pH treatment as an effective tool to select the functional and structural properties of yak milk caseins

H. N. Liu,* C. Zhang,* H. Zhang,† H. Y. Guo,†† P. J. Wang,* Y. B. Zhu,§ and F. Z. Ren*†¹

*College of Food Science and Technology Engineering, Gansu Agricultural University, Lanzhou 730070, China

†Key Laboratory of Functional Dairy, College of Food Science and Nutritional Engineering, China Agricultural University, Beijing 100083, China

††Beijing Key Laboratory of Nutrition, Health and Food Safety, Beijing 100083, China

§Beijing Higher Institution Engineering Research Center of Animal Product, Beijing 100083, China

ABSTRACT

Qula is made from yak milk after defatting, acidifying, and drying. Yak milk caseins are purified from Qula by dissolving in alkali solution. The effects of different pH treatments on the functional and structural properties of yak milk caseins were investigated. Over a broad range of pH (from 6.0 to 12.0), functional properties of yak milk caseins, including solubility, emulsifying activities, and thermal characteristics, and the structural properties, including 1-anilino-8-naphthalene-sulfonate fluorescence, turbidity and particle diameter, were evaluated. The results showed that the yak milk casein yield increased as the pH increased from 6.0 to 12.0. The solubility dramatically increased as the pH increased from 6.0 to 8.0, and decreased as the pH increased from 9.0 to 12.0. The changes in emulsifying activity were not significant. Caseins were remarkably heat stable at pH 9.0. The turbidity of the casein solution decreased rapidly as the pH increased from 6.0 to 12.0, and the results suggested that reassembled casein micelles were more compact at low pH than high pH. At pH values higher than 8.0, the yield of yak milk caseins reached more than 80%. The highest solubility was at pH 8.0, the best emulsification was at pH 10.0 and the greatest thermal stability was at pH 9.0. According to the functional characteristics of yak milk caseins, alkali conditions (pH 8.0–10.0) should be selected for optimum production. These results suggested that pH-dependent treatment could be used to modify the properties of yak milk caseins by appropriate selection of the pH level.

Key words: yak milk casein, pH, functional property, structural property

INTRODUCTION

Qula is made from yak milk with a traditional method: defatting, acidifying, and drying in air. Qula contains 7 to 12% moisture and approximately 80% protein. In the Qinghai-Tibet plateau areas, collection and large-scale processing of fresh yak milk is impossible, so the yak milk is made into Qula locally. Qula is the most important indigenous yak milk product, which contributes considerably to industrialized development and is economically important (Liu et al., 2012). Qula is collected and transported to the factory to make yak milk casein. The majority of yak milk casein is used for technical applications, such as in glues, high-esteem food ingredients, and pharmaceutical applications.

Caseins have a long history as essential ingredients in the food and non-food industries (Jahaniaval et al., 2000). Caseins lend structure or functionality, or both, to a broad variety of products; the functionality of caseins is based on their open and flexible structure. From the results of earlier studies, it is widely accepted that relationships exist between the processing-modified structure of caseins and their functionality (Vojdani and Whitaker, 1996; Dziuba and Darewicz, 2000).

Heat treatment and enzymatic hydrolysis have been attempted to improve the functionality of casein, and thereby improve its effect in some applications. For example, functional properties of casein and sodium caseinate have been studied by many investigators (Guo et al. 1989; Lee et al., 1992; Singh et al., 1993; Lieske and Konrad, 1994; Guo et al., 1996; Hustinx et al., 1997; Srinivasan et al., 2002; Flanagan and FitzGerald, 2003). Casein functionality is also adversely affected by pH. The most likely losses of functionality are associated with exposure to different pH values during the conversion of acidic casein to neutral caseinate (Rollemma, 1992). From the results of earlier studies, it is widely accepted that an alkaline pH also affects casein micelles (Chaplin, 1984; van Hooydonk et al., 1986; Dalgleish and Law, 1988; Huppertz et al., 2008; Madadlou et al., 2009b). The functionality of all yak milk caseins obtained from Qula critically depends on

Received January 23, 2013.

Accepted May 13, 2013.

¹Corresponding author: renfazheng@263.net

the alkaline conditions during manufacturing. Thus, pH values of the Qula solution is of great significance for the high quality of yak milk casein. Alkali solution conditions can improve the efficiency of casein production, depending on the different needs. In current casein production in China, solutions of different pH are known to result in different casein yields. However, the functional and structural changes of yak milk caseins at different pH have not been elucidated.

Therefore, the objectives of this work were to study the effects of different pH treatments on the functional and structural properties of yak milk caseins, and to propose a mechanism of the disruption of yak milk caseins induced by different pH values. The findings will broaden the application range of caseins in food, cosmetics, and medicine.

MATERIALS AND METHODS

Materials and Sample Preparation

Qula (from Gansu Province of the Qinghai-Tibetan Plateau) with the following composition was used in the present study: $80.07 \pm 0.06\%$ protein, $7.44 \pm 0.09\%$ moisture, $5.05 \pm 0.05\%$ lactose, $4.01 \pm 0.05\%$ fat, and $3.04 \pm 0.06\%$ ash. Qula (5 g) was dispersed in 100 mL of deionized water under vigorous stirring at 20°C , and the dispersions were stored at 4°C overnight to allow complete hydration. The pH was then adjusted to pH 6.00 ± 0.05 , 7.00 ± 0.05 , 8.00 ± 0.05 , 9.00 ± 0.05 , 10.00 ± 0.05 , 11.00 ± 0.05 , and 12.00 ± 0.05 with 1 M NaOH or 1 M HCl. Before analysis, alkalized samples were centrifuged at $4,000 \times g$ for 20 min. After centrifugation, the supernatant liquid was carefully removed and acidified (Yu, 2008) to obtain the casein in 0.05 M Tris-HCL buffer (pH 7.0); the initial concentration of the casein solution was 2 mg/mL.

Casein Yield

Casein yield was determined according to the method of Yu (2008), with slight modifications. The casein content and total protein content in Qula was determined by the micro-Kjeldahl method ($N \times 6.38$). The percentage casein yield was calculated as follows:

$$\text{Casein yield (\%)} = \frac{\text{casein weight}}{\text{total protein weight in Qula}} \times 100.$$

All determinations were conducted in triplicate.

The casein purity was checked using SDS-PAGE, and HPLC. The purity determination process closely followed the procedure described in a previous report (Shan et al., 2012).

Solubility

Casein solubility was determined according to the method of Wang et al. (2008), with slight modifications. Casein solution (6 mL) was stirred at room temperature for 2 min. It was then centrifuged at $12,000 \times g$ for 20 min at 20°C . The casein solubility in the supernatant was determined by the micro-Kjeldahl method ($N \times 6.38$). The percent of protein solubility was calculated as follows:

$$\text{Solubility (\%)} = \frac{\text{protein in supernatant}}{\text{total protein}} \times 100.$$

All determinations were conducted in triplicate.

Emulsifying Activity

The emulsifying activity index (EAI) and emulsion stability index (ESI) of the casein solution were determined according to the method of Pearce and Kinsella (1978), with slight modifications. Briefly, 6 mL of the 2 mg/mL protein solution dispersion and 2 mL of soybean oil were homogenized in an FJ-200 high-speed homogenizer (Shanghai Specimen Model Co., Shanghai, China) for 1 min at the maximum velocity. The emulsion (50 μL) was taken from the bottom of the tube, immediately (0 min) or 10 min after homogenization, and diluted (1:100, vol/vol) in 0.1% (wt/vol) SDS solution. The dilution was mixed by gentle inversion of the tube and the absorbance was read at 500 nm in the spectrophotometer. Emulsifying activity index and ESI values were calculated by the following formulas:

$$\text{EAI (m}^2/\text{g)} = \frac{2 \times 2.303 \times A_0 \times \text{dil}}{C \times \varnothing \times (1 - \theta) \times 10,000};$$

$$\text{ESI (min)} = \frac{A_0}{A_0 - A_{10}} \times 10,$$

where dil is the dilution factor (100), C the initial concentration of casein (g/mL), $\varnothing$ is the optical path (0.01 m), θ is the fraction of oil used to form the emulsion (0.25), and A_0 and A_{10} are the absorbance of the diluted emulsions at 0 and 10 min, respectively. All measurements were performed in triplicate.

Thermal Properties

The thermal characteristics of the casein solution were examined using a differential scanning calorimetry (DSC) thermal analyzer (DSC-60WS; Shimadzu Co., Tokyo, Japan) according to the procedure of Wang et

al. (2008), with some modifications. Approximately 0.5 g of a 2 mg/mL casein solution was added to coated aluminum pans. The pans were hermetically sealed and heated from 20 to 200°C at a rate of 5°C/min. The flow rate of nitrogen for protecting the samples was 20 mL/min. A sealed empty pan was used as a reference. Peak or denaturation temperature (T_d) and enthalpy of denaturation (ΔH) were computed from the thermograms by theta-60WS acquisition software. All experiments were conducted in triplicate.

1-Anilino-8-Naphthalene-Sulfonate Fluorescence

The 1-anilino-8-naphthalene-sulfonate (ANS) fluorescence of the casein solution (2 mg/mL) was measured according to Kato and Nakai (1980) using fluorescence probe ANS (Sigma Chemical Co., St. Louis, MO). Casein solutions (2 mg/mL) were centrifuged at $8,000 \times g$ for 20 min. The fluorescence experiments were performed at room temperature (25°C) with an RF-5301 PC fluorescence spectrometer (Shimadzu Co.) at 280 nm (excitation wavelength, slit 3 nm), 400 to 600 nm (emission wavelength, slit 3 nm), and a scanning speed of 10 nm/s.

Turbidity Measurements and Particle Analysis

The turbidity measurements were carried out with a Shimadzu 1601 PC UV-visible spectrometer. The turbidity of the casein solutions was monitored by the transmittance at 450 nm. A cuvette with 1-cm pathway was used. All the measurements were conducted at 25°C. Turbidity measurements were carried out as described by Madadlou et al. (2009a).

The mean particle diameter and size distribution of the casein solutions were measured with a laser-diffraction-based particle size analyzer (Malvern Mastersizer Hydro with program Mastersizer 2000S; Malvern Instruments Ltd., Malvern, UK). The experiments were performed on 5-fold diluted solutions over the range 20 nm to 15 μm . Just before the measurements, samples were diluted with deionized water (adjusted by addition of sodium hydroxide) to prevent their foaming during stirring and pumping in the instrument. Water was used as the dispersant with the refractive index was set at 1.330.

Statistical Analysis

Statistical analyses were performed using SPSS 13.0 for Windows XP software (2001; SPSS Inc., Chicago, IL). Samples were analyzed statistically using a one-way ANOVA statistical package. Tests of differences were performed using Duncan's multiple range method; $P < 0.05$ was considered significant.

RESULTS AND DISCUSSION

Casein Yield

Figure 1 shows the casein yield of the casein solution at different pH values. In our results, the casein purity in each group was higher than 90% (data not shown), which indicates that most of the composition is casein. For different pH treatments, the different yak milk caseins yielded 30.8, 59.5, 81.3, 86.2, 89.0, 90.6, and 93.5%, respectively. The results indicate that at pH values higher than 8.0, the yield of yak milk caseins reached more than 80%, and that a high yield was obtained in this range.

Solubility

Solubility is one of the most important functional properties of caseins; good solubility of casein is usually required. Figure 2 shows protein solubility profiles of the casein solution, as a result of different pH values, and indicates that solubility was significantly ($P < 0.05$) related to pH.

Our results show that the minimum solubility was observed at pH 6.0, which is near the isoelectric point of casein. The solubility dramatically increased as the pH increased from 6.0 to 8.0 (Figure 2), which is in close agreement with the results reported by Lee et al. (1992) for the same pH range. The solubility dramatically decreased as the pH increased from 8.0 to 12.0. Between

Figure 1. Casein yield as a result of different pH values. Means $\pm$ SE of triplicate analyses are given. Different letters (a–f) indicate significant differences ($P < 0.05$) among samples.

Figure 2. Solubility of casein as a result of different pH values. Means $\pm$ SE of triplicate analyses are given. Different letters (a–f) indicate significant differences ($P < 0.05$) among samples.

pH 6.0 and 8.0, casein exists as a polydispersed mixture of 4 major casein molecules: α_{s1} -CN, α_{s2} -CN, κ -CN, and β -CN (Lee et al., 1992; Jahaniaval et al., 2000). The increase in solubility of casein solutions is more likely because of the negative charge acquired by casein molecules in alkali solution that increase the dispersion of molecules (Whitaker, 1980). At pH >8.0 , proteins are more susceptible to denaturation in alkaline solution because of decreased stabilization of the tertiary structure by elimination of electrostatic interactions between carboxylate and protonated amino groups and hydrogen bonding between the hydroxyl group of tyrosine and carboxylate groups (Whitaker, 1980).

Emulsifying Capacity

Figure 3 shows the effect of casein solution of different pH values on the emulsifying properties (including EAI and ESI) of the casein solution. The results indicated that EAI of the casein solution were not significantly ($P < 0.05$) related to pH, except at pH 10.0, where pH 10.0 showed the highest values ($P < 0.05$). These observations do not agree with those of other reports (Jahaniaval et al., 2000; Tang et al., 2005). The results suggest that possible unfolding of proteins and subsequent exposure of hydrophobic groups by pH treatment improved the emulsifying properties of the casein solution (Pacheco-Aguilar et al., 2008; Shan et al., 2012).

The pH treatment resulted in slight fluctuations in ESI from pH 6.0 to 12.0 (Figure 3). These observations also do not agree with those of other reports (Jaha-

Figure 3. Emulsifying activity indexes (EAI; A) and emulsifying stability indexes (ESI; B) of the casein solution as a result of different pH values. Results are mean values $\pm$ SE of triplicate analyses. Different letters (a–c) at the top of the columns indicate significant differences ($P < 0.05$) among samples (at the same protein concentration) as a result of different pH values.

niaval et al., 2000; Wang et al., 2008). This may be attributed to a decrease in the molecular flexibility of the proteins resulting from aggregation, as molecular flexibility is an important factor influencing emulsion stability (Wang et al., 2008).

Usually, a protein or biopolymer with a higher molecular weight is more easily and more effectively distributed on the oil/water interface. However, if the molecular weight of biopolymers exceeds a certain value, in contrast, the biopolymers act as a bridge among oil droplets, and, consequently, disrupt the stability of the emulsion (Tang et al., 2005). These findings led us to surmise that no positive correlation exists between solubility and emulsifying properties.

Thermal Properties

The DSC technique has been widely used to investigate the denaturation of many proteins, such as soy globulins and wheat gluten (Sochava, 1997; Micard and Guilbert, 2000). Heat stability is an important functional property in applications. Because all the casein samples had almost the same moisture content, the differences in the DSC curves were the result of the polymerization of the casein solution by the different pH treatments. The thermal transition of the casein solution was investigated by DSC; the related DSC characteristics are listed in Table 1. Different DSC patterns were exhibited at different pH values. In general, the DSC thermogram of the casein solution has a relatively narrow endothermic peak, with the temperature varying from 133.01 to 148.76°C at different pH treatments. Therefore, pH (6.0–9.0) treatment can improve the thermal stability of the casein solution. The results suggest that the endothermic peak of the unpolymerized casein solution in the DSC profile was mainly because of the protein denaturation or the disruption of hydrogen bonds between protein and water (Wang et al., 2008).

The casein solution was remarkably heat stable at pH 9.0; the endothermic peak temperature of pH 9.0 reached 148.76°C. The results show that the caseins had strong thermal stability. Differences existed in the DSC curves of the casein obtained after different low-pH treatments in the current study, which could polymerize the casein to form higher-molecular-weight biopolymers, even supra-biopolymers (Tang et al., 2005). Obviously, caseins are suitable proteins for use in products in which higher thermal stability is required, but different temperatures may need to be selected or other applications.

The enthalpy change (ΔH) significantly decreased as the pH increased from 6.0 to 8.0 ($P < 0.05$; Table 1). The ΔH represents the extent of the ordered structure of a protein (Arntfield and Murray, 1981). Thus, the data suggest that the extent of the ordered structure of casein solution is gradually decreased by increasing the pH from 6.0 to 8.0, and increased by increasing the pH from 9.0 to 12.0.

ANS Fluorescence

1-Anilino-8-naphthalene-sulfonate has been widely used to monitor exposed hydrophobic patches on the surface of proteins during folding and to monitor conformational changes. Thus, ANS can be used to detect the accessibility of the hydrophobic microenvironment with affinity for ANS molecules on caseins at different pH values.

The fluorescence emission spectra of ANS in the casein solution with different pH values are shown in Figure 4A. The pH dependence of the maximum fluorescence intensity and wavelength of ANS in 2.0 mg/mL casein solution are shown in Figure 4B. In the pH range of 6.0 to 12.0, fluorescence intensity (I_f) decreased sharply, and the maximum wavelength ($\lambda_{\max}$) increased with the increase in pH.

The intensity of fluorescence reached a maximum at pH 6.0 and decreased sharply when the pH increased. The ANS data suggested that at pH 6.0, the caseins attain a conformation having the maximum fraction of their interior hydrophobic patches exposed to the solvent, hence facilitating ANS binding (Chakraborty and Basak, 2007). The electrostatic ionic interactions among ANS and casein decreased with an increase in pH, so the number of ANS molecules bound to casein micelles increased. Thus, the fluorescence intensity of ANS increased with the decrease in pH (Liu and Guo, 2008).

Turbidity and Particle Analysis

Solution turbidity has been used as a measure of protein-protein aggregation (Banavara et al., 2003). The results of casein solution turbidity are illustrated in Figure 5. Casein solution turbidity decreased rapidly as the pH increased from 6.0 to 7.0, with a marked but more modest linear-like decrease from 7.0 to 10.0. The solution turbidity decreased sharply in the pH range of 10.0 to 12.0. The turbidity value decreased as the pH increased (Figure 5, right axis). Vaia et al. (2006) reported that the extent of decrease in turbidity of reconstituted skimmed bovine milk at alkaline pH (8.0–11.0) increased with increasing pH. They indicated that in this alkaline pH range, the casein micelles were

Table 1. Differential scanning calorimetry (DSC) characteristics as a result of different pH values¹

pH	DSC characteristic ²	
	ΔT_d (°C)	ΔH (J/g)
6.0	137.06 ± 4.91 ^{abc}	−1,213.33 ± 9.86 ^d
7.0	139.90 ± 3.08 ^{bc}	−1,664.29 ± 9.74 ^e
8.0	141.67 ± 3.58 ^c	−1,750.37 ± 3.95 ^f
9.0	148.62 ± 3.81 ^d	−1,712.42 ± 9.08 ^b
10.0	133.21 ± 2.58 ^a	−722.68 ± 9.99 ^a
11.0	132.37 ± 2.18 ^a	−926.70 ± 6.32 ^b
12.0	134.04 ± 2.74 ^{ab}	−985.47 ± 12.55 ^c

^{a–f}Different superscript letters indicate significant differences ($P < 0.05$) within the same column.

¹Means ± SD of triplicate analyses are given.

² T_d = thermal denaturation temperature of the endotherm (or the major endotherm); ΔH = total enthalpy changes of 2 endothermic peaks.

Figure 4. Fluorescence emission spectra of 1-anilino-8-naphthalene-sulfonate (ANS: A) in casein (2.0 mg/mL) as a result of pH, and plots of fluorescence intensity (I_f) and maximum wavelength (λ ; B) as a result of pH.

disrupted. The decrease in turbidity of casein solutions is more likely because of the negative charge acquired by casein molecules in alkali solution that increase the dispersion of molecules (Whitaker 1980).

It is well known that turbidity depends upon 3 main factors: the size, concentration, and light-scattering properties of particles (Banon and Hardy, 1992). Therefore, for a given concentration and particle type, the particle-scattering factor plays a crucial role here. Our results are similar to those of other reports (Liu and Guo, 2008; Madadlou et al., 2009b); we infer that the most compact structure of casein micelles leads to the highest scattering factor of the particle and, hence, the turbidity of the casein solution is high at pH 6.0 to 7.0. The less-compact structure of the casein leads to the

lower scattering factor of the particles and, hence, a decrease in the turbidity of the casein solution at a pH higher than 10.0 (Madadlou et al., 2009b).

This decrease in turbidity was concomitant with increasing particle diameters (Figure 5, left axis). Particle analysis was used to further investigate the effect of pH on casein size. Figure 5 shows the effect of pH on casein particles. The particle diameter in the casein solution increased with pH in the pH range of 6.0 to 12.0, and there was a remarkably high particle value at pH 12.0. This is in agreement with the results obtained by Liu and Guo (2008), who indicated that assembled casein micelles were present at high pH.

CONCLUSIONS

This study demonstrated that alkalinization of Qula induced changes in yak milk caseins, which resulted in different yields and different functions of caseins. When the pH was higher than 8.0, the yield of yak milk caseins reached more than 80%. The highest solubility was at pH 8.0, the best emulsification was at pH 10.0, and the greatest thermal stability was at pH 9.0. Based on the functional characteristics of yak milk caseins, alkali solution conditions (pH 8.0–10.0) should be selected for optimum production. Reassembled casein micelles are more compact at low pH than high pH. These results suggest that pH-dependent treatment could be used to modify the properties of yak milk caseins by appropriate selection of the pH level.

ACKNOWLEDGMENTS

This study was supported by the International Science & Technology Cooperation Program of China (Beijing, China; 2011DFA32550), National Science

Figure 5. Particle size ($\diamond$) and turbidity ($\square$) of casein solution of reassembled casein micelles as a result of pH value. All means are statistically different ($P < 0.05$). NTU = nephelometric turbidity units.

and Technology Support Program (Beijing, China; 2012BAD12B08), and Natural Science Foundation of China (Beijing, China; no. 30960260).

REFERENCES

- Arntfield, S. D., and E. D. Murray. 1981. The influence of processing parameters on food protein functionality. 1. Differential scanning calorimetry as an indicator of protein denaturation. *Canadian Inst. Food Sci. Technol. J.* 14:289–294.
- Banavara, D. S., D. Anupama, and S. A. Rankin. 2003. Studies on physicochemical and functional properties of commercial sweet whey powders. *J. Dairy Sci.* 86:3866–3875.
- Banon, S., and J. Hardy. 1992. A Colloidal approach of milk acidification by glucono-delta-lactone. *J. Dairy Sci.* 75:935–941.
- Chakraborty, A., and S. Basak. 2007. pH-induced structural transitions of caseins. *J. Photochem. Photobiol. B* 87:191–199.
- Chaplin, L. C. 1984. Studies on micellar calcium-phosphate: Composition and apparent solubility product in milk over a wide pH range. *J. Dairy Res.* 51:251–257.
- Dalgleish, D. G., and A. J. R. Law. 1988. pH-induced dissociation of bovine casein micelles. 1. Analysis of liberated caseins. *J. Dairy Res.* 55:529–538.
- Dziuba, J., and M. Darewicz. 2000. Structural aspects of functional properties of milk proteins. *Natural Sci.* 4:257–272.
- Flanagan, J., and R. J. FitzGerald. 2003. Functional properties of *Bacillus* proteinase hydrolysates of sodium caseinate incubated with transglutaminase pre- and post-hydrolysis. *Int. Dairy J.* 13:135–143.
- Guo, M., P. F. Fox, A. Flynn, and K. S. Mohammad. 1989. Heat-induced changes in sodium caseinate. *J. Dairy Res.* 56:503–512.
- Guo, M. R., P. F. Fox, A. Flynn, and P. S. Kindstedt. 1996. Heat-induced modification of the functional properties of sodium caseinate. *Int. Dairy J.* 6:473–485.
- Huppertz, T., B. Vaia, and M. A. Smiddy. 2008. Reformation of casein particles from alkaline-disrupted casein micelles. *J. Dairy Res.* 75:44–47.
- Hustinx, J. C. A., T. K. Singh, and P. F. Fox. 1997. Heat-induced hydrolysis of sodium caseinate. *Int. Dairy J.* 7:207–212.
- Jahaniavali, F., Y. Kakuda, V. Abraham, and M. F. Marcone. 2000. Soluble protein fractions from pH and heat treated sodium caseinate: Physicochemical and functional properties. *Food Res. Int.* 33:637–647.
- Kato, A., and S. Nakai. 1980. Hydrophobicity determined by a fluorescence probe method and its correlation with surface properties of proteins. *Biochim. Biophys. Acta* 624:13–20.
- Lee, S.-Y., C. V. Morr, and E. Y. W. Ha. 1992. Structural and functional properties of caseinate and whey protein isolate as affected by temperature and pH. *J. Food Sci.* 57:1210–1229.
- Lieske, B., and G. Konrad. 1994. Thermal modification of sodium caseinate. 1. Influence of temperature and pH on selected physicochemical and functional properties. *Milchwissenschaft* 49:16–20.
- Liu, W. J., Z. H. Sun, Y. B. Zhang, C. L. Zhang, Menghebilige, M. Yang, T. S. Sun, Q. H. Bao, W. Chen, and H. P. Zhang. 2012. A survey of the bacterial composition of kurut from Tibet using a culture-independent approach. *J. Dairy Sci.* 95:1064–1072.
- Liu, Y., and R. Guo. 2008. pH-dependent structures and properties of casein micelles. *Biophys. Chem.* 136:67–73.
- Madadlou, A., M. E. Mousavi, Z. Emam-djomeh, M. Ehsani, and D. Sheehan. 2009a. Sonodisruption of re-assembled casein micelles at different pH values. *Ultrason. Sonochem.* 16:644–648.
- Madadlou, A., M. E. Mousavi, Z. Emam-Djomeh, D. Sheehan, and M. Ehsani. 2009b. Alkaline pH does not disrupt re-assembled casein micelles. *Food Chem.* 116:929–932.
- Micard, V., and S. Guilbert. 2000. Thermal behavior of native and hydrophobized wheat gluten, gliadin and glutenin-rich fractions by modulated DSC. *Int. J. Biol. Macromol.* 27:229–236.
- Pacheco-Aguilar, R., M. A. Mazorra-Manzano, and J. C. Ramírez-Suárez. 2008. Functional properties of fish protein hydrolysates from Pacific whiting (*Merluccius productus*) muscle produced by a commercial protease. *Food Chem.* 109:782–789.
- Pearce, K. N., and J. E. Kinsella. 1978. Emulsifying properties of proteins: Evaluation of a turbidimetric technique. *J. Agric. Food Chem.* 26:716–723.
- Rollema, H. S. 1992. Casein association and micelle formation. Pages 111–140 in *Advanced Dairy Chemistry, Vol. 1: Proteins*. P. F. Fox, ed. Elsevier Applied Science, Barking, UK.
- Shan, Y., M. Ma, X. Huang, Y. Guo, G. Jin, and Y. Jin. 2012. Simple pH treatment as an effective tool to improve the functional properties of ovomucin. *J. Food Sci.* 77:C740–C745.
- Singh, H., P. F. Fox, and M. Cuddigan. 1993. Emulsifying properties of protein fractions prepared from heated milk. *Food Chem.* 47:1–6.
- Sochava, I. V. 1997. Heat capacity and thermodynamic characteristics of denaturation and glass transition of hydrated and anhydrous proteins. *Biophys. Chem.* 69:31–41.
- Srinivasan, M., H. Singh, and P. A. Munro. 2002. Formation and stability of sodium caseinate emulsions: Influence of retorting (121°C for 15 min) before or after emulsification. *Food Hydrocoll.* 16:153–160.
- Tang, C., X.-Q. Yang, Z. Chen, H. Wu, and Z.-Y. Peng. 2005. Physicochemical and structural characteristics of sodium caseinate biopolymers induced by microbial transglutaminase. *J. Food Biochem.* 29:402–421.
- Vaia, B., M. A. Smiddy, and A. L. Kelly. 2006. Solvent-mediated disruption of bovine casein micelles at alkaline pH. *J. Agric. Food Chem.* 54:8288–8293.
- van Hooydonk, A. C. M., H. G. Hagedoorn, and J. Boerrigter. 1986. pH-induced physicochemical changes of casein micelles in milk and their effect on renneting 1. Effect of acidification on physicochemical properties. *Neth. Milk Dairy J.* 40:281–296.
- Vojdani, F., and J. R. Whitaker. 1996. Phosphorylation of proteins and their functional and structural properties. Pages 210–229 in *Macromolecular Interactions in Food Technology*. Vol. 650. N. Parris, A. Kato, L. K. Creamer, and J. Pearce, ed. American Chemical Society Press, Washington, DC.
- Wang, X.-S., C.-H. Tang, B.-S. Li, X.-Q. Yang, L. Li, and C.-Y. Ma. 2008. Effects of high-pressure treatment on some physicochemical and functional properties of soy protein isolates. *Food Hydrocoll.* 22:560–567.
- Whitaker, J. R. 1980. Changes occurring in proteins in alkaline solutions. Pages 146–160 in *Chemical Deterioration of Proteins*. Vol. 123. J. R. Whitaker and M. Fujimaki, ed. American Chemical Society Press, Washington, DC.
- Yu, M. 2008. Study on browning factors and process optimization of casein purified from Qula. MS Thesis. Harbin Institute of Technol., Harbin, China.