

Research Article

Combined Use of Trisodium Citrate and Transglutaminase to Enhance the Stiffness and Water-Holding Capacity of Acidified Yak Milk Gels

Lin Yang 

Food Science College, Tibet Agriculture & Animal Husbandry University, Nyingchi, Tibet 860000, China

Correspondence should be addressed to Lin Yang; yanglintibet@sina.com

Received 3 August 2018; Accepted 23 September 2018; Published 15 October 2018

Guest Editor: Xavier Cetó

Copyright © 2018 Lin Yang. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

In this research, the synergistic effect of trisodium citrate (TSC) and microbial transglutaminase (TGase) treatment on the textural properties of acidified yak skim milk gels was investigated. TSC was added to yak skim milk to concentrations of 0, 20, and 40 mmol/L, followed by adjusting the pH to 6.7. The samples were incubated with TGase for the cross-linking reaction, after which the samples were acidified with 1.4% (w/v) gluconodelta-lactone (GDL) at 42°C for 4 h to form gels. The stiffness and water holding capacity (WHC) of gels exhibited higher values at 20 or 40 mmol/L than without TSC. The final storage modulus (G') of yak milk gels was positively related to the concentration of TSC prior to TGase treatment. Cryoscanning electron microscopy observations showed that the gel networks were more rigid with higher TSC concentrations. Overall, TSC dissociated particles in yak milk into smaller ones. The newly formed particles in yak skim milk could form acid-induced gels with greater stiffness and higher WHC in the presence of TGase.

1. Introduction

Yak (*Bos grunniens*) milk is produced in the China Qinghai-Tibet Plateau area at an altitude of 3000 m [1]. It has become a new source of dairy products in China, due to its higher nutritional properties, less allergenicity, and better digestibility than cow milk [2–4]. Of the yak milk-based products, yogurt is the fastest growing one. However, textural defects including fragile structure (low stiffness) and syneresis (low water holding capacity) (WHC) usually occur in the yak yogurt gels, during storage or after mechanical damage. These defects significantly reduce the consumer acceptance of yak yogurt. Therefore, it is necessary to develop yak yogurt gels with higher stiffness and WHC.

The stiffness and WHC of yogurt gels are determined by their gel network structures, whose primary building blocks are caseins and whey proteins. In yak milk at native pH of 6.5–7.2, the casein molecules are noncovalent cross-linked by calcium phosphate, forming particles named casein micelles (about 200 nm in diameter) [5, 6]. On acidification,

dissociation of the micelles happens, resulting in the aggregation of caseins and thus forming a weak network structure through noncovalent interactions at pH~4.6 [7–10]. Whey proteins can be denatured and interacted with caseins after heated (higher than 70°C), which could further improve the textural properties of acid-induced milk gels [11, 12].

Introducing covalent bonds is an effective mean to improve the textural property of yogurt gels [13–15]. TGase has been widely used to generate covalent bonds among protein molecules [16–18]. TGase could catalyze the acyl transfer reaction between γ -carboxyl groups and ϵ -amino groups among protein molecules, leading to the intramolecular or intermolecular cross-linking of proteins [19, 20]. This could significantly improve the gel stiffness and WHC of yogurt gels [21]. Unfortunately, although lots of studies have been performed on the use of TGase to enhance the textural properties of yogurt, the defects have not been totally inhibited [22–25].

The calcium ions chelating agents, including trisodium citrate (TSC), ethylene diamine tetraacetic acid, and sodium

phosphates, were found to disrupt the casein micelles in milk [26–29]. This might alter the gel formation of TGase-treated caseins [18]. In this case, the cross-link bonds among milk proteins before or during acidification would be altered. Therefore, the TGase-treated milk proteins might create different gel network structures compared with the gels prepared from acidified TGase-treated native milk proteins.

In this research, with the aim to improve the textural properties of yak yogurt, we investigated the effect of TSC prior TGase treatment on the properties of acid-induced gel prepared from heated yak skim milk. Our finding will be beneficial to improve the textural properties of yak yogurt.

2. Materials and Methods

2.1. Materials. Yak milk was obtained from the Maqu grassland on the Qinghai-Tibetan Plateau in northwest China. The altitude of pastures is about 3450 m, while the temperature is $9.4 \pm 3.7^\circ\text{C}$. The ash, dry matter, protein, fat, and lactose in the yak milk were 0.81%, 17.38%, 5.14%, 5.47%, and 5.09% (w/v), respectively. To prevent bacterial growth, 0.04% (w/v) sodium azide was added into the yak milk. Calcium-independent TGase (200 U/g) was obtained from C&P Group GmbH (Rosshaupten, Germany). GDL and TSC were purchased from Sigma-Aldrich (St. Louis, MO, USA).

2.2. Preparation of Acid-Induced Yak Milk Gels. The fat in yak milk was removed by centrifugation at 4200 g at 25°C for 30 min, followed by denaturing the whey proteins at 80°C for 20 min. The skim milk was added with different amount of TSC powder to a concentration of 0, 20, and 40 mmol/L. After magnetic stirred for 10 min, the pH of heated yak skim milk was adjusted to 6.7 with 0.5 mol/L HCl. After adding the TGase to 10 U/g milk proteins, the yak skim milk was further magnetic stirred for 10 min. Then, all samples were stored at 42°C for 60 min, followed by acidification with 1.4% GDL (w/v) at 42°C for 4 h. The final pH of all the samples was between 4.3 and 4.6 [30]. Finally, all the gels were stored at 4°C for 6 h for further use.

For comparison, heated yak skim milk treated with 0, 20, and 40 mmol/L TSC (in the absence of TGase) was also investigated. The other procedures were performed as described in the above section.

2.3. Characterization of the Treated Milk or Gels. The particle size distribution was measured with a Zetasizer (Model Nano-ZS3600, Malvern, UK) at 25°C [31]. The skim milk was diluted 300-fold with ultrapure water.

The acid-induced gelation processes of yak skim milk were determined with a rheometer (AR1500ex, TA Instruments, USA). GDL was added into TSC- and TGase-treated samples to 1.4% (w/v). The samples were then transferred into the concentric cylinder. The acid-induced yak skim gels were oscillated at 0.1 Hz and with 1% of applied strain. The determination temperature was 42°C .

The stiffness of the acid-induced gels was determined by the analyzer (TMS-Pro, Food Technology Corp., Sterling, USA). The acid gels prepared in 2.2 were placed at room temperature for 60 min before determination. A cylinder probe (25 mm in diameter) moving into the gel to a distance of 10 mm at 30 mm/min was used for the penetration test.

The WHC of the gels was measured according to a modified procedure [32]. Forty millimeters of the TSC and TGase-treated yak skim milk were acidified at 42°C for 4 h in 50 mL centrifuge tubes, followed by centrifugation at 1500 g at 25°C for 15 min. The WHC was defined as the percentage of the weight of gels remaining in the centrifuge tubes to their initial weight.

The gel microstructures were observed by the cryo-scanning electron microscopy (S-3000N, Hitachi Co., Tokyo, Japan), based on the reported literatures [26].

2.4. Statistical Analysis. The experiments were performed in at least triplicate. Data analysis of variance (ANOVA) was used to check the significance of differences between means with $P < 0.05$ indicating significance.

3. Results and Discussion

3.1. Particle Size. The size distribution curves by number fraction of particles in heated yak skim milk are shown in Figure 1. In the absence of TGase, when the added TSC concentrations in the yak skim milk were 0, 20, and 40 mmol/L, the corresponding particle peaking diameters were 145.1 ± 7.0 , 56.9 ± 14.9 , and 34.2 ± 4.6 nm (mean \pm SD), respectively. In the presence of TGase, when the TSC concentrations in yak skim milk were 0, 20, and 40 mmol/L, the corresponding particle peaking diameters were 151.5 ± 10.8 , 61.4 ± 15.4 , and 29.2 ± 9.7 nm (mean \pm SD), respectively. This suggested that the particles in yak skim milk became smaller with increasing TSC concentrations. It is well established that calcium-chelating agents can disrupt the micellar framework by removing calcium from the micelles, leading to the dissociation of casein micelles. Therefore, it was expected that the citrate ions would dissociate the particles in yak skim milk into smaller particles.

3.2. Gelation Kinetics. The storage modulus (G') evolution of heated yak skim milk (after GDL addition) is shown in Figure 2. It can be clearly observed that both TSC and TGase had significant influence on the acid-induced gelation kinetics of yak milk. Gelation time of yak skim milk was positively related to the TSC concentrations, whether or not the TGase is in the presence. However, the influence of TSC on the final storage modulus (G') of gels was heavily dependent on the TGase. In the presence of TGase, the final G' of gels was higher at 20 or 40 mmol/L⁻¹ TSC than at 0 mmol/L⁻¹ TSC. As proved above, TSC favors the dissociation of particles in the yak skim milk into smaller particles. This could enhance the flexibility of the newly formed casein particles [10, 26] and thus favored the

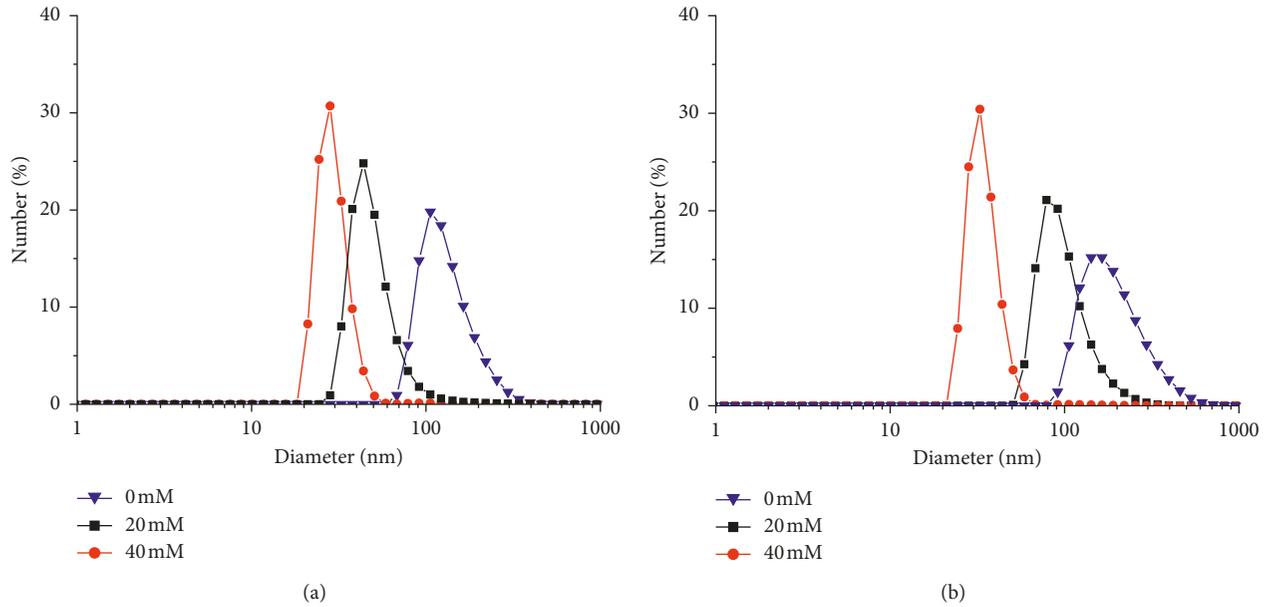


FIGURE 1: Particle size distribution curves of heated yak skim milk treated with different concentrations of TSC without (a) and with (b) TGase treatment.

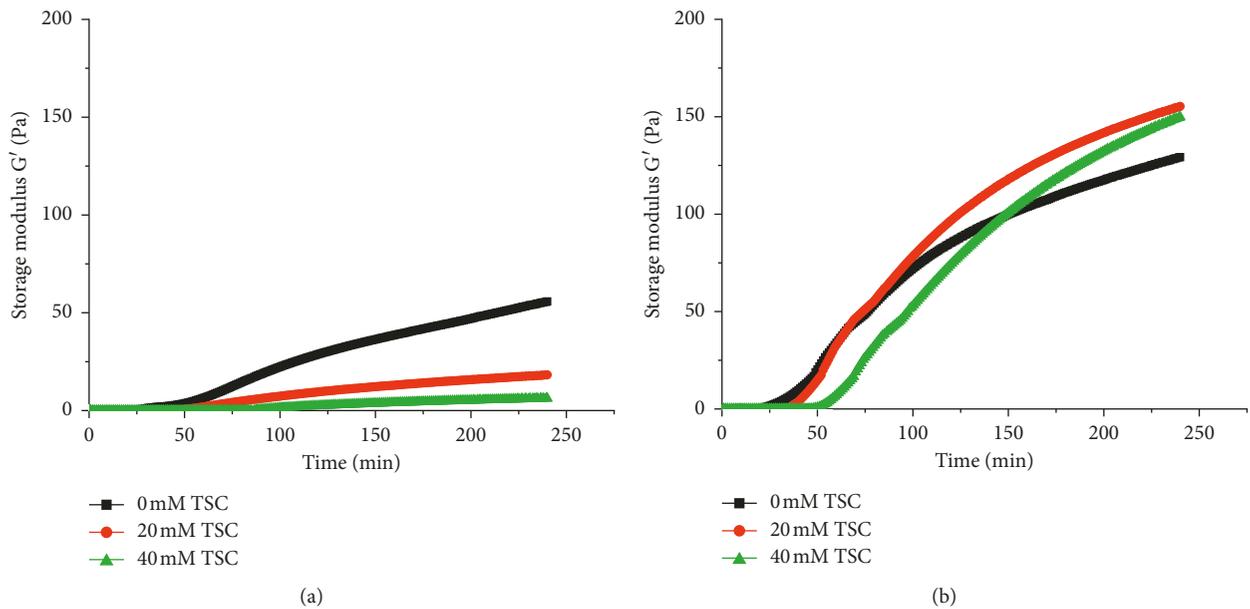


FIGURE 2: Effects of trisodium citrate on the storage modulus of acid-induced yak milk gels without (a) and with (b) TGase.

adequate rearrangement of caseins during gelation. This made the newly formed particles to be more susceptible to TGase cross-linking [33].

3.3. Stiffness and WHC. The stiffness of the acid-induced yak skim milk gels is shown in Figure 3. In the samples without TGase, it can be seen that the addition of TSC reduced the stiffness of acid-induced casein gels, which was consistent with the G' results. It was observed that the stiffness of gels prepared in the presence of TGase was higher

at 20 or 40 mmol/L⁻¹ TSC than those without TSC addition. The reasons for this can also be explained as described in the storage modulus (G') of gels. It can also be observed that, in the same TSC concentrations, the stiffness of gels prepared in the presence of TGase was significantly higher than those in the absence of TGase. This indicated that the TGase cross-linking played an important role in the gel stiffness.

The effect of TSC on the WHC of acid-induced yak skim milk gels is shown in Figure 4. In the same TSC concentration, the WHC of gels prepared in the presence of

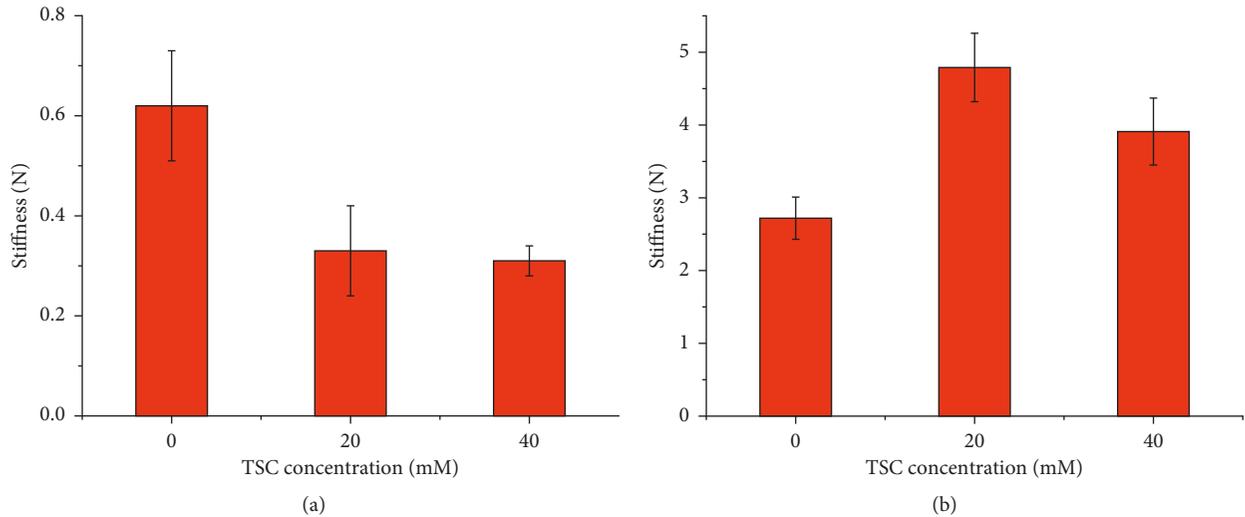


FIGURE 3: Effects of trisodium citrate on the stiffness (N) of acid-induced yak milk gels without (a) and with (b) TGase.

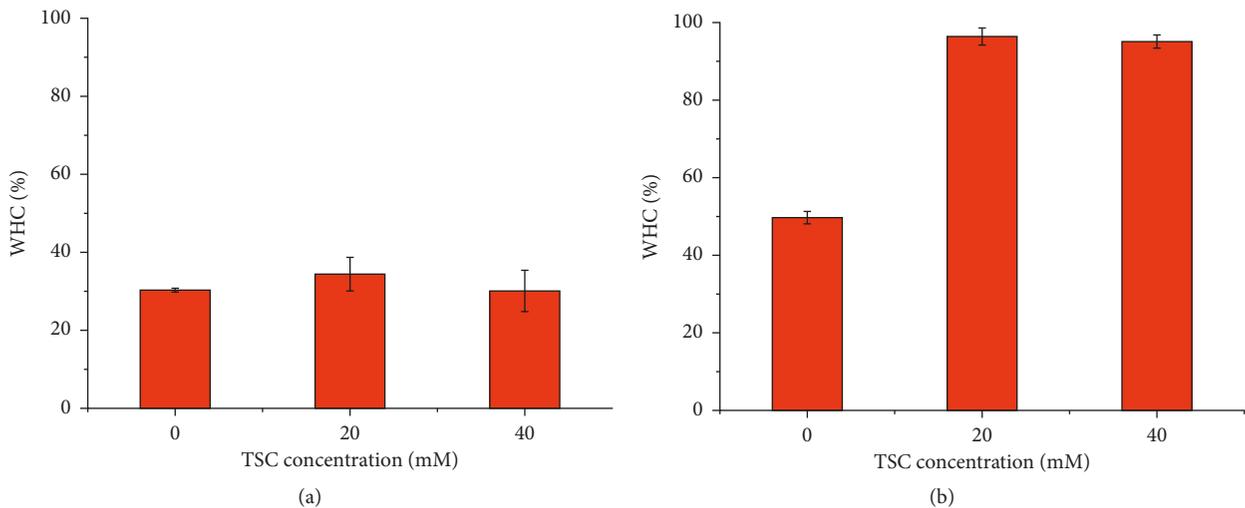


FIGURE 4: Effects of trisodium citrate on the water holding capacity of acid-induced yak milk gels without (a) and with (b) mTGase.

TGase was significantly higher than those in the absence of TGase. This indicated that the TGase cross-linking played a crucial role in improving the WHC. It can be seen that, in the presence of TGase, when the concentration of TSC was 20 or 40 mmol/L, little water in the final gels prepared was expelled after centrifugation. This indicated that the gels prepared from the newly formed smaller particles exhibited greater water retention in the presence of TGase. However, in the absence of TGase, more than 60% of water was expelled. This was identical with the results of gel stiffness because weak gels from noncovalent bonds are easier to shrinkage and subsequent expulsion of water.

3.4. Microstructure. The microstructures of acidified yak skim milk gels are exhibited in Figure 5. In the samples, in

the presence of TGase, it can be observed that network structures were more rigid with TSC addition than without TSC addition. This was consistent with the previous textural property results.

4. Conclusion

In this study, the effect of TSC on the stiffness of acid-induced, TGase-treated yak milk gels was investigated. Yak milk first treated by TSC (20 or 40 mmol/L) and then cross-linked with TGase resulted in gels with higher stiffness, WHC, and storage modulus (G'). TSC could dissociate the casein micelles in yak milk into smaller particles. In the presence of TGase, the newly formed particles formed acid-induced gels with higher stiffness, higher WHC, and storage modulus (G').

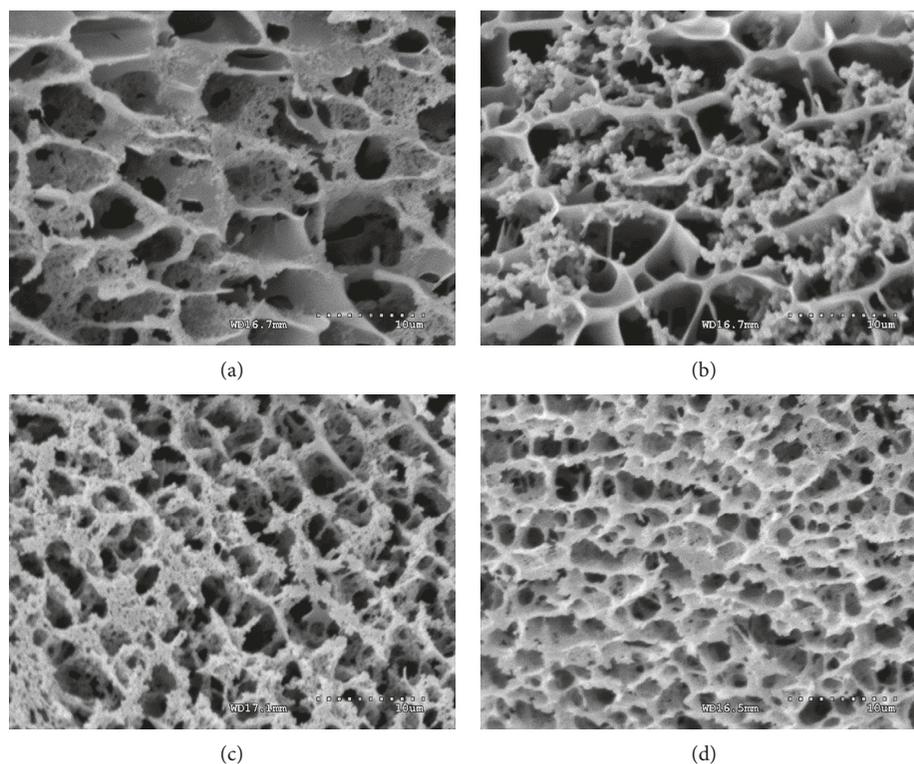


FIGURE 5: Effects of trisodium citrate on the microstructure of acid-induced yak milk gels without ((a) 0 mmol/L⁻¹ TSC; (b) 30 mmol/L⁻¹ TSC) and with ((c) 0 mmol/L⁻¹ TSC; (d) 30 mmol/L⁻¹ TSC) mTGase.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The author declares that there are no conflicts of interest regarding the publication of this paper.

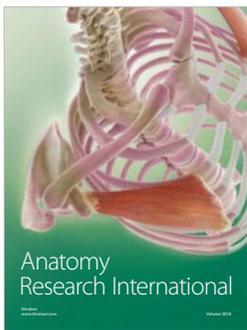
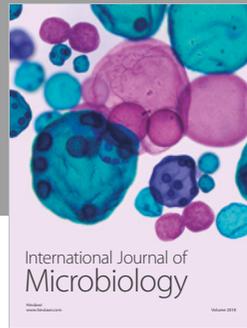
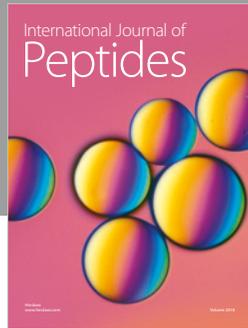
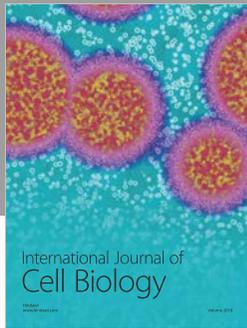
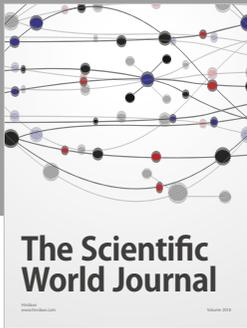
Acknowledgments

This study was supported by the key subject construction project of Food Science and Technology in Tibet Agriculture and Animal Husbandry University (502218009), the Development and Demonstration of Critical Processing Technics in Animal Products (2018XTCX-01), the Youth Teacher Research Cooperation Project between China Agricultural University and Tibet Agriculture and Animal Husbandry University (2017LHJJ-02), the teaching team construction project of Food Science and Technology in Tibet Agriculture and Animal Husbandry University (2016JXTD-01), Key Laboratory of Tibet Autonomous Region, the processing and storage team in special agricultural products in Tibet Autonomous Region (12018KYTD-02), and Key Laboratory in Tibet Autonomous Region (ZDSYS-02).

References

- [1] G. X. Cui, F. Yuan, A. A. Degen et al., "Composition of the milk of yaks raised at different altitudes on the Qinghai-Tibetan Plateau," *International Dairy Journal*, vol. 59, pp. 29–35, 2016.
- [2] H. Li, Y. Ma, J. Xiang et al., "Comparison of the immunogenicity of yak milk and cow milk," *European Food Research and Technology*, vol. 233, no. 4, pp. 545–551, 2011.
- [3] Q. Sheng, J. Li, M. S. Alam, X. Fang, and M. Guo, "Gross composition and nutrient profiles of Chinese yak (Maiwa) milk," *International Journal of Food Science and Technology*, vol. 43, no. 3, pp. 568–572, 2008.
- [4] H. Zhang, J. Xu, J. Wang et al., "A survey on chemical and microbiological composition of kurut, naturally fermented yak milk from Qinghai in China," *Food Control*, vol. 19, no. 6, pp. 578–586, 2008.
- [5] P. Wang, H. Liu, P. Wen, H. Zhang, H. Guo, and F. Ren, "The composition, size and hydration of yak casein micelles," *International Dairy Journal*, vol. 31, no. 2, pp. 107–110, 2013.
- [6] Y. Zhang, Y. Li, P. Wang, Y. Tian, Q. Liang, and F. Ren, "Rennet-induced coagulation properties of yak casein micelles: a comparison with cow casein micelles," *Food Research International*, vol. 102, pp. 25–31, 2017.
- [7] D. S. Horne, "Formation and structure of acidified milk gels," *International Dairy Journal*, vol. 9, no. 3, pp. 261–268, 1999.
- [8] G. H. Meletharayil, H. A. Patel, L. E. Metzger, C. Marella, and T. Huppertz, "Influence of partially demineralized milk proteins on rheological properties and microstructure of acid gels," *Journal of Dairy Science*, vol. 101, no. 3, pp. 1864–1871, 2018.

- [9] J.-L. Mession, S. Roustel, and R. Saurel, "Interactions in casein micelle - pea protein system (part II): mixture acid gelation with glucono- δ -lactone," *Food Hydrocolloids*, vol. 73, pp. 344–357, 2017.
- [10] T. Ozcan, J. A. Lucey, and D. S. Horne, "Effect of tetrasodium pyrophosphate on the physicochemical properties of yogurt gels," *Journal of Dairy Science*, vol. 91, no. 12, pp. 4492–4500, 2008.
- [11] G. Liu, T. C. Jæger, S. B. Nielsen, C. A. Ray, and R. Ipsen, "Physicochemical properties of milk protein ingredients and their acid gelation behaviour in different ionic environments," *International Dairy Journal*, vol. 85, pp. 16–20, 2018.
- [12] I. C. Torres, J. M. Amigo, J. C. Knudsen, A. Tolkach, B. O. Mikkelsen, and R. Ipsen, "Rheology and microstructure of low-fat yoghurt produced with whey protein microparticles as fat replacer," *International Dairy Journal*, vol. 81, pp. 62–71, 2018.
- [13] T. Huppertz and C. G. de Kruif, "Ethanol stability of casein micelles cross-linked with transglutaminase," *International Dairy Journal*, vol. 17, no. 5, pp. 436–441, 2007.
- [14] D. Jaros, U. Schwarzenbolz, N. Raak, J. Lobner, T. Henle, and H. Rohm, "Cross-linking with microbial transglutaminase: relationship between polymerisation degree and stiffness of acid casein gels," *International Dairy Journal*, vol. 39, no. 2, pp. 345–347, 2014.
- [15] S. J. Jiang and X. H. Zhao, "Transglutaminase-induced cross-linking and glucosamine conjugation of casein and some functional properties of the modified product," *International Dairy Journal*, vol. 21, no. 4, pp. 198–205, 2011.
- [16] L. Chen, Y. Li, J. Han, D. Yuan, Z. Lu, and L. Zhang, "Influence of transglutaminase-induced modification of milk protein concentrate (MPC) on yoghurt texture," *International Dairy Journal*, vol. 78, pp. 65–72, 2018.
- [17] S. M. T. Gharibzadeh and I. S. Chronakis, "Crosslinking of milk proteins by microbial transglutaminase: utilization in functional yogurt products," *Food Chemistry*, vol. 245, pp. 620–632, 2018.
- [18] N. F. N. Silva, F. Casanova, F. Gaucheron et al., "Combined effect of transglutaminase and sodium citrate on the microstructure and rheological properties of acid milk gel," *Food Hydrocolloids*, vol. 82, pp. 304–311, 2018.
- [19] C. Kleemann, I. Selmer, I. Smirnova, and U. Kulozik, "Tailor made protein based aerogel particles from egg white protein, whey protein isolate and sodium caseinate: influence of the preceding hydrogel characteristics," *Food Hydrocolloids*, vol. 83, pp. 365–374, 2018.
- [20] E. Lam, C. Holt, P. Edwards et al., "The effect of transglutaminase treatment on the physico-chemical properties of skim milk with added ethylenediaminetetraacetic acid," *Food Hydrocolloids*, vol. 69, pp. 329–340, 2017.
- [21] L. Zhang, L. Zhang, H. Yi et al., "Enzymatic characterization of transglutaminase from *Streptomyces mobaraensis* DSM 40587 in high salt and effect of enzymatic cross-linking of yak milk proteins on functional properties of stirred yogurt," *Journal of Dairy Science*, vol. 95, no. 7, pp. 3559–3568, 2012.
- [22] K. Hinz, T. Huppertz, and A. L. Kelly, "Susceptibility of the individual caseins in reconstituted skim milk to cross-linking by transglutaminase: influence of temperature, pH and mineral equilibria," *Journal of Dairy Research*, vol. 79, no. 4, pp. 414–421, 2012.
- [23] T. Huppertz and C. G. de Kruif, "Structure and stability of nanogel particles prepared by internal cross-linking of casein micelles," *International Dairy Journal*, vol. 18, no. 5, pp. 556–565, 2008.
- [24] T. Huppertz, M. A. Smiddy, and C. G. de Kruif, "Bio-compatible micro-gel particles from cross-linked casein micelles," *Biomacromolecules*, vol. 8, no. 4, pp. 1300–1305, 2007.
- [25] C. Schorsch, H. Carrie, and I. T. Norton, "Cross-linking casein micelles by a microbial transglutaminase: influence of cross-links in acid-induced gelation," *International Dairy Journal*, vol. 10, no. 8, pp. 529–539, 2000.
- [26] H. Li, C. Yang, C. Chen et al., "The Use of trisodium citrate to improve the textural properties of acid-induced, transglutaminase-treated micellar casein gels," *Molecules*, vol. 23, no. 7, p. 1632, 2018.
- [27] T. Ozcan-Yilsay, W. J. Lee, D. Horne, and J. A. Lucey, "Effect of trisodium citrate on rheological and physical properties and microstructure of yogurt," *Journal of Dairy Science*, vol. 90, no. 4, pp. 1644–1652, 2007.
- [28] P. Thomar and T. Nicolai, "Dissociation of native casein micelles induced by sodium caseinate," *Food Hydrocolloids*, vol. 49, pp. 224–231, 2015.
- [29] P. Wang, C. Chen, H. Guo, H. Zhang, Z. Yang, and F. Ren, "Casein gel particles as novel soft Pickering stabilizers: the emulsifying property and packing behaviour at the oil-water interface," *Food Hydrocolloids*, vol. 77, pp. 689–698, 2017.
- [30] P. Wang, N. Cui, J. Luo et al., "Stable water-in-oil emulsions formulated with polyglycerol polyricinoleate and glucono- δ -lactone-induced casein gels," *Food Hydrocolloids*, vol. 57, pp. 217–220, 2016.
- [31] P. Wang, S. Jin, H. Guo, L. Zhao, and F. Ren, "The pressure-induced, lactose-dependent changes in the composition and size of casein micelles," *Food Chemistry*, vol. 173, pp. 468–474, 2015.
- [32] M. Jacob, S. Nöbel, D. Jaros, and H. Rohm, "Physical properties of acid milk gels: acidification rate significantly interacts with cross-linking and heat treatment of milk," *Food Hydrocolloids*, vol. 25, no. 5, pp. 928–934, 2011.
- [33] C. H. Lee and C. Rha, "Microstructure of soybean protein aggregates and its relation to the physical and textural properties of the curd," *Journal of Food Science*, vol. 43, no. 1, pp. 79–84, 1978.



Hindawi

Submit your manuscripts at
www.hindawi.com

