T.C.
NİĞDE ÖMER HALİSDEMİR UNIVERSITY
GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES
DEPARTMENT OF ANIMAL PRODUCTION AND TECHNOLOGIES

IMPROVEMENT THE QUALITY OF FISH BY USE OF GELATIN FILM INCORPORATED WITH CITRUS SEED EXTRACT

AHLAM K. M. ABUIBAID

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Master Thesis

Supervisor

Assoc. Prof. Dr. İLKNUR UÇAK

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Ahlam ABUIBAID tarafından **Doç. Dr. İlknur UÇAK** danışmanlığında hazırlanan **"Improvement the Quality of Fish By Use of Gelatin Film Incorporated with Citrus Seed Extract"** adlı bu çalışma jürimiz tarafından Niğde Ömer Halisdemir Üniversitesi Fen Bilimleri Enstitüsü **Hayvansal Üretim ve Teknolojileri** Ana Bilim Dalı'nda Yüksek Lisans tezi olarak kabul edilmiştir.

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THESIS CERTIFICATION

I certify that the thesis has been written by me and that to the best of my knowledge and belief. All information presented as part of this thesis is scientific and in accordance with the academic rules. Any help I have received in preparing the thesis, and all sources used, have been acknowledged in the thesis.

Ahlam K. M. ABUIBAID

ÖZET

NARENCİYE ÇEKİRDEĞİ EKSTRAKTI İLE ZENGİNLEŞTİRİLMİŞ JELATİN FİLMLER KULLANILARAK BALIK KALİTESİNİN GELİŞTİRİLMESİABUIBAID,

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Bu çalışmada, %2 turunçgil tohumu ekstraktları ile zenginleştirilen yenilebilir jelatin filmlerle kaplanan levrek filetosunun 4±1oC'de 15 gün depolanması sırasında kalitesinde meydana gelen değişimler incelenmiştir. Bu amaçla levrek filetoları jelatin filmle kaplanan filetolar (CF), %2 narenciye çekirdeği ekstraktları ilaveli jelatin filmle kaplanan filetolar (portakal P2, limon L2) ve kaplama yapılmayan filetolar (kontrol, C) olarak dört gruba ayrılmıştır. Elde edilen sonuçlar doğrultusunda tüm gruplarda fiziko-kimyasal değerler depolama sonuna kadar artmış ve en yüksek değerler C ve CF gruplarında bulunmuştur. Depolama boyunca en düşük değerler ise L2 grubunda belirlenmiştir. En düşük mezofilik, psikrofilik bakteri ve Enterobacteriaceae sayısı limon çekirdeği ekstraktı ile zenginleştirilmiş filmlerle kaplanan gruplarda gözlenmiştir. Sonuç olarak, portakal veya limon çekirdeği ekstrakları ilavesi jelatin filmlerin etkinliğini arttırmış, bu filmlerle kaplanan levrak filetolarında lipit oksidasyonu, duyusal ve mikrobiyal bozulma geciktirilmiştir.

Anahtar Sözcükler: Gökkuşağı Alabalığı, Jelatin, Yenilebilir Film, Propolis, Raf Ömrü

SUMMARY

IMPROVEMENT THE QUALITY OF FISH BY USE OF GELATIN FILM INCORPORATED WITH CITRUS SEED EXTRACT

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In this study, the effect of edible gelatin films enriched with 2% of citrus seeds extracts on the quality of seabass fillets during refrigerated temperature at 4±1oC for 15 days of storage were determined. For this purpose samples were divided into four groups entitled as; fillets wrapping with gelatin film (CF), fillets wrapping with gelatin film with 2% of citrus seeds extract (orange O2, lemon L2) and fillets without coating (control, C). According to the results, physiochemical values were increased in all samples till the end of storage and the highest values were found in the C and CF groups. The lowest values were determined in L2 group during the storage. By the microbial results, the lowest mesophilic, psychrophilic bacteria and Enterobacteriaceae counts were observed in the groups wrapped with films incorporated with lemon seed extract. As a result it was determined that the supplementation of orange or lemon seeds extract raised the effectiveness of the gelatin films and the lipid oxidation, sensory and microbial deterioration were delayed in seabass fillets wrapped with these films.

Keywords: Seabass, Gelatin, Edible Films, Citrus Seeds, Shelf Life

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF PHOTOS

SYMBOLS AND ABBREVIATIONS

Symbols	Description
%	Percentage
°C	Degrees Celsius
kg	Kilogram
ppm	Parts per million
g	Gram
mg	Miligram
L	Litre
HCL	Hydrochloric acid
H_2SO4	Sulphuric acid
N	Nitrogen
KI	Potassium iodide

Abbreviations	Description
CFU	Colony Forming Unit
TAMB	Total Aerobic Mesophilic Bacteria
PDA	Potato Dextrose Agar
VRBA	Violet Red Bile Agar
PCA	Plate Count Agar
PV	Peroxide Value
TBARS	Thiobarbituric acid reactive substances

CHAPTER I

INTRODUCTION

Nowadays, the edible films and coating takes attention in food processing due to their benefits towards on food preservation, using natural products and materials which are environmental concern of disposal nonrenewable food packaging materials and giving capacity to create new products in market (Khan, 2013). They have been involved in many commercial applications such as confectioner's glaze produced from shellac (Alikonis, 1979), collagen casing for sausages (Hood, 1987), different waxes on fruits and gelatin coating for pharmaceutical purpose (Rose, 1987).

The edible films and coating approach was constructed from the needs of storage and preserve the fresh food when the transportation distribution was increased. Which means that, it takes time from transport food to reach the consumers. Therefore, the films are able to keep the food quality by inhibiting moisture losses, decline the load of spoilage microorganisms, and allowing the specific exchange of the important gasses for respiration processes such as, ethylene, oxygen and carbon dioxide. In addition, the consumers these days have been rising their awareness of bad environmental effects of synthetic packaging (Bourtoom, 2008; Donhowe and Fennema, 1993).

Edible film is different than edible coating by applying form into the products which the film used as outside wrapping while the coating applied directly from solution to the food surface itself (Cordeiro de Azeredo, 2012). Several researches proved that each method con improve the organoleptic characteristics of packaged seafood. Also, it can works to delay oxidation and microbial spoilage with the combination of antioxidant and antimicrobial agents.

Although the purpose of edible films and coatings is not to replace the traditional packaging, it is to enhance the ability of food protection by joining the direct packaging to the food (edible) and second packaging (nonedible). The need of nonedible packaging is to protect the food products from external hazards such as insects, chemicals, and uncleaned equipment in handling (Cordeiro de Azeredo, 2012).

CHAPTER II

GENERAL INFORMATIONS

2.1 Types of Edible Films

Edible films and coatings can be sorted based on materials type which they are derived. Polysaccharides, proteins and lipids based films are main types of edible films (Table 1.1). Each type has own pros and cons when applied for films. Polysaccharides are generally available and not costly. Widely, some of polysaccharides are used to produce films such as, starch, chitosan, and carrageenan. Most of polysaccharides are neutral charged, even though some gums are negatively charged. Because of the containing big amount of hydroxyl and different polar groups in their structure, the hydrogen bonds have important function in forming films and final characteristics. Gums have negative charged such as carboxymethyl cellulose (CMC), pectin, and alginate, relying on the pH, generally lead to have different properties (Qiu et al., 2014; Cordeiro de Azeredo, 2012).

The edible film formed from extracted protein from animals such as (gelatin, collagen, whey protein concentrate and isolates, egg albumin and casein) or plants such as (soybean, wheat, corn, cotton seed, rice and peanut). The chemical mechanism of protein film forming contain denaturation protein started by using chemical solvents, heat or a change in pH. The final step of this mechanism is forming intermolecular interaction when the mechanism is followed by using of groups of peptide chains. (Cordeiro de Azeredo, 2012). According to different research, the forming film protein showed good results in hydrophilic surfaces comparing to hydrophobic surfaces of meat. Moreover, it supports the diffusion of carbon dioxide and oxygen only without showing any support of water diffusion (Rodriguez-Turienzo et al., 2011; Min and Oh, 2009; Sánchez-Ortega et al., 2014).

Lipid-based edible films are not biopolymers and are not capable to form coherent films. Lipids can used for coatings or an included part to biopolymers to forming cohesive films, providing good water vapor barrier, because of their low polarity (Cordeiro de Azeredo, 2012). Films-forming lipid such as glycerol ester, resin and wax

are less widely applied in food industry due to their restrictive application. This is also because of rancidity and forming lipid oxidation which will change the sensory attributes of products during storage (Shinjie Lin, 2012).

Table 2.1. Examples of different edible sources use in forming films (Park et al., 2002b; Baldwin 2007; De Moura et al., 2009; Janjarasskul and Krochta 2010, Shinjie Lin 2012)

Protein	Polysaccharide	Lipid
• Gluten: wheat gluten • Collagen and gelatin • Corn zein • Vegetable source: peanut protein, rice protein, soy protein • Animal source: fish myofibrillar protein, milk protein (casein, whey), egg white protein	• Cellulose derivatives: hydroxypropyl methylcellulose, methyl cellulose • Starch: corn starch, amylose starch, tapioca, wheat • Chitosan • Pectin: high-methoxyl pectin, low-methoxyl pectin • Alginate • Carrageenan: α- carrageenan, ι-carrageenan, κ-carrageenan • Gums: Arabic gum, gellan gum, guar gum, karaya gum,	• Wax: rice brain wax, candelilla wax, beeswax • Resin: terpene resin, shellac resin • Glycerol ester: fatty acid ester, acetylated monoglyceride

2.1.1 Polysaccharide based edible film/coating

Polysaccharide consist of two types of subunits such as monosaccharide and disaccharide. The repeating polymer shape could be formed when the hydrogen bonds form monosaccharide and disaccharide attracted to other hydrophilic subunits. As a results, film forming polysaccharide have proper oxygen and oil barrier. However, the structure is disturbed with existence of moisture (Janjarasskul and Krochta 2010). The benefits to produce polysaccharide films in food industry is not need to use any toxic solvents, the sources are numerous, they are simple process and low in cost (Shinjie Lin, 2012). Polysaccharides and proteins are hydrophilic polymers that generally contain hydroxyl groups and some polyelectrolytes. Those kinds of ingredients also known as "hydrocolloids." Which are used widely in forming edible films solution because of their stabilizing impact on emulsions and increasing viscosity of the aqueous phase of edible films solution (Williams and Phillips 2000). There are some examples of polysaccharide can be used in forming edible films and coating.

2.1.1.1 Starch

Starch is generally applied in edible film-forming. Amylopectin and amylose are gives hydrogen bonding. Thus, film-forming starch is easy to dissolve in water and bound with other polar functional groups (Park et al., 2001; Bravin et al., 2006). The advantages of using starch in edible-forming are easy to prepare, not costly and have good barrier to oxygen and lipids but it has low water resistance.

Starch film-forming have been used to package bakery products and candies. As associate example of starch edible film application, wrapping the surface of candies with a thin layer of starch film to reduce unacceptable stickiness of the product (Tharanathan, 2003).

2.1.1.2 Cellulose derivatives

Cellulose is polysaccharides consist of ($C_6H_{10}O_5$) units. Water dissolution of highly crystalline cellulose cannot proceed without etherification. Dehydration of alcohol which known as etherification consider one of important steps because it enhances the separation between the hydrogen intramolecular force and the crystalline structure. Several cellulose derivatives have good barriers properties for reducing low oxygen transmission and oil uptake such as hydrocypropyl methylcellulose ($C_{56}H_{108}O_{30}$), carboxymethyl cellulose ($C_8H_{16}O_8$), and methyl cellulose ($C_{17}H_{32}O_{11}$) (Krochta et al., 1994).

2.1.1.3 Chitosan

Chitosan is linear polysaccharides derived from chitin which is extracted from crustaceans shell such as crawfishes, shrimps and crabs (by-products) by using hot water or alkali solutions. Chitosan has cationic characteristics. Thus, it is not soluble in water but it dissolves in acidic mediums such as formic, acetic, and citric acid. It was recognized for its biodegradable, antimicrobial, biomedical, biocompatible properties, and could be applied in food and health products (Jongrittiporn et al., 2001; Jayakumar et al., 2005; Jayakumar et al., 2006; Jayakumar et al., 2007). The antimicrobial properties of chitosan can eliminated the microorganisms by attracts the positive

charges of its amino group with negative charged polymers such as cholesterol, proteins and cell membrane of microorganisms. As a result, leaching of proteinaceous from the cell formed. Example on this, several studies done on chitosan has been success to inhibit Staphylococcus aureus, Listeria monocytogenes, Escherichia coli and Bacillus cereus (Coma et al., 2002; No et al., 2007). Thus, this antimicrobial activity can increase the chitosan applications in food industries. In previous researches, it extend of the shelf life of bread by reducing amount of starch retrogradation (Park et al., 2002a).

2.1.2 Lipid-based edible film/coating

Edible films from lipids are not common due to its limitations. Fatty acids have covalent bonds more than hydrogen bonds so they can't possess strong mechanical while they supply higher moisture barrier than hydrocolloid from edible film like, rice film. The materials of edible based lipid such as glycerol esters, resins and waxes have been rancidity and oxidized and this led to change which led to change the quality of selected products made with coatings or films. Also, these films can gives Also, nutritional concerns could change consumers acceptance of the products when a waxy flavor appeared on it due to the film. (Debeaufort and Voilley 2009; Janjarasskul and Krochta 2010).

2.1.3 Waxes

Waxes consists of two main parts which are alcohol and long chain fatty acids esters. Proficient water resistance when used in packaging materials. Normal waxes for example, candelilla, beeswax, carnauba waxes and rice brain are ordinarily utilized as edible coating materials, particularly on the vegetables surface and natural products. Also, some lipid films have indicated antimicrobial properties. Previous researchers determined that low convergence of ellagic acid (0.01%) blended with candelilla wax which extracted from Euphorbia antysyphilitica could decrease contamination of Colletotrichum gloesporioides on avocado, and lessening browning reaction during storage (Saucedo-Pompa et al., 2009).

2.1.4 Protein-based edible film/coating

The proteins used in edible films are produced from both animals and plants sources. The advantage of these films that they can used as an emulsifier between oil and water when it used as additives in food. Thusly, proteins and lipids could be combined together in forming films to increases barrier properties. In addition, polymer structures are balanced by functional groups of an amino acids which made a cross link between the chains. Also, the shelf life of protein-based edible films are longer compared to edible films from polysaccharide, since they are low sensitive to moisture (Barone and Schmidt 2006). Set up of protein based edible film done by dissolving extracted protein in selected solvent to utilize and modify it is properties such as plasticizer properties. In addition, protein types could influence some film properties such as crystallinity and hydrophobicity. Some of edible films protein listed below.

2.1.4.1 Collagen

The structure of collagen consists of proline, hydroxylysine, glycine, hydroxyproline, and other significant units of amino acids. Specifically, collagen considered as an animal protein and could be found in connective tissue of an animals such as intestine tract, skin, blood vessels, and tendons. It from edible film and had been used in sausage industry. Additionally, collagen explain the thermoplastic behavior by framing a fiber when it extends the hydrogen bonds in parallel structures (Janjarasskul and Krochta 2010). Shaping of meat into a tubular form utilized by collagen packaging. Finally, increasing of texture, juiciness, specificity and appearance all influenced by collagen edible films (Janjarasskul and Krochta 2010).

2.1.4.2 Gelatin

Gelatin is a denatured fibrous protein derived by different ways such as bones, animal skin and connective tissues. Formation of gelatin done by fractional hydrolysis of collagen (Morrison et al., 1999). Since the gelatin is a good hydrocolloid it had been used different fields of industry such cosmetic industry, food, photographic and pharmaceutical. Moreover, broad studies on gelation had been done last decades due to its ability to secure and cover food from light, oxidation by air and draying (Gómez-

Guillén et al., 2009). However, the film of gelation as other protein films can't resist the water vapor. So, gelatin could be modified by synthetic treatment to adjust and enhance the polymer network. The adjustment done by improving function of hydrocolloid film through cross-linking of the polymer chains (Rivero et al., 2009).

2.2 Methods of Producing Edible Films

There are several techniques of film-forming, including spraying/ brushing, casting and dipping.

2.2.1 Edible coatings

The appearance of edible films depend on its application. It can be transparent (clear) or colored films. Generally, the consumers are prefers clear coating products. Thin layers of edible film produced from sugar glazing, starch or waxes could made clear films. However, the starch can make off white coating by considering the thickness, the plasticizer used and type of polysaccharides (Chillo et al., 2008). The frequent method for coating applications in food industries is to direct the products into the edible solution and then solidify the coatings. The application of waxing vegetables and fruits used this technique and also in seafood processing. Spraying and brushing methods can apply different coating by a thin layer to the other side of a product. It tends to be applying consistently and it takes into consideration optional coatings. The benefit of these application is that the coating can totally cover products in all sides (Gaontard and Guilbert 1994).

2.2.2 Film formation

Dry and wet methods are two techniques utilized for forming edible film. Both these methods start by mixing the ingredients in a solvent until dissolved, and after that the drying remove the liquid phase (Peressini et al., 2003). Extrusion and compression are two models related to the dry methods and used to form an edible film. The dry method follows several processes and steps. The first one is to blend materials with a minimal moisture. Then, the temperature of this materials should be expanded in an extruder to pass the glass transition point (Tg). By this way, the mobility of materials increased due

to change from solid to morphology phases. Finally, the new mobile materials released from the extruder and cut into wanted shape (Peressini et al., 2003). Different wet methods are used to form edible film such as compression molding, draw down bar, and mold casting.

2.3 Function and Application of Edible Packaging

Edible films and coatings give protection, convenience, and functions as additive without effecting the ingredients of food products and their processing technique. Attaching totally to the product or turning into a part of food are two ways to process the edible film. Thus, edible packaging able to protect the product from microbial contamination, moisture loss, delayed respiration, improved appearance, fortified nutritional value, rate and aging, and mechanical properties (Janjarasskul and Krochta, 2010).

According to the properties of the polymeric matrix, which depends that the edible film can be utilized to combine flavors and spices for improving the organoleptic properties of the product. In addition, natural antioxidant and antimicrobial agents were integrated into edible films from polysaccharide (Park and Zhao, 2004). Edible films mixed with different additives, light absorbers or pigments are also applied to improving the appearance and the shelf life of different foods. Secondly, edible packaging control the texture of selected products by developing its mechanical strength. Thirdly, edible packaging can be used to apart food into individual portions (Janjarasskul and Krochta, 2010).

2.4 Citrus Seeds Composition

These days the researchers determined that citrus by-products are a source of bioactive compounds such as peels are high on secondary metabolites like, essential oil (Sahraoui et al., 2011) and phenolic compounds (Li et al., 2006; Ortuno et al., 1995; Khan et al., 2010; He et al., 2011). Additionally, previous researchers reported that citrus pomace contain natural antioxidants such as phenolic acids and flavonoids (Kim et al., 2008; Hayat et al., 2010). However, few studies are known about the bioactive potential of the seeds. Yusof et al. (1990) studied the content of naringin in a variety of Mexican citrus

have determined this flavanone in the seeds of Rough lime. Sun et al. (2010) investigated flavonoids composition of the different parts of the Chinese mandarin fruit and identified naringin, hesperidin, didymin, tangeretin and nobiletin in the seeds. Moulehi et al., 2012 determined the flavonoids content of citrus seeds ranged from 1.31to 2.52 mg CE/g DW. Also, they mentioned different phenolic compounds in citrus seeds such as flavonoids, benzoic acids, hydroxycinnamic acids and naringin.

The composition of citrus seeds contain 3.1 g/100g of crude protein and 5.50 g/100g of crude fiber. Also, the crude fats in citrus seeds is 52.0g/100g, while total ash is 2.5g/100g (Akpata and Akubor, 1999).

2.5 Utilization of Citrus Seeds

As previous researchers determined that Citrus juice contain high of vitamin C, which is main product of citrus processing industries and it's generally utilized for producing beverages rich in nutrients. However, it has only 50% yield of pure juice from its whole weight and rest is recognized as residues (pulp peel, and seeds) which contain 80% of moisture content (Garcia-Castello et al., 2011; Rezzadori, Benedetti, and Amante, 2012). The average citrus waste in the world is about 119.7 million tons per year (Anonymous, 2011). This waste could pollute environment when it dumped into lands, discharge to nearby rivers or burned. Moreover, this pollution could lower level of oxygen in the environment, and this happen frequently in contaminated water. (Wadhwa and Bakshi, 2013). This technique of waste management considered insufficient due to pollution of water and soils (Braddock, 1995; Martín, Siles, Chica, and Martín, 2010). On the other hand, the best way of management achieved by using some alternatives. This could be done by different ways likes feed animals with fortified nutrient, depends on organic fertilizers, try to extract all nutrients (micro and macro) from the by-product, produce ethanol and use of bio-oils, and get all benefits from rich components in confectionary products. All of these alternatives can lower the level of pollution in the environment and may add a profit to the industry.

The production amount of citrus fruits worldly is about 115 million tons in the 2010–11 season (FAO, 2013). The juice production of citrus fruits is 34% (Anonymous, 1996). Locally, the production of citrus fruits in Turkey is very critical for domestic

consumption and exporting to other countries. The citrus production in Turkey was 3.7 million metric Tons, including orange, lemon, mandarin, and grapefruit in marketing year 2010/11 (USDA, 2017). Turkey is the eighth ranked country in the world for citrus production. Adana province is famous in citrus production especially orange, mandarin and lemon (USDA, 2017). According to citrus juices manufacturing, the byproduct has been formed in huge amounts. The main residues are seeds and peels. The seeds contain essential attributes. For instance, Seeds have good amount of fatty acids, anti-carcinogenic/chemopreventive activities and can used to repair limonoids. Seeds like citrus fruit contain triterpenoid, giving strongly bitter taste (Braddock, 1995).

Phenolic compounds are found in many food types such as fruits, vegetables, and coffee (Clifford 1999). In addition, the effectiveness of extracting phenolic compounds as a natural antioxidants from plant sources has been tested on seafood products. For example, rosemary extracts and onion juice applied on sardine (Serdaroglu and Felekoglu, 2005), tea extracts used as antioxidant on pacific white shrimp (Nirmal and Benjakul, 2011). However, the effective of phenolic extracts is vary from plant species to another. There are some factors effect on antioxidant activities and phenolic content such as genotype or variety, growing season, temperature, climate, growing environment, light, soil type, postharvest storage and processing (Maqsood er al., 2014). The citrus seeds are promising source of phenolic compound such as flavonoids which are presents into two classes: the glycosylated flavanones and the polymethoxylated flavones. The health properties of citrus flavonoids have been investigated. Some of these properties are antiviral, anticancer, and anti-inflammatory activities, also they can effects on capillary fragility (Huet, 1982; Benavente- Garcia et al., 1997).

Recently, a lot of studies focused on antioxidant activities from different types of natural resources such as citrus fruits like lemon, sweet orange and grapefruit showed positive results. The antioxidant was utilized directly or extracted from the fruits (Williams and Harris, 1983; Piskur and Higgins, 1949); Pereira and Mancini- Filho, 1994; Sawamura et al., 1988; Kroyer, 1986; Ting and Newhall, 1965). In addition, production of films form from natural products with different types of biopolymers instead of petroleum derived bio-stable plastic due to possible damages to future mankind by petroleum derived bio-stable plastic (Guillet, 2012), (Dang et al, 2006). Consequently, this shift reduced the health and ecological problems of petroleum

derived bio-stable plastic. Production of films form with better physicochemical features agents likes alginate, cellulose, alginate, and chitosan could be better alternative than synthetic plastics. Moreover, production of edible films form could be done by using vegetables or animals' origin matrixes. Produced film are highly recommended for different types of applications like drug delivery, medical sciences, and food. Protein films prepared form fish gelation with antioxidant considered as example to extend the fish self-life during storage. The aim of my thesis is to improve the quality of seabass fillets with gelatin based edible films supplemented with citrus seeds extract.

CHAPTER III

LITERATURE REVIEW

3.1 Edible Films and Coating as Preservation Methods

Mohan et al. (2012). Examined the effects of edible coating of chitosan on the quality changes of Indian oil sardine (*Sardinella longiceps*) during frozen storage for 11 days. In this study, the chitosan coating was efficient on inhibition the bacterial growth and it reduced the growth of volatile bases and oxidation products. Also, it increased the water holding capacity, textural properties and drip loss significantly comparing to untreated sample. In addition, the taste quality remained good until 8 and 10 days for 1% and 2% chitosan treated samples respectively, while the untreated sample has been rejected from 6th day.

Rodriguez-Turienzo et al., (2011). They studied the impacts of ultrasound-treated whey protein coatings on frozen Atlantic salmon quality parameters. The trial was processed by utilizing an ultrasonic bath at a frequency of 35 kHz with three sonication times (1, 15 and 60 min). The results got in this study demonstrate whey protein coatings by using ultrasound can be valuable for the decreases the lipid oxidation of frozen salmon. These selected coatings could be another new sources in contrast to plastic packaging. The edible coatings can add to decrease the plastic packaging restrictions in frozen fish industry and protect the environment from contamination.

Jeon et al., (2002). In this study, the impact of chitosan with various molecular weights as coatings for shelf-life extension of fresh fillets of Atlantic cod (*Gadus morhua*) and herring (*Clupea harengus*) was assessed for 12 days of storage at refrigeration condition (4 ± 1 °C). Chitosan coating significant ($p<0.05$) decreased lipid oxidation in headspace volatiles, conjugated dienes, peroxide value, TBARS and chemical spoilage and also indicated in trimethylamine, and hypoxanthine, total volatile basic nitrogen, and growth of microorganisms as indicated in total plate count in both fish model systems contrasted with control samples. There was relation between chitosan viscosity and preservative efficacy; the efficacy of chitosan with viscosities of 57 and 360 cP was

resulted better than chitosan with a 14 cP viscosity. In this way, chitosan as edible coating could preserve seafood quality during storage.

Nowzari et al., (2013). The effect of combination of chitosan and gelatin in forming coating and film on developing the rancidity in rainbow trout (*Oncorhynchus mykiss*) fillets stored in refrigeration condition at 4 °C for 16 days was determined. Composite and bilayer coated and film covered fish samples were investigated occasionally for chemical (FFA, POV, TBARS, TVB-N) and microbiological (psychrotrophic count and total viable count) properties. The results showed that using chitosan in gelatin coating and film held their great quality properties and the shelf life of fish samples during storage was extended. The coating was more efficient to the film in diminishing lipid oxidation of fillets, while in control of contaminated bacterial was no significant difference between them.

Günlü and Koyun, (2013). They evaluated the ability of using chitosan films and vacuum packaging to improve the shelf life of fresh sea bass (*Dicentrarchus labrax*) fillets in refrigerated storage (4°C). The results observed that the control and vacuum-packaged groups were indicated as spoiled in 5 days, while the wrapped samples chitosan film and vacuum- packaged finished at 25 to 30 days. In this manner, the shelf life of sea bass fillets treated with chitosan films was extended around 20 days.

Rezaei, and Shahbazi, (2018). They applied three methods of edible packaging, (edible coating, direct addition, and composite film from sodium alginate carboxymethylcellulose (SA-CMC)) incorporated with different concentrations of apple peel extract (APE), zinc oxide nanoparticle (ZnO) and *Ziziphora clinopodioides* (ZEO) essential oil to improve the shelf life of sauced silver carp fillet (chemical, microbial, and sensory characteristics) and prevent the growth of *Listeria monocytogenes* at refrigerated storage for 15 days. All treated samples were delayed the increases in TVB-N, TMA-N, pH and PV. It can be observed that the SA-CMC coatings or films incorporated with APE 1% + ZnO 0.5%+ ZEO 0.5% and APE 1% + ZEO 0.5% can be applied as new alternative packaging materials to preserving sauced silver carp fillets.

3.2 Application of Edible Films/Coating Supplemented With Natural Products as Preservation of Seafood Quality

Volpe et al., (2015). This study determined that both carrageenan coating and carrageenan coating enriched with essential lemon oil (ELO) had good antimicrobial activity and could delayed the lipid oxidation of fresh rainbow trout (*Oncorhynchusmykiss*) fillets during refrigerated storage (4 ºC) for a period of two weeks. Fillets samples were tested for microbiological (Enterobacteriacea counts, H2S-producing bacteria, lactic acid bacteria, total viable count), biochemical (fatty acids content) and chemical (moisture, TVB-N, pH) properties. This investigation exhibits the viability of an edible active carrageenan coating in preserving fresh trout fillets from microbial growth and lipid oxidation. In conclusion of this study, the researchers mentioned that this study giving new alternative source of packaging which will attract the stakeholders if production and processing industries, due to the simple manufacturing methodology and the direct efficiency of ELO to extend the shelf life of trout fillets.

Kakaei, and Shahbazi, (2016). They evaluated the impacts of chitosan-gelatin film incorporated with different concentrations of grape seed extract (GSE) and *Ziziphora clinopodioides* essential oil (ZEO), the control and wrapped fillet samples were investigated for chemical, microbial and sensory properties. The most effective on bacterial growth, TVB-N and PV content were observed in fish samples treated in film enriched with 2% of (GSE + ZEO). The best organoleptic properties were observed in fillets enriched with GSE1% + ZEO2% and GSE2% + ZEO2%. In light of their discoveries, the antibacterial movement of chitosan-gelatin film incorporated with ZEO and GSE increased the shelf life of fish fillet.

Anvari and Rezaei, (2011). They applied gelatin coatings enriched with Cinnamon essential oil for maintaining the rainbow trout quality stored at refrigerated condition for 20 days. The samples were investigated for microbial (psychrotrophic count and aerobic plate count), chemical (TVB-N, TBA, FFA) and sensory properties. The obtained outcomes demonstrate that gelatin coating incorporated with cinnamon oil can be new alternative sources of packaging to preserve the quality of rainbow trout fillets and extended the shelf life of seafood during storage.

Albertos et al., (2017). They investigated the quality of cold smoked salmon wrapped with olive leaf as edible films. The antioxidant and antimicrobial ingredients of Olive leaf powder (OLP) and its extracts (water-ethanol extraction) (OLE) were determined against *Salmonella enterica, Escherichia coli* O157:H7, and *Listeria monocytogenes*. The antibacterial activity against L. monocytogenes could be indicated by OLP and OLE when agar diffusion tests were used. While, *S. enterica and E. coli* bacteria both did not show any effect by agar test. Increasing the concentration of OLE raised the antioxidant and antimicrobial activities of the films. On the other hand, the lightness of the film did not affect by OLE. Finally, the results showed a drop of pathogen growth on salmon fish while during storage when films were used.

Fadiloglu and Coban, (2018). Evaluate the quality of rainbow trout (stored at 4°C in refrigerator for 12 days) when it combined with the chitosan which coated when it combined with sumac. In his study he focused and evaluated the effects. In more details, the physicochemical (thiobarbituic acid [TBA], total volatile basic nitrogen [TVB-N], peroxide value [PV], microbiological (total viable counts [TVCs], and psychotropic bacteria, sensory analyzes and pH). The results indicated that the rainbow trout fillets' self-life increased by 6 days when sumac used comparing to control samples and it considered as natural preservative.

Teixeira et al. (2014). Prepared the films from proteins and supplemented by-products such as cape hake with tree essential oils (clove, garlic, and origanum). After preparation, he analyzed some properties such as antioxidant, antibacterial, physical and mechanical of prepared films. The results showed that the prepared films decreased in thickness, breaking force, elongation, and water solubility while it showed expand of free radical scavenging activity. Comparing to control film, the clove films had higher antibacterial activity against *Shewanella putrefaciens* and lower water vapor permeability. On the other hand, garlic films had the highest antioxidant activity and had the most yellowish color. Finally, the origanum films had almost the same properties of control films. To conclude, the prepared films from by-products protein such as cape hake with tree essential oils had sufficient properties with applicability in new preservation food packaging systems.

Jouki et al. (2014). Evaluated the shelf life films of rainbow trout when he prepared the films from quince seed mucilage film (QSMF) which include oregano (O) or thyme (T) oil and stored the films at (4 °C) for 18 days in the refrigerated storage. Jouki et al, (2014) focused on changing concentration of essential oils during films preparation so he used for different concentrations. Sample analysis done periodically for different variables like chemical (TVB-N, TMA-N, TBA), microbiological (aerobic and psychrotrophic count, Pseudomonas spp., lactic acid bacteria, H2S-producing bacteria, and Enterobacteriaceae), and sensory characteristics. The results showed that, for trout fillets stored in air the bacteria showed the highest and most quickly grown. The trout warped with QSMF had intermediate grown and the lowest grown for warped with QSMF + 2%T. in contact, the QSMF + 2%T had the highest antioxidant activities, and this could be due to composition of oregano essential oil. Finally, QSMF extended the microbial shelf life of rainbow trout fillets by 2 days, whereas the QSMF + 1%O, QSMF + 1.5%O, QSMF + 2%O, QSMF + 1%T, QSMF + 1.5%T and QSMF + 2%T resulted in a significant shelf life extension of the trout fillets by 3, 5, 9, 6, 10 and 11 days, respectively, as compared to the control samples.

Alsaggaf et al. (2017). In this study, they applied natural derivatives as new sources of preserving food. They applied fungal chitosan coating (ch) extracted from *aspergillus nigar* enriched with different concentrations of pomegranate peel extract (PPE) during refrigerated storage (4ºC) ror 1 month to maintain the quality of Nile tilapia (*Oreochromis niloticus*) fillets the fillets were investigated for microbial, chemical and sensory characteristics. The results observed sharp reduction in microbial growth during storage. Also, the treated fillets were retard the increases of lipid oxidation in TVB-N, PV and TBARS during storage. The increased concentrations from PPE enhance antimicrobial activity of coating film. The sensory panelists highly preferred for the sensory attributes of treated samples compared with control. PPE+ Ch could be recommended for extending the shelf life of seafood.

3.3 Application of Citrus Products With Edible Films/Coating on Seafood

Uranga et al. (2018). In this study, they prepared composite films of fish gelatin/chitosan enriched with citric acid. Citric acid could reduce the swelling values and swelling rate of films when it is added in film forming, which maintained their

integrity and resulted in flexible hydrated films. Additionally, citric acid acted as plasticizer, raising the elongation at break of the films. Moreover, films showed good UV barrier properties and combined citric acid and chitosan decreased the microbial growth (*E. coli*), particularly for 20% citric acid films, and these films can be new alternative as active food packaging. Also, films were determined by Fourier transform infrared (FTIR) analysis, which observed the different protein structure obtained as a function of citric acid and chitosan contents.

Alparslan et al. (2016). The effect of gelatin coating incorporated with orange leaves essential oil to maintain the quality of deep water pink shrimp stored at refrigerator was investigated. Microstructure characterization of the gelatin films was tested by Scanning Electron Microscopy. Melanosis and Sensory, microbiological [Enterobacteriaceae (EB), psychrotrophic bacteria counts (PBC), total viable counts (TVC)], chemical [sulphur dioxide (SO2), pH, TVB-N, TMA-N, TBA, PV] and color analysis were investigated for control and treated samples during 14 days of storage. The results observed that the addition of orange leaves essential oil to gelatin film could be efficient in extending the shelf life of shrimps and retard the chemical and microbial spoilage during storage.

Zarei et al. (2015). The coating efficacy of orange and pomegranate peel extract combined with chitosan nanoparticles (Nch) on the quality of silver carp (*Hypophthalmicthys molitrix*) fillets during refrigerated storage at 4◦C were investigated. Solutions of orange and pomegranate peel extracts (1%, w/v) was utilized for dip pretreatment, and Nano-chitosan solution (2%, w/v) was used for the coating. The control and the coated fish samples were analyzed periodically for microbiologic (total mesophilic and psychrotrophic count), physicochemical (pH, total volatile basic nitrogen, thiobarbituric acid reactive substances) and sensory attributes. The results showed that Nano-chitosan coating was effective for the preservation of silver carp fillets during refrigerated storage. While, a dip pretreatment in orange or pomegranate peel extract combined with Nano-chitosan coating were significantly lower TMC and TPC than the uncoated control and inhibited the development of lipid oxidation in fish samples.

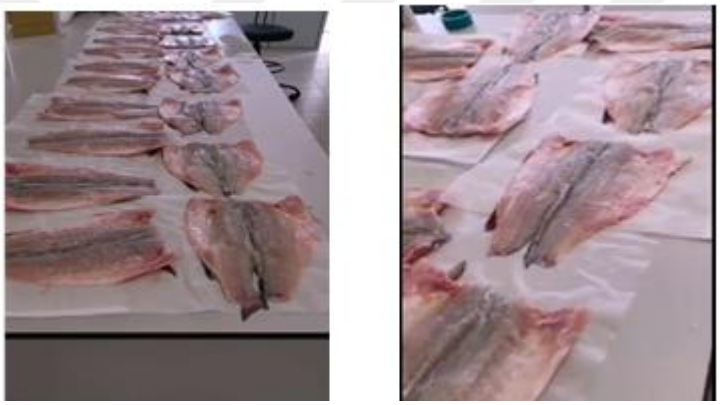
Ahmad et al. (2012). They investigated the quality changes of sea bass slices wrapped with gelatin film enriched with 25% (w/w) lemongrass essential oil (LEO) during 12 days and stored at 4 °C for 12 days. Fillets wrapped with gelatin film enriched with Lemongrass essential oil (LEO) delayed the microbial growth (lactic acid bacteria (LAB), Enterobacteriaceae, psychrophilic bacteria, and spoilage microorganisms) during storage. Also, the treated samples reduced the increases of chemical analysis TVB-N, TBARS, K value, and color. Thus, the antimicrobial and antioxidant properties of the film enhanced by adding LEO into gelatin film and maintained the quality of the sea bass slices stored at refrigerated conditions.

CHAPTER IV

MATERIALS AND METHODS

4.1 Materials

As a research material, seabass fillets, weighing 250 ± 10 g, were brought from the Niğde fish market to the laboratory within 30 minutes with ice in Styrofoam boxes. The citrus seeds used in the study was brought the fruits (orange and lemon) from Niğde market then squeezed the fruits and collected the seeds. After that, the seeds dried at 45 ^{o}C for 48 h under suitable conditions and stored at -80^{o}C until extraction.



(a) (b)

Photo 4.1. Seabass fillets preparation (a, b)

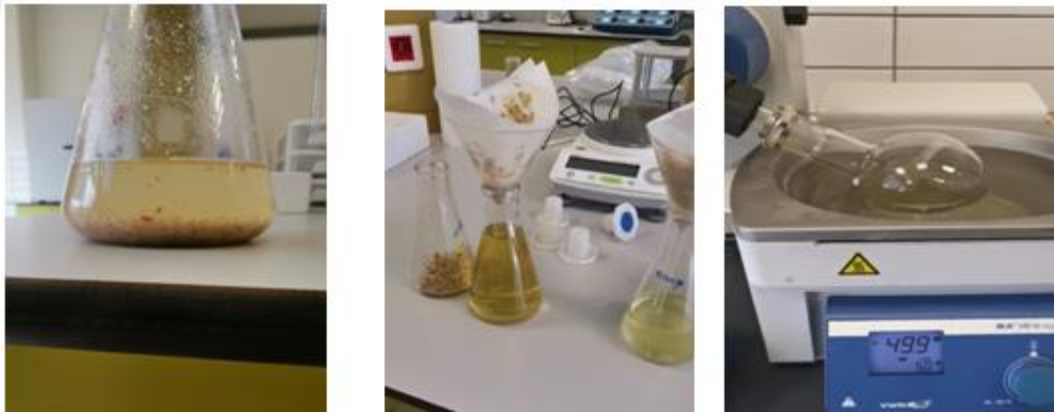
4.2 Methods

4.2.1 Extraction of citrus seeds

Citrus seeds (CS) was dried at 50 ^{o}C for 48 h and was grounded into powder with a blender. For extraction, 10 g of CS powder dissolved in 100 mL of 70% ethanol then stirred by ultrasonic water bath for 30 minutes and stored 24 hours at 4 °C. After extraction procedure, the extracts were filtered by using filter paper and evaporated by using rotary evaporator (IKA, HB-10 digital, Germany) at 45 °C under vacuum.

(a) (b) (c)

Photo 4.2. Squeezing fresh fruits (orange and lemon) (a) collecting seeds (b) citrus seeds powder(c)



(a) (b) (c)

Photo 4.3. Citrus seeds solution after stored 24 hrs at 4°C (a) The process of filtering the citrus seeds extract by filter paper (b) evaporating the ethanol from citrus extracts(c)

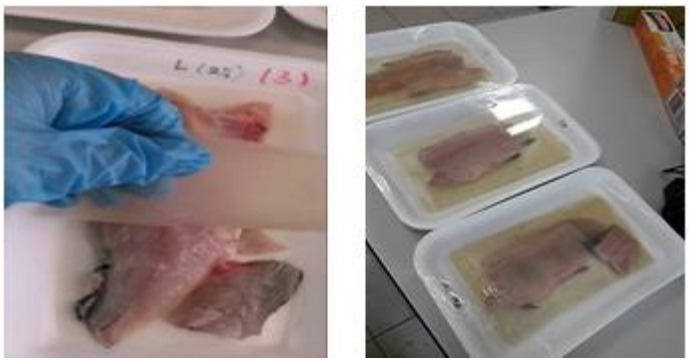
4.2.2 Preparation of gelatin films and application to fish fillets

Edible films were prepared according to (Gomez-Estaca et al., 2009) method. 8 g of fish gelatin was dissolved in 100 mL of distilled water for approximately 15 minutes at room temperature. Then, glycerol (0.1 mL per gram of gelatin) and D-sorbitol (0.15 g per gram of gelatin) were added to the gelatin solution and mixed for a further 15 minutes at 45^{o}C. 2% of prepared citrus extracts were added to the film solution (calculated on 8 grams of gelatin). One group was covered with gelatin film only, without adding the extract. Film solutions were homogenized for 1 minute by Homogenizator. Then, 40 mL of film solutions were poured into foam plates, and film coatings were obtained at 50% humidity at room temperature for 48 hours. Coating

method was applied according to the (Ahmad et al., 2012) method. The dried films were removed from the foam plates and sterilized by passing them under UV light on both sides. Samples were divided into four groups entitled as; fillets wrapping with gelatin film (CF), fillets wrapping with gelatin film with 2% of citrus seeds extract (orange O2, lemon L2) and fillets without coating (control, C). To cover the fillets, a piece of film was placed on a sterile foam plate, the seabass fillet was placed on the film then another sterile film was covered the other side of the fillet. Thus, both sides of each fillet are covered. The control group was not covered with any gelatin film. Then each group is covered with stretch film and stored in refrigerator at 4±1°C for 15 days.

(a) (b) (c) (d)

Photo 4.4. Preparation of edible gelatin films with citrus seeds (a, b, c, d)



(a) (b)

Photo 4.5. Trout fillets covered with gelatin film (a, b)

4.2.3 Determination of antioxidant activity in citrus seeds

A 7 mM ABTS solution containing 2.45 mM potassium persulfate was prepared and the radical solution (ABTS + •) was formed by storing 12-16 hours at room temperature and in the dark. In order to determine the antioxidant activity of the citrus seeds extract as a

trolox response, a series of extract concentrations and trolox were prepared. 10 µl of sample was added on 1 mL ABTS + and a decrease in absorbance was observed for 6 minutes. The slope was calculated from the graphs where the percent inhibition was plotted against the concentrations. The antioxidant activity determined by the ratio of the slope of propolis extract to the slope of trolox concentrations showed as 1 mM trolox response was determined. (Re et al., 1999). For each concentration, readings will be carried out in parallel and all spectrophotometric readings were carried out at 30°C using microwaves.

Sample slope / slope of trolox) x dilution factor = TEAC value µM trolox (4.1)

TEAC (Trolox Equivalent Antioxidant Capacity)

4.2.4 Determination of total phenolic compound in citrus seeds

In the analysis carried out using Folin Ciocalteau reagent, 900 µL of pure water, 5 mL of 0.2 N Folin-Ciocalteu reagent and 4 mL of saturated sodium carbonate solution (7.5 g / L) were added to 100 µL of the solution diluted from the extract. The mixture spectrophotometer, which was kept at room temperature and in the dark for 2 hours, was read against the corner at 765 nm. The results to be determined with the help of the previously determined gallic acid ribbon were evaluated as mg gallic acid/g (Spanos and Wrolstad, 1990).

4.2.5 pH measurement

In pH measurements, homogenized samples were mixed with distilled water in a 1: 1 ratio and measurements were made by immersing the pH-meter probe (Manthey et al., 1988). Care has been taken to carry out the measurements at the same temperature.

4.2.6 Peroxide analysis

Peroxide analysis in seabass oil samples was carried out according to the method specified by (AOAC, 1990). Approximately 2 g of oil in 30 mL of chloroform-glacelic acetic acid solution (3 chloroform: 2 glaciel acetic acid) was added with 1 mL of

saturated potassium iodide (KI) solution. After mixing, the solution will be kept in the dark for 5 minutes and 75 mL of distilled water and a few drops of starch solution are added and titrated with 0.1 M sodium thiosylphate ($Na_2S_2O_3$) solution. The peroxide values of the samples were calculated according to the formula below and expressed in

meq / kg. PV (meq/ kg) = K x (V-V_0) x 12.69 x 78.8 / w (4.2)

K to be spent on titration $Na_2S_2O_3$' starch concentration (mol/lt),

V spent in titration $Na_2S_2O_3$' amount of starch (mL),

w weight of fish oil (g)

4.2.7 Determination of thiobarbituric acid reactive substances (TBARS)

Spectrophotometric measurements were made based on the principle that the malondialdehyde in the fish oil samples color with the TBA reagent (AOCS, 1998). 5 mL of fish oil dissolved in n-butanol was taken and mixed with the same amount of TBA reagent. It was kept in a 95°C Water bath for 120 minutes to react. Rapidly cooled samples were measured spectrophotometrically at a wavelength of 530 nm and the results calculated by the formula given below were expressed as mg malondialdehyde / kg sample.

TBA = 50 x (The absorbance of lipid- The absorbance of blank) /

sample weight (mg) (4.3)

4.2.8 Microbiological analysis

Total aerobic mesophilic bacterial count

Total aerobic mesophilic bacterial count was calculated using the petri surface spreading method (ICMSF, 1982). It took 10 g of fish from each sample. These samples were homogenized for 2 minutes in a stomacher device by adding 90 mL of Ringer's solution. Then, by making decimal dilutions, 0.1 mL of each dilution was taken and spread to the surface of the petri dish containing PCA (Plate Count Agar). Petri dishes were taken into the incubator and incubated at 30ºC for 2 days. Then, TVC was calculated by looking at the colonies formed in petri dishes. Bacterial colonies in the

petri dish containing dilutions seen between 30 and 300 colonies were processed. The colony forming units (CFU/g) were calculated using the formula below.

$$Number\ of\ Colony\ Forming\ Units\ (CFU/g)\ = \frac{\text{colony number x Dilution factor}}{\text{Inoculation amount 2a}} \qquad (4.4)$$

Total psychrophilic bacteria count

Dilutions prepared for total psychrophilic bacteria count will be added to the Plate Count Agar (PCA) medium by smear culture method and left for 7 days of incubation at 8-10 °C (Anonymous, 1998). 10 g of fish meat were taken from each sample and this sample was mixed with 90 mL of Ringer's solution and homogenized in stomacher for 1 minute. Then dilutions were made with 9 mL Ringer's solution, and serial dilutions were prepared. Plate count agar (PCA) medium was used in microbiological analysis. PCA medium is a common medium and is widely used for total bacteria count. The total number of psychrophilic bacteria was determined by using the petri surface spreading method (ICMSF, 1982). Petri dishes were incubated at 10 ºC for 7 days.

Total yeast and mold count

To determine the number of yeast and mold, it was applied on Potato Dextrose Agar (PDA) medium, and added citric acid to adjusted pH to 3.5. It was calculated using the Petri surface spreading method (ICMSF, 1982). 10 g of fish were weighed from each sample. These samples were homogenized for 2 minutes in a stomacher device by adding 90 mL of Ringer's solution. Then, by making decimal dilutions, 0.1 mL of each dilution was taken and spread 2 parallel to the surface of the petri dish with Potato Dextrose Agar (PDA). The petri dishes were placed in the incubator and incubated for 5 days at 25 ± 1ºC.

Total Enterobacteriaceae count

The Enterobacteriaceae count in the samples was planted on Violet Red Bile Agar (VRBA) medium by using the Petri surface spreading method (ICMSF, 1982) and incubated at 37 ºC for 24-48 hours (Anonymous, 1998). 10 g of fish were weighed from

each sample. These samples were homogenized for 2 minutes in a stomacher device by adding 90 mL of Ringer's solution. Then, by making decimal dilutions, 0.1 mL of each dilution was taken and spread 2 parallel to the surface of the petri dish with Violet Red Bile Agar (VRBA). Petri dishes were taken into the incubator and incubated at 37 ºC at 24-48. Bacterial colonies in the petri dish containing dilutions seen between 30 and 300 colonies were processed.

4.2.9 Sensory evaluation

Fish fillets stored in the refrigerator were evaluated in terms of smell (fishy smell, bitter smell), texture (tight structure, elasticity, and water drop), color, appearance (color, gloss) and overall acceptance. 10 panelists with a fish consumption habit were included in every sensory analysis assessment performed. Before the panel, panelists was not been ınformed about the study, but explanations had been made about the criteria they will evaluate. Each sample was coded in three-digit numbers and presented to the panelists at random at room temperature, uncooked. Sensory analysis was performed using a 9-point hedonic scale. A score of 9-7 indicated ‘‘very good’’, a score of 6.9–4.0 ‘‘good’’, a score of 3.9-1.0 will be denoted as spoiled. (Table 4.1). The averages of the scores given by the panelists were taken and the total sensory quality was evaluated by summing the average scores of each characteristic (Amerina et al., 1975).

Table 4.1. Sensory evaluation form

Sample Code	Smell	Color	Texture	Apperance	Overall acceptane

Using a 9 points scale, the grades’ range of sensory attributes were: (9) like extremely, (8) like very much, (7) like moderately, (6) like lightly, (5) neither like nor dislike, (4) dislike lightly, (3) dislike moderately, (2) dislike very much, (1) dislike extremely,

4.2.10 Statistical analysis

All analysis were performed as triplicate. Statistical analysis were performed with SPSS software (Statistical Analysis System, Cary, NC, USA), and different applications were subjected to multiple comparison tests.

CHAPTER V

RESULTS AND DISCUSSIONS

5.1 Total Phenolic Content and Antioxidant Activity of Citrus Seeds

Phenolic compounds have been recognized for their nutritional and functional advantages, such as antioxidant and antimicrobial effects. The citrus seeds are alternative source of phenolic compound such as flavonoids which are presents into two classes: the glycosylated flavanones and the polymethoxylated flavones. The main phenolic compounds found in citrus seeds are flavonoids, benzoic acids, hydroxycinnamic acids, naringin, hesperidin, didymin, tangeretin and nobiletin. In this study, the antioxidant activity value of citrus seeds was found as 183.63 μmol trolox/g for lemon and 41.35 μmol trolox/g for orange, while the total phenolic substance content was 343.7 mg GAE / g for orange and 332.28 mg GAE/g for lemon. Malacrida et al. (2012) determined the phenolic content of orange seed oil and lemon seed oil were 1.15 mg GAE/g and 1.20 mg GAE/g, respectively. The citrus seed oil were extracted by lipid extraction using petroleum ether. The phenolic content vary depend on extraction method. Sultana et al., (2015) found the phenolic content of lemon seed extracted by using methanol was 98.23 mg GAE/g. Yerlikaya et al. (2015) reported the phenolic content of bitter orange peel extracted by 25% of ethanol was 0.51 mg GAE/100g and the antioxidant was 0.371 μM TEAC. Xi et al. (2017) found the phenolic content and antioxidant of lemon seed extracted by using 80 % methanol and dimethyl sulphoxide were 3.36 μg GAE/g and 11.97 μM/g TEAC.

5.2 pH Value

The difference in pH values of seabass fillets wrapped with gelatin films enriched with 2% concentration of orange seed and lemon seed extract are shown in Table 5.2.

Table 5.2. pH changes of seabass fillets wrapped with gelatin film incorporated with 2% of citrus seeds extracts (lemon (L2) and orange(O2)) compared with control samples and gelatin film without extracts

Storage (Day)	C	CF	O2	L2
0	6.43 ± 0.08^{Ae}	6.43 ± 0.08^{Ae}	6.43 ± 0.08^{Ac}	6.43 ± 0.08^{Ac}
3	6.48 ± 0.01^{Ae}	6.38 ± 0.01^{Be}	6.36 ± 0.01^{Cc}	6.33 ± 0.01^{Dd}
6	6.78 ± 0.02^{Ad}	6.67 ± 0.01^{Bd}	6.63 ± 0.02^{Bb}	6.37 ± 0.03^{Ccd}
9	7.25 ± 0.05^{Ac}	7.11 ± 0.01^{Bc}	6.72 ± 0.07^{Cab}	6.46 ± 0.02^{Dbc}
12	7.51 ± 0.02^{Ab}	7.30 ± 0.01^{Bb}	6.81 ± 0.01^{Ca}	6.54 ± 0.06^{Dab}
15	7.80 ± 0.05^{Aa}	7.49 ± 0.01^{Ba}	6.87 ± 0.09^{Ca}	6.61 ± 0.02^{Da}

Means indicated by different capital letters in the same row differ significantly ($P < 0.05$). Means indicated by different lowercase letters in the same column differ significantly ($P < 0.05$). C: control samples, CF film: samples wrapped with gelatin film, O2 film: samples wrapped with gelatin film incorporated with 2% of OSE, L2 film: samples wrapped with gelatin film incorporated with 2% LSE.

At the beginning of storage, the pH value of seabass fillets was 6.43 and raised in all groups until the end of storage time. According to previous studies (Li et al., 2012; Zarei et al., 2015), the increasing in pH values can be result of accumulation in volatile bases (ammonia, TMA) caused by either endogenous or microbial enzymes (Manat et al. 2005). There are significant differences ($P<0.05$) were noted between groups C, CF and groups wrapped with gelatin films prepared with citrus seeds extract. At day 15, the highest pH value was 7.80 and 7.49 in C and CF groups, respectively, the lowest pH value was found as 6.61 in L2 group. At the end of the storage, the pH values of L2 and O2 were still in acceptable limit of fresh fish according to Ludorf and Mayer (1973) reported the acceptable limit of pH value for fresh fish is between 6.8 and 7.0. While the value of C and CF have been exceeded the limit of fresh fish after 6^{th} day of storage.

Fadiloglu and Coban (2018) illustrated pH of rainbow trout fillets treated with chitosan coating as 7.07 in the beginning of storage and finally raised to 7.77 after 12 days. Ucak (2019) determined the initial pH of the rainbow trout fillets was 6.35 and pH of all samples were significantly raised during the storage period ($P<0.05$), whereas the lowest pH value was observed in trout fillets wrapped with gelatin film incorporated with 8% of garlic peel extract. Chaparro-Hernandez et al. (2009) reported that the decomposition of nitrogenous compounds in meat also caused an increase in pH. Yerlikaya et al. (2015) reported that the increases in pH values occurs due to increases in alkaline compounds, like ammonia, which formed from the microbial action during

fish spoilage. They determined the increases in pH values was significant ($p<0.01$) in all groups. While the pH value of bitter orange alfavedo was the lowest among all groups. In the present study, the lowest increase in the pH value of seabass fillets wrapped with gelatin film enriched with 2% LSE was noticed compared with unwrapped groups.

5.3 Peroxide Value (PV)

The differences in peroxide values (PV) of seabass fillets wrapped with gelatin films enriched with 2% concentration of orange seed and lemon seeds extract are shown in Table 5.3.

Table 5.3. Peroxide value (PV) changes of seabass wrapped with gelatin film enriched with 2% of citrus seeds extracts (lemon (LSE) and orange (OSE)) compared with control samples and gelatin film without extracts (meq/kg)

Storage (Day)	C	CF	O2	L2
0	1.00 ± 0.00^{Ae}	1.00 ± 0.00^{Ac}	1.00 ± 0.00^{Ac}	1.00 ± 0.00^{Ac}
3	3.00 ± 0.00^{Ad}	1.50 ± 0.71^{Ac}	1.50 ± 0.71^{Ac}	1.50 ± 0.71^{Ac}
6	5.50 ± 0.71^{Ac}	3.50 ± 0.71^{Bb}	$3.00\pm0.00B^{Cb}$	2.00 ± 0.00^{Cc}
9	6.00 ± 0.00^{Ac}	4.50 ± 0.71^{Bb}	3.50 ± 0.71^{Cb}	2.00 ± 0.00^{Dc}
12	7.00 ± 0.00^{Ab}	6.00 ± 0.00^{Ba}	4.00 ± 0.00^{Cb}	3.50 ± 0.71^{Cb}
15	8.50 ± 0.71^{Aa}	7.00 ± 0.00^{Ba}	5.47 ± 0.71^{Ca}	5.00 ± 0.00^{Ca}

Means indicated by different capital letters in the same row differ significantly ($P < 0.05$). Means indicated by different lowercase letters in the same column differ significantly ($P < 0.05$). C: control samples, CF film: samples wrapped with gelatin film, o2 film: samples wrapped with gelatin film incorporated with 2% of OSE, L2 film: samples wrapped with gelatin film incorporated with 2% LSE.

The peroxide value is one of the basic oxidation product and used to measure the initial level of lipid oxidation. Utilization of edible film and coating enriched with antioxidant provide new alternative application to limit oxidative degradation in food products (Yuan et al., 2016). The initial PV value of seabass fillets was illustrated as 1 meq/kg and raised in all samples during the storage time. The PV values were recorded as the wrapped samples were 7.00, 5.47 and 5.00 meq/kg in the fillets wrapped with gelatin alone (CF), O2 and L2 respectively, while this value was 8.50 meq/kg in the control, at the end of storage. The highest PV value was noted in control group compared with wrapped samples with gelatin film, while the lowest PV value was noticed significantly ($P < 0.05$) in seabass fillets wrapped with gelatin film enriched with citrus seeds extract.

Varlik et al. (1993) documented that a PV of less than 2 meq/kg fish as "very good," up to 5 meq/kg as "good," and 8-10 meq/kg as at acceptability limit. Thus, the PV value of 0 day for all samples was 1.00 meq/kg which indicate as very good. The control and samples wrapped with gelatin film increased to 8 and 7 meq/kg, respectively, which indicate as acceptable limit, while the O2 and L2 groups recognized as good at the end of storage time. Ucak, (2019) reported the PV value of rainbow trout fillets as 2 meq/kg at the beginning of storage time and this value increased to 8.99 meq/kg for control group at the end of storage (10 days). The lowest PV value was found 5.49 meq/kg in fillets wrapped with gelatin films containing 8% of garlic peel extract. Fadiloglu and Coban (2018) demonstrated lowest PV in trout fillets coated with chitosan films enriched with sumac than the control group. Similarly, Alparslan et al. (2014) documented that the PV of trout fillets coated with gelatin films enriched by laurel essential oil were lower than those of the control group. In the present study, incorporation of 2% CSE and gelatin film was much more effective in retarding the lipid oxidation of seabass fillets during refrigerated storage.

5.4 Thiobarbituric Acid Reactive Substances (TBARS)

The differences in TBARS of seabass fillets wrapped with gelatin films enriched with 2% concentration of orange seed and lemon seed extract are shown in Table 5.4.

Table 5.4. TBARS changes of seabass wrapped with gelatin film incorporated with 2% of citrus seed extracts (lemon (LSE) and orange (OSE)) compared with control samples and gelatin film without extracts (mg MDA/kg)

Storage (Day)	C	CF	O2	L2
0	1.49 ± 0.02^{Ac}	1.49 ± 0.02^{Ac}	1.49 ± 0.02^{Acd}	1.49 ± 0.02^{Ab}
3	1.50 ± 0.01^{Ac}	1.47 ± 0.03^{Ac}	1.46 ± 0.05^{Ad}	$1.29.\pm 0.04^{Bc}$
6	1.56 ± 0.07^{Ac}	1.55 ± 0.03^{Ac}	1.46 ± 0.01^{Ad}	1.29 ± 0.02^{Bc}
9	1.56 ± 0.05^{Ac}	1.64 ± 0.05^{Ab}	1.53 ± 0.02^{Ac}	1.37 ± 0.03^{Bc}
12	1.70 ± 0.04^{Ab}	1.67 ± 0.01^{Ab}	1.63 ± 0.02^{Ab}	1.47 ± 0.06^{Bb}
15	1.88 ± 0.05^{Aa}	1.82 ± 0.02^{ABa}	1.74 ± 0.02^{Ba}	1.61 ± 0.02^{Ca}

Means indicated by different capital letters in the same row differ significantly ($P < 0.05$). Means indicated by different lowercase letters in the same column differ significantly ($P < 0.05$). C: control samples, CF film: samples wrapped with gelatin film, O2 film: samples wrapped with gelatin film incorporated with 2% of OSE, L2 film: samples wrapped with gelatin film incorporated with 2% LSE.

Thiobarbituric acid reactive substances (TBARS) is indicator of lipid oxidation and used widely in determining secondary oxidation products such as aldehydes or carbonyls (Ucak et al., 2019). TBARS detected the free fatty acids, which are highly effected to oxidation and form lipid hydroperoxide. This hydroperoxides are ready to decompose to shorter chain products such as aldehydes (Benjakul et al., 2005). This free fatty acid formed because of psychotropic bacteria specially Pseudomonas species, which produce lipase and phospholipase, which helps to increases in free fatty acids (Koka and Weimer, 2001). TBARS value of seabass fillets was 1.49 mg MDA/kg and increased in all samples during the storage time. TBARS values of the control and the samples wrapped with gelatin film without extract (CF) were higher than those of the samples wrapped with gelatin film enriched with 2% of citrus seeds extracts. Whereas, the value of gelatin film without extracts (CF) showed slight lower values compared with control. This is because the gelatin film might function as a barrier to oxygen permeability and only a small amount of oxygen can enter and contact with samples (Ahmad et al., 2012). At the end of the storage TBARS values of the seabass fillets were noted as 1.88, 1.82, 1.74 and 1.61 mgMDA/kg in control, CF, O2 and L2 samples, respectively. During the storage, the lowest TBARS value was observed significantly ($P<0.05$) in fillets wrapped with gelatin film incorporated with 2% LSE. Ahmad et al., (2012) reported similar results of delay of lipid oxidation in seabass slices coated with gelatin films combined with lemongrass essential oil (LEO) as a result of the antioxidant property of LEO. Martinez et al. (2017) who noted that TBARS value of seabass fillets coated with chitosan and alginate films enriched with resveratrol as 0.62 mg MA/kg in start and documented that films are effect in preventing lipid oxidation. Fadiloglu and Coban (2018) reported addition of chitosan with sumac on rainbow trout fillets significantly reduced the lipid oxidation. According to this study, the citrus seeds extract incorporated in gelatin film can delay oxygen permeability by antioxidant characteristics of citrus seeds extract.

5.5 Total Aerobic Mesophilic Bacteria Count

The differences in total aerobic mesophilic bacteria count during the storage of seabass fillets wrapped with gelatin films enriched with 2% concentration of orange seed extract and lemon seed extract are shown in Table 5.5.

Table 5.5. Total mesophilic bacteria count changes of seabass wrapped with gelatin film incorporated with 2% of lemon seed extract (L2) and orange seed extract (O2) compared with control samples and gelatin film without extracts (CFU/g)

Storage (Day)	C	CF	O2	L2
0	1.28 ± 0.03^{Af}	1.28 ± 0.03^{Af}	1.28 ± 0.03^{Af}	1.28 ± 0.03^{Af}
3	3.14 ± 0.01^{Ae}	3.14 ± 0.01^{ABe}	3.04 ± 0.08^{Be}	2.32 ± 0.03^{Ce}
6	5.42 ± 0.03^{Ad}	5.27 ± 0.05^{Bd}	5.00 ± 0.02^{Cd}	3.81 ± 0.04^{Dd}
9	6.10 ± 0.04^{Ac}	5.98 ± 0.03^{Bc}	5.46 ± 0.01^{Cc}	5.05 ± 0.04^{Dc}
12	6.80 ± 0.02^{Ab}	6.76 ± 0.03^{Ab}	6.21 ± 0.03^{Bb}	5.61 ± 0.02^{Cb}
15	7.25 ± 0.07^{Aa}	7.07 ± 0.10^{Aa}	6.86 ± 0.02^{Ba}	6.36 ± 0.05^{Ca}

Means indicated by different capital letters in the same row differ significantly ($P < 0.05$). Means indicated by different lowercase letters in the same column differ significantly ($P < 0.05$). C: control samples, CF film: samples wrapped with gelatin film, O2 film: samples wrapped with gelatin film incorporated with 2% of OSE, L2 film: samples wrapped with gelatin film incorporated with 2% LSE.

The mesophilic bacteria count in 0 day for seabass fillet was 1.28 log CFU/g. After that, the mesophilic bacteria count of all samples increased continuously during this period of storage. Control and gelatin film samples were increased similarly with lower values in gelatin film counts which was 7.25 and 7.07 log CFU/g at the end of storage respectively. There is a noticeable changes with slight increases in the values of gelatin film samples enriched with orange and lemon seeds extracts were 6.86 and 6.36 log CFU/g at the day 15 of storage. According to (Sallam, 2007), the acceptable limit in total mesophilic aerobic bacteria count is 6 log CFU/g. During the storage period, it was observed that the samples of gelatin film enriched with citrus extracts did not exceeded the acceptable limit compared with control and CF samples.

Similar results was found by Ojagh et al. (2010) who coated the rainbow trout fillets with chitosan and chitosan + cinnamon oil solution and they determined that total viable counts of chitosan only and chitosan + cinnamon oil coated samples were less than control samples during storage of 16 days. In addition, Ucak (2019) reported the total viable count of trout fillets wrapped with gelatin film enriched with garlic peel extract (GPE) was 2.27 log CFU/g at the beginning of storage. After that, the values of control and gelatin film without extract have been increased and exceeded the acceptable limit while the samples wrapped with gelatin film enriched with (GPE) have not exceeded the limit at the end of storage. Andevari and Rezaei (2011) documented that the total viable number of trout fillets coated with gelatin films incorporated with cinnamon oil ranged

between 1.90-2.20 log CFU/g. They also observed that the total number of bacteria in fillets coated with films prepared with cinnamon oil was lower than that of the control group.

5.6 Total Psychrophilic Bacteria Count

The differences in total psychrophilic bacteria count during the storage of seabass fillets wrapped with gelatin films gelatin films enriched with 2% concentration of orange seed extract and lemon seed extract are shown in Table 5.6.

Table 5.6. Total Psychrophilic bacteria count changes of seabass wrapped with gelatin film incorporated with 2% of lemon seed extract (L2) and orange seed extract (O2) compared with control samples and gelatin film without extracts (CFU/g)

Storage (Day)	C	CF	O2	L2
0	2.25 ± 0.07^{Af}	2.25 ± 0.07^{Af}	2.25 ± 0.07^{Af}	2.25 ± 0.07^{Ae}
3	3.46 ± 0.01^{Ae}	3.28 ± 0.02^{Be}	3.15 ± 0.08^{Ce}	2.22 ± 0.01^{De}
6	5.46 ± 0.01^{Ad}	5.32 ± 0.03^{Bd}	4.25 ± 0.01^{Cd}	3.45 ± 0.02^{Dd}
9	6.48 ± 0.00^{Ac}	6.38 ± 0.04^{Bc}	5.44 ± 0.02^{Cc}	4.31 ± 0.02^{Dc}
12	7.48 ± 0.00^{Ab}	7.25 ± 0.09^{Bb}	6.19 ± 0.05^{Cb}	5.26 ± 0.01^{Db}
15	8.37 ± 0.04^{Aa}	8.15 ± 0.06^{Ba}	7.21 ± 0.06^{Ca}	6.25 ± 0.07^{Da}

Means indicated by different capital letters in the same row differ significantly ($P < 0.05$). Means indicated by different lowercase letters in the same column differ significantly ($P < 0.05$). C: control samples, CF film: samples wrapped with gelatin film, O2 film: samples wrapped with gelatin film incorporated with 2% of OSE, L2 film: samples wrapped with gelatin film incorporated with 2% LSE.

Psychrotrophic bacteria are a main group of microorganisms which important for the aerobic spoilage of fresh fish at cold storage (Sallam, 2007). The continuous increases in psychrophilic bacterial count in all samples were noticeable with increasing storage time up to day 15. The Total psychrophilic bacteria value of all samples at 0 day was 2.25 log CFU/g. Significant differences ($P <0.05$) were determined between the groups wrapped with gelatin film prepared by adding 2% of citrus extract for 15 days of storage. During storage, the control samples and gelatin film samples (CF) were increased significantly but CF samples were slightly lower than control values, which were 8.37 and 8.15 log CFU/g at day 15, respectively. The samples enriched with 2% of orange and lemon seeds extracts were significantly retarded the microbial growth

compared to control values which were 7.21 and 6.25 log CFU/g at day 15 of storage, respectively.

It has been documented that the majority of gram-negative psychrotroph bacteria are major microorganisms for the aerobic spoilage of fresh fish stored in the cold (Hubbs, 1991; Ibrahim Sallam, 2007). Ucak et al. (2018) that the psychrophilic bacteria count (PBC) of rainbow trout fillets enriched with onion peel extract (OPE) was 2.47 log CFU/g at the beginning of the storage. The PBC of OPE treated samples was lower than the value of control samples at the end of storage. Jouki et al. (2014) reported similar results in PBC of rainbow trout fillets which was 3.1 log CFU/g at the beginning of the storage. Whereas, the PBC of fillets wrapped with chitosan film added with oregano or thyme essential oil were lower than control group during storage period. Fadiloglu and Coban (2018), found the total number of PBC Of trout fillets coated with chitosan films incorporated with sumac as 3.18 log CFU/g in the control group. They determined that the application of sumac showed antimicrobial properties and retarded the growth of psychrophilic bacteria. Alparslan et al. (2014), documented that the total number of psychrophilic bacteria of trout fillets coated with gelatin films enriched with laurel essential oil was lower than that of the control and the group wrapped with film without addition of essential oil during the storage at 4 oC. Andevari and Rezaei (2011) found the initial total number of psychrophilic bacteria of trout fillets treated with gelatin coatings prepared by adding cinnamon oil as 2.36 log CFU/g. In addition, gelatin coatings enriched with cinnamon oil have been documented to inhibit bacterial growth by showing antimicrobial properties. The previous studies, the consumable limit values of the total number of psychrophilic bacteria in fresh fish have been recognized as 6-7 log CFU/g (Erkan, 2007; Sallam, 2007). In this study, the total number of psychrophilic bacteria of seabass fillets wrapped with gelatin films enriched with citrus seeds extract remained below the acceptable limit value due to antimicrobial compound of citrus seeds.

5.7 Total Yeast and Mold Count

The differences in total yeast and mold count during the storage of seabass fillets wrapped with gelatin films gelatin films enriched with 2% concentration of orange seed extract and lemon seed extract are shown in Table 5.7.

Table 5.7. Total yeast and mold count changes of seabass wrapped with gelatin film incorporated with 2% of lemon seed extract (L2) and orange seed extract (O2) compared with control samples and gelatin film without extracts (CFU/g)

Storage (Day)	C	CF	O2	L2
0	1.11 ± 0.10^{Af}	1.11 ± 0.10^{Ae}	1.11 ± 0.10^{Ae}	1.11 ± 0.10^{Ae}
3	1.35 ± 0.07^{Ae}	1.16 ± 0.02^{Be}	1.04 ± 0.06^{Be}	0.65 ± 0.07^{Cf}
6	2.50 ± 0.07^{Ad}	2.32 ± 0.03^{Bd}	2.15 ± 0.04^{Cd}	1.74 ± 0.06^{Dd}
9	3.58 ± 0.06^{Ac}	3.44 ± 0.01^{Ac}	3.24 ± 0.02^{Bc}	2.14 ± 0.09^{Cc}
12	4.51 ± 0.05^{Ab}	4.20 ± 0.04^{Bb}	3.41 ± 0.01^{Cb}	3.29 ± 0.05^{Db}
15	5.47 ± 0.01^{Aa}	5.27 ± 0.05^{Ba}	4.18 ± 0.04^{Ca}	3.65 ± 0.07^{Da}

Means indicated by different capital letters in the same row differ significantly ($P < 0.05$). Means indicated by different lowercase letters in the same column differ significantly ($P < 0.05$). C: control samples, CF film: samples wrapped with gelatin film, O2 film: samples wrapped with gelatin film incorporated with 2% of OSE, L2 film: samples wrapped with gelatin film incorporated with 2% LSE.

Significant differences ($P <0.05$) were determined in total yeast and mold count of seabass wrapped with gelatin film incorporated with 2% of citrus seeds extracts (lemon and orange) compared with control and CF samples. The initial yeast and mold count for all samples was 1.11 log CFU/g. the value of control samples was significantly increased at the end of storage, it is about 5.47 log CFU/g. whereas, the count of gelatin film samples (CF) was near to count of control samples with slight inhabitation in microbial count, with an approximate value of 5.27 log CFU/g at day 15 ($P<0.05$). For gelatin film samples with 2% of orange seeds extract (O2) had increased gradually during 15 days of storage, the value reached to 4.18 log CFU/g at day 15. Also, the count of samples enriched with Lemon seeds extracts (L2) was noticeable inhibit in microbial counts and did not exceed the value of 3.65 log CFU/g at day 15. In addition, it is significantly observed that the lowest value was L2 that due to its antimicrobial compound.

Duman and Özpolat (2015) found that the total number of yeasts and molds of fish fillets treated with propolis extract was lower than control group, and antifungal effect increased with increase of propolis extract concentration. Karaton Kuzgun (2014) reported the initial number of total yeast and mold count of Luciobarbus esocinus fillets wrapped chitosan film enriched with different essential oil 2.30 log CFU/g. Also, it was reported that the increase in the number of yeasts and molds of the covered fillets with essential oils was lower and remained at a relatively low level than the control group at

the end of storage. In this study, it was observed that the total yeast and mold counts of seabass fillets wrapped with gelatin films incorporated with citrus seeds extract were significantly ($P<0.05$) lower than the control group, and the films enriched with citrus seed extracts was more effective in inhibition of yeast and molds.

5.8 Total Enterobacteriaceae Count

The differences in total Enterobacteriaceae count during the storage of seabass fillets wrapped with gelatin films enriched with 2% concentration of orange seed extract and lemon seed extract are shown in Table 5.8.

Table 5.8. Total Enterobacteriaceae count changes of seabass wrapped with gelatin film incorporated with 2% of lemon seed extract (L2) and orange seed extract (O2) compared with control samples and gelatin film without extracts (CFU/g)

Storage (Day)	C	CF	O2	L2
0	0.87 ± 0.04^{Af}	0.87 ± 0.04^{Af}	0.87 ± 0.04^{Af}	0.87 ± 0.04^{Af}
3	2.35 ± 0.07^{Ae}	1.84 ± 0.03^{Be}	1.78 ± 0.04^{Be}	1.32 ± 0.03^{Ce}
6	3.91 ± 0.05^{Ad}	3.65 ± 0.05^{Bd}	3.18 ± 0.01^{Cd}	2.25 ± 0.03^{Dd}
9	5.44 ± 0.00^{Ac}	5.30 ± 0.01^{Bc}	4.89 ± 0.02^{Cc}	4.28 ± 0.03^{Dc}
12	6.42 ± 0.05^{Ab}	6.16 ± 0.02^{Bb}	5.17 ± 0.03^{Cb}	4.89 ± 0.02^{Db}
15	7.05 ± 0.04^{Aa}	6.77 ± 0.02^{Ba}	5.89 ± 0.02^{Ca}	5.53 ± 0.07^{Da}

Means indicated by different capital letters in the same row differ significantly ($P < 0.05$). Means indicated by different lowercase letters in the same column differ significantly ($P < 0.05$). C: control samples, CF film: samples wrapped with gelatin film, O2 film: samples wrapped with gelatin film incorporated with 2% of OSE, L2 film: samples wrapped with gelatin film incorporated with 2% LSE.

Enterobacteriaceae recognized as a hygiene indicator (Ahmad et al., 2012). Statistically significant differences ($P <0.05$) were determined in total Enterobacteriaceae count of seabass wrapped with gelatin film incorporated with 2% of citrus seeds extracts (lemon and orange) compared with control samples and gelatin film without extracts (CF). The initial enterobacteriaceae count of seabass fillets was 0.87 log CFU/g. The noticeable inhibition in Enterobacteriaceae count of samples enriched with 2% orange seed and 2% lemon seed extract was observed, and total Enterobacteriaceae values found as 5.89 and 5.53 log CFU/g at the end of the storage, respectively. The Enterobacteriaceae of control and gelatin film samples were increased sharply with an approximate values of 7.05 and 6.77 log CFU/g at day 15, respectively.

Aerobic microorganism growth could have delayed when the fillets were wrapped by gelatin film. Gelatin film was determined to have excellent oxygen barrier property (Chiou et al., 2008). Alparslan et al. (2014) reported the initial number of Enterobacteriaceae counts in fresh trout 4.0 log CFU/g. According to their results, gelatin film without laurel essential oil did not show an inhibitory effect on Enterobacteriaceae counts. The total number of Enterobacteriaceae counts in trout fillet wrapped with quinoa films was determined as 2.00 log CFU/g, and it was observed that the control group reached a higher value at the end of storage (Korkmaz, 2016). In trout fillets wrapped with gelatin films incorporated with garlic peel (GPE), the number of Enterobacteriaceae counts as 1.53 log CFU/g and it was observed that the control group was higher than the group wrapped with films adding GPE (Ucak, 2019). In this study, it was found that the Enterobacteriaceae counts of seabass fillets wrapped with gelatin films incorporated with citrus seeds extract were significantly ($P<0.05$) lower than the control group.

5.9 Sensory Evaluation

The sensory evaluation of seabass fillets wrapped with gelatin films enriched with 2% concentration of orange seed extract and lemon seed extract are shown in Table 5.9.

The sensory panelists' values refers, for the evaluation of texture, odor, color, appearance and overall quality of control and wrapped fish fillets, during storage for 15 days. By indicating the score (4) as the limit to accept fish sample as a good for human consumption. It could be evaluated that the unwrapped (control) samples were unacceptable after the 3rd day of storage in all sensory parameters. The scores were range in between (3.68 to 1) indicate the samples were spoiled but the panelists rejected the texture of control samples after 6th day of storage. Similarly, the panelists rejected the gelatin film samples (CF) after 6th day of storage in all parameters, the values were in between (3.50 to 1). It is noticeable that the gelatin film could extending the shelf life of the fillets three days more in contrast with control samples. On the other hand, the all gelatin films supplemented with citrus seeds extract wrapped fillets were considered as "acceptable" for human consumption up to 12 days of storage periods, the scores were in between (6.13 to 4.5). In addition, it was observed that the scores of lemon seeds extract samples (L2) got higher scores than orange seeds extract samples (O2) due to its

higher phenolic compound than orange seeds. Considering all sensory parameters, it was observed that the seabass fillets wrapped with gelatin film enriched with citrus seeds extracts got higher scores than those of the control and gelatin film wrapped without extracts samples ($P<0.05$). This determined that application of gelatin film enriched with citrus seeds extract showed antioxidant and antimicrobial effect and retarded spoilage of seabass fillets.

Jasour et al. (2015) observed similar results in the chitosan coated rainbow trout fillets compared with control samples and they reported 4 more days of shelf life than control samples. Jouki et al. (2014) documented the addition of oregano or thyme essential oil to edible film could extend the shelf life of trout fillets 8 days more than control samples. According to previous studies, it was reported that the application of edible films incorporated with plant extract or essential oil extended the shelf life of fish (Fadıloğlu et al., 2018; Alparslan et al., 2014; Ojagh et al., 2010; Alsaggaf et al., 2017; and Mohan et al., 2012). Ucak (2019) reported that the sensory quality of trout fillets wrapped with gelatin film enriched with garlic peel extract increased the shelf life as 5 days compared to the control group. In the present study, the citrus seed extracts were effective in extending the shelf life of seabass fillets up to 12 days of storage. However, the control and gelatin film groups are got rejected from 6th days and 9th days of storage, respectively.

Table 5.9. Sensory evaluation of seabass wrapped with gelatin film incorporated with 2% of lemon seed extract (L2) and orange seed extract (O2) compared with control samples and gelatin film without extracts

	Storage (Day)	C	CF	O2	L2
Odor	0	9.00 ± 0.00^{Aa}	9.00 ± 0.00^{Aa}	9.00 ± 0.00^{Aa}	9.00 ± 0.00^{Aa}
	3	7.13 ± 0.83^{Bb}	7.38 ± 0.52^{Bb}	7.75 ± 0.46^{Bb}	8.50 ± 0.53^{Aa}
	6	3.63 ± 0.52^{Cc}	4.50 ± 0.53^{Bc}	6.38 ± 0.52^{Ac}	6.50 ± 0.53^{Ab}
	9	1.38 ± 0.52^{Dd}	3.38 ± 0.52^{Cd}	5.63 ± 0.52^{Bd}	6.88 ± 0.64^{Ab}
	12	1.38 ± 0.74^{Bd}	1.50 ± 0.53^{Be}	4.63 ± 0.52^{Ae}	5.25 ± 0.89^{Ac}
	15	1.00 ± 0.00^{Bd}	1.00 ± 0.00^{Bf}	2.63 ± 0.52^{Af}	2.75 ± 0.46^{Ad}
Texture	0	9.00 ± 0.00^{Aa}	9.00 ± 0.00^{Aa}	9.00 ± 0.00^{Aa}	9.00 ± 0.00^{Aa}
	3	6.75 ± 0.46^{Bb}	6.88 ± 0.83^{Bb}	7.00 ± 0.53^{Bb}	8.50 ± 0.53^{Aa}
	6	5.00 ± 0.93^{Bc}	5.50 ± 0.53^{Bc}	6.50 ± 0.53^{Ab}	6.75 ± 0.46^{Ab}
	9	2.38 ± 0.52^{Dd}	3.50 ± 0.76^{Cd}	5.75 ± 0.46^{Bc}	6.50 ± 0.53^{Ab}
	12	1.13 ± 0.35^{Ce}	2.00 ± 0.00^{Be}	5.38 ± 0.52^{Ac}	5.25 ± 0.71^{Ac}
	15	1.00 ± 0.00^{Be}	1.00 ± 0.00^{Bf}	2.38 ± 0.52^{Ad}	2.50 ± 0.53^{Ad}
Color	0	9.00 ± 0.00^{Aa}	9.00 ± 0.00^{Aa}	9.00 ± 0.00^{Aa}	9.00 ± 0.00^{Aa}
	3	7.00 ± 0.53^{BCb}	6.50 ± 0.53^{Cb}	7.38 ± 0.52^{Bb}	8.50 ± 0.53^{Ab}
	6	3.25 ± 0.46^{Dc}	4.38 ± 0.52^{Cc}	6.50 ± 0.53^{Bc}	7.13 ± 0.35^{Ac}
	9	1.38 ± 0.53^{Cd}	2.50 ± 0.53^{Bd}	6.13 ± 0.64^{Ac}	6.13 ± 0.64^{Ad}
	12	1.00 ± 0.00^{Cd}	1.25 ± 0.46^{Cf}	3.50 ± 0.53^{Bd}	4.63 ± 0.52^{Ae}
	15	1.00 ± 0.00^{Cd}	1.00 ± 0.00^{Cf}	2.00 ± 0.00^{Be}	2.25 ± 0.46^{Af}
Appearance	0	9.00 ± 0.00^{Aa}	9.00 ± 0.00^{Aa}	9.00 ± 0.00^{Aa}	9.00 ± 0.00^{Aa}
	3	6.25 ± 0.46^{Cb}	6.50 ± 0.53^{Cb}	7.38 ± 0.74^{Bb}	8.50 ± 0.53^{Ab}
	6	3.25 ± 0.46^{Dc}	4.50 ± 0.53^{Cc}	6.50 ± 0.53^{Bc}	7.13 ± 0.35^{Ac}
	9	1.38 ± 0.52^{Cd}	2.25 ± 0.46^{Bd}	6.25 ± 0.46^{Ac}	6.50 ± 0.53^{Ad}
	12	1.13 ± 0.35^{Cd}	1.25 ± 0.46^{Ce}	4.50 ± 0.53^{Bd}	5.13 ± 0.64^{Ae}
	15	1.00 ± 0.00^{Bd}	1.00 ± 0.00^{Be}	2.38 ± 0.52^{Ae}	2.63 ± 0.52^{Af}
Overall acceptance	0	9.00 ± 0.00^{Aa}	9.00 ± 0.00^{Aa}	9.00 ± 0.00^{Aa}	9.00 ± 0.00^{Aa}
	3	6.25 ± 0.46^{Cb}	6.63 ± 0.52^{Cb}	7.38 ± 0.52^{Bb}	8.50 ± 0.53^{Ab}
	6	3.38 ± 0.52^{Dc}	4.38 ± 0.52^{Cc}	6.63 ± 0.52^{Bc}	7.13 ± 0.35^{Ac}
	9	1.38 ± 0.52^{Dd}	2.50 ± 0.53^{Cd}	5.75 ± 0.46^{Bd}	6.63 ± 0.52^{Ad}
	12	1.13 ± 0.35^{Cd}	1.25 ± 0.46^{Ce}	4.50 ± 0.53^{Be}	5.00 ± 0.53^{Ae}
	15	1.00 ± 0.00^{Cd}	1.00 ± 0.00^{Ce}	2.38 ± 0.52^{Bf}	2.75 ± 0.46^{Af}

Means indicated by different capital letters in the same row differ significantly ($P < 0.05$). Means indicated by different lowercase letters in the same column differ significantly ($P < 0.05$). C: control samples, CF film: samples wrapped with gelatin film, O2 film: samples wrapped with gelatin film incorporated with 2% of OSE, L2 film: samples wrapped with gelatin film incorporated with 2% LSE

CHAPTER VI

CONCLUSION

In this study, the application of citrus seeds and added in edible films in order to prevent oxidation in fish and protect its quality by phenolic compound and antioxidant activities of citrus seeds. For this purpose, gelatin film incorporated with 2% of citrus seeds (orange and lemon) extract was applied in wrapping the seabass fillets. The physiochemical changes and microbiological changes was determined. The conclusion obtained as a results of this study are listed below.

1. Differences were determined between the groups wrapped with gelatin films enriched with 2% of citrus seeds extracts (lemon and orange) compared with control samples and gelatin film without extracts (CF) ($P < 0.05$) during storage period. While the highest pH values were observed in the C and CF groups during storage, significant ($P < 0.05$), the lowest pH values were determined in the O2 and L2 groups.

2. The peroxide values, which are the primary oxidation products, the values were increased until the end of storage in all groups and statistically significant ($P<0.05$) to the highest values, respectively C (8.50 meq / kg) and CF (7.00 meq/kg) groups. The peroxide values of the O2 and L2 groups at the end of storage were found as 5.47and 5.00 meq / kg, respectively.

3. TBARS value of seabass fillets was determined as 1.49 mg MDA / kg at the beginning of storage and increased in all groups at the end of storage. The highest TBARS increase during storage was observed in the C and CF groups, respectively, and reached 1.88 and 1.82 mg MDA / kg at the end of storage. TBARS values of seabass fillets wrapped with films enriched with citrus seeds extract were found to be significantly low ($P<0.05$) and were found as 1.74 and1.61 mg MDA / kg in O2 and L2 groups, respectively.

4. The initial number of Total aerobic mesophilic bacteria was determined as 1.28 log CFU/g and increased in all groups during storage. The highest values were

observed in the groups wrapped with gelatin film (CF) and control group, while the lowest values were determined in the groups coated with films enriched with citrus extract.

5. The initial number of total psychrophilic bacteria was found as 1.93 log CFU/g in the seabass fillets covered with gelatin film enriched with citrus seeds extract. While the most significant ($P < 0.05$) psychrophilic bacteria count during storage was observed in the C and CF groups, respectively, the lowest values were found in the O2 and L2 groups. At the end of storage, the total number of psychrophilic bacteria is C (8.37 log CFU/g), CF (8.15 log CFU/g), O2 (7.21 log CFU/g) and L2 (6.25 log CFU/g) determined in groups.

6. At the beginning of storage, the total number of yeast and mold count in seabass fillets was determined as 1.11 log CFU/g and increased in all groups during storage. During storage, the highest yeast-mold number was observed in the control group and in groups covered with gelatin films (CF), and these values reached 5.47 and 5.27 log CFU/g at the end of storage. The lowest total yeast and mold number was found in the L2 group and at the end of the storage it became 3.65 log CFU/g.

7. The initial number of total Enterobacteriaceae count in seabass fillets was found to be 0.87 log CFU/g. At the end of storage, the highest value (7.05 log CFU/g) was found in the control group, while the lowest ($P < 0.05$) Enterobacteriaceae count were found in fillets wrapped with gelatin film enriched with 2% of citrus seeds (orange (O2) and lemon (L2) extract were 5.89 and 4.72 log CFU/g respectively.

8. As a results, the citrus seeds can be new alternative source of phenolic compound such as flavonoids which are performs into two classes: the glycosylated flavanones and the polymethoxylated flavones. Citrus seeds are able to retard the microbial growth due to its antimicrobial properties.

9. The sensorial panelists evaluated of seabass fillets according to odor, texture, color, appearance and overall acceptance criteria and the scores decreased

during storage. The control group was rejected after 3rd day and gelatin film group (CF) was rejected after 6th day. Whereas, the O2 and L2 groups were rejected on the 12th day.

In This study, all the results showed that the addition of citrus seeds extract increased the effectiveness of gelatin film, lipid oxidation, sensory and microbial deterioration were delayed in seabass fillets wrapped with these films. In recent years, there is an increasing search for alternative antioxidants and antimicrobial agents for extending the shelf life of seafood, it is also thought that gelatin films with citrus seeds can be used as an alternative natural source for extending the shelf life.

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