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Original article

The Effects of Microbial Transglutaminase (MTG) Enzyme on Sensory Properties of Hatay Cheese

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Abstract

Hatay Cheese is a kind of cheese produced in and around Hatay with its own shapes and sizes. The objective of this study was to evaluate the effect of the use of microbial transglutaminase enzyme on the sensory properties of Hatay cheese samples which are in the fresh cheese class. Enzymatic modification of milk proteins by microbial transglutaminase enzyme has been applied in the production of low-fat Hatay cheese with different pre-ripening time applications and some sensory properties of the cheeses have been investigated such as external appearance, internal appearance, texture, odor and taste during 60 days of storage. In this study, four different Hatay cheeses were produced; as control sample without enzyme treatment (A), sample pre-ripened with enzyme for 30 minutes (B), sample pre-ripened with enzyme for 45 minutes (C) and sample pre-ripened with enzyme for 60 minutes (D).

When the sensory properties of the cheeses were examined, it was observed that the application of different pre-ripening time significantly affected the external appearance, internal appearance, texture and taste scores of the cheese samples on the 60th day of storage ($p<0.05$), and storage time caused significant differences on the appearance scores of A, B and C cheeses ($p<0.05$). Storage time also caused significant differences on the internal appearance scores of all cheeses except cheese A ($p<0.05$). The effects of storage time on texture scores were not found significant ($p>0.05$). Additionally, the effects of different pre-ripening periods and storage time on the odor scores of the cheeses were not significant ($p>0.05$). Taste scores of cheese D were significantly affected by storage time ($p<0.05$). As a result of all sensory analyzes, it was found that the cheeses supplemented with MTG enzyme were more liked and the scores given to the cheeses increased as the pre-ripening time prolonged. As a result, the most admired cheese was D cheese.

Keywords: Microbial transglutaminase, Hatay cheese, Sensory properties.

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INTRODUCTION

Hatay cheese is an important cheese type which is peculiar to Hatay province. It has semi-hard texture, white color inside and light yellow color outside, porous structure and slightly salty taste.It is generally consumed without ripening or as fresh (Kesenkaş et al., 2012). According to the Turkish Food Codex Communiqué on Cheese, Hatay cheese is a fresh cheese which is produced by pasteurized milk and defined as fresh product on the food label (Anon., 2015). It is a local cheese produced in and around Hatay province in unique shapes and sizes. Unlike White cheese, Hatay cheese is usually produced without adding starter culture to milk. Only rennet enzyme is used for coagulation while production. Hatay cheese, which was produced only in small-scaled family enterprises in the past years, has recently started to be produced in industrially large-scale enterprises. It is freshly marketed as breakfast or pastry cheese (Çayır, 2018).

Transglutaminase enzyme is used to increase textural and functional properties of meat, fish, dairy, legumes and cereal products (Kuraishi et al., 2001; Gan et al., 2008; Gan et al., 2009; Yokoyama et al., 2010). It is accepted as GRAS in the USA and permitted by the US Department of Agriculture for use in meat, dairy, bread and fish products (Motoki and Kumazawa, 2000; Anonymous, 2001; Ercili-Cura, 2012). Transglutaminase forms high-molecular polymers by catalyzing covalent cross-linking through acyl-transferase reactions. Therefore, this enzyme has significant effects on the thermal stability, gel formation ability, water holding capacity, emulsification properties and nutritional properties of proteins (Motoki and Seguro, 1998; Kuraishi et al., 2001; Rahila et al., 2015).

In this study, microbial transglutaminase enzyme was used in the production of Hatay cheese in order to improve the textural structure and sensory properties of cheese. The effects of different pre-ripening periods on the sensory quality of cheese were investigated. In the study, four different Hatay cheeses were produced; as control sample without enzyme treatment (A), by pre-ripening with enzyme for 30 minutes (B), by pre-ripening with enzyme for 45 minutes (C) and by pre-ripening with enzyme for 60 minutes (D). The produced cheeses were stored for 60 days and the sensory properties of them were examined on the 1^{st}, 15^{th}, 30^{th} and 60^{th} days of storage.

Materials and Methods

In the production of Hatay cheese, a mixture of Holstein and Simental cows' milk which came to Güneydoğu Dairy Company was used. Ajinomoto-ACTIVA YG branded MTG enzyme was supplied from Barentz Food. NATUREN® MANDRA 175 (175 IMCU, 1/16.000 strength, minimum 80% Chymosin enzyme, maximum 20% Pepsin enzyme) rennet from Chr-Hansen was used in the study. Solvay brand calcium chloride ($CaCl_2$) and Saray Salt brand table salt from Aktentaş Aksaray Agriculture were used. 3 kilograms of polypropylene square bowl pack from Adana Doğan Plastic was used as packaging material.

In this research, cheese production was carried out in Güneydoğu Dairy Company with three replications and cheese analyzes were conducted in the Dairy Technology Research Laboratory of the Çukurova University Faculty of Agriculture Food Engineering Department. Raw milk was passed through the necessary preliminary tests (pH, titratable acidity, fat, solid non fat, protein) during intake and clarification process was applied to remove the milk from physical contamination. Afterwards, the fat content of the milk was standardized to 2% in the separator. In the production of Hatay Cheese, raw milk was heated to 82 °C for 6 minutes. At the end of the heat treatment, the milk was cooled to 42°C and taken to the vessel. In order to give the shape and mold dimensions of Hatay cheese, 450 L milk was taken to each vessel. $CaCl_2$ and MTG enzyme were added after the milk was taken to the vessels. The amount of MTG enzyme used in the research was 1 unit MTGase / g milk protein. This amount is the amount recommended by the manufacturer (Anon., 2018). In this context; four different processes were applied to obtain Hatay cheese samples as;

A: at 42°C without using MTG enzyme in milk,

B: at 42°C for 30 min with using MTG enzyme in milk,

C: at 42°C for 45 min with using MTG enzyme in milk,

D: at 42°C for 60 min with using MTG enzyme in milk.

At the end of the pre-ripening, the milk was incubated to break the clot in 50 minutes. The curd was broken to 1 cm^3 and after 5 minutes of clot precipitation, serum separation was performed by pressing of cheese. The cheese was kept under pressure until it reached the desired hardness and mold height (about 1 hour). Then the cheese was cut to size of 11x11x3 cm. After cheese cutting, 1.5% of salt was sprinkled on it and dry salting was performed. After waiting for half an hour, polypropylene square pans were filled with cheese and it was pasteurized. It was filled with 12% brine, cooled to 4 °C and stored at 4-6 °C. Sensory analyzes of the samples were performed on the 1^{st}, 15^{th}, 30^{th} and 60^{th} days of storage.

Sensory analyzes were performed by a panel consisting of 7 expert panelists using the sensory analysis form shown in Table 1. These features were evaluated by considering the specified features in TS 591; so they were evaluated as 5 points for external appearance, 5 points for internal appearance, 5 points for structure, 5 points for odor, 5 points for taste and totally 25 points for general acceptability (TSE, 2013).

Table 1. Sensory analysis chart of Hatay cheese

Cheese Properties	**Scores**	**Cheeses**			
External Appearance		**A**	**B**	**C**	**D**
Clean, bright and hard, white color	5				
Hard or soft, dull-looking, light yellow or cream colored	4				
Too hard or too soft, dull-looking, yellow	3				
Extremely hard or extremely soft, cracky and moldy appearance	1-2				
Internal Appearance					
Bright, homogeneous appearance, porcelain white	5				
Slight dull, inhomogeneous appearance, whitish or light yellow	4				
Dull, inhomogeneous appearance, cracked and porous, dark yellow or white	3				
Dull, inhomogeneous appearance, inhomogeneous white or dark yellow color	1-2				
Structure					
Smooth and homogeneous, smooth section, bright hard or soft, non-crumbling structure	5				
Straight section, slightly cracked or few holes, hard or soft	4				
Non-homogeneous structure, cracked and perforated, hard or soft structure	3				
Non-homogeneous, very cracked and porous, coarse structure and hard or soft, extremely elastic structure	1-2				
Odor					
Unique odor	5				
Normal odor or very slight uninspired or sour odor	4				
Slightly sour, soapy or moldy odor	3				
Extremely sour, moldy or soapy odor	1-2				
Taste					
Unique taste	5				
Very slightly foreign flavor, slightly salty	4				
Vapid or slightly foreign taste, sour, bitter or salty taste	3				
Extremely vapid, salty, sour, bitter or soapy taste	1-2				

Results and Discussion

There are many techniques to evaluate quality and development of a food product and measure its acceptability (Carbonell-Barrachina, 2007). Determination of sensory properties is important for specification of similarities and differences between food products, development of food products, investigation of scientific characteristics and measurement of consumer appreciation (Anon., 2010). The

scores which were given to the sensory properties of Hatay cheeses during storage were shown in Table 2.

Table 2. Sensory properties of Hatay cheese (n=3)

Properties	Storage time (Days)	Cheeses			
		A	B	C	D
External appearance	1	4.59±0.14^{aK}	4.46±0.16^{aK}	4.17±0.68^{aK}	4.58±0.12^{aKL}
	15	4.21±0.31^{aK}	4.00±0.37^{aL}	4.11±0.10^{aK}	4.35±0.18^{aKL}
	30	4.16±0.59^{aK}	4.07±0.12^{aKL}	4.47±0.32^{aK}	4.69±0.27^{aK}
	60	2.73±0.53^{bL}	4.22±0.16^{aKL}	4.54±0.39^{aK}	4.26±0.04^{aL}
Internal appearance	1	4.47±0.43^{aK}	4.08±0.84^{aK}	4.19±0.84^{aK}	4.24±0.88^{aK}
	15	4.28±0.39^{aK}	4.11±0.35^{aK}	3.92±0.25^{aK}	4.19±0.45^{aK}
	30	3.95±0.71^{aKL}	4.16±0.55^{aK}	4.52±0.32^{aK}	4.33±0.08^{aK}
	60	3.07±0.37^{bL}	4.37±0.02^{aK}	4.28±0.51^{aK}	4.40±0.28^{aK}
Structure	1	4.38±0.32^{aK}	4.11±0.87^{aK}	4.12±0.79^{aK}	4.24±0.88^{aK}
	15	4.11±0.62^{aK}	4.09±0.29^{aK}	4.19±0.35^{aK}	4.38±0.35^{aK}
	30	4.00±0.46^{aK}	4.28±0.32^{aK}	4.19±0.10^{aK}	4.35±0.31^{aK}
	60	3.57±0.24^{bK}	4.21±0.00^{aK}	4.40±0.27^{aK}	4.34±0.15^{aK}
Odor	1	4.33±0.57^{aK}	4.28±0.51^{aK}	4.33±0.57^{aK}	4.47±0.57^{aK}
	15	4.30±0.28^{aK}	4.16±0.17^{aK}	4.21±0.43^{aK}	4.14±0.44^{aK}
	30	4.19±0.29^{aK}	4.02±0.22^{aK}	4.28±0.32^{aK}	4.16±0.28^{aK}
	60	4.14±0.37^{aK}	4.04±0.45^{aK}	4.09±0.43^{aK}	3.90±0.45^{aK}
Taste	1	3.76±0.57^{aK}	3.68±0.41^{aK}	3.52±0.37^{aK}	3.73±0.55^{aKL}
	15	3.33±0.35^{aK}	3.90±0.16^{aK}	3.73±0.41^{aK}	3.35±0.37^{aL}
	30	4.02±0.73^{aK}	3.82±0.43^{aK}	3.70±0.30^{aK}	3.85±0.12^{aKL}
	60	3.26±0.04^{bK}	3.78±0.49^{abK}	3.83±0.53^{abK}	4.11±0.04^{aK}
Total	1	21.53	20.61	20.33	21.26
	15	20.23	20.26	20.16	20.41
	30	20.32	20.35	21.16	21.38
	60	16.77	20.62	21.14	21.01

a, b: Values that are shown in the same line with different exponential letters are different in terms of $p<0.05$ level of significance.

K, L: Values that are shown in the same column with different exponential letters are different in terms of $p<0.05$ level of significance.

When the total scores in terms of all sensory properties of Hatay cheeses were examined, it was determined that A cheese got the highest scores on the first day of storage, and it was followed by D, B and C cheeses, respectively. On the 15th day of storage, cheese D had the highest sensory property scores and it was followed by B, A, and C cheeses. On the 30th day of storage, cheese having the highest sensory property scores was D, whereas C cheese followed it with higher scores compared to the first fifteen

days. At this stage, cheese with the lowest scores was found as A cheese. On the 60th day of storage, cheese having the highest sensory property score was C and it was followed by D, B and A cheeses, respectively. On the 60th day, cheese A which is control sample had the lowest total score, and it showed that the addition of MTG enzyme improved the sensory properties of cheese during ripening. Similar results about improving sensory properties of cheeses by using MTG enzyme were specified by many researchers (Danesh et al., 2017; Darney et al., 2017; Romeih and Walker, 2017; Gharibzahedi et al., 2018; Abou-Soliman et al., 2020; Garcia-Gomez et al., 2020).

Although the cheese which was produced without enzyme had high scores on the first day of storage, it was seen that the rating decreased as the storage period progressed. On the contrary, in enzyme-added cheeses, as the storage time prolonged, the scores given to the sensory properties also increased or remained high. As a result of the sensory analyzes, it was found that the cheeses supplemented with MTG enzyme were more liked and the scores given to the cheeses increased as the pre-ripening period prolonged. Thus, the most admired cheese was cheese D.

When the external appearance scores of the cheeses were examined, it was determined that while cheese A had high scores in the first stages of storage, it decreased as storage progressed. It was found that both enzyme addition and storage time caused statistically significant differences on this decrease on the 60th day of storage ($p<0.05$). The effects of enzyme treatment and different pre-ripening periods were not significant on other days of storage ($p>0.05$). While changes in A, B and D cheeses were found significant during storage ($p<0.05$), cheese C was not significantly affected by storage time ($p>0.05$). The external appearance scores of cheeses clearly show that microbial transglutaminase enzyme improves the external appearance of cheeses. Karzan et al. (2016) reported that the addition of MTG does not have a statistically significant effect on the external appearance scores of cheeses.

The internal appearance scores of the cheeses are similar to the external appearance scores. The effects of enzyme treatment and storage time were significant on the decrease in internal appearance scores of cheese A on day 60 ($p<0.05$). The effects of enzyme treatment or different pre-ripening periods on other days of storage were not significant ($p>0.05$). It was found that the effects of storage time on the internal appearance scores of cheeses except cheese A were also not significant ($p>0.05$). At the end of the storage, internal appearance score of cheese which was produced without treatment of enzymes was significantly lower than the enzyme added cheeses. It showed that MTG enzyme improved the internal appearance of cheeses.

When the scores of structure characteristics of the cheeses were examined, it was determined that the highest score was given to cheese A on day 1, cheese D on days 15 and 30 and cheese C on day 60. The lowest scores were given to cheese B on the 1st and 15th days of storage, and to cheese A on the 30th and 60th days of storage. It was found statistically important that the structure scores of A cheese on 60th day of the storage was lower than other cheeses' scores ($p<0.05$). The effects of different pre-ripening

times on other days of storage and the effects of storage time on all cheeses were not statistically significant ($p>0.05$). The decrease in structure property scores of cheese A on the 60th day of storage, and the fact that the structure scores of other cheeses were close to each other during storage indicated that MTG enzyme improved the structure characteristics of cheeses. Karzan et al. (2016) reported that the scores given to the structure characteristics of MTG added cheeses were higher than the control samples. Mahmood and Sebo (2009) stated that the addition of MTG did not significantly affect the scores given to the textural properties of cheeses.

It was found that the scores of odor characteristics of cheeses generally decreased during storage. The scores of cheeses were close to each other during storage in terms of their odor characteristics. It is known that off-odors occur in cheese as storage progresses due to proteolytic activity. Decreasing in odor scores during storage might be associated with proteolysis. However, the effects of different pre-ripening periods and storage time were not significant on odor characteristics of cheese samples ($p>0.05$). Bulut Solak (2013), Badem (2015), Ehsannia and Sanjabi (2016), Kondyli et al. (2016), Paksoy (2016) and Miloradovic et al. (2017) reported that the odor scores of cheese samples decreased during storage.

In the first 30 days of storage, the taste scores of the cheeses were close to each other and the effects of different pre-ripening time were not significant ($p>0.05$). On the 60th day of storage, the taste score of cheese A was found considerably lower than the other cheeses and the effect of MTG enzyme on this value was significant ($p<0.05$). Storage time did not significantly affect the taste scores of A, B and C cheeses ($p>0.05$). Taste scores of cheese D decreased on the 15th day and then increased again. The effects of storage time on these changes were significant ($p<0.05$). In the study conducted by Mahmood and Sebo (2009), it was reported that the taste characteristics of the cheeses supplemented with MTG had higher scores than control sample.

It is known that changes in sensory properties of cheeses may occur due to proteolytic activity. Nitrogen compounds formed with a high level of proteolysis can cause malodour and bitter taste. The moderate level of proteolysis in cheeses makes the smell and taste characteristics become more appreciated (Al-Otaibiy and Wilbey, 2005). It was thought that the reason of low score in cheese A at the 60th day of storage might be high proteolytic activity of this cheese which could cause defects in taste.

Conclusion

As a result of the research, it was determined that the use of MTG enzyme may be preferable especially by producers because it causes to increase in cheese yield. It was determined that MTG enzyme improves the structural and textural characteristics of fresh and soft cheeses and preserves their structure during storage. In this case, the MTG enzyme served as fat substitutes and improved the

structural properties of cheeses. When the sensory properties of cheeses were examined, it was found that MTG enzyme improved the durability of cheeses and developed the external and internal appearances, structure and taste of cheese samples. In this study where different pre-maturation periods were tried, it was found that the application of a pre-maturation period of 45 minutes was advisable in terms of sensory properties of Hatay cheese samples.

Additional Declaration

Research and publication ethics principles were comply with in this study. Authors contributed equally to the study.

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