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EFFECTS OF EDIBLE COATING ON PHENOLIC CONTENT AND LIPID OXIDATION OF OLIVES DURING STORAGE

MASTER OF SCIENCE THESIS

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DECLARATION

I hereby declare that this thesis is my own work and that, to the best of my knowledge and belief, it contains no material previously published or written by another person nor material which has been accepted for the award of any other degree except where due acknowledgment has been made in the text.

17.06.2016

Feyziye Selen Aral

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LIST OF SYMBOLS AND ABBREVIATIONS

- OO Olive Oil
- OA Oleic Acid
- UV Ultraviolet
- ROS Reactive Oxygen Species
- EVOO Extra Virgin Olive Oil
- CIA Collagen- Induced Arthritis
- RA Rhematoid Arthitis
- PV Peroxide Value
- MUFA Monounsaturated Fatty Acid
- PUFA Polyunsaturated Fatty Acid

ABSTRACT

Aral F.S. (2016) Effects of Edible Coating on Phenolic Content and Lipid Oxidation of Olives During Storage. Yeditepe University, Institute of Health Science, Department of Nutrition and Dietetics, Master of Science Thesis, İstanbul.

Olive fruit which is called Olea *europaea L*. is the main food of Turkish breakfast culture. Olive consumption of the Turkey is the forth place in the world. This fruit consists of oil, fat, protein, sugar, water and microelements, has lots of beneficial constituents for the human health. Especially one of the microelement called phenolic is an organic compound is found in the olive fruit and can act as an antioxidant. This study aims to investigate the effects of storage temperatures (4° C, 20° C, 30° C, 60° C) and the effects of chitosan coating $(2.5\%$, w/v) on total phenolics level and lipid peroxidation value in green olive samples which were came upon unripe from Ayvalık, Balıkesir. Spectrophotometer and Folin Ciocalteau reagent used to specify the changes on phenolic contents and titration method with sodium thiosulphate used to determine the changes on lipid peroxidation levels in olive samples. For the standards, gallic acid curve were used to calculate total phenolics, codex alimentarius and previous studies were used as references of lipid peroxidation levels in olive. The recorded raw data were plotted versus time in order to investigate the occurance of the lipid peroxidation and downtrends of the total phenolics of olive fruit samples which accelerates decay of the fruit were drawn. The results show that maximum phenolic levels are 455,89 GAEµg /100g, 492,82 GAEµg /100g, 520,36 GAEµg /100g and 362,50 GAEµg /100g for the coated olives. Uncoated olives have 285,10 GAEµg /100g, 267,38 GAEµg /100g, 303,69 GAEµg /100g and 218,43 GAEµg /100g phenolic levels at 4° C, 20 $^{\circ}$ C, 30° C and 60° C. The final results of the phenolic contents are $30,18$ GAEug /100g, 95,10 GAEµg /100g, 167,20 GAEµg /100g, 92,64 GAEµg /100g for the coated samples and 18,17 GAEµg /100g, 18,69 GAEµg /100g, 18,17 GAEµg /100g, 11,15 GAEµg /100g for uncoated ones at $4^{\circ}C$, $20^{\circ}C$, $30^{\circ}C$ and $60^{\circ}C$. The final peroxide values are 16,19 mEqO₂/kg, 17,14 mEqO₂/kg, 18,57 mEqO₂/kg and 23.33 mEqO₂/kg for coated samples. For uncoated ones 18,57 mEqO2/kg, 25,95 mEqO2/kg, 33,57 mEqO2/kg and 36,67 mEqO₂/kg at 4° C, 20° C, 30° C and 60° C respectively. As it is seen from results, coated samples have higher in phenolic contents and lower in peroxide values than uncoated samples in several temperature degrees. It is determined that chitosan based edible

coating act as a barrier of lipid peroxidation in olive fruit thus, loss of phenolics could be decreased.

Key words; Phenolics of Olive, Lipid Peroxidation, Olive Coating, Chitosan

 ÖZET

Aral F.S. (2016) Zeytinlerin Saklanması Sırasında Yenebilen Kaplamaların Fenolik İçerik ve Lipid Peroksidasyon Üzerine Etkisi. Yeditepe Universitesi, Sağlık Bilimleri Fakültesi, Beslenme ve Diyetetik Bölümü, Master Tezi, İstanbul.

Latince *Olea europaea L*. adı verilen zeytin, Türk kahvaltı kültürünün en önemli ögesidir. Türkiye zeytin tüketimi açısından Dünyada dördüncü sıradadır. Bu meyve yağ, protein, şeker, su ve mikroelementler gibi birçok yararlı bileşenden oluşmaktadır. Özellikle fenolik adı verilen bir mikro element, zeytin meyvesinde organik olarak yüksek düzeyde bulunur ve antioksidan olarak görev alır. Bu çalışmanın amacı Balıkesir'in Ayvalık ilçesinden gelen olgunlaşmamış yeşil zeytinleri, değişik saklama sıcaklıklarının (4°C, 20°C, 30°C, 60°C) ve kitosan kaplamanın (% 2,5'luk v/w, %2 aseti asit ile v/v) lipid peroksidasyon ve total fenolikler üzerine olan etkisi araştırmaktır. Fenolik içerikteki değişimleri saptamak için spektrofotometre ve Folin-Ciocalteau ayracı kullanılmıştır ve lipid peroksidasyon değerindeki değişimlerini belirlemek için sodyum tiyosülfat ile titrasyon yöntemi kullanılmıştır. Zeytin için total fenoliklerin hesaplanmasında, galik asit eğrisi standart olarak kullanılmıştır, peroksidasyon değerleri için ise daha önce yapılan çalışmalar ile codex alimentarius referans olarak alınmış. Kaydedilen ham veriler zamana göre işaretlenerek, insan sağlığı açısından zaralı olan ve yiyeceklerin çürümesine sebep olan lipid peroksidasyon oluşumu ile fenoliklerin düşüşü araştırılmıştır. Fenolik değeleri 4 °C, 20 °C, 30 °C ve 60 °C'de kaplı olanlar için sırasıyla 455,89 GAEµg /100g, 492,82 GAEµg /100g, 520,36 GAEµg /100g ve 362,50 GAEµg /100g'dır. Kaplı olmayanlar için 285,10 GAEµg /100g, 267,38 GAEµg /100g, 303,69 GAEµg /100g ve 218,43 GAEµg /100g'dır. Fenolik içeriği son seviyede 4°C, 20°C, 30°C ve 60°C'de kaplı olanlar için 30,18 GAEµg /100g, 95,1 GAEµg /100g, 167,2 GAEµg /100g, 92,64 GAEµg /100g; kaplı olmayanlar için ise 18,17 GAEµg /100g, 18,69 GAEµg /100g, 18,17 GAEµg /100g ve 11,15 GAEµg /100g'dır. Deney sonundaki peroksit değerler açısından kaplı olanlar için 4°C, 20°C, 30°C ve 60 °C'de değerler sırasıyla 16,19 mEqO2/kg, 17,14 mEqO2/kg, 18,57 mEqO2/kg ve 23.33 mEqO2/kg'dır. Kaplı olmayanlarda ise 18,57 mEqO2/kg, 25,95 mEqO2/kg, 33,57 mEqO2/kg ve 36,67 mEqO2/kg'dır. Sonuçlardan da anlaşıldığı üzere; kaplı zeytinlerde her sıcaklık değeri için fenolik değerleri yüksek iken, peroksit değerlerinin daha düşük olduğu gözlemlenmiştir. Zeytin için kitosan kaplamanın lipid peroksidasyonu engellemede ve böylelikle fenolik kayıplarının azalmasında rol aldığı saptanmıştır.

Anahtar Sözcükler; Zeytinde Fenolik, Lipid Peroksidasyon, Zeytin kaplama, Kitosan

1. INTRODUCTION

Olive fruit has rich phenolic content and also has a significant role in Turkish breakfast culture. Olive fruit could be lost their phenolic compounds during storage because of the conditions. Olives can keep in an aqueous solutions thereby phenolics can be swiftly disappearead. In addition, olive decays so fast without interference. Olives were coated with the chitosan based edible film in order to prevent missing phenolics and protect against decays by preventing lipid oxidation.

Antioxidants are involved in the scavenging of reactive oxygen species (ROS) which are occured in an aerobic metabolism in cells are associated with aging, metabolic damages, neurodegerative diseases and inflammation in excessive production. In addition, lipid peroxidation can be caused some diseases and carcinogenesis in the body that could be prevented by polyphenol-rich diets. Avoiding from lipid peroxidation is not only important for the human body, but also substantial for the food industry. In recent years, food industry try to develop new methods for prohibiting the food decays and nutritional losses. Edible film coating which is one of the new method uses different organic materials such as chitosan is not harmful for the health and suitable for the consumption.

Phenolics that are basically found in vegetables and fruits have beneficial effects on the health by their antioxidant property. They are organic compounds and they could disappear quickly in view of storage conditions. Particularly, during storage food exposed to oxygen so there could be formed lipid oxidation and this leads to spoilage and get loss of phenolics as well. Therefore, the food industry aims to hold beneficial compounds at maximum level especially during storage and also trying to find new food covering techniques in food industry, which is called edible film coatings. This kind of coating uses as different types of materials to cover food and these materials are nontoxic and fully safe for the consumption.

Chitosan which is used constantly in recent years, is one of edible film coating material. Chitosan is derived from chitin. Chitin is abundant naturally occuring biopolymer and mainly found in exoskeleton of crustaceans.

In this thesis, lipid oxidation levels were determined by using titration with sodiumthiosulphate which is mentioned in codex alimentarius as a standard of lipid peroxidation in olive oils and phenolic contents were determined spectrophotometric method by using folin ciocalteau reagent. In addition for determination of moisture content, ash content and fat content were done at the beginging and at the end of the experiments.

Olives can oxidize especially at the storage conditions and can lose their phenolic content so covering olives with different materials could be avoided from the oxidation and phenolic loss.

Olives which were unripe, provided from Ayvalık, Balıkesir in Turkey and delivered to the laboratory immediately for the experiments. They were matured at the laboratory conditions and then were covered with chitosan based edible film.

The thesis covers the theoretical background about basic literature survey of olives, nutritional value of olives, phenolics of olives, lipid peroxidation in olives and edible film coatings especially chitosan coatings. Materials and methods were used to understand the relationship between lipid peroxidation levels and phenolic content of olive. Standard curve of gallic acid were used to understand phenolic content of olive and the spent of the sodiumthiosulphate in titration were used to understand lipid peroxidation of olives. The aim of this thesis is to prevent lipid peroxidation in the olive fruit by covering them within an edible film material chitosan during storage at different temperature degrees. Another purpose of the study is preventing the oxidation of olives keep their phenolic levels at the top level. So that one of the precious organic material is called phenolics which are found in olives were set at high levels by coating them during storage at different temperatures. Thus, preventing the lipid peroxidation and during the consumption of the olive fruit humans can gain more benefit from its nutritional components.

2. LITERATURE REVIEW

 2.1 Olive

Olive (Olea europaea L.) is one of the oldest fruit cultivated trees in the world, belongs to the family of oleacaea. Olive trees have approximately 600 species which are distributed in the costal areas of southern Europe, Asia, northern Africa and near by Caspian Sea [1]. Olive's fruit and leaves are used as a remedy for physical treatment and mental well being. According to the International Olive Council, approximately 97% of the world's olive cultivation is held by Mediterranean countries [IOOC, 2011]. Turkey produced 13.6% of the world's production of the table olives between 2010-2011 season and is the second greatest table olive producer in the world after Spain [1, 2]. Table 1.1 show the production of the table olives between 2005 to 2015 years in the world [3].

Table 1.1 Production of the table olives in the world [3]

Many table olive cultivars are grown in different areas in Turkey such as Memecik, Ayvalık, Gemlik, Domat, Uslu, Edincik and so on. If these cultivars have low oil and high sugar content, they are used as table olives, however some cultivars are suitable for both oil and table olive production [4]. The regional production of the table olive in Turkey is given at Figure 1.1

Figure 1.1 Production areas of table olive in Turkey [5]

1. Aegean 2. Marmara 3.Mediterranean 4. Southeast Anatolia 5.Blacksea (Euxine Sea)

Aeagen part is the most important region in production of table olives in Turkey. This region produces 30% of Turkey's table olives. Marmara, Mediterranean, Southeast Anatolia and Blacksea regions produce 28%, 23%, 11%, 8% of Turkey's table olives, respectively [5].

Not only the production of the olive is sustantial in Turkey, but also the consumption is noteworthy. Turkey has the fourth place in consumption of the olive in the world which is approximately 11% of the world's consumption [6]. Table 1.2 shows the consumption of the table olives between 2005-2015 years in the world [3].

Turkish cuisine has many recipies with using olive and olive oil, so that Turkey uses so much olive and olive oil in its culinary culture. According to one of the study, the factors that effects the consumption of the table olives in İzmir were investigated. The results were showed that gender, age, household size and location of the olive production could affect the consumption of the table olives [6].

Although the consumption of olive has a serious levels in Turkey, olive fruit cannot be consumed directly before maturation, because its oleuropein compound. It gives bitter taste of the fruit and it degraded in the water [7]. To remove this bitterness material from it, some of different processing methods related to maturation stage of green and black olive were used [8]. Although numerous processing methods are used around the world for the maturation of the table olive, processing is mainly conduced according to the four methods. These are called; Spanish-style green olives, Sicilian style green olives, Californian- style black olives and greek style ripe black olives also known as 'Natural style' or ' Kalamata' [8,9].

The method of the Spanish style, the fruits are harvested when became green. Olives were treated by Sodium hydroxide (NaOH) or an alkaline lye which penetrate 2/3 of the way through the skin. After fermentation, all residues and lye are removed then replaced by water and this process was repeated. After washing, olives were placed into the bottles containig %8-%10 brine [8,9,10]. In Sicilian style, like Spanish style, the fruit harvested when they are green then put into water or brine directly, without using any lye [9]. Californian style method, olives are harvested at the stowe stage were treated by 0.5% sodium hydroxide (NaOH) solution for three days which oxidizes the pigments and turns the black olives [8, 9]. The method of the Greek style (natural) olives are harvested completly mature and washed and only treated by 8%-10% sodium chloride (NaCl) brine (w/v) without help of preliminary alkaline lye [8,9,10]. All three methods of maturation, were used glass bottles and were tightly sealed in order to avoid olives to be exposed to air.

Approximately, 39.7 % of the world's production of olive includes green olive, 40% is black and 20.3 % is used for all other commercial types [2]. All methods using for the fermentation of olive are mainly based on degradation of oleuropein during the brining of fruit [1]. However, while doing fermentation and water treatment, nutritional constituents, especially sugar and phenolic content of the olives will be decreased [4, 11]. Although reduction of important constituents of the olive fruit during processing, fat content will be slightly increased [11]. For the Spanish-style green olive processing, one of the study showed that fermentation of washing water under acidic condition, concentration of the phenolics will slightly decreased during storage [12].

2.2 Nutritional Value of Olive

The olive is a *drupe* (fruit with stone), composed of kernel (endorcarp), pulp (mesocarp) and fruit coat (epicarp) [13]. There are many varieties of olive whose shape and colour differ, and it is a valuable product in terms of nutritional value. The average physical properties of the olive are given in Table 2.1.

Table 2.1 Average physical properties of olive fruit [14]

The main constituents of olive fruit are oil, sugar, proteins, water and phenolic compounds [4]. The maturity degree, the region of the olive cultivar and the olive type affect the composition of the olive [15]. The average composition of an olive drupe is given in Table 2.2

Composition	Quantity (%)
Water	50
Oil	22
Protein	1.6
Sugar	19.1
Cellulose	5.8
Minerals (Ash)	1.5

Table 2.2 Average composition of the olive drup [16]

Olives have rich source and wide range of fatty acids and micronutrients which are beneficial for human health. Also the phenolics, biologically active chemicals, are a major health benefit of olives [10, 17]. Hippocrates (the father of the medicine) is known to have used olive fruit for the treatment of patients in early 400 B.C. [18].

There is significant relationship between nutrition and health. The Mediterranean diet, for example, is rich in fruits, vegetables, grains and olive oil, and so includes a high amount of antioxidant agents which lower the risk of disease[1]. In the Mediterranean region, quality of life is increased and incidence of chronic disease decreased when compared with the other regions of the world because of this type of diet [19]. Mediterranean diets could lower morbidity and mortality from cardiovascular diseases and reduce the incidence of obesity and metabolic syndrome in comparision with other types of food intake [20]. Researchers have focused on the health benefits of the Mediterranean diet components, especially olive oil [21]. The consumption of olive oil protects cardiometabolic functions because of the potentially antioxidant phenolics and the anti- inflammatory and vasculo-protective properties of the monounsaturated fatty acids (oleic acid; OA, 18:1 n-9 cis) [20]. Galli and Visioli (1999) concluded that enough daily consumption of the table olives and olive oils could reduce the risk of diseases caused by generation of free oxygen, such as coronary heart disease [22]. Devi et. al. (2008) indicated that olive oil (OO), consisting of many antioxidant compounds, has beneficial properties like protecting against cancers. Studies have evaluated the effect of OO on B(a)P hydroxylase enzyme which mediates B(a)P pyrene a toxic material inducing oxidative stress. The results proved that OO had a protective role against $B(a)P$ – induced oxidative damage [23]. Oliveras- Lopez et. al (2014) showed that daily consumption of extra virgin olive oil (EVOO) which is rich in phenolic compounds, antioxidant activity and antioxidant enzymes, improved the antioxidant status of healthy adults and modified their antioxidant gene expression levels without affecting metabolic parameters [24]. Rosillo et. al. (2014) showed that oral administration of EVOO polyphenol extract could slow the arthritic process in collagen – induced arthritis (CIA) model of rheumatoid arthritis (RA) [25]. Oliveras- Lopez et. al. (2008) studied the mechanism of an olive oil (OO) which has high content in phenolics and antioxidants which had effect on the pancreatic islets of the liver in control mice. The results showed both a direct and an indirect effect of the extra-virgin OO on the pancreatic area of the mice. Direct effects were shown against oxidative stress while indirect effects were involved in the activity of certain enzymes with antioxidant function [26].

2.3 Phenolics in Olive

Phenolics, also known as phenols, consist of an hydoxyl group (-OH) bonded directly to an aromatic hydrocarbon group. Carbolic acid (C_6H_5OH) is the simplest class of the phenols. Phenolics were classified as simple polyphenols which are abundant in human diet by virtue of the number of phenolic units in the molecule [27]. Figure 2.1 shows the structure of the simplest of the phenols [27].

Figure 2.1 The simplest phenols structure [27]

 Phenolic compounds can be naturally occurring or synthetic [27]. Phenolic compounds which are synthesized by plants during their development are thought of as secondary metabolites [28]. These compounds are various groups of phytochemicals produced in response to stress conditions, such as injury, UV and infection. Phenolics may produce bitter taste, colour, odour, flavour and oxidative stability of food products, but are not homogenously dispersed in plant tissue cellular and subcellular levels, such that phenolics can be either soluble or insoluble. At the tissue level, the outer layers of the plant contain higher levels of phenolics than inner parts [29].

Plant phenolics which occur naturally include simple phenols, phenolic acids, flavonoids, lignans and lignins. In addition, these components can act as phytoalexines, antioxidants and UV protectors [30]. Especially, the duty of phenolics as antioxidants can inhibit or delay oxidation and are involved in scavenging of reactive oxygen species (ROS) [31, 32]. ROS that are formed as a natural by-product of the normal metabolism of oxygen in the cell, can be a contributing factor to heart disease, cancer, diabetes, stroke, and rheumatoid arthritis [33]. This structural class of mainly natural organic chemicals is characterized by the presence of large multiples of phenol structural units [34], and numerous studies have shown that polyphenolic compounds can be found in high concentration in fruit and vegetables, with the concomitant antioxidant and radical scavenging capacities. Consequently, the significance of the consumption of foods that contain antioxidants and health supporting phytochemicals is increased [35]. Olives include high concentration of phenolic matter, approximately 1% to 3% of its olive/pulp ratio contains phenolics [27]. Olives include some of the major phenolics which can be categorized into seven main groups: secoiridoids (oleuropein, ligustroside); phenolic alcohols (tyrosol, hydrotyrosol); phenolic acids; flavanoids; flavones; hydroxycinnamic acid (verbascoside); and anthocyanins [8, 27, 36]. Some and most abundant phenolic compounds structure of table olives were given at Figure 2.2. Concentration of individual phenolic compounds of Hurma, Erkence and Gemlik olive types were given at Table 3.8 (Appendix B)

Figure 2.2 Some of the table olive phenolics structure [37]

The phenolic fractions of table olives are very complicated and depend on processing methods, degree of maturation, growing conditions and cultivar [1, 37]. Studies about Greek table olives showed that every different type of table olive had different phenolic profile and these differences caused changes in quality and quantity of the polyphenols in olive compounds which in turn affected the total antioxidant capacity of the olive [38]. Unprocessed olives contain three main phenolics: hydroxytyrosal, tyrosol, and the most abundant, oleuropein [35]. The concentration of these phenolics depends upon the maturation degree and the treatment [39,40].

The bioavalibility of phenolic compounds of olive fruit is another important consideration. Kountouri et. al. (2007) studied the bioavailability of polyphenols in olive fruit, finding that table olive polyphenols were metabolised in the human body and were increased in the plasma, thus having good bioavalibility. Otherwise, these polyphenols can lead to increase in total antioxidant potential in the human body [41].

One of the studies on consumption of the polyphenol extract from olives was carried out by Filip et. al. (2014), and could be an example of the health protecting capacity of the polyphenolic compounds. The study showed that an olive polyphenol extract can affect serum osteocalcin levels which can stabilize lumbar spine bone mineral density in postmenopausal women with osteopenia. In addition, this intake of the polyphenolic extract could improve blood lipid profiles [42]. Khalid et. al. (2015) investigated antioxidant activity, total phenolic and flavonoid contents and possible protective effects of different cultivars of olive lipid peroxidation induced by iron in mice liver. The study showed that oxidative stress in the liver can be managed by dietary intake of olive fruit, which is rich in total phenolics and flavonoids [43]. Okcu and Keles (2009) studied the functioning of antioxidants in fruits and vegetables, including vitamins, minerals, phenolic compounds, and antioxidant enzymes which could be preventative of free radical damage resulting from metabolisim. As a result, the risk of certain diseases, such as cardiovascular disease and cancer, can be reduced by consumption of these [44].

2.4 Lipids in Olive

One of the best sources of energy is fats which are a sub-group of lipids, very large vaguely connected group of compounds [45]. They contain the elements carbon, hydrogen and oxygen, like carbohydrates, and they are the esters of glycerol and fatty acids. Glycerol has three -OH groups and is a trihydric alcohol, while fatty acid is RCOOH known as alkonoic acid and having a hydrocarbon chain represented by the R. The OH group of glycerol reacts with -COOH of the fatty acid to form a molecule of oil [46]. Fats and oils, which are both known as lipids, are chemically similar, however oils are liquid whereas fats are solid at room temperature [45]. One molecule of glycerol is combined with three molecules of fatty acid and triglycerides were emerged. Figure 3.1 were shown the equation of the formation of triglyceride.

Figure 3.1 Formation of triglyceride [47]

If all three fatty acids are the same, the triglyceride is called a simple triglyceride. If triglyceride contains two or three different fatty acids, it is called a mixed triglyceride. 40 different fatty acids have been found in foods. Generally though, there are two types of fatty acids, saturated and unsaturated.

Saturated fatty acids have a hydrocarbon chain which is saturated with hydrogen, and with each carbon atom attached by a single bond to the next atom. Unsaturated fatty acids, on the other hand, contain at least one double bond and a hydrocarbon chain which is not saturated with hydrogen. Saturated fatty acids were shown at Figure 3.2 and unsaturated fatty acids were shown at Figure 3.3

Figure 3.2 Saturated fatty acids [48]

Figure 3.3 Unsaturated fatty acids [48]

Unsaturated fatty acids can contain one double bond, in which case they are called monounsaturated fatty acids, or more than one double bond, in which case they are polyunsaturated fatty acids. Oleic and linolenic acids are monounsaturated fatty acid while linoleic acid is polyunsaturated fatty acid. Figure 3.4 was shown the structure of oleic acid, Figure 3.5 was shown the structure of linolenic acid and Figure 3.6 was shown the structure of linoleic acid.

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H_3C\underset{H_2}{\bigcup} \begin{array}{c} H_2\\ C\\ C\\ H_2\end{array} \begin{array}{c} H_2\\ C\\ H_2\end{array} \begin{array}{c} H_2\\ C\\ H_2\end{array} \begin{array}{c} H_1\\ C\\ H_2\end{array} \begin{array}{c} H_2\\ C\\ H_2\end{array} \begin{array}{c} H_2\\ C\\ H_2\end{array} \begin{array}{c} H_2\\ C\\ H_2\end{array} \begin{array}{c} H_2\\ C\\ H_2\end{array} \begin{array}{c} H_2\\ C\\ H_2\end{array} \begin{array}{c} H_2\\ H_2\end{array} \begin{array}{c} H_2\\ H_2\end{array}
$$

Figure 3.4 Structure of oleic acid [49]

Figure 3.5 Structure of linoleic acid [49]

$$
H_3C \underbrace{C-C}_{C-C} \underbrace{C-C}_{C-C} \underbrace{C-C}_{C} \underbrace{C-C}_{C} \underbrace{C}_{C} \underbrace{C}_{C} \underbrace{C}_{C} \underbrace{C}_{C} \underbrace{C}_{C} \underbrace{C}_{C}
$$

Figure 3.6 Structure of linolenic acid [49]

The composition of olives differs with their region, climate conditions, ripening degree and processing methods. One of the components is oil, which can be divided into two main categories: saponifiable (triacylglyserol) fractions, of which olive oil contains 98-99%; and unsaponifiable fractions (phenols, esters, terpenes, pigments, tocopherols), of which olive oil contains 2%. The total lipid composition of olives includes 80% oleic acid and monounsaturated fatty acids (MUFA) which are composed of saponifiable fractions. One of the polyunsaturated fatty acids (PUFA) found in olive oil is linoleic acid and the other major saturated fatty acid is palmitic acid. Because of these fatty acid compositions, olive oil has beneficial effects on human health. Studies were showed that higher monounsaturated fatty acids or omega 3 fatty acid consumption in the diet can reduce the risk of cardiovascular disease [50]. Oleic acid is the most common MUFA in Daily nutrition. The National Institute of Medicine, the United States Department of Agriculture, the European Food and Safety Authority and American Diabetes Association do not give any dietary recommendation for MUFA. However, for daily consumption, the Academy of Nutrition & Dietetics and the Canadian Dietetics Association both recommend more than 20% of MUFA in their guidelines [51].

 2.4 Lipid Peroxidation in Olives

Oxidation reactions play an important role in human physiology and the food industry [52]. The reactive oxygen species (ROS) which occur in an aerobic metabolism in cells are associated with aging, metabolic damage, neurodegerative diseases and inflammation in excessive production [52,53]. ROS could lead to oxidative stress which can cause oxidative damage of biomolecules resulting in lipid peroxidation, causing several chronic diseases and carcinogenesis [53,54] while free radicals generated during normal body function could cause damage to significant cellular structures [55,56]. Thus, these could contribute to several diseases like cancer and atherosclerosis.

Not only is lipid peroxidation important for human health, but also rancidity and oxidative spoilage of fruits and vegetables are important issues for the food industry [57,58]. The overall mechanism of lipid oxidation in food consists of three phases. The first phase is initiation and the formation of free radicals, the second phase is propagation and free-radical chain reactions, and the third phase is termination and the formation of nonradical products [59]. Figure 4.1 shows the scheme of the lipid peroxidation phases in food.

- Initiation: $RH + O2$ --> $R \cdot + \cdot OH$ $R + O2 \rightarrow + ROO$
- Propagation:

 $ROO⁺ + RH$ --> $R⁺$ + ROOH $ROOH\rightarrow RO + HO$

· Termination:

 $R \cdot + R \cdot - > RR$ $R + ROO \rightarrow ROO$ $ROO· + ROO· - > ROOR + O2$

Figure 4.1 Scheme of the mechanism of lipid peroxidation phases in food [59]

Human body has a great defense mechanism against free radicals which could be enzymatic such as catalase, superoxide dismutase or non-enzymatic such as vitamin C, carotenoids and flavonoids [60]. Dietary intake of antioxidants which are generally nonenzymatic components could be inhibit or prevent this oxidative damages [61]. Robertson et. al. (2003) showed that oxidative stress in pancreatic islets could be much more harmful than other tissues [62]. Facchini and Saylor (2003) indicated that polyphenol-rich diets could be protective effect on diabetes which is an pancreatic disease [63]. Rainis et. al. (2007) studied that oxidative stress is the main factor of carcinogenesis in the gastrointestinal tract [64]. Diet that was rich in antioxidant components like fruits and vegetables could be decrease the risk of the colonary heart disease and cancer [65]. Cook and Sammon (1996) also showed that high consumption of food and beverage rich in phenolics had a great correlation in reduction of the incidence of heart disease [66]. In a study antioxidant activity will be measured for understanding antioxidant property. There are various methods to evaluate antioxidant capacity [67].

2.5 Film-Forming and Chitosan Edible Films

The existence of oxygen in the environment can lead to degradation processes in food, like microorganism growth, lipid oxidation, enzymatic browning and nutritional losses especially vitamins and phenolics [68]. Oxidation causes deterioration of the pigments and lipids in the food. This leads to limit the shelf life of the food [69]. In order to prolong shelf life and decrease the loss of nutritional value, storage conditions and packaging are one of the main problems in the food industry. Moreover, browning of food is a major concern for extending the shelf life of the food [70]. Nowadays, consumers request safe foods with minimum impact and maximum benefit, so edible films and coatings of foods have become prominent [71]. Furthermore, oxidation could be reduced with the help of edible films and coatings by limiting oxygen permeability. In particular, polysaccharide or protein based hydrophillic films and coatings could provide a good barrier to oxygen transference [72]. Basically, edible films and coatings are a thin layer which can be consumed easily and provides a barrier against oxygen, moisture and microorganisms. It is safe for consumption, biodegradable and cannot change the sensory properties of the food, thereby ensuring the quality of the food. In addition, food should be preserved safely. It is important that the application of an edible film is determined by its mechanical, physico-chemical barrier properties and biological activity. Shelf life and nutritional value of fruits and vegetables could be improved by edible films by retarding oxygen penetration, limiting water vapor permability and inhibiting microorganisms [71]. Moreover, edible films can include antioxidant agents in their formula and this results in a better preservation quality. Temperature and relative humidty are the main factors that affect the oxygen permeability of the edible film material [73]. During processing, storing and dressing of the food, film coating could act as an oxygen and moisture barrier [74]. Many different materials have been tested as a protector against oxygen in both high and low moisture products [73]. These materials are natural polymers which are derived from polysaccharides (starch, cellulose, chitosan, alginate etc.), proteins (collagen, zein, gluten proteins etc.) and fats (bee wax, fatty acids, glycerols etc.) [75,76, 77,78]. Preparation of the films or coatings with these polymers are nontoxic and most of them have effective barriers against oxygen and carbondioxide [79]. Antioxidant film and coating applications on some fruit and vegetable products also prevent the enzymatic browning which is caused by oxidation of polyphenolic enzymes. Thus, the occurence of dark colour on fruits and vegetables is prevented [80]. Especially carbohdyrate derived edible films have hydrophillic effects exhibiting lower moisture barriers and can be produced more cheaply [80]. One of the materials particularly suited as film materail is chitosan, a cationic polysaccharide [81]. Lin et. al. (2011) used chitosan based edible coating for the litchi fruit which resulted in prevention of phenolic oxidation as compared with uncoated samples during storage [82]. Chitosan, a polymer of β- 1,4 linked 2 amino- Dglucosamine, is derived from chitin by deacetylation [83]. Chitin is a linear structure compound which is an abundant naturally occurring biopolymer (β- 1,4 linked N-acetyld- glucosamine) [84,85]. It is mainly found in the exoskeletons of crustaceans and also fungal cell walls. Chitin is the second most abundant linear polysaccharide [83, 86].

Chitosan is suitable for film forming material and chitosan films have antibacterial, antifungal, biocompability, biodegratibility and non toxic properties, also possessing a selective permeability to gasses [87]. Thanks to its properties, chitosan could be used in broad ranges such as biotechnology, agriculture, food science, drugs, cosmetics and so on [88]. Chitosan films can slow down the oxidation of phenolics and prevent browning of the material when used for coating foods. It also plays an important role in reducing microbial growth due to its antimicrobial properties [89]. Jiang and Li (2001) used the chitosan based edible film on post-harvested longan fruit and results were noteworthy. After coating, longan fruit respiration rate and weight loss were reduced, polyphenol oxidase activity was delayed, colour and eating quality were changed, and also decay of the fruit was partially inhibited during storage [90]. In another example of application of chitosan based edible film on post-harvested grapes, it was shown that decay was delayed [91]. Mango pulp is a sensitive fruit which can change its colour and flavour easily when sliced. Chien et al. (2007) carried out a study on the covering of mango fruit with chitosan based edible film to prolong shelf life. The results showed that coating with chitosan extends the shelf life and prolongs the quality of the sliced mango [92].

3. MATERIALS AND METHODS

 3.1 Sampling

Green table olives were harvested in September 2014 in an olive grove in Ayvalık, Turkey and immediately transported to the laboratory where only fruits without peel defects were selected. The green table olives were unripe and they were matured in the laboratory.

 3.2 Reagents & Solutions

Hexane, methanol, acetic acid, chloroform, sodium chloride, sodium hydroxide, potassium iodide, podium thiosuphate, petrolium ether, gallic acid, folin-ciocalteou reagent and starch (soluble) were used as chemicals in the experiments. All glassware were washed and placed drying oven overnight and all chemicals were purchased from Sigma-Aldrich.

3.3 Materials

Olives were picked over and cleaned then were put into the developing trays and filled with Sodium chloride (NaCl) solution and stored in the fridge temperature $(+4^{\circ}C)$. The solutions were changed every other day until the maturation of the table olives were existed.

After maturation, olives were put into 5 litre (L) plastic containers filled with freshly prepared 5% NaCl brine. Olives were brined with a fruit/brine ratio of 1.5 approximately (10kg/5L) and maintained at a controlled room temperature (20° C – 25° C) in room temperature then were separeted into two groups which one was coated and the other one was uncoated. Subsequently, this two groups were divided into four sub-groups for the different temperature conditions (4 \degree C, 20 \degree C, 30 \degree C, 60 \degree C) and stored.

3.4 Methods

3.4.1 Chitosan-Based Edible Film Preparation

Chitosan is derived from chitin, which is found in the exoskeleton of crustaceas, in fungal cell walls and in other biological materials, was used for coating of the green table olives. Film forming solutions were prepared as described by Souza et. al, (2009) [93]. Briefly, 2.5% chitosan solution (w/v) was dissolved in 1% acetic acid (v/v) until the solution were homogenized which is about 1.5h at 25° C with the help of magnetic stirrer. The coating material were applied on the surface of the table olives and then stored until the film materials were dried $(48h$ at 25° C).

3.4.2 Total Phenolic Content

Total phenolic determination of the olive extract s was based on the prosedure introduced by Gutfinger (1981) using the Folin-Ciocalteou reagent and also as a spectrophotometric method proposed by Singleton and Rossi (1965) [94, 95]. A sample (2.5g) was dissolved in 5 ml hexane and then phenolic compounds were extracted with 3ml methanol/water (60:40; v/v) for 2 minutes by using vortex system. Both phases separeted by centrifugation at 3500 rpm for 10 minutes and then hexane phase was reexcrated with 3ml methanol/water (60:40; v/v) in the same way. The methanolic extracts were combined and an aliquot (0,2 ml) of the methanolic phase was diluted with water to total volume of 2.5 ml followed by addition of Folin-Ciocalteau reagent. Briefly, 0.1 ml of extract, 5 ml Folin reagent and 1ml of distilled water were stayed for 3 minutes then 1ml Na2CO3 were added and filled with distilled water until 50 ml then were vortexed. The samples incubated for 90 minutes at room temperature, in dark conditions. The absorbance was measured at 725nm against a blank sample by using spectrophotometer UV-vis. Standard curve was prepared using 0, 40, 80, 120, 160 and 200 µg/mL solutions of gallic acid in water concentrations. Analysis was performed triplicated samples. The results expressed as µg GAE (gallic acid equivalents)/100g of sample.

3.4.3 Peroxide Value Determination

Although peroxide values (PV) could be determined by many different methods, European standard reference method (Commision Regulation EEC N-2568/91- Determination of PV) for the olive oil peroxide value is using starch as an indicator and sodium thiosulphate as a titrant so that peroxide values of the table olives were determined by AOCS Cd. 8-53 (1990) method [96]. 20 g of the olive sample were get into the petroleum ether (200 ml) for overnight in order to get an oil from experimental olives. Then the mixture were evaporated and oil were obtained from the olive samples.

In order to prepare 0.01 M (mol/L) sodium thiosulphate $(Na₂S₂O₃)$ which was using as standard at the titration of olive oil samples, 2 g of potassium iodate $(KIO₃)$ was dried in an oven at 90-100 °C for 1-2 hours. After 1-2 hours, 0.001 mol/L KIO₃ solutions (\approx 0.1070gr KIO₃/500 ml dH₂O) was prepared with potassium iodate (KIO₃) taken from the oven. The weight of $KIO₃$ was recorded. This prosedure could be let us to understand and be sure about the potassium iodine (KI) solution was made as a saturated form while using this solution in the titration of olive oil samples. To prepare 0.5 M (mol/L) sulphuric acid (H₂SO₄₎ solution, using 2.8 ml of H₂SO₄ (96% purity) which was diluted with 100 ml distilled water. For preparation of starch solution as an indicator; 1 g of starch was weighed and then dissolved in 10 ml of distilled water at room temperature. After that 90 ml of boiling distilled water was added into the starch solution and boiling phase continued for 5-6 minutes. So that 1% of starch indicator was ready for using in titration.

Before titration, to be sure about the molarity (M) of the sodium thiosulphate solution; 0.2 g of potassium iodine (KI) was weighed then 1 ml of 0.5 M $H₂SO₄$ and 50 ml of 0.001 M (mol/L) potassium iodate $(KIO₃)$ solutions were added into the potassium iodine (KI). Solution which was brown coloured was titrated with sodium thiosulphate (0.01M) until the solution has turned to its brown colour into the yellow colour. 2 ml of starch indicator solution was added into the yellow solution and titration was completed when the solution becomes colourless. Sodium thiosulphate spent during titration was recorded.

To calculate and understand the molality (M) of the sodium thiosulphate before titration of olive oil samples, these equations were used.

$$
M_{KIO3} = \frac{m_{KIO3}(g) / MW_{KIO3}(g/mol)}{m}
$$
 [96]

 V_{KIO3} (ml) solution

$$
M_{sodiumthiosulphate} = 6 \times M_{KIO3} (mol/L) \times V_{KIO3} (ml)
$$
 [96]

V sodiumthiosulphate (ml)

 m_{KIO3} = weight of KIO_3 (0.1070 g)

 $MW_{KIO3} = molecular weight of KIO₃ (214 g/mol)$

 V_{KIO} solution = total volume of $KIO₃$ solution (500 ml)

 V_{KIO3} = volume of KIO₃ solution (50 ml)

 V sodiumsulphate = amount of sodium thiosulphate used in titration (ml)

Approximately 5 g of olive oil, which was acquired from olive samples, 0.5 ml saturated potassium iodide (KI) solution and 30 ml of acetic acid and chloroform solvent mixture (3:2; v/v) were combined and mixed for a minute. This sample kept in dark conditions at room temperature for 5 minutes. Then 100 ml of distilled water and 0.5 ml starch solution (1%) which was using as an indicator, were added into the mixture. The colour of the mixture turned into blue-brown. Titration was carried against 0.01 mol/L (N) sodium thiosulphate $(Na₂S₂O₃)$ until the blue-brown colour turns into the colourless. The results were recorded and expressed in milliequivalents of oxygen per kg of olive oil. (meq O2/kg)

The calculation of PV was determined by using this equation.

PV =
$$
\underline{V}
$$
 (ml) x M (mol/L) x 1000 [96]
m(g)

 $V =$ volume of sodium thiosulphate solution spent during titration (ml)

 $M =$ molarity of sodium thiosulphate solution (0.01 mol/L)

 $M =$ the weight of the sample (g of olive oil)

3.4.4 Determination of Moisture Content

The olive samples were weighed about 5 g was placed on a desiccated, tared petri dish and recorded as 'wet weighed of sample' and the results were determined according to the AOAC (2000) 925.40 method [97]. These samples were dried at 105 \degree C for 4 hours in the oven. After drying petri dishes remove from the oven and were cooled in desiccator at room temperature $(25 \degree C)$. After cooling, samples were weighed again and recorded as the 'dry weighed of the sample'. This analysis were triplicated and results expressed as moisture %.

The moisture content of the sample is calculated using the following equation;

$$
\% \text{ Moisture} = (m_{\text{initial}} - m_{\text{dried}}) / m_{\text{initial}} \times 100 \quad [97]
$$

3.4.5 Determination of Ash Content

Ash content were analysed by using muffle furnace, which was set at 525 \pm 25 $^{\circ}$ C. The olive samples were prepared about 2 g and were placed crucibles. Crucibles were set in the muffle furnace until the ash was appeared (about 12h). The crucibles were removed from the muffle furnace and placed in desiccator for cooling about 2h. Crucibles were weighed and results were calculated as ash % content. The results were determined according to the method of AOAC (1980) [98].

Percentage of ash is determined by using the following equation;

% ash = (Wash **/** Wsample) x 100 [98]

Wash :Weight of ash left

Wsample :Weight of sample taken

3.4.6 Determination of Fat Content

For the fat analysis in olive, ISO 659-1988 (E) prosedure and Soxhlet extractor were used which was described as IUPAC (1979) [99]. The olive samples (10g) was weighed and placed in a cellulose thimble (22x88 mm) and the thimble was plugged with cotton-wool. The thimble were placed in Soxhlet chamber (500 ml) which was fitted to a distillation flask (vessel), containing 100 ml o *n*- hexane (Sigma- Aldrich) and boiling chips in it. The extraction were finished about 6h, then thimble was allowed to cool (approximately 24h) at room temperature $(25C^o)$. After cooling prosedure, the solvent was released with the help of rotary-evaporator and then thimbles and flash with the extract were put in an pre-heated oven at $100C^o$ for 2h, followed by cooling in desiccator and then were weighed.

The results were calculated as % fat content by using formula.

$$
F\% = (M_2 - M_1/E) \times 100 \quad [99]
$$

 $M₁$: Weight of dry empty vessel + boiling chips

 M_2 : Weight of vessel + boiling chips + fat residue after evaporation

E: Sample weight
4. RESULTS AND DISCUSSION

The moisture analysis of green olives is done with AOAC method by using an oven. Ash analysis is also done with AOAC method but using a muffle furnace. Fat analysis is accomplished by using the Soxhlet Extraction Method. AOAC method is used in the same way as moisture analysis. Measurements are obtained at the beginning and at the end of the experiments, which are run in triplicate. Table 4.1 shows the average levels of moisture, ash and fat content for coated and uncoated olives at the beginning of the experiments.

Table 3.1 Average levels of moisture, ash and fat content for coated and uncoated olives at the beginning of the experiments.

Samples	Moisture $(\%)$	Ash $(\%)$	Total Fat $(\%)$
Coated	58.54	1.83	26.13
Uncoated	58.30	1.82	26.01

Generally, initial values of moisture, fat and ash analysis are 58.54% moisture content, 1.83 % ash content and 26.13 % total fat content for the coated samples and 58.30% moisture content, 1.82% ash content and 26.01% total fat content for the uncoated samples, as seen in Table 3.1. Tanılgan et. al. (2007) studied the moisture content of five different olive samples from Turkey. The results showed that moisture content of olive fruit could vary between 35.30% to 64.72% [100]. Asık and Özkan (2011) found 58.16% moisture content for the conventional Memecik olive cultivar [101]. Findings from the experiments on moisture content of olive cultivars were similar to reports in the literature. When these initial samples were taken at different temperature degrees, the moisture content changed.

Temperature $\rm ^{(o}C$	Uncoated Moisture \mathcal{O}_0	Coated Moisture $(\%)$	Change $(\%)$
	53.79	58.50	8.39
20	53.26	58.36	9.14
30	30.58	34.03	10.68
60	21.26	30.73	36.43

Table 3.2 Moisture analysis results of coated and uncoated olive samples at the end of experiments and the percentages of change

Table 3.2 shows final moisture content of coated and uncoated samples at $4^{\circ}C$, 20° C, 30° C, and 60° C. Differences are observed in moisture content between uncoated and coated samples. The uncoated group has 53.79% while the coated ones have 58.50% moisture content at 4°C. At 20°C uncoated samples have 53.26% while coated ones have 58.36%. There is a big difference in moisture content by comparison with the initial values at 30° C. Uncoated samples have 30.58% and coated samples have 34.03% moisture ratios. The biggest variation between the moisture content of coated and uncoated samples is seen at 60° C: while the uncoated group has 21.26% moisture, the coated group has 30.73%.

Kilercioglu et. al. (2016) showed that the water content of Ayvalık, Light, Mega and Sele type olives, which were bought from stores and were ready to consume, ranged between 52% and 34% [102]. The Literature confirms that the range of fat content for table olives is very wide. It can be said therefore that previous analysis about olive cultivars is in agreement with the experimental analysis results.

The percentages change in moisture content between coated and uncoated samples based on increase in temperature. From these results, it would not be wrong to say that the coating material can act as a barrier against the loss of moisture. Table 3.3 shows the final ash content of coated olive samples and uncoated olives.

Temperature $(^{\circ}C)$ Uncoated Ash $(^{\circ}\%)$		Coated Ash (%)	Change $(\%)$
	1.78	1.80	1.12
20	1.77	1.81	2.24
30	1.76	1.83	3.90
	1.76	$\overline{.82}$	3.35

Table 3.3 Ash analysis results of coated and uncoated olive samples at the end of experiments and the percentages of change

According to the results, there is not a big difference between initial and end point levels. In addition, there is not a great difference between uncoated and coated samples either. Table 3.4 shows the initial and end values of the experiments for ash content. Ash ratio is 1.83% at initial level. The end value of ash content of uncoated and coated samples has percentages from 1.82% to 1.76%. Unal and Nergiz mentioned the initial ash content of 'green table' as being 1.42% of ash content before alkaline treatment [4]. Lanza et. al. (2013) mentioned that ash content of an Italian cultivar ranged 4.7% to 4.9% [103]. Tanılgan et. al. (2007) showed different types of olive fruits could have different ash contents between 0.6% to 1.2% [101]. From these results, it seems that ash content of olives could change depending on growing region.

Table 3.4 Total fat content of coated and uncoated olive samples at the end of experiments and the percentage change between coated and uncoated samples

Table 3.4 shows the final results of the fat content of coated and uncoated olive samples at given temperatures. At 4° C, uncoated samples have 28.19% fat content while coated ones have 30.07%. The percentages in total fat content between uncoated and coated samples are close to each other at 20° C, 30° C and 60° C. Fat content of uncoated

samples is 26.57%, 26.03% and 25.60%, and for coated samples 28.55%, 26.98% and 25.95% at 20° C, 30° C and 60° C, respectively. The reason for this change between samples is that the coating material can be used as an oxygen barrier, thus decreasing the prevalence of lipid oxidation, so that fat content of coated olive samples is much better than uncoated ones. From the literature review, fat content is increased to 15.28% then decreased to 14.82% during storage (4, 8, 12 months) [4]. Asık and Özkan (2011) showed that oil content of the conventional Memecik olive was 44.74% [101]. Lanza et. al. (2013) mentioned that fat content in Italian olives was 17.7% to 21.7% after maturation [102]. These literature results show that the range of fat content for table olives is wide. Also analyses and reviews show that fat content of olives could decrease during storage due to conditions which initiate oxidation.

Olive fruit has an important role in nutritive value because of its essential fatty acids composition and phenolic content. The olive samples were stored at at $4 \degree C$, $20 \degree C$, 30° C and 60° C and separated into two groups; coated and uncoated samples. Lipid peroxidation value analyses were done periodically at defined temperatures. The lipid peroxidation values were calculated by a given equation using the expenditure of sodium thiosulphate during titration. The results were expressed as $meqO₂/kg$ units. The results of these analyses were transferred into Excel charts and graphics were composed by using both coated and uncoated peroxidation values. Figure 6.5-6.8 (Appendix A) are the graphics for the peroxide values of uncoated olives and Figure 6.9-7.3 (Appendix A) are the graphics for peroxide values at given temperatures for coated olives. Figure 7.4-7.7 (Appendix A) are the graphics for peroxide values of both coated and uncoated olive samples at given temperatures. Oxidation was started on the 6th day in every sample. At every temperature degree coated olives had lower peroxide value than uncoated ones.

After all these analyses and graphical examinations, the peroxidation value analyses results can be summarized in a table. The results of the initial and maximum lipid peroxidation values and percentages of final peroxide value differences of coated and uncoated olive samples are given in Table 3.5

Table 3.5 Initial and final peroxide value of coated and uncoated samples and percentages of final peroxide value differences between coated and uncoated samples.

According to the results, the percentage differences of the final peroxide values between coated and uncoated samples are 13.70% at 4° C, 40.89% at 20° C, 51.60% at 30° C and 44.47% at 60° C. The biggest variation of peroxide values between coated and uncoated samples was at 30° C.

Tanılgan et. al. (2007) mentioned that peroxidation value of olive samples which were gathered as crude olives from Gemlik, Kilis, Uslu, Tirilye and Ayvalık ranged between 15.3 and 22.5 mEqO2/kg [100]. Krkic et. al. (2013) examined the effects of chitosan coating on lipid oxidation levels in dry fermented sausage. There were two sausage groups; one of them is coated with chitosan and the other one is the control group. Sausages were stored for approximately seven months. The coated group had lower lipid oxidation levels than the control group after storage [104]. Georgantelis et. al. (2007) mentioned about addition of chitosan individually or combination of some antioxidants (rosemary, α tocopherol) in beef burgers. Results demonstrated noteworthy effects on beef burgers following chitosan coating during frozen storage [105]. Suman et. al. (2010) investigated the influence of chitosan coating on lipid peroxidation of refrigerated groud beef in various modified atmosphere packaging systems. In all packages, coated samples had lower lipid oxidation than the control group [106]. Pasquariello et. al. (2015) observed the changes in coating of three different sweet cherry cultivars. From the results, it can be seen that chitosan inhibited some enzymatic activities and enhanced some antioxidant enzymes after storage at 2° C and 24° C. Thus, coating of sweet cherry samples prevented browning and extended the storage life at both temperatures [107]. Jeon et. al. (2002) reported effects of chitosan coating on fresh fillets of Atlantic cod and herring and explained the relation between coating and lipid oxidation. Samples were stored over 12 days at 4° C. Reduction of the lipid oxidation were shown in coated samples so that the quality of seafoods was enhanced during storage [108]. No study related to the coating of olives was found in the scientific literature review. The literature review and experiments show that the peroxidation value of olives varied with region and storage conditions. Chitosan is one of the best materials against oxidation of foods, a fact which is supported by other studies, and the degree of protection varies with storage conditions.

Phenolic compounds have antioxidant properties which are found in olive fruit and have a significant role in their nutrition. Phenolic content diminishes the effects of free radicals thus the presence of the phenolics are vital to inhibit the lipid peroxidation caused by the environment.

Total phenolic content is determined by Folin-Ciocalteu method and from the references, gallic acid curve is used for the standard. Figure 6.1 shows the standard curve of gallic acid which was prepared using different gallic acid concentrations.

Figure 5.1 Gallic acid curve for the standard

Phenolic levels of olives showed variation. The results of the initial, maximum and final phenolic contents of coated and uncoated olive samples and ratios of maximum and final phenolic changes between coated and uncoated samples are given in Table 3.6

Table 3.6 Initial, maximum and final phenolic contents of coated and uncoated samples and percentages of maximum and final level phenolic content differences between coated and uncoated samples

Temperature $(^{\circ}C)$	Samples	Initial Phenolic Content (GAE) μ g/100g)	Maximum Phenolic Content (GAE) μ g/100g)	Final Phenolic Content (GAE) μ g/100g)	Differences of Maximum Phenolic Content Between Coated and Uncoated Samples $(\%)$	Differences of Final Phenolic Content Between Coated and Uncoated Samples (%)
$\overline{4}$	Coated	51.94	455.89	30.18	46.10	49.68
	Uncoated	51.94	285.1	18.17		
	Coated	51.94	492.82	95.1	59.31	134.32
20	Uncoated	51.94	267.38	18.69		
	Coated	51.94	520.36	167.2	52.59	160.79
30	Uncoated	51.94	303.69	18.17		
60	Coated	51.94	362.5	92.64	49.60	157.03
	Uncoated	51.94	218.43	11.15		

The amount of total phenolics in the sample were calculated by using the gallic acid curve as a reference unit and the results were expressed as µg GAE/gr units. The curve was prepared for concentration values of 40 μ g/mL, 80 μ g/mL, 120 μ g/mL, 160 µg/mL and 200 µg/mL. Phenolic analysis results were plotted versus time in Excel charts and the graphical results obtained.

All samples had the same phenolic content at initial point. The highest final phenolic content was at 30° C within the coated samples. The biggest variation of maximum phenolic content was at 20°C between coated and uncoated samples. In addition, there was a huge difference between coated and uncoated olive fruits at several temperatures.

Andjelkovic et. al. (2009) mentioned that total phenolic content of refined olive oil from Ayvalık in 2005-2006 was between 104.6 GAEµg /100g and 110.3 GAEµg /100g [109]. Although olive phenolic content changed, the literature review showed that olives from Balıkesir and Ayvalık have hydroxytyrosol content between 517.78 GAE mg/100g and 167.41 GAE mg/100g in a specific season (September, October) [110].

Figure 5.2-5.5 (Appendix A) shows phenolic content of uncoated olive samples during storage at different temperature degrees and Figure 5.6-5.9 (appendix 1) shows the coated samples. Figure 6.1-6.4 (Appendix A) shows the changes of phenolic content for both coated and uncoated samples. Pre and post harvest factors and debittering methods influence the phenolic levels in olives. While the debittering process begins, oleuropein, which is one of the phenolics, decreases. Although decline of oleuropein is significant for the olive's phenolic profile, hydrolysis products hydroxytyrosol and tyrosol increased and the health benefits of table olives are also related to levels of hydroxytyrosol.

It can be understood from the results of the experiments that phenolic content of olives increased due to occurence of phenolic compounds after maturation. The differences of the percentages may be due to the presence of oxygen and storage in water brine. Some of the phenolics which are soluble in water could be lost while olives are kept in water. Generally, olives are stored in brine in order to extend their shelf life. However, this process lowers the nutritional value of olives. There are many beneficial phenolic compounds in olives which are dissolved in water, but the main issue is how to keep the nutritional value at the same level while preventing spoilage.

The Coating material chitosan enables the storage of olives without the need for brine, thus coated samples contain much more phenolic compounds than uncoated ones. Therefore, their nutritional value is optimized.

The other reason for the decline of phenolic content is environmental oxygen, one of the main issues of decay. Oxygen starts degeneration of the structure of olive samples. While oxygen initiates several reactions, the occurrence of oxidation is the most important. Especially, phenolics are reduced during oxidation, thus phenolic content goes down in normal storage conditions. However, using chitosan edible film inhibits the oxygen penetration of the table olives and this can lead to preclude the oxidation and reduce loss of phenolics. Lipid peroxidation is the main reason of substantial loss of phenolic content. Table 3.7 gives the ratios between maximum and final phenolic contents for coated and uncoated samples.

Temperature (°C)	Phenolic Content Differences Between Maximum and Final in Coated Olive Samples $(\%)$	Phenolic Content Differences Between Maximum and Final in Uncoated Olive Samples (%)
	175.16	176.03
20	135.30	173.87
30	102.73	177.42
60	118.58	180.57

Table 3.7 Percentage differences of coated and uncoated samples between maximum and final phenolic contents

The percentage differences of coated samples were 175.16 %, 135.30%, 102.73% and 118.58%; and for uncoated samples 176.03%, 173.87%, 177.42% and 180.57% at 4° C, 20° C, 30° C and 60° C. According to these results, coating material affects the percentage changes of phenolic contents between maximum and final beneficially. The comparison graphics of phenolics and peroxidation levels versus time were drawn simultaneously, as in Figure 7.8-8.6 (Appendix A). The primary lines show phenolic content changes and secondary lines show the peroxidation values.

The figures show that while phenolic content is at the maximum level, oxidation begins in every sample at several temperatures. Although oxidation starts on the 6th day in every sample, coated samples have lower peroxide value than uncoated ones. In addition to that, the final peroxidation values are lower in coated samples. Not only is the peroxide value at lower levels in coated samples, but also the phenolic content is higher than for uncoated samples at the beginning and at the end of the experiments. According to these results, the coating material chitosan acts as an oxygen barrier in table olive samples, thus preventing spoilage and stabilizing the nutritional values of olives.

Oxidation starts with concomitant decrease of phenolic content. Lipid peroxidation affects the phenolic contents in olives. Degeneration of olives causes loss of phenolic compounds and the related nutritional deficiencies can be seen. Chitosan based edible film prevents the spoilage of samples so that the nutritional value of coated olives reamins higher than for uncoated samples at the time of consumption.

5. CONCLUSION

In conclusion, from the nutritional side table olives could be complete food which has water, fat, carbohydrates, protein, fiber, phenolics, vitamins and minerals. According to these knowledge, moisture, ash, fat, total phenolics and peroxidation value analysis were done by several methods. It wouldn't wrong to be said that moisture content could be changed by temperature because of the loss of water content. Vaporization could be caused of this drying process of olives by the elevation of heat. There is a great difference between uncoated and coated samples end point moisture contents thus, this study shows that coating material could be used as a barrier of moisture loss in several temperatures. On the other hand, experimental findings of the fat analysis indicate diversities due to harvest time, cultivar, climate and ripeness degree.

The study is showed that after maturation, the olive phenolics content increased awhile because of the degredation of oleuropein and rise of hydroxytyrosol amount then decreased total phenolics level sharply due to existence of oxygen and water especially at high temperature conditions. And also decline of phenolic contents are in relation with occurence of oxidation which cause of the lipid peroxidation of the olive fruits is significant for nutritional value. After the formation of peroxidation, fruit lose all benefical phenolics swiftly. Furthermore this oxidation influence the olive fruits decay and humans which are consuming olive pass over all healthy phenolic source.

The experiments showed that coating material chitosan is suitable for using as coverage of olive by inhibiting lipid peroxidation. By coating olives we can decline of the loss of phenolics and also sighting of peroxidation could be prevented at given temperatures. Using coating material provides an advantage on table olives' nutrtitional value. Olives can be store without any necessity of water brine thus, phenolics which are loss in water are found at maximum level. In addition, coating with chitosan could block the oxygen penetration and diminishes oxidation in table olives.

6. REFERENCES

1. Uylaşer V, Yıldız G. The Historical Development and Nutritional Importance of Olive and Oil Constituted an Important Part of the Mediterranean Diet*. Food Science and Nutrition*, 54: 1092-1101, 2014.

2. Tetik D. H. Table Olive Technology in Turkey. Olivea, 27: 23-24, 1989.

3. International Olive Council (IOC) (n.d). world table olive figures, Retrieved 20.04.2016 from[\(http://www.internationaloliveoil.org/estaticos/view/132-world-table](http://www.internationaloliveoil.org/estaticos/view/132-world-table-olive-figures)[olive-figures\)](http://www.internationaloliveoil.org/estaticos/view/132-world-table-olive-figures)

4. Ünal K, Nergiz C. The Effect of Table Olive Preparing Methods and Storage on the Composition and Nutritive Value of Olive. *Grasas y Aceites*, 54(1):71-76, 2003.

5. Özışık S, Öztürk F. *Türkiyede Zeytincilik Sektörünün Mukayese Analizi*. Ulusal Zeytin Kongresi Bildiri Kitabı, pp 10-24, 22-25 Şubat 2011, Manisa.

6. Tumer IE. Table Olive Consumption By Socioeconomic and Demographic Groups of Consumers in Turkey. *Italian Journal of Food Science*, 25(4): 441-445, 2013.

7. Kıralan M, Yorulmaz A. Zeytin Meyvesinde ve Sızma Zeytinyağında Bulunan Başlıca Fenoller ve Bunları Etkileyen Bazı Faktörler. *Anadolu Üniversitesi Bilim ve Teknoloji Dergisi*, 7(2): 311-321, 2006.

8. Sahan Y, Cansev A, Gulen H. Effects of Processing Techniques on Antioxidative Enzyme Activities, Antioxidant Capacity, Phenolic Compounds, and Fatty Acids of Table Olives. *Food Science and Biotechnology*, 22(3): 613-620, 2013.

9. Colmagro S, Collins G, Sedgley M. Processing Technology of the Table Olive. *Horticultural Review*, 25: 238-241, 2001.

10. Pasqualone A, Nasti R, Montemurro C, Gomes T. Effect of Natural-Style Processing on the Oxidative and Hydrolytic Degradation of the Lipid Fraction of Table Olives. *Food Control*, 37: 99-103, 2014.

11. Ergönül Günç P, Nergiz C. Farklı Zeytin Çeşitlerinde Olgunlaşma Periyoduna Bağlı Olarak Kimyasal Kompozisyonunda Meydana Gelen Değişimler. *Türkiye Gıda Kongresi*, 10: 199-202, 2008, Erzurum.

12. Castro A, Brenes M. Fermentation of Washing Water of Spanish-Style Green Olive Processing. *Process Biochemistry*, 36: 797-802, 2001.

13. Rafehi H, Ververis K, Karagiannis TC. Mechanisms of Action of Phenolic Compounds in Olive. *Journal of Dietary Supplements*, 9(2): 96-109, 2012.

14. Berman P, Leshem A, Etziony O, Levi O, Parmet Y, Saunders M, Weisman Z. Novel ¹H Low Field Nuclear Magnetic Resonance Applications for the Field of Biodiesel. *Biotechnology and Biofuels*, 6(1): 55- 77, 2013.

15. Vinha A. F, Ferreres F, Silva B. M, Vanlentao P, Gonçalves A, Pereira J. A, Oliveria M.B, Seabre R. M, Andrade P. B. Phenolic Profiles of Portuguese Olive Fruits (*Olea europaea L.*) : Influence of Cultivar and Geographical Origin. *Food Chemistry*, 89(4): 561-568, 2005.

16. Zamora R, Alaiz M, Hidalgo J. F. Influence of Cultivar and Fruit Ripening on Olive (*Olea europaea*) Fruit Protein Content, Composition and Antioxidant Activity. *Journal of Agriculture and Food Chemistry*, 49(9): 4267-4270, 2001.

17. Caporaso N, Savarese M, Paduano A, Guidone G, De Marco E, Sacchi R. Nutritional Quality Assessment of Extra Virgin Olive Oil From Italian Retail Market: Do Natural Antioxidants Satisfy EFSA Health Claims. *Journal of Food Consumption and Analysis*, 40: 154-162, 2015.

18. Ross SM. Effects of Extra Virgin Olive Oil Phenolic Compounds and the Mediterranean Diet on Cardiovascular Health. *Herbals and Nutritional Supplements*, 27(5): 303-307, 2013.

19. Carter SJ, Robers MB, Salter J, Eaton CB. Relationship between Mediterranean Diet Score and Atherothrombotic Risk: Findings From the Third National Health and Nutrition Examination Survey. *Atherosclerosis,* 210: 630-636, 2010.

20. Scoditti E, Massaro M, Carluccio M, Pellegrino M, Wabitsch M, Calabriso N, Storelli C, De Caterina R. Additive Regulations od Adiponectin Expression by Mediterranean Diet Olive Oil Components Oleic Acid and Hydroxytyrosol in Human Adipocytes. *PLoSONE,* 10(6):e0128218, 2015.

21. Kastorini C, Haralampas J. The Effects of Mediterranean Diet on Metabolic Syndrome and its Components: A Meta-Analysis of 50 studies and 534906 Individuals. *Journal of American Collage Cardiology*, 57: 1299-1313, 2011.

22. Galli C, Visioli F. Antioxidant and Other Activities of Phenolics in Olives/Olive Oil, Typical Components of the Mediterranean Diet. *Lipids*, 34: 23-26, 1999.

23. Devi KP, Kiruthiga VP, Pandian KS, Archunan G, Arun S. Olive Oil Protects Rat Liver Microsomes Against Benzo(a) Pyrene – Induced Oxidative Damages: An in vitro study. *Molecular Nutrition and Food Research*, 52: 95-102, 2008.

24. Oliveras-Lopez J, Berna G, Jurado-Ruiz E, Lopez-Garcia de Serrana H, Martin F. Consumption of Extra-Virgin Olive Oil Rich in Phenolic Compounds Has Beneficial Antioxidant Effects in Healthy Human Adults. *Journal of Functional Foods*, 10: 475-484, 2014.

25. Rosillo MA, Alcaraz MJ, Sanchez- Hidalgo M, Fernandez- Bolonos JG, Alarcon-de-la Lastra, Ferrandiz ML. Anti-Inflammatory and Joint Protective Effects of Extra Virgin Olive-Oil Polyphenol Extract in Experimental Arthritis. *Food Science and Technology*, 25(12): 1275-1281, 2014.

26. Oliveras-Lopez JM, Berna G, Carneiro ME, Garcia de la Serrana HL, Martin F, Lopez CM. An Extra Virgin Olive Oil Rich in Polyphenolic Compounds has Antioxidant Effects in of 1Mice. *The Journal of Nutrition*, 138: 1074-1078, 2008.

27. Rappoport Z. *The Chemistry of Phenols*. 2nd ed. Wiley, England, pp 3-20, 2003.

28. Servili M, Montedoro G. Contribution of Phenolic Compounds in Virgin Olive Oil Quality. *European Journal of Lipid Science and Technology* 104: 602-613, 2002.

29. Naczk M, Shadihi F. Extraction and Analysis of Phenolics in Food. *Journal of Chromatography A*, 1054: 95-111, 2014.

30. Naczk M, Shadihi F. *Phenolics in Food and Nutraceuticals: Sources. Application and Health Effects*, CRC Press, FL, 2004.

31. Velioglu YS, Mazza G, Gao L, Domah BD. Antioxidant Activity and Total Phenolics in Selected Fruits, Vegetables and Grain Products. *Journal of Agricultural Food Chemistry*, 46: 4113-4117, 1998.

32. Khalid A, Ahmad DS, Sabir MS, Khan A. Antioxidant Activity and Inhibitory Effect of Cultivars of Olive (*Olea europaea*) Against Lipid Peroxidation in Mice Liver. *Journal of Biochemistry*, 40(2): 188-196, 2015.

33. Tripoli E, Giammanco M, Tabacchi G, Di Majo D, Giammanco S, La Guardia M. The Phenolic compounds of olive oil: structure, biological activity and beneficial effects on human health. *Nutrition Research Reviews*, 18: 98-112, 2005.

34. Filip R, Possemiers S, Heyerick A, Pinheiro I, Raszewski G, Davicco MJ, Coxam V. Twelve- Month Consumption of a Polyphenol Extract From Olive (*Olea europaea*) in Double Blind, Randomized Trial Increases Serum Total Osteocalcin Levels and Improves Serum Lipid Profiles in Postmenopausal Women with Osteopenia. *Journal of Nutrition, Health, Aging*, 19(1): 77-86, 2014.

35. Blekas G, Vassilakis C, Harizanis C, Tsimidou M, Boskou GD. Biophenols in Table Olives. *Journal of Food Agricultural and Food Chemistry*, 50: 3688-3692, 2002.

36. Tsantilli E. Quality Attributes and Their Relations in Fresh Black Ripe 'Kalamon' Olives (*Olea europaea L.*) for Table Use Phenolic Compounds and Total Antioxidant Capacity. *Food Science and Technology*, 49: 657-665, 2014.

37. Tuck LK, Hayball JP. Major phenolic compounds in olive oil: metabolism and health effects. *The Journal of Nutritional Biochemistry*, 13(11): 636-644, 2002.

38. Boskou G, Salta FN, Chrysostomou S, Mylona A, Chiou A, Andrikopoulos NK. Antioxidant Capacity and Phenolic Profile of Table Olives From the Greek Market. *Food Chemistry*, 94: 558-564, 2006.

39. Owen R, Haubner R, Mier W, Giacosa A, Hull WE, Spiegelhalder B. Isolation, Structure, Elucidation and Antioxidant Potential of the Major Phenolic and Flavonoid Compounds in Brined Olive Drupes. *Food and Chemical Technology*, 41: 703-717, 2003.

40. Romero C, Brenes M, Yousfi K, Garcia P, Garcia A, Garrido A. Effect of Cultivar and Processing Method on Contents of Polyphenols in Table Olives. J*ournal of Agricultural and Food Chemistry*, 52: 497-484, 2004.

41. Kountouri MA, Mylona A, Kaliora CA, Andrikopoulos N. K. Bioavalibility of Phenolic Compounds of the Fruits (drupes) of *Olea europaea* (olives): Impact on plasma antioxidant status in human. *Phytomedicine*, 14: 659-667, 2007.

42. Filip R, Possemiers S, Heyerick A, Pinheiro I, Raszewski G, Davicco MJ, Coxam V. Twelve- Month Consumption of a Polyphenol Extract From Olive (*Olea europaea*) in Double Blind, Randomized Trial Increases Serum Total Osteocalcin Levels and Improves Serum Lipid Profiles in Postmenopausal Women with Osteopenia. *Journal of Nutrition, Health, Aging*, 19(1): 77-86, 2014.

43. Khalid A, Ahmad DS, Sabir MS, Khan A. Antioxidant Activity and Inhibitory Effect of Cultivars of Olive (*Olea europaea*) Against Lipid Peroxidation in Mice Liver. *Journal of Biochemistry*, 40(2): 188-196, 2015.

44. Okcu Z, Keles F. Kalp-Damar Hastalıkları ve Antioksidanlar. *Atatürk Üniversitesi Ziraat Fakültesi Dergisi*, 40(1): 153-160, 2009.

45. Proudlove R. *The Science and Technology of Foods*. 5th ed. Great Britian (GB), Forbes Publications, pp:21-24, 2009.

46. Gaman PM, Sherrington KB. *The Science of Food*. 4th ed. Oxford (UK), Elsevier, pp:73-76, 2007.

47. Gresham HS. *Biology: Chemistry of life*. 6th ed. pp:35-41, 2007.

48. Department of Biological Sciences: Office of Technology for Education.

Introduction to Lipids Retrieved 12.05.2015

from[\(http://telstar.ote.cmu.edu/biology/index.html\)](http://telstar.ote.cmu.edu/biology/index.html)

49. Baggott J, Dennis ES. Fatty Acid Synthesis and Modification. *NetBiochem.*

*Fatty Acids,*1998 Revieved: 12.05.15 from

[\(http://library.med.utah.edu/NetBiochem/FattyAcids/index.html\)](http://library.med.utah.edu/NetBiochem/FattyAcids/index.html)

50. Gonzalez S, Jimenez J. Oil Content and Fatty Acid Profile of Spanish Cultivars During Olive Oil Fruit Ripening. *J.Am.Oil Chem. Soc*. , 88:1737:1745, 2011.

51. Schwingshackl L, Hoffmann G. Monounsaturated fatty acids, olive oil and health status: a systematic review and meta-analysis of cohort studies. *Lipids in Health and Disease,* 13(1): 154, 2014.

52. McDonald S, Prenzler DP, Antolovich M, Robards K. Phenolic Content and Antioxidant Activity of Olive Extracts. *Food Chemistry*, 73: 73-84, 2001.

53. Seraj AK, Shankhar M, Raju KD. Punam J, Anju P, Rajat KA. Antioxidants and Lipid Peroxidation Status in Women with Breast Cancer. *International Medical Journal Malaysia*, 14(1): 71-75, 2015.

54. Halliwell B. Antioxidants and Human Disease: A General Introduction. *Nutrition Review*, 55: 44-49, 1997.

55. Brahmi F, MEchri B, Dhibi M, Hammami M. Variation in Antioxidant Activity and Phenolic Content in Different Organs of Two Tunisian Cultivars of *Olea europaea L*. *Physiology of Plant*, 36: 169-178, 2014.

56. Rafehi H, Ververis K, Karagiannis CT. Mechanism of Action of Phenolic Compounds in Olive. *Journal of Dietary Supplements*, 9(2): 96-109, 2012.

57. Shahidi F, Wanasundara PK. Phenolic Antioxidants. C*ritical Reviews in Food Science and Nutrition*, 32: 67-103, 1992.

58. Basu T. K, Temple N. J, Garg M. L. *Antioxidants in Human Health and Disease*. 1st ed. CABI Publishing, New York, pp 89-100, 1999.

59. St. Angelo AJ. Lipid Oxidation in Food. *Critical Reviews in Food Science and Nutrition*, 36(3):175-224, 1996.

60. MatEs J. M, Perez-Gomez C, De Castro N. Antioxidant Enzymes and Human Disease. *Clinical Biochemistry*, 32: 595-603, 1999.

61. Dasgupta N, De B. Antioxidant Activity of Some Leafy Vegetables of India: A Comparative Study. *Food Chemistry*, 101: 471-474, 2006.

62. Robertson RP, Harmon J, Tron PO, Tanaka Y, Takahashi H. Glucose Toxicity in β-cells: Type-2 Diabetes, Good Radicals Gone Bad and the Glutathion Connection. *Diabetes*, 52: 581-587, 2003.

63. Facchini FS, Saylor KLA. Low Iron Available, Polyphenol Enriched Carbohydrate Restricted Diet to Slow Progression of Diabetic Nephropathy. *Diabetes*, 52: 1204-1029, 2003.

64. Rainis T, Maor I, Lanir A, Shnizer S, Lavy A. Enhanced Oxidative Stress and Leucocyte Activation in Neoplastic Tissues of the Colon. *Digestion Disease Science*, 52(2): 526-530, 2007.

65. Keys A. Mediterranean Diet and Public Health: Personal Reflections. *American Journal of Clinical Nutrition*, 61: 1321-1323, 1995.

66. Cook NC, Samman S. Flavonoids Chemistry, Metabolism, Cardioprotective Effects and Dietary Sources. *Journal of Nutritional Biochemistry*, 7: 66-76, 1996.

67. Robards K, Prenzler PD, Tucker G, Swatsitang P, Glover W. Phenolic Compounds and Their Role in Oxidative Processes in Fruits. *Food Chemistry*, 66: 401-436, 1999.

68. Ayranci E, Tunc S. A Method for the Measurement of the Oxygen Permeability and the Development of Edible Films to Reduce the Rate of Oxidative Reactions in Fresh Foods. *Food Chemistry*, 80: 423-431, 2003.

69. Liu F, Dai R, Zhu J, Di X. Optimizing Odor and Lipid Stability of Beef Patties with a Mixture Design Incorporating with Tea Cateching, Carnosine and alpha tocopherol. *Journal of Food Engineering*, 98: 170-177, 2010.

70. Shiekh A. R, Malik AM, Al-Thabaiti AS, Shiekh MA. Chitosan as a Novel Edible Coating for Fresh Fruits. Food Science and Technology, 19(2): 139-155, 2013.

71. Ackar D, Subaric J, Babic A, Jozinovic S, Jokic. Edible Films and Coatings – Production and Application. *Food Technology*, 16: 294-299, 2014.

72. Kestel JJ, Fennemo O. Edible Films and Coatings: a review. *Food Technology*, 40: 47-59, 1986.

73. Bonilla J, Atares L, Vargas M, Chiralt A. Edible Films and Coatings to Prevent the detrimental effect of oxygen on food quality: Possibilities and Limitations. *Journal of Food Engineering*, 110:208-213, 2012.

74. Xu WT, Hwang KL, Guo F, Qu W, Yang JJ, Liang ZH. Postharvest Grapefruit Seed Extract and Chitosan Treatments of Table Grapes Control Botrytis Cinerea. *Postharvest Biological Technology*, 46: 86-94, 2007.

75. Ayranci E, Tunc S. Cellulosed-based Edible Films and Their Effects on Fresh Beans and Strawberries. *Z. Lebens UntersForsh A*, 205(6): 470-473, 1997.

76. Xie L, Hittiarachchy NS, Ju ZY, Meullemet J, Wang H, Slavik MF, Janes ME. Edible Film Coating to Minimize Egg Shellbreakage Postwash Bacterial Contamination Measured by Penetratiton Eggs. *Journal of Food Science*, 1(9):27-42, 2002.

77. Casariego A, Souza BWS, Vicente AA, Teixeira JA, Cruz L, Diaz R. Chitosan Coating Surface Properties as Affected by Plasticizer, Surfactant and Polymer Concentrations in Relation to the Surface Properties of Tomato and Carrot. *Food Hydrocolloids*, 22: 1452-1459, 2008.

78. Saucedo- Pompa S, Rojas- Molina R, Aquilera- Carbo AF, Saenz- Galindo A, de La Garze H, Jasso- Cantu D, Aguila CN. Edible Film Based on Candelilla Wax to Improve Shelf-life and Quality of Avocado. *Food Resource International*, 4(42): 511- 515, 2009.

79. Singh PT, Chatli KM, Sahoo J. Development of Chitosan Based Edible Films: Process Optimization Using Response Surface Methodology. *Journal of Food Science and Technology*. 1007: 318-332, 2014.

80. Hiemenz PC, Rajagopalan R. *Surface Tension and Contact Angle*. 3rd ed. Marcel Dekker Inc., New York, pp. 248-255, 1997.

81. Dutta PK, Triphathi S, Mehrotra GK, Dutta J. Perspectives for Chitosan Based Antimicrobial Films in Food Applications. *Food Chemistry*, 114:1173-1182, 2009.

82. Lin B, Du Y, Liang X, Wang X, Yang J. Effect of Chitosan Coating on Respiratory Behaviour and Quality of Stored Litchi Under Ambient Temperature. *Journal of Food Engineering*, 102: 94-99, 2011.

83. Elsabee MZ, Abdou SE. Chitosan Based Edible Films and Coatings: A review. *Material Science and Engineering*, 33: 1819-1841, 2013.

84. Qin CQ, Du YM, Xiao L. Effect of Hydrogen Peroxide Treatment on the Molecular Weight and Structure of Chitosan. *Polymer Degradation and Stability*, 76(2):211-218, 2002.

85. Rinaudo M. Chitin and Chitosan: Properties and Application. *Progress in Polymer Science*, 31(7): 603-632, 2006.

86. Zheng X, Jiang W, Xing L, Pu J. Synthesis and Characterization of Low Molecular Weight Chitosan. *Bioresources*, 10(2): 2338-2349, 2015.

87. Chung YC, Wang HL, Chen YM, Li SL. Effects of Abiotic Factors on the Antibacterial Activity of Chitosan Against Waterborne Pathogens. *Bioresource Technology*, 88(3): 179-184, 2003.

88. Cheng M, Deng J, Yang F, Gang Y, Zhao N, Zhang X. Study an Physical Properties and Nerve Sell Affinity of Composite Films From Chitosan and Gelatin Solutions. *Biomaterials*, 24(17): 2871-2880, 2003.

89. No HK, Meyers SP, Prinyawiwatkul W, Xu Z. Application of Chitosan for Improvement of Quality and Shelf Life of Foods: A Review. *Journal of Food Science*, 72(5): 87-100, 2007.

90. Jiang Y, Li Y. Effects of Chitosan Coating on Postharvest Life and Quality of Longan Fruit. Food Chemistry, 73: 139-143, 2001.

91. Meng X. H, Li B. Q, Liu J, Tian S. P. Phsiological Responses and Quality Attributes of Table Grape Fruit to Chitosan Preharvest Spray and Postharvest Coating During Storage. *Food Chemistry*, 106: 501-508, 2008.

92. Chien PJ, Sheu F, Yang FH. Effects of Edible Chitosan Coating on Quality and Shelf Life of Sliced Mango Fruit. *Journal of Food Engineering*, 78: 225-229, 2007.

93. Souza BWS, Cerqueira MA, Martins JT, Casariego A, Teixeira JA, Vicente AA. Influence of electric fields on the structure of chitosan edible coatings. *Food Hydrocolloids*, 24: 330-335, 2010.

94. Singleton VL, Rossi JR. Colorimetry of total Phenolics with Phosphomolibdicphosphothungstic acid. *Amer Journal Enology Viticulture*, 16: 144-158, 1965.

95. Gutfinger T. Polyphenols in Olive Oils. *Journal of American Oil Chemists Society*, 58(11): 966-968, 1981.

96. AOCS. *Official Methods and Recommended Practices of the American Oil Chemists' Society*. 4th ed. American Oil Chemists' Society, Champaign, 1990.

97. AOAC. *Official Methods of Analysis. Association of Official Analytical Chemists*, Arlington, VA, 2000.

98. AOAC. *Official Methods of Analysis, Association of Official Analytical Chemists*, Washington DC, USA, 1980.

99. IUPAC. *Standard Methods for the Analysis of the oils, fats and derivatives*. 6th ed. Pergamon Press, Oxford, UK, 1979.

100. Tanılgan K, Özcan MM. Physical and chemical characteristics of five Turkish olive (*Olea europea L*.) varieties and their oils*. Grasas y Aceites*, 58(2):142-147, 2007.

101. Asık Ugurlu H, Özkan G. Physical, Chemical and Antioxidant Properties of Olive Oil Extracted from Memecik Cultivar. Akademik Gıda, 9(2): 13-18, 2011.

102. Kilercioglu M, Ozel B, Öztop HM. Characterization and Comparison of Turkish Table Olive Varieties with NMR Relaxometry and Magnetic Resonance Imaging. *Gıda*, 41(2):61-67, 2016.

103. Lanza B, Di Serio MG, Lannucci E. Effects of maturation and processing technologies on nutritional and sensory qualities of Itrana table olives. *Grasas y Aceites*, 64(3): 272-284, 2013.

104. Krkic N, Sojic B, Lazic V, Petrovic L, Mondic A, Sedej I, Tomovic V. Lipid oxidative changes in chitosan-oregano coated traditionally dry fermented sausage Petrovska Klobasa. *Meat Science*, 93:767-770, 2013.

105. Georgantelis D, Blekas G, Katikou P, Ambrosiadis I, Fletouris J D. Effects of rosemary extract, chitosan and α tocopherol on lipid oxidation and colour stability during frozen storage of beef burgers. *Meat Science*, 75: 256-264, 2007.

106. Suman S P, Mancini R A, Joseph P, Ramanathan R, Konda M K R, Dady G, Yin S. Packaging- specific influence of chitosan on color stability and lipid oxidation in refrigerated ground beef. *Meat Science*, 86:994-998, 2010.

107. Pasquariello M S, Di Patre D, Mastrobuani F, Zampella L, Scortichini M, Petriccione M. Influence of postharvest chitosan treatment on enzymatic browning and antioxidant enzyme activity in sweet cherry fruit. *Postharvest Biology and Technology*, 109:45-56, 2015.

108. Jeon Y J, Kamil J Y V A, Shahidi F. Chitosan as Edible Invisible Film for Quality Preservation of Herring and Atlantic Cod. *J. Agric. Food Chem*, 50(18): 5167-5178, 2002.

109. Yorulmaz A, Erinc H, Tekin A. Changes in Olive and Olive Oil Characteristics During Maturation. *Journal of American Oil Chemists* Society, 90: 647-658, 2013.

110. Andjelkovic M, Acun S, Van Hoed V, Verhe R, Van Camp J. Chemical Composition of Turkish Olive Oil- Ayvalık. *Journal of American Oil Chemists Society*, 86: 135-140, 2009.

111. Gresham HS. *Biology: Chemistry of life*. 6th ed. pp:35-41, 2007.

7. APPENDIX A

Figure 5.2 Phenolic value of uncoated samples at 4°C

Figure 5.3 Phenolic value of uncoated samples at 20°C

Figure 5.4 Phenolic value of uncoated samples at 30°C

Figure 5.5 Phenolic value of uncoated samples at 60°C

Figure 5.6 Phenolic value of coated samples at ^{4°}C

Figure 5.7 Phenolic value of coated samples at 20°C

Figure 5.8 Phenolic value of coated samples at 30°C

Figure 5.9 Phenolic value of coated samples at 60°C

Figure 6.1 Phenolic value of coated and uncoated samples at 4° C

Figure 6.2 Phenolic value of coated and uncoated samples at 20°C

Figure 6.3 Phenolic value of coated and uncoated samples at 30°C

Figure 6.4 Phenolic value of coated and uncoated samples at 60°C

Figure 6.5 Peroxide value of uncoated samples at ^{4°}C

Figure 6.6 Peroxide value of uncoated samples at 20°C

Figure 6.7 Peroxide value of uncoated samples at 30°C

Figure 6.8 Peroxide value of uncoated samples at 60°C

Figure 6.9 Peroxide value of coated samples at ^{4°}C

Figure 7.1 Peroxide value of coated samples at 20°C

Figure 7.2 Peroxide value of coated samples at 30°C

Figure 7.3 Peroxide value of coated samples at 60°C

Figure 7.4 Peroxide value of coated and uncoated samples at 4° C

 Figure 7.5 Peroxide value of coated and uncoated samples at 20°C

Figure 7.6 Peroxide value of coated and uncoated samples at 30°C

Figure 7.7 Peroxide value of coated and uncoated samples at 60°C

Figure 7.8 Phenolic and peroxide value of coated olive samples at 4^oC

Figure 7.9 Phenolic and peroxide value of coated olive samples at 20°C

Figure 8.1 Phenolic and peroxide value of coated olive samples at 30°C

Figure 8.2 Phenolic and peroxide value of coated olive samples at 60°C

 Figure 8.3 Phenolic and peroxide value of uncoated olive samples at 4°C

 Figure 8.4 Phenolic and peroxide value of uncoated olive samples at 20°C

 Figure 8.5 Phenolic and peroxide value of uncoated olive samples at 30°C

 Figure 8.6 Phenolic and peroxide value of uncoated olive samples at 60°C
APPENDIX B

Table 3.8 Concentration of individual phenolic compounds of Hurma, Erkence and Gemlik olive types [111]

Olive types were: H, Hurma; E, Erkence; G, Gemlik. The first number after the letter is the harvest year and second number is the harvest week.
MI, maturity index: TPC, total phenol content (averages of three measurements)

8. ÖZGEÇMİŞ

Kişisel Bilgiler

Öğrenim Durumu

İş Deneyimi (Sondan geçmişe doğru sıralayın)

Bilgisayar Bilgisi

Bilimsel Çalışmaları SCI, SSCI, AHCI indekslerine giren dergilerde yayınlanan makaleler

Diğer dergilerde yayınlanan makaleler

Uluslararası bilimsel toplantılarda sunulan ve bildiri kitabında (*Proceedings***) basılan bildiriler**

Hakemli konferans/sempozyumların bildiri kitaplarında yer alan yayınlar

Diğer (Görev Aldığı Projeler/Sertifikaları/Ödülleri)