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Process design for processed Kashar cheese (a pasta-filata cheese) by means of microbial transglutaminase: Effect on physical properties, yield and proteolysis

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ABSTRACT

Cheese industry has long been interested in improving the quality and functionality of cheese to meet consumers' demand as well as reducing the production costs by using different manufacturing strategies. In this study, the effects of microbial transglutaminase (mTG) on the yield, proteolysis and physical properties of processed Kashar cheese were evaluated. The mTG level used was 0.75 U/g protein and the concentrations of emulsifying salt (ES) mixture were 0.8, 1.0 and 1.2 g/100 g cheese curd (w/w). The increase in the moisture adjusted yield of cheeses were between 1.68 and 2.00% as relative change compared to control cheese. The cheeses had similar melting profile with the control cheese. The rheological and textural properties of the cheeses were different from the control cheese. The ES concentration and addition of mTG had an impact on the proteolysis but it did not affect meltability in current concentration. To conclude, the yield of processed Kashar cheese may well be increased to a satisfactory level without adversely affecting the physical characteristics of the end product.

1. Introduction

Enzymatic modification of milk proteins to produce cheese with optimized yield, shelf-life and quality characteristics have long been on the agenda of dairy industry. By now, a great number of commercial enzymes have been introduced into the dairy markets. Among these enzymes, microbial transglutaminase (mTG; protein-glutamine γ-glutamyltransferase, EC 2.3.2.13) which is a well-known food texture-promoting enzyme, has been subjected to a great number of scientific evaluations and is enjoying a commercial success today. It has long been established that mTG catalyses an acyl transfer reaction between γ-carboxyamide group of peptide-bound glutamine residue (acyl donor) and the primary amine group of glutamine and lysine residues (Liu & Damodaran, 1999; Moon, Hong, Huppertz, Fox, & Kelly, 2009; Romeih & Walker, 2017). This catalytic function leads to formation of additional intra- and inter-molecular covalent bonds between proteins (*i.e.*, α_s-, β-, κ-caseins) in which more free water is entrapped (Aaltonen, Huumonen, & Myllärinen, 2014). Additionally, smaller peptides may also be bound to proteins by mTG.

The application of mTG in cheese production is somehow more challenging than the other dairy products since optimum conditions of rennet and mTG in cheese milk do not overlap. For example, rennet reaches the optimum activity for raw milk at 42.5 °C and at pH 6.6, but mTG requires higher temperature (i.e., 50 °C) and slightly lower pH (i.e., 6.3) for its optimum activity. Therefore, a proper design of cheese-making process is required to make cheese environment suitable for optimum activities of both enzymes (Özer, Guyot, & Kulozik, 2012; Özer, Hayaloglu, Yaman, Gürsoy, & Şener, 2013). Primary manufacturing parameters that need to be considered in the optimization of use of mTG in cheese-making are initial milk pH, mTG dosage, renneting temperature, pre-heating of milk and sequence of mTG addition (*i.e.* before, after or simultaneously with rennet) (Bönisch, Heidebach, & Kulozik, 2008; Di Pierro et al., 2010; Özer et al., 2012). Although the mTG application conditions may vary depending on the type of cheese, slight reduction of initial milk pH (*i.e.* to 6.3), pre-heating under mild thermal conditions (*i.e.* 75-78 °C for 1–2 min) and simultaneously use of mTG with rennet are recommended (Desá & Bordignon-Luiz, 2010; Özer et al., 2012). Despite the fact that mTG improves the textural properties of cheese and increase cheese yield through partly fat and protein recovery, it may adversely affect some technological characteristics of cheese such as slow ripening, poor meltability and stretchability. Lower level of proteolysis was reported for mTG-treated White Brined Turkish cheese (Özer et al., 2013) and low fat Cheddar cheese (Hu et al., 2013). In order to overcome this handicap, Aaltonen

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et al. (2014) produced Edam cheese from milk of which protein level was adjusted to 12 g/100 g by ultrafiltration and then the retentate was treated with mTG at 17 °C for 2 h. This modification resulted in *ca.* 4 g/100 g increase in cheese yield with no considerable change in proteolytic profile of the resulting product.

Kashar cheese (a pasta-filata type Turkish cheese) is the second most commonly consumed cheese variety in Turkey. Traditionally, Kashar cheese is produced by scalding the cheese curd in hot brine (75–80 °C, 3–5 g/100 g NaCl, w/v) and then plasticized mass is moulded. Since the loss of protein and fat to the scalding solution is relatively high, this production method is largely abandoned in commercial practices. Currently, Kashar cheese is manufactured by dry scalding the cheese curd at *ca.* 70–80 °C in the presence of emulsifying salts (also called processed Kashar cheese). Dry scalding refers to the heat treatment to the blend of cheese curd, NaCl and emulsifying salts (citrate and phosphates based), by indirect and/or direct steam injection system with variable agitation speed by using twin-screw mechanical mixers to produce a homogeneous mixture with acceptable melting and stretching properties. Processed Kashar cheese is widely used in pizza- and toast-making. Therefore, melting properties are as important as the other characteristics of this variety (i.e. proteolysis). Whichever modification is made on the Kashar cheese manufacturing should primarily target to protect or improve the meltability of the end product. In processed Kashar cheese, yield is slightly higher than the traditional Kashar cheese but still needs a proper process design to minimize the loss of protein and fat during dry scalding. In this case, application of mTG to Kashar cheese milk may offer a practical solution to Kashar cheese industry. The present study was aimed to develop a new production protocol for processed Kashar cheese by incorporating mTG without adversely affecting physical properties of the resulting cheese and to evaluate yield and proteolysis. Since processed Kashar cheese is a high cooked pasta-filata cheese variety, the new production protocol developed for processed Kashar cheese could well be adapted to the manufacturing practices of other high cooked pasta filata type cheeses such as Kashkaval, Mozzarella or Provalone to improve yield and physical characteristics.

2. Materials and methods

2.1. Cheese making

Processed Kashar cheese was manufactured at Maysa Food R&D Center Pilot Dairy Processing Plant (Istanbul, Turkey). Cheese-making procedure and the photos of processing steps are given in Fig. 1 and Supplementary Fig. S1, respectively. Briefly, pasteurized milk (68 °C for 10 min, 150 L) was inoculated with a direct-vat-set freeze-dried starter culture of *Streptococcus thermophilus* (CT-333, Maysa Food, Istanbul, Turkey) which was added at a concentration of 1×10^8 cfu/mL of milk. When pH 6.3 was attained, rennet and microbial transglutaminase (mTG, 0,75 U/g protein, ACTIVA®YG, with declared enzyme activity of 100 U/g powder) was added to milk simultaneously. Then, the curd was cut into 1 cm^3 cubes and pressed to remove cheese whey until pH 5.10–5.15. Afterwards, the pressed curd was divided into three equal batches based on their weights. Each batch was transferred into a double wall twin screw cheese processing tank separately where the blocks of curd were cut into small pieces and steam was injected into the pieces. The temperature of the cheese mass was increased with a continuous mixing. Then, NaCl (1.5 g/100 g cheese curd, w/w) and the emulsifying salts (ES; Kasomel™ 3112 and 2185) were added by sprinkling (ES concentrations were 1.0 (for control cheese), 0.8 (first batch), 1.0 (second batch), and 1.2 (third batch) g/100 g cheese curd, w/w). Temperature of melted cheese was 70–72 °C. The cheese processing tanks had a twin screw mixer and the emulsifying salts were thoroughly mixed with the softened cheese mass for 5 min (Fig. S1). Emulsifying salts and calf rennet (MYSECOREN, 200 IMCU) was supplied from Maysa Food. Kasomel™ 3112 contains a blend of food grade phosphate salts (E331, E339, E452) with strong ion exchange and weak creaming capacity. It contributes to the hardening of the melted cheeses. Kasomel™ 2185 is also a blend of the same phosphate salts with medium ion exchange and weak creaming capacity. The major function of Kasomel™2185 is to enhance the re-melting properties. Kasomel™ 3112 and 2185 powders were mixed as ratio of 70:30 (w/w), then added to cheese curd as mentioned rate of above given, based on the recommendations of the manufacturer and industrial applications. Transglutaminase was kindly gifted by Ajinomoto Foods Europe S.A.S. (Mesnil, Saint-Nicaise, France). All the chemicals were supplied from Sigma-Aldrich Inc. (Sigma-Aldrich Inc. St.Louis, MO, USA) unless otherwise stated.

2.2. Compositional analysis and yield

The pH, moisture, protein and fat levels of the cheeses were determined as per Bulat and Topcu (2019). Gross composition of cheese milk and cheese whey was determined by Milkoscan™ Minor (Foss, Tekafos, Istanbul, Turkey). Actual yield (Y_a) and moisture-adjusted cheese yield (*MACY*) was calculated using the below equations according to Fox, Guinee, Cogan, and McSweeney (2000). For *MACY* calculation, reference moisture content was taken as 45 g/100 g cheese.

$$\textit{Actual yield}\ (Y_a,\ kg/100\ kg) = \frac{\textit{Cheese weight}\ (kg)}{\textit{Milk weight}\ (kg) + \textit{starter culture} + \textit{salts}} \times 100$$

$$MACY\ (kg/100\ kg) = \frac{100 - \textit{actual cheese moisture content}}{100 - \textit{reference cheese moisture content}} \times Y_a$$

2.3. Electrophoresis and HPLC analyses

The pH 4.6-soluble nitrogen fractions of the cheeses were prepared as per Kuchroo and Fox (1982) with a minor modification. Cheese was mixed with deionized water at a ratio of 1:5 (w/w) instead of 1:2 (w/w) and homogenized using an ultraturrax (Bulat & Topcu, 2019). The, pH of slurry was adjusted to 4.6 by using 2 N HCl. Then the mix was rested for 1 h at 40 °C, and centrifuged (5000 × *g*, 30 min, 4 °C). After removing the fat layer, the supernatant (soluble fractions) and pellet (insoluble fractions) were separated. The pH 4.6-insoluble fraction was freeze-dried for further use in urea-polyacrylamide gel electrophoresis (Urea-PAGE) and sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) analysis. The pH 4.6 soluble nitrogen fractions of the cheese samples were mixed with deionized water containing 0.2% TFA (trifluoroacetic acid, v/v) in a 1:1 ratio. The mixture was transferred to HPLC vials using a 0.45 μm cellulose acetate filter and analysed by a RP-HPLC using Spectra System HPLC (Thermo Finnigan Inc., CA, USA) as described by Topcu and Saldamli (2006). Separation was done using a C18 wide pore column (Jupiter 5 μm, 250 × 4.6 mm, Phenomenex, Torrance, CA) at 30 °C. The absorbance was monitored at 214 and 280 nm (Bulat & Topcu, 2019).

Urea-PAGE analysis were done according to Shalabi and Fox (1987). 10 mg of the freeze-dried pH-4.6 insoluble fraction was resolved in 1 mL of sample buffer. 8 μL of samples prepared was loaded onto the gels and the constant voltage of 300 V was applied. The gels were stained overnight with Coomassie Brilliant Blue G-250 (Blakesley & Boezi, 1977) and destained in distilled water. Quantification of β-casein and α_{s1}-casein was done by a densitometry combined with TotalLab TL100 1D gel analyses software (Version 2006, Nonlinear Dynamics, Newcastle-upon-Tyne, UK). The changes of casein fractions and serum proteins were also monitored by SDS-PAGE as described by Laemmli (1970) with some modifications as per Bulat and Topcu (2019). The freeze-dried pH-4.6 insoluble fraction was dissolved in sample buffer (10 mg/mL) and 6 μL of sample was run through the stacking and separating gels at 200 mV. Gels were kept in fixing solution (1.0:3.3:5.7 mixture of 100 g/100 mL TCA, methanol and deionized water) for

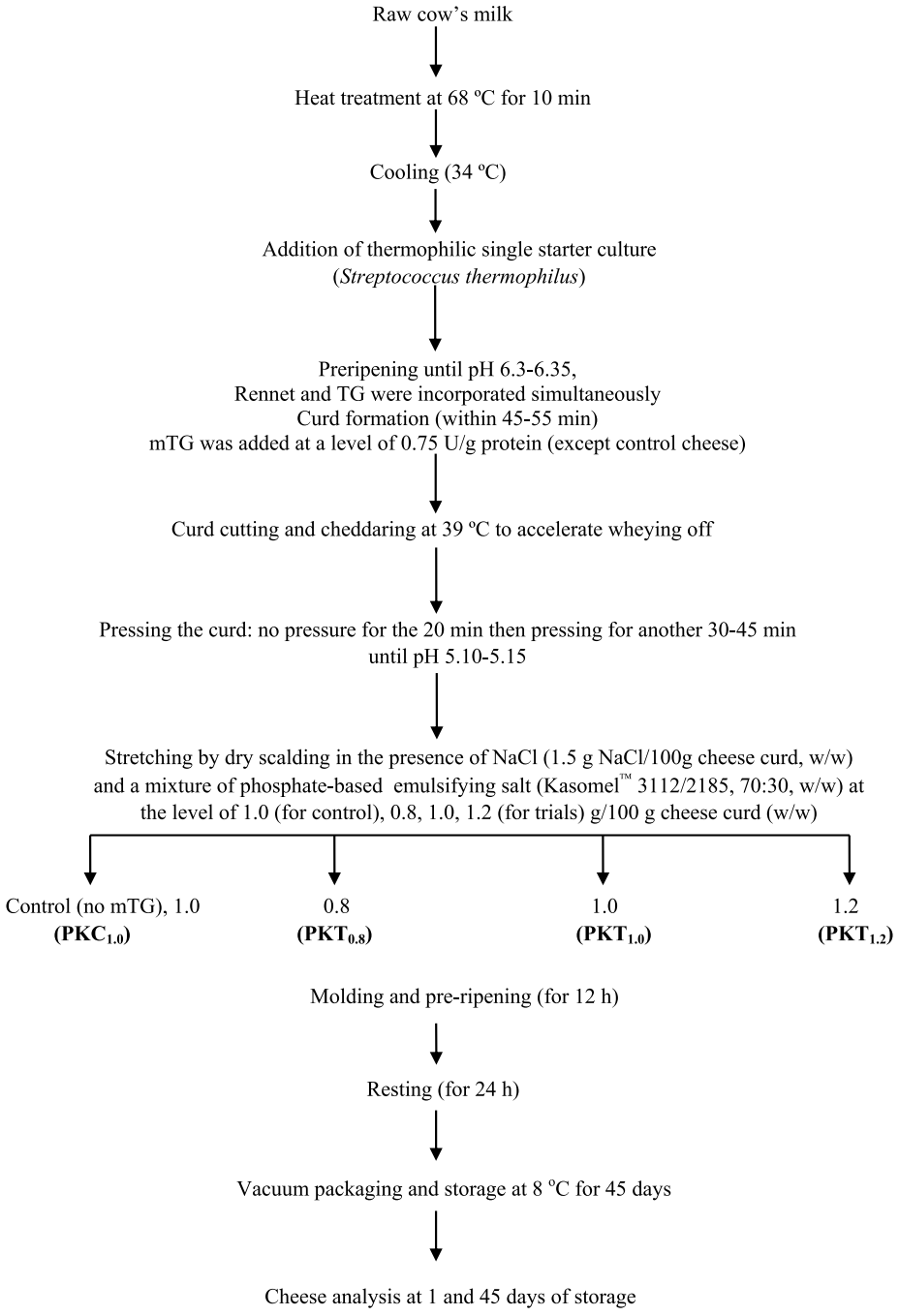

Fig. 1. Flow diagram of the processed Kashar cheese production.

60 min and then, similar staining and destaining method were applied as described above for urea-PAGE. Electrophoretic studies were carried out by using Protean II XL vertical electrophoresis units (Bio-Rad Laboratories Ltd., Watford, UK).

2.4. Physical analyses

2.4.1. Texture profile analysis

The large deformation physical properties of cheeses were monitored by means of a texture profile analyser (TA.XT2 model, Stable Micro Systems combined with an XT.RA dimension package, Blackdown Rural Industries, Surrey, UK). The analysis were done under the following conditions: cell load, 30 kg; diameter of cylinder probe, 35 mm; the sample surface area, 1635 mm^2, sample height, 30 mm; travel speed of the cylinder probe, 2 mm/s; total measuring time, 3.5 s and distance of compression, 7 mm. Each cheese block weighed 500 g and was square with ~50 cm^2 surface area. Three pieces from each cheese block were taken into analysis.

2.4.2. Temperature sweep tests

Temperature-dependent rheological properties of the samples were evaluated by means of a dynamic rheometer (Malvern Kinexus Lab Pro +, Malvern Instruments Ltd., Malvern, UK) with the following test conditions: set temperature range, 5–85 °C; normal force, 1.8 N; frequency, 0.8 Hz, shear strain, 0.5 percent; test time, 40 min; sampling interval, 30 s; sample size, 3 mm thickness and 20 mm diameter. Cheese discs were placed between the parallel plates of the rheometer. The diameter of the upper plate was 20 mm. In order to avoid drying, sunflower oil was applied around the edges of the discs. All the rheological experiments were done in triplicate.

2.4.3. Oscillation and creep/compliance experiments

The oscillation and creep/compliance tests were performed according to Messens, van de Walle, Dewettinck and Huyghebaert (2000) with slight modification in sampling. The cheese samples were cut into discs of 5 mm thickness and 20 mm diameter each. Then the samples were covered with an aluminium foil to avoid evaporation and kept at 15 °C until analysis. Measurements were achieved using parallel plate geometry with 2.5 cm diameter and a gap setting of 4 mm. All the oscillation studies were carried out within the linear viscoelastic region at 1 Hz and 1 percent strain. Creep measurements were achieved under the following conditions: 300 Pa of instantaneous stress for 120 s (creep time), followed by a recovery time of 120 s. Results were expressed in compliance units. A compression test was performed and the parallel plate geometry was completely filled with the sample after compression.

2.5. Statistical analysis

Data obtained were statistically processed with one-way analysis of variance. Different groups were determined by means of Duncan's multiple range test at a 0.05 significance level. The experiment was carried out in duplicate (n = 2) and statistical evaluations were done using the SPSS v16.0 (SPSS Inc., Chicago, IL, USA). Data obtained from RP-HPLC chromatogram from 5 to 80 min (serum proteins that eluted after 80 min were excluded) were subjected to the Principal Component Analysis (PCA) as described by Bulat and Topcu (2019).

3. Results and discussion

3.1. Impact of mTG on cheese composition and yield

The gross composition of raw cow's milk, cheese samples and cheese whey are given in Table 1. The moisture levels of the cheeses containing mTG were higher than the control sample ($P < 0.05$). The protein-in-dry matter levels of the cheeses treated with mTG varied between 44.60 and 44.89 g/100 g and were found to be higher than the control cheese (43.64 g/100 g). The cheese matrix modified by mTG retained more protein than the control cheese. Higher protein-in-dry matter values were also reported for the White brined Turkish cheeses made by mTG-treated milks (Özer et al., 2013). Neither emulsifying salt level nor mTG treatment had a clear effect on pH values and except for the cheese containing 0.8 g/100 g ES fat-in-dry matter levels of the cheeses.

Yield values of the cheeses are given in Table 2. The Y_a values of the cheeses containing mTG were higher than the control cheese. A similar trend was obtained in the *MACY* values of the cheeses with lower figures than the Y_a values. Compared with the control cheese, the highest increase in the *MACY* was obtained in the mTG-treated sample containing the highest level of emulsifying salt (ES) (1.2 g/100 g). Overall, the higher the level of emulsifying salt, the higher the Y_a and *MACY* in the end product. This fact was probably a result of more water retention in the cheese mass as a function of emulsifying salt mixtures or additional covalent bonds formed by mTG.

Table 2
Actual (Y_a, kg cheese/100 kg milk) and moisture-adjusted cheese yield (*MACY*, kg cheese/100 kg milk) (adjusted to the moisture of 45 g/100 g cheese) values of the experimental cheeses (n = 2).

	Y_a	Increase in Y_a (%)[a]	*MACY*	Increase in *MACY* (%)[a]
$PKC_{1.0}$	9.75	–	8.49	–
$PKT_{0.8}$	10.83	1.08	10.17	1.68
$PKT_{1.0}$	10.92	1.17	10.36	1.87
$PKT_{1.2}$	10.98	1.23	10.49	2.00

[a] % relative change compared to control cheese. For sample codes refer to Fig. 1.

3.2. Influence of mTG on electrophoresis patterns and peptide profiles of the cheeses

Urea-PAGE electrophoretograms of pH 4.6-insoluble fractions of the cheeses at day 1 and 45 were given in Fig. 2a. There were notable differences in urea-PAGE patterns of the cheeses. When compared with the control cheese, addition of mTG restricted degradation of α_{s1}-casein (CN). However, β-CN degradation was negligible in all cheeses. The residual α_{s1}-CN in the control cheese was lower than the rest of the samples (Fig. 2b). Addition of emulsifying salt at different concentrations did not have notable effect on casein degradation (Fig. 2a and b). Remaining chymosin in cheese matrix is primarily responsible for the hydrolysis α_{s1}-CN from the peptide bond between Phe_{23} and Phe_{24} to yield α_{s1}-CN (f1-23) and α_{s1}-CN (f24-199) also called α_{s1}-I-CN (McSweeney, Olson, Fox, Healy, & Højrup, 1993). In the cheeses treated with mTG, α_{s1}-CN remained nearly intact and α_{s1}-CN (f24-199) did not appear after 45 days of ripening. Hydrolysis of α_{s1}-and β-CN results in an increase in meltability and a decrease in the toughness and fibrous consistency of the pasta-filata type cheeses (Kindstedt, Hiller, & Mayes, 2010). The rate of primary proteolysis affects the physical functionality of pasta-filata type cheeses (*e.g.* processed Kashar cheese) (Kindstedt, Hillier, & Mayes, 2010). Therefore, advanced proteolysis in processed Kashar cheeses is usually undesirable for an optimum stretchability and meltability. Enzymatic crosslinking of caseins may decelerate the proteolysis in cheese probably due to the reduced availability of reactive sites of proteins for proteinase activity in the newly formed matrix. O'Sullivan, Kelly, and Fox (2002) showed that casein micelles in TGase-treated milks were resistant to hydrolysis by plasmin.

Table 1
Gross chemical composition of processed Kashar cheese (n = 2).

	Moisture (g/100 g)	Fat (g/100 g)	F/DM (g/100 g)	Protein (g/100 g)	Prt/DM (g/100 g)	pH
Raw milk	88.01 ± 0.01	3.50 ± 0.02	29.19 ± 0.02	3.16 ± 0.01	26.35 ± 0.01	6.67 ± 0.04
Cheeses						
$PKC_{1.0}$	47.94 ± 0.19^{c}	24.00 ± 0.04^{b}	46.10 ± 0.35^{a}	22.72 ± 0.10^{c}	43.64 ± 0.10^{a}	5.54 ± 0.01^{b}
$PKT_{0.8}$	52,67 ± 0.25^{a}	22.50 ± 0.12^{a}	47.53 ± 0.19^{b}	21.11 ± 0.23^{a}	44.60 ± 0.24^{b}	5.38 ± 0.01^{a}
$PKT_{1.0}$	51.37 ± 0.11^{b}	22.60 ± 0.08^{a}	46.47 ± 0.44^{a}	21.75 ± 0.05^{b}	44.72 ± 0.15^{b}	5.39 ± 0.00^{a}
$PKT_{1.2}$	52.53 ± 0.09^{a}	22.00 ± 0.33^{a}	46.34 ± 0.28^{a}	21.31 ± 0.16^{a}	44.89 ± 0.09^{b}	5.52 ± 0.03^{b}

Whey	Moisture (g/100g)	Fat (g/100g)	F/DM (g/100g)	Protein (g/100g)	Prt/DM (g/100g)
PKCw[a]	93.43 ± 0.07^{a}	0.24 ± 0.01^{a}	3.65 ± 0.01^{a}	1.12 ± 0.02^{a}	17.04 ± 0.11^{a}
$PKT_{MTG\text{-}W}$[b]	93.08 ± 0.12^{b}	0.27 ± 0.12^{a}	3.90 ± 0.07^{b}	1.04 ± 0.04^{b}	15.02 ± 0.08^{b}

Values are means ± standard deviation. Means in the same column having different superscript letters are significantly different ($P < 0.05$). F/DM: Fat in dry matter; Prt/DM: Protein in dry matter. For sample codes refer to Fig. 1.

[a] Cheese whey from the control sample containing no mTG.

[b] Cheese whey from the sample containing mTG at level of 0.75 U/g protein.

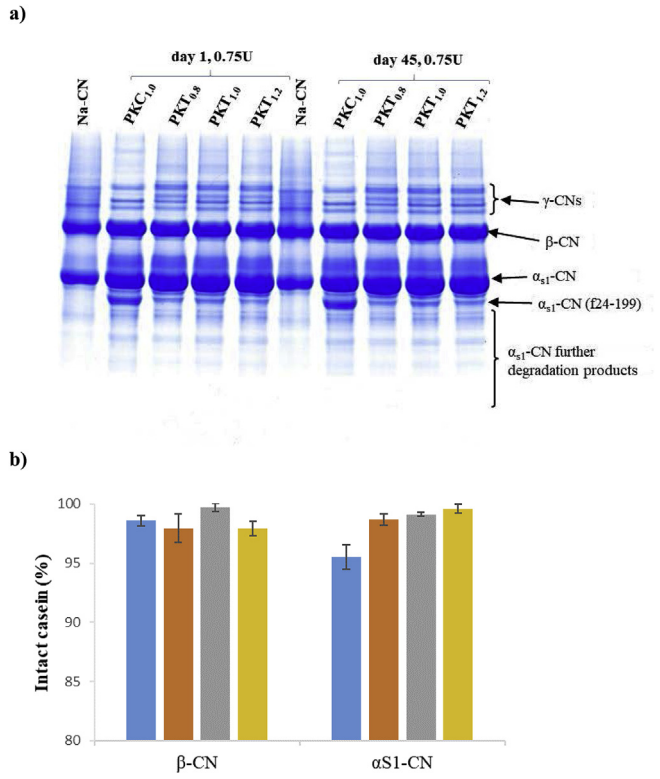

Fig. 2. (a) Urea-PAGE (%*T*:12.5, %*C*:4) electrophoretograms of pH4.6-insoluble fraction of processed Kashar cheese at 1 and 45 days of storage. Na–CN: sodium caseinate. For sample codes refer to Fig. 1. **(b)** Relative content of intact β- and α_{s1}-caseins of the cheese samples at 45 days of storage (as % of levels at 1 day) (■ $PKC_{1.0}$, ■ $PKT_{0.8}$, ■ $PKT_{1.0}$, ■ $PKT_{1.2}$).

Changes in the molecular weight and electrophoretic mobility of milk proteins after mTG treatment was also monitored by SDS-PAGE (Fig. 3). The densities of high molecular mass bands were limited in the mTG-treated samples, indicating low degree of cross-linking. A distinct disappearance of major casein monomers was not observed, probably depending on low concentration of mTG used, short reaction time and possible inactivation of mTG during scalding. Hinz, Huppertz, and Kelly (2012) reported that degree of polymerization caused by mTG is strongly dependent on the product's physicochemical parameters such as pH, temperature and mineral equilibria. In addition, cross-linking of casein in milk system is exclusively intra-micellar. In the present study, intra-micellar cross-linking and low degree of polymerization was observed which was important to keep functional properties in acceptable range during the storage (Fig. 3).

Since mTG increases the coagulation time and slows down the proteolysis, addition of mTG is not a common practice in the manufacture of hard cheeses (Romeih & Walker, 2017). However, retarding of ripening in processed Kashar cheese may be helpful to prevent over proteolysis and may extend shelf life of cheese.

The RP-HPLC peptide profiles of pH 4.6-soluble fractions of cheese samples at 214 and 280 nm showed qualitative and quantitative differences (Fig. S2). The peaks that were recorded at approximately 84, 88 and 89 min were α-lactalbumin, β-lactoglobulin B and β-lactoglobulin A, respectively. There was no clear changes in the concentration of those three peaks during ripening days or between the trials. It was reported that the whey proteins are not good substrates for mTG unless being unfolded by any means, *i.e.*, heat denaturation or adding reducing agents before enzymatic crosslinking (Romeih & Walker, 2017). The peak at 37 min represented tryptophan and the peaks following tryptophan represented hydrophobic peptides. As seen in Fig. S2, a decrease in the hydrophobic area (total peak number and area) of the mTG-treated cheeses was evident indicating a limited degree of proteolysis in the cheeses. For the determination of aromatic amino acids and their derivatives and peptides containing aromatic amino acids, 280 nm wavelength was preferred. Peptide bonds have much lower absorbance at 280 nm than at 214 nm (Bulat & Topcu, 2019). The more selective detection of presumably peptides of containing aromatic amino acids at 280 nm than at 214 nm might have been helpful to differentiate of cheese samples.

To investigate differences between the RP-HPLC profiles of cheese samples obtained at 214 nm or 280 nm for day 1 and day 45, PCA was performed after pre-processing HPLC data (Fig. 4a and b). The control cheese and mTG-treated cheeses were clearly differentiated on PCA based on pH 4.6-soluble fractions at both ripening days. In addition, PCA obtained at 280 nm more clearly differentiate the cheeses based on both mTG and emulsifying agent. Mayer (2001) found that overdose utilization of emulsifying agent may have led to hydrolysis of caseins which resulted in a high amount of water soluble peptides, and caused

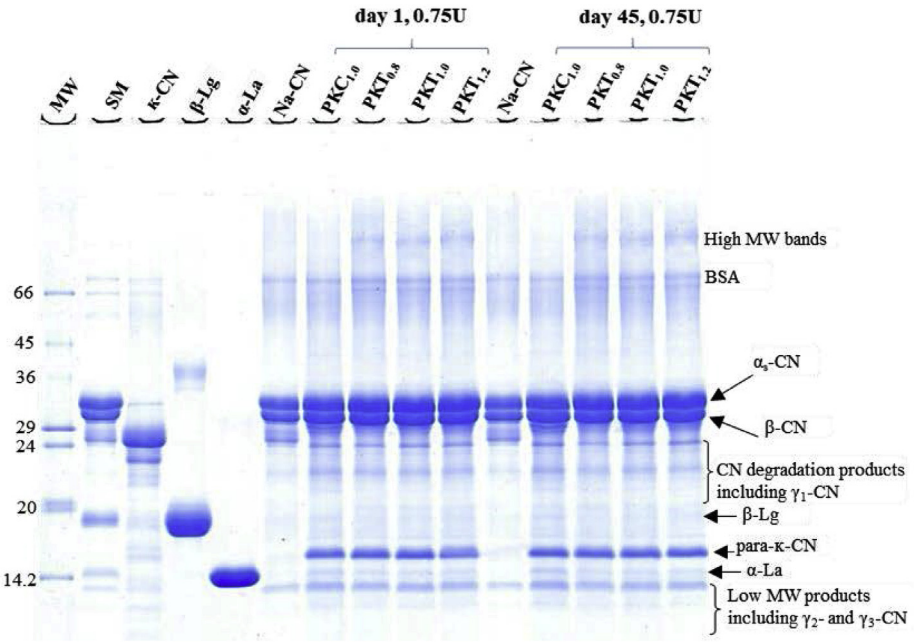

Fig. 3. SDS-PAGE (%*T*:12.5, %*C*:2.6) electrophoretograms of pH4.6-insoluble fractions of processed Kashar cheese at 1 and 45 days of storage. MW: Molecular weight marker (kDa), SM: skim milk, Na–CN: sodium caseinate. For sample codes refer to Fig. 1. The various protein bands have been identified based on their relative positions on the SDS-PAGE gel, with reference to literatures (Aaltonen et al., 2014; Soodam, Ong, Powell, Kentish, & Gras, 2015).

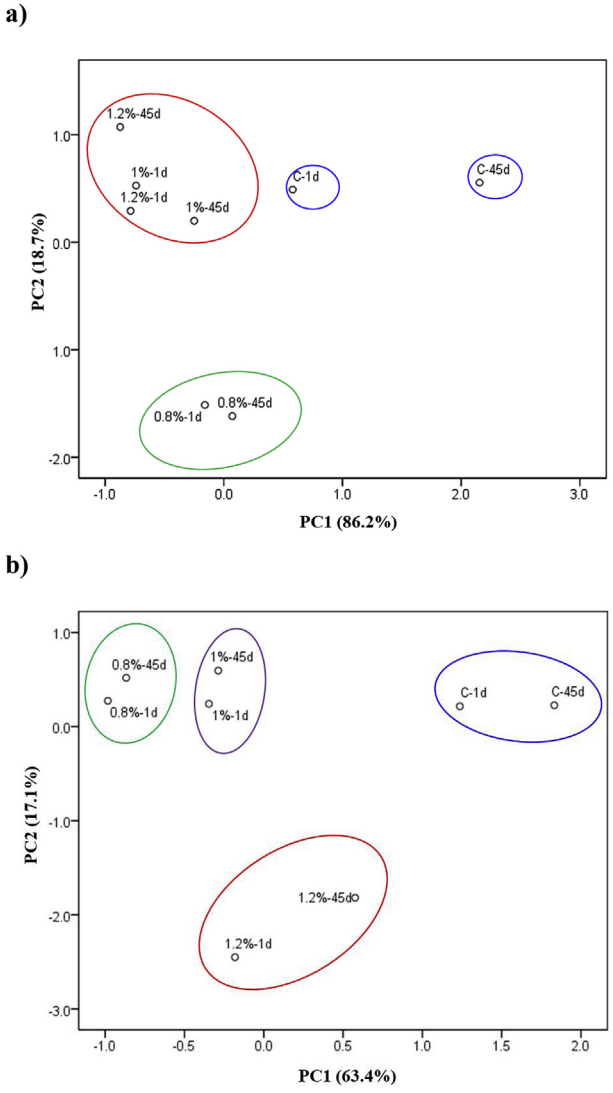

Fig. 4. Principal component analysis (PCA) of the processed peaks obtained from RP-HPLC analysis at 214 nm **(a)** or 280 nm **(b)** of pH 4.6-soluble fractions at 1 and 45 days of storage. (C refers control cheese that contain 1 g/100 g emulsifying salt but does not contain mTG; 0.8%, 1%, and 1.2% refers emulsifying salt concentrations (g/100 g) in mTG treated cheese samples).

bitterness defect. They concluded that type and concentration of the emulsifying agents were crucial in the manufacture of processed cheese. Utilization of mTG combined with emulsifying agents in processed type cheese may be a strategy to prevent that type of defects.

3.3. Effects of mTG on rheological and textural properties of cheeses

3.3.1. Temperature sweep profiles

Kashar cheese is a pasta-filata type cheese and is expected to flow and form a uniform continuous melt upon heating. In order to investigate the effects of mTG and/or emulsifying salts on the melting profile of the processed Kashar cheeses, a temperature sweep test was employed (Fig. 5a and b). Temperature sweep profiles of the cheeses were close to each other with lower starting values at higher emulsifying salt concentrations at both storage days, being more pronounced at day 45. In all cheese samples, a shoulder was observed in the G* profile at around 10 °C. At day 1, the combined effect of emulsifying salts and mTG led to slightly lower shear complex (G*) modulus values in the mTG-treated cheeses than the control cheese. This may be attributed to more water retention in the cheese matrices designed jointly

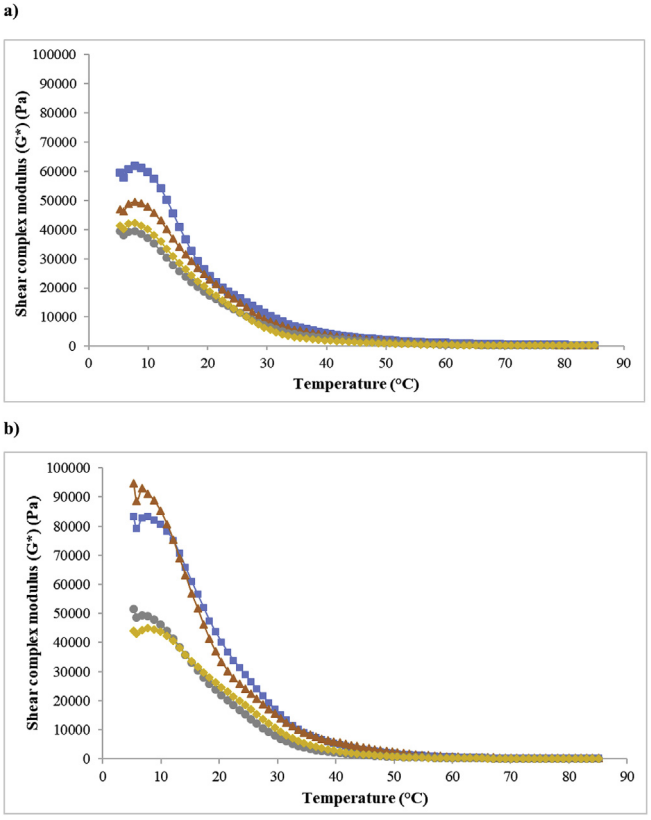

Fig. 5. Variation in temperature sweep profile of **(a)** 1 day old and **(b)** 45 days old processed Kashar cheeses (n = 2). (■) $PKC_{1.0}$, (▲) $PKT_{0.8}$, (●) $PKT_{1.0}$, (◆) $PKT_{1.2}$. Temperature range 5–85 °C, frequency 0.8 Hz, shear strain 0.5%. Error bars are smaller than symbol dimension. For sample codes refer to Fig. 1.

by emulsifying salts and mTG. At day 45, starting values of G* increased remarkably in the control cheese and the mTG-treated cheese added with ES at 0.8/100 g. This increase was rather limited in the mTG-treated samples added with emulsifying salt mixture at higher levels (1.0 g/100 g and 1.2 g/100 g ES). Ray, Gholamhosseinpour, Ipsen, and Hougaard (2016) compared the flow properties of Cheddar cheeses with different ages (3, 5, 7, 9, 12 or 15 months). The authors demonstrated that a time-dependent reduction in flow properties of Cheddar cheeses was evident as a result of dehydration, when they are aged under atmospheric conditions. However, in the present case, ripening period was rather short (45 days) and the cheeses were ripened in vacuum packages. With the increase in heating temperature, the overall number and strength of the protein bonds forming cheese matrix are reduced, resulting in lower dynamic moduli. Technically, melt occurs when viscous moduli (G″) of the cheese becomes dominant over the elastic moduli (G′). In the present case, no cross-over between G′ and G″ was observed in the cheeses applied with mTG. This might point out that the protein bonds formed by mTG partly prevented the cheese fully melted upon heating. It should also be borne in mind that such a cross-over might be seen at lower frequencies than that was applied in the present case.

The shear viscosities of the cheese samples are given in Table 3. With the increase in the emulsifying salt concentration, the shear viscosity of the samples decreased accordingly. Since the concentration of mTG was same in the samples added with ES, the variation in the shear viscosities among the samples was more related with the level of emulsifiers used. However, this does not exclude the role of mTG on viscosity of the cheeses as the control cheese and the cheese containing 1.0 g/100 g ES had the same level of emulsifying salt mixture but only the latter cheese contained mTG, and this had higher shear viscosity than the control sample. On the other hand, Chen, Wang, Zhan, Zhan,

Table 3
Shear viscosities of the experimental cheeses (n = 2).

	Shear viscosity (Pa.s)	
	Day 1	Day 45
$PKC_{1.0}$	400.8 ± 25.0^{bB}	310.0 ± 10.0^{bA}
$PKT_{0.8}$	480.2 ± 13.0^{cB}	345.4 ± 22.0^{cA}
$PKT_{1.0}$	470.1 ± 14.0^{cB}	336.7 ± 16.0^{cA}
$PKT_{1.2}$	275.8 ± 11.0^{aB}	$239.8 \pm 19.0^{a\ A}$

Values are means ± standard deviation. For shear viscosity, means in the same column having different superscript letters or same row having different superscript capital letters are significantly different ($P < 0.05$). For sample codes refer to Fig. 1.

and Ren (2019) showed that the effect of trisodium citrate on casein micelle size was limited when camel milk was incubated with mTG. This was related with the formation of additional covalent intra-micellar bonds triggered by mTG, as reported by Huppertz and de Kruif (2007). It is worth to note that the mTG concentration used by Chen et al. (2019) was much higher (10 U/g protein) than the mTG concentration employed in the present work (0.75 U/g protein).

3.3.2. Viscoelastic properties of cheeses

The viscoelastic properties of the cheeses were evaluated using dynamic small amplitude oscillatory shear (SAOS) test (Fig. 6). All the measurements were done within linear viscoelastic region. The G′ (elastic modulus) values of the cheeses were higher than the G″ (viscous modulus) at both storage days. The cheeses treated with ES + mTG had close G′ and G″ values but much lower than the control sample. This result was related with the higher moisture level of the former cheeses caused by more serum retention in the newly designed protein matrix by mTG. Interestingly, tan delta (tan δ) values of the 1 day old cheeses were 0.309, 0.280, 0.276 and 0.267 for the control cheese and the mTG-treated cheeses with ES levels of 0.8, 1.0, and 1.2 g/100 g, respectively. These figures decreased to 0.283, 0.275, 0.264 and 0.260 after 45 days of storage, in the same order. At both storage days, the tan δ values of the samples were close to each other indicating that similar protein bonds took place in cheese matrix formation but at varying degrees (*see* G′ and G″ values).

3.3.3. Creep/recovery profiles of cheeses

In order to further characterize the structural properties of the cheeses, a creep/recovery test was employed. The instantaneous creep/recovery compliances, *J(t)*, of the cheese samples are shown in Fig. 7. The instantaneous strain, the instantaneous recovery and constant residual strain of the mTG-treated cheeses were higher than the control

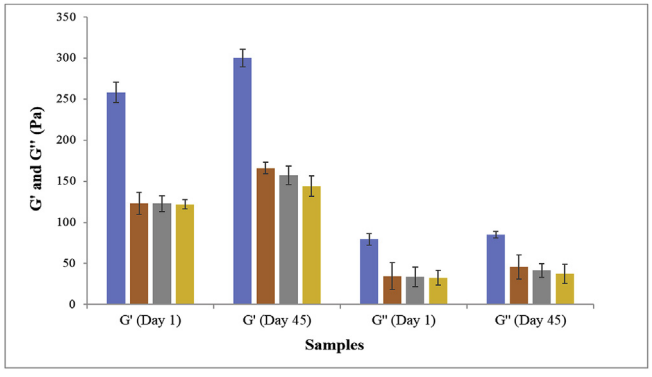

Fig. 6. Variations in the elastic (G′) and viscous (G″) modulus of the experimental cheeses (n = 2). (■) $PKC_{1.0}$, (■) $PKT_{0.8}$, (■) $PKT_{1.0}$, (■) $PKT_{1.2}$. Frequency 1 Hz (within linear viscoelastic region). For sample codes refer to Fig. 1.

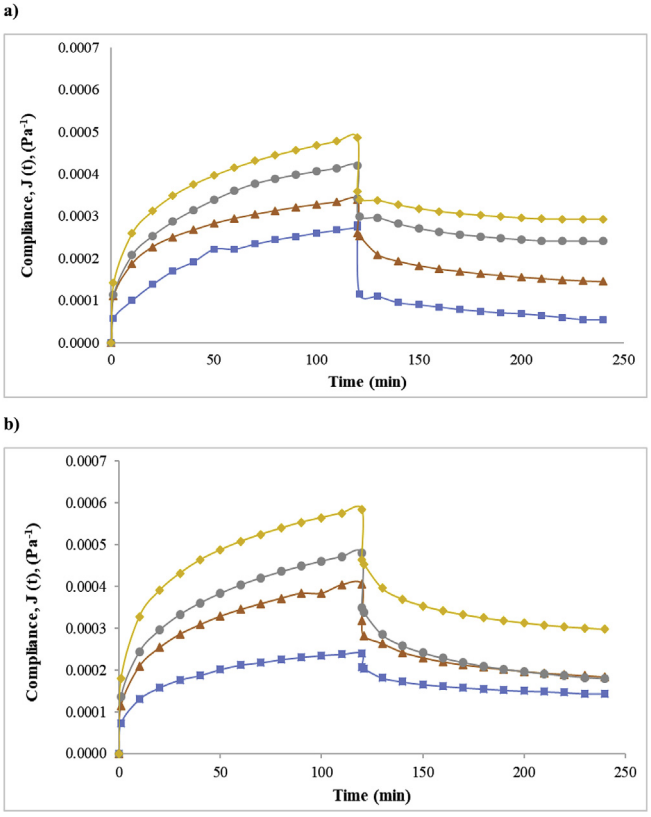

Fig. 7. Creep/recovery compliance curves as a function of time for **(a)** 1 day old and **(b)** 45 days old processed Kashar cheese at 25 °C (n = 2). Applied shear stress, 300 Pa (within linear viscoelastic region), creep time 120 s, recovery time 120 s (■) $PKC_{1.0}$, (▲) $PKT_{0.8}$, (●) $PKT_{1.0}$, (◆) $PKT_{1.2}$. Error bars are smaller than symbol dimension. For sample codes refer to Fig. 1.

cheese, indicating a less solid-like structure in the mTG-treated samples (Messens, van de Walle, Arevalo, Dewettinck, & Huyghebaert, 2000). The instantaneous compliance, J_0, shows the rigidity or integrity of the cheese network (Halim & Shoemaker, 1990). Over the ripening period, the J_0 values of the cheeses increased, indicating a slight loss of rigidity in the samples. As a result of protein degradation during cheese ripening, a decrease in J_0 value of the cheese is expected. However, in the present case, since the ripening period of the cheeses was short (45 days) and the cheeses were ripened in vacuum packages, the degree of proteolysis in the mTG-treated cheeses was limited (*see* section 3.4). Additionally, protein matrix in cheese designed by mTG is less accessible or less available for the action of proteolytic agents during ripening and proteolysis in mTG-treated cheeses continues at slower rates than the untreated cheeses, as previously reported by Özer et al. (2013) for white-brined cheese. No full recovery was observed for all cases. The creep/recovery test results were in harmony with the oscillatory rheological test results (Fig. 6).

3.3.4. Large deformation texture profiles of cheeses

The results of large deformation texture analysis of the samples are given in Table 4. The cheeses containing ES + mTG were harder than the control cheese at both storage days. A time-dependent increase in the hardness values of the cheeses were clear. A harder body in a cheese network is a result of more protein level and/or more protein junction points in the network (Özer, Robinson, & Grandison, 2003). It is the fact that more protein contact points in cheese network, increases the resistance of cheese matrix to deformation. In the present case, the protein-in-dry matter level of the mTG-treated cheeses were higher than the control cheese (*ca.* 1 percent, *see* Table 1). The degree of mTG-triggered protein polymerization in cheese matrix was not measured in

Table 4
Texture profile analysis (TPA) values of the experimental cheeses (n = 4).

	Hardness (N)		Springiness		Cohesiveness	
	Day 1	Day 45	Day 1	Day 45	Day 1	Day 45
$PKC_{1.0}$	1740.1 ± 39.5^{aA}	2837.2 ± 124.6^{aB}	0.930 ± 0.040^{bB}	0.802 ± 0.023^{aA}	0.680 ± 0.024^{aB}	0.579 ± 0.014^{aA}
$PKT_{0.8}$	4962.2 ± 74.9^{bA}	5342.9 ± 69.9^{bB}	0.900 ± 0.001^{aA}	0.895 ± 0.025^{bA}	0.782 ± 0.009^{bA}	0.793 ± 0.007^{bB}
$PKT_{1.0}$	5300.3 ± 73.3^{cA}	5411.7 ± 23.3^{bB}	0.930 ± 0.061^{bA}	0.935 ± 0.012^{cA}	0.808 ± 0.005^{cA}	0.821 ± 0.002^{cB}
$PKT_{1.2}$	5898.6 ± 95.3^{dA}	5919.2 ± 110.1^{cA}	0.945 ± 0.021^{bA}	0.926 ± 0.027^{bcA}	0.840 ± 0.001^{dB}	0.826 ± 0.011^{cA}

Values are means ± standard deviation. Means in the same column having different superscript letters are significantly different ($P < 0.05$). Different superscript capital letters for each parameters between ripening days within a row are significantly different ($P < 0.05$). For sample codes refer to Fig. 1.

the present work. However, by considering the general mode of action of mTG over milk proteins, it may be fair to assume that a harder body in the mTG-treated cheeses was more related with formation of additional inter and/or intra-micellar cross-links induced by mTG than the higher protein-in dry matter levels. This was further supported by the SDS-PAGE patterns of the cheeses (*see* bands representing high molecular weight components in the cheeses treated with mTG on Fig. 3). Similar conclusions were made by Özer et al. (2013) and Di Pierro et al. (2010). Li, Liu, Sun, Li, and Yu (2019) demonstrated that even though the mTG-treated Mozzarella cheese had higher moisture level (47.84%) than the control cheese (44.45%), the hardness, chewiness and adhesiveness values of the former cheese were higher than the control cheese. Similarly, De sá and Bordignon-Luiz (2010) showed that mTG-treated processed cheeses had higher hysteresis, indicating a firmer cheese matrix. Mleko, Gustaw, Glibowski, and Pielecki (2004) demonstrated that relaxation time and activation energy of Danbo cheese treated with mTG were higher than the untreated cheeses, pointing out restricted molecular mobility and more cross-linked protein network. There was no harmony between the results of the small deformation rheological tests (see Fig. 6) and the large deformation texture analyses. An in-depth investigation is needed to understand why combination of emulsifying salt and mTG yielded higher TPA-hardness and lower elastic modulus (G′) values in the resulting cheeses. The springiness values of the cheeses changed within a narrow margin during storage period with the exception of the control sample. The decrease in the springiness value of the control cheese was far more pronounced than the other samples. In general, one should expect that with an increase in hardness, the springiness should also decrease accordingly. Major factors affecting the springiness characteristics in cheese are protein hydrolyzation, reduction of free water and solidification of the milk fat. The limited changes in the springiness values of the cheeses treated with mTG were thought to be related with restricted proteolysis in these cheeses by mTG. A small variation in the springiness values of the mTG-treated White-Brined cheeses during 30 days of storage were also reported by Özer et al. (2013). The cohesiveness values of the cheeses increased with the increase in emulsifying salt concentration used. In the control sample, a decrease in the cohesiveness was evident; however, the cohesiveness values of the samples treated with mTG increased slightly during storage period.

4. Conclusion

Major problem associated with Kashar cheese production in the presence of mTG was reduced melting ability during scalding process. Additional cross-linking formed by mTG led to resistance of the Kashar cheese mass against melting. The rate of release of colloidal calcium from para-kappa-casein matrix is of critical importance in melting. In the preliminary studies, it was noted that pre-acidification of cheese milk was required for proper meltability of processed Kashar cheese during scalding process. Additionally, exceeding the mTG level of 0.75 U/g protein, adversely affected the meltability of Kashar cheese. The use of mTG clearly increased the yield of the processed Kashar cheese. Both mTG and emulsifying salts had effects on rheology and texture of the cheeses. They had also impact on the proteolysis, and the peptide profiles of pH 4.6-soluble fractions were clearly differentiated by principal component analysis. The present study demonstrated that the yield of processed Kashar cheese may well be increased to a satisfactory level without adversely affecting the physical characteristics of the end product. The future work should concentrate on the strategies to increase the level of protein retention in cheese network.

CRediT authorship contribution statement

Ali Topcu: Conceptualization, Writing - original draft, Formal analysis. **Tugba Bulat:** Formal analysis. **Barbaros Özer:** Conceptualization, Writing - original draft, Formal analysis.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.lwt.2020.109226.

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