The effect of heat treatment on the microstructure and functional properties of whey protein from goat milk

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ABSTRACT

This work investigated the effects of thermal processing methods commonly used in the dairy industry and prolonged treatment at different temperatures on the denaturation, microstructure, and functional properties of whey proteins (WP) from goat milk. The complete denaturation of WP was observed in goat milk treated at 85°C for 30 min, and at a higher temperature (>85°C), a considerable amount of WP was easily denatured. The low temperature, long time treatment had the least effect on the secondary structure, whereas ultra-high temperature treatment had the greatest effect, and the amount of regular structures decreased gradually with prolonged time. The most serious morphological damage occurred after treatment at 85°C for 30 min, which was consistent with the denaturation results. This result indicated that the denaturation degree, particle size, surface hydrophobicity, and microstructure had a strong influence on the functional properties of WP from goat milk after heat treatment. The heat treatment of goat milk at 65°C for 30 min and 85°C for 15 s increased the particle size, turbidity, zeta potential, and surface hydrophobicity of WP, and these increases ensured that the WP had a good emulsifying activity index, water-holding capacity, oil-holding capacity, foaming capacity, and foam stability. This study simulated the heat treatment conditions used in actual production, aiming to provide a theoretical basis for industry.

Key words: goat milk, whey protein, heat treatment, microstructure, functional properties

INTRODUCTION

Recently, goat milk has become popular with consumers because of its low lactose content, high protein content, high calcium content, and high proportion of more digestible fatty acids compared with cow milk (Haenlein, 2004; Kalyankar et al., 2016; Chen et al., 2018). In cow and goat milk, no significant difference was found in the total protein content. However, a significantly lower amount was found of α_{S1} -CN, which only exists in the casein micelle, and a higher concentrate of β -CN + κ -CN in the serum phase. Therefore, goat milk can be better digested in the human body compared with cow milk, which was found to be a main reason why the goat milk is relatively better in terms of nutritional utilization. As for mineral composition, goat milk is very similar to cow milk with regard to the content of Na, Fe, Zn, and Mb, but has higher amounts of Ca, K, Mg, P, Cl, and Mn. The good digestion of goat milk for humans also lies in its short- and medium-chain fatty acids. Results have shown both a higher mineral content and better utilization in goat milk (Ceballos et al., 2009). A comparative study stated that the hydrophobic sites on the micelle surface of goat milk were greater than those of sheep milk (Yuksel et al., 2012). Differences are also present in various goat breeds. The 2 most common methods used to analyze the differences are SDS-PAGE and reversed-phase HPLC. The ratio of α -LA/ β -LG presented a significant difference between sheep and goat WP in Greek breeds (Moatsou et al., 2005). However, a similarity between the 2 types of milk is that the content of α_{S1} -CN is very small (Costa et al., 2014). Heat treatment is one of the most common processing methods in the dairy industry and can kill microorganisms in raw milk, which can ensure food safety and extend the shelf life of products (McKinnon et al., 2009; Claeys et al., 2013). However, heat treatment can cause chemical changes, such as interactions among proteins, and many studies have indicated that

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the aggregation of whey protein (WP)/casein is one of the main chemical changes that occur during heat treatment in goat milk (Sharma and Dalglish, 1993; Vasbinder and Kruif, 2003). In addition, WP aggregates are composed of α -LA and β -LG in the serum phase (Donovan and Mulvihill, 1987). Native β -LG exists as a dimer, containing 2 disulfide bonds and 1 free thiol group buried inside the molecule (Wijayanti et al., 2014). α -Lactalbumin is stabilized by 4 disulfide bonds and lacks a thiol group, so α -LA cannot form aggregates unless in the presence of free thiol groups (Permyakov and Berliner, 2000). The order of ease of irreversible WP was Ig > serum albumin (SA) > lactoferrin (Lf) > β -LG > α -LA of caprine milk. The α -LA and β -LG unfold at 66 and 75°C, respectively (Clark et al., 1981). Upon reaching the denaturation temperature, the molecular structure of WP will be destroyed, and the disulfide bonds and thiol group will be exposed and available for reactions. Then, WP tends to associate with κ -CN in serum (Li et al., 2014a), forming β -LG aggregates and β -LG/ α -LA dimers and aggregates by -SH/S-S interchange interactions. Serum albumin, whose denaturation temperature is 66°C, can also associate with α -LA in a similar way (Havea et al., 2000; Considine et al., 2007). Some reports have clarified the pathways of β -LG/ α -LA aggregation and WP/CN complex formation, which occur through -SH/S-S interactions (Vasbinder and Kruif, 2003). Similar reactions were also found in goat milk; according to Felipe et al. (1997), the WP of goat milk was treated at 83°C for 1 min and then loaded to SDS-PAGE without adding 2-mercaptoethanol. As a result, WP and κ -CN did not appear in the running gel, but their larger size aggregates were found in the stacking gel, which proved that WP/ κ -CN complex was formed by disulfide bonds (Felipe et al., 1997). Furthermore, the soluble WP/CN complex has been proven to reduce the gel time of yak milk and improve the structure of yogurt gel (Xu et al., 2015). In this regard, WP can be effectively used as an ingredient in the food industry because of its outstanding interfacial properties and is usually used as an emulsifying and foaming agent (Anandharamakrishnan et al., 2008; Niculescu et al., 2008).

Previous studies have mainly focused on the heat treatment of WP below 100°C, and little information is available on the microstructure and functional properties of WP from goat milk after pasteurization and UHT treatment. Moreover, the molecular changes caused by heat treatment may have a positive or negative influence on the functional properties of WP (McSweeney et al., 2004). Therefore, it is necessary to clarify how heat treatment affects denaturation and the subsequent changes in the functional properties of WP from goat

milk. This study aims to determine the denaturation extent of WP from goat milk during thermal processing methods commonly used in dairy production and under prolonged treatment at different temperatures, as well as the changes in the WP microstructure and functional properties, to improve the utilization of WP by simulating the processing conditions in the food industry.

MATERIALS AND METHODS

Collection and Preparation of Goat Milk Samples

Fresh goat milk samples were collected from Laoshan goats at the Qingdao Aote Goat Breeding Farm in Shandong Province, China. All the goats were in the mid stage of lactation, showed no signs of acute mastitis or other clinical diseases, and were milked by machine at 1000 h. All milk samples were transferred on ice to the laboratory. The unheated samples served as the control group (CG), and other samples were treated according to Figure 1 referring to the heating method of Chen et al. (2019). The pasteurization/UHT group consisted of low temperature, long time (LTLT) at 65°C for 30 min (PG1), HTST at 85°C for 15 s (PG2), ultrapasteurization at 125°C for 4 s (PG3), and UHT at 135°C for 4 s (UG). The S1, S2, S3, S4, and S5 treatments were 65°C for 15 s, 65°C for 1 min, 65°C for 5 min, 65°C for 10 min, and 65°C for 15 min, respectively. The E1, E2, E3, E4, and E5 treatments were 85°C for 1 min, 85°C for 5 min, 85°C for 10 min, 85°C for 15 min, and 85°C for 30 min, respectively. The O1 and Q1 treatments were 105°C for 15 s and 125°C for 15 s, respectively. The samples were heated by laboratory miniature pasteurizer and laboratory UHT sterilizer (YC-02, Pilotech Company, Shanghai, China). The minimum pasteurization capacity is 1,000 mL, the temperature of pasteurization is 30 to 90°C, the accuracy of heating and temperature control is $\pm 1^\circ\text{C}$, and the external tap water or ice water is used for cooling. The minimum amount of UHT sterilization is 3 L, the highest sterilization temperature is 150°C, and the cooling temperature is 20°C.

Preparation of Whey Protein and SDS-PAGE

All samples were centrifuged at 4°C, $2,149 \times g$ for 15 min and the upper fat was discarded, then the skim milk was collected (Palmquist and Jenkins, 2003). The skim milk, adjusted by 10% acetic acid to pH 4.10, was centrifuged at 4°C, $1,986 \times g$ for 15 min, and then the supernatant was WP (Recio et al., 1997). The powder WP was obtained by vacuum freeze drying and stored

at -20°C in a refrigerator for the determination of micellar structure and functional properties.

The SDS-PAGE was performed according to the method of Sun et al. (2014). The WP powder samples were dissolved in water to a solution of 2 mg/mL. The WP solution was added to water in a 1:1 volume ratio and mixed evenly. Then 10 μL were loaded onto the gels composed by 12% separation gel concentration and 5% concentration gel, with 1 mm of gel thickness, and electrode buffer consisted of SDS buffer (pH 8.3) and Tris-Gly solution (Macklin Biochemical, Shanghai, China). Electrophoresis was carried out for approximately 3.5 h at 4°C , and gels were stained for 2 to 4 h with Coomassie Brilliant Blue R-250 (Biotopped Technology Company, Beijing, China) and decolorized until a clear background was achieved. The electropherograms were scanned with an optical density scanner, the scanned images were processed and analyzed in Lane 1D gel analysis software (Sagecreation Company, Beijing, China), and the relative optical density (IOD) of each protein band on the map was analyzed. The IOD was calculated as follows: integral optical density (IOD) = $\Sigma (\text{OD} \times \text{area})$, where OD is the optical density.

Determination of the Denaturation of WP by HPLC

Skimmed samples were centrifuged at 4°C , $20,893 \times g$ for 60 min, and serum phases were collected to be HPLC samples. A C4 column (250 mm $\times$ 4.6 mm, 5 μm , 300 $\text{\AA}$) was used in an RP-HPLC Agilent 1100 (Agilent Technologies, Santa Clara, CA); the detection wavelength was 214 nm; the flow rate was 0.8 mL/min; the column temperature was 30°C ; and the injection volume was 20 μL . Ultrapure water containing 0.1% TFA and acetonitrile containing 0.1% TFA were used as mobile phases A and B, respectively. Gradient elution: 0 to 12 min, B concentration of 30 to 36%; 12 to 30 min, B concentration of 36%; 30 to 45 min, B concentration of 36 to 40%; 45 to 60 min, B concentration of 40 to 43%; 60 to 61 min, B concentration of 43 to 100%; 61 to 63 min, B concentration of 100%; 63 to 68 min, B concentration of 100 to 30%; 68 to 80 min, B concentration of 30%. The denaturation degree of WP was calculated by the formula $(M1 - M2)/M1$, where M1 is the quantity of undenatured WP in fresh milk and M2 is the quantity of undenatured WP in heated milk.

Figure 1. Workflow for sample collection and preparation.

Determination of the Secondary Structure of WP

Fourier-transform infrared spectroscopy was used to measure the secondary structure of the WP. The WP powder made by freeze drying and potassium bromide were uniformly mixed at a ratio of 1:150, subjected to tableting treatment, and then tested over time. The test parameters were set as follows: 10 scans, resolution 4 cm^{-1} , and scan range from $4,000$ to 400 cm^{-1} . The infrared spectra obtained by scanning were compared; the characteristic amide I protein band was selected for analysis, and the secondary structure of the protein was further characterized by infrared desorption. Spectral acquisition, peak finding, and basic analysis were performed using Omnic 8.2 software (Thermo Fisher Scientific, Waltham, MA). Multipeak fitting was performed using Peakfit 4.12 software (Systat Software Inc., San Jose, CA) until the residual was minimal ($R^2 > 0.999$).

Determination of the Particle Size, Zeta Potential, Turbidity, and Surface Hydrophobicity of WP

The particle size and zeta potential were measured by Zetasizer Nano ZS90 (Malvern Panalytical Ltd., Malvern, UK), and the WP solutions were diluted to 10 mg/mL before measurement, as described by Lamb et al. (2013). The parameters were as follows: particle refractive index 1.450, particle absorption rate 0.001, dispersant refractive index 1.330, dispersant absorption rate 0.8872, and water as dispersant. The turbidities of the WP solutions (2 mg/mL) were determined by the absorbance at 633 nm using a UV spectrophotometer and a cell with a 1.0-cm path length (Partschfeld et al., 2007).

The surface hydrophobicity of the WP was measured by fluorescence intensity as an indicator. Twenty milliliters of WP (0.6 mg/mL) was added to $100\text{ }\mu\text{L}$ of 8-aniline-1-naphthalenesulfonic acid, shaken, and allowed to stand for 3 min for fluorescence scanning. The scanning parameters were set as follows: excitation wavelength: 368 nm ; emission wavelength: 390 to 600 nm ; scanning speed: 300 nm/min ; and slit: 5 nm .

Scanning Electron Microscopy of WP

A volume of $5\text{ }\mu\text{L}$ WP solution ($100\text{ }\mu\text{g/mL}$) was placed on a clean silicon chip and dried naturally. The silicon chip was placed in an evaporating dish, $50\text{ }\mu\text{L}$ of prepared 2.5% glutaraldehyde solution was dropped onto the silicon wafer, and the wafer was sealed with wrap film and fixed at 4°C overnight. A series of clean-

ing and gradient dehydration operations were then performed to dry the wafer. After coating with gold, the samples were observed by ASU8010 cold field emission scanning electron microscopy as described by Yang et al. (2014).

Determination of the Functional Properties of WP

The emulsifying activity index (EAI) and the emulsion stability (ES) were measured according to the method of Pearce and Kinsella (1978). The EAI and ES were calculated using the following formulas:

$$\text{EAI} = (2 \times 2.303A_0 \times N \times 10^{-4})/(\varphi L\rho),$$

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

where A_0 and A_1 are the absorbance of a blank sample and a mixed sample of WP and soybean oil, respectively; N is the dilution factor of the emulsion; φ is the volume fraction of the oil phase; L is the cuvette diameter (cm); and ρ is the concentration of the WP solution. A volume of 2 mL of WP solution (10 mg/mL) was placed in a 10-mL glass cylinder with a stopper. The initial height of the solution was recorded as V_0 , the cylinder was manually agitated for 1 min , and then the height of the foam was immediately recorded as V_1 . The height of the foam was recorded again after 5 min , and this value was named V_2 . The foaming capacity (FC) and foam stability (FS) were calculated as follows:

$$\text{FC} = \frac{V_1 - V_0}{V_0},$$

$$\text{FS} = (V_2 - V_0)/(V_1 - V_0).$$

The WP ($m = 0.1\text{ g}$) was accurately weighed in a 10-mL centrifuge tube, and the weight of the WP and centrifuge tube was recorded as m_1 . Five milliliters of distilled water or soybean oil was added, and then the tube was mixed thoroughly in vortex mixer for 1 min , allowed to stand at room temperature for 1 h , and centrifuged at $1,986 \times g$ for 30 min at room temperature. The supernatant was discarded, and the pellet was dried at 37°C for 30 min . The weight of the pellet and centrifuge tube was recorded as m_2 . The water-holding capacity (WHC) and oil-holding capacity (OHC) were calculated as follows:

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

Statistical Analysis

Data were analyzed by one-way ANOVA using SPSS software version 22 (IBM Corp., Armonk, NY). Significant differences between means were indicated when $P < 0.05$, and Origin 8.5 (OriginLab Corporation, Northampton, MA) was used to plot and report the data.

RESULTS AND DISCUSSION

Profile of Proteins in WP from Heat-Treated Goat Milk

Goat milk is characterized by a very low heat stability, which could be related to compositional factors (Morgan et al., 2001). As Figure 2 shows, β -LG and α -LA were the 2 most abundant components, accounting for approximately 70% of the total amount of WP. Changes in these 2 proteins could affect the properties of WP. Compared with the results for CG, the relative content of β -LG increased significantly ($P < 0.05$) in PG1 and reached a maximum in PG2. A previous study demonstrated the same change trend in β -LG from goat milk subjected to different heat treatments, according to liquid chromatography-MS and ELISA analysis (Chen et al., 2019). This may be because native β -LG changes from dimers to monomers at approximately 72°C: with increased heating time and temperature, the process became more obvious, and the -SH inside was exposed. Meanwhile, κ -CN gradually dissociated from casein micelles, and then β -LG could form a complex with κ -CN via -SH/S-S interchange. Further heating would result in a decrease in the pH of the solution, the pH of fresh goat milk was about 6.57, whereas the pH of LTLT, HTST, UP, and UHT decreased to 6.52, 6.55, 6.50, and 6.46, respectively. The previous research suggested that the heat-induced interaction of β -LG with κ -casein is of minor importance at natural pH, but is promoted at more elevated pH (Morgan et al., 2001). Also, the β -LG/CN would in turn combine with the surface of the casein micelles, which would result in a decrease in the serum content of the 2 proteins. In contrast to β -LG, α -LA decreased slightly in PG1 but maintained its original content in PG2 and increased significantly ($P < 0.05$) in PG3 and UG. The heat stability of α -LA was due to the binding of Ca^{2+} inside the molecule; therefore, the denaturation temperature was raised by 40°C. A previous study also suggested that the content of α -LA was maintained at 22 to 25% with HTST and UHT treatment (Qi et al., 2015). However, IgG, SA, and Lf, which are sensitive to heat, decreased significantly after HTST and UHT treatment ($P <$

0.05) compared with the values for CG, which is similar to the results of a previous study (Shimo et al., 2015).

Denaturation Degree of WP Under Different Processing Conditions

As shown in Figure 3C, under the instantaneous (<15 s) heating conditions, significant differences were observed among the treatments at 85, 105, 125, and 135°C. High-temperature treatment could result in a large amount of WP denaturation. At the same heating time, a significant difference was observed between S3 (65°C/5 min) and E2 (85°C/5 min; $P < 0.05$). There was also a significant difference between PG1 (65°C/30 min) and E5 (85°C/30 min). The change trend in the denaturation degree of WP in PG1 and E5 was similar to that in S3 and E2. That is, with increased heating temperature, the denaturation degree of goat milk WP

Figure 2. The SDS-PAGE profile (A) and content of protein components in whey protein (WP) of heat-processed goat milk (B). For A, lane M is the marker. The unheated samples served as the control group (CG), and other samples were treated according to Figure 1 referring to the heating method of Chen et al. (2019). Lf = lactoferrin; SA = serum albumin. For panel B, values of each group not sharing the same letters (a–c) are significantly different ($P < 0.05$), and error bars represent SD.

increased significantly. A previous study reported that the levels of denaturation of β -LG were generally higher than those for α -LA during HTST processing of model infant milk formula solutions (Joyce et al., 2017). The α -LA, SA, and IgG lost their natural conformation at approximately 64 to 66°C, whereas β -LG unfolded at 72°C. Considering the low proportion of SA and IgG in WP and the relatively strong heat resistance of α -LA, little denaturation occurred at 65°C. However, camel serum albumin showed substantial denaturation with increased temperature during heat treatment (Genene et al., 2019). One study suggested also that WP from goat milk were more denatured than those from cow milk in samples heated at temperatures higher than 75°C (Montilla et al., 1995). The WP denaturation reached 72.3% under treatment at 85°C for 5 min in this study, whereas a previous study reported a value of more than 81.2% under treatment at 85°C for 2 min (Joyce et al., 2017). The highest observed denaturation degree was the complete denaturation of WP under treatment at 85°C for 30 min, and a similar result was reported by Ruffin et al. (2014). However, the results showed that a considerable amount of WP was easily denatured at high temperatures (>85°C). Similarly, more WP would be denatured after a longer time at the same temperature. The reactive groups would be exposed when the denaturation temperature of each protein is reached (Li et al., 2014b).

Changes in the Secondary Structure of WP During Processing Under Different Conditions

After heat treatment, the most obvious changes occurred from 3,600 to 3,100 cm^{-1} and 1,700 to 1,600 cm^{-1} (Figure 4, amide I band). The infrared spectrum of WP in CG had an obvious characteristic absorption peak at approximately 3,310 cm^{-1} , which indicated abundant intermolecular and intramolecular hydrogen bonding in the WP. In the PG treatments and UG, from 3,600 to 3,100 cm^{-1} , different degrees of blueshift were observed, which represented damage to the molecular conformation of WP; this damage may have been caused by the destruction of hydrogen bonds that stabilized the conformation. The amide I band (1,700–1,600 cm^{-1}) is a sensitive region for protein secondary structure changes. The CG absorption peak was at 1,647.2 cm^{-1} , and little change occurred in UG, whereas a blueshift occurred in PG2 and PG3, which was essentially consistent with the changes in the bands from 3,600 to 3,100 cm^{-1} .

Multipeak fitting of the deconvoluted spectra showed that 9 to 10 subpeaks were obtained for each sample, and the fitting results are shown in Figure 4. The re-

Figure 3. High-performance liquid chromatography of standards (A) and samples (B) and denaturation degree of whey protein (WP) from goat milk under different heat-processing conditions (C). Peaks 1, 2, and 3 in A and B represent α -LA, β -LG A, and β -LG B, respectively. The unheated samples served as the control group (CG), and other samples were treated according to Figure 1 referring to the heating method of Chen et al. (2019). Different letters (a–g) indicate a significant difference ($P < 0.05$) among the different processing treatments, and error bars represent SD.

relationships between each subpeak in the amide I band and the secondary conformational structures were as follows: 1,650 to 1,660 cm^{-1} represented α -helix structures, 1,600 to 1,640 cm^{-1} and 1,682 to 1,700 cm^{-1} represented β -sheet structures, 1,661 to 1,681 cm^{-1} represented β -turn structures, and 1,641 to 1,650 cm^{-1} represented unordered structures (Barth, 2007). The relative contents of secondary structures in WP are displayed in Figure 5. In the PG samples and UG, the total contents of α -helix and β -sheet structures were reduced

Figure 4. Secondary structure original spectra and amide I band multimodal fitting spectra of whey protein (WP) from heat-processed goat milk. The unheated samples served as the control group (CG), and other samples were treated according to Figure 1 referring to the heating method of Chen et al. (2019).

Figure 5. The secondary structure content of whey protein (WP) from heat-processed goat milk. Different letters (a–e) indicate a significant difference ($P < 0.05$) among the different processing treatments. P/UHT group = pasteurization and UHT group, and error bars represent SD. The unheated samples served as the control group (CG), and other samples were treated according to Figure 1 referring to the heating method of Chen et al. (2019).

to different degrees, and irregular structures, including β -turn and unordered structures, increased (Figure 5A). Previous research has shown that α -helices and β -sheets are regular secondary structures in WP and that α -helices are the most stable (Parks and Player, 2007). The LTLT treatment had the least effect on secondary structure, whereas UG had the greatest effect. In the 65°C group, the regular-structure content decreased gradually with prolonged time, and significant decreases occurred in S4, S5, and PG1 (Figure 5B). Similar results were found in the 85°C group. In the instantaneous treatment group, the irregular-structure content increased with increasing temperature, which was essentially consistent with the results for the denaturation rate of WP. The results indicated that the secondary structure of WP was damaged to varying degrees and the damage was worsened with high temperature and prolonged heating time.

Morphological Analysis of WP During Processing Under Different Conditions

The WP of fresh goat milk exhibited a regular globular structure with slight aggregation, as shown in Figure 6 (CG). The WP in PG1 maintained this globular

structure, and the particle size increased slightly. Some globular-structured WP changed into flake-structured WP, probably due to α -LA and SA aggregation via $-\text{SH}/\text{S-S}$ interchange interactions under LTLT treatment. More obvious changes were observed in PG3 and UG, and almost all globular structures were destroyed and changed into a flake state. This was due to the much higher temperature of UP and UHT treatment, which destroyed intramolecular hydrogen bonds and disulfide bonds. Then, rapid and considerable denaturation occurred, which involved β -LG aggregation, β -LG/ α -LA aggregation, and β -LG/ κ -CN complexation via $-\text{SH}/\text{S-S}$ interchange. It has been suggested that denatured WP films are more stretchable due to covalent cross-linking (Khem et al., 2016).

Compared with PG1, no clear changes in the globular structure of S1 were observed in S3 (65°C/5 min), whereas in the 85°C/5 min group, the WP morphology possessed a less globular structure, and the particle size was observed to increase significantly. The most serious morphological damage occurred in E5 (85°C/30 min). The WP was almost completely changed into a flake state, and significant aggregation was observed, which was consistent with the high denaturation rate of 78% in this study. Several globular WP could still

be observed in O1 (105°C/15 s). However, more serious destruction was observed in O1 and UG. Therefore, during transient heating, a greater change would occur to the surface topography of WP with higher temperatures.

Changes in the Particle Size, Turbidity, Zeta Potential, and Surface Hydrophobicity of WP During Processing Under Different Conditions

As Figure 7A shows, the particle size of PG3 increased significantly ($P < 0.05$) to a maximum of 301 nm compared with that of CG, while the particle size of UG decreased significantly ($P < 0.05$). In the 65°C-treated group, the particle size increased significantly except for in S1, and no significant change was observed among the other 5 samples. In the 85°C-treated group, the particle size increased significantly ($P < 0.05$) in PG2 and E2 and then decreased with prolonged time. In the

15-s treated group, PG2 increased significantly, reached a maximum, and then decreased. There was a common trend among the 4 treated groups: with increasing temperature and prolonged time, the particle size rose first and then fell. The temperature of 65°C is close to the denaturation temperature of α -LA and SA, and α -LA/SA aggregation resulted in an increase in the PG1 particle size (Akkerman et al., 2016). β -Lactoglobulin changed into monomers and unfolded at 85°C, which is higher than its denaturation temperature. The -SH inside β -LG became exposed and then reacted with S-S contained in α -LA and κ -CN in serum, and WP/CN complexes appeared as the particle size increased (Wijayanti et al., 2014). With increasing temperature, when the level of protein polymerization had reached a certain extent, the aggregates failed to stabilize in the serum phase and then precipitated, and the particle size decreased (Genene et al., 2019). According to the study of Giroux et al. (2018), the agglomerates of WP could

Figure 6. Scanning electron micrographs of whey protein (WP) from heat-processed goat milk ($\times 120,000$). The unheated samples served as the control group (CG), and other samples were treated according to Figure 1 referring to the heating method of Chen et al. (2019).

improve the productivity of artisanal cheese plants by significantly increasing cheese yield without negative effects on the flavor of cheese. In this study, the turbidity was related to the molecular size of the WP. The change trend was similar to that of particle size (Figure 7B). Only the zeta potential of WP treated at 85°C had a significant difference, whereas that of other groups had no significant change (Figure 7C). It turned out that less turbid WP soluble aggregates of cow milk may be used to increase shelf life by reducing protein precipitation in beverages (Ryan et al., 2013). In this study, the turbidity of LTLT and HTST samples was significantly lower than other treated samples. The zeta potential of WP treated at 85°C/15 min and 85°C/30 min increased significantly, which was essentially consistent with the change trend in particle size in the 85°C groups in this study. The larger the particle size was, the more aggregated the molecules. In addition, the intermolecular forces of WP during heating affect the stability of WP in the system (Zhang et al., 2016). In the 65°C-treated group, no significant difference was observed. A temperature of 65°C would only destroy hydrogen bonds and block van der Waals forces, which would not have a strong impact on zeta potential.

The hydrophilic residues and hydrophobic side chains of WP were in a relatively balanced state in CG. Compared with the results in CG, the hydrophobicity of WP increased after heat treatment at 65°C/30 min and 85°C/15 s but decreased significantly after heat treatment at 125°C/4 s and 135°C/4 s (Figure 7D). The LTLT and HTST treatment resulted in more unfolding of WP molecules, thus exposing more hydrophobic groups from the cores of the globular proteins, thereby leading to an increase in surface hydrophobicity (Kim et al., 2005). Hydrophobic interactions would dominate again when the peptide chains are stretched to a certain extent and then reaggregated, resulting in a decrease in surface hydrophobicity. In the 65°C-heated group, the fluorescence intensity increased differently. The WP did not denature completely, but its peptide chains were gradually stretched at 65°C. The 85°C-treated group changed significantly ($P < 0.05$), and the fluorescence intensity increased and then decreased with increasing processing time. During continuous heating, reactions due to hydrophobic forces occurred, the WP aggregated, and fewer hydrophobic residues were exposed to water. A similar trend appeared in the 15 s-treated group. This study indicated that the heat treatment of goat

Figure 7. Changes in particle size, turbidity, zeta potential, and surface hydrophobicity of whey protein (WP) subjected to different processing treatments. The unheated samples served as the control group (CG), and other samples were treated according to Figure 1 referring to the heating method of Chen et al. (2019). For the pasteurization and UHT (P/UHT) group, 1 to 5 denote CG, PG1, PG2, PG3, and UG, respectively; for the 65°C group, 1 to 7 denote CG, S1, S2, S3, S4, S5, and PG1, respectively; for the 85°C group, 1 to 7 denote CG, PG2, E1, E2, E3, E4, and E5, respectively; and for the 15 s group, 1 to 5 denote CG, S1, PG2, O1, and Q1, respectively. Different letters (a–f) indicate a significant difference ($P < 0.05$) among different processing treatments, and error bars represent SD.

milk at 65 and 85°C increased the surface hydrophobicity of WP. A similar result has previously been noted: preheating WPI increased the surface hydrophobicity and viscosity of WPI dispersions (Ruffin et al., 2014).

Changes in the Functional Properties of WP During Processing Under Different Conditions

Emulsifying activity index is related to the surface hydrophobicity of molecules, and ES is related to the flexibility of proteins (Dickinson, 1997). As Figure 8A shows, EAI was increased significantly ($P < 0.05$) with the 4 heat treatments and reached a maximum in PG1. Under strong heating conditions (65°C for 30 min), the WP was completely unfolded, and a high EAI of the WP was observed; this result can be explained by the increase in surface hydrophobicity and decrease in particle size of WP from goat milk heated at 65°C for 30 min. This result occurred because WP unfolds and exposes the hydrophobic groups from the cores of the globular proteins (Croguennec et al., 2004; Ruffin et al., 2014). At higher temperatures, hydrophobic interactions became the dominant force, and the WP aggregated again, resulting in a decrease in EAI. It was also revealed that thermal processing at temperatures ranging from 60 to 90°C for 1,000 s had negative effects on EAI due to the failure of the large protein aggregates to absorb the fat droplets (Millqvist-Fureby et al., 2001). In terms of ES, the formation of protein complexes led to poor adsorption after heat treatment. Moreover, increased unfolding of proteins resulted in an increased flexibility of protein molecules, thus affecting the behavior of the interfacial adsorbed protein layer and the steric interactions between droplets and enhancing emulsion stability (Ruffin et al., 2014). The overall trend of EAI and ES showed a weak inverse relationship; that is, a higher EAI was associated with a lower ES under the same processing conditions (Figure 8A). In addition, the high solubility of the WP complexes caused by heat treatment contributed to the good emulsifying properties of the WP. As shown in Figure 8B, the WP samples from goat milk heated at 65°C for 30 min and 85°C for 15 s had a good WHC and OHC, respectively. The trend of OHC was similar to that of surface hydrophobicity. This indicated that LTLT should be applied to ensure an excellent WHC of WP and avoid the whey precipitation of yogurt. This study showed that pasteurization could improve emulsification performance, while the UHT process was not suitable for the processing of WP as an emulsifier. It also has been proved the soluble heat-induced WP/ κ -CN complex could increase WHC and firmness of yak yogurt (Xu et al., 2015). Whey protein is one of the

most common foaming agents. Foaming of WP can be evaluated by FC and FS. Foaming capacity is related to the volume of gas introduced into a WP solution, and FS is determined by the rate of foam disappearance over time (Nicorescu et al., 2008). From Figure 8C to 8F, we can see that the same results were obtained for FC and FS as for EAI, WHC, and OHC in this study: the WP of goat milk heated at 65°C for 30 min and 85°C for 15 s had a good FC and FS compared with those of goat milk samples from all other heat treatments. Figure 8C shows that both FC and FS in PG1 and PG2 increased significantly ($P < 0.05$), which can be explained by the stretching of protein peptide chains after heat treatment and the exposure of hydrophobic groups previously buried inside the protein structure, which could increase hydrophobic interactions and enhance the formation of foam (Lee et al., 1992). It has been reported that polymers can enhance FS due to their contribution to film viscosity and elasticity (Nicorescu et al., 2008). At higher temperatures, the formation of aggregates resulted in fewer proteins available to stabilize the gas-liquid interface (Richert et al., 1974; Bals and Kulozik, 2003). Therefore, when UP and UHT hydrophobic interactions dominated again, the WP reaggregated and failed to absorb to the gas-liquid interface, in accordance with the significant decrease in FC and FS ($P < 0.05$) in this study. With HTST, UP, and UHT treatment, the existence of large protein complexes made the WP unstable in solution, and FS decreased significantly ($P < 0.05$). From this, we can deduce that pasteurization improved foaming performance, whereas UHT treatment substantially damaged foaming performance.

CONCLUSIONS

In this study, the effects of thermal processing methods commonly used in the dairy industry and prolonged treatment at different temperatures on the denaturation, microstructure, and functional properties of WP from goat milk were investigated in detail. The results showed that the denaturation degree, particle size, surface hydrophobicity, and microstructure had a strong influence on the functional properties of WP from goat milk after heat treatment. Heat treatment of goat milk at 65°C for 30 min and 85°C for 15 s increased the particle size, turbidity, zeta potential, and surface hydrophobicity of WP, and these increases ensured that the WP had a good EAI, WHC, OHC, FC, and FS. Due to its functional properties, WP is commonly used as a food additive in the food industry. In this study, LTLT and HTST could be used preferentially in industry to improve the properties of WP, which could be

Figure 8. Changes in the functional properties of whey protein (WP) from heat-processed goat milk. Different letters (a–d) indicate a significant difference ($P < 0.05$) among different processing treatments, and error bars represent SD. EAI = emulsifying activity index; ES = emulsion stability; WHC = water-holding capacity; OHC = oil-holding capacity; FC = foaming capacity; FS = foam stability. The unheated samples served as the control group (CG), and other samples were treated according to Figure 1 referring to the heating method of Chen et al. (2019).

a key advancement for the dairy industry. This study simulated the heat treatment conditions used in actual production, aiming to provide a theoretical basis for industry. Further research is needed on the application of goat milk.

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