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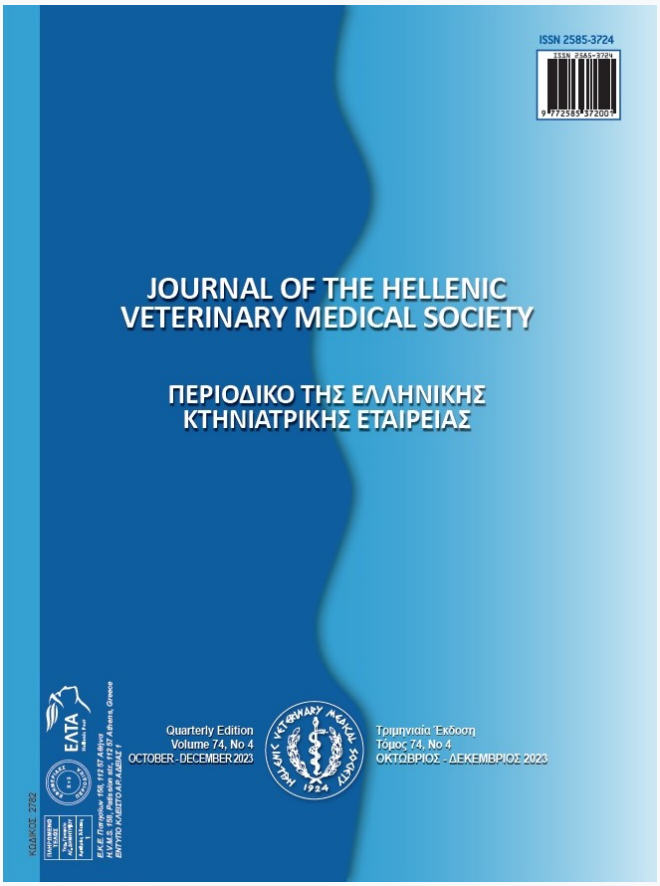

Effect of microbial transglutaminase addition on reconstructed frozen rainbow trout (Oncorhynchus mykiss) meatballs' quality

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Research article
Ερευνητικό άρθρο

Effect of microbial transglutaminase addition on reconstructed frozen rainbow trout (*Oncorhynchus mykiss*) meatballs' quality

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ABSTRACT: In this study, MTGase was added to rainbow trout meatballs in two groups (A and B) at two distinct concentrations (0.5%, 0.8%) in comparison to the control group without enzyme. The samples were stored at -18±1°C. 210 days were spent testing the trout meatballs' chemical (TVB-N, TBARs), physical (pH, water activity, color, texture, and cooking loss), microbiological (TMAB, TPAB, TYM, TCB, TAB, *E. coli*, and *S. aureus*), and sensory qualities. During frozen storage, the TVB-N and TBARs readings stayed within the permissible range. The control group's pH and cooking loss values were different from those of Groups A and B. In general, the water activity values stayed constant. MTGase was added, and this had an impact on the L* and a* values. The fish meatballs' sensory qualities and texture characteristics also improved. Except for the TCB values, none of the groups' limit values were surpassed during the frozen storage.

***Keywords*:** MTGase; trout ball; enzyme; quality; shelf-life

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INTRODUCTION

Frozen storage is regarded among the best preservation methods to preserve the nutritional value of fishery products, maintain quality characteristics, and guarantee safety (Cropotova et al., 2020). The freezing process is known to stop microbial growth effectively (Al-Bulushi et al. 2005) and reduce the bacterial load in fish by 60-90% (Shewan, 1954). In addition, it is reported that frozen storage adds to the shelf life by slowing down microbiological spoilage (Kong et al. 2016; Li et al. 2019). Thus, frozen storage increases the shelf life of the product by slowing down the sensory, chemical, and microbiological deterioration reactions. However, the long-term storage of frozen fish has some disadvantages such as the loss of protein solubility and decreased water-holding capacity (Nikoo et al. 2016; Jenkelunas and Li-Chan, 2018). In addition, it can cause chemical changes in seafood such as protein denaturation and lipid oxidation, leading to the loss of the functional properties of the product and adversely affecting consumer acceptability (Nikoo et al. 2016).

The alteration of food proteins using transglutaminase, an enzyme catalysing the crosslinking between glutamine (Gln) and lysine (Lys), has recently attracted attention in food processing. The use of transglutaminase in food products can prevent textural deformation by providing mechanical strength. Thus, the use of transglutaminase can be preferred to the use of food additives (Yuksel and Erdem, 2008). Microbial transglutaminase (MTGase) is widely used in the food industry to modify food proteins and improve the functional properties of food proteins. It modifies proteins by catalysing the formation of intramolecular and intermolecular cross-links between proteins or peptides (Gaspar and Goes-Favoni, 2015; Altan, 2020). The benefits of the addition of MTGase to fish and fish products include providing textural firmness and giving a more elastic structure to certain products (such as kamaboko, surimi fish ball, surimi crabstick), standardizing the quality of the final product, developing various products using various fish species, and increasing the binding property and stability without changing the sensory properties of the raw material (Kuraishi et al. 2001; Zhu et al. 1995; Miwa, 2020). MTGase is used for mixing and shaping minced meat, fish, chicken, and other food additives, and to produce meat products such as hamburgers, meatballs, meat, and fish balls that are packaged in pressure-resistant containers (Kuraishi et al. 2001; Altan, 2020).

Rainbow trout *(Oncorhynchus mykiss)* is a common fish species worldwide. Its total aquaculture production in Türkiye was reported to be 421411 tons. The share of rainbow trout (*Oncorhynchus mykiss*)in this production is 144283 tons. The inland waters supply 126101 tons of production while the marine waters supply 18182 tons (TUIK, 2021). It is generally consumed fresh in the domestic market and exported to the European countries in the form of chilled fresh or frozen fillets. The fish utilization that is produced in large quantities using alternative processing methods will contribute to the food industry. This can be achieved by creating a new processed product market in which farmed and exported trout in the Black Sea can be evaluated (Altan, 2020). Rainbow trout that is produced with aquaculture is frequently used in the production of fish balls due to its availability and fish meat quality (Keser and İzci, 2020). However, there is little literature on the application of MTGase, which can be offered directly to the consumer to produce fish balls using seafood (Ciftci, 2010; Altan, 2020).

The packaged raw, semi-cooked and fully cooked products in the frozen food market are in demand in the market because they require no prior preparation, can be stored for a long time, and are consumed by cooking without thawing. The aim of this study is to determine the effect of the MTGase enzyme, used as a binding agent in the production of frozen trout balls, on the physical, chemical, microbiological and sensory properties at -18±1°C for 210 days.

MATERIALS AND METHODS

The large rainbow trout (*Oncorhynchus mykiss*) (weighing more than 3 kg) was obtained from an aquaculture farm (Kızılırmak Seafood Company, Samsun, Türkiye). The fish samples were covered with crushed ice in styrofoam boxes and brought to the laboratory within an hour. About 15 kg of rainbow trout was used in the study.

Preparation of fish mince and trout balls

The rainbow trout were immediately headed, gutted, and filleted. The fillets were rinsed and filtered. Fillets were minced using a cutter (Mateka, RR-K30D). The fish mince was divided into three groups weighing 3250 g each. The three groups were referred to as the control group (trout ball without MTGase), Group A (0.5% MTGase added trout ball), and Group B (0.8% MTGase added trout ball). For the control group, 65.40% fish mince, 12.07% onion, 6.54% breadcrumbs, 6.03% egg, 6.03% sunflower seed oil,

1% tomato paste, 1% salt, 0.64% black pepper, 0.60% garlic, 0.34% cumin, and 0.30% powdered red pepper were used. On the other hand, 0.5% MTGase and 0.8% MTGase enzyme were added to Group A and Group B, respectively. All ingredients were mixed in the cuter. The trout mince was molded and then wrapped with stretch film to prevent cross contamination. Then, Group A and Group B were kept at 25±1ºC for 4 h for the activation of the enzyme. After 4 hours, restructuring samples were sliced (11x5x1.6 cm^3) and were vacuum packaged using a device (Abant Group, MG42, Hellas, Sydney, Australia). The vacuum bag was 30-40 cm in size and 80 microns in thickness(Aksedef, Türkiye). Shock freezing was applied for 24 hours after vacuum packaging and stored at -18±1 °C for 210 days.

Analyses

For the chemical, physical, microbiological and sensory analysis, the samples were analyzed after thawing at +4ºC for 18 hours.

Chemical analyses

Total Volatile Basic Nitrogen (TVB-N)

The TVB-N content was determined using the method proposed by Antonacopoulos and Vyncke (1989). For TVB-N, trout ball (10 g) was homogenized for 3 min. The homogenized sample was put into the Kjeldahl tube. Then 1 g of magnesium oxide (MgO) and a few drops of paraffin to prevent foaming and 100ml distilled water were added. The distillate was collected in the receiver, containing 10ml boric acid (3%) and six drops of tashiro indicator and was filled to 100 ml with distilled water. The distillate was titrated with 0.1N hydrochloric acid (HCl) until the grey neutral point was reached. The amount of TVB-N was calculated as mg100/g of trout ball.

Thiobarbituric acid reactive substances (TBARs) value

The TBARs values were determined using the method proposed by Erkan and Ozden (2008). The samples were placed in a 50 ml centrifuge tube into which 16 mL of a 5% (w/v) solution of trichloroacetic acid and 100 µlof butylated hydroxytoluene were added and homogenized for 2 min in the Ultra-Turrax for at high speed. The mixture was filtered through Whatman No. 1 filter paper. One ml of a 0.01M aqueous solution of TBA and 5 ml of the filtrate were mixed. The mixture was heated in a boiling water bath for 30 min until the pink colour has fully developed. The mixture was then cooled, and the optical density was determined at 532 nm on a Rayleigh VIS-723G spectrophotometer. TBARs values were expressed as mg of malondialdehyde (MDA)/ kg of fish meat.

Physical analyses

pH

pH analysis was carried out using a pH meter. A 5 g of homogenized trout ball sample was transferred to the sample bottle. The measurement was carried out by immersing the pH meter probe into the sample.

Water activity (Aw)

The water activity value was determined according to the AOAC (1980).The measurements were done using a water activity device at 25ºC. Approximately, 5 g of the homogenized sample was used in each measurement.

Cooking loss (%)

Cooking loss analyses were modified from Wang et al. (2019). Raw trout ball samples were weighed in an analytical balance. Then, the samples were cooked in an oven (Ulubas/UEO-TT/1400W) at 180°C for 20 min. After the cooking treatment, the remaining amount of trout balls was recorded. The cooking loss was calculated using the following equation:

Cooking loss (%) = (a-b/b) x100

a= *weight of sample before cooking*

b= weight of sample after cooking

Colour analysis (L^*, a^*, b^*)

A colorimeter device (Konica Minolta/CR-A 33a, Osaka, Japan) was used for colour measurements. The L^*, a^*, b^* values were measured according to the CIE (1976). L* describes the lightness of the sample, a* intensity in red (a* >0), and b* intensity in yellow (b* >0). The colour measurements of the samples were done directly on the surface of the trout balls. Measurements were made from different regions of each sample (n=6). Sample means for L*, a* and b* values were calculated and recorded.

Texture Profile Analysis (TPA)

Brookfield CT3 Texture Analyzer (USA) was used for texture profile analysis comprising the parameters of hardness, adhesiveness, chewiness, resilience, cohesiveness, gumminess, and springiness (Anony-

mous, 2021). A cylindrical probe (12 mm diameter) was used for analysis and the device was set to 60% target, 0.05N trigger load, 0.50 mm/s test speed, 2 mm/s pre-test speed, 20 points/sec data rate, and 50 kg load cell.

Sensory evaluation

Sensory analyses were performed following the method proposed by Tseng et al., (2000) with minor modifications. An average of 20 g of trout ball samples from each group were cooked in the oven (Ulubas/UEO-TT/1400W) at 180ºC for 20 min. Then, the samples were presented to six trained panellists for sensory evaluation. The samples were evaluated and scored by the panellistsin terms of their appearance, odour, flavour, texture, spicy flavour, onion flavour, garlic flavour, and overall acceptance. A 5-point hedonic scale was used during the evaluation, with a score of 5 indicating very good, a score of 4 indicating good, a score of 3 indicating neither good nor bad, a score of 2 indicating consumable, and a score of 1 indicating very bad (spoiled). Sensory analyses were carried out in two replications and two series.

Microbiological analysis

For all microbiological analyses, 10 g of samples were collected under aseptic conditions. The samples were homogenized in a stomacher (Mayo HG400, Italy) for 4 minutes using 90 ml peptone water. Dilutions of up to 10^6 were prepared for each batch of samples and pipetted onto the relevant agar surface. The total mesophilic aerobic bacteria (TMAB) and total psychrophilic bacteria (TPB) counts were determined using plate count agar (PCA). The TMAB and TPB were incubated at 37°C for 2 days and at 7°C for 10 days, respectively (AOAC, 2000). For the counts of total yeast and mold (TYM), the samples were incubated in PDA at 28°C for 3 days. For total Enterobacteriaceae, anaerobic bacteria, and *Staphylococcus aureus*, counts, the VRB agar, RC agar, and BP agar were used, respectively, and the samples were incubated at 37°C for 1 day (Halkman, 2005).Anaerobic bacteria were performed by the pouroverlay plate method and other analyzes were performed by the surface plate method. Microbial counts were given in log cfu/g.

Statistical analysis

The statistical analysis of the samples was performed using one-way analysis of variance (ANOVA) and the Minitab 21 software program (Minitab Inc., State College, PA, USA). The differences between the mean were examined using Tukey's test. Mean and standard error values were used for data analysis. The significance level was set at 0.05.

RESULTS AND DISCUSSION

The TVB-N and TBARS values of the groups during the frozen storage period

Figure 1 shows the TVB-N and TBARS values of the trout balls during the frozen storage period. The limit value for TVB-N is specified to be 35 mg/100 g for fishery products (Varlik et al., 1993). Values above this threshold may render the product inconsumable. The TVB-N value of the raw trout was found to be 19.08 mg/100g. Although no statistical difference was observed between the groups on day zero, the TVB-N values of Group B were different from that of the other groups at the end of storage ($p<0.05$). However, the consumable limit values were not exceeded in all groups during the frozen storage. The number of studies examining the frozen storage ofMTGase is limited (Aref et al. 2016; Aref et al. 2018; Moreno et al. 2010; Yang et al. 2020; Tokay et al. 2021). Moreover, the few studies on the issue differ from our study in terms of the parameters they examined. For example, the TVB-N values were not investigated in these studies. Similar to our study, various studies were carried out at the temperature of 4°C. Yerlikaya et al. (2015) reported that the addition of MTGase to mackerel mince prevented the formation of volatile bases. Another study found that increasing the enzyme concentration inhibited the formation of TVB-N (Yerlikaya et al. 2017). Similarly, Altan (2020) reported that the increase in TVB-N was slower in trout ball groups with MTGase and the increase in enzyme concentration had a more suppressive effect on TVB-N. In the current study, the TVB-N values fluctuated in all groups during the frozen storage. While the limiting effect of the enzyme on TVB-N was more evident in the first period of frozen storage, this limiting effect decreased after this period. This is consistent with the results obtained in the study on the examination of trout ball 4°C(Altan, 2020).

The TBARS value represents the oxidation in fish meat and TBARS values below 3 mg/kg indicate that the product is of very good quality (Schormüller, 1969). The TBARS value of fresh trout is quite low(0.26). On the first day of storage, the TBARS values of the trout balls were 0.44, 0.45, and 0.43 in the control group, Group A, and Group B, respectively ($p>0.05$). These values increased during storage

but remained within very good quality limits. No statistical difference was observed between the groups, except for on day 60 ($p>0.05$). The low TBA values were attributed to low levels of lipid oxidation (Moreno et al. 2010). Tokay et al. (2021) determined the totox (total oxidation) value of restructured fish meat by applying MTGase within its consumable limit values after 5 months of frozen storage. The results revealed thatthe combined use of different ingredients and applications with MTGase during frozen storage is beneficial in delaying lipid oxidation (Rodriguez-Turienzo et al. 2013), but more research is needed on the effect of MTGase alone. However, the researchers reported that the application of MTGase at 4°C was more successful than the control samples and was effective in suppressing lipid oxidation (Tokay, 2015; Altan 2020).

The pH, aw, and cooking loss values of the groups during the frozen storage period

Table 1 shows the pH, water activity (aw), and cooking loss values of the trout balls during the frozen storage period. The pH value of fresh trout was 7.57. The pH values of the control group, Group A,

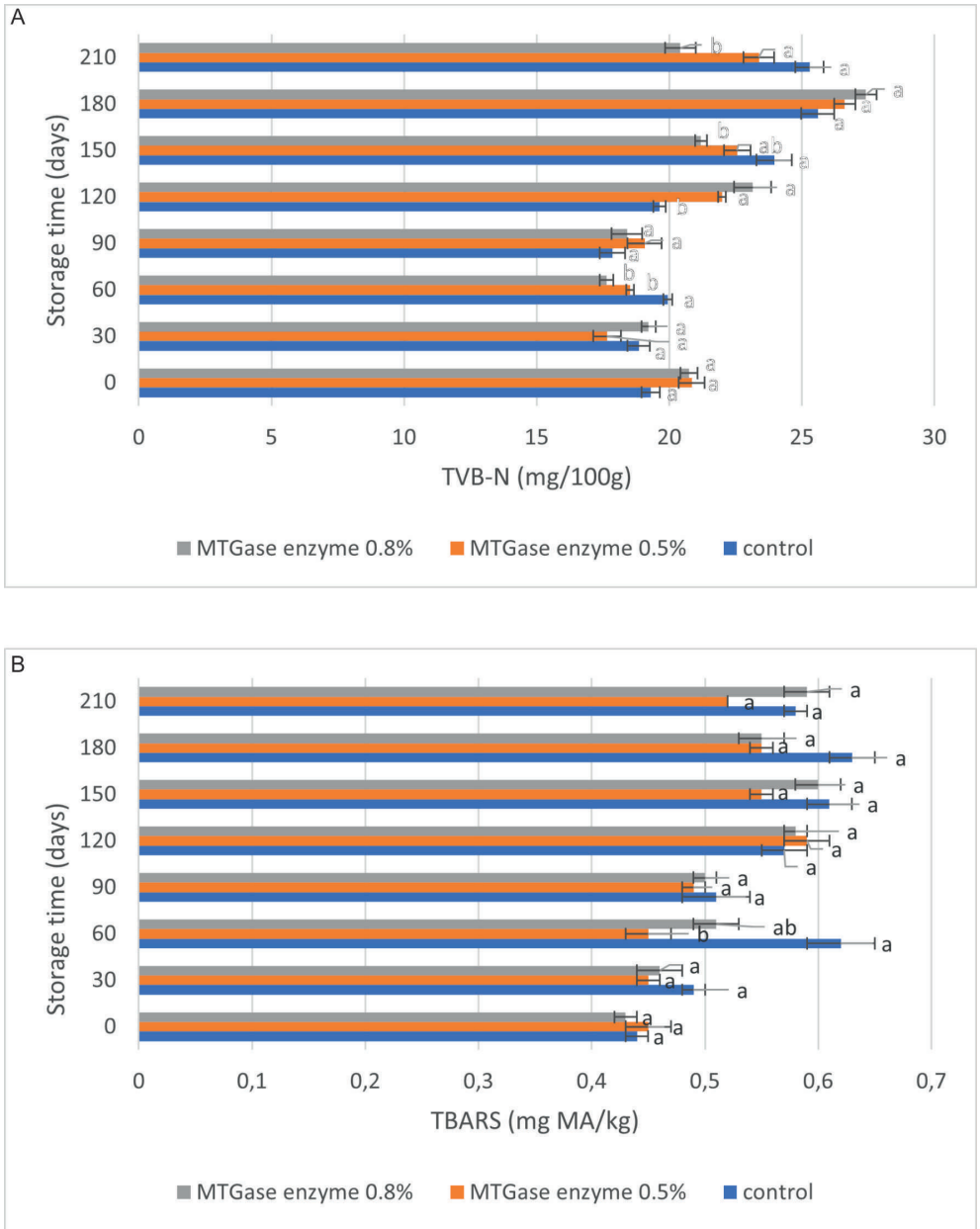

Figure 1. TVB-N and TBARs values in groups during frozen storage period. mean±SE (n=4), Control: Trout meatball without MTGase enzyme; A: 0.5% MTGase enzyme added trout meatball; B: 0.8% MTGase enzyme added trout meatball a, b, c,…f: the difference between groups with different lowercase letters is significant ($p\leq0.05$)

and Group B on the first day of storage were 7.13, 7.30, and 7.28, respectively. These values decreased to 6.14, 6.24, and 6.34 at the end of the storage, respectively. During the frozen storage period, the pH values of the control group were different from that of groups A and B ($p<0.05$). In addition, a statistical difference was observed between Group A and Group B, except for days 0, 150, and 210 of storage ($p<0.05$). Uran and Yılmaz (2018) reported that the addition of MTGase did not affect the pH value of chicken burgers. However, Karina and Setiadi (2020) stated that the supplementation of this enzyme to fish meat increased the pH value. Although the data obtained in the current study support this statement, more research is needed to be exact.

The water activity (aw) value was found to be 0.97 in the fresh trout and on day zero of storage in all groups. At the end of the frozen storage, the aw values decreased to 0.96 in all groups. The aw value of the control group on day 90 of storage was statistically different from that of Group B ($p<0.05$). Except for this difference, no statistical difference was observed in all groups during storage. Martelo-Vidal et al. (2016) obtained reduced-salt restructured products from European hake (*M.merluccius*), added MTGase at different rates, and found that the use of MTGase at different rates did not change aw compared to the control group. Altan (2020) investigated the effects of using MTGase at different rates at +4°C in trout balls and reported that the water activity values at the end of the storage period were similar to each other. This is congruent with the results of our study. Our results indicated that enzyme vacuum packaging was also effective in the general stability of the aw values.

Table 1. pH, aw and cooking loss values in groups during frozen storage period

pH			
Storage period (days)	Control	A	B
0	7.13 ± 0.01^{Ba}	7.30 ± 0.01^{Aa}	7.28 ± 0.00^{Aa}
30	6.21 ± 0.00^{Cb}	6.26 ± 0.00^{Bc}	6.33 ± 0.00^{Ac}
60	6.19 ± 0.00^{Cbcd}	6.25 ± 0.00^{Bc}	6.32 ± 0.00^{Acd}
90	6.19 ± 0.00^{Cbc}	6.24 ± 0.00^{Bc}	6.29 ± 0.00^{Ad}
120	6.17 ± 0.01^{Cbcd}	6.33 ± 0.01^{Bb}	6.42 ± 0.01^{Ab}
150	6.14 ± 0.00^{Bde}	6.19 ± 0.00^{Ad}	6.21 ± 0.00^{Ae}
180	6.10 ± 0.00^{Ce}	6.16 ± 0.00^{Bd}	6.23 ± 0.00^{Ae}
210	6.14 ± 0.01^{Bcde}	6.24 ± 0.01^{ABc}	6.34 ± 0.01^{Ac}
aw			
Storage period (days)	Control	A	B
0	0.97 ± 0.00^{Aa}	0.97 ± 0.00^{Aa}	0.97 ± 0.00^{Aa}
30	0.97 ± 0.00^{Aa}	0.97 ± 0.00^{Aab}	0.97 ± 0.00^{Aa}
60	0.96 ± 0.00^{Aab}	0.97 ± 0.00^{Aabc}	0.97 ± 0.00^{Aa}
90	0.96 ± 0.00^{Bbc}	0.96 ± 0.00^{ABc}	0.97 ± 0.00^{Aab}
120	0.96 ± 0.00^{Aabc}	0.96 ± 0.00^{Aabc}	0.97 ± 0.00^{Aab}
150	0.96 ± 0.00^{Aabc}	0.97 ± 0.00^{Aabc}	0.97 ± 0.00^{Aab}
180	0.97 ± 0.00^{Aa}	0.96 ± 0.00^{Aabc}	0.96 ± 0.00^{Aab}
210	0.96 ± 0.00^{Ac}	0.96 ± 0.00^{Abc}	0.96 ± 0.00^{Ab}
Cooking loss (%)			
Storage period (days)	Control	A	B
30	21.69 ± 0.98^{Ab}	19.16 ± 0.29^{Abc}	19.10 ± 0.22^{Abc}
60	19.60 ± 0.03^{Abc}	16.99 ± 0.62^{Ac}	16.66 ± 0.94^{Acd}
90	15.39 ± 0.03^{Ac}	10.77 ± 0.02^{Bd}	15.71 ± 0.67^{Acd}
120	32.61 ± 0.95^{Aa}	25.60 ± 0.48^{Ba}	23.42 ± 0.16^{Bab}
150	30.74 ± 0.54^{Aa}	23.80 ± 0.57^{Bab}	24.35 ± 0.38^{Ba}
180	30.00 ± 0.75^{Aa}	27.44 ± 0.87^{Aa}	25.92 ± 0.80^{Aa}
210	24.03 ± 0.70^{Ab}	18.61 ± 0.81^{Bc}	14.15 ± 0.05^{Bd}

Mean (n=4) ±SE

A, B, C (→): The difference between groups with different capital letters is significant ($p\leq0.05$).

a, b, c,…f (↓): The difference between days with different lowercase letters is significant ($p\leq0.05$)

Control: Trout meatball without MTGase enzyme; A: 0.5% MTGase enzyme added trout meatball; B: 0.8% MTGase enzyme added trout meatball

Cooking loss is an important parameter and reducing it is desired in meat and meat products (Erdem et al. 2020). On day 30 of storage, cooking loss (%) was lower in the enzyme-added groups than in the control group. However, no statistical difference was observed ($p>0.05$). The group with the highest enzyme addition had the lowest cooking loss, followed by the other enzyme-added group, and the control group, respectively. At the end of the storage, the cooking loss in the enzyme-added trout balls was statistically different from that in the control group ($p<0.05$).

Altan (2020) reported that the addition of enzymes to trout balls was very effective in cooking loss, with cooking loss correspondingly decreasing with decreasing enzyme concentration. Our results agree with this finding. In another study, the researchers added different amounts of transglutaminase to beef, chicken, and turkey meatball samples and found that cooking loss decreased in all transglutaminase-added groups (Erdem et al. 2020). There are several studies examining the positive effects of transglutaminase enzyme on cooking loss in the literature (Pietrasik et al. 2007; Uran et al. 2013; Ersoz et al. 2021).

The L^*, a^*, b^* values of the groups during the frozen storage period

The determination of colour perception using instrumental devices is of importance considering its variability from person to person. Colour perception can also be determined using sensory analysis methods, but measurements with instrumental devices can yield results that are more precise (Kocatepe and Corapci, 2019).

Table 2 shows the L^*, a^*, b^*values of the trout balls during the frozen storage period. The L^*, a^*, b^*values of the fresh trout samples were measured to be 63.70, 12.49, and 22.78, respectively. The coloration of cultured fish can be obtained by adding natural or synthetic colorants to the feed (Emir Coban and Tuna Kelestemur, 2011). The colour values in this study, especially the redness value (a^*), were higher than those in other studies (Corapci, 2017; Altan, 2020).This was attributed to the substances used in nutrition during aquaculture, which might have colored the fish meat. The different sizes (feeding times) of the compared fish samples may also be a factor. The L^* values both at the beginning and end of the storage were different in all groups and generally decreased during the storage period ($p<0.05$). At the end of storage, 0.8% MTGase-containing Group B had the highest L^* value. However, the lowest a^* value at the beginning and end of storage was obtained in Group B as well ($p<0.05$). No statistical difference was observed between the b^* values measured at the beginning and end of storage in all groups ($p>0.05$). However, on days 90 and 120 of storage, the values of groups A and B were different from those of the control group ($p<0.05$).

Luo et al. (2020) reported that the use of MTGase added to the decreases in the L* values in the case of cross-links. In addition, Tokay et al. (2021) has attributed the decreases in the L values to the quality loss during frozen storage. In this study, the addition of MTGase to the trout balls could have decreased the L^* values at the beginning of storage ($p<0.05$). During the frozen storage, the L^* values of the control group were lower than the enzyme-containing groups, thus leading to the conclusion that the addition of MTGase

Table 2. L^*, a^*, b^* values in groups during frozen storage period

Storage period (days)	Colour								
	Control			A			B		
	L	a	b	L	a	b	L	a	b
0	75.35 ± 0.05^{Aa}	6.58 ± 0.13^{Bd}	22.00 ± 0.36^{Ac}	69.58 ± 0.24^{Ca}	7.98 ± 0.07^{Abc}	24.32 ± 0.90^{Ac}	73.29 ± 0.28^{Ba}	6.50 ± 0.04^{Bc}	22.44 ± 0.29^{Ad}
30	61.57 ± 0.34^{Cf}	8.73 ± 0.27^{Ab}	26.81 ± 0.65^{ABab}	64.10 ± 0.19^{Bf}	8.00 ± 0.08^{Ab}	28.01 ± 0.25^{Aa}	67.61 ± 0.39^{Ad}	8.50 ± 0.13^{Aa}	26.06 ± 0.18^{Babc}
60	65.38 ± 0.39^{Cb}	10.03 ± 0.18^{Aa}	28.35 ± 0.44^{Aa}	68.31 ± 0.30^{Bab}	8.97 ± 0.14^{Ba}	27.17 ± 0.28^{Aab}	74.43 ± 0.21^{Aa}	8.82 ± 0.09^{Ba}	27.78 ± 0.13^{Aa}
90	64.38 ± 0.30^{Cbcd}	8.37 ± 0.07^{Abc}	27.40 ± 0.20^{Aab}	67.13 ± 0.31^{Bbc}	7.65 ± 0.09^{Bbc}	25.94 ± 0.24^{Bbc}	70.60 ± 0.29^{Ab}	7.31 ± 0.11^{Bb}	25.48 ± 0.27^{Bbc}
120	62.67 ± 0.11^{Cef}	8.53 ± 0.14^{Abc}	27.65 ± 0.17^{Aab}	66.20 ± 0.15^{Bcd}	7.78 ± 0.04^{Bbc}	25.82 ± 0.20^{Bbc}	70.55 ± 0.20^{Ab}	7.43 ± 0.07^{Bb}	25.69 ± 0.14^{Bbc}
150	64.89 ± 0.30^{Cbc}	7.82 ± 0.04^{Ac}	26.67 ± 0.43^{Aab}	66.69 ± 0.07^{Bcd}	7.50 ± 0.07^{Abc}	26.18 ± 0.23^{Aabc}	69.98 ± 0.20^{Abc}	7.14 ± 0.09^{Bbc}	26.11 ± 0.21^{Aabc}
180	62.75 ± 0.14^{Cdef}	8.13 ± 0.08^{Abc}	27.47 ± 0.25^{Aab}	64.55 ± 0.19^{Bef}	7.37 ± 0.03^{Bc}	26.70 ± 0.18^{ABab}	68.88 ± 0.21^{Acd}	7.35 ± 0.08^{Bb}	26.45 ± 0.21^{Bab}
210	63.38 ± 0.45^{Cde}	8.75 ± 0.15^{Ab}	25.72 ± 0.65^{Ab}	65.71 ± 0.26^{Bde}	8.62 ± 0.13^{Aa}	26.28 ± 0.62^{Aabc}	69.82 ± 0.44^{Abc}	7.68 ± 0.14^{Bb}	24.47 ± 0.69^{Acd}

Mean ±SE (n=6)

A, B, C (→): The difference between groups with different capital letters is significant ($p\leq0.05$).

a, b, c,…f (↓): The difference between days with different lowercase letters is significant ($p\leq0.05$)

Control: Trout meatball without MTGase enzyme; A: 0.5% MTGase enzyme added trout meatball; B: 0.8% MTGase enzyme added trout meatball

affected the L values of the trout balls. Erdem et al. (2020) stated that the addition of transglutaminase at different rates did not affect the colour values of the beef, chicken, and turkey meatballs. Altan (2020) added transglutaminase at different rates to trout balls, stored the samples at 4°C, and found close a^* and b^* values to those in our study during storage.

In the present study, the addition of MTGase (except on day zero of storage) decreased the a^* values in the enzyme-containing groups compared to the values of the control group. The b^*values slightly increased in all groups during the frozen storage. Although the b* values were different in certain months compared to those of the control group, it is difficult to conclude that MTGase dominantly affected the b^* values.

The results of the texture profile analysis of the groups during the frozen storage period

Table 3 shows the TPA values of the trout balls during the frozen storage period. At the end of the storage (day 210), the results of the hardness analysis (N) of the control group, Group A, and Group B were1.27, 1.39, and 1.72 N, respectively ($p<0.05$). Increases were observed in all groups at the end of the storage period compared to the initial results ($p<0.05$). Slight fluctuations were observed in all groups during the storage period. The hardness values of the control group were lower than those of both enzyme-containing groups from the second month to the end of the research period ($p<0.05$). The results revealed that the hardness values increased with increasing enzyme concentration ($p<0.05$). Moreno et al. (2010) reported that the addition of MTGase increased the hardness parameters of rainbow trout (*O. mykiss*) and hake (*M. merluccius*) minces and determined that the hardness values increased with increasing concentrations of the enzyme. Similarly, Altan (2020) reported that the addition of MTGase affected the hardness values of rainbow trout (*O. mykiss*) during storage and found that the hardness values increased with increasing concentrations of the enzyme ($p<0.05$). Moreover, some researchers have noted that when MTGase was added at equal concentrations to the groups, salt ratios had a decisive role in the changes in the hardness parameters, with increasing salt levels leading to higher hardness values (Tzikas et al., 2015; Kunnath et al., 2015).

The adhesiveness (mJ) values at the end of the storage period (day 210) revealed that the group control (1.10 mJ) had significantly higher adhesiveness than the enzyme-containing groups. Significant decreases were observed in the enzyme-containing groups starting from month four. Altan (2020) attributed the decrease in adhesiveness to the physically reduced surface stickiness due to the addition of the enzyme, particularly by the end of the storage period. Andres-Bello et al. (2011) reported that the adhesiveness values of both 0.3% and 0.6% MTGase-added groups of sea bream (*S.aurata*) mince significantly decreased ($p<0.05$), with the adhesiveness values decreasing more with the increasing enzyme ratio. Higher resilience values were observed in Group B until the end of day 150. The resilience values of the control group decreased after five months, with close values in the last two months (6^{th} and 7^{th} months) to each other ($p>0.05$). Altan (2020) determined that the control group had the lowest resilience values during the 24-day storage period and reported that the resilience values increased with increasing enzyme concentration.

The addition of salt, MTGase, and their various combinations can affect the texture parameters of restructured products of such as water-holding capacities, cooking losses, and volumetric changes (Uresti et al., 2004; Cardoso et al., 2007; Ramirez et al., 2007; Cardoso et al., 2010). The activity of MTGase is slightly dependent on salt concentration in restructured meat products, with the combined use of MTGase and salt directly and positively affecting the mechanical properties. As seen in Table 3, the springiness value of the control group was higher than that of the enzyme-containing groups on the day of production (day0) and the first day of analysis (day 30) due to the use of salt without enzyme causing undesired and uncontrollable volume gaining (increase in height). Moreover, breadcrumbs and other ingredients held water in all groups, but the addition of the enzyme slowed the volumetric increase, leading to obtaining a more stable product in the following days, especially in terms of its springiness parameters. All groups had slight fluctuations during the storage period, but the values of Group B were higher than those of the other groups at the end of the storage period. Some researchers reported that the presence of MTGase increased the springiness values, with values increasing with increasing enzyme concentration (Moreno et al., 2009; Andres-Bello et al., 2011; Kunnath et al., 2015; Tzikas et al., 2015; Lametta and Lametta, 2018; Altan, 2020). The cohesiveness value of the control group was higher (0.49) than that of the other groups and significantly decreased from the production date

to the first month. Fluctuations were observed in all groups during the storage period. After the third month, the values of all groups tended to decrease, except for the control group. Some researchers have found higher cohesiveness values in enzyme-containing groups (Moreno et al., 2008; Tzikas et al., 2015), while others did not find any difference between the enzyme-containing and non-enzyme-containing control groups (Kunnath et al., 2015; Lametta and Lametta, 2018). During the entire research, the highest enzyme-containing group (Group B) had the highest gumminess value ($p<0.05$), leading to the conclusion that the use of higher concentrations of MTGase directly affected the increase in the gumminess. Beginning from month four, the gumminess value of Group A significantly decreased, continued to decrease until the end of the sixth month, and began tore-increase until the end of the storage period. Similarly, Kunnath et al. (2015) has determined close gumminess results for pangasius fish (*P. hypophthalmus*) mince in groups containing % 0.5 MTGase, with the values increasing at the end of the 11-day storage period and fluctuating between the first and eleventh days. The chewiness values (N. mm) of the enzyme-containing groups momentously changed compared to the control group, especially during the first two months. The comparison of the values on the day of production (day 0) and at the end of storage (day 210) revealed that the chewiness values of the control group control slightly decreased ($p<0.05$) while the values of the enzyme-containing groups (groups A and B) remained unchanged ($p>0.05$). Andres-Bello et al. (2011) stated that chewiness increased with the use of MTGase depending on the increase in enzyme concentration.

The results of the sensory analyses of the groups during the frozen storage period

Figure 2 shows the results of the sensory analyses of the trout balls during the frozen storage. All groups received a score of 5.00 in terms of all sensory criteria (appearance, odor, flavor, texture, spicy flavor, onion flavor, garlic flavor, and overall acceptance) on the first day of the sensory analysis. The appearance scores were 3.60, 4.40, and 4.52 in the control group, Group A, and Group B on the last day of storage, respectively. Thus, the score of the control group was lower than that of the enzyme-containing groups ($p<0.05$). Similarly, the odor scores of the control group were lower compared to the enzyme-added groups ($p<0.05$). However, no statistical difference was observed between the scores of the enzyme-containing groups($p>0.05$). The texture-

Table 3. Texture Profile Analysis (TPA) of trout meatballs during frozen storage period

		Days							
Analyses	Groups	0	30	60	90	120	150	180	210
Hardness (N)	*Control*	0.63 ± 0.01^{dC}	1.04 ± 0.02^{cB}	1.18 ± 0.00^{bC}	1.19 ± 0.01^{bB}	1.31 ± 0.04^{aB}	1.37 ± 0.03^{aB}	1.30 ± 0.02^{aC}	1.27 ± 0.03^{abC}
	A	0.77 ± 0.01^{eB}	0.88 ± 0.03^{dC}	1.38 ± 0.01^{cB}	1.43 ± 0.03^{bcA}	1.52 ± 0.04^{abA}	1.55 ± 0.02^{aA}	1.46 ± 0.01^{abcB}	1.39 ± 0.01^{cB}
	B	1.07 ± 0.02^{eA}	1.25 ± 0.02^{dA}	1.47 ± 0.01^{cA}	1.50 ± 0.02^{cA}	1.54 ± 0.01^{cA}	1.64 ± 0.01^{bA}	1.65 ± 0.02^{abA}	1.72 ± 0.01^{aA}
Adhesiveness (mJ)	*Control*	2.23 ± 0.09^{aB}	0.47 ± 0.07^{eB}	0.60 ± 0.12^{deB}	1.30 ± 0.10^{bAB}	1.23 ± 0.07^{bcB}	0.97 ± 0.07^{bcdA}	0.87 ± 0.03^{cdA}	1.10 ± 0.06^{bcA}
	A	2.97 ± 0.07^{aA}	0.83 ± 0.03^{eA}	1.37 ± 0.07^{bA}	1.37 ± 0.03^{bA}	1.50 ± 0.06^{bA}	0.77 ± 0.03^{cdA}	0.73 ± 0.03^{cdA}	0.57 ± 0.07^{dB}
	B	1.60 ± 0.06^{aC}	1.00 ± 0.06^{bcA}	0.73 ± 0.03^{dB}	1.07 ± 0.03^{bB}	1.13 ± 0.03^{bB}	0.83 ± 0.03^{cdA}	0.77 ± 0.03^{dA}	0.70 ± 0.00^{dB}
Resilience	*Control*	0.02 ± 0.00^{eA}	0.03 ± 0.00^{deB}	0.04 ± 0.00^{cdeB}	0.04 ± 0.00^{cdB}	0.04 ± 0.00^{cdeB}	0.05 ± 0.00^{cB}	0.06 ± 0.00^{bA}	0.08 ± 0.00^{aA}
	A	0.02 ± 0.00^{eA}	0.04 ± 0.00^{bA}	0.04 ± 0.00^{bAB}	0.04 ± 0.00^{bB}	0.04 ± 0.00^{bB}	0.04 ± 0.00^{bB}	0.04 ± 0.00^{bB}	0.06 ± 0.00^{aB}
	B	0.02 ± 0.00^{dA}	0.04 ± 0.00^{cA}	0.05 ± 0.00^{cA}	0.06 ± 0.00^{bcA}	0.06 ± 0.00^{bcA}	0.07 ± 0.00^{abA}	0.07 ± 0.00^{abA}	0.08 ± 0.01^{aA}
Springiness (mm)	*Control*	12.97 ± 0.16^{aA}	8.18 ± 0.05^{bA}	5.86 ± 0.10^{cB}	5.19 ± 0.17^{dB}	5.48 ± 0.10^{cdB}	5.56 ± 0.05^{cdA}	4.68 ± 0.03^{eB}	4.52 ± 0.06^{eB}
	A	8.66 ± 0.10^{aB}	6.55 ± 0.10^{bC}	6.46 ± 0.06^{bA}	6.07 ± 0.08^{cA}	6.08 ± 0.04^{cA}	5.50 ± 0.07^{dA}	5.25 ± 0.03^{dA}	4.48 ± 0.03^{eB}
	B	6.59 ± 0.18^{bC}	7.06 ± 0.02^{aB}	5.81 ± 0.06^{cB}	5.27 ± 0.03^{dB}	6.00 ± 0.07^{cA}	4.38 ± 0.01^{fB}	4.84 ± 0.08^{eB}	5.06 ± 0.05^{deA}
Cohesiveness	*Control*	0.49 ± 0.01^{aA}	0.31 ± 0.02^{cB}	0.39 ± 0.01^{bB}	0.38 ± 0.00^{bC}	0.34 ± 0.01^{bcA}	0.30 ± 0.01^{cB}	0.39 ± 0.01^{bA}	0.39 ± 0.02^{bA}
	A	0.36 ± 0.01^{bB}	0.35 ± 0.01^{bB}	0.42 ± 0.00^{aAB}	0.43 ± 0.00^{aB}	0.36 ± 0.01^{bA}	0.34 ± 0.01^{bAB}	0.34 ± 0.01^{bB}	0.33 ± 0.00^{bB}
	B	0.40 ± 0.01^{cB}	0.50 ± 0.01^{aA}	0.45 ± 0.01^{bA}	0.47 ± 0.01^{abA}	0.37 ± 0.01^{cdA}	0.36 ± 0.01^{cdA}	0.34 ± 0.01^{dB}	0.33 ± 0.00^{dB}
Gumminess (N)	*Control*	0.32 ± 0.01^{dB}	0.36 ± 0.02^{cdB}	0.45 ± 0.02^{abB}	0.47 ± 0.02^{abB}	0.45 ± 0.02^{abB}	0.43 ± 0.02^{bcB}	0.53 ± 0.01^{aA}	0.51 ± 0.01^{abB}
	A	0.27 ± 0.01^{dC}	0.24 ± 0.01^{dC}	0.50 ± 0.01^{aAB}	0.50 ± 0.01^{aB}	0.37 ± 0.01^{cC}	0.36 ± 0.01^{cB}	0.36 ± 0.02^{cB}	0.43 ± 0.01^{bC}
	B	0.41 ± 0.00^{dA}	0.49 ± 0.01^{cA}	0.55 ± 0.01^{bA}	0.64 ± 0.01^{aA}	0.54 ± 0.01^{bcA}	0.64 ± 0.02^{aA}	0.53 ± 0.01^{bcA}	0.65 ± 0.01^{aA}
Chewiness (N.mm)	*Control*	4.01 ± 0.11^{aA}	2.64 ± 0.20^{bB}	2.67 ± 0.11^{bB}	2.36 ± 0.07^{bB}	2.43 ± 0.16^{bB}	2.26 ± 0.13^{bB}	2.35 ± 0.08^{bB}	2.25 ± 0.17^{bB}
	A	2.37 ± 0.09^{deB}	2.02 ± 0.07^{eC}	3.79 ± 0.08^{aA}	3.76 ± 0.10^{aA}	3.29 ± 0.02^{bA}	2.90 ± 0.08^{cA}	2.58 ± 0.05^{cdAB}	2.06 ± 0.03^{eB}
	B	2.80 ± 0.13^{deB}	4.37 ± 0.02^{aA}	3.86 ± 0.07^{bA}	3.75 ± 0.07^{bcA}	3.44 ± 0.07^{cA}	2.56 ± 0.04^{eAB}	2.71 ± 0.03^{deA}	2.91 ± 0.05^{dA}

Mean ±SE (n=8)

A, B, C (↓): The difference between groups with different capital letters is significant ($p\leq0.05$).

a, b, c,…f (→): The difference between days with different lowercase letters is significant ($p\leq0.05$)

Control: Trout meatball without MTGase enzyme; A: 0.5% MTGase enzyme added trout meatball; B: 0.8% MTGase enzyme added trout meatball

scores of the control group, Group A, and Group B were 3.10, 4.10, and 4.90 on the last day of storage, respectively. The texture scores increased with the increasing concentrations of the enzyme in the trout balls ($p<0.05$). The flavor values were close to each other in the enzyme-containing groups($p>0.05$) while the control group received lower scores, ($p<0.05$). Group B received higher scores in terms of the onion, garlic, and spicy flavor criteria compared to Group A and the control group. However, the statistical difference between the enzyme-containing groups was not significant ($p>0.05$) while the score of the control group was significantly different ($p<0.05$). The overall acceptability scores were 3.50, 4.40, and 4.80 in the control group, Group A, and Group B on the last day of storage. Group B had received the highest overall acceptability scores, which contained the highest enzyme concentration ($p<0.05$). The general acceptability scores of the groups indicated that the quality of the enzyme-added groups was good while the control group had neither good nor bad quality at the end of storage.

Figure 2. Sensory analysis results in groups during the frozen storage period. mean±SE (n=4), Control: Trout meatball without MTGase enzyme; A: 0.5% MTGase enzyme added trout meatball; B: 0.8% MTGase enzyme added trout meatball a, b, c,…f:the difference between groups with different lowercase letters is significant ($p\leq0.05$)

The results revealed that the addition of MTGase improved the sensory quality of the trout balls, with the increasing concentrations of the enzyme positively affecting the sensory properties. The results of a study on mackerel mince revealed that the panellists preferred groups that contained higher concentrations of MTGase in sensory analysis (Yerlikaya et al. 2015). Aref et al (2018) reported that the addition of the MTGase enzyme improved the textural and sensory properties of the fish fingers, especially its combined use with other tested additives. Various studies in the literature have shown that the sensory properties of the product are positively affected by the addition of MTGase (Erdem et al. 2020; Tokay et al. 2021; Aref et al. 2016). The results of this study agree with those reported in the literature.

The results of the microbiological analyses of the groups during the frozen storage period

Figure 3 shows the total mesophilic aerobic bacteria, total psychrophilic aerobic bacteria, total yeast-mold, and total coliform bacteria results of the trout balls during the frozen storage. The TMAB load of the fresh trout sample was 2.15 log cfu/g. This value is similar to that obtained by Altan (2020) but lower than those reported by Volpe et al. (2015), Kocatepe et al. (2016), and Rezaeifar et al (2020). At the beginning of storage, the TMAB values of the trout balls were 2.91 log cfu/g, 2.88 log cfu/g, and 2.75 log cfu/g in the control group, Group A, and Group B, respectively. These values increased to 6.33 log cfu/g, 6.06 log cfu/g, and 6.00 log cfu/gin the same groups, respectively, at the end of storage. On days 30, 60, 150, and 180 of storage, the counts of the MTGase-containing groups were statistically different from those of the control group ($p<0.05$). No statistical difference was observed between the groups on the last day of storage ($p>0.05$). ICMSF (1986) has reported that the microbiological shelf life ends when the counts reached the range of 7-8 log cfu/g. Thus, the counts of the trout balls in our study remained within the consumable limit values at the end of storage. Altan (2020) reported that the TMAB count of the trout balls on the first day of storage was 3.04 log cfu/g in the control group, 3.17 log cfu/g in the 0.5% MTGase group, and 3.15 log cfu/g in the 1% MTGase group. In our study, these values were higher than the values on the first day. This is attributable to many factors such as ingredients, additives, processing and thawing procedures. Within the scope of the study, frozen ball samples were thawed in the refrigerator for analysis. It can also be said that the 18-hour thawing step of the samples under the refrigerator conditions was effective in increasing the bacterial count.

No studies that investigate trout ball, MTGase, and frozen storage together were found in the literature. However, there are studies examining the effect of MTGase on total viable bacteria in different fish species and different storage temperatures (Tzikas et al. 2015, Moreno et al. 2013).

The initial TPAB count of fresh trout was 2.27 log cfu/g, which is lower than those reported by other researchers for fresh trout (Kocatepe et al., 2016; Altan, 2020; Rezaeifar et al. 2020). The TPAB counts of the control group, Group A, and Group B were 2.29 log cfu/g, 2.38 log cfu/g and 2.27 log cfu/g on the first day of storage, respectively. At the end of storage, these values were 4.95 log cfu/g, 4.69 log cfu/g, and 4.80 log cfu/g, respectively. No statistical difference was observed between the groups ($p>0.05$), except for the 90^{th}, 120^{th}, and last day of storage while the values of the enzyme-added groups were statistically different from that of the control group on the last day of storage ($p<0.05$). Yerlikaya et al. (2017) reported that the increasing concentrations of MTGase inhibited the growth of TPABs. In our study, Group B (the group with the highest enzyme concentration) was more effective on TPAB during storage, except for the last day of storage. However, despite the slightly lower TPAB value of Group A than Group B on the last day of storage, no statistical difference was observed between the two groups. Although our findings partially support the results reported by Yerlikaya et al. (2017), there is a need for long-term studies on the frozen storage of MTGase-added products.

The TYM load of fresh trout was 1.73 log cfu/g in the study while it was reported to be 2.61 by Altan (2020) and Yerlikaya et al. (2017) found no yeast or moldin the trout mince samples. At the beginning of storage, the TYM values of the trout balls were 1.93 log cfu/g, 1.54 log cfu/g, and 1.45 log cfu/g in the control group, Group A, and Group B, respectively. These values increased to 4.88 log cfu/g, 4.69 log cfu/g, and 4.41 log cfu/g, respectively, in the same groups at the end of storage. No difference was observed between the groups both at the beginning and end of storage. Altan (2020) reported the TYM counts of the trout balls on the first day of storage to be 3.22 log cfu/g in the control group, 3.05 log cfu/g in the 0.5% MTGase group, and 3.15 log cfu/g in the 1% MTGase group. Altan (2020) also stated that the ad-

dition of MTGase positively affected the reduction of the TYMs count and the use of higher concentrations of the enzyme further increased its positive effect. The findings of our study support their conclusion. Other researchers have also reported the inhibitory effect of MTGase on the yeast-mold load (Tokay, 2015). The initial TCB load of fresh trout was 2.21 log cfu/g, which was higher than the value reported by Altan (2020). However, the initial TCB load of mackerel mince is similar to the load found in this study (Yerlikaya et al. 2015).The maximum permissible limit of total coliform bacteria is 100 cfu/g for fish (ICMSF, 1986). The TCB load of fresh trout was slightly above this limit value. The initial loads of trout balls were 2.63 log cfu/g, 2.62 log cfu/g, and 2.58 log cfu/g in the control group, Group A, and Group B, respectively. These values decreased to 2.56 log cfu/g, 2.34 log cfu/g, and 2.10 log cfu/g, respectively, at the end of the frozen storage. No statistical difference was observed between the groups during the storage period ($p>0.05$). However, the enzyme-containing groups had lower TCB loads than the control group. Similarly, Yerlikaya et al. (2015) reported that the addition of the enzyme proportionately inhibited the growth of total coliform bacteria. On the first day of storage and after the 180th day, the total coliform counts in the trout balls exceeded 100 cfu/g. This high TCB load after the production of the trout balls is attributable to the sources of contamination in the laboratory environment such as equipment, tools, additives, and personnel. However, frozen storage decreased the TCB count while the count increased after day 180. The microbiological analysis revealed no anaerobic bacteria, *E. coli,* and *S. aureus* counts.

CONCLUSION

The TVBN and TBARS values of the trout balls during 210-day frozen storage did not exceed the consumable limit values. The pH values of the MTGase-added groups were different from that of the control group. Except on day 90, no statistical difference was observed between the water activity values of the groups. The cooking losses in the enzyme-added trout balls were statistically different from that of

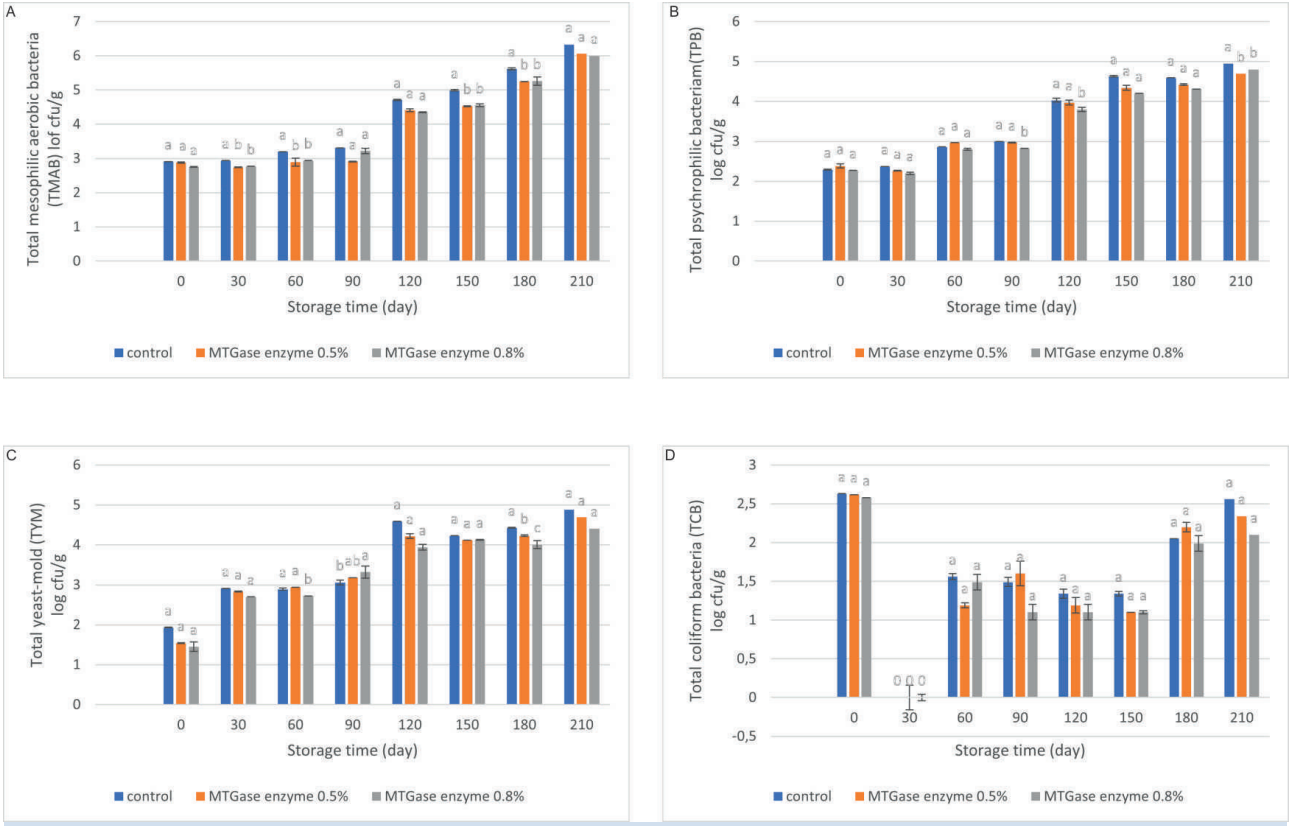

Figure 3. Microbiological analysis results in groups during the frozen storage period. mean±SE (n=4), Control: Trout meatball without MTGase enzyme; A: 0.5% MTGase enzyme added trout meatball; B: 0.8% MTGase enzyme added trout meatball
a, b, c,…f:the difference between groups with different lowercase letters is significant ($p\leq0.05$)

the control group at the end of storage. Although the b* values were not significantly affected by the addition of MTGase, MTGase affected the L* and a* values. Both textural and sensory properties contributed to the properties of the MTGase-added trout balls. The increasing concentrations of the enzyme increasingly contributed to these properties. The counts of TMAB, TPAB, and TYM remained within the limit values during the frozen storage. However, the TCB counts exceeded the limit value on day 180 of the frozen storage. No total anaerobic bacteria, *E. coli,* and *S. aureus* were detected during the frozen storage.

In conclusion, the addition ofMTGase improved and contributed to the cooking loss, colour, texture, and sensory properties of the trout balls. The sensory analyses revealed that Group B, which contained the highest MTGase concentration, was preferred more by the panellists than the other groups. A storage period of 180 days is recommended for the storage of restructured frozen trout balls at -18±1ºC in terms of microbiological safety.

CONFLICT OF INTEREST

The authors have declared no conflicts of interest for this article.

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