appears to be affected by the levels of caseins and whey protein in the serum phase. Therefore, it appears that the dissociation of the caseins and their interactions with denatured whey proteins were the major factors in governing yak milk properties (Anema, 1998, 2007; Anema & Li, 2003; Singh & Fox, 1985).

The turbidity of the yak milk increased concomitantly with temperature. At pH 6.4 it was particularly high. Low levels of whey proteins and caseins were found in the serum phase at pH 6.4, indicating that the interactions between the whey proteins and caseins are likely to be associated with micelles. Whereas at pH 6.6–7.0, the amounts of dissociated caseins and whey proteins complexes was increasing in the serum phase, resulting in the size of those complexes being smaller than the micelle colloidal particles, thus giving low levels of scattering (Jeurnink, 1992).

The viscosity decreased with increasing heating temperature from 20 to 85 °C giving the minimum values at 85 °C, but then increased noticeably with further increasing in temperature. Since the largest drop in whey proteins, particularly β-Lg, in the serum phase occurred at 80–85 °C, the sudden changes in the viscosity at ~85 °C indicates that the shift of the distribution of whey protein/κ-casein complexes from the serum phase to the colloidal phase changed the interactions between the colloidal particles.

4. Conclusions

The effected of yak milk heat stability by pH was similar with bovine milk and the dissociated from casein and whey proteins denaturation were showed a pH-dependent. The changes in protein distribution upon heating of skim yak milk at natural pH, α-La and SA in serum reduce at 65 °C, the most significant reduction of β-Lg occurred between 75 and 85 °C. At 95 °C, the amounts of β-Lg and κ-CN in the serum were around ~30%. At a pH below 6.4, the reduction of whey proteins in serum with increasing heat temperature and the levels of caseins were decreased. With increasing pH from 6.6 to 7.0, the levels of caseins increased in the serum phase at all temperatures. These results suggest that the dissociation of caseins from the micelle colloidal phase increased with increasing pH, and the distribution of β-Lg and κ-CN complexes also shifted towards more in the serum phased as demonstrated by the increasing levels of β-Lg and κ-CN.

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