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THE EFFECT OF OREGANO AND ROSEMARY ESSENTIAL OILS ON SHELF LIFE OF PISTACHIO NUT PUREE

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EDA ADAL

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THE EFFECT OF OREGANO AND ROSEMARY ESSENTIAL OILS ON SHELF LIFE OF PISTACHIO NUT PUREE

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in

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Supervisor

Prof. Dr. Sami EREN

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Eda ADAL

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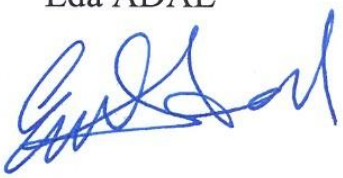

ABSTRACT

THE EFFECT OF OREGANO AND ROSEMARY ESSENTIAL OILS ON SHELF LIFE OF PISTACHIO NUT PUREE

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In this study, the effect of adding essential oil from different plants on prolonging shelf life of pistachio puree was investigated. For this purpose, Rancimat Method, which is one of Accelerated Shelf-Life Test (ASLT) method, was used to determine the effect of oregano (OO) and rosemary (RO) essential oils on the oxidative stability of pistachio puree. Butylated hydroxyanisole (BHA) and control sample was used for the comparison. 150, 300 and 600 ppm and 150 ppm concentrations were used for essential oils and synthetic antioxidant, respectively. The conductivity was followed at three temperatures, 110, 120 and 130ºC, with the addition of essential oils and BHA to pistachio puree during ASLT. Pistachio puree with essential oils showed protection against the lipid oxidation process. As the temperature increased induction time was significantly decreased during ASLT ($p<0.05$). The same concentration of essential oils and BHA were also added into pistachio puree to observe the change in peroxide value (PV) and free fatty acid (FFA) value under normal storage temperature 15, 25 and 40ºC. The PV and FFA increase were lower in RO, OO and BHA added pistachio puree samples during 8 month storage ($p<0.05$). 600 ppm of RO added sample had antioxidative effect as much as 150 ppm BHA added sample for both ASLT and normal storage conditions. These essential oils could be used as natural antioxidants in foods with high lipid content increasing the shelf life of these products.

Key Words: Pistachio puree, lipid oxidation, antioxidant, oregano, rosemary, essential oil, BHA, stability.

ÖZET

KEKİK VE BİBERİYE UÇUCU YAĞLARININ FISTIK FÜRESİNİN RAF ÖMRÜNE ETKİSİ

ADAL, Eda

Yüksek Lisans Tezi, Gıda Müh. Bölümü

Tez Yöneticisi: Prof. Dr. Sami EREN

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Bu çalışmada farklı bitkilerden elde edilen uçucu yağların fıstık füresinin raf ömrünü uzatmadaki etkisi incelenmiştir. Bu amaçla Hızlandırılmış Raf Ömrü Testlerinden (HRÖT) biri olan Ransimat metodu kekik ve biberiye uçucu yağlarının fıstık püresinin oksidatif stabilitesi üzerine etkisi incelenmek üzere kullanılmıştır. Bütillendirilmiş hidroksianisol ve kontrol örnekleri karşılaştırma için kullanılmıştır. Uçucu yağlar 150, 300 ve 600 ppm ve BHA 150 ppm konstantrasyonlarda fıstık pürelerine eklenmiştir ve induksiyon süreleri Ransimat cihazında 3 farklı sıcaklıkta (110, 120 ve 130°C) ölçülmüştür. Uçucu yağ içeren örneklerin yağ oksidasyonuna karşı koruyucu etki gösterdiği bulunmuştur. HRÖT analizlerinde sıcaklık arttıkça indüksiyon süresinin önemli ölçüde azaldığı gözlemlenmiştir ($p<0.05$). Peroksit değeri (PD) ve Serbest yağ asitliği değerindeki (SYAD) değişmeler aynı konsantrasyondaki uçucu yağ ve BHA'nın fıstık püresine eklenerek 15, 25 ve 40°C'de depolanmasıyla incelenmiştir. 8 ay süresince PD ve SYAD deki artış uçucu yağ ve BHA içeren örneklerde daha az gözlemlenmiştir ($p<0.05$). HRÖT ve normal depolama sonuçlarına göre 600 ppm biberiye yağı eklenmiş fıstık püresi 150 ppm BHA eklenmiş fıstık püresi kadar antioksidatif etkiye sahiptir. Kullanılan uçucu yağlar, yüksek yağ oranı içeren gıda ürünlerinde raf ömrünü uzatmak üzere doğal antioksidan olarak kullanılabilir.

Anahtar Kelimeler: Fıstık füresi, yağ oksidasyonu, antioksidant, kekik, biberiye, uçucu yağ, BHA, kararlılık

To My Father and Mother

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LIST OF SYMBOLS/ABBREVIATIONS

AAI	Antioxidant Activity Index
ANOVA	Analysis of Variance
AOAC	Association of Official Analytical Chemists
ASLT	Accelerated Shelf-Life Test
BHA	Buthylated hydroxyanisole
BHT	Buthylated hydroxytoluene
BS	British Standard
CIE	Commission Internationale de léclairage
COI	International Olive Oil Council
FAO	Food and Agriculture Organization
FFA	Free Fatty Acid
GC	Gas chromatography
GC-MS	Gas chromatography-Mass spectroscopy
NaOH	Sodium Hydroxide
OIP	Oxidative Induction Period
OO	Oregano essential oil
OSI	Oxidative Stability Index
PV	Peroxide value
RO	Rosemary essential oil
R^2	Correlation coefficient
SPSS	Statistical package for social sciences
g	gram

h	hour(time)
kcal	kilocalorie
kg	kilogram
L	litre
mL	millilitre
mm	millimetre
μm	micrometre
ppm	parts per million
sec.	second
v/v	volume/volume ratio
$	Dollar
°C	the degree Celsius
Cont’d	Continued

CHAPTER I
INTRODUCTION

Pistachio is one of the most important nuts in the world due to its high nutritional value and unique flavor as snack and a food ingredient. Turkey is one of the leading country that produce pistachio nuts with an annual output 128,000 tons (FAO, 2011). So, pistachio nuts are great economic value for Turkey.

Pistachios are generally marketed as split for snack food. Nonsplit nuts are used for processing. The food industry uses pistachios for cakes, biscuits, pies, candies, ice cream and pistachio butter (Omid et al., 2009). They are also used as the main ingredient of many Turkish desserts. Pistachio puree, the main material of this study, is made by pasting roasted pistachio kernel and used as filling material or additive in pastry, cookery and confectionary.

The most important problem of pistachio puree that has high amount of oil content (50-60%) and less water activity (0.2-0.3%) is lipid oxidation. Such oxidation leads to significant loss of food's nutritional value, since it involves a loss of vitamins and essential fatty acids. It also affects the food's sensory quality, changes in color, texture and taste, which shortens its shelf-life and can result in rejection on the part of consumers (Martos et al., 2009).

In the food industry, the rate of auto-oxidation is reduced by freezing, refrigeration, packaging under inert gas in the absence of oxygen and vacuum packaging (Daker et al., 2008). In cases where these methods are neither economic nor practical from the nutritional and technological points of view, it is highly desirable to control oxidation by the addition of antioxidants, inhibitory substances that do not reduce food quality (Gramza et al., 2006).

Because of health concerns surrounding synthetic antioxidants, there has been an increasing interest in natural antioxidants as agents for enhancing the shelf life of foods. Many sources of antioxidants of a plant origin have been studied in recent years. Among these, many aromatic plants and spices have been shown to be effective in retarding the process of lipid peroxidation (Kulisic et al., 2004; Singh, 2005).

The aim of this study was to investigate the availability of usage of rosemary (*Rosmarimus officinalis* L.) and oregano (*Origanum onites L.*) plant essential oils, two spice plants widely produced in Turkey, on prolonging shelf life of pistachio puree. For this purpose, the essential oils were added to pistachio puree with different concentration. The synthetic antioxidant, Buthylated hydroxyanisole (BHA), and control sample were used for comparison. The oxidative stability values of pistachio puree samples with or without antioxidants were provided by Rancimat method, which is one of the Accelerated Shelf – Life Test (ASLT) method. The effect of natural antioxidants on the change in peroxide value and free fatty acid value were followed at normal storage conditions of pistachio puree oil of samples.

CHAPTER II
LITERATURE REVIEW

2.1. Pistachio

Pistachio nut (*Pistacia vera L.*) is one of the most important and delicious tree nuts of the world. Several species of the genus Pistacia are referred to as pistachio, but only the fruits of Pistachia vera attain sufficiently large size to be acceptable to consumers as edible nuts (Shokraii and Esen, 1988).

Pistachio nuts are a rich source of fat around 56%, depending on the variety and stage of harvest, and contain fatty acids such as oleic, linoleic and linolenic acids (Table 2.1), which are essential for the human diet (Küçüköner and Yurt, 2003). They also contain around 23% protein, 19% carbohydrate, 5% moisture, minerals (Ca, Mg, K, P, Cu etc.) and vitamins (A, B_1, B_2, B_6, etc.) (Küçüköner and Yurt, 2003; Gamlı and Hayoğlu, 2007).

Table 2.1. Fatty acid composition of Turkish pistachio nuts (%) (Küçüköner and Yurt, 2003).

Fatty acid	Uzun	Kırmızı	Siirt	Ohadi	Halebi
Palmitic acid (C16:0)	9.20	8.22	8.47	9.67	8.32
Palmitoleic acid (C16:1)	0.68	0.56	0.65	0.65	0.67
Stearic acid (C18:0)	2.01	1.59	1.85	0.94	1.78
Oleic acid (C18:1)	70.14	72.01	74.01	56.66	69.79
Linoleic acid (C18:2)	17.35	22.67	14.37	31.00	18.49
Linolenic acid (C18:3)	0.18	0.30	0.19	0.27	0.24

Due to its high nutritional value, unique flavor and split shell, pistachio is an increasingly important nut crop consumed as raw, salted or roasted. Roasted and salted or unsalted pistachio nuts are usually directly consumed as snack foods. The non-split pistachio form is used in the production of pistachio oil, pistachio paste, pistachio chocolate and pistachio halva. It is also added as ingredient in the sausage, confectionery and sauces industries (Ardakani, 2006).

The major cultivars of nuts grown in Turkey are Siirt, Ohadi, Halebi, Kırmızı and Uzun. Producers prefer Kırmızı and Uzun cultivars for the production of baklava and nut paste due to their special green kernel color, flavor and texture (Gamlı and Hayoğlu, 2007).

2.1.1. Importance of Pistachio in the World Market

The pistachio tree (*Pistacia vera*) is native to the Middle Eastern region and has been naturalized in many parts of the world (Shahidi and Miraliakbari, 2005). Turkey is one of the main pistachio nuts producing country in the world. Anatolia is surely one of the main origins of pistachio species. The leading countries in pistachio nut production are Iran, the United States of America and Turkey. As can be seen in the Tables 2.2, the largest producer is Iran, with an annual output of 446,647 tons (49%) that followed by United States of America with 213,000 tons (23.3%, Sacramento and San Joaquin Valleys in California) and Turkey with 128,000 tons (14%) (FAO, 2011).

Table 2.2. Production of pistachio nuts in the world (FAO, 2011).

Country	**Annual production amount (tons)**
Iran	446,647
U.S.A	213,000
Turkey	128,000
Syrian	57,300
China	48,700
Greece	9000
Total (World)	**912,179**

Pistachio nut is also one of the important agricultural products, which is exported as well as being largely consumed in Turkey. In the 2009, the annual export amount of pistachio nut grown in Turkey was 3,574,000 kg which value of 39,382,255 $ (TurkStat, 2010). So, it also plays an important role in the economy of Turkey.

2.1.2. Processing of Pistachio Puree

The whole green and pink-green (mawardi) pistachio kernel are used to make pistachio puree by producer Asım Samlı Agricultural Products Export, Import And Trading, Sa, Gaziantep. The average length, height and width of pistachio nut ranges from 15-18 mm, 6-8 mm and 7-9 mm, respectively and contains less amount of oil (45-55%). The green pistachios contain less amount of oil than the others. Because when pistachios ripen, the color of pistachio kernel becomes yellow-brownish and oil content increase up to 50-60% (Seeram et al., 2008).

As shown in Figure 2.1 raw pistachio nuts are firstly separated from the shell and sorted according to size. After size sorting, pistachios are sorted from foreign matters like shell, stone, insect, glass etc. and contaminated kernels by using several machines and workers at critical control points. Then, the pistachios are roasted to improve the flavor, color, texture and overall acceptability of product. Temperature and time of roasting are important to develop roasted flavor and aroma. The roasting time and temperature can be changed according to initial moisture content of pistachio nuts. The final moisture content should be equal or less then 1.5-2% (wet basis) to make pistachio puree. The pistachio kernels are roasted at 130-135°C for 30 minutes in two-step roasting process. The roasted pistachios are pasted using pre-pasting machine up to 150-170 μm and then pasted up to 40-60 μm in size by pasting machine that include iron rolls inside. These iron rolls provide minimize size of pre-pasted pistachio and homogenization of puree. Particle size is changeable that depends on customer demand. Finally, pistachio puree is filled plastic bags and sealed (Anonyms, 2010a).

2.1.3 Composition of Pistachio Puree

The composition of pistachio puree is present in Table 2.3 (Anonyms, 2010b).

2.1.4 Usage of Pistachio Puree in Food Industry

Pistachio puree is used as a flavoring in cookery and confectionery and also favored, because of the deep green color of their kernels, in the ice cream and pastry industry (Ardakani, 2006).

Table 2.3. The compositions of pistachio puree (Anonyms, 2010b).

Property	Typical (per 100 g)
Energy (kJ)	2391
Energy (kcal)	571
Protein (g)	21.3
Carbohydrate (g)	27.7
Fat (g)	46
SAFA (g)	5.6
MUFA (g)	24.2
PUFA (g)	13.9
Trans fat (g)	0
Linoleic acid (Omega 6) (g)	13.636
α-Linolenic acid (omega 3) (g)	0.262
Fibre (g)	10.3
Calcium (mg)	110
Copper (mg)	1.3
Iron (mg)	4.2
Magnesium (mg)	120
Phosphorus (mg)	500
Potassium (mg)	21
Sodium (g)	10
Water (g)	2
Ash (g)	3

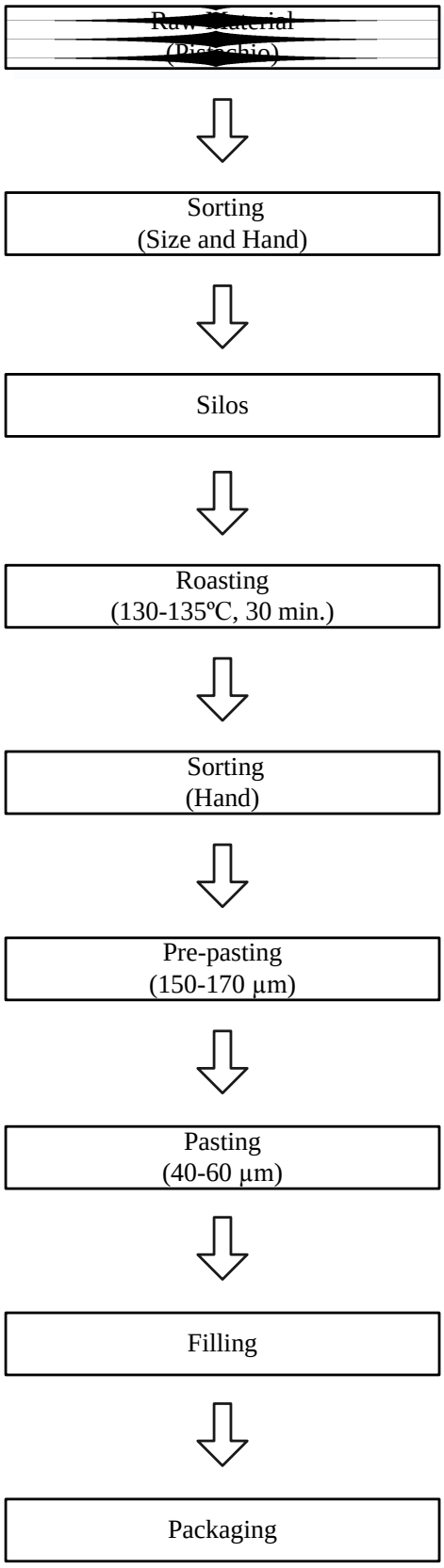


Figure 2.1. Flow charts of Pistachio puree production (Anonyms, 2010a).

2.2. Deterioration of lipids and lipid-containing foods

The least stable macro components in foods are the lipids. The problem of oxidative deterioration is of greatest economic importance in the production of lipid containing foods. Depending on the degree of unsaturation, lipids are highly susceptible to oxidation resulting in the development of rancidity. When this occurs, the food becomes unacceptable and is rejected by the consumer. In addition to the development of oxidized off-flavors, many of the oxidized products of rancidity are now considered to be unhealthy (Eskin and Przybylski, 2001).

2.2.1. Rancidity

Rancidity can be considered to be based on the subjective organoleptic appraisal of the off-flavor quality of food. It is associated with characteristic, unpalatable odor and flavor of the oils. The off-flavor can be caused by the absorption of the taints into the food or by contamination. The lipids act as reservoirs for the off-flavors. The rancidity can be caused by the changes that occur from reaction with atmospheric oxygen called oxidative rancidity. Finally, the off-flavors can be produced by hydrolytic reactions which one catalyzed by enzymes called hydrolytic rancidity. The hydrolytic reactions and the absorption effects can be minimized by cold storage, good transportation, careful packaging and sterilization, but oxidative rancidity, sometimes referred to as autooxidation, is not stopped by lowering the temperature of food storage. This is because autooxidation is a chemical reaction with low activation energy, 4-5 kcal mol^{-1}, for the first step and 6-14 kcal mol^{-1} for the second step (Hamilton, 1994).

2.2.2.1. Hydrolytic rancidity

Hydrolysis is the reaction of fats and oils with water. Moisture promotes the splitting of triacylglycerols to form free fatty acids, mono- and diacylglycerols (Figure 2.2), which result in increase of refining losses directly related to the free fatty acid content of oils and fats (List et al., 2005).

Essentially, hydrolysis is the reverse of making a fat molecule. This process typically requires a fat-soluble catalyst, high temperature (>100°C), and long time (several hours). Partial hydrolysis of lipids typically occurs because of improper seed storage and handling, such as high moisture content, high temperature, and seed mechanical

damage. Such reaction is usually catalyzed by the lipases naturally present in the oilseeds. The conditions used during oil extraction can also significantly affect such lipase catalyzed reaction (List et al., 2005).

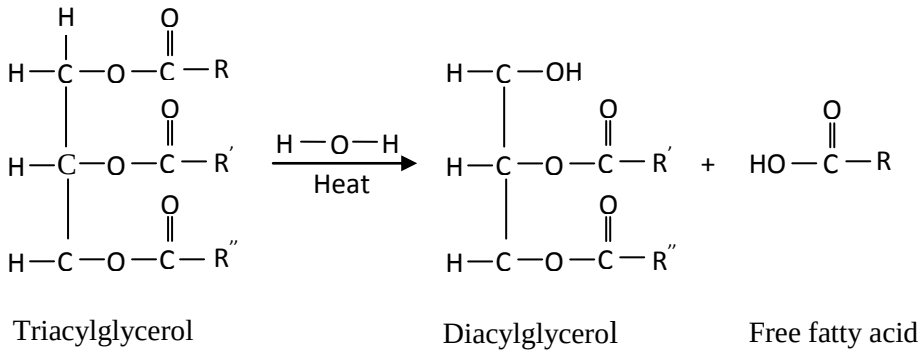


Figure 2.2. Hydrolysis of fats and oils (List et al., 2005).

2.2.2.2. Oxidative rancidity

Oxidation is the chemical reaction in which oxygen combines with another substance with the liberation of heat. Oxidation is mostly responsible for much more of the deterioration of fats and oils than hydrolysis. The initial step in the oxidation is the addition of oxygen at or near the double bond of fatty acid chain to form unstable compounds generally designated as peroxides (List et al., 2005).

2.2.2.2.1. Autooxidation

Lipid oxidation can be initiated by light, temperature, metals, metalloproteins, pigments and air pollutants as well as microorganisms. Lipid oxidation generates free radicals which are catalyzers of this process. The primary substrates for these reactions are polyunsaturated fatty acids and oxygen (Figure 2.3). The free radical mechanism of lipid oxidation is three-stage process: (1) initation, (2) propagation, and (3) termination. During initation stage, lipid radicals are formed directly from both unsaturated fatty acids in the presence of light, heat, other radicals react with oxygen to form peroxy radicals ($LOO^{\bullet}$), which in turn abstract a hydrogen atom from another lipid molecule to form hydroperoxides (LOOH) and another lipid radical (Figure 2.3). These generated radicals cause this process to become autocatalytic.

During the termination phase, free radicals interact with each other to form nonradical products (Eskin and Pryzybylski, 2001).

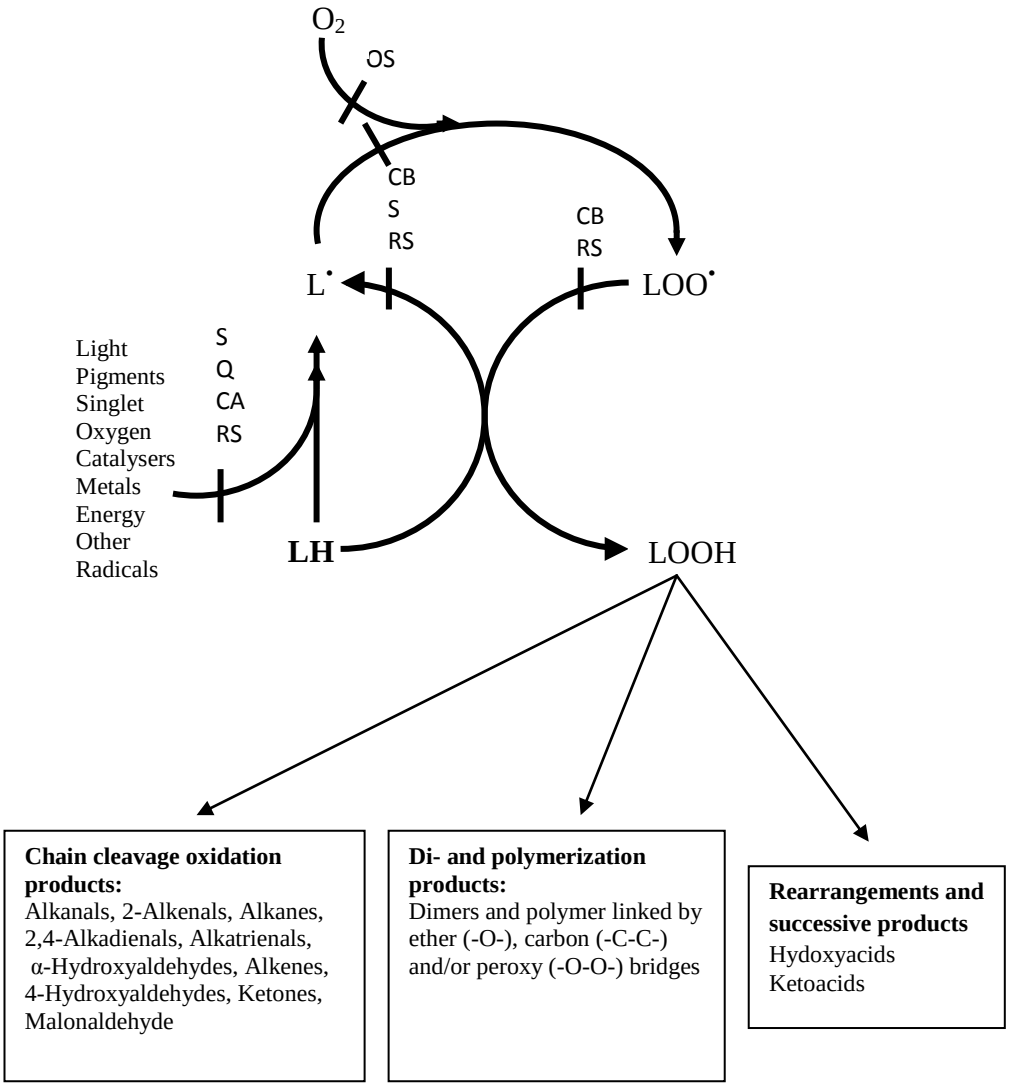


Figure 2.3. Mechanism of unsaturated fatty acids oxidation. LH= lipid component; L= lipid radical; LOO= lipid peroxy radical; LOOH= lipid hydroperoxide. Antioxidants: OS=oxygen scavengers; CB= chain reaction breakers; S=synergist; Q= quenchers; CA= chelating agents; RS=radicals scavengers/blockers (Eskin and Pryzybylski, 2001).

Any components that prevent or interfere with propagation of oxidation by deactivating free radicals in the system play a key role in the termination mechanism.

Chain breaking antioxidants, such as phenolic compounds, react with lipid radicals by donating a hydrogen atom to the lipid radicals, thereby stopping propagation by forming inactive components (Simic et al., 1992). Examples of phenolic antioxidants include tocopherols, Buthylated hydroxyanisole, buthylated hydorxytoluene, and propyl gallate (Eskin and Pryzybylski, 2001).

The LOOH formed are unstable and decompose into a wide range of volatile and non-volatile products (Figure 2.3). These volatile and non-volatile products are themselves unstable and undergo further oxidation and/or decomposition to a range of oxidized products responsible for the off-flavors associated with rancid oils (Eskin and Pryzbylski, 2001).

The susceptibility of fatty acids to oxidation depends on their ability to donate a hydrogen atom. Unsaturated fatty acids with more than one double bond are particularly susceptible to oxidation due to presence of methylene interrupted bond configuration, where a methylene carbon atom is located between two double bonds. The relative oxidation rates for oleic, linoleic, and linolenic acids were reported to be in the order of 1:12:25 based on the peroxides formed (Eskin and Pryzybylski, 2001).

2.2.2.2.2. Photooxidation

Photooxidation is a far more detrimental to the stability of vegetable oils than free-radical oxidation. Most oils contain photosensitizers, natural pigments such as chlorophyll and its degradation products, heme and related compounds, methylene blue, fluorescein derivatives, erythrosine, and polycyclic aromatic hydrocarbons capable of transferring energy from light to chemical molecules. The following reactions outline the process of photooxidation:

$$\text{Sensitizer}_{\text{Ground}} + h\nu \longrightarrow \text{Sensitizer}_{\text{Excited}} \qquad (2.1)$$

$$\text{Sensitizer}_{\text{Excited}} + \mathbf{LH} \longrightarrow \text{Sensitizer}_{\text{H}} + \mathbf{L^{\bullet}} \qquad (2.2)$$

$$\text{Sensitizer}_{\text{Excited}} + {}^{3}O_2 \longrightarrow \text{Sensitizer}_{\text{Ground}} + \mathbf{{}^{1}O_2} \qquad (2.3)$$

$${}^{1}O_2 + LH \longrightarrow \mathbf{LOO^{\bullet}} + {}^{3}O_2 \qquad (2.4)$$

Energy (hv) is transferred from light to sensitizer ($Sensitizer_{Excited}$), which may react directly with lipid (LH) forming radicalss ($L^{\bullet}$) , thereby initating autoxidation (Equation 2.2). The direct formation of lipid radicals is less likely to occur due to higher energy requirement. The more damaging reaction is between the excited sensitizer and ground state oxygen to form singlet oxygen (Equation 2.3). Singlet oxygen has been shown to react with linoleic acid 1500 times faster than ground state oxygen. This very reactive component is considered the most important initiator of the free-radical aoutoxidation of fatty acids. Exposure to light in the presence of photosentisizer and oxygen can cause the formation of singlet oxygen and free radicals (Equation 2.2 and 2.3). This process initiates free-radical reactions in which lipid radicals are formed and autoxidation started (Eskin and Pryzybylski, 2001).

2.3. Measurement methods of lipid oxidation

Numerous analytical methods are routinely used for measuring lipid oxidation in foods. However, there is no uniform and standard method for detecting all oxidative changes in all food systems. Therefore, it is necessary to select a proper and adequate method for a particular application. The available methods to monitor lipid oxidation in foods can be classified into five groups based on what they measure:

- The absorption of oxygen
- The loss of initial substrates
- The formation of free radicals
- The formation of primary oxidation products
- The formation of secondary oxidation products

A number of physical and chemical tests, including instrumental analyses, have been employed in laboratories and the industry for measurement of various lipid oxidation parameters. These include the weight-gain and headspace oxygen uptake method for oxygen absorption; chromatographic analysis for changes in reactants; iodometric titration, ferric ion complexes, and Fourier transform infrared (FTIR) method for peroxide value; spectrometry for conjugated dienes and trienes, 2-thiobarbituric acid TBA) value, p-anisidine value (p-AnV), and carbonyl value; Rancimat and Oxidative Stability Instrument (OSI) method for oil stability index; and electron spin resonance

(ESR) spectrometric assay for free-radical type and concentration. Other techniques based on different principles, such as differential scanning calorimetry (DSC) and nuclear magnetic resonance (NMR), have also been used for measuring lipid oxidation. In addition, sensory tests provide subjective or objective evaluation of oxidative deterioration, depending on certain details (Shahidi and Zhong, 2005).

2.3.1. Measurement of oxygen absorption

2.3.1.1. Weight gain

Consumption of oxygen during the initial stage of autoxidation results in an increase in weight of fat or oil, which theoretically reflects its oxidation level. Heating an oil and periodically testing for weight gain is one of the oldest methods for evaluating oxidative stability. In this method, oil samples (about 2.0 g) are weighed into Petri dishes; then traces of water are removed by placing the samples overnight in a vacuum oven at 35°C and over a desiccant. Samples are then reweighed and stored in an oven at a set temperature. The weight gain of the samples may be recorded at different time intervals (Shahidi and Wanasundara, 2008).

Olcott and Einset (1958) reported that marine oils exhibit a fairly sharp increase in their weight at the end of the induction period and are rancid by the time they gain 0.3%–0.5% in weight (at 308°C-608°C). Ke and Ackman (1976) reported that this method is simple, has a satisfactory reproducibility, and may be used to compare oxidation of lipids from different parts of fish. Recently, Wanasundara and Shahidi (1994, 1996) used this method to compare storage stability of vegetable and marine oils as affected by added antioxidants and were able to compare relative activity of antioxidants employed. However, surface exposure of the sample to air is an important variable in determining the rate of oxidation. Therefore, use of equal size containers to store samples is essential when carrying out such experiments (Shahidi and Wanasundara, 2008).

The weight gain method also suffers from certain disadvantages: (1) the weighing frequency hinders monitoring of fast kinetics (a higher frequency would involve nocturnal weighing), and low or moderate temperatures require long analysis times for stable samples; (2) discontinuous heating of the sample (which must be cooled before weighing) may give rise to nonreproducible results, so the heating and cooling

intervals must be accurately controlled; (3) the method involves intensive human participation; and (4) the working conditions (sample size, shape of container, and temperature) may influence the results. Nevertheless, this method offers advantages, such as low instrumentation cost as well as unlimited capacity and speed for sample processing (Shahidi and Wanasundara, 2008).

2.3.1.2. Headspace oxygen uptake

Oxygen consumption can also be measured directly by monitoring the drop of oxygen pressure. In this method, an oil sample is placed in a closed vessel also containing certain amount of oxygen at elevated temperatures, commonly around 100°C. The pressure reduction in the vessel, which is due to oxygen consumption, is monitored continuously and recorded automatically. The induction period as the point of maximum change in rate of oxygen uptake can be calculated (Velasco and Dobarganes, 2002). A commercial instrument for this method, known as Oxidograph, is available. In the Oxidograph, the pressure change in the reaction vessel is measured electronically by means of pressure transducers (Shahidi and Zhong, 2005).

The headspace oxygen method is simple and reproducible and may be the best analytical method to evaluate the oxidative stability of fats and oils. Its application in measurement of lipid oxidation in food products other than fats and oils, however, is limited because protein oxidation also absorbs oxygen (Shahidi and Zhong, 2005).

2.3.2. Measurement of reactant change

Methods that measure primary changes of lipids may be classified as those that quantify loss of reactants (unsaturated fatty acids). Measurement of changes in fatty acid composition is not widely used in assessing lipid oxidation because it may require total lipid extraction from food and subsequent conversion to derivatives suitable for gas chromatographic analysis. Separation of lipids into neutral, glycolipid, phospholipid, and other classes may also be necessary. However, it has been proven that this method serves as a useful technique to identify class of lipids and fatty acids that are involved in the oxidative changes and also to assess lipid oxidation induced by different metal complexes that afford a variety of products. On the other hand, changes of fatty acid composition cannot be used in more saturated

oils because this indicator reflects only the changes that occur in unsaturated fatty acids during oxidation. Similarly, changes in iodine value due to loss of unsaturation during accelerated oxidation studies may be used as an index of lipid oxidation (Shahidi and Zhong, 2005).

2.3.3. Measurement of Primary products of oxidation

2.3.3.1. Peroxide value (PV)

Oxidation of lipids is a major cause of their deterioration, and hydroperoxides formed by the reaction between oxygen and unsaturated fatty acids are the primary products of this reaction. Hydroperoxides have no flavor or odor but breakdown rapidly to form aldehydes, which have a strong, disagreeable flavor and odor. The peroxide concentration usually expressed as peroxide value, is a measure of oxidation or rancidity in early stages. Peroxide value (PV) measures the concentration of substances (in terms of miliequivalents of peroxide per 1000 grams of sample) that oxidize potassium iodide to iodine (O'Brien, 2009).

The iodometric method for determination of PV is applicable to all normal fats and oils, but it is highly empirical and any variation in procedure may affect the results. This method also fails to adequately measure low PV because of difficulties encountered in determination of the titration end point. Therefore, the iodometric titration procedure for measuring PV has been modified in an attempt to increase the sensitivity for determination of low PV. The modification involves the replacement of the titration step with an electrochemical technique in which the liberated iodine is reduced at a platinum electrode maintained at a constant potential. PV ranging from 0.06 to 20 meq/kg has been determined in this manner, but it is essential to deaerate all solutions to prevent further formation of peroxides. Several other chemical methods have also been suggested for monitoring PV. Colorimetric methods based on the oxidation of Fe^{2+} to Fe^{3+} and determination of Fe^{3+} as ferric thiocyanate, and a 2,6-dichlorophenol-indophenol procedure are reported in the literature. In studies on the oxidation of biological tissues and fluids, measurement of fatty acid hydroperoxides is more common than measurement of their decomposition products. Fatty acid hydroperoxides can be analyzed by high-performance liquid chromatography (HPLC) or their corresponding hydroperoxy acid reduction products

may be determined by gas chromatography mass spectrometry (GC–MS) (Shahidi and Wanasundara, 2008).

Fluorescence methods have also been developed to determine hydroperoxides by allowing them to react with substances such as luminol and dichlorofluorescein, which form fluorescent products. Although determination of PV is common, its usefulness is generally limited to the initial stages of lipid oxidation (Shahidi and Wanasundara, 2008).

2.3.3.2 Conjugated dienes and trienes

Oxidation of polyunsaturated fatty acids is accompanied by an increase in the ultraviolet absorption of the product. Lipids containing methylene interrupted dienes or polyenes show a shift in their double bond position during oxidation due to isomerization and conjugate formation. The resulting conjugated dienes exhibit an intense absorption at 234 nm; similarly conjugated trienes absorb at 268 nm.

Farmer and Sutton (1946) indicated that the absorption increase due to the formation of conjugated dienes and trienes is proportional to the uptake of oxygen and formation o f peroxides during the early stages of oxidation. St. Angelo et al. (1972) studied the autoxidation of peanut butter by measuring the PV and absorption increase at 234 nm due to the formation of conjugated dienes. Shahidi et al. (1994) and Wanasundara et al. (1995) found that conjugated dienes and PV of marine and vegetable oils correlate well during their oxidation. These authors concluded that the conjugated diene method may be used as an index of stability of lipids in place of or in addition to PV. However, carotenoid containing oils may give high absorbance values at 234-236 nm because of the presence of double bonds in the conjugated structure of carotenoids. The conjugated diene method is faster than PV determination, is much simpler, does not depend on chemical reactions or color development, and requires a smaller sample size. However, presence of compounds absorbing in the same region may interfere with such determinations (Shahidi and Wanasundara, 2008).

2.3.4. Measurement of Secondary products of oxidation

2.3.4.1. Thiobarbituric Acid (TBA) Test

One of the oldest and most frequently used tests for assessing lipid oxidation in foods and other biological systems is the 2-thiobarbituric acid (TBA) test. The extent of lipid oxidation is reported as the TBA value and is expressed as milligrams of malonaldehyde (MA) equivalents per kilogram sample or as micromoles MA equivalents per gram sample. MA is a relatively minor product of oxidation of polyunsaturated fatty acids that reacts with the TBA reagent to produce a pink complex with an absorption maximum at 530-532 nm. The adduct is formed by condensation of two molecules of TBA with one molecule of MA (Tarladgis et al., 1964).

Other products of lipid oxidation, such as 2-alkenals and 2,4-alkadienals, also react with the TBA reagent. However, the exact mechanism of their reaction with the TBA reagent is not well understood. There are several procedures for the determination of TBA values. The TBA test may be performed directly on the sample, its extracts, or distillate. In case of the distillation method, volatile substances are distilled off with steam. Then the distillate is allowed to react with the TBA reagent in an aqueous medium. The advantage of the distillation method is the absence of interfering substances. In the extraction method, TBA-reactive substances (TBARSs) are extracted from food material into an aqueous medium (i.e., aqueous trichloroacetic acid) prior to color development with the TBA reagent (Shahidi and Wanasundara, 2008).

The main disadvantages of both of these methods are long assay time and possibility of artifact formation. In the direct assay method, lipid sample (oil) reacts with the TBA reagent and the absorbance of the colored complex so prepared is recorded. The direct assay method is simple and requires less time for sample preparation Nonetheless, it is preferable to quantitative the extent of lipid oxidation by a complementary analytical procedure in order to verify the results (Shahidi and Wanasundara, 2008).

Several attempts have been made to establish a relationship between TBA values and the development of undesirable flavors in fats and oils. It has been shown that flavor

threshold values correlate well with the TBA results of vegetable oils, such as those of soybean, cottonseed, corn, safflower (Gray, 1978) and canola (Hawrysh, 1990).

2.3.4.2. p-Anisidine Value (p-AnV)

The p-anisidine value (p-AnV) measures the amount of α and β unsaturated aldehydes present in the oil. The method is based on the fact that in the presence of acetic acid, p-anisidine reacts with the aldehydic compounds in oil, producing yellownish reaction products. The color intensity depends not only on the amount of aldehydic compounds present, but also on their structure. Thus, it has been found that the double bound in the carbon chain conjugated to the carbonyl double bond enhances the molar absorbance at 350 nanometers by a factor of 4 or 5. Anisidine value is a measure of secondary oxidation, or the history of oil, and therefore is useful in determining the quality of crude oils and the efficiency of processing procedures, but it is not suitable for the detection of fat oxidation. Anisidine values below 2 to 3% after deodorization are indicative of good oil stability (Mag, 1990; O'Brien, 2009).

2.3.4.3. Totox value

The totox value is a measure of the total oxidation, including primary and secondary oxidation products. It is combination of PV and p-AnV:

$$\text{Totox value} = 2\text{PV} + \text{p-AnV}$$

During lipid oxidation, it is often observed that PV first rises, then falls as hydro-peroxides decompose. PV and p-AnV reflect the oxidation level at early and later stages of oxidation reaction, respectively. Totox value measures both hydroperoxides and their breakdown products, and provides a better estimation of the progressive oxidative deterioration of fats and oils. However, Totox value has no scientific basis because it is a combination of two indicators with different dimensions (Shahidi and Zhong, 2005).

2.3.4.4. Accelerated stability tests

2.3.4.4.1. Schall oven test

The Schaal Oven test involves heating an oil sample to 50-60°C in an oven. The end-point of oxidation can be detected either by sensorial characteristics or by suitable end-point detection (PV, TBA value). Because this test uses relatively low

temperatures, the oil is exposed to mild oxidative stress. The Schaal Oven test correlates well with actual shelf life predictions. This method is time and labor-consuming and thus impractical as a routine method, which requires a short analysis time (Verleyen et al., 2005).

2.3.4.4.2 Active Oxygen Method (AOM Test)

In the official AOM test, purified air is bubbled through a fat sample held in a heated oil bath at 97.8°C. At various time intervals, aliquots of oil sample are taken out of the flask for determination of the PV. The PV is plotted against time and the time required to reach a PV of 100 mEq/kg fat is reported as the AOM time. Because lipid oxidation is a dynamic process, the PV must be determined at regular time intervals. Estimation of the time required to reach a PV of 100 mEq/kg should be based on two peroxide measurements ranging between 75 and 175 mEq/kg. If a PV > 175 mEq/kg is obtained, the analysis should be restart. Several modifications to the official AOM method were made over the years.

The AOM test has several disadvantages; it is a rather time-consuming and labor-intensive test. Problems with the AOM test may also arise from shortcuts introduced by the operator but that are also partly inherent in the procedure. The AOM test requires at least two titrations of the PV on one oil sample. However, in some quality control laboratories, the method is substituted by a pass/fail system in which a sample is analyzed for a predetermined time range and a single PV is determined. If the resulting PV is below a fixed value, the sample passes the test and is deemed to meet the specification (Verleyen et al., 2005).

Another major deficiency is the determination of the end-point during the rapid oxidation process. During the rapid and accelerated oxidation phase, the reaction is dependent upon the oxygen supply. Variations in oxygen supply can result in poor reproducibility between duplicate samples. Data obtained from an interlaboratory study published an actual coefficient of variation (CV) of 35%. This means that independent laboratories would report an AOM value of 100 ± 35 h for an oil sample with an AOM specification of 100 h (Jebe et al., 1993). The combined effect of these problems is a large variability in the AOM time reported for any particular sample.

Consequently, alternative methods were developed to replace the AOM test as an accelerated method to study the stability of oils and fats (Verleyen et al., 2005).

2.3.4.4.3 Oxidative Stability Index (Rancimat and OSI)

The same basic principle lies behind the Rancimat and OSI. These instruments differ only slightly in design and operating convenience. OSI operate by a stream of purified air passing through a sample of fat or oil that is held in a thermostated aluminum heating block. The air distribution system does not heat the air before being bubbled into the oil. The incoming air is regulated with a needle valve to control the flow rate. After passing through the oil, the effluent air is passed into a detection cell that contains deionized water. OSI use the formation of volatile oxidation products as a marker to detect the induction point in the lipid oxidation process. The effluent air containing volatile organic acids from oil oxidation increases the conductivity of the water in the detection cell. Initially, a manual integration of the induction point on the conductivity curve was required. Currently, the conductivity measurement is linked to a computer software program, which allows an automated selection of the induction point in the conductivity curve (Jebe et al., 1993).

At the start of the oxidation experiment, the conductivity of the water in the detection cell is very low. Heating the oil and simultaneously passing air through it will accelerate the oxidation process. Initially, peroxides will be formed which are unstable and break down to secondary oxidation products. Different secondary oxidation products will be formed depending on the type of oil. Many of these secondary oxidation products have a relatively low volatility. They will not be distilled over into the water in the detection cell, but will remain in the oil. Aldehydes will be further oxidized to short-chain fatty acids. These short-chain acids are volatilized and will condense in the water of the detection cell, increasing its conductivity. Consequently, the conductivity of the water has a direct relation to the degree of oil oxidation (Verleyen et al., 2005).

The induction time reported by OSI depends on several operational parameters. The most important parameter influencing the induction time is clearly the operating

temperature because lipid oxidation is a chemical reaction, which is temperature dependent. At higher temperatures, lipid oxidation will proceed more quickly, leading to a lower induction point. The temperature of the heating block in the Rancimat and OSI instrument can be varied between 50 and 160°C. Generally, the oxidative stability of oils and fats is determined at 100 or 110°C. Accelerated tests on saturated fats are often carried out at temperatures of 120–130°C to reduce the analysis time. On the other hand, lipids sensitive to oxidation are often analyzed at lower temperatures in the OSI, ranging between 60 and 80°C. Fish oil, for example, is frequently analyzed at 68°C (Mendez *et al.* 1996; Verleyen et al., 2005).

2.3.5. Free fatty acid and acid value

Hydrolytic rancidity occurs as a result of splitting of the triglyceride molecule at ester linkage with the formation of free fatty acid (FFA), which can contribute objectionable odor, flavor, and other characteristics. The flavor resulting from FFA development depends on composition of the fat. Both acid value and FFA are measures of the free fatty acid content of fats and oils. Acid value is the amount of potassium hydroxide required for neutralization, whereas FFA utilizes sodium hydroxide for neutralization. FFA results may be expressed in terms of acid value by multiplying the FFA percent by 1.99. FFA is calculated as free oleic acid on a percentage basis for most fats and oils sources, although for coconut and palm kernel oils it is usually calculated as lauric acid and for palm oil as palmitic acid (O'Brien, 2009).

Free fatty acid content monitoring during and after all processes, including storage provides process control results that identify potential problems for which corrective actions can be initiated on a timely basis. FFA is the result of hydrolysis of the fat and oil. Moisture must be present for hydrolysis to develop. This reaction is accelerated with heat and pressure, as are most reaction (O'Brien, 2009).

2.3.6. Shelf life and rancidity studies pistachio puree like products in the literature

Knowing the spoilage mechanism of a food product is the first step in the process of determining its shelf life. Essentially, how a food spoils and hence how long its shelf life is going to be influenced by a number of factors. This shelf life influencing

factors are the properties of the final product and of the environment in which it is to be manufactured, stored distributed and used. These factors can be divided into the groups of intrinsic factors (raw product, composition and structure, a_w, pH etc.) and extrinsic factors (packaging material, storage time, temperature, humidity, light). Temperature, the single most important environmental factor, influences all mechanisms of food spoilage, so the effects of temperature must be evaluated in all shelf life studied (Sedaghat, 2010).

There are very limited studies in shelf life or rancidity of pistachio, pistachio puree and pistachio paste in literature. However, shelf life studies about the peanut and hazelnut products are more available than pistachio products.

Meugo- Gnanasekharan and Resurreccion (1992) stated that when a detectable oxidized flavor was used as the indicator of storage deterioration, peanut paste had a predicted shelf life of 152 days at 30°C; 98 days at 40°C; and 96 days at 50°C.

Maskan and Karataş (1999) stored pistachio nuts at ambient conditions (20-30°C), under air and CO_2 at 10, 20, 30°C. The peroxide value on ambient storage increased rapidly from 0.651 to 14.9, whereas peroxide value of other conditions did not show a rapid increase.

In one study, a peanut line had been developed at the University of Florida with about 80% oleic and 3% linoleic acid. Volatiles and sensory characteristics of roasted normal (55% oleic acid) and high oleic acid peanuts stored at 25°C were compared. Peroxide values were lower for high oleic peanuts than normal peanuts during storage at 25°C and 40°C and the hexanal content of the peanuts was higher for normal than high oleic acid peanuts. Peanutty flavor was more stable for high oleic acid peanuts than normal after 6 week storage. Painty and cardboard flavors were higher in normal peanuts than high oleic peanuts during storage. Shelf life was estimated from sensory data to be two times longer in high oleic acid peanuts (Braddock et al., 1995).

Özçelik (2000) studied the effect of rosemary extracts on prolonging shelf life of hazelnut puree. For this purpose, Stability of hazelnut puree was observed by,

Differential Scanning Colorimetry (DSC), Rancimat test and Schaal Oven test. Induction periods of hazelnut puree oils with or without antioxidants were measured at 100, 110, 120, 145, 155 and 165°C at DSC; at 100, 110 and 120°C at Rancimat; at 30, 40 and 50°C at Schaal Oven Test. Induction time of hazelnut puree oil was found as 12.8 and 14.5 in DSC and Rancimat results (120 °C), respectively. In Schaal oven test, peroxide value, p-anisidine value and conjugated diene values of samples were increased with increasing temperature during storage.

Nepote et al (2006) determined the oxidative stability in honey roasted peanuts and roasted peanuts stored at -15, 23 and 40°C. The chemical analyses (peroxide, thiobarbituric acid reactive substance (TBARS)) and descriptive analyses were performed during 126 days of storage. It was observed that addition of honey coating provided protection against lipid oxidation. Peroxide value reached 10 meqO_2 kg-1 after 6 days in roasted peanuts and 36 days in honey roasted peanuts at 23 °C.

Çapanoğlu and Boyacıoğlu (2008) examined the effect of adding stabilizer, antioxidant mixture and maltose syrup to prevent undesirable quality changes of almond paste during storage at 4°C and 30°C. For this purpose, peroxide value, free fatty acid and Rancimat analyses have been carried out during storage. It was observed that peroxide and free fatty acid values increased as the storage time progressed at both temperatures. Induction period of the samples stored at 30 °C were significantly lower ($p<0.05$) than those of samples stored 4°C. Also, induction periods of control samples (0.29-4.16 h) were significantly less than those of samples containing antioxidant in their formulation (4.20-36.28 h) for both 4°C and 30°C.

Arranz et al. (2008) determined the induction time of walnut, almond, hazelnut, peanut and pistachio nut oils by use of Rancimat method which operated with 15 L/h air flow rate at 100°C. The oxidative stability of nut oils was found as 4.7, 21.8, 52.7, 14.6 and 44.4 hour, respectively.

Olmedo et al. (2009) analyzed peroxide value, p-anisidine value, conjugated diene measurements and descriptive analyses on the fried salted peanuts, fried salted peanuts with oregano essential oil and fried- salted peanuts with oregano essential oil and olive oil during 112 days of storage. Fried- salted peanut with olive oil and

oregano essential oil showed higher stability. Peroxide value and p- anisidine value were lower in all cases. It was also observed that the oxidized and cardboard flavor intensities increased more in fried-salted peanuts durind storage than the other products while the roasted peanutty flavor also decreased more in fried-salted peanuts.

In another study, Peroxide value, Free Fatty Acid and Induction time of nuts were investigated at 21%, 8%, and <2%O_2 and different storage temperature (5, 20, 35 and 45°C). Samples were experimented at 4, 6, 8, 10 and 12 weeks by use of split-plot design and the modeling shelf of this product at various conditions. Results showed that the Induction time under factors of temperature and storage time were high significant but under factor of O_2 was significant and optimum storage condition for raw dried pistachio nuts determined at 20°C and <2%O_2 based on Induction time (Sedaghat, 2010).

Riveros et al. (2010) compared the chemical (peroxide and p-anisidine and conjugated dienes) and sensory (roasted peanutty, oxidized and cardboard flavours) stability of peanut paste prepared with high-oleic peanuts with that of peanut paste prepared with normal peanuts stored at 4, 23 and 40°C from Argentina. It was found that peanut paste prepared with high-oleic peanuts had four (at 4°C), two (at 23°C) and three (at 40°C) times longer shelf-life than peanut paste prepared with normal peanuts.

Shakerardekani et al. (2011) studied the effect of roasting conditions (90-190°C temperature and 5-65 min. times interval) on hardness, moisture content and colour of pistachio kernels by response surface methodology (RSM). Roasting of whole-kernels is an important step in the production of pistachio paste. In this study, it was found that the recommended range of roasting temperature and time of whole-kernel for the production of pistachio paste were 130-140°C and 30-40 minute, respectively.

2.4. Antioxidants

Antioxidants are any substance that when present at low concentrations compared to those of an oxidizable substrate significantly delays or prevents oxidation of that substrate. While antioxidants are associated primarily with inhibition of lipid

peroxidation, free radicals can also damage other components, so that "oxidizable substrate" includes almost everything found in foods and in living tissues such as proteins, lipids, carbohydrates, and DNA. In general, antioxidants do not prevent oxidation, but rather extend or retard the induction period (Wanasundara and Shahidi, 2005).

Antioxidants can act at different steps in the oxidation sequence, depending on their mode of action (Figure 2.4). The primary or chain-breaking antioxidant reacts with lipid radicals to yield more stable products and such antioxidants are known as free radical interceptors. The secondary or preventative antioxidants reduce the rate of chain initiation by a variety of mechanisms which include metal inactivators, hydroperoxide decomposers, oxygen scavengers, and synergists.

A primary antioxidant rapidly donates a hydrogen atom to a lipid radical, or is converted to other stable products. Free-radical interceptors inhibit two important steps in the free-radical chain sequence of lipid oxidation. They react with peroxyl radicals (LOO*) to stop chain propagation, thus inhibiting formation of peroxides (Equation 2.5), and with alkoxyl radicals (LO*) to decrease the decomposition of hydroperoxides to harmful degradation products (Equation 2.6).

$$LOO^{*} + AH \longrightarrow LOOH + A^{*} \qquad (2.5)$$

$$LO^{*} + AH \longrightarrow LOH + A^{*} \qquad (2.6)$$

Metal inactivators or chelating agents act as preventative antioxidants by removing or deactivating metal ions, which act as initiators as well as catalyze the decomposition of hydroperoxides. Decomposers of hydroperoxides transfer them into stable hydroxy compounds by reduction, while oxygen scavengers react with oxygen to deplete the supply of oxygen needed for autoxidation. Synergism can be expected between substances with differing modes of action, so that multicomponent antioxidant systems exhibit much greater antioxidant activity beyond that expected from the additive effects of individual antioxidants. Synergists generally extend the life of primary antioxidants by acting as hydrogen donors to their radicals, thereby regenerating the primary antioxidants or inactivating metal ions (Eskin and Pryzybylski, 2001).

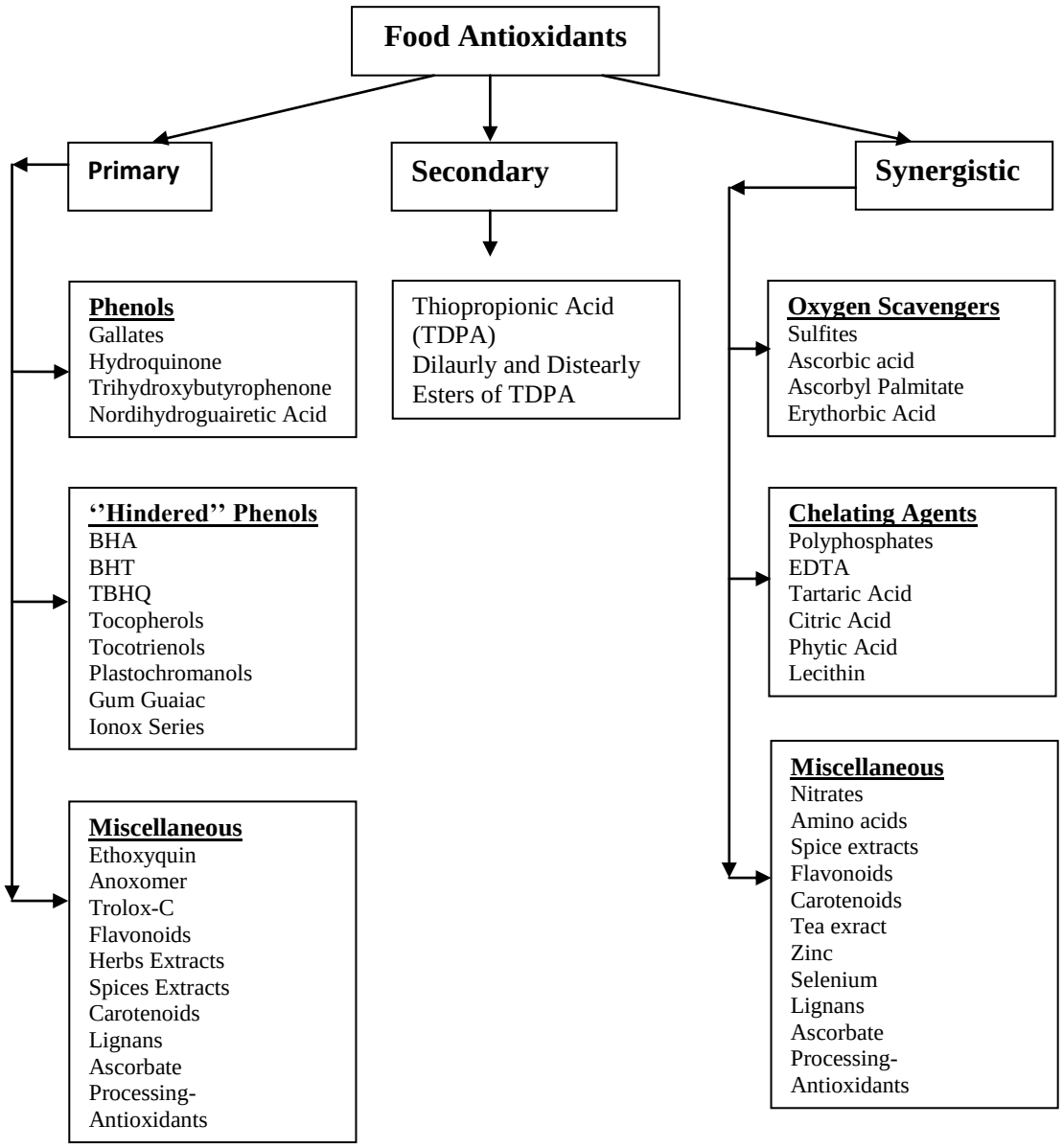


Figure 2.4. Types of Antioxidants (Eskin and Pryzybylski, 2001).

2.4.1. Food Antioxidants

Antioxidants, as defined by the U.S. Food and Drug Administration (FDA), are substances used to preserve food by retarding deterioration by rancidity or discoloration due to oxidation. They are compounds present in small quantities capable of preventing or retarding oxidation of oils and fats.21 Antioxidants can act in cell membranes and/or food products by: (1) scavenging free radicals, which initiates oxidation; (2) inactivating metal ions; (3) removing reactive oxygen species such as oxygen radicals; (4) breaking the initiated chain of reactions; (5)

quenching/scavenging singlet oxygen; (6) destroying peroxides to prevent radical formation; and (7) removing oxygen and/or decreasing local oxygen concentration/pressure (Labuza, 1982; Eskin and Pryzybylski, 2001).

They can be classified as primary, secondary, or synergists, depending on their particular function (Figure 2.4). Primary antioxidants, which break the chain reaction of oxidation by hydrogen donation and generation of more stable radicals, and, secondary antioxidants, which slow the oxidation rate by several mechanism. A number of synthetic and natural antioxidants are used commercially to stabilize food products or pure animal fats and vegetable oils. An antioxidant acceptable for food use must meet several essential requirements: effectiveness at low concentrations, compatibility with substrate, and absence of sensory influence, non-toxic, and not affecting physical properties of food products (Schuler, 1990; Eskin and Pryzybylski, 2001).

2.4.1.1. Primary antioxidants

This group of antioxidants donates hydrogen atoms to free lipid radicals to terminate free radical chain reactions by forming stable products. Such antioxidants include polyhydroxy phenolics as well as the hindered phenolic components listed in Figure 2.4. In addition, many natural phenolic compounds including flavonoids, eugenol, and other components from herbs and spices also form part of this group (Eskin and Pryzybylski, 2001).

2.4.1.2. Secondary antioxidants

Secondary antioxidants function by decomposing lipid peroxides into more stable end products. This group includes thiopropionic acid and it derivatives (Eskin and Pryzybylski, 2001).

2.4.1.3. Synergists

Synergistic antioxidants are primarily oxygen scavengers and metal chelators. They operate by a number of mechanisms including regeneration of primary antioxidants by donating hydrogen atoms to phenoxyl radicals or by proving a more stable acid environment for these antioxidants. Ascorbic acid, sulfites, and erythorbic acid are examples of oxygen scavengers, while EDTA, citric acid, and phosphates function as

metal chelators. Compounds listed under miscellaneous are capable of functioning as primary antioxidants and synergists (Figure 2.4). A large number of compounds found in animal and plant tissues, also available as synthetic molecules, are used in food applications. These include tocopherols and ascorbic and citric acids, often used in combination with each other or other antioxidants to take advantage of synergistic effects. Although commonly used for years as a spice or flavoring agent, extracts of rosemary leaves have become the subject of increasing interest as inhibitors of lipid oxidation, and are used commercially. Rosemary extract contains many different compounds including carnosol, carnosic acid, and rosmanol thought to act synergistically as antioxidants and now available commercially (Eskin and Pryzybylski, 2001).

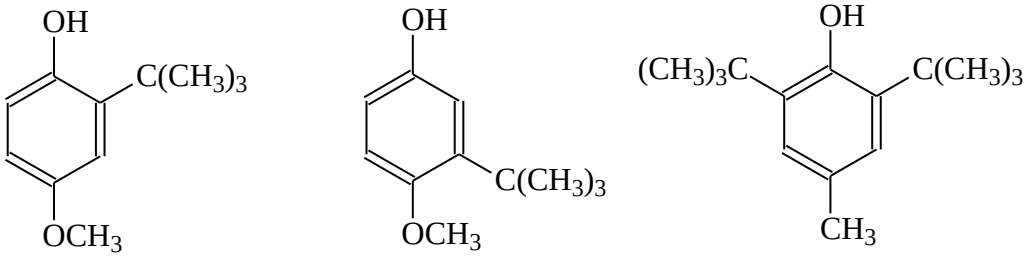
2.4.2. Synthetic Antioxidants

The most commonly used antioxidants are butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), *tert*-butyl hydroquinone (TBHQ), and propyl gallate (PG), often used in combinations. Various regulations exist in different countries t control the amount or application of use of food antioxidants. In Canada, TBHQ has only recently been permitted for use in foods, even though it has been used for a number of years in other countries. Toxicological data have led to concerns regarding the potential toxicity of BHA such as the development of carcinoma in rodents if consumed routinely. Some of these studies were criticized for their use of excessive concentrations and thereby overestimating the hazards. Nevertheless, there is a worldwide trend to reduce or avoid use of synthetic food antioxidants (Eskin and Pryzybylski, 2001).

2.4.2.1. BHA (Butylated Hydorxyanisole) and BHT (Butylated Hydoxytoluene)

The most widely used synthetic antioxidants are BHA and BHT, both monohydroxy phenols (Figure 2.5). BHA is a mixture of two isomers, 3-tertiary-butyl-4-hydroxyanisole (90%) and 2-tertiary-butyl-4-hydroxyanisole (10%). Both antioxidants are fat soluble and exhibit good carry-through effects; although BHA is slightly better. BHT is particularly effective in protecting animal fats from oxidation while BHA is more effective in vegetable oils. BHA is particularly effective in protecting the flavor and color of essential oils. It also protects effectively short-chain fatty acids, such as those found in cereal and confectionery products, from

oxidation. Because of their volatility, these antioxidants are not effective in frying. BHA and BHT are both used in packaging materials as they can migrate into the food. Together they act synergistically and are used as such in many food antioxidant formulations (Eskin and Pryzybylski, 2001).



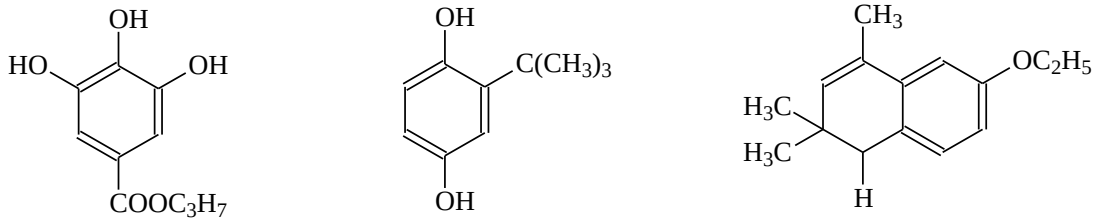
2-BHA **3-BHA**

Butylated Hydroxyanisole (BHA) **Butylated Hydroxytoluene (BHT)**

Figure 2.5. Structure of synthetic antioxidants (Eskin and Pryzybylski, 2001).

2.4.2.2. Propyl Gallate (PG)

Produced commercially by esterification of gallic acid with propyl alcohol, PG acts synergistically with BHA and BHT (Figure 2.6). Because PG chelates metal ions such as Fe to form a blue–black complex, it is used with a chelator such as citric acid to prevent food discoloration. PG, like BHA and BHT, loses its effectiveness with heat due to evaporation and is therefore unsuitable in frying oils (Eskin and Pryzybylski, 2001).



Propyl Gallate (PG) **TBHQ** **Ethoxyquin**

Figure 2.6. Structure of propyl gallate, tertiary-butyhydroquinone (TBHQ) and ethoxyquin (Eskin and Pryzybylski, 2001).

2.4.2.3. Tertiary-Butylhydroquinone (TBHQ)

Because of its excellent carry-through properties, TBHQ is considered by far the best antioxidant for use in frying oils (Figure 2.6). TBHQ does not chelate metal ions and is used in combination with citric acid. Commercially, TBHQ is used alone or with BHA and BHT at a maximum level of 0.02% or 200 ppm depending on the fat content of the food. As a diphenolic antioxidant, TBHQ reacts with the peroxy radicals forming a semiquinone resonance structure (Eskin and Pryzybylski, 2001).

2.4.2.4. Ethoxyquin

Ethoxyquin, chemical name 6-ethoxy-1,2-dihydro-2,2,4-trimethylquinoline, is used mainly as a feed antioxidant (Figure 2.6). However, it is also used to protect fish products and fish oil, poultry fats, potatoes, apples, and pears during storage. Ethoxyquin was found to effectively protect the pigments of paprika (Vinas et al., 1991). It was shown to minimize nitrosoamine formation in bacon when added at a level of 20 ppm (Bharucha et al., 1987).

2.4.3. Natural Antioxidants

Most natural antioxidants, with the exception of tocopherols, are phenolic compounds, containing *ortho*-substituted active groups, whereas synthetic antioxidants, with the exception of gallates, are *para*-substituted. The term "phenolic compound" includes a large number of secondary plant products which differ in chemical structure and reactivity, ranging from simple compounds to highly polymerized compounds. Many properties of plant products are associated with the presence of different polyphenolic compounds. Phenolic compounds possess an aromatic ring bearing one or more hydroxyl groups together with a number of other constituents. At least 5000 phenolic compounds have been identified with an early reference classifying plant phenolics into 15 groupings (Eskin and Pryzybylski, 2001).

2.4.3.1. Tocopherols and Tocotrienols

Tocopherols and tocotrienols, known as chromanols, are well recognized for their efficient protection against lipid oxidation in food and biological systems. These components are synthesized by plants and provide essential nutrients for humans and animals. Tocopherols are present in green parts of higher plants, leaves, and oil seeds

(Hess, 1993). There are eight structurally different compounds in the tocopherol family (Figure 2.7); four known as tocopherols and four known as tocotrienols. α Tocopherol is present mainly in the plant cell chloroplasts, while the other three isomers are found outside of these organelles. Tocopherols are present in refined vegetable oils at levels ranging from 60 to 110 mg/100 g. Tocotrienols are absent or present in only very small amounts in most vegetable oils with the exception of palm .They are found in the bran and germ parts of cereals and some seeds.

The basic structure of all eight of these compounds is similar (Figure 2.7), consisting of a 6-chromanol aromatic ring system containing a hydroxyl group and a 16-carbon phytol side chain. Tocotrienols differ from tocopherols by the presence of three double bonds in the phytol side chain. Tocopherols and tocotrienols both consist of α, β, γ, and δ isomers, which differ in the number of methyl groups present in the aromatic ring (Figure 2.7) (Eskin and Pryzybylski, 2001).

Chromanols are probably the most efficient lipid antioxidants produced by nature. The antioxidant activity of these components is related to the following: (1) phytyl chain with phenolic ring make them lipid soluble; (2) lipid radicals react with them several times faster than with other lipid radicals; and (3) one tocopherol molecule can protect about 103 to 108 molecules of polyunsaturated fatty acid molecules at low peroxide values. Tocopherols act as antioxidants by donating a hydrogen atom from the hydroxyl on the ring system to a free radical (Eskin and Pryzybylski, 2001).

Unsubstituted phenols are not hydrogen donors, while the reactivity of substituted phenols is mainly attributed to two factors: (1) inductive effects of electron-releasing substitutes in the position *ortho*- and *para*- to the hydroxy group/function, and (2) stereoelectronic effects related to the orientation of substituents to the aromatic ring. Electron releasing substituents present in the *ortho*- and *para*-position increases the electron density of the active center(s) promoting release of hydrogen from hydroxyl group and improving reactivity with peroxy radicals. The antioxidant activity of tocopherols is 250 times greater than BHT mainly due to the heterocyclic ring — hydrogen donor part and chroman moiety — responsible for fat solubility. Phenols with an oxy substituent in the para position to the hydroxy group produced more stable phenoxyl radicals and exhibited higher activity as antioxidants (Burton et al.,

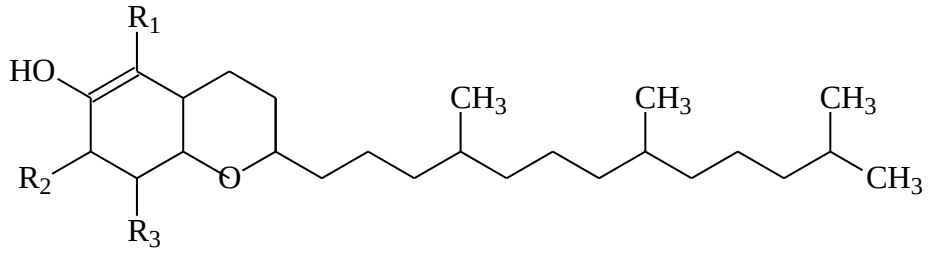
1985). More methyl substitutes in phenolic ring improved the relative antioxidant activity of tocopherol isomers but also made these isomers more soluble in fats/oils (Eskin and Pryzybylski, 2001).

2.4.3.2. Phenolic acids

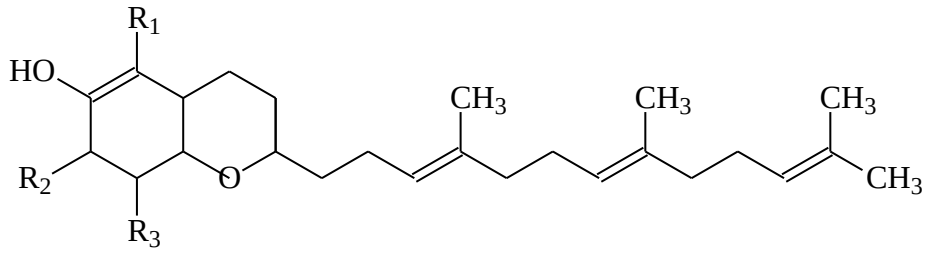
Natural antioxidants are primarily plant phenolic compounds including flavonoids, phenolic acid derivatives, coumarins, tocopherols, and polyfunctional acids. Phenolic compounds function as primary antioxidants by performing the role of free radical terminators. They interfere with lipid oxidation by rapidly donating a hydrogen atom to the lipid radicals, and the efficiency of these antioxidants (AH) increases with decreasing A-H bond strength. Phenolic antioxidants are excellent hydrogen or electron donors, and their radical intermediates are relatively stable due to resonance delocalization and general lack of suitable sites for attack by molecular oxygen. The reaction of a phenol with a lipid radical forms a phenoxy radical, which is stabilized by delocalization of unpaired electrons around the aromatic ring (Shahidi and Nazck, 1995).

Widespread interest in natural sources of antioxidants has generated an enormous amount of research to assess the antioxidant potential of novel sources of phenolic compounds as well as recognize the inherent activity of commonly consumed foods. This has been combined with the role of lipid peroxidation in human health through modulation with food sources of antioxidants. The average daily consumption of food phenolics has been reported to range from 25 mg to 1 g. (Shahidi and Nazck, 1995).

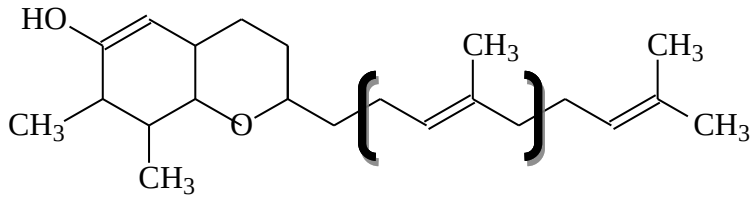
Using an oxygen electrode, Kumamoto and Sonda (1998) evaluated the antioxidant activity of 25 different kinds of tea. They correlated antioxidant activity with the amount of the four major tea catechins (epicatechin, epigallocatechin, epicatechin gallate, and epigallocatechin gallate). A correlation (r) of 0.6402 indicated that catechins contributed significantly to the antioxidant activity of green tea. However, the intercept at regression line was greater than 1, suggesting other compounds must also be involved such as other polyphenols as well as vitamin C.



Tocopherol



Tocotrienol



Plastochromanol-8

Isomer	R_1	R_2	R_3
α	**CH_3**	**CH_3**	**CH_3**
β	**CH_3**	**H**	**CH_3**
ϒ	**H**	**CH_3**	**CH_3**
δ	**H**	**H**	**CH_3**

Figure 2.7. Structure of plastochromanol and isomers of tocopherol and tocotrienol (Eskin and Pryzybylski, 2001).

Wanasundra and Shahidi (1998) compared different flavonoids with commercial antioxidants BHA, BHT, and TBHQ on the stability of marine oils during storage at 65°C. The synthetic and natural antioxidants were added to refined, bleached, and deodorized sea blubber and menhaden oils at equivalent concentrations of 200 ppm and for α-tocopherol, 500 ppm. In the case of sea blubber oil, added rutin, kaemferol, quercetin, morin, and myrcetin proved more effective than α-tocopherol, BHA, and BHT in extending the induction period. However, myricetin proved to be the most effective of all the flavonoids and was more effective than TBHQ. Flavonoids reduced the peroxide values (PV) in both sea blubber and menhaden oils, particularly the flavonols, kaemferol, morin, myricetin, quercetin, and rutin. Myricetin reduced the PV in both oils by 50%. The flavonones, naringenin and naringin, were effective to a lesser degree.

A similar pattern was observed for production of TBARS, with TBHQ the most effective antioxidant (Figure 2.8). However, myricetin, performed very similar to TBHQ throughout the storage period of both oils. Flavonols were far more effective in inhibiting TBARS formation than flavones and flavononols as shown by the following order: myricetin > quercetin > morin > rutin > kaemferol > naringenin > naringin > apigenin > taxifolin (Pratt and Hudson, 1990).

2.4.3.3. Herbs and Spices

Herbs and spices have traditionally been used to enhance the flavor of foods. Among the important members of the Labiatae family are rosemary, sage, oregano, and thyme. Almost half a century ago, Chipault (1950) investigated the antioxidant activity of a number of these spices reporting rosemary and sage to be the most effective ones in lard, while cloves proved more effective in an oil-in-water emulsion. Subsequent research by Chipault (1951) screened 17 different spices for antioxidant activity in mayonnaise- type products and reported oregano as the most beneficial. These studies pointed to the importance of identifying the type of food system used in the evaluation process. In recent years there has been a flurry of activity in identifying the antioxidant active components in herbs and spices, the

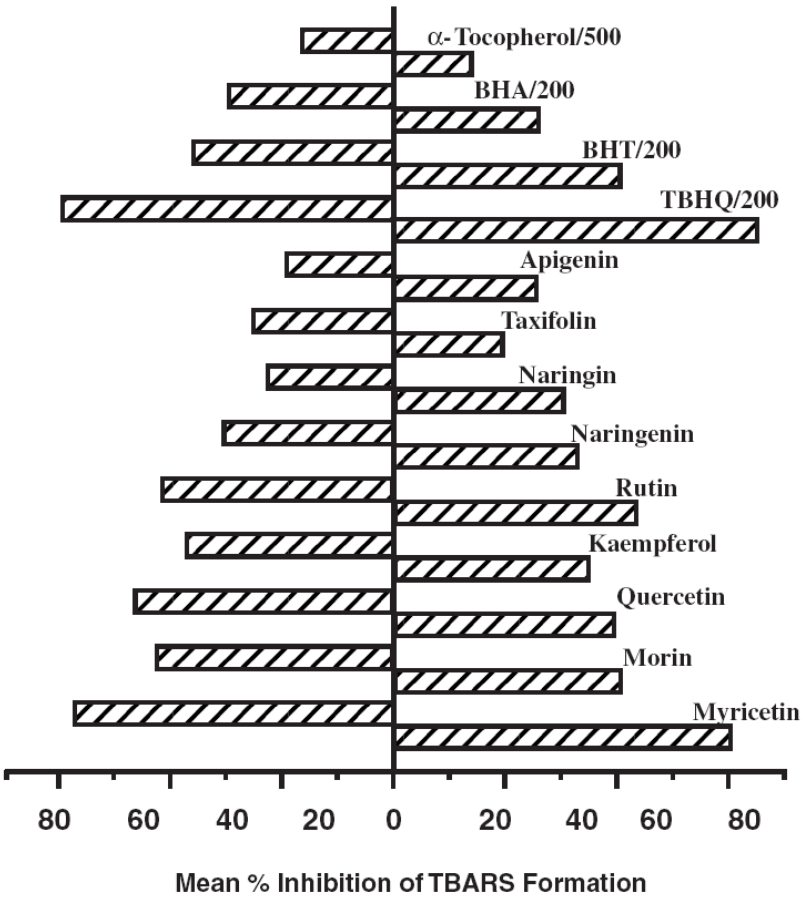
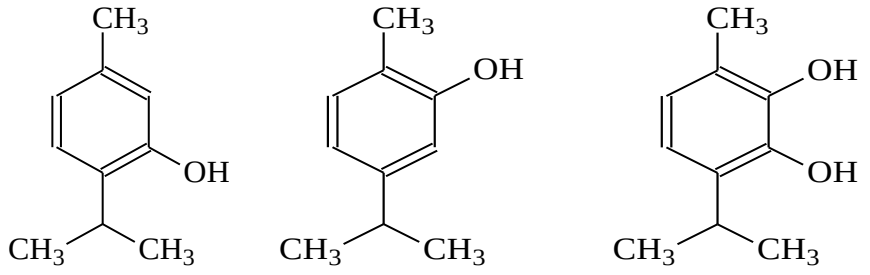


Figure 2.8. Effect of flavonoids and commercial antioxidants on 2-thiobarbituric acid reactive products (TBARS) of refined, bleached, and deodorized seal blubber and menhaden oils during accelerated storage at 65°C without light presence (Pratt and Hudson, 1990).

majority of which appear to be phenolic compounds. Since herbs and spices have been used for centuries, their antioxidant active components are considered harmless (Kim et al., 1994).

Aeschbach et al. (1994) first established that the efficacy of thymol and cavracol isomers to inhibit peroxidation of liposome phospholipids was concentration dependent. These antioxidants were identified in the essential oils from plants of the oregano species (Lagouri and Boskou, 1995). Yanishleva et al. (1999) examined the antioxidant activity and mechanism of action of thymol and carvacrol on the autoxidation of purified triacylglycerols of lard and sunflower oil. At ambient temperature, thymol was reported to be an effective antioxidant.

A number of studies examined the antioxidant properties of essential oil of thyme (Farag et al., 1989). These properties were attributed to the phenolic components, cavracol and thymol. Schwartz and Ernst (1996) studied the anitoxidant properties of a non-polar fraction isolated from thyme leaves. Besides carvacrol and thymol, these researchers isolated a new phenolic compound, *p*-cymene-2,3-diol (2,3-dihydroxy-4-isoprpropyl- 1-methylbenzene) (Figure 2.9). Using the Rancimat and Schaal Oven test (*p*-cymene-2,3-diol proved to be the most potent antioxidant and was far more effective in retarding oxidation of lard compared to α-tocopherol, BHA (Shahidi and Nazck, 1995).



Thymol **Carvacrol** **p-Cymene-2,3,-diol**

Figure 2.9. Structure of natural antioxidants (Eskin and Pryzybylski, 2001).

2.4.3.3.1. Rosemary (Rosmarinus officinalis L.)

Rosemary (*Rosmarinus officinalis L.*), which belongs to Labiatae family, is one of the most effective spices widely used in food processing (Figure 2.10) (Moss et al., 2003; Yanishlieva et al., 2006). It is used as an additive in traditional Mediterranen cusine. The fresh and dried leaves have a bitter, astringent taste which complements a wide variety of foods. Owing to its antioxidant properties of leaves, *Rosmarinus officinalis* L. has been widely accepted as one of the spices with the highest antioxidant activity (Peng et al., 2005).

Figure 2.10. The image of rosemary plant in nature

In previous studies, the main components of rosemary essential oil were found as 1,8-cineole, α-pinene, camphor, camphene, borneol, ß-caryophyllene, bornyl acetate, verbenone, linalool, limonene, sabinene and α-terpineol (Bayrak and Akgül, 1989; Wang et al., 2008; Jiang et al., 2011) and rosemary extracts were carnosol, carnosic acid, rosmanol, rosmaridiphenol, rosmarinic acid and rosmariquinone (Figure 2.11) (Bracco et al., 1981; Houlihan at al., 1985). The antioxidant activity of rosemary essential oils and extracts has been associated with the presence of these monoterpenes and phenolic components which break free radical chain reactions by hydrogen atom donation (Figure 2.11) (Aruoma et al., 1992; Basaga et. al., 1997; Georgantelis et al., 2007).

Several researchers have reported that the effectiveness of rosemary extracts for achieving higher sensory scores and retarding lipid oxidation in various food: Stoick et al. (1991) used 500-1000 ppm rosemary extract in beef steaks; Shahidi and Wanasundara (1992) recommended concentration ranging between 200 and 1000 ppm in various foods, while Sebranek et al. reported that the addition of 1000 ppm of rosemary extract was effective as BHA/BHT in maintaining low TBARS values of precooked-froozen sausage.

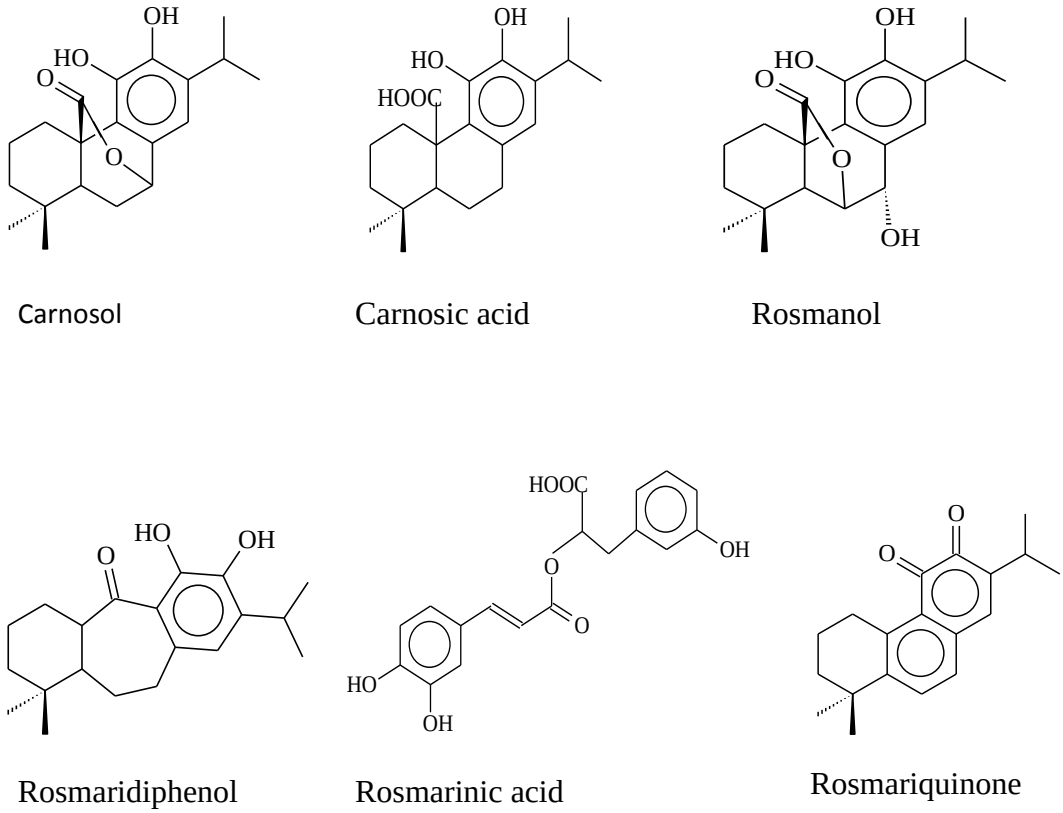


Figure 2.11. Chemical structures of antioxidatively acting compounds Rosemary (Yanishlieva et al., 2006).

Nowadays, essential oils and their components from plants are gaining increasing attention due to its relatively safe status, their wide acceptance by consumers, and possibility of their exploitation for potential multi-purpose functional uses (Viuda-Martos et al., 2009).

The antioxidant, antimicrobial, anticarcinogenic, cognition-improving and certain glucose level lowering properties of rosemary essential oil was studied by several researches (Fahim et al., 1999; Debersac et al., 2001; Oluwatuyi et al., 2004; Wang, 2007; Fu et al., 2007; Okoh et al., 2011; Jiang et al., 2011).

Wang et al. (2007) examined that the antioxidant activities of *R. officinalis* L. essential oil compared to different components (1,8- cineole, α-pinene, β- pinene). The oil and components antioxidant activities were measured by 2,2- diphenyl-1-picryhydrazyl (DPPH) assay and β-carotene bleaching test. It was found that *R.*

officinalis L. essential oil showed greater activity than its components in both systems, and the antioxidant activities of all tested samples were mostly related to their concentrations.

In another study, the antioxidant activity of five essential oil was evaluated using five different methods, 2,2- diphenyl-1-picryhydrazyl (DPPH), Ferric reducing antioxidant power (FRAP), Thiobarbituric acid reactive species test (TBARS), Ferrous ion-chelating ability assay (FIC) and Rancimat assay. In all methods, rosemary essential oil considered good sources of natural compound with significant antioxidant activity (Viuda-Martos et al., 2009).

Özcan (2011) determined the antioxidant effects of essential oils from rosemary, clove and cinnamon on hazelnut and poppy oils. The essential oils were added 0.25% and 0.50% ratio in oils, respectively and compared with 0.02% BHA and control oils during storage at 50°C. Rosemary essential oil showed significant antioxidant activity in crude oil but less than other essential oils and BHA.

2.4.3.3.2. Oregano (*Origanum onites L.*)

Oregano is another plant of the Labiatae family that used as a spice and its flavor is highly favorable to consumers all over the world. It is valued also for its antimicrobial and antioxidant properties (Figure 2.12) (Yanishlieva et al., 2006).

Figure 2.12. The image of oregano plant in nature

The genus Origanum (Labiatae) is represented in Turkey by 22 species or 32 taxa, 21 being endemic and the ratio of endemism in the genus is 65.2 %. Oregano plays a primary role among culinary herbs in world trade and it is produced mainly in France, Greece, Spain, and Turkey in Europe, and Chile, Mexico and Peru in America. *Origanum onites, Origanum minutiflorum, Origanum majorana, Origanum syriacum var. bevanii, Origanum vulgare var. hirtum* are exported from Turkey (Baser et al., 1993; Kirimer et al., 2003; Toncer et al., 2009). Turkish Oregano (*O. onites*) is the most exported Origanum species from Turkey to the entire world) and is commonly known as "Izmir kekiği", "Bilyalı kekik", ''Mercanköşk'', ''Ak kekik'','' İstanbul kekiği''or ''Peynir kekiği'' and it includes 2-3 % of essential oil. Oregano has been used as a stimulant, analgesic, antitussive, expectorant, sedative, antiparasitic, and antihelminthic in Turkish folk medicine, and it is mostly used for gastrointestinal complaints (Dundar et al., 2008; Toncer et al., 2009).

The composition of essential oil in plants was affected by genetical and environmental conditions. The main components of essential oil of *O. onites* are carvacrol, thymol, 1,8 cineol, p-cymene, and gamma-terpinene, borneol, linalool, alpha-terpinene (Yaldiz et al., 2005; Ceylan et al., 1999; Kacar et al., 2006; Baydar et al., 2004; Demirci et al., 2004 ; Toncer et al., 2009). However, Kokkini et al. (2004) stated that Turkish oregano had higher amounts of sabinene; a monoterpene that is used in manufacturing of fragrance and flavor concentrates of all types. Biological activities of Oregano depended mainly on carvacrol and thymol (Figure 2.9). Carvacrol is an oxygenated monoterpene with multiple pharmacological actions. (Toncer et al., 2009).

Several studies have been reported about the oregano, its oils and extracts retard the lipid oxidation in different media that may be related to the presence of thymol and carvacrol (Figure 2.9) (Bendini et al., 2002; Cervoto et al., 2000; Damechki et al., 2001; Viudo-Martos 2009). Abdalla and Roozen (1999) reported that oregano acetone extract was more active in sunflower oil than in its 20% oil-in-water emulsion during oxidation in the dark 60°C. Yanishlieva et al. (1999) evaluated that difference in mechanisms of inhibition action of thymol and carvacrol at room temperature, which depended on the character of the lipid medium. Thymol was a better antioxidant in triacylglycerols of sunflower oil than in triaglycerols of lard.

Kulisic et al. (2004) investigated that antioxidant activity of oregano essential oil with three different methods: the ß-carotene bleaching (BCB) test, the 2,2- diphenyl-1-picryhydrazyl (DPPH) assay, and the thibarbituric acid reactive species (TBARS) assay. It was found that the total essential oil, its fraction as well as its pure constituents had significant antioxidant effect when tested by each method, respectively. Generally, the antioxidant activity of the oregano essential oil was less effective than the ascorbic acid but comparable with the α-tocopherol and BHT.

In another study, it was observed that dietary oregano essential oil supplementation exerted antioxidative effects, the supplementation being most effective in retarding lipid oxidation in stored raw and cooked chicken at the 100 mg oregano essential oil kg^{-1} feed (Botsoglou et al., 2002). Batsoglou (2003) also reported that Oregano oil at 200 mg kg^{-1} was significantly more effective in delaying lipid oxidation of raw and cooked turkey compared to the level of 100 mg kg^{-1}, equivalent to α-tocopherly acetate at 200 mg kg^{-1}.

Pizzale et al. (2002) showed that the extracts of *Origanum intercedens* and *Origanum onites* had an antioxidant activity lower than that of the 1:1 (w/w) mixture of BHA and BHT in the Rancimat test.

Olmedo et al. (2009) analysed the peroxide value, p-anisidine value and conjugated diene measurements and descriptive analysis of fried-salted peanuts flavored with 200 ppm oregano essential oil and olive oil during 112 days of storage. Peroxide value and p-anisidine value at the end of storage were lower in fried-salted peanut flavored with oregano essential oil and olive oil followed by peanut flavored with only oregano essential oil, respectively.

CHAPTER III
MATERIALS AND METHODS

3.1. Materials

Main material pistachio puree was obtained from the Asım Samlı Agricultural Products Export, Import And Trading, Sa, (Gaziantep, Turkey). Pistachio puree was made using roasted (130-135°C) pistachio kernels, mawardi (Nizip, August 2010 crop) type, 17 mm length, 8 mm height and 7 mm width in size.

The essential oils from oregano, *Origanium onites L.*, (Antalya, 2010) and rosemary, *Rosmarinus officinalis L.*, (Aydın, 2010) plants were supplied from TİKTA A.Ş., Tarımsal İklimleme ve Teknolojik Araştırmalar Anonim Şirketi, (Ankara, Turkey). BHA was purchased from Merck Chemical Co. (Darmstadt, Germany).

All chemicals used in this study were of analytical grade and purchased from Merck Chemical Co. (Darmstadt, Germany).

3.2. Methods

3.2.1. Preliminary determination of antioxidant activity

Before study, pistachio oil was obtained from the roasted pistachio nuts by cold pressing (Kardeşler press, İstanbul). 150 ppm, 300 ppm and 600 ppm of essential oils and BHA were added to roasted pistachio oil samples to determine antioxidant activity of essential oils. Control oil was used for comparison. In order to obtain induction time, a rancimat device Methrohm 743 (Herisau, Switzwerland) was used. 3 g of each sample was placed into a Rancimat standard tube in dublicate and subjected to the normal operation of the test by applying 120°C and a flow of air of 20 L/h. 60 mL of ultra distilled water was put in each measuring vessel. The end point of the induction time was characterized by the sudden increase of water conductivity, due to the dissociation of volatile carboxylic acids.

The antioxidant activity index (AAI) was calculated from the measured induction time according to following formula (Viudo-Martos et al., 2009).

AAI=induction period of oil with antioxidant/induction time of pure oil

3.2.2. Sample preparation and storage conditions

The essential oils from rosemary (*R. officinalis L.*) and oregano (*O. onites L.*) plants were added to pistachio puree at three different concentration (150, 300 and 600 ppm) on the basis of oil content of puree. BHA was added as 150 ppm for the comparison due to 300 and 600 ppm exceed limit of usage BHA according to the regulation in the world. Control sample was also used to observe effect of adding antioxidants on prolonging shelf life of pistachio puree. All samples were tightly sealed in the glass jars and two parts of them were placed to incubators (Daihan WIG-150, Korea) at 25 and 40°C. The another part was stored in a cold room at 15°C for analysis. The samples were taken at proper time intervals to observe chemical changes during storage.

3.2.3. Oil extraction for chemical analysis

100 grams of each sample were extracted with 300 mL of chloroform/methanol (2/1, v/v) mixture at appropriate time intervals. After mixing sample and solvent with a glass rode, the mixture was waited 1-2 hour in dark place and filtered through Whatman No:42 filter paper by twice. The residue was re-extracted twice by same solvent; the filtrates from three extractions were combined. The solvent-oil mixture was then passed through over anhydrous sodium sulphate placed over a filter paper in a funnel. The solvent was removed using rotary evaporator (Heidolph Laborota 4000 Efficient, Germany) at 40°C. The resulting oil was stored at 4°C until use (Miraliakbari and Shahidi, 2008).

3.2.4. Chemical analysis

3.2.4.1. Moisture content determination

The moisture content was determined by the oven method according to Official method of analysis (AOAC, 1995). The measurements were carried out in triplicate.

3.2.4.2. Protein determination

The protein content was determined by the Kjeldahl method. The nitrogen rate in samples was measured, the obtained number for sample was multiplied in protein factor 6.25 and the amount of protein in sample was obtained according to Official method of analysis (AOAC, 1995). The measurements were carried out in triplicate.

3.2.4.3. Oil content determination

The oil content of pistachio pure was determined according to TS 973 EN ISO 659 using Solvent extractor (Ser 148-6, Velp Scientifica, Italy) (Turkish Standard, 2000). The measurements were carried out in triplicate.

3.2.4.4. Fatty acid composition of pistachio puree oil

The fatty acid composition of pistachio puree was analyzed on Agilent GC7890A gas chromatography equipped with a flame ionization detector and capillary column 50 m in length and 0.25 mm in diameter according to method of International Olive Oil Council. The analytical conditions were as seen Table 3.1 (COI, 2001).

Table 3.1. GC operating condition for free fatty acid determination (COI, 2001)

Parameters	**Condition**
Flow rate of carrier gas (helium)	1.2 mL/minute
Detector temperature	260°C
Injector temperature	250°C
Column temperature	165°C(15 min.), 5°C/min. to 200°C
Injection volume	1μL
Column type	Agilent, CP Sil 88 Capillary column coated with cyanopropylsilicone

3.2.4.5. Peroxide value determination

5 grams of the each oil sample, in duplicate, was weighed in a glass 250 mL of erlenmeyer flask. 10 mL of chloroform was added and dissolved quickly by stirring. Then, 15 mL of glacial acetic acid and 1 mL of saturated potassium iodide (KI) were added. The flask was immediately closed, stirred for 1 minute and left to stay 5 minute in a dark place at ambient temperature. After that time 75 mL of distilled water was added to mixture and by addition 0.5 ml 1% starch solution was titrated with 0.01N sodium thiosulphate ($Na_2S_2O_3$) solution. A blank test was carried out Results were expressed as milliequivalents of peroxide per kilogram of sample (BS 684, 1987). The peroxide value (PV) was calculated as follows;

$$PV = \frac{(S - B)xNx1000}{m}$$

where;

S= volume of sodium thiosuphate used for sample.

B= volume of sodium thiosuphate used for blank.

N= normality of sodium thiosuphate used for titration.

m=mass of sample, in grams

3.2.4.6. Free fatty acid determination

20 mL of alcohol (95%) was mixed with 20 mL of diethyl ether by addition 1 mL of phenolphthalein indicator in a glass 250 mL of erlenmeyer flask. The mixture was neutralized by adding 0.1 N NaOH by burette. 5 grams of sample was weighed out in another flask and mixed with the solvent solution. The mixture was titrated with 0.1 N NaOH until a pink color persists for 30 sec. (Bozkurt and Göğüş, 2004). The % free fatty acid (oleic acid) was calculated as follows;

$$\% \text{ Free fatty acids} = \frac{V \times 28.2 \text{ X } N}{m}$$

where;

V= volume of 0.1 N NaOH used for sample.

N= normality of NaOH used for titration.

m=mass of sample, in grams

3.2.4.7. Color measurement of pistachio puree

The color measurements of pistachio puree were performed using HunterLab ColorFlex (A60-1010-615 Model Colorimeter, HunterLab, and Reston VA) during storage. The L*a*b* color space (also referred to as CIELAB) was used to expressed the color changes. The color values are expressed as L* (darkness/whiteness), a* (greenness/redness), b* (blueness/yellowness) values and yellowness index (YI). Three measurements were taken for sample. The instrument was calibrated against the standard reference white tiles (L*= 93.41, a* =-1.12, b* =1.07).

3.2.5. Gas chromatography-mass spectroscopy (GC-MS) analysis of essential oils

The analysis of the essential oils of rosemary and oregano performed using Agilent 7890 A GC and Agilent 5975 C VL MSD with triple axis detector equipped with Agilent HP Innowax (60m x0.25mm i.d.; film thickness 0.25 μm) column. The instrument conditions were as seen Table 3.2.

Table 3.2. GC-MS operating condition for chemical determination

Parameters		**Condition**
Quadropole temperature		150°C
Source temperature		230°C
Split ratio		1:40
Total flow rate		64.5 ml/min.
Injection volume		1μL
GC oven ramp		
	Initial	60°C 10 min. hold
	Ramp1	4°C/min. to 220°C
	Ramp2	1°C/min. to 240°C

3.2.6. Oxidative Induction Period (OIP) measurement

A metrohm Rancimat model 743 (Herisau, Switzerland) capable of operating over a temperature range of 50-220°C was used in this study. The glassware was rigorously cleaned between each run to avoid any contamination that would catalyze the peroxidation. The tubes were cleaned with acetone after each run and then washed off with washing liquid in hot water. The washed tubes were rinsed with distilled water and dried in oven. Measuring vessels, electrodes, and connecting tubes were cleaned several times with alcohol and distilled water.

Determination of the oxidative stability of pistachio puree by the Rancimat method was carried according to AOCS Cd 12b-92 method (AOCS, 1995). A stream of air was bubbled into 3 g of oil samples in a reaction vessel placed in an electric heating block. Effluent air containing volatile organic acids from the oil sample were collected in a measuring vessel containing distilled water (60 mL). The conductivity of water was measured automatically as oxidation proceeded. Filtered, cleaned, dried air was allowed to bubble through the hot oil at rates of 20 L/h. The induction time of the oil samples were automatically recorded at 110, 120, and 130°C. For each time studied, eight samples were accommodated in the equipment and analyzed simultaneously (Farhoosh, 2007).

3.2.7. Statistical Analysis

The results were compared by multifactor analysis of variance (multifactor ANOVA) to test for significant differences. Means of the groups the groups were compared using Duncan‘s multiple range test using a SPSS statistical packet (Version 16, 2007, Polar Engineering and Consulting, Nikiski, USA). Differences among sample means were reported to be significant when $p<0.05$ and Microsoft Office program used for drawing plots. The simple regression equations for the shelf life prediction of pistachio puree sample with and without antioxidants were calculated also using SPSS statistical packet (Version 16, 2007, Polar Engineering and Consulting, Nikiski,USA).

CHAPTER IV
RESULTS AND DISCUSSION

4.1. Characteristics of pistachio puree

Some characteristics of pistachio puree were presented in Table 4.1. The moisture content of pistachio puree was 1.36% in wet basis. There is no available standard for the composition of pistachio puree but according Turkish standard of hazelnut puree (TS 10938), similar processed product; it should be less than 3%. Also from the producer side it should be less than 2% based on customer demand (Anonyms, 2010a). The protein content of sample was 20.5%. In a study carried out by Küçüköner and Yurt (2003), the amount of protein cultivars of Uzun, Kırmızı, Siirt, Ohadi and Halabi pistachios was reported 22.67%, 20.93%, 22.45% , 23.62% and 20.18%, respectively. It is possible that low amounts of protein obtained in this study have been influenced by the kind of pistachio nuts. The amount of oil was obtained as 48%. The result in the study was less than previous studies obtained by Okay (2002) and Küçüköner and Yurt (2003) due to kind of pistachio nut. In the fatty acid composition, main fatty acids were oleic acid 71.05%, linoleic acid 16.55% and palmitic acid 8.52% that are relatively consistent with the results of other studies (Maskan and Karataş, 1999; Okay, 2002; Chahed et al., 2008).

The L^* (whiteness or darkness), a^* (redness/greenness), b^* (blueness/yellowness) and YI (yellowness index) value which represent color value of pistachio puree was measured as 41.08, 1.84, 41.03 and 96.32, respectively.

4.2. Compositions of essential oils

The compositions of oregano (*O.onites* L.) and rosemary (*R. officinalis* L.) essential oils were present in Table 4.2 and Table 4.3, respectively. The composition was analysed by Agilent 7890 A GC and Agilent 5975 C VL MSD device. The results are relatively consistent with results of other studies (Bayrak and Akgül, 1989; Wang et al., 2008; Jiang et al., 2011; Yaldiz et al., 2005; Ceylan et al., 1999; Kacar et al., 2006; Baydar et al., 2004; Demirci et al., 2004 ; Toncer et al., 2009).

Table 4.1. Composition of pistachio puree

Moisture content (% wb.)	1.36
Protein (% wb.)	20.50
Fat (% wb.)	48.00
Fatty acid composition (%)	
Caproic acid	ND*
Caprylic acid	ND*
Capric acid	ND*
Lauric acid	ND*
Myristic acid	0.10
Palmitic acid	8.52
Palmitoleic acid	0.52
Margaric acid	ND*
Heptadecanoic acid	0.07
Stearic acid	1.88
Oleic acid	71.05
Linoleic acid	16.55
Linolenic acid	0.34
Arachidic acid	0.19
Eicosanoic acid	0.49
Behenic acid	0.01
Erucic acid (C22:1)	0.01
Erucic acid (C22:2)	0.01
Ligroceric acid	0.04
Nervonic acid	0.02

Table 4.2. The compositions of oregano (*O.onites* L.) essential oil

Components	Amount (%)
Cis-Salvene	6.4
α-Thujene	1.1
Myrcene	1.4
ϒ-Terpinene	4.2
p-Cymene	2.6
Trans-Sabinene hydrate	1.1
Linalool	10.2
Linalyl acetate	1.2
B-Bisabolene	3.7
Thymol	1.6
Carvacrol	56.3
B-caryophyllene	2.4
Borneol	1.0
Germacrene D	1.0
Total*	94.2

*Values <1% not included.

Table 4.3. The compositions of rosemary (*R. officinalis* L.) essential oil

Components	Amount (%)
α-Pinene	14.0
Camphene	4.3
B-Pinene	4.5
δ-3-Carene	1.2
Myrcene	2.1
Limonene	3.5
1.8-Cineole	25.8
ϒ-Terpinene	1.3
p-Cymene	1.5
Camphor	6.8
Linalool	2.1
Isopinocamphone	1.2
Bornyl acetate	2.3
Terpinen-4-ol	1.5
B-Caryophyllene	5.0
α-Terpineol	1.6
Bomeol	7.3
Verbenone	2.0
Total*	88.0

*Values <1% not included.

4.3. Preliminary determination of antioxidant activity

The Rancimat test is a very easy and inexpensive method, which requires small sample volumes and achieves reproducible results. Although this technique has been questioned, it is commonly used in the food industry and governmental analytical laboratories. Table 4.4. gives the related antioxidant activity index (AAI) of pistachio oils with the essential oils added.

The higher the induction period of the pistachio oil with the essential oils added, compared with the control (pure pistachio oil) the better the antioxidant activity of that compound. The antioxidant activity index, as determined by the Rancimat method, decreased in the order: BHA > Rosemary (*R.officinalis* L.) essential oil > Oregano (*O. onites* L.) essential oil.

Table 4.4. Antioxidant acitivity of rosemary, oregano essential oils and BHA at 120°C.

Antioxidants	AAI		
	150 ppm	300 ppm	600 ppm
Rosemary	1.60^{aB}	1.95^{abB}	2.25^{bB}
Oregano	1.23^{aA}	1.33^{aA}	1.43^{aA}
BHA	2.04^{aC}	2.30^{aB}	2.75^{bC}

Means followed by the same small letter within same line are not significantly different at 0.05 significance level according to Duncan's multiple range test.

Means followed by the same capital letter within same column are not significantly different at 0.05 significance level according to Duncan's multiple range test.

According to this method, oregano and rosemary essential oils showed antioxidant activity, but less than the activity of synthetic antioxidant. At the maximum concentration, rosemary essential oil showed the highest ($p < 0.05$) antioxidant activity index (2.25) of all the essential oils analyzed. At all concentrations (150, 300 and 600 ppm) oregano essential oils showed the lowest ($p < 0.05$) antioxidant activity indices.

4.4. Oxidative Induction Period (OIP) measurements

4.4.1.Oxidative Induction time (OIP) of pistachio puree samples with or without antioxidants at different temperature

Oxidative stability is an important parameter for the quality assessment of fats and oils. Autoxidation is affected by atmospheric oxygen and the oxidation process proceeds via free radical reactions involving unsaturated fatty acids (Wanasundara and Shahidi, 2005). The OIP of pistachio puree samples with or without antioxidants at different temperature are presented in Table 4.5.

The induction time of pistachio puree oil was 26.22 hour at 110°C. The induction time of puree oil significantly increased to 50.34 (BHA-150ppm), 39.84 (RO-150ppm), 45.72 (RO-300ppm), 54.79 (RO-600ppm), 27.66 (OO-150ppm), 29.03 (OO-300ppm) and 34.48 (OO-600ppm) as hour by adding antioxidants ($p<0.05$). In samples with essential oils, there was significant increase in induction time with increasing concentration of essential oils ($p<0.05$). The highest induction time (54.79 h) was observed in sample which contain 600 ppm rosemary essential oil and lowest one (27.66 h) in sample containing 150 ppm oregano essential oil. The effect of 150 ppm BHA was lower than the 600 ppm rosemary essential oil in lowering oxidation rate whereas it was higher than other concentration of both essential oil.

The induction time of pistachio puree oil was 13.23 hour at 120°C. The induction time of puree oil significantly increased to 25.87 (BHA-150ppm), 22.44 (RO-150ppm), 23.94 (RO-300ppm), 27.32 (RO-600ppm), 14.3 (OO-150ppm), 13.73 (OO-300ppm) and 17.46 (OO-600ppm) as hour by adding antioxidants ($p<0.05$). In samples with essential oils, there was significant increase in induction time with increasing concentration of essential oils ($p<0.05$). The highest induction time (27.32 h) was observed in sample which contains 600 ppm rosemary essential oil and lowest one (13.73 h) in sample containing 150 ppm oregano essential oil. The effect of again 150 ppm BHA was lower than the 600 ppm rosemary essential oil in lowering oxidation rate whereas it was higher than other concentration of both essential oil.

The induction time of pistachio puree oil was 6.68 hour at 130°C. The induction time of puree oil significantly increased to 12.08 (BHA-150ppm), 10.52 (RO-150ppm), 12.03 (RO-300ppm), 12.85 (RO-600ppm), 7.31 (OO-150ppm), and 7.31 (OO-

600ppm) as hour by adding antioxidants ($p<0.05$). In samples with essential oils, there was significant increase in induction time with increasing concentration of essential oils except 300 ppm oregano essential oils added sample ($p<0.05$). The highest induction time (12.85 h) was observed in sample which contain 600 ppm rosemary essential oil and lowest one (6.32 h) in sample containing 300 ppm oregano essential oil. The effect of 150 ppm BHA was lower than the 600 ppm rosemary essential oil in lowering oxidation rate whereas it was higher than other concentration of both essential oil. The induction time of samples containing both essential oils and BHA was close or having less difference between each other at 130°C. It shows us that at 130°C increasing concentration of essential oils above a level does not any change in induction time of pistachio puree samples.

The induction time of all pistachio puree oil samples was decreased by increasing operating temperature of Rancimat device (Figure 4.1). As would expect, the increasing rancimat operating temperature significantly decreased OIP of sample ($p<0.05$). These observations indicate that the conditions under which the oil sample oxidizes vary with temperature. Similar results were found by Farhoosh (2007).

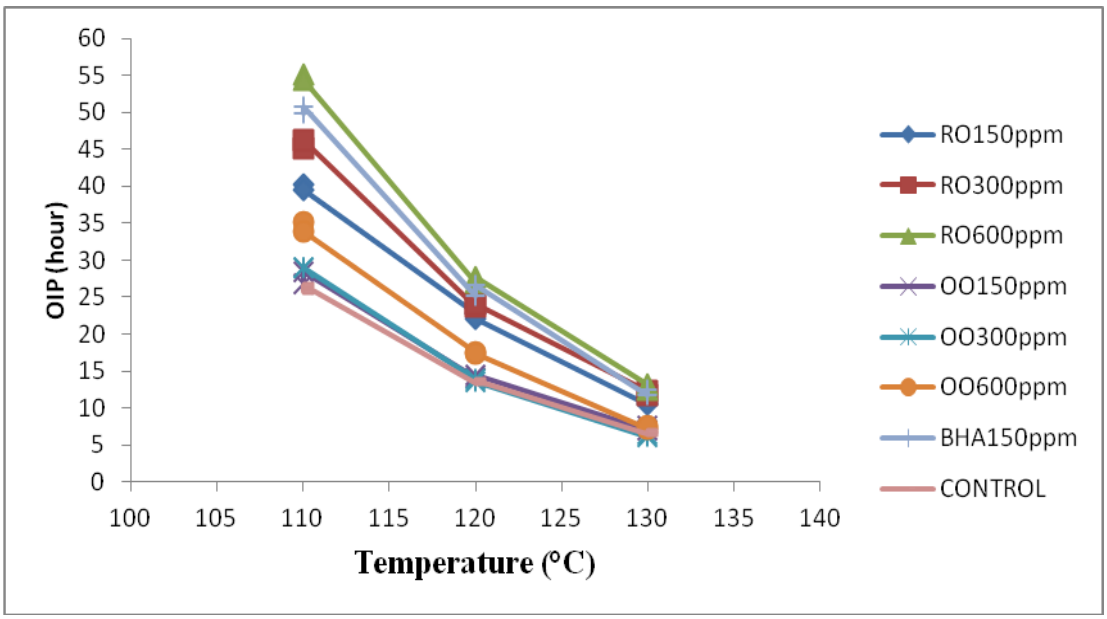


Figure 4.1. Plot of OIP (hour) versus Rancimat operating temperature for samples with or without antioxidants.

Table 4.5. The OIP of pistachio puree samples with or without antioxidants at different temperature.

Antioxidant type	**Amount (ppm)**	**Temperature (°C)**		
		110	120	130
Rosemary *(R. officinalis L.)*	150	39.84^{dA}	22.44^{cB}	10.52^{cC}
	300	45.72^{eA}	23.94^{dB}	12.03^{dC}
	600	54.79^{gA}	27.32^{fB}	12.85^{eC}
Oregano *(O. onites* L.)	150	27.66^{abA}	14.30^{aB}	7.31^{bC}
	300	29.03^{bA}	13.73^{aB}	6.32^{aC}
	600	34.48^{cA}	17.46^{bB}	7.31^{bC}
BHA	150	50.34^{fA}	25.87^{eB}	12.08^{deC}
Control	0	26.22^{aA}	13.23^{aB}	6.68^{abC}

Means followed by the same small letter within same column are not significantly different at 0.05 significance level according to Duncan's multiple range test.

Means followed by the same capital letter within same line are not significantly different at 0.05 significance level according to Duncan's multiple range test.

4.4.2. Shelf life prediction of pistachio puree samples with or without antioxidants

The Rancimat method may also provide some other useful information regarding oxidative stability of edible fats and oils and fat-containing foods. By plotting the logarithms of OIPs versus elevated temperatures and extrapolating to room temperature, we can predict the shelf-life of the sample at ambient conditions. However, these predictions may result in overestimation or underestimation, but sometimes acceptable results. The slope of the curves represents the temperature coefficients for oil samples (Farhoosh, 2007).

The data calculated from the linear relationship between the natural logarithm of the OIP and the temperature for the treatment combinations in Table 4.5 were shown in Table 4.6.

Table 4.6. The linear regression results calculated from the linear relationship between the natural logarithm of the OIP assessed by the Rancimat test.

Samples	**Log OIP=A(T)+B**			
	A±SE	B±SE	R^2	$OIP_{25°C}$(days)
Rosemary (150ppm)	-0.029±0.001	4.795±0.145	0.993	490
Rosemary (300ppm)	-0.029±0.000	4.853±0.520	0.999	559
Rosemary (600ppm)	-0.031±0.001	5.208±0.72	0.999	1129
Oregano (150ppm)	-0.029±0.001	4.623±0.105	0.996	329
Oregano (300ppm)	-0.033±0.000	5.107±0.049	0.999	797
Oregano (600ppm)	-0.034±0.001	5.258±0.157	0.994	1066
BHA (150ppm)	-0.031±0.001	5.118±0.102	0.997	918
Control	-0.030±0.001	4.691±0.067	0.999	364

As seen in Table 4.6 the shelf life of pure pistachio puree was found as 364 days. The shelf life of pistachio puree samples significantly increased to 918 (BHA-150ppm), 490 (RO-150ppm), 559 (RO-300ppm), 1129 (RO-600ppm), 797 (OO-300ppm), 1066 (OO-600ppm) as days ($p<0.05$). It was obvious from the results (Table 4.6) that the adding essential oils significantly increased the shelf life of pistachio puree. The pistachio puree with 600 ppm rosemary essential oil had the highest shelf life where as 150 ppm oregano essential oils added pistachio puree had lowest shelf life. It seemed 150 ppm oregano essential oil was no effect on prolonging shelf life of pistachio puree. The difference between pistachio puree and pistachio puree with 150 ppm oregano essential oil was not significant ($p>0.05$). Published information on the study about shelf life prediction of pistachio puree or relative products is not available in the literature. But, Frankel (1998) stated that the extrapolation of the stability results obtained by the Rancimat test to ambient storage leads to either over prediction or under prediction of the actual shelf life depending on the type of oil. Mendez et al. (1996) attributed this to different mechanisms of peroxidation under accelerated conditions of Rancimat test from the corresponding mechanisms at ambient storage conditions. In another study, the shelf-life prediction of an infant formula based on such extrapolation provides reasonably acceptable results (Presa-Owens et al., 1995). As a result, choosing the levels of operational parameters in the Rancimat method depending on the type of the product may result in least possible difference between predictions from long-term storage studies and OSI test (Farhoosh, 2007).

4.5. Normal storage results of pistachio puree samples

4.5.1. Peroxide value (PV) changes during storage

Lipid oxidation involves the continuous formation of hydroperoxides as primary oxidation products that may break down to a variety of nonvolatile and volatile secondary products. The formation rate of hydroperoxides outweighs their rate of decomposition during the initial stage of oxidation, and this becomes reversed at later stages. Therefore, the peroxide value (PV) is an indicator of the initial stages of oxidative change. The PV represents the total hydroperoxide content and is one of the most common quality indicators of fats and oils during production and storage (Shahidi and Zhong, 2005).

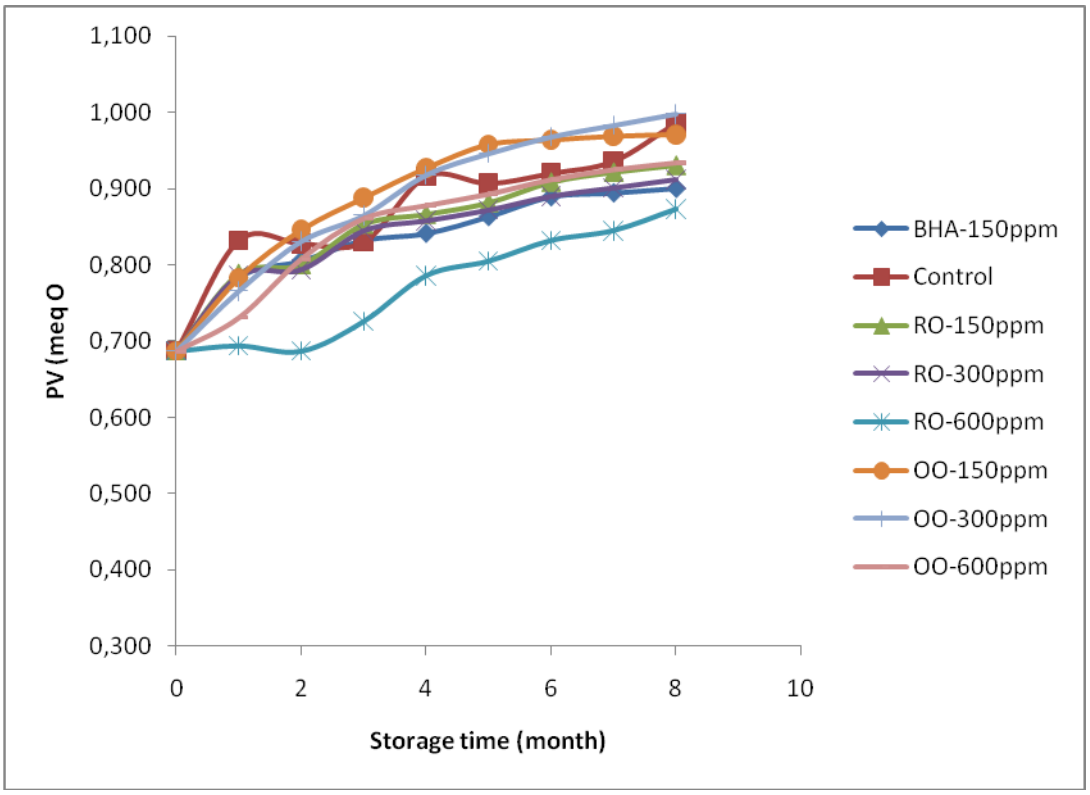


Figure 4.2. Peroxide values (meq O_2/kg) from different pistachio puree oil of samples: pistachio puree control oil and pistachio puree oil of samples added with different concentration (150,300 and 600ppm) of RO (*R.officinalis* L. essential oil), OO (*O. onites* L. essential oil) and BHA (Butylated Hydroxyanisole) at 15°C ($p<0.05$, all samples with antioxidants significantly different from control).

The changes in peroxide values of pistachio puree oil of samples with or without antioxidants during storage at 15°C are shown in Figure 4.2. In general, PV of all sample oils increased significantly ($p<0.05$) with storage time. The PVs of pistachio puree oils were from 0.687 meq O_2/kg (day 0) to 0.986 (month 8) in control, from 0.687 to 0.971 in OO-150ppm, from 0.687 to 0.998 in OO-300ppm, from 0.687 to 0.934 in OO-600ppm, from 0.687 to 0.930 in RO-150ppm, from 0.687 to 0.912 in RO-300ppm, from 0.687 to 0.873 in RO-600ppm and from 0.687 to 0.900 in BHA-150ppm added samples. After third month, control sample oil had higher PV than the other sample oils except OO-150ppm and OO-300ppm added sample oils. The PV increase was lower in RO, OO and BHA added samples than in control sample oil. Among pistachio puree oil of samples with essential oils and BHA, RO-600ppm had lower in PV increase. The lowest increase among all samples was observed in RO-600ppm sample. But, statistically there was no difference between the RO-600ppm

and BHA-150ppm added samples according to results of Duncan's multiple range test at 15°C.

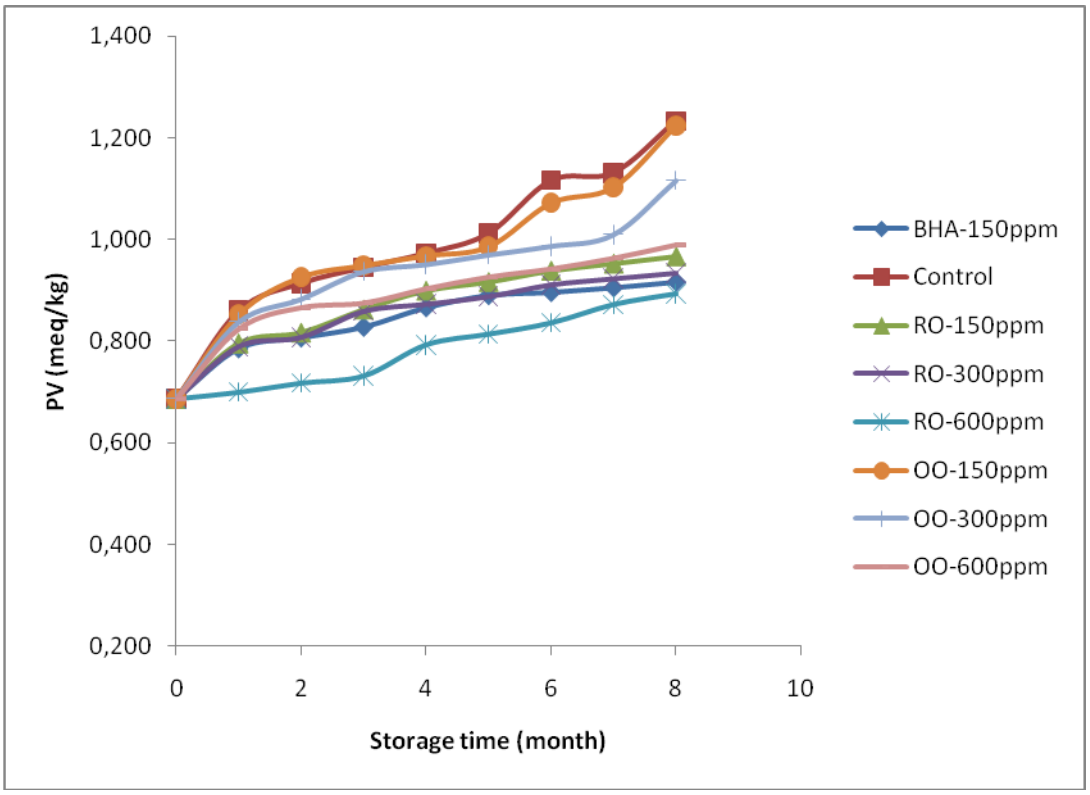


Figure 4.3. Peroxide values (meq O_2/kg) from different pistachio puree oil of samples: pistachio puree control oil and pistachio puree oil of samples added with different concentration (150,300 and 600ppm) of RO (*R.officinalis* L. essential oil), OO (*O. onites* L. essential oil) and BHA (Butylated Hydroxyanisole) at 25°C ($p<0.05$, all samples with antioxidants significantly different from control).

The changes in peroxide values of pistachio puree oil of samples with or without antioxidants during storage at 25°C are shown in Figure 4.3. In general, PV of all sample oils increased significantly ($p<0.05$) with storage time. The PVs of pistachio puree oils were from 0.687 meq O_2/kg (day 0) to 1.233 (month 8) in control, from 0.687 to 1.224 in OO-150ppm, from 0.687 to 1.116 in OO-300ppm, from 0.687 to 0.989 in OO-600ppm, from 0.687 to 0.966 in RO-150ppm, from 0.687 to 0.934 in RO-300ppm, from 0.687 to 0.893 in RO-600ppm and from 0.687 to 0.916 in BHA-150ppm added samples. After third month, control sample oil had higher PV than the other sample oils. The PV increase was lower in RO, OO and BHA added samples than in control sample. Among pistachio puree oil of samples with essential oils and

BHA, RO-600ppm had lower in PV increase. The lowest increase among all samples was observed in RO-600ppm sample.

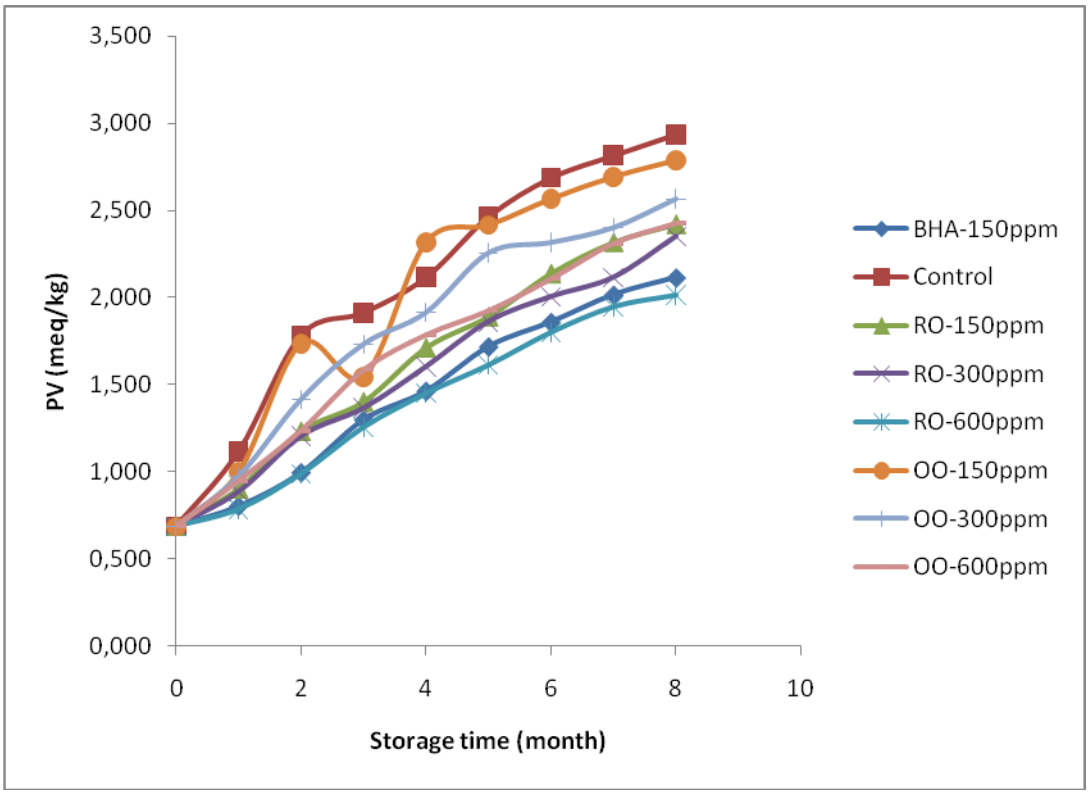


Figure 4.4. Peroxide values (meq O_2/kg) from different pistachio puree oil of samples: pistachio puree control oil and pistachio puree oil added with different concentration (150,300 and 600ppm) of RO (*R.officinalis* L. essential oil), OO (*O. onites* L.essential oil) and BHA (Butylated Hydroxyanisole) at 40°C ($p<0.05$, all samples with antioxidants significantly different from control).

The changes in peroxide values of pistachio puree oil of samples with or without antioxidants during storage at 40°C are shown in Figure 4.4. In general, PV of all sample oils increased significantly ($p<0.05$) with storage time. The PVs of pistachio puree samples were from 0.687 meq O_2/kg (day 0) to 2.936 (month 8) in control, from 0.687 to 2.787 in OO-150ppm, from 0.687 to 2.565 in OO-300ppm, from 0.687 to 2.428 in OO-600ppm, from 0.687 to 2.420 in RO-150ppm, from 0.687 to 2.350 in RO-300ppm, from 0.687 to 2.016 in RO-600ppm and from 0.687 to 2.115 in BHA-150ppm added samples. Generally, after third month, control sample oil had higher PV than the other sample oils. The PV increase was lower in RO, OO and BHA added samples than in control sample. Among pistachio puree oil of samples with

essential oils and BHA, RO-600ppm had lower in PV increase. The lowest increase in PV among all samples was observed in RO-600ppm sample.

These results indicate that pistachio puree oil of samples with *R.officinalis* L. essential oil and *O. onites* L.essential oil had better resistance to lipid oxidation than control samples. In addition, RO-600ppm added sample showed highest antioxidant activity that very close to BHA-150ppm added sample at all storage temperature. Other researches (Bendini et al., 2002; Cervoto et al., 2000; Damechki et al., 2001; Viudo-Martos 2009; Wang et al., 2007; Kulisic et al., 2004; Nepote et al., 2006; Olmedo et al., 2009; Özcan, 2011) observed antioxidant activity of oregano and rosemary essential oil measured by different methods. The antioxidant activity of rosemary essential oils and extracts has been associated with the presence of these monoterpenes (α-pinene, 1,8-cineole and camphor) and phenolic components which break free radical chain reactions by hydrogen atom donation (Aruoma et al., 1992; Basaga et. al., 1997; Georgantelis et al., 2007). Several studies have been reported about the oregano, its oils and extracts retard the lipid oxidation in different media that may be related to the presence of thymol and carvacrol (Bendini et al., 2002; Cervoto et al., 2000; Damechki et al., 2001; Viudo-Martos 2009).

4.5.2. Free fatty acid (FFA) value changes during storage

Hydrolysis process in fats and oils results in the formation of FFAs, mono- and di-glycerides, and glycerol. This hydrolysis is a deterioration process of lipid and it can be estimated measuring the FFAs (Gertz, 1996).

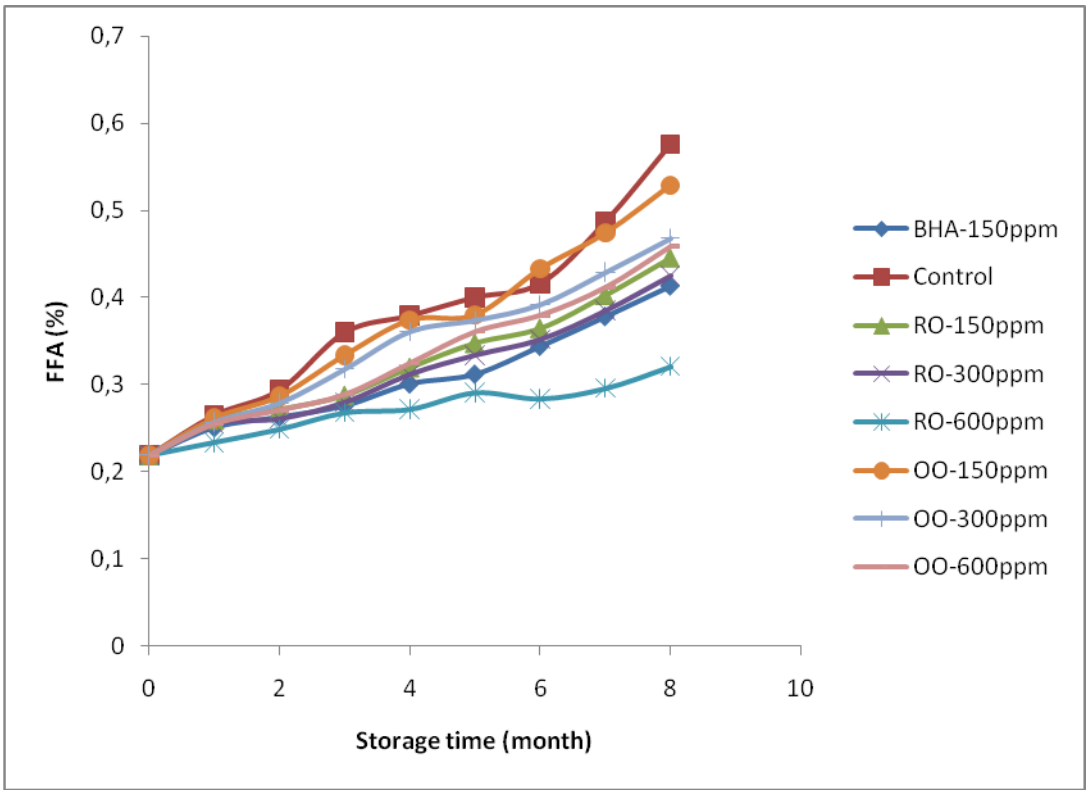


Figure 4.5. Free fatty acid values (%) from different pistachio puree oil of samples: pistachio puree control oil and pistachio puree oil of samples added with different concentration (150,300 and 600ppm) of RO (*R.officinalis* L. essential oil), OO (*O. onites* L.essential oil) and BHA (Butylated Hydroxyanisole) at 15°C ($p<0.05$, all samples with antioxidants significantly different from control).

The changes in free fatty acid values of pistachio puree oil of samples with or without antioxidants during storage at 15˚C are shown in Figure 4.5. In general, FFA of all sample oils increased significantly ($p<0.05$) with storage time. The FFAs of pistachio puree oils were from 0.219 (%) as oleic acid (day 0) to 0.576 (month 8) in control, from 0.219 to 0.529 in OO-150ppm, from 0.219 to 0.468 in OO-300ppm, from 0.219 to 0.459 in OO-600ppm, from 0.219 to 0.446 in RO-150ppm, from 0.219 to 0.425 in RO-300ppm, from 0.219 to 0.321 in RO-600ppm and from 0.219 to 0.413 in BHA-150ppm added samples. The FFA increase was lower in RO, OO and BHA added samples than in control sample. Among pistachio puree oil of samples with

essential oils and BHA, RO-600ppm had lower in FFA increase. The lowest increase among all samples was observed in RO-600ppm sample.

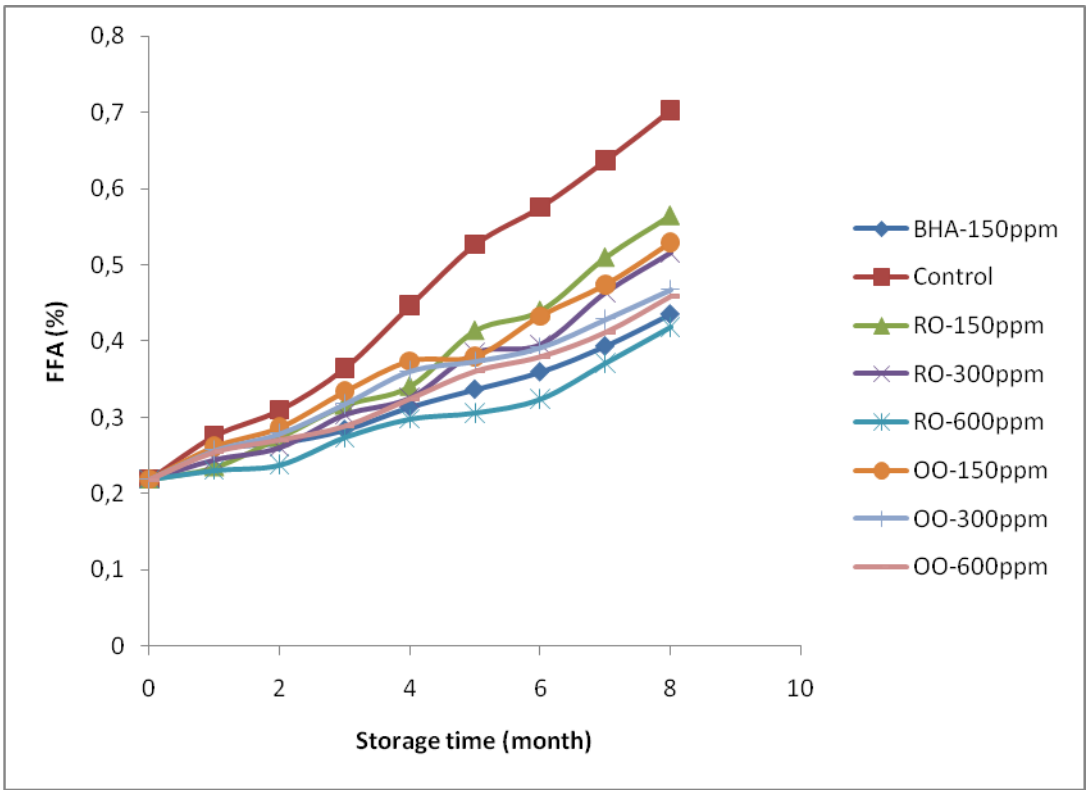


Figure 4.6. Free fatty acid values (%) from different pistachio puree oil of samples: pistachio puree control oil and pistachio puree oil of samples added with different concentration (150,300 and 600ppm) of RO (*R.officinalis* L. essential oil), OO (*O. onites* L.essential oil) and BHA (Butylated Hydroxyanisole) at 25°C ($p<0.05$, all samples with antioxidants significantly different from control).

The changes in free fatty acid values of pistachio puree oil of samples with or without antioxidants during storage at 25˚C are shown in Figure 4.6. In general, FFA of all sample oils increased significantly ($p<0.05$) with storage time. The FFAs of pistachio puree oils were from 0.219 (%) as oleic acid (day 0) to 0.703 (month 8) in control, from 0.219 to 0.615 in OO-150ppm, from 0.219 to 0.584 in OO-300ppm, from 0.219 to 0.571 in OO-600ppm, from 0.219 to 0.565 in RO-150ppm, from 0.219 to 0.516 in RO-300ppm, from 0.219 to 0.418 in RO-600ppm and from 0.219 to 0.436 in BHA-150ppm added sample. The FFA increase was lower in RO, OO and BHA added samples than in control sample. Among pistachio puree oil of samples with essential oils and BHA, RO-600ppm had lower in FFA increase. The lowest increase among all samples was observed in RO-600ppm sample.

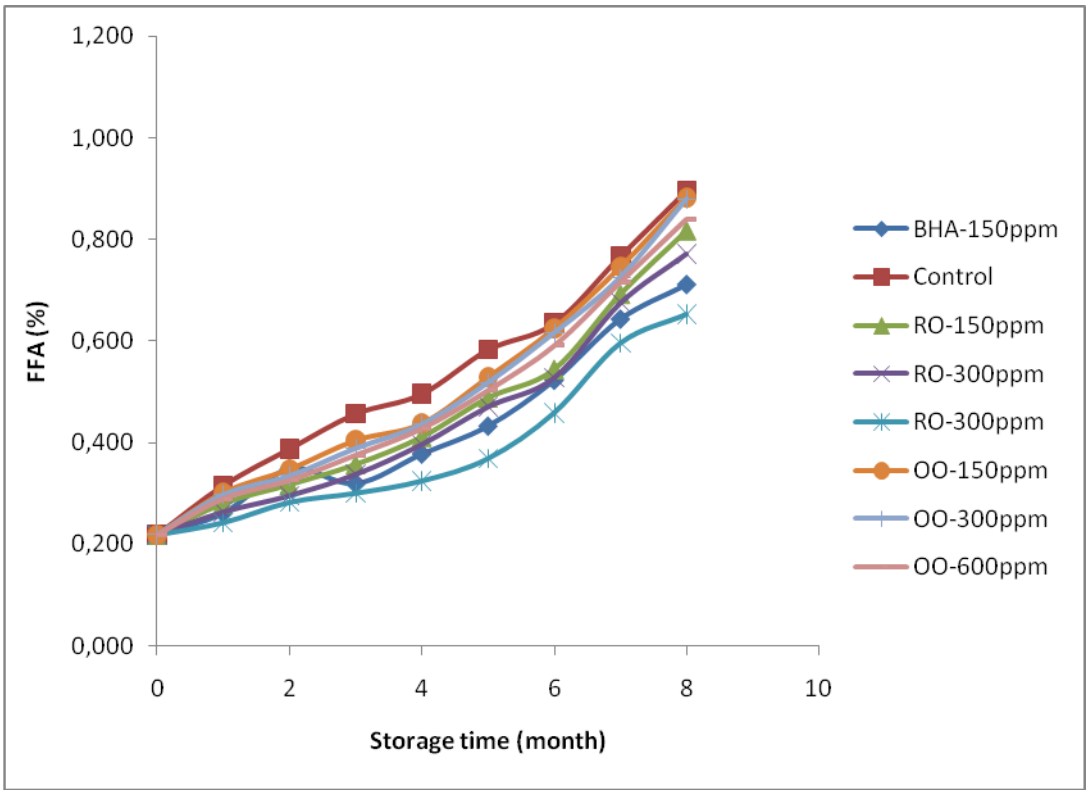


Figure 4.7. Free fatty acid values (%) from different pistachio puree samples: pistachio puree control and pistachio puree added with different concentration (150,300 and 600ppm) of RO(*R.officinalis* L. essential oil), OO (*O. onites* L.essential oil) and BHA(Butylated Hydroxyanisole) at 40°C ($p<0.05$, all samples with antioxidants significantly different from control).

The changes in free fatty acid values of pistachio puree oil of samples with or without antioxidants during storage at 40˚C are shown in Figure 4.7. In general, FFA of all oils increased significantly ($p<0.05$) with storage time. The FFAs of pistachio puree oils were from 0.219 (%) as oleic acid (day 0) to 0.896 (month 8) in control, from 0.219 to 0.883 in OO-150ppm, from 0.219 to 0.879 in OO-300ppm, from 0.219 to 0.840 in OO-600ppm, from 0.219 to 0.818 in RO-150ppm, from 0.219 to 0.772 in RO-300ppm, from 0.219 to 0.653 in RO-600ppm and from 0.219 to 0.711 in BHA-150ppm added sample. The FFA increase was lower in RO, OO and BHA added samples than in control sample. Among pistachio puree oil of samples with essential oils and BHA, RO-600ppm had lower in FFA increase. The lowest increase among all samples was observed in RO-600ppm sample.

Ayadi et al. (2009) reported that the addition of grounded leaves and flower from aromatic plant (rosemary, lavender, sage, lemon, and thyme) in olive oil caused a

slight increase in FFA. Asensio et al. (2011) also found that the oregano essential oil addition in olive oil for different treatment didn't increase the FFA.

In general, PV and FFA of pistachio puree samples increased as the storage time progressed at all storage temperature. However, the increases in PV values were much higher in samples stored at 40°C ($p<0.05$) as expected since the oxidation mechanism slows down at lower temperatures (Labuza, 1982). Combination of low temperature storage and addition of antioxidant resulted in low peroxide values.

During storage of present study, increasing temperature also increase oil separation. Low oxidation rate in those samples also may be related the prevention of oil phase separation and less exposure to oxygen in combination with the protective effect of antioxidant (Çapanoğlu and Boyacıoğlu, 2008).

CHAPTER V
CONCLUSION

The results of the present work indicate that the addition of essential oils of rosemary and oregano on pistachio puree that improves the stability of the product preventing lipid oxidation and development of rancid flavors. These antioxidant activities could be mainly associated with α-pinene, 1,8-cineole and camphor for rosemary and thymol and carvacrol for oregano essential oil. Therefore, these essential oils can be added as natural antioxidants in pistachio puree.

Rosemary essential oil showed higher antioxidant effect on the stability of this product with respect to the synthetic antioxidant, BHA, and oregano essential oil during accelerated conditions and normal storage conditions. The highest antioxidant effect was observed between the sample with 600 ppm rosemary essential oil and 150 ppm BHA added sample at all conditions. Synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxitoluene (BHT) and propyl gallate (PG) are used in many foods to prevent rancidity but there is growing concern for the potential health hazards because of their synthetic conditions. Therefore, natural antioxidants from essential oil can be replaced by synthetic ones.

This study also provides equations to estimate shelf-life of pistachio puree with and without antioxidants using Rancimat method. By this method, estimated shelf life of pistachio pure was found as 364 days which very close to actual shelf life of pistachio puree. Actual shelf life of pistachio puree changes between 6-12 month depending on composition of pistachio, production parameters (roasting time and temperature) and storage conditions. The PV and FFA results didn't reach the limit of pistachio puree which is 2 meq O2/kg for PV and 1(%) as oleic acid for FFA at the end of eight month storage time except 40°C. Therefore, it should be better storage of pistachio puree at 15 and 25°C than 40°C to get lower oxidation rate.
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As indicated present study, using natural antioxidants in lipid or lipid containing foods increase shelf life of the product. However, the intense herb flavor that may limit some applications for food products. On the contrary to increase in shelf-life, aspects such as toxic or pathogenic activity and intense herb flavor should be considered.

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APPENDICES

Table A.1. ANOVA result of preliminary determination of antioxidant activity.

Dependent Variable:AAI

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	4,198[a]	8	,525	43,504	,000
Intercept	63,346	1	63,346	5,252E3	,000
EO	3,223	2	1,611	133,601	,000
Amount	,806	2	,403	33,419	,000
EO * Amount	,169	4	,042	3,499	,055
Error	,109	9	,012		
Total	67,653	18			
Corrected Total	4,306	17			

a. R Squared = ,975 (Adjusted R Squared = ,952)

Table A.2. Multiple range test result for 150 ppm essential oils and BHA added samples of preliminary determination of antioxidant activity.

AAI

Duncan

EO	N	Subset for alpha = 0.05		
		1	2	3
2*	2	1,2323		
1*	2		1,6032	
3*	2			2,0384
Sig.		1,000	1,000	1,000

Means for groups in homogeneous subsets are displayed.

(*1=Rosemary essential oil, 2=Oregano essential oil, 3=BHA)

Table A.3. Multiple range test result for 300 ppm essential oils and BHA added samples of preliminary determination of antioxidant activity.

AAI

Duncan

EO	N	Subset for alpha = 0.05	
		1	2
2*	2	1,3310	
1*	2		1,9527
3*	2		2,2989
Sig.		1,000	,051

Means for groups in homogeneous subsets are displayed.

(*1=Rosemary essential oil, 2=Oregano essential oil, 3=BHA)

Table A.4. Multiple range test result for 600 ppm essential oils and BHA added samples of preliminary determination of antioxidant activity.

AAI

Duncan

EO	N	Subset for alpha = 0.05		
		1	2	3
2*	2	1,4265		
1*	2		2,2549	
3*	2			2,7457
Sig.		1,000	1,000	1,000

Means for groups in homogeneous subsets are displayed.

(*1=Rosemary essential oil, 2=Oregano essential oil, 3=BHA)

Table A.5. Multiple range test result for rosemary essential oils added samples of preliminary determination of antioxidant activity.

AAI

Duncan

Amount	N	Subset for alpha = 0.05	
		1	2
150	2	1,6032	
300	2	1,9527	1,9527
600	2		2,2549
Sig.		,060	,084

Means for groups in homogeneous subsets are displayed.

Table A.6. Multiple range test result for oregano essential oils added samples of preliminary determination of antioxidant activity.

AAI

Duncan

Amount	N	Subset for alpha = 0.05
		1
150	2	1,2323
300	2	1,3310
600	2	1,4265
Sig.		,110

Means for groups in homogeneous subsets are displayed.

Table A.7. Multiple range test result for BHA added samples of preliminary determination of antioxidant activity.

AAI

Duncan

Amount	N	Subset for alpha = 0.05	
		1	2
150	2	2,0384	
300	2	2,2989	
600	2		2,7457
Sig.		,121	1,000

Means for groups in homogeneous subsets are displayed.

Table A.8. Multiple range test result for Oxidative Induction Period (OIP) results at 110°C.

OIP

Duncan

Exprment	N	Subset for alpha = 0.05						
		1	2	3	4	5	6	7
8*	2	26,2200						
4*	2	27,6600	27,6600					
5*	2		29,0250					
6*	2			34,4800				
1*	2				39,8350			
2*	2					45,7150		
7*	2						50,3350	
3*	2							54,7900
Sig.		,087	,101	1,000	1,000	1,000	1,000	1,000

Means for groups in homogeneous subsets are displayed.

(*1=RO-150ppm, 2=RO-300ppm, 3=RO-600ppm, 4=OO-150ppm, 5=OO-300ppm, 6=OO-600ppm, 7=BHA-150 ppm, 8=Control)

Table A.9. Multiple range test result for Oxidative Induction Period (OIP) results at 120°C.

OIP120

Duncan

Exprment	N	Subset for alpha = 0.05					
		1	2	3	4	5	6
8*	2	13,6300					
5*	2	13,7250					
4*	2	14,3000					
6*	2		17,4600				
1*	2			22,4400			
2*	2				23,9350		
7*	2					25,8650	
3*	2						27,3200
Sig.		,207	1,000	1,000	1,000	1,000	1,000

Means for groups in homogeneous subsets are displayed.

(*1=RO-150ppm, 2=RO-300ppm, 3=RO-600ppm, 4=OO-150ppm, 5=OO-300ppm, 6=OO-600ppm, 7=BHA-150 ppm, 8=Control)

Table A.10. Multiple range test result for Oxidative Induction Period (OIP) results at 130°C.

OIP130

Duncan

Exprme nt	N	Subset for alpha = 0.05				
		1	2	3	4	5
5*	2	6,3200				
8*	2	6,6750	6,6750			
6*	2		7,3050			
4*	2		7,3100			
1*	2			10,5150		
2*	2				12,0250	
7*	2				12,0800	12,0800
3*	2					12,8500
Sig.		,329	,113	1,000	,876	,054

Means for groups in homogeneous subsets are displayed.

(*1=RO-150ppm, 2=RO-300ppm, 3=RO-600ppm, 4=OO-150ppm, 5=OO-300ppm, 6=OO-600ppm, 7=BHA-150 ppm, 8=Control)

Table A.11. Multiple range test result for RO-150ppm added sample of Oxidative Induction Period (OIP) results.

RO150

Duncan

Exprme nt	N	Subset for alpha = 0.05		
		1	2	3
130*	2	10,5150		
120*	2		22,4400	
110*	2			39,8350
Sig.		1,000	1,000	1,000

Means for groups in homogeneous subsets are displayed.

(*Rancimat operating temperature)

Table A.12. Multiple range test result for RO-300ppm added sample of Oxidative Induction Period (OIP) results.

R0300

Duncan

Exprme nt	N	Subset for alpha = 0.05		
		1	2	3
130*	2	12,0250		
120*	2		23,9350	
110*	2			45,7150
Sig.		1,000	1,000	1,000

Means for groups in homogeneous subsets are displayed.

(*Rancimat operating temperature)

Table A.13. Multiple range test result for RO-600ppm added sample of Oxidative Induction Period (OIP) results.

R0600

Duncan

Exprme nt	N	Subset for alpha = 0.05		
		1	2	3
130*	2	12,8500		
120*	2		27,3200	
110*	2			54,7900
Sig.		1,000	1,000	1,000

Means for groups in homogeneous subsets are displayed.

(*Rancimat operating temperature)

Table A.14. Multiple range test result for OO-150ppm added sample of Oxidative Induction Period (OIP) results.

OO150

Duncan

Exprme nt	N	Subset for alpha = 0.05		
		1	2	3
130*	2	7,3100		
120*	2		14,3000	
110*	2			27,6600
Sig.		1,000	1,000	1,000

Means for groups in homogeneous subsets are displayed.

(*Rancimat operating temperature)

Table A.15. Multiple range test result for OO-300ppm added sample of Oxidative Induction Period (OIP) results.

OO300

Duncan

Exprme nt	N	Subset for alpha = 0.05		
		1	2	3
130*	2	6,3200		
120*	2		13,7250	
110*	2			29,0250
Sig.		1,000	1,000	1,000

Means for groups in homogeneous subsets are displayed.

(*Rancimat operating temperature)

Table A.16. Multiple range test result for OO-600ppm added sample of Oxidative Induction Period (OIP) results.

OO600

Duncan

Exprme nt	N	Subset for alpha = 0.05		
		1	2	3
130*	2	7,3050		
120*	2		17,4600	
110*	2			34,4800
Sig.		1,000	1,000	1,000

Means for groups in homogeneous subsets are displayed.

(*Rancimat operating temperature)

Table A.17. Multiple range test result for BHA-150ppm added sample of Oxidative Induction Period (OIP) results.

BHA

Duncan

Exprment	N	Subset for alpha = 0.05		
		1	2	3
130*	2	12,0800		
120*	2		25,8650	
110*	2			50,3350
Sig.		1,000	1,000	1,000

Means for groups in homogeneous subsets are displayed.

(*Rancimat operating temperature)

Table A.18. Multiple range test result for control sample of Oxidative Induction Period (OIP) results.

CONTROL

Duncan

Exprment	N	Subset for alpha = 0.05		
		1	2	3
130*	2	6,6750		
120*	2		13,6300	
110*	2			26,2200
Sig.		1,000	1,000	1,000

Means for groups in homogeneous subsets are displayed.

(*Rancimat operating temperature)

Table A.19. ANOVA result of Peroxide value changes depend on temperature, antioxidant type and antioxidant amount.

Tests of Between-Subjects Effects

Dependent Variable:PV9

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	24,646[a]	23	1,072	101,359	,000
Intercept	90,285	1	90,285	8,540E3	,000
Temperature	19,936	2	9,968	942,884	,000
antioxidanttype	,557	2	,279	26,346	,000
amount	,232	2	,116	10,966	,000
Temperature * amount	,119	4	,030	2,805	,048
Temperature * antioxidanttype	,244	4	,061	5,773	,002
antioxidanttype * amount	,006	2	,003	,264	,770
Temperature * antioxidanttype * amount	,030	4	,007	,707	,595
Error	,254	24	,011		
Total	129,283	48			
Corrected Total	24,900	47			

a. R Squared = ,990 (Adjusted R Squared = ,980)

Table A.20. Multiple range test result of Peroxide value changes of each treatment.

Duncan

Experiment	N	Subset for alpha = 0.05				
		1	2	3	4	5
6*	2	,87300				
9*	2	,89300				
1*	2	,90000				
5*	2	,91200				
2*	2	,91600				
4*	2	,93000				
8*	2	,93400				
15*	2	,93400				
7*	2	,96600				
13*	2	,97100				
22*	2	,98600				
18*	2	,98900				
14*	2	,99800				
17*	2	1,11600	1,11600			
16*	2		1,22400			
23*	2		1,23300			
12*	2			2,01600		
3*	2			2,11500		
11*	2				2,35000	
10*	2				2,42000	
21*	2				2,42800	
20*	2				2,56500	
19*	2					2,78700
24*	2					2,93600
Sig.		,056	,293	,345	,066	,160

(*1=BHA-150ppm at 15°C, 2=BHA-150ppm at 25°C, 3= BHA-150ppm at 40°C, 4=RO-150ppm at 15°C, 5=RO-300ppm at 15°C, 6=RO-600ppm at 15°C, 7= RO-150ppm at 25°C, 8= RO-300ppm at 25°C, 9= RO-600ppm at 25°C, 10= RO-150ppm at 40°C, 11= RO-300ppm at 40°C, 12= RO-600ppm at 40°C, 13= OO-150ppm at 15°C, 14= OO-300ppm at 15°C, 15= OO-600ppm at 15°C, 16= OO-150ppm at 25°C, 17= OO-300ppm at 25°C, 18= OO-600ppm at 25°C, 19= OO-150ppm at 40°C, 20= OO-300ppm at 40°C, 21= OO-600ppm at 40°C, 22=Control at 15°C, 23= Control at 25°C, 24= Control at 40°C)

Table A.21. ANOVA result of Free Fatty Acid value changes depend on temperature, antioxidant type and antioxidant amount.

Tests of Between-Subjects Effects

Dependent Variable:FFA9

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	1,371[a]	23	,060	81,089	,000
Intercept	15,157	1	15,157	2,062E4	,000
Temperature	,869	2	,435	591,215	,000
antioxidanttype	,149	2	,074	101,296	,000
amount	,060	2	,030	41,026	,000
Temperature * amount	,001	4	,000	,246	,009
Temperature * antioxidanttype	,007	4	,002	2,255	,003
antioxidanttype * amount	,016	2	,008	11,026	,000
Temperature * antioxidanttype * amount	,002	4	,001	,684	,610
Error	,018	24	,001		
Total	18,903	48			
Corrected Total	1,388	47			

a. R Squared = ,987 (Adjusted R Squared = ,975)

Table A.22. Multiple range test result of Free Fatty Acid value changes of each treatment.

Duncan

Experiment	N	Subset for alpha = 0.05										
		1	2	3	4	5	6	7	8	9	10	11
6*	2	,32100										
1*	2		,41300									
9*	2		,41800									
5*	2		,42500									
2*	2		,43600									
4*	2		,44600									
15*	2		,45900	,45900								
14*	2		,46800	,46800								
8*	2			,51600	,51600							
13*	2				,52900	,52900						
7*	2				,56500	,56500	,56500					
18*	2				,57100	,57100	,57100					
22*	2				,57600	,57600	,57600					
17*	2					,58400	,58400					
16*	2						,61500	,61500				

Table A.22. Cont'd

12*	2							,65350	,65350			
23*	2								,70300			
3*	2								,71100			
11*	2									,77200		
10*	2									,81800	,81800	
21*	2										,84000	,84000
20*	2											,87900
19*	2											,88300
24*	2											,89600
Sig.		1,000	,088	,057	,057	,080	,110	,168	,055	,103	,425	,069

(*1=BHA-150ppm at 15°C, 2=BHA-150ppm at 25°C, 3= BHA-150ppm at 40°C, 4=RO-150ppm at 15°C, 5=RO-300ppm at 15°C, 6=RO-600ppm at 15°C, 7= RO-150ppm at 25°C, 8= RO-300ppm at 25°C, 9= RO-600ppm at 25°C, 10= RO-150ppm at 40°C, 11= RO-300ppm at 40°C, 12= RO-600ppm at 40°C, 13= OO-150ppm at 15°C, 14= OO-300ppm at 15°C, 15= OO-600ppm at 15°C, 16= OO-150ppm at 25°C, 17= OO-300ppm at 25°C, 18= OO-600ppm at 25°C, 19= OO-150ppm at 40°C, 20= OO-300ppm at 40°C, 21= OO-600ppm at 40°C, 22=Control at 15°C, 23= Control at 25°C, 24= Control at 40°C)

Table A.23. OIP results of each treatment.

Report

Experiment		OIP110	OIP120	OIP130
1*	Mean	39,8350	22,4400	10,5150
	N	2	2	2
	Std. Deviation	,55861	,60811	,12021
	Std. Error of Mean	,39500	,43000	,08500
2*	Mean	45,7150	23,9350	12,0250
	N	2	2	2
	Std. Deviation	,81317	,24749	,28991
	Std. Error of Mean	,57500	,17500	,20500
3*	Mean	54,7900	27,3200	12,8500
	N	2	2	2
	Std. Deviation	,46669	,43841	,50912
	Std. Error of Mean	,33000	,31000	,36000
4*	Mean	27,6600	14,3000	7,3100
	N	2	2	2
	Std. Deviation	1,18794	,29698	,46669
	Std. Error of Mean	,84000	,21000	,33000
5*	Mean	29,0250	13,7250	6,3200
	N	2	2	2
	Std. Deviation	,02121	,17678	,19799
	Std. Error of Mean	,01500	,12500	,14000
6*	Mean	34,4800	17,4600	7,3050
	N	2	2	2
	Std. Deviation	,96167	,08485	,27577
	Std. Error of Mean	,68000	,06000	,19500
7*	Mean	50,3350	25,8650	12,0800
	N	2	2	2
	Std. Deviation	,67175	,96874	,46669
	Std. Error of Mean	,47500	,68500	,33000

Table A.23. Cont'd

8*	Mean	26,2200	13,6300	6,6750
	N	2	2	2
	Std. Deviation	,60811	,25456	,16263
	Std. Error of Mean	,43000	,18000	,11500
Total	Mean	38,5075	19,8344	9,3850
	N	16	16	16
	Std. Deviation	1,05558E1	5,52533	2,66717
	Std. Error of Mean	2,63894	1,38133	,66679

(*1=RO-150ppm, 2=RO-300ppm, 3=RO-600ppm, 4=OO-150ppm, 5=OO-300ppm, 6=OO-600ppm, 7=BHA-150 ppm, 8=Control)