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Araştırma Makalesi / Research Paper

Physical Properties of Half-Fat Kashar Cheese Manufactured with and without Transglutaminase

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ABSTRACT

Transglutaminase (TG) is a transferase that forms both inter and intra-molecular isopeptide bonds in and between many proteins by cross-linking of the amino acid residues of protein bound glutamine and lysine. The cross-linking affects the functional properties of proteins, thus enabling new ways of structure formation. TG enzyms catalyzes the covalent cross-linking formation in different proteins and provides an important treatment for food processing. These reactions lead to changes in protein functional properties and enable product formation with better rheological and sensory properties. In this study, half-fat Kashar cheeses were produced using the TG enzyme and it was investigated that the effect of TG on Kashar cheese. It was determined some physico-chemical analysis such as pH, titratable acidity (oSH), total solids, salt in solids, fat and fat in solids values. Hardness, springiness, gumminess, chewiness and cohesiveness parameters were determined with texture analysis by TA.XT.plus texture analyzer. When the results of physico-chemical and texture analyzes are compared, it wasn't observed that statistically significant differences between the samples except fat in solids values of Kashar cheese samples during maturation. As a result, it was determined that TG enzyme addition had no effect on properties of half-fat Kashar cheese.

Keywords: Textural properties, transglutaminase, Kashar cheese

Transglutaminaz İlave Edilerek ve Edilmeksizin Üretilen Yarım Yağlı Kaşar Peynirlerinin Fiziksel Özellikleri

ÖZ

Transglutaminaz (TG), proteine bağlı glutamin ve lisin amino asit kalıntılarının çapraz bağlanmasıyla birçok protein arasında hem molekül içi hem de moleküller arası izopeptit bağları oluşturan bir transferazdır. Çapraz bağlama ile proteinlerin fonksiyonel özellikleri etkilenir ve böylelikle yapı oluşumunda yeni yollar sağlanır. TG farklı proteinlerde kovalent çapraz bağ oluşumunu katalize eder. Bu reaksiyonlar sonucunda, proteinlerin fonksiyonel özelliklerinde değişiklik meydana gelerek daha iyi reolojik ve duyusal özelliklere sahip ürün oluşumu sağlanır. Bu çalışmada, TG kullanılarak yarım yağlı Kaşar peyniri yapılmış ve TG'nin Kaşar peyniri üzerine etkisi araştırılmıştır. Bu etkileri belirlemek için Kaşar peyniri örneklerinin, fiziko-kimyasal analizlerden pH, titrasyon asitliği (oSH), toplam kurumadde, yağ, kurumaddede yağ ve kurumadde de tuz değerleri belirlenmiştir. TA.XT.plus tekstür cihazı ile sertlik, esneklik, sakızımsılık, iç yapışkanlık ve çiğnenebilirlik gibi tekstür parametreleri incelenmiştir. Olgunlaşma süresince Kaşar peyniri örneklerinin kurumadde de yağ değerleri haricinde fiziko-kimyasal ve tekstür analiz sonuçları karşılaştırıldığında istatistiksel bakımdan örnekler arasında belirgin farklılık görülmemiştir. Sonuç olarak; TG enzimi ilavesinin yarım yağlı Kaşar peyniri üzerinde olumlu ya da olumsuz bir etkisinin olmadığı belirlenmiştir.

Anahtar Kelimeler: Tekstürel özellikler, transglutaminaz, Kaşar peyniri

INTRODUCTION

Cheese is made from milk and nutritious food which has an ancient history. Ripe and freshly consumed cheeses have an important place in human nutrition with its rich food content (Sert, 2004). There are more than 1000 types of cheese in the world. Approximately 40-50 types of cheeses are produced in Turkey. Kashar cheese is the most produced cheese variety, after Beyaz cheese in Turkey (Şahan and Kaçar, 2003; Kurultay et al., 2004; Keçeli et al., 2006). Kashar cheese is produced under different names in some Balkan and European countries. Kashkaval in Bulgaria, Kassari in Greece, Kachkawalj in Yugoslavia cheeses are similar to Kashar cheese (Scott, 1981).

TG can cross-link with many food proteins, leguminous proteins, wheat gluten, egg yolk and egg white proteins, actinins, myosin, fibrin, casein α-lactalbumin, β-lactoglobulin dairy proteins, and also with many other albumin (Öner, 2004). Among the dairy proteins, the casein fraction represents a favorable substrate for TG because of its flexible and open bond structure (Bönisch et al., 2006). In recent years, trials have been made to improve the properties of half-fat or reduced-fat dairy products using TG (Yokoyama et al., 2004; Özer et al., 2007).TG enzyme provides increase in the yield of fresh dairy products, cream and cheese making without the addition of chemical additives, improves textural properties and makes gel formation possible (Özrenk, 2006; Özer et al., 2007; Gustaw et al., 2008; Demirkaya and Ceylöan, 2009; Desá and Bordignon-Luiz, 2010). This enzyme is also used as a functional ingredient which affects and improves the structure of products such as gelation, stability, viscosity, emulsification and water binding, and it has an affect on the processing properties of foods (Feargamand et al., 1998). In many reviews, casein has been shown to have high potency to change texture properties in dairy products by cross-linking with TG (Nielsen, 1995; Lorenzen and Schlimme, 1998; Motoki and Seguro, 1998; De Jong and Koppelman, 2002; Jaros et al., 2006).

It has been observed that yoghurt production is the most advantageous area for the use of TG in dairy products.TG is used for yoghurt to prevent releasing of water and to increase firmness (Lorenzen et al., 2002). The role of TG in the improvement of the textural properties of half-fat or non-fat yoghurt has been well shown (Faergemand et al., 1999; Lorenzen et al., 2002; Özer et al.,2007). Many patents point out that TG application has high economic potential due to it is effect on the increase of cheese yield and improvement of cheese functionality (Miwa et al., 2002). There are some studies that show the effects of TG on rennet coagulation properties and the properties of processed cheese. TG improves the physical properties of processed cheeses with the formation of enzymatic cross-links (Desá and Bordignon-Luiz 2010).

The formation of additional covalent cross-linking by TG is a promising method to improve the functional properties of casein-based dairy products, especially half-fat cheeses (Özrenk, 2006). There are many studies in the literature on dairy products related to TG enzyme, but studies on semi-hard and hard type cheese have not been found much.

In this study, TG enzyme was added to half-fat Kashar cheese which was made from semi-hard cheese after that 0, 30, 60 and 90 days of ripening period it was compared with the control sample (without TG). The effect of TG enzyme on half-fat Kashar cheese was investigated during the three months ripening period by analyzing the change in the textural characteristics of cheese.

MATERIAL AND METHODS

Material

The TG use purchased from TG AktivaTM YG, was supplied by Ajinomoto Co., Inc., Tokyo, Japan. The mean enzymatic activity was 100 U/g-1 protein. Raw milk was obtained from the factory. Milk was standardized for making half-fat to 2.2%.

Kashar Cheese Manufacture

Two different half-fat Kashar cheeses (TG-treated and untreated cheeses) were made in Süleyman Demirel University Faculty of Engineering Department of Food Engineering Laboratory. Half-fat milk was pasteurized at 60oC for 30 min. and then cooled to 32-33oC. At this temperature, 0.02% calcium chloride and 1% starter culture were added and incubated for 30 min. Then rennet was added and TG was added to milk after 7 min adding the rennet (2 U g-1protein) (Desá and Bordignon-Luiz, 2010). The first clot formed after about 15 min. and it was ready to cut after about 75 min. The pressing process was carried out to remove the whey from the clot. When the acidity of the cheese was 45-50 oSH, the curd was boiled in water at 65oC. Thereafter the kashar cheeses was allowed to mature at room temperature for 5 days. The cheese blocks were packed under vacuum and ripened at 4℃ for 90 days. The cheeses were conducted in triplicate and the analyzes were made in two parallel. Analaysis were made in 0, 30, 60 and 90 days.

Pyhsico-Chemical Analysis

In this study the pyhsico-chemical analyzes were carried out at 0, 30, 60 and 90 days of ripening. pH, titratable acidity (oSH), total solids, salt in solids, fat and fat in solids values were determined with the physico-chemical analyzes, of half-fat Kashar cheese samples. The pH value was determined by the inolab WTW digital pH meter. Titratable acidity and total solids of samples were measured according to the Turkish Standard TS 591 (Anon., 2006). Fat and fat solids were determined by Gerber method as described in Turkish Standard TS 591 (Anon.,1995). Salt analysis was made by the method of Mohr (Bradley et al., 1992).

Textural Analysis

Rheologyof materials such as cheese can be defined as examination of its deformation and flow, when subjected to a stress or strain (Ahmed et al., 2015). In case of cheeses are exposed to stress or strain, their behaviour is expressed in terms such as hardness, springiness (Ahmed et al., 2015). In this study the texture profile analysis (TPA) was carried out at 0, 30, 60 and 90 days of ripening. TPA including the measurement of hardness, springiness, gumminess, chewiness and cohesiveness was performed on cheese samples (0, 30, 60 and 90 days old) using a TA.XT.plus Texture Analyzer with a 50 mm cylindrical flat probe. Each sample was obtained from the central part of the cheese, cut into cubes (1.5 cm3) and equilibrated at room temperature (~20℃) for 1 h prior to analysis (Hu et al., 2013).

Statistical Analysis

Results were subjected to analysis of variance (ANOVA) using SPSS 16.0 statistical software and evaluated at 95% confidence interval according to Duncan test.

RESULTS AND DISCUSSION

Pyhsico-Chemical Properties

The chemical composition of the Kashar cheese samples was shown in Table 1. The total solids contents of the cheeses obtained in this study ranged from 45.8%-61% while the typical total solids content in a Kashar cheese is in the range of 60%. The fat contents for control Kashar cheese were slightly higher than TG added Kashar cheese.

Table 1. Pyhsico-chemical properties of Kashar cheese samples.

Ripening Days	pH	°SH	TS%	Fat	Fat% in TS	Salt% in TS
Control cheese						
0	5.0±0.31^{Aa}	68.6±6.65^{Da}	45.8±3.24^{Ba}	15.1±2.94^{Ca}	32.9±4.32^{DEa}	3.46±2.51^{Ba}
30	5.01±0.16^{Aa}	124.3±6.02^{Aa}	60.1±4.45^{Aa}	23.2±3.31^{ABa}	38.5±2.79^{BCa}	9.03±1.52^{Aa}
60	4.55±0.24^{Ba}	111.0±6.55^{BCa}	61.0±4.46^{Aa}	24.3±3.54^{ABa}	39.6±3.22^{ABCa}	10.81±1.76^{Aa}
90	3.75±0.23^{Ca}	100.3±6.80^{Ca}	59.5±5.57^{Aa}	26.3±4.02^{Aa}	44.01±3.08^{Aa}	9.25±1.88^{Aa}
TG added Kashar cheese						
0	4.96±0.14^{ABa}	73.0±3Da	45.8±2.68^{Ba}	13.7±3.32^{Ca}	29.7±5.60^{Eb}	3.45±2.1^{Ba}
30	4.92±0.06^{ABa}	123.0±5.19^{Aa}	60.2±3.87^{Aa}	21.3±3.37^{Ba}	35.2±3.81^{CDb}	8.19±2.03^{Aa}
60	4.62±0.32^{ABa}	113.6±3.21^{ABa}	60.8±3.71^{Aa}	22.91±3.36^{ABa}	37.4±3.65^{BCDb}	8.22±1.19^{Aa}
90	3.72±0.25^{Ca}	102.6±9.50^{BCa}	59.3±4.77^{Aa}	24.41±4.06^{ABa}	40.9±4.48^{ABb}	9.74±2.63^{Aa}

Means within the same column with different superscripts are significantly different (Duncan test, $p<0.05$). The results are expressed as mean ± standard deviation.
$^{a, b}$ Different lowercase superscripts depict the statistical difference between means for Kashar cheese samples
$^{A-E}$ Different uppercase superscripts depict the statistical difference between ripening time for Kashar cheese samples
TS:Total Solids

Differences in total solids, acidity, pH, fat, salt in solids values between control and enzyme treated Kashar cheese samples except fat in solids values were not significant ($p>0.05$). Similar results were observed for Cheddar cheese with half-fat content and Labneh (Aloğlu and Öner 2013; Hu et al., 2013). There were no significant differences in the moisture and fat contents for TG added Cheddar cheese samples during the 90

days of ripening (Hu et al., 2013). It was determined that TG application in Labneh production did not affect the chemical composition such as pH, total solids, salt in solids, fat and fat in solids of the Labneh samples (Aloğlu and Öner 2013). However, the total solids contents of the Beyaz cheeses supplemented with TG were determined lower than the control cheeses.

The role of TG in the system is the formation of bonds between lysine and glutamine (Rachel and Pelletier, 2013). Thus, forming a more complex matrix where additional water molecules can be held (Salinas-Valdés et al., 2015). In fact, TG is used in the yoghurt to prevent syneresis and in the case of cheese, the enzyme positively affects the Panela cheese, Edam cheese, Beyaz cheese and Cheddar cheese moisture content (Şener, 2012; Hu et al., 2013; Aaltonen et al., 2014; Salinas-Valdés et al., 2015). However, a similar case had not been observed in Kashar cheese.

Textural Properties

During ripening proteolysis by enzymes led to a reduction in the levels of intact casein in the cheese and therefor may contribute to the overall weakening of this struture in 90 days. The changes in the texture parameters value of half-fat Kashar cheese samples in the ripening process is presented in the Fig. 1.

Moisture and yield decreased in half-fat Kashar cheese when adding TG however, in studies conducted in different cheese such as Edam cheese TG-added moisture increase was due to the enzyme and increased the cheese yield 4% (Fig. 1). Increased water content softens the texture of cheese, but TG did not reduce the hardness of cheese because of protein cross-linking (Aaltonen et al, 2014). Proteolysis rate, pH, total solids and salt effect the texture in cheese (Lawrence vd., 1987). Hardness is related to the moisture and salt content in the cheese. As the moisture content of the cheese increases, the hardness decreases, as the salt content increases, on the contrary the hardness increases (Kaya, 2002).

Maximum strain applied to the cheese at first compression is hardness (Kim et al., 2004). The hardness of Cheddar cheese treated with half-fat TG that is increased during the 90 days of ripening period. The hardness of cheeses treated with TG that is determined to be more than untreated cheese up to about 60 days of the ripening (Hu et al., 2013). Unlike these the results, in our study, the hardness values of both cheeses were demonstrated an increase from the first day to the sixtieth day of maturation and then a decrease to the ninetieth day of maturation (Fig. 1). This decrease is caused by proteolysis occuring in Kashar cheese (Koca, 2002). When the hardness values of control sample and TG added half-fat Kashar cheese are compared, it is found that values are close to each other during maturation period, as the Fig.1 shows ($p>0,05$). Şener (2012) showed in Beyaz cheese, hardness values of TG-added samples were higher than the control samples and found significant the application of the enzyme ($p<0.05$). Similarly, springiness, gumminess, chewiness and cohesiveness parameters values were determined higher than control cheese.

Springiness can be defined as recuperation to original dimension of the object, after it is applied deforming force to the object (Guerra-Martınez et al., 2012). In our study, it was determined that the springiness values of both cheeses are close to each other and similar situation continues during storage time (Fig. 1). As a result of statistical analysis, it was determined that differences between the springiness values of these cheeses aren't at a significant level ($p>0.05$). Similarly, it was detected that the springiness values of Cheddar cheese treated with half-fat TG during maturation very slowly increased and undulated (Hu et al., 2013). The reason of that can depend on protein intermolecular cross-linking (Kwan and Easa, 2003).

Another important texture parameter in cheese samples is cohesiveness. The cohesiveness is explained as the ratio of the shape of the resistance to the second compression to the behaviour of the compression (Koca, 2002). The high springiness and cohesiveness values can depend on cross-linking ability of TG which led to a compact protein network and increased the association between casein micelles (Gauche et al., 2009). Compared to TG and soybean-fortified, control samples, the cohesiveness value in Panela cheese is higher. The reason of that is internal bonds occuring in the cheese (Salinaz-Valdés et al., 2015). However, in our study, Fig.1 shows that TG addition didn't affect on the cohesiveness value in half-fat Kashar cheese samples($p>0,05$).

Gumminess is described as breaking force required to ingest a semi-solid food easily (Raphielides et al., 1995). It was found that the gumminess values of the cheeses are close to each other and similar situation continues during storage time (Fig. 1). It was observed that the gumminess values of both cheeses increased during maturation. It wasn't found that difference as statistical between the gumminess values of the cheeses ($p>0.05$).

Chewiness is defined as required chewing force to make a solid food ready for ingesting (Raphielides et al., 1995). The chewiness is related to the hardness, springiness, and cohesiveness (Salinaz-Valdés et al.,

2015). In a study in Beyaz cheese, it was detected that the chewiness values of TG added samples are higher than the control sample. In our study, the values of chewiness increased in both cheeses during maturation (Fig. 1.). In this study, the chewiness values of both cheeses increased during the maturation (Fig. 1.), but difference between these cheeses that was insignificant as statistical (p>0,05). Similarly, In a study in Panela cheese, it was determined that the chewiness values was higher in cheeses treated with TG, but this difference wasn't significant compared to control cheese (p>0,05) (Salinaz-Valdés et al., 2015).

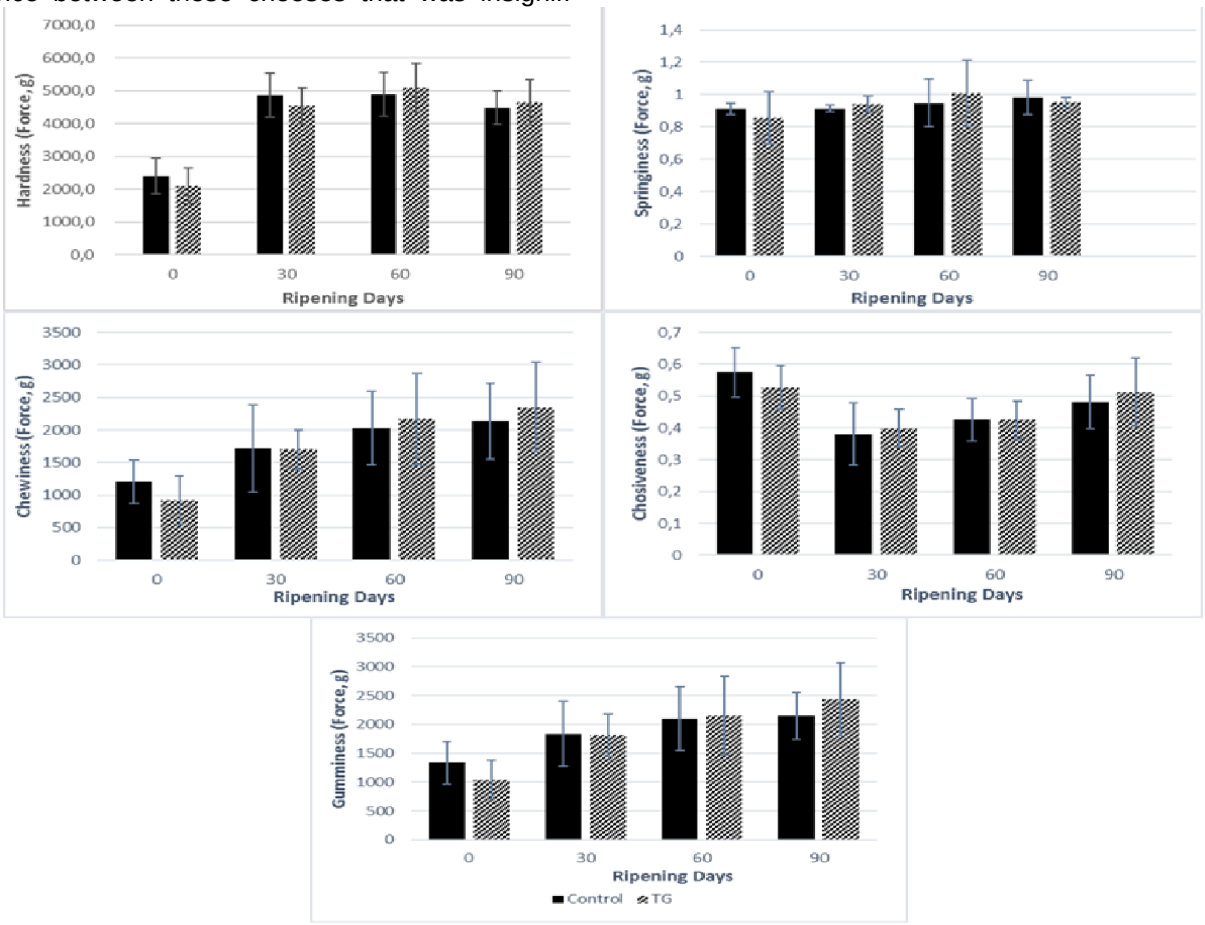

Figure 1. The hardness, springiness, chewiness, chosiveness, gumminess of Kashar cheese samples

CONCLUSIONS

In this study, as a result, it was determined that TG enzyme addition had no effect on half-fat Kashar cheese. It was also considered that the yield can be increased using cross-linking property of protein, in contrast TG enzyme had no affect on the yield of Kashar cheeses. It was decided found that when the textural parameters of half-fat, TG supplemented Kashar cheesewere compared with the textural parameters of control cheese, differences between these values during maturation weren't significant (p>0,05). Also, it has been found that the use of TG is not important for improving the textural properties and increasing the fat recovery in Kashar cheese, which is half-fat and semi-hard type cheese. Many researchers should be made on use of TG in Kashar cheeses.

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