Journal of Animal and Veterinary Advances 11 (13): 2238-2246, 2012
ISSN: 1680-5593

The Influence of Transglutaminase Treatment on Functional Properties of Strained Yoghurt

Nayil Dinkci
Department of Dairy Technology, Faculty of Agriculture, Ege University,
35100 Bornova, Izmir, Turkey

Abstract: The aim of this study was to investigate the influence of Transglutaminase (TG) on the physicochemical, microbiological and sensory properties of strained yoghurt. Strained yoghurt samples were produced with four different enzyme concentrations; C (Control), T1 (0.74 Unit TG g^{-1} protein), T2 (1.29 Unit TG g^{-1} protein) and T3 (1.85 Unit TG g^{-1} protein). The samples were evaluated regarding chemical composition, proteolysis, texture profile, viscosity, water holding capacity, microbiological counts and sensory properties. Cross linking of milk proteins by TG enzyme improved the physical properties of the yoghurts before straining. Surprisingly the textural parameters of the strained yoghurts were not affected by the enzyme but the water holding capacity was improved. Higher treatments of the TG enzyme decreased the proteolytic activity and acidity with increasing storage time. On the contrary enzymatic cross linking had no significant effect on the microbiological properties and the sensory attributes were not unfavorable affected.

Key words: Transglutaminase, cross-linking, strained yoghurt, proteolysis, TPA, sensory

INTRODUCTION

Strained yoghurt (labneh) is a traditional fermented milk product. The popularity and consumption has increased during recent years and it is now produced widely industrially. Strained yoghurt is derived from yoghurt by draining its water and water-soluble compounds into a specified total solid content in cloth bags.

Similar products are manufactured in different countries for example, Labneh or yoghurt cheese (Middle East), Laban Zeer (Egypt), Torba or Suzme (Turkey), Besa (Bulgaria), Skyr (Irland), Ymer (Denmark) and Chakka and Shrikhand (India) (Yazici and Akgun, 2004; Nsabimana *et al*., 2005).

Straining of yoghurt whey is one of the important processes during strained yoghurt production. Compositional and textural variations occur during the removal of whey. Water soluble nutrients are lost from yoghurt with the whey. This concentration process allows further fermentation by lactic acid bacteria which effects the flavor of the final product (Guler and Sanal, 2009).

Important properties for consumer acceptance are textural characteristics, aroma formation and acidity besides nutritional properties. Textural properties and water holding capacity could be changed by formation of new covalent bonds in the yoghurt gel whereas yoghurt gel network is stabilized mainly by weak noncovalent interactions. These weak interactions can be modified by formation of new covalent bonds in the gel. As a cross-linking enzyme for catalyzing the covalent bond formation between protein molecules can transglutaminase (TG, EC 2.3.2.13, protein-glutamine γ-glutamyl-transferase) be used (Fernandes De Sa and Bordignon-Luiz, 2010). Transglutaminase catalyses acyl-transfer reactions between γ-carboxyamide groups of glutamine residues as acyl donors and the ε-amino group of lysines as acyl acceptors. The enzyme is capable to form inter and intramolecular crosslinks in many proteins. While lysyl residues act as acyl acceptors intra and intermolecular ε-(γ-glutamyl)-l-lysine isopeptide crosslinks are formed by the enzyme reaction and they result in the polymerization of proteins (Nielsen, 1995; Seguro *et al*., 1996; Ozrenk, 2006). In the absence of lysine residues and other primary amines, water reacts as a nucleophile for the deamination of glutamines (De Jong and Koppelman, 2002). Several studies have shown that the casein is an excellent substrate for TG, therefore it's predominantly applied to casein based milk products (Lauber *et al*., 2000; Gauche *et al*., 2009; Kulozik, 2009; Fernandes De Sa and Bordignon-Luiz, 2010).

The effect of traditional methods; such as increasing the total solids in the milk with skim milk powder whey protein concentrates, etc., adding stabilizers, e.g., pectin, gelatin; to improve the textural and physical properties of yoghurt is well known. Emerging trends are novel stabilizers, various types of milk derived ingredients, membrane concentrates and fractions, specific cultures

(specific exopolysaccharide producing types) and specially the enzymatic cross-linking of milk proteins with transglutaminase (Farnsworth *et al*., 2006). Transglutaminase has recently been applied in different dairy products, e.g., stirred and set type yoghurts, cheese, ice cream, kefir and milk powder, mainly to improve their structure. The use of TG increased the gel strength and decreased the syneresis of set type yoghurts (Farnsworth *et al*., 2006; Gauche *et al*., 2009; Sanli *et al*., 2011). The enzyme (TG) was postulated to improve the structure of stirred yoghurt at a reduced addition or without addition of protein ingredients such as skim milk powder, whey protein concentrate or sodium caseinate (Bonisch *et al*., 2007). The enzyme was also used to improve the physical and sensory properties of non-fat set yoghurts (Ozer *et al*., 2007). Furthermore, by using TG-treated skim milk powder only half of the protein addition was required to obtain an equivalent viscosity compared to the control skim milk yoghurt (Guyot and Kulozik, 2011). Bonisch *et al*. (2007) reported that the protein cross-linking was enhanced by TG and higher apparent viscosity and higher degrees of protein polymerisation were measured in yoghurt gel. The influence of TG on rennet coagulation properties was investigated which enabled obtaining a rennet gel with modified functional properties (Bonisch *et al*., 2007; Fernandes de Sa and Bordignon-Luis, 2010). TG has been used for the microencapsulation of probiotics, to protect microorganisms from damage due to a low level of pH (Heidebach *et al*., 2009). The use of TG decreased the kefirs protein immunoreactivity while it obtained higher sensory scores for overall quality than the control one (Wroblewska *et al*., 2009). Recently, TG has been applied for ice cream production and it was observed that the TG increased the consistency index and favored the pseudoplastic behavior of the samples (Rossa *et al*., 2011). Although, the manufacturing methods, chemical compositions, rheological properties and losses of nutrients of concentrated yoghurt have been studied by several researchers (Ozer *et al*., 1998; Nsabimana *et al*., 2005; Guler and Sanal, 2009) however, no data is available on the effect of TG on functional, chemical and microbiological properties of strained yoghurt.

The aim of this study was to investigate the influence of TG treatment on the functional, chemical, microbiological and sensory properties of modified strained yoghurt.

MATERIALS AND METHODS

Transglutaminase: Microbial transglutaminase was obtained from Ajinomoto (Ajinomoto Europe Sales GMBH Hamburg, Germany). The enzyme (Activa MP) was used in the original form.

Production of strained yoghurt: Strained yoghurt production was carried out in Sakipaga Dairy and Food Co., Izmir, Turkey. The flow diagram of the strained yoghurt production is shown in Fig. 1. As it can be shown in Fig. 1. After the standardization, evaporation, homogenization and pasteurization steps, the milk used for strained yoghurt production was divided in 4 equal portions. One portion was used to produce Control (C) strained yoghurt sample without TG addition while the other portions T1-T3 separately mixed with 0.74, 1.29 and 1.85 Unit g^{-1} milk protein, respectively. The amount of TG added to strained yoghurt milk was calculated according to the suggested amounts (0.2-0.5 g L^{-1} milk) by the enzyme producer (Activa MP, Ajinomoto Europe Sales GMBH Hamburg, Germany). TG induced samples were incubated for 2 h at 40°C before enzyme inactivation at 75°C for 1 min. While the K milk was reheated the enzyme treated milks were cooled to 45°C and inoculated with 2% (w/v) of thermophilic starter culture (*Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus,* Yo-Mix 500 Wisby-Danisco). Incubated at 43°C until 4.7 pH, stored for 24 h at 4°C, strained in cloth bags at 4°C for 10-14 h, mixed gently and packaged in 500 g cups and storaged for 14 days at 4°C.

Chemical characterization: The raw milk and strained yoghurt samples were analyzed for total solids (g/100 g),

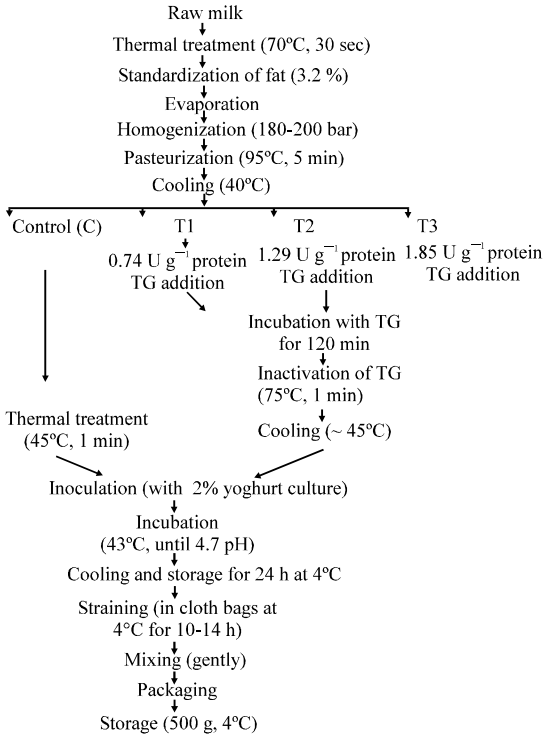

Fig. 1: Flow diagram of strained yoghurt production

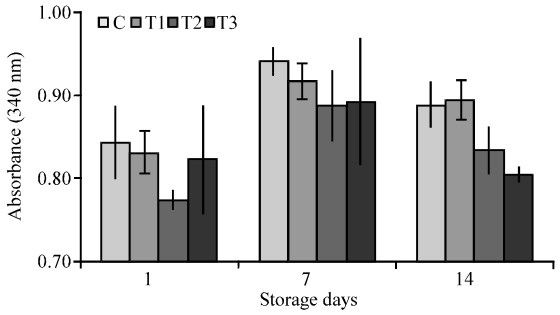

Fig. 2: Variations in the pH values during storage of the strained yoghurts. The added transglutaminase concentrations were as follows: C (control, 0 U g^{-1} protein), T1 (0.74 U g^{-1} protein), T2 (1.29 U g^{-1} protein), T3 (1.85 U g^{-1} protein). Standard deviations are given as error bars of the means (n = 3)

ashes (g/100 g) and fat (g/100 g) according to AOAC (2005), total protein (g/100 g) by the Kjeldahl Method. The lactose content was determined spectrophotometrically (Kurt *et al*., 1999) and the pH of the samples was measured by using a pH-meter (Hanna model 211, Hanna Instruments Woonsocket RI USA) (Fig. 2).

Enumeration of the yoghurt starters: The colony counts of *Lactobacillus delbrueckii* ssp. *Bulgaricus* and *Streptococcus thermophilus* were enumerated in each sample following serial dilutions in Ringer's solution. MRS agar was used for the enumeration of *L. delbrueckii* ssp. *bulgaricus* incubated anaerobically at 42°C for 48 h; M17 agar for the enumeration of *S. thermophilus* incubated aerobically at 37°C for 48 h. Yeasts and moulds were enumerated with YGC agar and incubated at 25°C aerobically for 3-5 days.

Determination of the proteolytic activity: The influence of TG treatment on the proteolytic activity of the strained yoghurts were determined by measuring liberated amino acids and peptides using the O-Phtaldialdehyde (OPA) method as described by Donkor *et al.* (2006) with some modifications. Samples (1 g) were mixed with 5 mL of 0.75% (w/v) trichloroacetic acid and vacuum filtered using a Whatmann # 1 filter paper (Whatmann International Ltd., Kent, England). The filtrate (150 μL) was added to 3 mL of OPA reagent. The absorbance was measured in a Spekol 1300 spectrophotometer (Analytic Jena AG, Jena Germany) at 340 nm after 2 min at room temperature (20°C). The proteolytic activity was expressed as the free amino groups measured as the difference in absorbance between samples.

Physical properties of the samples

Water holding capacity: The water holding capacity of the samples were determined according to the methodology proposed by Bhullar *et al.* (2002) and Sodini *et al.* (2005). A sample about 20 g of strained yoghurt was centrifuged for 10 min at 5000 g, 20°C (Sigma Model No: 3-16 K, Sigma Laborzentrifugen Ostreode am Harz, Germany). The supernatant was weighed. The Water Holding Capacity (WHC) was calculated as:

$$\text{WHC}(\%) = \frac{(\text{Yoghurt weight} - \text{Supernatant weight})}{\text{Yoghurt weight}} \times 100$$

Penetrometer index of the samples: The penetrometer index of the samples were measured with a Gerber Instruments penetrometer Model FT II (Gerber Instruments AG Switzerland) using 180 g of weight, fixed 20 sec time and variable penetrate distance (max. 3 cm).

Apparent viscosity: The apparent viscosity of samples was measured with a rotational Brookfield DV-II+Pro viscometer (Middleblro, MA USA) with a helipath stand and T-bar spindle (T-F) at 0.6 rpm. The helipath stand allowed the spindle to be lowered slowly while rotating into the sample, eliminating the channeling effect normally experienced with highly viscous materials. The data were evaluated using Rheocalc V 3.2 Build 46-1, Brookfield Engineering Labs.

Texture profile analysis: The texture analysis were carried out with a TA-XT Plus Texture Analyzer (Stable Micro Systems LTD., Vienna Court, Godalming, Surrey GU7 1YL UK), equipped with a 35 mm diameter disc and extension bar using 5 kg load cell. Tests were carried out in the original container. A constant crosshead velocity of 1 mm sec^{-1}, to a sample depth of 30 mm and a post test speed of 10 mm sec^{-1} was used. From the resulting force-time curves the values for texture attributes were obtained. The peak or maximum force was taken as a measurement of firmness (g), the higher the value the firmer is the sample. The area of the curve up to this point was taken as a measurement of consistency (g, s), the higher the value the thicker the consistency of the sample. The negative region of the graph, produced on probe return is a result of the weight of sample which is primarily on the upper surface of the disc on return, i.e., due to back extrusion and hence gives again an indication of consistency/resistance to flow off the disc. The maximum negative force is taken as an indication of the cohesiveness (g) of the sample, the more negative the value the more cohesive the sample. The area of the negative region of the curve is referred as the Index of Viscosity (g s), the higher the value the more resistant to withdrawal the sample.

Sensory evaluation: The sensory evaluation of the test yoghurts were carried out by 8 panelists from the Department of Dairy Technology who were familiar with strained yoghurt and had previous taste panel experience. The strained yoghurt samples were organoleptically examined for appearance, consistency, odour, aroma/flavour and overall acceptance by a mixed-point system with a rating scale of 1 min to 5 (max) scores. The samples were coded with three digit random numbers and presented to the panelists in their original packages. Orders of serving were completely randomly. Panelists rinsed their mouth with water before tasting each sample. Mean scores for each attributes were used for comparison of the samples.

Statistical analysis: The effect of the enzyme on the chemical, physical and rheological properties of strained yoghurt samples was carried out by Analysis of Variance (ANOVA) and the mean differences were analyzed using Duncan's Multiple Range Test when a 0.05% difference in the level of these values was verified. The interactions of the instrumental texture parameters were evaluated by Principal Component Analysis. All analyses were evaluated by SAS V8 (SAS Institute Inc., NC, USA). Each experiment was performed in triplicate.

RESULTS AND DISCUSSION

Chemical composition: The chemical characteristics of the raw milk, yoghurt and strained yoghurt are shown in Table 1. The gross composition of the yoghurt samples was suitable with the legal Turkish regulation for yoghurt. While the addition of TG showed no significant ($p<0.05$) effect on the chemical properties of strained yoghurt it increased significantly ($p<0.05$) the protein and total solids in the yoghurt samples. These results are consistent with the study of Fernandes De Sa and Bordighon Luiz (2010), similar results were also found by Cozzolino *et al*. (2003). The total solids, protein and ash contents did not significantly differ among the strained yoghurt samples. There were significant ($p<0.05$) differences in the fat and lactose contents of the strained yoghurt samples which was caused by the straining process. The chemical composition of the strained yoghurt samples was within ranges of values reported by Ozer *et al*. (1997), Akin (1999), Tamime and Robinson (2007) and Kesenkas (2010).

The acidity of the strained yoghurt samples was affected by the TG enzyme. The differences between the samples were statistically significant ($p<0.05$) while the samples T2 and T3 had similar pH values during the 14 days of storage, the control sample had the lowest pH values.

The pH's of the enzyme treated samples were proportionally with the enzyme concentration, the lower the enzyme concentration the lower was the pH. The storage period affected the pH values also significantly ($p<0.05$). These findings are in good agreement with Ozer *et al*. (2007) who reported that the enzyme led to an imbalance of the protosymbiosis growth of the yoghurt culture. As it is known there is a symbiotic growth between the yoghurt starter cultures. In the 1st step, *S. thermophilus* ferments the lactose to lactic acid and grows until a pH of 5.5. In the 2nd step, *Lactobacillus delbrueckii* ssp. *bulgaricus* stimulates the growth of Streptococci by their proteolytic activity on milk proteins and concurrent peptisation, forming amino acids. The liberation of amino acids into the milk is essential for the growth of *S. thermophilus* and for the production of lactic acid (Walstra *et al*., 1999).

Microbiological properties: The viable counts of yoghurt bacteria and yeast-moulds of the strained yoghurt samples during storage are shown in Table 2. The viable counts of *L. delbrueckii* ssp. bulgaricus generally decreased during the storage in all samples except T1 ($p<0.05$). There were no significant differences between the control and the treated samples ($p<0.05$). The counts of *S. thermophilus* increased significantly in all samples except T3 on every analyze date during storage ($p<0.05$).

Table 1: Chemical characteristics of the raw milk, yoghurt and strained yoghurt samples

Samples	Total solids (g/100 g)	Fat (g/100 g)	Total proteins (g/100 g)	Lactose (g/100 g)	Ashes (g/100 g)
Raw milk	11.79±0.00	3.50±0.00	2.71±0.01	3.79±0.03	0.71±0.02
Yoghurt					
C	15.80±0.02^{c}	4.80±0.14^{a}	4.58±0.10^{b}	5.00±0.29^{a}	1.03±0.00^{a}
T1	16.33±0.08^{a}	4.80±0.14^{a}	5.05±0.07^{a}	5.18±0.14^{a}	1.03±0.01^{a}
T2	16.13±0.00^{b}	5.10±0.14^{a}	4.98±0.06^{a}	4.94±0.45^{a}	1.02±0.00^{a}
T3	16.16±0.01^{b}	5.10±0.14^{a}	5.08±0.10^{a}	5.03±0.25^{a}	1.03±0.02^{a}
Strained yoghurt					
C	21.25±0.76^{a}	7.90±0.17^{bc}	7.63±0.74^{a}	4.66±0.27^{ab}	0.96±0.02^{a}
T1	21.14±0.79^{a}	8.50±0.35^{a}	7.06±0.00^{a}	4.65±0.08^{ab}	1.00±0.01^{a}
T2	21.12±0.43^{a}	8.10±0.30^{ab}	6.94±0.26^{a}	4.91±0.36^{a}	0.99±0.03^{a}
T3	21.93±0.41^{a}	7.50±0.00^{c}	7.90±0.32^{a}	4.45±0.08^{b}	1.01±0.03^{a}

The values correspond to the mean values±SD obtained from three repetitions. Different letters on the same column and sample represent statistically significant differences between values ($p<0.05$) by the Duncan multiple range test. C = Control (0.00 U TG g^{-1} protein); T1 = 0.74 U TG g^{-1} protein; T2 = 1.29 U TG g^{-1} protein; T3 = 1.85 U TG g^{-1} protein

Table 2: Viability of yoghurt bacteria and yeasts moulds in strained yoghurt samples during 14 days of cold storage

Microorganism	Sample	Day 1	Day 7	Day 14
Lactobacillus bulgaricus	C	8.77 ± 0.09^{aX}	8.29 ± 0.25^{bX}	8.08 ± 0.07^{bX}
	T1	8.74 ± 0.13^{aX}	8.25 ± 0.35^{aX}	8.44 ± 0.17^{aX}
	T2	8.83 ± 0.11^{aX}	8.41 ± 0.20^{bX}	8.38 ± 0.13^{bX}
	T3	8.81 ± 0.10^{aX}	8.39 ± 0.27^{bX}	8.35 ± 0.17^{bX}
Streptococcus thermophilus	C	8.26 ± 0.06^{bX}	8.52 ± 0.05^{aX}	8.47 ± 0.08^{aY}
	T1	6.75 ± 0.21^{cY}	8.43 ± 0.14^{bX}	8.81 ± 0.16^{aX}
	T2	8.26 ± 0.14^{cX}	8.51 ± 0.03^{bX}	8.78 ± 0.08^{aX}
	T3	8.47 ± 0.28^{aX}	8.51 ± 0.21^{aX}	8.84 ± 0.07^{aX}
Yeasts-moulds	C	2.21 ± 0.18^{cX}	2.73 ± 0.18^{bX}	3.72 ± 0.11^{aX}
	T1	1.46 ± 0.28^{bX}	1.78 ± 0.44^{bY}	4.26 ± 1.01^{aX}
	T2	1.60 ± 0.56^{bX}	1.54 ± 0.47^{bY}	3.30 ± 0.19^{aX}
	T3	1.50 ± 0.17^{bX}	1.68 ± 0.25^{bY}	3.43 ± 0.17^{aX}

The values correspond to the mean values±SD obtained from three repetitipns. $^{a-c}$Means in the same row with different superscripts represent statistically significant differences between the values ($p<0.05$). $^{X-Z}$Means in the same column with different superscripts represent statistically significant differences between the values ($p<0.05$). Treatment codes: C (control, 0 U TG g^{-1} protein), T1 (0.74 U TG g^{-1} protein), T2 (1.29 U TG g^{-1} protein), T3 (1.85 U TG g^{-1} protein)

No important differences could be detected between the samples for *S. thermophilus*, only the control sample had significantly lower viable counts on the 14th day of storage. It's apparent that TG had no toxic side effect on yoghurt bacteria. In contrary, Ozer *et al.* (2007) reported that the growth rate of the yoghurt starter bacteria was reduced in the presence of TG, dependent on the enzyme concentration in yoghurt. The ratio of *S. thermophilus* to *L. delbrueckii* ssp. *Bulgaricus* was approximately 1:1 for all samples. Kesenkas (2010) found also a ratio of 1:1 in strained yoghurt samples and similar viable counts of starter bacteria. While Ozer and Robinson (1999) reported higher viable counts of *S. thermophilus*, Akkaya *et al.* (2009) enumerated lower counts of starter bacteria in strained yoghurt samples. Strained yoghurt is a suitable media for the growth of yeast and moulds because of its concentrated contents, low pH and limited access to air at packaged, refrigerated storage conditions. When the traditional production method is used and Good Manufacturing Practices (GMP) are not fully observed the contamination of strained yoghurt by yeast-mould can not be prevented. As it can be shown from Table 2, the counts of yeast and mould increased in the control sample constantly ($p<0.05$) while the enzyme treated samples were stabile until the 14th day of the cold storage. There were no significant differences between the samples only the control sample had on the 7th day of storage significantly ($p<0.05$) higher yeast-mould counts. These yeast-mould counts are in accordance with earlier reports of Uysal (1993) and Kesenkas (2010).

Proteolytic activity: The proteolytic activity of strained yoghurts was determined by measuring the free amino groups using the spectrophotometric O-Phtaldialdehyde (OPA) method. During fermentation milk proteins are hydrolised by proteinases which were produced by lactic acid bacteria. This result in an increase in the amount of the free amino groups and these amino groups are quantified by the OPA method (Kesenkas, 2010). The

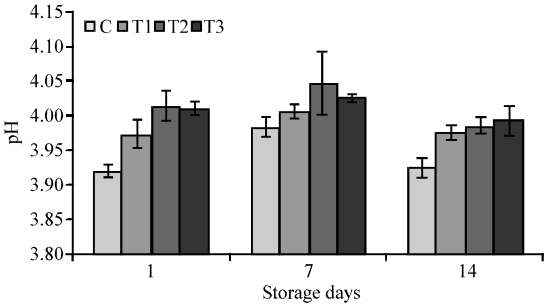

Fig. 3: Variations in the proteolytic activity values during storage of the strained yoghurts. The added transglutaminase concentrations were as follows: C (control, 0 U TG g^{-1} protein), T1 (0.74 U TG g^{-1} protein), T2 (1.29 U TG g^{-1} protein), T3 (1.85 U TG g^{-1} protein). Standard deviations are given as error bars of the means (n = 3)

proteolytic activities of TG enzyme treated strained yoghurt samples in comparison to the control sample are shown in Fig. 3. The proteolysis in all samples followed a similar, significant ($p<0.05$) increasing trend throughout the cold storage, except T3. There were no significant differences in proteolytic activities among yoghurt samples with the exception of the 14th day. Only at the 14th day was a significant difference ($p<0.05$) between the samples. Control and T1 had similar and higher results than T2 and T3. It could be inferred that higher treatments of the enzyme decreased the proteolytic activity with increasing storage time.

Yuksel and Erdem also reported a significant ($p<0.05$) decrease in the proteolytic activity and acidity of TG treated set yoghurts.

Physical properties of the strained yoghurt samples: Texture profile analysis and apparent viscosity of strained yoghurt samples during refrigerated storage is shown in Table 3. TPA parameters such as firmness, consistency, cohesiveness index of viscosity and also the apparent

Table 3: TPA and apparent viscosity parameters of strained yoghurt samples during 14 days of cold storage

Parameters	Sample	Day 1	Day 7	Day 14
Firmness (g)	C	348.18±66.25^{b}	516.36±32.86^{a}	571.62±42.58^{a}
	T1	310.18±61.25^{b}	510.74±16.87^{a}	565.75±7.41^{a}
	T2	339.01±33.18^{b}	518.75±59.83^{a}	517.94±54.35^{a}
	T3	325.72±16.18^{b}	548.30±29.75^{a}	447.27±123.39^{ab}
Consistency (g.s)	C	6936.25±1553.01^{b}	10680.05±908.07^{a}	11936.94±1040.89^{a}
	T1	6368.99±1270.04^{b}	10574.73±135.08^{a}	11868.08±256.19^{a}
	T2	6942.56±592.18^{b}	10909.10±1135.97^{a}	11375.48±307.32^{a}
	T3	6478.28±236.82^{b}	11633.75±263.89^{a}	11003.13±104.47^{a}
Cohesiveness (g)	C	290.22±63.83^{b}	349.51±21.32^{ab}	415.67±47.29^{a}
	T1	282.91±16.12^{c}	366.84±15.52^{b}	405.85±21.69^{a}
	T2	274.93±27.35^{b}	388.21±37.35^{a}	366.30±19.95^{a}
	T3	254.06±21.44^{a}	377.89±3.63^{a}	356.02±105.51^{a}
Index of viscosity (g.s)	C	465.05± 75.99^{b}	729.39±127.99^{a}	594.94±62.46^{ab}
	T1	433.42±7.67^{b}	673.98±18.32^{a}	722.63±115.06^{a}
	T2	423.53±44.55^{a}	686.62±179.35^{a}	438.27±80.56^{a}
	T3	412.59±17.80^{b}	678.71±91.93^{a}	655.48±47.05^{a}
Apparent viskosity (Pa.s)	C	1001.90±143.08^{a}	985.49±69.39^{a}	900.14±30.53^{a}
	T1	1016.36±123.52^{a}	1015.99±42.99^{a}	801.34±100.48^{a}
	T2	1032.18±46.62^{a}	965.04±61.07^{ab}	867.60±67.34^{b}
	T3	1134.95±36.35^{a}	973.81±24.07^{b}	923.62±57.16^{b}

The values correspond to the mean values±SD obtained from three repetitions. $^{a-c}$Means in the same row with different superscripts represent statistically significant differences between the values ($p<0.05$). $^{X-Z}$Means in the same column with different superscripts represent statistically significant differences between the values ($p<0.05$). Treatment codes: C (control, 0 U TG g^{-1} protein), T1 (0.74 U TG g^{-1} protein), T2 (1.29 U TG g^{-1} protein), T3 (1.85 U TG g^{-1} protein)

viscosity of the strained yoghurt samples were evaluated. The addition of transglutaminase enzyme had surprisingly no significant ($p<0.05$) effect on the TPA parameters of the strained yoghurt samples. Since there is no data for TPA parameters of TG enzyme treated strained yoghurt. Many researchers suggested that the addition of TG enzyme shows a significant increase in almost all textural parameters of yoghurt, since the basic function of TG is to cross-link milk proteins covalently which results in a finer and stronger gel network (Ozer *et al*., 2007; Gauche *et al*., 2009; Sanli *et al*., 2011).

The reason, therefore could be the mixing procedure before packaging of the strained yoghurts. Ozer *et al*. (2007) demonstrated also that when the activity of the enzyme was allowed during and after fermentation the development of gel stiffness was stimulated in yoghurt. In the present case, the enzyme activity was inactivated prior to fermentation. On the other hand similar to the results of Gauche *et al*. (2009), the consistency of the yoghurt samples (not strained) was significantly ($p<0.05$) effected by the enzyme. The higher the enzyme treatment the higher was the consistency. The consistency values are 8319.01, 10175.73, 9793.41 and 11099.17 g.s for the yoghurt samples C, T1, T2 and T3, respectively on the 1st day (data not given). Textural parameters of strained yoghurts were significantly effected ($p<0.05$) by the storage period. Nearly all of the strained yoghurt samples showed significantly higher ($p<0.05$) values for all TPA parameters on the 7th day of storage and apparent viscosity values decreased significantly ($p<0.05$) in the same period in the samples T2 and T3.

Table 4: Penetrometer values of the strained yoghurt samples during 14 days of cold storage (mm 20 sec^{-1})

Samples	Day 1	Day 7	Day 14
C	8.00±1.00^{a}	4.33±2.31*	4.33±1.53^{b}
T1	10.00±2.65^{a}	18.00±10.15^{a}	5.66±1.53^{a}
T2	8.00±1.73^{a}	11.33±3.21^{a}	8.00±3.00^{a}
T3	7.00±3.00^{a}	6.33±2.31^{a}	4.33±0.58^{a}

The values correspond to the mean values±SD obtained from three repetitions. $^{a, b}$Means in the same row with different superscripts represent statistically significant differences between the values ($p<0.05$). Treatment codes: C (control, 0 U TG g^{-1} protein), T1 (0.74 U TG g^{-1} protein), T2 (1.29 U TG g^{-1} protein), T3 (1.85 U TG g^{-1} protein). *The used weight for this sample was 50 g instead of 180 g

Penetrometer values of the strained yoghurts during cold storage are shown in Table 4. Differences among the penetrometer values of the strained yoghurt samples were found also statistically not significant ($p<0.05$), similar as the TPA parameters. But the enzyme treated samples had generally higher values especially on the 7th day of cold storage. While there was a significant decrease ($p<0.05$) in the penetrometer values for the control sample during cold storage, the enzyme treated samples were not effected significantly and the differences between the enzyme treated samples showed a decrease in the values in proportion to the enzyme treatment. When the enzyme activity is inactivated prior to fermentation, as it is in the present case, the enzyme can form only intra-molecular cross-linking between milk proteins. However when the enzyme is active during the fermentation, the enzyme can form also inter-molecular cross-linking between milk proteins because colloidal stability casein micelles changes and collision distance between the micelles decrease with increasing acidity. This could be one of the reasons why the textural properties of the strained yoghurt samples were not significantly different.

Table 5: Water holding capacities of the strained yoghurt samples during 14 days of cold storage

Samples	Day 1	Day 7	Day 14
C	71.19±1.02^{Z}	70.78±1.68^{Z}	72.84±1.50^{Z}
T1	78.85±0.67^{Y}	78.43±1.00^{Y}	77.11±1.23^{Y}
T2	83.45±0.61^{X}	82.13±0.67^{X}	81.66±1.56^{X}
T3	83.63±1.25^{X}	82.24±2.96^{X}	79.70±0.80^{X}

The values correspond to the mean values±SD obtained from three repetitions. $^{X-Z}$Means in the same column with different superscripts represent statistically significant differences between the values ($p<0.05$). Treatment codes: C (control, 0 U TG g^{-1} protein), T1 (0.74 U TG g^{-1} protein), T2 (1.29 U TG g^{-1} protein), T3 (1.85 U TG g^{-1} protein)

Another factor for textural properties and a parameter effecting consumers' acceptance of fermented milk products is whey separation. It's also called water holding capacity. Whey separation can be defined as the appearance of the serum on the gel surface. Classical methods like the enrichment of dry matter or protein content as well as addition of hydrocolloids and the use of stabilizers are common means of avoiding whey syneresis. One of the emerging trends is the enzymatic cross-linking of milk proteins with transglutaminase (Farensworth *et al*., 2006; Jarosh *et al*. 2007). Table 5 shows the impact of TG treatment and cold storage period on the water holding capacities of the strained yoghurt samples. As it can be shown from Table 5, the WHC of the strained yoghurt samples was affected by the TG enzyme. The differences between the samples were statistically significant ($p<0.05$) while the samples T2 and T3 had similar WHC values during the 14 days of storage, the control sample had the lowest WHC values. The average increase in the WHC caused by cross-linking was approximately 15%. The effect of TG enzyme on WHC is comparable with the results published by Farnsworth *et al*. (2006), Jarosh *et al*. (2007), Ozer *et al*. (2007) and Sanli *et al*. (2011) for yoghurt.

Principal component analysis: The principal component analysis applied, aided a description of the correlation of the groups of instrumental textural parameters and enzyme treatments (Fig. 4). Figure 4a shows PCA correlations of the yoghurt samples before straining. It can be observed that 4 groups occurred in correlation with the enzyme level added. All the variables were well represented. The T3 treatment with the highest TG enzyme level showed the highest values for textural parameters like viscosity, firmness, consistency, cohesiveness and viscosity index. Figure 4b shows the PCA correlations of the strained yoghurt samples. In contrary to the yoghurt samples the PCA demonstrates that the textural parameters firmness, consistency, cohesiveness and viscosity index are negatively correlated with the viscosity of the strained yoghurt samples and a grouping according to the enzyme level could not be indicated from the PCA. This confirms the results obtained by variance analysis.

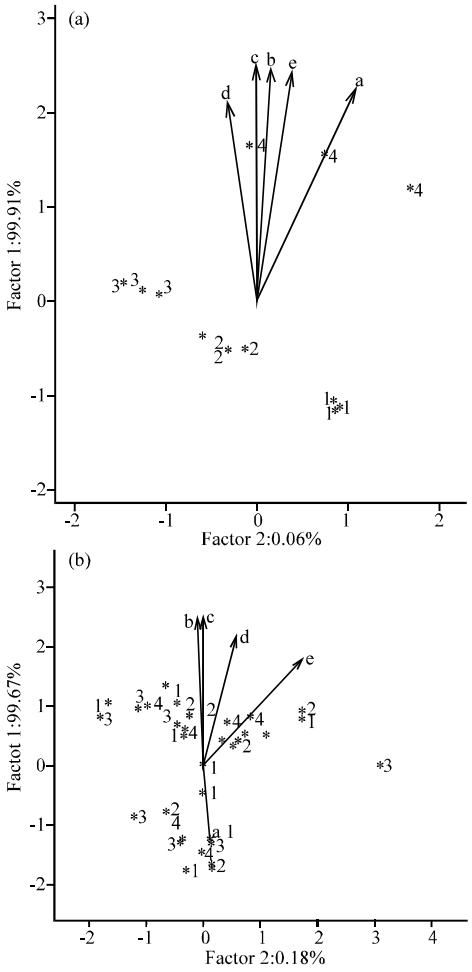

Fig. 4: The principal component analysis of instrumental texture parameters obtained from yoghurt (a) and strained yoghurt (b) samples submitted to enzymatic treatment with transglutaminase 1: (control, 0 U TG g^{-1} protein), 2: T1 (0.74 U TG g^{-1} protein), 3: T2 (1.29 U TG g^{-1} protein), 4: T3 (1.85 U TG g^{-1} protein). The textural parameters are coded as; a: viscosity, b: firmness, c: consistency, d: cohesiveness, e: viscosity index

Sensory evaluation: Sensory attributes as appearance, texture, aroma, taste and overall acceptability of the strained yoghurt samples were evaluated. The results of 1, 7 and 14 days stored strained yoghurt samples are shown in Fig. 5a-c, respectively.

In general, TG enzyme treatment had no significant ($p<0.05$) effect on the sensory attributes of the samples. No significant differences ($p<0.05$) could be detected regarding the storage period in terms of appearance and texture. From the sensory map in Fig. 5c, it is noticeable

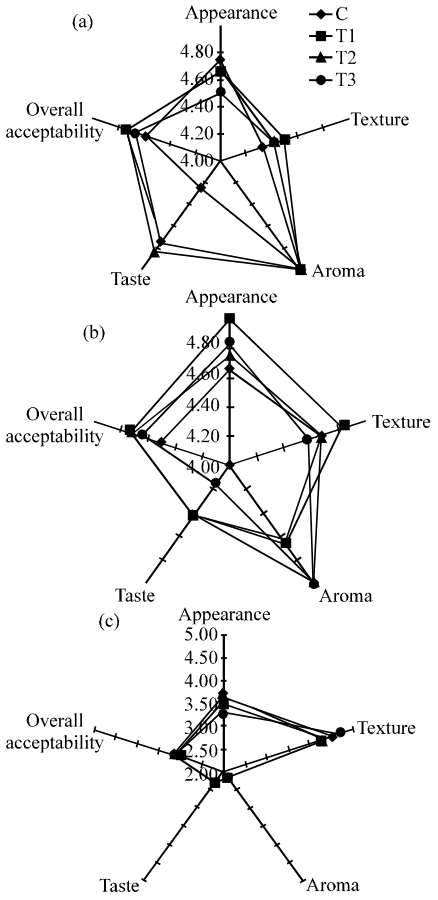

Fig. 5: Sensory scores of: a) 1 day; b) 7 days; c) 14 days stored strained yoghurt samples. C: (control, 0 U TG g^{-1} protein), T1 (0.74 U TG g^{-1} protein), T2 (1.29 U TG g^{-1} protein), T3 (1.85 U TG g^{-1} protein). The values correspond to the mean values obtained from three repetitions

that the storage time had an adverse impact on the aroma and taste parameters at the end of storage which also effected the overall acceptability of the samples ($p<0.05$). Panelists noted a yeasty flavour defect in all of the samples at the end of the storage period. Al-Kadamany *et al*. (2002) and Kesenkas (2010) reported also an increased yeasty flavour during the storage of concentrated yoghurt. The sensory results are also consistent with the microbiological and instrumental TPA results.

CONCLUSION

Enzymatic cross-linking of milk proteins by transglutaminase enzyme appears to be an effective way to improve the physical properties of yoghurt. But surprisingly no significant effect on the textural parameters of strained yoghurt could be detected. The reason therefore could be the inactivation of the TG enzyme prior to fermentation and the mixing procedure before packaging. However, the water holding capacity of the strained yoghurt samples could be significantly improved approximately 15% by TG enzyme treatment. Higher treatments of the TG enzyme also decreased the proteolytic activity with increasing storage time. This was also consistent with the acidity values. Furthermore; enzymatic cross linking showed no significant effect on the microbiological properties and the sensory attributes were not unfavorable affected. The microbiological and sensory results showed that the shelf life of strained yoghurt should be <14 days because of yeast and mould increment. Consequently, it could be suggested to use the TG enzyme in T2-T3 levels to enhance the functional properties of yoghurt. The use of the enzyme by strained yoghurt production with active enzyme during fermentation should be investigated.

ACKNOWLEDGEMENT

This research was supported by Ege University Scientific Research Fund (BAP) (Project Number: 2007-ZRF-035). The researcher thanks grateful to the Ege University Research fund for their financial support.

REFERENCES

AOAC, 2005. Official Methods of Analysis of AOAC International. 18th Edn., AOAC, Maryland, USA.

Akin, N., 1999. Inek ve koyun sutunden uretilen bazi konsantre fermente sut urunlerinin sertligi ve duyusal ozellikleri. Tr. J. Vet. Anim. Sci., 23: 583-590.

Akkaya, L., R. Telli and O. Sagdic, 2009. Growth-death kinetics of *Lysteria monocytogenes* in strained yogurt. I. J. Food Proper., 12: 705-712.

Al-Kadamany, E., I. Toufeili, M. Khattar, Y. Abou-Jawdeh, S. Harakeh and Y. Haddad, 2002. Determination of shelf life of concentrated yogurt (labneh) produced by in-bag straining of set yogurt using hazard analysis. J. Dairy Sci., 85: 1023-1030.

Bhullar, Y.S., M.A. Udin and N.P. Shah, 2002. Effects of ingredients supplementation on textural characteristics and microstructure of yoghurt. Milchwissenschaft, 57: 329-332.

Bonisch, M.P. M., Huss, K. Weitl and U. Kulozik, 2007. Transglutaminase cross-linking of milk proteins and impact on yoghurt properties. Int. Dairy J., 17: 1360-1371.

Cozzolino, A., P. Di Pierro, L. Mariniello, A. Sorrentino, P. Masi and R. Porta, 2003. Incorporation of whey proteins into cheese curd by using transglutaminase. Biotechnol. Applied Biochem., 38: 289-295.

De Jong, G.A.H. and S.J. Koppelman, 2002. Transglutaminase catalysed reactions: Impact on food applications. J. Food Sci., 67: 2798-2806.

Donkor, O.N., A. Henriksson, T. Vasiljevic and N.P. Shah, 2006. Effect of acidification on the activity of probiotics in yoghurt during cold storage. Int. Dairy J., 16: 1181-1189.

Farnsworth, J.P., J. Li, G.M. Hendricks and M.R. Guo, 2006. Effects of transglutaminase treatment on functional properties and probiotic culture survivability of goat milk yogurt. Small Ruminant Res., 65: 113-121.

Fernandes De Sa, E.M. and M.T. Bordignon-Luiz, 2010. The effect of transglutaminase on the properties of milk gels and processed cheese. Int. J. Dairy Technol., 63: 243-251.

Gauche, C., T. Tomazi, P.L.M. Barreto, P.J. Ogliari and M.T. Bordignon-Luiz, 2009. Physical properties of yoghurt manufactured with milk whey and transglutaminase. Food Sci. Technol., 42: 239-243.

Guler, Z. and H. Sanal, 2009. The essential mineral concentration of torba yoghurts and their wheys compared with yoghurt made with cows, ewes and goats milks. I. J. Food Sci. Nutr., 60: 153-164.

Guyot, C. and U. Kulozik, 2011. Effect of transglutaminase-treated milk powders on the properties of skim milk yoghurt. I. Dairy J., 21: 628-635.

Heidebach, T., P. Forst and U. Kulozik, 2009. Microencapsulation of probiotic cells by means of rennet-gelation of milk protein. Food Hydrocolloids, 23: 1670-1677.

Jarosh, D., C. Heidig and H. Rohm, 2007. Enzymatic modification through microbial transglutaminase enhances the viscosity of stirred yogurt. J. Texture Stud., 38: 179-198.

Kesenkas, H., 2010. Effect of different probiotic cultures on properties of torba (strained) yoghurt. Mljekarstvo, 60: 19-19.

Kulozik, U., 2009. Optimierung der Proteinquervernetzung durch Transglutaminase in geruhrtem Joghurt. AÝF 15044 N Schlussbericht Forschungskreis der Ernaehrungsindustrie E.V.

Kurt, A., S. Cakmakci and A. CAglar, 1999. Analysis and examination Methods for Dairy Products Guidebook. Ataturk University Faculty of Agriculture, Erzurum, Turkey.

Lauber, S., T. Henle and H. Klostermeyer, 2000. Relationship between the crosslinking of caseins by transglutaminase and gel strength of yoghurt. Eur. Food Res. Technol., 210: 305-309.

Nielsen, P.M., 1995. Reactions and potential industrial applications of transglutaminase. Food Biotechnol., 9: 119-156.

Nsabimana, C., B. Jiang and R. Kossah, 2005. Manufacturing, properties and shelf life of labneh: A review. Int. J. Dairy Technol., 58: 129-137.

Ozer, B.H., R.K. Robinson, A.S. Grandison and A.E. Bell, 1997. Comparison of techniques for measuring the rheological properties of labneh (Concentrated Yoghurt). Int. J. Dairy Technol., 50: 129-134.

Ozer, B.H., A.E. Bell, A.S. Grandison and R.K. Robinson, 1998. Rheological properties of concentrated yoghurt (labneh). J. Texture Stud., 29: 67-79.

Ozer, B.H. and R.K. Robinson, 1999. The behaviour of starter cultures in concentrated yoghurt (labneh) produced by different techniques. Lebensm. Wiss. Technol., 32: 391-395.

Ozer, B., H.A. Kirmaci, S. Oztekin, A. Hayaloglu and M. Atamer, 2007. Incorporation of microbial transglutaminase into non-fat yogurt production. Int. Dairy J., 17: 199-207.

Ozrenk, E., 2006. Review: The use of transglutaminase in dairy products. Int. J. Dairy Technol., 59: 1-7.

Rossa, P.N., E.M.F. De Sa, V.M. Burin and M.T. Bordignon-Luiz, 2011. Optimization of microbial transglutaminase activity in ice cream using response surface methodology. Food Sci. Technol., 44: 29-34.

Sanli, T., E. Sezgin, O. Deveci, E. Senel and M. Benli, 2011. Effect of using transglutaminase on physical, chemical and sensory properties of set-type yoghurt. Food Hydrocolloids, 25: 1477-1481.

Seguro, K., N. Nio and M. Motoki, 1996. Some Characteristics of a Microbial Protein Cross-Linking Enzyme: Transglutaminase. In: Macromolecular Interactions in Food Technology, Parris, N., A. Kaito, L.K. Creamer and J. Pearce (Eds.). America Chemical Society, Washington, DC., USA., pp: 271-280.

Sodini, I., A. Lucas, J.P. Tissier and G. Corrieu, 2005. Physical properties and microstructure of yoghurts supplemented with milk protein hydrolysates. I. Dairy J., 15: 29-35.

Tamime, A.Y. and R.K. Robinson, 2007. Tamime and Robinson's Yoghurt Science and Technology. 3rd Edn., Woodhead Publishing Limited, Cambridge, UK., Pages: 791.

Uysal, H.R., 1993. Investigations on comparison with classical method and making strained yoghurt from milk concentrated by vacuum and ultrafiltration techniques. Ph.D. Thesis, Faculty of Agriculture, Ege University, Izmir, Turkey.

Walstra, P., T.J. Geurts, A. Noomen, A. Jellema and V.A.J.S. Van Boekel, 1999. Dairy Technology. Marcel Dekker Inc., New York, Pages: 727.

Yazici, F. and A. Akgun, 2004. Effect of some protein based fat replacers on physical, chemical, textural and sensory properties of strained yogurt. J. Food Eng., 62: 245-254.