Comparative study on quality characteristics of yogurt from goats' milk processed by various heating treatments

DI CHEN – MING CHENG – CUNFANG WANG – XIAOQING SHAO – XIAOXUE FAN –XIAONING ZHANG– YUSI QIN

Summary

This study investigated the effects of various heating processes on the quality of goats' yogurt. The degree of goats' milk protein denaturation increased significantly with increasing the heating temperature and time ($P < 0.05$). α-Lactalbumin (α-LA) and β-lactoglobulin (β-LG) showed a similar change trend in 5 samples after heat treatment, as did α-casein (α-CN) and β-casein (β-CN), but that of κ-casein (κ-CN) was different. In general, yogurt heated at 85 ± 1 °C for 15 s or at 120 ± 1 °C for 4 s showed good water holding capacity and rheological properties, and the gel network microstructure had a more compact, greater degree of crosslinking and smaller voids. Thus, milk heat-treated at 85 ± 1 °C for 15 s or at 120 ± 1 °C for 4 s had better indicator parameters than other groups.

Keywords

goats' milk; yogurt; heat treatment; rheological properties

Goats' milk is a very good food source for human nutrition, being rich in lipids, proteins and minerals. The former involve short chain fatty acids, which are bound in acylglycerols forming small fat globules and are characterized by good digestibity [1–4]. However, goats' milk tends to form weak curds in yogurt due to its lack of αs1-casein. Thus, the dairy industry encounters some challenges regarding goat milk yogurt production [5, 6].

The basis of yogurt is its gel network. The structure and type of this network are affected by temperature, pH and mineral components, which contribute to textural characteristics of yogurt [5]. Casein plays an important role in goats' milk yogurt. Casein micelles can be affected by temperature and pH substantially [7]. κ-Casein (κ-CN) on the surface of casein micelles provides the micelle with a stable layer to maintain its spatial and negative charge stability.

Gel strength and firmness are important parameters of yogurt [7, 8]. Additionally, proper heat treatment of milk can have a profound influence on the gel strength and water holding capacity of yogurt [9]. During the heating process, κ-CN on the micelle surface dissociates and then combines with the denatured whey protein to form a soluble complex, thus increasing the viscosity and firmness of the yogurt gel [10]. Compared with unheated milk, the casein micelles associated with denatured whey protein interact more with each other after heating, increasing the number and strength of bonds between proteins, thus increasing the gel strength [11]. A previous study revealed that the structural properties of yogurt can be improved by increasing total solids or using polymerized whey protein, whey protein isolate or heat-treated whey protein concentrate [12]. Clearly, heat treatment affects important physical properties of goats' milk gel, improves the acid gelation of milk, and is largely applied in acid gels manufacturing [10].

Despite some studies reported data on the influence of heat treatment of goats' milk on casein properties and physico-chemical characteristics of functional goats' milk yogurt, the related research was not comprehensive enough. In particular, only few studies were devoted to changes of all proteins influenced by various kinds of heat processing [10, 13]. The heat treatment of milk is generally carried out by pasteurization (low temperature,

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long time, LTLT; high temperature, short time, HTST), and processes yielding ultra-pasteurized (UP) milk or ultra-high temperature (UHT) milk have also become very popular recently. Accordingly, the aim of the present study was to investigate the effects of various heating processes commonly used in industrial production. Effects of prolonged heating time at the same temperature on pH, viscosity, elastic modulus, water holding capacity and microstructure of goats' milk yogurt were studied. This study provides a reference for goats' yogurt to adopt heating processes that are conducive to the formation of goats' milk gel and to provide ideas for the development of high-quality dairy products.

MATERIALS AND METHODS

Sample preparation

Goats' milk samples were collected from healthy adult goats at the Qingdao Aote Goat Breeding Farm in Shandong Province, China. They were transferred to the laboratory by a cold chain. The first group was untreated and served as the control group (CG). Six groups were heated in a dairy processing system according to the technical specification for dairy processing (NY/T 5050-2001) and served as the heated groups. The samples of heated groups were homogenized (20 000 Pa) before the heat treatment. The heating conditions are given in Tab. 1. The heat treatment was carried out in triplicate, that is each group contained 3 samples. The samples were stored at –80 °C for 1 day.

Using the 7 groups of differently treated goats' milk, yogurt samples were prepared by inoculation with 10 g·l^{-1} starter cultures (*Lactobacillus bulgaricus, Streptococcus thermophilus, Lactobacillus acidophilus* and *Lactobacillus casei*, 7 log CFU·ml^{-1}; Beijng Chuanxiu Technology, Beijing, China). The samples were incubated at 43 ± 0.5 °C in incubator SPX-70 (Zhong Yi Guo Ke Technology, Beijing, China) until the pH reached 4.4.

Tab. 1. Heat treatment conditions and designation of samples.

Sample designation	Temperature [°C]	Time
CG	0	0
PG1	65 ± 1	30 min
PG2	85 ± 1	15 s
PG2'	85 ± 1	30 s
PG3	120 ± 1	4 s
PG3'	120 ± 1	10 s
UG	135 ± 1	4 s

Denaturating electrophoretic separation of proteins

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was performed in 5 % concentration gel at 15 mA for 30 min and then in 12 % separation gel at 40 mA until the bromophenol blue dye reached the bottom of the gel as described by CHEN et al.[14].

Liquid chromatography-mass spectrometry

The liquid chromatograph-mass spectrometer (LC-MS) settings were based on the study of CHEN et al. [15]. The samples were loaded onto a reversed-phase trap column Acclaim PepMap100, nanoViper C18 (particle size 3 μm, length 20 mm, diameter 0.1 mm (Thermo Fisher Scientific, Waltham, Massachusetts, USA) connected to a C18 reversed-phase analytical column Thermo EASY (particle size 3 μm, length 100 mm, diameter 0.075 mm; Thermo Fisher Scientific). Buffer A was 0.1 % formic acid, while buffer B contained 84 % acetonitrile and 0.1% formic acid. A flow rate of 300 nl·min^{-1} was used with the following program: 0–55 % buffer B for 110 min, 55–100 % buffer B for 5 min and 100 % buffer B for 5 min. The survey scan of the mass spectrometer (MS) data was performed at 300–1 800 m/z. Taking all the proteins in each sample as a whole, the proportion of individual proteins, i. e. α-lactalbumin (α-LA), β-lactoglobulin (β-LG), α-casein (α-CN), β-casein (β-CN) and κ-casein (κ-CN), was calculated as in Eq. 1, where the percentage of α-LA ($P_{\alpha LA}$) in CG is used as an example.

$$P_{\alpha LA} = \frac{C_{\alpha LA}}{\left(C_{\alpha LA} + C_{\beta LG} + C_{\alpha CN} + C_{\beta LA} + C_{\kappa CN}\right)} \times 100 \quad (1)$$

$C_{\alpha LA}$, $C_{\beta LG}$, $C_{\alpha CN}$, $C_{\beta CN}$, $C_{\kappa CN}$ are the contents of α-LA, β-LG, α-CN, β-CN and κ-CN, respectively.

Thermal denaturation degree determination

The degree of thermal denaturation of milk proteins after treatment was determined using ultra-high performance liquid chromatography (UHPLC) according to ZHAO et al. [16] with slight modifications. Briefly, a C4 column (250 mm × 4.6 mm, 5 μm, 30 nm;, Thermo Fisher Scientific) was used in U3000 chromatograph (Thermo Fisher Scientific). The column temperature was 35 °C. Buffer A was water and 0.1% trifluoroacetic acid (TFA), buffer B contained acetonitrile and 0.1% TFA. A flow rate of 1 ml·min^{-1} was used with the following program: 30.0–40.1 % buffer B for 1 min, 40.1–40.2 % buffer B for 30 min, 40.2–40.7 % buffer B for 10 min, 40.7–45.0 %

buffer B for 2 min, 45.0–51.0 % buffer B for 2 min, 51.0–100.0 % buffer B for 2 min, and buffer B for 2 min. The standards from Sigma-Aldrich (St. Louis, Missouri, USA) used included α-LA (> 85 %), β-LG (> 90 %), α-CN (> 70 %), β-CN (> 98 %) and κ-CN (≥70 %). The denaturation degree of protein (*Dp*) was calculated by Eq. 2 and expressed in percent

$$Dp = \frac{M_1 - M_2}{M_1} \times 100 \quad (2)$$

where M_1 is the quantity of protein in the fresh milk group, and M_2 is the quantity of protein in the heated milk group.

pH and rheological characteristics determination

The pH value of the yogurt was determined using a calibrated digital pH meter FE28 (Mettler Toledo, Columbus, Ohio, USA) by direct insertion of the electrode into the samples. The measurements were carried out every hour. A rheometer MCR 302 (Anton-Paar, Graz, Austria) was used to determine the viscosity and elastic modulus (including storage modulus *G*' and loss modulus *G*") of the yogurt. The instrument operated at room temperature 25 °C at a shear rate (γ) of 0.01–100.00 s^{-1}.

Water holding capacity determination

Water holding capacity (*WHC*) of the samples was measured using the centrifugation method described by Xu et al [8]. The samples were centrifuged in Allegra X-30R centrifuge (Beckman Coulter, Fullerton, California, USA) at 5 000 ×*g* and 4 °C for 10 min.. The supernatant was discarded and the sediment was weighed. *WHC* was calculated using Eq. 3.

$$WHC = \frac{W_1 - W_2}{W_1} \times 100 \quad (3)$$

where W_1 is weight of sample and W_2 is weight of the expelled whey.

Scanning electron microscopy

The operating conditions and procedures of the field emission scanning electron microscopy (SEM) FESEM SU8010 (Hitachi, Tokyo, Japan) were the same as described by Chen et al [17]. The goats' milk protein was dissolved in 100 g·ml^{-1} phosphate buffered saline solution (PBS; 0.1 mol·l^{-1}, Na_2HPO_4-NaH_2PO_4, pH 7.0). Five microliters of the protein solution were placed on a silicon wafer and dried at room temperature 25 °C. The silicon wafers were covered with 50 μl of 2.5% glutaraldehyde and kept overnight at 4 °C. The cells were washed 3 times with PBS. Then, dehydration treatment was carried out with 10 %, 30 %, 50 %, 70 %, 90 %, 95 % and 100 % ethanol at intervals of 15 min, followed by drying and observation.

Statistical analysis

Statistical analysis was performed by one-way analysis of variance (ANOVA) using IBM SPSS Statistics 23.0 (IBM, Armonk, New York, USA). Experimental data were recorded and expressed as mean ± standard deviation by Excel 2016 (Microsoft, Redmond, Washington, USA). The average difference was considered to have a significance level of $\alpha = 0.05$.

RESULTS AND DISCUSSION

Goats' milk protein profiles

Changes in milk proteins in duplicate samples for each group were observed at a good reproducibility by SDS-PAGE (Fig. 1A), which indicated that the samples could be further analysed by LC-MS. As shown in Fig. 1A and Fig. 1B, protein bands from milk samples processed by different heat treatments varied greatly. While the difference between PG2 and PG2' was not obvious, the difference between PG3 and PG3' was obvious (Fig. 1B). This showed that when the heating time was extended, the effect of high temperature (120 ± 1 °C, duration increased from 4 s to 10 s) on the milk proteins was more obvious than that of the lower temperature (85 ± 1 °C, duration increased from 15 s to 30 s). Our previous results [17] indicated that there were significant differences in β-LG, β-CN and κ-CN between different heat treatments ($P < 0.05$), which is consistent with the results on the protein profile obtained in this study.

Results of LC-MS analysis

The changes in five proteins (α-LA, β-LG, α-CN, β-CN and κ-CN) in the control group and four heated groups (PG1, PG2, PG3 and UG) were analysed by LC-MS (Fig. 2). The base peak diagram mainly reflected the chromatographic resolution, peptide signal strength and complexity of the protein composition of the samples. It can be seen from Fig. 2A that there were many peaks eluted at different times, and the relative abundances of the proteins were high, which indicated that there were robust quantitative results from the LC-MS analysis, by which overall change trends of individual proteins could be observed after different heat treatments (Fig. 2B).

As Fig. 2B showed, after the different heat

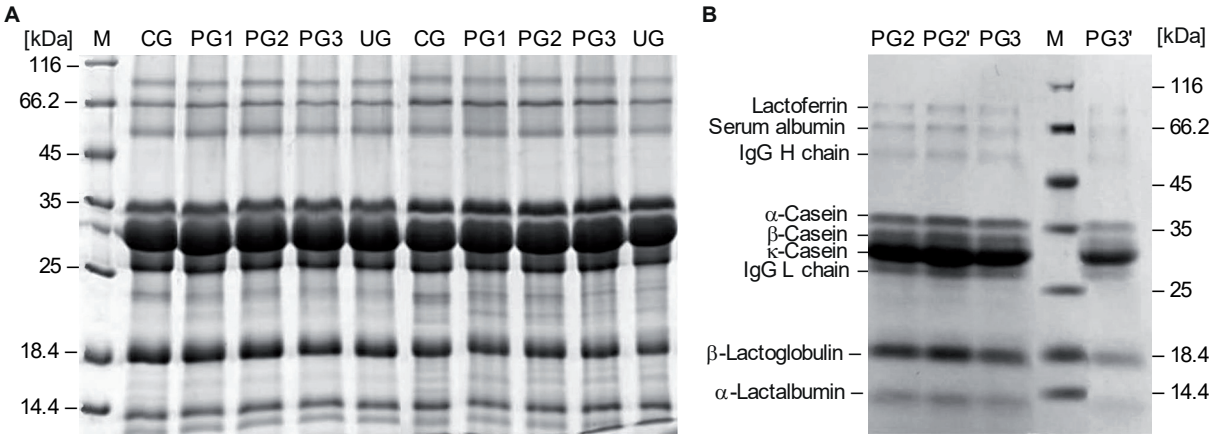

Fig. 1. SDS-PAGE of milk samples processed by different heating treatments.

A – different heat treatments, B – different heating time.
Designation of samples is given in Tab. 1. M – marker.

processing, there were the same change trends for the concentration of α-LA and β-LG in 5 groups. The higher thermal stability of α-LA was well established compared to β-LG. For casein, the changes trend in the concentration of α-CN and β-CN were completely the same, but that of κ-CN was slightly different, which may be related to the casein micelle structure. A previous study [18] found that α-CN and β-CN are located in casein micelles, but κ-CN is located on the micelle surface. This may be the reason why the thermal sensitivity of κ-CN is different from that of α-CN and β-CN.

When the goats' milk is heated at the beginning of processing, the milk protein is unfolded by the heat, the β-LG spiral structure disappears, the dimer dissociates, the hydrophobic side chain groups present in the natural structure are exposed, and α-LA is denatured to expose the sulfhydryl group [19]. Therefore, the concentration of all five monomers increases. There are hydrophobic forces and hydrogen bonds between the denatured proteins, and with the increase in the intensity of heat treatment, denatured β-LG forms aggregate by themselves, α-LA and β-LG aggregate as a complex, and the two proteins will also bind to the casein micelle surface [20]. In this study, the monomer content showed a decreasing trend basically as the temperature increased, except for UG.

Denatured whey proteins also bind to free casein monomers such as κ-CN [21, 22]. Proteins combine with each other to form aggregates, and as the temperature increases, larger aggregates are formed, resulting in a decrease in their respective concentration. The largest casein micelles were discovered after treatment at 85 °C for 5 min, but their size decreased at the treatment of 95 °C for 5 min. [13, 23]. It can be inferred that when the temperature continues to increase, the form of the aggregate changes resulting in a difference in protein content during the heating process.

Denaturation degree of goats'milk protein

Through LC-MS analysis, it was found that, during the process of heat treatment, all five milk proteins (α-LA, β-LG, α-CN, β-CN and κ-CN) changed with changes in heat treatment temperature (Fig. 2). With these five proteins as representatives, the effects of different heat treatments on goats' milk proteins could be preliminarily analysed by UHPLC, and the degree of thermal denaturation of the goats'milk proteins can be determined (Fig. 3). Unheated fresh milk was used as a baseline. This study showed that the degree of goats' milk protein denaturation increased significantly with increasing the heating temperature and duration of heating ($P < 0.05$).

Compared with CG, PG1, PG2, PG3 and UG, UHT had the greatest effect on milk protein among the different heating processes used in the dairy industry. When the temperature was lower (85 °C) and the heat treatment duration was increased, the difference in protein denaturation was not obvious. However, when the temperature was high (120 °C), the degree of protein denaturation changed significantly with the increase in the duration of the heat treatment ($P < 0.05$). This is consistent with our previous research results [15], that is, on the effects of different heat treatment intensities on thermal denaturation of the whey protein. Zhao et al. [16] noted that, with increased

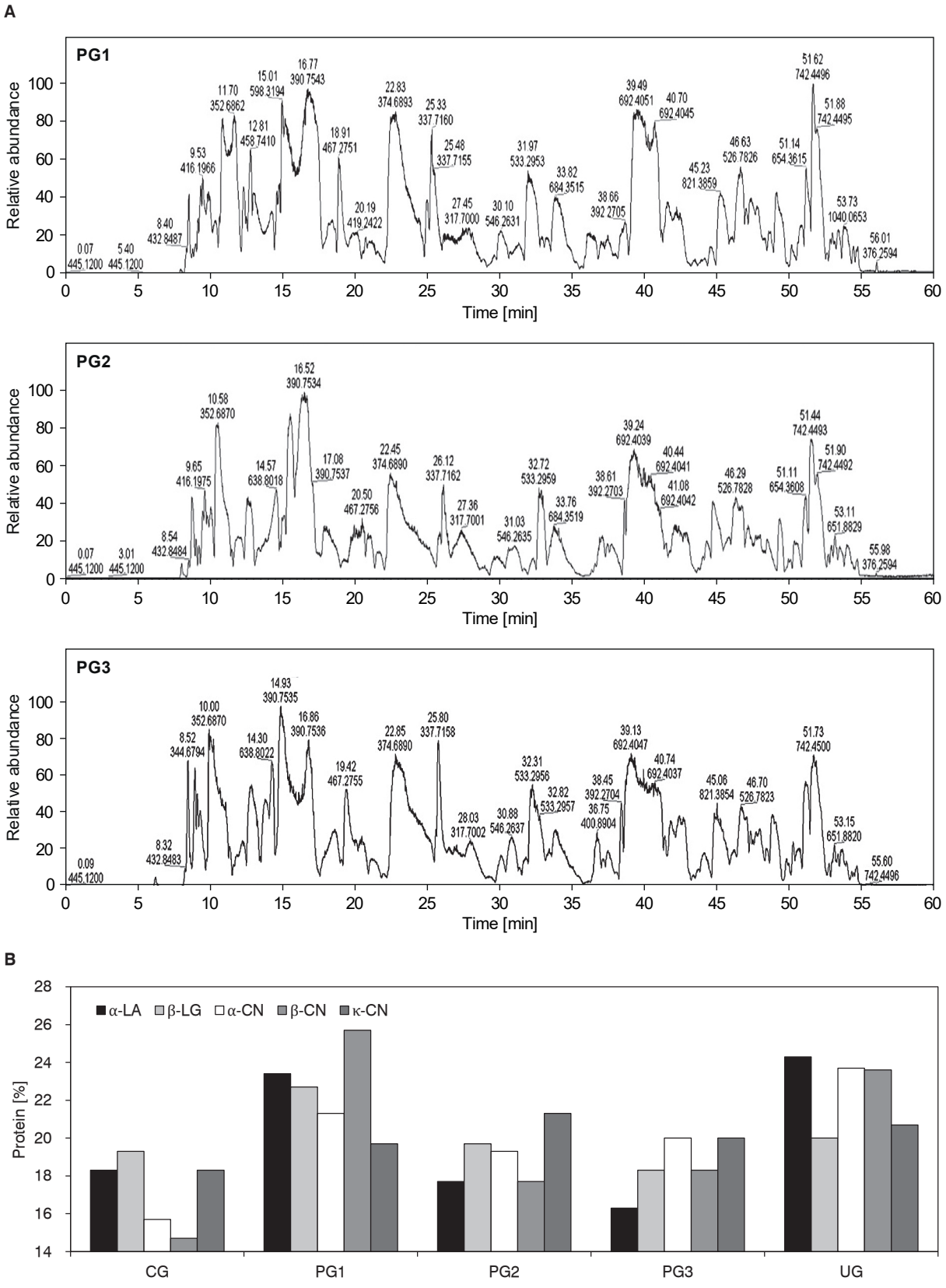

Fig. 2. Separation of goats' milk by LC-MS.

A – chromatograms, B – patterns of five major proteins.
α-LA – α-lactalbumin, β-LG – β-lactoglobulin, α-CN – α-casein, β-CN – β-casein, κ-CN – κ-casein. Designation of samples is given in Tab. 1.

heating temperature, the degree of goats' milk whey protein denaturation increased significantly, reaching 72.3 % under treatment at 85 °C for 5 min. The study of QIAN et al. [21] also showed that the degree of whey protein denaturation and that of the combination of whey protein with casein increased with the increase in the heat treatment temperature and duration.

pH and water holding capacity of yogurt

This study revealed changes in the pH values of 7 groups of goats' milk yogurt after different heat treatments during 0–7 h fermentation process (Tab. 2). The pH values of the goats' milk yogurt samples were pH 4.4 after 7 h. During the fermentation process, the pH of the 7 groups of yogurts decreased, which was faster in the early stage and slightly slower in the later stage. XU et al. [24] found that there were no significant effects of heat-induced aggregation between κ-CN and β-LG at different pH values on the final pH of yak milk acid gel. In accordance with that, consistent results without significant differences in pH values between the 7 groups were obtained in this study.

The water holding capacity can reflect the ability of yogurt to prevent water from exuding and indicate yogurt gelation and quality. This study showed that *WHC* of the PG2, PG2' and PG3 yogurts was higher than that of the CG yogurt, but *WHC* values of PG1, PG3' and UG yogurst were

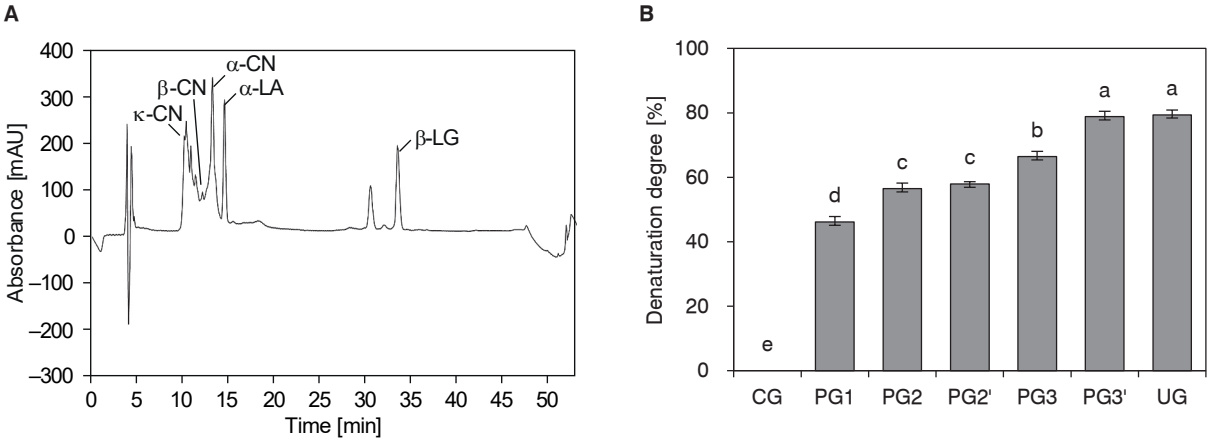

Fig. 3. Denaturation degree of goats' milk protein as determined by UHPLC.

A – chromatogram, B – denaturation degree of goats' milk protein.
Different letters indicate a significant difference ($P < 0.05$) among the different heat treatments.
Designation of samples is given in Tab. 1. α-LA – α-lactalbumin, β-LG – β-lactoglobulin, α-CN – α-casein, β-CN – β-casein, κ-CN – κ-casein.

Tab. 2. Effects of heating processes on pH and water-holding capacity of acid gels.

Fermentation time	CG	PG1	PG2	PG2'	PG3	PG3'	UG
pH							
1 h	6.14	6.18	6.12	6.12	6.10	6.06	6.07
2 h	5.79	5.84	5.82	5.80	5.71	5.73	5.71
3 h	5.39	5.46	5.28	5.25	5.15	5.23	5.18
4 h	4.78	4.86	4.84	4.80	4.79	4.83	4.78
5 h	4.69	4.75	4.67	4.65	4.60	4.66	4.62
6 h	4.55	4.64	4.53	4.51	4.50	4.53	4.51
7 h	4.43	4.51	4.41	4.41	4.42	4.46	4.43
WHC [%]							
	24.4 ± 0.3^{b}	18.8 ± 1.6^{d}	28.4 ± 2.1^{a}	28.9 ± 3.0^{a}	28.9 ± 1.7^{a}	22.0 ± 1.7^{bc}	20.4 ± 1.2^{c}

WHC – water holding capacity (values are presented as mean ± standard deviation ($n = 3$), different letters in superscript indicate a significant difference ($P < 0.05$) among the different heat treatments).
Designation of samples is given in Tab. 1.

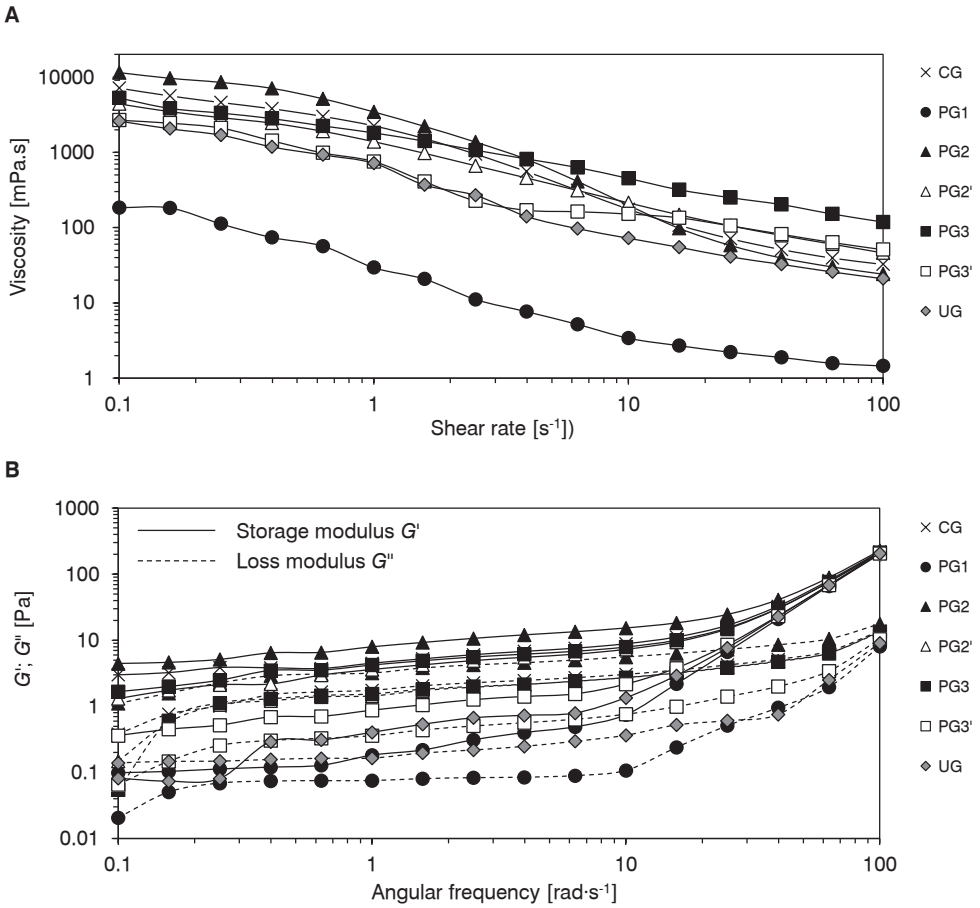

Fig. 4. Rheological characteristics of yogurt.

A – viscosity, B – elastic modulus.
Designation of samples is given in Tab. 1.

lower than that of CG ($P < 0.05$; Tab. 2). This result was related to the microgel network structure of yogurt. It is possible that goats' milk protein denaturation and the formation of heat-induced complexes create new porous structures into which water influxes and gets immobilized. The proper heat-induced whey protein/κ-casein complexes can promote the formation of homogenous porosity. The heat treatment of the milk promotes aggregation of whey protein and casein, thereby providing the formation of firmer gels, which will affect the texture and viscosity of the yogurt [5, 25]. Reports stated that the amount of soluble heat-induced whey protein/κ-casein complexes was predominant in increasing *WHC* of yogurt compared with micelle-bound protein complexes [26]. However, if the heating temperature was too high or the heating time too long, they were not strong enough to form a gel.

Viscosity and elastic modulus of yogurt

The yogurt behaved as a shear thinning non-Newtonian fluid. The rheological properties of yogurt are of great significance in product design, development, processing, quality control, storage and transportation. In this study, viscosity, storage modulus *G*' and loss modulus *G*'' of the 7 groups of samples were compared to preliminarily evaluate favourable heat treatment conditions (Fig. 4). The overall trend showed that as the shear rate increased, viscosity of the 7 groups of yogurts decreased non-linearly, indicating that the samples were non-Newtonian fluids that is to say shear thinning (Fig. 4A).

Fig. 4B shows the determined storage modulus and loss modulus values of the samples during the shear process. In general, the storage modulus *G*' and loss modulus *G*'' of all samples showed an increasing trend, and *G*' of the 7 groups of samples was higher than *G*''. Therefore, the yogurt had

a good elastic properties of gel. Fig. 4A shows that PG1 had the lowest viscosity, PG2 and PG3 had high viscosities, viscosities of PG2' and CG were similar, and that viscosity of PG3' was close to that of UG. The resulting *G*' and *G*" were consistent with viscosity (Fig. 4B). This may be due to the changes in covalent disulfide bonds and thiol-disulfide conversion during the heating process. In our previous study [15] on comparative proteomics of goats'milk during heat processing, we found, by cluster analysis of the different groups, that PG2 and PG3 clustered together first, followed by UG and CG, while PG1 was the most distant group. It is possible that goats' milk proteins were denatured to a large extent under the heat treatment of PG1, and this would not be conducive to casein gel formation. For PG2 and PG3, the interaction between whey proteins and casein was favourable, which might have promotedformation of a protein gel in the yogurt and resulted in a relatively stable viscosity.

The viscosity of PG3' fluctuated, perhaps because the goats' milk proteins denatured to a greater extent than under other treatments, giving the heated milk slightly chyme-like properties, and then causing the clots to be unevenly dispersed. Wang et al. [12] added denatured proteins, such as polymerized whey protein, whey protein isolate or heat-treated whey protein concentrate, to increase viscosity of yogurt and ultimately improve its quality. Soluble whey protein/κ-casein complexes were found to predominate in increasing the storage modulus, *WHC* and firmness of the acid gel compared with micelle-bound complexes [8, 27]. That is consistent with the results of this study.

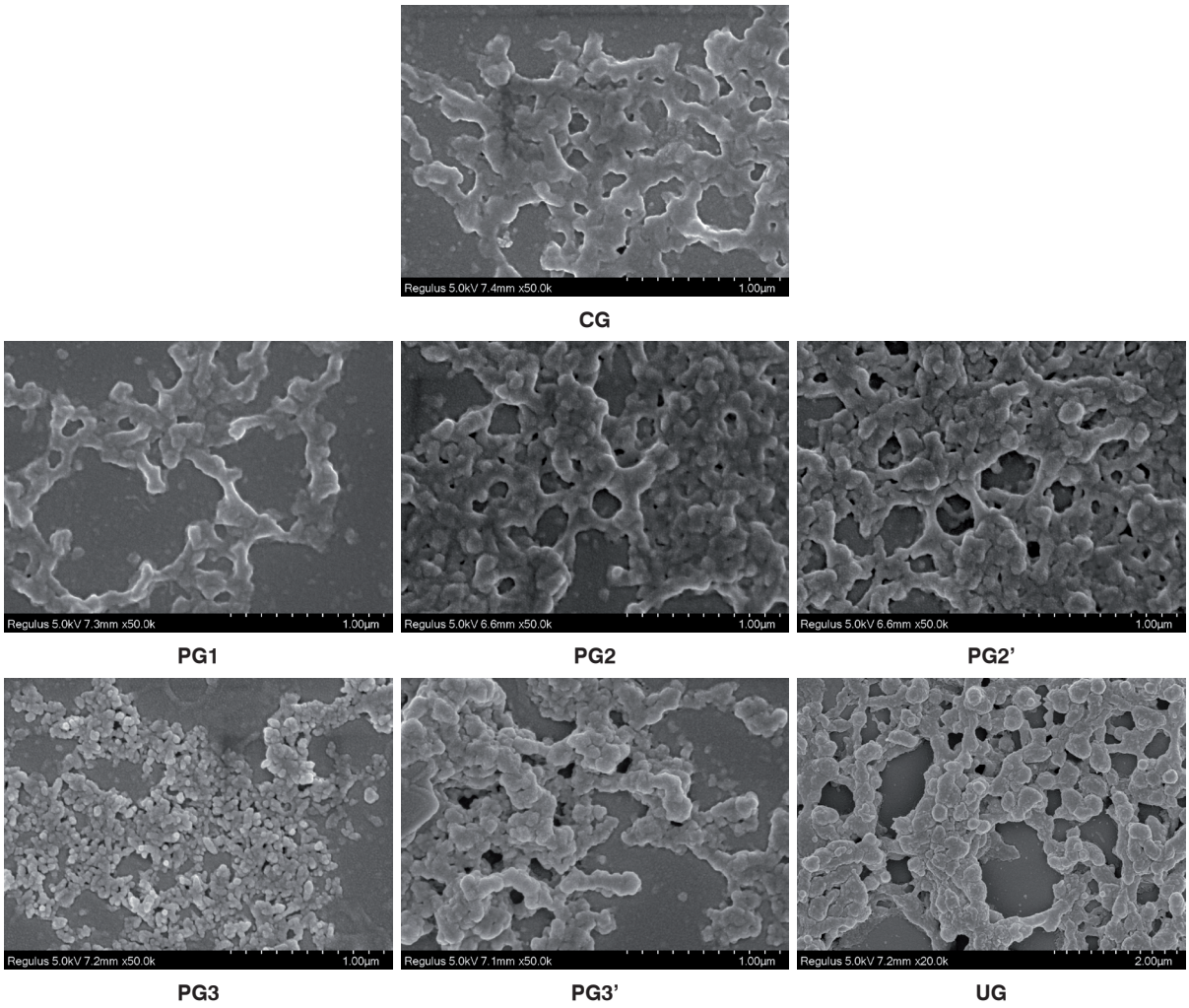

Fig. 5. Scanning electron microscopy images of yogurt.

Designation of samples is given in Tab. 1.

Microstructure of yogurt

As shown in Fig. 5, there were very significant differences between the yogurt samples from goats' milk processed by different heating treatments. The yogurt gel structures of PG1 and UG were looser and had inhomogeneous larger voids than in the control, while for the other 4 groups, the network structure was more compact. Compared with PG3 and PG3', the protein networks in PG2 and PG2' were distributed compactly and uniformly throughout the yogurt, which effectively improved the hardness, viscosity and *WHC* of these yogurts (Fig. 4, Tab. 2). Clearly, a weak gel network structure led to low *WHC*. This may be because the heat treatment breaks the disulfide bonds in β-LG, resulting in a loss of the tight spherical shape and to denaturation of the protein [15].

The casein subunits (α-CN, β-CN and κ-CN) in the micelles are held together by an internal calcium phosphate bridge [5]. When the temperature rises, κ-CN on the surface of the micelles dissociates. The original structure of the micelle changes, because of the change in electrostatic force and the increase in hydrophobic force, and then various complexes are formed [11]. Denatured proteins can form better network gels, increase the viscosity between proteins and increase the amount of water that can be combined a correct term should be used here , thus improving the texture of yogurt [8].

As in previous research [5, 7], SEM was used to analyse the microstructure of non-fat goats' milk yogurt. Heat-treated whey protein concentrate was found to interact with casein micelles to form a relatively compact network in the yogurt gel. However, when the temperature was high (120 °C) and the heating time was prolonged (from 4 s to 10 s), then protein denaturation took place and the goats' milk appeared to be in a chylous state, finally leading to the failure of proteins to form gel structures in the fermentation process.

CONCLUSIONS

This study comprehensively examined the effects of heat treatment conditions at industrial production of goats' milk yogurt. Among the six differently heated groups, the yogurt from milk processed at 120 °C for 10 s had the poor *WHC*, viscosity and elastic modulus. The degree of goats' milk protein denaturation increased significantly with intensity of heat treatment. Yogurt from milk heated at 85 °C for 15 s or 120 °C for 4 s showed good quality characteristics. So, we recommended conditions of 85 °C for 15 s and 120 °C for 4 s as the most suitable for goats' milk yogurt. Analysing the protein changes at different heating treatments allowed us to elucidate the specific effects of heat treatment on yogurt. Fully understanding the effects of goats' milk heat treatment on rheological properties of yogurt will help to provide theoretical guidance to the yogurt industry.

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