Quality attributes of set-style skimmed yoghurt affected by the addition of a cross-linked bovine gelatin

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Atributos cualitativos del preparado de yogur desnatado afectado por la adición de gelatina bovina reticulada

Se reticuló gelatina bovina utilizando peroxidasa de rábano picante, glucosa oxidasa y glucosa, después se utilizó en la elaboración de preparado de yogur desnatado. Las dos gelatinas bovina y reticulada se añadieron a la leche para yogur a 2 g/kg, mientras que la leche para yogur compuesta de azúcar común de 60 g/kg se fermentó mediante un iniciador comercial directo a 42°C durante 5 h. A las muestras de yogur que se almacenaron a 4°C durante 1 y 7 d se les examinó sus composiciones e índices de calidad. En comparación con el que contenía gelatina bovina, el yogur que contenía gelatina reticulada obtuvo unos índices químicos similares en términos proteínicos, reducción de azúcares y contenido total de sólidos, aunque exhibió una menor sinéresis, una mayor hysteresis loop area, higher apparent viscosity, elastic and viscous moduli, and improved microstructure. Cross-linked gelatin could delay yoghurt post-acidification slightly. Cross-linked gelatin was thus proved to be a potential stabilizer better than bovine gelatin to improve the quality of set-style skimmed yoghurt.

ABSTRACT

Bovine gelatin was cross-linked using horseradish peroxidase, glucose oxidase, and glucose, and then used in the preparation of set-style skimmed yoghurt. Both bovine and cross-linked gelatins were added into yoghurt milk at 2 g/kg, whilst the yoghurt milk composed of table sugar of 60 g/kg was fermented by a commercial direct vat set starter at 42°C for 5 h. Yoghurt samples stored at 4°C for 1 and 7 d were assessed for their compositions and quality indices. In comparison with that contained bovine gelatin, the yoghurt contained cross-linked gelatin had similar chemical indices in terms of protein, reducing sugars, and total solids contents, but exhibited lower syneresis, larger hysteresis loop area, higher apparent viscosity, elastic and viscous moduli, and improved microstructure. Cross-linked gelatin could delay yoghurt post-acidification slightly. Cross-linked gelatin was thus proved to be a potential stabilizer better than bovine gelatin to improve the quality of set-style skimmed yoghurt.

Introduction

Yoghurt is one of the most important fermented dairy products due to its good nutritive value and potential health-care function. Textural feature of yoghurt is important in the view of processing development and quality control (Benezech & Maingonnat, 1994), which may ensure desired product acceptability to the consumers. Yoghurt texture is associated with yoghurt’s chemical compositions, such as the total solids and used additives (Shaker, Jumah, & Abu Jdayil, 2000). Some technological approaches have been used to improve yoghurt quality and especially to decrease the so-called syneresis (i.e. whey drainage); for example, by modifying the properties of milk proteins and/or adding food stabilizers such as gelatin, pectin, and various starch products. Cross-linking of milk proteins is one of the most important approaches to modify yoghurt quality. Treatment of yoghurt milk by transglutaminase (TGase, EC 2.3.2.13) can result in lower syneresis (Şanlı, Sezgin, Deveci, Şenel, & Benli, 2011) and better gelling properties (Gauche, Tomazi, Barreto, Ogliari, & Bordignon-Luiz, 2009). In the presence of glucose, horseradish peroxidase (HRP, EC 1.11.1.7) and glucose oxidase (EC 1.1.3.4) can induce an enzymatic cross-linking of milk proteins (Chang & Zhao, 2012). Using this enzymatic approach for whole and skimmed milk can confer the yoghurt samples with lower syneresis and finer texture (Chang, Kong, & Zhao, 2014; Han, Fu, & Zhao, 2015). Yoghurt quality can also be enhanced by addition of food stabilizers in yoghurt milk. Addition of food stabilizers like gelatin or other hydrocolloids that function as gelling agents or thickener will provide good stability and desirable textures for yoghurt (Routray & Mishra, 2011). Food stabilizers have two basic functions in yoghurt in terms of water binding and texture improvement (Kumar & Mishra, 2004), and thus enable improved consistency (increased viscosity) and reduced syneresis (Lucy, 2002). Addition of food stabilizers also has extra beneficial impacts on yoghurt, such as better appearance and mouthfeel (Fiszman & Salvador, 1999; Tasneem, Siddique, Ahmad, & Farooq, 2014). In practical,
yoghurt quality therefore can be efficiently improved by the addition of food stabilizers (Celik & Bakirci, 2003).

Gelatin as one of the widely used protein ingredients is derived from the fibrous protein collagen via partial denaturation and hydrolysis of native collagen extracted from skins, bones, and connective tissues of animals like bovine and porcine (Yilmaz et al., 2013). Gelatin consists of a heterogeneous peptide mixture of various molecular weights, and is a good food hydrocolloid and stabilizer used in food industry (Schrieber & Gareis, 2007). Physiochemical properties of gelatin can be modified by chemical and enzymatic cross-linking, for example, using glutaraldehyde (Matsuda, lwata, Se, & Ikada, 1999) and TGase (Weng & Zheng, 2015).

A recent study reports that if HRP, glucose oxidase, and glucose are used to induce cross-linking of bovine gelatin, the resultant cross-linked gelatin has better colloidal stabilization and gelation (Han & Zhao, 2016). However, whether this cross-linked gelatin is capable of improving yoghurt quality is still not assessed.

In this study, bovine gelatin and respective cross-linked gelatin both were used in the preparation of set-style skimmed yoghurt. The obtained yoghurt samples were assessed for their chemical compositions (including acidity, protein, total solids, and reducing sugars), textural and rheological properties, and microstructural features. The objective of this study was to show if cross-linked gelatin was also a good additive for yoghurt production.

**Materials and methods**

**Materials and chemicals**

Bovine gelatin (type B) was bought from Shandong Yimin Biological Technology Co. Ltd. (Binzhou, Shandong Province, China), and determined with a protein concentration of 957.8 g/kg (on dry basis). Skimmed bovine milk powder used in yoghurt preparation was bought from Fonterra Trading (Shanghai) Co. Ltd. (Shanghai, China), and determined negative in antibiotic residues. Glucose oxidase (type X-S, with an activity of 130 kU/g) from Aspergillus niger was obtained from Sigma-Aldrich Co. (St. Louis, MO, USA), whilst HRP (with an activity of 20 kU/g) was bought from Shanghai Guoyuan Biotech, Inc. (Shanghai, China). A direct vat set (DVS) starter (YO-MIX 499) consisting of Streptococcus thermophilus and Lactobacillus bulgaricus was a commercial product of Danisco GmbH. (Beijing, China). The table sugar was provided by Dalian Minyipin Trading Co., Ltd. (Dalian, China). Other chemicals used were of analytical grade. Distilled water was used throughout this study.

**Preparation of cross-linked gelatin and yoghurt samples**

Cross-linked gelatin was prepared using the bovine gelatin and conditions as previously described (Han & Zhao, 2016). In brief, bovine gelatin was dispersed in water at a protein concentration of 55 g/kg and then adjusted to pH value of 7.0 using 0.2 mol/L NaOH solution. After that, 100 U HRP, 1 U glucose oxidase and 0.025 mmol glucose for 1 g protein were added to the gelatin dispersion. The gelatin dispersion was kept at 37°C for 3 h with constant agitation to carry out gelatin cross-linking, and rapidly heated at 85°C for 10 min to inactivate the enzymes. The gelatin dispersion was then subjected into freeze-drying to obtain cross-linked gelatin.

Set-style skimmed yoghurt samples were prepared from skimmed milk as per the method (Lee & Lucey, 2010) with minor modification. The skimmed milk powder was dissolved in water to obtain skimmed milk with a protein concentration of 30 g/kg, which was adjusted into a pH value of 6.8 using 0.2 mol/L NaOH solution. Bovine gelatin or cross-linked gelatin was added to the skimmed milk at a level of 2 g/kg, whilst the table sugar was added at a level of 60 g/kg. All yoghurt milk samples were heated at 90°C for 10 min, rapidly cooled to about 42°C, and mixed with the DVS starter at 0.06 g/kg milk as recommended by the producer. The yoghurt milk samples were then poured into glass containers of 100 mL under aseptic conditions, and fermented at 42°C until their pH values fell to near 4.5. The yoghurt contained bovine gelatin was assigned as Yoghurt I, whilst that contained cross-linked gelatin was assigned as Yoghurt II. All yoghurt samples were stored at 4°C for 1 and 7 d, respectively, and then selected randomly for the assays mentioned below.

**Chemical analyses**

The yoghurt samples were assayed for their protein, total solids contents, titratable acidities using the respective Kjeldahl, oven drying and titration methods (AOAC, 1999). Total reducing sugar contents were assayed as the recommended methods (James, 1995). A pH meter (Mettler, Toledo, DELTA-320 pH, Shanghai, China) was used to detect the pH values of these samples.

**Assays of yoghurt texture and syneresis**

Textural features of the yoghurt samples were measured using a reference method (Sandoval-Castilla, Lobato-Calleros, Aguirre-Mandujano, & Vernon-Carter, 2004). The measurement was conducted at a Stable Micro Systems Texturometer (Model TA-XT2i, Stable Micro Systems Ltd, Surry, UK) using a 5 kg load cell. The yoghurt samples (60 mm diameter × 60 mm height) were placed in the container after the samples had been equilibrated to ambient temperature. A 35 mm diameter solid rod (A/BE 35) was plugged into the container holding the yoghurt samples. Other parameters using in this measurement were the same to those previously described (Shi, Li, & Zhao, 2016). After that, the values of four textural attributes in terms of hardness, adhesiveness, springiness, and cohesiveness were obtained by using the XT.RA Dimension Ver. 3.7 software (Stable Micro Systems Ltd, Surry, UK).

Syneresis of the yoghurt samples was measured by a centrifugation method as per the study (Han et al., 2015). Yoghurt samples of 20 g were held in centrifuging tubes of 50 mL, and centrifuged at 640 × g for 10 min. The supernatants were collected and weighed. Syneresis was calculated as the supernatant amount per initial sample weight, and shown as percentage.

**Rheological analyses**

The measurement of apparent viscosity was performed at 25°C as the previously described (Singh & Muthukumarappan, 2008). The yoghurt samples were
equilibrated to ambient temperature and then stirred by rotating 10 times to ensure them in uniform state before the measurement. The measurement was conducted on a Bohlin Gemini II Rheometer (Malvern Instruments Limited, Worcestershire, UK) equipped with a cone-plate geometry (diameter 40 mm, cone angle 4°, gap 0.15 mm). Viscoelastic behaviours of the yoghurt samples were also assessed in the Rheometer using frequency sweeps of 0.1 – 10 Hz and 0.5% strain, to obtain the elastic and viscous moduli (G’ and G”).

Thixotropy of the yoghurt samples was measured as per the method (Debon, Prudêncio, & Petrus, 2010) at the Rheometer. In brief, the yoghurt samples were loaded on the inset plate. After that, the samples were sheared at 500 1/s for 1 min. Flow curves of the yoghurt samples were obtained by increasing shear rates from 0.1 to 100 1/s within 30 min, holding the shear rate at 100 1/s for 3 min, and then decreasing shear rates from 100 to 0.1 1/s within 30 min. Hysteresis loop areas of the yoghurt samples were calculated using the Rheo Win Pro software (Malvern Instruments Limited, Worcestershire, UK).

### Microstructure observation

Scanning electron microscopy was used to observe yoghurt microstructure as the previously described (Sandoval-Castilla et al., 2004) with slight modification. A portion of the yoghurt samples (4 mm × 4 mm × 3 mm) was obtained from about 10 mm below the sample surface, and then fixed by glutaraldehyde solution of 25 g/kg for 24 h at 4°C. The samples were dipped in 0.1 mol/L phosphate buffer (pH 6.8) for 10 min, washed three times with the buffer for 10 min interval. The samples dehydrated in a graded ethanol series (50–90%, 15 min in each), dipped in ethanol, ethanol/tert-butyl, and tert-butyl alcohol for 15 min interval, and then dried in liquid nitrogen for 48 h. After being mounted onto the holders by an adhesive carbon membrane and coated with gold in a Hummer VI sputtering system (Matsushita Electric Industrial Co., Osaka, Japan), microstructural features of the yoghurt samples were observed and photographed using a HitachiS-3400 N Scanning Electron Microscope (Hitachi High-Technologies Co., Tokyo, Japan) at an accelerating voltage of 5 kV.

### Statistical analysis

All experiments and analyses were carried out three times. The data were reported as means or means ± standard deviations. The differences between the mean values of multiple groups were assessed by the one-way analysis of variance (ANOVA) with the Duncan test, using the SPSS 13.0 software (SPSS Inc., Chicago, IL, USA).

### Results and discussion

#### Chemical compositions of the two yoghurt samples

Main chemical compositions of Yoghurt I and Yoghurt II after storage time of 1 d were assayed. The results revealed that they had similar protein, total solids, and reducing sugar contents (p > 0.05), in respective levels about 32.4–32.7, 88.1–88.3, and 132.9–133.0 g/kg. Yoghurt I and Yoghurt II also had similar acidity after a short-time storage (i.e. 1 d), as their titratable acidities and pH values showed insignificant difference (p > 0.05) (Table 1). However, the two yoghurt samples after a storage time of 7 d had increased titratable acidities and decreased pH values (Table 1), especially in the case of Yoghurt I. After storage time of 7 d, Yoghurt I had a higher acidity than Yoghurt II (p < 0.05) (Table 1). This result indicated that cross-linked gelatin was stronger than bovine gelatin to delay the well-known post-acidification during yoghurt storage. Cross-linked gelatin contains more polymers with greater molecular weights, and is more able than bovine gelatin to increase viscosity of the respect dispersion (Han & Zhao, 2016). This property might make a contribution to the delayed post-acidification, as higher viscosity has been proved to have adverse impact on fermentation yet (Renan et al., 2009).

#### Textural features of the two yoghurt samples

The measured values of the textural indices of Yoghurt I and Yoghurt II are listed in Table 2. In general, the results show that they had no difference in the four indices (p > 0.05), and storage time also had unclear impact on these indices (p > 0.05). This indicated that using bovine gelatin...
gelatin or cross-linked gelatin in yoghurt production as food stabilizers had little influence on yoghurt texture. However, addition of cross-linked gelatin showed a beneficial impact on yoghurt syneresis, as Yoghurt II was detected to have much lower syneresis than Yoghurt I (28.0% versus 31.5% at the first day, or 7.0% versus 10.7% at the seventh day). Addition of cross-linked gelatin into yoghurt milk was thus proved able to reduce yoghurt syneresis effectively.

Gelatin can improve yoghurt texture (Ares et al., 2007), resulting in improved gel network, decreased syneresis, increased water holding capacity along with increased viscosity, and firmness (Andic, Boran, & Tuncturk, 2013). Using stabilizers in yoghurt production can increase yoghurt firmness (Kucukcetin, Demir, Comak, Asci, & Zengin, 2011), and decrease syneresis significantly (Kiros, Seifu, Bultosa, & Solomon, 2016). In this study, Yoghurt I and Yoghurt II both were added with the respective stabilizers at the same level; and therefore, the differences in these measured indices reflected the impacts of bovine gelatin and cross-linked gelatin on yoghurt quality. Cross-linked gelatin shows better gelling properties than bovine gelatin, as it contains polypeptides with higher molecular weights (Han & Zhao, 2016). Yoghurt II was thus able to confer a uniform gel structure on yoghurt, which consequentially resulted in lower syneresis. However, it is unknown why Yoghurt II totally had textural indices similar to Yoghurt I, although adhesiveness of Yoghurt II stored for 7 d showed increased but statistically insignificant value.

Rheological properties of the two yoghurt samples

Rheological properties of Yoghurt I and Yoghurt II were also verified (Figure 1). The results showed that using cross-linked gelatin led to Yoghurt II higher apparent viscosity, elastic and viscous moduli than Yoghurt I (Figure 1(a–c)). A storage time of 7 d resulted in both Yoghurt I and Yoghurt II with increases in these three indices; however, Yoghurt II always exhibited higher index values than Yoghurt I. At the same time, Yoghurt II was detected to have larger hysteresis loop area than Yoghurt I (Figure 1(d)). After being stored for 1 d, Yoghurt I and Yoghurt II showed respective hysteresis loop areas of (138 ± 6) and (148 ± 3). After a storage time of 7 d, the respective values increased into (154 ± 6) and (173 ± 6). These results indicated cross-linked gelatin once used in yoghurt production was more potential than bovine gelatin to increase both rheological and thixotropic properties.

Gelatin can develop a three-dimensional network in yoghurt, which therefore shows higher apparent viscosity (Ares et al., 2007). Dityrosine formation in cross-linked gelatin results in more covalent bonds within and between the gelatin molecules (Han & Zhao, 2016). These covalent bonds were found to be more stable than those formed by bovine gelatin, leading to an increase in both rheological and thixotropic properties.

**Figure 1.** Apparent viscosity (a), elastic modulus ($G'$) (b), viscous modulus ($G''$) (c), and thixotropy loop areas (d) of Yoghurts I and II stored for 1 and 7 d, respectively. Yoghurt I and Yoghurt II contained bovine gelatin and cross-linked gelatin of 2 g/kg, respectively.

**Figura 1.** Viscosidad aparente (a), modulo elástico ($G'$) (b), modulo viscoso ($G''$) (c) y áreas de ciclos tixotrópicos (d) de los Yogures I y II almacenados durante 1 y 7 días, respectivamente. El Yogur I y el Yogur II que contenian gelatina bovina y gelatina reticulada de 2 g/kg, respectivamente.
bonds thus provide stronger interaction for cross-linked gelatin at molecular level (Wang, Liu, Ye, Wang, & Li, 2015). Larger hysteresis loop area reflects higher stabilization of yoghurt (Shi et al., 2016). The added cross-linked gelatin composed of more polypeptides of greater molecular weights, Yoghurt II was reasonable to have larger values than Yoghurt I in apparent viscosity, elastic and viscous moduli, and hysteresis loop area. Yoghurt storage increases hydration of these macromolecules and add stabilizers; as a consequence, yoghurt has improved viscosity and consistency (Tamime & Robinson, 2000). After being stored for 7 d, both Yoghurt I and Yoghurt II were not surprised to have increases in the values of apparent viscosity, elastic and viscous moduli, and hysteresis loop area.

Microstructural characteristics of the two yoghurt samples

Microstructural characteristics of Yoghurt I and Yoghurt II are described in Figure 2. After being stored for 1 d, Yoghurt II showed a uniform structure than Yoghurt I (Figure 2(b) versus Figure 2(a)). Yoghurt II was observed to have smaller pores, more particle clusters, and less free ends (Figure 2(b)). Yoghurt I and Yoghurt II also showed microstructural difference after being stored for 7 d (Figure 2(d) versus Figure 2(c)). These results also supported that Yoghurt II had lower syneresis than Yoghurt I. It is thus concluded that cross-linked gelatin was more potential than bovine gelatin to improve yoghurt microstructure.

Conclusion

In the production of set-style skimmed yoghurt, replacing of bovine gelatin with cross-linked gelatin as food stabilizer did not impact yoghurt’s textural parameters, but enhanced apparent viscosity, elastic and viscous moduli, and thixotropy. Using cross-linked gelatin also had other benefits on yoghurt in terms of delayed post-acidification, reduced syneresis, and improved microstructure. It is concluded that cross-linked gelatin generated by this enzymatic method was a potential stabilizer better than bovine gelatin for the production of set-style skimmed yoghurt.

Fiszman, Lluch, and Salvador (1999) had investigated the effect of gelatin addition on microstructure of acidified milk gels and yoghurt, and found that gelatin retained aqueous phase and reduced syneresis efficiently. Gelatin modifies yoghurt microstructure via forming flat sheets or surfaces with interacted casein matrix, or enclosing casein granules in several zones (Fiszman et al., 1999). As a consequence, gelatin confers yoghurt with a uniform microstructure. Cross-linked gelatin was more potential than bovine gelatin to form a three-dimensional network in yoghurt, as it had more polypeptides of larger molecular weights (Han & Zhao, 2016). Yoghurt II (but not Yoghurt I) was thus detected to have better microstructure.
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