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PERFORMANCE OF MICROBIAL TRANSGLUTAMINASE AND BUTTERMILK POWDER ON FUNCTIONAL PROPERTIES OF FREE-FAT SET YOGHURT

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ABSTRACT

Free-fat yoghurt manufactured from skim milk treated with transglutaminase (TG; 1 $Ug^{-1}_{\text{milk protein}}$) and buttermilk powder (BMP; 1% and 2%); either individually or in combination, was investigated. For comparison, the full-fat and free-fat variants without TG or BMP addition were also studied. The results reveal that TG did not interfere with the pH reduction during fermentation progress, while BMP addition had fairly accelerated the pH drop during incubation time. Although TG treatment or BMP addition has significantly (P<0.05) improved the yoghurt gel strength by means of improving the water holding capacity functionality, the free-fat yoghurts made from TG-treated milk in combination with BMP addition obtained the highest values which were similar to that of full-fat control yoghurt. Furthermore, addition of BMP enhanced the reactivity of TG as indicated by the extended and excessive appearance of high molecular weight protein polymers bands in electrophoreses patterns. This result was confirmed by scanning electron microscope (SEM) analysis as a more compact and dense structure accompanied by tortuous clusters of protein aggregates were observed within micrographs of free-fat yoghurt made with combination of TG and BMP. Addition of TG or BMP individually had a marked affirmative impact on free-fat gel network, represented in a denser and more homogeneous systematic protein aggregate network characterised by a finer-meshed network. It is worthwhile to state that this impact was more pronounced within BMP addition (either 1% or 2%) than for TG treatment. Free-fat yoghurts of individually BMP addition exhibited the most desirable organoleptic attributes as indicated by the assessors and were similar to the full-fat yoghurt perception, whereas, the combined TG-BMP treatments received fairly criticized scores due to its firmer and crumbly mouth-feel. Overall, addition of TG or BMP appears to be a valuable alternative in free-fat yoghurt production, and BMP can be worthy considered as a source of extra protein level, which in-turn offers a promising option to develop innovative functional free-fat yoghurt.

Keywords: Free-fat yoghurt, transglutaminase, cross-linking, buttermilk, microstructure.

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 Dairy products sector that possesses a vital role in public daily diet. Yoghurt is the most popular fermented milk produced in Egypt and worldwide, due to its known nutrients that promote health and enhance the immune system. Any method disturbing the balance among the milk components has a direct effect on the textural/rheological properties of yogurt. As milk fat plays a crucial role in yoghurt quality attributes, therefore, fat reduction can cause some defects 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 the reduced-fat yoghurt, various efforts have been made to improve the texture of low-fat variants that still regarded as having inferior quality. To date, wheying-off or syneresis continues to be one of the main issues in yoghurt production, especially in low- and non-fat yoghurt (Ozer et al., 2007). Increasing non-fat solids level of milk and/or addition of natural or synthetic gums as stabilizers into milk are among the conventional methods that are employed to improve the yoghurt texture. However, since addition of stabilizers into yoghurt milk is being highly criticised in many countries, investigations of alternative methods of achieving satisfying texture of low-fat yoghurt have been of interest in recent years (Gauche et al., 2009).

The enzymatic cross-linking (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 to generate 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 demonstrate the high potential of modifying the texture properties of casein-based dairy products by means of TG cross-linking (De Jong and Koppelman, 2002; Ozrenk, 2006)

Buttermilk, a byproduct of butter making released during churning of cream, is very rich in milk fat globule membrane (MFGM), and has been used as 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; Spitsberg, 2005).

Consumers are increasingly demanding clean-label products containing very little or no additives or stabilizers. The addition of buttermilk does not only give an added nutritional value and health promotion compounds, but also approved to reduces the wheying-off which may eliminate the need for using other stabilizers for low- and free-fat yoghurt production. Accordingly, in the study presented herein, the influence of protein cross-linking by means of TG and the addition of deferent concentration of buttermilk powder (BMP) on the functional characteristics and organoleptic properties of free-fat yoghurt, was investigated. These two means (TG and BMP) have been proposed to overcome the inferior quality attributes of free-fat yoghurt were explored either individually or by their interactions.

MATERIALS AND METHODS

Fresh buffalo milk was obtained from the Dairy Unit of Faculty of Agriculture, Cairo University. A quantity of whole milk standardized to 6.4 % fat was taken as control, and the remaining amount of the milk was subjected to separation giving skim milk of 0.2 % fat. A freeze-dried yoghurt culture (YO-MIX^{TM}) consists of *Str. thermophilus* and *Lb. 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 yoghurt 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, Ohio 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. Protein content of BMP was considered through the preparation of TG powder addition within their respective experiments.

Yoghurt preparation was carried out in seven treatments. The experimental design was performed to compare full-fat yoghurt and fat-free yoghurt as controls (without addition of TG or BMP and coded as F and S treatments, respectively) with five different fat-free yoghurt 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; where are represented in codes 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 (3 levels), and yoghurt treatment (7 levels). All milks were heat treated at 90ºC/10 min, followed by cooling to 43ºC and inoculated by yoghurt culture (1 %, w/w) as described in above. The inoculated treatments were then packed in plastic cups and incubated at 43ºC until coagulation (till pH value reaches 4.6). All yoghurt treatments were stored in the refrigerator at 5 ± 1ºC.

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 yoghurt milks after heat treatment step. The pH values were estimated using a digital pH-meter JENWAY (JENWAY 3505, Bibby-Scientific Ltd, Staffordshire, UK). All samples were analyzed in triplicate.

The serum binding capacity was assessed as the fraction of serum after centrifugation. 50 g yoghurt was filled in centrifugation tubes and centrifuged at 3000 $_g$ at 20 ºC for 15 min (HERMLE Z323K, HERMLE Labortechnik Co., Wehingen, Germany). The amount of supernatant yoghurt serum was determined gravimetrically and the relation between the weights of serum m_{Serum} and original yoghurt sample $m_{Yoghurt}$ (50 g) gives the serum loss SL (w/w) in percent:

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

All measurements were carried out in triplicate.

Scanning Electron Microscopy (SEM): from the yoghurt stored overnight at refrigerator of the third experimental batch, small cylindrical pieces (approximately 3mm in diameter and height) were prepared from the center of the yoghurt cup within at least 2 cm deep of yoghurt gel. The protein was fixed overnight in 4% (v/v) glutaraldehyde in 0.1 M sodium cacodylate buffer. Samples were washed several times in 0.1 M sodium cacodylate buffer for 15 min intervals, then post fixed in 2% (w/v) osmium tetraoxid (OsO_4) in 0.1 M sodium cacodylate for 1-2 hr for fat fixation. The yoghurt cylinders were re-washed several times in 0.1 M sodium cacodylate buffer for 15 min intervals, followed by dehydration in increasing concentrations of aqueous ethanol solutions (25%, 50%, 75%, 90% and 100%, 15 min in each). Samples were then dried to critical point using CO_2 in a Critical Point Dryer (Polaron, Waterford, England), and mounted on aluminum SEM stubs, sputter-coated with gold (Spi module sputter coater, spi supplies division of structure probe.). Samples were examined at 25 KV through scanning electron microscope (JEOL–jsm 5200, Faco Europe Sarl, 84120 Pertuis, France) and magnification of 1500x.

The possible formation of protein cross-links was analyzed by Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis **(**SDS-PAGE) according to (Laemmli, 1970). SDS-PAGE under reducing conditions was carried-out by a Mini-Protean II unit (Bio-Rad Laboratories Ltd., Hercules, California, 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 within the third experimental batch of this work.

Organoleptic assessment of the yoghurt was carried out within the three replicate batches by a seven-member panel of the Dairy Science Department's staff selected on the basis of interest and experience in sensory evaluation of yoghurt and fermented milks. The panel was asked to evaluate the coded samples of the seven yoghurt 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 yoghurt samples (e.g. lumpiness, bitterness; yeasty flavor, whey off). All samples were served within randomly order in plastic cups after overnight storage at refrigerator.

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 ($P \leq 0.05$) between treatments were determined.

RESULTS AND DISCUSSION

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

The reduction of pH of all experimental yoghurt samples during incubation at 43ºC and after 1 day of storage at refrigerator are shown in Fig. 1. The incubation of yoghurt samples was terminated when pH 4.6 was attained. The control yoghurts (F and S testaments) reached the target pH within 270 ± 7 min. Addition of cross-linking enzyme has 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 that no interference of TG with starter bacteria during fermentation of yoghurt, and no differences in fermentation time were observed 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.

Table (1). Chemical composition of buffalo milks used in the manufacture of full-fat and experimental free-fat yoghurt treatments.

	%Fat	%Protein	%TS	%Ash	Serum loss %
F	$6.33^{a} \pm 0.12$	$3.87^{d} \pm 0.09$	$17.18^{a} \pm 0.16$	$0.78^{d} \pm 0.02$	$5.29^{f} \pm 0.23$
S	$0.23^{b} \pm 0.06$	$4.00^{cd} \pm 0.10$	$11.17^{d} \pm 0.17$	$0.83^{c} \pm 0.01$	$18.03^{a} \pm 0.58$
STG	$0.23^{b} \pm 0.06$	$4.13^{c} \pm 0.08$	$11.15^{d} \pm 0.08$	$0.83^{c} \pm 0.01$	$8.73^{d} \pm 0.21$
S-BMP1	$0.23^{b} \pm 0.06$	$4.47^{b} \pm 0.09$	$12.28^{c} \pm 0.10$	$0.89^{b} \pm 0.01$	$13.25^{b} \pm 0.71$
S-BMP2	$0.27^{b} \pm 0.06$	$5.00^{a} \pm 0.08$	$13.36^{b} \pm 0.13$	$0.96^{a} \pm 0.02$	$10.54^{c} \pm 0.14$
STG-BMP1	$0.23^{b} \pm 0.06$	$4.61^{b} \pm 0.10$	$12.19^{c} \pm 0.11$	$0.90^{b} \pm 0.01$	$6.74^{e} \pm 0.15$
STG-BMP2	$0.27^{b} \pm 0.06$	$5.15^{a} \pm 0.09$	$13.37^{b} \pm 0.06$	$0.96^{a} \pm 0.01$	$5.58^{f} \pm 0.11$
LSD	**0.056**	**0.153**	**0.106**	**0.011**	**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 differ (*P* < 0.05).
LSD: least significant difference

However, compared with the control yoghurts, the pH reduction of free-fat yoghurt 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 the four free-fat yoghurt treatments (with individually BMP or its combination with TG additions) show 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. (2011). The slight faster drop in pH that also represented in shorter fermentation time (~ 15 min less), compared to the reference yoghurts might be attributed to availability of low molecular weight peptides and/or amino acids; provided by buttermilk protein content, that are required by *Str. 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 and propagation needs further investigation.

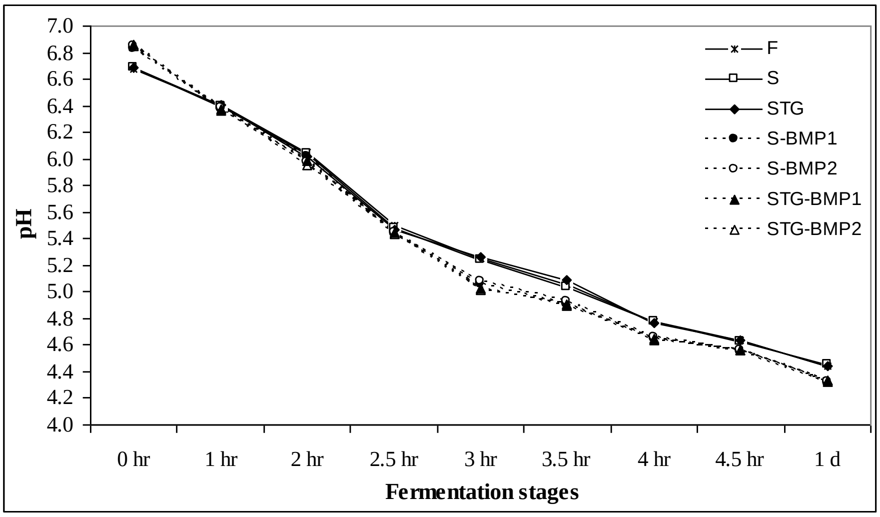

Fig. (1): Decrease in pH values of full-fat and free-fat experimental yoghurts during fermentation and after 1 day storage at refrigerator. Values are means of triplicate yoghurt batches.

Regarding the Serum binding capacity, centrifugally forced serum loss percentage (SL %) was measured to monitor the water holding capacity of the modified yoghurt gel as a function of TG and/or BMP addition. As it can be seen from the data of Table 1 and Fig. 2, the control free-fat yoghurt treatment shows the highest SL% with almost 3.5-fold as the full-fat control yoghurt, reflecting the crucial role of milk fat in yoghurt gel functionality. As shown in Table 1, addition of TG to free-fat milk (STG treatment) significantly improved serum binding capacity and reduced serum loss by 51.6% relatively compared with the base value of free-fat 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 yoghurt gel permeability which in turn prevent yoghurt whey expulsion (Lorenzen et al., 2002). Decrease in yoghurt gel permeability causes a more compact microstructure with smaller pores embedded in clusters of protein, and, consequently more water is entrapped in the yoghurt gel network (Moon and Hong, 2003). This would be further explored in the present work by scanning electron microscope.

The data depicted in Fig. 2 clearly revealed that all free-fat yoghurt manufactured with addition of BMP were also significantly (*P*<0.05) less in SL% compared to the control free-fat yoghurt. Extend of SL% reduction has increased with increasing BMP concentration. Free-fat yoghurt of S-BMP1 and S-BMP2 samples showed reduction in SL% by 26.5 and 41.5 %, respectively, in relation to the control free-fat 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 yoghurt gel resulted by 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 binding yoghurt serum.

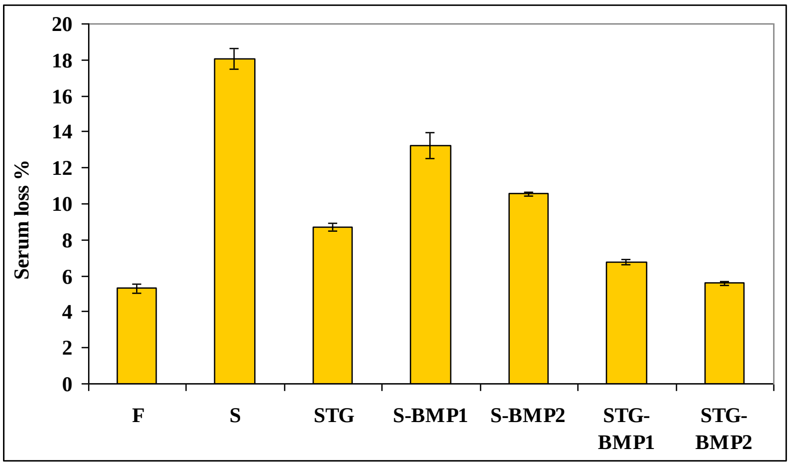

Fig. (2): Serum loss% of full-fat and free-fat experimental yoghurts. Values are means of triplicate analyses of three individual yoghurt samples (*n* = 9) with their standard deviations represented by vertical bars. Column heading by a different letter differ significantly (*P*<0.05).

In this context, the interaction effect of addition of TG simultaneously with BMP (STG-BMP1 and STG-BMP2) shows an extensive impact and significantly extended reduction on SL% in comparison to the addition of TG or BMP individually to free-fat yoghurts; STG, S-BMP1 and S-BMP2 treatments, respectively. This finding would be expected, since the buttermilk powder contains considerable protein content (34.3%), which in-turn promotes the TG reactivity. This interaction impact is clearly represented within free-yoghurt sample of higher BMP addition (STG-BMP2) that showed no significant difference (*P*<0.05) in SL% compared with full-fat control yoghurt (Fig. 2), as well as, improved the serum holding capacity of yoghurt gel by 69% reduction in SL% relatively to free-fat control yoghurt (Table 1).

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. Fig. 3 distinctly shows the influence of enzymatic cross-linking on molecular mass of milk proteins of 1 d-old yoghurts, which represented by the formation of intensively high molecular weight (HMW) protein polymers bands (lanes 3, 6 and 7), compared with the expected HMW protein bands that appeared in all yoghurt treatments as a result of excessive thermal treatment of milk (Lucey et al., 1999). However, it is worthy to note that there are no bands representing the whey proteins within all treatments, which is most probably attributed to the demutualization 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-yoghurt manufactured from thermally and high pressure processed milk.

Fig (3): SDS-PAGE gel of the experimental free-fat yoghurts. Lanes; (1) Molecular weight marker (KDa), (2) free-fat control yoghurt, (3) free-fat yoghurt + TG, (4) free-fat yoghurt + BMP 1%, (5) free-fat yoghurt + BMP 2%, (6) free-fat yoghurt + TG +BMP1% and (7) free-fat yoghurt + TG + BMP2%.

Microstructure characterization: The impacts of TG and/or BMP addition on microstructure of the experimental yoghurts are shown in Fig. 4. The protein matrix (gray area) formed a continuous phase permeated by an amorphous system of voids filled with serum (black area), which inturn revealed the spatial dimensions of these images. As these micrographs show, an obvious variation in the yoghurt microstructural properties was obtained between full-fat and free-fat treatments (Figs. 4A and 4B). An extremely porous, open and spongy-like structure free of fat globules was obtained in free-fat yoghurt, whereas a continuous phase of tortuous protein aggregate network characterized by a more compacted and dense structure accompanied by less voids revealed in the full-fat yoghurt where the spherical fat globules were obviously dispersed throughout the protein matrix.

As it might be seen in Fig. 4C, addition of TG (STG yoghurt) promoted regularly aggregated protein matrices characterised by a finer-meshed network accompanied by systematic small pores that obviously much less in size compared, to that of free-fat control yoghurt (Fig. 4B). Moreover, noticeably clusters of cross-linked strands and a fibrous-like protein aggregates (black arrows) were observed, indicating the cross-linking impact of TG that performed by the formation of both inter- and intra-molecular isopeptide protein bonds (Lorenzen et al., 2002). This finding 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 interconnectivity of the network.

The manifested microstructure in Fig. 4 clearly revealed that the free-fat yoghurt manufactured with BMP (Figs, 4D - G) exhibited a compact and dense structure accompanied by meandrous clustered protein folds (pointed with white arrows). The relatively fused protein matrices 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. Within this point of view, 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 and Ong et al., 2010). A closer observation of the microstructure details in these micrographs revealed that the impact of BMP on yoghurt microstructure characteristics was more pronounced as BMP concentration increased, and was even greater and further intense by combined TG-BMP addition as illustrated in SEM micrographs of STG-BMP1 and STG-MBP2 yoghurt treatments (Figs. 4F and 4G, respectively). These microstructure modifications could be mainly attributed to the induced capability of TG towards protein cross-linking, which resulted from the relatively high contents of protein and MFGM components in BMP.

Fig (4): Scanning electron micrographs of full-fat and free-fat experimental yoghurts; (A) F, (B) S, (C) STG, (D) S-BMP1, (E) S-BMP2, (F) STG-BMP1 and (G) STG-BMP2 treatments.

Apparently, the microstructure properties of free-fat yoghurt made with BMP addition markedly differed from those of free-fat yoghurts without BMP (S and STG treatments), whereas they are to-some extent resemble to full-fat control yoghurt with respect to compacted matrices and dense structure attributes, reflecting the functional properties of buttermilk in dairy product structures. These microstructural characteristics are in conformity with the polymerization reaction and cross-linking activities obtained by SDS-PAGE analysis, which may considered together, in explaining the results trend of serum binding capacity measurement (SL%).

A comparison of the sensory data for the seven experimental yoghurts after 1 d of storage at refrigerator is given in Table 2. A significant difference (P<0.05) was observed between F and S yoghurts, reflecting the generally recognized negative effect of fat reduction on appearance scores of cheeses. However, the free-fat yoghurts manufactured with BMP gaineda higher appearance score than the STG yoghurt, and were similar to the full-fat control yoghurt (F treatment). Nevertheless, STG yoghurt exhibited significant appearance preference (P<0.05) compared to free-fat control yoghurt (S treatment). Concerning the acidity score, there was no marked differences obtained among the free-fat yoghurts. Whilst, the full-fat control yoghurt (F treatment attained the highest desirable acidity score, and does not significantly differ from the free-fat yoghurts made with individually BMP (S-BMP1 and S-BMP2 treatments).

Table (2). Effect of TG and/or BMP addition on yoghurts at day 1 of cool storage as indicated by the analysis of variance from data obtained by a seven member evaluation panel.

	Appearance	Acidity	Consistency	Overall acceptability
F	$8.48^{a} \pm 0.15$	$8.42^{a} \pm 0.21$	$8.29^{a} \pm 0.10$	$8.54^{a} \pm 0.21$
S	$6.92^{c} \pm 0.92$	$7.21^{c} \pm 0.46$	$6.79^{c} \pm 0.36$	$6.63^{d} \pm 0.38$
STG	$7.78^{b} \pm 0.58$	$7.35^{c} \pm 0.98$	$7.72^{b} \pm 0.32$	$7.81^{b} \pm 0.69$
S-BMP1	$8.18^{ab} \pm 0.08$	$8.06^{ab} \pm 0.16$	$8.11^{a} \pm 0.15$	$8.28^{a} \pm 0.51$
S-BMP2	$8.21^{ab} \pm 0.04$	$7.98^{ab} \pm 0.32$	$8.23^{a} \pm 0.29$	$8.42^{a} \pm 0.42$
STG-BMP1	$8.03^{ab} \pm 0.50$	$7.57^{bc} \pm 0.58$	$7.73^{b} \pm 0.60$	$7.42^{c} \pm 0.42$
STG-BMP2	$8.07^{ab} \pm 0.27$	$7.65^{bc} \pm 0.46$	$7.88^{b} \pm 0.20$	$7.33^{c} \pm 0.29$
LSD	**0.70**	**0.51**	**0.22**	**0.27**

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 differ (P < 0.05).
LSD: least significant difference

With regard to textural attributes, free-fat control yoghurt (S treatment) received the lowest (P<0.05) consistency score and described as brittle and weak structure with observed whey-off on yoghurt surface as indicated by the panelists notes. Whereas, experimental free-fat yoghurts made with TG and/or BMP showed a markedly significant improvement (P<0.05) in consistency score compared to the reference free-fat yoghurt. It is worthwhile to note that the body consistency of free-fat yoghurt made with individually BMP was significantly resembled (P<0.05) to that of full-fat counterpart (F treatment). As it can be seen in Table 2, there was a significant negative effect (P<0.05) on overall acceptability score by fat reduction in yoghurt milk, which is expected result. In contrast, the overall acceptability scores clearly reflected the constructive impact of TG and/or BMP on the production of free-fat set yoghurt. These free-fat yoghurts revealed a dry (no whey-off), smooth and white shining surface as described by the panelists. BMP concentration has no significant effect (P<0.05) in all sensory attributes evaluated in this study. Theses findings are in agreement with the data reported by Lorenzen et al. (2002) and Ozer et al. (2007) for yoghurt made from TG-treated milk, as well as, Trachoo and Mistry (1998) for yoghurt made with ultrafiltered sweet buttermilk. Additionally, sensory analysis performed by Faergemand et al. (1999) have shown that, it was possible to reduce both the fat and protein content and to obtain products similar in texture to the full-fat control yoghurt by TG treatment, due to the fat and protein simulating effect of TG action.

It is interesting to note that the free-fat yoghurts made with individually BMP addition exhibited the highest (P<0.05) perceived overall-acceptability among all experimental free-fat yoghurts, and were similar to that of full-fat yoghurt. S-BMP1 and S-BMP2 treatments exhibited creamy mouth-feel and a homogenous good texture, and showed the most desirable organoleptic attributes indicated by the assessors among all free-fat treatments. Whereas, free-fat yoghurts made with combined TG-BMP were described as grainier and excessively firm texture with crumply mouth-feel, which could mainly attributed to relatively higher protein content and the excessive cross-linking and polymerization extent of milk proteins obtained by TG action as indicated by SDS-PAGE analysis .

CONCLUSION

There is an increased demand for low-fat dairy products that matched the quality of its full-fat counterparts with little or no synthetic additives or stabilizers. As an alternative, the applications of enzymatic cross-linking protein and buttermilk powder in production of novel yoghurt were investigated in this study. Results indicate that TG has no effects on gross composition of yoghurt as well as on fermentation time and pH development, whereas, addition of BMP relatively increased the protein content of yoghurt and fairly accelerated fermentation time. Moreover, TG appeared to be an effective means of improving the water holding capacity of free-fat yoghurt gel, and its impact was even higher than those obtained by individually BMP addition of different concentration. Besides, the combined effect of TG-BMP addition revealed an extensive serum loss reduction in free-fat yoghurt, which was resembled to that of full-fat yoghurt. As indicated by SDS-PAGE analysis, BMP addition enhanced the reactivity of TG. Concerning sensory evaluation, TG as well as BMP has markedly altered the functional properties of the free-fat yoghurt. Nevertheless, free-fat yoghurt of individually BMP addition received the most desirable organoleptic attributes as indicated by the assessors and was similar to the full-fat yoghurt perception. Interestingly, the combined effect of TG-BMP addition revealed an extensive serum loss reduction in free-fat yoghurt and excessive high molecular weight protein polymers bands which demonstrated more cross-links. The SEM analysis confirms these findings through the presence of much compact and dense protein network accompanied by clusters of cross-linked strands attached to protein matrices which were further, agglomerated into meandrous clustered protein folds within structure of free-fat yoghurt of combined TG-BMP implantation.

Finally, cross-linking of milk proteins by means of TG appears to be an acceptable alternative instead of addition of stabilizer or extra protein in free-fat yoghurt. Also, addition of BMP offers a promising option to develop innovative functional free-fat yoghurt of much promoting health compounds. However, the addition of TG combined to BMP is not recommended due to the excessive cross-linking occurs as the BMP promotes and enhance the TG activity.

REFERENCES

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

Bönisch, M. P., Heidebach, h. C. and Kulozik, U. (2008). "Influence of transglutaminase protein cross-linking on the rennet coagulation of casein". Food Hydrocolloids, 22: 288-297.

Bönisch, M. P., Lauber, S. and Kulozik, U. (2007). “Improvement of enzymatic cross-linking of casein micelles with transglutaminase by glutathione addition”. International Dairy Journal, 17: 3–11.

De Jong, G. A. H. and Koppelman, S. J. (2002). “Transglutaminase catalyzed reactions: Impact on food applications”. Journal of Food Science, 67: 2798–2806.

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

Faergement, M., Sorensen, M. V., Jorgensen, U., Budolfsen, G. and Qvist, K. B. (1999). “Transglutaminase: Effect on instrumental and sensory texture of set style yoghurt”. Milchwissenschaft, 54: 563–566.

Farnsworth, J. P., Li, J., Hendricks, G. M. and Guo, M. R. (2006). “Effects of transglutaminase treatment on functional properties and probiotic culture survivability of goat milk yogurt”. Small Ruminants Research, 65: 113–121.

Gauche, C., Tomazi, T., Barreto, P. L. M., Ogliari, P. J. and Bordignon-Luiz, M. T. (2009). “Physical properties of yoghurt manufactured with milk whey and transglutaminase”. LWT - Food Science and Technology, 42: 239-243.

Haque, Z. U. and Ji, T. (2003). “Cheddar whey processing and source: II. Effect on non-fat ice cream and yoghurt”. International Journal of Food Science and Technology, 38: 463–473

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

International Dairy Federation (IDF) (1993). "Milk-determination of nitrogen content (Kjeldahl method)." Standard 20B (pp. 1-12). Brussels, Belgium.

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

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

Le, T. T., Camp, J., Pascual, P. A. L., Meesen, G., Thienpont, N., Messens, K., Dewettinck, K. (2011). “Physical properties and microstructure of yoghurt enriched with milk fat globule membrane material”. International Dairy Journal, 21: 798-805.

Ling, E. R. (1963). “A text book of dairy chemistry”. Vol. 2 (3rd ed.). Chapman & Hall (eds.), London.

Lopez, C. (2005). Focus on the supramolecular structure of milk fat in dairy products. Reproduction Nutrition Development, 45: 497–511.

Lopez, C., Camier, B. and Gassi, J. Y. (2007). “Development of the milk fat microstructure during the manufacture and ripening of Emmental cheese observed by confocal laser scanning microscopy”. International Dairy Journal, 17: 235–247.

Lorenzen, P. C., Neve, H., Mautner, A. and Schlimme, E. (2002). “Effect of enzymatic cross-linking of milk proteins on functional properties of set-style yoghurt”. International Journal of Dairy technology, 55: 152-157.

Lucey, J. A., Munro, P. A. and Singh, H. (1999). “Effects of heat treatment and whey protein addition on the rheological properties and structure of acid skim milk gels”. International Dairy Journal, 9: 275–279.

Martinovic, A.; Moe. K, M.; Romeih, E.; Aideh, B.; Vogensen, F.K.; Østlie, H.; Skeie, S. (2013). Growth of adjunct *Lactobacillus casei* in Cheddar cheese differing in milk fat globule membrane components. International Dairy Journal, 31: 70-82.

Moon, J. H., & Hong, Y. H. (2003). “Electron microscopic property of transglutaminase added milk”. Journal of Food Science of Animal Resources, 23: 350–355.

Morin, P., Jimenez-Flores, R. and Pouliot, Y. (2007). Effect of processing on the composition and microstructure of buttermilk and its milk fat globule membranes. International Dairy Journal, 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. Journal of Dairy Science, 91: 871–882.

Myllärinen, P., Buchert, J. and Autio, K. (2007). “Effect of transglutaminase on rheological properties and microstructure of chemically acidified caseinate gels”. International Dairy Journal, 17: 800-807.

Ong, L., Dagastine, R. R., Kentish, S. E., & Gras, S. L. (2010). The effect of milk processing on the microstructure of the milk fat globule and rennet induced gel observed using confocal laser scanning microscopy. Journal of Food Science, 75: 135-145.

Özer, B. H., Kırmacı, H. A., Öztekin, S., Hayalo_glu, A. and Atamer, M. (2007). "Incorporation of microbial transglutaminase into non-fat yogurt production". International Dairy Journal,17:199-207.

Özrenk, E. (2006). "The use of transglutaminase in diary products". International Journal of Dairy Technology, 59: 1-7.

Romeih, E. A.; Moe, K. M. and Skeie, S. (2012). "The influence of fat globule membrane material on the microstructure of low-fat Cheddar cheese". International Dairy Journal, 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, Denmark, pp. 371–375.

Schorsch, C., Carrie, H. and Norton, I. T. (2000). "Cross-linking casein micelles by a microbial transglutaminase: influence of cross-links in acid-induced gelation." International Dairy Journal 10: 529-539.

Spitsberg, V. L. (2005). Bovine milk fat globule membrane as a potential nutraceutical. Journal of Dairy Science, 88: 2289–2294.

Trachoo, N. and Mistry, V. V. (1998). Application of Ultrafiltered Sweet Buttermilk and Sweet Buttermilk Powder in the Manufacture of Nonfat and Low Fat Yogurts. Journal of Dairy Science, 81: 3163–3171.

Tsevdou, M. S., Eleftheriou, E. G. and Taoukis, P. S. (2013). "Transglutaminase treatment of thermally and high pressure processed milk: Effects on the properties and storage stability of set yoghurt". Innovative Food Science and Emerging Technologies, 17: 144–152.

Turcot, S., St-Gelais, D., & Turgeon, S. L. (2002). Ripening of low-fat Cheddar cheese made from milks enriched with phospholipids. *Lait*, 82: 209-223.

تـأثير إنـزيم الترانسـجلوتامينيز واللـبن الخـض المجفـف علـى الخـواص الوظيفيـة لليوغورت خالى الدهن

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تم دراسة إمكانية إنتاج يوغورت خالي الدهن باضافة اللبن الخض المجفف (١% و ٢%) و إنزيم الترانس جلوتامينيز (١ وحدة / جرام بروتين) ومقارنـة الخـواص الوظيفيـة والتركيـب البنـائي الـدقيق مـع كـل مـن اليوغـورت كامـل وخـالي الـدهن المقـارن. وقـد أظهـرت النتـائج أن إضـافة إنـزيم الترانس جلوتامينيز لم تؤثر علي معدل إنخفاض الـpH أثناء فترة التحضين في حين أن إضـافة اللـبن الخض المجفف أدت الي إسراع نسبي في معدل إنخفاض درجة الـpH أثناء فترة التحضين . وقد لوحظ ان إضافة اللبن الخض المجفف أو المعاملة بإنزيم الترانس جلوتامينيز خفضت بدرجة معنوية من قابليـة الخثـرة لطـرد الشـرش . فـي حـين أن إضـافة اللـبن الخـض مـع المعاملـة بـإنزيم الترانس جلوتامينيز كانت أكثر تأثيرا من إضافة كل منهما علي حدة ، حيث انه لم يظهر أي فروق معنوية مقارنة مع الزبادي كامل الدسم.

وأشـارت النتـائج المتحصـل عليهـا بواسـطة التفريـد الكهربـي (SDS-PAGE) الـي أن إضافة اللبن الخض المجفف أدت الـى تحسين نشـاط و زيـادة عمـل الإنـزيم والـذي إنعكس فـي ظهـور حزم بروتينية (Bands) كثيفة نتيجة تكوين بـولميرات بروتينيـة عاليـة الـوزن الجزيئـي . وقد اكدت نتـائج التركيـب الـدقيق بواسـطة الميكروسـكوب الاليكترونـي الماسـح النتـائج السـابقة ، حيـث ظهـر التركيب البنائي الدقيق للخثرة المتكونة من إضافة اللبن الخض المجفف مع إنزيم الترانس جلوتـامينيز أكثر تماسكا وكثافة مع ظهور تكتلات بروتينية مندمجة في هذا التركيب البنائي . ومن الجدير بالـذكر أن إضافة أي من إنزيم الترانس جلوتـامينيز أو اللبن الخض المجفف أدت إلـي تـأثير إيجـابي معنـوي موضحا بظهور تركيب بنائي دقيق أكثر كثافة وتجانسا للشبكة البروتينية . وقد كـان هذا التـأثير أكثر وضوحا في حالة إضافة اللبن الخض المجفف عنه في حالة المعاملة بإنزيم الترانس جلوتـامينيز. وقد اشارت النتائج الي أن معاملات اليوغورت خالي الدهن المصنعة باضافة اللبن الخض المجفف كانـت أكثر قبولا لدي المحكمين حيث حصلت علي أعلي درجات التقييم الحسي والتي لم تختلف معنويا عن اليوغورت كامل الدهن المقـارن. في حين أن معـاملات اليوغـورت خـالي الـدهن الناتجـة عن التـأثير المضاعف لإضافة اللبن الخض المجفف مع إنزيم الترانس جلوتامينيز لم تكن علي نفس درجة القبول لدي المحكمين.

وبصفة عامة يمكن استنتاج ان إستخدام انزيم الترانس جلوتامينيز أو اللبن الخض المجفف أدي الي تحسين الصفات الوظيفية لخثرة اليوغورت خالي الدهن. بالإضافة الي أنه يمكن إعتبار اللبن الخض المجفف مصدر جيد لرفع الجوامد الكلية ونسبة البروتين فـي صـناعة اليوغـورت خـالي الـدهن بالاضـافة الـى تـوفيره خيـارات واعدة لتطـوير منتجـات لبنيـة وظيفيـة خاليـة الـدهن ذات قـيم داعمـة للصحة.

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