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The aggregation behavior and interactions of yak milk protein under thermal treatment

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ABSTRACT

The aggregation behavior and interactions of yak milk protein were investigated after heat treatments. Skim yak milk was heated at temperatures in the range of 65 to 95°C for 10 min. The results showed that the whey proteins in yak milk were denatured after heat treatment, especially at temperatures higher than 85°C. Sodium dodecyl sulfate-PAGE analysis indicated that heat treatment induced milk protein denaturation accompanied with aggregation to a certain extent. When the heating temperature was 75 and 85°C, the aggregation behavior of yak milk proteins was almost completely due to the formation of disulfide bonds, whereas denatured α-lactalbumin and β-lactoglobulin interacted with κ-casein. Besides, when yak milk was heated at 85 and 95°C, other noncovalent interactions were found between proteins including hydrophobic interactions. The particle size distributions and microstructures demonstrated that the heat stability of yak milk proteins was significantly lowered by heat treatment. When yak milk was heated at 65 and 75°C, no obvious changes were found in the particle size distribution and microstructures in yak milk. When the temperature was 85 and 95°C, the particle size distribution shifted to larger size trend and aggregates were visible in the heated yak milk.
Key words: yak milk protein, thermal aggregation, disulfide bonds, hydrophobicity, SDS-PAGE

INTRODUCTION

The yak, which is distributed on the Qinghai-Tibetan Plateau at altitudes above 3,000 m, is a rare and valuable bovine species. Yak milk products are gaining in popularity due to their special nutritional value. To promote the industrialization of yak milk products, it is necessary to investigate the physico-chemical properties of yak milk, particularly the heat stability characteristics of yak milk proteins (Li et al., 2014).

Heat treatment is one of the essential steps widely used in industrial dairy processes for either improving the technological–functional properties of dairy products or ensuring their safety and shelf life (Oldfield et al., 2000). It can reduce the number of microorganisms, but may result in a certain degree of protein denaturation. Inappropriate thermal intensity can lead to product browning, protein precipitation, and other quality issues (Taterka and Castillo, 2015). The effect of thermal treatment on the stability of milk proteins mainly concentrate on the denaturation of whey proteins and cross linking between whey proteins and casein micelles. Heat-induced casein/whey protein interactions in cow milk have been reported (Rattray and Jelen, 1997; Boye and Alli, 2000; Oldfield et al., 2000). The β-LG of bovine milk will be denatured and crosslinked with κ-casein at a certain heating intensity (Anema, 2000; Guyomarc'h et al., 2003). When heating intensity increases further, α-LA also begin to denature, form complexes with denatured β-LG, and attach to the surface of casein micelles (Corredig and Dalgleish, 1996). It has been shown that the main contributor to thickening of milk after heating at low pH is the variation in the ratio of casein to whey protein when the serum mineral composition is the same. The higher the proportion of whey proteins in total protein composition, the lower the gelation temperature, and the more susceptible the milk is to thickening (Singh et al., 2015). Casein is capable of controlling the aggregation behavior of heat-induced denatured whey proteins, even at acidic pH values (O'Kennedy and Mounsey, 2006). Besides, the heat stability of low-heat skim milk powder could be improved by reducing free Ca^{2+} concentration of the original milk, suggesting that free Ca^{2+} concentration in milk can be used as an indicator of the heat stability of skim milk (Faka et al., 2009).

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The content of TS (16.9–17.9%), proteins (4.9–5.9%), and fat (5.5–7.5%) of yak milk is higher than that in cow milk (Li et al., 2010). With regard to protein fractions, yak milk contains more protein and caseins than cow milk (Li et al., 2010). Total casein content of yak milk (40.2 $g\cdot L^{-1}$ on average) is 1.5 times the concentration of cow milk. Compared with cow caseins, yak caseins contain more α_{S2}- and β-casein and less α_{S1}- and κ-casein (Wang et al., 2013). The ratio of whey protein in the total protein of yak milk is higher than that of cow milk as well (Li et al., 2010). Because whey proteins are more heat-sensitive than caseins, the whey protein ratio differences may influence the interactions between whey proteins and caseins in heated yak milk, which leads to aggregation behavior variances. Besides, the major mineral contents of yak milk are much higher than those of cow milk, including calcium (Li et al., 2011). The existence of more calcium may reduce the solubility of proteins and induce protein aggregation (Vyas and Tong, 2004). Therefore, due to the differences of protein composition and calcium content between cow milk and yak milk, the heat stability and proteins aggregation of yak milk may also be different. Therefore, it is necessary to investigate the heat stability of yak milk protein and demonstrate the differences from cow milk.

Until now, little research has been conducted on the heat stability of yak milk protein, including the effect of heat treatment temperature and duration on yak micellar casein and the effect of pH on yak milk proteins (Li et al., 2014; Yang et al., 2014; Xu et al., 2015). But the effect of thermal treatment on yak milk system still need to be explored. Therefore, the objective of this study was to assess the effect of thermal treatment on the stability and interactions of yak milk proteins. Whey protein denaturation degree, surface hydrophobicity, surface sulfhydyl content, and heat-induced denaturation and interactions of yak milk proteins after different heat treatments were investigated. The particle size distribution and protein aggregation microstructures after heat treatment were also evaluated. Our findings may provide a good understanding about thermal stability of yak milk, which could be helpful to the technological design of yak milk processing.

MATERIALS AND METHODS

Materials and Reagents

Skim yak milk was provided by Aba Ruoergai county (Sichuan, China). 8-Anilino-1-naphthalenesulfonic acid (**ANS**) and 5,5′-dithio-bis2-nitrobenzoic acid (**DTNB**) were obtained from Sigma Chemical Co. (St. Louis, MO). All other chemicals were reagent grade.

Heat Treatment of Skim Yak Milk

Skim yak milk was heated in 5-mL aliquots at 65, 75, 85, and 95°C, respectively, for 10 min in test tubes in a temperature-controlled water bath. After treatment, the yak milk was immediately cooled to room temperature with ice-water bath.

Determination of Whey Protein Nitrogen Index

Aliquots of 5-mL heated yak milk were added with 2 g of NaCl, sealed with stoppers, and stored in a water bath at 37°C for 30 min. The tubes were shaken for 15 min and then kept at a standstill. The mixture was filtered through S & S no. 602, 9-cm-filter paper without cooling. The filtrates were added with saturated NaCl solution (1.8:4, vol/vol), and the diluted solutions were capped and slowly inverted. Then 2 mL of 10% HCl (wt/vol) was added, the solutions were kept at a standstill for 10 min, subsequently poured into the cuvette, and the absorbance was measured at 420 nm. The measured values were corrected with the buffer (saturated NaCl solution with corresponding amount of 10% HCl).

SDS-PAGE Analysis

The protein composition of yak milk was determined by SDS-PAGE according to the method of Laemmli (1970). Heated yak milk was diluted 3-fold by deionized water, then prepared under reducing conditions with β-mercaptoethanol (**βME**; **SDS-R-PAGE**) and nonreducing conditions (without βME; **SDS-NR-PAGE**) to determine the role of disulfide bonds. The dispersions were vortexed, boiled for 5 min, and cooled down. Ten microliters of protein solution containing 30 μg proteins was injected to all wells. The samples were electrophoresed with a 5% stacking gel and a 12.5% polyacrylamide resolving gel using a Mini-Protean electrophoresis system (Bio-Rad Laboratories Inc., Hercules, CA). After electrophoresis, the gels were stained with Coomassie Blue R-250 (Bio-Rad Laboratories Inc.) and then destained with a water-methanol-acetic acid mixture (8:1:1, vol/vol/vol) until the background was clear. PageRuler Unstained Protein Ladder (Thermo Scientific, Waltham, MA) consisting of proteins in the range from 10 to 200 kDa was used as a molecular weight standard.

Determination of Surface Sulfhydyl Content

Surface sulfhydryl (**SH**) content in the heated yak milk was determined using Ellman's reagent according to the method described by Beveridge et al. (1974).