Utilization of konjac glucomannan as a fat replacer in low-fat and skimmed yogurt

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ABSTRACT

Konjac glucomannan (KGM) has been reported to be beneficial to human health, as well as having potential functional properties as a fat replacer in dairy products. In this study, 0.5% KGM solution was added to prepare low-fat (LFKGM) and skimmed (SKKGM) yogurts, and their physicochemical properties were compared with those of full-fat yogurt control (FFC), low-fat yogurt control (LFC), and skimmed yogurt control (SKC). Properties and composition were determined and the microscopic structures of all yogurts were observed during storage at 4°C for 21 d. Generally, addition of KGM to yogurts had no significant effect on composition, pH, and titratable acidity at each storage day. The LFKGM and SKKGM had higher whiteness, greenness, and yellowness hues compared with those of the LFC and SKC. The proteolysis of LFKGM and SKKGM was similar to that of FFC, whereas it was lower than in LFC and SKC after 14 d of storage. Addition of KGM had no positive effects on the water-holding capacity, but led to a decrease in syneresis and spontaneous whey separation in LFKGM and SKKGM compared with those of LFC and SKC. The spontaneous whey separation of LFKGM was similar to that of FFC. Presence of KGM in skimmed yogurt affected textural characteristics, while having little effect on texture of low-fat yogurt. Additionally, LFKGM and SKKGM showed stronger and more stable gel structures than those of FFC, LFC, and SKC. Overall, no substantial changes were found in the characteristics for each yogurt during storage, except for pH and gel structures. Results indicated that KGM may be a good fat replacer to develop reduced-fat yogurts with desired characteristics.

Key words: konjac glucomannan, yogurt, physiochemical property, texture, structure

INTRODUCTION

Yogurt is a popular dairy product obtained by lactic acid fermentation of milk. High milk fat diets are associated with high serum cholesterol levels, digestive disorders, heartburn, and stress (Ünal et al., 2003). The awareness of the harmful effects of excess fat consumption on human health has led consumers to demand low-fat or nonfat dairy products (Aziznia et al., 2008). However, the presence of fat in dairy products is important for their physical properties, rheological, and textural characteristics; in addition, fat influences other product characteristics such as appearance, flavor, and mouthfeel (Brennan and Tudorica, 2008). Separation of whey and variations in viscosity are 2 primary defects in yogurts, especially in low-fat yogurt. Some reports suggest that certain dairy ingredients and hydrocolloids could be added to combat these defects (Keogh and O'Kennedy, 1998). Carbohydrate-based fat replacers can be used in yogurt for improving its functionality, such as binding of water and increasing viscosity (Tammime and Robinson, 1999).

Fat-free yogurt with 1% inulin added showed similar characteristics to a control yogurt containing 3% milk fat, but increased addition of inulin (2 and 3%) had negative effects on some physical properties, such as whey separation, consistency, and organoleptic scores (Güven et al., 2005). Other studies report that addition of gum tragacanth did not improve the firmness of nonfat yogurt. Increasing amount of gum led to softer gels with more syneresis, and coarser and more open structure compared with the control nonfat yogurt (Aziznia et al., 2008). Native maize starch was added to reduced-fat yogurt, resulting in lower syneresis than in full-fat yogurt, and contributing to the formation of more stable dispersed acidified milk gelled systems (Lobato-Calleros et al., 2014). However, we were unaware of any published report on the use of konjac glucomannan (**KGM**) in the manufacture of low-fat or skimmed yogurt.

Konjac glucomannan is extracted from the tubers of *Amorphophallus konjac*, a member of the family

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Araceae found in East Asia, and it is a neutral heteropolysaccharide that has been widely used in food products (Nishinari et al., 1992). Konjac glucomannan is a linear random copolymer of 1,4-linked β -D-mannose and β -D-glucose at a molar ratio of 1.6:1 with a low degree of acetyl groups at the C-6 position and also certain short-chain branches at C-3 position of the mannoses (Kato and Matsuda, 1969). Konjac glucomannan is dispersible in hot or cold water and forms a highly viscous solution (Vanderbeek et al., 2007). Konjac glucomannan is beneficial to human health and is reported to prevent some diseases (Vuksan et al., 1999; James and Follett, 2000; Fang and Wu, 2004). It is reported that KGM has antiobesity and antihyperglycemic properties due to promotion of satiety, delay in stomach emptying, retarded bowel transit time, and subsequent reduced rate of dietary sugar absorption; these can reduce the elevation of postprandial glucose and insulin surge levels (Fang and Wu, 2004; Kraemer et al., 2007; Chua et al., 2010). As a soluble dietary fiber, KGM also exhibits an anti-hypercholesterolemia property by transferring bile acids bound with KGM out of human body; this leads to the body to convert more cholesterol into bile acids (Fang and Wu, 2004; Chua et al., 2010; Abhyankar et al., 2011). Additionally, it was reported that KGM also has laxative effect, prebiotic, and anti-inflammatory properties (Li et al., 2005; Chua et al., 2010; Abhyankar et al., 2011). These properties of KGM make it a potential fat replacer for use in dairy products.

In this study, KGM was added to milk to make low-fat and skimmed yogurts to investigate the possibility of using KGM as a fat replacer. The effects of KGM addition on physicochemical properties, texture parameters, and serum expulsion of low-fat and skimmed yogurt during storage were investigated.

MATERIALS AND METHODS

Materials

Fresh whole, low-fat, and skimmed cow milk were obtained from a local milk company (Farm Milk Company Limited, Hong Kong). The protein and fat contents of each kind of milk were 3.6, 3.8, and 3.6 g/100 mL and 5.1, 2.5, and 0.7 g/100 mL, respectively. Konjac purified flour (KJ30, KGM content $\geq 85\%$) was obtained from Hubei Konson Konjac Gum Co., Ltd. (Wuhan, Hubei Province, China).

Activation of Starter Culture

Streptococcus thermophilus (ST) 1303 and *Lactobacillus bulgaricus* (LB) 859 were originally obtained from

the Starter Culture Collection of Dairy Innovation Australia Ltd. (ASCC, Werribee, Victoria, Australia; now Chr. Hansen, Werribee, Victoria, Australia). The stock of ST 1303 and LB 859 were first activated by growing in sterile M17 broth and de Man, Rogosa, Sharpe broth 3 times, respectively, and then propagated using 12% (wt/wt) sterile reconstituted skim milk at 37°C for 24 h in triplicate. Enumeration of each bacterium was carried out using plate count method before used as starters in following yogurt preparation part. For the plate count determination, serial 10 $\times$ dilutions of ST 1303 and LB 859 stocks were carried out in sterile 0.85% saline. Subsequently, 50- μ L aliquots of ST 1303 or LB 859 serial dilutions were poured into M17 or de Man, Rogosa, Sharpe agar plates, respectively, and then the plates were incubated at 37°C for 24 h. Plates showing 25 to 250 colonies were counted and results were expressed as colony-forming units per milliliter of the inoculated sample.

Yogurt Preparation

Konjac glucomannan solution (0.5%) was prepared by dissolving konjac purified flour in sterile Milli-Q water and stored overnight at 4°C before use. Yogurt mix was prepared by mixing 28.5 mL of milk and 1.5 mL of Milli-Q water or 0.5% KGM solution in a 50-mL tube at 22°C for 2 h using a shaker according to Table 1. Full-fat yogurt control (FFC), low-fat yogurt control (LFC), skimmed yogurt control (SKC), low-fat yogurt with KGM (LFKGM), and skimmed yogurt with KGM (SKKGM) were then made as follows: yogurt mixes were heated at 85°C for 30 min using a water bath with a shaker and cooled to 45°C, followed by inoculating with 1% (vol/vol) activated ST 1303 and 1% (vol/vol) LB 859 and incubation at 42°C until pH decreased to about 4.5. Both ST 1303 and LB 859, which were enumerated by plate count as mentioned above, were diluted to 1 $\times$ 10⁹ cfu/mL with sterile reconstituted skim milk before inoculation. The yogurts were stored at 4°C; properties and composition were tested at 0, 7, 14, and 21 d during storage. All yogurts produced were set-style.

Composition and Color Analysis

Compositional analysis was carried out after 24 h of storage after yogurt preparation. Protein, fat, and TS were analyzed using the Kjeldahl, Babcock, and oven drying methods, respectively (AOAC International, 1999). The L* (lightness), a* (red/greenness), and b* (yellow/blueness) values were measured using a Minolta Chroma Meter at 4°C (CR-300 Series, Minolta, Osaka, Japan).

pH and Titratable Acidity Measurement

A pH meter (model 250A, Science International Corporation, Hong Kong) was used to measure the changes in pH of the product. The pH meter was calibrated with fresh pH 4.00 and 7.00 standard buffers and samples were tempered to 22°C before measurement.

Titratable acidity (**TA**) of yogurts was measured according to the AOAC titration method (AOAC International, 1999). Briefly, 9.0 g of yogurt sample was diluted with equal amount of Milli-Q water and 0.1 *M* NaOH was used for titration using phenolphthalein as indicator. The TA was expressed as percent lactic acid as follows:

$$\text{Lactic acid \%} = V \times 0.009/W \times 100,$$

where *V* is the volume of 0.1 *M* NaOH (mL) and *W* is the weight of yogurt (g).

Extent of Proteolysis

Filtrates of the yogurt samples were prepared as per Ramchandran and Shah (2010) by centrifuging the yogurts at $4,000 \times g$ for 30 min at 4°C, and then the supernatants were filtered through 0.45-μm PTFE Acrodisc CR syringe membrane filters (Pall Corporation, New York, NY) and stored at −20°C until assayed.

The extent of proteolysis was determined by the free AA content in filtrates of yogurt samples using the *o*-phthaldialdehyde (**OPA**) method according to Church et al. (1983) with some modification. Briefly, 50 μL of filtrate was added to 1 mL of OPA reagent and vortexed for 5 s, followed by measuring absorbance value at 340 nm within 2 min using a UV-VIS spectrophotometer (Shimadzu Scientific Instruments, Kyoto, Japan). The readings of the reagent blank were deducted from the corresponding readings of yogurt samples to obtain the amount of free AA released as a consequence of the proteolytic capacities of the starter cultures during fermentation and storage.

Whey Expulsion Measurement

Water-Holding Capacity. The water-holding capacity (**WHC**) of yogurts was measured according to Sahan et al. (2008) with some modification. To determine WHC of the yogurts, 5 g of yogurt samples was centrifuged at $4,713 \times g$ (Hitachi high-speed refrigerated centrifuge, model GR22G, Hitachi Ltd., Tokyo, Japan) for 30 min at 4°C. After centrifugation, the supernatant was collected and weighed. The WHC was calculated as 1 minus the percent weight of the supernatant over the initial weight of the yogurt samples.

Syneresis. Measurement of syneresis was carried out by the drainage method according to Tamime et al. (1996) with some modifications. Briefly, about 25 g of yogurt sample was weighed on a no. 4 filter paper (qualitative, catalog no. 1004 125, Whatman, Maidstone, UK) placed on the top of a funnel. Syneresis of whey was measured by gravity at 4°C for 120 min and the syneresis value was calculated as the percent weight of whey collected in a flask divided by the initial weight of the yogurt sample.

Spontaneous Whey Separation. Spontaneous whey separation (**SWS**) of undisturbed set yogurts was carried out by siphon method according to Lucey et al. (1998) with some modifications. Briefly, the weight of a tube of yogurt taken out from 4°C was recorded immediately and then the collected whey at the side of the tube was siphoned out with a pipette within 10 s by keeping the tube at an angle of approximately 45°. The SWS is expressed as the percent weight of the whey over the initial weight of the yogurt.

Texture Analysis

Texture measurements of yogurt were carried out with a TA.XT Plus model Texture Analyzer (Stable Micro Systems, Godalming, Surrey, UK) with a 5-kg load cell. A tube of yogurt sample was taken out from 4°C, and the whey on the surface was siphoned out. A 20-mm-diameter probe (A/BE20) was used, and when

Table 1. Yogurt mix composition before fermentation

Sample	Yogurt mix composition				
	Full-fat milk (mL)	Low-fat milk (mL)	Skim milk (mL)	Water (mL)	0.5% KGM ¹ solution (mL)
Full-fat yogurt control	28.5	—	—	1.5	—
Low-fat yogurt control	—	28.5	—	1.5	—
Skimmed yogurt control	—	—	28.5	1.5	—
Low-fat yogurt with KGM	—	28.5	—	—	1.5
Skimmed yogurt with KGM	—	—	28.5	—	1.5

¹KGM = konjac glucomannan.

a 10-g surface trigger was attained, the probe started to penetrate to a depth of 30 mm at a constant cross-head speed of 1.0 mm/s. From the resulting force-time curve, the values for texture attributes were analyzed using the Exponent version 6.1.4.0 equipment software (Lotun Science Co Ltd., Beijing, China).

Microscopic Structures

Microscopic structures of yogurt were observed by a confocal laser scanning microscopy (CLSM) with multiphoton system (LSM710 NLO, Carl Zeiss, Jena, Germany). For staining yogurt samples, 20 μ L of Nile red (1 mg/mL in ethanol) and 20 μ L of fluorescein isothiocyanate (1 mg/mL in ethanol) were mixed with a 1-mL yogurt sample for 30 min. Afterward, 50 μ L of stained sample was pipetted to a glass slide and sealed with a coverslip before observation. Nile Red and fluorescein isothiocyanate were excited at the excitation/emission wavelengths of 534 nm/500 to 600 nm and 488 nm/495 to 559 nm, respectively.

Statistical Analysis

Data reported are mean $\pm$ standard deviation for triplicate determinations of each yogurt. One-way ANOVA and Tukey's test at 95% confidence intervals ($P < 0.05$) were performed using IBM SPSS Statistics 20.0 (Armonk, NY). Tukey's tests were applied for different yogurt at each storage time and for each yogurt at different storage times.

RESULTS AND DISCUSSION

Composition and Color

The composition of yogurt samples is shown in Table 2. The TS of FFC was higher than other yogurt samples, whereas there was no significant difference among LFC, SKC, LFKGM, and SKKGM. Fat

content of FFC was higher than other yogurt samples, with no significant differences among fat contents of LFKGM and SKKGM as compared with those of LFC and SKC. Protein contents of yogurt samples ranged between 4.82 and 5.03%, which were higher than those in milk. Aziznia et al. (2008) and Dinkci (2012) also reported the higher protein content in yogurt than in milk. The increased protein content in yogurt may be because of the evaporation during and increased bacterial mass during fermentation (Deeth and Tamime, 1981). The bacterial cell mass constitutes about 1% of the DM in yogurt, which is a rich source of EAA (Erdman et al., 1977; Deeth and Tamime, 1981; Park, 2000). The evaporation could cause the loss of weight of yogurt, which may have also resulted in an increase of protein percentage in the yogurt. The composition results indicated that the overall composition was not changed by the low level addition of KGM in low-fat or skimmed yogurt. According to our previous work (unpublished data), when the concentration of KGM solution was more than 0.5%, a metastable or unstable state of the KGM-milk mixtures was observed at the level of 95% milk. Additionally, phase separation was observed in yogurts prepared with the metastable or unstable KGM-milk mixtures; this would be unacceptable in practice.

Color plays an important role in food choice for consumers. The color attributes of yogurt samples are also shown in Table 2. The L^* value represents lightness (100) and blackness (0), and is an estimation of food whiteness (Zare et al., 2011). No difference was present among L^* values of FFC, LFKGM, and SKKGM and they were higher than those of LFC and SKC. No difference was found in L^* values of LFC and SKC. The results illustrated that the addition of KGM in low-fat or skimmed yogurt enhanced the whiteness of yogurts, which provided whiteness similar to that of full-fat yogurt. The a^* and b^* values represented red-green (positive-negative) and yellow-blue (positive-negative) hues of the yogurt samples (Sanz et al., 2008). Param-

Table 2. Composition and color of yogurts¹

Sample ²	TS (%)	Fat (%)	Protein (%)	Color		
				L^*	a^*	b^*
FFC	17.69 $\pm$ 1.79 ^a	5.78 $\pm$ 0.27 ^a	4.93 $\pm$ 0.05 ^{ab}	84.46 $\pm$ 1.27 ^{ab}	-5.70 $\pm$ 0.10 ^a	2.98 $\pm$ 0.21 ^b
LFC	10.53 $\pm$ 0.46 ^b	2.65 $\pm$ 0.28 ^b	4.82 $\pm$ 0.04 ^b	79.79 $\pm$ 1.45 ^{bc}	-5.53 $\pm$ 0.06 ^a	1.07 $\pm$ 0.18 ^c
SKC	10.73 $\pm$ 0.56 ^b	0.83 $\pm$ 0.28 ^c	4.92 $\pm$ 0.03 ^{ab}	76.62 $\pm$ 0.77 ^c	-5.73 $\pm$ 0.19 ^a	1.59 $\pm$ 0.74 ^c
LFKGM	10.85 $\pm$ 0.08 ^b	1.65 $\pm$ 0.29 ^{bc}	4.96 $\pm$ 0.04 ^a	84.49 $\pm$ 1.61 ^{ab}	-6.14 $\pm$ 0.21 ^b	3.71 $\pm$ 0.97 ^{ab}
SKKGM	10.19 $\pm$ 0.39 ^b	0.66 $\pm$ 0.28 ^c	5.03 $\pm$ 0.07 ^a	85.92 $\pm$ 0.83 ^a	-6.24 $\pm$ 0.02 ^b	4.85 $\pm$ 0.41 ^a

^{a-c}Means in each parameter without common superscripts are significantly different ($P < 0.05$).

¹Values are expressed as mean $\pm$ SD, $n = 3$.

²FFC = full-fat yogurt control; LFC = low-fat yogurt control; SKC = skimmed yogurt control; LFKGM = low-fat yogurt with KGM; SKKGM = skimmed yogurt with KGM; KGM = konjac glucomannan.

Table 3. pH and titratable acidity (%) of yogurts during 21 d of storage at 4°C¹

Sample ²	Period of storage (d)			
	0	7	14	21
pH				
FFC	4.48 ± 0.01 ^A	4.31 ± 0.02 ^B	4.23 ± 0.02 ^C	4.20 ± 0.02 ^C
LFC	4.50 ± 0.04 ^A	4.32 ± 0.04 ^B	4.23 ± 0.05 ^B	4.20 ± 0.04 ^B
SKC	4.49 ± 0.03 ^A	4.33 ± 0.04 ^B	4.24 ± 0.05 ^B	4.23 ± 0.03 ^B
LFKGM	4.53 ± 0.04 ^A	4.35 ± 0.03 ^B	4.26 ± 0.04 ^{BC}	4.22 ± 0.03 ^C
SKKGM	4.50 ± 0.05 ^A	4.35 ± 0.06 ^{AB}	4.27 ± 0.06 ^B	4.23 ± 0.04 ^B
Titratable acidity (%)				
FFC	0.782 ± 0.057 ^{a,B}	0.868 ± 0.003 ^{a,AB}	0.894 ± 0.011 ^{a,A}	0.882 ± 0.003 ^{a,A}
LFC	0.816 ± 0.028 ^{a,A}	0.857 ± 0.025 ^{ab,A}	0.897 ± 0.008 ^{a,A}	0.904 ± 0.011 ^{a,A}
SKC	0.806 ± 0.079 ^{a,A}	0.861 ± 0.017 ^{a,A}	0.881 ± 0.025 ^{ab,A}	0.888 ± 0.013 ^{ab,A}
LFKGM	0.801 ± 0.066 ^{a,A}	0.837 ± 0.019 ^{ab,A}	0.893 ± 0.022 ^{ab,A}	0.843 ± 0.012 ^{ab,A}
SKKGM	0.759 ± 0.078 ^{a,B}	0.802 ± 0.008 ^{b,AB}	0.843 ± 0.017 ^{b,A}	0.847 ± 0.007 ^{b,A}

^{A-C}Means of the same yogurt sample stored for different days without common superscripts are significantly different ($P < 0.05$).

^{a,b}Means of different yogurt samples stored at the same day without common superscripts are significantly different ($P < 0.05$).

¹Values are expressed as mean ± SD, $n = 3$.

²FFC = full-fat yogurt control; LFC = low-fat yogurt control; SKC = skimmed yogurt control; LFKGM = low-fat yogurt with KGM; SKKGM = skimmed yogurt with KGM; KGM = konjac glucomannan.

eters a^* and b^* showed that all the yogurt samples had greenish (negative a^* values) and yellowish (positive b^* values) characteristics. No difference was found in a^* values of LFKGM and SKKGM and these were more negative than those of FFC, LFC, and SKC, which presented with a greener hue than the control yogurts. No difference was present in a^* values of FFC, LFC, and SKC, indicating that the fat content had no effect on the greenness of yogurt. The b^* values of LFKGM and SKKGM were higher than those of FFC, LFC and SKC, which indicated that the addition of KGM increased yellowness of yogurts. The increased yellowish hue of yogurts with KGM may be because of the Maillard reaction in milk-KGM mixture when heated at 85°C for 30 min for pasteurization and the light brownish color of KGM powder used in our study. Additionally, Park (1996) also reported that the addition of konjac flour increased lightness and yellow hue (b^*) of surimi gels, whereas the green hue (negative a^*) was not affected by the addition of konjac flour.

Changes in pH and TA

The pH and TA of yogurts during storage at 4°C are shown in Table 3. The pH of all the yogurts decreased during 21 d of storage, with a significant decrease occurring at d 7, and stable pH after 14 d of storage. Similar observations were reported by Guven et al. (2005) and Sahan et al. (2008) in skimmed yogurt with addition of inulin and β -glucan, respectively. García-Pérez et al. (2005) also reported that pH of yogurt with and without orange fiber decreased with storage time, with the decrease more evident during the first 14 d of storage. Additionally, Beal et al. (1999) observed that the pH

decreased mainly by d 7, and was due to the increase in lactic acid caused by the high bacterial metabolic activity due to hydrolysis of lactose. Similarly, the level of TA increased slightly during the 21 d of storage at 4°C for all the yogurts. The addition of KGM did not affect the trend of pH and TA changes in low-fat or skimmed yogurt, likely because it did not adversely affect the activity of starter bacteria in yogurt (Barrantes et al., 1994).

Extent of Proteolysis

Extent of proteolysis, representing the proteolytic capabilities of bacteria, is related to the growth of bacteria and acidification of yogurts (Ramchandran and Shah, 2010). Proteolysis of all the yogurt samples increased during 21 d of storage (Figure 1) and had no significant differences at d 14 and 21 among treatment. The LFC showed continued increase in proteolysis at d 7 and 14, but the increase in proteolysis occurred only at d 7 for LFKGM and SKKGM, and only at d 14 for FFC. Proteolysis of SKC showed no significant differences between d 0 and 7, d 7 and 14, and d 14 and 21 of storage. Proteinase and peptidases in lactic acid bacteria are related to the proteolysis of caseins (Shihata and Shah, 2000) and this function could provide peptides and AA for the survival of ST 1303 and LB 859 in yogurts continuously. Those proteinase and peptidases could hydrolyze the milk proteins, which resulted in an increase in the amount of the free amino groups that was quantified by the OPA method. Generally, the proteolysis of yogurts increased during the early 14 d storage and then became stable at d 21 (Figure 1). It may be because the bacteria were unable to use all peptides

and AA that were liberated from casein, which resulted in increased accumulated peptides and AA during early 14 d storage (Li and Shah, 2015). Furthermore, the liberation and consumption of peptide and AA were balanced, which may result in stable proteolysis during the storage between d 14 and 21. Proteolysis of FFC was the lowest among the yogurt samples during 21 d of storage. No difference was found in the proteolysis of LFC as compared with that of SKC, and no differences were found between LFKGM and SKKGM at d 7, 14, and 21. This indicated that fat content did not affect the proteolytic capability of the reduced-fat yogurt after 7 d of storage, whereas proteolysis of LFKGM and SKKGM showed no differences compared with those of LFC and SKC at d 0 and 7, respectively. The LFKGM and SKKGM had lower proteolytic capability than those of LFC and SKC, and had similar proteolysis with FFC at d 14 and 21. Perols et al. (1997) reported that the protein release from KGM hydrogel remained low, and the contraction of the KGM gel solidifies droplets, thus prohibiting the proteolytic enzyme release, and the low enzyme release in whey led to premature decrease in proteolysis. These may be the reason that the proteolysis of yogurts with KGM was lower than that without KGM after 14 d of storage in our study.

Expulsion of Serum

Water-Holding Capacity. The WHC measured by centrifugation method represents the level of whey separated from the collapsed gels as a result of centrifugal force (Amatayakul et al., 2006). Higher WHC represents less whey separated from the yogurt by centrifugation. The WHC of FFC was the highest whereas that of SKC was the lowest among the yogurt samples during 21 d of storage (Figure 2). The WHC of LFC was higher than those of SKC, LFKGM, and SKKGM during 21 d of storage, except that of LFKGM at d 21, which had no significant difference with LFC, whereas WHC of SKKGM was similar to SKC during 21 d of storage, except that the WHC at d 14 was higher than SKC. The results illustrated that fat content was the main factor that influenced the WHC of collapsed gels in yogurts, and KGM had no positive effect on WHC in low-fat or skimmed yogurt. Centrifugation method is likely to accelerate the phase separation between milk protein and polysaccharide in yogurt. The whey collected as a result of centrifugation would not only be influenced by the cross-linking of milk proteins with polysaccharides but also some factors such as rigidity and rheological properties of yogurt gels (Amatayakul et al., 2006). In our study, the final percentage of KGM in our yogurt was 0.025%, which could be too low to show the acceleration effects of centrifugation. Addi-

Figure 1. Changes in proteolysis (absorbance at 340 nm; A_{340}) of yogurts during 21 d of storage at 4°C. Values are expressed as mean $\pm$ SD, $n = 3$. KGM = konjac glucomannan; FFC = full-fat yogurt control; LFC = low-fat yogurt control; SKC = skimmed yogurt control; LFKGM = low-fat yogurt with KGM; SKKGM = skimmed yogurt with KGM. Means of the yogurt sample at the same storage time without common letters (a–c) are significantly different ($P < 0.05$). Means of the same yogurt sample stored for different days without common letters (A–C) are significantly different ($P < 0.05$).

tionally, centrifugation might break the binding of free water with KGM and subsequently resulted in releasing the water from yogurt as whey. All these complicated factors may be the reason why KGM did not increase WHC in yogurts in our study. The WHC of LFC was stable and for those of other yogurt samples WHC increased at d 21 as compared with that at d 0. The WHC of FFC was stable over 14 d storage and increased at d 21, whereas that of SKKGM increased at d 7 and was stable after that. For other yogurt samples, WHC increased slightly during storage. The results were in agreement with those of Sahan et al. (2008) and Guven et al. (2005) that addition of β -glucan hydrocolloidal composite or inulin did not change the WHC of nonfat yogurts or set-type low-fat yogurts reported in their papers, respectively.

Syneresis. Syneresis represents the level of whey separated from yogurts under the influence of gravity (Amatayakul et al., 2006). Similar to the WHC results, syneresis of FFC was the lowest, whereas those of SKC were the highest among the yogurt samples during 21 d of storage (Figure 3). Results of FFC, LFC, and SKC indicated that fat contents in yogurts could be a reason to determine the syneresis. In addition to the fat content in yogurt, another reason why FFC had the lowest syneresis may be because of its higher TS compared with other samples (Harwalkar and Kalab, 1986). Syneresis values of LFKGM were lower than those of LFC during 21 d of storage, but were not different compared

Figure 2. Water-holding capacities of yogurts measured by centrifugation method during 21 d of storage at 4°C. Values are expressed as mean $\pm$ SD, $n = 3$. KGM = konjac glucomannan; FFC = full-fat yogurt control; LFC = low-fat yogurt control; SKC = skimmed yogurt control; LFKGM = low-fat yogurt with KGM; SKKGM = skimmed yogurt with KGM. Means of the yogurt sample at the same storage time without common letters (a–d) are significantly different ($P < 0.05$). Means of the same yogurt sample stored for different days without common letters (A–C) are significantly different ($P < 0.05$).

with LFC at d 0 and 7. Similarly, overall syneresis of SKKGM was lower than SKC during storage, but had no significant differences with SKC at d 7 and 14. The results indicated that the addition of KGM in low-fat or skimmed yogurt could partly decrease the syneresis of yogurts. This may be because of the high WHC of KGM that could absorb the whey released by the gel. A similar observation was reported by García-Pérez et al. (2005), that syneresis decreased when 1% orange fiber was added because of the high water-holding capability of the fiber. Syneresis of all the yogurt samples decreased at d 21 compared with those at d 0. However, the syneresis of FFC, LFC, and SKC was stable during storage, and that of LFKGM and SKKGM decreased at d 7 compared with that at d 0 and then kept stable during storage. The difference in WHC and syneresis detected from each method suggests that these methods measured different data. The whey was separated by gravity in this study. The centrifugation method is likely to accelerate the phase separation between milk protein and polysaccharide in yogurt as mentioned above, and gravity is a slow and gentle force to act on yogurts compared with that of centrifugation force. Gravity is not strong enough to break the cross-linking between milk protein and KGM, and also the binding between KGM and free water in yogurt. Guven et al. (2005) reported that syneresis of low-fat yogurt was

Figure 3. Index of syneresis of yogurts measured by drainage method during 21 d of storage at 4°C. Values are expressed as mean $\pm$ SD, $n = 3$. KGM = konjac glucomannan; FFC = full-fat yogurt control; LFC = low-fat yogurt control; SKC = skimmed yogurt control; LFKGM = low-fat yogurt with KGM; SKKGM = skimmed yogurt with KGM. Means of the yogurt sample at the same storage time without common letters (a–d) are significantly different ($P < 0.05$). Means of the same yogurt sample stored for different days without common letters (A–C) are significantly different ($P < 0.05$).

reduced with inulin addition during storage, which was similar to our results.

Spontaneous Whey Separation. Spontaneous whey separation represents the level of spontaneous whey separated on the surface of yogurt gels (Amatayakul et al., 2006). The SWS of FFC were the lowest among the yogurt samples during 21 d of storage (Figure 4). In general, the SWS decreased due to an increase in solids content (Amatayakul et al., 2006), this may be the reason why FFC had the lowest SWS. The LFC and SKC, which had similar TS contents, did not differ in SWS during 21 d of storage, whereas LFKGM and SKKGM were similar to each other at d 7, 14, and 21. These results illustrated that the fat content had no obvious effect on SWS for reduced-fat yogurt. The SWS of LFKGM was lower than that of LFC during storage and had no significant differences with FFC at d 7, 14, and 21, which indicated the KGM addition in low-fat yogurt could decrease the whey separated from yogurt surface spontaneously similar to the SWS of full-fat yogurt. Similar results were reported by Miao et al. (2011) that yogurts with addition of water-soluble polysaccharides from truffles had lower SWS than yogurts without the polysaccharides. Sahan et al. (2008) also reported that addition of β -glucan composite to skimmed yogurt caused a decrease in SWS values. The addition of KGM in skimmed yogurt did not change

Figure 4. Spontaneous whey separation of yogurts measured by siphon method during 21 d of storage at 4°C. Values are expressed as mean $\pm$ SD, $n = 3$. KGM = konjac glucomannan; FFC = full-fat yogurt control; LFC = low-fat yogurt control; SKC = skimmed yogurt control; LFKGM = low-fat yogurt with KGM; SKKGM = skimmed yogurt with KGM. Means of the yogurt sample at the same storage time without common letters (a–d) are significantly different ($P < 0.05$). Means of the same yogurt sample stored for different days without common letters (A, B) are significantly different ($P < 0.05$).

the SWS much as the results showed no significant differences between SKC and SKKGM at d 7, 14, and 21. The changes of SWS in all the yogurt samples were not significant during 21 d of storage, except FFC had a decreased SWS value at d 7. Similar results were observed by Ramchandran and Shah (2010) for the low-fat yogurts.

Texture

The FFC had higher firmness and consistency, and more negative cohesiveness and viscosity index than all of the other yogurt samples, whereas the lowest firmness and consistency, and the least negative cohesiveness and viscosity index were observed for SKC throughout the 21 d of storage (Table 4). Additionally, SKKGM had higher firmness, consistency, cohesiveness (negative), and viscosity index (negative) than SKC during storage. However, the textural characteristics of LFC, LFKGM, and SKKGM were not significantly different among each other over 21 d of storage. The KGM addition to yogurt had positive effects on the textural characteristics of the skimmed yogurts, but was ineffective for the low-fat yogurts. This may be because of the complicated interaction between protein, fat, and polysaccharide during fermentation. Similar results were reported by Ramchandran and Shah (2009) that

the inclusion of inulin to low-fat yogurt prepared with exopolysaccharide-producing cultures did not influence the firmness.

No significant variation was found in the firmness of FFC, SKC, and LFKGM throughout the storage period, whereas that of LFC and SKKGM increased at d 21 and 7, respectively. Consistency and cohesiveness (negative) of all the yogurt samples kept stable, except that of SKKGM increased at d 7. Index of viscosity of FFC, LFC, LFKGM, and SKKGM was stable, whereas that of SKC became more negative during 21 d of storage. Similar results were observed and explained by Seçkin and Baladura (2012). In their study, the storage did not affect viscosity index values of all yogurt samples with addition of different fibers, and the changes in cohesiveness and consistency did not follow any particular pattern during storage, likely due to the complicated physical, biochemical, and chemical reactions occurring during the storage. This might also be the reason for the results achieved in our study.

Microscopic Structure

Structures of yogurts observed by CLSM at d 0 and 21 are shown in Figure 5. Denser protein aggregates appeared light green, and the black areas represented serum pores (Cruz et al., 2009; Nguyen et al., 2014; Li and Shah, 2015). All the yogurt samples at d 0 showed homogeneous structures containing small serum pores. Folkenberg et al. (2006) reported that yogurts should have medium-size pores in the matrix to provide stable structures. However, SKC had more and larger areas of serum pores than other yogurt samples, which may be because of the higher whey expulsion from the yogurts that were observed in the Expulsion of Serum section. The serum pores were less and smaller in LFKGM and SKKGM compared with those of LFC and SKC, indicating the stronger and more stable gel structures in LFKGM and SKKGM. Galesloot (1958) and Ciron et al. (2010) reported that the weak gel structure (firmness) of yogurt contributed to its low viscosity and low water retention. Weak gels had a strong tendency to flow and were more prone to shrinkage and whey expulsion (Pereira et al., 2003; Serra et al., 2008). Thus, the stronger and more stable structures of LFKGM and SKKGM may be attributed to the higher viscosity of KGM and subsequent higher viscosity of yogurts. Additionally, the structures may due to high water-holding capability of KGM and subsequent less whey expulsion from the yogurts.

After 21 d of storage, microscopic structures showed much larger areas of serum pores in FFC, LFC, and SKC compared with those at d 0. The protein aggregated and small serum pores that formed at d 0 could

stimulate later protein aggregation in the yogurts during storage (Li and Shah, 2015). Additionally, LFKGM and SKKGM showed denser protein matrix at d 21 than d 0, and the serum pores at d 21 were similarly homogeneous and medium-size as those at d 0. The sizes of serum pores in yogurts at d 21 were in the order of SKC > LFC > FFC > SKKGM > LFKGM, which indicated that the reduction of fat content in yogurt negatively influenced the stability of gel structures, whereas the addition of KGM could enhance the stability of gel structures. The results accorded with Ciron et al. (2010) and Lanciotti et al. (2004), who reported that the texture and stability of yogurt primarily depended on the levels of TS, fat, and protein. Guggisberg et al. (2009) reported that additional inulin accelerated the protein aggregation in set yogurt with the increasing concentrations of inulin. Additionally, in the study of Li and Shah (2015), the fermented milk with addition of polysaccharide extracted from *Pleurotus eryngii* at different levels showed denser protein aggregates and contained larger serum pores than that of fermented milk without *P. eryngii* during 21 d of refrigerated storage. Similarly, our previous work (unpublished data) showed that the aggregation of milk and KGM increased with the increasing concentrations of KGM in milk-KGM

mixtures before fermentation. When the concentration of KGM solution was more than 0.5%, a metastable or unstable structure of the milk-KGM mixtures induced by the aggregation of the milk and KGM particles would be observed at the level of 95% (vol:vol) milk. However, the additional KGM improved the structures of low-fat and skimmed yogurt during 21 d of storage because the KGM-milk mixtures used in this study had a stable structure in which the concentration of KGM solution was 0.5%. Moreover, the high water holding capability of KGM and the subsequent lower whey expulsion and improved textural attributes observed in the Expulsion of Serum and Texture sections might be other factors that improved the CLSM structures in LFKGM and SKKGM during 21 d of storage.

CONCLUSIONS

The addition of KGM in low-fat or skimmed yogurt did not affect the composition or the changes of pH and TA during storage. However, yogurts with additional KGM had higher whiteness, greenness, and yellowness hues compared with those without KGM. The presence of KGM weakened the proteolytic capability in yogurts, but provided similar proteolysis as in full-fat yogurt

Table 4. Texture attributes of yogurts during 21 d of storage at 4°C¹

Sample ²	Period of storage (d)			
	0	7	14	21
Firmness (g)				
FFC	112.63 ± 3.02 ^{a,A}	112.34 ± 6.95 ^{a,A}	126.45 ± 5.73 ^{a,A}	117.44 ± 3.73 ^{a,A}
LFC	79.82 ± 3.68 ^{bc,B}	81.93 ± 2.52 ^{b,B}	81.07 ± 5.48 ^{bc,B}	83.55 ± 9.36 ^{abc,A}
SKC	68.27 ± 2.94 ^{c,A}	66.71 ± 0.86 ^{c,A}	73.22 ± 2.38 ^{c,A}	73.94 ± 4.09 ^{c,A}
LFKGM	82.25 ± 2.75 ^{b,A}	85.91 ± 5.71 ^{b,A}	89.14 ± 2.08 ^{b,A}	88.31 ± 5.68 ^{bc,A}
SKKGM	78.75 ± 0.94 ^{bc,B}	94.89 ± 8.00 ^{b,A}	91.45 ± 4.94 ^{b,AB}	95.86 ± 2.97 ^{b,A}
Consistency (g-s)				
FFC	2,837.18 ± 93.09 ^{a,A}	2,776.87 ± 172.79 ^{a,A}	3,039.32 ± 233.62 ^{a,A}	2,946.26 ± 110.35 ^{a,A}
LFC	1,730.25 ± 24.79 ^{bc,A}	1,825.79 ± 79.69 ^{b,A}	1,756.53 ± 129.24 ^{bc,A}	1,855.75 ± 90.42 ^{c,A}
SKC	1,518.93 ± 147.17 ^{c,B}	1,540.45 ± 68.68 ^{c,AB}	1,705.85 ± 86.52 ^{c,AB}	1,788.00 ± 44.95 ^{c,A}
LFKGM	1,906.06 ± 81.62 ^{b,A}	1,981.75 ± 44.78 ^{b,A}	2,080.40 ± 24.29 ^{ab,A}	2,036.93 ± 97.93 ^{bc,A}
SKKGM	1,852.30 ± 13.78 ^{b,B}	2,164.84 ± 119.09 ^{b,A}	2,150.01 ± 157.23 ^{bc,AB}	2,258.94 ± 120.40 ^{b,A}
Cohesiveness (g)				
FFC	-46.99 ± 0.51 ^{c,A}	-46.39 ± 1.03 ^{c,A}	-47.62 ± 0.71 ^{c,A}	-46.91 ± 1.41 ^{c,A}
LFC	-36.28 ± 5.40 ^{b,A}	-36.35 ± 0.22 ^{b,A}	-34.58 ± 5.09 ^{b,A}	-37.38 ± 5.44 ^{ac,A}
SKC	-21.41 ± 2.57 ^{a,AB}	-18.61 ± 0.63 ^{a,A}	-25.64 ± 1.26 ^{a,C}	-22.67 ± 0.43 ^{a,BC}
LFKGM	-30.53 ± 0.24 ^{b,A}	-32.17 ± 3.66 ^{b,A}	-33.84 ± 2.14 ^{b,A}	-32.78 ± 2.82 ^{ab,A}
SKKGM	-29.46 ± 1.74 ^{b,A}	-36.50 ± 2.23 ^{b,BC}	-38.14 ± 3.08 ^{b,AC}	-39.87 ± 2.32 ^{bc,C}
Index of viscosity (g-s)				
FFC	-54.31 ± 5.75 ^{c,A}	-43.10 ± 4.62 ^{c,A}	-52.88 ± 7.98 ^{c,A}	-53.26 ± 4.34 ^{c,A}
LFC	-22.85 ± 1.26 ^{b,A}	-25.41 ± 5.03 ^{ab,A}	-18.89 ± 1.96 ^{a,A}	-26.34 ± 4.36 ^{ab,A}
SKC	-6.73 ± 1.90 ^{a,A}	-4.16 ± 0.36 ^{a,A}	-14.87 ± 1.53 ^{a,C}	-10.16 ± 0.03 ^{a,B}
LFKGM	-27.13 ± 2.31 ^{b,A}	-28.81 ± 2.99 ^{bc,A}	-30.63 ± 0.17 ^{b,A}	-30.07 ± 2.42 ^{b,A}
SKKGM	-24.90 ± 1.97 ^{b,A}	-30.70 ± 3.73 ^{bc,A}	-33.39 ± 3.02 ^{b,A}	-31.43 ± 4.65 ^{b,A}

^{a-c}Means of the yogurt sample at the same storage time without common superscripts are significantly different ($P < 0.05$).

^{A-C}Means of the same yogurt sample stored for different days without common superscripts are significantly different ($P < 0.05$).

¹Values are expressed as mean ± SD, n = 3.

²FFC = full-fat yogurt control; LFC = low-fat yogurt control; SKC = skimmed yogurt control; LFKGM = low-fat yogurt with KGM; SKKGM = skimmed yogurt with KGM; KGM = konjac glucomannan.

Figure 5. Confocal laser scanning microscopy (CLSM) of yogurts at d 0 and 21. FFC = full-fat yogurt control; LFC = low-fat yogurt control; SKC = skimmed yogurt control; LFKGM = low-fat yogurt with KGM; SKKGM = skimmed yogurt with KGM; KGM = konjac glucomannan.

after 14 d of storage. The KGM added to yogurts had no positive effect on WHC, but could decrease the syneresis and SWS during storage; the SWS of LFKGM was similar to those of FFC during storage. Textural characteristics were positively influenced by additional KGM in skimmed yogurt, but no effects were found in low-fat yogurt. Additional KGM improved the structures of yogurts during storage, as the yogurt with KGM showed stronger and more stable gel structures than that of control yogurts. Overall, the storage time had no substantial effects on the characteristics of the yogurts except the gel structures. Our study indicated that KGM may be considered as a fat replacer added to milk before fermentation to improve the characteristics and health benefits of reduced-fat yogurts.

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