ABSTRACT

The dissociation of yak casein (CN) micelles was evaluated by scanning electron microscopy, particle size, fluorescence properties, and soluble mineral and CN molecule content at pH 4.6 to 8.2. The results showed that the size of CN micelles remained constant with decreasing pH from 8.2 to 5.8 but sharply increased at pH ≤5.4. Casein micelles began to aggregate at pH 5.4, and the serum magnesium, potassium, iron, zinc, copper, and manganese levels had their minimum values at this pH level. During acidification, colloidal calcium phosphate dramatically disassociated from yak CN micelles, but the soluble CN monomer content decreased slightly. During alkalization, the soluble calcium and phosphorus content decreased below pH 6.8 but increased with pH increases from 6.8 to 8.2. However, the soluble CN content increased markedly during alkalization. The emission wavelength of 8-anilino-1-naphthalenesulfonic acid sodium salt fluorescence decreased during both acidification and alkalization from pH 6.6, whereas the opposite was found for intrinsic fluorescence.

Key words: yak milk, casein micelle, mineral, fluorescence property

INTRODUCTION

Yak milk is a unique kind of milk with high protein and mineral contents, and it is particularly rich in αs2-CN (4.80 and 2.68 g/L in yak and bovine milk, respectively), β-CN (18.20 and 9.60 g/L in yak and bovine milk, respectively), calcium (198–227 mg/100 g of yak milk; 114 mg/100 g of bovine milk), and phosphorus (154–170 mg/100 g of yak milk; 103 mg/100 g of bovine milk; Li et al., 2010; Yang et al., 2014a; Cui et al., 2016). It is also a crucial material for herdsmen on the Qinghai-Tibet Plateau. Several yak milk products are manufactured, such as CN, yogurt, and milk powder. Yak CN has higher emulsifying activity, foam stability, foaming capacity, and water absorption than cow CN, but its emulsion stability is much lower than that of cow CN (Yang et al., 2014a). Yak CN and caseinate are the major products made from qula, which is a yak milk product made by a traditional method: defatting, acidifying, and drying in air (Liu et al., 2013). Qula contains approximately 80% protein, and it is much easier to collect, store, and transport than fresh yak milk because of the remote location and poor transportation on the Qinghai-Tibet Plateau. As an important industrial material, qula is melted in water with pH 8 to 10 and then acidified by hydrochloric acid to pH 4.6 to extract the CN products. However, the processing of qula is crude due to a lack of knowledge about the effects of pH on yak milk, resulting in low yields and poor functional properties of the products.

It is well known that pH, as well as heat treatment, can alter the microstructure and physicochemical behavior of CN micelles significantly (Liu and Guo, 2008). Therefore, much research has been undertaken on the effect of pH on bovine milk, combined with heating. It has been shown that CN micelles transform from a loose to a compact microstructure during acidification (Liu and Guo, 2008; Liu et al., 2017). At pH <5.4, the formation of CN aggregates with complex behavior occurs, followed by milk gel formation at pH 4.8 (McMahon et al., 2009; Anema and Li, 2015). It has further been demonstrated that bovine CN particle size has a significant negative correlation with pH (Taterka and Castillo, 2015).

Colloidal calcium phosphate (CCP) contributes to the conformation and stability of CN micelles (de Kruif et al., 2012). The trend of change in CN micelles throughout acidification can be estimated from the distribution of calcium and phosphorus between serum and the micellar phase. During acidification, the micellar contents of colloidal calcium, phosphorus, and magnesium decrease, whereas those of sodium and potassium first decrease and then increase sharply around pH 5.8 or 5.5 (Law and Leaver, 1998; Silva et al., 2013; Liu et al., 2017). Meanwhile, the micellar CN molecule content changes slightly with decreasing pH (Anema and Klostermeyer, 1997; Law and Leaver, 1998).
Although there are many reports of the influence of pH on bovine milk, it is doubtful whether their results could be applied to yak milk processing and whether yak milk is deviating from the general knowledge on bovine CN micelles because of the great differences between yak and bovine milk in terms of their constituents and properties (Wang et al., 2013; Yang et al., 2014a,b). Previous studies on the influence of pH on characteristics of yak milk have proven that the heat stability of yak milk protein reaches a maximum at approximately pH 6.8, that heating leads to a marked increase in serum κ-CN content, and that particle size decreases with increases in pH, with similar, though less marked, changes in α-CN and β-CN (Li et al., 2014; Wang et al., 2017). The dissociation degree of α-CN, β-CN, and κ-CN in heating bovine milk with different temperature has the lowest value under pH 6.5. According to a report of qula’s properties across a large pH range, its solubility dramatically increases as the pH increases from 6.0 to 8.0 and decreases as the pH increases from 9.0 to 12.0, but the solubility of yak CN changes little as the pH increases from 6.0 to 12.0 (Liu et al., 2013; Yang et al., 2014a). However, there is no report of the microstructure and dissociation of CCP and other minerals from yak CN micelles at different pH levels. Moreover, the dissociation of CN molecules and minerals across a wide pH range without heating has not been discussed in detail.

In this study, the dissociation of yak CN monomers and 8 minerals was measured at pH 4.6 to 8.2. The microstructure and fluorescence properties of yak CN micelles at pH 5.0 to 8.2 are presented, and the relationship between soluble calcium and phosphorus increases in supernatant and yak CN micellar size is analyzed. The aim of this study was to provide basic data and reference information for industrial processing and manufacture of qula and yak milk products.

**MATERIALS AND METHODS**

**Materials**

Yak milk was collected from Zhuaxixiulong Town of the Tianzhu grassland, on the Qinghai-Tibetan Plateau, in northwest China. The Tianzhu grassland is a typical pasturing area for yak production. After milking, 0.02% (wt/vol) sodium azide was added to the milk to inhibit bacterial growth. Yak milk was then placed in sterile plastic bottles and stored in a box filled with ice, which was transported to the laboratory within 6 h. 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 (Yang et al., 2015).

**pH Adjustment**

Raw skim yak milk samples (50 mL) were pH adjusted from 4.60 ± 0.02 to 8.20 ± 0.02 using 0.1 to 1 M NaOH or 0.1 to 1 M HCl at intervals of 0.4. The samples were allowed to equilibrate for at least 2 h, and minor readjustments were made to achieve a stable pH.

**Particle Size Measurement**

Sample aliquots of 50 μL were diluted with distilled water to 15 mL for particle size measurement. The average diameter of particles was determined using a Malvern Zetasizer (Malvern Instruments Ltd., Malvern, UK) at 25°C (Chandrapala et al., 2012).

**Scanning Electron Microscopy**

Samples were diluted and dropped onto silicon chips. The silicon chips were freeze-dried for 24 h using a vacuum freeze-drying machine (GLZ-0.4, Su Yuan Zhong Tian Scientific Inc., Beijing, China), then coated with gold. Images of typical structures were recorded at a magnification of 20,000 using an S-4800 microscope (Hitachi Ltd., Tokyo, Japan) operating at 5 kV.

**Fluorescence Spectroscopy**

Skim milk samples (1.5 mL) were diluted to 5 mL with distilled water for fluorescence measurements. Intrinsic fluorescence experiments were performed with an RF-5301PC luminescence spectrometer (Japan Shimadzu Co., Kyoto, Japan) for solutions in a 1-cm path length quartz cell at room temperature (20–24°C). The excitation and emission slits were fixed at 5 nm, the excitation wavelength was set at 280 nm, and the emission spectra were collected from 290 to 450 nm.

A volume of 200 μL of 8-anilino-1-naphthalenesulfonic acid sodium salt (ANS; 8.0 × 10⁻⁵ M) was mixed with 8 mL of diluted milk and allowed to stand for 3 min. The excitation and emission slits were fixed at 5 nm, the excitation wavelength was set at 280 nm, and the emission spectra were collected from 290 to 450 nm (Yang et al., 2015).

**Protein Analysis of Supernatant**

To separate protein aggregates and CN micelles, acidified skim milk was centrifuged at 120,000 × g for 40 min at 20°C using a Beckman Optima XL-100K refrigerating ultracentrifuge (Beckman Coulter, Brea, CA). The supernatant was collected for analysis of protein and mineral contents. Supernatant (4 mL) was
added to 4 mL of buffer solution (8 mol/L of urea, 44 mmol/L of sodium citrate, and 0.3% (vol/vol) β-mercaptoethanol). Protein standards (20 mg) were separately dissolved in 10 mL of buffer solution as above.

The protein content of the supernatant was measured by reversed-phase HPLC apparatus (Agilent 1100; Agilent Technologies, Santa Clara, CA) equipped with a C18 column (150 mm × 4.6 mm, 300-Å pores, 5-µm particles; Agilent) and a UV/visible detector at 220 nm. Solvents A and B were solutions of acetonitrile–water–trifluoroacetic acid of different ratios (100:900:1 and 900:100:1 by volume, respectively). After filtering through a 0.45-µm filter (Membrana, Wuppertal, Germany), 20 µL samples were analyzed.

The analysis was carried out by applying a binary gradient profile of the mobile phase. The gradient elution program was run at a constant flow rate of 1.0 mL/min and was set as follows: 0- to 13-min linear gradient from 20 to 31.3% B, followed by a 3-min isocratic elution of 31.3% B; 17- to 19-min linear gradient from 31.3 to 34.7% B, followed by a 3-min isocratic elution of 34.7% B; 23- to 24-min linear gradient from 34.7 to 36.0% B, followed by a 5-min isocratic elution of 36.0% B; 30- to 31-min linear gradient from 36.0 to 38.2% B, followed by a 5-min isocratic elution of 38.2% B; 37- to 38-min linear gradient from 38.2 to 40.8% B; and 39- to 45-min linear gradient from 40.8 to 46.0% B. The temperature of the column was maintained at 25°C.

Mineral Analysis of Supernatant

The supernatants were hydrolyzed by hydrogen peroxide and nitric acid. The calcium, magnesium, phosphorus, and potassium contents were determined using an Optima 7000DV inductively coupled plasma optical emission spectrometer (Perkin Elmer, Waltham, MA). The iron content was determined by a GGX-800 atomic absorption spectrometer (Haiguang Instrument Ltd. Co., Beijing, China). The manganese, copper, and zinc contents were measured by an ICPQc inductively coupled plasma mass spectrometer (Thermo Fisher, Waltham, MA).

Statistical Analysis

All data were expressed as mean ± standard deviation from at least 3 independent trials. The differences were assessed by 1-way ANOVA and Duncan’s multiple range tests. Statistical significance was set at $P < 0.05$. The data were analyzed using PASW Statistics 18.0 software (SPSS Inc., Chicago, IL) and Origin 8.0 (OriginLab Corporation, Northampton, MA).

RESULTS AND DISCUSSION

Effect of pH on Particle Size of Yak Skim Milk

The Z-average diameter of skim milk at different pH values is shown in Figure 1. The CN micelles reached a maximum size at pH 5.0, which was close to the isoelectric point of CN. The size of CN micelles in yak skim milk at pH 5.4 was 144 nm smaller than at pH 5.0. A sharp increase in particle size indicated the beginning of the aggregation phase of CN at pH ≤5.4, which has been identified in the milk of different species (Moitzi et al., 1996; Jaubert et al., 1999; Liu et al., 2017). Higher polydispersity index and multimodal size distribution were observed at the same pH in bovine skim milk (Liu et al., 2017). As shown in Figure 1, pH had no effect on the Z-average diameter of CN micelles in the pH range 5.8 to 8.2. It has been reported that the size of yak CN micelles was in the range of 193 to 212 nm at natural pH levels (Wang et al., 2013; Li et al., 2014; Yang et al., 2014b). However, a size of 149.9 ± 0.2 nm at pH 6.6 was observed in the present study, possibly due to the use of different measurement techniques and the sourcing of yak milk from a different geographical area.

The steady particle size was found when increasing pH from 5.8 to 8.2 (Figure 1), and other researchers have found an almost constant particle size in yak skim milk when increasing pH over the range of 6.4 to 7.6 (Li et al., 2014). Therefore, it could be deduced that pH does not significantly influence the size of CN micelles in yak skim milk over the pH range of 5.8 to 8.2. The
same conclusion has been deduced in cow skim milk from pH 5.4 to 6.7 (Liu et al., 2017).

Generally, the Z-average diameter of CN micelles is a complex colloidal property. The change of CN micellar structure might be determined by a combination of size and various structural parameters whose relative contributions would change during acidification and alkalization (Liu et al., 2017). Therefore, the topography, fluorescence properties, and dissociation of CN molecules and minerals from CN micelles were measured to reveal the structural change of CN micelles during acidification and alkalization.

**Topography of CN Micelles in Yak Skim Milk at Different pH Levels**

The topography of yak CN micelles, obtained by scanning electron microscopy, at different pH levels proved that the size of the micelles changed little with increasing pH from 5.8 to 8.2 (Figure 2). During acidification, micelles seemed to maintain their integrity until the pH decreased below 5.4 (Figure 2B, C, D). Scanning electron microscopy indicated that CN micelles came closer to each other and aggregated before clusters with a network microstructure were formed at pH 5.0 (Figure 2A). Although there was no obvious aggregation at pH 5.4, the stretched structure of the micelles became denser and small units of aggregates appeared (Figure 2B).

According to scanning electron microscopy images, the aggregation of yak CN micelles with acidification could be divided into 3 stages. The first stage was at pH 6.6 to 5.8, when the micelles maintained their original shape and individual characteristics, although there were a few micelles connected to each other, which could be due to the high micelle content and the particles not being dispersed very well. With continuous acidification to around pH 5.4, the second stage, a few CN micelles formed small aggregates but most retained their individuality, consistent with the Z-average diameter results. It seemed that significant aggregation occurred at pH 5.0 (the beginning of the third stage), when micelles lost their individual shape and linked to each other, thus achieving the network microstructure. This observation was consistent with the results by Gonzalez-Jordan et al. (2015), who found a sharp increase in density and the formation of large flocs at pH 5.0 in bovine milk by confocal laser scanning microscopy. Compared with the initial aggregation pH of yak CN micelles of 5.4, that of bovine CN micelles was reported to be 5.8 (Gastaldi et al., 1996). Therefore, yak CN micelles are more stable than bovine CN micelles because of their higher calcium and phosphorus contents.

During alkalization, yak CN micelles appeared to retain their shape and individuality (Figure 2E, F, G, H). It also seemed that some small micelles became smaller still due to the slight dissociation of CN molecules, whereas larger particles changed little with increasing pH from 7.0 to 8.2. Moreover, the size of CN micelles decreased slightly throughout alkalization according to scanning electron microscopy, contrary to the Z-average size values (Figure 1). Although the size of aqueous CN micelles remained stable with pH change, the interaction among CN molecules in micelles was changed; the CN micelles shrank to a smaller size during dry treatment for scanning electron microscopy.

**Fluorescence Spectroscopy of Yak Skim Milk at Different pH Levels**

The tyrosine, tryptophan, and phenylalanine residues of protein usually fluoresce. The fluorescence spectrum of protein is sensitive to the microenvironment because of these chromophores. According to Figure 3A, the intrinsic fluorescence spectrum of yak skim milk changed with pH alteration. Figure 3B showed the maximum intrinsic fluorescence intensity (\(I_f\)) and its emission wavelength (\(\lambda_{max}\)) in yak skim milk at different pH values. It showed that the \(I_f\) increased sharply when pH decreased from 6.6 to 5.0. During alkalization, \(I_f\) decreased at pH 7.0, then slightly increased with increasing pH. The \(I_f\) values at pH <6.6 were much higher than those at pH >6.6. The \(\lambda_{max}\) of intrinsic fluorescence increased both when pH increased from 6.6 to 8.2 and when it decreased from 6.6 to 5.8. When pH decreased at pH ≤5.8, \(\lambda_{max}\) remained constant.

With acidification, the protonation of AA led to stronger hydrogen bonds between CN micelles. In addition, cation–π interaction occurred between the cationic and aromatic side chains of CN (Liu and Guo, 2008). Therefore, the structure of CN micelles became more compact, resulting in tryptophan residues being buried in a hydrophobic domain and the \(I_f\) increasing. Although \(\lambda_{max}\) increased at the beginning of acidification, the increase was less than 2 nm. During alkalization, deprotonation of the carboxylic groups created a loose CN micellar structure, which led to the dissociation of CN from micelles. Thus, the content of soluble CN increased, and a red shift of \(\lambda_{max}\) and increase of \(I_f\) were observed (except for at pH 7.0).

In terms of the ANS fluorescence spectrum of yak skim milk at different pH levels, \(\lambda_{max}\) presented an opposite trend to that of intrinsic fluorescence, which decreased during both acidification and alkalization from the natural pH of 6.6 (shown in Figure 3C). The trend of \(I_f\) in the ANS fluorescence spectrum was similar to that of intrinsic fluorescence (shown in Figure 3D).
Figure 2. The topography of CN micelles in yak skim milk at different pH levels (scanning electron microscope; ×20,000).
The ANS anion bound readily to the cationic groups of lysine, arginine, and histidine side chains. During acidification, the negative charge decreased and ANS binding to CN increased; thus, $I_f$ increased. In addition, acidification changed the structure of CN micelles (as intrinsic fluorescence showed), and the surface hydrophobicity of yak CN micelles increased. The blue shift of $\lambda_{\text{max}}$ with acidification could be attributed to compaction of the CN micelle structure. With alkalinization, the dissociation of minerals and CN molecules weakened the steric hindrance between ANS and CN, which could lead to an increase of $I_f$. Moreover, the change of whey-CN interaction and the slight disruption of CN micelles would make the hydrophobicity of yak CN micelles increased during alkalinization (Chandrapala et al., 2013). However, the state transformation of phosphate and the addition of sodium made the internal structure of micelles more compact; thus, $\lambda_{\text{max}}$ shifted toward the lower wavelength. A different ANS fluorescence spectrum trend was found in bovine CN micelles (Liu and Guo, 2008). It could be concluded that minerals and whey protein have a significant effect on both intrinsic and ANS fluorescence spectra of milk.

**Effect of pH on Dissociation of Protein from CN Micelles in Yak Skim Milk**

The degree of protein dissociation in yak skim milk supernatant against pH is presented in Figure 4 by means of protein concentration. The $\kappa$-CN concentration decreased with acidification from pH 6.6 to 5.0, as did the $\beta$-CN concentration. The content of $\alpha$-CN es-
sentially changed little from pH 5.0 to 6.6 except for at pH 5.8. The slight decreases in κ-CN and β-CN content during acidification implied that they had isoelectrically aggregated. In other words, the CN molecule did not dissociate from the micelle during acidification. Law and Leaver (1998) reported that the extent of dissociation of the CN, especially κ-CN, increased significantly as cow skim milk was acidified between pH 6.7 and 5.5 at 20°C. The higher stability of yak CN micelles should be due to their higher content of CCP compared with cow CN micelles (Wang et al., 2013).

Whey protein followed a different trend from the CN, with its content increasing when pH decreased from 6.6 to 5.8 and then decreasing. The content of the 3 CN in skim milk, especially β-CN, increased dramatically with alkalization from pH 6.6 to 8.2. However, the whey protein content remained constant throughout alkalization.

The α-CN content followed a pH-independent profile during acidification, attributed to its higher level of phosphorylation and lower isoelectric point compared with other CN molecules (McMahon et al., 2009). The marked increase in CN content throughout pH increase from 6.6 to 8.2 indicated the dissociation of CN micelles. It has been reported that κ-CN content in yak skim milk supernatant increased slightly across the pH ranges 6.4 to 7.6 (Li et al., 2014), 6.6 to 7.4 (Xu et al., 2015), and 6.0 to 7.0 (Wang et al., 2017). The pH had no effect on the whey protein content of yak skim milk supernatant in the pH range 6.6 to 8.4. The trend of whey protein content observed in the present study was similar to that in other analyses of yak skim milk (Li et al., 2014; Xu et al., 2015; Wang et al., 2017). The soluble protein content in the milk of other species has been widely reported, and the soluble protein components identified in yak skim milk in the present study are comparable with those previously reported (Anema and Klostermeyer, 1997; Anema et al., 2007; Pesic et al., 2014; Anema and Li, 2015; Liu et al., 2017).

It was likely that β-CN had a higher concentration because of its higher content in skim milk and lower level of phosphorylation. Although the κ-CN content of skim milk was much lower than that of α- and β-CN, its content was slightly lower than that of α-CN at pH 5.8 to 7.4 because κ-CN had the lowest phosphorylation and was located on the surface of CN micelles. Therefore, the order of preferential dissociation from CN micelles was κ > β > α, which correlated with their levels of phosphorylation.

**Effect of pH on Dissociation of Minerals in Yak Skim Milk at Different pH Levels**

Calcium and phosphorus are the main and most important minerals in milk. Two-thirds of calcium and one-third of phosphorus are in the micellar phase, presenting as CCP or micellar calcium phosphate (de Kruif et al., 2012). Casein molecules link together by bridging CCP to form CN micelles (Mata et al., 2011). Calcium was released from CN micelles rapidly ($P < 0.05$) when pH decreased from 6.6 to 4.6 (shown in Figure 5A). Additionally, the phosphorus content increased throughout acidification. It was shown that the soluble calcium and phosphorus contents reached a maximum at pH 4.6 and a minimum at pH 6.8 and then increased with increasing pH. The calcium and phosphorus contents in serum at pH 8.2 were similar to those at pH 5.4.

Sharp increases in soluble calcium and phosphorus in the serum of skim milk acidified with HCl were observed between pH 6.0 and 4.6 in most previous works (Gastaldi et al., 1996; Gonzalez-Jordan et al., 2015; Koutina and Skibsted, 2015; Liu et al., 2017), suggesting increased solubilization of CCP. Organic and inorganic phosphates are protonated during acidification of milk, while, simultaneously, the serum phase is no longer saturated with calcium phosphate, thus leading to the gradual solubilization of CCP until a new steady state is achieved. A different result reported by Law and Leaver (1998) indicated that acidification with HCl did not markedly affect the solubilization of CCP in bovine skim milk over most of the pH range 6.7 to 4.6, which was measured immediately and after re-equilibration for 18 h at 4°C and 22°C after acidification to preset pH values (Law and Leaver, 1998; Zhao...
The calcium and phosphorus contents in yak skim milk supernatant decreased in the pH range 6.6 to 7.0 and then increased as the pH range increased from 7.0 to 8.2, which was consistent with results obtained for cow milk and similar to those for buffalo milk with a turning point of pH 7.8 (Ahmad et al., 2009).

With pH increase, inorganic phosphorus changes from $\text{HPO}_4^{2-}$ toward $\text{PO}_4^{3-}$. The $\text{PO}_4^{3-}$ state has a greater affinity for calcium ($2.88 \times 10^6 \text{ M}^{-1}$) than does $\text{HPO}_4^{2-}$ ($642 \text{ M}^{-1}$; Ahmad et al., 2009). As the calcium phosphate in the aqueous phase of the milk was saturated, precipitates of this salt formed. Thus, the calcium and phosphorus contents decreased. However, this precipitate was too light to remove by ultracentrifugation, but it was small enough to associate with proteins that would contain nucleation points or incorporate into micellar calcium (Ahmad et al., 2009).

A considerable amount of sodium was added to the skim milk during continuous alkalization. The addition of sodium was likely to have affected the electrostatic interaction among CN micelles, as seen with the addition of NaCl in the literature (Huppertz and Fox, 2006; Nguyen et al., 2017). Sodium replaced some calcium bound to CN micelles via phosphoseryl residues, thus increasing the calcium content in the supernatant. In addition, the dissociation of CN micelles (shown in Figure 3) caused CCP dissolution, resulting in increases in soluble calcium and phosphorus.

The calcium and phosphorus contents of supernatant followed a polynomial function for pH values of 4.6 to 8.2 (Figure 5A). A linear relationship between increases in soluble calcium and phosphorus in yak skim milk at pH 4.6 to 6.6 was identified, and its function was shown in Figure 5B, which was consistent with previous findings for bovine milk at pH 4.9 to 6.7 (Liu et al., 2017). The molar ratios between colloidal calcium and inorganic phosphorus of bovine milk were reported to be in the range of 1.7 to 1.9 (Silva et al., 2013). The molar ratio of 1.87 in yak milk indicated the same basic type of CCP as was found in bovine milk.

The magnesium, potassium, iron, zinc, copper, and manganese contents in supernatant and in yak skim milk are presented in Table 1. During acidification, the levels of most minerals in serum first increased and then sharply decreased at pH 5.4, followed by further increase. In other words, all minerals reached their minimum content at pH 5.4. Scanning electron microscopy has shown that small yak CN micelle aggregates formed at pH 4.6, which should be presumed to absorb the minerals and were precipitated by ultracentrifugation. In addition, a compact microstructure and elastic properties of bovine CN micelles were found at pH 5.5, and the loss tangent and viscosity reached a minimum at approximately pH 5.3 according to rheology (Gastaldi et al., 1996; Liu and Guo, 2008). Casein micelles are hypothesized to pass through a transition state to compensate for the loss of interaction with CCP not only by new protein–protein interactions but also by electrostatic interactions between minerals and AA residues. This is the most likely reason for the minimum mineral levels observed in yak milk supernatant at pH 5.4.

During alkalization, the mineral content first decreased and then markedly increased ($P < 0.05$). The increasing number of negative AA residues strongly associated with cations during alkalization, thus decreas-
Table 1. Total and soluble mineral content of yak skim milk at different pH levels

<table>
<thead>
<tr>
<th>pH</th>
<th>Mg (mg/L)</th>
<th>K (mg/L)</th>
<th>Fe (mg/L)</th>
<th>Zn (mg/L)</th>
<th>Cu (µg/L)</th>
<th>Mn (µg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>155.03 ± 11.67</td>
<td>1,535.61 ± 29.95</td>
<td>7.79 ± 0.24</td>
<td>4.73 ± 0.13</td>
<td>122.80 ± 13.95</td>
<td>199.80 ± 2.95</td>
</tr>
<tr>
<td>4.6</td>
<td>137.04 ± 3.57a</td>
<td>1,387.21 ± 9.81a</td>
<td>2.56 ± 0.11ab</td>
<td>4.32 ± 0.17ab</td>
<td>62.64 ± 2.89a</td>
<td>118.18 ± 6.64a</td>
</tr>
<tr>
<td>5.0</td>
<td>120.94 ± 2.12abc</td>
<td>1,358.14 ± 18.12abc</td>
<td>2.86 ± 0.19bc</td>
<td>2.82 ± 0.22abc</td>
<td>49.93 ± 3.80abc</td>
<td>116.23 ± 4.25abc</td>
</tr>
<tr>
<td>5.4</td>
<td>100.65 ± 1.61c</td>
<td>1,216.10 ± 9.06c</td>
<td>2.54 ± 0.21bc</td>
<td>1.60 ± 0.36bc</td>
<td>44.40 ± 2.36bc</td>
<td>103.72 ± 4.11bc</td>
</tr>
<tr>
<td>5.8</td>
<td>120.23 ± 4.19b</td>
<td>1,377.92 ± 16.78b</td>
<td>3.85 ± 0.07bc</td>
<td>2.31 ± 0.16bc</td>
<td>55.67 ± 1.94bc</td>
<td>133.72 ± 8.45bc</td>
</tr>
<tr>
<td>6.2</td>
<td>106.49 ± 2.68c</td>
<td>1,407.52 ± 6.89c</td>
<td>2.86 ± 0.11c</td>
<td>1.55 ± 0.22c</td>
<td>56.96 ± 3.58c</td>
<td>115.57 ± 4.83c</td>
</tr>
<tr>
<td>6.6</td>
<td>103.45 ± 2.96cd</td>
<td>1,405.57 ± 6.42d</td>
<td>4.39 ± 0.18de</td>
<td>1.42 ± 0.25d</td>
<td>47.47 ± 3.12d</td>
<td>107.34 ± 4.98d</td>
</tr>
<tr>
<td>7.0</td>
<td>94.15 ± 3.16e</td>
<td>1,194.11 ± 6.62e</td>
<td>3.03 ± 0.11c</td>
<td>1.21 ± 0.13c</td>
<td>45.55 ± 2.19c</td>
<td>92.49 ± 5.42c</td>
</tr>
<tr>
<td>7.4</td>
<td>90.23 ± 3.01f</td>
<td>1,216.41 ± 6.73f</td>
<td>3.16 ± 0.19f</td>
<td>1.21 ± 0.13f</td>
<td>45.48 ± 2.19f</td>
<td>92.49 ± 5.42c</td>
</tr>
<tr>
<td>7.8</td>
<td>104.43 ± 4.08e</td>
<td>1,341.22 ± 3.57e</td>
<td>6.66 ± 0.32f</td>
<td>2.07 ± 0.13f</td>
<td>57.13 ± 1.76e</td>
<td>166.60 ± 9.98e</td>
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<tr>
<td>8.2</td>
<td>119.67 ± 1.33f</td>
<td>1,447.18 ± 5.96f</td>
<td>7.02 ± 0.11g</td>
<td>3.17 ± 0.13f</td>
<td>91.40 ± 1.76f</td>
<td>182.75 ± 6.18f</td>
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<tr>
<td>4.6</td>
<td>137.04 ± 3.57a</td>
<td>1,387.21 ± 9.81a</td>
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<td>2.86 ± 0.19bc</td>
<td>2.82 ± 0.22abc</td>
<td>49.93 ± 3.80abc</td>
<td>116.23 ± 4.25abc</td>
</tr>
<tr>
<td>5.4</td>
<td>100.65 ± 1.61c</td>
<td>1,216.10 ± 9.06c</td>
<td>2.54 ± 0.21bc</td>
<td>1.60 ± 0.36bc</td>
<td>44.40 ± 2.36bc</td>
<td>103.72 ± 4.11bc</td>
</tr>
<tr>
<td>5.8</td>
<td>120.23 ± 4.19b</td>
<td>1,377.92 ± 16.78b</td>
<td>3.85 ± 0.07bc</td>
<td>2.31 ± 0.16bc</td>
<td>55.67 ± 1.94bc</td>
<td>133.72 ± 8.45bc</td>
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<td>6.2</td>
<td>106.49 ± 2.68c</td>
<td>1,407.52 ± 6.89c</td>
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<td>1.55 ± 0.22c</td>
<td>56.96 ± 3.58c</td>
<td>115.57 ± 4.83c</td>
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<td>6.6</td>
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<td>4.39 ± 0.18de</td>
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</tr>
<tr>
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<td>90.23 ± 3.01f</td>
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<td>57.13 ± 1.76e</td>
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</tr>
<tr>
<td>8.2</td>
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<td>1,447.18 ± 5.96f</td>
<td>7.02 ± 0.11g</td>
<td>3.17 ± 0.13f</td>
<td>91.40 ± 1.76f</td>
<td>182.75 ± 6.18f</td>
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*#Means within a column with different superscripts differ (P < 0.05).

ACKNOWLEDGMENTS

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REFERENCES

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