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The Trout Journal of Atatürk University Araştırma/Research

Purification, characterization and determination of kinetic features of carbonic anhydrase from turbot (*Psetta maxima) muscle* tissue

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Abstract: In this study, carbonic anhydrase purification from turbot (*Psetta maxima*) muscle tissue, together with analysis of the kinetic behavior and some enzyme properties, is described. The purification steps comprised hemolysate preparation, Sepharose-4B-L tyrosine-sulfanilamide affinity gel chromatography, and dialyzing. The yield was 69.05%, and the enzyme was found to have a specific activity of 755.2 EU/mg protein. The overall purification was about 50.65-fold. A temperature of +4 °C was maintained during the purification process. The molecular mass of the subunit was determined to be 29.7 kDa by SDS polyacrylamide gel electrophoresis (SDS-PAGE). The enzyme had an optimal pH of 8.0, a stable pH of 8.0, and an optimal temperature of 30 °C. Km and Vmax for p-nitrophenylacetate as a substrate were also determined.

Keywords: Fish, carbonic anhydrase, muscle, turbot

INTRODUCTION

Turbot, a member of the flatfish order, consists of 14 families and 716 subspecies (Munreo, 2005). As a habitat, they spread to the region, extending from the Icelandic and Baltic coasts in the north to the Scandinavian coasts and the Moroccan coasts in the south, to the Black Sea and the Mediterranean. The length of the turbot fish living on the bottom and in muddy and sandy areas at depths of 70-100 meters reach 1 meter in places. Turbot fish migrate from mid-March to mid-May in coastal waters to lay eggs (Hara, 2001).

Turbot fish have eyes on one side and are blind on the other. One of the long and simple ventral fins is on the eye side, and the other is on the blunt side. There are approximately 11-12 spine-like structures on its gills (Memis, 2010). In order to survive, they feed on small fish such as rockfish, silverfish, anchovies, fry, and mainly crustaceans (Samsun, 2004). The egg productivity of turbot fish may differ from each other.

While the average egg number of Atlantic shields is 3.5-4.2 million for adult individuals, the total egg quantity of Black Sea shields is 9 million annually (Jones, 1974; Samsun, 2004). Turbot production varies between 10100 and 14100 tons per year in Europe (Sevgili and Nezaki, 2010). In China, turbot production through aquaculture has reached levels of 50000–60000 tons in recent years (FAO, 2010).

The catalytic mechanism of the carbonic anhydrase enzyme has been tried to be clarified as a result of the studies carried out in the last sixty years. It has been understood that the CA enzyme has advantageous properties such as being extremely important in metabolism, being stable in the solution environment, and being kept for a long time without losing its activity under suitable conditions (Supuran and Scozzafava, 2001).

Today, it is known that the carbonic anhydrase (carbonate hydrolase, EC: 4.2.1.1, CA) is present in all animals, organisms with photosynthetic cells, and all organisms such as bacteria, and has 14 different isoenzymes depending on the proteins to which it is attached (Chegwitten et al., 2000, Solis et al., 1999, Clare and Supuran, 2000).

Carbonic anhydrase (CA) reversibly catalyzes the CO_2 hydration and the HCO_3^- dehydration in organisms; it also plays a role in the H+ and HCO_3^- accumulation in kidney, gastric mucosa, and eye lens tissues (Supuran et al., 2003, Şentürk et al., 2009). In addition, the important roles of this enzyme have been proven in the gills and glandular organs of fish, in some insects and bacteria, in the production of shells of crustaceans and in the formation of egg shells, in algae, and in terrestrial plant chloroplasts (Graham et al., 1984, Tsuzuki and Miyachi, 1989, Badger and Price, 1994).

The important function of the CA enzyme in the gill membrane of cartilaginous fish is related to pH and/or CO_2 sensitivity in the respiratory system, in addition, it ensures the continuity of CO_2 excretion. (Henry et al., 1988).

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Our aim is to determine the kinetic properties of the carbonic anhydrase (CA) enzyme from the muscle tissue of turbot fish for the first time.

MATERIALS AND METHODS

Chemicals

Chemicals used in our studies, p-nitrophenyl acetate, N,N,N',N'-tetramethylethylene diamine (TEMED), Sepharose-4B, a dialysis bag, standard serum albumin, and L-tyrosine from Sigma Chemical Comp. sodium hydroxide, sulfanilamide, sodium bicarbonate, sodium sulfate, trihydroxymethylaminomethane (Tris), sodium acetate, sodium perchlorate, 2-mercaptoethanol, sulfuric acid, bromine thymol blue, glycine, phosphoric acid, hydrochloric acid, sodium barbital, methanol, ethanol, isoproponal, acetone, sodium nitrogenate, acrylamide, N,N'-methylene bisacrylamide, R-250 from E. Merk AG, coomassie brillant blue G-250; carbon dioxide gas and sodium hydroxide

Procurement of turbot muscle tissue and preparation of homogenate

Ten turbot fish (*Scophthalmus maximus*) were purchased from a fish farm in the Samsun province on the Black Sea coast of Turkey. Turbot fish were taken to the laboratory under cold-chain conditions, and muscle tissue was removed. Then, the blood and other impurities in the tissue samples were eliminated by washing with 0.9% NaCl, and this process was repeated 3 times. In order to prepare the tissue homogenate, the sample was first cut into small pieces with the help of a scalpel. It was then disintegrated in liquid nitrogen and homogenized in 10 volumes of 25 mM Tris HCl / 0.1 M Na_2SO_4 (pH= 8.7) buffer. The prepared suspension was centrifuged at 10,000 rpm for 30 minutes, and the supernatant was used for analysis. All experiments were performed in triplicate (Wistrand, 2002).

Carbonic anhydrase purification

The supernatant sample was added to a buffer solution containing Tris-HCl/Na_2SO_4 (25 mM/0.1 M) at pH 8.7 for kinetic studies. The pH-adjusted homogenate was loaded onto the Sepharose-4B-l-tyrosine-sulphanilamide affinity column and was washed with Na_2SO_4 (22 mM), in Tris–HCl buffer solution (25 mM, pH8.7). At the end of this process, the carbonic anhydrase enzyme was retained in the column. Then, 0.1 M $NaCH_3COO.3H_2O$/0.5 M $NaClO_4$ (pH=5.6) solution was applied to the column to obtain the CA enzyme. The flow rate of the column was fixed at 20 ml/h using a peristaltic pump (Kucuk and Gulcin, 2016).

Measurement of carbonic anhydrase activity

CA was detected by changes in absorbance at 348 nm of p-nitrophenyl acetate to p-nitrophenolate over a period of 3 min at 25°C using a spectrophotometer. The enzymatic reaction performed contained 0.4 mL Tris–SO_4 buffer solutions (0.05 M, pH 7.4), 0.36 mL of p-nitrophenyl acetate (3 mM), 0.22 mL of water, and 0.2 mL of enzyme solution in a total volume of 1 mL. An enzyme solution was not added to the control sample (Kucuk and Gulcin, 2016).

SDS-PAGE study

After the purification of the enzymes was completed, the purity level of the purified enzymes was examined by applying 3-8% batch SDS-PAGE as described by Laemmli (1970). SDS polyacrylamide gel electrophoresis was performed to confirm the enzyme purity following the purification steps. The running and stacking gels contained 3% acrylamide, 10% acrylamide, and 0.1% SDS. A 20-µg sample was applied to the electrophoresis medium. Gels were stained for 1.5 h in 0.1% Coommassie Brilliant Blue R-250 in 10% acetic acid and 50% methanol (Şentürk et al., 2009).

Qualitative Protein Determination

Qualitative protein determination is achieved by determining the maximum absorbance of tryptophan and tyrosine in the structure of proteins at 280 nm (Segel, 1968). Qualitative protein determination in equal volume fractions obtained in chromatography processes was performed with the help of this method.

Protein determination by the Bradford method

The protein amounts in the homogenate and the enzyme solution purified by affinity chromatography were determined by this method. Protein detection during purification was performed spectrophotometrically at 595 nm according to the Bradford method. Bovine serum albumin was used as a standard. (Bradford, 1976).

Optimum pH study for the carbonic anhydrase enzyme

The activity values of the CA enzyme were measured in 20 mM potassium phosphate (KH_2PO_4) solutions with a pH between 7.0-9.0 and pH 5.0-8.0 and 20 mM Tris-HCl (Ceyhun et al., 2011).

Ionic strength study for the carbonic anhydrase enzyme

The pH of Tris-HCl and potassium phosphate buffers was taken at 8.0 from turbot muscle tissue, where the activity of the CA enzyme is at its optimum level. Esterase activity measurement for CA enzyme was performed in Tris-HCl buffer with ionic strength between 10 mM and 1000 mM, and then the results were brought in graphic form (Ceyhun et al., 2011).

Studies related to finding the stable pH of the carbonic anhydrase enzyme

SO_4-Tris buffer with a pH of 7.0-9.0 was used to detect the pH at which enzymes are stable. After mixing 500 µl of buffer solutions with 500 µl of enzyme solution in the pH range shown, it was kept at 4 °C. Activity measurements were made with an interval of 2 days, and the pH value at which the enzyme was stable was detected. The values of the activity

values corresponding to the incubation time were plotted (Ceyhun et al., 2011).

Investigation of the effect of temperature on muscle tissue carbonic anhydrase enzyme

In order to determine the effect of temperature on CA enzyme activity, enzyme activities were measured between 20 ºC and 70 ºC, and the optimum operating temperature was determined at its optimum pH and optimum ionic strength value (Ceyhun et al., 2011).

Studies on finding KM and Vmax values for p-nitrophenyl acetate substrate

Using five different concentrations of p-nitrophenyl acetate, the activity of CA enzymes purified from turbot muscle tissue was evaluated under different optimum conditions. Then the Lineveawer-Burk graph was drawn, and calculations were made by looking at the KM and Vmax values from this graph (Ceyhun et al., 2011).

RESULTS and DISCUSSION

There are many studies carried out in the form of carbonic anhydrase enzyme purification and characterization in many living tissue species in order to determine how and where the enzyme is located in a living organism and how it functions (Söyüt et al., 2008; Göçer et al., 2015; Göksu et al., 2014; Maheshwari et al., 2019; Jo and Hwang, 2019; Del Prete et al., 2016). However, no study has been found on the purification and kinetic properties of the CA enzyme in turbot muscle tissue. Carbonic anhydrase (CA) reversibly catalyzes CO_2 hydration and HCO_3^- dehydration in organisms; it also plays a role in H+ accumulation and HCO_3^- in tissues (Supuran et al., 2003; Şentürk et al., 2009). The important roles of this enzyme have been proven in the gills and glandular organs of fish, in some insects and bacteria, in the production of shells of crustaceans and in the formation of eggshells, in algae, and in terrestrial plant chloroplasts (Graham, et al., 1984, Tsuzuki and Miyachi, 1989, Badger and Price, 1994). The main function of the CA enzyme in the gill membrane of cartilaginous fish is related to pH and/or CO_2 sensitivity in the respiratory system; in addition, it ensures the continuity of CO_2 excretion. (Henry et al., 1988). Many studies have been conducted on the purification of CA enzymes in different organisms. In our study, we purified and characterized the CA enzyme from turbot muscle tissue using a sepharose-4B-l-tyrosine-sulphanilamide affinity column. With the help of the preparation of CA enzyme homogenate from turbot muscle tissue and Sepharose 4B-tyrosine-sulfanilamide affinity chromatography methods, the enzyme with a specific activity of 755.2 EU/mg protein was purified 69.05 times with a yield of 50.65% (Table 1).

Table 1. Enzyme unit, specific activity and purification values of CA enzyme purified from turbot muscle tissue from affinity column

Purification steps	Total volume (mL)	Protein (mg/mL)	Total protein (mg)	Total activity (EU)	Specific activity (EU/mg)	Yield (%)	Purification factor
Homogenate	8	17.6	140.8	2100	14.91	100	1
Sepharose-4B-tyrosine-sulfanilamide affinity column chromatography	6	0.32	1.92	1450	755.20	69.05	50.65

SDS-polyacrylamide gel electrophoresis was performed to examine the purity of the CA enzyme obtained from turbot muscle tissue. Standard protein markers with known molecular weights were used (29 kDa bovine carbonic anhydrase; 16.5 kDa egg white lysozyme), and isoenzymes obtained by purification from humans were applied to SDS-polyacrylamide gel electrophoresis. Photographed after the proteins became apparent. A purified enzyme was obtained, showing a single band in SDS-PAGE (Figure 1).

The molecular mass of the subunit was determined to be 29.7 kDa by SDS polyacrylamide gel electrophoresis (SDS-PAGE). Our findings were similar to those of previous studies (Demirdağ et al., 2013; Kucuk and Gulcin, 2016). When the studies on CA purification in different organisms and tissues are examined, CA is 30.5 kDa in the gills of sea bream, CA 29 kDa in the erythrocytes of zebrafish, 28 kDa in the gills of Antarctic icefish, and 29.4 kDa in the liver tissues of rainbow trout (Kaya et al., 2013; Rizzello et al., 2007; Peterson et al., 1997). A step affinity chromatographic technique was used (Ceyhun et al., 2011; Ekinci et al., 2010). It has been determined that the enzyme is stable at pH = 8.0, the optimum ionic strength is 20 mM Tris-HCl at pH = 8.0, and the

temperature with the highest activity is 20°C (Figs. 2, 3, 4, 5, 6).

Similar findings were found in other studies (Öztürk Sarikaya et al., 2011; Ceyhun et al., 2011; Bayram et al., 2008; Ekinci et al., 2010). With their catalytic versatility, alpha-CAs phosphatases can act as esterases and paraoxonases. (Demirdağ et al., 2013). Thus, we have researched the esterase activity of the turbot enzyme with 4-nitrophenyl acetate (NPA) as a substrate. The KM and Vmax values were calculated for NPA hydrolysis catalyzed by the fish enzyme by means of Lineweaver-Burk graphs (Fig. 7). In the present study, at pH 8.0, the Vmax value was 0.253 EU/ml and the KM value was 0.344 mM for p-nitrophenyl acetate (Fig. 7).

Consequently, we purified carbonic anhydrase from turbot muscle tissue for the first time and analyzed the properties of this enzyme.

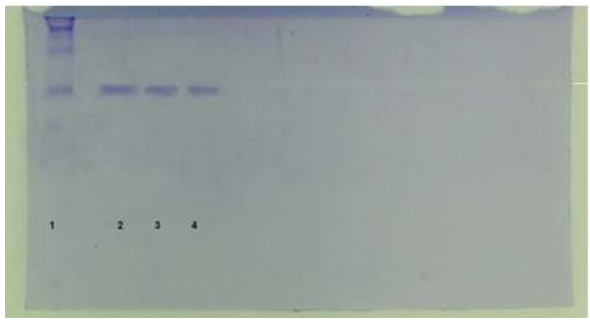

Figure 1. SDS-polyacrylamide gel electrophoresis photograph of turbot muscle carbonic anhydrase enzyme purified by affinity chromatography ((1) standard proteins (E. coli β-galactosidase 116 kDa, rabbit phosphorylase B 97 kDa, bovine serum albumin 66 kDa, and bovine erythrocyte CA 29 kDa), (2), (3) and (4) Muscle CA)

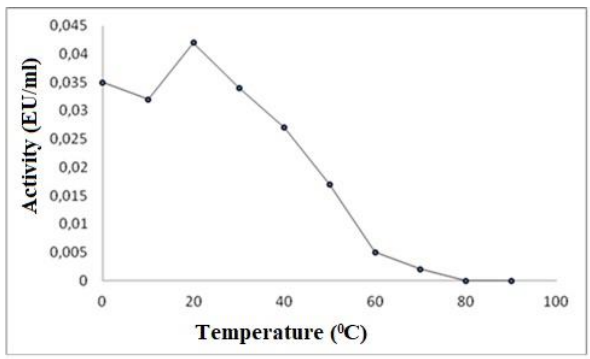

Figure 2. Optimum temperature measurement for turbot muscle CA enzyme and the temperature-activity graph of the values drawn

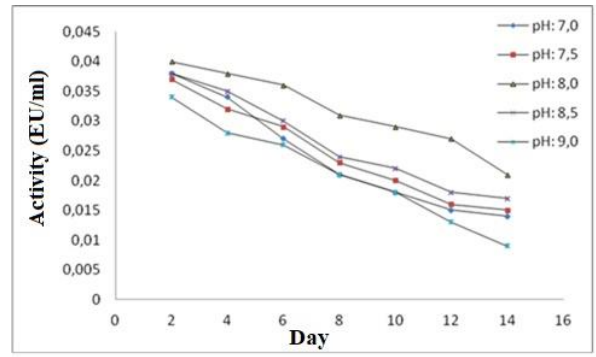

Figure 3. Stable pH graph for turbot muscle CA enzyme obtained using 20mM Tris HCl buffer solution at different pH

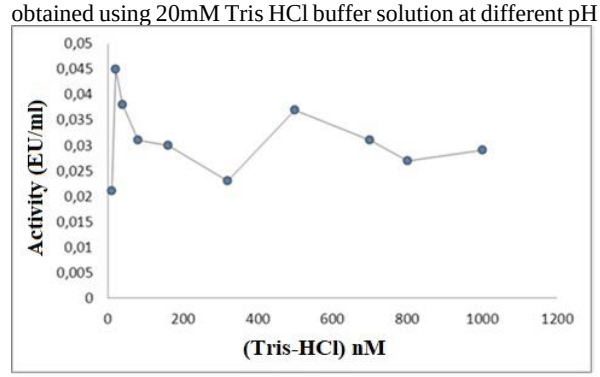

Figure 4. Ionic strength-activity graph for turbot muscle tissue CA enzyme obtained using Tris-HCl buffer solutions at different concentrations

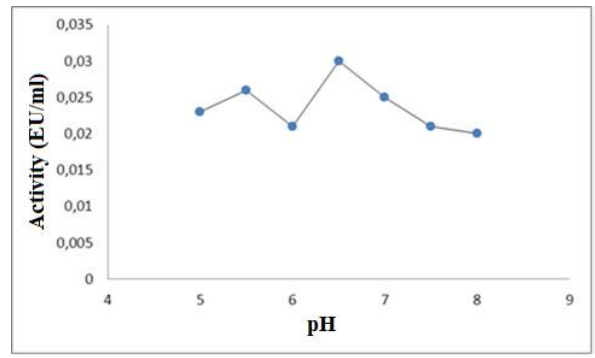

Figure 5. Activity measurement graph using 20mM phosphate buffer solution for optimum pH of turbot muscle CA enzyme

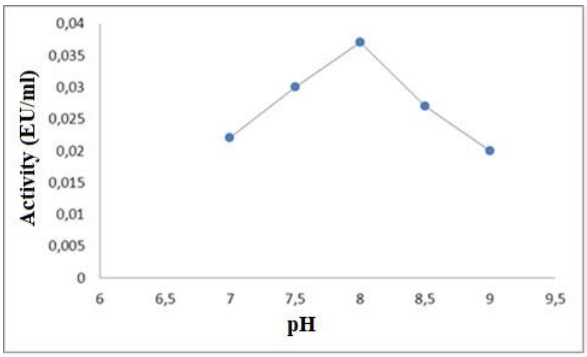

Figure 6. Activity measurement graph created using 20 mM Tris-HCl buffer solution for optimum pH of turbot muscle CA enzyme

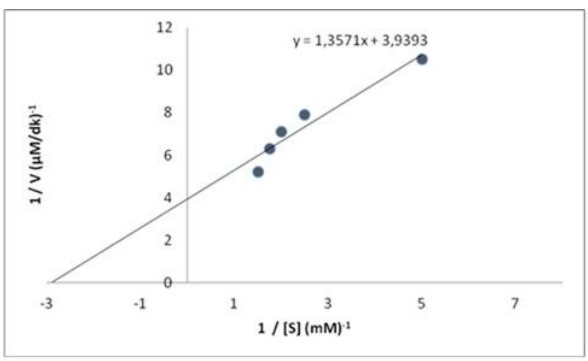

Figure 7. Lineweaver-Burk curves for turbot muscle CA enzyme

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CONFICT OF INTEREST

The authors declare no competing interests

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Ethics committee approval is not required.

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Antibiofilm activities and *in vitro* susceptibility testing of eucalyptus (*Eucalyptus camaldulensis*) essential oil (EO) against fish pathogen *Pseudomonas* species

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Abstract: Essential oils are concoctions of aromatic and volatile chemicals extracted from several plant species. These mixes have been used by society for a variety of reasons, and they play significant functions in nature. This study aimed to analyze the biological properties of essential oil extracted from *Eucalyptus camaldulensis* leaves, including its antipseudomonal and antibiofilm effects. Antibiotics have been heavily used both to treat bacterial infections and to stimulate fish growth, which has led to the emergence of germs that are resistant to the drugs. The study inoculums have been defined by the McFarland 0.5 standard and disk diffusion method has been used to analyze antimicrobial activity. The essential oils from *E. camaldulensis* possessed antibacterial activity against tested *Pseudomonas aeruginosa*, *Pseudomonas fluorescens*, and *Pseudomonas putida* at 5-10 µg/disc. The antibacterial effect has been established to be dependent on the concentration. Our findings showed that *E. camaldulensis* essential oil has been a great source of antipseudomonal, and also exhibited inhibition of *Pseudomonas* species biofilm formation. Based on its antibacterial and antibiofilm potential, *E. camaldulensis* essential oil shows promise as a potential source of antibacterial agents. Therefore, the use of *E. camaldulensis* essential oil in applications may have the potential to be a natural antibacterial agent against pathogenic and spoiling microorganisms.

Keywords: Antibacterial activity, antibiofilm activity, essential oil, Eucalyptus, *Pseudomonas*

INTRODUCTION

Bacterial diseases which caused by various bacterial pathogens are the main cause of high mortalities and economic losses among fish farms (Austin and Austin, 2007; Algammal et al., 2020).

Pseudomonads are one of the most prevalent bacterial species naturally present in almost all aquatic environments. They only become pathogenic when the fish is exposed to unfavorable environmental conditions like poor sanitation and water quality. *Pseudomonas* infections in fish mostly result in ulcerative syndrome and hemorrhagic septicemia (Oh et al., 2019; Narvaez et al., 2021; Eissa et al., 2010).

To control the diseases in fish that are mainly infectious, a wide range of chemicals like antimicrobials have been widely used in fish farming (Mohamed et al., 2000). Intensive use and misuse of antimicrobial agents in the aquaculture industry cause antimicrobial resistance, which results in not only treatment failures but also limits sustainable food animal production and animal welfare (Schar et al., 2020).

The rise in bacterial resistance, which has become a major concern worldwide, has focused the attention of researchers on natural products that could have similar effects on bacteria and that they could use instead of conventional antibiotics. Essential oils (EO) derived from plants seem to be a potential alternative because of their anti-inflammatory, antimicrobial, and antioxidant properties (Yap, 2014; Wińska et al., 2019).

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Eucalyptus species are commonly used in traditional medical applications for their antimicrobial properties. Essential oils (Eos) derived from this plant have been widely examined (Asiaei et al., 2017; Aleksic Sabo and Knezevic, 2019).

Park et al. (2016) reported antimicrobial activity of the essential oil obtained from *Eucalyptus globulus* against seven fish pathogenic bacteria, and they claimed that this EO can be used in fish farms as an antimicrobial agent in cases of bacterial infections.

To respond to changing environmental conditions, one of the strategies for bacteria to adapt and survive is forming multicellular communities known as biofilms (Čabarkapa et al., 2019). Bacterial biofilms are aggregates of microorganisms attached to surfaces or to each other and embedded in a self-assembled matrix of extracellular polymeric substances (Vestby et al., 2020).

Bacteria can protect themselves from hosts' defenses and antibacterial agents through the formation of biofilms and they also tend to develop their resistance mechanisms in many ways, such as through physical, physiological, and gene-related factors. Planktonic forms of bacteria are much more susceptible to antimicrobial agents than the bacteria that are inside a biofilm. Antimicrobial resistance, caused by biofilm-forming bacterial pathogens, not only results in treatment failures in cases of bacterial fish diseases but also causes recurrent exposure of fish to infections (Sundell and Wiklund, 2011; Abebe, 2020; Dinçer et al., 2019).

Antimicrobial susceptibility testing (AST) is an important task for the microbiology laboratories that are commonly used to determine possible drug resistance and antimicrobial susceptibility against common pathogens (Jorgensen and Ferraro, 2009). Although a variety of methods exist, the Kirby-Bauer agar diffusion method is well documented, cost-effective, more accurate, fast screening, and a standardized method for determining antibiotic susceptibility (Liu et al., 2016; Nassar et al., 2019).

The aim of the present study is to determine the antibiofilm activities and antimicrobial susceptibilities of eucalyptus (*Eucalyptus camaldulensis*) EOs against the fish pathogen *Pseudomonas* species.

MATERIALS AND METHODS

Plant material and preparation of EO

Eucalyptus (*Eucalyptus camaldulensis*) fresh leaves used in the study were harvested from the trees growing wild in Sinop, Turkey (42°02'43.4" N, 35°02'27.9" E) during June. Samples were cleaned to remove any dust and impurities, then dried at room temperature before use (Insuan and Chahomchuen, 2020). For the complete extraction of the essential oil, a total of 500 g of the dried sample was crushed and exposed to hydro-distillation using a Clevenger's apparatus. The EOs evaporated together with water vapor and passed through the refrigerant before being collected into the condensation flask. After the liquid phase was removed, the essential oil was collected in a glass vial and stored at 4 °C until further testing and analysis (Ghalem and Mohamed, 2008; Mazumder et al., 2020).

Gas Chromatography-Mass Spectrometry Analysis

Analysis was carried out in Eskisehir Anadolu University Medicinal Plants, Drugs and Scientific Research Center (AUBİBAM). The EO was subjected to Gas chromatography (Hewlett Packard system, HP 5973) and Mass spectrometry (GC-MS 6890 GC system). Agilent HP innowax column (60 m in length, inner diameter of 0.25 mm, film thickness of 0.25 µm) was used. As a carrier gas, helium was used. The injection temperature was 250 °C and the oven temperature was kept at 60 °C for 10 minutes, then programmed to 220 °C at a rate of 4 °C/min, kept constant at 220 °C for 10 min and then programmed to 240 °C at a rate of 2 °C/min for 40 minutes. Retention time (RT) was measured in minutes, and relative quantities of the described components were represented in percentages. (Sevindik et al., 2016).

Bacterial strains

P. aeruginosa (ATCC 9027), *P. fluorescens* (BC 7324), and *P. putida* (BC 1617) were obtained from the Microbiology Laboratory of the Department of Food Engineering at Atatürk University in Erzurum, Turkey, for use as test organisms (Cetin et al., 2011).

Antibacterial assay

Antimicrobial disc diffusion assays were performed for the screening of essential oils efficacy. After 24 h of bacterial culture at 37C in nutrient Broth, a diffusion test for Eucalyptus Essential Oil was performed. The bacterial cultures of different *Pseudomonas* species were visually adjusted to 0.5 McFarland standard with sterile saline. The bacterial suspensions were swabbed on the surface of Nutrient Agar plates and left to stand for 3 minutes before testing. Sterile Whatman No. 1 filter paper discs (6 mm, Biolife) were loaded with following concentration of EO (5 and 10 µl/disc) and placed on the surface of the freshly inoculated medium. As positive controls, Cefoperazone + Sulbactam (105 µg), Oxolinic acid (2µg), and Chloramphenicol (C30 µg) were employed. For 20 hours, the plates were incubated at 37 °C. The antibacterial activity was measured by measuring the diameter of the zones of

inhibition surrounding each of the disks (Bauer, 1966; Merghni et al. 2016; Andoğan et al., 2002).

Determination of biofilm inhibition

Using EEOs, a biofilm inhibition experiment was conducted against *P. aeruginosa* (ATCC 9027), *P. fluorescens* (BC 7324), and *P. putida* (BC 1617) in 96-well culture plates. The strains were kept at 37 °C for 24 hours while being cultured in 10 mL of tryptic soy broth that contained 1% glucose. The creation of dilutions equal to the 0.5 McFarland standard value came next. One hundred mi lliliters of eucalyptus oil, with final concentrations ranging from 7.8 to 125 µg/ml, 90 µl of growth medium (TSB with 1% glucose), and 10 µl of test bacterial dilutions were combined in each well of the plates.

While the negative control simply included growth media, the positive controls combined 10 µl of the bacterial dilutions with 190 µl of growth medium. The 96-well plate was incubated for 24 hours at 37 °C, then the unattached planktonic cells were washed out three times with distilled water to remove them. The remaining adherent sessile cells were then dyed with 200 µl of 0.4% crystal violet for 30 min, the excess dye was poured out, and the wells were then three times washed with distilled water. The leftover colored biofilm was dissolved in 200 µl of 70% ethanol and left to stand for 30 minutes. A microplate reader (Thermo Scientific Inc., Multiscan GO, Finland) was used to read the wells' optical density (OD) at 570 nm (Bai et al., 2019).

The following equation was used to compute biofilm inhibition:

Biofilm inhibition (%) = [(Control OD570nm – Test OD570nm) / Control OD570nm] x 100

RESULTS and DISCUSSION

The industries employ a variety of naturally occurring antimicrobials derived from plants and spices to minimize or eradicate harmful bacteria, improve the overall quality of products, and prolong the shelf life of goods. In the current study, the antibacterial capacity of 5 and 10 µl/disc of essential oil of *E. camaldulensis* was tested against *P. aeruginosa*, *P. fluorescens*, and *P. putida*.

The results obtained are shown in Table 1. On each of the studied bacteria, the EO inhibited it to a different extent. The zone of inhibition was 7–16 mm wide. The highest inhibition zone (16 mm) was observed against *P. putida* at a concentration of 10 µl/disc. The diameter of inhibition increased as the concentration of EO increased, indicating that the inhibition was dependent on dose concentration. *P. aeruginosa* was shown to be more resistant to the examined antibiotics and EEOs than the other two microorganisms.

In our previous study, the GC-MS analyses resulted with the identification of 18 components above 0.63%, representing 86.68% of the eucalyptus EO. The major constituents and amounts were detected as p-cymene (20.09%) and α-phellandrene (18.61%), respectively and antibacterial effect against *Aeromonas caviae* LipT51 also reported (Bektaş & Özdal, 2022).

EOs rich in carvacrol, p-cymene, and γ-terpinene showed strong inhibitory activity on the growth of all tested pathogenic bacteria (Anastasiou et al., 2019).

The primary compounds of EEO are terpenes and alcohol. As reported by Barbosa et al. (2016), D-limonene, 3-carene, myrcene, and α-pinene are terpenes that are linked to EEO's antibacterial effect. A relative increase in terpene content may lead to increased antibacterial activity. In actuality, it's also conceivable that substances with lower concentrations may work in a synergistic manner with other active substances.

Bioactive compounds and EOs derived from aromatic plants are alternative antibacterial agents that are thought to be safe and promising.

According to research by Chen et al. (2007) and Lu et al. (2022), biofilms provide difficulties for water treatment systems and have a specific influence on food industry. When compared to their planktonic counterparts, bacteria in biofilms can be up to 1000 times more resistant to antibiotic treatment (Simoes et al., 2009). *Pseudomonas* species, an opportunistic pathogen with a high degree of viability in environments such as water, air, soil, and food, are widely colonized (Osman et al., 2019).

Table 1. Antibacterial activity of essential oil of *E. camaldulensis* against *Pseudomonas* species

	Zones of inhibition (mm)		
	P. aeruginosa	*P. putida*	*P. fluorescens*
EO 5 µl/disc	7	10	12
EO 10 µl/disc	10	16	14
Cefoperazone + Sulbactam (105 µg)	16	27	30
Oxolinic acid (2µg)	11	13	15
Chloramphenicol (C30 µg)	13	20	16

P. fluorescens and *P. putida* biofilms were found to be suppressed by the anti-biofilm activity of EEO at low doses. Our findings show that *P. fluorescens* and *P. putida* are more vulnerable to EEO than *P. aeruginosa*. When EEO at concentrations of 7.81, 15.62, 31.25, 62.5, and 125 µg/mL were used, 0, 0, 0, 6, and 39.2% of *P. aeruginosa* biofilm formation was inhibited, respectively (Figure 1). Likewise, the same concentrations of EEO (7.81, 15.62, 31.25, 62.5, and 125 µg/mL) prevented 35, 41, 68, 99, and 99,8% of biofilm formation by *P. fluorescens*. For *P. putida* at the same concentrations, these values were measured as 46, 67, 98, 99, and 100.

Many studies have demonstrated the antibiofilm activity of EEOs against *Staphylococcus aureus* (Merghni et al., 2018), *Streptococcus mutans* (Goldbeck et al., 2014), *Actinobacillus pleuropneumoniae* (Rodrigues et al., 2022), *Listeria monocytogenes*, *P. aeruginosa*, *Escherichia coli*, *Pectobacterium carovotorum* (Caputo et al., 2020).

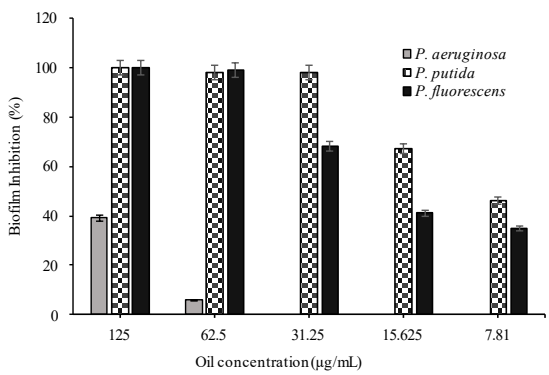

Figure 1. Inhibition of biofilm formation by *P. aeruginosa*, *P. fluorescens*, and *P. putida* using EEO.

CONCLUSIONS

According to these observations, Eucalyptus essential oil's antibacterial activity may thus point to its potential value as a microbiostatic, antiseptic, or hygienic agent, particularly against Gram-negative bacteria. The current study gives information that EEO have antibacterial and antibiofilm properties when it comes to *Pseudomonas* species. There is an urgent need to find effective solutions to battle Pseudomonads due to the growth of multidrug-resistant strains and the predominance of biofilm formation, and EOs have come to light as a promising solution. Numerous EOs have been demonstrated to be efficient antimicrobials and antibiofilm agents, enabling them to be employed either alone or in conjunction with well-established antibiotics in therapeutic formulations.

COMPLIANCE WITH ETHICAL STANDARDS

CONFLICT OF INTEREST

The authors declared no conflict of interest. All the authors read and approved the final manuscript.

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CONSENT FOR PUBLICATION

Applicable.

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Araştırma/Research

Investigation of the effect of chlorpyrifos-ethyl and pendimethalin on *Desmodesmus communis* (E.Hegewald) E. Hegewald

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Abstract: This study was carried out to determine the effect of herbicides and insecticides on *Desmodesmus communis.* Pendimethalin and chlorpyrifos-ethyl were applied to isolate *D. communis* from microalgae collected from natural environment. The experimental was regulated control group (C), herbicide [H1 (19 mgL^{1}), H2 (110 mgL^{-1}) and H3 (280 mgL^{-1})] and insecticide [I1 (10 mg/l), I2 (30 mg/l) and I3 (115 mg/l) 30 mgL^{-1})] and each group 3 times was repeated. The highest biomass in herbicide application was determined on first day. The highest values according to the groups (H1, H2 and H3) was found 0.40±0.09 μgL^{-1}, 0.47±0.18 μgL^{-1} and 0.49±0.15 μgL^{-1} respectively. In the case of insecticide application, the highest value was calculated on the starting day in all groups. According to the groups (I1, I2 and I3) biomass values were determined as 0.33±0.01 μgL^{-1}, 0.37±0.00 μgL^{-1} and 0.38±0.01 μgL^{-1}, respectively. At the end of the experiment, it was observed that pesticide groups with nitrogenous compound in the development of *D. communis* were more active than the group of pesticides with phosphorous compound, and thus the decrease in cell numbers was less.

Keywords: Pendimethalin, chlorpyrifos-ethyl, biomass, *D. communis*

INTRODUCTION

Agriculture is the oldest and most well-known method of meeting the nutritional needs of the world. As a result of population growth, countries have started to search for ways to get more efficiency from the unit area in order to meet the need for quality and cheap food. Pesticides have been used to combat pests such as weeds and insects for a long time (Solmaz et al., 2010).

Intensive fertilization and pesticide activities are affected on freshwater ecosystems as a serious threat. Pendimethalin is the active ingredient of herbicide used in the control of weeds. It has been determined that 10-20% of this herbicide, which is used in terrestrial areas, evaporates in the first weeks after application. The half-maximum spread time or half-dose (LD50) of pendimethalin has been reported to last from a few days to 4200 days. As a result of the experiments carried out in the field and laboratory, it was determined that the concentration of pendimethalin reached 6 mgL^{-1} in fresh waters. The lethal concentration (LC_{10}) value for *Daphnia* was found to be 6 mgL^{-1}. It has been reported that soil microbiota is affected by pendimethalin for 4 weeks after application (Strandberg and Scott-Frodsmand, 2004). According to the Environmental Quality Standard, the limit value that can be found in rivers and lakes has been reported as 0.5 μgL^{-1} (Anonim, 2014).

According to the Environmental Impact Standard, the limit value of chlorpyrifos-ethyl, which can be found in rivers and lakes, is 0.5 $\mu g/L^{-1}$ (Anonim, 2014). In our country, pesticide derivatives used in plant protection, whose active ingredient is chlorpyrifos-ethyl, are prohibited in imported products according to the Veterinary Services Plant Health and Feed Law No. 5996 in 2016 (Anonim, 2016).

The aim of this research was isolate *D. communis* from lakes, and investigate of the effect pendimethalin and chlorpyrifos-ethyl active substances on this species.

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MATERIAL AND METOD

Phytoplankton samples were collected from Lake Tortum with a plankton net (55Ø) in June 2016 and October 2016. It was brought to the laboratory in 100 ml plastic sample storage containers. Then, the samples were incubated for 10 days in a 250 ml glass Erlenmeyer in Bold Basal 11 medium enriched with agar. The microalgae that grew most at the end of the incubation period were isolated under invert microscope (Zeiss) with 600X magnification, and *D. communis* was transferred to 10 ml glass tube using the method reported by Andersen (2005).

In the experiments the herbicide concentration, which is the trade name Herbimat 330 EC (active ingredient pendimethalinden), was prepared as 19 mgL^{-1}, 110 mgL^{-1} and 280 mgL^{-1}. The insecticide concentration, which is the trade name was Chlorfet 48 EC (active ingredient chlorpyrifos-ethyl), was prepared as 10 mgL^{-1}, 30 mgL^{-1} and 115 mgL^{-1}. Stock solutions were stored in the refrigerator at +4 °C until the experiment started. All of experiments was carry out in Atatürk University Faculty of Fisheries Algae Unit. It was carried out by applying 2% CO_2 every day to all groups and the room temperature was fixed to 26 °C. The research took a total of 32 days, with 7 days of the experimental phase and 25 days of the isolation, identification and production of *D. communis* before the experiment.

Experimental Procedure

In this research has been carried out in Laboratory of Atatürk University, Faculty of Fisheries, Basic Science Research Unit. Initially, microalgae were grown in 10 ml tubes. The microalgae that developed in the tubes were 250 ml Erlenmeyer flasks, and were grown at 26 ºC, in a 120 $\mu molm^{-2}s^{-}$ lighting and 110 rpm shaking incubator (JRS Lab 32 brand) in a 16:8-hour day-night photoperiod. For intensive production of microalgae, they were taken into 5L glass containers, where it was started to apply insecticide and herbicide with lids in the Algae Unit in the research unit.

In the experiment, modified Bold Bazal 11 Medium was used as nutrient medium. It is contained: $NaNO_3$ (1.5 gL^{-1}), $K_2HPO_4.3H_2O$ (40 gL^{-1}), $MgSO_4.7H_2O$ (75 gL^{-1}), $CaCl_2.2H_2O$ (36 gL^{-1}), Na_2CO_3 (20 gL^{-1}), $MgNa_2EDTA.H_2O$ (1.0 gL^{-1}), trace metal solution (1 ml) (Andersen, 2005).

Dry Biomass Analysis

Samples (50 ml) taken from all groups in the experiment were filtered using Whatman GF/C filter paper, then the filter papers were kept at 100 °C until they reached a constant weight. The samples were weighed by placing them in the tared petri dishes on a balance with a sensitivity of 0.001 g. The dry matter content was calculated by taking the differences between the wet and dry weights of the samples (Vonshak, 1997).

D. communis Cell Count

The number of *D. communis* cells in the experimental groups was counted daily under the Zeiss Primo Star AxioCam ERc 5s model binocular microscope (with 400X magnification).

Phytoplankton cell count (cell/ml) = N x DF x 10.000

In this;
N = *D. communis* cell numbers (cell),
DF = Dilution factor (mm^3)
10.000 = The coefficient used in converting the counting result in 0.1 mm^3 to the number in 1 ml

D. communis Biomass

D. communis biomass in the experimental groups were measured every day on spectrophotometer (Beckman Coulter DU730) at a wavelength of 680 nm. *D. communis* biomass was calculated from the following formula (Kang et al., 2005);

Biomass (μgL^{-1}) = $0.713 \times OD_{680}$

Statistical Analysis

The variation of *D. communis* biomass, cell count, pH, water temperate and dry biomass depending on groups and days was determined by One-Way (ANOVA) test using IBM SPSS 20. The significance of the differences was evaluated according to the DUNCAN test. Checks all of dates by Kesici and Kocabaş (2007).

RESULTS AND DISCUSSION

In this study was planned as 27 groups into control (C), pendimethalin – treated groups [H1 (19 mgL^{-1}), H2 (110 mgL^{-1}) and H3 (280 mgL^{-1})] and chlorpyrifos-ethyl - treated groups [I1 (10 mgL^{-1}), I2 (30 mgL^{-1}) l) and I3 (115 mgL^{-1})]. There were performed measurement of pH, temperature and light, with analysis of cell count, dry matter amount and biomass during the experiment.

Change of pH in Pendimethalin and Chlorpyrifos-Ethyl - Treated Groups

In this experiment, the change in pH value between the groups depending on the days was found to be statistically significant ($p<0.05$). In the control group, the highest pH value was measured on the 6th day (8.41±0.40), while the lowest value was determined on the 7th day (7.49±0.02). The mean pH value was 7.54±0.17 in the pendimethalin – treated groups, and 7.53±0.15 in the chlorpyrifos-ethyl - treated groups (Figure 1, Figure 2).

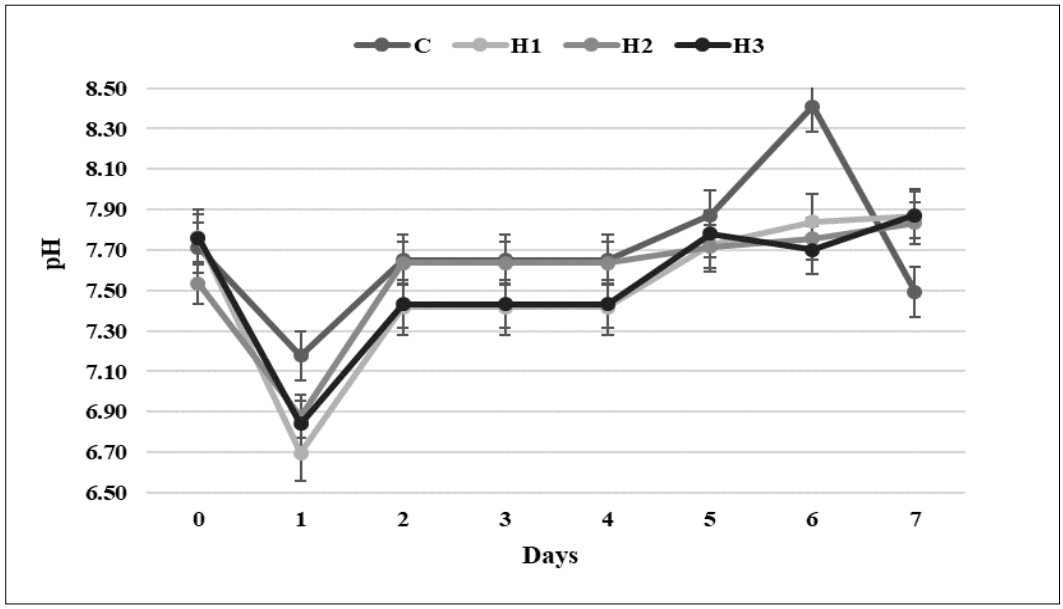


Figure 1. pH change depending on the day and groups in pendimethalin – treated groups.

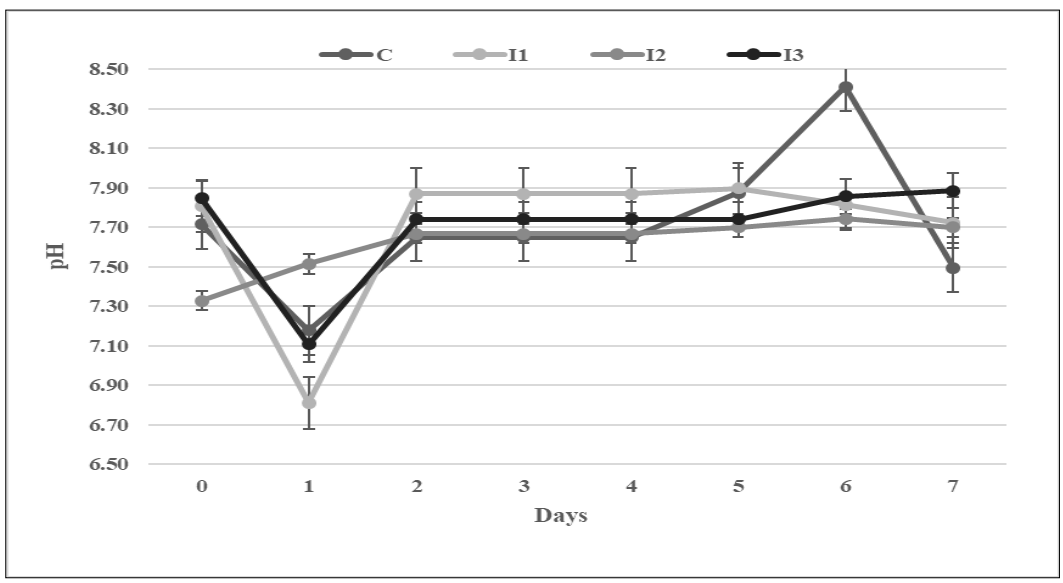


Figure 2. pH change depending on the day and insecticide (chlorpyrifos-ethyl) application groups

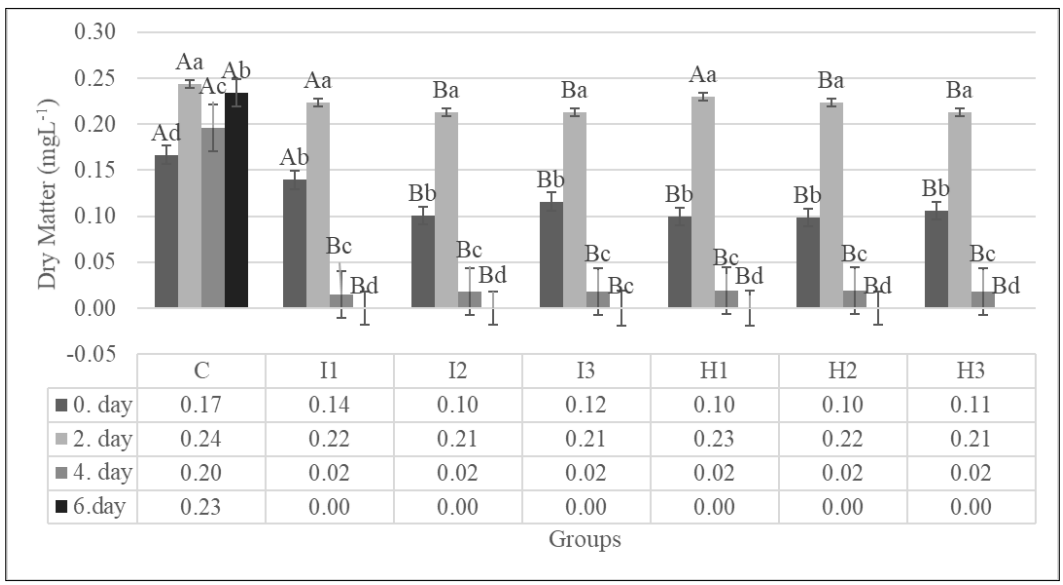
The changes in water temperature and light intensity of the pendimethalin – treated groups and chlorpyrifos-ethyl - treated groups and the control group were measured every morning at 9:00 am. The relationship between the untreated groups was found to be statistically insignificant ($p>0.05$).

The most important factor in the effects of pesticides on aquatic system is changed the pH value in a direction that increases or decreases. It was reported that the effects of dichlorodiphenyldichloroethane (DDD) and dichlorodiphenyldichloroethylene (DDE) change depending on the pH value, at the same time, cell uptake was highest when the pH value was 7 for both pesticides and cell death began after the 4th day (Luo et al., 2014). In this study was determined that the highest pH value was measured on the 6th day when *D. communis* cells died.

Dry Matter of Pendimethalin and Chlorpyrifos-Ethyl - Treated Groups

The variation of the dry matter amount depending on the days and the relationship between the groups were found to be

statistically significant ($p<0.05$). It was determined that the amount of dry matter increased suddenly in the weighing made on the 2nd day, and then a decrease was observed on the 4th day, although it increased again on the 6th day in the control group. It was calculated that dry matter values increased approximately two times on the 2nd day in the pendimethalin and chlorpyrifos-ethyl - treated groups. However, this groups were observed a sudden decrease on the 4th day, and the death of all cells on the 6th day (Figure 3).



	C	I1	I2	I3	H1	H2	H3
0. day	0.17	0.14	0.10	0.12	0.10	0.10	0.11
2. day	0.24	0.22	0.21	0.21	0.23	0.22	0.21
4. day	0.20	0.02	0.02	0.02	0.02	0.02	0.02
6.day	0.23	0.00	0.00	0.00	0.00	0.00	0.00

Figure 3. Changes of dry matter (mgL^{-1}) [ABCDE Capital letters indicate the difference of each group within days ($p<0.05$), and abc Lowercase letters indicate the difference between each group and the other groups ($p<0.05$)]

In this study, the amount of dry matter in the pendimethalin and chlorpyrifos-ethyl - treated groups increased approximately two times on the 2nd day, while it showed a sudden decrease on the 4th day. Change of dry matter values depend on days was observed similar with variation of *D. communis* biomass depend on days. This situation was found similar with *C. vulgaris* experiment, which was investigated effect of in waste water contaminated with organic substances on chlorophyll-a and cell count of *C. vulgaris* (Shelknanloymilan et al., 2012).

***D. communis* Cell Counting**

During the experiment, *D. communis* cell count between groups and days was found to be statistically significant ($p<0.05$). The highest *D. communis* numbers was found pendimethalin – treated groups. *D. communis* numbers (H1, H2 and H3) were calculated as 130.67±7.02 cell/ml, 133.67±28.10 cell/ml and 153.67±19.40 cell/ml, respectively (Table 1). The highest *D. communis* numbers were determined in chlorpyrifos-ethyl - treated groups (I1, I2 and I3) as 95.67±6.66 cell/ml, 134.67±14.57 cell/ml and 125.33±8.02 cell/ml, respectively (Table 2).

In this experiment, the least *D. communis* cell counts were determined on the 6th day. It was reported that *C. vulgaris* cell count was determined as 63.5±3.9 μgL^{-1}, and at the end of the 4th day, the cell number was 29.7±1.0 μgL^{-1}, and this situation was caused by abiotic factors such as sunlight and ventilation (Hultberg et al., 2016).

It has been reported that *Monoraphidium contortum* numbers was found to be affected by increasing the dose concentrations of herbicides [(l.1-dimethyl-4.4-bipydridyldiylium dichloride), 2.4-D (2.4-dichlorophenoxyacetic acid) and daminozide (N-dimethyl amino succinic acid)], for instance gradual decreases were observed in the amount of water up to the 96th hour. At the 96th hour, it was observed that the 1.0 ppm dose affected the number of organisms less than the 0.5 ppm dose (Dere and Sıvacı, 2003). In this study, *D. communis* cell numbers were decrease in pendimethalin – treat groups until the 3rd day, while the number of cells (20.00±3.61 cell/ml) in the H3 group (280 mgL^{-1}) was determined on the 3rd day. It was determined that the number of cells in the H1 group (19 mgL^{-1}) was higher (7.67±0.58 cell/ml). In the groups treated with chlorpyrifos-ethyl on the same day, the number of cells in group I1 (10 mgL^{-1}) (3.33±3.21 cell/ml) was higher than the number of cells in group I3 (115 mgL^{-1}).

Table 1. Change in the number of *D. communis* cells count (cell/ml) depending on the day and groups in pendimethalin - treated groups (n=3, Mean±SD)

Days	Groups			
	C	**H1**	**H2**	**H3**
0	142.00 ± 14.00^{Aa}	130.67 ± 7.02^{Ab}	133.67 ± 28.10^{Ab}	153.67 ± 19.40^{Aa}
1	156.00 ± 7.00^{Aa}	74.67 ± 11.93^{Bc}	101.00 ± 19.40^{Ab}	120.67 ± 7.00^{Ab}
2	78.67 ± 20.50^{Ba}	11.67 ± 3.79^{Cc}	16.00 ± 0.58^{Bc}	40.67 ± 5.29^{Ab}
3	45.00 ± 3.00^{Ca}	7.67 ± 0.58^{Dc}	11.00 ± 2.00^{Bb}	20.00 ± 3.61^{Bb}
4	45.00 ± 3.00^{Ca}	2.33 ± 0.58^{Ec}	3.67 ± 1.53^{Cc}	6.33 ± 1.53^{Cb}
5	23.00 ± 10.58^{Da}	1.33 ± 0.58^{Eb}	1.00 ± 0.58^{Cb}	1.33 ± 0.00^{Db}
6	25.67 ± 2.52^{Da}	0.67 ± 0.58^{Eb}	0.00 ± 0.58^{Db}	0.67 ± 0.00^{Eb}
7	12.67 ± 2.08^{Ea}	0.00 ± 0.00^{Eb}	0.00 ± 0.00^{Db}	0.00 ± 0.00^{Eb}

*ABCDE Capital letters indicate the difference of each group within days, and the difference between means with different capital letters in the same column is statistically significant ($p<0.05$).
**abc Lowercase letters show the difference between each group and the other groups, and the difference between means with different lowercase letters in the same row is statistically significant ($p<0.05$).

Table 2. Change in *D. communis* cell count (cell/ml) depending on the day and groups in chlorpyrifos-ethyl - treated groups (n=3, Mean±SD)

Days	Groups			
	C	**I1**	**I2**	**I3**
0	142.00 ± 14.00^{Aa}	95.67 ± 6.66^{Ab}	134.67 ± 14.57^{Aa}	125.33 ± 8.02^{Aa}
1	156.00 ± 7.00^{Aa}	79.67 ± 13.01^{Ab}	106.00 ± 13.45^{Aa}	92.00 ± 5.29^{Bb}
2	78.67 ± 20.50^{Ba}	10.33 ± 6.66^{Bc}	27.67 ± 8.50^{Bb}	19.67 ± 0.58^{Cb}
3	45.00 ± 3.00^{Ca}	3.33 ± 3.21^{Cb}	1.33 ± 0.58^{Cc}	1.00 ± 0.00^{Dc}
4	45.00 ± 3.00^{Ca}	3.33 ± 3.21^{Cb}	1.33 ± 0.58^{Cc}	1.00 ± 0.00^{Dc}
5	23.00 ± 10.58^{Da}	1.67 ± 0.58^{Cb}	1.00 ± 0.00^{Cb}	1.33 ± 0.58^{Db}
6	25.67 ± 2.52^{Da}	0.33 ± 0.5^{Db}	0.67 ± 0.58^{Db}	1.00 ± 0.00^{Db}
7	12.67 ± 2.08^{Ea}	0.00 ± 0.00^{Db}	0.00 ± 0.00^{Db}	0.00 ± 0.00^{Eb}

*ABCDE Capital letters indicate the difference of each group within days, and the difference between means with different capital letters in the same column is statistically significant ($p<0.05$).
**abc Lowercase letters show the difference between each group and the other groups, and the difference between means with different lowercase letters in the same row is statistically significant ($p<0.05$).

D. communis Biomass

During the experiment, *D. communis* biomass between groups and days was found to be statistically significant ($p<0.05$). The lowest biomass value was measured on the 5^{th}, 6^{th} and 7^{th} days (0 μgL^{-1}) between the pendimethalin – treated groups; However, the highest biomass value was calculated as 0.40 ± 0.09 μgL^{-1} on the 1^{st} day. In H2 group, the lowest and highest values were determined as 0 μgL^{-1} on the 5^{th} day and 0.47 ± 0.18 μgL^{-1} on the 1^{st} day, respectively in H3 group, the lowest value was 0 μgL^{-1} on the 5^{th} day, and the highest value was 0.49 ± 0.15 μgL^{-1} on the 1^{st} day (Table 3).

Table 3. Change of *D. communis* biomass (μgL^{-1}) depending on the day and the groups in the pendimethalin – treated groups (n=3, Mean±SD)

Days	Groups			
	C	H1	H2	H3
0	0.33±0.01^{aA}	0.37±0.01^{aA}	0.35±0.01^{aA}	0.34±0.00^{aA}
1	0.40±0.01^{aA}	0.40±0.09^{aA}	0.47±0.18^{aA}	0.49±0.15^{aA}
2	0.38±0.02^{aA}	0.34±0.25^{aA}	0.11±0.01^{bB}	0.14±0.03^{bB}
3	0.38±0.02^{aA}	0.01±0.01^{bB}	0.03±0.01^{bC}	0.01±0.00^{bC}
4	0.38±0.01^{aA}	0.01±0.01^{bB}	0.01±0.00^{bC}	0.00±0.00^{cD}
5	0.22±0.02^{aB}	0.00±0.00^{bC}	0.00±0.00^{bD}	0.00±0.00^{bD}
6	0.22±0.02^{aB}	0.00±0.00^{bC}	0.00±0.00^{bD}	0.00±0.00^{bD}
7	0.02±0.01^{aC}	0.00±0.00^{bC}	0.00±0.00^{bD}	0.00±0.00^{bD}

*ABCDE Capital letters indicate the difference of each group within days, and the difference between means with different capital letters in the same column is statistically significant ($p<0.05$).
**abc Lowercase letters show the difference between each group and the other groups, and the difference between means with different lowercase letters in the same row is statistically significant ($p<0.05$).

The lowest *D. communis* biomass were determined as 0 μgL^{-1} on the 6th day in all chlorpyrifos-ethyl – treated groups. The highest value of *D. communis* biomass was calculated on the first day in all groups. According to the groups (I1, I2 and I3), the *D. communis* biomass were 0.33±0.01 μg/l, 0.37±0.00 μg/l and 0.38±0.01 μg/l, respectively (Table 4).

Table 4. Change of *D. communis* biomass (μgL^{-1}) depending on the day and groups in chlorpyrifos-ethyl – treated groups (n=3, Mean±SD)

Days	Groups			
	C	I1	I2	I3
0	0.33±0.01^{aA}	0.33±0.01^{aA}	0.37±0.00^{aA}	0.38±0.01^{aA}
1	0.40±0.01^{aA}	0.31±0.01^{aA}	0.37±0.05^{aA}	0.33±0.01^{aA}
2	0.38±0.02^{aA}	0.10±0.16^{bB}	0.16±0.04^{bB}	0.21±0.11^{bB}
3	0.38±0.02^{aA}	0.02±0.02^{bC}	0.02±0.01^{bC}	0.01±0.01^{bC}
4	0.38±0.01^{aA}	0.02±0.00^{bC}	0.03±0.00^{bC}	0.03±0.03^{bC}
5	0.22±0.02^{aB}	0.00±0.00^{cD}	0.01±0.01^{bC}	0.02±0.00^{bC}
6	0.22±0.02^{aB}	0.00±0.00^{bD}	0.00±0.00^{bD}	0.00±0.00^{bD}
7	0.02±0.01^{aC}	0.00±0.00^{bD}	0.00±0.00^{bD}	0.00±0.00^{bD}

*ABCDE Capital letters indicate the difference of each group within days, and the difference between means with different capital letters in the same column is statistically significant ($p<0.05$).
**abc Lowercase letters show the difference between each group and the other groups, and the difference between means with different lowercase letters in the same row is statistically significant ($p<0.05$).

In this study was carried out under the laboratory condition. The *D. communis* biomass was determinate lower in the pendimethalin – treated groups than in the chlorpyrifos-ethyl – treated groups. The lowest *D. communis* biomass was found on the 4th day in the pendimethalin – treated groups, while the lowest *D. communis* biomass was calculated on the 5th day in the chlorpyrifos-ethyl – treated. It was reported that galactopyranoside-treated on *Pseudokirchneriella subcapitata*, *Scenedesmus acutus*, *Scenedesmus quadricauda* and *Coelastrum reticulatum* had no effect on this species growth up to concentration of 5 mgL^{-1}, but inhibited the growth of *P. subcapitata* at a concentration of 10 mgL^{-1} (Nakajima vd., 2007).

It was determinate that TRIA (triacontanol chloroform) affected increase in chlorophyll and biomass of *Chlorella vulgaris*, and basic physiological mechanisms (Aminfarzaneh, 2010). It was found that the growth phase of pesticide-treated *C. vulgaris* and *Desmodesmus communis* were short period, but the stagnation and collapse phases take longer, and the cultures collapse more slowly (Öterler and Albay, 2010). In this study, *D. communis* biomass was detected suddenly increase in all groups within one day. However, the stationary phase and collapse phases were realized within two days contrary to the expectations. It is thought that the applied active substances accelerate the biomass increase, but reach the saturation in a short time and damage the cells.

Herbicides and insecticides are important chemicals for increase production quality used to agricultural field. However, the mixing of these chemicals into aquatic ecosystems without decomposition, except for the pests

they affect, harms many living things living in these areas. These chemicals cause accumulation in aquatic organisms. Therefore, depending on the concentration of the chemical and the water quality values of the environment, it causes cell deformation and/or death in these organisms.

As a result of this study, both pesticide derivatives increased the cell numbers of *D. communis* until the 3rd day, but decreased it rapidly from the 4th day. It was observed that *D. communis biomass* was affected more rapidly in the groups exposure with pendimethalin than in the groups exposure with chlorpyrifos-ethyl. In our opinion that *D. communis* bimass is more affected by pesticide groups containing nitrogen compounds (Pendimethalin) rather than the pesticide group containing phosphorus compounds (Chlorpyrifos-Ethyl). For this reason, the correct adjustment of the application doses and methods of pesticides used in agricultural areas is important in order to preserve all of organism in the aquatic ecosystem.

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The Trout Journal of Atatürk University Araştırma/Research

Healthy consumption of seafood by cooking at the right temperature and time: Proven by rich physical and chemical parameter support

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Abstract: Monitoring certain factors both before and after cooking makes it possible to ensure the healthfulness of the food. The most significant chemical and/or physical changes that take place when food is heated are those caused by denaturing proteins. This study's goal is to assess the degree of protein denaturation (PD) in rainbow trout (*Oncorhynchus mykiss*) fillets that have been prepared using the most common time and temperature combinations. A few physicochemical characteristics, including area shrinkage, cooking loss, L*, a*, and b* values, thiobarbituric acid reactive substance, pH, total volatile basic, differential scanning calorimeter, texture profile analysis, and Fourier transform infrared investigations, were observed in fish flesh (cooked and raw samples) in this context.

Except for pH, there were statistically significant differences ($p<0.05$) within the treatment temperature groups. The results showed that short-term, low-temperature treatments had the best effects on the metrics measuring area shrinkage, cooking loss, and protein degradation. Despite the fact that deterioration was being watched in all groups based on the circumstance (time and/or temperature), it was determined that PD was developing faster at higher temperatures and protein coagulation became more obvious.

Keywords: Healthy eating, FTIR, DSC, protein degradation, fish, fillet

INTRODUCTION

Fish and other seafood are fundamental nutritional ingredients for a healthy life that are enjoyed globally. Besides, the high degree of spoilage of these products has led to the development of a prevalent variety of preservation, processing, and analytical techniques in this sector. At the same time, since healthy nutrition has become a philosophy of life in our century, the importance of cooking food in a healthy way has also emerged. In line with this trend, interest in baking foods with traditional methods is also increasing. Parallel efforts continue at the same pace in seafood, researchers from different disciplines (gastronomy, aquaculture producers, aquaculture engineers, etc.) come together and conduct research. Due to the unique characteristics of fish meat, the cooking stage becomes more important.

Although the purpose of different baking methods is to give the fish different aromas, it has become a priority in order to increase durability and reduce the rate of nutrient loss during baking.

Tasty foods are prepared through a number of processes in order to meet the nutritional needs of people. In one of these processes, foods, including those in the industrial field, are subjected to baking, known as controlled heat treatment, before they are made available for consumption. Conventional baking methods allow heat transfer through conduction, convection, and radiation. Of these methods, steaming and dry-heat baking are the most preferred conventional methods (Devine and Dikeman, 2014).

Meat and meat products, as well as aroma formation, some physical (brittleness, color, size, etc.), chemical (protein and lipid denaturation), and microbiological changes caused by the meat quality, affect the nutritional value. One of the most important of these events is protein denaturation, which is considered the first stage of changes in protein oxidation reactions (Bastıoğlu et al., 2011).

With its high essential fatty acid-amino acid, mineral substance, and vitamin content, seafood is one of the foods with high nutritional value, which has an important function for the development of children as well as for adult people to lead a healthy life. In this study, physical and chemical studies

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were used to investigate the impact of generally favored temperatures and cooking durations on rainbow trout fillet quality. In addition to the classical methods targeting protein denaturation, the integration of techniques that are considered quite new in the food sector has been taken into consideration, and it is aimed at establishing the infrastructure for what can be done for high quality criteria in future studies.

In this work, rainbow trout fillets were baked at two different temperatures and times that are often employed in dry heat (oven baking), and protein denaturation in the fillets was examined using a variety of assays. For this purpose, besides the classical methods used at protein denaturation, the integration of the new techniques in the food sector is taken into consideration, and it is aimed to create the infrastructure for the ones that can be done for high quality criteria in future studies.

MATERIALS AND METHODS

Experimental Design and Cooking procedure

Rainbow trout which was grown under routine conditions in the Atatürk University Fisheries Faculty Fresh Water Fish Production and Treatment Center, was used as fish material in the research. The fillets obtained from the marked sized rainbow trout were transferred to the Atatürk University Fisheries Faculty Seafood Processing Laboratory. The baking process was started on the fillets by taking into consideration the commonly used baking temperatures and times. The fillets, which were stored at 4 ºC were heat treated (cooked) in the oven (convection oven) at 180 ºC (30 min and 20 min), and at 200 ºC (20 min and 30 min).

Physical/chemical analyses [pH, cooking loss, area shrinkage, color, TBARS: thiobarbituric acid reactive substance, TVB-N: total volatile basic-nirogen, DSC: Differential Scanning Calorimeter, TPA: texture profile analysis, and FTIR: Fourier Transform Infrared] were done on the fillets before and after baking (Bayram, 2019).

pH

10 g raw and cooked fish flesh samples were taken and distilled water (100 ml) was added then pH values were measured in homogenized mixture.

Cooking loss

The weights of raw and cooked fillets are weighed with precision scale (Scaltec, SBA 41) and cooking loss is calculated proportionally with the formula of Ovissipour et al. (2017).

Cooking loss= ((final weight-first weight) ÷first weight) ×10

Area shrinkage

Area shrinkage of raw and cooked fillets were determined by scale and area shrinkage ratios were calculated proportionally (Ovissipour et al., 2017).

Color measurement

In the color measurement of samples, colour [value of L*, a* and b*] were examined by using Minolta (CR-200, Minolta Co, Osaka, Japanese) calorimeter. The color values are measured based on three-dimensional color measurement according to the criteria of CIELAB (Commision Internationele de I'E Clairage).

In this criterion, these values show color intensity as; (L*); L*=0; L*=100 white (darkness / clearance); (a*); +a* = red, -a* = green and (b*); +b*=yellow, -b* = blue (Bayram, 2019; Atamanalp et al., 2021).

Texture profile analyze

Texture Analysis Device (England, Stable Micro System, model TA-XT plus) with 75 mm probe was used to determine the TPA (textural properties test) of the samples taken from fish fillets. Without removing the skin, 30 mm length, width, and approximately 12 mm in height samples were taken from single fillet of each fish. From the obtained TPA curve; cohesiveness, flexibility, hardness, adhesion, gum and chewiness parameters were measured.

Determination of Total volatile basic nitrogen (TVB-N)

Minor modifications were done in the method given by Malle and Tao (1987). The TVB-N value was calculated by taking into account the H_2SO_4 solution spent in titration (Bayram, 2019; Atamanalp et al., 2021).

Determination of Thiobarbituric Acid Reactive Substance (TBARS)

TBARS is an important biomarker of the lipid peroxidation process strongly associated with lipid degragation levels. In this investigation, the method given by Lemon (1975) was used to determine the value of TBARS and the TBARS value was calculated as µmol Malonaldehit /kg tissue.

Differential scanning calorimeter (DSC)

For the DSC analysis, approximately 10 mg of tissue were taken from fish flesh samples and weighed in aluminum DSC containers (40 µL) and the containers were hermetically sealed. Sample containers placed in DSC (using empty container as reference) were heated from 20 °C to 90 °C at a heating rate of 5 °C / min. Enthalpy and temperature parameters of the thermal changes of proteins were monitor from the provided thermograms. The DSC-60 Plus (Japanese) was calibrated using indium (ΔH: 28,5 j/g; T: 156.6 ºC) for temperature and heat flow prior to analysis (Akköse, 2017).

Fourier transform infrared (FTIR)

A FTIR spectrometer (PerkinElmer precisely) with the specular reflection principle was used for both groups of samples from fish fillets and different time / temperature heat-treated fillets (1 cm x 0.5 cm x 0.2 cm). The samples were placed in direct contact with the ATR crystal cell, and the spectrum was taken from 4000 to 400 cm^{-1}. For each sample, 10 spectra were collected at room temperature (nearly 22 °C) (Ovissipour et al., 2017).

RESULTS AND DISCUSSION

Food is an important source for sustaining human life in healthy conditions. It is also known to the consumer that food safety is as vital as food sustainability (Sridhar et al., 2021). Global aquaculture production is estimated to reach 201 million metric tons in 2030 (Lundebye et al., 2021). Baking in the oven is one of the traditional cooking methods used since ancient times and is still preferred more frequently. In this study, physical and chemical changes and protein degradation with DSC were examined by applying different temperatures to rainbow trout at different times with this cooking method. In our study, it was observed that undesirable loss rates and protein degradation increased with high cooking temperatures. Since the new analysis methods that emerged with the development of technology give faster and more reliable results about product quality, the possibilities of their use in the food sector should definitely be investigated. As a matter of fact, our findings from protein denaturation and physical changes revealed that it is a safe, high-quality product that can be used to eliminate cooking errors. It has also been revealed that the selected temperature values in our study are not suitable for the analysis of protein denaturation with DSC, and lower temperature ranges should be tried.

pH

It is known that pH is an important factor in determining the quality of seafood and increasing its endurance after the process. In our study, pH was found to be significant ($p < 0.05$) in raw samples and statistical differences were not significant ($p > 0.05$) in heat treated samples (Table 1).

Table 1. pH measurements of raw and baking fillets with different temperature applied at different times (Mean±Standard deviation)

Group	Before heat process*	After heat process ^{NS}
180 ºC / 20 min.	6.30±0.29^{a}	6.05±0.12^{a}
180 ºC / 30 min.	6.29±0.29^{a}	6.15±0.11^{a}
200 ºC / 20 min.	6.21±0.29^{ab}	6.13±0.04^{a}
200 ºC / 30 min.	6.19±0.29^{b}	6.14±0.10^{a}

*p<0,05, NS: Not significant, there is no statistical difference between the values shown in the same column with the same letter (a, b).

In the raw fillet samples, the highest pH value was found as 6.30±0.29 and the lowest value was 6.19±0.29. These differences are depending on factors such as fish species and habitat, muscle type, nutritional status, rigor process, storage conditions and the buffering capacity of meat (Chaijan et al., 2013).

At the end of the heat treatments, the highest pH was obtained at 180 ºC during 30 min group (6.15±0.11) and the lowest value at 180 ºC during 20 min group (6.05±0.12). Our current research pH values and changes of the raw and cooked fillets, are consistent with previous studies which done with rainbow trout (Alak et al., 2010; Alak et al., 2011).

Öz et al. (2007) reported that the pH value of the heat-treated rainbow trout increased but Kotan (2015) research findings had showed that pH value decreased as a result of baking the same fish species in the oven (180 ºC / 10 min). Our study findings are compatible with Kotan (2015), but at the different with Öz et al. (2007) is thought to be affected by the baking technique and duration.

Cooking loss and area shrinkage results

The initial and post-baking weight measurements of the fillets with different baking temperature and times are carried out and the % changes are presented in the Table 2. Temperature and duration were statistically significant between groups ($p < 0.05$). The results demonstrated that cooking loss increased significantly ($p < 0.05$) due to the time and temperature of the heat treatment. The area shrinkages were determined to be statistically significant ($p < 0.05$) in raw and cooked (different time and temperature) filets and presented in Table 2. The highest area shrinkage was obtained as 40.23±4.93 in 200 ºC /30 min. cooked filets, and the lowest value was as 24.84±3.33 in 180 ºC / 20 min group fillets.

Table 2. Cooking loss and area shrinkage results of raw and baking fillets with different temperature applied at different times (Mean±Standard deviation)

Group	Cooking loss (%)*	Area shrinkage (%)*
180 ºC / 20 min.	15.37±5.69 [ab]	24.84±3.33[c]
180 ºC / 30 min.	17.96±4.90 [ab]	33.07±8.75[ab]
200 ºC / 20 min.	13.61±5.04 [b]	29.53±9.65[bc]
200 ºC / 30 min.	20.22±5.13[a]	40.23±4.93[a]

*p<0,05, there is no statistical difference between the values shown in the same column with the same letter (a, b).

Considering the results of these studies, the reason why these losses do not rise to very high levels is that fish meat contains high levels of myofibrillary protein and connective tissue (Ovissipour et al., 2013). Again, as known, more than 85% of the baking loss is moisture, while the rest forms lipids, muscle fragments, and some byproduct (aggregated sarcoplasmic proteins, collagen or gelatin etc) (Kong et al., 2007a; Skipnes et al., 2008; Skipnes et al., 2011; Ovissipour et al., 2013). Baking of meat results in denaturation of proteins, loss of water/lipid and shrinkage. The most important point in the application of heat treatment is the last temperature of the center (the coldest point) and how long it remains. Our findings are consistent with previous studies done with various fish species (Kong et al., 2007a; Kong et al., 2007b; Skipnes et al., 2011; Ovissipour et al., 2013; Cao et al., 2016).

When the sources of variation of our study were taken into consideration, it was observed that the cooking loss rate of 180 ºC and 200 ºC was more in 30 min group. In comparison of the cooking loss rate of the two different temperatures for 30 minutes, it was determined that the loss of 200 ºC was higher. This can be attributed to a reduction in the water holding capacity due to excess water loss of the fillets exposed to high temperatures for a long time.

The process that is effective in area shrinkages; is explained by the loss of moisture due to changes in the functional properties of the protein and as a result of the muscle protein denaturation and collection by heat treatment (Ovissipour et al., 2013; Cao et al., 2016). Also during the heat treatment, the diameter of the muscle fibers and the length of the sarcomere may bereduced due to the discarded water, soluble proteins, lipids and PD (Ovissipour et al., 2017). Numerous studies have been found to be related to protein denaturation of baking and area shrinkages and parallel to our study data (Barbera and Tassone, 2006; Kong et al., 2007a,b; Ovissipour et al., 2013; 2017). In addition to this, it has been reported that the fish meat is narrowed and thickening of the vertical section accompanied by protein denaturation (Liu et al., 2013).

In the description of the area shrinkages in the cooked samples, the breakdown of hydrogen bonds at high temperature and the shortening of the collagen molecule by opening and loosening of the fibrillary structure (shrinkage of up to one-third of the first length) is considered.

Color measurement results

The results of the color values (L*, +a*, and +b*) of the raw and different time / temperature cooked filets are given in Table 3.

L*, +a*, and +b* values of the heat treatment groups were 67,85-79,13, 0,40-1,36, and 16,28-22,42, respectively. When L (brightness) values are examined, it is seen that the lowest values are 67,85 in 200 ºC/ 30 min group and the highest (77,18) in 180 ºC / 20 min. group. High temperature application for long time affected the brightness of the materials negatively.

The highest +a value (1,36) was obtained in 200 ºC / 30 min. group and the lowest (0,40) was in 180 ºC /20 min. group. Haard (1992) was met with a similar situation in one of his researches, and reported that, due to denaturation of proteins, carotenoids and sugar between the oxidation of the fish proteins was attributed to the mallard reaction.

When the + b (yellow) data were examined, it was found that the lowest value (16.28) in 200 ºC / 20 min and, the group of 200 ºC/30 minutes was reached the highest value (22.42). During the baking of rainbow trout fillets, the color of the fish meat was masked by a rapid increase in L* value and a* sharp decrease in a value ($p < 0.05$). As a known condition, the formation of L*, a* and b* values caused by temperature can disrupt the colour balance in the cooked samples. Studies done with Pacific salmon (*Oncorhynchus keta*) (Bhattacharya et al., 1994) and pink salmon (Kong et al., 2007a) reported similar trends with our results. Whitening is the result of rapid denaturation of both proteins and carotenoid oxidation, while the browning phase is related to the Maillard reaction between sugars, fish proteins and amines (Haard, 1992). In this study, the determined browning phase is thought to be caused by high test temperatures. Because browning is usually related to process temperature and time (Whistler and Daniel, 1990; Kong et al., 2007a).

Table 3. Color measurements of raw and baking fillets with different temperature applied at different times (Mean±Standard deviation)

L*	**Raw fillet**	37.10±2.82[d]
	180 ºC / 20 min.	77.18±5.15 [ab]
	180 ºC / 30 min.	71.36±7.48 [bc]
	200 ºC / 20 min.	79.13±3.95 [a]
	200 ºC / 30 min.	67.85±7.35 [c]
a*	**Raw fillet**	2.62±1.93 [a]
	180 ºC / 20 min.	0.40±0.64 [b]
	180 ºC / 30 min.	0.58±1.07 [b]
	200 ºC / 20 min.	0.58±0.99 [b]
	200 ºC / 30 min.	1.36±1.18 [ab]
b*	**Raw fillet**	7.06±2.38 [d]
	180 ºC / 20 min.	19.28±1.91 [b]
	180 ºC / 30 min.	19.03±1.55 [b]
	200 ºC / 20 min.	16.28±1.82 [c]
	200 ºC / 30 min.	22.42±0.44 [a]

$p<0,05$, there is no statistical difference between the values shown in the same column with the same letter (a, b).

Texture profile results

The data of the textures of the raw and cooked filets (different time / temperature) are given in Table 4. Kong et al. (2007a) gave the most suitable peak temperature of the tissue for optimum baking time for the pink salmon as 100 and 131.1 °C. Since the temperature of protein denaturation in fish muscle is reported to be between 40 and 80 °C (Skipnes et al., 2008), tissue changes are probably due to the denaturation of myofibrillary proteins and the gelation of collagen (Kong et al., 2007a). Myosin and actin are very important due to the low collagen content in gelation for fish muscles (Skipnes et al., 2011). In this study, the hardening phase time was decreased with increasing temperature, and at higher temperatures, protein denaturation rate was determined to be faster than protein collection. This situation is consistent with other findings of the study (FTIR and DSC). Again, the temperature applied during baking can change the physical properties of the fish meat by affecting the protein denaturation. Especially the deterioration of myosome is more effective in this process (Sahin and Sumnu, 2001; Liu et al., 2013).

Our data showed that different cooking treatments significantly reduced elasticity and gummy. Textural structure of fish meat (due to myofibrillar proteins) does not show a high texture strength as high as meat and chicken meat and especially processed products have been reported to affect the textural properties of protein and lipid quality (Dinçer et al., 2017). According to Kerr et al. (2005); some tissue modifier situations act on the binding and stiffness of proteins.

Table 4. Texture results of raw and baking fillets with different temperature applied at different times (Mean±Standard deviation)

	Groups				
Texture					
	Raw filet	**180°C/20 min**	**180°C /30 min**	**200°C /20 min**	**200°C /30 min**
Hardness (N)*	8.45±0.97^{a}	7.60±0.35^{a}	12.37±2.93^{b}	9.15±1.63^{a}	18.38±3.28^{c}
Stickiness (N.s)^{NS}	0.68±0.39^{a}	0.52±0.33^{a}	0.18±0.13^{a}	0.56±0.23^{a}	0.28±0.29^{a}
Flexibility ^{NS}	0.27±0.35^{a}	0.12±0.03^{a}	0.11±0.03^{a}	0.11±0.01^{a}	0.11±0.01^{a}
Cohesiveness ^{NS}	0.29±0.05^{a}	0.35±0.06^{a}	0.33±0.03^{a}	0.34±0.04^{a}	0.33±0.03^{a}
Elasticity (mm)*	2.61±0.46^{c}	1.98±0.33^{a}	2.04±0.53^{ab}	1.68±0.18^{a}	2.37±0.27^{bc}
Gummy (N)*	2.43±0.18^{a}	2.65±0.46^{a}	4.12±0.96^{b}	3.14±0.67^{ab}	6.23±1.40^{c}
Chewiness (N.mm)*	6.34±1.12^{ab}	5.32±1.46^{a}	8.02±0.72^{b}	5.18±0.69^{a}	14.68±3.11^{c}

*p<0,05, NS: Not significant, there is no statistical difference between the values shown in the same column with the same letter (a, b).

TVB-N and TBARS results

TVB-N analyze is a chemical method used to determine the quality and freshness of seafood (Çetinkaya et al., 2014). TVB-N value changes depending on many factors, such as fish species, the hunting season, degree of maturity, sex, age, processing technology and storage time.

While the average TVB-N value of the raw fillets was 9.18 ± 1.69 mg / 100 g, this value showed an increase due to the time and temperature. The highest value was found in 200 ºC /20 min group as 17.64±1.70mg/100 g and at 200 ºC/ 30 min group as 18.76±1.69 mg/100 g.

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In this study, TVB-N measurements were made in the raw and different time / temperature heat treated filet samples. Total volatile basic nitrogen (TVB-N) value in fish is an important criterion in determining freshness. Lipid oxidative products lead to protein oxidation. In addition, lipid oxidation and protein oxidation can occur independently or together. TVB-N; is a nitrogen-containing group of compounds originating from protein degradation by enzymes and bacteria, including NH3, amines (Duan et al., 2018).
Kolsarıcı and Özkaya (1998) reported that cold and hot smoking of rainbow trout, caused changes in TVB-N values. They also found that temperature can increase TVB-N levels in microbial and enzymatic activation. The high amounts of TVB-N obtained in our study can be explained by the applied heat levels.

The TVB-N changes caused by different applications were determined as significant at $p < 0.05$ level and the differences are presented in Table 5.

Table 5. TVB-N and TBARS values of raw and baking fillets with different temperature applied at different times (Mean±Standard deviation)

Group	TVB-N (mg/100g)*	TBARS (µmol malonaldehyde /kg tissue)*
Raw filet	9.18±1.68^{b}	0.95±0.29^{c}
180 ºC / 20 min.	13.72±1.67^{ab}	1.89±0.56^{bc}
180 ºC / 30 min.	16.95±1.69^{a}	2.60±0.83^{b}
200 ºC / 20 min.	17.64±1.70^{a}	2.01±0.55^{bc}

*p<0,05, there is no statistical difference between the values shown in the same column with the same letter (a, b).

The number of thiobarbituric acid (TBA) is a method used in meat and meat products to determine the aggravation caused by autoxidation in lipids. The oxidative reaction during the storage of meat and meat products in cold or frozen conditions results in the formation of various oxidation products. These products are hydroperoxide, peroxide, aldehyde and ketones, respectively. The most important aldehyde formed is malonaldehyde (MA) (Yeo and Shibamoto, 1992). This aldehyde is an important parameter in determining the degree of oxidation. The amount of MA determined by TBA analysis is directly proportional to the oxidation of meat.

In this study, changes in the TBARS values of the fillets exposed to different temperature / time applications were determined. This change in TBARS values of raw/heat treated fillets was shown in Table 5 and found significant at $p < 0.05$ level. The mean TBARS value of raw filets was found as 0.95±0.29 µmol malonaldehyde/kg tissue. The highest TBARS value after baking was obtained as 5.54±2.26 µmol malonaldehyde/kg tissue in 200 ºC - 30 min group.
Secondary oxidation products were evaluated in order to determine the course of the lipid oxidation process. TBARS is one of the classical methods of monitoring secondary products of lipid oxidation (Qiu et al., 2016). In TBARS; it is well known that oxidants such as radicals form protein and lipid damage in processed aquatic products, especially fish containing excess polyunsaturated fatty acids, are critical for shortening shelf life (Duan et al., 2018).

The TBARS values of the samples cooked 30 min. at 180 ºC and 200 ºC were found to be higher compared to the shorter periods baking of the same temperatures. Our study was tried to cook with the same technique in the same fish species. This result coincides with the effect of heat treatment on the TBARS value. Similarly, it is known that TBARS is lower in raw meat and reactive compounds formed by baking react with various compounds such as protein and amino acids found in meat to increase TBARS value (Meinert et al., 2007; Sanchez del Pulgar et al., 2012; Dominguez et al., 2014).
In this study, the increase in lipid oxidation at high temperature for long periods of baking was found as marginal. When the formation of secondary oxidation products was evaluated, it was concluded that TBARS value was below the acceptable limit value (2-2.5 mg MDA/ kg meat) in all cooked samples except for high temperature long-term application, this situation could not be defined as increased lipid oxidation (Al-Sagir et al., 2004).

Thermal stability results

The thermal properties of the raw and heat treated (different time-temperature) fillets are given in Table 6. In the thermograms obtained by thermal analyzes, three different peaks were determined for the control sample (raw fillet) and these peaks were respectively myosin (T_1, 〖ΔH〗_1), and sarcoplasmic proteins with collagen (T_3, 〖ΔH〗_3) and actin (T_4, 〖ΔH〗_4) was taken into consideration in the denaturation (Table 6).

Table 6. DSC results of control and different temperature/time applied groups

Group	1. peak		2. peak		3. peak	
	Temperature (C)	**ΔH (J/g)**	**Temperature(C)**	**ΔH (J/g)**	**Temperature (C)**	**ΔH (J/g)**
Raw fillet	41.83	1.11	65.93	0.08	75.65	0.49
	41.59	0.76	65.68	0.07	75.05	0.25
180 ºC/20 min	-	-	-	-	-	-
180 ºC /30 min	-	-	-	-	-	-
200 ºC/ 20 min	-	-	-	-	-	-
200 ºC /30 min	-	-	-	-	-	-

However, in the cooked samples myosin, actin and sarcoplasmic proteins with no longer observed that the peaks of collagen denaturation. In order to estimate the degree of protein denaturation of fish meat during baking, the activation energy must be known. Peak temperature (Tmax) and heating rate (b) are two parameters used in the DSC method and indispensable for interpretation (Liu et al., 2013).

For DSC analysis, there is no data in rainbow trout so it is defined as similar temperature ranges with reference to the data of Skipnes et al. (2008) and Liu et al. (2013) in different fish species.

In this respect, the myosin (40-50 °C) and actin (70-80 °C) proteins were taken into account and in the prediction of the degree of protein denaturation, the concentration of myosin and actin detected during heating for the denaturation of these two proteins was focused.

As is known, there is a connection between reduction in tissue, increase in proteolytic activity and myosin denaturation. The deterioration of tissue and high temperature are thought to have negative effects in the absence of peaks (Sahin and Sumnu, 2001).

The temperature in protein denaturation of the tissue is considered to be the main cause, since the physical properties of fish meat during the baking process are partially damaged by protein denaturation (Liu et al., 2013). When consider the effects of different cooking time/temperature on the protein degradation, it is not surprising that temperature enthalpy is decreased and eventual coagulation to the cooking samples.

TBARS level, other important parameters, showed a significant increase under high temperature exposure compared to the control and the other treated groups.

The enthalpy values that cannot be taken can be explained by a decrease in the thermal stability of proteins. In our study, the TVB-N results, which are examined as validation parameters, are in parallel with the DSC results. The amount of TVB-N, which increases with temperature, is directly proportional to the increase in compounds such as amine and ammonia. The increase in the amount of compounds such as amine and ammonia in the medium strengthens the result of protein denaturation.

Fourier transform infrared (FTIR)

In this study, wave motion measurement of raw and cooked (different time-temperature) fillets was determined by FTIR readings and the changes were given in Figure 1.

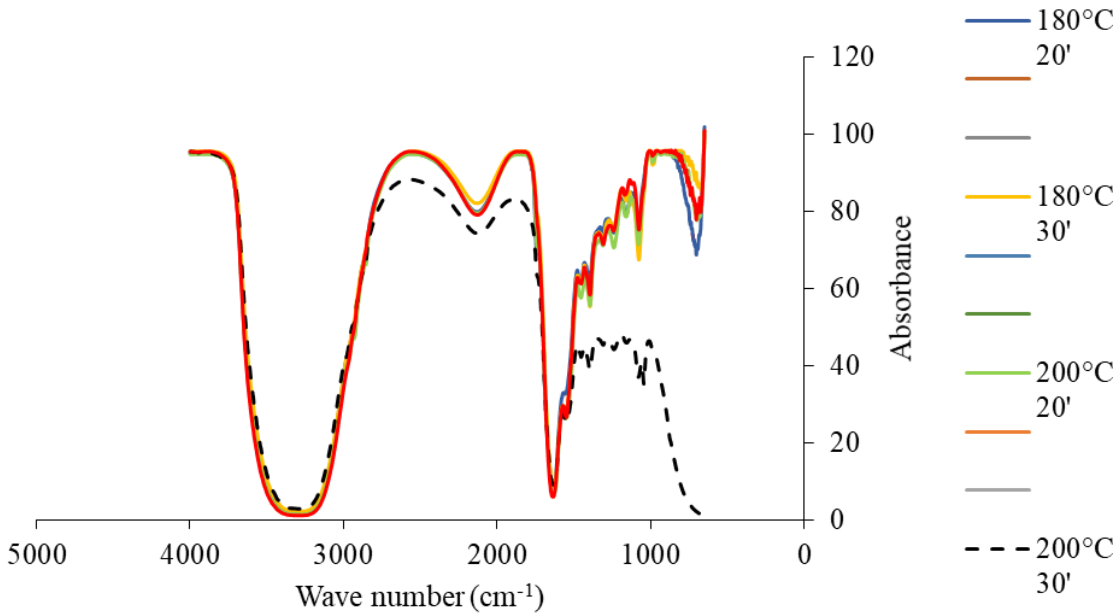

Figure1. FTIR results of raw and baking fillets with different temperature applied at different times

Due to the high-water activity in fish fillets, there were no significant differences in the FTIR graphs of raw and heat-treated groups. In this case, it is also believed that the amine groups formed depending on the temperature have an effect. The presence of amine groups and high-water activity as a known condition make it difficult to read the other bonds on the spectrophotometer in the graphs.

Amid I (1700-1600 cm^{-1}) in the Myofiber spectrum is known as the most dominant and most useful group in studies. This structure is also widely used to study the secondary structure of protein. In this common use, hydrogen bonding patterns, dipole-dipole interaction and susceptibility to the geometry of the polypeptide backbone are quite important (Carton et al., 2009; Astruc et al., 2012). The changes with the effects of heating or salting in Amid I spectrum were examined in various muscles (Carton et al., 2009; Ojagh et al., 2011; Astruc et al., 2012). In this study, the highest absorbance read in the fillets of the original spectrum was recorded as values of 3271.71 cm^{-1} - 3288.04 cm^{-1}, and recorded as the indicating the presence of amine groups. This absorbance gave increase/decrease frequencies depending on the amount of water, heating temperature and time.

The determined minimum frequencies, sample-layer structures in the heated samples can be attributed to the increased aggregation at the intermolecular level in the cooked fillets (Carton et al., 2009; Ojagh et al., 2011). The same trend may also be related to changes in intramolecular antiparallel β-layer structures in heated samples (Ovissipour et al., 2017).

When the graphs were examined in general terms, functional groups were determined according to the energy values, with 2127.83 cm^{-1} and 2127.31 cm^{-1}, ether groups defining bonds were determined with high rate in all graphs.

Although multiple confirmation tests were used to determine denaturation in our study, it should be supported by different studies (different fish species, different processing technologies, different environmental conditions and different temperatures).

There is a need for new researches in order to increase the studies on the loss of basic components with different cooking techniques and to reduce the lipid and protein denaturation that occur during cooking and storage to the minimum or to observe the optimum.

CONCLUSION

The food and food processing industry worldwide requires novel products and novel processes to customize products. In this study, it was determined that different temperature/time applications in the oven caused changes of fillets and protein degradation in rainbow trout. This study demonstrates the ability of low temperature and time to protect rainbow trout fillet from quality damage induced by high temperature exposure.

Our findings from protein denaturation and physical changes have shown that it can be used to improve safety and high-quality products and improve baking errors. In our study, it was also revealed that protein denaturation of the selected temperature values was not suitable for DSC analysis and that lower temperature ranges should be tried. In this study,

although multiple verification tests are used to determine denaturation should be supported with different studies (different fish species, different processing technologies, different ambient conditions and different temperatures). Considering all data we can say that;

1.In our study, it was observed that the highest pH value was 6.30±0.29 and the lowest value was 6.19±0.29 in the untreated samples. As a result of heat treatment applications, the highest pH was found to be 6.15±0.11 at 180 °C/30 min and the lowest value was 6.05±0.12 at 180 °C/20 min.

2.Considering the sources of variation, it was determined that the rate of weight loss at 180 °C and 200 °C was higher in 30 minutes. In the comparison of two different temperatures, it was determined that the weight loss rates were higher at high temperatures.

3.The surface area losses of the fillets cooked raw and cooked at different temperatures/times were found to be statistically significant. The highest surface area shrinkage was 40.23±4.93% at 200 °C/30 min. The lowest loss in cooked fillets is 24.84±3.33% at 180 °C/20 min. obtained in cooked fillets. Another remarkable point in this parameter is that the maximum surface area shrinkages are determined in long time periods.

4.The range of color values of the heat treatment groups are L*; 67.85-79.13, a*: 0.40-1.36, and b*: 16.28-22.42, respectively. When the L* (brightness) values are examined, the lowest is seen at 67.85 at 200 °C in 30 minutes, and the highest at 77.18 at 180 °C in 20 minutes. High temperature application for a long time adversely affected the gloss of the materials. The highest +a* value was determined at 1.36 to 200 °C in 30 minutes and the lowest at 0.40 to 180 °C in 20 minutes. Finally, when the +b* value was examined, it was seen that it reached the lowest value with 16.28 in 20 minutes at 200 °C and the highest value with 22.42 in 30 minutes at 200 °C.

5.It had been observed that the applied temperatures and times cause changes for each parameter (hardness, stickiness, flexibility, cohesiveness, elasticity, gumminess and chewiness) on the texture, especially hardness, elasticity, gumminess and chewiness parameters.

6.While the average TVB-N value of raw fillets was 9.18±1.69 mg/100 g, this value increased depending on time and temperature as a result of cooking. The highest values were found as 17.64±1.70 mg/100 g in the 200 °C/20 min group and 18.76±1.69 mg/100 g in the 200 °C/30 min group.

7.In the TBARS value, the average TBARS value of the raw fillets was 0.95±0.29 µmol Malonaldehyde/kg tissue, and the highest TBARS value after cooking was 5.54±2.26 µmol Malonaldehyde/kg tissue at 200 °C/30 min.

8.Three different peaks were determined for the control sample (raw fillet) in the thermograms obtained as a result of the thermal analysis, however, the peaks of the targeted proteins could not be read because protein denaturation (denaturation of collagen with myosin, actin and sarcoplasmic proteins) occurred in the cooked samples.

9.In FTIR measurements, it was determined that the high water ratios and amine contents in both raw samples and cooked fillets prevented the appearance of small molecule bonds by making some shadows.

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CONFICT OF INTEREST

The authors declare no competing interests

ETHICAL APPROVAL

Ethics committee approval is not required.

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CONSENT FOR PUBLICATION

Not applicable.

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The Trout Journal of Atatürk University Araştırma/Research

Paketleme ve antimikrobiyal madde uygulamalarının su ürünlerinde kullanımı

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Özet: Besleyici özellikleri açısından gıdalar arasında önemli bir yere sahip olan su ürünleri, mikrobiyal bozulmaya karşı çok hassas oldukları için yakalanması, saklanması ve uygun tekniklerle işlenmesi gereklidir. Bozulmayı yavaşlatırken taze tutmanın en etkili yolu soğuk depolama teknolojisidir. Ayrıca dondurma, tütsüleme, kurutma, tuzlama, ısıl işlem muhafaza, asitleme, paketleme teknikleri (vakum ve modifiye atmosfer paketleme (MAP) vb.), gıda katkı maddelerinin kullanımı gibi su aktivitesinin azaltılması da raf ömrünü uzatır. Antimikrobiyal ajanlar, çevredeki her türlü mikroorganizmayı öldürmek, üremelerini veya aktivitelerini engellemek için koruyucu olarak eklenen katkı maddelerinden biridir. Bu maddeler arasında yaygın olarak kullanılan sorbik asit ve tuzları, depolama sırasında bakterilerin üremesini ve istenmeyen kötü tat ve aromaya neden olan bileşiklerin oluşumunu yavaşlatarak engeller. Bu derleme, vakum paketleme, modifiye atmosfer paketleme ve antimikrobiyal sorbik asidin su ürünlerinin raf ömrü üzerindeki etkilerini çeşitli bilimsel kaynakları kullanarak özetlemektedir.

Anahtar Kelimeler: Antimikrobiyal, balık eti, raf ömrü, MAP, vakum

Usage of packaging and antimicrobial substance applications in aquaculture

Abstract: Aquatic products, which have an important place among foods in terms of their nutritional properties, need to be caught, stored and processed with appropriate techniques because they are very sensitive to microbial spoilage. The most effective way to keep it fresh while slowing down deterioration is with cold storage technology. In addition, reducing water activity such as freezing, smoking, drying, salting, heat treatment preservation, pickling, packaging techniques (vacuum and modified atmosphere packaging (MAP) etc.), the use of food additives also extends the shelf life. Antimicrobial agents are one of the additives added as preservatives to kill all kinds of microorganisms in the environment, inhibiting their reproduction or activity. Among these substances, commonly used sorbic acid and its salts inhibit the growth of bacteria during storage and the formation of compounds that cause unwanted bad taste and aroma by slowing them down. This review summarizes the effects of vacuum packaging, modified atmosphere packaging, and antimicrobial sorbic acid on the shelf life of aquaculture using a variety of scientific sources.

Keywords: Antimicrobial, fish meat, shelf life, MAP, vacuum

GİRİŞ

Balıkların, yüksek besleyici özelliğinden dolayı gıdalar arasında çok önemli bir yere sahip oldukları bilinmektedir (Turan vd., 2006). Tüketicilere taze, kaliteli balık sağlamak, bozulma mekanizması veya bozulmaya neden olan faktörlerin bilinmesine ve alınması gereken önlemlere bağlıdır (Huss, 1988). Gıdaların bileşim ve özelliklerinde meydana gelen istenmeyen değişikliklerden kaynaklanan bozulmaların önlenmesi ve raf ömrünün uzatılması için gıda işleme ve muhafazaya yönelik farklı yöntemler geliştirilmiştir. Bununla birlikte, soğuk depolama teknolojisine ek olarak su aktivitesinin azaltılması için tuz ve şeker ilavesi, kurutma, pastörizasyon ve sterilizasyon ile vakum ve modifiye atmosfer paketleme gibi uygulamalar yapılmaktadır (Evren vd., 2006). Yiyeceklerin lezzetli olması

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ve uzun süre muhafaza edilmesi için formülasyonlarına "gıda katkı maddesi" adı verilen bazı maddeler eklenir. Bu gıda katkı maddelerinden biri de koruyucu olarak kullanılan antimikrobiyal maddelerdir (Çakmakçı ve Çelik, 1994). Asetik asit ve asetatları, benzoik asit ve tuzları, propionik asit ve tuzları, nitrit ve nitrat bileşikleri, nisin, sorbik asit ve tuzları, kükürt dioksit ve çeşitli sülfitler gıda endüstrisinde yaygın olarak kullanılan antimikrobiyal ajanlardır (Gökalp ve Çakmakçı, 1991; Çakmakçı ve Çelik, 1994; Küçüköner, 2006). Günümüzde gıda koruyucu olarak önemli ölçüde kullanılan sorbik asit ve potasyum güvenli olarak kabul edilen maddelerdir ve kolayca sindirilebilirler (Gülyavuz ve Ünlüsayın, 1999; İnanlı, 2003). Suda az çözünen sorbatların antimikrobiyal aktivitesi, kullanılan gıdanın içerdiği diğer katkı maddeleri, pH'sı, besin madde içeriği, nem miktarı, işleme metodu, paketleme metodu, muhafaza sıcaklığı ve raf ömrü gibi birçok faktöre bağlıdır. Sorbatlar maksimum aktivitelerini pH 6,0-6,5 arasında gösterirler ve düşük pH'lı gıdalarda daha etkilidirler (Sofos ve Busta, 1981; Çakmakçı ve Çelik, 1994). Sorbik asit ve tuzları küf ve maya gelişimine karşı antimikrobiyal ajanlar olarak kullanılmaktadırlar. Ayrıca patojenler de dahil olmak üzere birçok saprofitik bakteriye karşı da etkilidirler (Kıvanç, 1991; Thakur ve Patel, 1994; Yetim, 1996).

Sorbatlar doğrudan ürüne katılma, daldırma, püskürtme, toz halinde serpme, ambalaj malzemesine veya ambalaj kağıdına katılma gibi çeşitli yöntemlerle gıdalara uygulanmaktadırlar (Yetim, 1996). Dünya Sağlık Örgütü (WHO), gıda konservelerinde sorbatlar için maksimum kabul edilebilir günlük alım miktarını (ADI) 25 mg/kg olarak belirlemiştir (Sofos ve Busta, 1981). Balık etinin bozulmadan uzun süre saklanamaması, hemen işlenmesini veya ön işleme tabi tutulmasını ve muhafaza edilmesini gerektirmektedir (Huss, 1988).

Bu derlemede, paketleme yöntemleri ve bir antimikrobiyal ajan olan potasyum sorbatın balık muhafazasında kullanımına ilişkin araştırmalar tartışılmaktadır.

1.1.Balıkçılık endüstrisinde paketleme yöntemleri

Gıdaların bileşim ve özelliklerinde meydana gelen istenmeyen değişiklikler nedeniyle bozulmayı önlemek ve raf ömrünü uzatmak için gıda işleme ve muhafazaya yönelik farklı yöntemler geliştirilmiştir. Su ürünlerini muhafaza etmenin ana yöntemleri dondurma, konserveleme, tütsüleme, tuzlama ve kurutma iken, günümüzde su ürünlerinin taze olarak muhafazasında vakum ve modifiye atmosfer paketleme teknolojileri de kullanılmaktadır (Üçüncü, 2000; Kılınç ve Çaklı 2001, 2004; Evren vd., 2006).

Bozulmayı geciktirerek gıdaları taze tutmanın en uygun ve etkili yolu soğuk depolama teknolojisidir. Bununla birlikte, soğuk depolama teknolojisine ek olarak ısıl işlemlerden pastörizasyon ve sterilizasyon, su aktivitesinin azaltılması için kurutma, tuz ve şeker ilavesi ve vakum ve modifiye atmosfer gibi paketleme tekniklerinin kullanımı giderek artmıştır. Özellikle kontrollü atmosfer, modifiye atmosfer (MAP), vakum, yenilebilir film, akıllı ve aktif paketleme gıda muhafazasında kullanılmaktadır (Evren vd., 2006).

1.1.1.Modifiye Atmosfer Paketleme

Modifiye atmosfer paketlemede prensip; paketleme ortamına gaz karışımları uygulayarak hakim mikrofloranın metabolizma hızını düşürmek, ürünün solunum hızını düşürmek, enzimatik ve oksidatif bozunma reaksiyonlarını minimum seviyede tutarak mikrobiyal bozunmayı geciktirmektir (Özoğul vd., 2006).

Modifiye atmosfer paketleme ürünlerin potansiyel raf ömrünü 1,5 ila 4 kat artırmak, ekonomik kayıpları azaltmak, ürünlerin daha düşük dağıtım maliyetleri ile daha uzun mesafeler kat etmesini sağlamak ve daha kaliteli ürünler sunmak gibi birçok avantajlar sunmaktadır (Farber, 1991).

Ancak çözünmüş karbondioksit kontrolü, ambalaj yapısının bozulması, her ürün için farklı gaz bileşiminin gerekliliği, taşıma sırasında ambalajlarda meydana gelebilecek yırtılma, delinme gibi fiziksel hasarlardan ürün güvenliğinin azalması gibi dezavantajlara da yol açabilmektedir (Kılınç ve Çaklı, 2004). Farklı gıdaları paketlemek için değişik gaz karışım konsantrasyonları uygulanmaktadır (Özoğul vd., 2006).

Karbondioksit (CO_2) gazı fungistatik ve bakteriyostatik özelliklerinden dolayı balıkların modifiye atmosferde paketlenmesinde önemlidir ve bozulmaya neden olan bakterilerin gelişmesini yavaşlatmaktadır. Maksimum antimikrobiyal etki elde etmek için, MAP ürününün saklama sıcaklığı mümkün olduğunca düşük tutulmalıdır (Farber, 1991). Oksijen (O_2) genel olarak aerobik bakterilerin büyümesini teşvik etmekte ve anaerobik bakterilerin büyümesini engellemektedir. Düşük oksijen seviyesinde (<% 0,5), et ve et ürünlerinde kahverengi veya kahverengi/gri renk meydana gelmektedir. Azot (N_2), antimikrobiyal aktiviteye sahip hoş olmayan inert bir gaz olup, su ve yağda karbondioksite göre çok az çözündüğü için dolgu gazı olarak kullanılmaktadır. Nitrojen ayrıca ambalajda oksijenin yerini almakta, oksidasyonu yavaşlatmakta ve aerobik mikroorganizmaların büyümesini engellemektedir (Sivertsvik vd., 2002).

1.1.2. Vakum Paketleme

Vakum paketleme yapılan gıdaların ambalajında hava olmadığı için bakterilerin üremesi ve ürünlerin oksidasyonu engellenmektedir. Fakat vakum ürünlerinde su aktivitesi ve pH gibi faktörlere bağlı olarak anaerobik ve fakültatif türler ile *Lactobacillus* türleri gelişebilmektedir (Kılınç ve Çaklı, 2001). Balıklar ve diğer su canlılarda vakum paketleme kullanımı donan yağlı balıklardan (örn. somon, uskumru, sardalya) kaynaklanan donma hasarını azaltmaya yardımcı

olmaktadır. Vakum paketlemenin bir diğer önemli avantajı da paket hacminin ürünün hacmine eşit olması ve paket içinde boş alan olmamasıdır. Vakum paketleme ile karşılaştırıldığında, MAP daha çok yönlüdür, çünkü ilki yalnızca hava gidermeye dayanır, ikincisi belirli gıdalarda kullanılabilmektedir. Raf ömrünü uzatmak için farklı gazlar bir karışım içinde ve farklı konsantrasyonlarda kullanılmaktadır (Dikel, 2022).

2. Balık Muhafaza Yöntemleri ve Depolama Süresi Üzerine Etkileri ile İlgili Yapılan Çalışmalar

Parkin vd. (1981) modifiye atmosferde ambalajlamada hava ile ambalajlamaya göre trimetil amin (TMA), aerobik bakteri sayısı ve pH değerlerinin daha düşük olduğunu, modifiye atmosferle ambalajlamanın 13 gün süreyle kaya balığı (*Sebastes* spp.) fileto kalitesini koruduğunu bildirmektedirler. Fey ve Regenstein (1982) taze berlam (*Merluccius merluccius*) ve somon (*Salmo* spp.) balıklarında modifiye atmosfer ve potasyum sorbatlı buz kullanımının raf ömrünü artırdığını tespit etmişlerdir.

Lynch ve Potter (1982) 7 °C'de yapılan depolama süresince %0,5 potasyum sorbat içeren morina balığı (*Gadus* spp.) kıymalarında toplam aerobik bakteri sayısını kontrol grubuna göre daha düşük bulmuşlardır. Regenstein (1982) gaz karışımı ve gaz karışımı + potasyum sorbat uygulanmış mezgit balığı (*Melanogrammus aeglefinus*) filetolarında hava ve vakum paketlemenin duyusal özellikleri olumlu yönde etkilediğini bildirmiştir.

Bremner ve Statham (1983) taraklarda (*Pecten alba*) vakum ambalajlama ve potasyum sorbat uygulamasının başlangıç *Vibrio* florasını depolama süresince düşürdüğünü, sorbat uygulanan numunelerde ise önce düşüş daha sonra yükseliş rapor etmişlerdir. Statham vd. (1985) derin deniz levreklerinde (*Nemadactylus macropterus*, jackassfish) vakum amabalajlamanın potasyum sorbatın %100 CO_2 atmosferinden daha etkili olduğunu belirtmişlerdir. Taze beyaz göl balığı (*Coregonus clupeaformis*) filetolarında %5 potasyum sorbat uygulamasının raf ömrünü uzattığı tespit edilmiştir (Sharp vd., 1986). Sazan balıklarında (*Cyprinus carpio*) sorbat muamelesinin raf ömrünü uzattığı saptanmıştır (Gelman vd., 1990).

Barnett vd. (1987) modifiye atmosfer paketlemede yarı geçirgen ambalajlama materyali kullanıldığında alabalık (*Salmo gairdneri*) filetolarının raf ömrünün uzadığı, potasyum sorbat uygulamasının modifiye atmosfer paketlemeden önce yapılmasının ise mikrobiyal gelişmeyi düşürdüğünü bildirmektedirler. Harrison ve Heinsz (1989) karideslerde (*Penaeus aztecus*) raf ömrünün potasyum sorbatlı buz kullanımı ile uzadığını ve bozulmaya sebep olan bakterilerin lag ya da adaptasyon fazını uzattığını rapor etmişlerdir.
Vakum ve modifiye atmosferde ambalajlanan ve 0 °C'de depolanan morina balığı (*Gadus morhua*) filetolarında raf ömrünün, vakum ambalajlama ile karşılaştırıldığında modifiye atmosferde %100 CO_2 kullanımının 2-3 gün, %48 CO_2 kullanımının ise 6-7 gün uzadığı kaydedilmiştir (Dalgaard vd., 1993). Tilapia (*Tilapia* spp.) balıklarında raf ömrünün hava ile ambalajlanan filetolarda 9 gün, %25 N_2, %50 CO_2 gaz karışımında 13 gün, %50 CO_2+%50 N_2 gaz karışımında 23 gün ve %25 CO_2+%75 N_2 gaz karışımında ise 30 gün olduğu saptanmıştır (Reddy vd., 1994). İzgi (1995) tarafından gökkuşağı alabalığında (*Oncorhynchus mykiss*) kontrol grubunda 7 gün, %50 CO_2+%50 N_2 gaz karışımında 9 gün, %50 CO_2+%45 N_2+%5 O_2 ile %100 CO_2 gruplarına ait örneklerde ise 12 gün raf ömrü olduğu ve en iyi sonucu %50 CO_2+%45 N_2+%5 O_2 gaz karışımının verdiği bildirilmiştir.

Soğukta muhafaza edilen kedi balığı (*Ictalurus punctatus*) filetolarında laktik asit kültür, potasyum sorbat ve sodyum asetat uygulamanın Gram negatif bakteri gelişimini 6 günden daha fazla bir süre inhibe ettiği tespit edilmiştir (Kim ve Hearnsberger, 1994).

Modifiye atmosferde ambalajlanan morina (*Gadus morhua*) filetolarında bakteri gelişimini sınırlayıcı bir etkiye sahip olduğu ve toplam uçucu baz (TVB) ve TMA değerlerini düşürdüğü bildirilmiştir (Debevere ve Boskou, 1996).

Gökkuşağı alabalığı (*S. gairdneri*) ve Baltık ringa balığı (*Clupea harengus membras*) filetoları vakum ve modifiye atmosferde paketlenerek 2 °C'de depolanmış ve mikrobiyolojik analizlere tabi tutulmuştur. Mezofilik bakterilerin vakum ve gaz ambalajlamada, koliform grubu bakterilerin ise gaz ambalajlamada gelişiminin yavaş olduğu tespit edilmiştir (Randell vd., 1997).

Potasyum sorbat, sodyum glukonat ve poatsyum sorbat-sodyum glukonatın her ikisi ile birlikte potasyum muamele edilerek modifiye atmosferde ambalajlanan çipura (*Sparus aurata*) filetolarında sodyum-glukonatın birlikte kullanımının potasyum sorbatın tek başına kullanımından daha etkili olduğu, glukonat muamelesinin Gram pozitif mikroorganizmaların gelişimini sınırlandırdığı saptanmıştır (Drosinos vd., 1997).

López-Gálvez vd. (1998) %20 CO_2 ile ambalajlanan dil balığı (*Solea solea*) filetolarında normal raf ömrünün 4 gün olduğunu, ancak %40 CO_2+%60 O_2 gaz karışımı ile paketlemenin raf ömrünü 8 günün üzerine çıkardığını tespit etmişlerdir.

Potasyum sorbatla muamele edilen taze hamsilerin (*Engraulis encrasicolus*) depolama ve taşınması esnasında kimyasal ve mikrobiyolojik kalitenin daha uzun süre korunduğu saptanmıştır (Yapar ve Yetim, 2000). Vakum ve modifiye atmosferde ambalajlamanın atlantik ringa balığında (*Clupea harengus*) buzda depolamaya göre raf ömrünü uzattığı bildirilmiştir (Özoğul vd., 2000).

Shalini vd. (2001) tarafından potasyum sorbatla muamele edilerek vakum ambalajlanan ve buzdolabı sıcaklığında depolanan imparator balığında (*Lethrinus lentjan*) mikrobiyal gelişmenin yavaşladığı, bozulmanın geciktiği ve dolayısıyla raf ömrünün uzadığı belirtilmiştir.

Potasyum sorbata daldırılmış kolyoz (*Scomber japonicus*) filetolarının daha düşük mikrobiyal yüke sahip olduğu vurgulanmıştır (Erkan vd., 2001).

Potasyum sorbat uygulamasının vakum ve MAP ile ambalajlanan ve soğukta muhafaza edilen gökkuşağı alabalığı (*O. mykiss*) filetolarında kimyasal kalitenin daha uzun süre korumasına katkıda bulunabileceği ve bakteriyel gelişmeyi sınırlandırdığı bildirilmiştir (Çarbaş, 2008).

Gümüş levrek (*Bidyanus bidyanus*) balığında potasyum sorbat uygulamasının bakteri sayısındaki artışı önemli ölçüde azalttığı belirlenmiştir (Gelman vd., 2001).

Patır vd. (2001) potasyum sorbat uygulanmış tuz kürü aynalı sazan (*C. carpio*) filetolarında potasyum sorbat uygulanmış ve %10 oranında tuzlanmış örneklerde mikroorganizma sayısının nispeten daha az olduğunu saptamıştır.

Giménez vd. (2002) modifiye atmosferde ambalajlamanın gökkuşağı alabalığı (*O. mykiss*) filetolarında raf ömrünü önemli ölçüde uzattığını tespit etmişlerdir.

Erkan vd. (2002) tuzlanmış alabalığın (*O. mykiss*) raf ömrünün %5 O_2+%35 CO_2+%60 N_2 gaz karışımında 60 gün, %30 CO_2+%70 N_2 gaz karışımında ise 75 gün olduğunu bildirmektedir.

Aras Hisar (2002) tarafından gökkuşağı alabalıklarında (*O. mykiss*) modifiye atmosferde ambalajlamanın raf ömrünü uzattığı tespit edilmiştir.

Tuzu giderilmiş morina (*G. morhua*) balıklarında sitrik asit/potasyum sorbat kombinasyonunun, izolatlar üzerinde farklı derecede inhibisyon etkisi gösterdiği belirlenmiştir (Pedro vd., 2004).

Özoğul vd. (2004) sardalyanın (*Sardina pilchardus*) raf ömrünün modifiye atmosferde 12, vakum ambalajlamada 9 ve havada 3 gün olduğunu, bakteri gelişiminin modifiye atmosferde oldukça yavaş seyrettiğini rapor etmiştir.

Taze morina (*G. morhua*) filetolarında vakum ve %65 N_2+%30 CO_2+%5 O_2 gaz karışımı uygulanarak ambalajlanmış ve 4 °C'de depolanmış örneklerin raf ömrünün daha uzun olduğu ve depolama sıcaklığı düştükçe bakteriyel gelişme oranının azaldığı ve lag fazının uzadığı tespit edilmiştir (Corbo vd., 2005).
Sardalya (*S. pilchardus*) balıklarında sitrik ve sorbik asitin raf ömrünü olumlu yönde etkileyen katkı maddeleri olduğu belirlenmiştir (Altın, 2006).

Tüketime hazır morina (*G. morhua*) balıklarında mikrobiyal gelişmenin normal paketlerde daha hızlı olduğu, modifiye atmosfer uygulamasının bu gelişmeyi önemli oranda azalttığı, ayrıca sitrik asit ve sorbat ya da sitrik asit-sorbat solüsyonu ile işlem görmüş filetolarda modifiye atmosferin daha etkin olduğu belirlenmiştir (Magnússon vd., 2006).

Modifiye atmosferde ambalajlamanın sardalyanın (*S. pilchardus*) raf ömrünü uzattığı bildirilmiştir (Erkan vd., 2006). Sardalya balıklarında (*S. pilchardus*) mikrobiyal floranın *Shewanella putrefaciens, Pseudomonas, B. thermosphacta,* Enterobacteriaceae ve laktik asit bakterilerinden oluştuğu, bakteri gelişiminin hava ile ambalajlamada hızlı, modifiye atmosferde yavaş seyrettiği saptanmıştır (Stamatis ve Arkoudelos, 2007).

Abosrea (2007) potasyum sorbat/*Bifidobacterium* kombinasyonunu uygulanan Nil tilapia balığı (*Oreochromis niloticus*) filetolarının fizikokimyasal, mikrobiyolojik ve duyusal özelliklerini -18 °C'de 24 hafta, 4 °C'de ise 12 gün koruduğunu tespit etmiştir.

Siyah pomfret (*Parastromateus niger*) ve yeşil çiklit (*Etroplus suratensis*) balıklarında vakum uygulanarak ambalajlanmış örneklerde raf ömrünün sırasıyla 8 ve 10 gün olduğu, sodyum asetat ve potasyum sorbatla muamele edilen vakum ambalajlanmış siyah pomfret balığı örneklerinde bu sürenin 16 gün, aynı uygulamaların yapıldığı yeşil çiklit balık örneklerinde ise 15 gün olduğu rapor edilmiştir (Manju vd., 2007).

Pantazi vd. (2008) hava ile ambalajlanmış kılıç balığında (*Xiphias gladius*) *Pseudomonas* spp. ve H_2S üreten bakterilerin dominant olduğunu, vakum ve modifiye atmosferde ambalajlamanın bakterileri gelişimini kısmen inhibe ettiğini, Enterobacteriaceae ve laktik asit bakterilerinin de mikrobiyal florada bulunduğunu belirlemişlerdir.

Potasyum sorbat uygulamalarının vakumla paketlenmiş inci kefali (*Chalcalburnus tarichi*) filetolarında toplam aerobik mezofilik bakteri, psikrotrofik bakteri, Enterobacteriaceae, maya-küf sayıları, TVB-N değerleri, feniletilamin, putresin, triptamin ve pH değerleri üzerine önemli derecede etkisinin olduğu belirlenmiştir. Özellikle %5 seviyesinde potasyum sorbat uygulaması ve vakumlu paketlemenin soğukta depolanan filetoların biyojenik aminlerini, mikrobiyolojik ve kimyasal kalitelerini daha uzun süre korumalarına yardımcı olduğu sonucuna varılmıştır (Genççelep vd., 2014).

Gandotra vd. (2014) tarafından gümüş sazanı (*Hypophthalmichthys molitrix*) üzerinde yürütülen çalışmada, örnekler A (kontrol-işlenmeden alüminyum folyo kaplanmış) ve B (%5 potasyum sorbat çözeltesine 15 dakika daldırılıp, alüminyum folyo kaplanmış) gruplarına ayrılarak hava geçirmez kaplarda -12±2 ºC'de 30 gün süreyle depolanmış ve depolamanın 0, 10, 20 ve 30. Günlerinde biyokimyasal ve

mikrobiyolojik analizlere tabi tutulmuştur. B grubu filetoların TBA, FFA (serbest yağ asidi), toplam bakteri, koliform grubu bakteri ve psikrotrofik bakteri sayısı kontrol (A) grubundan daha düşük değerler gösterdiği belirlenmiştir. %5 potasyum sorbat muamelesinin dondurulmuş filetoların raf ömrünü korumada etkili olduğu, depolama süresinde kimyasal ve mikrobiyolojik bozulmanın geciktirildiği, filetoların depolamanın son gününde (30) bile iyi durumda olduğu tespit edilmiştir.

Kocatepe vd. (2016) tarafından yürütülen araştırmada, farklı CO_2/N_2 (MAP) gaz konsantrasyonlarının (atmosferik hava (kontrol) (A), %60CO_2 + %40N_2 (B), %75CO_2 + %25N_2 (C), %40CO_2 + % 60N_2 (D)) 3±1 ºC'de depolanan alabalık kıymasının kimyasal/duyusal stabilite, renk özellikleri ve mikrobiyolojik özellikleri üzerindeki etkisi incelenmiştir. Analizler depolamanın 1., 4., 8., 12., 16., 20., 24. ve 28. Günlerinde yapılmıştır. Üç modifiye atmosfer gazı kompozisyonu arasında en iyi koruma yöntemi ve alabalık kıyması için en uygun gaz konsantrasyonu %75 CO_2 içeren C grubunda bulunmuştur. Depolamanın 12. Gününde kontrol (A) grubunun, 16. Gününde MAP B grubunun, 24. Gününde MAP C grubunun ve 16. Gününde MAP D grubunun tüketilemeyeceği tespit edilmiştir.

Fadıloğlu ve Çoban (2020) yaptıkları araştırmada, potasyum sorbat (%1) ve karanfil yağının (%1) 2 °C'de 20 gün depolama süresince kerevitlerin (*Astacus leptodactylus*) kalite özellikleri üzerindeki antioksidan ve antibakteriyel etkilerini karşılaştırmışlardır. Depolamanın 10. Günden sonuna kadar potasyum sorbat uygulamasının kerevit örneklerinin TBA değerleri üzerinde kontrol ve karanfil yağı uygulanmış örneklere göre önemli etkisinin olduğunu ($p<0,05$), hem potasyum sorbat hem de karanfil yağı uygulamasının, 20 günlük depolama boyunca lipid oksidasyonunu geciktirdiğini tespit etmişlerdir.

Buzdolabı sıcaklığında 15 gün boyunca depolanan domuz balığı (çotira) (*Alosa immaculata*, Bennett, 1838) filetolarının fizikokimyasal (renk ve doku, pH ve total uçucu baz azotu-TVB-N), mikrobiyolojik (toplam bakteri sayım, psikrotrofik, sülfür indirgeyen bakteriler ve asit-laktik bakteriler), duyusal kalite özellikleri ve ve raf ömrü üzerinde farklı paketleme türlerinin (havada (kontrol), vakumda (VP) ve modifiye atmosferde (MAP) etkilerinin araştırıldığı bir çalışmada, numuneler 0. gün (taze balık), 5., 10. (8 ve 12. duyusal analiz için) ve 15. günlerde analiz edilmiştir. MAP, kontrol grubundaki filetolara kıyasla TVB-N içeriklerindeki artışı engellemiştir. Kontrol grubundaki filetoların (toplam bakteri sayısı ve psikrotrofik bakteri sayısı, 10. Günde 7 log (kob/g)'ı aşarken, vakum ve modifiye atmosferde paketlenmiş filetolarda bu sınırın altında kalmıştır. Kontrol grubu filetoların raf ömrü 8 gün iken, vakum ve modifiye atmosferde paketlemenin raf ömrünü sırasıyla 15 ve 12 güne kadar uzattığı tespit edilmiştir (Esteves vd., 2021).

SONUÇ

Yapılan çalışmalar göz önüne alındığında balık etlerinin muhafazasında paketleme metotlarının başarıyla uygulandığı ve raf ömrü üzerine olumlu etkiler yaptığı rapor edilmiştir. Ayrıca antimikrobiyal maddelerin kullanımında balık etlerinde kimyasal ve mikrobiyal bakımdan olumsuz değişikliklere neden olmaksızın raf ömrünü uzattığı bildirilmiştir. Sonuç olarak balık etlerinin muhafazasında gelecekte yeni katkı maddeler kullanılarak teknoloji ışığında paketleme materyalleri ya da yöntemleri geliştirilmesi sektörün gelişmesi bakımından önem arz etmektedir.

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Yazarlara Önemli Not

Tüm yazarlar:

Sunulan makalenin yazar(lar)ın orijinal çalışması olduğunu, tüm yazarların bu çalışmaya bireysel olarak katılmış olduklarını ve bu çalışma için her türlü sorumluluğu aldıklarını, sunulan makalenin tüm yazarlarından makaleyle ilgili tüm mali hakları Atatürk Üniversitesi Alabalık Dergisine devrettiklerini, formlardaki taahhütleri kabul ettiklerini, doğmuş veya doğabilecek tüm uyuşmazlıklardan sorumlu olacaklarını, tüm yazarların sunulan makalenin son halini gördüklerini ve onayladıklarını, tüm yazarlarla ilgili e-mail ve posta adreslerinin dergi sistemine doğru girildiğini (sonradan olan değişikliklerin ivedi olarak bildirilmesini), makalenin yazılması sırasında kullanılan metin işleme çizim fotoğraflama analiz gibi her türlü bilgisayar programının telif haklarını çiğnemediklerini, makalenin başka bir yerde basılmadığını veya basılmak için sunulmadığını, makalede bulunan metnin şekillerin ve dokümanların diğer şahıslara ait olan telif haklarını ihlal etmediğini, sunulan makale üzerindeki mali haklarını özellikle işleme, çoğaltma, temsil, basım yayım, dağıtım ve internet yoluyla iletim de dahil olmak üzere her türlü umuma iletim haklarını Atatürk Üniversitesi Alabalık Dergisi yetkili makamlarınca sınırsız olarak kullanılmak üzere Atatürk Üniversitesi Alabalık Dergisine devretmeyi kabul ve taahhüt eder. Buna rağmen yazar (lar)ın veya varsa yazar (lar)ın işvereninin patent hakları, yazar (lar)ın gelecekte kitaplarında veya diğer çalışmalarında makalenin tümünü ücret ödemeksizin kullanma hakkı, makaleyi satmamak koşuluyla kendi amaçları için çoğaltma hakkı gibi fikri mülkiyet hakları saklıdır. Bununla beraber yazar(lar) makaleyi çoğaltma, postayla veya elektronik yolla dağıtma hakkına sahiptir.

Makalenin herhangi bir bölümünün başka bir yayında kullanılmasında Atatürk Üniversitesi Alabalık Dergisi'nin yayımcı kuruluş olarak belirtilmesi ve dergiye atıfta bulunulması şartıyla izin verilir. Sorumlu yazar olarak, telif hakkı ihlali nedeniyle üçüncü şahıslarca istenecek hak talebi veya açılacak davalarda Atatürk Üniversitesi Alabalık Dergisi ve dergi editörlerinin hiçbir sorumluluğunun olmadığını, tüm sorumluluğun sorumlu yazara ait olduğu taahhüt edilir. Ayrıca makalede hiçbir suç unsuru veya kanuna aykırı ifade bulunmadığını, araştırma yapılırken kanuna aykırı herhangi bir malzeme ve yöntem kullanılmadığı, çalışma ile ilgili tüm yasal izinlerin alındığı ve etik kurallara uygun hareket edildiği taahhüt edilir. Yayınlanan makalelere ayrıca telif ücreti ödenmez.