Quality attributes of the set-style skimmed yoghurt containing enzymatic cross-linked or thermal polymerized whey protein isolate

Jia Shi, Dan Li and Xin-Huai Zhao

*Key Laboratory of Dairy Science, Ministry of Education, Northeast Agricultural University, Harbin, PR China; †Synergetic Innovation Center of Food Safety and Nutrition, Northeast Agricultural University, Harbin, PR China; ‡College of Food Science, Heilongjiang Bayi Agricultural University, Daqing, PR China

ABSTRACT

Impacts of replacing of milk proteins with two modified whey protein isolates (WPIs) on quality of set-style skimmed yoghurt were assessed. WPI dispersion (35 g protein/kg) was cross-linked by transglutaminase for 5–10 min or polymerized at 90°C or 100°C for 10–30 min, mixed with skimmed milk (35 g protein/kg) at 1:2 (v/v), and fermented by a commercial starter at 42°C for 5 h. Compared with the control yoghurt generated from skimmed milk only, these yoghurt samples showed no difference in main chemical compositions, but using the cross-linked WPI somewhat delayed yoghurt fermentation. These yoghurt samples had enhanced values of hardness, adhesiveness, springiness, and cohesiveness but decreased syneresis, especially using long-time and high-temperature treatment for WPI. The cross-linked and polymerized WPIs both conferred yoghurt samples with enhanced viscosity, elastic and viscous moduli, and finer microstructure. In general, the polymerized WPI was better than the cross-linked WPI to enhance these quality attributes.

Introduction

Yoghurt is one of the fermented dairy products manufactured from full-fat, reduced-fat, or skimmed milk and is well known for its nutritional value and health-care benefits. However, yoghurt quality is very important in terms of the practical preference of the consumers, for example textural characteristics and syneresis (i.e. whey drainage). Traditional methods used to improve yoghurt texture and to decrease yoghurt syneresis include enrichment of dry matter (total solids) and/or protein contents, as well as the use of food hydrocolloids like gelatin (Lorenzen, Neve, Mautner, & Schlimme, 2002). In addition, yoghurt quality can also be improved at a molecular level via forming covalent bonds between or within milk proteins (Færgemand, Sørensen, Jørgensen, Budolfson, & Qvist, 1999). For example, horse-radish peroxidase (EC 1.11.1.7) is potential to improve yoghurt quality through inducing protein cross-linking (Wen, Kong, & Zhao, 2014; Wen, Liu, & Zhao, 2012).

Another enzyme transglutaminase (TGase, EC 2.3.2.13) has also been utilized to cross-link food proteins (Beermann & Hartung, 2012). TGase is efficiently used to modify protein properties via the formation of the so-called ε-(γ-glutamyl) lysine bonds (Motoki & Seguro, 1998), which finally results in dramatic changes in protein size, stability, and conformation. TGase has been proved as a potential ingredient in yoghurt production for quality improvement (Gauhe, Tomazi, Barreto, Ogliari, & Bordignon-Luiz, 2009). TGase treatment of milk proteins before yoghurt fermentation has other beneficial effects on yoghurt quality, such as increasing gel hardness (Færgemand & Qvist, 1997; Gauche et al., 2009), decreasing syneresis (Şanlı, Sezgin, Deveci, Şenel, & Benli, 2011), and enhancing viscosity (Özer, Kirmaci, Oztekin, Hayaloglu, & Atamer, 2007) and elastic modulus (Anema, Lauber, Lee, Henle, & Klostermeyer, 2005). Cross-linking of milk proteins by TGase prior to yoghurt fermentation can
also improve gel structure (i.e. protein network) of set-style yoghurt (Færgemand & Qvist, 1997; Farnsworth, Li, Hendricks, & Guo, 2006).

Cheese processing results in whey protein as by-product, which is now available in food industry as protein ingredient in terms of whey protein concentrate and isolate (WPC and WPI). WPC and WPI both can be used in yoghurt production in order to enrich protein content. Whey protein addition has been observed to increase strength of yoghurt gels (Krzeminski, Grosshable, & Hinrichs, 2011; Lucey, Munro, & Singh, 1999), decrease syneresis (Küçükcetin, 2008), and enhance viscosity (Damin, Alcántara, Nunes, & Oliveira, 2009). It is known that thermal treatment of whey protein induces protein denaturation and more importantly polymerization of whey protein (Fitzsimons, Mulvihill, & Morris, 2008), which brings about modified properties to whey protein. During thermal treatment of yoghurt milk, whey protein (primarily β-lactoglobulin) is denatured; after then, β-lactoglobulin interacts with κ-casein through the disulfide bridges and hydrophobic interactions (Haque & Kinsella, 1988), resulting in polymerization of milk proteins. This polymerization is very important and desired in yoghurt production; for example, it will lead to short fermentation time (Labropoulos, Collins, & Stone, 1984; Thomopoulos, Tzia, & Milkas, 1993) and decrease yoghurt syneresis (Sanli et al., 2011).

As it has been discussed above, cross-linking of milk proteins is one of the most important approaches to enhance yoghurt quality. When WPI is used in yoghurt production, it is unknown that whether prior TGase and thermal treatments of WPI might have impacts on yoghurt quality. In this study, a commercial WPI was therefore subjected into TGase-induced cross-linking or thermal polymerization and then used in the preparation of set-style skimmed yoghurt. The enzymatic cross-linked and thermal-polymerized WPIs both were intended added into skimmed milk, in order to partially replace milk proteins in the skimmed milk. Some quality attributes of the prepared yoghurt samples in terms of acidity, rheological and textural properties were assessed and compared with those of a control yoghurt generated from the skimmed milk only. The aim of this study was to assess the applicability of the two treatments on the WPI used for yoghurt production as well as potential effects of the treated WPIs on yoghurt quality.

**Materials and methods**

**Materials and chemicals**

Skimmed bovine milk powder, negative in antibiotic residues, was obtained from Fonterra Trading (Shanghai) Co. Ltd. (Shanghai, China) and used to prepare skimmed milk. WPI with protein content of 87.95% (on dry basis) and negative in antibiotic residues was purchased from Brewster Dairy (Brewster, OH, USA). TGase was produced by Jiangsu Yiming Fine Chemical Industry Co. Ltd. (Jiangsu, China) with a measured activity of 110 units (U) per gram. A direct vast set (DVS) starter (YO-MIX 499) consisting of Streptococcus thermophilus and Lactobacillus bulgaricus was purchased from Danisco GmbH (Beijing, China). The used table sugar (i.e. commercial sucrose) was a product from Dalian Minyipin Trading Co., Ltd. (Dalian, China). Other chemicals used were of analytical grade. The water used in the experiments was redistilled water.

**WPI treatments and yoghurt preparation**

WPI was reconstituted in water to achieve a protein concentration of 35 g/kg and heated to 40°C at a water bath. TGase was added to the WPI dispersion at a level of 10 U/g protein. The mixture was then kept at this temperature for 5, 7.5, and 10 min with constant agitation, respectively. After the reaction, the mixture was rapidly heated to 85°C for 5 min in order to inactivate the TGase and cooled to room temperature. The obtained products were enzymatically cross-linked WPIs and therefore assigned as WPI I, WPI II, and WPI III, respectively.

WPI was reconstituted in water as above, after then heated at 90°C or 100°C. The used treating times for the WPI dispersion were 10, 20, and 30 min with constant agitation. After then, the mixture was cooled to room temperature. The obtained products were thermal-polymerized WPIs and assigned as WPI IV, WPI V, and WPI VI (90°C), or WPI VII, WPI VIII, and WPI IX (100°C), respectively.

The preparation of set-style skimmed yoghurt followed the method of Lee and Lucey (2010) with slight modification. The skimmed milk powder was dispersed in water to obtain skimmed milk with a protein concentration of 35 g/kg, which was used to produce the control yoghurt. At the same time, the prepared WPIs I–IX were mixed with the skimmed milk at a fixed volume ratio of 1:2 and then used to produce yoghurt samples I–IX, respectively. The table sugar was added into yoghurt milk at a fixed level of 60 g/kg milk. All yoghurt milk samples were heated at 90°C for 10 min, cooled to about 42°C, and inoculated with the DVS starter at a level of 0.06 g/kg milk as recommended by the supplier. After then, the yoghurt milk samples were poured into glass containers with capacity of 100 mL under aseptic conditions and incubated at 42°C for about 5 h until an acidity of pH 4.5. Afterward, the 10 yoghurt samples control yoghurt and yoghurt samples I–IX were stored at 4°C for 24 h and selected randomly for the assays mentioned below.

**Chemical analyses**

All yoghurt samples were assayed for their protein and total solids contents, and titratable acidity using the Kjeldahl method, oven drying method. and titration method described in AOAC (1999), respectively. A pH meter (Mettler, Toledo, DELTA-320 pH, Shanghai, China) was used to monitor the pH values of the yoghurt samples.

**Texture profile and syneresis analyses of yoghurt samples**

Four textural parameters in terms of springiness, hardness, adhesiveness, and cohesiveness of the yoghurt samples were assayed as per the method (Sandoval-Castilla, Lobato-Calleros, Aguine-Mandujano, & Vernon-Carter, 2004) using the texture profile analysis test, which was carried out at a Stable Micro Systems Texturometer (Model TA-XT2), Stable Micro Systems Ltd, Surry, UK) with a 5-kg load cell. The samples (60 mm diameter × 60 mm height) were placed in the sample container after the samples were equilibrated to room temperature. A cylindrical probe with 35 mm diameter (A/BE 35) was used in this assay. Other assaying
conditions used were the same to those used in a previous study (Han, Fu, & Zhao, 2015). The values of the four textural parameters were calculated using the XT.RA Dimension Ver. 3.7 software (Stable Micro Systems Ltd, Surry, UK).

All yoghurt samples were assessed for their syneresis using a centrifugation method (Farnsworth et al., 2006). Samples about 20 g were centrifuged at 640 × g for 10 min. The supernatants were collected and weighed carefully. Syneresis (expressed as percentage) was calculated as the amount of the supernatant per initial sample weights.

Rheological evaluation

Apparent viscosity of the yoghurt samples was assayed at 25°C using a reported method of Singh and Muthukumarappan (2008). The samples were stirred gently by rotating 10 times to make themselves in homogeneous state prior the measurement. The assay was then conducted at a Bohlin Gemini II Rheometer (Malvern Instruments Limited, Worcestershire, UK) using a cone-plate geometry with diameter of 40 mm, cone angle of 4°, and gap of 0.15 mm. Elastic and viscous moduli (G’ and G”) values of the samples were also measured at the Rheometer, using frequency sweep in 0.1–10 Hz at 0.5% strain and the same cone-plate geometry.

Thixotropy of the yoghurt samples was detected at 25°C as per the method (Debon, Prudêncio, & Petrus, 2010), using the Rheometer and cone-plate geometry. The analyzed samples were loaded onto the plate and sheared at 500 1/s for 1 min. The flow curves were obtained via detecting shear stress as a function of increasing shear rates (0.1–100 1/s) within 30 min, holding the shear rate 100 1/s for 3 min, and then deceeding the shear rates from 100 to 0.1 1/s within 30 min. Hysteresis loop areas of the samples were then calculated using the Rheo Win Pro software (Malvern Instruments Limited, Worcestershire, UK).

Observation of yoghurt microstructure

Microstructure evaluation followed the reported method (Sandoval-Castilla et al., 2004) with minor modification. A portion of the yoghurt samples (4 mm × 4 mm × 3 mm) was separated from 10 mm below the yoghurt surface and fixed at 4°C in 0.1 mol/L phosphate buffer (pH 6.8) containing 25 g/kg glutaraldehyde solution for 24 h. The fixed samples were dipped in the buffer for 10 min, washed three times with the buffer for 10 min-interval, and dehydrated in a graded ethanol series (50–90%, 15 min in each). Afterwards, the treated samples were dipped in ethanol, ethanol/tert-butyl, and tert-butyl alcohol for 15-min interval, respectively, and dried for 48 h in liquid nitrogen. The dried samples were mounted onto the holders using an adhesive carbon membrane and coated with gold using a Hummer VI sputtering system (Matsushita Electric Industrial Co., Osaka, Japan). Microstructural features of the samples were observed and photographed using a HitachiS-3400N Scanning Electron Microscope (Hitachi High-Technologies Co., Tokyo, Japan) at an accelerating voltage of 5 kV.

Statistical analysis

All preparations and analyses were carried out at three times. All data reported in this study were expressed as mean values or mean values ± standard deviations. The differences between the mean values of multiple groups were assessed using one-way analysis of variance with Duncan’s multiple range tests. SPSS 13.0 software (SPSS Inc., Chicago, IL, USA) was used in data analysis.

Results and discussion

Chemical compositions of yoghurt samples

The data listed in Table 1 report the pH values and titratable acidity of these prepared yoghurt samples. In comparison with the control yoghurt (pH of 4.21 and titratable acidity of 0.82%), yoghurt samples IV–IX (containing the thermal-polymerized WPIs) showed no difference in terms of these two parameters (p > 0.05) as they had pH values of 4.21–4.23 and titratable acidity of 0.80–0.83%. The yoghurt samples I–III (containing the enzymatic cross-linked WPIs) were detected to have less acid production, as they had higher pH values (4.29–4.44) and lower titratable acidity (0.69%–0.75%) than the control yoghurt (p < 0.05). This indicates that using the thermal-polymerized WPIs to replace milk proteins in skinned milk had no adverse impact on acid production during yoghurt fermentation. However, using the enzymatic cross-linked WPIs somewhat delayed yoghurt fermentation. When TGase was used to treat yoghurt milk, development of titratable acidity was reduced down (Ozer et al., 2007). This finding proved that yoghurt samples I–III would have higher pH values and lower titratable acidity than the control yoghurt.

The control yoghurt had a protein content of about 34.2 g/kg that was very close to those of yoghurt samples I–IX (34.1–34.5 g/kg, p > 0.05). Total solids content of the yoghurt samples (4 mm × 4 mm × 3 mm) was observed and photographed using a Hitachi S-3400N Scanning Electron Microscope (Hitachi High-Technologies Co., Tokyo, Japan) at an accelerating voltage of 5 kV.

Table 1. The pH values and titratable acidities of the control yoghurt and yoghurt samples I-IX.

| Samples          | pH values | Titratable acidity (lactic acid %) |
|------------------|-----------|-----------------------------------|
| Control yoghurt  | 4.21 ± 0.03<sup>a</sup> | 0.82 ± 0.01<sup>b</sup>          |
| Yoghurt I        | 4.29 ± 0.01<sup>b</sup> | 0.75 ± 0.01<sup>b</sup>          |
| Yoghurt II       | 4.35 ± 0.01<sup>c</sup> | 0.73 ± 0.01<sup>c</sup>          |
| Yoghurt III      | 4.44 ± 0.01<sup>d</sup> | 0.69 ± 0.01<sup>d</sup>          |
| Yoghurt IV       | 4.23 ± 0.02<sup>e</sup> | 0.80 ± 0.01<sup>e</sup>          |
| Yoghurt V        | 4.22 ± 0.01<sup>e</sup> | 0.80 ± 0.01<sup>e</sup>          |
| Yoghurt VI       | 4.22 ± 0.02<sup>e</sup> | 0.82 ± 0.01<sup>e</sup>          |
| Yoghurt VII      | 4.21 ± 0.02<sup>e</sup> | 0.82 ± 0.01<sup>e</sup>          |
| Yoghurt VIII     | 4.22 ± 0.02<sup>e</sup> | 0.83 ± 0.01<sup>e</sup>          |
| Yoghurt IX       | 4.23 ± 0.01<sup>e</sup> | 0.81 ± 0.01<sup>e</sup>          |

Yoghurt samples I–III contained the WPIs cross-linked by TGase for 5, 7.5, and 10 min, respectively. Yoghurt samples IV–VI contained the WPIs polymerized at 90°C for 10, 20, and 30 min, respectively. Yoghurt samples VII–IX contained the WPIs polymerized at 100°C for 10, 20, and 30 min, respectively. Different lowercase letters after the data as the superscripts in the same column indicate that one-way ANOVA of the mean values is significantly different (p < 0.05). Muestras de yogur I–III que contienen WPI reticulados mediante TGase durante 5, 7.5 y 10 min, respectivamente. Las muestras de yogur IV–VI que contienen WPI polimerizados a 90°C durante 10, 20 y 30 min, respectivamente. Las muestras de yogur VII–IX que contienen WPI polimerizados a 100°C durante 10, 20 y 30 min, respectivamente. Las distintas letras minúscula después de los datos como los superíndices en la misma columna indican que ANOVA de un único sentido de los valores promedio es significativamente diferente (p < 0.05).
Textural characteristics and syneresis values of yoghurt samples

Four textural parameters (hardness, adhesiveness, springiness, and cohesiveness) of these yoghurt samples were measured to show their textural features. The results are listed in Table 2. Replacement of milk proteins with the enzymatic cross-linked and thermal-polymerized WPIs had clear impacts on these textural properties of yoghurt samples I–IX, as these yoghurt samples were significantly different in terms of all evaluated parameters. In comparison with the control yoghurt, enhanced hardness, adhesiveness, cohesiveness, and springiness values were obtained in yoghurt samples I–IX. It was also demonstrated that long-time treatment of WPI resulted in yoghurt samples with increased parameter values. Generally, thermal polymerization of WPI (especially at 100°C for 30 min) was more efficient than enzymatic cross-linking of WPI to enhance the textural parameters. Among the yoghurt samples containing the cross-linked WPIs, yoghurt sample III had the highest parameter values. On the other hand, yoghurt sample IX had the highest parameter values among the yoghurt samples containing the polymerized WPIs. Syneresis of the control yoghurt was about 30.0%, whilst those of yoghurt samples I–IX were 12.2–24.6% (Table 2). Long-time thermal polymerization of WPI at 100°C resulted in yoghurt samples with lower syneresis, as yoghurt sample IX had the lowest value. Among the yoghurt samples containing the enzymatic cross-linked (or thermal-polymerized) WPIs, yoghurt sample III (or IX) showed the greatest enhancements in terms of these textural parameters and the lowest syneresis. Yoghurt samples III and IX were thus selected as two typical samples and subjected for further evaluations of their rheological properties and microstructural characteristics.

The hardness of yoghurt gels can be enhanced by TGase-induced cross-linking of milk proteins (Faergemand & Qvist, 1997). Jacob, Nöbel, Jaros, and Rohm (2011) had observed that the yoghurt prepared from cross-linked milk via TGase had higher maximum hardness than the control yoghurt. Cross-linking of milk proteins using a mixture containing horseradish peroxidase, glucose oxidase, and glucose can bring about higher hardness and adhesiveness to the yoghurt (Chang, Kong, & Zhao, 2014). The results from two past studies indicated that the yoghurt gels generated from the TGase-treated milk had significantly lower syneresis than the control yoghurt gels (Lorenzen et al., 2002; Şanlı et al., 2011). Cross-linking of milk proteins with three oxidases (Hiller & Lorenzen, 2011), or with the mixture containing horseradish peroxidase, glucose oxidase, and glucose (Chang et al., 2014) before yoghurt fermentation, has been proved to result in decreased whey drainage. It has also been found that WPC after thermal treatment was able to improve water-holding capacity and reduce syneresis of yoghurt, via increasing the bridging degree between protein particles (Akalin, Unal, Dinkci, & Hayaloglu, 2012). These mentioned studies give support to the present results about textural properties and syneresis of these yoghurt samples.

Rheological properties of yoghurt samples

Three rheological properties of the control yoghurt, yoghurt samples III, and IX were evaluated. The results (Figure 1(a–c)) indicated that the three selected yoghurt samples had significant differences in terms of apparent viscosity, elastic and viscous moduli. Yoghurt sample IX (containing the thermal-polymerized WPI) exhibited the highest values in viscosity and two other moduli, followed by yoghurt sample III (containing the enzymatic cross-linked WPI), and the control yoghurt. This fact points out that partial replacement of milk proteins with the cross-linked and polymerized WPIs had significant impacts on rheological properties of the yoghurt samples. During the enzymatic cross-linking and thermal polymerization of WPI, it could be expected that some protein polymers generated. The cross-linked and polymerized WPIs both thus had higher degree of protein polymerization with larger molecular sizes than the original WPI. Consequently, replacing milk proteins with the cross-linked and polymerized WPIs brought about changes in the rheological properties of the yoghurt samples. It has been proved that the formation of covalent bonds in milk proteins via cross-linking can produce yoghurt gels with enhanced rheological properties (Guyot & Kulozik, 2011; Ozer, Grandison, Robinson, & Atamer, 2003). Pretreatment of the milk with TGase may lead to increased apparent viscosity for yoghurt samples (Farnsworth et al., 2006). Heat treatment of WPC

Table 2. The evaluated textural parameters, syneresis, and hysteresis loop areas of the control yoghurt and yoghurt samples I–IX.

| Samples       | Hardness (g) | Adhesiveness (g s) | Springiness | Cohesiveness | Syneresis (%) | Hysteresis loop areas |
|---------------|--------------|--------------------|-------------|--------------|---------------|----------------------|
| Control yoghurt | 95.6 ± 2.5  | 610.9 ± 11.5       | 0.922 ± 0.002 | 0.273 ± 0.003 | 30.0 ± 0.9   |                     |
| Yoghurt I     | 106.2 ± 2.6b | 641.1 ± 14.2       | 0.946 ± 0.003 | 0.321 ± 0.014 | 23.6 ± 1.0  | 134.1 ± 2.3b        |
| Yoghurt II    | 112.3 ± 1.3bc| 815.3 ± 10.8       | 0.950 ± 0.002 | 0.354 ± 0.010 | 21.3 ± 1.3   | 176.1 ± 4.2         |
| Yoghurt III   | 115.5 ± 0.5c | 896.7 ± 6.2        | 0.958 ± 0.002 | 0.368 ± 0.004 | 17.4 ± 1.1   | 201.6 ± 3.2         |
| Yoghurt IV    | 119.4 ± 5.9d | 1146.4 ± 12.5      | 0.927 ± 0.002 | 0.279 ± 0.003 | 24.6 ± 1.1   | 142.5 ± 3.1         |
| Yoghurt V     | 142.0 ± 7.6e | 1280.9 ± 17.4      | 0.932 ± 0.003 | 0.344 ± 0.006 | 21.5 ± 0.5    | 187.8 ± 9.4         |
| Yoghurt VI    | 164.4 ± 5.6f | 1347.1 ± 93.9      | 0.941 ± 0.002 | 0.360 ± 0.005 | 19.6 ± 1.3   | 223.2 ± 4.5         |
| Yoghurt VII   | 125.9 ± 2.6e | 1416.8 ± 10.3      | 0.950 ± 0.002 | 0.316 ± 0.011 | 17.1 ± 1.1   | 214.5 ± 3.7         |
| Yoghurt VIII  | 166.6 ± 6.5f | 1438.7 ± 11.1      | 0.954 ± 0.001 | 0.346 ± 0.012 | 15.0 ± 1.9   | 236.9 ± 1.5         |
| Yoghurt IX    | 182.4 ± 3.5f | 1486.5 ± 11.6      | 0.963 ± 0.003 | 0.415 ± 0.011 | 12.2 ± 0.8   | 267.5 ± 2.8         |

Yoghurt samples I–III contained the WPIs cross-linked by TGase for 5, 7.5, and 10 min, respectively. Yoghurt samples IV–VI contained the WPIs polymerized at 90°C for 10, 20, and 30 min, respectively. Yoghurt samples VII–IX contained the WPIs polymerized at 100°C for 10, 20, and 30 min, respectively. The different lowercase letters after the data in the same column indicate different significantly (p < 0.05).

Muestras de yogur I–III que contienen WPI reticulados mediante TGase durante 5, 7.5 y 10 min, respectivamente. Muestras de yogur IV–VI que contienen WPI polymerizados a 90°C durante 10, 20 y 30 min, respectivamente. Muestras de yogur VII–IX que contienen WPI polymerizados a 100°C durante 10, 20 y 30 min, respectivamente. Las distintas letras minúscula después de los datos en la misma columna indican que ANOVA de un único sentido de los valores promedio es significativamente diferente (p < 0.05).

CYTA – JOURNAL OF FOOD 37
brings about protein denaturation and also results in higher viscosity during protein coagulation (Unal & Akalin, 2012). TGase-induced cross-linking of whey protein is able to confer the respective dispersion with enhanced apparent viscosity, $G'$, and $G''$ values (Gauche, Vieira, Ogliari, & Bordignon-Luiz, 2008). These mentioned studies show the impacts of protein cross-linking and polymerization on rheological properties of protein gels, which are consistent with our results of this study.

Figure 1(d) describes the detected thixotropy loops of the three selected yoghurt samples, which could be used to reflect their stability. The control yoghurt and yoghurt sample IX were considered to be the least and most stable, respectively, as the respective hysteresis loop areas of the control yoghurt, yoghurt samples III, and IX were 126.5, 201.6, and 267.5 (Table 2). Hysteresis loop is assigned to be the difference between the energies required for structural breakdown and rebuilding (Damin et al., 2009). Larger hysteresis loop areas of the yoghurt samples III and IX reflected that greater energy was required for their structural breakdown. That is, partial replacement of milk proteins with the cross-linked and polymerized WPIs could enhance hysteresis loop areas (i.e. stability) of the set-style skimmed yoghurt. Some previous studies had shown a conclusion same to the present study, as cross-linking of milk proteins by horseradish peroxidase (Wen et al., 2012, 2014) and TGase (Dickinson & Yamamoto, 1996) also confers yoghurt samples with greater hysteresis loop areas than the non-cross-linked yoghurt controls.

**Microstructural characteristics of yoghurt samples**

Microstructural characteristics of the three selected yoghurt samples are depicted in Figure 2. In comparison with the control yoghurt (Figure 2(a)), yoghurt samples III and IX both possessed smaller voids and showed a denser and finer protein network structure (Figure 2(b,c)). At the same time, yoghurt sample IX (containing the polymerized WPI, Figure 2(c)) showed much finer protein network than yoghurt sample III (containing the cross-linked WPI, Figure 2(b)). This proves that partial replacement of milk proteins with the cross-linked and polymerized WPIs did not bring any adverse impact on yoghurt microstructure and the polymerized WPI was more efficient than the cross-linked WPI to generate finer yoghurt microstructure. This result also confirmed the prior evaluated syneresis results; that is, yoghurt sample IX with much finer microstructure had the lowest syneresis value.

Færgemand and Qvist (1997) reported that TGase-treated casein was helpful to form gels with more homogeneous microstructure and smaller pores. Lauber, Henle, and Klostermeyer (2000) found that TGase treatment of skimmed milk cross-linked proteins, which then induced yoghurt gels with firmer texture. Using horseradish peroxidase to cross-
link milk proteins was also proved able to improve yoghurt microstructure (Han et al., 2015; Wen et al., 2014). It was assumed in this study that the occurred WPI cross-linking and polymerization made contribution to the improved microstructure of these yoghurt samples. The cross-linked and polymerized WPIs both contained much protein polymers than the original WPI. Yoghurt samples III and IX were reasonable to have finer microstructure than the control yoghurt.

**Conclusion**

TGase cross-linked WPI and especially thermal-polymerized WPI could be used to replace milk proteins in skimmed milk for the production of set-style yoghurt. Using the cross-linked and polymerized WPIs had no effective impact on main chemical compositions of the yoghurt samples; however, the cross-linked WPI might somewhat delay yoghurt fermentation. The yoghurt samples got enhanced textural parameters but decreased syneresis, especially when long-time and high-temperature treatment was used for WPI. Using the cross-linked and polymerized WPIs both could bring enhanced viscosity, elastic and viscous moduli values as well as finer microstructure for the yoghurt samples. Finally, the polymerized WPI showed better effects than the cross-linked WPI to enhance these yoghurt attributes. Thermal polymerization is thus a more appropriate treatment for the WPI used in yoghurt production.

**Acknowledgments**

This study was supported by the Open Research Fund for Key Laboratory of Dairy Science, Ministry of Education of China (Project No. 2012KLDSQF-04), and the National High Technology Research and Development Program (‘863’ Program) of China (Project No. 2013AA102205). The authors also thank the anonymous reviewers and editors for their valuable advices.

**Disclosure statement**

No potential conflict of interest was reported by the authors.

**Funding**

This study was supported by the Open Research Fund for Key Laboratory of Dairy Science, Ministry of Education of China [Project No. 2012KLDSQF-04] and the National High Technology Research and Development Program (‘863’ Program) of China [Project No. 2013AA102205].

**ORCID**

Xin-Huai Zhao http://orcid.org/0000-0001-9682-5426

**References**

Akalin, A.S., Unal, G., Dinkci, N., & Hayaloglu, A.A. (2012). Microstructure, texture and sensory characteristics of probiotic yogurts fortified with sodium calcium caseinate or whey protein concentrate. *Journal of Dairy Science, 95*, 3617–3628. doi:10.3168/jds.2011-5297
Anema, S.G., Lauber, S., Lee, S.K., Henle, T., & Klostermeyer, H. (2005). Rheological properties of acid gels prepared from pressure- and transglutaminase-treated skim milk. Food Hydrocolloids, 19, 879–887. doi:10.1016/j.foodhyd.2004.12.001

AOAC. (1999). Official methods of analysis of AOAC international (16th ed.). Gaithersburg, MD: AOAC International.

Beermann, C., & Hartung, J. (2012). Current enzymatic milk fermentation procedures. European Food Research and Technology, 235, 1–12. doi:10.1007/s00217-012-1733-8

Chang, C.-H., Kong, B.-H., & Zhao, X.-H. (2014). Quality attributes of the set-style yoghurt from whole bovine milk as affected by an enzymatic oxidative cross-linking. CyTA-Journal of Food, 12, 249–255. doi:10.1080/19476337.2013.837963

Damin, M.R., Alcântara, M.R., Nunes, A.P., & Oliveira, M.N. (2011). Transglutaminase: Effect on rheological properties, microstructure and permeability of set style acid skim milk gel. Food Hydrocolloids, 11, 287–292. doi:10.1016/j.foodrc.2010.02.008

Dickinson, E., & Yamamoto, Y. (1996). Rheology of milk protein gels and protein-stabilized emulsion gels cross-linked with transglutaminase. Journal of Agricultural and Food Chemistry, 44, 1371–1377. doi:10.1021/jf950705y

Debon, J., Prudêncio, E.S., & Petrus, J.C.C. (2010). Rheological and physico-chemical characterization of prebiotic micro-filtered fermented milk. Journal of Food Engineering, 99, 128–135. doi:10.1016/j.jfoodeng.2010.02.008

Færgemand, M., & Qvist, K.B. (1998). Transglutaminase: Effect on rheological properties, microstructure and permeability of set style acid skim milk gel. Food Hydrocolloids, 11, 287–292. doi:10.1016/0958-6946(98)00058-6

Færgemand, M., Sørensen, M.R., Jørgensen, U., Budolfsen, G., & Qvist, K.B. (2005). Physical properties of yoghurt manufactured with milk whey and transglutaminase. LWT-Food Science and Technology, 38, 287–292. doi:10.1016/j.lwt.2003.09.019

Dickinson, E., & Yamamoto, Y. (1996). Rheology of milk protein gels and protein-stabilized emulsion gels cross-linked with transglutaminase. Journal of Agricultural and Food Chemistry, 44, 1371–1377. doi:10.1021/jf950705y

Debon, J., Prudêncio, E.S., & Petrus, J.C.C. (2010). Rheological and physico-chemical characterization of prebiotic micro-filtered fermented milk. Journal of Food Engineering, 99, 128–135. doi:10.1016/j.jfoodeng.2010.02.008

Motoki, M., & Seguro, K. (1998). Transglutaminase and its use for food processing. Trends in Food Science and Technology, 9, 204–210. doi:10.1016/S0927-0573(98)01331-6

Ozer, B., Grandison, A.S., Robinson, R., & Atamer, M. (2003). Effects of lactoperoxidase and hydrogen peroxide on the rheological properties of yoghurt. Journal of Dairy Research, 70, 227–232. doi:10.1017/S0022029903006149

Ozer, B., Kircim, H.A., Oztekin, S., Hayaloglu, A., & Atamer, M. (2007). Incorporation of microbial transglutaminase into non-fat yogurt production. International Dairy Journal, 17, 199–207. doi:10.1016/j.idairyj.2006.02.007

Sandoval-Castilla, O., Lobato-Calleros, C., Aguirre-Mandujano, E., & Milkas, D. (2004). Microstructure and texture of yoghurt as influenced by fat replacers. International Dairy Journal, 14, 151–159. doi:10.1016/j.idairyj.2003.10.004

Unal, G., & Akalin, A.S. (2011). Effect of using transglutaminase on physical, chemical and sensory properties of set-type yoghurt. Food Hydrocolloids, 25, 1477–1481. doi:10.1016/j.foodhyd.2010.09.028

Singh, G., & Muthukumarappan, K. (2008). Influence of calcium fortification on sensory, physical and rheological characteristics of fruit yogurt. LWT–Food Science and Technology, 41, 1145–1152. doi:10.1016/j.lwt.2007.08.007

Thomopoulos, C., Tzia, C., & Millas, D. (1993). Influence of processing of solids-fortified milk on coagulation time and quality properties of yoghurt. Milchwissenschaft-Milk Science International, 48, 426–430.

Unal, G., & Akalin, A.S. (2012). Influence of fortification with sodium-calcium caseinate and whey protein concentrate on microbiological, textural and sensory properties of set-type yoghurt. International Journal of Dairy Technology, 66, 264–272.

Wen, Y., Kong, B.-H., & Zhao, X.-H. (2014). Quality indices of the set-yoghurt prepared from bovine milk treated with horseradish peroxidase. Journal of Food Science and Technology, 51, 1525–1532. doi:10.1007/s13197-012-0680-5

Wen, Y., Liu, N., & Zhao, X.-H. (2012). Chemical composition and rheological properties of set yoghurt prepared from skimmed milk treated with horseradish peroxidase. Food Technology and Biotechnology, 50, 473–478.