EFFECTS OF CHITOSAN COATING ON MOISTURE LOSS, MICROBIAL GROWTH AND LIPID OXIDATION KINETICS OF MINCED ANCHOVY MEAT DURING STORAGE

by

Müge Kırtış

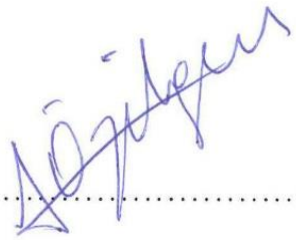
Submitted to Graduate School of Natural and Applied Sciences
in Partial Fulfillment of the Requirements
for the Degree of Master of Science in
Biotechnology

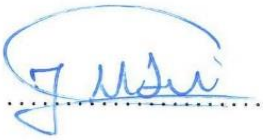
Yeditepe University

2017

EFFECTS OF CHITOSAN COATING ON MOISTURE LOSS, MICROBIAL GROWTH AND LIPID OXIDATION KINETICS OF MINCED ANCHOVY MEAT DURING STORAGE

APPROVED BY:

Assoc. Prof. Dr. Zehra Sibel Özilgen ..... 
(Thesis Supervisor)

Assoc. Prof. Dr. Mahmut Şeker ..... 

Assist. Prof. Dr. İskender Karaltı ..... 

DATE OF APPROVAL: ..../..../2017

To My Mother...

ACKNOWLEDGEMENTS

First I would like to thank Assoc. Prof. Dr. Sibel Özilgen, my thesis advisor who provided me endless support, guidance and motivation in my work and showed immense patient during our work together since the first day of my master's degree.

I would like to thank Prof. Dr. Seyda Bucak, Head of Chemistry Department of Yeditepe University for the support in the use of laboratory facilities and for the guidance during analysis stage of my study; my dear friend Elçin Yenigül who helped me again in the analysis stage and Assist. Prof. Dr. İskender Karaltı from Nutrition and Dietetics Department of Yeditepe University for his support in microbiological analysis.

I would like to express my sincere thanks to Nicolas H. C. Cartier and Fahri Demir, dear instructor chefs of Yeditepe University Gastronomy and Culinary Arts Department, giving all kinds of support to me and providing the use of laboratories.

I would like to thank Merve Buğdaypınarı for her kind helps and support.

And finally I would like to thank my family and my mum who have never denied their full unconditional support to me.

ABSTRACT

EFFECTS OF CHITOSAN COATING ON MOISTURE LOSS, MICROBIAL GROWTH AND LIPID OXIDATION KINETICS OF MINCED ANCHOVY MEAT DURING STORAGE

Fish is commonly consumed raw, and also used in the food industry to produce fish oil, fish flour, frozen fish, canned fish and fish ball. Different techniques have been used to prevent loss of quality that may occur in sea food during storage. One of these techniques is coating food with edible films. Kinetics of lipid oxidation, and the effects of coating on quality loss in foods have been extensively studied in the literature. However, there are only a few studies on the protective effects of edible coating on the lipid oxidation kinetics of minced fish meat. In this study, the effects of chitosan coating on oxidation kinetics, microbial load, and moisture content of anchovy minced meat, stored at different temperatures, 20 ℃, 4 ℃, -1 ℃ and -18 ℃, were studied. Spread plate technique was used to analyze the total viable count. Thiobarbituric acid and malondialdehyde complex was determined by using UV-visible spectrophotometer to measure lipid oxidation. Logistic equation simulated lipid oxidation in the fish samples. Numerical values of the model parameters were used to analyze the effect of coating on lipid oxidation in the samples. The k values obtained from the slopes of the logistic equation were used in the Arrhenius equation to analyze the temperature dependence of the reaction rate. Chitosan coating decreased the rate of propagation stage of lipid oxidation in all samples compared to the corresponding uncoated samples. The coating process decreased the maximum malondialdehyde value (C_{max}) in the samples compared to the uncoated samples, stored at the same temperature. The rate of malondialdehyde formation was found to be depended on the storage temperature and followed Arrhenius equation in all samples. Chitosan coating decreased the percent moisture loss and the rate of moisture loss for all storage temperatures.

ÖZET

HAMSİ BALIĞI KIYMASINDA KİTOSAN KAPLAMANIN NEM KAYBI, MİKROBİYAL BÜYÜME VE LİPİT OKSİDASYON KİNETİĞİ ÜZERİNDEKİ ETKİLERİ

Balık, gıda endüstrisinde ham madde olarak balık yağı, balık unu, dondurulmuş balık ve konsenserve balık gibi farklı gıda ürünlerinde yaygın bir şekilde tüketilmektedir. Depolama sırasında meydana gelebilecek kalite kayıplarını önlemek amacıyla, birçok teknik geliştirilmiştir. Bu tekniklerden birisi de yenilebilir film kaplama tekniğidir. Lipit oksidasyon kinetiği, kaplamanın gıdadaki kalite kayıbı üzerindeki etkisi yaygın olarak literatürde görülmektedir. Ancak balık kıymasına uygulanan kaplamanın lipit oksidasyon kinetiğindeki koruyucu etkisi hakkındaki çalışmalar azdır. Bu çalışmada, farklı sıcaklılarda, 20 ℃, 4 ℃, -1 ℃ ve -18 ℃, depolanan hamsi kıymasında kitosan kaplamanın, oksidasyon kinetiği, mikrobiyal yükleri ve nem miktarlarındaki etkileri çalışılmıştır. Toplam canlı sayımını analiz etmek için yayma plaka tekniği kullanılmıştır. Lipit oksidasyonu ölçmek için, Tiyobarbitürik asit ve malondialdehit kompleksi UV-görünür spektofotometre kullanılarak tayin edilmiştir. Lojistik denklem, balık örneklerinde meydana gelen lipit oksidasyona simüle edilmiştir. Örneklerde, kaplamanın lipid oksidasyona etkisi, model parametrelerinin sayısal değerleri kullanılarak analiz edilmiştir. Lojistik denklemin eğiminden elde edilen bu k değerleri, Arrhenius denkleminde kullanılarak, reaksiyon hızının sıcaklığa bağlı olup olmadığı incellenmiştir. Tüm örneklerde kitosan kaplama, lipid oksidasyondaki yayılma basamağındaki hızını, kaplanmamış örneklere kıyasla düşürmüştür. Tüm örneklerde kaplama işlemi, maksimum malonaldehit değerini (C_{max}), kaplanmamış örneklere kıyasla düşürmüştür. Malonaldehit oluşma hızı, tüm numunelerde depolama sıcaklığına bağlı olduğu Arrhenius denklemine göre açıklanmıştır. Kitosan kaplama tüm depolama sıcaklıklarında nem yüzdesini ve nem kaybı hızını düşürmüştür.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF SYMBOLS/ABBREVIATIONS

CFU	Colony unit-forming
DHA	Docosahexaenoic asit
EPA	Eicosapentaenoic asit
FAO	Food and Agriculture Organization
MDA	Malondialdehyde
MUFA	Monounsaturated fatty acids
PUFA	Polyunsaturated fatty acids
SFA	Saturated fatty acid
SPSS	Statistical Package for the Social Sciences
TBA	Thiobarbituric acid
TBC	Total Bacteria Count
TCA	Trichloroacetic acid
TEP	1.1.3 Tetrahoxypropane
TÜİK	Türkiye İstatistik Kurumu
UV	Ultraviolet

1. INTRODUCTION

Today, seafood is at the top of the list of foods that are preferred the most in many countries due to its cost-efficiency and rich nutrient content. According to the statistics of Turkish Statistics Institute, in 2015 total seafood production was 397.731 tonnes, farmed seafood production was 240.334 tonnes and fresh water farmed seafood production was 34.176 tonnes [1]. Total average fish consumption is 24 kg in the European Union and 7.6 kg in Turkey [2]. In the developed countries fresh fish consumption constitutes 5 %, frozen fish constitutes 42 %, smoked fish constitutes 15% and canned fish consumption constitute 5 % of the total fish consumption [3].

Fish is highly rich in two types of polyunsaturated fatty acid (PUFA) which are not produced in the body and therefore need to be obtained from food: EPA (Eicosapentaenoic acid) and DHA (Docosahexaenoic acid). EPA and DHA are known as polyunsaturated fatty acids that contain essential omega-3 fatty acids. Polyunsaturated fatty acids, essential amino acids, minerals and vitamins in seafood makes it a valuable food in a healthy and balanced diet [4].

In the industry, commonly, fish is cleaned right after it is caught, then packaged in air tight packages and stored for further process. Smoking, salting, drying, canned and frozen storage methods are used commonly to extend the shelf life today. When these methods are not applied properly, very serious adverse changes occur in the chemical, biological and sensory profile of fish [5]. Shelf life of fish is around 3 days when stored in a refrigerator (+5 °C). Storage time in a freezer (-18 °C) is 3 months for small fish such as European anchovy, sardine, and 5-6 months for gilthead bream and bluefish [6]. Oxidation of fatty acids and associated discoloration, off odours and off-flavours, undesirable microbial growth, loss of moisture and associate changes in texture are the most common adverse changes. These changes cause both economic and nutrient losses [7]. Studies have demonstrated that the oxidation rate of fatty acids depends on the oxygen concentration in the environment, the food's surface area in contact with oxygen, types and amounts of fatty acids in the food, temperature and humidity of the environment and to light and metals [8].

Edible coatings have been used in some food such as fruits, meat and meat products to prolong their shelf lives and demonstrated to be effective in delaying certain biological and chemical reactions leading to spoilage [9]. Studies have observed that edible film coatings reduce fatty acid oxidation by creating an oxygen barrier in fish rich in essential unsaturated fats, reduce loss of moisture by creating a barrier, and show an antimicrobial effect and prolong shelf life [10].

This study aims to study the effects of chitosan coating on the kinetics of lipid oxidation, and also loss of moisture, and microbial growth in minced anchovy fish stored at different temperatures.

2. LITERATURE REVIEW

2.1. FISH CONSUMPTION AND NUTRIENT COMPOSITION

Seafood production increased by 25.1 % and reached to 672.241 tonnes in Turkey in 2015 as shown in Figure 2.1. Sea fish constitutes 51.4 % , other seafood constitutes 7.7 %, fresh water fish constitutes 5.1 % and farmed fish constitutes 35.8 % of the production [1].

Average annual fish consumption is around 7 kg in Turkey. When compared with the EU countries, despite being in the 7th position in fish production, Turkey is at the bottom for consumption [11].

More than 80 % of marine products are used for human food in our country. Remaining part is used for fish flour, fish oil and other purposes. This consumption has the values of 75 % fresh fish, 4 % frozen fish and 2 % processed fish [12].

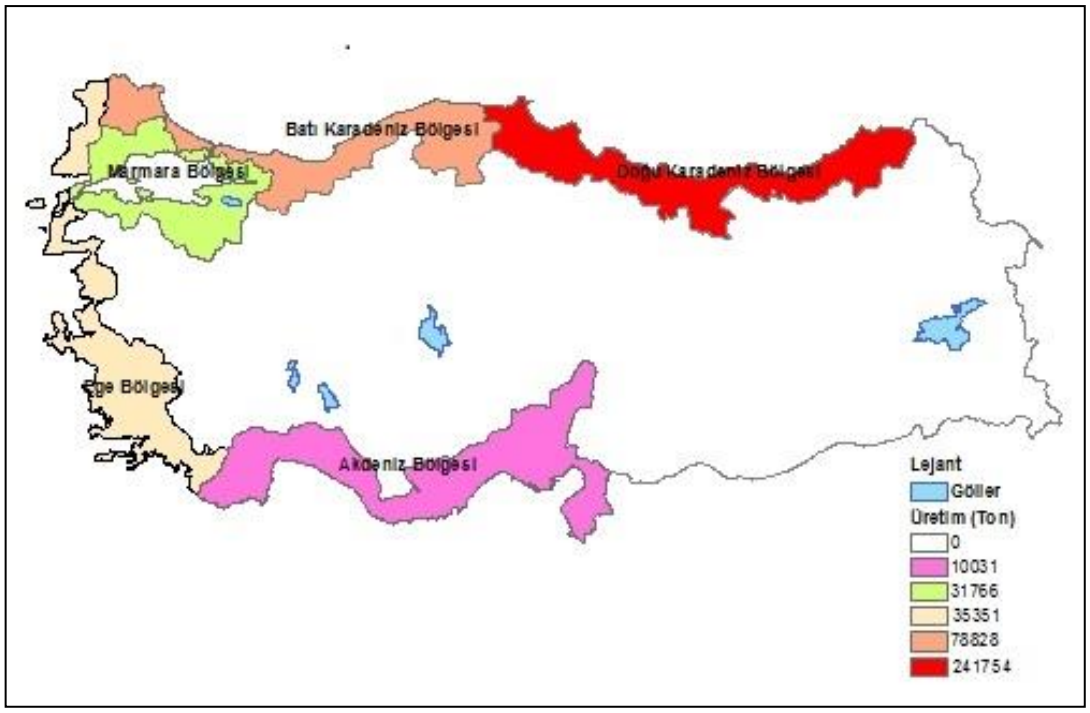


Figure 2.1. Production of seafood in Turkey in 2015

Fish meat is a perfect food in for its nutrient values. It contains all essential amino acids (leucin, lysine, valine, methionine, phenylalanine, threonine, tryptophane, histidine, arginine) required for the protection and growth of body tissues [13].

Fish meat is also rich in fat soluble vitamins, A, D and E; and thiamine (B1), riboflavin (B2), pyridoxine (B6), niacin (B3) and cobalamin (B12), vitamin B group which are soluble in water [14] .

Fish and other seafoods have distinctive importance in healthy diet model for their rich mineral content, such as iodine, selenium, phosphorus, magnesium and zinc [15].

Another major nutrition component of seafood is fat. Fat content of fish meat vary greatly; for the same fish species not only depending on the fish species but also on the seasonal conditions, feeding characteristics, salt percentage in the water. Fish that have a fat content less than 2 % are generally classified as not oily and fish that have a fat content higher than 5 % are classified as oily fish [16].

Average composition of fish was shown in Table 2.1 [17].

Table 2.1. Average composition of fish

Component	**Minumum (%)**	**Average Range (%)**	**Maximum (%)**
Protein	6,0	16-21	28
Fat	0,1	0,2-25	67
Carbohydrate	-	< 0,5	-
Ash	0,4	1,2-1,5	1,5
Water	28,0	66-81	96

A large portion of the fat in the fish meat is found in the form of triglyceride which is an ester derived from glycerol and three fatty acids as shown in Figure 2.2 [18].

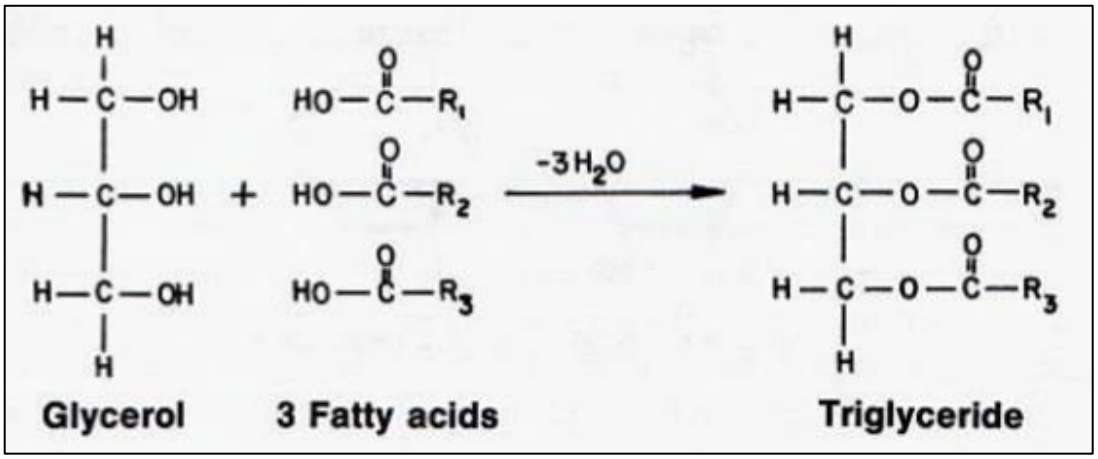


Figure 2.2. Formation of triglycerides

Fatty acid classification is primarily done based on the number of bond between the carbon atoms in long hydrocarbon chain. A saturated fatty acid is type of fatty acid which the fatty acids have single bonds in the carbon chain. An unsaturated fatty acid is type of fatty acid which the fatty acids have one or more double bonds in the carbon chain. A monounsaturated fatty acid (MUFA) has just one double bond, a polyunsaturated fatty acid (PUFA) has two or more double bonds in the carbon chain.as shown in Figure 2.3 [19].

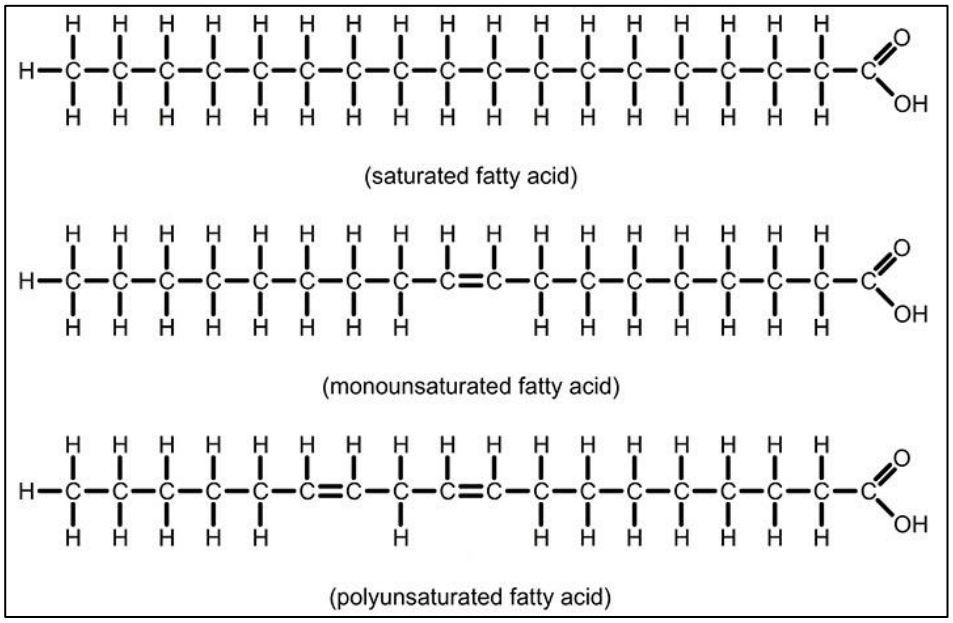


Figure 2.3. Chemical structure of saturated and unsaturated fatty acids

Unsaturated fatty acids are found in nature are omega-9, omega-6 and omega-3 and they are called oleic, linoleic and linolenic, respectively as shown in Table 2.2 [20]. Two very important fatty acids, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), are found in all seafood [21].

Saturated fatty acids (SFA) and monounsaturated omega-9 fatty acids are able to be synthesized in human body. Therefore, it is not compulsory to take these fatty acids from external food sources. Polyunsaturated fatty acids (PUFA) including more than one double bonds are categorized as two subgroups omega -3 and omega-6 and both fatty acids must be taken externally because they cannot be synthesized in human body. These fatty acids are accepted as "essential" and their consumption by foods has great importance for healthy nutrition [22].

Table 2.2. Unsaturated fatty acids and numerical formulas

Fatty acid	**Molecular Formula**	**Structure Formula**
Oleic acid (Omega 9)	$C_{18}H_{34}O_2$	$CH_3.(CH_2)_7.CH=CH(CH_2)_7.COOH$
Linoleic (Omega 6)	$C_{18}H_{32}O_2$	$CH_3.(CH_2)_4.CH=CH.CH_2.CH=CH.(CH_2)_7.COOH$
Linolenic (Omega 3)	$C_{18}H_{30}O_2$	$CH_3.CH_2.CH=CH.CH_2.CH=CH.CH_2.CH=CH.(CH_2)_7.COOH$

Fatty acids and their saturation levels in various seafood are listed in Table 2.3 [23].

Table 2.3. Fatty acid amount in some seafood

Type	Fat (g/100g)	SFA (g/100g)	MUFA (g/100g)	PUFA (g/100g)	EPA (g/100g)	DHA (g/100g)	Cholesterol (mg)
European anchovy	4.8	1.3	1.2	1.6	0.5	0.9	-
Herring	9.0	2.0	3.7	2.1	0.7	0.9	60
Mackerel	13.0	2.5	5.9	3.2	1.0	1.2	53
Tuna	6.6	1.7	2.2	2.0	0.4	1.2	38
Carp	5.6	1.1	2.3	1.3	0.2	0.1	67
Codfish	0.7	0.1	0.1	0.3	0.1	0.2	43
Hake	1.6	0.3	0.3	0.6	0.2	0.2	-
Halibut	2.3	0.3	0.8	0.7	0.1	0.3	32
Sole Fish	1.2	0.3	0.4	0.2	Tr	0.1	50
King Salmon	10.4	2.5	4.5	2.1	0.8	0.6	-
Pink Salmon	3.4	0.6	0.6	1.4	0.4	0.6	-
Rainbow trout	3.4	0.6	1.0	1.2	0.1	0.4	57

2.1.1. European Anchovy

Anchovy (*Engraulis*) generally lives in all tropical and subtropical waters and together form dense schools close to the shore [24].

The anchovy species which are widely caught are *Engraulis anchoita* (Argentine anchovy), *Engraulis australis* (Australian anchovy), *Engraulis capencis* (South African Anchovy), *Engraulis encrasicolus* (European anchovy, the species in Turkey), *Engraulis eurystole* (silver anchovy), *Engraulis japonicus* (Japanese anchovy), *Engraulis mordax* (Californian anchovy), *Engraulis ringes* (Peruvian anchovy) [25].

E. engrasicolus is known as European anchovy in the world as shown in Figure 2.4 [26]. *Engraulis encrasicolus* is pelagic fish that belongs to the *Clupeiformes* team and Engraulidae family of the Actinopterygii class. This species is widely distributed from Eastern Atlantic shores stretching to West Africa and throughout Mediterranean, Black Sea, Marmara Sea and the Azak Sea. It is widely distributed in all the seas surrounding Turkey [27].

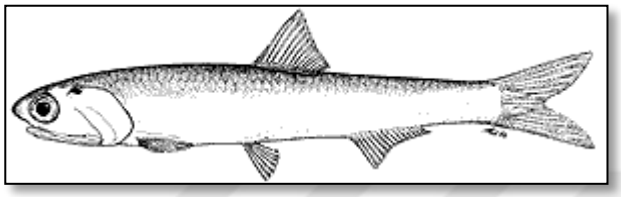


Figure 2.4. Image of Engraulis encrasicolus

European anchovy is a natural food source caught with purse seining especially in the Black Sea in winter months.

In terms of volumes caught, anchovy (Engraulis encrasicolus) is the number one fish species in Turkey and makes more than 50 % of sea fish harvest. Anchovy makes almost 30 % of Turkey's seafood production with 179.615 tonnes and a major portion, around 76.190 tonnes of this amount are used as a by-product in fish flour and oil factories [28]. Anchovies caught are consumed fresh in Turkey and processed in many processing plants throughout Turkey using various techniques and then introduced both to the local and international markets [29].

2.2. LIPID OXIDATION MECHANISM

Lipid oxidation is defined as reactions of free radicals and oxidative degradation of unsaturated fatty acids. Main form of oxidation is autoxidation which results in generation of hydroperoxides. Lipid oxidation is a chain reaction that occurs in three stages; initiation, propagation and termination [30]. Figure 2.5 shows the basic steps of lipid oxidation. Where RH is a fatty acid, R is a alkyl radical, RO_2 is a peroxide radical, RO_2H is a hydroperoxide, RO_2R is a oxidation product as shown in Figure 2.5 [31].

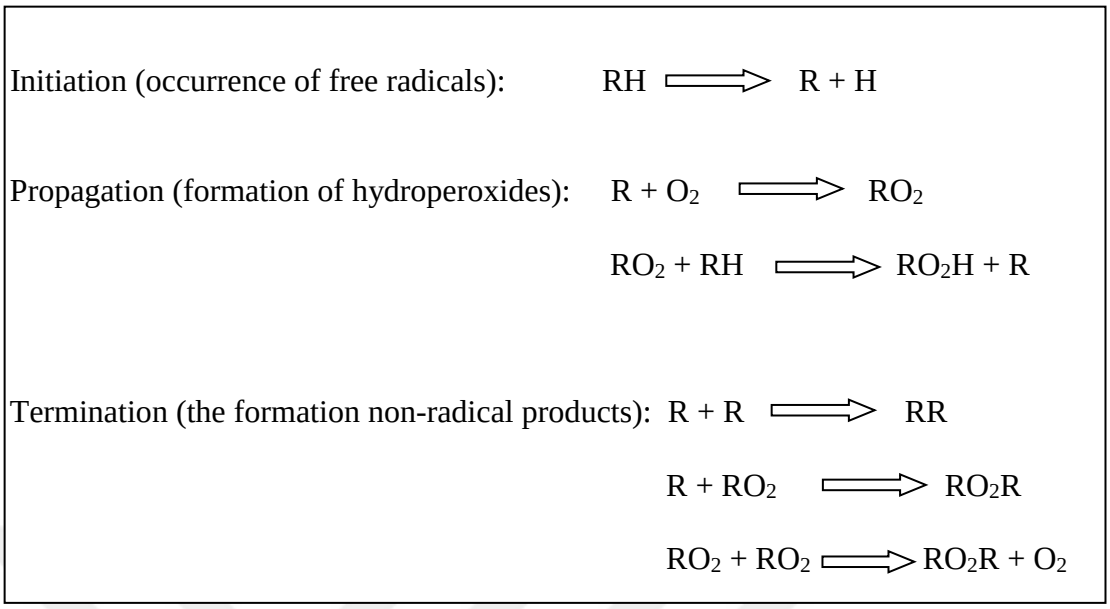


Figure 2.5. Lipid oxidation mechanism

At the initiation stage of the reaction, unsaturated fatty acid, which is bonded to carbon atom (adjacent to dual bond) and contains unstable hydrogen atom, breaks into free radicals by removing hydrogen atom with effect of oxygen, light, heat and heavy metal.

At the propagation stage of the reaction, after sufficient free radicals are formed, the chain reaction starts with the reaction of R with oxygen. As a result peroxide radicals (RO_2) are formed. These peroxide radicals react with hydrogen atoms of α-methylenic groups of fatty acid molecules that were not in reaction to generate hydroperoxides RO_2H and new free radicals R. New free radicals reacts with oxygen and the reaction cycle repeats itself.

At the termination stage, two free radicals react and termination occurs. At this stage, oxidation products such as aldehydes, ketons, alcohols, acids, and hydrocarbons which causes off flavour and odour in the product are generated [32].

Lipid oxidation is affected from some factors. These factors are explained as below:

Fatty acid chemical structure; lipid oxidation rate mainly depends on the fatty acid distribution in lipid. As the number of double bonds in a fatty acids increases, induction time for lipid oxidation decreases and the oxidation rate increases. Fats that contain high

amount of PUFA are much more prone to oxidation [31]. *Oxygen*; oxygen contentration is important for oxidation reactions. The oxidation becomes faster when the active oxygen contentration is very much. The effect of oxygen concentration on oxidation rate is also affected by other factors such as temperature, surface area [33]. *Energy and temperature*; oxidative reactions of lipids start by the action of an energy source such as heat, light or ionizing radiation. Heat accelerates oxidation. Generally, an increase of 15 ℃ in temperature doubles the oxidation rate. High temperature converts food molecules into free radicals and increases further breakdown of hydroperoxides into secondary oxidation products [34]. *Moisture*; lipid oxidation rate is strongly depended on water activity. Water forms hydrogen bonds with hydroperoxides. High water activity accelerates oxidation. Surface area; Oxidation rate gets higher by the increase in surface area of lipid that is in contact with air. *Enzyme*; Lipoxygenase and peroxidase catalyses lipid oxidation [35]. *Metals*; Most foods contain sufficient amount of heavy metals to catalyze lipid oxidation. Important metals such as iron, copper, cobalt, manganese and nickel act as lipid oxidation catalysts in foods. *Antioxidants*; Antioxidants are classified as products which slow or delay reaction during lipid oxidation in food systems [36].

2.3. LIPID OXIDATION KINETICS

Many chemical reactions occur in foods. Some of these chemical reactions are needed to obtain the compounds that provide the flavour, texture and nutritive values and some reactions cause the spoilage of the foods or the undesired subtances. If the activation energy is high enough, the chemical reactions are formed by the collisions of the molecules that cause the breaking down or the formation of the chemical bonds. Chemical kinetics deals with the rates of the chemical reactions and provides the the correlations for the mathematical modeling and the mechanisms [37].

The kinetic model of lipid oxidation in foods by Ozilgen and Ozilgen simulated the lipid oxidation in food systems using the logistic equation. Nearly complete correlation was achieved between the logistic model and literatural data of seventeeth sets. These literatural data contains egg yolk, restructured beef and pork nuggets, cooked pork meat, iced gutted dogfish, frozen mackerel fillets and ground raw poultry meat. A basic

method was generated to evaluate the model parameter values. The changes in the values were also related to variable pre-treatment and storage conditions [38].

Sergio Gomez had an experimental design to produce a kinetic model for the autoxidation reaction of triacylglycerols in olive oil stored in dark environment at a temperature range between 25–75 ℃. Then, a pseudo first order reaction was obtained when secondary oxidation products were formed. The rate constant belonging to secondary oxidation products with temperature was produced as fitted to Arrhenius equation [39].

In an other study, the kinetic modeling of lipid oxidation was investigated for soybean oil with addition of mono-olein, stearic acid and iron by A. Çolakoğlu. The addition of mono-olein, stearic or iron into soybean oil increased the lipid oxidation rate during the storage. Peroxide decomposition became higher due to iron. Lipid oxidation in soybean oil was determined as first order reaction and the addition of mono-olein, stearic acid and iron did not influence the lipid oxidation reaction kinetics [40].

The kinetics of the alteration on freshness of grass carp for various temperature values during storage was studied to analyze the quality change in cold chain circulation by L. Zhan et al. . The kinetical modeling of sensorial evaluation, total aerobic counts, k-value, total volatile basic nitrogen and TBA versus storage duration and temperature were performed depending on Arrhenius equation. The higher regression coefficient meant acceptable first order reaction and Arrhenius modeling to predict the quality variation of grass carp [41].

A study to verify the model applications was performed by using the sausage samples by F. Wenjiao et al. . The change in thiobarbituric acid reactive substances were followed for the samples for the storage temperatures of 5, 15, 25, 30 and 35 °C, while the control temperatures were specified as 10 ℃ and 20 ℃. The use of these predictive models was verified by the obtainment of first order kinetic model fitting of TBARS data at constant temperature. A first order kinetic modeling corresponded the TBARS data at constant temperature with equation $B = B_0e^{kBt}$ [42].

As another article studied the lipid oxidation kinetics of ozone-processed shrimp during iced storage using peroxide value measurements by G. Bono et al. . In iced storage of

shrimp samples, the lipid oxidation equations were not affect to oxidizing substrate concentration rate values [43].

The formation of hydroperoxides was also studied by fitting lipid oxidation data of microencapsulated and bulk chia oil stored at water activity values of 0.105 – 0.765 and temperatures of 25, 35 and 40 ℃ by L. Escalona et al. . Zero, first and second order reaction models were obtained in the study [44].

A project comprising the study of peroxide value, lipid oxidation and shelf life for pistachio powder was carried out by H. Tavakolipour et al. . Storage temperatures were 15, 25, 35, and 40 ℃ and relative humidities were 11, 33, 73 and 87 %. The lipid oxidation kinetics were found out to be first order reaction [45].

2.4. LIPID OXIDATION IN SEAFOOD

Fish products are susceptive to lipid oxidation due to their high unsaturated lipid concentration [46].

In one of the studies, 0.06 % rosemary extract, tocoferol and propyl gallate as antioxidant were added to Australian red crayfish (*Cherax quadricarinatus*.Then, crayfish was frozen and stored at -20 ℃. The chemical changes were investigated at 1st, 3rd and 6th months of storage by freezing. TBARS of antioxidant added groups were found to be higher with respect to control group [47].

The investigation of quality variations in anchovy patties stored at of 4°C was studied by P. Yerlikaya et al. [48]. An increase in total volatile basic nitrogen and thiobarbutiric acid contents, and a decrease in, acidity and sensory scores were observed during the storage. The peroxide value of anchovy patties significantly increased until the fifth day of storage then decreased on 5th and 6th days of storage [48].

In another study, dead nettle *(Urtica diocia)* containing natural antioxidant was added on steelhead *(Oncorhynchus mykiss)* fillets by O. Hisar et al. [49]. Three different concentration of dead nettle extract (0.4 %, 0.8 % and 1.61 %) and propyl gallate were used as study groups. Total antioxidant activity, TBARS, TVB and pH values were

followed during storage. It was stated that dead nettle extracts increased the shelf life of steelhead fillets stored aerobically and decreased lipid oxidation [49].

A Rodriguez et al. studied coho salmon (Oncorhynchus kisutch) was stored for 9 months at -18 ℃ with high technology uniting vacuum and polyphenolic rich film. Lipid hydrolysis, lipid oxidation and antioxidant content variation in salmon muscles [50]. It was observed that free fatty acid formation was not affected by packaging conditions. Furthermore, vacuum packaging conditions partially influenced the inhibition of primary and secondary lipid oxidation development [50].

Al-Bandak et al. studied the addition of Majorana syriaca as a natural antioxidant source to minced tuna fish (*Thunnus albacares*); then investigated antimicrobial and antioxidant anti-oxidative effect by storing at 0 ℃. Peroxide and TBARS analyses were carried out to observe oxidation changes. It was reported that lipid oxidation was inhibited by the increase of extract concentration [51].

I. Aydın et al. studied the effects of temperature and time of freezing on lipid oxidation in anchovy at during frozen storage. The fish were stored at different temperatures; -20 ℃, -40 ℃, -80 ℃. Peroxide value and thiobarbutiric acid values were significantly affected by temperature. Low freezing temperature resulted in low oxidation level. During the storage, oxidation value increased. The best freezing temperature in terms of delaying of lipid oxidation was found to be -80 ℃ [52].

2.5. EDIBLE COATINGS

Edible films are one of the most sought out packaging materials because they are cheap, they require simple production technologies and made of natural components, they have varied functionalities and they are biodegradable [53]. They mainly control carbon dioxide, moisture and lipid migration and improve mechanical properties of the food system, protects food from external factors, prevents quality deterioration, reduce the loss of flavour and odour, preserve vitamins and ions that stop browning reactions, pigments, antioxidants, antimicrobial agents in the product to improve the quality and shelf life [54].

Edible films and coatings are made of non-synthetic natural sources, coated on the surface of a food or applied between layers of food components to prevent quality loss in food and increase its shelf life [55].

There are mainly three types of coating in edible films.

1. Lipid-based coatings (include animal fat and vegetable oils, cross linked triglycerides and wax).

2. Polysaccharide based coatings (include starch, cellulose, gums, chitin/chitosan and similar materials).

3. Protein based coatings (corn zein, wheat gluten, soya protein, keratin, collagen, gelatin, casein and whey protein) [56].

Dipping, spraying, foaming, and enrobing techniques are used to apply edible coatings. A coating technique that does not harm the product should be chosen [57]. Therefore, the non-use of abundant coating material, homogeneous distribution of coating material on product and drying must be performed carefully [58] .

2.6. CHITIN AND CHITOSAN

Chitin and chitosan are the most common polysaccharide based biopolymers after cellulose. Chitin is the main component of shellfish such as crabs and shrimps, and found in the bones of insects and cell walls of fungus. Chitosan is a modified and natural carbohydrate polymer obtained through partial deacetylation of chitin [59]. Chemical structure of chitin and chitosan were given in Figure 2.6 [60].

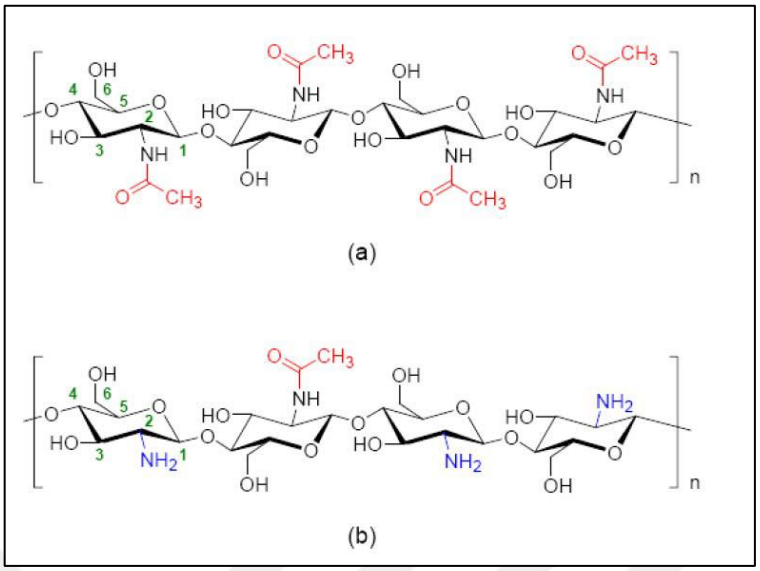


Figure 2.6. Chemical structure of chitin (a) and chitosan (b)

Chitosan is a white colour, odourless, tasteless semi-transparent particles or powder and resistant to digestive enzymes. On the other hand, it is degraded by some bacteria. It is insoluble in water, and soluble in acidic solutions (<6.0 pH). Organic acids such as acetic, formic, lactic are used to dissolve chitosan. Dissolution with inorganic acids is limited (dissolved in 1% hydrochloric acid, does not dissolve with sulfuric and phosphoric acid). Chitosan solutions are destabilized at pH 7.0 and over. Similarly, storage at room temperature for an extended period of time can have a negative effect on the stability of solutions [61].

Chitosan is very sensitive to environmental conditions, so it is sugessted to store in closed containers at low temperatures (2–8 °C). Exposure to high temperatures (above 40 °C) was found to cause a significant loss of moisture (dehydration of chitosan powder) [62].

2.6.1. Use of Edible Coatings On Seafood

Although fish and other seafood are rich in nutrients, their shelf lives are quite short when compared to red meat and poultry products. New preservation techniques and applications are developed every day in order to maintain their shelf lives and quality

properties. One of these techniques is edible films which are widely used on other foodstuff [63].

S. Dursun et al. stated that when red meat, poultry meat and fish slices are placed in plastic packages, leaking liquid makes the product unattractive for potential buyers [64]. Edible coatings hold the liquid inside, prevent leakage, improve product presentation and therefore no absorbent pads are required in packages.

A. Gennadios et al. stated that volatile flavour loss and development of off-odours in red meat, poultry meat and seafood can be limited with edible coatings [65]. Coating applied on fish, poultry meat and red meat can reduce oil absorption during frying and improve their nutrient.

Chitosan based films and coatings are commonly used in marine products. Jeon et al. stated that chitosan based coatings decreased the lipid oxidation and moisture loss [66].

Sathivel reported that chitosan-based film coated salmon fish minimized lipid oxidation and dropping loss [67]. Coatings with 1 % and 2 % chitosan solution were effective in reducing about 50 % relative moisture loss compared with the uncoated fillets.

Lopez-Caballero et al. studied on coating of gadoid fish with edible film [68]. They used three groups: non-chitosan coated control group, powder chitosan added group and chitosan-gelatin coated group. It was stated that powder chitosan addition into minced meat did not affect the bacterial growth. Sensory properties of chitosan-gelatin coating were fine and then the coatings retarded the food spoilage in minced meat by decreasing total volatile basic nitrogen and number of Gram (-) bacteria.

W. Fan et al. stated that the effect of chitosan coating on shelf life and quality of carp was studied [69]. The fish samples were coated with 2 % chitosan and stored at -3 ℃ for 30 days. It was stated that quality properties were preserved and shelf life was increased.

Alak et al. explained that chitosan based films increased the shelf life of bonito fish (*Sarda sarda*) due to antimicrobial effect [70]. The chitosan may absorb the necessary nutrients for bacteria. Also, chitosan may interact with negative charge of cell membrane by increasing membrane permeability.

In another study, V. Charmanara et al. stated that fish fillets were coated with chitosan and stored at 2 ℃ for 3 weeks and at -20 ℃ for 3 months [71]. TBARS values decreased, total bacteria count was inhibited and total lipid and omega-3 fatty acid amounts were increased and dropping losses were decreased.

3. MATERIALS AND METHODS

3.1. MATERIALS

Ten kg of fresh anchovy (*Engraulis encrasicholus)* were purchased from the İzmit fish market hall in Kocaeli. The fish was boxed with crushed ice and brought to the laboratory (Figure 3.1). Fishbones were removed, fish head, tail and viscera were seperated and cleaned. Then, fileto of fish were passed through a meat grinder. Minced fish meat was put on 1 cm depth stainless steel trays uniformly. Chitosan was smeared onto some of minced fish meat samples and caoted samples were dried for 3 days at 4 ℃. Uncoated samples were specified as control group. Trays were covered with layers of plastic wrap and aluminium foil. Samples were stored at 4 ℃, -1 ℃, -18 ℃ and room temperature, respectively.

Figure 3.1.Fish filetos in boxed with crushed ice

3.2. REAGANTS

Chitosan, TEP (1.1.3 Tetrahoxypropane), hexane, ethanol, hydrochloric asit (HCl), thiobarbituric acid (TBA), trichloroacetic acid (TCA), butylated hydroxytoluene (BHT), acetic acid, sulfuric acid (H_2SO_4) were purchased Sigma-Aldrich. Methyl red, bromocresol green, boric acid, sodium hydroxide (NaOH), Kjedahl tablet, Plate Count Agar, saline solution, crystal violet, lugol and safranine were purchased from Merck. Blood Agar / Eosin Methylene Blue Agar was purchased from Salubris.

3.3. METHODS

3.3.1. Chitosan Solution and Coating

2 % (w/v) of chitosan was prepared in 1000 mL of 1 % (v/v) acetic acid solution. Chitosan was gradually added to acetic acid, and stirred with magnetic stirrer at room temperature until completely dissolved (2.5 h) [72].

3.3.2. Determination of Protein Content

Protein analysis were done by using Kjeldahl nitrogen method [73]. The fish samples (3 g) were weighed and taken into digestion tube. Two Kjeldahl tablets and 20 mL of concentrated H_2SO_4 (98 %) were added into each digestion tube with fish sample, and blank and were heated for 2-3 h at 450 ℃ until samples were neon green in colour. The digestion tubes were cooled to room temperature. The digested samples in digestion tubes were placed into the distillation unit and 40 % (w/v) NaOH was added. The samples were distilled until 100 mL of distillate were collected in 100 mL of 4.0 % (w/v) boric acid for 10 minutes. For preparing 4.0 % (w/v) boric acid, 20 g boric acid was solved in 250 mL boiling water and was added to 5 mL bromocosol green solution and 3.5 mL methyl red solution. Distilled of samples were titrated it with 0.1 M HCl until pH of 4.2 and colour pink [73].

The percentage protein content was calculated as below:

$$\%\,\mathrm{N}: \mathrm{Normality\ HCl} \times \left[\frac{\mathrm{V1} - \mathrm{V2}}{\mathrm{w}}\right] \times (14\ \mathrm{g\ N} / \mathrm{mol} \times 100) \qquad (3.1)$$

$$\%\ \mathrm{Protein} = \%\ \mathrm{N} \times \mathrm{Protein\ factor} \qquad (3.2)$$

Where V_1 is volume HCl (mL) for sample, V_2 is volume HCl (mL) for blank, W is weight of sample (g) and protein factor is 6.25 .

3.3.3. Determination of Lipid Content

Fat analysis were done by soxhlet extraction method [74]. The fish samples (5 g) were weighed and taken into a cellulose thimble, and the thimble was plugged with cotton-wool. The thimble were placed in soxhlet extraction chamber, which was placed into a distillation flask containing 250 mL *n*- hexane. The extraction was continued for 6 h, then thimble was cooled for 24 h at room temperature. A residue of solvent was evaporated at 45 ℃. The sample was cooled in desiccator and after, percentages of fat content was determined according to the weighing difference [74].

The percentage fat content was calculated as below:

$$\% \text{ Fat } = \left(\frac{\text{W2} - \text{W1}}{\text{M}}\right) \text{x } 100 \tag{3.3}$$

Where W_1 is weight of flask (g), W_2 is weight of flask and oil (g), M is sample weight (g).

3.3.4. Determination of The Moisture Content

5 g of minced fish sample was weighed in the Petri dish, with lid. The Petri dish was left in an oven at 105 ℃ for 4 h. Then, the samples were removed from the oven, and transferred into the desiccator. After the samples were cooled, they were weighed. Then, fish samples were reheated in a oven at 105 ℃ for ½ h. They were cooled in dessicator an weighed again. This process was repeated until reading between measurements do not differ more than 1 mg [75].

The percentage moisture content was calculated as below:

$$\% \text{ Moisture } = \left[\frac{(\text{W1} - \text{W2})}{(\text{W1} - \text{W0})}\right] \text{x } 100 \tag{3.4}$$

Where W_0 is weight of pan (g), W_1 is weight of pan and wet fish sample (g) and W_2 is weight of pan and dried fish sample (g).

3.3.5. Determination of the Ash Content

Crucibles dried at 100 ℃ for 2 h before the experiment. 5 g fish sample was placed into a tared crucible. Three crucibles were ignited at 550 ℃ for 4 h in a cool muffle furnace. Then, muffle furnace was turned off and waited to open it until the temperature was decreased to at least 250 ℃. Crucibles were transferred to desiccator until samples are weighed (AOAC, 920.153) [76].

The percentage ash content was calculated as below:

$$\% \text{ Ash} = \frac{(\text{Weight after ashing (g)} - \text{ Tare weight of crucible(g) })}{(\text{Original sample weight (g)})} \times 100 \qquad (3.5)$$

3.3.6. Microbial Analysis

All samples were cultured and observed microbiological growth. One g of fish sample was mixed with 10 mL sterilized distilled water into a tube and, were homogenized by vortex. 100 µL of homogenized sample was pipetted onto surface of Plate Count Agar Petri dishes and sample was spread evenly over surface of agar using sterile glass spreader by spread plate technique to analyze the total viable count. Homogenized sample was inoculated to Blood Agar / Eosin Methylene Blue Agar Petri dishes for aerobic bacteria incubated for 2 days at 37 ℃. Isolation aerobic microorganizms. Then all Petri dishes were put in the incubator and put into gas packs to identify anaerobic bacteria. The indicator was added to gas pack to eliminate the oxygen and anaerobic conditions were provided [77]. All samples were kept at 37 ℃ in the incubator for 3 days. After incubation, cfu (colony forming-unit) values were calculated in terms of log10 with counting colonies in Petri dishes. Then colonies in Petri dishes were inoculated to media for identifying microorganisms and applied different microbiological method for identification.

3.3.6.1. Gram Staining

Gram staining was performed to identify Gram (+) and Gram (-) microorganizms. Gram staining is a common method used to differences Gram (+) and Gram (-) bacteria. Gram (+) bacteria stain violet due to presence of a thick layer of peptidoglycan in their cell walls. Gram (-) has a thinner bacteria peptidoglycan layer. One drop of saline solution and microorganism sample were smeared onto the slide. then, the solution was dried on the slide. Crystal violet stain solution was added on the slide and waited for 1 minute. The slide was washed with large amount of distilled water. Lugol stain solution was added on the slide and waited for 1 minute. The slide was washed with large amount of distilled water. Alcohol was added on the slide and waited for 15-20 seconds. The slide was washed with distilled water again. The safranine was added on the slide and waited for 30 seconds. After drying, the slide was observed by microscope. Purple color microorganisms were identified as Gram (+) and pink color microorganisms were identified as Gram (-) bacteria.

3.3.6.2. Catalase and Oxidase Test

Catalase test was applied on Gram (+) colonies. Catalase is detected by adding 3 % H_2O_2 into test tube. A sterile wooden stick was used take several colonies of the 18 to 24 hours test organism and immerse in the hydrogen peroxide solution. When catalase is positive, air bubbles was observed. When catalase is negative, air bubbles was not observed. [78]. Catalase (+) microorganisms were identified as *Staphyloccus* species, catalase (-) microorganisms were *Streptococcus* species.

Oxidase test was applied on Gram (-) colonies. Strip of filter paper are soaked in 1% solution of tertramethyl-p-phenylene-diamine dihydrochloride. The strips are freeze dried and stored in a dark bottle tightly sealed with a screw cap. For use, a strip is removed, laid in a Petri dish and moistened with distilled water. The colony to be tested is picked up with a platinum loop and smeared over the moist area. A positive reaction is indicated by an intense deep-purple hue, appearing within 5-10 seconds, a “delayed positive” reaction by colouration in 10-60 seconds, and a negative reaction by absence of colouration or by colouration later than 60 seconds [79]. Oxidase (+) microorganisms were non-fermentative, oxidase (-) microorganisms were *Enterobacteriaceae.*

Then, Biomerieux Vitec 2.0 (France) system was used for species identification. Vitec 2.0 is a system to identify the bacteria according to biochemical properties.

3.3.7. Determination of Lipid Oxidation by UV-Visible spectrophotometer

In this study, UV-Visible Spectrophotometry was used to quantify TBA-MDA complex, formed as a result of lipid oxidation, in ground fish meat (Figure 3.2) .

Figure 3.2. Image of UV-visible spectrophotometer

The extent of lipid peroxidation in foods was measured by using TBA test which is the most commonly measuring method. The absorption spectrum obtained with oxidized fatty foods is quite similar to the spectrum obtained when TBA and MDA react. MDA is three carbon dialdeyde. It has been known longly that unsaturated oil and fats produce MDA at the last steps of the autoxidation and it is the secondary oxidation product. Formation of MDA-TBA_2 complex occurs by a nucleophilic attack comprising carbon-5 of TBA and carbon-1 of MDA, followed by dehydration and similar reaction with a second molecule of TBA producing a red pigment (Figure 3.3). The intensity of the pink pigment formed through MDA-TBA condensation displays the extent of lipid peroxidation. The velocity of the reaction depends on the temperature, pH and concentration of TBA [80].

Ultraviolet - visible spectrophotometer is performed by passing light through the solution and determining the amount of light absorbed by the solution, to quantify the extend of lipid oxidation [81].

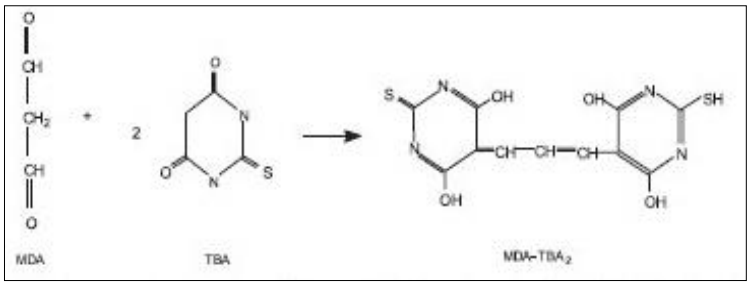


Figure 3.3. Reaction between the MDA and TBA to form the MDA-TBA_2 pigment

Lipid oxidation was measurement by TBA value with spectrophotometer [82].

For preparing MDA standart curve, 18 µL TEP was dissolved in 25 mL HCl (0,1 M) to final stock solution 3,2 mmol/L, and kept for 2 h at room temperature in the dark. Then, stock solution was diluted with 7,5 % TCA in water to the concentration of 1, 2, 4, 8, 16, 32 µmol/L.

MDA was extracted from the fish samples with TCA solution as follows; 3 g fish sample, 9 mL of the 7,5 % TCA solution, and 50 µL of 7,2 % BHT were added into graduated cylinder, and were homogenized at homogenizer (16,000 rpm) for 1 min. Then, the homogenate was centrifuged at 2090 g for 15 min and filtered through a Whatman No. 4 filter paper.

Then, 1 mL of MDA standard, MDA extract and 7,5 % TCA solution (blank) was taken into different tubes and 1 mL 0,02 M TBA solution was added in each these tubes The tubes were heated in a boiling water at 90 ℃ for 30 min and cooled for 10 min. All the solutions were diluted to 1:5 ratio. Absorbance of the MDA-TBA complex was measured at 532 nm on a UV-visible spectrophotometer (Thermo Scientific, evolution 201) [82].

3.3.8. Mathematical Modelling

The logistic model was used to simulate the lipid oxidation reaction steps occuring in the samples and stated as:

$$\frac{dC}{dt} = kC\left[1 - \frac{C}{C_{max}}\right] \tag{3.6}$$

where C is the concentration of total oxidation products, C_{max} is the maximum accessible value of parameter C at the end of lipid oxidation process, k is the reaction rate constant, and t is time. In initial stages of lipid oxidation, when $C << C_{max}$ the term $1 - \left(\frac{C}{Cmax}\right)$ of equation 3.6, the logistic equation gets similar to first oder reaction and it may be assumed as $\frac{dC}{dt} = kC$. When C equals to C_{max} in termination step, $1 - \left(\frac{C}{Cmax}\right)$ becomes zero. Correspondingly, the logistic equation is simplified as $\frac{dC}{dt} = 0$ which means the process is ended and the reaction is completed [38].

According to logistic equation, in this study, the effect of chitosan coating on the lipid oxidation kinetics of minced anchovy meat was modeled.

Equation 3.6 was integrated as below;

$$kt = \left[\ln\left(\frac{Cmax}{C}\right) - 1\right] + \ln\left[\left(\frac{x}{1-x}\right)\right] \tag{3.7}$$

Where $x = \frac{C}{Cmax}$.

TBA data obtained from minced fish samples stored at 20 ℃, 4 ℃, -1 ℃ and -18 ℃ were modeled as explained above. ln (x/(1-x)) values were calculated for each temperature and ln (x/(1-x)) versus time graphs were plotted (Figure A.1- A.8 in Appendix A).

The slopes were analyzed from the equations obtained from graphics. The slope values stand for the reaction rate constant. The rate constants of minced fish stored at different temperatures were compared.

Arrhenius equation expresses the temperature dependence of reaction rate constants as below [83] :

$$\ln k = \ln k_0 - \left(\frac{Ea}{RT}\right) \tag{3.8}$$

where k is reaction rate constant, T is temperature (K) and E_a (kJ/mol) is the activation energy. Ln k versus 1/T values were plotted for coated and uncoated minced fish samples [83].

4. RESULTS AND DISCUSSION

4.1. COMPOSITION ANALYSIS

Initial protein, fat, ash contents of minced fish are given in Table 4.1.

Table 4.1 Initial composition of minced fish

Component	**%**
Protein	19,70
Fat	5,20
Ash	1,14
Moisture	77,68

In the literature, compositional contents of protein, fat,and ash in the anchovy from our country analyzed were 16.60 - 22.10 %, 5.00 - 17.51 %, 0.94 % - 1.72 %, respectively. [84]. In this study, composition of anchovy were a similar to the literature. These variations may arise from differences in the species, age, gender, nutrition environment, reproduction and migration season [85].

4.2. LIPID OXIDATION, MOISTURE LOSS, MICROBIAL ANALYSIS

Lipid oxidation was measured with TBA method. Standard curve is given in Figure 4.1.

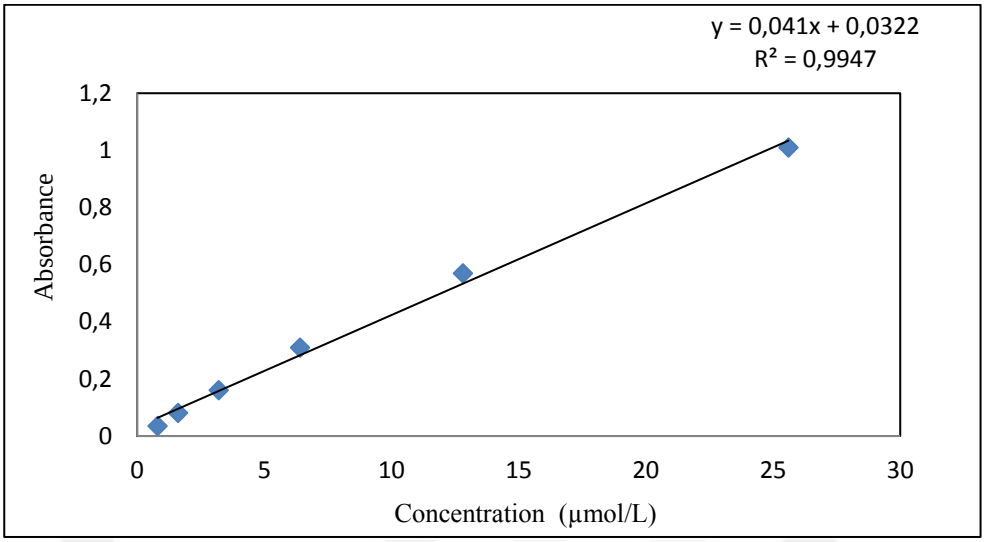


Figure 4.1 .MDA standart curve

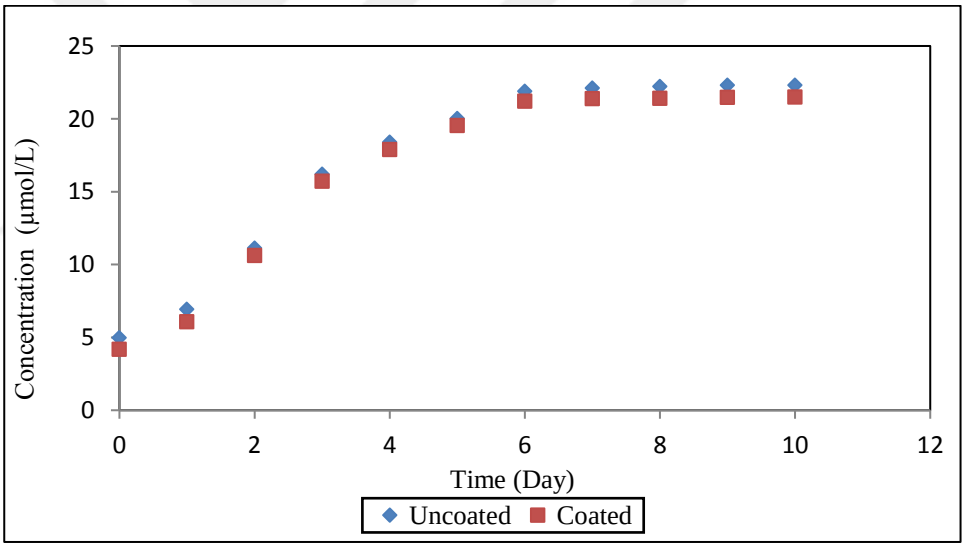


Figure 4.2. Comparison of TBA – MDA complex values of uncoated and coated sample stored at 20 ℃

TBA value is a parameter for measuring secondary products of lipid oxidation [86]. Figure 4.2 shows the changes in TBA values with time in uncoated and coated samples stored at 20 ℃. It was observed that free radicals concentration increased from the first day of the storage in both samples. Therefore, the coating did not retard the lipid oxidation in samples stored at 20 ℃. According to statistical analysis, there was a significant difference between uncoated and coated samples at propagation stage ($p < 0.001$).

TBA values of minced fish samples stored at 20 ℃ were used to generate a predictive model to validate the applicability of kinetic models. Logistic equation simulated lipid oxidation in those samples (Figure A.1-A.2 in Appendix A). Numerical values of the model parameters were used to analyze the effect of coating on lipid oxidation in the samples. C_{max} is the maximum accessible value of parameter, C at the end of lipid oxidation process, k is the reaction rate constant, and t is time. The k values obtained from the slopes of the logistic equation. When the rate constants were compared for uncoated and coated minced fish samples stored at 20 ℃, uncoated samples have higher rate constant than coated samples, 0.88 and 0.81, respectively. Chitosan coating decreased the rate of reaction lipid oxidation. C_{max} of uncoated samples was 22.32 on 10^{th} day. C_{max} of coated samples was 21.49 on 10^{th} day. At uncoated and coated samples, time for C_{max} was not change. Both samples reached C_{max} at the same time. But the chitosan coating reduced the C_{max} value.

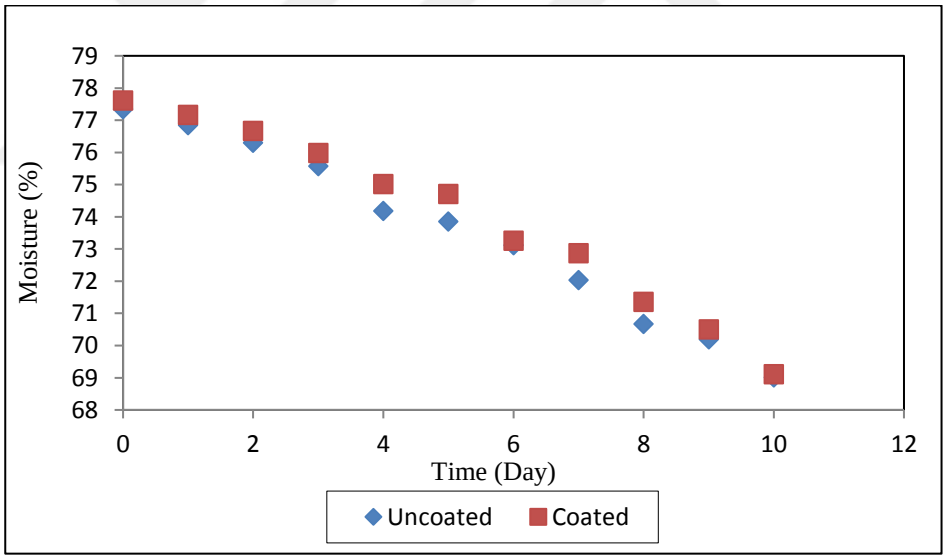


Figure 4.3. Comparison of moisture content of uncoated and coated samples at 20°C

The effect of coating on the moisture loss was also analyzed (Figure A.9 – A.10 in Appendix A). The results for uncoated samples showed that moisture rate constant was 1.0961, for coated samples that moisture rate constant was 1.0906. Chitosan coating decreased the percent moisture loss and the rate of moisture loss.

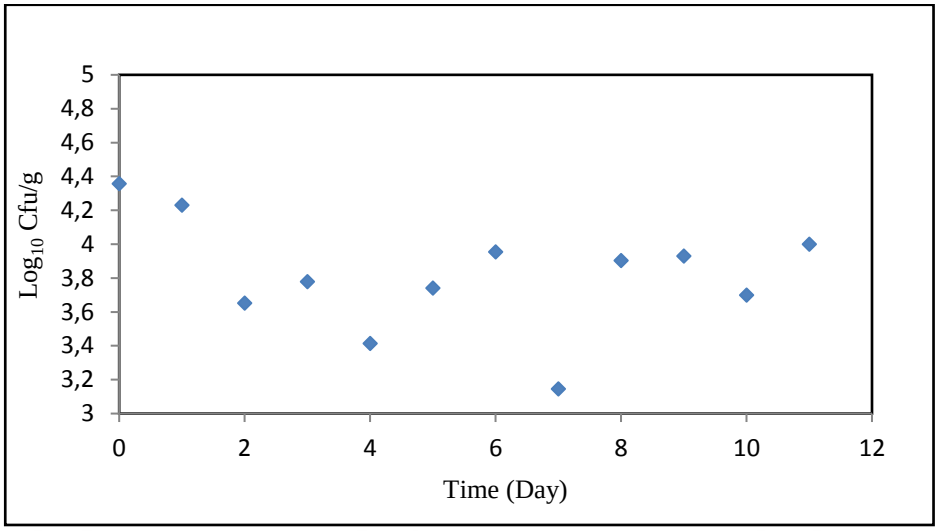


Figure 4.4. Colony counts of uncoated samples stored at 20 ℃

Figure 4.4 shows the changes in numbers in total viable count with time in uncoated samples stored at 20 ℃. Initial total bacteria count (TBC) was found as 4.23 $\log_{10}$Cfu/g in uncoated samples. On 2nd day, TBC changed as 3.65 $\log_{10}$Cfu/g. Minimum TBC value was recorded as 3.14 $\log_{10}$Cfu/g on 7th day during storage. At the end of storage, TBC might have increased as a result of contamination during analysis.

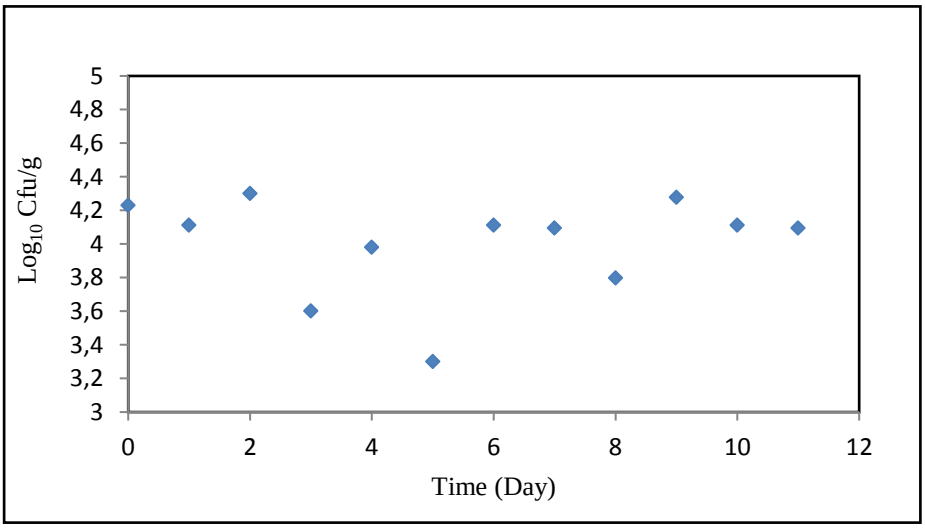


Figure 4.5. Colony counts of coated samples stored at 20 ℃

Figure 4.5 shows the changes in numbers of total viable count with time in coated samples stored at 20 ℃. Initial TBC was found as 4.23 $\log_{10}$Cfu/g in coated samples.

Minimum TBC value was recorded as 3.30 log_{10}Cfu/g on 5th day of storage. TBC value was constant during storage with the effect of chitosan coating.

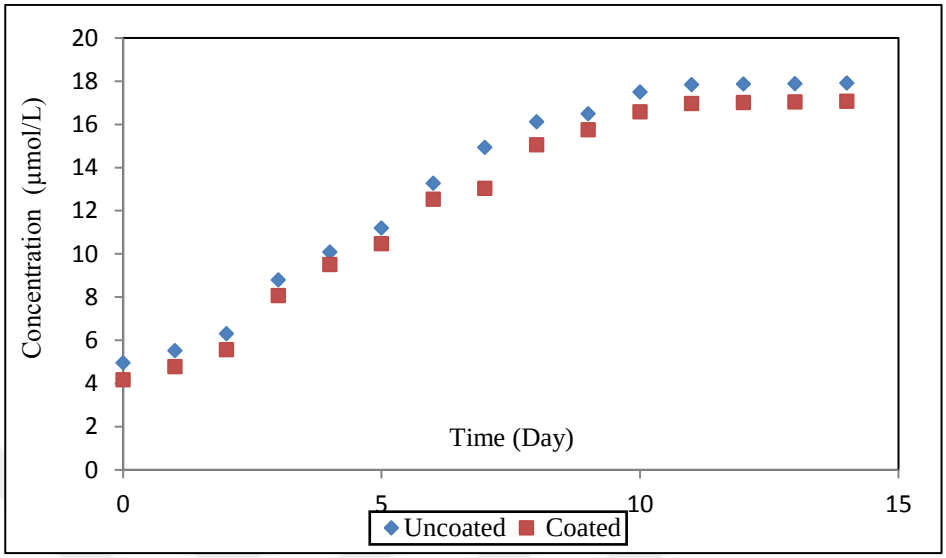


Figure 4.6. Comparison of TBA – MDA complex values of uncoated and coated samples stored at 4 °C

Figure 4.6 shows the changes in TBA values with time in uncoated and coated samples stored at 4 ℃. In uncoated and coated samples, lipid oxidation was terminated on 12th day. According to statistical analysis, there was a significant difference between uncoated and coated samples at propagation step ($p < 0.001$).

TBA values of minced fish samples stored at 4 ℃ were used to generate a predictive model to validate the applicability of kinetic models. Logistic equation simulated lipid oxidation in those samples (Figure A.3-A.4 in Appendix A). When the rate constants were compared for uncoated and coated minced fish samples stored at 4 ℃, uncoated samples had higher rate constant than coated samples, 0.62 and 0.60, respectively. Chitosan coating decreased the rate of reaction lipid oxidation. C_{max} of uncoated samples was 17.92 on 14th day. C_{max} of coated samples was 17.08 on 14th day. For uncoated and coated samples, time for C_{max} was not different. Both samples reached C_{max} at the same time. But the chitosan coating reduced the C_{max} value.

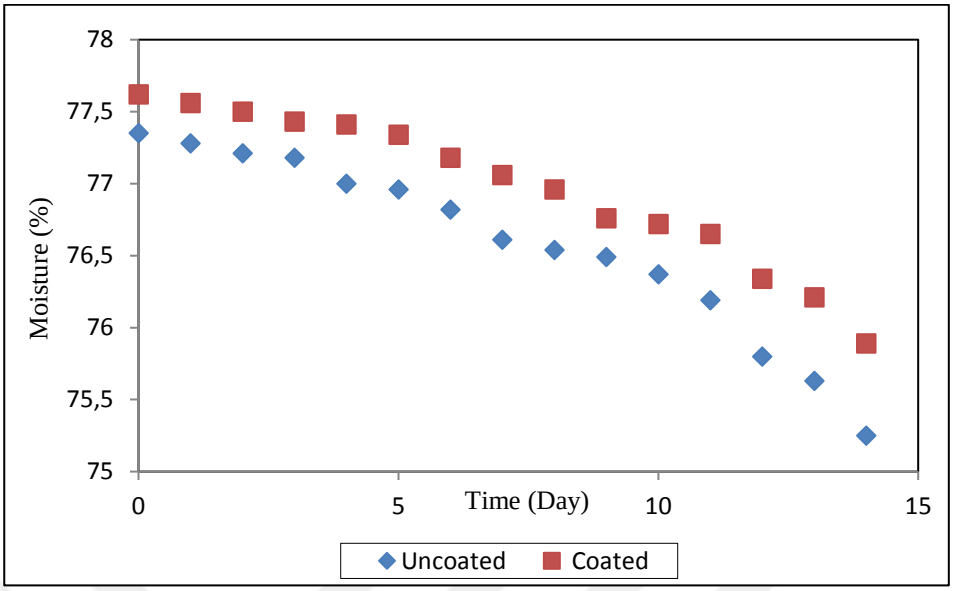


Figure 4.7. Comparison of moisture content of uncoated and coated samples stored at 4℃

The effect of coating on the moisture loss was also analyzed (Figure A.11 – A.12 in Appendix A). The results for uncoated samples showed that moisture rate constant was 0.1788, for coated samples that moisture rate constant was 0.1499. Chitosan coating decreased the percent moisture loss and the rate of moisture loss.

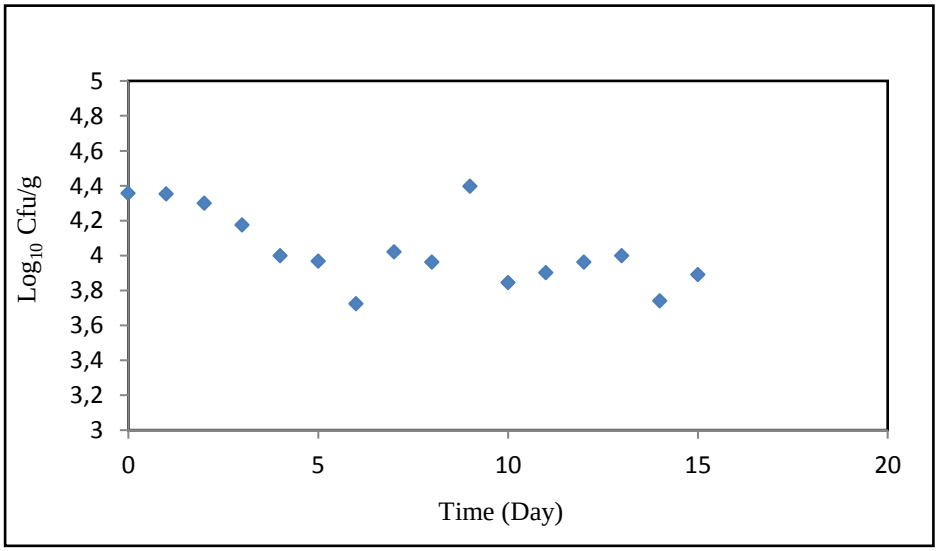


Figure 4.8. Colony counts of uncoated samples stored at 4 ℃

Figure 4.8 shows the changes in numbers of total viable count with in uncoated samples stored at 4 ℃. Initial total bacteria count (TBC) was found as 4.35 $\log_{10}$Cfu/g in

uncoated samples. On 4th day, TBC changed as 4 log_{10}Cfu/g. Minimum TBC value was recorded as 3.72 log_{10}Cfu/g on 6th day during storage. At the end of storage, TBC might have increased as a result of contamination during analysis.

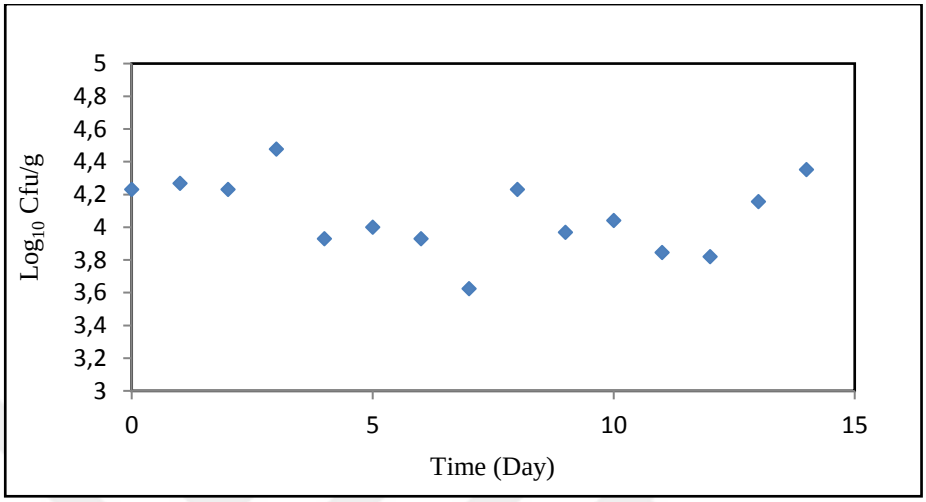


Figure 4.9. Colony counts of coated samples stored at 4 ℃

Figure 4.9 shows the changes in numbers of total viable count time in coated samples stored at 4 ℃. Initial TBC was found as 4.26 log_{10}Cfu/g in coated samples. Minimum TBC value was recorded as 3.62 log_{10}Cfu/g on 7th day during storage. The effect of chitosan coating was observed to be decreased from 12th day and increase in TBC value was observed.

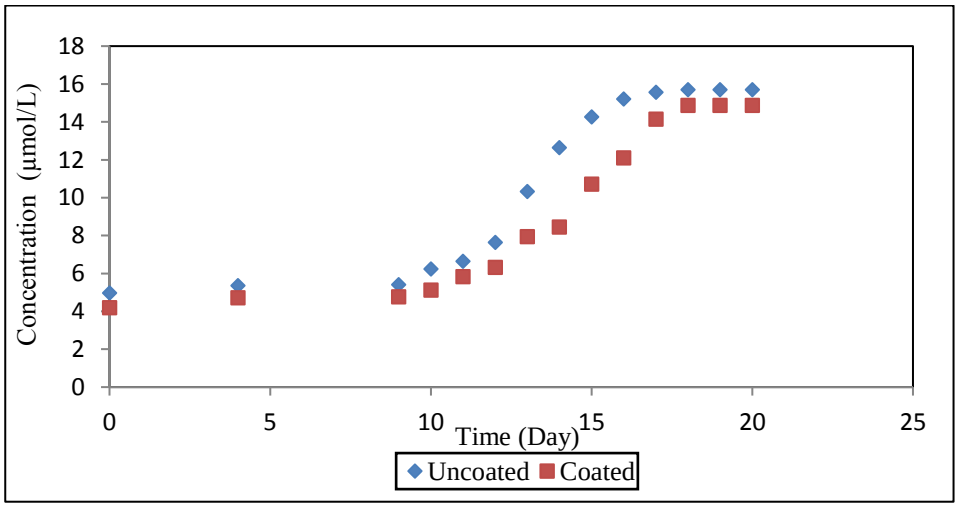


Figure 4.10. Comparison of TBA – MDA complex values of uncoated and coated samples stored at -1°C

Figure 4.10 shows changes in TBA values with time in uncoated and coated samples stored at -1 ℃. During first 10 days, the lipid oxidation occurred in initial stage. After ten days, the lipid oxidation accelerated, and the propagation stage began. In both samples, lipid oxidation was determined to begin to stop as from 18th day. According to statistical analysis, there was a no significant difference between uncoated and coated samples at propagation stage ($p > 0.001$).

TBA values of minced fish samples stored at -1 ℃ were used to generate a predictive model to validate the applicability of kinetic models. Logistic equation simulated lipid oxidation in those samples (Figure A.5-A.6 in Appendix A.) When the rate constants were compared for uncoated and coated minced fish samples stored at -1 ℃, uncoated samples had higher rate constant than coated samples, 0.56 and 0.52, respectively. Chitosan coating decreased the rate of reaction lipid oxidation. C_{max} of uncoated samples was 15.69 on 20th day. C_{max} of coated samples was 14.86 on 20th day. At uncoated and coated samples, time for C_{max} was not change. Both samples reached C_{max} at the same time. But the chitosan coating reduced the C_{max} value.

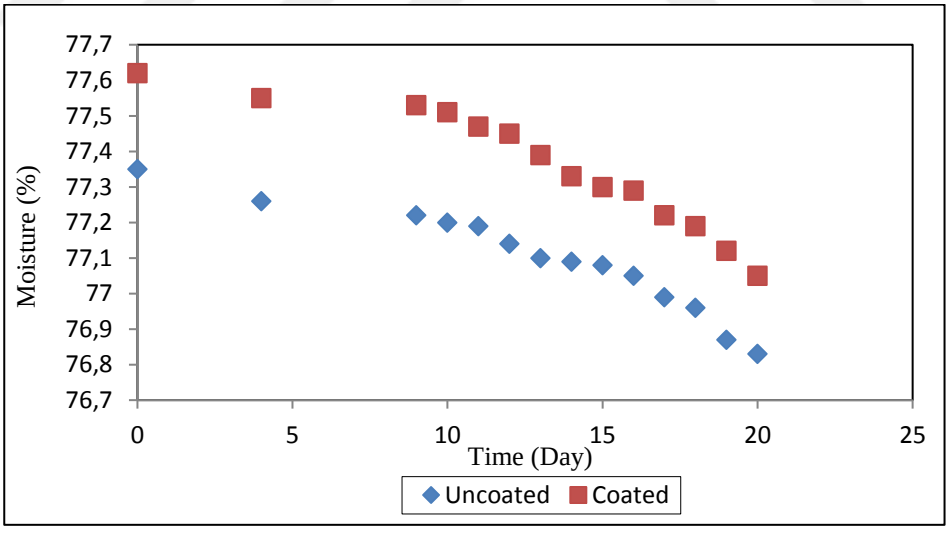


Figure 4.11. Comparison of moisture content of uncoated and coated samples stored at -1 ℃

The effect of coating on the moisture loss was also analyzed (Figure A.13 – A.14 in Appendix A). The results for uncoated samples showed that moisture rate constant was

0.0319, for coated samples that moisture rate constant was 0.0367. Chitosan coating did not decrease the percent moisture loss and the rate of moisture loss.

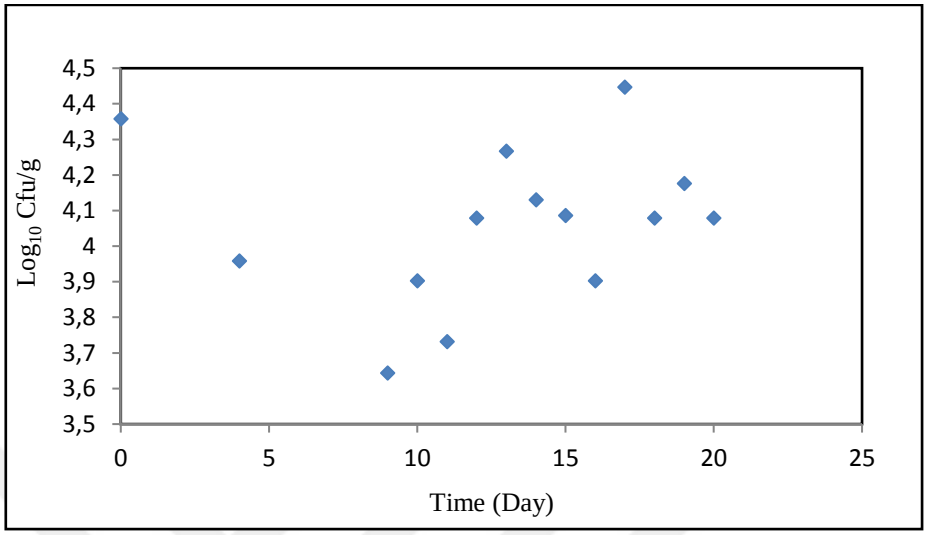


Figure 4.12. Colony counts of uncoated samples stored at -1 ℃

Figure 4.12 shows the changes in numbers of total viable count with time in uncoated samples stored at 1 ℃. Initial total bacteria count (TBC) was found as 3.95 $\log_{10}$Cfu/g in uncoated samples. On 9th day, TBC changed as 3.64 $\log_{10}$Cfu/g. Minimum TBC value was recorded as 3.64 $\log_{10}$Cfu/g on 9th day during storage. At the end of storage, expected decrease in TBC was seen due to storage at low temperature effect.

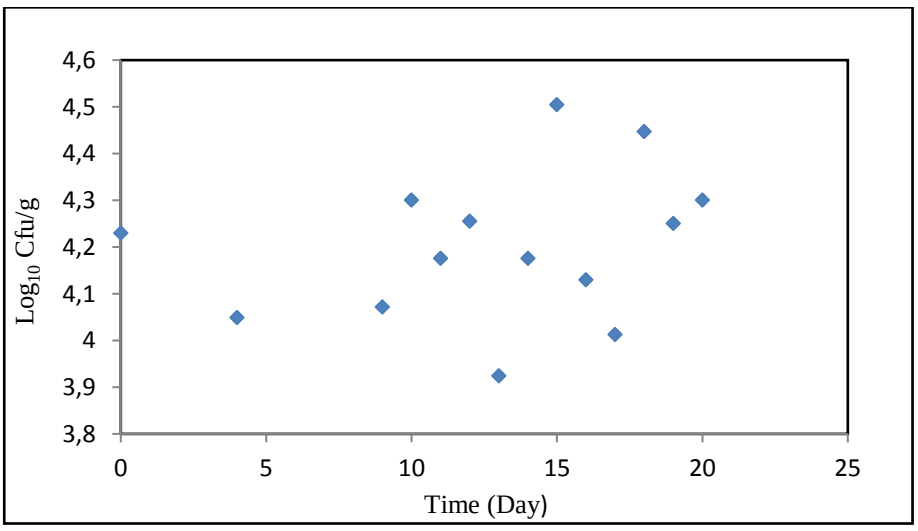


Figure 4.13. Colony counts of coated samples stored at -1 ℃

Figure 4.13 shows the changes in numbers of total viable count with time in coated samples stored at 1 ℃. Initial TBC was found as 4.04 log_{10}Cfu/g in coated samples. Minimum TBC value was recorded as 3.92 log_{10}Cfu/g on 13th day during storage. The effect of chitosan coating was observed to be decreased from 19th day and increase in TBC value was observed.

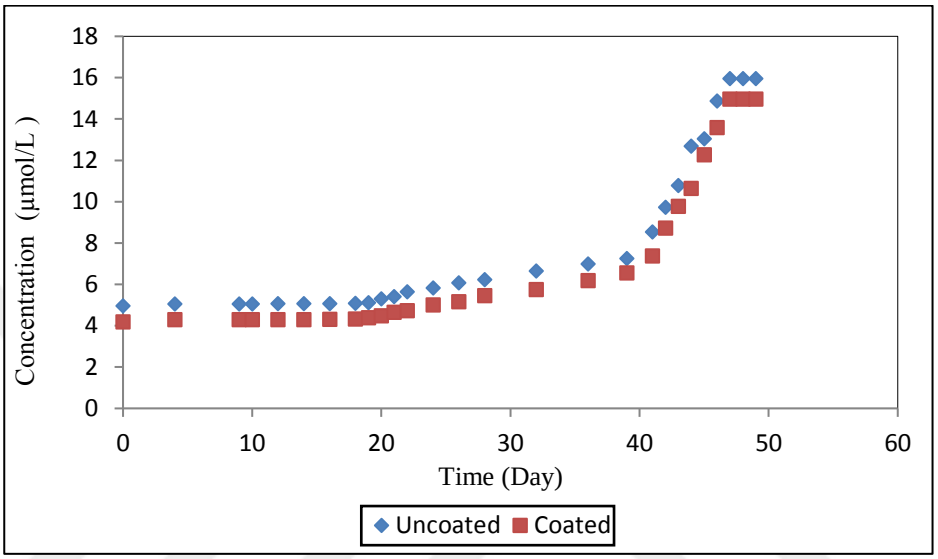


Figure 4.14. Comparison of TBA – MDA complex values of uncoated and coated samples stored at -18 °C

Figure 4.14 shows changes in TBA values with time in uncoated and coated samples stored at -18 ℃. During first 40 days, the lipid oxidation occurred in initial stage. After 40 days, the lipid oxidation accelerated and the propagation stage began. In both samples, lipid oxidation was determined to begin to stop as from 47th day. According to statistical analysis, there was a significant difference between uncoated and coated samples at propagation step ($p < 0.001$).

TBA values of minced fish samples stored at -18 ℃ were used to generate a predictive model to validate the applicability of kinetic models. Logistic equation simulated lipid oxidation in those samples (Figure A.7-A.8 in Appendix A). When the rate constants were compared for uncoated and coated minced fish samples stored at -18 ℃, uncoated samples have higher rate constant than coated samples, 0.38 and 0.35, respectively. Chitosan coating decreased the rate of reaction lipid oxidation. C_{max} of uncoated samples was 15.96 at 49th day. C_{max} of coated samples was 14.97 at 49th day. At

uncoated and coated samples, time for C_{max} was not change. Both samples reached C_{max} at the same time. But the chitosan coating reduced the C_{max} value.

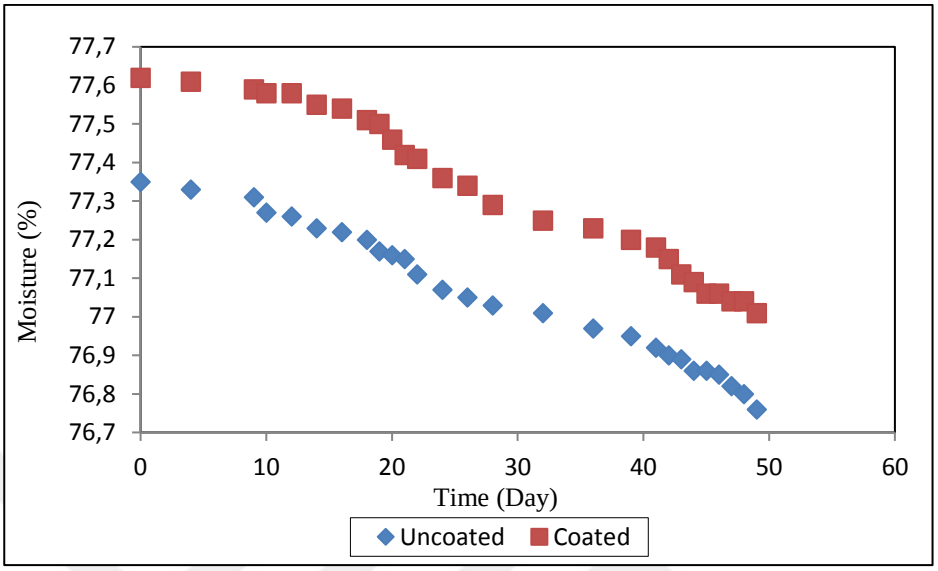


Figure 4.15. Comparison of moisture content of uncoated and coated samples stored at 18 ℃

The effect of coating on the moisture loss was also analyzed (Figure A.15 – A.16 in Appendix A). The results for uncoated samples were showed that moisture rate constant was 0.0155, for coated samples were showed that moisture rate constant was 0.0179. Chitosan coating non decreased the rate of moisture loss.

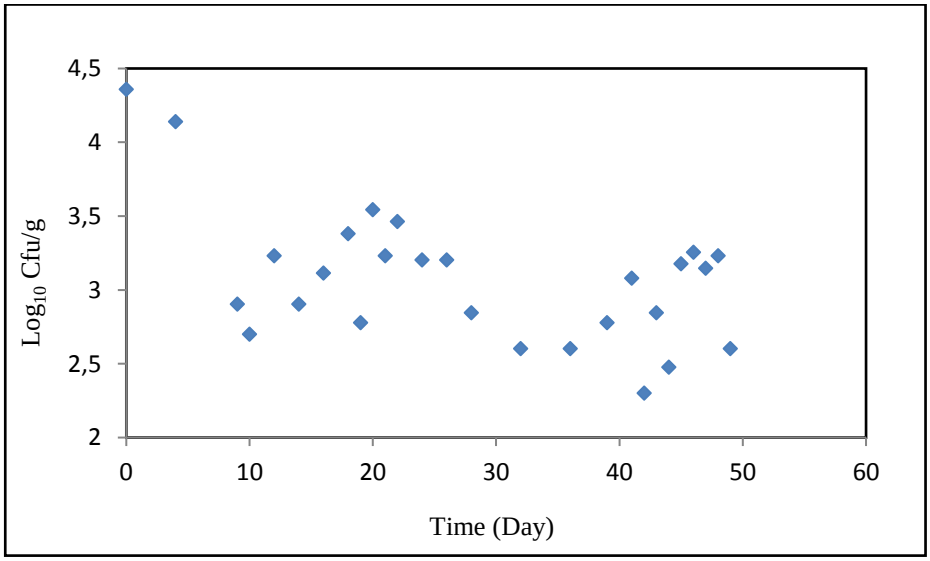


Figure 4.16. Colony counts of uncoated samples stored at -18 ℃

Figure 4.16 shows the changes in numbers of total viable count with time in uncoated samples stored at 18 ℃. Initial total bacteria count (TBC) was found as 4.13 log_{10}Cfu/g in uncoated samples. On 10th day, TBC changed as 2.69 log_{10}Cfu/g. Minimum TBC value was recorded as 2.30 log_{10}Cfu/g on 42th day during storage. At the end of storage, an effective decrease in TBC was seen due to storage at low temperature effect.

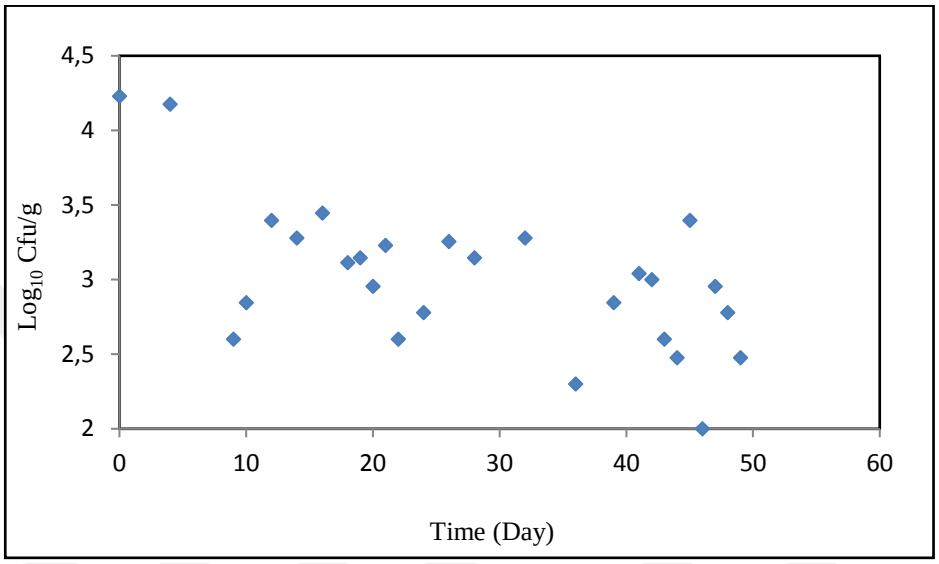


Figure 4.17. Colony counts of coated samples stored at -18 ℃

Figure 4.17 shows the changes in numbers of total viable with in coated samples stored at -18 ℃. Initial TBC was found as 4.17 log_{10}Cfu/g in coated samples. On 10th day, TBC changed as 2.60 log_{10}Cfu/g. Minimum TBC value was recorded as 2 log_{10}Cfu/g on 46th day during storage. At the end of storage, an effective decrease in TBC was seen due to storage at low temperature effect.

According to results of lipid oxidation, chitosan coating was not observed to retard the lipid oxidation when TBA analysis results as an indication of lipid oxidation in fish. Initial TBA value was found to be 4.185 µmol/L in chitosan-coated minced fish meat and 4.965 µmol/L in uncoated minced fish meat. During storage, TBA value increased rapidly at high temperatures. TBA value was related to storage time and temperature.

TBA values of minced fish samples stored at 20 ℃, 4 ℃, -1 ℃ and -18 ℃ were used to generate a predictive model to validate the applicability of kinetic models.

High regression coefficients shows the validity of the modeling by applying logistic equation (Table 4.2).

Table 4.2. Values of model parameters used for the simulation of the lipid oxidation data obtained at different temperatures for model minced fish.The values are expressed as mean ± Standart deviation for three samples (n = 3).

Temperature (℃)	**Sample**	**Onset of lipid oxidation time (d)**	**C_{max}**	**Time for C_{max} (d)**	**k (d^{-1})**	**R^2**
20	Uncoated	1	22.32±0.015	10	0.88	0.9678
	Coated	1	21.49±0.017	10	0.81	0.9903
4	Uncoated	3	17.92±0.019	14	0.62	0.9151
	Coated	3	17.08±0.012	14	0.60	0.9131
-1	Uncoated	10	15.69±0.016	20	0.56	0.9265
	Coated	11	14.86±0.012	20	0.52	0.8829
-18	Uncoated	39	15.96±0.012	49	0.38	0.9048
	Coated	39	14.97±0.006	49	0.35	0.9109

Table 4.2 shows that the k has the maximum value of 0.88 for uncoated minced fish samples stored at 20 ℃ while minimum value of 0.38 was obtained for uncoated minced fish samples stored at -18 ℃. The k has the maximum value of 0.81 for coated minced fish samples stored at 20 ℃ while minimum value of 0.35 was obtained for coated minced fish samples stored at -18 ℃. C_{max} increased to correlated with the storage time and temperature as shown in Table 4.2.

When the rate constants were analyzed for uncoated and coated minced fish samples, it was figured out that temperature was directly proportional to rate constant (k). In all samples, k values from the logistic equation were observed to increase in higher temperatures.

The dependence of oxidation rate on temperature was explained with Arrhenius kinetics. The slope of regression line was obtained by plotting ln k versus 1/Temperature (K^{-1}) for rate constants calculated for different temperatures (Figure 4.18 and Figure 4.19).

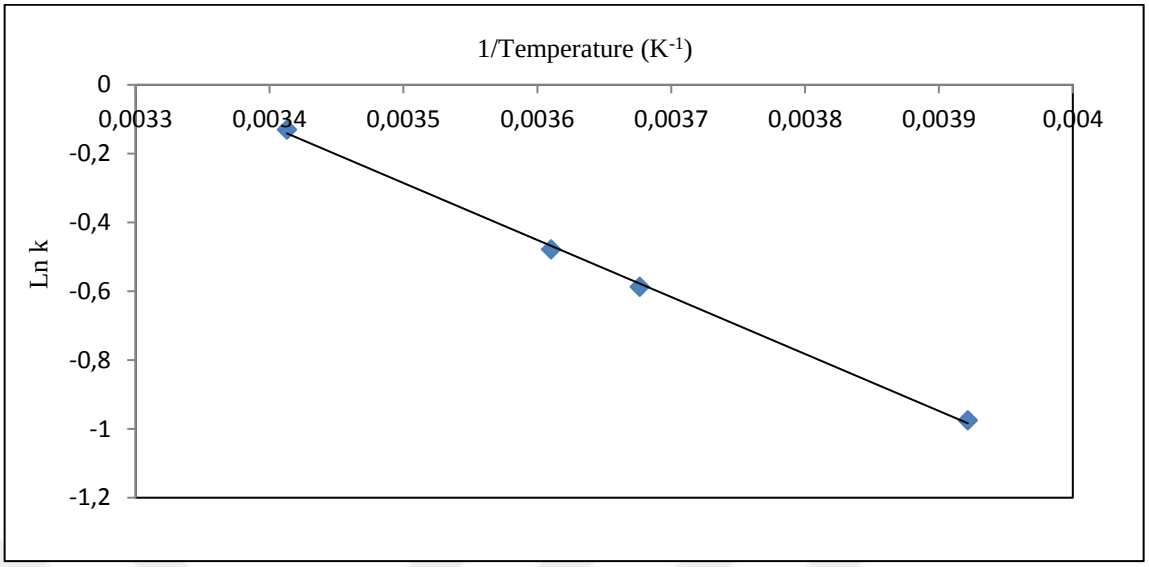


Figure 4.18. Arrhennius equation for all uncoated samples

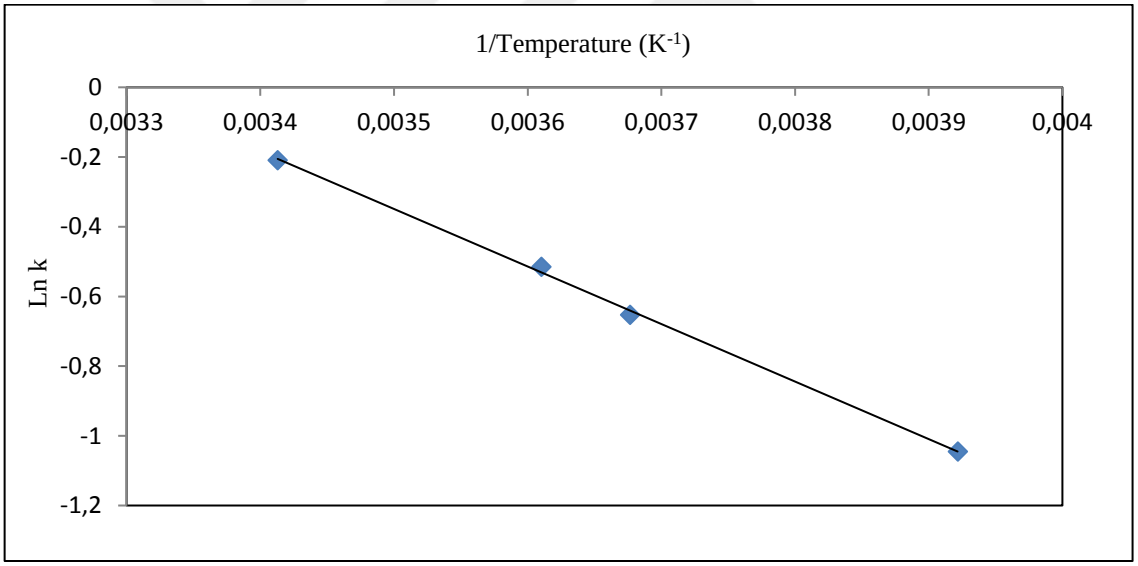


Figure 4.19. Arrhennius equation for all coated samples

The moisture content was observed to be decreasing significantly during the storage at 20 ℃, 4 ℃, and -1 ℃ and -18 ℃. According to moisture content determination, the moisture content decreases as the storage temperature increases when the samples kept at different storage temperatures were compared.

The effect of coating on the moisture loss was also analyzed (Figure A.9 – A.16 in Appendix A). The maximum moisture loss resulted in uncoated and coated minced fish

samples stored at 20 ℃. Rate of moisture loss in coated samples was lower than uncoated samples.

The effect of chitosan on moisture loss during storage was observed. Some researchers stated that although chitosan film was a strong barrier resistant to oxygen, its water vapour barrier properties were weak [86].

S. Sathivel studied the effects of chitosan and protein based coating of salmon and freezing on moisture content and lipid oxidation during storage duration. Similar to our results, the chitosan coating was observed to lower moisture loss in chitosan-coated fish samples rather than uncoated fish samples [87].

Image of colonies in Petri dishes of uncoated and coated minced fish meat samples after removal of internal organs stored at different temperatures were given in Figure B.1 - B.8 in Appendix B.

According to analysis results, *Streptococcus pseudoporcinus, Kocuria kristinae, Staphylococcus kloosii, Lactobacillus plantarum* bacteria species were identified in uncoated and coated fish samples in analyses performed with Vitec 2.0 system. Anaerobic growth was not observed in the samples.

Microbiological studies showed that storage at low temperatures affected the fish samples positively. Total Viable Count exhibited an increased tendency in uncoated and coated fish samples at 4 ℃, uncoated fish samples stored at 20 ℃ and coated fish sampled stored at -1 ℃ at the end of storage duration. These numbers remained constant in coated fish samples stored at 20 ℃, they showed a decreasing tendency in uncoated fish samples stored at -1 ℃. TBC revealed a significant decrease in uncoated and coated fish samples stored at -18 ℃.

T. Özbay and D. Ayas searched the effect of coating with 1 % chitosan and 1 % acetic acid solution on stored sardine fillets. An important decrease in microbial load and enzymatic oxidation was observed in coated fish in comparision to uncoated fish. Chitosan applications were recommended for sardine freezing [88].

5. CONCLUSION

In this study, the effect of chitosan coating on lipid oxidation, moisture loss and microbial growth of minced fish meat stored at different temperatures was analyzed; and the storage temperature and durations were optimized in order to increase the shelf life of fish by performing kinetic modeling. The results show that TBA-MDA complex of the uncoated and coated fish samples increased during stored at all tempeartures. Chitosan coating decreased the rate of propagation stage of lipid oxidation in all samples compared to the corresponding uncoated samples. The TBA-MDA complex increased to correlated with temperature. Logistic equation simulated lipid oxidation in all samples. C_{max} increased to correlated with the storage time and temperature in all samples. Chitosan coating decreased lipid oxidation, moisture loss, and microbial growth for all storage tempeartures.

REFERENCES

1. Turkish Statistical Institute, Seafoods. http://www.tuik.gov.tr/PreHaberBultenleri.do?id=21720 [retrieved 5 June 2017].

2. P. Oğuzhan and F. Yangilar. Position and Importance in Catering of Seafoods. *Journal of Science and Technology*, 7-1:65-76, 2014.

3. Food and Agricultural Organisation, Statistics. http://www.fao.org. [retrieved 5 June 2017].

4. H. Turan, Y. Kaya and G. Sonmez. Position in Human Health and Food Value of Fish Meat. *Ege Üniversitesi Su Ürünleri Dergisi*, 23:505-8, 2006.

5. N. Gokoglu. Aquaculture Processing Technology. Water Foundation Publications, Bilge Publishing. Istanbul, Turkey, 2002.

6. Food and Drug Administration-Refrigerator & Freezer Storage Chart. https://www.fda.gov/downloads/Food/ResourcesForYou/HealthEducators/UCM109315.pdf [retrieved 18 March 2017].

7. A. Demirkaya. Lipid Oxidation in Minced Meat and Chicken Meats Consumed in Bilecik *Academic Food Journal*, 12:26-29, 2014.

8. B. Özçelik and Ö. Evranuz. Lipid Oxidation in Oil Seed: Effective Factors and Measurement Factors. *Gıda* , 23:221-227, 1998.

9. D. Suput, V. L. Lazic, S. Popovic and N. Hromis. Edible Films and Coatings: Sources, Properties and Aapplication. *Food and Feed Research*, 42:11-22, 2015

10. T. Bourtoom. Edible Films and Coatings: Characteristics and Properties. *International Food Research Journal*, 15:237-48 , 2008.

11. S. Kutlu. Seafood Head Office Research Institute. *Food, Agriculture and Animal Directorate Publication,* ISSN-2147-2947, Trabzon, 2016.

12. M. Dağtekin and O. Ak. Consumption of Seafoods in the Eastern Black Sea Region, Export and Import Potential. *Yunus Research Bulletin*, 7:3, 2007.

13. H. Atar and Z. Alçiçek. Consumption of Seafoods and Health. *TAF Preventive Medicine Bulletin*, 8:173-176, 2009.

14. J. Burt. The Effects of Drying and Smoking on the Vitamin Content of Fish Smoking and Drying. In: Fish Smoking and Drying: the Effect of Smoking and Drying on the Nutritional Properties of Fish. Elsevier Applied Fish Science Publishers Ltd., London and New York, 53-60, 1988.

15. T. Can. The Importance in Human Health and Nutrition of Fish. *Food Safety Bulletin*, 3: 4-6 , Ordu, 2007.

16. S. Kaushik, G. Corraze, J. Radunz-Neto, L. Larroquet and J. Dumas. Fatty Acid Profiles of Wild Brown Trout and Atlantic Salmon Juveniles In The Nivelle Basin. *Journal of Fish Biology*, 68:1376-87, 2006.

17. Food Technology, Meat and Products Analysis-1. T. C. Ministry of Education, Ankara, 2013.

18. Triglycerides and Chemistry of Fatty Acid. https://www.slideshare.net/nurfvirtual/fatty-acids-and-triglycerides [retrieved 1 June 2017].

19. The Association of the British Pharmaceutical Industry, Lipids: Triglycerides and Fatty Acids, Available at: https://www.abpischools.org.uk/topic/chemistryoflife/7/0 [retrieved 23 May 2017].

20. H. Karabulut and İ. Yandı. Effects on Health and Importance of Omega-3 Fatty Acids in Seafood. *Journal of Fisheries and Aquatic Sciences*, 23:339-342, 2006.

21. Y. Kaya, H. Duyar and M. Erdem. The Importance of Fish Fatty Acids on Human Health. *Journal of Fisheries and Aquatic Sciences*, 21:365– 370, 2004.

22. S. Mol. Consumption of Fish Oil and Its Effects on Human Health. Review Article, *Journal of Fish Sciences*, 2:601-607, 2008.

23. G. Misir. Lipid and Fatty Acid Content of Fish and Their Some Important Metabolic Functions. *Yunus Research Bulletin*, 51-61, 2014.

24. T. Doğan. Fısh Fauna of Fethiye Bay (Mugla, Turkey). Adnan Menderes Univeristy, 2007.

25. H. Örek and F. Bingel. Black Sea Anchovy and Types of Anchovy. http://www.yelkenli.com/bilgi/hamsi.pdf [retrieved 9 September 2016].

26. Food and Agricultural Organisation, Fisheries and Aquaculture. http://www.fao.org/fishery/species/2106/en. [retrieved 23 May 2017].

27. S. Özdemir, Y. Erdem, H. Satilmiş, Z. Özdemir and E. Erdem. Determination of School Structure and Catch Efficiency of Anchovy (Engraulis encrasicolus, L.) Fishing by Pelagic Pair Trawl in Two Fishing Areas. Fırat University, *Journal of Science and Engineering*, 19:33-40, 2007.

28. O. Yıldırım. Circumstances of Fish Meal-Oil Fabrics in Sinop and Their Position in Turkish Fish Meal-Oil Production. Fırat University, *Journal of Science and Engineering*, 18 :197-203, 2005.

29. B. Tufan, S. Koral and S. Kose . Changes During Fishing Season in the Fat Content and Fatty Acid Profile of Edible Muscle, Liver and Gonads of Anchovy (*Engraulis*

encrasicolus) Caught in the Turkish Black Sea. *International Journal of Food Science and Technology*, 46:800-10 , 2011.

30. E. Wasowicz, A. Gramza, M. Hes and H. Jelen. Oxidation of Lipids in Food. *Polish Journal of Food and Nutrition Sciences*, 13/54, SI 1: 87–100, 2004.

31. K. Tozer. Quality Improvement and Shelf-life Extension of Fish Filets from Three Aquaculture Species. The Faculty of Graduate Studies of the University of Guelph, Master Thesis, 2001.

32. A. Soyer. Effect of Glazing and Storage Time on Lipid Oxidation of Frozen Chub Mackerel (Scomber japonicus). *Journal. of Veterinary and Animal Sciences*, 23:575-584 1995.

33. D. R. Johnson and E. Decker. The Role of Oxygen in Lipid Oxidation Reactions: A Review. *Food Science and Technology*, 6:171-90, 2015.

34. A. Şimşek. Stability of Frying Oils and Determination of Quality by Thermal Methods. Çukurova Univeristy, Institue of Natural and Applied Sciences, Department of Chemistry, Master Thesis, Adana, 2008.

35. C. Schneider, D. Pratt, N. Porter, and A. Brash. Control of Oxygenation in Lipoxygenase and Cyclooxygenase Catalysis. *Chemistry and Biology*, 14:473-88, 2007.

36. E. Choe and D. Min. Mechanisms of Antioxidants in the Oxidation of Foods. *Comprehensive Reviews in Food Science and Food Safety*, 8, 2009

37. R. Cavalieri and J. Reyes De Corcuera. Kinetics of Chemical Reactions in Foods. Encyclopedia of Life Support Systems, Food Engineering, 1, 2009.

38. S. Ozilgen and M. Ozilgen. Kinetic Model of Lipid Oxidation in Foods. *Journal of Food Science*, 55: 498–536, 1990.

39. S. Gómez-Alonso, V. Mancebo-Campos, M. Desamparados Salvador and G. Fregapane. Oxidation Kinetics in Olive Oil Triacylglycerols Under Accelerated Shelf-life Testing. *Journal of Lipid Science and Technology*, 106:369-75,2004.

40. A. Colakoglu. Oxidation Kinetics of Soybean Oil in the Presence of Monoolein, Stearic Acid and Iron. *Food Chemistry,* 101:724–728, 2007.

41. L. Zhan, X. Li, W. Lu, H. Shen and Y. Luo. Quality Predictive Models of Grass Carp (*Ctenopharyngodon idellus*) at Different Temperatures During Storage. *Food Control*, 22: 1197–1202, 2011.

42. F. Wenjiao, Z. Yongkui, C. Yunchuan, S. Junxiu and Y. Yuwen. TBARS Predictive Models of Pork Sausages Stored At Different Temperatures. *Meat Science*, 96:1-4, 2014.

43. C. Okpala, G. Bono, M. Geraci, G. Sardo, S. Vitale and C. Schaschke. Lipid Oxidation Kinetics of Ozone-Processed Shrimp During Iced Storage Using Peroxide Value Measurements. *Food Bioscience*, 16:5-10, 2016.

44. L. Escalona-Garcia, R. Pedroza-Islas , R. Natividad, M. Rodríguez-Huezo, H. Carrillo-Navas and C. Pérez-Alonso. Oxidation Kinetics and Thermodynamic Analysis of Chia Oil Microencapsulated in A Whey Protein Concentrate - Polysaccharide Matrix. *Journal of Food Engineering*, 175:93-103, 2016.

45. H. Tavakolipour, M. Mokhtarian and A. Ashtari. Lipid Oxidation Kinetics of Pistachio Powder During Different Storage Conditions. *Journal of Food Process Engineering*, 40, 3, 2016.

46. M. P. Richards, H. O. Hultin and J. Agric. Contributions of Blood and Blood Components to Lipid Oxidation in Fish Muscle. *Journal of Agricultural and Food Chemistry*, 50: 555-564, 2002.

47. Y. Tseng, Y. Xiong, and C. Webster. The Preservation of the Quality of the Muscle in Frozen Australian Red Claw Crayfish (Cherax quadricarinatus) by Pre-storage Anti-oxidant Dipping Treatments. *International Journal of Food Science and Technology*, 40:841-848, 2005.

48. P. Yerlikaya, N. Gökoğlu and H. Uran. Quality Changes of Fish Patties Produced from Anchovy During Refrigerated Storage. *European Food Research and Technology*, 220: 287- 291, 2005.

49. Ş. A. Hisar, O. Hisar, G. Alak and T. Yanık. The Effects of Stinging Nettle (Urtica diocia L.) on Chemical Properties of Filetos of Rainbow Trout (Oncorhynchus mykiss). *1. National Trout Symposium*, 2008.

50. A. Rodríguez, M. Trigo, R. Pérez, J. Cruz, P. Paseiro and S. P. Aubourg. Lipid Oxidation Inhibition in Frozen Farmed Salmon (Oncorhynchus kisutch): Effect of Packaging. *Czech Journal of Food Science*, 27:182-184, 2009.

51. G. Al-Bandak, T. Tsironi, P. Taoukis and V. Oreopoulou. Antimicrobial and Antioxidant Activity of Majorana Syriaca in Yellowfin Tuna. *International Journal of Food Science and Technology*, 44:373-9,2009.

52. I. Aydin and N. Gokoglu. Effects of Temperature and Time of Freezing on Lipid Oxidation in Anchovy (*Engraulis encrasicholus*) During Frozen Storage. *European Journal of Lipid Science and Technology*, 8, 2014

53. T. Mchugh, C. Weller and J. Krochta . Edible Coatings and Films Based On Proteins. Edible Coatings and Films to Improve Food Quality. In: A. Elizabeth, J. Krochta and O. Nisperos-Carriedo, editors, CRC Press, 1994.

54. A. Pavlath and W. Orts. Edible Films and Coatings: Why, What, and How? In Edible Films and Coatings for Food Applications. In: M. Embuscado, C. Huber, editors, Springer Dordrecht Heidelberg London New York, 403, 2009.

55. J. Krochta and D. Mulder-Johnston. Edible and Biodegradable Polymer Films: Challenges and Opportunities. Food Technology, USA, 1997.

56. M. Khan, M Adrees, M. Tariq and M Sohaib. Application of Edible Coating for Improving Meat Quality: A Review. *Journal of Food Sciences* , 2:71-79, 2013.

57. E. Kapetanakou, S. Manios and P. Skandamis. Application of Edible Films and Coatings on Food. In: Ioannis S. Boziaris editors. Novel Food Preservation and Microbial Assessment Techniques, CRC Press, Chapter 9, 237:265, 2014.

58. N. Turhan. Methods Used in the Coating of Foods. *Journal of Science and Technical*, 31, 2006.

59. M. Rinaudo. Chitin and Chitosan: Properties and Applications. Progress in Polymer Science. *Elsevier Science*, 31:603-32, 2006.

60. M. Struszczyk. Chitin and Chitosan, Part1- Properties and Production. Industrial Chemistry Research Institute, Polimery,47: 396-403, 2002.

61. P. Yıldız, F. Yangılar. Use of Chitosan in Food Industry. Erciyes University, *Journal of Science Institute*, 30:198-206, 2014.

62. E. Szymanska, K. Winnicka and P. Laurienzo. Stability of Chitosan - A Challenge for Pharmaceutical and Biomedical Applications. *Marine Drugs*, 13: 1819–1846, 2015.

63. N. Erkan, H. Dogruyol and Ali Gunlu, I. Genc. Use of Natural Preservatives in Seafood: Plant Extracts, Edible Film and Coating. *Journal of Food and Health Science*, 1: 33-49, 2015.

64. S. Dursun and N. Erkan. Edible Protein Films and Their Use in Seafoods. *Journal of Fisheries Sciences*, 3:352-73, 2009.

65. A. Gennadios, M. Hanna and L. Kurth. Application of Edible Coatings on Meats, Poultry and Seafoods: A Review. *LWT-Food Science and Technology*, 30:337-50, 1997.

66. Y. Jeon, J. Kamil and F. Shahidi. Chitosan as an Edible Invisible Film for Quality Preservation of Herring and Atlantic Cod. *Journal of Agricultural and Food Chemistry*, 50:5167−5178, 2002.

67. S. Sathivel. Chitosan and Protein Coatings Affect Yield, Moisture Loss, and Lipid Oxidation of Pink Salmon (*Oncorhynchus gorbuscha*) Fillets During Frozen Storage. *Journal of Food Science*, 70:455-459, 2005.

68. M. Lopez-Caballero, N. Gomez-Guille, M. Perez-Mateos and P. Montero. A Chitosan–Gelatin Blend as a Coating for Fish Patties. *Food Hydrocolloids*, 19:303–311, 2005.

69. W. Fan, J. Sun, Y. Chen, J. Qiu, Y. Zhang and Y. Chi. Effects of Chitosan Coating on Quality and Shelf Life of Silver Carp During Frozen Storage. *Food Chemistry,* 115: 66 – 70, 2009.

70. G. Alak, S. Hisar, O. Hisar, G. Kaban and M. Kaya. Microbiological and Chemical Properties of Bonito Fish (*Sarda sarda*) Fillets Packaged with Chitosan Film, Modified Atmosphere and Vacuum. Kafkas University, *Journal of Veterinary Faculty*, 16:73-80, 2010.

71. V. Chamanara, B. Shabanpour, M. Khomeiri and S. Gorgin. Shelf-Life Extension of Fish Samples by Using Enriched Chitosan Coating with Thyme Essential Oil. *Journal of Aquatic Food Product Technology*, 22: 3–10, 2013.

72. C. Mohan, C. Ravishankar, K. Lalitha and T. S. Gopal. Effect of Chitosan Edible Coating on the Quality of Double Filleted Indian Oil Sardine (*Sardinella longiceps*) During Chilled Storage. *Food Hydrocolloids*, 26:167-74, 2012.

73. S.S. Nielsen. Food Analysis Laboratory Manual. Food Science Texts Series, DOI 10.1007/978-1-4419-1463-7_5, Springer Science, Business Media, LLC 2010.

74. S.S. Nielsen. Food Analysis Laboratory Manual. Food Science Texts Series, DOI 10.1007/978-1-4419-1463-7_4, Springer Science, Business Media, LLC 2010.

75. Manual of Methods of Analysis of Foods, Meat and Meat Products - Fish and Fish Products-Lab. Manual 6 , 2012.

76. Official Methods of Analysis, Association of Official Analytical Chemists, Washington DC, USA, 2012.

77. T. Acharya. Cultivation of Aerobic and Anaerobic Bacteria. https://microbeonline.com/cultivation-of-aerobic-and-anaerobic-bacteria/ [retrieved 9 June 2017].

78. S.Aryal. Catalase Test - Principle, Uses, Procedure, Result Interpretation with Precautions. 2015.

79. S.Aryal. Oxidase Test - Principle, Uses, Procedure, Result Interpretation with Precautions. 2015.

80. R. Guillen-Sans and M. Guzman-Chozas. The Thiobarbituric Acid (TBA) Reaction in Foods: A Review. *Food Science and Nutrition*, 38:315-50, 1998.

81. T. Owen. Fundamentals of UV-Visible Spectroscopy: A Primer: Hewlett Packard. 1996.

82. S. Jung, K. Nam and C. Jo. Detection Of Malondialdehyde in Processed Meat Products without Interference from the Ingredients. *Food Chemistry*, 209:90-4, 2016.

83. A. Kaczmarek, R. Cegielska-Radziejewska, T. Szablewski and J. Zabielski . TBARS and Microbial Growth Predicative Models of Pork Sausage Stored at Different Temperatures. *Journal of Food Science*, 33:320-5, 2015.

84. F. Koç. Investigations on Chemical Composition of Anchovy. Ankara University Faculty of Veterinary, Ankara, 1969.

85. N. Ustun and S. Turhan. Variation of Physical and Chemical Properties of Anchovies *(Engraulis encrasicolus)* with Different Length During Fishing Period. *Gıda*, 22:295-9, 1997.

86. X. Xu, K. Kim, M. Hanna and D. Nag. Chitosan-Starch Composite Film: Preparation and Characterization. *Industrial Crops and Products an International Journal*, 21:2, 185-192, 2005.

87. S. Sathivel. Chitosan and Protein Coatings Affect Yield, Moisture Loss, and Lipid Oxidation of Pink Salmon (*Oncorhynchus gorbuscha*) Fillets During Frozen Storage. *Journal of Food Science*, 70, 2005.

88. T. Özbay and D. Ayas. The Effects of the Use of Chitosan and Asetic Acid on the Quality of Frozen Sardine Fillets (Sardinella aurita, Valenciennes). Suleyman Demirel University, *Journal of Aquaculture Faculty*, 7:2, 2011.

APPENDIX A: GRAPHS FOR LOGISTIC EQUATION AND MOISTURE LOSS

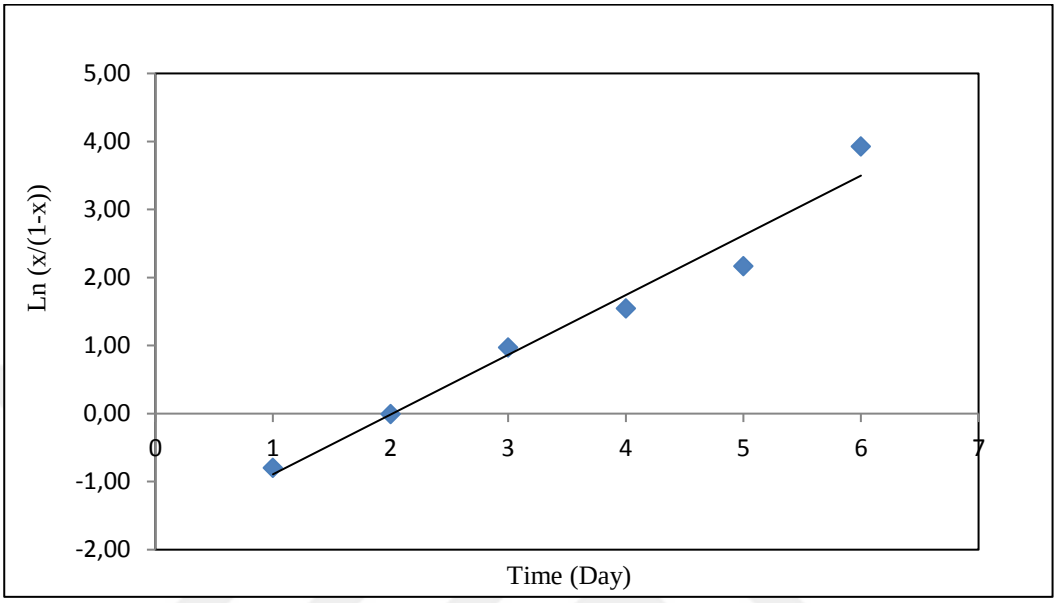


Figure A.1. Logistic equation of uncoated samples stored at 20 ℃

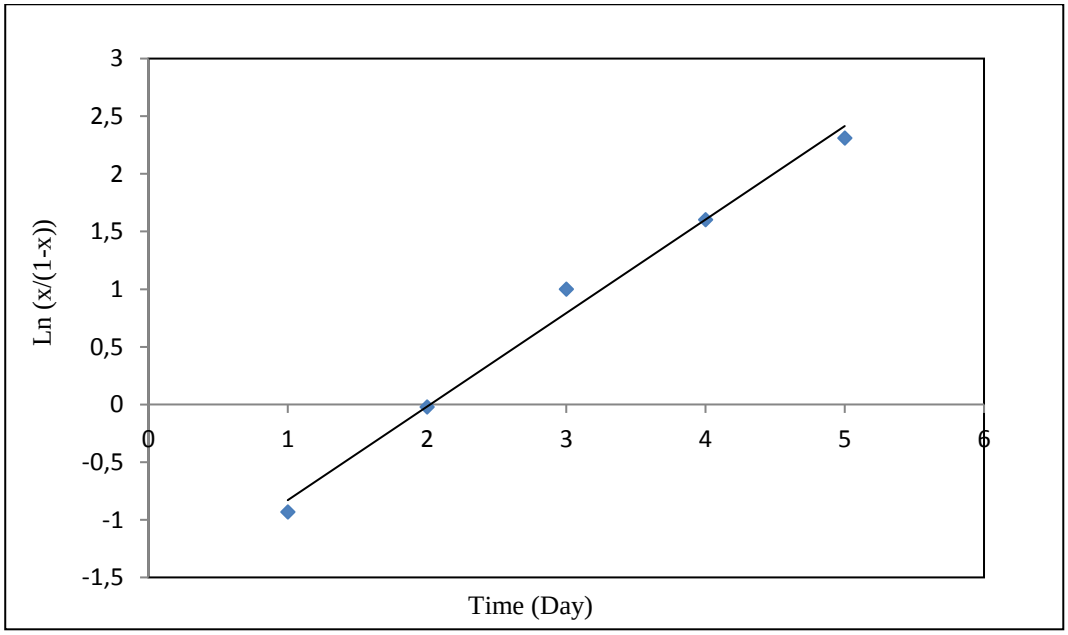


Figure A.2 Logistic equation of coated samples stored at 20 ℃

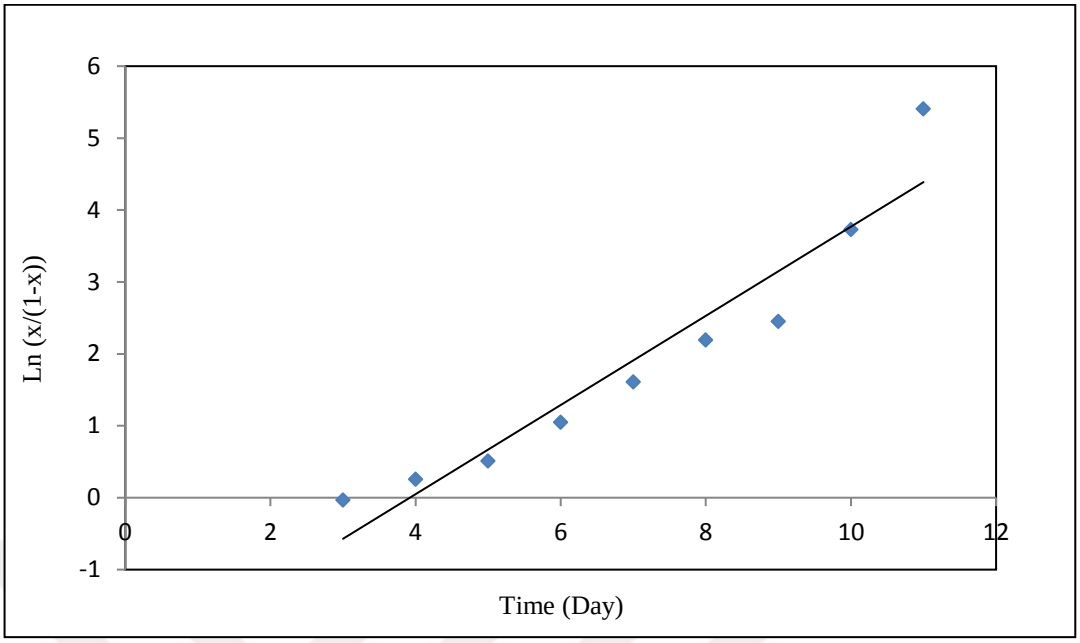


Figure A.3 Logistic equation of uncoated samples stored at 4 ℃

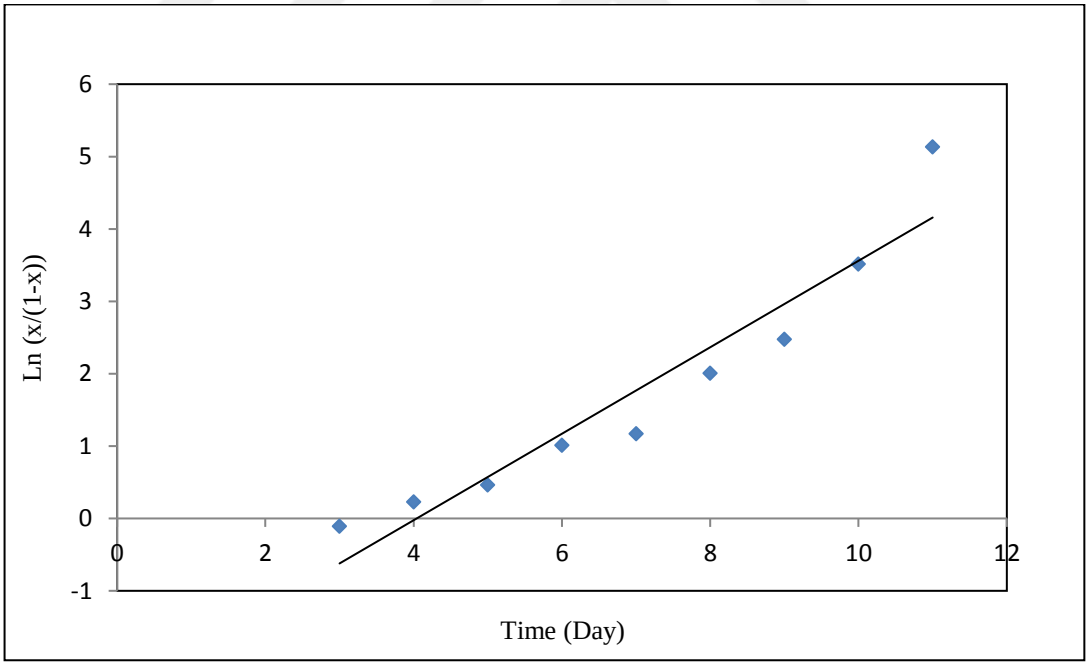


Figure A.4 Logistic equation of coated samples stored at 4 ℃

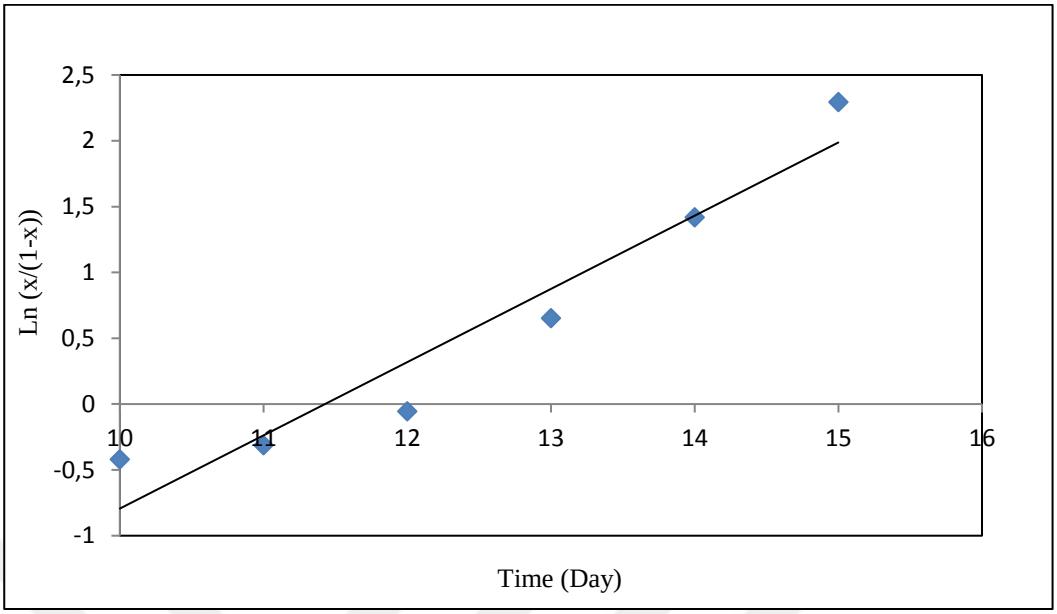


Figure A.5 Logistic equation of uncoated samples stored at -1 ℃

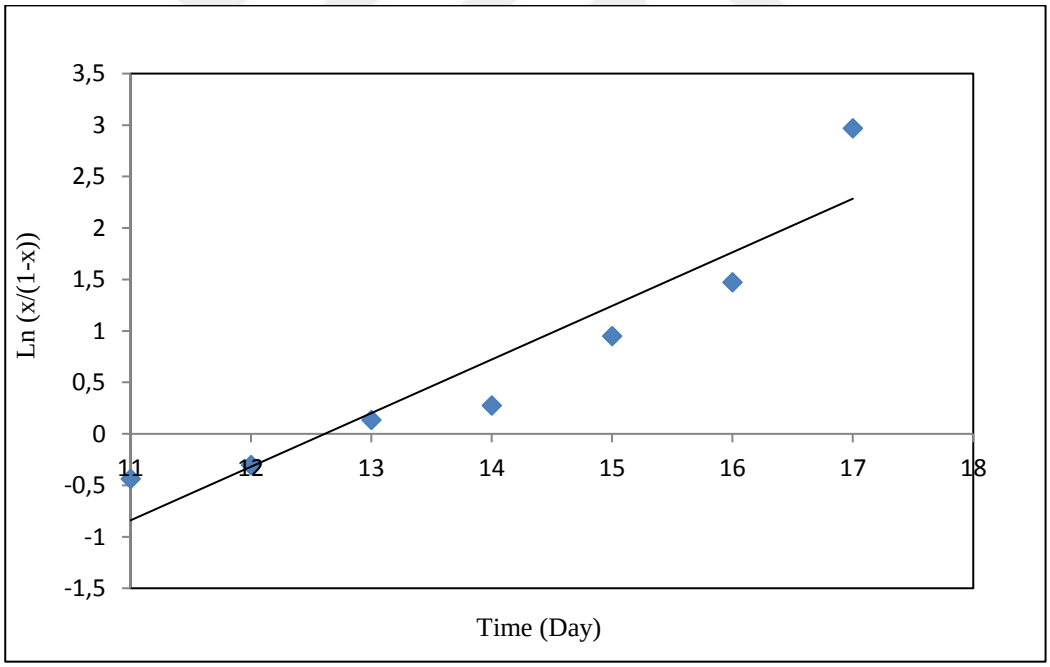


Figure A.6 Logistic equation of coated samples stored at -1 ℃

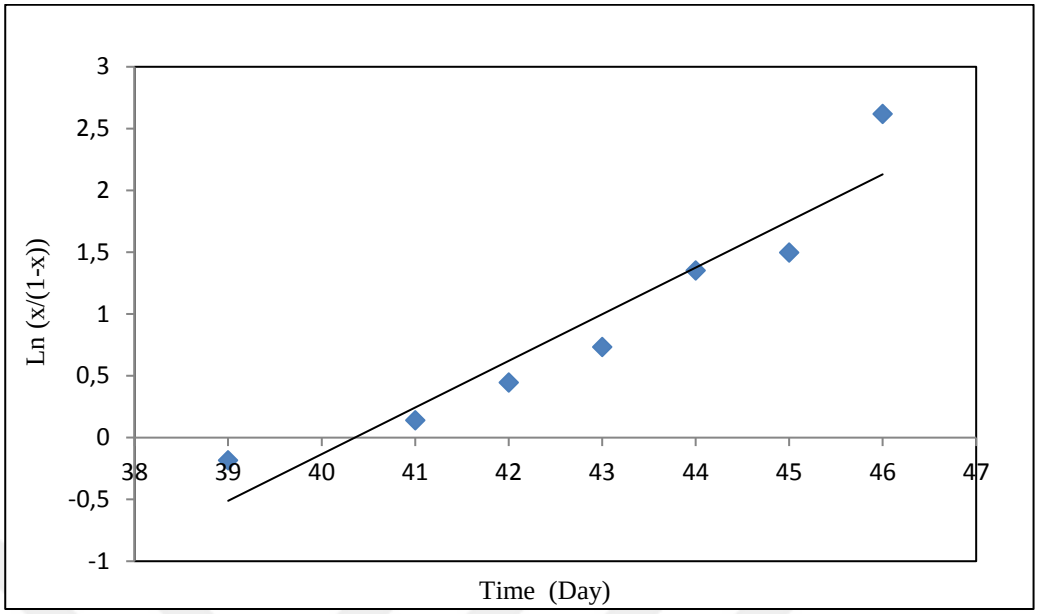


Figure A.7 Logistic equation of uncoated samples stored at -18 ℃

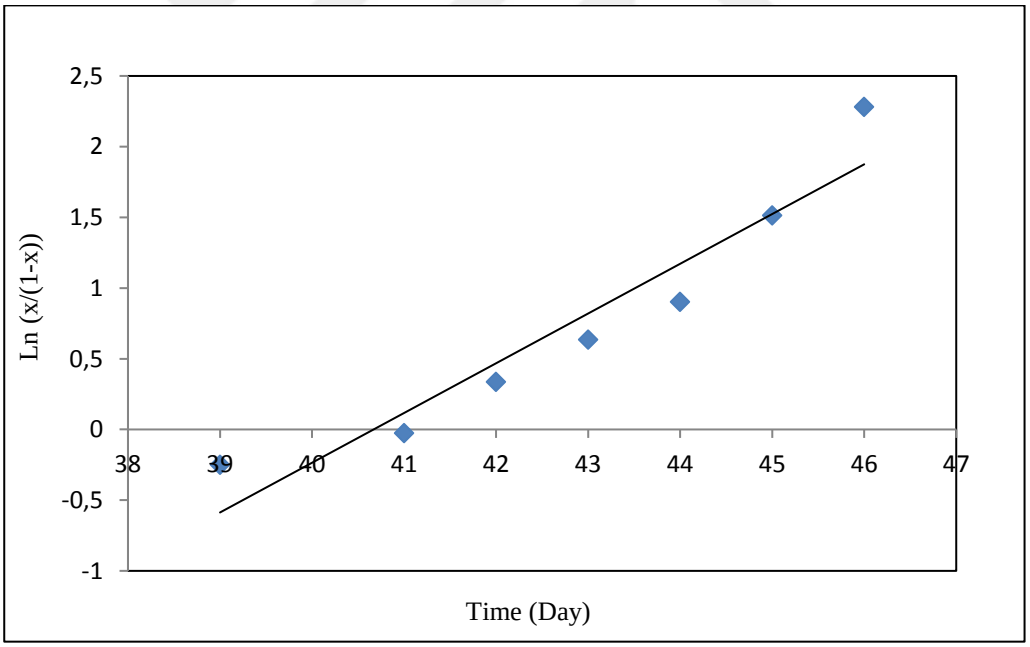


Figure A.8 Logistic equation of coated samples stored at -18 ℃

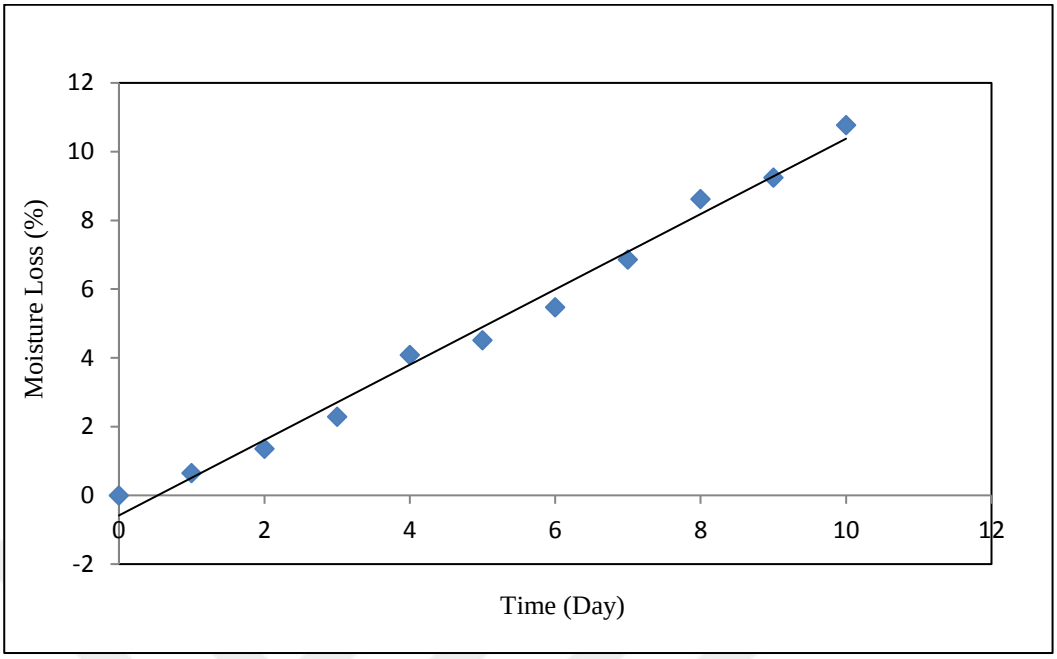


Figure A.9 Moisture loss of uncoated samples stored at 20 ℃

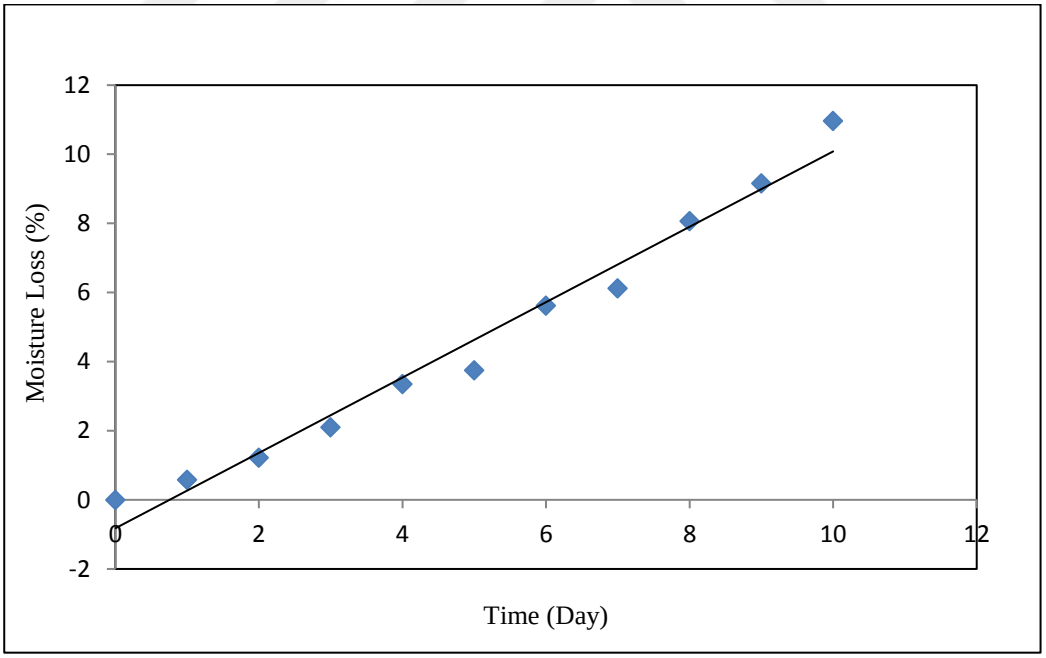


Figure A.10 Moisture loss of coated samples stored at 20 ℃

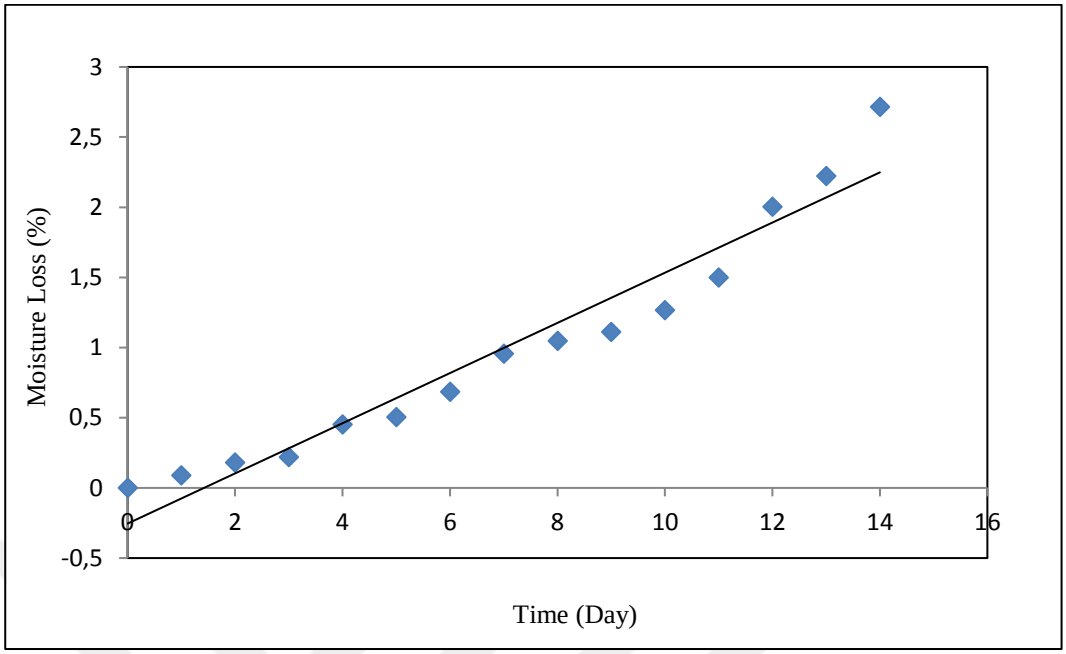


Figure A.11 Moisture loss of uncoated samples at 4 °C

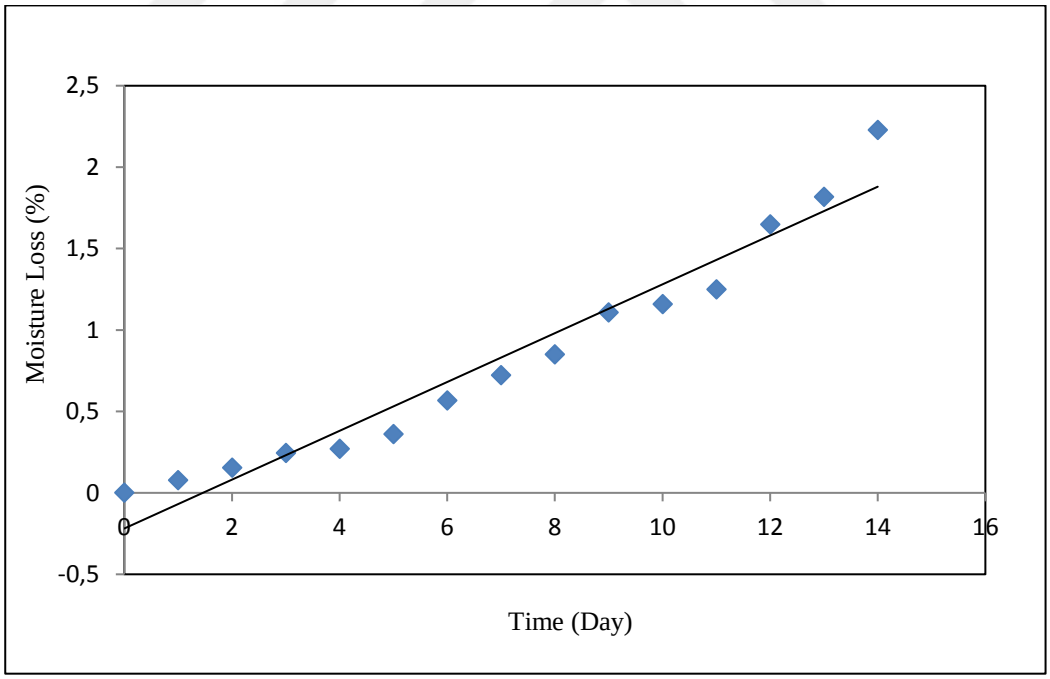


Figure A.12 Moisture loss of coated samples stored at 4 °C

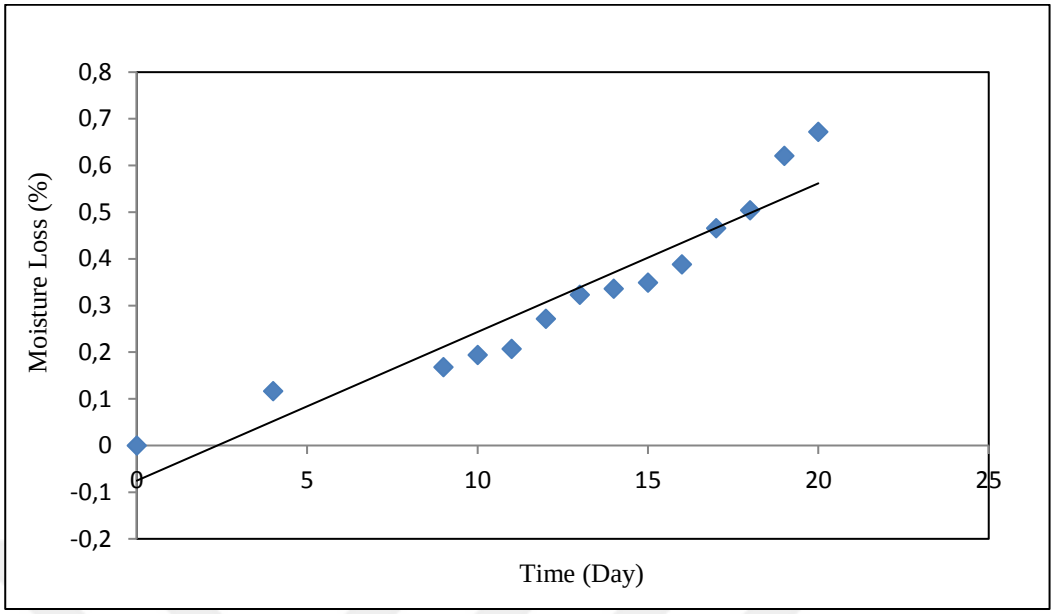


Figure A.13 Moisture loss of uncoated samples stored at -1 ℃

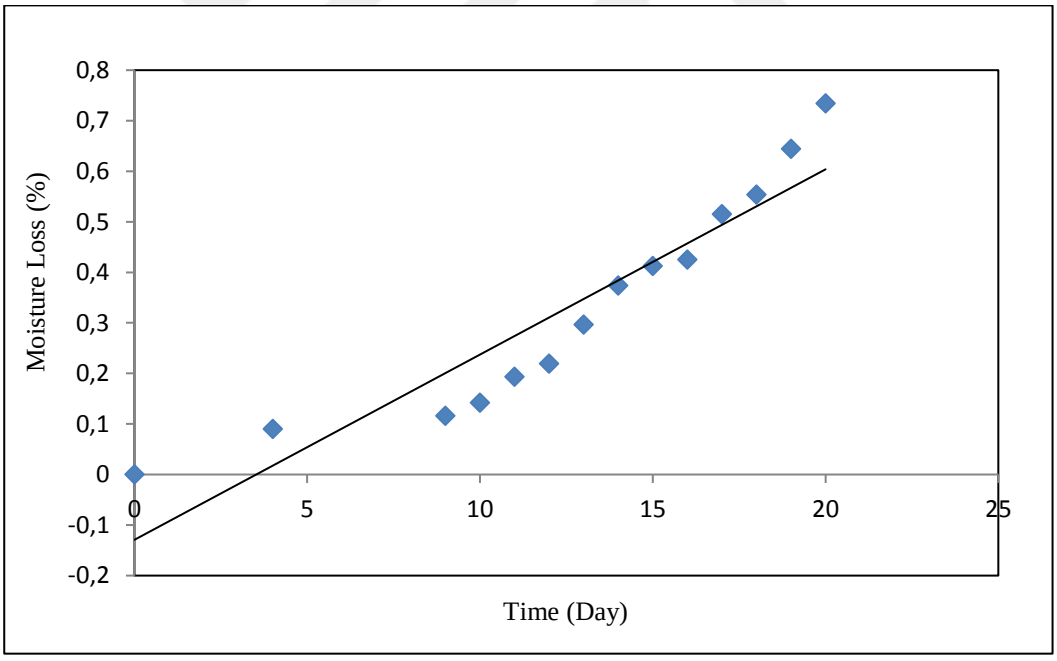


Figure A.14 Moisture loss of coated samples stored at -1 $^{\circ}C$

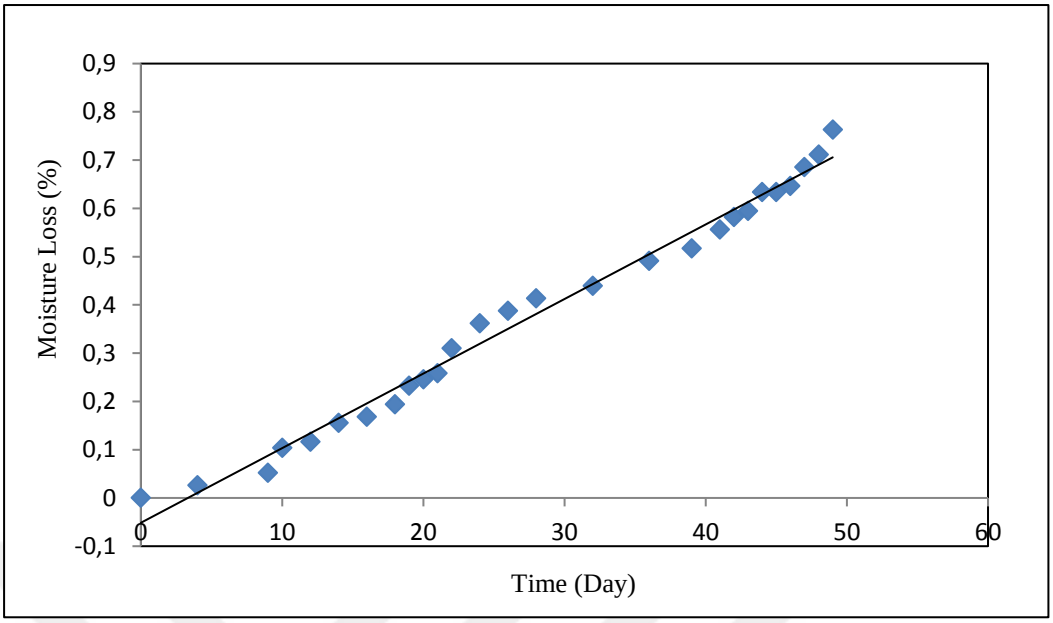


Figure A.15 Moisture loss of uncoated samples stored at -18 ℃

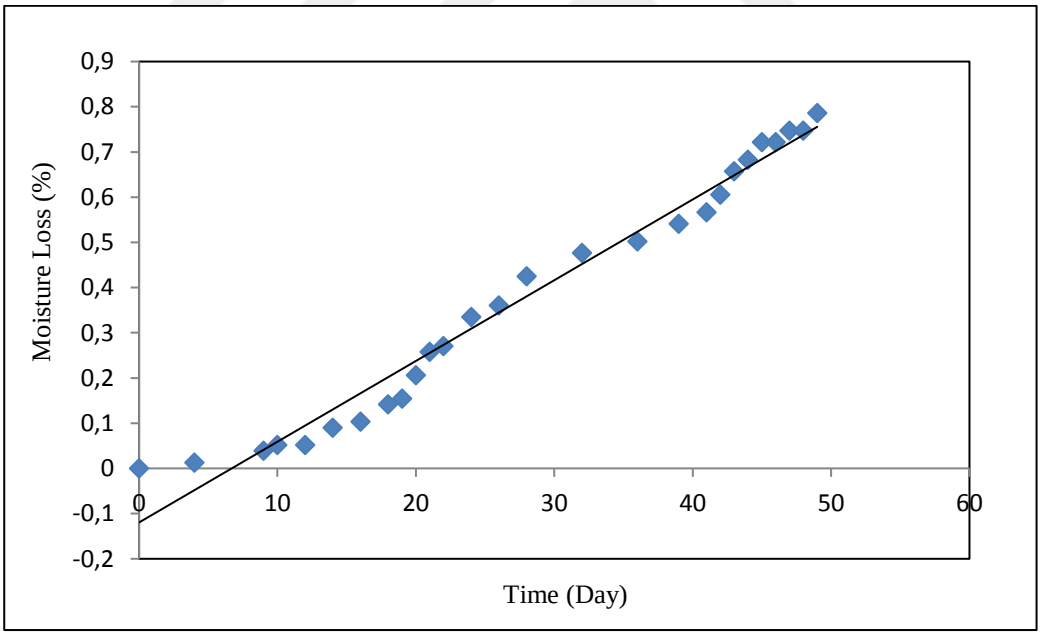


Figure A.16 Moisture loss of coated samples stored at -18 ℃

APPENDIX B: IMAGES OF COLONIES IN PETRI DISHES

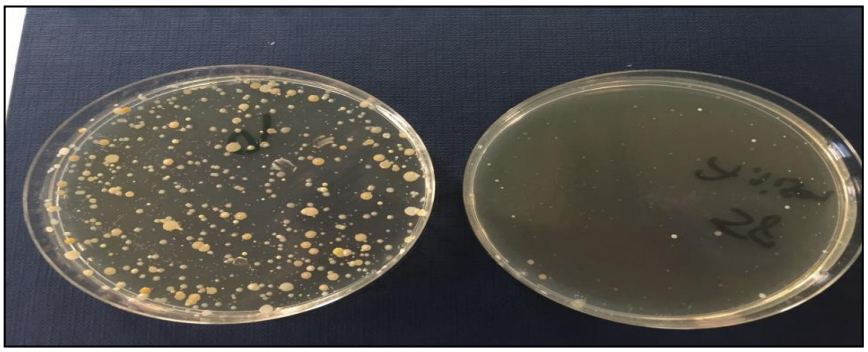


Figure B.1 Beginning and finally occured microorganzims of uncoated samples stored at 20 ℃

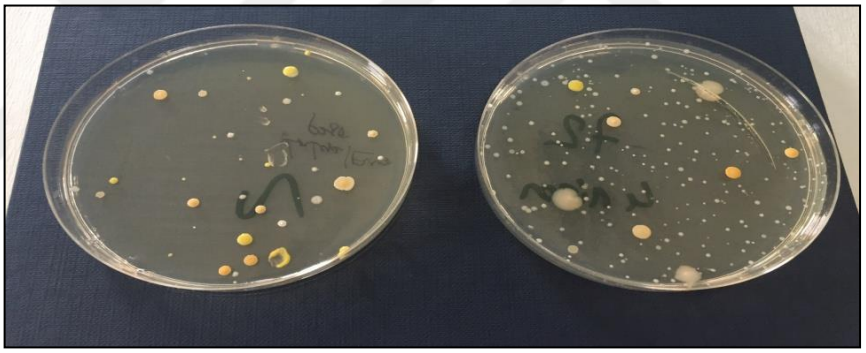


Figure B.2 Beginning and finally occured microorganzims of uncoated samples stored at 4 ℃

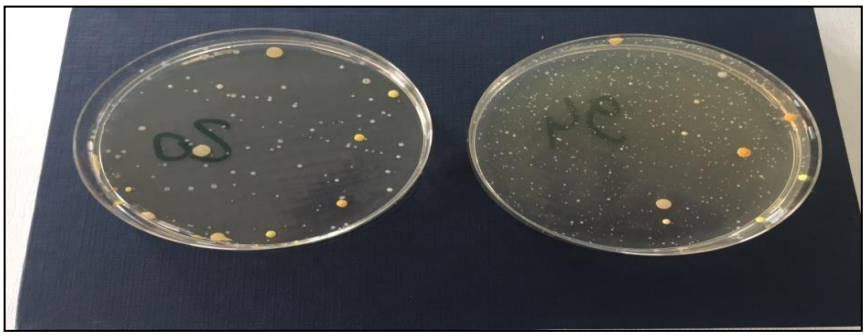


Figure B.3 Beginning and finally occured microorganzims of uncoated samples stored at -1 ℃

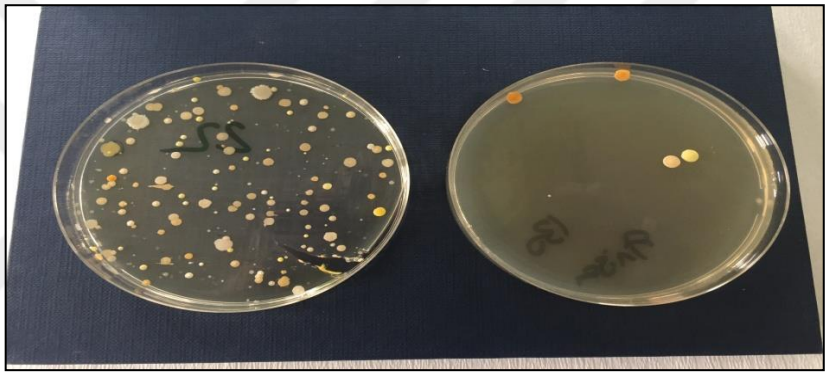


Figure B.4 Beginning and finally occured microorganzims of uncoated samples stored at -18 ℃

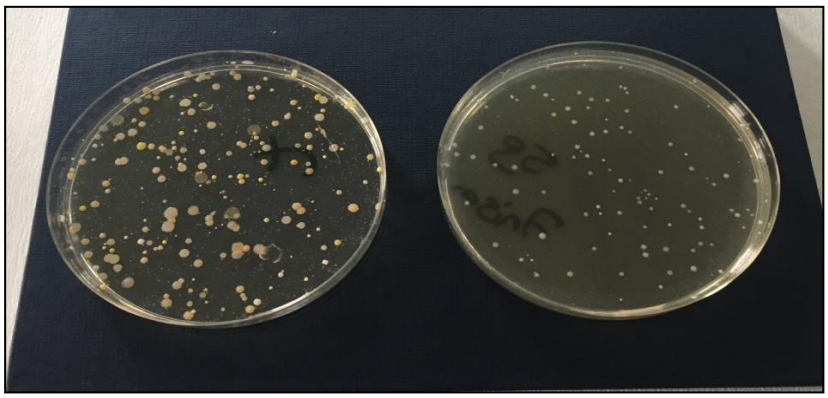


Figure B.5 Beginning and finally occured microorganzims of coated samples stored at 20 ℃

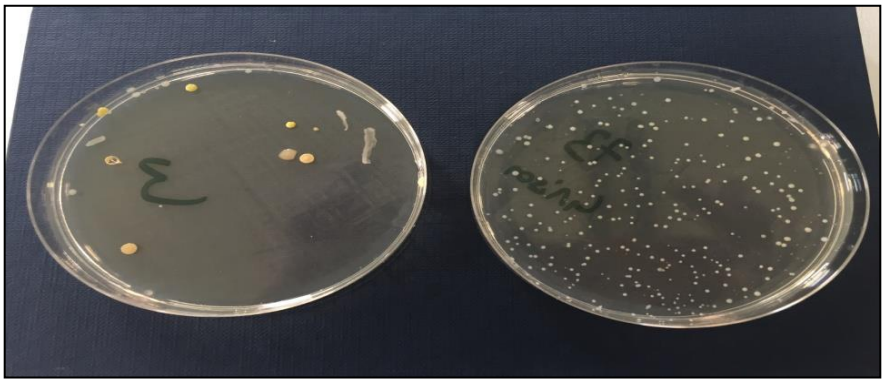


Figure B.6 Beginning and finally occured microorganzims of coated samples stored at 4 ℃

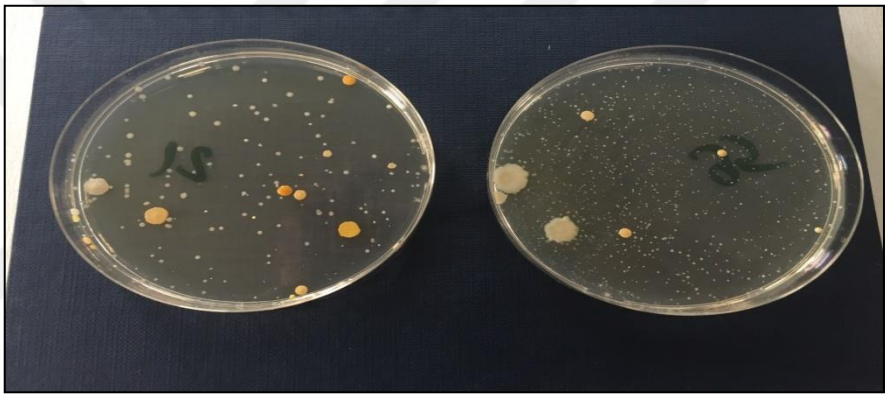


Figure B.7 Beginning and finally occured microorganzims of coated samples stored at -1 ℃

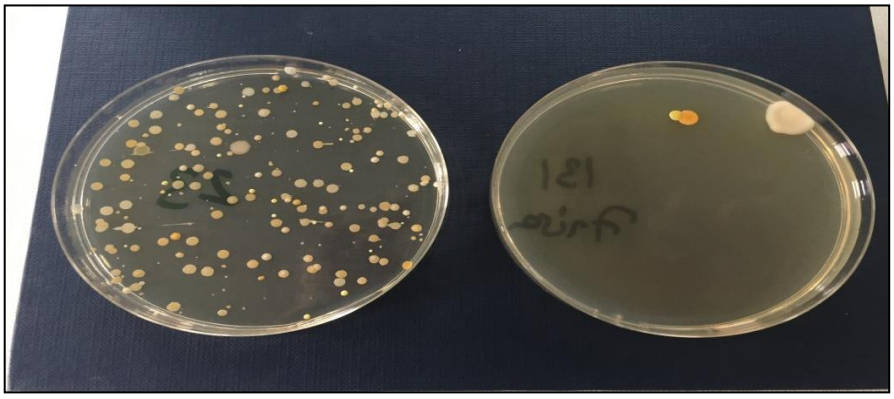


Figure B.8 Beginning and finally occured microorganzims of coated samples stored at -18 ℃