The addition of buttermilk powder and transglutaminase improves textural and organoleptic properties of fat-free buffalo yogurt

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ORIGINAL PAPER

The addition of buttermilk powder and transglutaminase improves textural and organoleptic properties of fat-free buffalo yogurt

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Abstract To meet the increased consumer demand for yogurt with reduced fat content, various efforts have been made to improve the quality of reduced-fat variants that still is regarded as having inferior quality. As an alternative, a yogurt was produced from buffalo skim milk with the addition of microbial transglutaminase (TG; 1 $U.g^{-1}_{protein}$) and buttermilk powder (BMP; 1 and 2% "*w*/*w*"). For comparison, a fat-free variant without TG or BMP and a full-fat yogurt were studied. Monitoring the pH drop during incubation time revealed that TG did not interfere with the pH reduction, while BMP addition accelerated the decline in pH. TG treatment or BMP addition substantially improved the water holding capacity functionality of the yogurt gel. Electrophoretic analysis revealed that the addition of BMP enhanced the reactivity of TG as indicated by the appearance of high molecular weight protein polymer bands. These results were confirmed by scanning electron microscope analysis. The addition of TG and BMP, either individually or in combination, showed a marked impact on gel network, resulting in a more compact and dense gel structure accompanied by irregular agglomerated clusters of protein aggregates. Fat-free yogurts of individually BMP addition exhibited the most desirable organoleptic attributes as indicated by a sensory panel and were perceived as similar to the full-fat yogurt perception. Overall, the addition of TG or BMP appeared to have potential as a valuable alternative in fat-free yogurt production, and BMP can be used as a source of extra protein, which in turn offers promising option to develop innovative functional fat-free yogurt.

Keywords Fat-free yogurt · Transglutaminase · Buttermilk · Microstructure

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1 Introduction

The demand for foods low in calories and foods enriched with nutrients that have health-promoting and/or disease-preventing properties has increased notably over the last two decades. This challenges food manufacturers to develop such variants of foods with acceptable physical and sensory characteristics.

The above concepts are fully true with respect to the dairy products sector that possesses a vital role in public daily diet. Yogurt is the most popular fermented milk produced in Egypt and worldwide, due to its known nutrients that promote health and enhance the immune system. Buffalo milk, known by its high fat content that ranges from 6–7% (Elagamy 2003), is most commonly used for yogurt production as it is preferred by Egyptian consumers. Milk fat plays a crucial role in yogurt quality attributes, and therefore, fat reduction can cause some undesirable qualities in yogurt such as lack of flavor, weak body, and poor texture (Haque and Ji 2003). In line with the increase in consumers' demand for reduced-fat yogurt, various efforts have been made to improve the texture of low-fat variants that still is regarded as having inferior quality. To date, whey off or syneresis continues to be one of the main defects in yogurt, especially in low- and non-fat yogurt (Ozer et al. 2007). Increasing the non-fat solid level of milk and/or addition of natural or synthetic gums as stabilizers in milk are among the conventional methods that are employed to improve the yogurt texture. However, the addition of stabilizers in yogurt milk is being highly criticized in many countries, and consumers are increasingly demanding clean-label products containing very little or no additives or stabilizers. Therefore, investigations of alternative methods to achieve desirable texture of low-fat yogurt have been of interest in recent years (Gauche et al. 2009).

The enzymatic cross-linking (transglutaminase, TG) is one of the several attempts that have been carried out to overcome the problems and quality defects linked to the low-fat dairy products during the last decade. TG (EC 2.3.2.13) permits the generation of novel gel-like network structures by forming both inter- and intra-molecular isopeptide bonds in and between all milk protein types ((Bönisch et al. 2008; Jaros et al. 2010). Various reviews have demonstrated the high potential of modifying the texture properties of casein-based dairy products by means of TG cross-linking (Ozrenk 2006; Ardelean et al. 2013).

Buttermilk, a byproduct of butter making released during churning of cream, is very rich in milk fat globule membrane (MFGM), and it has been used as a natural functional ingredient in many food products. The MFGM fragments have previously been shown to carry many beneficial health effects, i.e., may inhibit colon cancer, suppress gastrointestinal pathogens, and may be involved in stress responses (Dewettinck et al. 2008; Parodi 2001).

In addition to the high nutritional value of buttermilk that is considered as health-promoting compounds (Spitsberg 2005), it has a high water holding capacity (Turcot et al. 2001) which in turn may reduce or eliminate the whey off. Accordingly, adequate addition of buttermilk may offer a promising replacement to stabilizers in low- and fat-free yogurt production. The objective of this study was to investigate the influence of buttermilk powder (BMP) addition and protein cross-linking by TG on the functional characteristics and organoleptic properties of fat-free set yogurt produced from buffalo skim milk. The performance of BMP and TG was examined either individually or combined, through a comparative evaluation to the full-fat and fat-free yogurt counterparts.

2 Material and methods

2.1 Milk, culture, BMP, and TG enzyme

Fresh buffalo milk was obtained from the Dairy Unit of Faculty of Agriculture, Cairo University. Whole milk standardized to 6.4% fat was kept as control (see Section 2.2), and the remaining amount of the milk was separated, giving skim milk of 0.2% fat. A freeze-dried yogurt culture (YO-MIX^{TM}) consisting of *Streptococcus* thermophilus and *Lactobacillus delbrueckii* subsp. bulgaricus was obtained from Danisco, 75017 Paris, France. The culture was reactivated and grown at 43 °C for 7 h followed by refrigeration overnight before yogurt manufacture. The activated starter culture was added to milk at a level of 1% (*w*/*w*).

A commercially available sweet buttermilk powder (BMP; Barry Farm Foods Co., Wapakoneta, OH 45895, USA) was used in this study. This BMP contained 34.3% protein, 5.8% fat, 3.4% moisture, 49.1% lactose, and 7.4% ash. BMP was added to raw skim milk of the respective treatments at concentration of 1 and 2% (*w*/*w*).

Activa® YG microbial transglutaminase enzyme (E.C. 2.3.2.13) was obtained from Ajinomoto Foods Europe S.A.S. (Hamburg, Germany) which had a declared specific activity of 100 $U.g^{-1}$ powder. TG was added, at a concentration of 1 $U_{TG}.g^{-1}$ milk proteins, to raw skim milk of the respective treatments for 30 min of incubation at 40 °C, prior to heat treatment of milk. The final protein levels of the individual yogurt milks were considered when calculating the level of individual TG addition.

2.2 Experimental design and yogurt preparation

Seven different yogurts were produced. The experimental design was performed to compare full-fat yogurt and fat-free yogurt as controls (without addition of TG or BMP and coded as F and S treatments, respectively) with five different fat-free yogurt consisted of the following treatment: skim milk with addition of TG, skim milk with addition of 1% BMP, skim milk with addition of 2% BMP, skim milk with addition of TG+1% BMP, and skim milk with addition of TG+2% BMP; and they were coded STG, S-BMP1, S-BMP2, STG-BMP1, and STG-BMP2, respectively. For STG-BMP1 and STG-BMP2 treatments, BMP was added simultaneously with TG to raw skim milk. The factorial design was made with two factors: replicate block (three levels) and yogurt treatment (seven levels).

All milks were heat treated at 90 °C for 10 min followed by cooling to 43 °C and inoculated with yogurt culture (1%, *w*/*w*) as described in Section 2.1. The inoculated treatments were then packed in plastic containers and incubated at 43 °C until coagulation (until pH value reaches 4.6). All yogurts were stored in the refrigerator at 5±1 °C.

2.3 Chemical and physicochemical analysis of milk

The total nitrogen content (TN%) was measured by the Kjeldahl method (International Dairy Federation (IDF) 1993). Total protein content was calculated by multiplying the TN% by 6.38. Milk fat content was determined by the Gerber

method according to (Ling 1963). Milk total solids (TS%) and ash contents were determined according to (AOAC 1990). Gross composition was conducted for yogurt milks after heat treatment step. The pH values were measured using a digital pH-meter JENWAY (JENWAY 3505, Bibby-Scientific Ltd, Staffordshire, UK). All samples were analyzed in triplicate.

2.4 Serum binding capacity

The serum binding capacity was assessed as the fraction of serum after centrifugation. Fifty grams of yogurt was filled in centrifugation tubes and centrifuged at 3,000×g at 20 °C for 15 min (HERMLE Z323K, HERMLE Labortechnik Co., Wehingen, Germany). The amount of supernatant yogurt serum was determined gravimetrically and the relation between the weights of serum m_{Serum} and original yogurt sample m_{Yogurt} (50 g) gave the serum loss SL (w/w) in percent:

$$\mathrm{SL} = \frac{m_{\mathrm{Serum}}}{m_{\mathrm{Yogurt}}} \times 100\%$$

All measurements were carried out in triplicate.

2.5 Scanning electron microscopy

From the yogurt stored overnight at 5±1 °C, small cylindrical pieces (approximately 3 mm in diameter and height) from the center of the yogurt container were prepared as described by Romeih et al. (2012). After drying to critical point using CO_2 in a Critical Point Dryer (Polaron, Waterford, England), samples were then mounted on aluminum scanning electron microscopy (SEM) stubs and sputter-coated with gold (SPI module sputter coater, SPI supplies division of structure probe). Samples were examined at 25 kV with the use of a scanning electron microscope (JEOL–jsm 5200, Faco Europe Sarl, 84120 Pertuis, France) at a magnification of 1500x. SEM analysis was performed for the third replicate block of this work.

2.6 Sodium dodecyl sulfate polyacrylamide gel electrophoresis

The possible formation of protein cross-links was analyzed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) technique according to (Laemmli 1970). SDS-PAGE under reducing conditions was carried out by a Mini-Protean II unit (Bio-Rad Laboratories Ltd., Hercules, CA, USA). Gels were stained with Coomassie Brilliant Blue (Sigma-Aldrich, UK). Protein classes were determined according to their molecular weight by comparison with a molecular weight marker. SDS-PAGE analysis was performed for the third replicate block of this work.

2.7 Sensory evaluation

Organoleptic assessment of the yogurt was carried out by a seven-member panel of the Dairy Science Department's staff selected on the basis of interest and experience in sensory evaluation of yogurt and fermented milks. The panel was asked to evaluate the coded samples of the seven yogurt treatments using a graduated scale from 1 to 9 (1 for

extremely undesirable to 9 for extremely desirable) for appearance, acidity, consistency, and overall acceptability. Panel members were also instructed to report any defects of sensory characteristics for the yogurt samples (e.g., lumpiness, bitterness, yeasty flavor, whey off). All samples were served within random order in plastic cups after overnight storage at 5±1 °C.

2.8 Statistical analysis

Data were analyzed by one-way analysis of variance (ANOVA), followed by assessment of differences by LSD post hoc test. All statistical calculations were performed using MSTAT-C (ver. 2.10, Michigan State University, USA), and significant differences ($P<0.05$) between treatments were determined.

3 Results and discussion

3.1 Chemical characterization

The average compositions of yogurt milk treatments after heat treatment are given in Table 1. The gross composition of yogurt milk samples was in conformity with the legal Egyptian standard for yogurt. The total solids of fat-free yogurt milk samples were significantly ($P<0.05$) reduced as a result of skimming. However, the addition of TG had no significant ($P>0.05$) effect on the chemical composition of fat-free yogurt milk samples. Farnsworth et al. (2006) have reported similar impact of TG on milk chemical composition. In contrast, the addition of BMP increased the protein, total solids, and ash contents in skim milk samples significantly ($P<0.05$), which correlated to its relatively high levels of protein, lactose, and ash (Section 2.1). Also, increasing BMP concentration was expected to cause similar trend of significant changes ($P<0.05$) in chemical composition.

Table 1 Chemical composition of buffalo milks used in the manufacture of the different full-fat and experimental fat-free yogurts

	%Fat	%Protein	%TS	%Ash	pH*	Serum loss %
F	$6.33^{a}\pm0.12$	$3.87^{d}\pm0.09$	$17.18^{a}\pm0.16$	$0.78^{d}\pm0.02$	$4.44^{a}\pm0.07$	$5.29^{f}\pm0.23$
S	$0.23^{b}\pm0.06$	$4.00^{cd}\pm0.10$	$11.17^{d}\pm0.17$	$0.83^{c}\pm0.01$	$4.45^{a}\pm0.08$	$18.03^{a}\pm0.58$
STG	$0.23^{b}\pm0.06$	$4.13^{c}\pm0.08$	$11.15^{d}\pm0.08$	$0.83^{c}\pm0.01$	$4.44^{a}\pm0.06$	$8.73^{d}\pm0.21$
S-BMP1	$0.23^{b}\pm0.06$	$4.47^{b}\pm0.09$	$12.28^{c}\pm0.10$	$0.89^{b}\pm0.01$	$4.31^{a}\pm0.08$	$13.25^{b}\pm0.71$
S-BMP2	$0.27^{b}\pm0.06$	$5.00^{a}\pm0.08$	$13.36^{b}\pm0.13$	$0.96^{a}\pm0.02$	$4.32^{a}\pm0.05$	$10.54^{c}\pm0.14$
STG-BMP1	$0.23^{b}\pm0.06$	$4.61^{b}\pm0.10$	$12.19^{c}\pm0.11$	$0.90^{b}\pm0.01$	$4.32^{a}\pm0.07$	$6.74^{e}\pm0.15$
STG-BMP2	$0.27^{b}\pm0.06$	$5.15^{a}\pm0.09$	$13.37^{b}\pm0.06$	$0.96^{a}\pm0.01$	$4.33^{a}\pm0.08$	$5.58^{f}\pm0.11$
LSD	0.056	0.153	0.106	0.011	0.161	0.309

Values are means of triplicate analyses of three individual milk samples ($n=9$), ±standard deviation. Means with different superscripts in the same column are significantly different ($P<0.05$) *LSD* least significant difference * pH values of all experimental yogurt samples after 1-day storage at 5±1 °C

3.2 Changes in pH during fermentation

The reduction of pH of all experimental yogurt samples during incubation at 43 °C is shown in Fig. 1 and the pH values after 1 day of storage at 5±1 °C are presented in Table 1. The incubation of yogurt samples was terminated when pH 4.6 was attained. The control yogurts (F and S testaments) reached the target pH within 270±7 min. The addition of TG had no effect on fermentation time as indicated from the fermentation curve and pH drop of STG treatment. This finding is in agreement with that of Schey (2003) and Bönisch et al. (2007) who demonstrated no interference of TG with starter bacteria during fermentation of yogurt and no differences in fermentation time as a result of TG addition. Additionally, Ozer et al. (2007) concluded that it is unlikely that the TG interfered with the pathway of lactic acid metabolism in any way.

However, compared with the control yogurts, the pH reduction of fat-free yogurt with BMP addition (S-BMP1, S-BMP2, STG-BMP1, and STG-BMP2 treatments) was slightly faster even though those treatments showed slightly higher initial pH values. The fermentation curves of these four fat-free yogurt treatments showed a typical decrease in pH from 6.8 to 4.6 within 255±5-min fermentation time. This result is in accordance with that of Le at al. (Le et al. 2011). The slightly faster drop in pH that also represented in shorter fermentation time (~15 min less) compared to the reference yogurts may be attributed to the availability of low molecular weight peptides and/or amino acids, provided by buttermilk protein content, that are required by *S. thermophilus* for its growth (Trachoo and Mistry 1998). Furthermore, the increased amount of milk fat globule membrane in buttermilk showed an improvement in the growth and metabolism of starter bacteria used in Cheddar cheese ripening as discussed by Martinovic et al. (2013). However, the impact of buttermilk components on the pathway of culture metabolism needs further investigation.

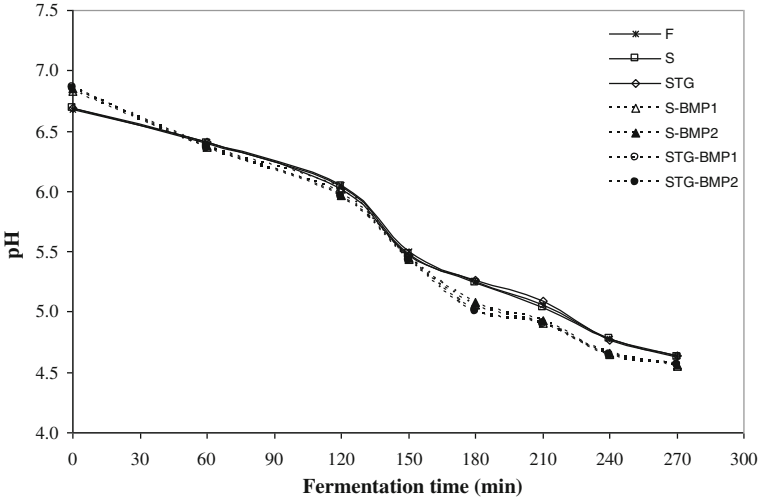

Fig. 1 Decrease in pH values of full-fat and fat-free experimental yogurts during fermentation. Values are means of triplicate yogurt batches

3.3 Serum binding capacity

The centrifugally forced serum loss percentage (SL%) was measured to monitor the water holding capacity of the modified yogurt gel as a function of TG and/or BMP addition. As it can be seen from the data of Table 1, the control fat-free yogurt treatment shows the highest SL% with almost 3.5-fold as the full-fat control yogurt, reflecting the crucial role of milk fat in yogurt gel functionality. As shown in Table 1, the addition of TG to fat-free milk (STG treatment) significantly ($P<0.05$) improved serum binding capacity and reduced serum loss by 51.6% compared with the base value of fat-free reference sample. This finding is in agreement with that of Schorsh et al. (2000) and Ozer et al. (2007). The cross-linking of milk proteins triggered by TG leads to a stabilization of the three-dimensional network and a decrease in yogurt gel permeability which in turn prevent yogurt whey expulsion (Lorenzen et al. 2002). Decrease in yogurt gel permeability causes a more compact microstructure with smaller pores embedded in clusters of protein, and consequently, more water is entrapped in the yogurt gel network (Moon and Hong 2003). This will be further explored in the scanning electron microscope section of this paper (see Section 3.5).

Table 1 shows that all fat-free yogurt manufactured with addition of BMP had also significantly ($P<0.05$) less SL% compared to the control fat-free yogurt. Extent of SL% reduction increased with increasing BMP concentration. Fat-free yogurt of S-BMP1 and S-BMP2 samples showed reduction in SL% by 26.5 and 41.5\%, respectively, in relation to the control fat-free sample. This is mainly attributed to the increased hydration capacity of buttermilk components with particular respect to its protein and phospholipid contents (Le et al. 2011; Romeih et al. 2012). Phospholipids are known to have high water holding capacity due to their amphiphilic characteristic (Morin et al. 2008). These results are in accordance with those of Trachoo and Mistry (1998) and Turcot et al. (2002). Nevertheless, it should be noted that the serum binding capacity of yogurt gel resulting from BMP addition (S-BMP1 and S-BMP2) was significantly ($P<0.05$) lower than that of TG addition (STG), reflecting the excessive influence of modified protein network, i.e., cross-linking protein aggregates and formation of high molecular protein polymers resulted by TG (Myllärinen et al. 2007), on yogurt serum binding.

In this context, the interaction effect of addition of TG simultaneously with BMP (STG-BMP1 and STG-BMP2) showed an extensive impact and significantly ($P<0.05$) extended reduction of SL% in comparison to the addition of TG or BMP individually to fat-free yogurts (STG, S-BMP1, and S-BMP2). This finding would be expected, since the buttermilk powder contains considerable protein content (34.3%) which in turn promotes the TG reactivity. This interaction was apparent in free-yogurt samples with high BMP addition (STG-BMP2) which showed no significant difference ($P>0.05$) in SL% compared to the full-fat yogurt (Table 1).

3.4 Electrophoresis of the yogurts

The SDS electrophoresis profile was performed to evaluate the polymerization extent of the milk protein chains as a function of TG and/or BMP addition. Figure 2 distinctly shows the influence of TG on molecular mass of milk proteins of 1-day-old yogurts,

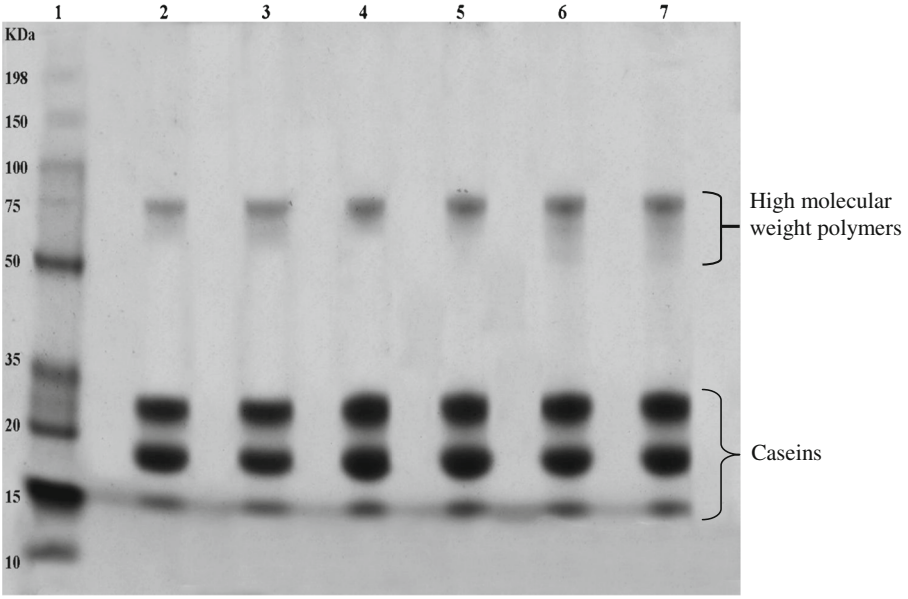

Fig. 2 SDS-PAGE gel of the experimental fat-free yogurts. Lanes: (*1*) molecular weight marker (KDa), (*2*) fat-free control yogurt, (*3*) fat-free yogurt + TG, (*4*) fat-free yogurt + BMP 1%, (*5*) fat-free yogurt + BMP 2%, (*6*) fat-free yogurt + TG + BMP 1%, and (*7*) fat-free yogurt + TG + BMP 2%

which is represented by the formation of high molecular weight (HMW) protein polymer bands (lanes 3, 6, and 7), compared with the expected HMW protein bands that appeared in all yogurt treatments as a result of excessive thermal treatment of milk (Lucey et al. 1999). However, it is worth to note that there are no bands representing the whey proteins within all treatments, which is most probably attributed to the denaturation effect caused by the thermal treatment used in this study. This result is in close agreement with the observation of Tsevdou et al. (2013) for set yogurt manufactured from thermally and high-pressure-processed milk.

Moreover, the SDS-PAGE patterns (Fig. 2) clearly revealed that the addition of BMP resulted a noticeable increase in the caseins band sizes (S-BMP1, S-BMP2, STG-BMP1, and STG-BMP2 treatments; lanes 4–7), which is attributed to their relatively higher protein contents of these yogurts (Table 1). In this context, Lopez et al. (2007) have stated that cross-links can be formed between MFGM material and the casein matrix. Likewise, it has been reported that excessive heat treatment of milk might promote interactions between MFGM proteins with both milk caseins and whey proteins (Morin et al. 2007). It is worth to mention that the intensity of HMW polymers bands was more pronounced in fat-free yogurt manufactured with TG combined with BMP additions (lanes 6 and 7) than those of individually BMP addition (lanes 4 and 5). This result indicates susceptibility of BMP materials toward TG cross-linking as BMP consisted of 34.3% protein (Section 2.1). These findings are in correlation with the serum binding capacity results (Section 3.3). In a previous report, Hinz et al. (2007) has stated that the milk fat was not interfered or affected by the extent of the TG cross-linked milk proteins; therefore, the full-fat yogurt sample was excluded from the SDS-PAGE analysis of the present work.

3.5 Microstructure

The impacts of TG and/or BMP addition on microstructure of the experimental yogurts are shown in Fig. 3. The protein matrix (gray area) formed a continuous phase permeated by an amorphous system of voids filled with serum (black area), which in turn revealed the spatial dimensions of these images. The micrographs show obvious variations in the yogurt microstructural properties between full-fat and fat-free treatments (Fig. 3a, b). An extremely porous, open, and sponge-like structure free of fat globules was obtained in fat-free yogurt, whereas a continuous phase of protein aggregate network characterized by a more compacted and dense structure accompanied by less voids was revealed in the full-fat yogurt where the spherical fat globules were obviously dispersed throughout the protein matrix.

As it might be seen in Fig. 3c, the addition of TG (STG yogurt) promoted regularly aggregated protein matrices characterized by a finer-meshed network accompanied by small pores that was much smaller in size compared to that of fat-free control yogurt (Fig. 3b). Moreover, noticeable clusters of cross-linked strands and fibrous-like protein aggregates (black arrows) were observed, indicating the cross-linking impact of TG. This finding is consistent with the observations of Schorsch et al. (2000) and Myllärinen et al. (2007) who have stated that TG can improve the homogeneity of caseinate gel structure and promotes the interconnectivity of the network.

The manifested microstructure in Fig. 3 clearly reveals that the fat-free yogurt manufactured with BMP (Fig. 3d, e, f, g) exhibited a compact and dense structure accompanied by irregularly clustered protein folds (pointed with white arrows). The relatively high levels of fused proteins and the increased agglomerated clusters obtained by addition of BMP were most probably attributed to its high levels of MFGM components as well as total protein content. Lopez (2005) has stated that milk caseins are able to associate with the fat globule membrane, forming a protein layer which in turn enables the newly formed phase to behave as pseudo-protein particles, becoming an integral part of the protein matrix during coagulation. Moreover, it has been reported that MFGM fragments could induce direct physical and chemical interactions with casein (CN) by folding CN micelles inside reconstituted aggregates (Morin et al. 2008; Ong et al. 2010). A closer observation of the microstructure details in these micrographs revealed that the impact of BMP on yogurt microstructure characteristics was more pronounced and further intensified by the combined TG-BMP addition as illustrated in SEM micrographs of STG-BMP1 and STG-MBP2 yogurt treatments (Fig. 3f, g, respectively). These structural modifications could be attributed to the induced capability of protein cross-linking by TG, which resulted from the relatively high contents of protein and MFGM components in BMP. These microstructural characteristics are in conformity with the polymerization reaction and cross-linking activities obtained by SDS-PAGE analysis, which may be considered together, in explaining the results trend of serum binding capacity measurement (SL%).

3.6 Sensory evaluation

A comparison of the sensory data for the seven experimental yogurts after 1 day of storage at refrigerator is given in Table 2. Fat-free control yogurt (S treatment) received the lowest ($P<0.05$) preference values in all organoleptic attributes, reflecting the

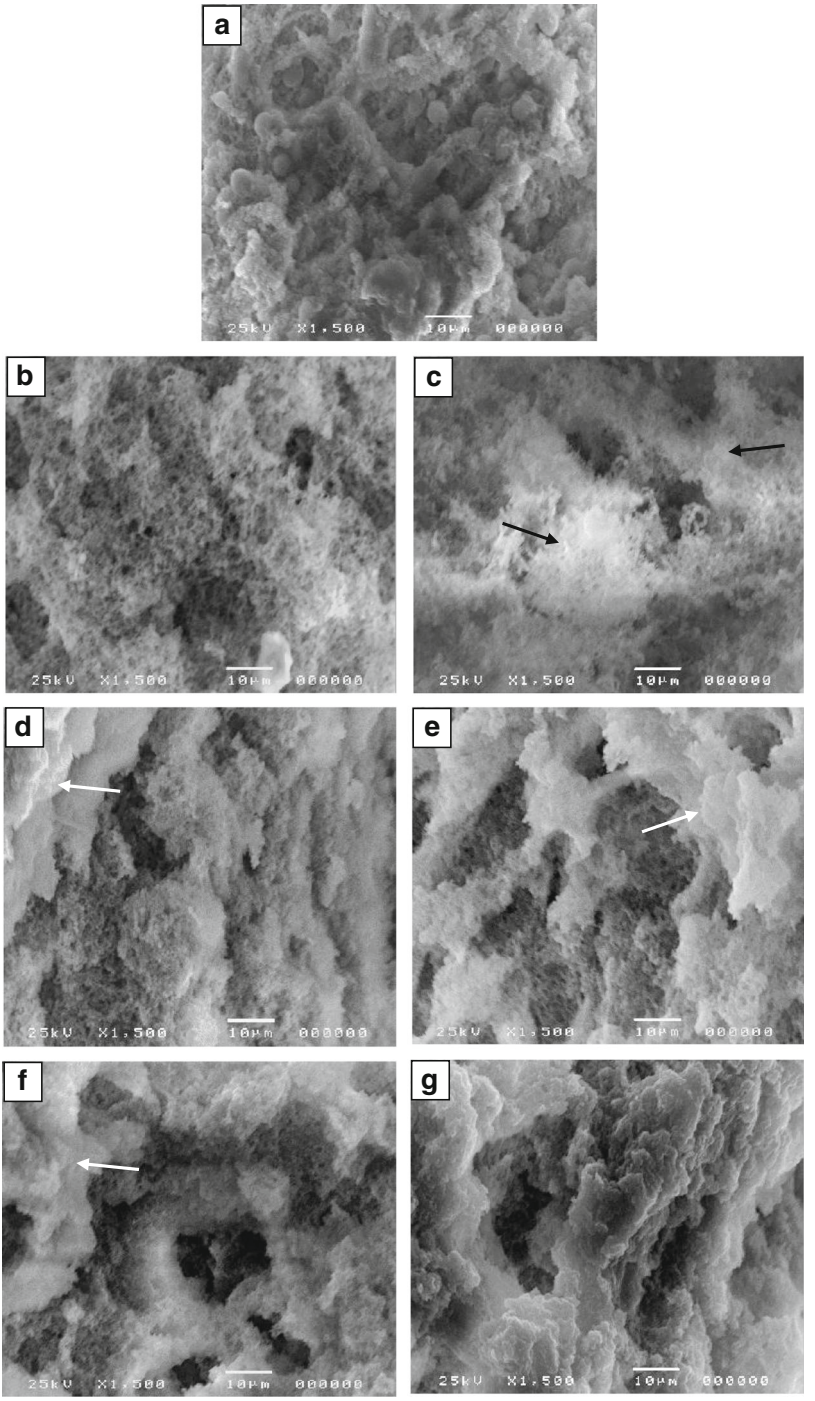

Fig. 3 Scanning electron micrographs of full-fat and fat-free experimental yogurts; **a** full-fat control yogurt, **b** fat-free control yogurt, **c** fat-free yogurt + TG, **d** fat-free yogurt + BMP 1%, **(e)** fat-free yogurt + BMP 2%, **f** fat-free yogurt + TG + BMP 1%, and **g** fat-free yogurt + TG + BMP 2%. *Black arrows* indicate fibrous-like protein aggregates and *white arrows* indicate clusters of protein folds. Scale bar is 10 μm

Table 2 Effect of TG and/or BMP addition on yogurts at day 1 of cold storage as indicated by the analysis of variance from data obtained by a seven-member evaluation panel

	Appearance	Acidity	Consistency	Overall acceptability
F	$8.48^{a}\pm0.15$	$8.42^{a}\pm0.21$	$8.29^{a}\pm0.10$	$8.54^{a}\pm0.21$
S	$6.92^{c}\pm0.92$	$7.21^{c}\pm0.46$	$6.79^{c}\pm0.36$	$6.63^{d}\pm0.38$
STG	$7.78^{b}\pm0.58$	$7.35^{c}\pm0.98$	$7.72^{b}\pm0.32$	$7.81^{b}\pm0.69$
S-BMP1	$8.18^{ab}\pm0.08$	$8.06^{ab}\pm0.16$	$8.11^{a}\pm0.15$	$8.28^{a}\pm0.51$
S-BMP2	$8.21^{ab}\pm0.04$	$7.98^{ab}\pm0.32$	$8.23^{a}\pm0.29$	$8.42^{a}\pm0.42$
STG-BMP1	$8.03^{ab}\pm0.50$	$7.57^{bc}\pm0.58$	$7.73^{b}\pm0.60$	$7.42^{c}\pm0.42$
STG-BMP2	$8.07^{ab}\pm0.27$	$7.65^{bc}\pm0.46$	$7.88^{b}\pm0.20$	$7.33^{c}\pm0.29$
LSD	0.70	0.51	0.22	0.27

Values are means of triplicate analyses of three individual milk samples (n=21), ±standard deviation. Means with different superscripts in the same column are significantly different (P<0.05) *LSD* least significant difference

generally recognized negative effect of fat reduction in yogurt milk. Moreover, fat-free control yogurt exhibited brittle and weak structure with observed whey off on yogurt surface as described by the panelists' notes. In contrast, TG and/or BMP additions clearly presented a positive impact on all sensory perceptions of their respective fat-free yogurts, which were characterized by smooth texture, no whey off and white shining surface as described by the assessors.

It is interesting to note that the fat-free yogurts made with only BMP addition exhibited the highest (P<0.05) perceived body consistency and overall acceptability among all experimental fat-free yogurts and were not judged significantly different (P>0.05) from that of the full-fat counterpart (F treatment). Nevertheless, BMP concentration had no significant effect (P>0.05) on the organoleptic parameters evaluated in this study. These findings are in agreement with those of Faergemand et al. (1999), Lorenzen et al. (2002), and Ozer et al. (2007) for yogurt made from TG-treated milk, as well as, Trachoo and Mistry (1998) for yogurt made with ultrafiltered sweet buttermilk. It is worth to add that S-BMP1 and S-BMP2 treatments exhibited creamy mouth-feel and a homogenous good texture as described by the assessors, and showed the most desirable organoleptic attributes among all fat-free treatments (Table 2). Whereas, fat-free yogurts made with combined TG-BMP were described as excessively firm texture with crumbly mouth-feel, which could be attributed to the relatively high protein content and the excessive cross-linking and polymerization extent of milk proteins obtained by TG action.

4 Conclusion

In this study, it has been shown that TG did not interfere with the pH development of yogurt, whereas the addition of BMP decreased the fermentation time. Moreover, TG appeared to be an effective means of improving the water holding capacity of fat-free yogurt gel, and its impact was even higher than those obtained by individual BMP additions. However, the combined effect of TG-BMP addition revealed an extensive

serum loss reduction in fat-free yogurt, which was resembled to that of the full-fat counterpart. Furthermore, the combined TG-BMP addition showed intensive high molecular weight polymer bands as a result of higher protein cross-linking. The SEM analysis confirmed these findings through the presence of a much more compact and dense protein network accompanied by clusters of cross-linked strands attached to protein matrices. The protein network was further agglomerated into irregular clusters of protein folds within the structure of fat-free yogurt added both TG and BMP. TG as well as BMP had markedly altered the organoleptic properties of the fat-free yogurt. Nevertheless, fat-free yogurt with only BMP added received the best sensory attributes score as indicated by the assessors and was similar in quality to the full-fat yogurt.

In general, the addition of BMP offers a promising option to develop novel functional fat-free yogurt with health-promoting compounds. Also, cross-linking of milk proteins by means of TG appeared to be an acceptable alternative to overcome the inferior quality of fat-free variants. However, the addition of TG combined with BMP is not recommended by the organoleptic assessors, which could mainly be attributed to the excessive cross-linking and polymerization of the milk proteins.

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References

AOAC (1990) Association of official analytical chemists. Official methods of analysis, 15th edn. Association of Anal. Chem INC Suite 400, 2200, Wilson Boulevard, Arlington, Virginia 22201, USA

Ardelean AI, Jaros D, Rohm H (2013) Influence of microbial transglutaminase cross-linking on gelation kinetics and texture of acid gels made from whole goats and cows milk. Dairy Sci Technol 93:63–71

Bönisch MP, Lauber S, Kulozik U (2007) Improvement of enzymatic cross-linking of casein micelles with transglutaminase by glutathione addition. Int Dairy J 17:3–11

Bönisch MP, Heidebach HC, Kulozik U (2008) Influence of transglutaminase protein cross-linking on the rennet coagulation of casein. Food Hydrocoll 22:288–297

Dewettinck K, Rombaut R, Thienpont N, Le TT, Messens K, Van-Camp J (2008) Nutritional and technological aspects of milk fat globule membrane material. Int Dairy J 18:436–457

Elagamy EI (2003) Properties of milk and milk fats from species other than cow. In: Rossell B (ed) Oils and Fats, Vol 3 - Dairy Fats, Leatherhead International Ltd, Leatherhead, UK

Faergement M, Sorensen MV, Jorgensen U, Budolfsen G, Qvist KB (1999) Transglutaminase: effect on instrumental and sensory texture of set style yogurt. Milchwissenschaft 54:563–566

Farnsworth JP, Li J, Hendricks GM, Guo MR (2006) Effects of transglutaminase treatment on functional properties and probiotic culture survivability of goat milk yogurt. Small Rumin Res 65:113–121

Gauche C, Tomazi T, Barreto PLM, Ogliari PJ, Bordignon-Luiz MT (2009) Physical properties of yogurt manufactured with milk whey and transglutaminase. LWT Food Sci Technol 42:239–243

Haque ZU, Ji T (2003) Cheddar whey processing and source: II. Effect on non-fat ice cream and yogurt. Int J Food Sci Technol 38:463–473

Hinz K, Huppertz T, Kulozik U, Kelly AL (2007) Influence of enzymatic cross-linking on milk fat globules and emulsifying properties of milk proteins. Int Dairy J 17:289–293

IDF (1993) Milk-determination of nitrogen content (Kjeldahl method). Standard 20B, International Dairy Federation, Brussels, Belgium

Jaros D, Jacob M, Otto C, Rohm H (2010) Excessive cross-linking of caseins by microbial transglutaminase and its impact on physical properties of acidified milk gels. Int Dairy J 20:321–327

Laemmli U (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227:680–685

Le TT, Camp J, Pascual PAL, Meesen G, Thienpont N, Messens K, Dewettinck K (2011) Physical properties and microstructure of yogurt enriched with milk fat globule membrane material. Int Dairy J 21:798–805
Ling ER (1963) text book of dairy chemistry. Vol. 2 3rd edn. Chapman & Hall, London
Lopez C (2005) Focus on the supramolecular structure of milk fat in dairy products. Reprod Nutr Dev 45:497–511
Lopez C, Camier B, Gassi JY (2007) Development of the milk fat microstructure during the manufacture and ripening of Emmental cheese observed by confocal laser scanning microscopy. Int Dairy J 17:235–247
Lorenzen PC, Neve H, Mautner A, Schlimme E (2002) Effect of enzymatic cross-linking of milk proteins on functional properties of set-style yogurt. Int J Dairy Technol 55:152–157
Lucey JA, Munro PA, Singh H (1999) Effects of heat treatment and whey protein addition on the rheological properties and structure of acid skim milk gels. Int Dairy J 9:275–279
Martinovic A, Moe KM, Romeih E, Aideh B, Vogensen FK, Østlie H, Skeie S (2013) Growth of adjunct *Lactobacillus casei* in Cheddar cheese differing in milk fat globule membrane components. Int Dairy J 31: 70–82
Moon JH, Hong YH (2003) Electron microscopic property of transglutaminase added milk. J Food Sci Anim Resour 23:350–355
Morin P, Jimenez-Flores R, Pouliot Y (2007) Effect of processing on the composition and microstructure of buttermilk and its milk fat globule membranes. Int Dairy J 17:1179–1187
Morin P, Pouliot Y, Britten M (2008) Effect of buttermilk made from creams with different heat treatment histories on properties of rennet gels and model cheeses. J Dairy Sci 91:871–882
Myllärinen P, Buchert J, Autio K (2007) Effect of transglutaminase on rheological properties and microstructure of chemically acidified caseinate gels. Int Dairy J 17:800–807
Ong L, Dagastine RR, Kentish SE, Gras SL (2010) The effect of milk processing on the microstructure of the milk fat globule and rennet induced gel observed using confocal laser scanning microscopy. J Food Sci 75:135–145
Özer BH, Kırmacı HA, Öztekin S, Hayalo GA, Atamer M (2007) Incorporation of microbial transglutaminase into non-fat yogurt production. Int Dairy J 17:199–207
Özrenk E (2006) The use of transglutaminase in diary products. Int J Dairy Technol 59:1–7
Parodi PW (2001) Cow's milk components with anti-cancer potential. Aust J Dairy Technol 56:65–73
Romeih EA, Moe KM, Skeie S (2012) The influence of fat globule membrane material on the microstructure of low-fat Cheddar cheese. Int Dairy J 26:66–72
Schey A (2003) Texture improvement of fermented dairy products by enzymatic crosslinking with transglutaminase. IDF seminar on aroma and texture of fermented milks, Kolding, Denmark, 371–375
Schorsch C, Carrie H, Norton IT (2000) Cross-linking casein micelles by a microbial transglutaminase: influence of cross-links in acid-induced gelation. Int Dairy J 10:529–539
Spitsberg VL (2005) Bovine milk fat globule membrane as a potential nutraceutical. J Dairy Sci 88:2289–2294
Trachoo N, Mistry VV (1998) Application of ultrafiltered sweet buttermilk and sweet buttermilk powder in the manufacture of nonfat and low fat yogurts. J Dairy Sci 81:3163–3171
Tsevdou MS, Eleftheriou EG, Taoukis PS (2013) Transglutaminase treatment of thermally and high pressure processed milk: effects on the properties and storage stability of set yogurt. Innov Food Sci Emerg 17: 144–152
Turcot S, Turgeon SL, St-Gelais D (2001) Effect of buttermilk phospholipid concentrations in cheese milk on production and composition of low-fat Cheddar cheese. Dairy Sci Technol 81:429–442
Turcot S, St-Gelais D, Turgeon SL (2002) Ripening of low-fat Cheddar cheese made from milks enriched with phospholipids. Dairy Sci Technol 82:209–223