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Effect of pH on dissociation of casein micelles in yak skim milk

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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 α_{S2}-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).

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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^{-3} 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 390 nm, and the emission spectra were collected from 400 to 650 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