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ORIGINAL ARTICLE

Physico-chemical, microstructural and rheological properties of camel-milk yogurt as enhanced by microbial transglutaminase

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Abstract Camel milk produces watery texture when it is processed to yogurt. Despite the extensive studies on microbial transglutaminase (MTGase) in dairy research, the effect of this enzyme on the properties of yogurt made from camel milk has not been studied. This study aims to investigate the impact of MTGase with and without bovine skimmed milk powder (SMP), whey protein concentrate (WPC),or β-lactoglobulin (β-lg) on physico-chemical, rheological, microstructural, and sensory properties of camel-milk yogurt during 15 days of storage period. MTGase treatment markedly reduced the fermentation time of unfortified and SMP-fortified camel milk. The fortification of camel milk without MTGase failed to give set-type yogurt. The treatment of unfortified milk with MTGase enormously improved the viscosity and the body of yogurt samples. Fortification of MTGase-treated milk impacted positively on the viscosity, the water holding capacity, and the density of the protein matrix in the gel microstructure, which were influenced by the type of dairy ingredients. All MTGase-treated yogurts differed from each other in hardness and adhesiveness values. Electrophoresis results showed that the susceptibility of the individual milk proteins to MTGase varied, and there were differences among the treatments toward the enzyme. SMP-fortified yogurt was the most accepted product. Generally, the addition of MTGase preparation at a concentration of 0.4%, simultaneously with starter culture, to fortified camel milk was considered an effective tool to solve the challenges of producing set-type yogurt from this milk.

Keywords Camel milk · Set-type yogurt · Dairy ingredients · Microbial transglutaminase

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Introduction

According to the recent statistics of FAO for 2014, the total population of camels in the world is 27,735,941 head, which produce about 2.9 million tons milk per year. In Egypt, camel is considered the most important animal for Bedouins as a main source of animal food, primarily in the form of camel milk. Nowadays, different dairy products made from camel milk have spread not only to Gulf area and Mauritania (El-Agamy et al. 2009) but also to the European markets (EICMP 2017).

Despite the uniqueness of camel milk in terms of nutritional and therapeutic properties (Al haj and Al Kanhal 2010; Kaskous 2016; Khan and Alzohairy 2011), it does not form a desirable curd by lactic acid fermentation process. The fermented camel milk products have a watery consistency and have a fragile and poor structure (Abdel Rahman et al. 2009). This behavior is probably more due to the large size of casein micelles (Kamal et al. 2017), the relative distribution of casein fractions (Al haj and Al

Kanhal 2010) andthe absence of β-lg in camel milk (El-Agamy et al. 2009) compared with bovine milk. The small size of camel fat globule might be another cause for the poor structure in fermented camel milk (El-Zeini 2006).Because yogurt texture is an important characteristic that affects its quality and consumer acceptance, many attempts were conducted to solve the problems related to poor texture of fermented camel milk and to produce a set-type yogurt from this milk. Of which, fortification of camel milk with skimmed milk powder (Salih and Hamid 2013), or the addition of hydrocolloids and stabilizers (Al-Zoreky and Al-Otaibi 2015). Despite all these efforts, no published studies are found to improve the quality of camel's milk network during acid fermentation by using microbial transglutaminase (MTGase), which is one of the most promising ways of improving the properties of fermented dairy products. Several studies showed the positive impact of MTGase on the textural, microstructural and physical properties of yogurt gel (Abdulqadr et al. 2015; Farnsworth et al. 2006; Ozer et al. 2007).

Transglutaminase (TGase, EC 2.3.2.13) is an enzyme that catalyzes an acyl transfer reaction between γ-carboxyamide groups of peptide-bound glutamine residues (acyl donor) and the ε-amino group (acyl receptor) of lysine residues in particular proteins. This reaction will lead to the formation of new intra- and intermolecular ε-(γ-glutamyl) lysine bonds, which can modify the structure and functionality of proteins (Motoki and Seguro 1998) without any impact on the nutritional quality or the digestibility of milk proteins (de Souza et al. 2009). MTGase, isolated from *Streptoverticillium mobaraense,* has been approved by food industries (Kuraishi et al. 2001) in many other countries than EU member states, including, but not limited to, Canada, Brazil, Japan, Korea, China and Thailand. As a processing aid in a legal sense its commercial preparation ACTIVA® YG was Generally Recognized as Safe (GRAS) in USA by the Food and Drug Administration (FDA) for use as a cross-linking agent in food in general at the lowest levels necessary to achieve the desired technical effects since 2001.

The aim of this study was to investigate the influence of MTGase on physico-chemical, rheological, microstructural, and sensorial characteristics of yogurt and the possibility of producing a set-type yogurt from camel milk. The research also aims to examine the impact of adding some dairy ingredients such as bovine skimmed milk powder (SMP) (2%, w/w), whey protein concentrate (WPC) (2%, w/w), or β-lactoglobulin (β-lg) (0.5%, w/w) to fortify camel milk, with MTGase on these properties.

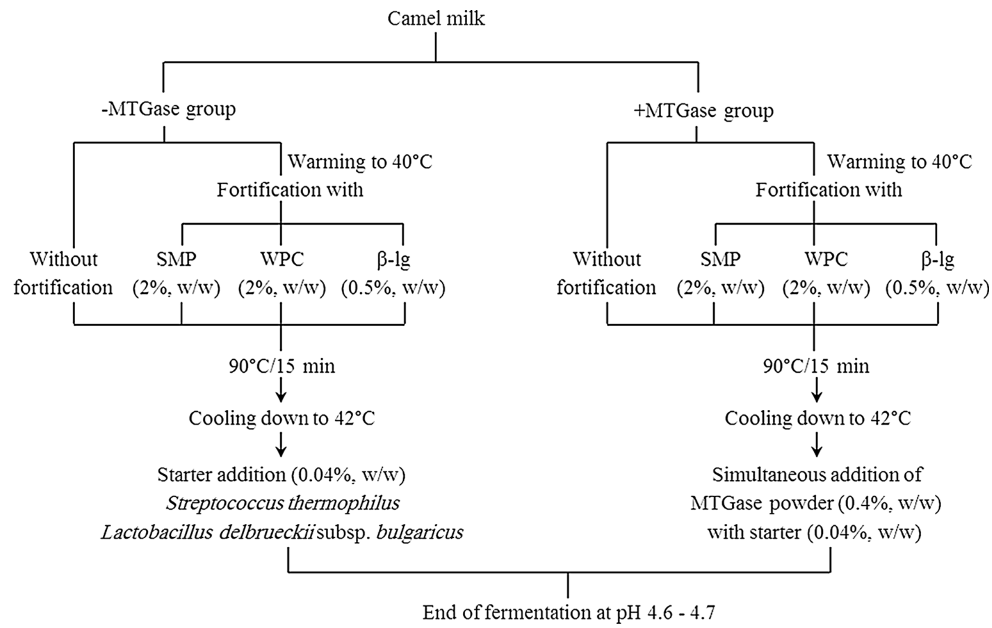
Materials and methods

Materials

Dromedary camel milk was collected from the herd of Camel Research Center, Marsa Matrouh, Egypt. A microbial preparation of transglutaminase from *S. mobaraense*, commercially available as ACTIVA® YG was used. The enzyme preparation with a specific activity of 100 U/g was a gift from Ajinomoto Europe Sales GmbH, Hamburg, Germany. Commercially available lyophilized culture (Express 0.2, DVS) was obtained from Chr. Hansen Laboratories, Copenhagen, Denmark. SMP with 1.25% fat, 36% protein, 51% lactose, and 4% moisture was obtained from Arla Foods, Sweden. In addition, WPC with 3–4.5% fat, 34–36% protein, 48–52% lactose, and 3–4.5% moisture was supplied as a gift from the U.S. Dairy Export Council. BioPURE β-lg (97.8% protein/dry basis, 93.6% β-lg [% of protein], 0.3% fat and 5% moisture) was provided by Davisco Foods International, Inc., Minnesota, USA.

Yogurt preparation

Camel milk was divided into eight batches. Four batches, which considered as controls, were processed into yogurts without MTGase treatment (−MTGase group). The other four batches, MTGase powdered preparation was added to milk, simultaneously with starter culture (+MTGase group), at concentration of 0.4% (w/w). The preparation of yogurts is illustrated in the flowchart below. Yogurt was manufactured according to the protocol proposed by Tamime and Robinson (2007) in triplicate.



When the pH value reached to 4.6–4.7, the samples of yogurt were stored at refrigerator temperature (4 ± 1 °C) for 15 days. The samples were analyzed on the 1st, 7th and 15th day of storage period in triplicate. The physico-chemical properties of camel milk and fortified camel milk, which yogurts made from, were determined in triplicate and presented in Table 1.

Yogurt analyses

Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE)

SDS-PAGE, 12.5% T, was carried out using the discontinuous buffer system described by Laemmli (1970). All yogurts at the end of fermentation time and milk samples were diluted (1:3) with a sample buffer. The injected volumes were 7.5 and 10 µl for milk and samples of yogurt, respectively. Whey samples from yogurts at the end of fermentation time were obtained by centrifugation at 3300×*g*/10 min by Sigma 2–16 PK, Germany and diluted (1:1) with a sample buffer, the injected volume was 10 µl.

Apparent viscosity

The apparent viscosity of the samples of yogurt was measured using two different Brookfield viscometers (Brookfield Lab., USA). Those samples without MTGase were measured at 15 ± 2 °C after 24 h of cold storage using LV spindle ULA rotation of 5 rpm and using viscometer model DV-II + viscometer. In addition, the viscosity of yogurts treated with MTGase was measured at 15 ± 2 °C after 1 and 15 days of cold storage with RV spindle (No. 4) rotation of 200 rpm using viscometer model DV-II + Pro.

Microstructure

After 1 day of cold storage, the microstructure of MTGase-treated samples of yogurt was examined by scanning electron microscope (SEM). Cubes of 1 mm were fixed at room temperature in a 2.5% glutaraldehyde solution in phosphate buffer (0.1 M, pH 4.6). The fixed samples were washed with 0.1 M phosphate buffer and dehydrated in a graded ethanol series (15, 30, 50, 70, 80,

Table 1 Physico-chemical properties (Mean ± SD) of camel milk and fortified camel milk

Parameters	Unfortified milk	SMP-fortified milk (2%)	WPC-fortified milk (2%)	β-lg fortified milk (0.5%)
pH	6.67	6.58	6.46	6.60
Acidity (%)	0.168 ± 0.01	0.209 ± 0.00	0.220 ± 0.00	0.189 ± 0.00
Total solids (%)	12.81 ± 0.01	14.62 ± 0.08	14.45 ± 0.12	13.25 ± 0.02
Fat (%)	4.1 ± 0.00	4.1 ± 0.00	4.0 ± 0.07	4.0 ± 0.00
Total protein (%)	2.33 ± 0.07	2.73 ± 0.03	2.82 ± 0.03	2.69 ± 0.06

95 and 100%, 30 min in each). Dried sections were mounted on aluminum SEM stubs with double-sided adhesive tape, and vacuum gold coated using S150A Sputter coater-Edwards-England. Microstructure of the samples of yogurt was examined with a Quanta SEM model FEG 250 electron probe microanalyzer using a magnification of ×4000.

Chemical analyses

The pH values of milk and MTGase-treated samples of yogurt were measured during storage period using a digital pH meter with a glass electrode (Jenway 3305, England). The titratable acidity (%) was determined by titration with 0.1 N NaOH, using phenolphthalein as an indicator. The total solids and total protein (%) were determined according to (AOAC 2000a, b). Fat (%) was estimated by Gerber method according to Ling (1963). Briefly, 5 ml of diluted ammonia solution (one part of concentrated ammonia to four parts of distilled water) was added to 100 g of yogurt sample and homogenized well before fat determination. Then, fat was determined by Gerber's method and the percentage of fat in yogurt samples was calculated by using the following equation:

$$\mathrm{Fat}(\%) = \left(\text{fat column reading on Gerber's tube}\right) \times \left(\frac{105}{100}\right).$$

Water holding capacity (WHC)

The WHC of MTGase-treated samples of yogurt was determined during storage period using centrifugation method as described by Saffon et al. (2013). Yogurt samples (approx. 25 ml) were fermented directly in 50 ml Falcon tubes and centrifuged at $222 \times g$ for 10 min at 4 °C. The clear supernatants were poured off and weighed. WHC (%) was calculated from the following equation:

$$\mathrm{WHC}(\%) = 1 - (\text{Supernatant weight (g)}/\text{yogurt weight (g)}] \times 100$$

Hardness and adhesiveness

Both the hardness and the adhesiveness of MTGase-treated samples of yogurt were performed during storage period by one-bite penetration test using the Texture Analyzer (FT, USA) with the 30 mm diameter probe and operated at a crosshead speed of 1 mms^{-1} and penetration distance of 20 mm.

Sensory evaluation

A scoring method based on a 100-point-scaled scorecard was used for judging and grading of MTGase-treated samples of yogurt. Samples of yogurt were presented cooled in individual plastic cups for sensory evaluation after 1, 7, and 15 days of cold storage by ten trained panelists from the staff members of Dairy Science Department, Faculty of Agriculture, Cairo University, Egypt and Department of Dairy Science and Technology, Faculty of Agriculture, Alexandria University, Egypt. Panelists independently evaluated each sample for flavor, body and texture, acidity, and color and appearance out of 45, 30, 10, and 15 score points, respectively. Panel members were also instructed to report any comments describing the differences between the samples of yogurt.

Statistical analysis

The data were analyzed by a general linear model procedure of the Fisher's protected least-significant difference (PLSD) test using SAS (SAS Institute, Inc., Cary, NC). This test combines ANOVA with comparison of differences between the means of the treatments at the significance level of $P \leq 0.05$.

Results and discussion

Preliminary studies were conducted to find the appropriate concentration of MTGase preparation to be added (from 0.025 to 0.4% (w/w)) and to determine the most effective way of adding MTGase (the simultaneous addition of MTGase with starter culture, pretreatment of milk with MTGase for 1, 2 and 3 h., and then the inhibition of the enzyme prior to fermentation or pretreatment of milk with MTGase for 1 h without enzyme inhibition prior to fermentation). Furthermore, the concentrations of SMP (1 and 2%), WPC (1 and 2%) or β-lg (0.4 and 0.5%) were studied to establish the optimum fortification to be used with camel milk. From the obtained data (Data not shown), the optimum concentration of MTGase preparation was 0.4%; the simultaneous addition of MTGase with the starter culture was the most effective way; and the best concentration of dairy ingredients was 2, 2, and 0.5% for SMP, WPC and β-lg, respectively. Given these findings, the experiment was designed.

Fermentation time

The impact of adding MTGase and fortification with SMP, WPC, or β-lg on the fermentation time of yogurt samples made from camel milk was investigated. The fermentation time of all the yogurts was stopped when the pH value was 4.6–4.7. Concerning MTGase-untreated yogurts, namely; unfortified, SMP-, WPC-, and βlg-fortified yogurts, they reached pH value of 4.62 ± 0.02 within different times. The time required to attain this pH was the longest for unfortified samples (240 min), whereas the shortest time was 215 min for those of βlg-fortified. Fortifying milk with β-lg reduced the fermentation time. Previously, Hallén (2008) found that concentration of β-lg in milk was negatively associated with clotting time at acid-induced coagulation of milk. The fermentation times of SMP- and WPC-fortified yogurts were 234 and 237 min, respectively.

The addition of MTGase to unfortified milk expedited markedly the fermentation time, which was 195 versus 240 min compared with MTGase-untreated counterpart. Also, MTGase treatment reduced the fermentation time of SMP-fortified yogurt to 210 min rather than to234 min in MTGase-untreated SMP-fortified yogurts. While, fermentation time did not differ because of enzymatic treatment whether in β-lg-fortified milk (216 min) or WPC-fortified milk (237 min) compared with the fermentation time of MTGase-untreated counterparts. The last finding is compatible with that of Lorenzen et al. (2002) who found that the simultaneous use of MTGase with starter culture had no influence on the fermentation time.

SDS-PAGE electrophoresis

SDS-PAGE electrophoretic patterns of unfortified and fortified camel milks from which yogurts were made are presented in Fig. 1a. In unfortified milk, casein was separated into two major fractions, α_s- and β-caseins (α_s- and β-CNs), and minor fraction, κ-casein (κ-CN). In addition, β-lg the major whey protein found in other livestock ruminant milk, was absent (El-Agamy et al. 2009). Fortification of camel milk with SMP resulted in the appearance of β-lg as a faint band, which became observable by adding WPC and which increased in density when β-lg was added.

The impact of MTGase on camel-milk proteins of unfortified and fortified yogurts at the end of fermentation time are shown in Fig. 1b. Samples of yogurt made from MTGase-untreated milks had almost the same electrophoretic patterns of milk samples from which these yogurts are made (Fig. 1a). However, SDS–PAGE profiles of MTGase-treated samples of yogurt revealed an obvious decrease in the intensities of casein and monomeric whey proteins bands as well the disappearance of κ-CN and blood serum albumin (BSA) compared with MTGase-untreated samples. All the changes in the bands were accompanied by a large mass of aggregates, which did not enter the stacking gel. Such changes have occurred due to the action of MTGase, which induced chemical crosslinks and thus the formation of large molecular mass of covalently linked polymers as described by Farnsworth et al. (2006). The decrease in band intensities of caseins indicates that these proteins are good substrates for the enzyme due to their flexible open structure (Monogioudi et al. 2009). The high susceptibility of κ-CN towards crosslinking is partly due to its peripheral position in the casein micelles (Sharma et al. 2001) and due to the high reactivity of caseinomacropeptide towards MTGase (Tolkach and Kulozik 2005). Furthermore, the disappearance of BSA of MTGase-treated yogurts means that this protein is an excellent substrate for the enzyme.

The changes that occurred to camel-milk whey proteins due to the treatment with MTGase were monitored (Fig. 1c). It is clear that the resultant whey from unfortified or SMP-fortified yogurt had almost identical electrophoretic patterns, and there was a marked difference between them and the other two treatments i.e. whey from

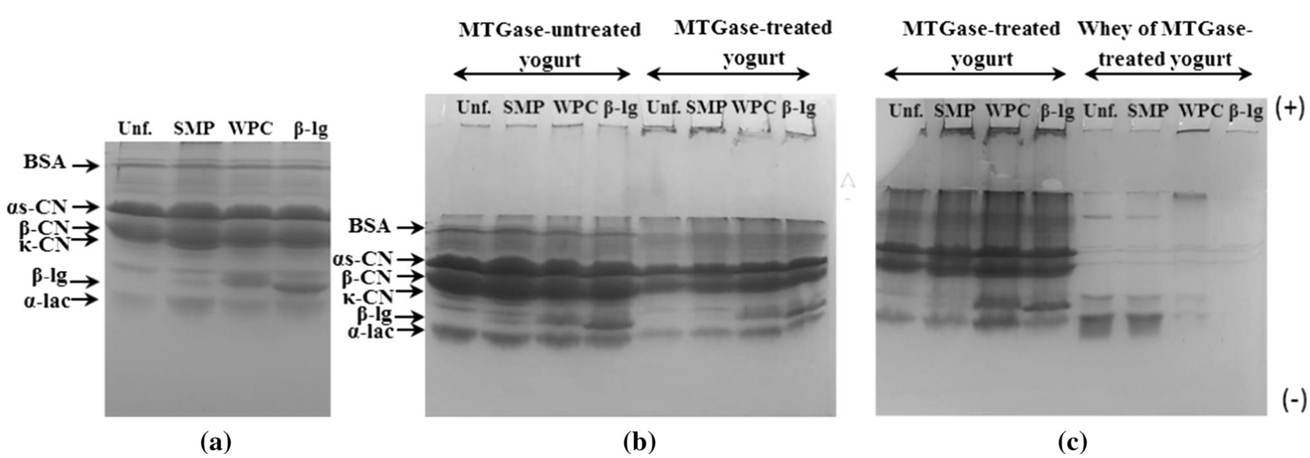


Fig. 1 SDS-PAGE (12.5%T) of unfortified and fortified camel-milks (**a**), MTGase-untreated and -treated yogurt samples (**b**), and MTGase-treated yogurts and their whey (**c**), Unf.: milk or yogurt without fortification; SMP, WPC or β-lg: fortification of milk or yogurt with SMP, WPC or β-lg

WPC- and β-lg-fortified yogurts. Furthermore, the electrophoretic pattern of whey, which separated from β-lg-fortified yogurt contained very faint bands of caseins, while the bands that concerning whey proteins were vanished. This is probably due to the different behavior of β-lg-fortified milk towards MTGase action, where almost all proteins entered in the interaction and consequently whey proteins did not appear on the resolving gel. In this study, thermal treatment of milk before reaction with MTGase improved the reactivity of whey proteins towards protein cross-linking because of a set of interactions arising from heat treatment, as described by Rodriguez-Nogales (2006).

Impact of fortification on the viscosity of MTGase-untreated yogurts

The impact of fortifying camel milk with SMP, β-lg or WPC on the apparent viscosity of MTGase-untreated yogurts at the first day after production is shown in Table 2. The lowest viscosity was recorded for yogurt made from the unfortified milk. Our finding is compatible with the result reported by Abdel Rahman et al. (2009) who observed that the viscosity of camel-milk yogurt was very low and that its consistency was watery. Increasing the total solids of milk by adding either SMP or WPC (2%) led to a slightly improvement ($P \leq 0.05$) in the viscosity of yogurt samples, which still had the watery texture, whereas the fortification of milk with β-lg (0.5%) gave more viscous product. The watery texture of fermented camel-milk products may be related to the absence of β-lg in camel milk. The significant increase in the viscosity when β-lg was added to camel milk, as its ratio in bovine milk, confirmed this assumption. Compared with bovine milk, the remarkable differences of camel-milk casein micelle i.e., the micelle size and the relative distribution of individual caseins especially for β- and κ-caseins (Al haj and Al Kanhal 2010; Kamal et al. 2017) as well the different behavior of camel-milk casein during acidification (Kherouatou et al. 2003) could be other reasons for the pseudo curd from camel milk.

Table 2 Apparent viscosity (Mean ± SD) of yogurt samples made from MTGase-untreated and -treated camel milk during 15 days of storage period

Yogurt sample	Apparent viscosity (cP)	
	1st day	15th day
−MTGase group		
Unfortified	26.37 ± 2.27^{D}	ND
SMP-fortified	47.59 ± 4.47^{B}	ND
WPC-fortified	31.87 ± 3.12^{C}	ND
β-lg-fortified	109.59 ± 1.53^{A}	ND
+MTGase group		
Unfortified	137.05 ± 5.20^{Db}	236.75 ± 11.99^{Ca}
SMP-fortified	577.91 ± 62.08^{Ba}	489.30 ± 34.30^{Bb}
WPC-fortified	471.70 ± 54.72^{Ca}	477.22 ± 28.05^{Ba}
β-lg-fortified	769.83 ± 53.11^{Aa}	633.91 ± 90.59^{Ab}

Means in the same column having different capital superscript letters are significantly different at $P \leq 0.05$. Means in the same raw having different small superscript letters are significantly different at $P \leq 0.05$

ND not determined

Based on the appearance of yogurts and viscosity measurements, those samples of yogurt without MTGase treatment had watery texture; in addition, the fortification of camel milk with SMP, WPC, or β-lg failed to give set-type yogurts (the aim of the study),which were unacceptable by the panelists. Therefore, MTGase-untreated yogurts have been excluded from all analyses.

MTGase-treated yogurts analyses

Viscosity

The influence of using MTGase in unfortified and fortified camel milk on the apparent viscosity of yogurt samples during 15 days of storage period is presented in Table 2. Treatment of camel milk with MTGase enormously increased ($P \leq 0.05$) the viscosity of yogurt samples compared with MTGase-untreated ones due to permanent, strong and irreversible cross linking induced by the enzyme (Aprodu et al. 2011).

The fortification of MTGase-treated milk impacted positively on the viscosity of yogurt. The viscosity of MTGase fortified yogurts was significantly higher ($P \leq 0.05$) due to the variation in composition, particularly the protein content compared with unfortified ones (Table 3). Protein fortification enhanced the degree of protein polymerization and the apparent viscosity of yogurts (Bönisch et al. 2004). Furthermore, the type of fortification led to significant differences ($P \leq 0.05$) in the viscosity among samples of yogurt. Yogurt fortified with β-lg showed the highest viscosity followed by SMP, WPC, and unfortified yogurt. The highest viscosity of β-lg-fortified yogurt may be due to the combined effects of disulfide and ε-(γ-glutamyl) lysine crosslinks (Eissa and Khan 2003). This finding is consistent with the electrophoresis results (Fig. 1c).The electrophoresis results showed that in MTGase β-lg-fortified yogurt, almost all proteins are inserted in the interaction, thus increasing protein polymerization and consequently increasing the viscosity. The studies about the replacement of SMP by WPC on the viscosity of yogurt are conflicting. Some studies mentioned the positive influence of WPC on yogurt viscosity. Remeuf et al. (2003) stated that when milk was fortified with WPC

Table 3 Physico-chemical properties, WHC, hardness and adhesiveness (Mean ± SD) of MTGase-treated yogurt samples during 15 days of storage period

Parameter	Unfortified yogurt	SMP-fortified yogurt	WPC-fortified yogurt	βlg-fortified YOGURT
1st day				
pH	4.35 ± 0.02^{Ca}	4.44 ± 0.01^{Aa}	4.41 ± 0.00^{Aba}	4.36 ± 0.03^{BCa}
Titratable acidity (%)	0.784 ± 0.01^{Cb}	0.909 ± 0.02^{Ac}	0.892 ± 0.01^{Aa}	0.818 ± 0.01^{Bb}
Total solids (%)	13.46 ± 0.23^{Aa}	14.70 ± 0.01^{Ab}	14.94 ± 0.08^{Ab}	13.84 ± 0.06^{Bb}
Fat (%)	4.3 ± 0.12^{Aa}	4.2 ± 0.07^{Aa}	4.3 ± 0.07^{Aa}	4.2 ± 0.07^{Aab}
Total protein (%)	2.28 ± 0.05^{Ca}	2.88 ± 0.13^{Aba}	2.96 ± 0.11^{Aa}	2.72 ± 0.10^{Ba}
WHC (%)	96.5 ± 0.25^{Aa}	98.4 ± 1.82^{Aa}	98.0 ± 0.54^{Aa}	97.0 ± 0.97^{Aa}
Hardness (g)	61.00 ± 6.93^{Bc}	72.67 ± 4.16^{Ab}	31.33 ± 2.31^{Ca}	56.00 ± 1.73^{Ba}
Adhesiveness (g mm)	23.51 ± 3.96^{ABb}	27.18 ± 3.57^{Ab}	15.14 ± 0.85^{Ca}	21.77 ± 1.96^{Bb}
7th day				
pH	4.13 ± 0.01^{Cc}	4.28 ± 0.02^{Ab}	4.28 ± 0.01^{Ac}	4.18 ± 0.01^{Bc}
Titratable acidity (%)	0.843 ± 0.00^{Ca}	0.965 ± 0.01^{Ab}	0.917 ± 0.04^{Ba}	0.839 ± 0.02^{Cb}
Total solids (%)	13.58 ± 0.08^{Da}	15.21 ± 0.02^{Aa}	15.07 ± 0.09^{Bab}	13.83 ± 0.02^{Cb}
Fat (%)	4.3 ± 0.01^{Aa}	4.1 ± 0.00^{Ba}	4.2 ± 0.10^{Aba}	4.1 ± 0.06^{Bb}
Total protein (%)	2.28 ± 0.05^{Ca}	2.88 ± 0.09^{Aa}	2.97 ± 0.10^{Aa}	2.71 ± 0.01^{Ba}
WHC (%)	95.8 ± 0.28^{Aab}	96.3 ± 0.97^{Aa}	95.9 ± 0.82^{Ab}	96.1 ± 1.65^{Aa}
Hardness (g)	74.67 ± 8.33^{Bb}	110.50 ± 16.26^{Aa}	37.67 ± 7.23^{Da}	57.00 ± 4.00^{Ca}
Adhesiveness (g mm)	41.7 ± 3.49^{Aa}	38.30 ± 0.80^{Aba}	26.16 ± 11.15^{Ba}	50.34 ± 3.02^{Aa}
15th day				
pH	4.25 ± 0.02^{Cb}	4.29 ± 0.01^{Bb}	4.32 ± 0.01^{Ab}	4.30 ± 0.02^{Bb}
Titratable acidity (%)	0.869 ± 0.03^{Ca}	0.995 ± 0.01^{Aa}	0.929 ± 0.02^{Ba}	0.862 ± 0.00^{Ca}
Total solids (%)	13.69 ± 0.11^{Ca}	15.19 ± 0.05^{Aa}	15.20 ± 0.02^{Aa}	14.06 ± 0.1^{Ba}
Fat (%)	4.2 ± 0.00^{Aa}	4.2 ± 0.10^{Aa}	4.3 ± 0.06^{Aa}	4.3 ± 0.06^{Aa}
Total protein (%)	2.34 ± 0.05^{Ca}	2.86 ± 0.06^{Aa}	2.96 ± 0.07^{Aa}	2.75 ± 0.02^{Ba}
WHC (%)	94.5 ± 1.07^{Bb}	95.3 ± 0.89^{Aba}	96.9 ± 0.78^{Aab}	95.7 ± 0.36^{Aba}
Hardness (g)	100.33 ± 2.31^{Ba}	112.0 ± 2.83^{Aa}	38.00 ± 0.00^{Da}	61.00 ± 5.66^{Ca}
Adhesiveness (g mm)	26.75 ± 2.92^{Ab}	24.73 ± 4.07^{Ab}	15.17 ± 1.20^{Ba}	26.65 ± 0.21^{Ab}

Means in the same row having different capital superscript letters are significantly different at $P \leq 0.05$. Means in the same column having different small superscript letters are significantly different at $P \leq 0.05$

(34–80% wt/wt protein), heating led to a high level of cross-linking within the gel network, which increased yogurt viscosity and water-holding capacity. Other studies found that the viscosity of yogurt was similar or weaker when SMP was replaced by WPC (Guinee et al. 1995; Guzman-Gonzalez et al. 1999).

Through the storage period, there were different trends concerning the changes in viscosity for all yogurts (Table 2). There was a significant ($P \leq 0.05$) increase in the viscosity of unfortified yogurt. Whereas the viscosity of yogurt fortified by SMP or β-lg declined, the viscosity of WPC-fortified yogurt remained stable. The increment in the viscosity of unfortified yogurts was perhaps due to the continuing formation of covalently cross-linked protein polymers (Aprodu et al. 2011), since the enzyme retained part of its activity. In this study, MTGase was added simultaneously with starter cultures and thus enzyme activity continued during fermentation time and storage period; however, this activity decreased gradually with time as reported by Ozer et al. (2007). In addition, Abu-Jdayil and Mohameed (2002) found that throughout storage, protein rearrangement was continuing, and more protein–protein contacts were being established, leading to increasing viscosity during storage. Decrease in the viscosity of SMP-fortified yogurt was perhaps due to the combined effect of its high acidity and the development of acidity through the storage period (Jooyandeh et al. 2015).Concerning β-lg-fortified yogurt, the presence of β-lg in the yogurt network increase charge but remain in the soluble phase not in the colloidal one (Anema 2015) which may be play a role in demineralization of casein/whey protein network causing a decrease in the acidified camel milk viscosity during the storage period (Kherouatou et al. 2003). With regard to WPC-fortified yogurt, the reason for non-alteration in its viscosity may be related to the steadiness of its acidity during the storage period. Generally, the spontaneous interactions between casein micelles and whey proteins in dairy products are often difficult to be analyzed and understood because they are affected by any small changes to milk environment (Anema 2015).

Microstructure

The scanning electron micrographs and the body of MTGase-treated samples of yogurt after 1 day of storage period are presented in Fig. 2. It is obvious that the treatment of camel milk with MTGase improved the body of yogurt dramatically compared with that of MTGase-untreated yogurts. Several studies demonstrated that treatment of milk with MTGase improved the microstructure of the yogurt gel via inserting permanent ε-(γ-Glu) Lys bonds between milk proteins and stabilizing the three-dimensional networks of yogurt (Farnsworth et al. 2006; Kuraishi et al. 2001).

The addition of dairy ingredients increased the density of the protein matrix in the gel microstructure. In addition, the gel microstructure of MTGase camel-milk yogurt was influenced markedly by the type of dairy ingredients (Fig. 2). Supavititpatana et al. (2009) reported that dried dairy ingredients exhibited diverse functional and structural characteristics of yogurt. As for the unfortified yogurt, a thick compact structure with large holes was observed compared with fortified ones. Adding SMP enhanced the interactions between particles and increased the density of the matrix. The structure of WPC and β-lg fortified yogurts is homogenous and showing the presence of small aggregates linked together with a finer network compared with unfortified and SMP-fortified samples of yogurt. Moreover, the gel of the WPC-fortified yogurts had the most regular structural distribution and the finest network compared with those of other samples of yogurt. Remeuf et al. (2003) reported that the gels of WPC-enriched yogurts showed a very fine network. Fortification with WPC or β-lg decreases the ratio of casein/whey proteins; hence, it decreases the pore size of the yogurt gel (Bönisch et al. 2007).

Physico-chemical properties

The physico-chemical properties of MTGase-treated yogurts are shown in Table 3. There were significant differences ($P \leq 0.05$) in titratable acidity among samples. Yogurt made from SMP- or WPC-fortified milk had the highest acidity, while samples prepared from unfortified or βlg-fortified milk had the lowest ones. The elevation in the acidity of SMP- and WPC-fortified samples of yogurt is related to the high acidity of the milk used in the manufacture (Table 1) due to the high lactose content of both the powders and the buffering action of the supplemental proteins, phosphates, citrates and other milk constituents (Tamime and Robinson 2007).

During the storage period, there was a slow development of acidity ($P \leq 0.05$) in all samples except WPC-fortified yogurt, whose acidity did not change. The presence of the enzyme, particularly at high concentration, reduced the post-acidification process. This may be due to the influence of MTGase on starter culture growth because of the insertion of low molecular weight peptides and amino acids, which are required for starter growth, in the cross-links induced by the enzyme (Ozer et al. 2007). On the 15th day of storage, the highest acidity was recorded for SMP-fortified yogurt.

In this study, dairy ingredients were added to fortify camel milk because of its low percentage of total solids, especially total protein as illustrated in Table 1. Table 3 shows that samples of yogurt varied significantly ($P \leq 0.05$) in the content of total solids and total protein due to the percentage of addition and protein content of the ingredients. The highest content of total solids and protein was observed with SMP- and WPC-fortified yogurts compared with other treatments. Storage period did not influence ($P \leq 0.05$) the protein content of yogurt samples but resulted in a negligible increase in the total solids. As for the fat content, all processed samples contained almost the same percent, and there were no significant differences ($P \leq 0.05$) between the samples and during storage period.

Water holding capacity

The WHC during 15 days of storage period is shown in Table 3. All samples of yogurt exhibited great ability to bind water through the storage period because of the action of MTGase. Treatment with MTGase led to a decline in the pores size and the permeability of the protein matrix of the yogurt; consequently, more free water is entrapped in the gel network (Abdulqadr et al. 2015; Kuraishi et al. 2001).

On the 1st and 7th day of storage, no significant differences ($P \leq 0.05$) were observed based on the ability to bind water for all four treatments. However, on the 15th day of storage, there were small differences ($P \leq 0.05$) in WHC between yogurt made from WPC-fortified milk (the highest capacity) and that made from unfortified milk (the lowest capacity). There were no significant differences among the samples produced from SMP-, WPC-, or βlg-fortified milk. The high ability of WPC-fortified samples to bind water was attributable to the nature of its network, i.e. the finest one (Fig. 2), due to the action of enzyme as well as the shifting of casein/whey protein ratio when WPC was added. The finer gel microstructure increased the capacity for holding water (Farnsworth et al. 2006). In addition, the high WHC of fortified yogurts may be attributed to the high

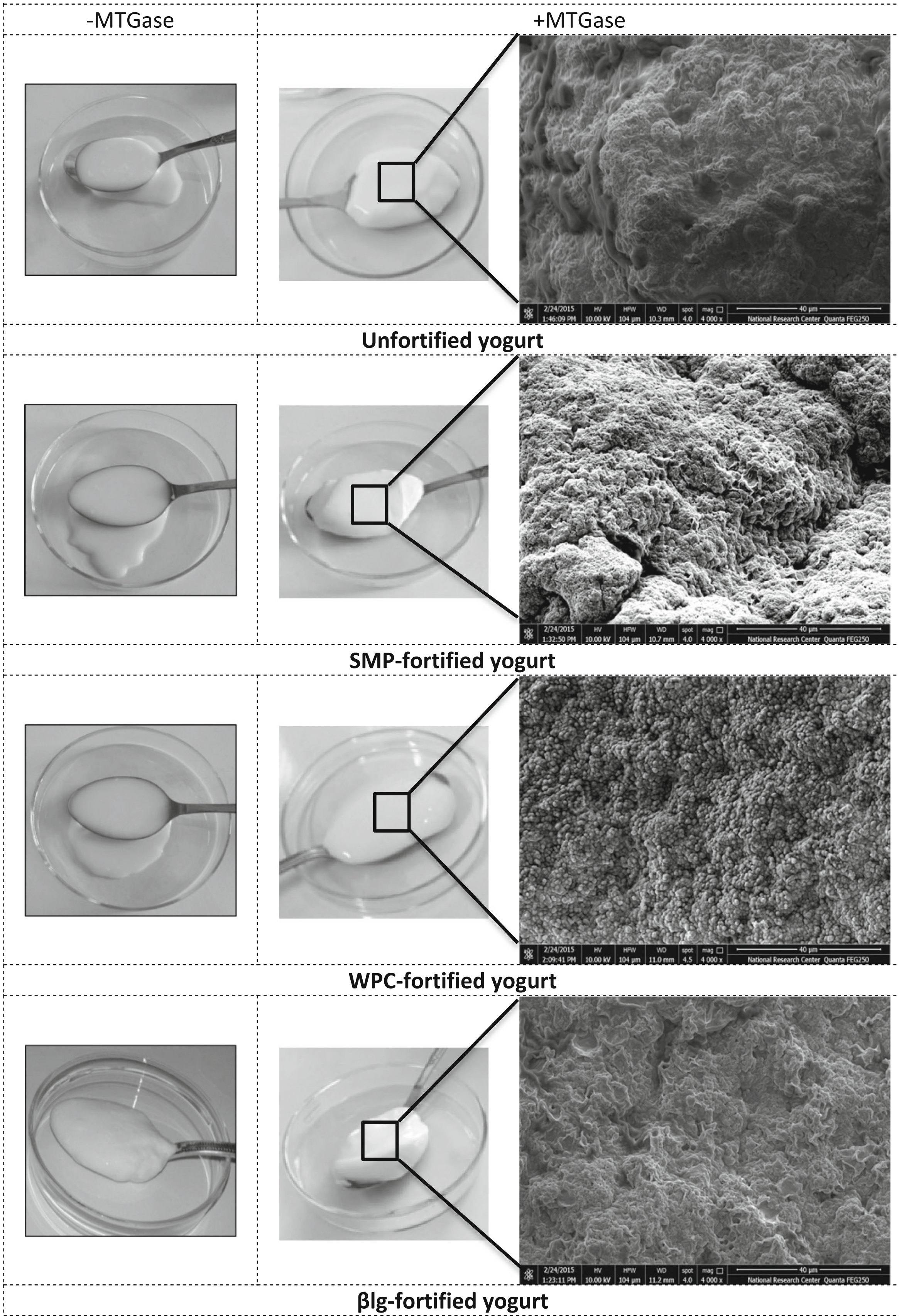


Fig. 2 Scanning electron micrographs (×4000) and body of yogurts made from MTGase-treated camel milk at the first day of storage period

viscosity of these samples compared with unfortified ones. However, the lowest WHC of unfortified yogurt was perhaps due to its structure as well due to its low content of total solids and total protein compared with fortified samples. Jooyandeh et al. (2015) mentioned that syneresis of MTGase-treated yogurt was declined markedly ($P < 0.05$) by increasing the amount of milk solids not fat.

Hardness and adhesiveness

Camel-milk yogurts without MTGase treatment had watery texture and could not be measured by texture analyzer. The results of fresh MTGase-treated yogurts showed that samples differed from each other in hardness values (Table 3) due to the method of fortification (Tamime and Robinson 2007). The highest hardness value ($P \leq 0.05$) was recorded in samples made from SMP-fortified milk and the lowest value was observed in WPC-fortified yogurt compared with unfortified ones. While there were no significant differences ($P \leq 0.05$) in hardness between β-lg-fortified samples of yogurt and unfortified ones. The combined procedure of fortification with SMP and MTGase treatment enhanced the yogurt hardness (Ardelean et al. 2012). On the other hand, Amatayakul et al. (2006) mentioned that the aggregation of denatured whey proteins at the surface of casein micelles along with the decline in the casein/whey protein ratio might prevent the coalescence of casein micelles and network formation; hence, it may decrease the hardness of the yogurt. In this study, even with the MTGase treatment, the WPC-fortified yogurt was much weaker and had the lowest hardness compared with other treatments (Table 3).

On the 7th and 15th day of storage period, there were significant differences in hardness among all samples of yogurt, and the order of hardness is as follows: SMP-fortified → unfortified → β-lg-fortified → WPC-fortified yogurts. At the end of storage period, the hardness ($P \leq 0.05$) increased significantly in unfortified and SMP-fortified samples; however, the increase in hardness in WPC- and β-lg fortified ones was not significant. Isleten and Karagul-Yuceer (2008) stated that the hardness values of the bovine yogurt fortified with SMP significantly increased, but the hardness values of samples with whey protein isolate remained constant during storage. Likewise, Herrero and Requena (2006) found no change in the hardness values of goat yogurts fortified with WPC during the cold storage. The increase in hardness during storage is due to the remaining activity of the enzyme, which allowed the cross-linking to occur during fermentation process and continued during storage period (Abdulqadr et al. 2015; Ozer et al.2007).

The highest adhesiveness value was found in fresh yogurt of milk fortified with SMP while the lowest value was noticed in WPC-fortified yogurt. In addition, there were no significant ($P \leq 0.05$) differences between unfortified and β-lg-fortified fresh samples of yogurt. On the 7th and 15th day of storage period, there were no significant differences in adhesiveness values among all treatments except WPC-fortified samples, which differed from the rest of samples and had the lowest values of adhesiveness. There was also a significant ($P \leq 0.05$) increase in adhesiveness after 7 days of storage for all the samples of yogurt except WPC-fortified yogurt, which its adhesiveness did not change during all stages of storage period. The increasing values of adhesiveness in the yogurt that was produced using MTGase-treated milk are related to the increase in the cross linking between proteins. After 15 days of storage, there was a ($P \leq 0.05$) reduction in the adhesiveness for unfortified-, SMP- and β-lg-fortified samples of yogurt. In addition, there were no significant differences between adhesiveness values in 15-day-old samples and in fresh samples for all treatments.

Sensory evaluation

The average sensory attributes scores of different MTGase-treated camel milk yogurts during the 15 days of storage period are presented in Table 4. Generally, all samples of yogurt had an excellent appearance, homogenous structure, white color and no free water on the surface until the end of cold storage period. The total score of samples of yogurt was significantly ($P \leq 0.05$) enhanced by the storage period to reach its maximum average for set-yogurt made from SMP-fortified milk (94.67 ± 3.67) on the 15th day. There was a 17.6% positive increase in total score for SMP-fortified yogurt compared with unfortified ones. This high increment is directly related to the high scores recorded for flavor, body, and texture. These data were parallel to the comments reported by panelists. Some of them said there was a strange but not distinctive flavor on the first day, especially for unfortified yogurt, and this flavor disappeared completely during storage. Also, there was a slightly salty taste and it is normal for camel milk but fortification of camel milk especially with SMP resulted in masking of this taste. On the other hand, the total score for WPC-fortified yogurt was recorded to be the lowest (74.78 ± 9.31) among treatment on the first day with a 1.32 percent decrease from unfortified sample of yogurt. Some panelists mentioned that the body and texture for WPC fortified yogurt was weak.

Concerning the acidity and color scores, there were no noticeable differences between treatments and during storage period for all samples of yogurt. The non-significant ($P \leq 0.05$) changes in acidity during storage period due to the impact of MTGase on the starter culture which

Table 4 Sensory evaluation (Mean ± SD) of MTGase-treated yogurt samples during 15 days of storage period

Parameters	Storage period (days)	Unfortified yogurt	SMP-fortified yogurt	WPC-fortified yogurt	βlg-fortified yogurt
Flavor (45)	1	31.00 ± 6.98^{Ab}	35.44 ± 6.86^{Ab}	32.11 ± 7.66^{Ab}	33.20 ± 5.94^{Ab}
	7	39.29 ± 4.46^{Aa}	40.86 ± 1.07^{Aa}	40.14 ± 4.41^{Aa}	40.71 ± 4.89^{Aa}
	15	34.25 ± 10.75^{Bab}	42.50 ± 1.64^{Aa}	38.17 ± 4.67^{ABab}	38.20 ± 4.60^{ABab}
Body and texture (30)	1	24.67 ± 4.00^{BCa}	27.44 ± 1.33^{Ab}	22.56 ± 2.96^{Ca}	26.40 ± 1.51^{ABa}
	7	23.86 ± 2.80^{Ba}	26.57 ± 1.40^{Ab}	26.00 ± 2.45^{Aa}	27.29 ± 1.38^{Aa}
	15	25.00 ± 6.68^{ABa}	29.33 ± 0.82^{Aa}	22.50 ± 4.76^{Ba}	26.80 ± 1.30^{ABa}
Acidity (10)	1	7.56 ± 1.81^{Aa}	8.22 ± 1.99^{Aa}	7.56 ± 2.07^{Aa}	7.90 ± 1.91^{Aa}
	7	8.29 ± 1.70^{Aa}	8.57 ± 1.51^{Aa}	8.14 ± 1.57^{Aa}	8.57 ± 1.51^{Aa}
	15	7.00 ± 1.41^{Aa}	8.50 ± 1.05^{Aa}	7.83 ± 1.47^{Aa}	7.80 ± 1.10^{Aa}
Color and appearance (15)	1	12.56 ± 2.60^{Aa}	13.44 ± 1.59^{Aa}	7.56 ± 2.07^{Bb}	13.10 ± 1.91^{Aa}
	7	13.14 ± 1.77^{Aa}	13.57 ± 1.27^{Aa}	13.43 ± 1.62^{Aa}	13.57 ± 1.27^{Aa}
	15	14.25 ± 1.50^{Aa}	14.33 ± 1.21^{Aa}	12.83 ± 1.84^{Aa}	13.40 ± 1.34^{Aa}
Total (100)	1	75.78 ± 8.86^{Bb}	84.56 ± 8.79^{Ab}	74.78 ± 9.31^{Bb}	80.60 ± 6.08^{ABb}
	7	84.57 ± 6.27^{Aa}	89.71 ± 3.73^{Aab}	87.71 ± 7.20^{Aa}	90.14 ± 6.82^{Aa}
	15	80.50 ± 7.51^{Bab}	94.67 ± 3.67^{Aa}	81.33 ± 7.58^{Bab}	86.20 ± 5.45^{Bab}

Means in the same row having different capital superscript letters are significantly different at $P \leq 0.05$. Means in the same column having different small superscript letters are significantly different at $P \leq 0.05$

led to a lower post acidification of yogurt products during storage (Lorenzen et al. 2002).

Conclusion

Camel milk produces watery texture when it is processed to yogurt. Fortification of camel milk with dairy powder ingredients such as SMP, WPC or β-lg did not improve the texture of yogurt, whereas the treatment of camel milk with MTGase preparation, added simultaneously with starter culture at a concentration of 0.4%, significantly enhanced the quality of yogurt (i.e. consistency/viscosity of the coagulum, to meet consumer demands and enabled the production of set-type yogurt from camel milk). The type of milk protein used for fortification influenced positively the physical, textural properties, and the microstructure of yogurts. The total acceptability scores of the sensory evaluation showed that the MTGase treated SMP-fortified yogurt was the most accepted product. In addition, the treatment of camel milk with MTGase reduced the fermentation time of unfortified and SMP-fortified yogurts. In order for the MTGase-treated samples to reach the final pH faster than the untreated samples, the effect of MTGase on fermentation time is not understandable, and it requires further research. In sum, this study successfully produced set-type yogurt from camel milk.

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