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Impact of modified atmosphere packaging on the microbiological and physicochemical analyses of mirror carp (*Cyprinus Carpio L.*, 1758) fish balls

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ABSTRACT: In this study, the impacts of dissimilar modified atmosphere packaging treatments on the microbiological and physicochemical quality of fish balls from mirror carp were investigated. In the study, one control group (K: under air) and three experimental groups (D1: 55% CO_2 + 15% O_2 + 30% N_2, D_2: 65% CO_2 + 10% O_2 + 25% N_2, D3: 75% CO_2 + 10% O_2 + 15% N_2) consisted of fish ball samples prepared using fillets from freshly caught mirror carp. After undergoing the respective MAP treatments, the cheese samples were stored at 4°C. Evaluations of their quality were carried out on storage days 0, 3, 6, 9, 12, 15, and 18. During the storage period, the D2 and D3 groups exhibited lower counts of *Enterobacteriaceae*, coliform bacteria, and yeast-mold in comparison to other groups (P≤0.05). The levels of total volatile base nitrogen and the thiobarbituric acid index witnessed a progressive rise across all groups, with discernible differences noted among them (P≤0.05). Sensory evaluation outcomes divulged that the shelf life of fish balls extended to 9 days for the control group, 15 days for the D1 group, and 18 days for both the D2 and D3 groups (P≤0.05). Based on these conclusions, it can be inferred that fish balls subjected to high CO_2 MAP have a notably prolonged shelf life when stored under refrigeration.

Keyword: Fish ball; mirror carp; microbiological-physicochemical parameters; modified atmosphere packaging; shelf-life.

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INTRODUCTION

The mirror carp (*Cyprinus carpio L*., 1758) is one of the most significant kinds of farmed freshwater fish because of its fast growth, soft-textured meat, and low number of scales (Du et al., 2020; Li et al., 2023). According to FAO (2018), *Cyprinus carpio* is the third most freshwater species grown worldwide in fish farming. Due to the decrease of marine fish species, which is a global problem, and the increase in demand for fish products, the mirrored carp species constitutes the main ingredient used to obtain fish products (Maas et al., 2019). These fish, which are sold whole and fresh, are offered to the market in the form of ready-made products with small packages due to the different consumption habits and developing technologies (Liu et al., 2013).

Fish holds a significant economic and nutritional value, attributed to its rich content of essential amino acids (EAA) and polyunsaturated fatty acids (PUFAs) (Maulu et al., 2021). The chemical composition of fish meat varies depending on the age, gender, seasonal, and environmental factors of the fish. It is stated that fish contains 1-3% glycogen, 0.8-2% mineral matter, 0.1-22% fat, 15-24% protein, and 66-84% water (Arslan, 2002). However, fish meat containing high level of protein (especially free amino acids), nitrogenous compounds, and unsaturated fatty acids can readily disrupt because of its high pH value and water content (Metin et al., 2002; Abraha et al., 2018; Beltrán and Bellés, 2019; Jääskeläinen et al., 2022). For this reason, all aquatic products, including fish, must be transported, processed, and preserved by appropriate methods immediately after fishing (Gulyavuz and Unlusayin, 1999; Metin et al., 2002; Budaraga et al., 2021)

Surimi falls under the category of ready-to-eat foods, encompassing an assortment of products like fish balls, fish cakes, fish chips, fish crackers, fish ham, and fish sausages among others. Their widespread consumption can be attributed to their high level of protein content, low levels of cholesterol, and distinctive textural characteristics (Gao et al., 2020; Budaraga et al., 2021; Fan et al., 2022). Mincing is one of the preferred methods for evaluating fish meat as a processed product. One of the traditional products of fish mince is fish balls (Capkin, 2008). Fish balls are produced by mixing cleaned, minced, and boiled fish with spice additives (starch, spices) (Budaraga et al., 2021).

The MAP technology is frequently used in the aquaculture industry. The composition and ratio of the gas mixture used in MAP is bound up with the fish species (Aktas and Kaya, 2010; Chan et al., 2021). Lately, many researchers have focused on the MAP method for a longer shelf life (Chan et al., 2021; Jääskeläinen et al., 2022). In addition to being one of the popular food preservation methods recently, MAP also prevents biochemical, microbiological, and enzymatic reactions that may occur in foods by changing the gas composition (CO_2, N_2, and O_2) in packaged foods (Tsironi and Taoukis, 2018; Chan et al., 2021). The carbon dioxide gas is the most important gas in the MAP due to its fungistatic and bacteriostatic properties. Qiu et al. (2012) indicated that for a significant suppression of microbial proliferation, the CO_2 concentration in the gas blend should exceed 20%-30%. Thus, the quality of the food product is preserved for a certain period of time with extended shelf life (Sivertsvik et al., 2002; Mendes et al., 2008; Gun et al., 2009; Yesudhason et al., 2014; Feng et al., 2017). This extension largely hinges on the initial microbial load present in the fish meat and the conditions under which the fish is processed (Sezer et al., 2022).

Fish meat, once cleaned and minced, is transformed into fish balls by incorporating a mix of spices and additives, subsequently being packaged in assorted forms. Various studies have been conducted on the evaluation and shelf life of fish meat processed in this way. For instance, raw and boiled fish ball samples were prepared from anchovy fish and they stated that it has a shelf life of 9 days when stored at 4°C (Akkuş et al., 2004). Oksuztepe et al. (2010) stated that the addition of sodium lactate to fish ball samples increased the shelf life of the samples. In another study conducted on fish balls, it was stated that the shelf life of fish ball samples packaged with the MAP is extended up to 52 days (Yongji et al., 2010).

In the present research, we systematically examined the alterations in the quality of mirror carp (*Cyprinus carpio L*., 1758) fish balls subjected to the MAP with varying gas combinations, stored at 4°C. The investigation spanned two primary dimensions: microbiological and physicochemical attributes. The overarching goal of this study is to furnish theoretical insights beneficial for the quality maintenance of mirror carp fish balls throughout their storage duration.

MATERIAL AND METHODS

Since in many studies on fish, fish cakes and modified atmosphere packaging (Akkuş et al., 2004;

Arashisar et al., 2004; Yilmaz, 2004; Cakli et al., 2006; Rosnes et al., 2006; Baygar et al., 2008; Capkın, 2008; Del Nobile et al., 2009; Patir et al., 2009; Liu et al., 2010; Provincial et al., 2010; Oguzhan, 2011; Torrieri et al., 2011; Guran et al., 2015; Qian et al., 2015; Rodrigues et al., 2016; Smaldone et al., 2017; Antunes-Rohling et al., 2019; Kaya, 2019; Wang et al., 2019; Nayma et al., 2020; Mahdavi and Ariaii, 2021) samples were stored at 4°C and MAP packaged products were stored at 4°C in markets, we preferred this temperature in our study. However, we did not find it appropriate to work at -18°C since bacterial growth would slow down or not show any growth. We created our current working groups based on studies that used similar gases and gas mixtures and obtained good results (Arashisar et al., 2004; Yilmaz, 2004; Cakli et al., 2006; Del Nobile et al., 2009; Yesudhason et al., 2009; Liu et al., 2010; Provincial et al., 2010; Yesudhason et al., 2014; Qian et al., 2015; Zhu et al., 2016; Smaldone et al., 2017; Nayma et al., 2020; Mahdavi and Ariaii, 2021; Chan et al., 2021).

Raw material

The fresh mirror carp (*Cyprinus carpio L.*, 1758) was obtained from Elazig Keban Dam Lake. For each repetition, approximately 2-3 kg of fresh mirror carp was used. The fish were brought to the Fırat University Faculty of Veterinary Medicine laboratory in an ice box and kept at refrigerator temperature (4°C) until processing. In the laboratory, fish samples were descaled, skinned, and the head was removed, and their internal organs were thoroughly cleaned (gutting). Following the removal of the fish bones (descaling), the fillets were processed into a minced form using a meat grinder and, washing.

Preparation of fish balls and creation of experimental groups

The fish balls contained 86% fish (raw), 3% sunflower oil (Komili, Türkiye), 3% corn flour, 2% salt (Billur, Türkiye), 1.5% garlic, 1% thyme (Bagdat, Türkiye), 1% of chopped onion, 1% red pepper powder (Bagdat, Türkiye), 0.5% cumin (Bagdat, Türkiye), 0.5% ginger (Bagdat, Türkiye), and 0.5% black pepper powder (Bagdat, Türkiye) (Guran et al., 2015). In the laboratory, the homogeneously prepared mixture was formed into fish balls, each weighing approximately 25 grams, and then four fish balls were placed on packaging plates. In a factory, the mixture was divided into 4 groups by applying modified atmosphere packaging [Control (78.5% N_2 + 20.8% O_2 + 0.7% other gases in the air) (K); 55% CO_2 + 15% O_2 + 30% N_2 (D1); 65% CO_2 + 10% O_2 + 25% N_2 (D2); 75% CO_2 + 10% O_2 + 15% N_2 (D3)]. The samples were placed in a packaging container (Foiltherm Packaging, Dimensions: length 190mm, width 144mm, depth 43mm. Construction features, polyethylene-thermoplastic black, FT355-H50, İstanbul/Türkiye) and placed in a modified atmosphere packaging device (Multivac T 200, Australia) and packaged with packaging film (Construction features: polyethylene transparent, FP1040 BOPET/HB4, Istanbul/Türkiye). The samples were stored in the laboratory in the refrigerator at 4°C. Microbiological and physicochemical evaluations were undertaken on days 0, 3, 6, 9, 12, 15, and 18 of storage. On each designated day of analysis, samples were assessed sensorially, considering attributes like aroma, flavor, and appearance. Only those sample groups that met the sensory acceptance criteria underwent further analysis. Those deemed sensorially unacceptable were excluded from testing. For each day of analysis, samples were tested in duplicate, and the entire study was replicated three times (n=3).

Microbiological analysis

On each designated sampling day for microbiological assessment, 10 g each of fillet and fish ball samples were collected from every group into a sterile stomacher bag. To this, 90 mL of sterile 0.1% peptone water (Acumedia 7365A, UK) was added, and the mixture was then homogenized using a stomacher (Stomacher 400, UK), achieving a $^1/_{10}$ (10^{-1}) dilution of the samples. Subsequent dilutions were derived from this primary dilution, ensuring the consistent use of the same diluent. Each dilution was plated in two sets using 1 mL aliquots. After the incubation period, only petri dishes that harbored between 30 and 300 colonies were taken into consideration for evaluation, in line with the standards detailed by Halkman (2005). For microbial analysis, the study of Guran et al. (2015) was taken as reference and the parameters therein were examined.

Plate Count Agar (PCA) (Merck 1.05463.0500, Darmstadt, Germany) was used in counting total aerobic mesophilic bacteria (TAMB) incubated at 30°C for 72 hours (ISO, 2013). Psychrotrophic bacteria were counted on Plate Count Agar (PCA) (Merck 1.05463.0500, Darmstadt, Germany). The plates were incubated at 5±1°C for 7 days (USDA/FSIS, 2011). Violet Red Bile Glucose Agar (VRB-GA) (Merck 1.10275.0500, Darmstadt, Germany) was used in counting *Enterobacteriaceae* incubated at 37±1°C for 24 hours. After incubation, dark red

colored colonies were counted, and the oxidase test (Merck 1.00181.0002, Darmstadt, Germany) was applied to 5 randomly selected colonies for biochemical confirmation (ISO, 2017). Coliform bacteria were counted on Violet Red Bile Agar (VRB) (Merck 1.01406.0500, Darmstadt, Germany) and incubated at 30±1°C for 24 hours (ISO, 2006). Yeast and mold were counted on Dichloran Rose Bengal Chloramphenicol Agar (DRBCA) (Merck 1.00466.0500, Darmstadt, Germany) incubated at 25±1°C for 5 days (ISO, 2008).

Physicochemical analysis

For physicochemical analysis, the study of Guran et al. (2015) was taken as reference and the parameters therein were examined. The pH of both the mirror carp fillet and fish ball samples was ascertained following the AOAC (1990) guidelines. To start, 10 g of the sample was combined with 90 mL of distilled water, followed by homogenization using a Bag Mixer Interscience homogenizer (78860 St. France-Stochmaher). Subsequently, the pH values of fillet and fish ball samples were determined using a pH meter (HI 11310, Hanna Instruments, USA).

Total volatile base nitrogen (TVB-N) was determined according to the method described by Commission Regulation (EC) No: 2074/2005 (EC, 2005). After the fish balls were homogeneously shredded using a blender, 10 g samples were taken and 90 mL of 6% perchloric acid ($HClO_4$) (Merck 1.00519.2501, Darmstadt/Germany) solution was added and homogenized by mixing in a high-speed mixer for 2 minutes. After homogenization, the sample was filtered through a paper filter (Whatman No. 1). Fifty mL of the obtained extract was taken and a few drops of phenolphthalein (Merck 107233, Darmstadt/Germany) (1 g/100 mL of 95% ethanol (Merck 100983, Darmstadt/Germany)) solution, anti-foaming agent (silicone), and 6.5 mL of 20% sodium hydroxide solution (NaOH) (Merck 106498.1000, Darmstadt/Germany) were added and placed in the steam distillation unit. Hundred mL of 3% boric acid (Merck 100160.5000, Darmstadt/Germany) solution, 3-5 drops of indicator solution (2 g of methyl red (Merck C.I. 13020, Darmstadt/Germany) and 1 g of methylene blue (Merck C.I. 52015, Darmstadt/Germany) dissolved in 1000 mL of 95% ethanol) were added to the erlenmeyer flask, and then 100 mL of distillate is obtained in 10 minutes by using unit distillation. Finally, the obtained distillate was titrated with 0.05 N hydrochloric acid (HCl) (Merck 100317, Darmstadt/Germany).

Thiobarbituric acid index (TBA) was performed using the spectrophotometric method suggested by Tarladgis et al. (1960). The 10 g of homogenized fish ball samples were taken and placed in a Kjeldahl tube and added to 2.5 mL of 1:2 HCl and 97.5 mL of distilled water. Then, it was distilled until 200 mL of distillate was collected in the Erlenmeyer flask. For fish ball samples, 5 mL of distillate and 5 mL of freshly prepared TBA reagent (Merck 108180.0025 2, Darmstadt/Germany) were placed in each tube. For the blind, 5 mL of distilled water and 5 mL of TBA reagent were placed in a capped glass tube. These tubes were kept in boiling water bath for 35 minutes then allowed to cool and absorbances were determined with a Chebios UV/VIS spectrophotometer (Optimum One, Chebios s.r.l., Rome, Italy) at a wavelength of 538 nm. The readings were multiplied by 7.8 and the TBA value in the sample was determined as mg malonaldehyde/kg.

The dry matter analysis for both fillet and fish ball samples was conducted in line with the TSI 1743-ISO 1442/April 2001 standards as set out by TSI (2001).

Statistical analysis

In the statistical evaluation of the study, the significance of the intra-group and inter-group differences were analyzed with the SPSS22 package program (IBM SPSS, IBM Corporation, USA) (Version 22). Microbiological data were calculated logarithmically and expressed as $\log_{10}$CFU/g. Before analysis, Kolmogorov-Smirnov test was applied to all data obtained to determine the normal distribution and Levene test was applied to determine homogeneous distribution. After the test of normality, the data were subjected to the One-Way ANOVA test. The post hoc Duncan test was utilized for multiple comparisons of the groups. Data are presented as mean ± standard deviation. The statistical significance level was considered when $P \leq 0.05$ (Collins et al., 2009).

RESULTS AND DISCUSSION

Table 1 and Table 2 show the microbiological and physicochemical alterations observed in fish balls produced from mirror carp (*Cyprinus carpio L.*, 1758) when packaged with the MAP, and the samples were stored in the laboratory in the refrigerator at 4°C.

Microbiological results

In Table 1, the TAMB count of mirror carp fillet was $\log_{10}$CFU/g 4.42. The number of TAMB in the

Table 1. Microbiological analyses results of fillet and fish balls during storage at 4±1°C (log_{10} CFU/g) (mean ± standard deviation)

			Storage time (days)						
Microorganism	**Fillet**	**Groups**	**0**	**3**	**6**	**9**	**12**	**15**	**18**
Total aerobic mesophilic bacteria	4.42±0.19	K	4.17±0.13^{cA}	4.87±0.34^{cA}	5.97±0.53^{bA}	7.44±0.35^{aA}	NA	NA	NA
		D1	4.17±0.13^{dA}	4.79±0.23^{dA}	5.66±0.51^{cA}	6.20±0.60^{cB}	7.01±0.14b^{A}	7.93±0.35^{aA}	NA
		D2	4.17±0.13^{gA}	4.69±0.22^{fA}	5.16±0.26^{eA}	5.64±0.32^{dB}	6.23±0.25^{cB}	6.95±0.10^{bB}	7.86±0.17^{aA}
		D3	4.17±0.13^{eA}	4.60±0.26^{eA}	5.28±0.43^{dA}	5.62±0.28^{cdB}	5.98±0.22^{cB}	6.73±0.27^{bB}	7.52±0.12^{aA}
Psychrophilic bacteria	4.02±0.16	K	3.79±0.04^{dA}	4.35±0.07^{cA}	5.27±0.05^{bA}	6.75±0.08^{aA}	NA	NA	NA
		D1	3.79±0.04^{fA}	4.12±0.07^{eB}	4.61±0.14^{dB}	5.36±0.21^{cB}	6.02±0.19^{bA}	7.11±0.09^{aA}	NA
		D2	3.79±0.04^{gA}	4.12±0.06^{fBC}	4.24±0.07^{eC}	4.78±0.10^{dC}	5.36±0.17^{cB}	6.05±0.08^{bB}	6.83±0.06^{aA}
		D3	3.79±0.04^{fA}	3.94±0.12^{efC}	4.12±0.09^{eC}	4.71±0.18^{dC}	5.26±0.27^{cB}	5.97±0.10^{bB}	6.92±0.14^{aA}
Enterobacteriaceae	2.71±0.14	K	2.50±0.15^{dA}	3.18±0.12^{cA}	3.76±0.14^{bA}	4.48±0.45^{aA}	NA	NA	NA
		D1	2.50±0.15^{fA}	3.04±0.78^{eA}	3.45±0.16^{dB}	3.86±0.20^{cB}	4.24±0.11^{bA}	4.91±0.57^{aA}	NA
		D2	2.50±0.15^{eA}	3.02±0.08^{dA}	3.23±0.14d^{BC}	3.61±0.09^{cB}	3.77±0.18^{cB}	4.25±0.21^{bB}	4.86±0.10^{aA}
		D3	2.50±0.15^{fA}	2.97±0.07^{eA}	3.18±0.03^{dC}	3.53±0.13^{cB}	3.65±0.07^{cB}	4.10±0.14^{bB}	4.75±0.09^{aA}
Coliform	3.43±0.09	K	3.31±0.08^{dA}	4.16±0.36^{cA}	5.24±0.12^{bA}	6.17±0.11^{aA}	NA	NA	NA
		D1	3.31±0.08^{eA}	4.06±0.23^{dA}	4.54±0.28^{cB}	4.95±0.26^{bcB}	5.34±0.11^{bA}	6.30±0.13^{aA}	NA
		D2	3.31±0.08^{eA}	3.97±0.18^{dA}	4.30±0.18^{cdB}	4.59±0.20^{bcB}	4.86±0.28^{bA}	5.41±0.20^{aB}	5.66±0.18^{aA}
		D3	3.31±0.08^{fA}	3.94±0.23^{eA}	4.25±0.34^{deB}	4.61±0.20^{dB}	4.98±0.09^{cA}	5.39±0.21^{bB}	5.80±0.11^{aA}
Yeast and mould	2.75±0.17	K	3.12±0.45^{cA}	3.74±0.37^{bcA}	4.30±0.2^{6bA}	5.08±0.06^{aA}	NA	NA	NA
		D1	3.12±0.45^{dA}	3.54±0.34^{dA}	3.88±0.51^{cdA}	4.46±0.08^{bcAB}	5.02±0.04^{abA}	5.33±0.08^{aA}	NA
		D2	3.12±0.45^{dA}	3.46±0.35^{cdA}	3.64±0.49^{cdA}	3.97±0.49^{cdB}	4.30±0.62^{bcA}	4.83±0.09^{abB}	5.22±0.06^{aA}
		D3	3.12±0.45^{dA}	3.50±0.45^{cdA}	3.59±0.43^{cdA}	3.46±0.37^{cdB}	4.19±0.32^{bcA}	4.65±0.23^{abB}	5.00±0.24^{aA}

abcdefg: means in the same line with different letters are significantly different from the others (P<0.05); **ABC:** means in the same column with different letters are significantly different from the others (P<0.05); **NA:** Not analyzed; **K:** control; **D1:** 55% CO_2 + 15% O_2 + 30% N_2; **D2:** 65% CO_2 + 10% O_2 + 25% N_2; **D3:** 75% CO_2 + 10% O_2 + % 15 N_2

fish ball samples increased from the 3rd day during storage. It increased to 7.44 log_{10} CFU/g on day 9 of storage in the K group, 7.93 log_{10} CFU/g on day 15 in the D1 group, and 7.86 and 7.52 log_{10} CFU/g on day 18 in the D2 and D3 groups, respectively. Overall, TAMB counts increased during storage in all groups. The important differences (P≤0.05) were determined between groups on days 9, 12, and 15 (Table 1) (Figure 1). A significant difference was found statistically between the days within the group during storage period, beginning from day 6 in the K, D1, and D3 groups, and on all analysis days in the D2 group (P≤0.05) (Table 1). The K group had high levels of TAMB compared to other groups. Although limits are specified for some pathogens in seafood in the Turkish Food Codex Microbiological Criteria Regulation, no value is specified for the TAMB number (TFC, 2011). There are no criteria in the International Commission on Microbiological Specification for Foods (ICMFS, 1986) for prepared fish products. However, it stated that the maximum aerobic microorganism counts for fresh, frozen, and chilled fish is 7 log_{10} CFU/g (ICMFS, 1986). In this study, it was determined that the detected TAMB count exceeded the acceptable limit value on the 9th day in the K group, on the 15th day in the D1 group, and on the 18th day in the D2 and D3 groups. Thus the MAP treatment can be considered effective on TAMB inhibition. It is thought that this fact is caused by the different CO_2 ratios in the gas mixtures of the groups and the antimicrobial effect of CO_2 (Yesudhason et al., 2014; Chan et al., 2021). These results are also similar to the research findings of Altan (2020) and Yongji et al. (2010).

The psychrophilic bacteria number of mirror carp fillet was determined to be 4.02 log_{10} CFU/g. This count was consistently increased in all groups during storage, and it was detected that counts reached at the max level of 6.75 log_{10} CFU/g on day 9 in the K group, 7.11 log_{10} CFU/g on day 15 in the D1 group, 6.83 and 6.92 log_{10} CFU/g on day 18 in the D2 and D3 groups, respectively (Table 1) (Figure 2). There are statistically significant differences between the groups on the 3, 6, 9, 12, and 15 days (P≤0.05)

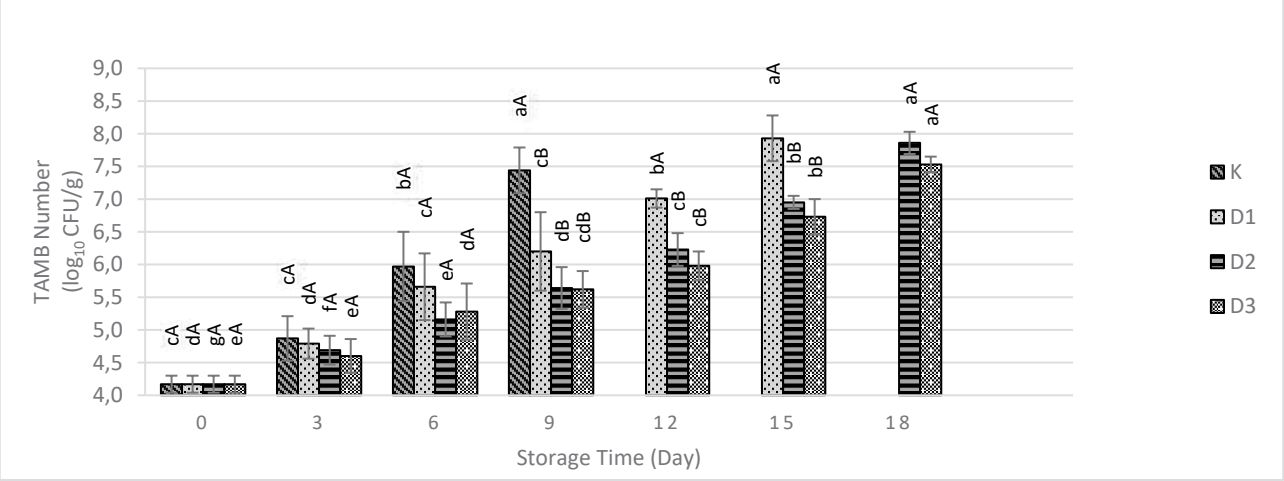

Figure 1. Changes in the total number of aerobic mesophilic bacteria in fish balls during the storage process (log_{10} CFU/g ± standard deviation).

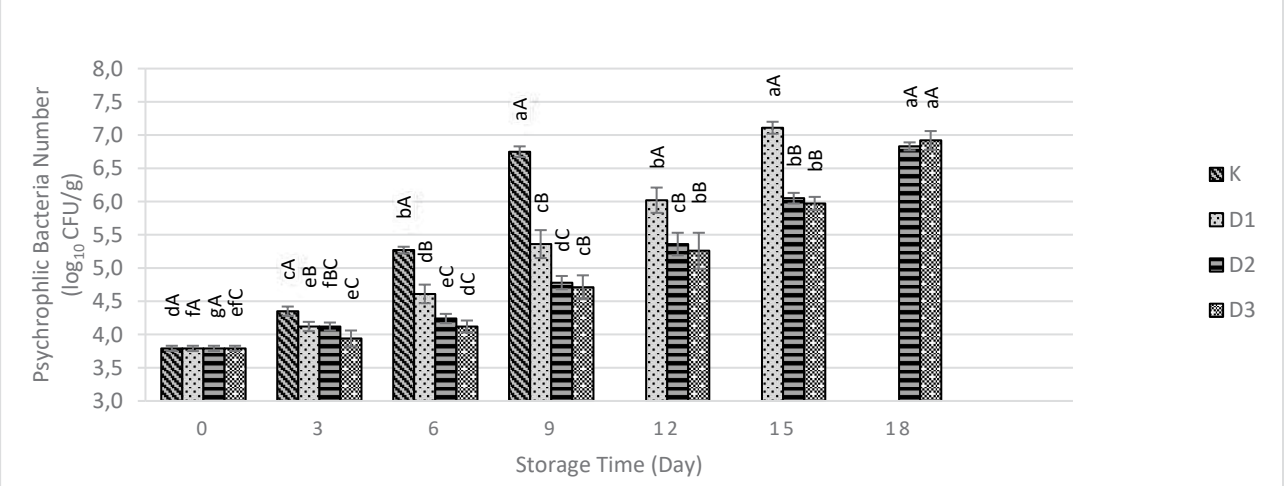

Figure 2. Changes in the total number of psychrophilic bacteria in fish balls during the storage process (log_{10} CFU/g ± standard deviation).

(Table 1). Throughout the entire storage period, an important difference was statistically found between the days in all groups (P≤0.05). Studies by Kaya (2019) and Altan (2020), showed similarities in terms of inhibiting the growth of psychrotrophic bacteria in groups with higher CO_2 ratios, although the compositions of the MAP gas mixtures and fish balls are different in this study.

The *Enterobacteriaceae* count is accepted as a hygiene indicator in food products. *Enterobacteriaceae* counts were determined at 2.71 log_{10} CFU/g in the fillet (Table 1) (Figure 3). The number of *Enterobacteriaceae* in all groups increased in parallel with the storage period. *Enterobacteriaceae* count was determined as 4.48±0.45 log_{10} CFU/g in the K group on day 9, 4.91±0.57 log_{10} CFU/g in the D1 group on day 15, 4.86±0.10 log_{10} CFU/g in the D2 group and 4.75±0.09 log_{10} CFU/g in the D3 group on day 18. There were also statistical differences observed between all groups on days 6, 9, 12, and 15 (P≤0.05). During storage period, it was determined that there was a statistically significant difference between the periods within the group, starting from day 6 in the K group, all analyses during storage in the D1 group, and on all days except for the 12th day in the D2 and D3 groups (P≤0.05). In studies conducted on rainbow trout fillets (Arahisar et al., 2004; Yılmaz, 2004), hake (*Merluccius merluccius*)

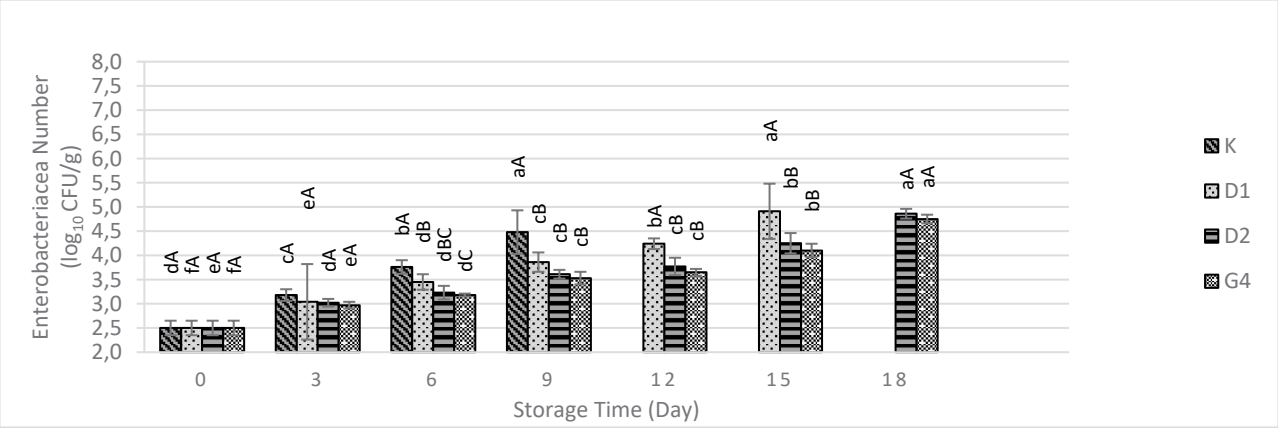

Figure 3. Changes in the total number of *Enterobacteriaceae* in fish balls during the storage process (log_{10} CFU/g ± standard deviation).

fillets (Antunes-Rohling et al., 2019), it was reported that the number of *Enterobacteriaceae* in raw trout fillets packaged using MAP technology with different gas compositions increased in parallel with the storage period in all groups. In the same studies, it was stated that the *Enterobacteriaceae* numbers of fillets packaged with MAP technology were lower than those in the control group (normal air) and vacuum packages and that as the CO_2 content in the packages increased, the growth rate slowed down and the shelf life extended. Our study results are similar to these results although the material used (fillet) was different.

Coliform bacteria, which were determined as 3.43 log_{10} CFU/g in the fillet, started to increase in all groups from the first day of storage. It was determined that coliform counts reached at the maximum level of 6.17 log_{10} CFU/g on day 9 in the K group, 6.30 log_{10} CFU/g on day 15 in the D1 group, 5.66 and 7.80 log_{10} CFU/g on day 18 in the D2 and D3 groups, respectively (Table 1) (Figure 4). It was observed that there was a closeness between the D2 and D3 groups in terms of the number of bacteria in the coliform group as the CO_2 ratio increased; however, there were important differences between the other groups including the K group. During storage, there was a statistically significant difference between the days within the group on all analysis days in the K, D1, and D3 groups, and on the other analysis days in the D2 group, except for the 18th day (Table 1) ($P \leq 0.05$). In the study conducted by Altan (2020), it was reported that the coliform bacteria count in-

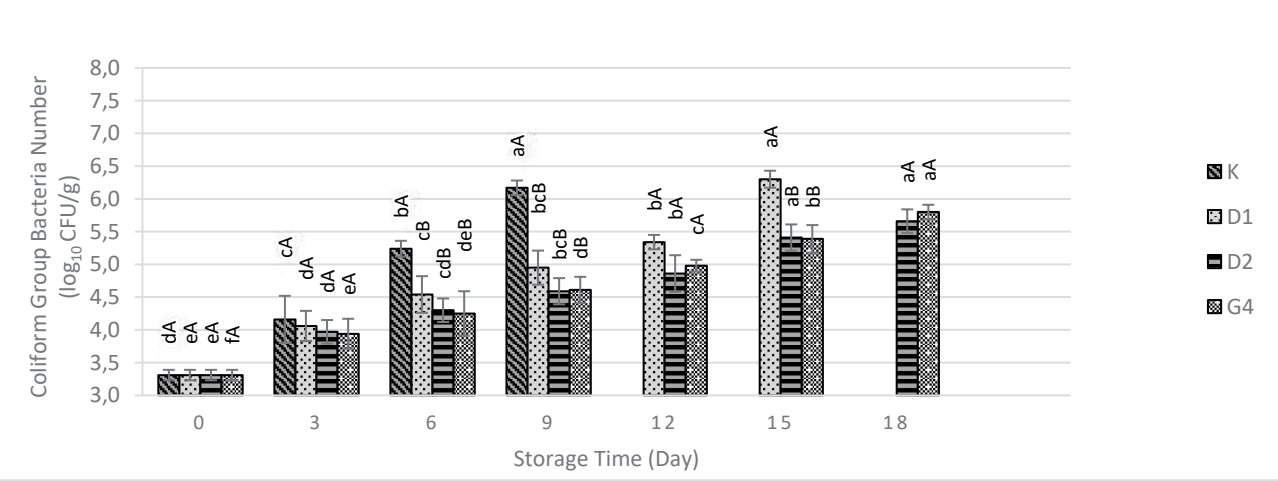

Figure 4. Changes in the total number of coliform group bacteria in fish balls during the storage process (log_{10} CFU/g ± standard deviation).

creased at the end of the storage. These findings are similar to our study. In the study performed on trout fish balls (Baygar et al., 2008; Guran et al., 2015), it was reported that the coliform group bacteria count decreased at the end of the storage period in the control group (normal air) and MAP2 (35% CO_2-60% N_2-5% O_2) groups, and increased in the group MAP1 (25% CO_2-70% N_2-5% O_2). The values in some groups of related studies differ in comparison to our findings. It is thought that these differences are due to the different gas combinations we use, the composition of the fish balls, and the kind of fish.

Yeast and molds are not found in the natural flora of fish meat. Yeast and molds are usually soil-based and are messed with from water, contact surfaces, tools, and equipment after being caught (Goktan, 1990; Patir et al., 2005; Patir et al., 2009). Yeast and mold count was determined as 2.75 $\log_{10}$ CFU/g in the fillet. This count increased in all groups, including the control, depending on the progression of the preservation (Table 1) (Figure 5). The yeast and mold numbers of the D2 and D3 groups were statistically lower ($P \leq 0.05$) than the other groups. During storage, significant differences were found in all analysis days in the K group, beginning from day 6 in the D1 group, and excluding days 6 and 9 in the D2 and D3 groups ($P \leq 0.05$). These findings are similar to the results of some studies (Kaya, 2019; Altan, 2020) stating that the number of yeast and mold increases during storage.

When evaluated in terms of microbiological data, the differences occurred between the current study and similar studies (Arashisar et al., 2004; Yilmaz, 2004; Cakli et al., 2006; Del Nobile et al., 2009; Yesudhason et al., 2009; Liu et al., 2010; Provincial et al., 2010; Yesudhason et al., 2014; Quian et al., 2015; Zhu et al., 2016; Smaldone et al., 2017; Nayma et al., 2020; Mahdavi and Ariaii, 2021; Chan et al., 2021) due to many factors as, production hygiene, gas combinations used, the composition of fish balls, microbial quality of fish meat, additives and spices, and storage conditions of fish balls.

Physicochemical results

One of the initial chemical alterations observed in fish meat is the fluctuation in its pH value (Baygar et al., 2008). Several factors, such as fish species, its dietary habits, the season, and muscle structure, can cause variations in the pH of live fish. Such variations can considerably influence the quality of the fish meat post-capture. Generally, while the pH of live fish muscle hovers around 7.2-7.3, it shifts to a range of 6.0-6.5 in freshly caught fish (Anonymous, 2013). In this study, the pH values of fish balls are presented in Table 2. The pH value of the mirror carp fillet was found to be 6.63. The pH value decreased in parallel with the storage period in all groups (Table 2) (Figure 6). At the end of the storage period, the pH values in all fish ball groups were observed to range between 5.77-6.10 with no statistical difference ($P > 0.05$) between groups. After the fish is cut, anaerobic respiration is activated and the lactic acid formed causes the pH of the fish meat to decrease (Liu et al., 2013). This is thought to be the reason for the drop in pH in the control group, also the decrease in pH value occurring in MAP groups is thought to be due to the carbonic acid formed (Dermiki et al.,

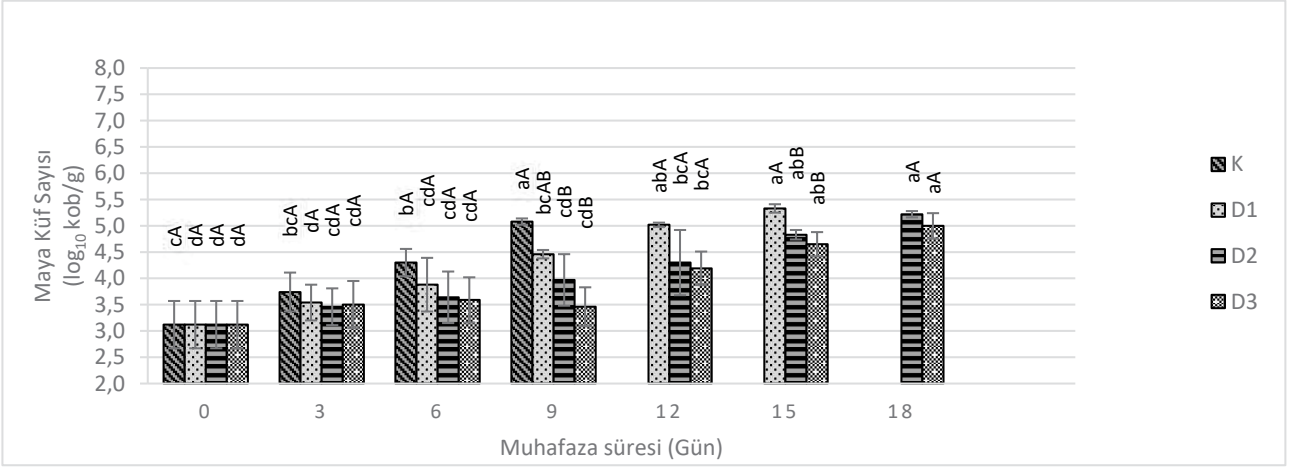

Figure 5. Changes in the total number of yeast and mold in fish balls during the storage process ($\log_{10}$ CFU/g ± standard deviation).

Table 2. Chemical analyses results of fish balls during storage at 4±1°C (mean ± standard deviation)

			Storage time (days)						
Analysis	**Fillet**	**Groups**	**0**	**3**	**6**	**9**	**12**	**15**	**18**
pH	6.63±0.11	K	6.44±0.08^{aA}	6.37±0.03^{abA}	6.23±0.13^{bcA}	6.10±0.06^{cA}	NA	NA	NA
		D1	6.44±0.08^{aA}	6.35±0.11^{abA}	6.30±0.06^{abA}	6.26±0.04^{bA}	5.98±0.03^{cA}	5.77±0.08^{dA}	NA
		D2	6.44±0.08^{aA}	6.34±0.12^{aA}	6.33±0.10^{aA}	6.24±0.11^{abA}	6.04±0.30^{abA}	5.83±0.5^{cA}	5.79±0.02^{cA}
		D3	6.44±0.08^{aA}	6.40±0.19^{abA}	6.36±0.08^{abA}	6.20±0.16^{bcA}	6.06±0.21^{cdA}	5.96±0.09^{cdA}	5.82±0.11^{dA}
TVB-N	20.20±1.08	K	17.80±0.24^{cA}	19.53±0.76^{cA}	19.53±0.76^{cA}	30.56±0.76^{aA}	NA	NA	NA
		D1	17.80±0.24^{eA}	19.99±0.34^{dA}	22.52±0.48^{cA}	23.98±1.52^{cB}	31.07±0.61^{bA}	36.31±0.47^{aA}	NA
		D2	17.80±0.24^{fA}	19.14±0.90^{fA}	21.69±1.30^{eA}	24.51±0.78^{dB}	28.98±0.97^{cB}	33.74±0.57^{bB}	36.52±0.88^{aA}
		D3	17.80±0.24^{gA}	19.37±1.12^{fA}	23.25±1.35^{eA}	25.07±0.31^{dB}	29.34±1.00^{cB}	33.46±0.21^{bB}	35.55±0.71^{aA}
TBA	0.32±0.04	K	0.39±0.02^{dA}	0.48±0.03^{cA}	0.82±0.06^{bA}	1.39±0.04^{aA}	NA	NA	NA
		D1	0.39±0.02^{eA}	0.51±0.05^{eA}	0.89±0.07^{dA}	1.35±0.12^{cA}	1.56±0.06^{bA}	2.09±0.05^{aA}	NA
		D2	0.39±0.02^{fA}	0.43±0.07^{fA}	0.70±0.04^{eB}	1.14±0.09^{dB}	1.33±0.07^{cB}	1.85±0.13^{bB}	2.25±0.08^{aA}
		D3	0.39±0.02^{fA}	0.44±0.05^{fA}	0.60±0.05^{eB}	0.97±0.09^{dB}	1.22±0.09^{cB}	1.74±0.06^{bB}	2.20±0.03^{aA}
Dry Matter	22.78±1.58	K	36.37±0.16^{aA}	36.27±0.22^{aA}	36.38±0.14^{aA}	36.45±0.35^{aA}	NA	NA	NA
		D1	36.37±0.16^{cA}	36.21±0.27^{cA}	36.46±0.16^{bcA}	36.43±0.06b^{cA}	36.78±0.18^{abA}	36.86±0.21^{aA}	NA
		D2	36.37±0.16^{aA}	36.56±0.21^{aA}	36.78±0.29^{aA}	36.86±0.26^{aA}	36.62±0.23^{aA}	36.92±0.12^{aA}	37.02±0.15^{aA}
		D3	36.37±0.16^{bA}	36.38±0.17^{bA}	36.73±0.26^{aA}	36.88±0.18^{aA}	36.76±0.20^{aA}	36.94±0.10^{aA}	36.88±0.05^{aA}

abcdefg: means in the same line with different letters are significantly different from the others (P<0.05); **ABC:** means in the same column with different letters are significantly different from the others (P<0.05); **NA:** Not analyzed; **K:** control; **D1:** 55% CO_2 + 15% O_2 + 30% N_2; **D2:** 65% CO_2 + 10% O_2 + 25% N_2; **D3:** 75% CO_2 + 10% O_2 + % 15 N_2

2008). In this study, no statistical difference was found between the samples considering the control and the MAP groups (P>0.05). These results are consistent with the values reported in trout balls (Caklı et al., 2006; Baygar et al., 2008; Kaya, 2019) packed with MAP technology.

The total volatile base nitrogen (TVB-N) is used as an important indicator among the freshness criteria of seafood. As fish deteriorates, there's a noticeable rise in the concentration of volatile basic nitrogenous compounds. This uptick can be attributed to the action of both bacteria and endogenous enzymes in the fish (Anonymous, 2013). Varlik et al. (1993) described in quality classification that the amount of TVB-N as up to 25 mg N/100 g "very good", up to 30 mg N/100 g "good", up to 35 mg N/100 g "marketable", more than 35 mg N/100 g as "deteriorated". When the TVB-N level in fish meat reaches 35-40 mg TVB-N/100 g, it is considered that the food has spoiled (Fan et al., 2008). In this study, the TVB-N value in raw fish meat used in the production of fish balls was determined as 20.20±1.08 mg N/100g. The TVB-N value increased during storage in all groups (Table 2). The TVB-N

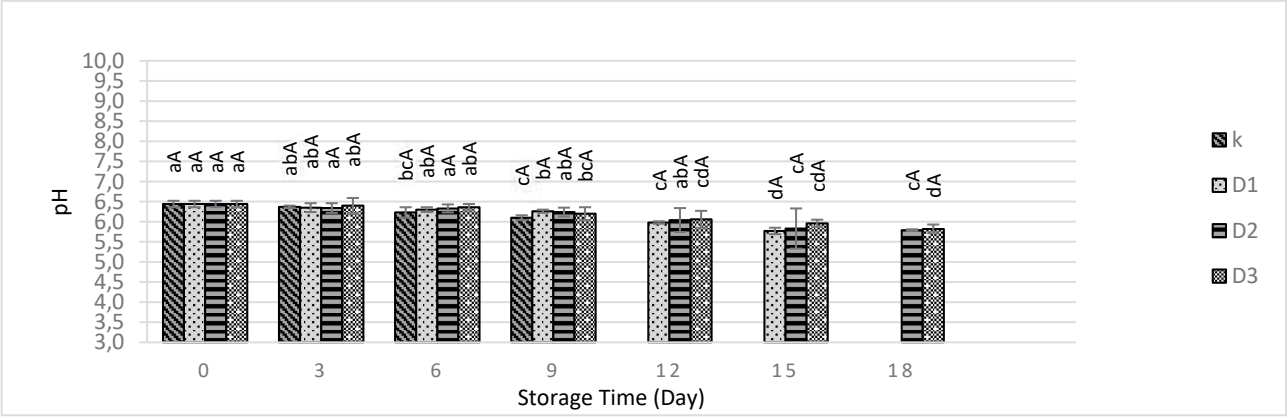

Figure 6. Changes in pH value of fish balls during storage (mean ± standard deviation).

value was detected as 17.80±0.24 mg N/100g on day 9 in the K group, 36.31±0.47 mg N/100g on day 15 in the D1 group, 36.52±0.88 mg N/100g on day 18 in the D2 group, and 35.55±0.71 mg N/100g in the D3 group. Significant differences (P≤0.05) were detected between the groups on days 9, 12, and 15 (Table 2) (Figure 7). It is thought that the reason for this increase may be a result of the oxidation of amines, their degradation by microbial activity, the presence of autolytic enzymes of nucleotides, and the deamination of free amino acids (Ocano-Higuera et al., 2011). The reported studies are similar to the other results (Altan, 2020) found in fish products in which MAP technology has been applied.

Thiobarbituric acid index (TBA), which is an indicator of lipid oxidation in fatty food products, is a criterion used to determine the freshness of seafood products. The TBA shows variabilities according to the kind of fish, the quantity of fat, the season in which it is caught, and the storage conditions (temperature, humidity, light, etc.). It plays an important role in the occurrence of oxidation reactions (Ruiz-Capillas and Moral, 2001). The TBA, which is the rancidity index of fish and fish products resulting from the oxidation of fats, should be less than 3 mg malonaldehyde/kg in a very good product, and it should not be more than 5 mg malonaldehyde/kg in a good product. It is stated that the limit value of the consumption the TBA should be between 7-8 mg malonaldehyde/kg (Varlık et al., 1993; TFC, 2011). In this study, the TBA in fish ball samples was determined as 0.32±0.04 mg malonaldehyde/kg. On day 3rd of the storage, the TBA levels were found to be 0.48±0.03 mg malonaldehyde/kg for the K group, 0.51±0.05 mg malonaldehyde/kg for the D1 group, 0.43±0.07 mg malonaldehyde/kg for the D2 group, and 0.44±0.05 mg malonaldehyde/kg for the D3 group. The TBA value increased during storage in all groups (Table 2) (Figure 8). The TBA levels increased from 0.39 to 1.39 mg malonaldehyde/kg in the K group, from 0.39 to 2.09 mg malonaldehyde/kg in the D1 group, from 0.39 to 2.25 mg malonaldehyde/kg in the D2 group, from 0.39 to 2.20 mg malonaldehyde/kg in the D3 group at the end of the storage. There were significant differences between the groups on days 6, 9, 12, and 15 of storage (P≤0.05) (Table 2). In a study performed on trout fish balls (Kaya, 2019; Altan, 2020), it was stated that TBA incremented in all groups during storage. The TBA in our study shows parallelism with Altan's study (2020).

In the present research, the initial DM content of the mirror carp fillet was found to be 22.78%. During storage, the DM levels displayed variations across different groups: by the 9th day, the K group registered 36.45%, the D1 group marked 36.86% on the 15th day, while the D2 and D3 groups documented 37.02%, and 36.88%, respectively, by the 18th day (as depicted in Table 2) (Figure 9). However, it's noteworthy that the variances in DM content among these groups during storage were not statistically significant (P>0.05). Our findings align with those of previous research on fish products packaged using the MAP technology (Altan, 2020).

CONCLUSIONS

In conclusion, the application of the MAP in fish balls from mirror carp significantly extends their

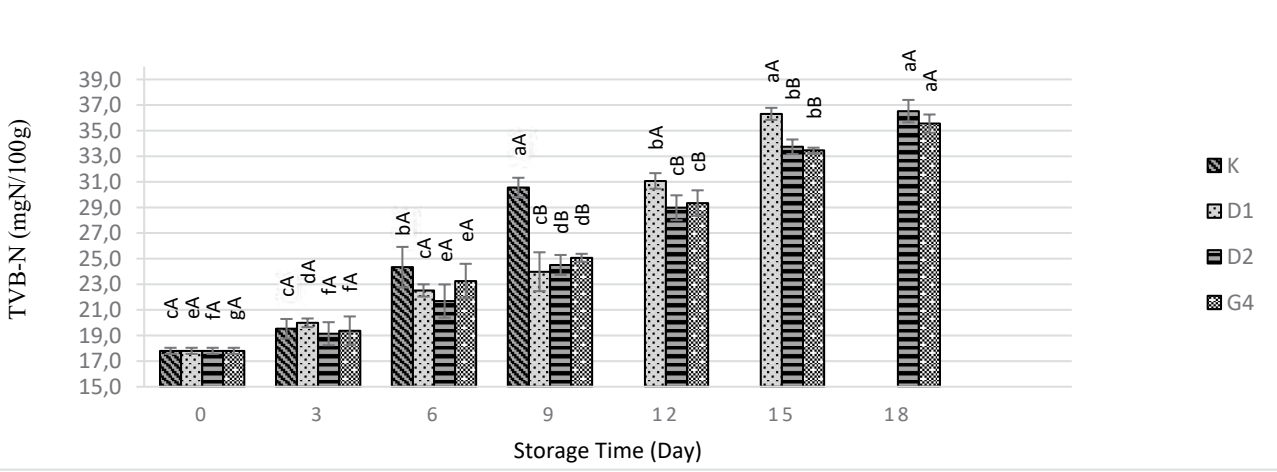

Figure 7. Changes in TVB-N value of fish balls during storage (mean ± standard deviation).

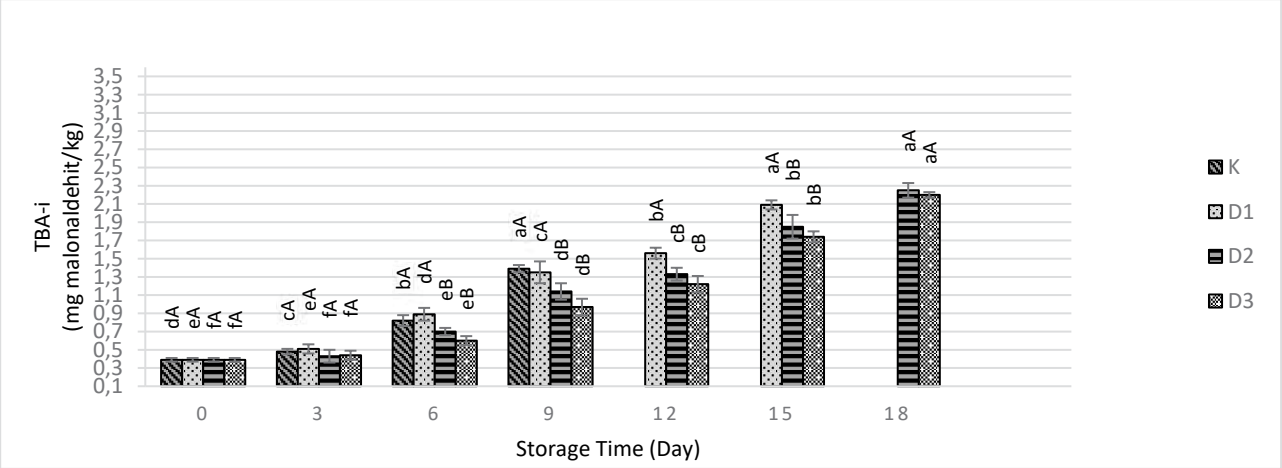

Figure 8. Changes in the TBA levels of fish balls during storage (mean ± standard deviation).

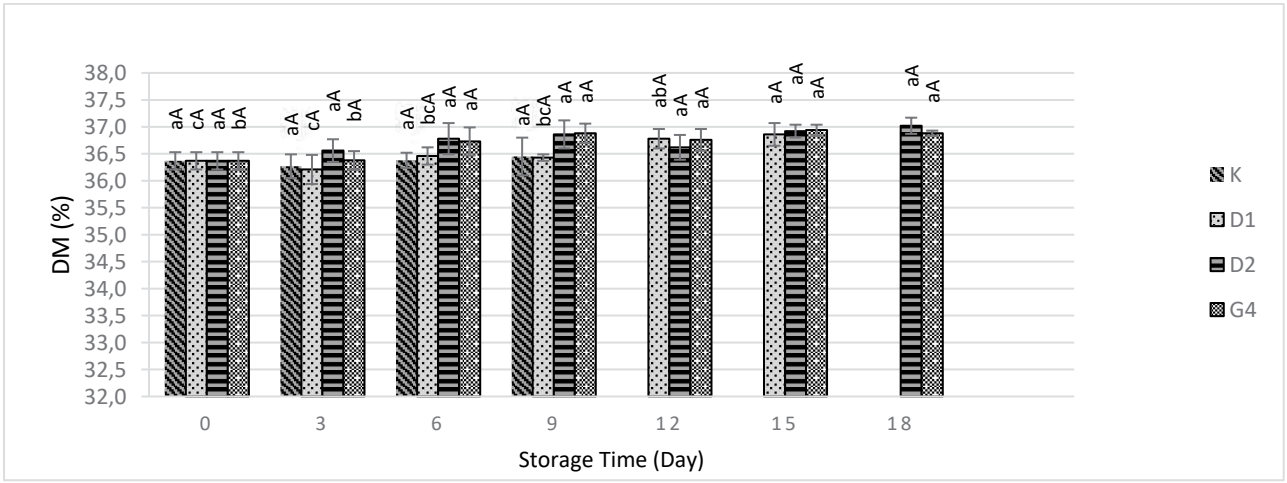

Figure 9. Changes in DM content of fish balls during storage (mean ± standard deviation).

shelf life. This is attributed to the increase in CO_2 and decrease in O_2 levels within the packages, leading to reduced microbial growth and declines in TVB-N and the TBA values. Notably, treatments with 65% CO_2, 10% O_2, and 25% N_2 (D2) and 75% CO_2, 10% O_2, and 15% N_2 (D3) yielded optimal results, allowing the fish balls to be preserved for up to 18 days at 4ºC. Employing MAP, especially with a high CO_2 and low O_2 composition, can effectively prolong the shelf life of processed mirror carp fish balls to 18 days, eliminating the need for chemical additives. This offers a healthier and alternative option to traditional meatballs made from red meat and poultry. Furthermore, harnessing MAP technology in packaging not only enhances the longevity of mirror carp products but also stands to benefit the broader aquaculture economy. In the future, combined MAP methods can be developed and contributed to the literature by adding different types of fish and various preservatives.

CONFLICT OF INTEREST STATEMENT

The Authors declared that there are no conflicts of interest. There has been no significant financial support for this study. This article is derived from Erdoğan DOĞAN's Master thesis. A part of this study was presented as a summary in the form of an oral presentation at 9th International European Conference on Interdisciplinary Scientific Research (January 19-21, 2024 / Valencia, Spain).

AUTHORS' CONTRIBUTIONS STATEMENT

ED, PB, and AA performed conceptualization, methodology, formal analysis, investigation, writing - original draft, writing- review and editing, visualization.

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