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The Impact of Microbial Transglutaminase on the Quality and Antioxidant Activity of Camel-Milk Soft Cheese

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Abstract

This study aimed at investigating the impact of adding microbial transglutaminase (MTGase) after rennet addition on some properties of fresh soft cheese made from camel milk. MTGase was added to milk at concentration of 80, 100 and 120 U/L after 20 and 30 min of renneting. The chemical composition, yield, hardness, antioxidant activity and sensory properties of cheese were estimated. Enzymatic protein crosslinking was analyzed by SDS-PAGE. Results revealed that MTGase-treated cheeses were higher in moisture and lower in protein content compared to control. In addition, the concentration of MTGase and time of addition significantly ($P < 0.05$) impacted these parameters. Among treated cheeses, samples with 80 U of MTGase and addition time of 20 min were the highest in total solids and protein content. Adding MTGase significantly ($P < 0.05$) increased the cheese yield, however, increased MTGase concentration at any time of addition did not improve it. The electrophoretic patterns of MTGase-cheese proteins showed a reduction in the intensity of caseins bands and the appearance of new protein fractions with high molecular weights. However, the changes in the intensity of the whey proteins bands were not sufficiently clear as caseins. The cheese hardness was significantly ($P < 0.05$) affected by adding MTGase. Cheese containing 80 U of MTGase had the highest hardness value compared to control and other treated samples. The antioxidant activity of cheese was negatively influenced by adding the enzyme. The use of MTGase enhanced the mouthfeel, texture and overall acceptability of cheese. However, the effect of MTGase concentration and addition time was not significant ($P > 0.05$) on the sensory attributes. In conclusion, adding MTGase to milk at concentration of 80 U after 20 min of renneting is recommended to improve the yield, textural and some sensory properties of fresh soft cheese made from camel milk.

Keywords

Camel Milk, Soft Cheese, Transglutaminase, Texture and Yield, Antioxidant Activity

1. Introduction

Camel milk plays an important role in nutrition, particularly in hot and dry zones, in which milk is consumed fresh or as fermented milk. Recently and in conjunction with the global trend of consuming functional foods, several studies have been carried out to emphasize the therapeutic and healthy role of camel milk for humans [1]. Nevertheless, camel milk is difficult to process into dairy products that are acceptable to consumers and can compete commercially due to the problems facing the manufacture of these products, particularly cheese.

Camel milk is characterized by its poor rennetability; it shows long rennet coagulation time [2]. Camel milk fails to form an actual curd structure, but just flakes that lack firmness are produced [3] as well as the cheese yield is low [4]. All of these problems are related to the nature of camel milk. Compared to cow milk, camel milk often has a lower content of dry matter, particularly casein [3]. It contains smaller fat globules [5]. Besides, the casein composition of camel milk is qualitatively and quantitatively different from that of cow milk. It contains a greater number of large micelles [3]. The cleavage site of chymosin in camel milk κ-casein (Phe^{97}-Ile^{98}) is different from that of cow milk (Phe^{105}-Met^{106}) as reported by [6]. Additionally, the ratios of casein fractions to whole casein in camel milk can negatively impact the curd coagulation and firmness, as β-, α_{s1}- and κ-caseins constitute about 65%, 21% and 3.47%, respectively of whole casein [6].

Several strategies have been reported to accelerate camel milk coagulation and to improve the cheese properties and yield, for instance, using ultrafiltration technique [7], fortifying camel milk with the milk of other ruminants [4] or using camel gastric enzymes instead of the commercial rennet [8]. Recently, camel chymosin (CHY-MAX® M), which introduced to the market since 2008, has shown good results for camel milk coagulation [9]. In addition, the utilization of microbial transglutaminase (MTGase) has been addressed to improve the properties of soft cheese [10].

MTGase isolated from *Streptoverticillium mobaraense* is an enzyme that catalyzes acyl-transfer reactions, which results in formation of new isopeptide bonds that can modify the structural and functional properties of proteins without any impact on the bioavailability of lysine residue [11]. MTGase is a useful tool for processing dairy products with new features due to the enzyme-induced reactions. There are a number of patents in this regard that are listed by [12]. MTGase has been approved by food industries, today almost cheese varieties are produced using this enzyme [13]. The benefits of using MTGase in cheese man-

ufacture have been reviewed [12] [14]. Briefly, MTGase helps to enhance the yield, water-holding capacity, texture, rheology and sensory properties of cheese. In addition, this enzyme has nutritional, economic and environmental importance [11] [15]. Also, it can play an important role in extending the shelf life of cheese [15].

MTGase has been used to enhance the properties of rennet-coagulated cheese. However, sometimes the competitive interactions between MTGase and rennet during rennet coagulation may constitute an obstacle during cheese manufacture, depending on the step at which MTGase was added. Four methods have been suggested for adding MTGase during cheese making. The first method is to add the enzyme in cheese milk prior to adding the rennet. The second is to add MTGase simultaneously with rennet. The third is the addition of MTGase after a certain time of adding rennet. The last method is to add MTGase after curd cutting. The use of the first method prevented or considerably delayed milk coagulation [10] [17]. In this case, the enzymatic cross-linking impacted the primary [18] or the secondary stage of rennet coagulation [19] or both stages [20]. The simultaneous addition of MTGase and rennet significantly decreased the cheese hardness as well as increased the loss of proteins and fat in the whey [10] [15]. However, other studies recommended this method [21] [22]. The third method [17] [23] and the fourth one [10] [15] [16] have been recommended, as they positively influenced the composition, yield and sensory properties of produced cheese. Concerning camel-milk cheese, data are scarce for cheese made with MTGase [10]. This study recommended adding MTGase after curd cutting. However, there is no data available on the effect of MTGase on the properties of camel-milk soft cheese as a result of adding this enzyme to milk at a certain time after rennet addition.

On the other side, milk contains considerable amounts of antioxidants such as caseins, whey proteins, certain peptides, sulfur-rich amino acids, vitamins C, A and E, carotenoids, some minerals and enzyme systems [24]. Dairy products, particularly yogurt and cheese, have higher antioxidant properties as compared to the normal milk. Intake of these products reduced the risk of various types of cancer and other chronic diseases [25]. Additionally, the antioxidant activity of milk and its products is important for extending their shelf life [26]. Processing, packaging, storage conditions and other factors have an obvious influence on the proportions of antioxidants, which is directly relevant to oxidative stability of dairy products [25] [27]. Numerous researches respecting the impact of processing on the antioxidant activity of milk and dairy products have been conducted [28]. However, until now, rare studies have been carried out on the effect of MTGase on the antioxidant activity of dairy products [29] and there is no available data on this topic regarding camel milk products.

The aim of the present study was to investigate the effect of adding MTGase, with different concentrations, after renneting on the yield, texture, quality and antioxidant activity of soft cheese made from camel milk. MTGase was added to cheese milk at a concentration of 80, 100 and 120 U/L after 20 and 30 min of

rennet addition. The chemical composition, yield, hardness, DPPH radical scavenging activity, ferric ion reducing antioxidant power (FRAP) and sensory properties of fresh cheese were evaluated. Also, SDS-PAGE was performed to monitor the protein crosslinking by MTGase.

2. Material and Methods

2.1. Materials

Dromedary camel milk was collected from the herd of Camel Research Center, Marsa Matrouh, Egypt. Microbial Transglutaminase (MTGase, EC 2.3.2.13) of *Streptoverticillium mobaraense*, (ACTIVA®YG) with a specific activity of 100 U/g was obtained from Ajinomoto Europe Sales GmbH, Hamburg, Germany. Commercially available lyophilized culture (Express 0.1, DVS) and pure camel chymosin (Far-M®) were supplied from Chr. Hansen Laboratories, Copenhagen, Denmark. 1, 1-diphenyl-2-picryl-hydrazyl (DPPH) was purchased from Sigma–Aldrich (Munich, Germany). Gallic acid, ferric chloride and potassium ferricyanide were obtained from Loba Chemie, Mumbai, India.

2.2. Cheese Preparation

Camel-milk soft cheese was prepared according to the method of [30] with some modifications. Camel milk (pH, 6.58 ± 0.01; acidity, 0.16% ± 0.01%; total solids, 12.97% ± 0.04%; protein, 3.16% ± 0.21% and fat, 3.95% ± 0.07%) was heated to 65˚C for 30 minutes, then the temperature of the milk was brought down to 40˚C. DVS starter culture (0.2 g/L) was added and incubated for 30 min, then $CaCl_2$ (0.2 g/L) and camel chymosin (0.6 mL/L) were added and gently mixed. MTGase in the range of 80, 100 and 120 U/L of milk was added after 20 and 30 min of rennet addition. The cheese milk was left undisturbed to coagulate for approximately 3 h. The whey drainage was performed by pouring the cheese curd into a plastic mold lined with a cheese cloth. After drainage of whey, the curd was pressed for 12 h. The salt was distributed on cheese surface at a level of 3% of cheese curd. Cheese without MTGase was considered as control.

2.3. Chemical Composition

Milk and fresh cheese were analyzed for moisture, protein and fat (%) using the AOAC procedures [31]. Titratable acidity (lactic acid %) of milk was evaluated by titration with 0.1 N NaOH in the presence of phenolphthalein as an indicator. The pH of milk was measured using a digital pH meter (Martini, Italy). All analyses were performed in triplicate.

2.4. Yield Calculations

The actual yield of cheese was determined as the quantity of cheese obtained from 100 Kg of milk as the formula given by [32].

$$\text{Actual yield}(\%) = \frac{\text{Cheese}(\text{Kg})}{\text{Milk}(\text{Kg})} \times 100$$

Moisture-adjusted cheese yield expressed as the quantity (kg) of cheese produced with 60% moisture was calculated as follows:

$$\text{Adjusted yield}(\%) = \frac{\text{Actual yield} \times (100 - \text{Actual percentage of moisture})}{(100 - \text{Desired percentage of moisture})}$$

2.5. Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE)

SDS-PAGE, 12.5% T, was conducted under reducing conditions using the discontinuous buffer system described by [33]. SDS-PAGE was performed on cheese samples using a Mini-PROTEAN electrophoresis cell (Bio-Rad Laboratories, Hercules, CA, USA). One gram of each cheese sample was stirred with 15 mL of cold acetone for 10 min to get rid of the cheese fat and left to dry at room temperature. The dried powdered cheese (20 mg) was mixed with 500 µL of sample buffer. Samples were denatured by boiling for 5 min, and then 7 µL of each sample was injected. The data were analyzed by Total Lab software (V1.11).

2.6. Hardness

Cheese samples for texture analysis were obtained from the middle of the whole cheese block rather than from the surface to avoid surface effects. Cheese cubes (20 × 20 × 20 mm) were placed in plastic cups, sealed (to prevent dehydration) and tempered to 10˚C ± 0.5˚C prior to analysis. Hardness was performed using the texture analyzer (Stable Micro Systems ltd. Vienna court, Lammas TA.XT. Plus) with cylindrical probe (30 mm diameter) and operated at a crosshead speed of 1 mms^{-1} and compressed distance of 10 mm. Hardness was evaluated in duplicate according to the procedure reported by [34].

2.7. Determination of Antioxidant Activity

2.7.1. Preparation of Cheese Water-Soluble Extracts

Water-soluble extracts (WSE) were prepared using the method described by [35]. Briefly, 10 g of cheese sample was suspended in 30 mL of distilled water and kept at 40˚C for 1 h under gentle stirring. Then, the homogenates were centrifuged at 10,000 ×g at 4˚C for 30 min. Thereafter, the top layer of fat was removed, the supernatant was filtered using Double Rings filter paper No. 102, and kept at −20˚C until analysis.

2.7.2. DPPH Scavenging Activity

Scavenging activity of the stable 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical was determined according to the procedure described by [36]. Two milliliters of 0.15 mM DPPH, dissolved in methanol, were added to 1 mL of extracts, mixed well and left in the dark for 30 min at room temperature. Absorbance (A) was measured at 517 nm against distilled water as a blank using UV/Visible spectrophotometer, Pharmacia-LKB-Ultrospec III (Pharmacia, USA). The control was prepared by adding 2 mL of DPPH to 1 mL of methanol. The determination was carried out in triplicate. The results were expressed as a percentage of radical

scavenging activity.

$$\text{Radical scavenging activity}(\%) = \frac{\left(A_{\text{control}} - A_{\text{sample}}\right)}{A_{\text{control}}} \times 100$$

2.7.3. Ferric ion Reducing Antioxidant Power

Ferric ion reducing antioxidant power (FRAP) was determined according to [37]. One milliliter of extracts was added to 2.5 mL of phosphate buffer (0.1 M, pH 6.6) and 2.5 mL of potassium ferricyanide (1% w/v). The mixture was then incubated in a water bath at 50˚C for 20 min followed by cooling to room temperature and adding 2.5 mL of trichloroacetic acid (10% w/v). The contents of the tubes were centrifuged at 10,000 ×g at 4˚C for 10 min. Next, 2.5 mL of supernatant was removed from each tube, and mixed with 2.5 mL of distilled water and 0.5 mL of ferric chloride solution (0.1% w/v). The mixtures were allowed to stand for 30 min then absorbance was measured at 700 nm using UV/Visible spectrophotometer, Pharmacia-LKB-Ultrospec III (Pharmacia, USA). The assay was done in triplicate. The FRAP values, expressed in mg gallic acid equivalents (GAE)/mL extract, were derived from a standard curve.

2.8. Sensory Evaluation

Cheese samples were evaluated for organoleptic characteristics (color, flavor, taste, and texture), and overall acceptability. The evaluation was conducted by 10 panelists (6 males and 4 females) from the Department of Dairy Science and Technology, Faculty of Agriculture, Alexandria University, with ages ranging from 23 to 65 years; they have a good experience in the sensory evaluation of dairy products. Cheese samples (20 g) were presented in white plastic cups coded randomly with 3-digit numbers, at a temperature of 20˚C. Water was provided between evaluations of samples for mouth rinsing. The evaluation was identified using a 5-point hedonic scale (1 dislike extremely, 5 like extremely).

2.9. Statistical Analysis

Data were analyzed for statistical differences by a two-way analysis of variance (ANOVA) (first factor: MTGase concentration, second factor: time of addition). Means were compared by Duncan's test at the significance level of $P < 0.05$. Statistical analyses were carried out using SAS, 2004 (SAS Institute, Inc., Cary, NC).

3. Results and Discussion

Adding MTGase during cheese manufacture is considered an important step for producing high-quality cheese with good coagulation properties [15] [17]. In the current study, MTGase was added to milk 20 and 30 min after rennet addition for two reasons. The first one is to give rennet an adequate time to cleave κ-casein because casein micelles are not able to aggregate until about 60% - 80% of their κ-casein is degraded [38]. The second reason is to prevent the competitive interactions between MTGase and rennet during the primary stage of rennet

coagulation due to the high susceptibility of κ–casein to MTGase. The insertion of κ-casein in the crosslinks induced by MTGase may prevent cleavage of κ-casein hence suppress the primary stage of coagulation [22].

3.1. Chemical Composition

The chemical composition (moisture, protein, fat, protein/dry matter (P/DM) and fat/dry matter (F/DM)) of camel-milk soft cheese with and without MTGase is presented in Table 1. The results of a two-way ANOVA test reveal that the moisture content of MTGase-treated cheese samples was significantly ($P < 0.05$) higher than that of control cheese (without MTGase). The cross-linking induced by MTGase increased the free volume inside the curd matrix and produced finer protein network, causing the curd to retain more water [39] [40]. Similar results have been obtained by [21] [41], who found a marked increase in the moisture content of white cheese treated with MTGase compared with the control.

Moreover, the MTGase concentration had an obvious influence on the cheese moisture content. Among MTGase-treated cheese, samples with 80 U of MTGase had the lowest moisture content. Increasing MTGase concentration to 100 U significantly ($P < 0.05$) increased the moisture content of cheese samples. While, the continuous increase of MTGase concentration up to 120 U led to a slight, but significant, decrease in cheese moisture content. Gaspar and de Góes-Favoni [42] have reported that the appropriate concentration of MTGase is associated with an increase in water holding capacity. At this concentration, MTGase provides stable and highly porous gels, which able to retain water more efficiently. While increasing the concentration beyond the optimum level range of MTGase may cause a reduction in the water holding capacity due to reduced protein-water interactions and increased the number of inter- and intramolecular isopeptide bonds [42].

Table 1. Effect of MTGase concentration and time of addition on the chemical composition of camel-milk soft cheese (Two-way ANOVA).

Factors	Parameters				
	Moisture (%)	Protein (%)	Fat (%)	P/DM (%)	F/DM (%)
MTGase concentration (U/L milk)					
Control	58.3 ± 0.84^{d}	15.3 ± 0.25^{a}	21.0 ± 0.60^{a}	36.8 ± 0.56^{a}	50.4 ± 0.04^{a}
80	67.2 ± 0.24^{c}	11.3 ± 0.48^{b}	16.3 ± 0.50^{b}	34.4 ± 1.23^{b}	49.6 ± 1.36^{a}
100	69.2 ± 1.20^{a}	10.4 ± 0.34^{c}	16.0 ± 0.82^{b}	33.9 ± 0.39^{b}	52.1 ± 3.02^{a}
120	68.7 ± 1.09^{b}	10.6 ± 0.21^{c}	15.3 ± 0.50^{b}	33.9 ± 0.61^{b}	48.7 ± 1.62^{a}
Time of addition (min)					
20	67.6 ± 0.53^{B}	11.1 ± 0.49^{A}	16.0 ± 0.63^{A}	34.2 ± 1.00^{A}	49.5 ± 1.90^{A}
30	69.1 ± 1.32^{A}	10.5 ± 0.33^{B}	15.7 ± 0.82^{A}	33.9 ± 0.54^{A}	50.7 ± 2.95^{A}

F/DM: Fat/dry matter; P/DM: Protein/dry matter. Mean values (± standard deviation) with different small letters within the MTGase concentration are significantly different at $P < 0.05$; means with different capital letters within the time of addition are significantly different at $P < 0.05$.

Also, the time of adding MTGase significantly ($P < 0.05$) affected the moisture content of cheese samples. As evident, the addition of MTGase to milk after 30 min of adding rennet increased the cheese moisture content compared to those samples to which the enzyme was added after 20 min of renneting. This may be attributed to the difference in the incubation time of milk with MTGase. In other words, cheese milk was left to coagulate in 180 min. Thus, the addition of MTGase to milk after 30 min of renneting reduced the incubation time of milk with MTGase until the end of coagulation, *i.e.* 150 min versus 160 min when MTGase added after 20 min of renneting. Gharibzahedi *et al.* [14] have declared that MTGase concentration as well as the time and temperature of incubation are the main factors affecting the cheese moisture content.

In terms of protein content, the control cheese possessed the highest content of protein and P/DM, due to the decrease in its moisture content, compared to MTGase-treated samples. This finding agrees with those obtained by [21] [43] [44]. On the contrary, other studies showed that the use of MTGase increased the protein content in cheese samples to which the enzyme was added in comparison with control [10] [23]. Concerning treated cheeses, samples containing 80 U of MTGase were the highest in their protein content ($P < 0.05$). Increasing the concentration of MTGase resulted in a significant decrease in the protein content. This is due to the high moisture content of these samples, as the decrease in the protein content was accompanied by a high percentage of moisture. However, there were no significant differences in protein content between samples containing 100 and 120 U of MTGase. Moreover, the time of addition significantly ($P < 0.05$) impacted the protein content of cheeses at all MTGase concentrations. The protein content increased in the samples in which the enzyme was added after 20 min of renneting. The higher content of protein in these samples is likely due to the prolonged incubation period (160 min) of cheese milk with MTGase; thus an increase in the binding of protein was occurred, resulting in an increase in the protein content. The concentration of MTGase and the addition time had no impact on the P/DM content of cheese samples.

The results also show that the control cheese had the highest fat content, due to its lower moisture content, compared to MTGase-treated samples. However, there were no significant differences ($P > 0.05$) in F/DM content between the control and MTGase-cheese samples. In addition, the concentration of MTGase and the time of addition had no effect on the fat and F/DM content of treated cheese samples.

3.2. Cheese Yield

The yield of camel-milk soft cheese as a result of adding MTGase is illustrated in **Figure 1**. The addition of MTGase at all concentrations to cheese milk after renneting significantly ($P < 0.05$) increased the cheese yield. MTGase free cheese possessed the lowest yield compared to other treated samples. Other studies observed the low yield of cheese from camel milk [4] [10]. The actual yields of all cheeses treated with MTGase were in the range of 19.0% - 20.5%, an increase of

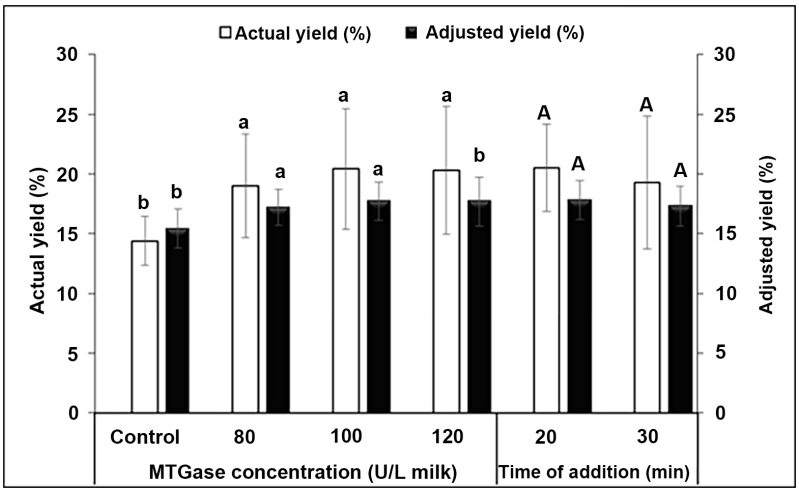

Figure 1. Effect of MTGase concentration and time of addition on the yield of camel-milk soft cheese (Two-way ANOVA). Mean values (± standard deviation) with different small letters within the MTGase concentration are significantly different at $P < 0.05$; means with different capital letters within the time of addition are significantly different at $P < 0.05$.

31.9% - 42.4%, respectively with respect to control. Similarly, the adjusted yield was increased by 11.7%, 14.9% and 14.9% when it added at a concentration of 80, 100 and 120 U compared to control. The increment in the yield of MTGase-cheeses mainly attributed to their high moisture content due to the addition of MTGase. These results are consistent with the previous studies by [15] [21] [43]. Ibrahim and Khalifa [10] found that adding 100 U of MTGase/L milk after curd cutting significantly increased the yield value of camel-milk soft cheese, it was 15.13 versus 11.60% for control.

Increasing the concentration of MTGase from 80 to 120 U did not improve the cheese yield. Besides, adding MTGase 20 or 30 min after renneting had the same impact on cheese yield. Other investigations have declared that the addition of MTGase increased the yield of cheeses in a concentration-dose manner [45]. However, [17] found that the addition of MTGase up to 60 U to cow milk after renneting resulted in a noticeable increase in the cheese yield, followed by a slight decrease in the yield due to increased MTGase concentration over 60 U. In the present study, the increase in the moisture content of cheese treated with MTGase was accompanied by a decrease in protein content (**Table 1**); therefore the effect of enzyme concentration on increasing cheese yield was not significant. In conclusion, Adding MTGase after renneting significantly increased the yield of cheese. It is recommended to use MTGase at a concentration of 80 U after 20 min of rennet addition to improve the yield of soft cheese made from camel milk.

3.3. SDS-PAGE Electrophoresis

SDS-PAGE is considered an acceptable method to monitor the action of MTGase due to it shows cross-linking caused by the enzyme through the forma-

tion of new high molecular weight bands [46]. The impact of MTGase on the protein patterns of fresh soft cheese made from camel milk is illustrated in **Figure 2**. The electrophoretic patterns of cheese proteins showed the appearance of several bands on the gel, differed in their migration positions and band intensity. In all cheese samples, it is clear that casein was resolved into two major bands, α_s- and β-caseins, however, the intensity of these bands was higher in control than MTGase-treated samples. Further, there were new fractions of protein with different molecular weights appeared with samples containing MTGase. These new fractions were formed due to the action of MTGase, which catalyzes formation of chemical crosslinks within milk proteins, so formation of polymers with high molecular weight. These results agree with other studies, in which MTGase had the same effect on camel milk proteins [39]. The decrease in the intensity of caseins bands on the gel elucidated that these proteins are preferred substrate for MTGase due to their flexible open structure [47] and due to the absence of disulphide bonds in α_{s1}- and β-caseins, leaving the reactive groups exposed to the enzyme [48].

Regarding whey proteins, the changes in the intensity of the whey proteins bands were not sufficiently clear as caseins. This mostly attributed to the low-temperature heat treatment used in current study; cheese milk was pasteurized at 65˚C/30 min. This thermal treatment is insufficient to improve the reactivity of whey proteins towards MTGase. At this temperature, studies showed that no effect was found on the whey proteins of camel milk [49] [50]. The denaturation of whey proteins is necessary for MTGase to work effectively. The existence of whey proteins, particularly α-lactalbumin, in their globular shape impedes the action of the enzyme. Gauche [51] reported that native whey proteins are poor substrates for TGase, denaturation process leads to exposure of more sites for TGase-specific reaction. Recently, [39] declared that thermal treatment of camel milk before reaction with MTGase (90˚C/15 min) significantly enhanced the reactivity of whey proteins towards protein cross-linking due to a series of interactions arising from heating.

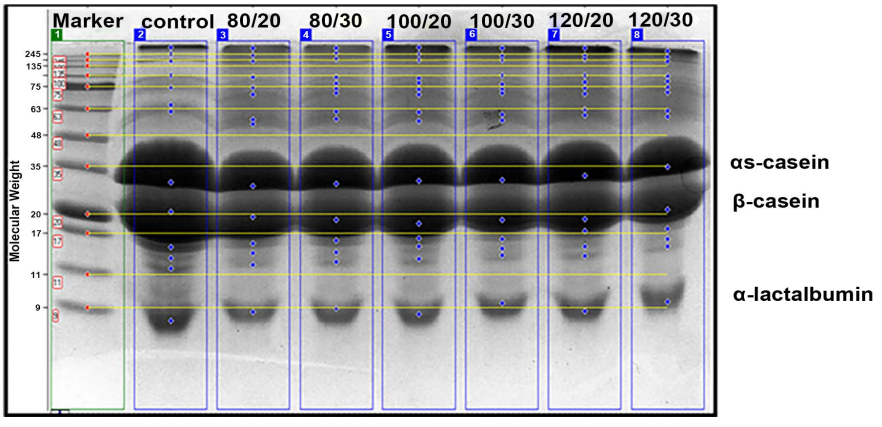

Figure 2. SDS-PAGE (12.5%T) of camel-milk soft cheese treated with MTGase at different concentration (80, 100 and 120 U/L of milk) and addition time (20 and 30 min after renneting).

The effect of MTGase concentration on the milk proteins is almost the same except for the treatment that contains 80 U of the enzyme. In addition, the density of new polymers with high molecular weight decreased by increasing the time of addition at all MTGase concentration. This behavior is due to the long incubation time of milk with MTGase when it added 20 min after renneting. Overall, the electrophoretic pattern of the sample with 80 U of MTGase and addition time of 20 min was somewhat differed from that of other treated samples as the new bands were more intense as evidenced by Total Lab software.

3.4. Hardness

Hardness is the force required for compressing a cheese between the molars [52]. The results in **Figure 3** clearly show that the hardness was significantly ($P < 0.05$) increased in all cheeses that made using milk treated with MTGase when compared to control. This is due to the fact that MTGase could modify the textural properties of cheese through the formation of covalent bonds of ε-(γ-glutamyl) lysine, which were catalyzed by the enzyme [15] [41] [45]. The intra- and intermolecular crosslinks induced by this enzyme yielded a strong three-dimensional gel network thus increased the hardness of cheeses containing MTGase [53].

Regarding the MTGase-treated samples, it can be observed that the hardness was the highest ($P < 0.05$) in the sample made using 80 U of the enzyme. There was a significant reduction in hardness with increasing the dosage of MTGase more than 80 U. Moreover, there were no significant differences in hardness between the samples with 100 and 120 U of MTGase. Similar results have also been obtained with paneer, as further increase in the concentration of MTGase

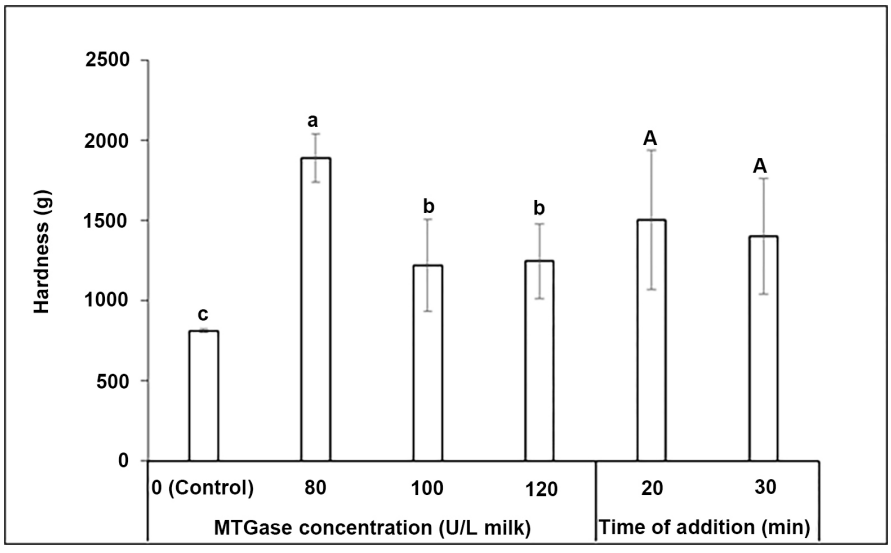

Figure 3. Effect of MTGase concentration and time of addition on the hardness of camel-milk soft cheese (Two-way ANOVA). Mean values (± standard deviation) with different small letters within the MTGase concentration are significantly different at $P < 0.05$; means with different capital letters within the time of addition are significantly different at $P < 0.05$.

significantly ($P < 0.05$) decreased the hardness of the samples [45]. Likewise, [15] found that adding MTGase after curd cutting from 12 up to 60 U/L milk significantly increased the hardness value of cow-milk soft cheese, increasing the enzyme concentration to 72 U/L led to a remarkable drop in hardness. A study by [10] found that adding MTGase (0 - 100 U/L milk) after curd cutting caused an improvement in the hardness of camel-milk soft cheese that increased with MTGase concentration, the maximum hardness was recorded in cheese contained 100 U of the enzyme.

The higher protein content of cheese containing 80 U of MTGase, together with the action of the enzyme, could be the reason for the harder texture of this cheese in comparison with other treated samples. Earlier, [54] reported that proteins play a significant role in cheese texture since they represent the only continuous solid phase of the product. Hence, any modification of the nature or the amount of the protein will alter the texture of cheese. Whereas, the cause of low hardness in samples with 100 and 120 U of MTGase could be returned to the higher moisture content of these samples. The water content of the product is a determining factor for texture; therefore, small variations in the water content will highly impact the firmness [54]. Lately, [21] have reported that increased the serum pores number in the microstructure of MTGase-treated cheese resulted in lowering of the hardness with respect to the control cheese due to the plasticizer role of water, which give the cheese structure more flexibility.

With regard to the addition time of MTGase, the results show that adding the enzyme either 20 or 30 min after adding rennet had no significant impact on the hardness of camel milk soft cheese.

3.5. Antioxidant Activity

Antioxidant capacity assays are beneficial to determine the overall antioxidant activity in foods [25]. In the current study, the antioxidant activity of cheese water-soluble extracts was measured by two different assays, which are DPPH radical scavenging activity and FRAP. These assays were used to evaluate the antioxidant activity from amino acids in milk and dairy products that can act as hydrogen donors [25].

The influence of MTGase on the antioxidant activity of fresh soft cheese is illustrated in **Figure 4**. As shown, the water extract of control samples exhibited the highest DPPH radical scavenging activity and FRAP value ($P < 0.05$) compared to MTGase-treated samples. As expected, the antioxidant activity of cheese decreased by adding MTGase. Cheese possesses antioxidant capacity because it contains antioxidants, primarily casein. However, cheeses show variation of this activity due to many factors of which, the processing conditions as well as the difference in their composition and structure [26]. MTGase as a transferase forms new inter- and intramolecular ε-(γ-glutamyl) lysine bonds by cross-linking lysine and glutamine residues [11], result in the polymerization of almost milk proteins and peptides [39]. The polymerization process could

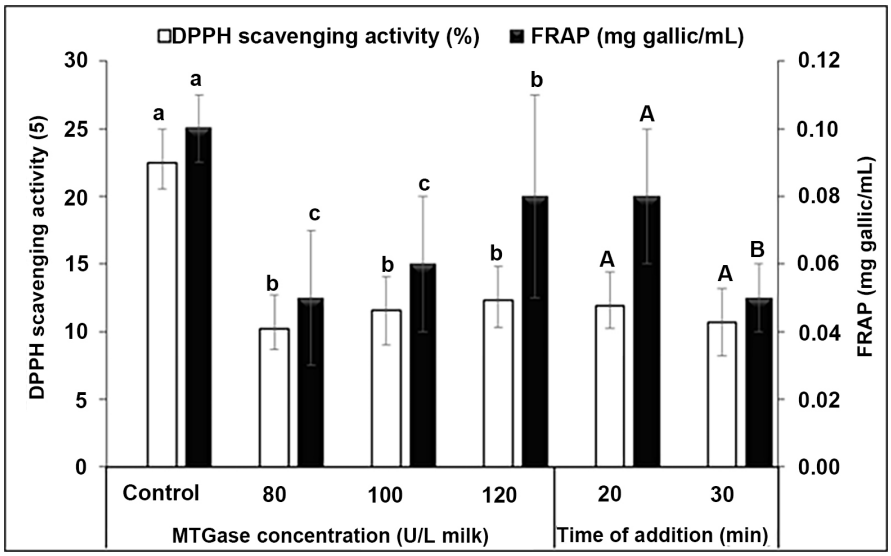

Figure 4. Effect of MTGase concentration and time of addition on the antioxidant activity of camel-milk soft cheese (Two-way ANOVA). Mean values (± standard deviation) with different small letters within the MTGase concentration are significantly different at $P < 0.05$; means with different capital letters within the time of addition are significantly different at $P < 0.05$.

decrease the antioxidant activity of the final product via inserting specific amino acid residues with antioxidant properties such as lysine in the interaction. It has been reported that the amino acid profile determines the antioxidant activity of proteins [25]. Also, the reduction in the antioxidant activity of MTGase-treated cheese may be due to the nature of the curd microstructure. The additional cross-linking induced by MTGase produces a dense protein network [39]. Recently, [55] have mentioned that the microstructure of the curd can influence its antioxidant properties, as the densest network showed the lowest antioxidant capacity due to the presence of protein segments not flexible enough to act as antioxidants. Beyond that, the decrease in protein content may be another reason for the decrease in antioxidant activity of MTGase-treated cheese in comparison to control.

In addition, the DPPH scavenging activity of treated cheese was not affected by the increased concentration of MTGase. Similarly, Increasing the MTGase concentrations from 80 to 100 U did not affect the FRAP value. However, increasing the enzyme dose above 100 U resulted in a slight, but significant, increase in the value of FRAP. Moreover, no significant differences ($P > 0.05$) were found between the treated cheeses in terms of DPPH scavenging activity due to the time of addition. On the contrary, the time of addition significantly ($P < 0.05$) affected the FRAP values. The FRAP value for samples to which MTGase was added 20 minutes after rennet addition was higher ($P < 0.05$) than those for which the enzyme was added after 30 minutes. There is no explanation for this behavior; it is assumed that adding MTGase 20 min after rennet addition provides a longer incubation time for MTGase with milk that may reduce or even

not affect the antioxidant activity of the final product as in DPPH scavenging activity.

3.6. Sensory Evaluation

The sensory evaluation of the control and MTGase-treated cheese samples was carried out for color, flavor, taste, texture and overall acceptability (Table 2). The results revealed that no significant ($P > 0.05$) differences were found between the untreated and MTGase-treated cheese samples in terms of color, flavor and taste. Similar data for soft cheese were reported regarding the color [15] [43] and the flavor [15]. Except that [43] found that MTGase has a positive effect on the cheese flavor. Concerning taste, it is worth mentioning that despite there were no statistical differences between the control and treated samples in the taste scores, the panelists were able to discern a creamy taste in the MTGase-treated cheese which was not found in control. Other investigations have confirmed this finding [15] [43].

With regard to texture, the control recorded the lowest rating for texture in comparison with MTGase-treated samples. This result confirms the previous results concerning the positive influence of this enzyme on the texture of soft cheese [43] [56]. The enhancement of texture in treated cheese could be due to its high water content and the cross-linking bonds between protein molecules, which caused by the enzyme as reported by [44] [56]. On the otherwise, [15] found that the texture of soft cheese was not significantly impacted by the addition of MTGase. The results of sensory evaluation also reflected that MTGase concentration did not impact the texture of cheese samples.

Also, results revealed that MTGase-treated samples had higher rating compared with control for overall acceptability, particularly those samples containing

Table 2. Effect of MTGase concentration and time of addition on the sensory properties of camel-milk soft cheese (Two-way ANOVA).

Factors	Parameters				
	Color (5)	Flavor (5)	Taste (5)	Texture (5)	Overall acceptability (5)
MTGase concentration (U/L milk)					
Control	4.3 ± 0.82^{a}	3.9 ± 1.07^{a}	3.6 ± 1.11^{a}	2.9 ± 1.24^{b}	3.2 ± 0.91^{b}
80	4.6 ± 0.63^{a}	4.0 ± 1.11^{a}	4.0 ± 1.01^{a}	3.8 ± 0.85^{a}	3.8 ± 0.72^{ab}
100	4.7 ± 0.47^{a}	4.3 ± 0.91^{a}	4.3 ± 1.09^{a}	3.6 ± 0.72^{a}	3.6 ± 0.72^{ab}
120	4.6 ± 0.50^{a}	4.2 ± 0.97^{a}	4.2 ± 0.99^{a}	4.0 ± 0.57^{a}	4.1 ± 0.63^{a}
Time of addition (min)					
20	4.7 ± 0.48^{A}	4.2 ± 0.89^{A}	4.2 ± 0.90^{A}	4.0 ± 0.58^{A}	4.0 ± 0.66^{A}
30	4.6 ± 0.58^{A}	4.1 ± 1.09^{A}	4.1 ± 1.12^{A}	3.6 ± 0.82^{A}	3.8 ± 0.75^{A}

Mean values (± standard deviation) with different small letters within the MTGase concentration are significantly different at $P < 0.05$; means with different capital letters within the time of addition are significantly different at $P < 0.05$.

120 U of MTGase. No significant ($P > 0.05$) differences were found among MTGase-treated samples as a result of increasing the dose of the enzyme. Moreover, the addition of MTGase 20 or 30 min after adding rennet had the same impact on the sensory characteristics of all cheese samples. In sum, inclusion of MTGase enhanced the texture and overall acceptability of fresh soft cheese made from camel milk.

4. Conclusion

The cross-linking between camel-milk proteins caused by MTGase enhanced the properties of soft cheese but negatively influenced its antioxidant activity. Moreover, the concentration of MTGase and time of addition highly impacted some properties of cheese. Despite the increase in the yield of MTGase cheese was mainly related to the high moisture content, this increase in moisture did not negatively affect the properties of cheese; the treated cheese was superior in its texture and overall acceptability compared to control. MTGase added to milk at a concentration of 80 U after 20 min of renneting is recommended for improving the yield, textural and some sensory properties of soft cheese made from camel milk.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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