Influence of transglutaminase treatment on the physicochemical, rheological, and melting properties of ice cream prepared from goat milk

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Abstract

This study was conducted to evaluate the effects of the transglutaminase enzyme on the physicochemical characteristics, overrun, melting resistance, rheological and sensorial properties of ice cream made from goat's milk. Different enzyme units (0.5, 1, 2, and 4 U/g milk protein) and treatment times (20 min and 60 min) were applied to determine the optimum process conditions. Treatment of the transglutaminase in the ice cream mix significantly affected the rheological and melting properties of the ice cream samples. The samples prepared with higher enzyme units and enzyme-treatment times showed higher melting resistance, consistency index, and viscoelastic modulus (G') than the ice cream mix. The correlation coefficient between melting resistance and viscoelastic modulus was found to be high (0.76). The apparent viscosity of all samples decreased with increasing the shear rate, indicating that all samples exhibited non-Newtonian shear thinning flow behavior. The sensory, overrun, and physicochemical properties of samples were not affected by the enzyme treatment. This study showed that treatment times and enzyme units are essential factors in the processing of the transglutaminase enzyme for improving the rheological and melting properties of ice cream mixes. Another significant result was that desired melting resistance could be achieved for ice cream with lower stabilizer and fat content.

Key words: goat's milk ice cream, transglutaminase, rheological properties

Introduction

Ice cream is a complex colloidal system that consists of ice crystals, air bubbles, partially coalesced fat globules, and aggregates that is surrounded by an unfrozen continuous matrix of sugars, proteins, salts, polysaccharides, and water. The heat and melting resistance and rheological behavior of ice cream is a crucial characteristic. These behaviors are highly related to the formulation of ice cream, especially milk proteins. The proteins at the interfaces that contribute to increased viscosity and the textural quality of the ice cream (Goff, 2002). Transglutaminase enzymes can cause modifications in solubility, emulsification capacity, foam forming, and protein gelling by causing cross-linking reactions (Faergermand

et al., 1998). The cross-linking of proteins improves the structure of protein-rich foods and increases functionality (Motoki and Seguro, 1998). Milk proteins are widely considered to be a suitable substrate for transglutaminase reactions (Farnsworth et al., 2006; Lorenzen, 2002; Schorsch et al., 2000). Transglutaminase enzymes catalyze acyl-transfer reactions between the γ-carboxamide group of a residue of peptide-linked glutamine (acyl donor) and primer amino groups (acyl acceptor) of a residue of glutamine and lysine (Sharma et al., 2001). This enzyme causes cross-linking reactions, which result in the formation of high molecular weight polymers (Motoki and Seguro, 1998). As a result of the cross-linking of proteins with transglutaminase

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enzymes, the water-binding capacity, gelling features, emulsification features, and rheological features of ice cream have been found to improve (O'Sullivan et al., 2002; Lorenzen and Schlimme, 1998). The cross-linking ratio catalyzed by the enzyme depends on the molecular structure of the protein acting as the substrate.

Farnsworth et al. (2003) identified the effect of transglutaminase on the texture and consistency of yogurts produced from goat's milk. In their study, the addition of enzymes was increased the viscosity of yogurt, decrease serum separation, and to had no effect on chemical properties such as total dry matter, pH, and protein. Similarly, Şanlıdere Aloğlu and Öner (2013) produced labneh from transglutaminase enzyme-treated goat's milk and determined that the enzyme addition increased gel strength and showed a positive effect on texture. In both studies, the researchers reported that enzymatic cross-linking formed by transglutaminase enzymes was a suitable application for improving texture and consistency in products manufactured from goat's milk. Yüksel and Erdem (2010) found that dairy products such as cheese and ice cream with low fat content and low dry-matter content could be produced with transglutaminase enzymes. Kuraishi et al. (2001) reported in their study that the usage of transglutaminase enzymes had a positive effect on the quality improvement of frozen dairy desserts.

Unlike the case with milk fat, there is no difference between goat's milk and cow's milk regarding protein amount, but there is a significant difference in milk proteins, especially amounts of casein fractions. According to Silanikove and colleagues (2010), the casein micelles of cow's milk (60-80 nm) are smaller than in goat milk's (100-200 nm). The most notable difference between milk types regarding the concentration of casein fractions are visible in the κ-casein and $αs_1$-casein concentrations of goat's milk. The amount of $αs_1$-casein in goat's milk is lower than in cow's milk (approximately 5 g/100 g total casein), while the amount of κ-casein (approximately 20 g/100 g total casein) is higher (Hayaloğlu and Özer, 2011). This situation may be observed in transglutaminase enzyme treatment. Goat's-milk proteins were found to be susceptible to cross-linking reactions when it contains transglutaminase; especially significant improvements in such reactions may be seen when the milk is preheated (Rodriguez-Nogales, 2006).

Goat's milk has been an important part of human nutrition for centuries because of the similarity between milk from goats and humans, the softer curd formation, the higher proportion of small milk-fat globules, and different allergenic properties compared with milk from cows (Clark and Mora García, 2017). Ice cream from goat's milk belongs to such products. In general, people tend to enjoy ice cream produced from goat's milk. Ribeiro and Ribeiro (2010) found that ice cream made from goat's milk has a softer structure and specific melting properties than ice cream from other forms of milk.

Limited research has been conducted thus far on the effects of transglutaminase on several quality criteria of ice cream from goat's milk. This study aimed to evaluate the effects of transglutaminase enzymes on the melting and rheological properties of this ice cream and to determine the most suitable process conditions based on the different characteristics of the ice cream.

Material and methods

Goat's milk was supplied from a goat's-milk producer in the Kırklareli region of Turkey. Milk obtained from morning milking sessions was brought to the laboratory and processed into ice cream. The chemicals used in the analysis were highly pure and were obtained from Merck (Darmstadt, Germany).

Ice cream production

The ice cream was produced according to the following procedure. First, stabilizer (5 %) was dispersed in the milk and left for 6 hours to completely hydrate while applying constant vigorous stirring. Next, emulsifier (2.5 %), sugar (30 %), cream (35 %, of 50 % fat content), and vanillin (0.1 %) were added to form the ice cream mixture. The mixture was pasteurized at 85 °C for 15 minutes by continuous stirring and then cooled to 50 °C. Five different ice cream mixtures were produced according to enzyme unit. To determine the effect of the enzyme treatment time and the unit, control sample was prepared; we did not treat the sample with the enzyme. After incubation (20 and 60 minutes), heat treatment was applied at 85 °C for 1 minute to inactivate the enzyme. The ice cream mixture was

homogenized at 510 x 10 rpm then ripened at +4 °C for 24 hours. Freezing was done in an ice cream machine (Delonghi, ICK 5000, China) using batch production. The resulting ice creams were packed into lidded plastic containers and allowed to freeze to -20 °C for one day, and then analyses were performed. All the ice cream samples were produced in triplicate.

Physicochemical analysis

Titratable acidity was measured in °SH, pH values were determined electrometrically with a pH meter (Hanna HI 2211 Ph/ORP Meter), fat content was determined via the Gerber method, and ash and dry-matter content were obtained gravimetrically (AOAC, 1990). Protein bands were determined via sodium dodecyl sulfate (SDS) gel-electrophoresis (Laemmli, 1970).

Overrun

The overrun values of the ice cream samples were determined according to the equation below:

$$Overrun(\%) = \left[\frac{(V_I - V_M)}{V_M} \right] x \, 100 \qquad (1)$$

where V_M represents the weight of the ice cream mixture per unit volume and V_I represents the weight of the soft serve ice cream per unit volume.

Meltdown rate analysis

A total of 50 g of ice cream was placed on a 2-mm stainless-steel screen with a funnel and graduated cylinder beneath to collect the melted ice cream; the first dripping and complete melting times were then recorded. The amount of melting was determined by the melting of the ice cream in a graduated cylinder and by weighing the melted parts in 15, 30, 45, 60, and 75 minute increments and calculated from the initial weight of the ice cream (Cottrell et al., 1979). The analysis was performed at 25 °C. The quantity of melting (%) = (weight of melted ice cream / initial weight of ice cream) x 100.

Rheological properties

Steady shear rheological properties

The rheological analysis was then performed on the ice cream mix samples. The steady shear and dynamic rheological behavior of the samples was determined using a strain/stress-controlled rheometer (Anton Paar, MRC 302, Austria) equipped with a Peltier temperature controller. The steady shear analysis was conducted using a parallel plate configuration (plate diameter 35 mm, angle 4°, gap size 0.5 mm) within the shear rate range of 0.1-100 s^{-1} at a constant measurement temperature (25 °C), according to Karasu et al.'s directions (2015). The measurement was started immediately after 1 mL of the ice cream mix sample was placed between plates. Each measurement was conducted in triplicate. Consistency coefficient (K) and flow behavior index (n) values were calculated according to the following model:

$$\sigma = K\gamma^n \qquad (2)$$

where σ is the shear stress (Pa), K is the consistency coefficient (Pa s^n), γ is the shear rate (s^{-1}), and n is the flow behavior index (dimensionless).

Dynamic rheological flow behavior

The dynamic rheological properties of the ice cream samples were determined using a parallel plate configuration. An amplitude sweep test was conducted with a strain range of 0.1-100 % at 1 Hz to determine the linear viscoelastic region (LVR). A frequency sweep test was conducted at 0.1 % strain (within LVR) over a frequency (ω) range of 0.1-10 Hz at constant temperature (25 °C) to record the storage (G') and loss modulus (G'') values. A non-linear regression was applied to the plots of the G' and G'' data versus ω to calculate the magnitudes of intercepts (K', K''), slopes (n' and n''), and R^2 using the following equations (Yoo, 2004; Rao and Cooley, 1992):

$$G' = K'(\omega)^{n'} \qquad (3)$$

$$G'' = K''(\omega)^{n''} \qquad (4)$$

Sensory analysis

Sensory analysis of the ice cream samples was performed according to the method described by Dertli et al. (2016). To summarize the method, sensory evaluation of the ice cream samples was conducted by ten selected panelists at Kirklareli University (five females and five males). Each panelist was trained before the analysis to become familiarized with the sensory analysis, samples, and methodology. For the presentation, 150 mL ice cream samples were served in glass beakers of 100 mL capacity covered with glass dishes. Sensory analysis was conducted in a 25 °C room. The ice cream samples were randomly coded using three-digit numbers and stored for 12 hours in a freezer at -18 °C before the analysis. All coded ice cream samples were evaluated for color, icy texture, melting resistance, cream taste, different taste and general acceptance on a scale ranging from 1 to 9 points, in which 1 reflected disliked qualities and 9 showed desirable qualities.

Statistical analysis

An evaluation of the findings from the experiments on the three replications was conducted using SPSS 21.0 software; ANOVA was also performed to determine the differences between samples. The Duncan multiple comparison test was applied to determine the difference between averages. The relation between shear rate and shear stress was also evaluated; the data were fitted to the power law model using the Statistica 8.0 software package program (StatSoft Inc., Tulsa, OK, USA).

Results and discussion

Physicochemical properties of the ice creams

The physicochemical properties of the ice cream samples are shown in Table 1. As the table shows, the pH values ranged from 6.69 to 6.75. Transglutaminase enzyme treatment did not significantly affect titratable acidity or pH ($p>0.05$). Kırımhan (2011) also reported that transglutaminase treatment did not significantly affect the acidity and pH values of yogurt-based ice cream samples. Total solids, ash, and fat of the ice cream samples are presented in Table 1; the changes in these values were not significant ($p>0.05$). The transglutaminase enzymes had no effect on these values in the ice cream mix. Similar results have also been reported in other studies (e.g., Şanlıdere Aloğlu and Öner, 2013; Rossa et al., 2012).

Milk proteins' cross-linking reactions to transglutaminase enzymes can be viewed via SDS gel electrophoresis. This method is preferable for viewing the formation of new high molecular weight bands following cross-linking reactions (Rossa et al., 2011). The protein bands from the ice creams produced from the transglutaminase-treated and non-treated samples are shown in Figure 1. A difference regarding textural and sensorial properties was observed especially in samples treated with the enzyme for 60 minutes, but the high molecular weight protein bands that caused this difference were not visible in the gel. Although no clear bands from the enzyme-treated mix (60 min) were found in the gel, this may only be because these bands were invisible.

Table 1. Mean values for chemical composition of control and enzyme-treated ice cream samples (n=3).

Enzyme treatment time	Ice cream sample	Total Solids (%)	Ash (%)	Fat (%)	Titration Acidity (%)	pH
-	Control	35.944±0.94	0.555±0.03	16.693±0.01	0.229±0.01	6.71±0.01
20 min	05U	36.766±0.65	0.549±0.03	16.319±0.45	0.250±0.01	6.75±0.01
	1U	37.304±1.17	0.547±0.03	16.084±0.04	0.234±0.01	6.71±0.01
	2U	36.862±0.74	0.560±0.03	16.277±0.12	0.235±0.01	6.72±0.01
	4U	37.509±1.30	0.556±0.03	15.996±0.01	0.230±0.03	6.73±0.00
60 min	05U	37.189±0.80	0.556±0.03	16.134±0.03	0.273±0.03	6.72±0.01
	1U	36.751±0.36	0.550±0.01	16.326±0.06	0.271±0.02	6.71±0.02
	2U	37.469±1.12	0.581±0.06	16.013±0.15	0.270±0.03	6.71±0.03
	4U	38.989±0.28	0.623±0.03	15.389±0.11	0.244±0.01	6.69±0.01

Means values±standard deviations of trials

Increasing the treatment time with transglutaminase enzymes for the mixture or the milk might increase band formation.

Overrun

Overrun affects not only the texture of ice cream but also its mouthfeel characteristics, durability, product yield, and nutritional quality (Yangılar, 2015). The overrun rates are shown in Table 2. As shown in the table, the overrun values ranged from 3 % to 9 %. The overrun values could be affected by the ice cream composition, production parameters, or production methods (Güven et al., 2010; Metwally, 2007). In this study, the low overrun rates may have been caused by the manufacturing equipment.

As Table 2 shows, transglutaminase enzyme treatment decreased the overrun, but enzyme dosage and enzyme treatment times were not significantly affected ($p>0.05$). In a similar finding to our study, Kırımhan (2011) reported that using enzyme-treated yogurt caused a significant decrease in overrun results. Our lower overrun result probably originated from the increased protein layer adsorbed by the fat globule surface due to cross-linking between proteins and the transglutaminase enzyme. In contrast, Metwally (2007) found that transglutaminase enzyme increased overrun in enzyme-treated ice creams that had low fat and stabilizer content; the study determined that fat destabilization had increased by enzyme treatment and that the interactions of proteins formed by cross-linking with fat globules and stabilizer promoted more stable foam-forming via the
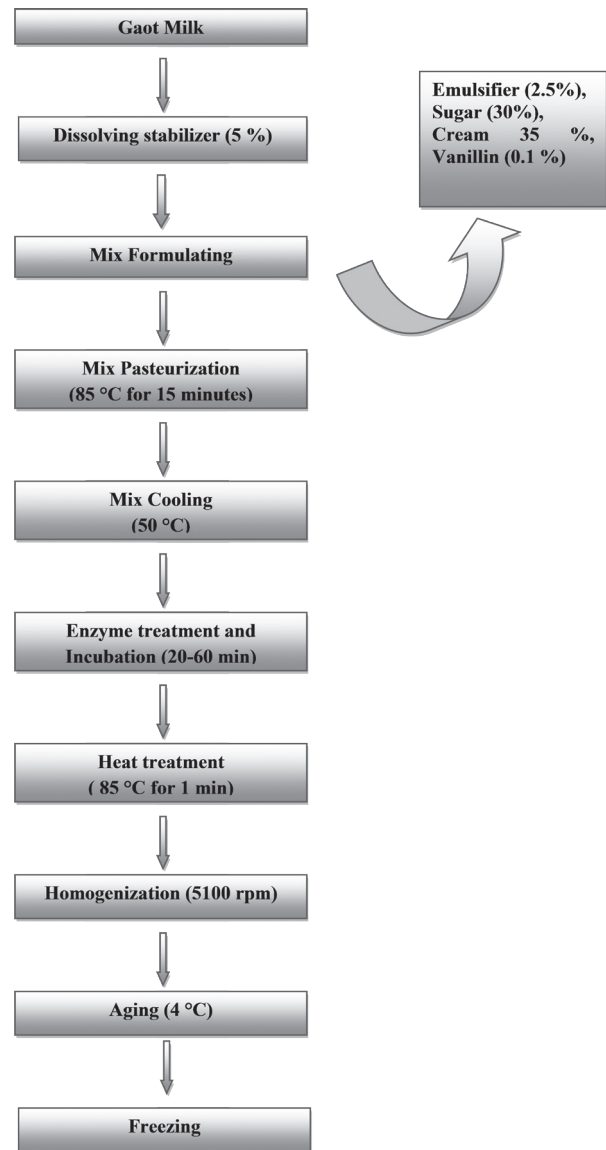
Figure 1. Ice cream production flow chart

Table 2. Overrun rates, first dripping and melting time of ice cream (n=3)

Enzyme treatment time	Ice cream sample	Overrun %	First dripping time (min)	Complete melting time (min)
-	Control	9±2.11[*a]	8±0.71[b]	44±2.83[b]
20 min	05U	6±4.62[ab]	9±3.79[b]	54±5.51[a]
	1U	3±2.13[b]	13±2.63[a]	52±6.18[a]
	2U	4±2.30[b]	14±4.76[a]	52±3.70[a]
	4U	3±0.66[b]	13±5.91[a]	52±4.12[a]
60 min	05U	5±3.16[ab]	12±4.04[ab]	56±3.79[a]
	1U	4±2.75[b]	13±5.66[a]	55±1.41[a]
	2U	4±1.15[b]	10±2.83[ab]	50±4.95[a]
	4U	4±1.30[b]	11±2.00[ab]	49±2.12[a]

Means values±standard deviations of trials.
*Different small letter depict the statistical difference between means for ice cream samples ($p<0.05$).

positive cohesion features of air bubbles, which were interpreted as larger overrun. Rossa et al. (2012) found that decreasing the fat content of ice cream promotes air bubbles in the structure, which was previously suggested by other researchers (Alamprese et al., 2002; Adapa et al., 2000).

In the present study, even though the overrun results were lower than in the control groups, enzyme treatment did not affect the physical features of ice cream. Metwally (2007) argued that the effect of enzyme treatment on the physical properties of ice cream is the same as the effect of the presence of high dry-matter content, which increases overrun, smoothness, and resistance to melting.

Effect on the melting properties of ice cream

The first dripping time provides information about the structure and the melting resistance of ice cream. To determine the melting resistance of ice cream, the first dripping and complete melting times were determined, as presented in Table 2.

Transglutaminase enzyme treatment had a significant effect on the first dripping times and full melting times ($p < 0.05$). Ice creams that showed the longest first dripping times were enzyme-treated samples. Following heat treatment, more stable structures were formed (depending on the denaturation of the serum proteins and their interactions with casein micelles) in the enzyme-treated ice creams (Sharma et al., 2001). This results were related to the binding of water by stabilizers and the polymerization of milk proteins by transglutaminase (Rossa et al., 2011). Control samples had the lowest first dripping times and complete melting times.

Rossa et al. (2012) found in their study that enzyme-treated ice creams had increased melting resistance compared with the control samples. Metwally (2007) reported that the enzymatic cross-linking of milk proteins resulted in high-mix viscosity when compared with the control groups, and the ice cream's resistance to melting increased with enzyme treatment. Similarly, Kırımhan (2011) reported that the extension of the first dripping time of yogurt samples was due to the increase in the water-binding capacity of milk proteins after enzymatic modification; that study also reported that the first dripping times in the enzyme-treated samples containing fat were higher than those of the non-fat ice cream. Muse and Hartel (2004) determined that fat destabilization significantly influenced the melting properties of ice cream. Increased fat destabilization had a positive effect on melting properties. Fat destabilization was associated with the partial coalescence and increase of fat globules in enzyme-treated ice creams (Rossa et al., 2012).

The melting amounts of the ice cream samples at 15, 30, 45, and 60 minutes and the ratios (%) of these values are shown in Table 3. The effect of transglutaminase enzyme treatment on the melting time of ice cream was found to be statistically significant ($p < 0.05$). While a significant portion of melting had occurred in the control group by the 45th minute, such melting did not occur until the 60th minute in the enzyme-treated ice cream samples, indicating that the enzyme treatment improved the melting resistance of the ice cream samples. According to the data we obtained, neither enzyme dosage nor treatment time affected the melting amount. An increase in the melting rate of the ice cream

Table 3. Melting amount of ice cream samples (%) (n=3)

Enzyme treatment time	Ice cream sample	15th minute	30th minute	45th minute	60th minute
-	Control	13.79±3.36*[a]	60.95±8.65[a]	96.79±1.72[a]	98.68±0.45[a]
20 min	05U	5.63±0.32[b]	39.26±4.67[b]	83.23±1.97[a]	98.28±1.35[a]
	1U	4.42±0.29[b]	36.14±1.11[c]	82.39±1.87[a]	97.78±0.98[a]
	2U	3.40±0.23[b]	33.95±1.67[c]	80.66±1.35[a]	98.95±1.97[a]
	4U	2.39±0.56[c]	32.93±1.18[d]	79.44±1.37[a]	97.50±0.52[a]
60 min	05U	3.83±0.06[b]	42.91±0.02[b]	78.11±1.39[a]	98.26±1.01[a]
	1U	3.63±0.51[b]	35.15±8.35[c]	84.03±12.41[a]	97.81±0.41[a]
	2U	2.82±0.03[c]	33.36±0.00[c]	80.22±0.01[a]	97.85±0.00[a]
	4U	2.67±0.02[c]	30.94±3.35[d]	79.56±0.07[a]	97.35±0.00[a]

Means values±standard deviations of trials.
*Different small letter depict the statistical difference between means for ice cream samples ($p < 0.05$).

samples from transglutaminase treatment has also been reported in previous studies (D a n e s h et al., 2017; M e t w a l l y, 2007). R o s s a et al. (2012) reported that transglutaminase treatment increased the stability of ice cream samples, which showed greater resistance to ice cream meltage compared to the control (i.e., without transglutaminase). This result was associated to the polymerization of the milk proteins by the action of transglutaminase.

S a k u r a i et al. (1996) and H a r t e l et al. (2003) found that ice creams with high overrun melted more slowly than ice creams with low overrun, which showed good melting resistance. Both studies reported that this situation could have resulted from the reduction in heat transfer with increased ice cream volume and the tracing of melted liquid in a "bumpy" way when flowing. We found that, despite showing less overrun than the control samples, the enzyme-treated ice cream had a low melting quantity.

Rheological properties of the ice cream mix
Steady shear properties

Figure 2 shows the steady shear rheological behavior of the ice cream mixture samples subjected to different transglutaminase enzyme concentrations and treatment times. As the figure shows, the apparent viscosity of all samples decreased with increased shear rate, which indicates that all the samples exhibited non-Newtonian shear thinning flow behavior. This flow behavior has also been reported from previous studies (e.g., D e r t l i et al., 2016; D o g a n et al., 2013; T o k e r et al., 2013).

A power law model was applied to model the shear rate versus shear stress data. The model parameters - the consistency coefficient (K), flow behavior index (n), and R^2 values - are presented in Table 4. As presented, the n values of all samples were lower than 1, which indicates typical pseudoplastic flow behavior. The K values ranged between 11.280 and 16.404 Pas^n. K values were significantly affected by transglutaminase enzyme unit and treatment times. At each enzyme unit, the K values were found to be significantly higher than those of the control sample for the 60 min treatment time. In contrast, the 20 min transglutaminase treatment did not significantly affect either the K or n values for all transglutaminase units, which indicates that treatment time is an important factor in the processing of transglutaminase enzymes for improving the rheological properties of ice cream mixtures.

Enzyme concentration also significantly affected the K and n values. As Table 4 shows, the K values increased with increased enzyme concentrations, while the n values decreased; this indicates that the
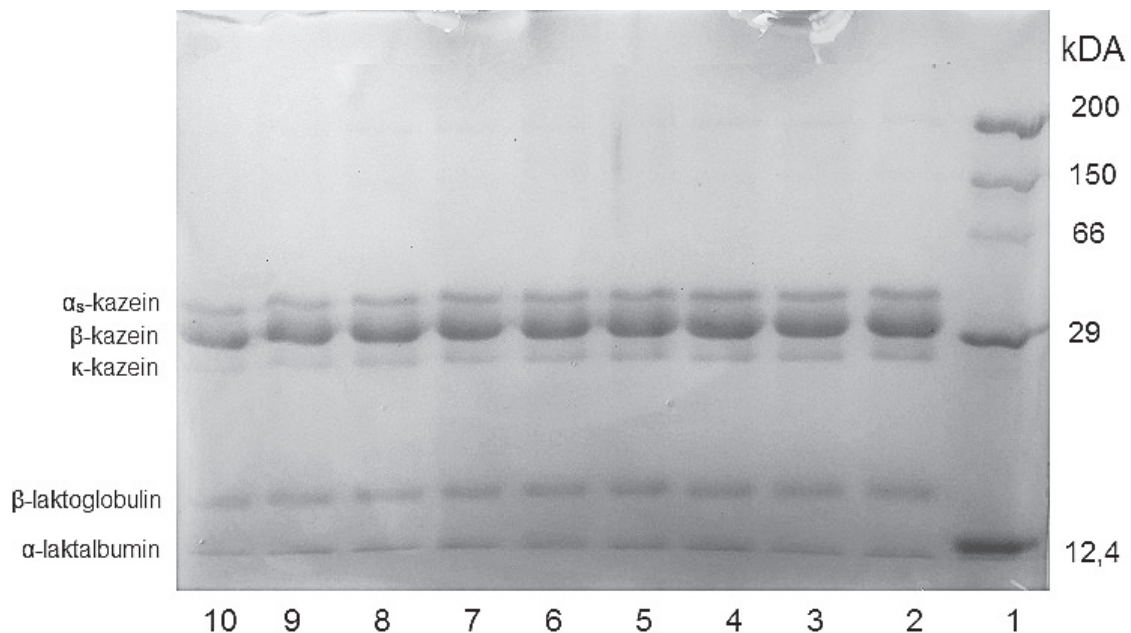

Figure 2. SDS gel electrophoresis image of ice cream samples (1: marker; 2: control; 3: 0.5 U 20 min; 4: 1U 20 min; 5: 2U 20 min; 6: 4U 20 min; 7: 0.5 U 60 min; 8: 1U 60 min; 9: 2U 60 min; 10: 4U 60 min)

pseudoplastic characteristic increased with higher transglutaminase concentrations. K values can also be used as an indicator of the recrystallization and resistance to melting of ice cream (Bolliger et al., 2000). The increasing K values observed in our study suggests that the desired qualities of melting time and low-recrystallization behavior may be obtained by treatment with transglutaminase. To summarize our results, transglutaminase treatment improved the rheological properties of the ice cream mix and the pseudoplastic character can be increased with increased transglutaminase concentrations and treatment times. The increase in K values can be attributed to cross-linking of the milk protein (Gauche et al., 2008). The increase in the K value of ice cream mix by transglutaminase treatment has also been reported by Rossa et al. (2011). Our study reported the optimum transglutaminase unit, incubation time, and incubation temperature to be 4U, 90 min, and 56.8 °C, respectively. Rossa et al.

(2012) reported similar results. In a finding that was similar to that of our study, they reported that the treatment of transglutaminase increased pseudoplastic character, and they found that 4U was the optimum transglutaminase concentration. Other studies have also reported improved rheological properties for other dairy products by the use of transglutaminase (Iličić et al., 2014; Iličić et al., 2013).

Dynamic rheological properties

The dynamic rheological properties of the ice cream mix subjected to different transglutaminase concentrations and times are shown in Figure 3. As shown, the storage modulus (G') of the samples increased with increased angular frequency (ω) and differed according to transglutaminase concentrations and treatment times. The G' values of the samples subjected to higher enzyme concentrations and longer treatment times were higher than the other samples' values.
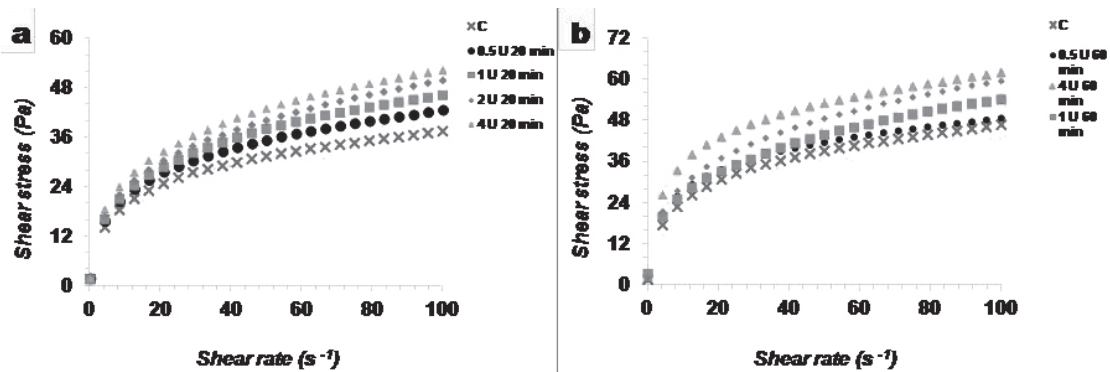

Figure 3. Steady state rheological behavior of the ice cream mix samples subjected to different transglutaminase enzyme unit and incubation time (a: Effect of enzyme concentration, b: Effect of incubation time)

Table 4. Steady state rheological parameters of ice cream mix subjected to different transglutaminase enzyme unit and time

Enzyme treatment time	Ice cream sample	K (Pas^n)	n	R^2
-	Control	12.321±0.081[*Ab]	0.292±0.001[Aa]	0.9941
20 min	05U	11.852±0.075[Ab]	0.303±0.000[Aa]	0.9958
	1U	12.018±0.941[Ab]	0.308±0.001[Aa]	0.9981
	2U	12.280±0.111[Ab]	0.3175±0.001[Aa]	0.9979
	4U	12.762±0.154[Ab]	0.3154±0.005[Aa]	0.9975
60 min	05U	13.114±0.260[Ba]	0.267±0.001[Ab]	0.9914
	1U	14.577±0.825[Ba]	0.2703±0.000[Ab]	0.9989
	2U	15.145±1.100[Aa]	0.2799±0.002[Ab]	0.9981
	4U	16.404±0.115[Aa]	0.2625±0.003[Ab]	0.9906

*Different superscript uppercase letter shows statistical differences between samples subjected to different enzyme unit (p<0.05)
*Different superscript lowercase letter shows statistical differences between samples subjected to different enzyme time (p<0.05)

G' and G'' data versus ω were fitted to the power law model and model parameters; namely, K', K'' n', n'', and the regression coefficient (R^2) were calculated using non-linear regression. The model parameters are shown in Table 5. As the table shows, the K' values were higher than K'' for all samples, which indicates that the ice cream mixture samples showed solid-like behavior. These results were in accordance with previously published studies (Kurt et al., 2016; Dogan et al., 2013; Toker et al., 2013). The K' values ranged from 7.265 to 22.511 Pas^n and were significantly affected by treatment time and enzyme concentration. As Table 5 shows, the K' values of the samples increased with increased treatment times, and the transglutaminase concentrations suggest that the solid-like character of the samples could be improved by optimizing transglutaminase concentrations and treatment times. The increase in the K values may be attributed to cross-linking of

the protein due to transglutaminase activity. Stabilizers and fat can create a more solid-like character or viscoelasticity of the ice cream mix (Adapa et al., 2000; Bolliger et al., 2000). Transglutaminase subjection can reduce the need for stabilizer and milk-fat usage in ice cream mix (Iličić et al., 2013).

As expected, the n' and n'' values decreased with an increase in K' and K'' values, which represents typical shear thinning behavior. Figure 4 shows the combined effect of the enzyme unit and treatment time on the rheological and melting resistance of the ice cream samples. Both enzyme unit and treatment time increased the melting resistance, consistency index, and elastic character of the samples. We can concluded that both the steady shear and viscoelastic properties of the ice cream mix samples could be improved by the treatment of transglutaminase and enzyme concentrations, and treatment time should be adjusted to obtain the desired mix quality.
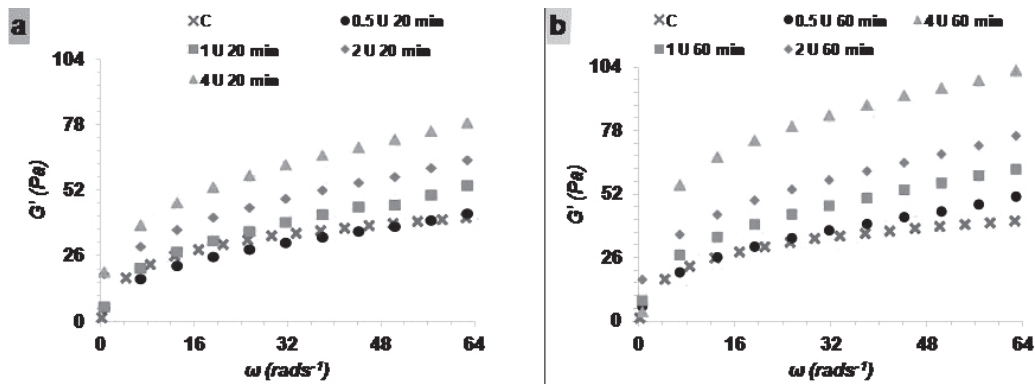

Figure 4. Dynamic rheological properties of ice cream mix subjected to different enzyme concentration and incubation time (a: Effect of enzyme concentration, b: Effect of incubation time)

Table 5. Dynamic rheological parameters of ice cream mix subjected to different transglutaminase enzyme unit and time

Enzyme treatment time	Ice cream sample	K' (Pas^n)	n'	R^2	K'' (Pas^n)	n''	R^2
-	Control	8.05±0.01 *[Ab]	0.414±0.03[Aab]	0.9995	7.351±0.73[Ab]	0.247±0.01[Aa]	0.9989
20 min	05U	7.265±0.11[Db]	0.423±0.01[Aa]	0.9991	6.419±1.02[Cc]	0.251±0.01[Aa]	0.9949
	1U	10.597±0.60[Cb]	0.388±0.00[Bb]	0.9988	7.525±0.05[Cb]	0.243±0.00[ABa]	0.9963
	2U	14.701±1.02[Bb]	0.335±0.01[Cb]	0.9911	10.151±1.00[Ba]	0.236±0.04[Ba]	0.9998
	4U	19.205±1.08[Ab]	0.344±0.02[Cb]	0.9992	12.205±0.85[Ab]	0.204±0.03[Cb]	0.9992
60 min	05U	9.689±1.02[Da]	0.403±0.01[Ac]	0.9991	7.677±0.08[Ca]	0.255±0.05[Aa]	0.9952
	1U	12.468±0.36[Ca]	0.378±0.00[Ab]	0.9998	10.362±1.10[Ba]	0.229±0.05[Bb]	0.9992
	2U	17.525±0.81[Ba]	0.314±0.01[Bc]	0.9984	11.701±0.51[Ba]	0.223±0.01[Bb]	0.9951
	4U	22.511±1.11[Aa]	0.313±0.05[Bc]	0.9718	15.912±1.15[Aa]	0.184±0.01[Cc]	0.9983

The different superscript uppercase letter shows statistical differences between samples subjected to different enzyme unit (p<0.05)
*Different superscript lowercase letter shows statistical differences between samples subjected to different enzyme time (p<0.05)
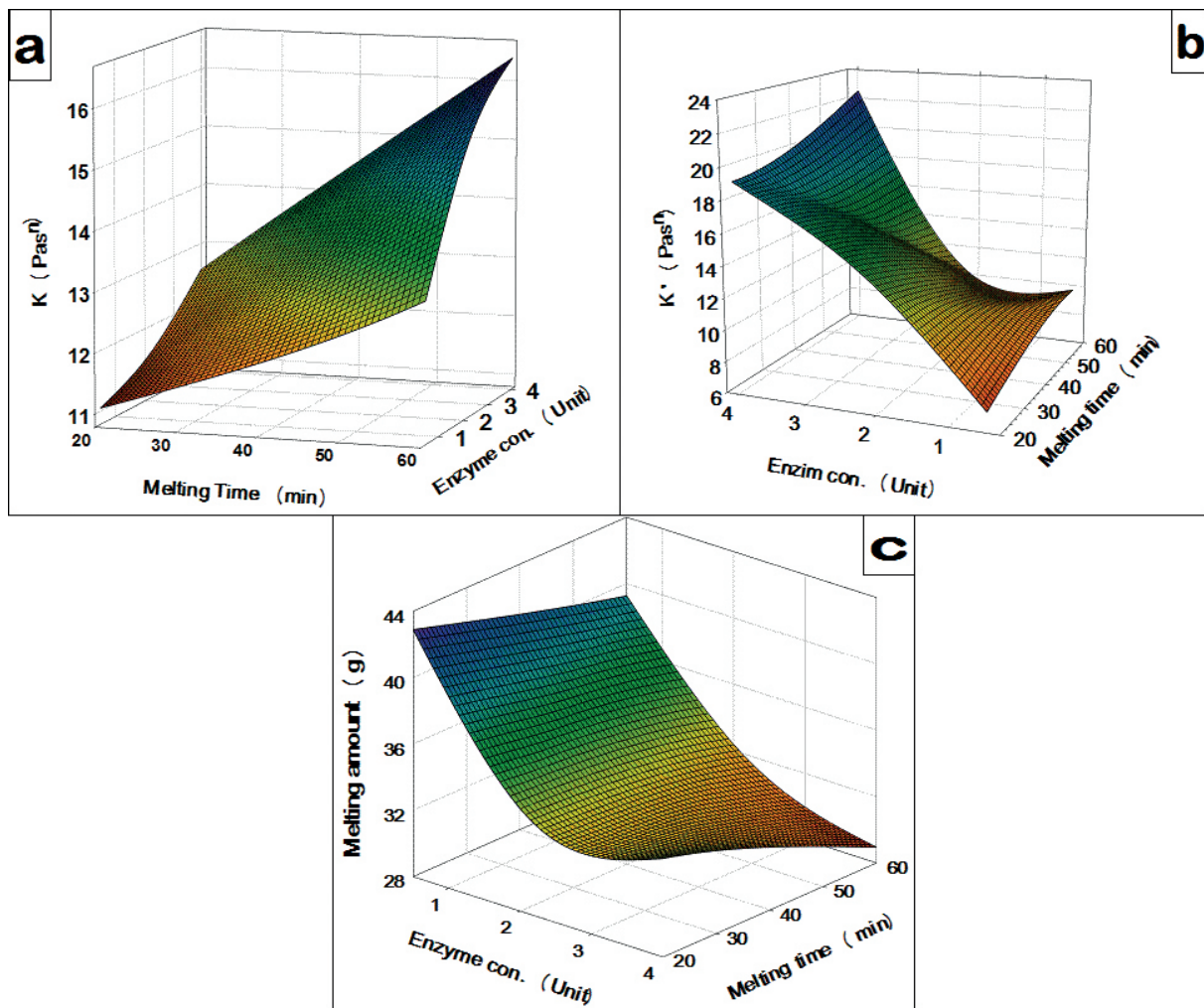
Figure 5. Combined effect of the treatment time and enzyme unit on the melting and rheological properties (a: Effect of melting time and enzyme concentration on K value, b: Effect of melting time and enzyme concentration on K' value, c: Effect of melting time and enzyme concentration on melting amount)

Table 6. Sensory analysis of ice cream samples (n=3)

Enzyme treatment time	Ice cream sample	Color and appearance	Icy structure and consistency	Foreign taste and smell	Cream taste	Melting resistance	General acceptance
-	Control	8.50±0.05	6.00 ±0.03[b]	8.00±0.10	8.00±0.10	5.00±0.05[c]	7.00±0.20[*b]
20 min	05U	8.50±0.07	6.00±0.05[b]	8.00±0.09	8.00±0.20	6.50±0.05[bc]	7.50±0.05[b]
	1U	8.50±0.09	8.00±0.05[ab]	8.00±0.20	7.00±0.20	6.50±0.10[bc]	7.70±0.05[b]
	2U	9.00±0.20	8.00±0.03[ab]	8.00±0.10	8.50±0.05	7.50±0.10[b]	8.00±0.01[ab]
	4U	9.00±0.09	8.00±0.09[ab]	7.50±0.10	7.50±0.10	8.50±0.10[ab]	8.00±0.10[ab]
60 min	05U	8.00±0.05	9.00±0.05[a]	8.00±0.20	8.00±0.10	6.50±0.20[bc]	8.00±0.05[ab]
	1U	9.00±0.09	9.00±0.03[a]	7.40±0.09	8.50±0.20	7.50±0.05[b]	8.00±0.02[ab]
	2U	9.00±0.05	9.00±0.03[a]	8.00±0.05	8.00±0.05	8.50±0.09[ab]	8.50±0.10[a]
	4U	9.00±0.05	9.00±0.03[a]	8.00±0.10	8.00±0.05	9.00±0.09[a]	9.00±0.05[a]

The average of the scores given by the panelists
*Different small letter depict the statistical difference between means for ice cream samples (p<0.05)

Sensory analysis

In our studies, flavor/aroma differed in transglutaminase-treated fermented dairy products when compared to control groups (Oner et al., 2008). When considered from this point of view, although ice cream is not a fermented product, we did examine the effect of enzyme additions on sensorial properties; the evaluation results are presented in Table 6. As the table shows, the enzyme-treated ice cream samples were the same as the control group in both, color and appearance. Accordingly, the transglutaminase enzyme treatment did not cause a significant difference among the tested samples. The actual impact of the transglutaminase enzyme on texture and consistency was as expected. Thus, the enzyme treatment time was a more significant factor than enzyme dosage. Ice cream produced from 60 minutes of enzyme treatment had a point regarding texture and consistency. These samples had a firmer texture and evident elasticity while no crystallization was detected. In contrast, a loose texture was found in the control groups. Such results might be due to improvements in textural properties with an increase in the water-binding capacity of the milk proteins as a result of the enzymatic modifications. It was obvious that enzyme treatment did not have any adverse effects regarding taste and smell. With the increase of enzyme dosage and treatment times there was a decrease in perception of ice cream sweetness. The enzyme-treated ice cream samples had higher general acceptance scores than the non-treated samples.

Ice creams were thawed and frozen again during storage and textural changes were examined during that period. The textures of the control groups and the enzyme-treated groups were found to be different. While recrystallization in the control groups was evident, no perceivable recrystallization occurred, especially in ice creams treated for 60 minutes.

Conclusion

In this study, transglutaminase enzyme application in ice cream mix had no significant impact on the physicochemical and sensory properties of ice cream, while it significantly affected the rheological and melting properties of the ice cream samples. The consistency index and viscoelastic modulus values increased from the enzyme treatment. A positive correlation was observed between the melting resistance of the ice cream and the viscoelastic modulus of the ice cream mix. This result indicates that the melting resistance and pseudoplastic character of the ice cream could be increased by treatment with this enzyme for low-fat ice cream without changing the functional, sensorial, and rheological properties of the ice cream.

Acknowledgments

Utjecaj tretmana transglutaminazom na fizikalno-kemijska i reološka svojstva te topivost sladoleda od kozjeg mlijeka

Sažetak

U radu je ispitivan utjecaj dodatka enzima transglutaminaze na fizikalno-kemijska svojstva, porast volumena, otpor prema topljenju, reološka i senzorska svojstva sladoleda od kozjeg mlijeka. Enzim je dodavan u različitim koncentracijama (0,5, 1, 2, i 4 U/g proteina mlijeka) te su ispitivana različita vremenska razdoblja enzimatskog tretmana (20 min i 60 min) kako bi se odredili optimalni parametri procesa. Tretman sladoledne smjese dodatkom transglutaminaze rezultirao je statistički značajnim učincima na reološka svojstva i topivost ispitivanih uzoraka. Uzorci pripremljeni dodatkom većih koncentracija enzima i duljim vremenima tretiranja pokazivali su veći otpor prema topljenju, veći indeks konzistencije i veću viskoznost (G') nego kontrolni. Koeficijent korelacije između otpora prema topljenju i koeficijenta viskoznosti bio je visok (0,76). Prividna viskoznost svih uzoraka opadala je s porastom brzine smicanja, što je ukazivalo na nenewtnovski tip tečenja u svim uzorcima. Dodatak enzima nije utjecao na fizikalno-kemijska, senzorska i reološka svojstva, kao niti na porast volumena sladoleda. Ovo je istraživanje pokazalo da su vremena tretmana i primijenjene koncentracije enzima transglutaminaze ključni faktori za poboljšanje

reoloških svojstava i topljivosti sladolednih smjesa. Također se pokazalo da primjena ovog enzima omogućuje postizanje željenih otpora prema topljenju u sladolednim smjesama s nižim udjelom stabilizatora i mliječne masti.

Ključne riječi: sladoled od kozjeg mlijeka, transglutaminaza, reološka svojstva

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