

Effects of Using Transglutaminase and Fat Replacer on Functional Properties of Non-Fat Yoghurt ^[1]

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Abstract

The aim of this study was to investigate the effectiveness of microbial transglutaminase (mTGase, EC 2.3.2.13) as compared with using of a commercial fat replacer (Dairy-Lo) in the manufacture of non-fat set yoghurt. For this purpose, two types of non-fat yoghurt supplemented with mTGase and Dairy-Lo and control non-fat yoghurt without additive as a control sample were produced and stored at 4°C for 20 days. Physical properties of the non-fat yoghurt were improved by mTGase during 20-day storage, moreover; addition of mTGase did not have any effect on the acetaldehyde content of yoghurt. While the sample supplemented with Dairy-Lo showed the lowest serum separation, the gel strength of this sample was weaker than those made with mTGase. Sensory results indicated that non-fat yoghurt with mTGase had taste and aroma similar to that of the control yoghurt. In addition, the incorporation of Dairy-Lo had negative effect on sensory properties of non-fat yoghurt. According to the results obtained, the use of mTGase could be suggested for the production of non-fat yoghurt with reduced dry matter content without adversely affecting the textural properties of the end product.

Keywords: Acid gels, Enzymatic modification, Fat replacer, Non-fat yoghurt, Transglutaminase

Transglutaminaz ve Yağ İkame Maddesi Kullanımının Yağsız Yoğurdun Fonksiyonel Nitelikleri Üzerine Etkileri

Özet

Bu çalışmanın amacı yağsız yoğurt üretiminde mikrobiyal transglutaminaz (mTGase, EC 2.3.1.13) ile ticari bir yağ ikame maddesinin (Dairy-Lo) etkinliğini karşılaştırmalı olarak araştırmaktır. Bu amaçla, mTGase ve Dairy-Lo ilavesi ile iki tip yağsız yoğurt ve herhangi bir katkı ilave edilmeksizin kontrol yağsız yoğurt üretilmiş ve +4°C'de 20 gün süreyle depolanmıştır. Yağsız yoğurt örneklerinin fiziksel özellikleri mTGase ile 20 günlük depolama süresince gelişmiş, ilaveten; mTGase ilavesinin yoğurdun asetaldehit içeriği üzerine olumsuz herhangi bir etkisi olmamıştır. Duyusal analiz sonuçları da mTGase ile yağsız yoğurdun kontrol yoğurdununkine benzer tat-aroma profiline sahip olduğunu desteklemektedir. Dairy-Lo ilavesi edilen yoğurtta en düşük serum ayrılması görülürken, aynı örnekte pıhtı sıklığı mTGase ilavesi ile üretileninkinden daha düşük olmuştur. İlaveten, Dairy-Lo ilavesi yağsız yoğurdun duyusal özelliklerini olumsuz yönde etkilemiştir. Elde edilen sonuçlara göre, mTGase kullanımı, son ürünün tektürel özelliklerine olumsuz etkisi olmadan düşük kurumaddeli yağsız yoğurt üretimi için tavsiye edebilir.

Anahtar sözcükler: Asit jeller, Enzimatik modifikasyon, Yağ ikame maddesi, Yağsız yoğurt, Transglutaminaz

INTRODUCTION

In recent years, low fat and non-fat dairy products including yoghurt have gained popularity because of consumer awareness about health concerns related to decreasing the risks connected with obesity and coronary heart diseases ^[1]. However, the partial or total removal of fat from yoghurt decreases the overall quality perceived

by the consumers ^[2]. It was reported that reduction of fat content in yoghurt resulted in lower gel strength and firmness than full fat yoghurt, as a consequence of lower number of fat globules embedded in the protein network ^[3].

To improve textural and functional properties of non-fat yoghurt, the use of additives has been widely investigated ^[4]. Fat replacers can be successfully used in



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the manufacture of reduced fat dairy products such as cheese, ice cream and yoghurt [5,6]. Fat replacer is an ingredient that can be used to provide some or all of the function of fat, yielding fewer calories [7]. Also, fat replacers can be used to solve some physical and textural problems originating from low-fat level in the dairy products. Dairy-Lo is a protein-based fat replacer which has a GRAS (Generally Recognized as Safe) status derived from whey protein concentrate [5,8].

Enzymatic cross-linking of protein by mTGase modifies the techno-functional properties of proteins and is reported as an innovative way of producing novel milk gels [9-11]. The mTGase which catalyzes the acyl-transfer (acyl donor) reaction between the γ -carboxamid group of peptide or protein-bound glutamyl residues and primary amines (acyl acceptor), is a transferase [12-14]. MTGase catalyzes the reactions which cause to the formation of cross-links in food proteins [15,16]. In this way, intermolecular cross-linking of proteins results in high molecular weight polymers which have different functional properties to improve the techno-functional properties of foods [10,17,18]. Milk proteins, especially caseins, are good substrates for cross-linking with mTGase [18-21]. The effect of cross-linking of milk proteins on various functional properties has been investigated [9,10]. It was reported that cross-linking of the proteins in milk improved gels firmness and reduced serum separation of acid-induced milk gel, mainly set-type yoghurts [17,22-25]. Özer et al. [26] also expressed that the mTGase added into milk may be an alternative method instead of addition of extra protein and stabilizer in non-fat yoghurt.

The present study was carried out to examine the effects of TGase and commercial fat replacer (Dairy-Lo) on some chemical, microstructural and textural properties of non-fat yoghurts.

MATERIAL and METHODS

Materials

Raw cow's milk was obtained from the Ankara University, Agricultural Faculty Dairy Farm. The raw milk contained 11.5 g/100 g total dry matter, 3.65 g/100 g protein and 3.5 g/100 g fat. Lyophilized-mixed yoghurt culture containing *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* coded Bulk Set Y 502 (Danisco Deutschland GmbH, Niebüll, Germany) was used as starter culture. The mTGase was supplied by Ajinomoto Co. (Japan, with declared activity of 100 units/g ActivaMP) at an enzyme/substrate ratio of 1 unit/g milk protein. The commercial protein based fat replacer Carbelac Dairy-Lo (Carbery Group, Ireland) was used at a level of 1.5% (w/v) as recommended by the manufacturer. The chemicals which were supplied from Merck Chemicals Ltd. (Merck, UK) were of analytical grade.

Production of Yoghurt

Raw milk was standardized to maximum 0.15% fat content and then divided three parts. First part was used for the production of control yoghurt (sample A) without any additive. Second part (sample B) was incubated with mTGase at 50°C for 60 min after pasteurization (85°C for 15 min) in order to improve so that the gel strength and to decrease the syneresis in yoghurt [25]. Dairy Lo was added to the third part of milk at the ratio of 1.5% before homogenization (sample C). Yoghurt production is outlined in Fig. 1.

Milk samples were inoculated with commercial yoghurt starter culture (2%, v/v), and then, were incubated at 43°C until pH 4.6 was attained. After incubation, yoghurt samples were cooled down to room temperature and kept in refrigerator at 4°C for 20 days. Samples were analysed at the 1st, 10th and 20th day of storage. Total dry matter contents of the samples A, B and C were 10.18%, 10.21% and 10.98%, respectively.

Chemical and Physical Analysis

The acidity of yoghurts was determined by titration and expressed as SH [27]. The pH was measured by a digital pH meter (MP 225, Mettler-Toledo GmbH, Giessen, Germany). Fat contents of the samples were determined by the Gerber method, while dry matter and protein contents were detected by oven drying and Kjeldahl methods, respectively [27]. For determination of tyrosine value, *spectrophotometric* method was used as reported by Hull [28].

Viscosity measurements were carried out using a viscometer (181/VTR 24, Thermo Haake GmbH, Karlsruhe, Germany) at +4°C. Gel firmness was measured by using a penetrometer (Model 17310-0, Stanhope-Seta Ltd., Surrey, England) which equipped with a 25 g conical (45°C) probe. Penetration depth of the probe into the yoghurt gel within 1 s of duration was referred to the value of penetrometer as millimeter.

Serum separation was measured by transferring twenty five gram of yoghurt samples into a funnel with filter paper placed on a flask. The volume of serum collected after draining at 4°C for 2 h was measured as serum separation value [29].

Determination of Carbonyl Compounds

Carbonyl compounds (i.e. acetaldehyde, acetone and diacetyl) were determined by headspace method using the procedure reported by Ulbert [30]. Five grams of yoghurt samples transferred into headspace vials (Agilent, made in USA, 20 mL flat bottom) and capped using crimper. Samples were kept at -18°C until the analysis was conducted. Prior to analysis, frozen samples were held at 70°C for 20 min in an oven. Then, the gas sample in

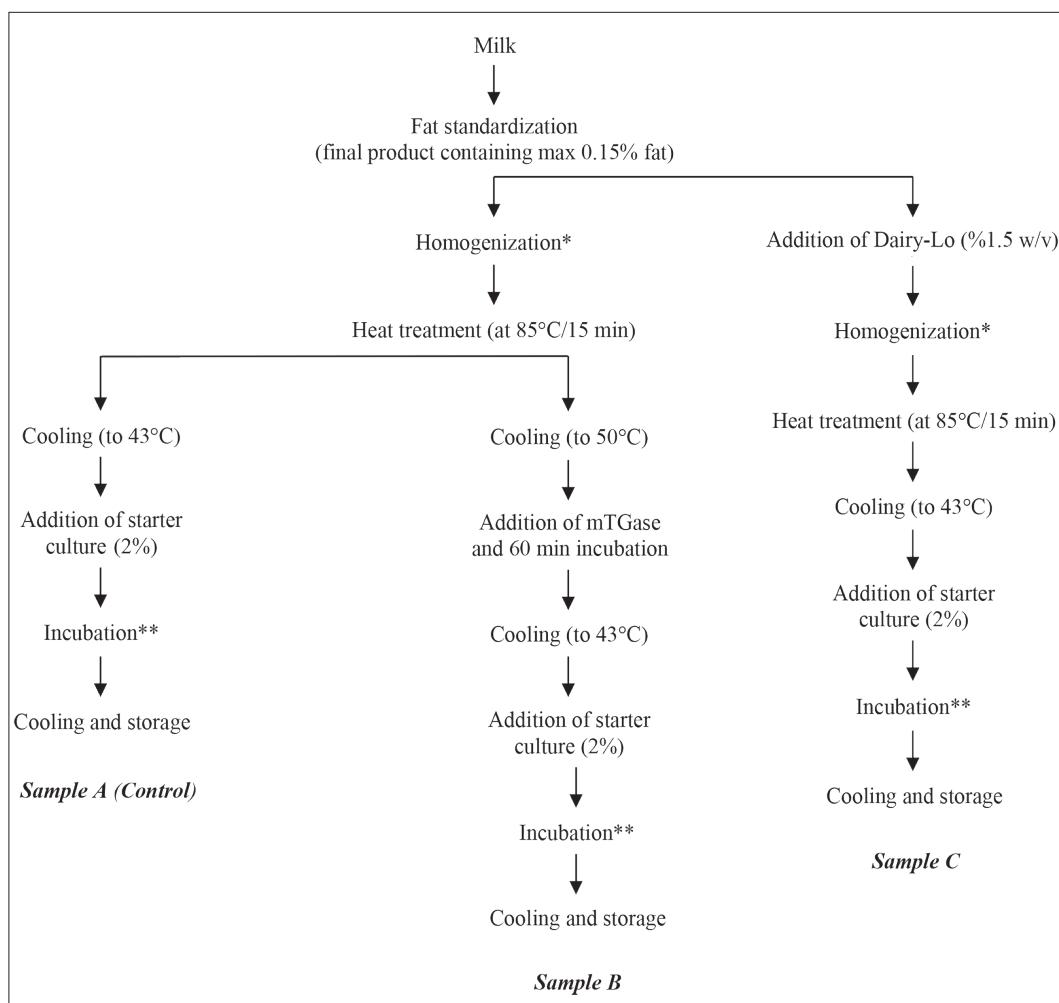


Fig 1. Flow diagram of yoghurt samples (*50°C 175 bar;** at 43°C until pH 4.6)

Şekil 1. Yoğurt üretim şeması (*50°C 175 bar;** 43°C'de pH 4.6'ya kadar)

headspace of vial was injected with a gas-tight syringe (1.000 µL) to GC equipped with a FID detector and Innowax polyethylene glycol capillary column (30 m long, 320 µm in diameter, 0.25 µm film thickness) (Agilent Technologies Inc., CA, USA). Operation conditions: temperature; injection block 80°C and FID 250°C; flow rates (mL min⁻¹); make-up gas (nitrogen) 30 mL/min, hydrogen 40 mL/min, air 400 mL/min and carrier gas (nitrogen) 0.7 mL/min. The programme of oven temperature was as follows: raised to 50°C for 0.5 min followed by increasing to 60°C at a rate of 4°C/min then kept for 0.5 min, increased to 70°C at a rate of 4°C/min and kept for 0.5 min, then increased to 180°C at a rate of 20°C/min and kept for 0.2 min.

Concentrations of the standard mix solutions for each carbonyl compound were 25, 50, 75 and 100 ppm. Calibration curves were prepared by plotting the peak area against the mass of each carbonyl compound.

Sensory Analysis

Sensory analysis of the yoghurt samples was performed by 10 experienced panelists using a 0-5 point scale for

appearance, consistency, odor and taste on 1, 10 and 20 days of analysis [31]. The yoghurt samples in 200 g plastic cups which were coded with three digit numbers were tempered to 10°C before serving to assessors.

Determination of Microstructural Properties of Yoghurts

Microstructure of the yoghurt samples was determined by scanning electron microscope. Samples were prepared by fixing on stapes by using carbon coated bands according to the method proposed by Skriver et al. [32] and Hayat [33]. Samples were then coated with pure gold using Polaron SC 502 sputter coater (Quorum Technologies, New Haven, UK) and examined with a Jeol JSM 6060 LV scanning electron microscope (Jeol Ltd, Tokyo, Japan).

Statistical Analyses

Data were analyzed using Minitab 13.0 statistical software (Minitab INC., PA, USA). The comparison of differences between the samples were determined by one-way analysis of variance (ANOVA) at P<0.01 [34].

RESULTS

The titratable acidity (SH) and pH values of the yoghurts are shown in *Table 1*. Sample supplemented with Dairy-Lo had significantly higher acidity level than the other samples ($P<0.01$).

The viscosity and penetrometer values (consistency) of the yoghurt samples are given *Table 1*. The sample treated with mTGase had significantly higher viscosity and consistency values than the other samples ($P<0.01$). These results can be attributed to the formation of cross-linking of milk proteins induced by mTGase which leads to the decrease in gel permeability, resulting in more stable and firm structure [17,25,26]. However, viscosity and consistency of the yoghurt sample added with Dairy-Lo was similar to the control yoghurt.

One of the factors affecting the acceptance of yoghurt by consumer is serum separation [37]. A gel formed with ϵ -(γ -glutamyl)lysine bonds improves water holding capacity of set type of yoghurt made from milk treated with mTGase, which results in reduction in serum separation [21]. These results confirmed that serum separation of the yoghurt

sample treated with mTGase was significantly ($P<0.01$) reduced compared with the control sample. However, the lowest level of serum separation was determined in the sample added with fat replacer.

Scanning electron micrographs (SEM) (x2.500 and x 5.000) of yoghurt gels are shown in *Fig. 2*. Microstructure of the non-fat yoghurts consisted of a protein network composed by chain and aggregates of fused casein micelles, where the streptococci and lactobacilli are easily distinguished (*Fig. 2-A2*). The protein network of the control sample (*Fig. 2-A2*) was less dense and more open as a consequence of smaller fused casein micelles aggregate, and probably absence of fat globules. Besides, SEM related to the protein matrices of the sample treated with mTGase (B1 and B2) was relatively more compact than the control sample (*Fig. 2 A1* and *A2*). This result was in an agreement with the results obtained from Lorenzen et al. [17], Faergemand and Qvist [18], Şanlı et al. [25].

Sensory evaluation results of yoghurts are given in *Table 2*. Significant differences were observed in consistency and appearance of the yoghurt samples during storage period. Consistency scores were the highest in the

Table 1. Physical and chemical characteristics of non-fat yoghurt samples during storage period¹

Tablo 1. Depolama sürecinde yağsız yogurt örneklerinin fiziksel ve kimyasal karakteristikleri¹

Parameters	Days	A	B	C
Titratable acidity (SH)	1	35.03±1.51 ^c	39.03±1.51 ^b	45.05±2.39 ^a
	10	38.14±1.89 ^b	40.00±1.01 ^b	52.36±1.01 ^a
	20	42.05±2.39 ^b	43.02±0.2 ^b	52.09±0.00 ^a
pH	1	4.37±0.00 ^a	4.11±0.07 ^b	4.06±0.04 ^b
	10	4.15±0.08 ^a	3.89±0.03 ^b	3.85±0.04 ^b
	20	4.03±0.08	3.88±0.00	3.85±0.01
Tyrosine (g/5 g)	1	0.51±0.17 ^b	0.46±0.08 ^c	0.57±0.00 ^a
	10	0.60±0.01 ^b	0.53±0.00 ^c	0.61±0.01 ^a
	20	0.71±0.06	0.70±0.08	0.74±0.01
Penetrometer value (mm/s)	1	46.05±0.42 ^a	33.75±0.63 ^b	45.15±1.20 ^a
	10	46.30±0.56 ^a	30.25±1.34 ^b	45.95±0.28 ^a
	20	40.05±0.21 ^b	27.80±1.27 ^c	44.58±1.94 ^a
Whey separation (mL/25 g)	1	9.12±0.17 ^a	7.25±0.00 ^b	4.00±0.00 ^c
	10	8.00±0.35 ^a	5.12±0.17 ^b	2.75±0.35 ^c
	20	7.75±0.35 ^a	5.62±0.53 ^b	3.25±0.35 ^c
Viscosity (Pas)	1	7.75±3.54 ^b	19.75±3.54 ^a	8.00±0.00 ^b
	10	11.00±0.00 ^b	27.00±1.41 ^a	12.00±1.41 ^b
	20	11.50±7.00 ^b	28.50±7.04 ^a	13.50±7.07
Acetaldehyde (ppm)	1	82.11±2.90	84±42±6.97	86.54±1.08
	10	55.71±3.37 ^b	61.10±1.27 ^b	72.56±1.99 ^a
	20	55.04±0.96	64.35±4.70	65.75±6.51
Diacehtly (ppm)	1	18.96±1.65	18.59±0.29	16.30±0.50
	10	19.81±2.13	19.63±1.63	20.45±1.46
	20	18.95±3.44	20.45±1.46	18.88±4.31
Acetone (ppm)	1	4.31±0.08 ^a	4.58±0.13 ^a	6.44±0.13 ^b
	10	4.62±0.17 ^a	4.00±0.20 ^a	6.40±0.83 ^b
	20	4.93±0.29 ^a	5.38±0.004 ^a	7.8±0.18 ^b

¹ Presented values are the means (±SD) of two replicates

^{a-b-c} Different letters in the same line indicate significantly different means at $P<0.01$

A: Control, B: Pasteurized milk was incubated with TGase at 50°C for 1 h, C: Prepared from milk added 1.5% of Dairy Lo before homogenization

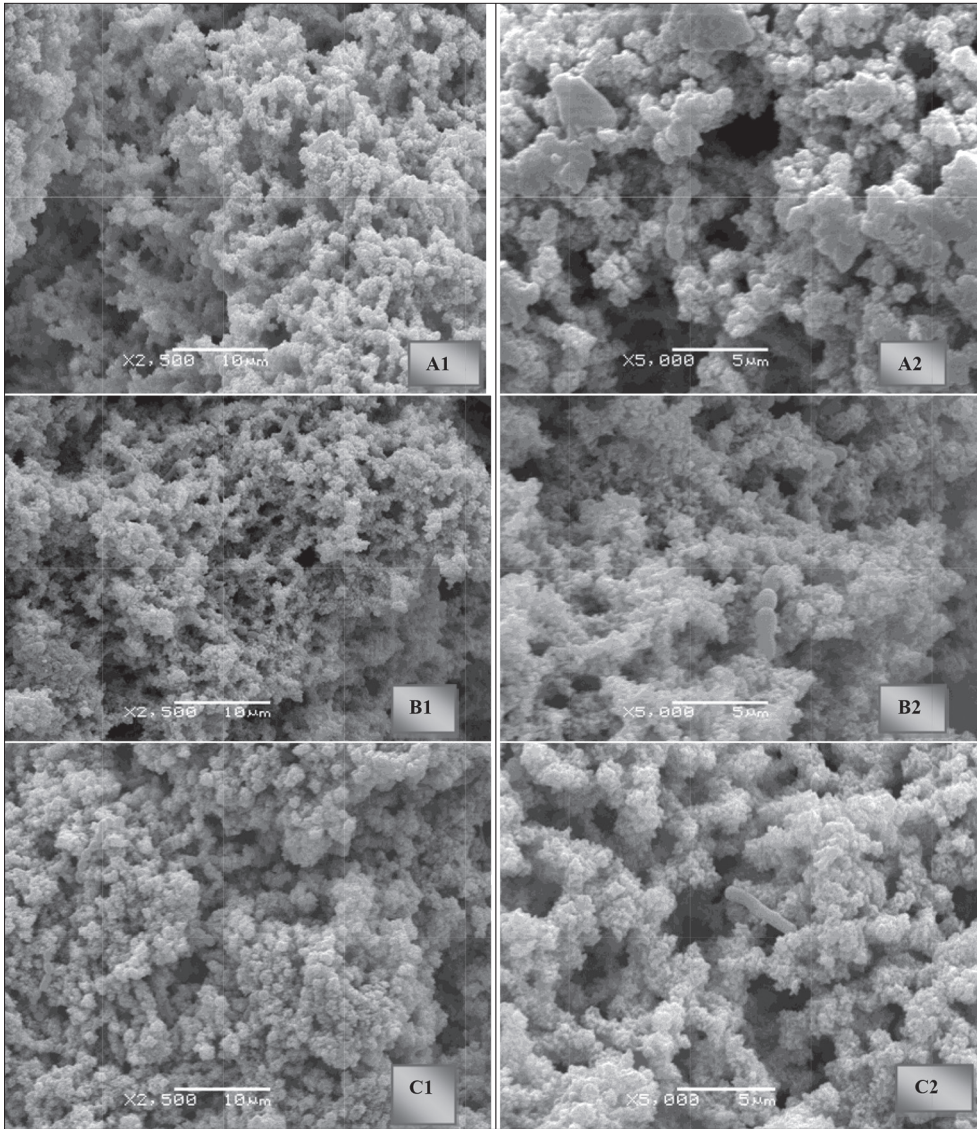


Fig 2. SEM micrographs of yoghurts. A1-B1-C1: Magnification is x2500, scale bar 10 µm. A2-B2-C2: Magnification is x5000, scale bar 5 µm. A- Control, B- Pasteurized milk was incubated with TGase at 50°C for 1 h, C- Prepared from milk added 1.5% of Dairy Lo before homogenization

Şekil 2. Yoğurtların SEM mikrofotoğrafları. A1-B1-C1: Büyütme x2500, ölçek çubuğu 10 µm. A2-B2-C2: Büyütme x5000, ölçek çubuğu 5 µm. A- Kontrol, B- Pastörize süt TGase ile 50°C'de 1 saat inkübe edilmiştir, C- Homojenizasyondan önce süte %1.5 Dairy Lo ilave edilmiştir

Table 2. Sensory properties of non-fat yoghurt samples during storage period¹

Tablo 2. Depolama sürecinde yağsız yogurt örneklerinin duyu özellikleri¹

Variables (Scores)	Days	A	B	C
Appearance	1	4.50±0.14 ^a	4.50±0.14 ^a	2.80±0.14 ^b
	10	4.10±0.14 ^a	4.15±0.07 ^a	2.55±0.07 ^b
	20	3.70±0.00 ^b	4.10±0.00 ^a	3.00±0.00 ^c
Consistency	1	3.05±0.07 ^b	4.15±0.07 ^a	2.85±0.07 ^c
	10	2.90±0.00 ^b	4.45±0.63 ^a	2.40±0.14 ^b
	20	2.70±0.00 ^b	4.35±0.07 ^a	2.60±0.00 ^b
Odour	1	3.85±0.07	4.10±0.00	4.05±0.07
	10	3.95±0.07 ^a	3.75±0.07 ^a	2.95±0.07 ^b
	20	4.05±0.00 ^a	4.00±0.07 ^a	3.60±0.00 ^b
Taste	1	2.65±0.07 ^a	2.80±0.00 ^a	2.00±0.00 ^b
	10	2.55±0.07 ^a	2.20±0.00 ^b	1.75±0.07 ^c
	20	2.35±0.07 ^a	2.40±0.00 ^a	1.85±0.00 ^b

¹ Presented values are the means (±SD) of two replicates

^{a-b-c} Different letters in the same line indicate significantly different means at P<0.01

A: Control, B: Pasteurized milk was incubated with TGase at 50°C for 1 h, C: Prepared from milk added 1.5% of Dairy Lo before homogenization

sample B ($P < 0.01$), while the samples A (control) and C received similar scores for consistency. These results were consistent with the instrumental analysis results. Panelists could not detect any difference in odors of the samples at the beginning of storage time; however, the sample C was found different from the other samples in terms of odors at days 10 and 20 ($P < 0.01$).

The variations in the acetaldehyde, acetone and diacetyl levels of the yoghurt samples during storage period are presented in *Table 1*. The acetaldehyde levels of the yoghurt samples at 10 d of storage were found significantly different ($P < 0.01$). The highest acetaldehyde level was detected at the sample C added with Dairy-Lo. This may be associated with the increase in protein content of yoghurt which acts as a precursor compound for the formation of acetaldehyde in yoghurt [8]. However, there was no significant difference between the sample B and the control sample regarding the level of acetaldehyde.

DISCUSSION

The acidity of the yoghurt increased with addition of Dairy-Lo; however, the presence of mTGase in yoghurt did not affect the acidity. Also, Lorenzen and Schlimme [22] did not detect any significant difference among the yoghurts with and without mTGase in regard to acidity during the storage period of 14 days. Moreover, the titratable acidity and pH values of the sample treated with mTGase was higher than the control sample ($P < 0.01$) at 1st day of storage. On the contrary, some authors reported that the treatment with mTGase caused slower production of acidity in yoghurt [17,26]. In addition, the mTGase application did not cause any delay in fermentation period of non-fat yoghurt production. The incubation of the yoghurt samples was terminated when the pH reached to 4.6. Fermentation time were 225 and 215 min in the samples A (control) and sample B (containing mTGase), respectively. Similar results were reported by Schey [35] that there is no interaction between mTGase and starter bacteria through the fermentation of yoghurt. The acidities of all samples increased throughout storage.

Tyrosine is an indicator of the level of proteolysis. Tyrosine value of the sample treated with mTGase was found to be the lowest during storage. These findings indicate that cross-linking of proteins catalyzed by the enzyme results in proteins become more stable against to proteolysis. The level of tyrosine increased during storage as a consequence of the proteolytic activity of yoghurt starter culture. However, the increment in the tyrosine content of the sample treated with TGase was slightly slower than the other samples. Yüksel and Erdem [36] reported that mTGase active yoghurt samples had lower peptide content and tyrosine values than those without mTGase and mTGase inactive samples.

The viscosity and consistency values of all samples increased during the storage time and the highest levels were observed at 20 day of storage. These increases during storage period could be as a result of protein rearrangement and protein-protein interactions [26]. However, the remarkable increase was determined in the sample treated with mTGase. This result could indicate that activity of enzyme continued after fermentation. Similar findings were reported by Özer et al. [26].

Scanning electron microscopy images confirmed that gel strength of yoghurt made from milk treated with mTGase was higher due to a more regular distribution of protein network with smaller pores, leading to less serum separation during storage [18]. Also, some researchers reported that decrease in gel porosity resulted in the decrease in yoghurt whey expulsion because of the fact that the cross-linking of protein chains can stabilize the three dimensional network of yoghurt gel [18,26]. Scanning electron micrographs of yoghurt made with Dairy-Lo showed that the addition of protein based-fat replacer caused to differences in arrangement of the gel network. It can be explained that protein based-fat replacer integrated into the aggregates and as a result of these interactions between denatured whey proteins and the surface casein micelles were prevented in milk. Thus, the microstructure of the yoghurt including Dairy-Lo was coarser and fluffier (*Fig. 2 C1-C2*).

The mTGase treatment did not have a negative effect on aroma and flavour. Panelists reported that the flavour of the yoghurt treated with mTGase (sample B) was the same as the flavour of the control sample. However, Dairy-Lo had a negative impact on the taste. Sample C added with Dairy-Lo was perceived lower taste scores by the panelists than the other samples (A and B) ($P < 0.01$). The difference in the appearance of the yoghurt samples was found to be significant ($P < 0.01$). Dairy-Lo added sample had the lowest appearance score than the other samples.

There is a general agreement in literature that the aroma and flavour of yoghurt consist mainly of non-volatile and volatile acids and carbonyl compounds. One of the major flavour carbonyl compounds in yoghurt is acetaldehyde [37]. Acetaldehyde concentration in all yoghurt samples were declined with storage time. This decrease in the level of acetaldehyde could arise from the alcohol dehydrogenase activity of yoghurt starters. This enzyme transforms acetaldehyde to ethyl alcohol during storage [37]. No significant difference was observed among the yoghurt samples regarding diacetyl contents (*Table 1*). In addition, the presence of mTGase in the yoghurt did not have any effect on the acetone levels. However, the acetone level of the sample C was higher than the other samples (sample A and sample B).

The results indicated that mTGase enzyme may be useful for production of non-fat yoghurt without adversely

affecting the sensory properties of the end product. The rheological data and SEM indicate that mTGase had significant effects on the protein microstructure of non-fat yoghurt. This study also showed that the use of Dairy-Lo could negatively affect texture development and sensory properties of non-fat set yoghurts.

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