Change in the structural and functional properties of goat milk protein due to pH and heat

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ABSTRACT

This study was carried out to investigate the effects of pH and heat on the structure and function of milk proteins by comparing goat milk treated under different pH and temperature conditions. The results showed that pH had a significant effect on the thermal stability of goat milk proteins, and the proteins were least thermally stable at pH 7.7. Except for the pH 6.9 goat milk, the surface hydrophobicities of the milk proteins at various pH values reached their maxima at 85°C. The particle size, zeta potential, and content of regular secondary structure also decreased significantly at 85°C, and the turbidity of milk proteins under alkaline pH conditions was lower than that under acidic conditions. It was concluded that alkaline conditions resulted in better emulsion stability and oil-holding capacity, and acidic conditions offered better foaming ability, foam stability, and water-holding capacity for goat milk protein during heat processing. It can also be seen that 85°C was the key temperature for milk proteins after changing the pH of the milk. This paper provides a theoretical basis for optimizing the processing conditions for goat milk and the applications of goat milk proteins.

Key words: pH-heat induction, goat milk protein, structural property, functional property

INTRODUCTION

Goat milk is widely used in dairy products because it is highly digestible, is hypoallergenic, and has high nutritional value (Lou et al., 2018; Chen et al., 2019a). The lower content of α_{S1} -casein and lactose in goat milk might allow β -LG and lactose to have better digestion and be less allergenic than cow milk (Bevilacqua et al., 2001; Lara-Villoslada et al., 2004; Chen et al., 2018a).

Milk protein is the most important nutritional component in goat milk, and is a good standard for evaluating and testing the nutritional quality of dairy products (García et al., 2014; Chen et al., 2018a; Zhu et al., 2018). The protein in goat milk is composed of 70% water-insoluble casein, 25% water-soluble whey protein, and 5% fat-soluble milk fat globule membrane protein (Chen et al., 2019a). In the processing and production of dairy products, the proteins in milk are prone to flocculation and precipitation, which has a serious effect on the quality of goat milk products. Therefore, the changes in goat milk proteins during processing are a very important issue.

The stability of milk protein is determined by its structural properties, and the structural properties are related to the functional properties. The changes in the functional properties of milk proteins during processing are the macroscopic effects of changes in the structural properties. Acidification and heat treatment are commonly used in process milk to dairy products (Montilla and Calvo, 1997; Yuksel et al., 2010; Li et al., 2014), and these processes can rapidly change the spatial structure of proteins and the interactions between proteins, affecting the structural and functional properties of milk proteins (Xu et al., 2015; Lajnaf et al., 2017; Chen et al., 2019b). Previous work has investigated the effects of heat treatment on milk protein functionality at emulsion interfaces, which appear to be important with respect to the physico-chemical properties of milk-protein-stabilized emulsions (Raikos, 2010). Understanding the changes in the structural and functional properties of milk proteins during acidification and heat treatment will facilitate the optimization of the production process and improve the quality of milk products.

The mechanism and the pH dependence of heat-induced instabilities of the milk protein have been the subject of previous research, most of which has been performed on micellar casein systems of cattle milk (Sauer and Moraru, 2012; Stănciuc et al., 2012; Nair et al., 2013). Some research has also focused on the

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effects of succinylation or other modification mechanisms on the physicochemical and functional properties of proteins (Shilpashree et al., 2015). Previous works have shown the potential of varying the structural and functional properties of milk proteins as affected by disodium phosphate addition, diafiltration, and high-pressure homogenization (Meena et al., 2017; Yu et al., 2018). However, a detailed understanding of the changes in the structural and functional properties of goat milk that occur during pH and heating treatment has not been reported. The objective of this study was to elucidate the effects of pH and heat treatment on the structural and functional properties of goat milk proteins. In particular, this research focused on the effect of partial acidification of goat milk to pH 6.5 and basification of goat milk to pH 7.7 on proteins during heat processing. It is important to fully understand the effect of the parameter on the structural properties of goat milk proteins and the potential of modifying processing techniques to achieve specific functionalities.

MATERIALS AND METHODS

Sample Collection and Preparation

Fresh goat milk samples were collected from Tai'an Three Xi goat farms in Shandong Province, China. This farm raises the goats in freestall barns and feeds a TMR silage diet. Feces are cleared manually, and machine milking is performed at 1000 h. A total of 200 dairy goats at the peak of lactation showing no signs of acute mastitis or other clinical diseases were involved in this study. The milk samples were transported to the laboratory under refrigeration.

All milk samples were centrifuged at 4°C and $1,365 \times g$ for 20 min to remove fat, then were adjusted to pH levels of 6.5, 6.9, 7.3, and 7.7 with 10% acetic acid or 1 mol/L sodium hydroxide solution as necessary. These milk samples were heated at 65, 85, 105, and 125°C for 15 s, respectively, and served as the treated groups, referring to the study of Chen et al. (2019a). As Figure 1 shows, the fresh goat milk samples were used as the control group (CG).

Determination of the Thermal Stability of Goat Milk Protein

The thermal stabilities of goat milk the protein treated at different pH values were evaluated using differential scanning calorimetry, as described by Pugliese et al. (2016) with some modifications. In this study, the flow rate of nitrogen was 60 mL/min as a shielding gas and 40 mL/min as a reaction gas. The thermal

denaturation temperature and enthalpy change (ΔH) of the protein were analyzed via the peak temperature and peak area using the method described previously.

Determination of the Surface Hydrophobicity of Goat Milk Protein

Changes in the surface hydrophobicity of the goat milk protein in the treated and control samples were determined by fluorescence probe techniques, as described by Yuksel et al. (2010) with some modifications. The concentration of the 8-anilino-1-naphthalenesulfonic acid (Aladdin, Shanghai, China) fluorescent probe was 8 mmol/L. The fluorescence intensities of the samples were measured by a fluorescence photometer with an excitation wavelength of 380 nm, an emission wavelength of 470 nm, and excitation and emission slit widths of 5 nm.

Determination of the Average Particle Size, Zeta Potential, and Turbidity of Goat Milk Protein

A 200- μ L aliquot of sample was diluted to 10 mL with 0.1 mol/L $\text{Na}_2\text{HPO}_4\text{-NaH}_2\text{PO}_4$ (pH 7.0) buffer solution. After mixing thoroughly, the samples were centrifuged at $1,078 \times g$ for 5 min at room temperature to remove dust and impurities. The particle size of the proteins was determined by a nanometer particle size analyzer according to the method described by Yang et al. (2018). The relative parameters were as follows: measuring temperature 25°C, scattering angle 90°, particle refractive index 1.450, particle absorptivity 0.8872, using water as a dispersant, and with a dispersant refractive index of 1.330. Using the method described by O'Brien et al. (1995), the zeta potential of the goat milk samples was determined using a Ma Erwen (Malvern, UK) nanometer particle size analyzer with laser Doppler microelectrophoresis. The turbidity of the goat milk protein samples was determined by spectrophotometry, and the absorbance (A) of each sample was determined using a UV spectrophotometer at room temperature with a maximum absorption wavelength of 633 nm (Partschfeld et al., 2007).

Determination of the Secondary Structure of Goat Milk Proteins

The secondary structure of goat milk protein samples was determined by Fourier-transform infrared spectroscopy, as described by El Oudiani et al. (2017). Considering that potassium bromide sample was scanned as background, the sample powder obtained by freeze-drying was ground evenly with potassium bromide and

then pressed into flaking, and the secondary structure was determined by infrared spectrometry. The detection window was 4,000 to 400 cm^{-1} , the spectral resolution was 4 cm^{-1} , and the number of scans was 32.

Scanning Electron Microscopy of Goat Milk Protein

A 100 $\mu\text{g/mL}$ solution of goat milk protein was prepared, and 5 μL of this solution was dripped on a single silicon wafer, dried, and 50 μL of glutaraldehyde fixed solution (2.5%) was added. The samples were sealed with fresh-keeping film and fixed at 4°C for at least 5 h. The samples were cleaned with $\text{Na}_2\text{HPO}_4\text{-NaH}_2\text{PO}_4$ buffer solution, dehydrated in graded ethanol solutions, allowed to dry naturally, and then coated with gold to make them electrically conductive. The samples were observed by an ASU8010 cold-field emission scanning electron microscope as described by Min et al. (2014).

Determination of the Emulsifying Properties of Goat Milk Protein

The sample solution ($\rho = 1 \text{ mg/mL}$) was mixed evenly with soybean oil at a 3:1 ratio and emulsified by a FJ200 high-speed dispersing machine (Biaoben model factory, Shanghai, China) for 1 min (10,000 rpm). Then, 400 μL from the bottom of the emulsified sample solution was extracted and diluted with 16 mL of 0.1% SDS solution. Finally, the sample solution was measured using an UV spectrophotometer at 500 nm (Pearce and Kinsella, 1978). The 0.1% SDS solution was used as a blank to measure the absorbance value, A_0 . The sample solution was allowed to stand for 10 min and then diluted again to determine the absorbance value, A_1 . The emulsifying activity index (EAI) and emulsion stability (ES) were calculated by the following formula. In the formula, N is the dilution factor

Figure 1. Workflow for sample collection and preparation. CG = control group; TG = treated group.

of the milk protein, φ is the volume fraction of the oil phase in the sample emulsion, and L is the diameter of the colorimetric cup (cm).

$$\text{EAI} = \left(2 \times 2.303A \times N \times 10^{-4} \right) / (\varphi L \rho);$$

$$\text{ES (\%)} = A_1/A_0 \times 100\%.$$

Determination of the Foaming Properties of Goat Milk Protein

A 3-mL aliquot of sample was transferred with a micropipette into a 10-mL mixing cylinder with a stopper, and it was vigorously oscillated by hand for 1 min to achieve foaming. The foam height was immediately recorded (0 min) as the initial foam height, V_0 . After setting in the measuring cylinder for 30 min, the foam height (V_1) was recorded again. The ratio of the initial height to the sample content was taken as an indicator of foaming capacity (**FC**). The ratio of foam height after 30 min (V_1) to the V_0 was used as an indicator of foam stability (**FS**; Meena et al., 2017).

Determination of the Water-Holding Capacity and Oil-Holding Capacity of Goat Milk Proteins

The protein sample ($m = 0.05$ g) was placed into a 2.5-mL Eppendorf tube, and the total mass (m_1) was determined. Then, 1 mL of distilled water or 1 mL of soybean oil was added. After mixing thoroughly, the mixture was allowed to stand for 30 min and then centrifuged at room temperature at $1,078 \times g$ for 30 min. Finally, the supernatant was removed and the precipitate inverted for 30 min, and the mass of the goat milk protein and the Eppendorf tube was determined again (m_2). The water-holding capacity (**WHC**) and the oil-holding capacity (**OHC**) were calculated by the following formula:

$$\text{WHC (OHC)} = (m_2 - m_1)/m.$$

Data Processing and Analysis

All the data were recorded and statistically processed using Excel 2016 (Microsoft Corp., Redmond, WA), and Origin 8.0 (OriginLab, Hampton, MA) was used for drawing. The averages, standard deviations, and significance ($P < 0.05$) of experimental data from different combinations of pH and temperature treatments were calculated and analyzed by IBM SPSS Statistics 23.0 (IBM Corp., Armonk, NY). The experimental results are expressed as means $\pm$ standard deviation.

RESULTS AND DISCUSSION

Analysis of the Thermal Stability of Goat Milk Protein as Affected by pH and Heat

Differential scanning calorimetry can be used to assess the structural changes in samples during the thermal treatment and concomitant performances changes (Ptiček Siročić, 2017). As shown in Figure 2, the pH had a significant effect on the thermal stability of goat milk proteins ($P < 0.05$). The pH value of the treated group with pH 6.5 was close to that of the CG, and the denaturation temperature did not change significantly. Among another 3 treated groups with pH 6.9, 7.3, and 7.7, the thermal denaturation temperature of the goat milk proteins decreased gradually with the increasing of pH value. The thermal denaturation temperature of the goat milk protein at pH 7.7 was significantly lower than that of CG ($P < 0.05$). The results showed a lower thermal stability for the treated groups with pH 6.9, 7.3, and 7.7 compared with CG. This may be explained by the lower protein stability due to the higher intramolecular repulsion of the deprotonated groups in the protein. On the other hand, the reactivity of the free thiol groups is expected to increase with increasing pH (Leeb et al., 2018). Higher reactivity of the thiol groups might enhance intermolecular thiol-disulfide exchange reactions and a subsequent irreversible denaturation of the protein (Hoffmann and Van Mil, 1999).

Surface Hydrophobicity Analysis of Goat Milk Protein as Affected by pH and Heat

As shown in Figure 3A, the surface hydrophobicity of the goat milk protein treated at 85°C and above was significantly greater ($P < 0.05$) than that of the CG and reached maximum at 85°C, indicating that the hydrophobic groups exposed on the surface of the milk protein increased when the heating temperature was increased to 85°C. This may be explained by the fact that when the temperature was lower than 85°C, the heating caused exposure of the thiol group, the whey protein conformation changes and the protein structure unfolds, resulting in the exposure of the hydrophobic site, and thus the surface hydrophobicity is increased (Vasbinder and de Kruif, 2003; Choudhary et al., 2018). With increasing heating temperature (85–125°C), interaction between β -LG and cysteine containing whey protein such as α -LA led to the formation of whey protein aggregates, and β -LG and κ -casein formed casein-whey protein aggregation by thiol-disulfide bond exchange reactions, which resulted in irregular surface structures of the casein and improvements in surface hydrophobic-

Figure 2. Differential scanning calorimetry (DSC) curves of goat milk proteins as affected by pH and heat. CG = control group. Values of each group not sharing the same letters are significantly different ($P < 0.05$).

ity (Vasbinder and de Kruif, 2003; Choudhary et al., 2018). However, the polymerization between proteins caused hydrophobic groups to be embedded in the polymer. In addition, denatured β -LG combined with α -LA such that the surface irregularities of the casein were filled, and the surface became smooth, resulting in a decrease in hydrophobicity (Michel et al., 1997).

As seen from Figure 3B, with the exception of pH 7.7, the surface hydrophobicities of the goat milk protein at different pH levels were significantly lower

than that of fresh milk ($P < 0.05$), which indicated that changing the pH could change the hydrophobicity of goat milk proteins. The surface hydrophobicities of goat milk proteins at different pH conditions were significantly affected by the temperature used during heat treatment ($P < 0.05$), and the trend was generally consistent with fresh milk (Figure 3A). The surface hydrophobicity of goat milk protein at pH 6.9 reached its maximum at 105°C, whereas at other pH values, the maximum was observed at 85°C, indicating that

Figure 3. Changes induced by pH and heat on the surface hydrophobicity of goat milk proteins. Values of each group not sharing the same letters (a-e) are significantly different ($P < 0.05$), and error bars represent SD.

pH can change the characteristics of the hydrophobic groups in the goat milk proteins during heating. Jean et al. (2006) suggested that the surface hydrophobicity of casein and whey protein aggregates produced during treatment at high temperatures were very high under neutral pH conditions, which was similar to the results of the current study. It was found that ionic calcium, pH, and total soluble divalent cations decreased as temperature increased between 20 and 110°C in both dialysates and ultrafiltration permeates (On-Nom et al., 2010). In addition, the change in pH leads to the different conformations in the goat milk proteins and the distribution of hydrophobic groups inside and outside of the molecule, resulting in a change in the surface hydrophobicity of the goat milk protein during heating (Lam and Nickerson, 2015).

Analysis of the Particle Size, Zeta Potential, and Turbidity of Goat Milk Protein as Affected by pH and Heat

As shown in Figure 4A and 4C, compared with those of CG, the goat milk protein samples treated at different temperatures had significantly different particle sizes and zeta potentials ($P < 0.05$). The average particle size and zeta potential initially decreased and then increased with increasing treatment temperature. However, Figure 4B showed the particle size of goat milk protein was not significantly affected by pH ($P > 0.05$). Figure 4A and 4C show that the particle size and zeta potential of goat milk protein treated at different pH values decreased significantly at 85°C ($P < 0.05$), which indicated that 85°C was the key point of the change for the goat milk protein after pH adjustment. When the heated temperature is less than 85°C, whey protein denatured and dissociated from oligomer to monomer, casein micelle depolymerized, κ -casein dissociated on micelle surface, and the degree of dissociation increased with increasing temperature. The micelle morphology was broken and became loose, resulting in a decrease in particle size, an increase in the net negative surface charge, and a decrease in the zeta potential (Alexander et al., 2002). When the heated temperature is higher than 85°C, whey protein monomers formed polymers through disulfide bonds, and β -LG and κ -casein formed casein-whey protein aggregation by thiol-disulfide bond exchange reactions. The aggregation of casein itself and the binding of casein to whey protein were higher than the degree of depolymerization of casein itself, resulting in an increase in protein particle size (del Angel and Dalgleish, 2006; Lin et al., 2018).

In addition, the change of trend in the particle sizes of goat milk protein at pH 6.5 and 7.7 after heat treatment were basically the same as those of fresh goat

milk (Figure 4A). When the pH was 6.9, the smallest goat milk protein particle size was observed at 85°C and the largest was at 105°C. At pH 7.3, the average goat milk protein particle size gradually decreased with increasing temperature. It can be hypothesized that near neutral pH, β -LG oligomers depolymerized into monomers and reacted more easily with other proteins during heat treatment. Previous research has also demonstrated that changes in pH before heating affect the formation and composition of goat milk protein aggregates during heat treatment (Vasbinder and de Kruif, 2003; Nair et al., 2013).

As shown in Figure 5A, above 85°C, the turbidity of the goat milk protein was significantly higher than that of the CG ($P < 0.05$), indicating that heat treatment can result in the denaturation of the goat milk proteins to varying degrees. With the exception of pH 7.7, the turbidity of goat milk protein at various pH values increased significantly with increasing temperature ($P < 0.05$). The trend in the turbidity found in this study was similar to that of Anema and Klostermeyer (1997). The diameter and light scattering coefficient of the milk protein particles changed, and the milk proteins were dissociated and aggregated to different degrees. As shown in Figure 5B, pH had a significant effect on the turbidity of the goat milk proteins ($P < 0.05$). In addition, the turbidity of the goat milk proteins under basic conditions (pH 7.3 and 7.7) was lower than under acidic conditions (pH 6.5 and 6.9) during heat processing (Figure 5A). These results showed that changes in pH caused changes in the thermal induction of goat milk protein. Lower pH values resulted in greater degrees of milk protein aggregation. At a lower pH, the whey protein/casein polymer formed on the micelle surface when the whey protein and casein micelle interacted with each other during heat treatment, which resulted in an increase in turbidity. At higher pH values, the proteins interact to form soluble whey protein/ κ -casein complexes, which mainly exist in the whey phase and result in decreased turbidity (Vasbinder and de Kruif, 2003; Vasbinder et al., 2004; Nair et al., 2013).

Structural Analysis of Goat Milk Protein as Affected by pH and Heat

Fourier-Transform Infrared Spectroscopy of Goat Milk Protein as Affected by pH and Heat.

As shown in Figure 6, the infrared spectra of the treated samples were similar to that of the CG, and the characteristic absorption peaks at 1,700 to 1,600 cm^{-1} exhibited obvious redshifts and blueshifts. The 1,700 to 1,600 cm^{-1} band is the most commonly used amide I band for protein analysis, as it is characteristic of the vibrational stretching of C = O moieties in the protein (Morand et

Figure 4. Changes induced by pH and heat on the particle size and zeta potential of goat milk proteins. Values of each group not sharing the same letters (a–c) are significantly different ($P < 0.05$), and error bars represent SD.

al., 2011). Compared with the CG, the 1,700 to 1,600 cm^{-1} absorption band of goat milk protein at pH 6.9 showed a significant redshift, indicating that pH had a significant effect on the secondary structure of goat milk protein (Figure 6B). The absorption peaks of pH 6.5 and 6.9 goat milk treated at 105°C were observed at 1,649.9 and 1,654.1 cm^{-1} , respectively, correspond-

ing to a significant redshift (Figure 6C and 6G). The results indicated that milk protein was unstable to pH and heat cotreatment. When the AA residues formed intermolecular hydrogen bonds, the electron density on the C = O moieties decreased, and the absorption peak shifted toward lower wavenumbers (Jackson and Mantsch, 1995). For 105°C treated samples, the absorption

Figure 5. Changes induced by pH and heat on the turbidity of goat milk proteins. OD = optical density. Values of each group not sharing the same letters (a–e) are significantly different ($P < 0.05$), and error bars represent SD.

Figure 6. Fourier-transform infrared spectra of goat milk proteins as affected by pH and heat. CG = control group.

peaks of the goat milk protein samples treated at pH 6.5 and 6.9 were 1,649.9 and 1,654.1 cm^{-1} (Figure 6C and 6G), whereas for 85°C, the samples at pH 7.3 and 7.7 were observed at 1,657.9 and 1,661.6 cm^{-1} , respectively, corresponding to a significant blueshift (Figure 6H and 6I). Thus, we can see that the different pH treatments and temperatures influenced the conformations of the proteins, as they disrupted the intramolecular hydrogen bonds, causing the peaks to shift toward higher wavenumbers (Barreto et al., 2003).

Fitting of the Amide I Bands of Goat Milk Protein as Affected by pH and Heat. The number and position of characteristic peaks can be determined from the second derivative of the amide band I, and the deconvolution and multipeak fitting operations were carried out by peak fit (Figure 6D, 6E, 6F, 6J, 6K, and 6L). The secondary structures of the protein include regular α -helix (1,650–1,660 cm^{-1}), β -sheet (1,600–1,640 cm^{-1} and 1,682–1,700 cm^{-1}), and β -turn (1,661–1,681 cm^{-1}) structures, and the irregular structures include

an irregular curl ($1,641\text{--}1,650\text{ cm}^{-1}$). According to the relationship between the secondary structure and the subpeak in the amide I band, the relative contents of the secondary structures in all samples were calculated based on the integrals (Table 1).

The secondary structure in the CG was mainly β -sheets, accounting for 34.45% of the total, but the percent of regular structures in the pH treated and unheated groups decreased to varying degrees, and it was the lowest at pH 6.9, and the content of α -helix at pH 6.9 was significantly reduced compared with the CG. The acidic environment may increase the electrostatic interactions among proteins due to neutralization of the charge, affecting the stability of the hydrogen bonds and reducing the content of the α -helices (Fennema, 1996; Damodaran, 2008). The changes in electrostatic interactions and hydrogen bond stability could in turn contribute to the loss of α -helix content under acidic conditions (Liu et al., 2008). When goat milk samples at different pH levels were heated, the content of irregular structures in the milk proteins were observed the trend of declining and then increasing. The pH value of solution is a very important factor affecting the formation or stability of α -helix structure. Where acidic or alkaline AA accumulate, the same charge repulsion on side chains will cause α -helix instability. The mecha-

nism of the interaction of pH and temperature on the stability of α -helix structure is still unclear. However, it can be seen that pH and heat cotreatment changed the secondary structure of goat milk proteins from ordered to disordered, which resulted in a decrease in their stability to varying degrees. At pH 6.5 and 6.9, the content of regular structures, particularly α -helix, decreased upon heating to 105°C . It has been previously reported that the α -helix structures of protein molecules are negatively correlated with hydrophobicity (Kato et al., 1981). When the α -helix content decreases, the exposure of hydrophobic moieties embedded in the molecules increases, resulting in stronger surface hydrophobicity, which corresponds to the hydrophobicity results in this study. In addition, for milk at pH 7.3 and 7.7, the content of regular structures, especially β -turns, decreased dramatically when heated to 85°C . It could be concluded that alkaline treatment could decrease the contents of α -helix and β -sheet structures, whereas heat treatment formed thermal aggregates; the β -sheets between molecules were easily transformed into β -turns, and a portion of the β -turns were converted into irregular structures, which lead to a decrease in the β -turn content and an increase in the irregular structures. These results are in agreement with previous findings by Liu et al. (2008).

Table 1. The secondary structure contents of goat milk proteins as affected by pH and heat (%); values shown as means $\pm$ SD

Group ¹	Treatment method		α -Helix	β -Sheet	β -Turn	Irregular curl
CG	Unadjusted pH	Unheated	32.23 ± 1.44^a	34.45 ± 3.62^a	20.02 ± 2.65^{ab}	13.29 ± 0.47^b
TG1	Unadjusted pH	65°C	33.24 ± 0.16^a	27.89 ± 0.13^b	22.75 ± 0.13^a	16.12 ± 0.13^a
		85°C	32.95 ± 0.54^a	28.97 ± 0.61^b	21.67 ± 0.04^a	16.40 ± 1.11^a
		105°C	32.41 ± 0.64^a	29.46 ± 0.62^b	24.56 ± 2.95^a	13.56 ± 1.69^b
		125°C	32.99 ± 0.45^a	30.38 ± 1.05^b	22.61 ± 1.30^a	14.02 ± 0.20^b
		Unheated	27.04 ± 2.80^{ab}	35.70 ± 3.98^a	17.58 ± 0.25^{ab}	19.67 ± 6.53^b
TG2	pH 6.5	65°C	31.72 ± 0.42^a	30.20 ± 0.69^b	23.20 ± 0.69^a	14.87 ± 1.80^c
		85°C	25.91 ± 5.35^b	32.34 ± 0.11^a	18.42 ± 1.55^b	28.19 ± 1.93^b
		105°C	17.31 ± 0.32^d	26.91 ± 0.13^c	24.22 ± 0.14^a	31.56 ± 0.15^a
		125°C	30.97 ± 1.80^a	31.67 ± 1.42^a	22.37 ± 2.38^a	14.99 ± 1.99^c
		Unheated	24.57 ± 3.73^b	25.13 ± 3.85^b	14.26 ± 5.18^b	36.04 ± 5.06^a
TG3	pH 6.9	65°C	21.91 ± 10.01^{ab}	31.82 ± 1.87^a	21.60 ± 0.89^b	24.67 ± 9.04^{ab}
		85°C	20.07 ± 0.05^{ab}	31.23 ± 0.24^a	21.94 ± 0.01^b	26.77 ± 0.29^{ab}
		105°C	15.92 ± 4.29^b	32.03 ± 1.76^a	16.98 ± 0.60^a	35.45 ± 5.06^a
		125°C	25.32 ± 7.68^{ab}	28.69 ± 4.99^a	21.54 ± 0.62^b	24.45 ± 2.07^{ab}
		Unheated	28.10 ± 2.90^{ab}	36.10 ± 4.58^a	23.72 ± 0.49^a	12.07 ± 2.17^b
TG4	pH 7.3	65°C	22.97 ± 0.13^b	31.88 ± 1.90^{ab}	25.50 ± 4.21^a	19.65 ± 5.98^b
		85°C	19.19 ± 2.93^b	30.14 ± 2.19^b	16.59 ± 7.10^a	34.08 ± 6.36^a
		105°C	21.99 ± 0.47^b	27.73 ± 3.55^b	17.99 ± 3.04^a	32.29 ± 7.07^a
		125°C	21.66 ± 1.56^b	35.57 ± 0.20^a	20.16 ± 0.44^a	22.61 ± 0.91^b
		Unheated	25.31 ± 1.26^b	30.28 ± 0.05^b	24.64 ± 2.59^a	19.77 ± 3.89^b
TG5	pH 7.7	65°C	16.06 ± 2.99^c	29.15 ± 1.88^b	29.46 ± 4.93^a	25.32 ± 3.82^b
		85°C	21.01 ± 0.09^b	28.96 ± 0.28^b	6.91 ± 0.01^c	43.12 ± 0.18^a
		105°C	34.42 ± 2.58^a	31.12 ± 2.27^{ab}	19.34 ± 0.70^b	15.11 ± 5.56^c
		125°C	22.51 ± 0.01^b	33.66 ± 0.10^a	19.89 ± 0.06^b	23.93 ± 0.05^b

^{a-d}Different letters indicate a significant difference ($P < 0.05$) among different pH and heat treatment groups.

¹CG = control group; TG = treated group.

Analysis of the Surface Structure of Goat Milk Protein as Affected by pH and Heat

As shown in Figure 7A, the scanning electron microscopy results showed that the fresh goat milk proteins were spherical, smooth, fine particles. Figure 7B suggested that the size, distribution, and surface morphology of the goat milk proteins heated at 65°C/15 s were uniform and regular, and the particle size of the micelles clearly decreased compared with Figure 7A. The size difference might be resulted by the structural rearrangement or shielding of charge. After heat treatment at 125°C/15 s, due to the increased heating temperature, the interactions among the proteins caused aggregation, and the protein particles were partially agglomerated. Compared with Figure 7A, the particle size was obviously increased, the proteins had an irregular shape, and a dense network structure was observed in Figure 7C. At pH 6.5, the surface morphology appeared to be similar to that of the original spherical micellar morphology, which was conducive to the stability of the micelles (Figure 7D). Compared with the CG, the surface of the milk protein particles was not smooth, but the distribution of the particles was more uniform. At pH 6.9, most of the milk protein particles were spherical. However, the smoothness of the milk protein particles was reduced, the molecular spacing was obviously small, and the distribution of the milk protein particles was uneven (Figure 7E). At pH 7.3, the milk protein molecules showed slight adhesion, and a small number of milk protein particles were connected to each other to form small micelles. These micelles were connected to each other, forming a small number of relatively dense network structures (Figure 7F). At pH 7.7, a large number of dense network structures were formed by interconnection of micelles (Figure 7G). The scanning electron microscopy results indicated that as the pH of the milk increased, the milk protein became less stable, which was consistent with the differential scanning calorimetry results in this study.

Analysis of the Functional Properties of Goat Milk Protein as Affected by pH and Heat

As shown in Figure 8A, a significant difference was observed in the EAI of goat milk proteins between pH 6.5 and 7.3, which may be related to the hydrophobicity of the surface of the goat milk proteins (Pacheco-Aguilar et al., 2008). According to Figure 8B, the EAI values of goat milk proteins at pH 6.5 and 7.7 were significantly affected by temperature ($P < 0.05$), and the changes in the other groups were not significant ($P > 0.05$). The milk protein at pH 6.5 had the highest EAI under different treatment conditions except 85°C.

It can be concluded that pH 6.5 provided the greatest EAI for the goat milk protein. Wagner and Gueguen (1999) also suggested that using an acidic pH could expand the protein structure, expose hydrophobic groups, increase the hydrophobicity of the surface, and increase the EAI, which was consistent with the results of the present study. According to Figure 8C, the ES values of the treated groups were significantly affected by temperature ($P < 0.05$). The EAI and ES change trend of goat milk proteins with different pH values were inversely proportional to each other following different heat treatments.

Figure 7. Scanning electron micrographs of goat milk proteins as affected by pH and heat ($\times 12,000$). A and H denote the control group. B and C denote the samples heated at 65°C for 15 s and 125°C for 15 s, respectively. D, E, F, and G denote the sample with pH 6.5, 6.9, 7.3, and 7.7, respectively.

As displayed in Figure 9A, the FC of the goat milk proteins treated at 85°C and above were significantly lower than that of the CG ($P < 0.05$), and the FC also changed significantly at pH 6.9 (Figure 9B). With the exception of pH 7.3, the FC values of the treated groups and the CG followed the same trends with increasing temperature. Compared with the unheated and 65°C treatment groups, when the temperature was above 85°C, significant differences were present (Figure 9A). A previous study showed that heat treatment below 85°C increased the FC of proteins, whereas heat treatments above 85°C reduced the FC, which was consistent with the conclusions of this study (Lajnaf et al., 2018). In addition, changes in the pH changed the ionization of the proteins, the distribution of hydrophobic groups in the solution, the protein structure, the adsorption at the gas-liquid interface, and the FC (Shan et al., 2012). The FS values of goat milk proteins at pH 6.9 and 7.3 were significantly affected by temperature ($P < 0.05$), and the trends were entirely different (Figure 10A). As shown in Figure 9B and 10B, the FC and FS values of CG were inversely proportional, whereas the milk proteins at pH 6.5 had better FC and FS values than those of other pH-treated groups, which

indicated that changes in the pH could improve the foaming properties of goat milk proteins at different temperatures (Figure 9A and 10A). Thus, compared with other pH treatment groups, under slightly acidic conditions, such as pH 6.5, goat milk proteins not only have good foaming abilities but also good FS when heat treated at different temperatures.

From Figure 11A, the WHC was lower under weakly alkaline conditions and higher under acidic conditions, indicating that weak acidity resulted in goat milk protein with a better WHC. The trend in the WHC in this study was similar to that of Chen et al. (2018b). The WHC of goat milk proteins at different pH values were significantly affected by temperature ($P < 0.05$), and the trends were different (Figure 11B). The WHC of goat milk protein at pH 6.5 and 6.9 were significantly higher than those of the samples at pH 7.3 and 7.7 during heat treatment, especially the WHC of pH 6.5 goat milk protein, which was the highest at 85°C (Figure 11B). This result showed that acidic conditions were optimal for the WHC of goat milk proteins during heat processing. The pH influenced the degree of dissociation of the protein molecules, the surface charge, and the interactions among proteins and the change of

Figure 8. Changes induced by pH and heat on the emulsifying properties of goat milk proteins. EAI = emulsifying activity index; ES = emulsion stability. Values of each group not sharing the same letters (a-e) are significantly different ($P < 0.05$), and error bars represent SD.

Figure 9. Changes induced by pH and heat on the foam capacity of goat milk proteins. Values of each group not sharing the same letters (a–c) are significantly different ($P < 0.05$), and error bars represent SD.

divalent cations, thus changing the ability of the proteins to bind water. At the same time, an appropriate heat treatment can cause the milk protein molecules to form soluble β -LG/ κ -casein polymers, which promote the formation of a protein gel network, and prevent water loss and thus improve the WHC of the proteins.

The OHC of goat milk proteins after heat treatment above 85°C were significantly higher than that of fresh goat milk protein ($P < 0.05$; Figure 11C). The reason might be that the appropriate heat treatment promoted the unfolding of the highly organized structure of the protein, exposure of the hydrophobic groups, increased flexible molecular segments, and more orderly arrangement of molecules, resulting in an increase in the OHC (Oliyae et al., 2017). The OHC of goat milk proteins at pH 6.9 and 7.3 were superior to samples subjected to other pH conditions (Figure 11D). As a result, goat milk proteins at neutral pH had the best OHC. The OHC of the treated groups were significantly affected by temperature, and the trend was different ($P < 0.05$).

With the exception of 85°C, the goat milk proteins at pH 6.5 and 7.7 showed relatively good OHC during heat processing, and the goat milk protein at pH 6.5 had the highest OHC at 105°C. Zhang et al. (2017) indicated that the OHC of proteins were influenced by their hydrophobic groups. The pH influenced the distribution of hydrophobic groups on the inside and outside of the protein molecule. At the same time, different heat treatments can cause the dissociation of the protein molecules or heat-induced aggregation, which results in the exposure or protection of hydrophobic sites and affects the binding between proteins and oil, influencing the OHC (Alizadeh-Pasdar and Li-Chan, 2000).

CONCLUSIONS

In this study, the combination of pH and heat treatment can significantly change the structural and functional properties of goat milk proteins, which were

Figure 10. Changes induced by pH and heat on the foam stability of goat milk proteins. Values of each group not sharing the same letters (a–c) are significantly different ($P < 0.05$), and error bars represent SD.

Figure 11. Changes induced by pH and heat on the water-holding capacity and oil-holding capacity values of goat milk proteins. Values of each group not sharing the same letters (a–e) are significantly different ($P < 0.05$), and error bars represent SD.

closely related to the dissociation and aggregation of casein and whey proteins and the interactions between them. The thermal stability of goat milk protein was significantly affected by pH, and the thermal stability was the lowest when the pH was 7.7. The pH and heat treatments had significant effects on the structural properties of goat milk the protein, such as their surface hydrophobicity, zeta potential, turbidity, secondary structure, and surface structure. In addition, under weakly acidic conditions, the goat milk proteins subjected to different heat treatments had better ESI, FC, FS, and WHC values. Under basic and neutral conditions, goat milk proteins subjected to different heat treatments had better ES and OHC values. In conclusion, the structural and functional properties of goat milk proteins were influenced by pH and heat. The processing conditions of goat milk proteins can be optimized by tuning the pH and heat conditions, which provides a theoretical basis for research into the applications of goat milk proteins.

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