DECEMBER 2019 M.Sc. in Food Engineering HİLAL ALAK

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EFFECT OF CARVACROL ON OXIDATIVE STABILITY OF SUNFLOWER SEED OIL DURING STORAGE

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FOOD ENGINEERING

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HİLAL ALAK

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EFFECT OF CARVACROL ON OXIDATIVE STABILITY OF SUNFLOWER SEED OIL DURING STORAGE

M.Sc. Thesis

in

Food Engineering

Gaziantep University

Supervisor

Prof. Dr. Medeni MASKAN

by

Hilal ALAK

December 2019

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GRADUATE SCHOOL OF NATURAL & APPLIED SCIENCES
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Hilal ALAK

ABSTRACT

EFFECT OF CARVACROL ON OXIDATIVE STABILITY OF SUNFLOWER SEED OIL DURING STORAGE

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Natural antioxidants recently have gained popularity since synthetic ones have toxic and carcinogenic effects. In the present study, carvacrol was used as a natural antioxidant and compared with a synthetic antioxidant BHT. In this study, the shelf-life of oils was determined both under normal conditions and using accelerated tests. Rancimat and PetroOXY devices are used to investigate the oxidative stability and shelf-life of edible oils. Therefore, the purpose of this study was to predict shelf-life of mid-oleic sunflower seed oil by Rancimat and PetroOXY test devices. The study was performed at 110, 120, 130°C with both Rancimat and PetroOXY devices as accelerated methods. In addition, sunflower seed oil was stored at normal storage temperatures of 20, 30 and 40°C. It was determined that carvacrol was not as protective as BHT in both normal storage conditions and accelerated methods. The induction periods (IP) with Rancimat and PetroOXY devices were measured as 5.45, 2.86 and 1,45 and 2.23, 1.12 and 0.62 hours at temperatures of 110, 120 and 130°C, respectively. At normal storage temperatures, the IP values were measured by tangent method in terms of days. The peroxide values (PV) at the determined IP values were 12.08, 29.24 and 33.09 at 20, 30 and 40 ^{o}C, respectively. The induction periods predicted from Rancimat and PetroOXY devices for normal storage temperatures of 20, 30 and 40°C were 88.71, 48.85 and 23.22 and 30.17, 15.91 and 8.38 days, respectively. The shelf-life prediction for normal storage temperatures from Rancimat method was seems more reliable than those of PetroOXY device. Also, there was a good correlation between two accelerated methods and it was observed that PetroOXY was a faster method than Rancimat.

Key Words: Rancimat, PetroOXY, Oxidation Stability, Induction Period, Storage

ÖZET

KARVAKROL'UN AYÇIÇEK YAĞININ DEPOLAMA SIRASINDAKI OKSIDASYON DAYANIKLILIĞINA ETKISI

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Doğal antioksidanlar son zamanlarda popülerlik kazanmıştır, çünkü sentetik olanların toksik ve kanserojen etkileri vardır. Bu çalışmada Karvakrol doğal bir antioksidan olarak kullanılmış ve sentetik bir antioksidan BHT ile karşılaştırılmıştır. Bu çalışmada, hem normal koşullar altında hem de hızlandırılmış testler kullanılarak yağların raf ömrü belirlenmiştir. Rancimat ve PetroOXY cihazları, yenilebilir yağların oksidatif stabilitesini ve raf ömrünü araştırmak için kullanılır. Bu çalışmanın amacı, Rancimat ve PetroOXY test cihazları ile orta oleik ayçiçeği tohumu yağının raf ömrünü tahmin etmektir. Çalışma 110, 120 ve 130°C 'de hem Rancimat hem de PetroOXY cihazlarıyla hızlandırılmış yöntemler olarak yapıldı. Ek olarak, ayçiçeği yağı 20, 30 ve 40 ° C'lik normal saklama sıcaklıklarında depolandı. Hem normal depolama koşullarında hem de hızlandırılmış yöntemlerde Karvakrol'ün BHT kadar koruyucu olmadığı belirlenmiştir. Rancimat ve PetroOXY cihazları ile indüksiyon süreleri (IP), sırasıyla 110, 120 ve 130°C sıcaklıklarda 5,45, 2,86, 1,45 ve 2,23, 1,12 ve 0,62 saat olarak ölçüldü. Normal depolama sıcaklıklarında, IP değerleri gün olarak teğet yöntemle ölçülmüştür. Belirlenen IP değerlerinde peroksit değerleri (PV), sırasıyla 20, 30 ve 40 °C 'de 12,08, 29,24 ve 33,09 olarak tespit edilmiştir. Rancimat ve PetroOXY cihazlarından 20, 30 ve 40 °C'lik normal depolama sıcaklıkları için öngörülen indüksiyon süreleri sırasıyla 88,71, 48,85 ve 23,22 ve 30,17, 15,91 ve 8.38 gündü. Rancimat yöntemiyle normal depolama sıcaklıkları için raf ömrü tahmini, PetroOXY cihazına göre daha güvenilir olduğu görülmüştür. Ayrıca, iki hızlandırılmış yöntem arasında iyi bir korelasyon olduğu ve PetroOXY'nin Rancimat'tan daha hızlı bir yöntem olduğu gözlendi

Anahtar Kelimeler: Rancimat, PetroOXY, Oksidasyon Stabilitesi, İndüksiyon Süresi, Depolama

"Dedicated to my family"

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LIST OF ABBREVIATIONS

AOM	Active Oxygen Method
BHA	Butylated Hydroxyanisole
BHT	Butylated Hydroxytoluene
TBA	Thiobarbituric Acid
TBHQ	Tertiary-butylatedhydroquinone
TOTOX	Total Oxidation
DSC	Differential Scanning Calorimetry
FTIR	Fourier Transform Infrared Spectroscopy
GC	Gas Chromotography
IP	Induction Period
IV	Iodine Value
MA	Malonaldehyde
NMR	Nuclear Magnetic Resonance
***p*-AnV**	Para Anisidine Value
PG	Propyl Gallate
PV	Peroxide Value
PUFA	Polyunsaturated Fatty Acid
OSI	Oil Stability Index

CHAPTER I

INTRODUCTION

1.1 Motivation of the Present Study

Lipid oxidation is a significant factor that can occur in process stages such as storage, cooking and frying and may be the basic reason of degradation in the quality of food. Therefore, companies that produce fat-containing products see oxidative degradation as a major economic problem for themselves. Oxidation of unsaturated lipids not only provides undesirable taste to fat products, but also produces reagents related to carcinogenesis, aging and cardiovascular disorders. If the quantity of unsaturated/polyunsaturated fatty acids in the fats & oils are high, it decompose readily and making them unacceptable to consumers. Antioxidant is used to stop these deterioration reactions. Synthetic antioxidants are widely used, although they are known to show carcinogenic effects when used for a long time. Due to this negativity, the demand for natural antioxidants has increased recently (Inanç, 2012)

The amount of polyunsaturated fatty acids in mid-oleic sunflower is higher than high-oleic sunflower oil. In this study, mid-oleic sunflower oil was utilized which is easily accessible.

Consumers want to know the estimated shelf-life when they buy products. Therefore, food producers should also determine the shelf life of the products in a shorter time and in a correct way. For this reason, several accelerated shelf-life testing methods have been developed to ensure a reasonable measurement of the shelf life of oils in a shorter time. The end or shelf-life or induction time of product is determined quickly and reliably with commercially available devices. Today, Rancimat device is widely used in this area (Farhoosh, 2007).

In this study, Carvacrol and butylated hydroxytoluene (BHT) were used at the same concentrations as natural and synthetic antioxidants respectively. Then, peroxide, FFA, p-*Av*, TOTOX values were measured during normal storage (20-30-40ºC). Two

accelerated shelf-life testing devices were used. The first one was Rancimat, the other was PetroOXY device. These two devices were worked with high temperatures (110, 120, 130 ºC) and induction times were evaluated for each temperatures for control oil, Carvacrol and BHT added oils. On the other hand, shelf-life was predicted by working with normal storage temperatures (20, 30, 40 ºC) in terms of peroxide value by tangent method. As a result, the estimated shelf-life found in PetroOXY, Rancimat, and normal storage were compared in order to determine whether the results of a new device, PetroOXY, are reliable.

1.2 Mid-Oleic Sunflower Oil

Sunflower oil, as one of the significant consumable oils all over the world, is greatly consumed on a daily basis due to the large amount of polyunsaturated fatty acids (PUFA, 85–95%) (Noreen & Ashraf, 2010). Due to the high amount of linoleic acid, which occupies 68-72% of total fatty acid and shows hypocholesterolemic effect, diminishing the cardiovascular risk (Upadhyay and Mishra, 2015). At the same time there are several natural antioxidants in the oil, which increase its oxidative stability (Choi et al., 2013). Apart from these positive properties, due to the large amount of PUFA, the oils & fats is more exposed to oxidative decomposition which may produce rancid smells, diminish the quality of foods (Chen et al., 2014).

The amounts of linoleic acid in sunflower oil are quite high in order to some processing, but in high oleic acid sunflower oil the level of linoleic acid are low (PUFA), for this reason more stable than mid-oleic sunflower and sunflower oil (Table 1.1.)

Table 1.1 Fatty acid composition of sunflower oil (Codex Stan 2010-1999)

Fatty Acids	Sunflower Seed Oil	Sunflower Seed Oil (High-Oleic Acid)	Sunflower Seed Oil (Mid-Oleic Acid)
C6:0	ND	ND	ND
C8:0	ND	ND	ND
C10:0	ND	ND	ND
C12:0	ND-0.1	ND	ND
C14:0	ND-0.2	ND-0.1	ND-0.1
C16:0	5.0-7.6	2.6-5.0	4.0-5.5
C16:1	ND-0.3	ND-0.1	ND-0.05
C17:0	ND-0.2	ND-0.1	ND-0.05
C17:1	ND-0.1	ND-0.1	ND-0.06
C18:0	2.7-6.5	2.9-6.2	2.1-5.0
C18:1	14.0-39.4	75-90.7	43.1-71.8
C18:2	48.3-74.0	2.1-17.0	18.7-45.3
C18:3	ND-0.3	ND-0.3	ND-0.5
C20:0	0.1-0.5	0.2-0.5	0.2-0.4
C20:1	ND-0.5	0.1-0.5	0.2-0.3
C20:2	ND	ND	ND
C22:0	0.3-1.5	0.5-1.6	0.6-1.1
C22:1	ND-0.3	ND-0.3	ND
C22:2	ND-0.3	ND	ND-0.09
C24:0	ND-0.5	ND-0.5	0.3-0.4
C24:1	ND	ND	ND

ND: Not detected

1.3 Lipid Oxidation

Lipid oxidation is a significant reaction that directly influences the quality and shelf-life of fats & oils. Lipid oxidation reactions begin to accelerate in the existence of oxygen. Fats and oils can subject to some types of oxidation in some cases .A series of reactions, which is described as initiation, propogation and termination, start with the oxygen attacks on the double bonds of the triacylglycerol molecules initiated by heat during storage (Shahidi, 2005).

Lipid oxidation is generally a very rapid reaction. In the initiation stage (Eqn. 1.1), which is slow to formation, unsaturated lipids (RH) decompose into the free radicals (R•) by a catalysts (metals, oxygen, light, etc.) in contact with the oil. Free radical formation accelerates with heat.

$$RH \rightarrow R\bullet + H \qquad (1.1)$$

Once a free radical is produced, it joins with oxygen in order to create a peroxy-free radical (Eqn.1.2), and then the peroxide and new free radicals are produced, thus the propagation step begins. At the first step, oxidation normally proceeds slowly, hydroperoxides increases slowly, the time in order to reach a rapid rise in oxidation rate is called to as the induction time (Velasco et al., 2004; Shahidi, 2005). In this stage, formation of a hydroperoxide (ROOH) and another free radical is formed by reacting the free radical with an oxygen molecule that produces a peroxy radical (ROO•) which breakdown the weak C-H bonds on triacylglycerol (RH). This free radical reacts with oxygen to produce the peroxy radical. Through the chain reactions, more hydroperoxides and free radicals are produced (Eqn. 1.3) (Shahidi, 2005).

$$R\bullet + O2 \rightarrow ROO\bullet \tag{1.2}$$

$$ROO\bullet + RH \rightarrow ROOH + R\bullet \tag{1.3}$$

The primary oxidation product (Hydroperoxides) occured in the first step can be separated from smaller volatile compounds such as aldehydes, ketones, alcohols etc. These secondary oxidation products cause oxidative chain reactions to proceed to a more stable structure, and thus may bring about many diseases (Yanishlieva and Marinova, 2001).

If free radicals react with each other (Eqns. 1.4-1.6) in order to produce inactive and more stable products, termination may occur after propagation.

$$R\bullet + R\bullet \rightarrow R - R \tag{1.4}$$

$$ROO\bullet + R\bullet \rightarrow ROOR \tag{1.5}$$

$$ROO\bullet + ROO\bullet \rightarrow \text{Non-radical products} \tag{1.6}$$

1.4 Prevention of Oil Deterioration by Antioxidants

Oxidative deterioration has a great economic importance due to the fact that it has an impact on limiting shelf-life of lipid-containing foods beside of sensory and nutritional quality. Therefore, lipid oxidation prevented not only economical but also important for human health. There are several ways to prevent or delay lipid oxidation in food products. For instance, vacuum packaging, modified atmosphere packaging and refrigeration/freezing (Naz et al., 2004). Fats with a proportion of polyunsaturated fatty

acids are hydrogenated in order to become more stable and thus degraded later. This application is not very preferred because it may harm to human health. Another, fats with high amount of PUFA, is blended with saturated fats, which is stable, to becomes more resistance to deterioration. For example, palm olein oil is used generally for frying purpose, after mixing a small amount of palm oil to palm olein to obtain more stable product.

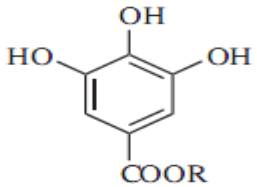
Antioxidants are classified into two types, primary and secondary, depending on their mechanism used to prevent degradation process (Inanç, 2012). The primary antioxidants are generally known as free radical scavengers, while secondary antioxidants are known as peroxide scavengers or hydroperoxide disintegrants (Brewer, 2011).

Two kinds of antioxidants are available: synthetic and natural.

1.4.1 Synthetic Antioxidants

Synthetic antioxidants are widely used in the world because of cheap, easy to obtain and their high performance for the purpose of delay lipid oxidation. Synthetics antioxidants are mixed with the appropriate oils & fats types according to the amount within the legal limits. Prolonged use of synthetic antioxidants may cause cancerogenic effect. Example of the synthetics antioxidants are butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), propyl gallate (PG), and tertiary-butylhydroquinone (TBHQ) (Akoh and Min, 2002).

Propyl gallate (Figure 1.1) is a powerful synthetic antioxidant in order to increase shelf-life of edible fats at approximately of 100 - 200 ppm (Richard, 2004). PG is not heat stable antioxidant, for this reason it is not used for frying process.



R = C_3H_7	Propyl Gallate
= C_8H_{17}	Octyl Gallate
= $C_{12}H_{25}$	Dodecyl Gallate

Figure 1.1 Chemical structures of different alkyl gallates (Shahidi, 2005).

TBHQ is the most forceful sythetic antioxidants in fats generally consumed for frying purposes (Figure 1.2). It has excellent antioxidant properties when used with citric acid (Akoh and Min, 2002; Shahidi, 2005).

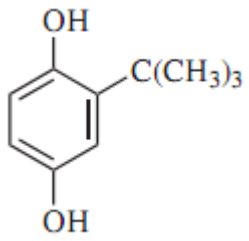


Figure 1.2 Chemical structure of TBHQ (Shahidi, 2005)

BHA is a waxy, monophenolic, white solid so fat-insoluble (Figure 1.3). It is not effective for vegetable oils whereas effective in animal fats (Akoh and Min, 2002; Shahidi, 2005).

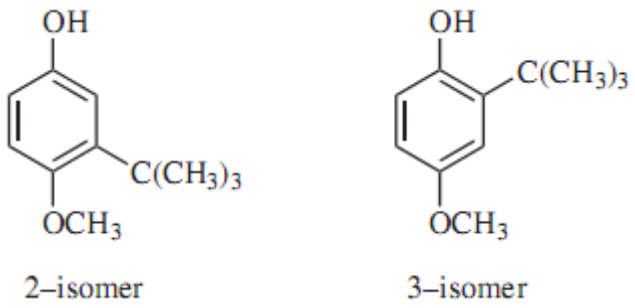


Figure 1.3 Chemical structures of BHA molecules (Shahidi, 2005)

BHT (Figure 1.4) shows similar physical properties with BHA. It is suitable for high temperature use, but not as stable as BHA (Inanç, 2012).

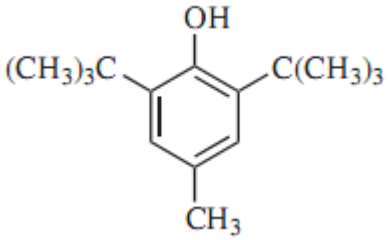


Figure 1.4 Chemical structure of BHT (Shahidi, 2005)

In spite of the cheap and more stable synthetic antioxidants, long-term use of synthetic antioxidants increases the use of natural antioxidants over time due to carcinogenic effects on human health (Bandoniene et al., 2002).

1.4.2 Natural Antioxidants

Most common natural antioxidants are tocopherols (vitamin E). The amount and types of tocopherols vary according to the oil types. Most vegetable oils have high vitamin E content. For this reason, using of tocopherols may cause to prooxidant effect (Akoh and Min, 2002; Shahidi, 2005). Ascorbic acid is another natural antioxidant produced synthetically. Ascorbic acid has primary or secondary antioxidant properties. Carotenoids are also naturally producing antioxidants, removing primary free radicals and showing primary antioxidant properties. Many plants are known as the source of natural antioxidants because of their phenolics content. Spices, herbs, teas, oils, seeds, cereals, cocoa shell, grains, fruits and vegetables are defined as the source of natural antioxidants (Inanç, 2012). Because of their content of phenolic acids and flavonoids, spices and herbs have antioxidant activity. They act as a free radical scavengers. Due to the strong aroma of spices or herbs, there is a handicap. Therefore, some studies have been carried out in order to prevent this negative effect. (Akoh and Min, 2002; Shahidi, 2005).

1.5 Methods for Measuring Lipid Oxidation

Lipid oxidation can be evaluated by many ways. However, these methods may not be standard for each food to determine oxidative changes. In other words, it is necessary to select the suitable way according to the convenient food (Akoh and Min, 2002; Shahidi, 2005). The present methods to show lipid oxidation in foods can be categorized into five groups based on what they evaluate: the absorption of oxygen, the loss of initial substrates, the formation of free radicals, and the formation of primary and secondary oxidation products (Shahidi, 2005; Dobarganes, 2000). Stability of fats and oils are generally accepted as the storage life/shelf-life of the product. Oxidative rancidity is usually the basic concern, even though other types of decomposition may occur at the same time and make the problem more complex (Richard, 2004). Several ways have been improved for commenting the long-term stability of fats and oils, which by exposing the sample to appropriate conditions in order to accelerate the normal oxidation process. Since the oxidation of fats and oil

samples occurs via a complex mechanism, a direct relationship between these assessments may not be possible. The overall lipid oxidation mechanism is summarized as shown in Figure 1.5

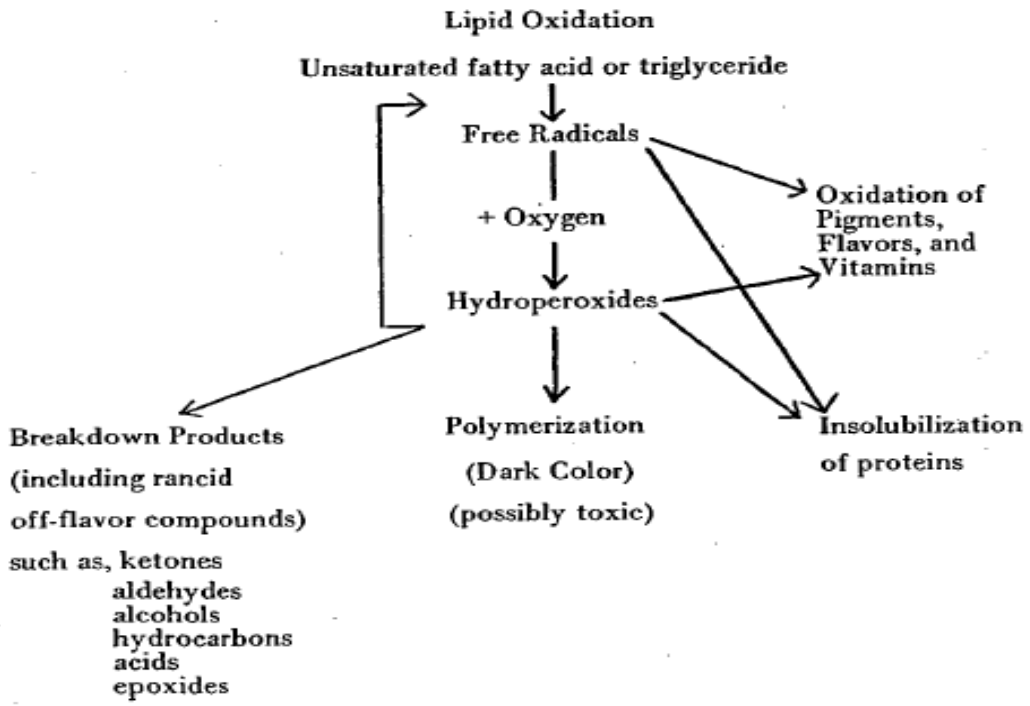


Figure 1.5 Overall mechanism of lipid oxidation (Labuza, 1971)

1.5.1 Measurement of Oxygen Absorption

Weight gain method is largely well-known that consumption of oxygen at lipids and producing of hydroperoxides is measurable throughout first steps of autoxidation. Sample is weighed and waited at suitable temperature after remove the volatile matters or water from the sample. The weight gain is measured periodically and the weight gain is plotted during the holding time to obtain the Induction Time (IP) (Shahidi, 2005). There are many disadvantages of applying this method. For instance; some products that require low or medium temperature, require more analysis time. Amount of sample size and temperature affect the results. On the other hand, this method offers advantages due to low equipment costs. In addition, this method is useful for oils with high unsaturated fatty acid composition.

Another method to weight method is headspace oxygen uptake. The oil sample is placed in a closed container containing a certain amount of oxygen at high temperatures (Shahidi, 2005). The pressure drop in the vessel, which is because of

oxygen usage, is measured. The induction time, which is the stage of the maximum change in the rate of oxygen usage, can be measured (Velasco, 2004). The measurement of lipid oxidation by this method is limited because protein oxidation, other than fats and oils, also absorbs oxygen (Melton, 1983).

1.5.2 Measurement of Reactant Change

Unsaturated fatty acids are the basic reactants in foods containing fats and oils and its composition varies during oxidation (Shahidi, 2005). Lipids extracted from foods are converted to fatty acid methyl esters by chromatographic analysis, usually gas chromatography (GC) and fatty acid compositions are determined (Fruhwirth et al., 2003). However, this method is not suitable for oils with a large amount of saturated fatty acid due to measurement of the change in unsaturated fatty acid composition (Shahidi, 1997). For this reason, this method is appropriate for measuring the change in fatty acid contents change of the marine oils and highly unsaturated vegetable oils during oxidation. Similarly, iodine value indicating the degree of unsaturation is also one of the methods used to measure lipid oxidation (Hudson et al., 1983).

1.5.3 Measurement of Primary Products of Oxidation

Summary, principles and application areas of methods for analysis of primary oxidation products are shown in Table 1.2. Hydroperoxides, the primary oxidation product during lipid oxidation, are continuously formed and may be converted into secondary oxidation products (Dobarganes, 2002). Throughout the first step of oxidation, producing rate of hydroperoxides are more than their decomposition. For this reason, peroxide value (PV) is an index of primary steps of oxidation. Several ways have been improved for assignation of PV such as iodometric titration, ferric ion complex measurement spectrophotometry and infrared spectroscopy (Shahidi, 2005). The most important factors affecting peroxide formation are temperature, time and type of oils due to fatty acid composition.

Iodometric titration method, which is one of the most commonly used methods to determine PV value, is based on oxidation of iodine ion (I^-) by hydroperoxides (ROOH) (Shahidi, 2005). Saturated iodine solution is added to oil sample and free iodine is then titrated with sodium thiosulfate and use starch as indicator (Akoh and Min, 2002). The PV is calculated. Disadvantages of this method are difficulties in determining the titration endpoint. The method needs high care and precision.

Ferric ion complexes are another chemical method to measure the lipid oxidation spectrophotometrically. It is defined as the oxidation of ferrous ion to ferric ion in an acidic medium (Shahidi, 2005).

Fourier transform infrared spectroscopy (FTIR) has been known that the characteristic O-H tension absorption band is measured by ınfrared spectroscopy, which leads to the determination of the hydroperoxides. An absorption band at about 2.93 µm demonstrates the formation of hidroperoxides, while the changing of a hydrogen atom on a double bond or polymerization accounts for the disappearance of a band at 3.2 µm (Shahidi, 2005). The FTIR spectroscopy is a fast and reliable method to measure lipid oxidation.

The position of the double bond in polyunsaturated fatty acids shifts due to the isomerization and formation of conjugated dienes during the oxidation. The formation of conjugated dienes exhibits an intense absorption at 234 nm; similarly conjugated trienes absorb at 268 nm (Akoh and Min, 2002). An increase in UV absorption indicates increase in the production of primary oxidation products.

Table 1.2 Summary of methods for analysis of primary oxidation products (Shahidi, 2005)

Methos	Principle	Measurement	Sensivity	Applications
Iodometric titration(PV)	Reduction of ROOH with KI and measurement of I_2	Titration with $Na_2S_2O_3$	~0.5 meq/kg fat	Fats and oils
Ferric ion complexes (PV)	Reduction of ROOH with Fe^{2+} and producing of Fe^{3+} complexes	Absorption at 500-510 nm of the red complex with SCN^-	~0.1 meq/kg fat	Fats, oils and food lipids
FTIR (PV)	Reduction of ROOH with TPP	Absorption at 542 cm^{-1} of TPPO	~0.2 meq/kg fat	Fats and oils
UV spectroscopy (Conjugated dienes and trienes)	Prediction of conjugated dienes and trienes	Absorption at 230-234 nm and 268 nm	~0.2 meq/kg fat	All samples

1.5.4 Measurement of Secondary Products of Oxidation

Summary, compounds measured and application areas of methods for analysis of secondary oxidation products are shown in Table 1.3. Hydroperoxides are not stable and decomposed to reach more stable structure such as aldehydes, ketones, alcohols, volatile organic acids.

Thiobarbituric acid (TBA) test is the one of the oldest method and largely used to determine oxidative decomposition of fats&oils (Shahidi, 2005). Throughout the lipid oxidation, malonaldehyde (MA) small oxidation product of PUFA reacting with TBA reagent to form a pink complex that is evaluated spectrophotometrically at its absorption maximum at 530–532 nm (Jardine et al., 2002)

Hydroperoxides are decomposed during oxidation to produce aldehydes and the amount of the aldehydes are measured by the para-anisidine value (*p*-AnV). The *p*-anisidine standard reacts with aldehydes under acidic medium turns yellowish that absorb at 350 nm (Akoh and Min, 2002)

Totox value is called sum of oxidation of primary and secondary products (Eqn.1.7). It is a assembling of:

$$\text{Totox value} = 2\text{PV} + p\text{-AnV} \qquad (1.7)$$

Totoks value gives information about the oil's present (PV) and past history (Para-anisidine). However, in spite of its advantages, Totox value has no scientific foundation for why it consist of two distinct extends (Akoh and Min, 2002).

Secondary oxidation products, including aldehydes, ketones and carbonyl groups formed by degradation of hydroperoxides, cause off-flavors and rancidity food products (Shahidi, 2005). Total carbonyl content is determined by a colorimetric 2,4-dinitropheniylhydrazone procedure (DPNH).

Oil stability index (OSI) method evaluate the producing of volatile acids which is formed as secondary oxidation products at high temperature during lipid oxidation by modify in electrical conductivity principle (Gordon et al., 2001). During the oxidation, volatile organic acids are formed, the conductivity increases and the rate of oxidation changes reach to the maximum point. This point is defined as OSI (Miura at al., 2002).

To achieve measurable results with this method, it is necessary to work high degree of oxidation. For this reason, the oil sample is exposed to elevated temperature and excess amount of air while measuring under laboratory conditions (Shahidi, 2005). Although both active oxygen method (AOM) and OSI methods use the principles of accelerated oxidation, the OSI method is the development of the AOM method. The AOM method measures the peroxide value, but the OSI method measures the varying conductivity due to volatile organic acid ions (Akoh and Min, 2002).

Two commercially equipment are available to determine the OSI value; the Rancimat and Oxidative Stability Instrument (Shahidi, 2005). The induction period (IP) in the Rancimat device is determined by the automatically measurement of the continuous change of time with the conductivity.

Table 1.3 Summary of Measurement Methods of Secondary Oxidation Products (Shahidi, 2005)

Method	Compounds	Comments	Applications
TBA	TBARS, mainly malonaldehyde	Spectrometry technique	All samples, especially fish oils
p-Anisidine	Aldehydes, mainly alkenals	Absorption at 350-nm	Fats and oils
Carbonyls	Total carbonyls or specific carbonyl compound formed	Spectrometry technique and HPLC for total or specific carbonyl compounds	Fats and oils
OSI method (Rancimat&Oxidative Stability Instrument)	Volatile organic acid	Monitoring changes in conductivity	Fats and oils

1.5.5 Analysis of Free Radicals

In the first stage of lipid oxidation, free radicals are formed which are nonstable. These free radicals are determined the oxidation level of fats and oils. Electron spin resonance (ESR) is called as electron paramagnetic resonance (EPR) is a device used to

determine the free radicals formed throughout the first step of oxidation (Velasco et al., 2004).

1.5.6 Other Measurement Methods

Thermal reactions occur during lipid oxidation of fat or oil materials for example, transfer of oxygen molecule to unsaturated fatty acids. Thus, it can be measured in thermal analyzes in accelerated oil stability tests.

The differential scanning calorimetry (DSC) method can be used for this purpose. It gives information about energy that analyse temperature and heat flows changes related to lipid oxidation in terms of time and temperature (Shahidi, 2005). The endpoint can be determined at the time when a fast exotermic reaction between oil and oxygen appears and induction period (IP) detected by intersection of extrapolated baseline and tangent line of the exotherm (Velasco et al., 2004).

High-resolution nuclear magnetic resonance (NMR) is used to assessment deterioration fats and oils by determining the hydrogen atoms in the triacylglycerol (TAG) (Akoh and Min, 2002). In this method, H atoms in powerful magnetic field absorb energy, at the radiofrequency zone in which varies according to the environmental conditions of the molecule. These varietys can be measured by NMR as the level of oxidation of oils. This method provides information about the oxidative regions of the TAG molecules, while both primary and secondary oxidation changes are determined (Shahidi, 2005).

1.6 Accelerated Methods and Prediction of Shelf-Life

Shelf life is identified as the term of compliance with any label declaration, as well as maintaining the desired sensory, chemical, physical and biological properties during certain food storage conditions (Monzocco et al. 2010). Assessing / estimating shelf life is important to confirm how long the product will last before it is elevated to a grade unsuitable for consumption. Shelf-life evaluation methodology of oxidized fats are addressed, based on the identification of the admissibility limit defined by appropriate oxidative index and shelf-life test strategies.

The detection of the deterioration of oils are time-consuming and expose to error analyzed under ambient conditions, therefore oxidative stability is quickly determined

by accelerated tests. The shelf life of the sample may be predicted by analyzing the product to an accelerated oxidation test under standardized states. Various parameters are available in order to enhance the rate of degradation, such as increasing the temperature, the amount of metal catalyst, the oxygen pressure. The shelf-life of refined edible oils is usually 12-18 months at room conditions, whereas these may be estimated long time in analysis performed at high temperatures (Farhoosh, 2007). During the shelf life of oil, the peroxide value is stated to be up to 10 milliequivalent of active oxygen/kg oil (Codex Stan 2010-1999). Since the oxidation rate is concerned to temperature, the shelf-life of an oil diminishes logarithmically with increasing temperature (Figure 1.6). So, the shelf life of the sample at room temperatures may be predicted in a short time by drawing an appropriate stability end point logarithm against high temperatures and extrapolating to room temperature (Kaya, 1993; Farhoosh, 2007).

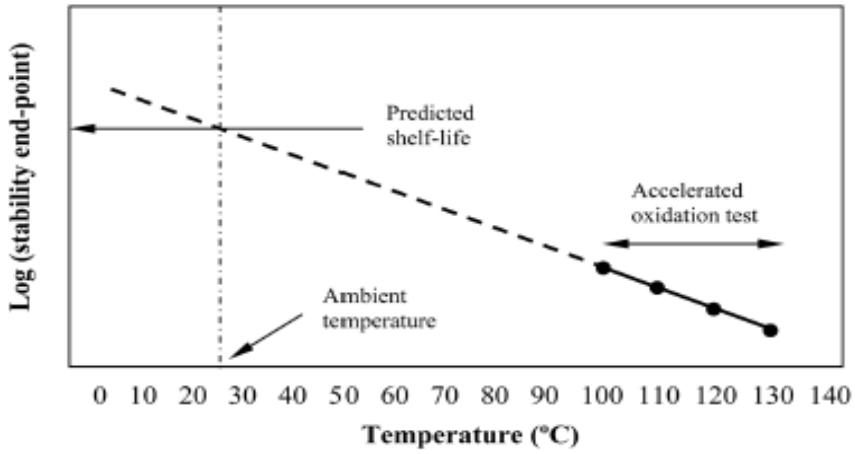


Figure 1.6 A Graph of shelf-life depends on extrapolating a convenient end point of stability to ambient temperature (Farhoosh, 2007)

Several accelerated techniques have been described to evaluate the oxidative stability of oils in a shorter period such as, Active Oxygen Method (AOM), Rancimat, new method of PetroOXY test. The shelf-life of products assessment is expressed in three basic stages. First, an important parameter that leads to a reduction in product quality must be defined. Finally, modeling is done with the data obtained to estimate/predict shelf-life of foods (Figure 1.7)

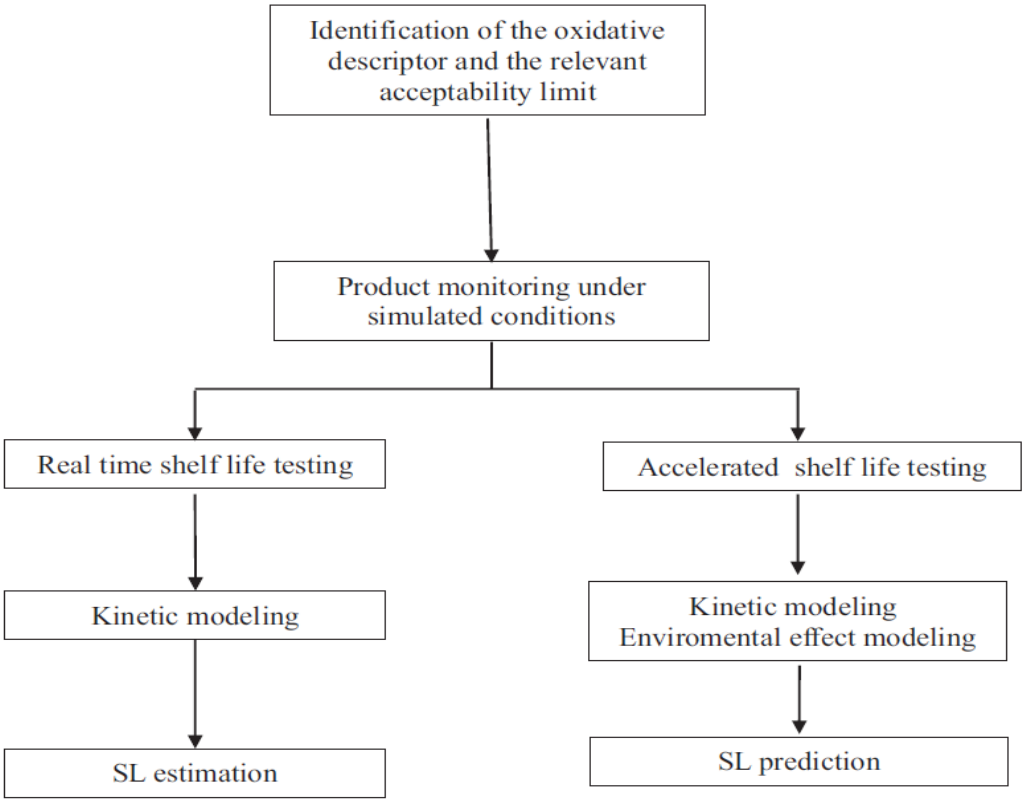


Figure 1.7 Basic steps in shelf-life evaluation schematics of fats/oils-based foods (Monzocco, 2016).

It is stated that zero orders are utilized to plot peroxide changes in products. For example, potatoes, chips, biscuits (Monzocco, 2016).

After the induction time, indicating that the oxidative reaction indicates the end, peroxides gradually rise before reaching maximum level (Figure 1.8). Two methods can be carried out in order to evaluate shelf life basis on the degree of peroxide value selected as the limit of acceptability. Two strategies can be applied to assess shelf life depending on the level of peroxide value selected as the limit of acceptability . The first approach can be detecting change, i.e., shelf life. It is defined as the time when the increase in peroxide value is measured. In this case, the induction time can be considered as the product shelf life (SL1 in Figure 1.8). A constant change level attempt when a selected peroxide value is selected as the limit of acceptability (SL2a in Figure 1.8). Furthermore, changes in peroxide value during storage can be modeled

by defining an experimental model that fits the curve (SL2b in Figure 1.8) (Monzocco, 2016).

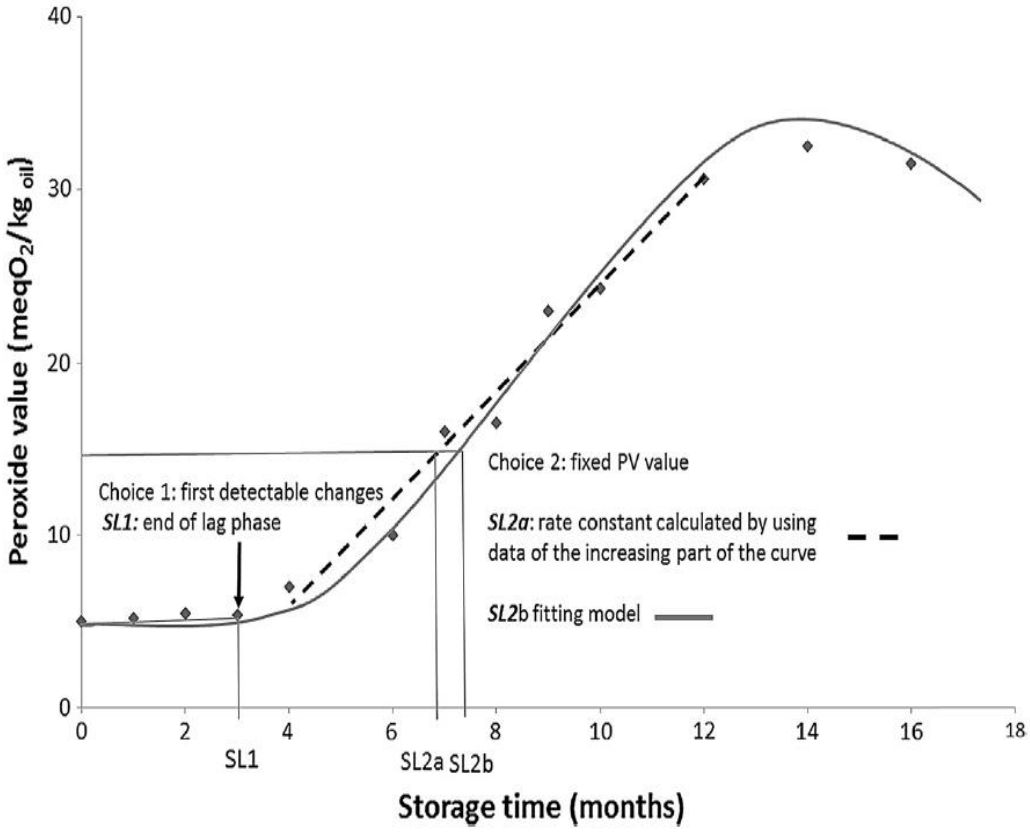


Figure 1.8 Graphical exhibition of changes of peroxide value as a function of storage time and potential method in order to define shelf-life (Monzocco, 2016).

CHAPTER II

LITERATURE REVIEW

2.1 Summaries of the Recent Studies

Mechanisms of antioxidants in different lipid systems may be different from each other. For example, Yanishlieva et al. (1999) examined the autoxidation of purified triacylglycerols of lard and sunflower oil (TGL and TGSO) containing 0.02, 0.05, 0.10 and 0.20% Thymol and Carvacrol(natural antioxidants) at ambient temperature. As a result, it was found that Thymol was more effective antioxidant than Carvacrol during the autoxidation of lipids at ambient temperature and both antioxidants were found to be different in their inhibitory effect mechanisms depending on the character of the lipid medium. Thymol was found to be a better antioxidant in TGSO than TGL. This is thought to be due to more steric inhibition of the phenolic group in Thymol than Carvacrol.

It is very important to determine the optimum parameters of the method used in order to predict the shelf life and it is a factor affecting the results of the analysis. Farhoosh (2007) investigated the effect of Rancimat's operational parameters for determination of oxidation stability measurements and to estimate shelf life of soybean oil. It was understood that the operational parameters in Rancimat studies which are amount of oil, air flow rate and temperature, affected the temperature coefficient, determination of OSI, Q_{10} value and shelf life estimation of products. The results presented that at 100 ^{o}C and amount of oil of 3 g, the air flow rates of 10 and 15 L h^{-1} give the same OSI. On the other hand, OSI at an airflow rate of 20 L h^{-1} was higher. There was no important distinction between the 10, 15, 20 h^{-1} air flow rates for 6 g oil and 3 g samples with 10, 15 h^{-1} air flow rates which showed air-saturated conditions. As the amount of fat increases from 3 g to 12 g exhibited that the OSIs increase from 15.82 to 16.65. At the end of the study it was reported that Rancimat device is used to detect quickly the oxidative stability of oils. This may

supply good results than other techniques in the estimation of shelf life of products when the levels of the operational factors of the device are appropriately selected.

Many studies have been conducted in order to detect the shelf life of oils. In this regard, Shim and Lee (2011) studied the shelf life estimation of perilla oil. The perilla oil was stored at different temperatures (25, 40, 50, 60, 75 ^{0}C) and the level of lipid oxidation was determined with respect to peroxide values (PV). For each temperature, the PV were measured at certain periods and the graph of PV versus time was obtained. The concurrents of the two lines were defined basis of time and PV at the IP. Estimation of shelf-life was made with kinetic modeling that is Arrhenius-like equations. The PV values at all temperatures increased over time. The inclination of two part before and after the concurrent period (IP, PV_{IP}) identified the rate constants k_1 and k_2 in the induction and propagation steps, respectively. Shelf life graph showed IP zone, safe zone and non-secure zone at PV levels. In this study, the equations that may be identified as the intersection of two lines. The PV values of perilla oil were estimated from kinetics models. The predicted values corresponded almost to experimental values and verified by using of the equations.

The mechanism of lipid oxidation varies with respect to temperatures. Farhoosh et al. (2013) studied the oxidative stability of variety of olive oils with various chemical combinations at low and high temperatures by accelerated method (IP, Rancimat at 100-110-120-130 ^{0}C) and normal storage. Some regression models were developed to set up the correlation between oxidative stability measures (IP or OSI) and combinational variables and shelf life of olive oils under soft storage terms predicted with developed empirical examples. Olive oil samples which consist of different chemical compositions were used and chemical analyzes of these oils were made, such as ratio of monounsaturated fatty acid and polyunsaturated fatty acid M/P, Peroxide value PV, conjugated diene value CDV, acid value AV , total polar compound change TPC , total phenolic content TP, total tocopherol content TT. The kinetics of hydroperoxides and conjugated dienes occuring throughout the oxidation of the olive oil at high temperaure were investigated PV and CDV showed a trend to rise over time and two linear lines were defined as initiation and termination. The oxidation rate constants (k_iPV, k_iCDV, k_pPV, k_pCDV) were determined. Findings showed that producing rate constants of conjugated dienes were significantly lower in each of the

two oxidation stages than in hydroperoxides. This may be because of all the hydroperoxides formed as primary oxidation products do not meanwhile include conjugated diene structures. During the oxidation of oil samples at 50 ^{0}C, the IP values of the formation of hydroperoxides and conjugated dienes correlated well. The Olive oil samples at 50 ^{0}C and accelerated stability tests determined that the IP values are different from each other. Due to the different kinetics of lipid oxidation at high temperatures used in acceleration tests, estimations from the accelerated stability tests do not often correlate with stability at ambient temperatures.

PetroOXY, accelerated method device to determine the oxidation stability, has been used in some studies. For example, Inanç (2012) has used this device to investigate the antioxidant effect of various herbal extracts on corn and palm oils during frying. The stability of seven plant essential oils (cinnamon, clove, rosemary, sage, turmeric, thyme, and oregano enriched with 80% carvacrol) and four active components of plants (curcumin, thymol, carvacrol, and cinnamaldehyde) were studied. The essential oils used between 1500-5000 ppm while active ingredients (Carvacrol and Thymol) used was 60-350 ppm. Firstly, IP values of corn oils, which contain different concentrations of active components and essential oils, were determined by PetroOXY device. The results showed that essential oils other than oregano fortified with 80% carvacrol had no important antioxidant influence on corn oil. Furthermore, carvacrol was found to be the only active components that significantly increased the oxidative stability of oils at the frying temperature. For this reason, frying of potatoes was carried out in corn and palm oil at optimum frying temperature of 150°C from which maximum induction times were obtained and optimum carvacrol and synthetic antioxidant BHT concentrations (200 ppm) were used. After these studies, optimum amounts of natural antioxidants (200 ppm) and temperature values (150°C) were estimated and used for subsequent frying experiments. The results showed that the induction periods were high when Carvacrol is added to the oil at 150°C 1462 and 3379 sec for corn and palm oil, respectively. These induction periods were greater than those when BHT was used. In this study, it was stated that peroxide value was not considered to be a useful test for the assessment of oxidative stability of frying oils. Although the effect of carvacrol on PV is not clearly understood, it is stated that it is very effective in reducing FFA in oils and is more effective than BHT. The Studies have shown that carvacrol had more effect on reducing pAV of corn and palm oils than

synthetic antioxidant BHT. Also, the IP values determined by the accelerated test device PetroOXY were found to be consistent with the experimental studies.

Tinello et al. (2017) studied the comparison of two accelerated methods to asses the oxidative stability in frying oils. Induction times of 15 frying oil samples were measured using OXITEST and RANCIMAT accelerated oxidation methods. Temperatures were set to 110^0C for both instrument. As a result, OXITEST and RANCIMAT devices obtained the similar results despite distincts measurement methods. Statistical analysis of the IP data of the oils were evaluated with OXITEST and RANCIMAT methods showed an important positive relationship between the IP values of these different methods. The IP measured by the OXITEST method are nearly two times lower than measured when using the RANCIMAT device. The OXITEST method can be compared to RANCIMAT to evaluate the relative oxidative stability of frying oils.

One of the new methods used in oxidation stability measurements, PetroOXY has been compared with Rancimat method in many studies. Also, it was compared the oxidation stability of different types of oils with conventional methods. The PetroOXY method was not used directly for shelf life determination. For example, Duert et al., (2018) was compared the thermal and oxidative stability of moringa oil with olive and canola oils. In this study Rancimat and PetroOXY accelerated methods and Schaal analysis were used. When the induction times determined by the PetroOXY method were evaluated, it was found that moringa oil had the highest oxidation stability, while the values of the induction times determined in the Rancimat methods exhibited an oxidative stability the same as olive oil. Both the moringa and olive oils were importantly more stable than canola oil. In this study, the author stated that accelerated methods for determining oxidative stability were evaluated for changes in oils when exposed to accelerated aging conditions that were completely different from actual aging cases. It is stated that the estimation of shelf life is difficult because the peroxide value is low and also the rate of decomposition of peroxides and the producing of renewed peroxide cannot be predicted. In this case, they say why the estimate does not encounter to the actual shelf life. One of the advantages of the PetroOXY method with respect to Rancimat is that the PetroOXY equipment gives faster results. In this study, the author evaluated the induction time measured by the Rancimat test with a highly

degradable oil sample including aldehydes, ketones and carboxylic acids, as well as peroxide and hydoperoxides, whereas the induction time in the PetroOXY test was defined by a partially oxidized sample. Finally, the difference in induction times in oil samples is explained by the working principle of accelerated methods and the differences in their fatty acid compositions.

CHAPTER III

MATERIALS & METHODS

3.1 Materials

In the present study, the oil used was refined , mid-oleic sunflower oil. It was obtained from a factory established in Gaziantep. The chemical reagents (Hexane, Acetic Acid, Chloroform, Sodium Thiosulfate, Ethyl Alcohol 95%, Sodium Hydroxide, I) and Carvacrol ($C_{10}H_{14}O$), which has a molecular weight of 150.22 g / mol and is 98% purity, colorless liquid were purchased from Sigma Aldrich Co. (St. Louis, MO).

3.2 Methods

3.2.1 Determination of Fatty Acids Composition of Mid-Oleic Sunflower Oil by Gas Chromatography

The fatty acid compositions (saturated, unsaturated fatty acid content) of the oils used were evaluated by gas chromatographic analysis of the corresponding methyl esters (FAMEs) (Ichihara et al., 1996). Analysis was performed using an Agilent 789A GC (Agilent Technologies, Cernusco sul Naviglio, Milan, Italy) system. This equipment has a autosampler and a Flame Ionization Detector (FID). First, the FAMEs were prepared by taking 40 mg of the sample in a tube and adding 2 mL of hexane and 200 µL of 2 M KOH. Then, all reagents were mixed well at room temperature and allowed to wait after adding about 0.5 g Na_2SO_4. Aliquot of the upper hexane layer (1 µL) was injected into the capillary column in split mode (1:50). Helium was utilized as carrier gas. The injector and FID temperatures were set to 250 °C, and isolation of the different FAMEs at 100°C was performed using oven temperature. Different fatty acids were determined with respect to their retention times. The fatty acid composition was determined as a percentage relative to the total of the peak areas detected.

3.2.2 Testing Oxidative Stability of Carvacrol in Mid-Oleic Sunflower Oil by Accelerated Oxidation Method

The oxidation stability of sunflower oil was tested by using natural and synthetic antioxidants, Carvacrol and BHT, respectively. For testing the activity of both, 200 ppm antioxidant was used from each. The oxidative stability of the oils at different temperatures was determined by using PetroOXY (Petrotest Instruments GmbH & Co. KG, Dahlewitz, Germany) and Rancimat (Model 892, Metrohm, Heriasu, Switzerland) devices with respect to induction period/time. The induction time is defined as the time until the acceleration of lipid oxidation begins exponentially. High induction period means that the product has a high oxidative stability, which means that the shelf-life of the product is high.

3.2.2.1 Determination of Induction Period by PetroOXY Method.

In the present study, the induction times of the oils were determined using PetroOXY equipment which is one of the accelerated oxidation tests. The PetroOXY method evaluates the oxidation stability of all volatile and non-volatile oxidation compounds of the sample. It has a small test chamber which is sealed hermetically. A small amount of sample, about 3 mL, in the test chamber is subjected to fresh oxygen at a pressure of about 700 kPa and heated to the set temperature. When the oil sample oxidizes, it depletes the oxygen in the closed test chamber, causing a pressure drop. The induction time is defined as the time passed between the start of the analysis and the break point defined as a pressure drop below 10% of the maximum pressure determined in the chamber while heating to the analysis temperature defined by Neumann et al. (2008). The induction period of oil samples (Carvacrol, BHT and Control) at different temperatures (110-120-130 °C) was determined by using the PetroOXY under 700 kPa. A representative plot of determination of induction period by PetroOxy devise is shown in Figure 3.1.

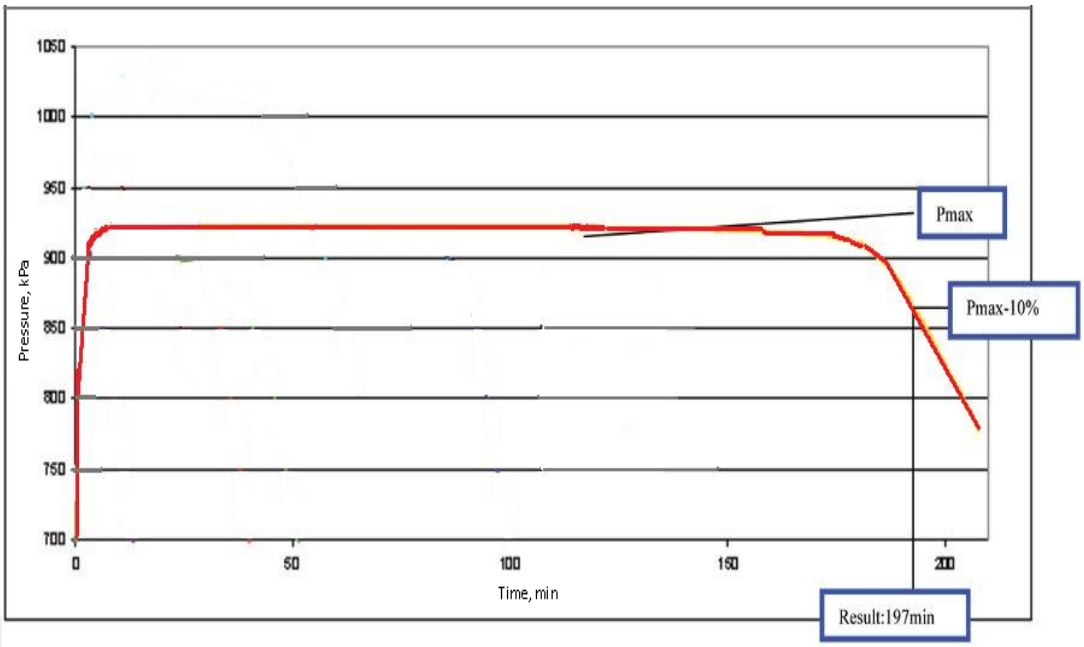


Figure 3.1 A schematic plot exhibiting measurement of induction period by PetroOXY (Neumann et al., 2008)

3.2.2.2 Determination of Induction Period by Rancimat Method

The measurement principle of Rancimat systems is based on oxidation under natural flow of oxygen in the air at constant temperature. The system is a closed-circuit system. The "accelerated" oxidation process is based on the blowing of volatiles formed due to oxidation of oil into a container filled with pure water. At the same time the conductivity of water in the container is monitored against the time and the conductivity change called "induction time" is determined (Figure 3.2). The induction time determines the time of occurrence of volatile reaction products (typically volatile fatty acids), which is the most critical step of the degradation process, and allows the monitoring of the occurrence of these components on the instrument as a change of conductivity (Farhoosh, 2007).

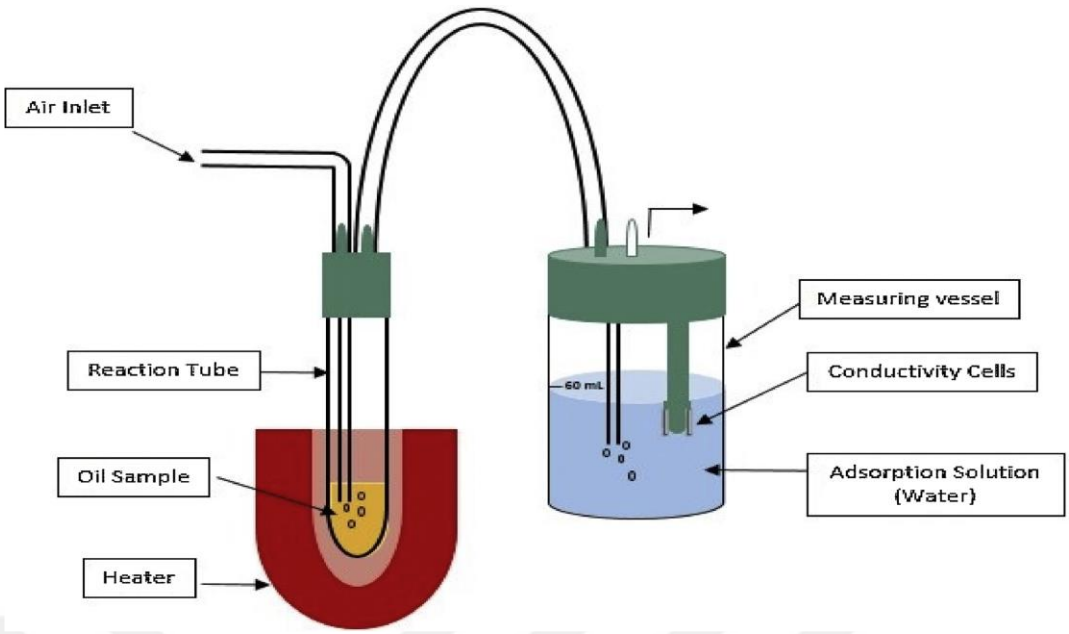


Figure 3.2 Schematic representation of the Rancimat equipment (Kurtulbaş et al., 2018)

Accelerated oxidation terms in oil samples (3 g) were set up using an air flow rate of 20 L/h and temperatures of 110, 120 and 130°C. Conductivity cells (60 mL) containing pure water, max. 2.5 µs conductivity, were continuously followed automatically for rise in conductivity throughout the analysis. Finally, the IP of the oils was automatically recorded as an appropriate endpoint to estimate oxidative stability (Figure 3.3). The change in conductivity of 60 ml distilled water versus time was recorded, and the IP was calculated using Metrohm software (Metrohm Ltd, CH-9101 Herisau, Switzerland). Each test was performed in duplicate for carvacrol, BHT and control. The log(IP) versus temperature was plotted to make oxidation stability prediction at higher temperatures (> 100°C). The shelf life of the oil was also estimated at low temperatures by extrapolation (Farhoosh 2007; Upadhyay and Mishra 2015a) from the information derived at accelerated temperatures.

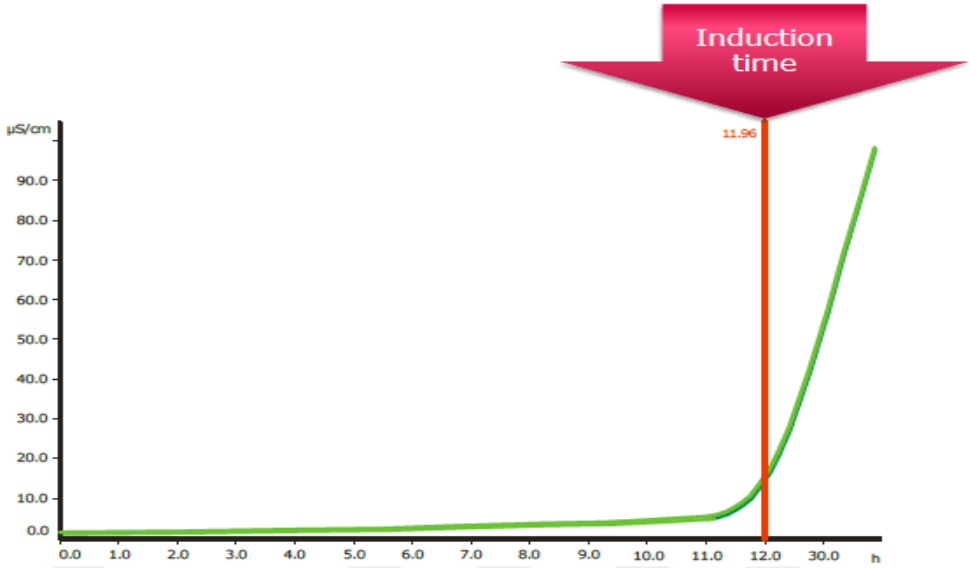


Figure 3.3 Schematic representation of induction time by Rancimat method (X axis represents time(h), Y axis represents conductivity (µs/cm))

Up to the time of induction, as summarized in Figure 3.4; fatty acids are oxidized, peroxide is formed, and the reaction rate is slow. While the accelerated oxidation time begins, the reaction is much faster, sample deteriorates, bad smell and polymerization begins.

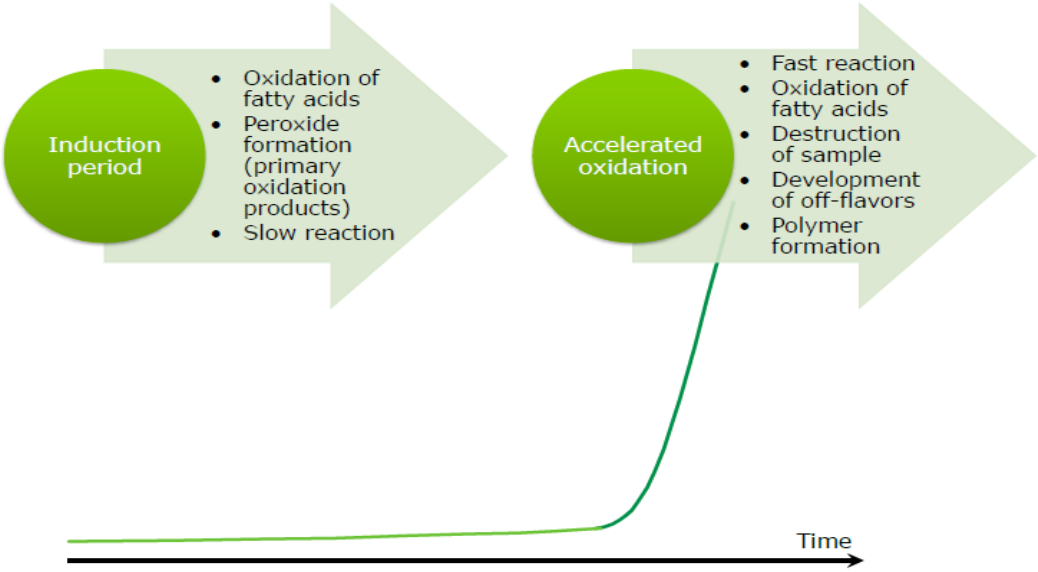


Figure 3. 4 Summary of oxidation by Rancimat method

3.2.3 Testing Oxidative Stability of Carvacrol in Mid-Oleic Sunflower Oil by Normal Storage Method

The primary and secondary lipid oxidation products are determined as peroxide value and p-*Av* value analyses to measure the lipid oxidation degree in oils during storage. The oxidation process of oil under normal storage conditions, i.e., the change in the amount of peroxide was followed. Induction period was calculated by Tangent method (Upadhway, 2016)

Oils are physically and chemically deteriorated at various storage temperatures. Various analyses in each designated time plan; peroxide value, free fatty acid value, para-anisidine value, TOTOX value were used to assess the deterioration of oils.

Mid-oleic sunflower oil samples (sample containing 200 ppm carvacrol, sample containing 200 ppm BHT, control sample: no antioxidant added sample) were prepared in 60 ml amber bottles and placed in ovens pre-set to 20, 30, 40 °C. Samples were taken from the incubator for analysis at planned times. For each analysis period one sample bottle was used and rejected. The analysis were performed at every 15 and 3 days intervals for the oils stored at 15 and 30-40°C respectively.

3.2.3.1 Peroxide Value

Peroxide values of oils at 20-30°C were measured in the determined time periods. Approximately 5 g of oil sample was weighed into an erlenmayer flask . Then, 30 mL of acetic acid / chloroform solution (3: 2) was added and agitated to dissolve. 0.5 g of saturated KI solution was added and the solution was stand to remain in the dark place for 5 minutes. Then, 30 mL of distilled water and 1 mL of 1% starch solution were added. The solution was titrated with 0.01 N sodium thiosulfate, $Na_2S_2O_3$. Same processes were performed without sample (blank test). The results were calculated from Eqn. 3.1 and expressed as milliequivalents (meq) of peroxide per kilogram of oil (AOAC, 1990).

$$PV = ((S - B) * N * 1000)/m \tag{3.1}$$

where,

PV: peroxide value, meq / kg oil

S: ml of 0.01 N $Na_2S_2O_3$ used for the sample

B: ml of 0.01 N $Na_2S_2O_3$ used for the blank

N: normality of $Na_2S_2O_3$ solution

m: mass of the test sample, in grams.

3.2.3.2 Free Fatty Acids

5 g of oil sample was weighed into a flask. First, 50 mL of diethyl ether and 50 mL of ethyl alcohol (95%) were added to dissolve the oil. After adding 1 mL of 1% phenolphthalein, the solution was titrated with 0.01 N sodium hydroxide (NaOH) solution. The amount of free fatty acid of the sample was determined as % oleic acid (Eqn.3.2) as described by Ozbayram (2000).

$$FFA(\%\ oleic\ acid) = (V * N * 28.2)/m \qquad (3.2)$$

where,

V: ml of 0.01 N NaOH used for the sample

N: normality of NaOH

m: mass of the sample, in grams

3.2.3.3 Para-Anisidine Value

2 g of the sample was added to a flask and dissolved in 25 mL isooctane. The absorbance of this solution was measured at 350 nm using a spectrophotometer (Novaspec, Pharmacia Biotech, Cambridge, England). Then, 5 mL of this prepared solution was mixed with 1 mL of 0.25% para-anisidine in acetic acid (w/v) and waited for 10 minutes, then the absorbance of this solution was measured at 350 nm. Para-anisidine value of the oil was calculated by using Eqn.3.3 (Zhang et al. 2010).

$$pAv = (25 * (1.2 * As - Ab))/m \qquad (3.3)$$

where,

As: absorbance of the oil solution after reaction with the para-anisidine reagent

Ab: absorbance of the oil solution

m: mass of the sample, in grams

3.2.3.4 Total Oxidation (TOTOX) Value

TOTOX value was calculated with respect to the Eqn. 3.4 (Allen and Hamilton, 1994).

$$TOTOX = pAV + 2 * PV \quad (3.4)$$

3.2.3.5 Statistical Analysis

All analyses were conducted in duplicate and the results shown are the average of the values obtained. The results were compared by analysis of variance (ANOVA) to test for significant differences between parameters (Peroxide, Free Fatty Acid, Para Anisidine Value) of Control group, BHT and Carvacrol added oils stored at 20, 30 and 40ºC at 5% significance level. Means of the groups were compared by Duncan's multiple range test using SPSS 16.0 program. Trends were considered significant when means of compared pairs differed at $p<0.05$ level.

3.2.4 Prediction of Shelf-Life

The shelf life of the oils at room temperature was estimated by plotting the logarithms of IP's against accelerated temperatures and extrapolating to room temperature From Eqn.3.5, the slope of the graph indicate the temperature coefficients for the products.

$$Log(OSI\ or\ IP) = A(T) + B \quad (3.5)$$

CHAPTER IV

RESULTS & DISCUSSION

In the present study, firstly, the oxidative stability of Carvacrol and BHT added mid-Oleic sunflower oils and control oil were tested by PetroOXY and Rancimat device at 110, 120 and 130°C. Then, oxidation stability of these samples was tested at normal storage temperatures (20, 30, 40°C). Induction time at each temperature was determined. Graphics of samples stored at 20, 30 and 40°C were plotted as peroxide values versus time. Then, the induction periods were estimated from these graphes using tangent method. Finally, the shelf-life of the oils at room temperature was estimated by plotting the logarithm of IP's against temperature. Then, the shelf life at any temperature (lower temperatures, 20, 30, 40°C) was estimated by extrapolation using the same plot. Thus, induction times determined by the PetroOXY method were compared with the results obtained from Rancimat methods. Also, the results obtained from extrapolation to estimate the shelf life at lower temperatures were compared with the normal storage shelf lives.

4.1 Determination of Fatty Acid Compositions of Mid-Oleic Sunflower Oil by Gas Chromatography

The type of fatty acid of oil is important. If the amount of unsaturated fatty acids is higher in the fats and oils, the oil will deteriorate more quickly. Fatty acid composition of the sample was determined as shown in Table 4.1

Table 4.1 Fatty acid composition of Mid-Oleic Sunflower oil sample

Fatty Acids	Composition (%)
C6:0	ND
C8:0	ND
C10:0	ND
C12:0	ND
C14:0	ND
C16:0	4.2
C16:1	0.04
C17:0	ND
C17:1	0.05
C18:0	3.3
C18:1	**53.1**
C18:2	**37**
C18:3	0.4
C20:0	0.3
C20:1	0.25
C20:2	ND
C22:0	1.01
C22:1	ND
C22:2	0.05
C24:0	0.3
C24:1	ND

ND: Not Detected

As can be seen from the results, the amount of oleic acid was 53.1 % and polyunsaturated fatty acid, primarily linoleic acid (18:2), in the sample was 37%. Accelerated Rancimat tests showed that induction times are higher in high-oleic than mid-oleic sunflower oil. For instance, the author (Vidrih et al., 2010), determined that the induction time of sunflower oil at 110 °C and oleic acid (72.84%) amount to 12.61 hours, while the induction time of sunflower oil with 29.92% oleic acid was determined as 6.19 hours. In our study, for our sample containing 53.1% oleic acid, the induction time at the same operational parameters was determined as 5.45 hours.

Fatty acid content is a significant factor in estimating product stability and shelf life. It is well known that oils with a high proportion of polyunsaturated fatty acids are more sensitive to oxidative decomposition and thus have a shorter shelf life. High saturated or monounsaturated fatty acids are known to maintain more stable, nutritious and

sensory properties (Duarte et al., 2018). Inanç (2012) determined the induction times of corn and palm oil with PetroOXY, an accelerated method, and the results were consistent with each other. At each working temperature (140-185°C), the IP values of corn oil was lower than that of palm oil. The high stability character of palm oil can be explained by the high palmitic acid and low linoleic acid content in palm oil compared to corn oil.

4.2 Testing Oxidative Stability of Carvacrol in Mid-Oleic Sunflower Oil by Accelerated Oxidation Methods

The relationship between different temperature values (110, 120, 130°C) and the response (induction time) was determined using PetroOXY and Rancimat devices. Thus, the effect of carvacrol and BHT on oxidative stability of oil samples was determined by accelerated methods. In addition, it was investigated whether there was correlation between the data obtained from both the accelerated methods.

4.2.1 Measurement of Induction Period by PetroOXY Method

The PetroOXY test has been rarely used to measure the oxidative stability of edible oils. An important benefit of the PetroOXY test is to provide elevated pressure to reduce oil volatility by raising boiling points. The kinetics of the pressure drop curves show three different stages: induction, acceleration and deceleration (Figure 3.1). Oxidation stability is generally associated with the length of the induction phase, also known as induction time. That is the longer the induction time, the better the oxidation stability .

The induction periods of the sample was determined using PetroOXY for each temperature as shown in Table 4.2. It can be easily realized that increasing temperature decreased the induction time of all the samples. BHT showed the best protective effect at all three temperatures. Carvacrol was found to be not as protective as BHT at high temperature.

Table 4.2 Induction time of sample using PetroOXY device

Induction Time Of PetroOXY (h)			
Temperature(°C)	Carvacrol (200 ppm)	BHT(200 ppm)	Control
110	2.38	3.33	2.23
120	1.20	1.66	1.11
130	0.66	0.86	0.61

The antioxidant activity of plants usually varies depending on the composition of the active ingredients in the plant. Various studies have shown that the extracts (e.g., carvacrol) have high antioxidant activity due to the content of the high active ingredients. However, Tomaino et al. (2005) stated low resistance of carvacrol to heat in their study. In the current study, the effect of Carvacrol was not as high as expected. Some studies have shown that BHT is the best antioxidant when used in olive oil, while the antioxidant properties of BHT on sunflower oil have shown that carvacrol is not as prominent as the more important antioxidant effect (Miguel et al., 2003).

The results obtained in this study showed the opposite results. The differences between different studies were likely may be due to genotypic and environmental difference within species (Shan et al., 2005). Furthermore, carvacrol is known to have high volatility at high temperatures (Tomaino et al., 2005). The volatility causes loss of quantity of carvacrol and, dependently, its activity.

The induction times obtained from PetroOXY device at different temperatures is shown in Figure 4.1 for control, carvacrol and BHT oil samples. It can be said that there was a good correlation between induction time and temperature because of R^2 >0.99 for all samples.

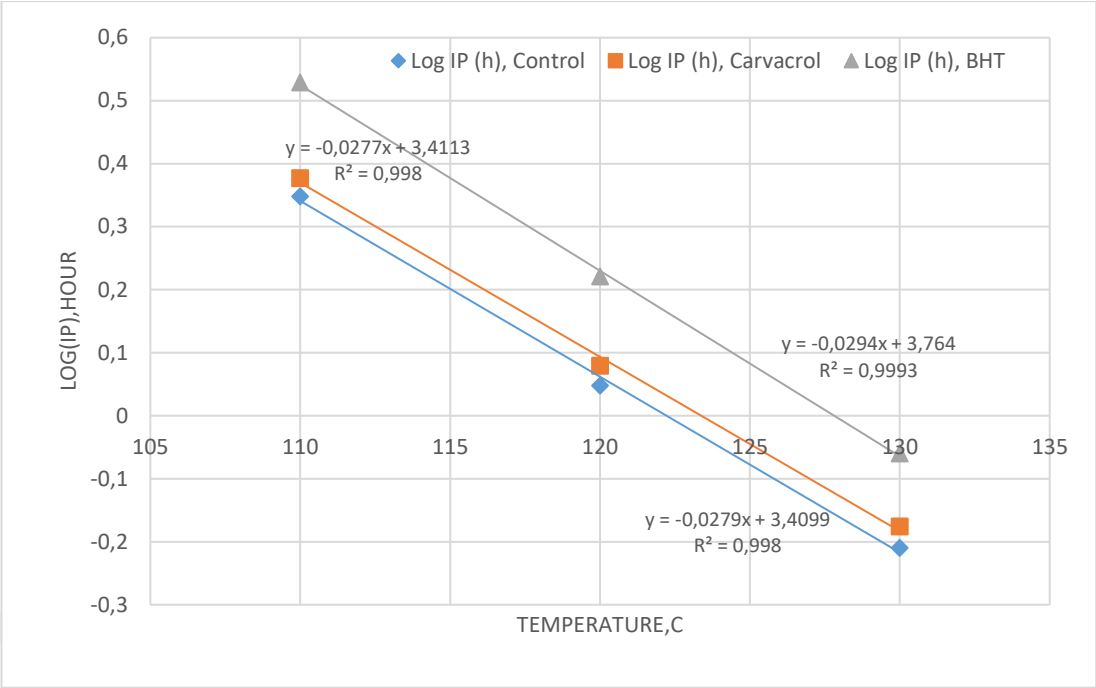


Figure 4.1 Induction time vs Temperature by using PetroOXY (For Control , Carvacrol, BHT samples)

4.2.2 Measurement of Induction Period by Rancimat Method

Because of the determination of the stability and shelf-life of edible sample under ambient conditions is quite time-consuming and labor-intensive work, in many studies, accelerated tests (the most commonly used in Rancimat methods) are used to define the stability of oils under normal cases. In this study, it was observed that BHT protects better than Carvacrol at all three temperatures studied and the induction periods of the control samples were detected as 5.45, 2.85, 1.45 hours at temperatures of 110, 120, 130°C, respectively (Table 4.3). Both Rancimat and PetroOXY devices showed that Carvacrol does not have as much protective effect as BHT antioxidant. The reason for this is that the antioxidant loses its effectiveness at high temperature and that the antioxidant is obtained may vary according to the soil and genetic characteristics of the plant (Inanç, 2012).

Table 4.3 Induction time of samples using Rancimat device

Induction Time Of Rancimat (h)			
Temperature(°C)	Carvacrol (200ppm)	BHT(200 ppm)	Control
110	5.72	6.52	5.45
120	2.98	3.49	2.85
130	1.51	1.7	1.45

The induction times obtained from Rancimat device at different temperatures is shown in Figure 4.2 for control, carvacrol and BHT oil samples (For control samples, Figure B1-B3). Referring to Figure 4.2, the logarithm of the induction time versus the temperature shows that these induction times determined at different temperatures are consistent with each other for all the samples. However, in a study, (Farhoosh, 2007) it has been stated that under accelerated conditions, lipid oxidation mechanisms may differ from normal storage conditions, which may lead to errors in shelf-life estimations. During measurement, it automatically determines the time before the maximum oxidation rate changes by transferring volatile acids (mainly carrying short chain acids) from the heated sample (100-130 °C) to deionized water with dry air bubbled and measuring the conductivity of the water. This time, commonly referred to as the induction period (IP) or oil stability index (OSI). When the results were examined, induction times at all three temperatures were found to be consistent both within each other and/or separately. In this study, operational parameters other than temperature were kept constant, amount of oil and air flow rate are 3 g and 20 lt/hour, respectively. Farhoosh (2007) stated that the operational parameters of Rancimat had an effect on the measurement of oxidative stability and thus prediction of the shelf-life. Tinello et al. (2018) measured the frying oils' IPs with 3 grams of sample and 20 liters / hour air flow by using Rancimat device. On the other hand, Farhoosh et al. (2013) used 25 liters / hour air flow for IP measurement of olive oil sample. Namely, operational conditions may vary depending on the type of sample used, so optimum conditions can be achieved by considering previous study.

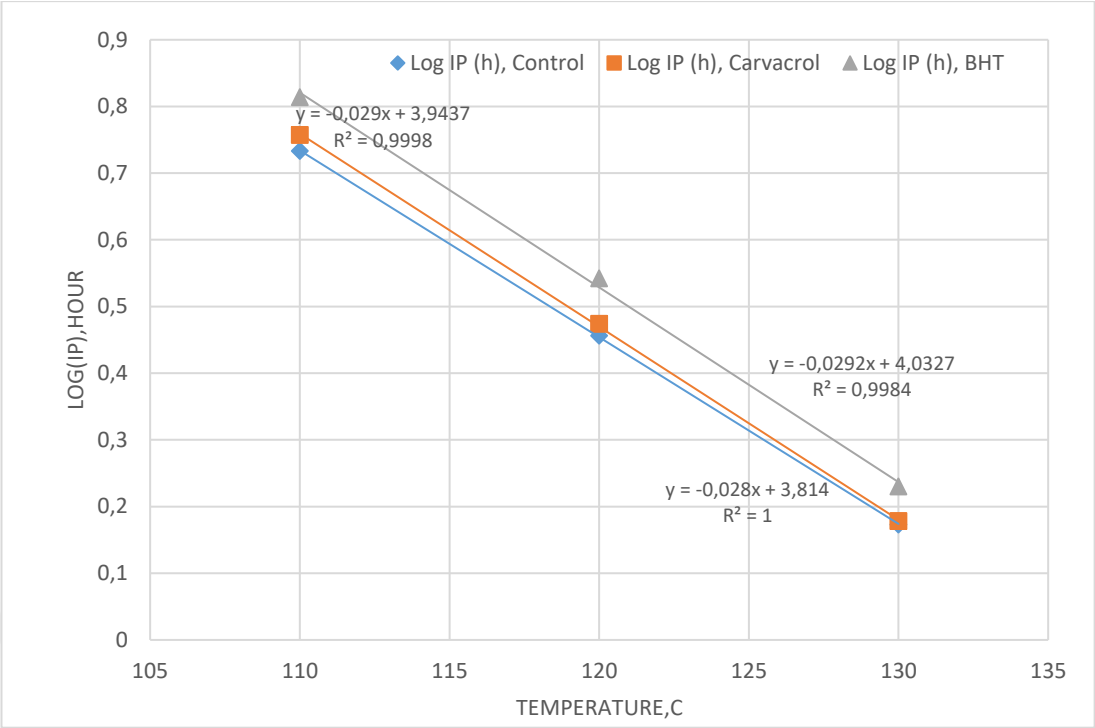


Figure 4.1 Induction time vs Temperature by using Rancimat (Control oil, Carvacrol, BHT)

In addition, both of the antioxidants were observed to show greater positive effect in sunflower oil. Thus, higher induction period values were obtained from samples which contain antioxidants.

4.3 Testing Oxidative Stability of Carvacrol in Mid-Oleic Sunflower Oil at Normal Storage Conditions

The oxidation degree of oil samples were measured at normal storage temperatures of 20, 30 and 40°C at previously specified time intervals. Peroxide, para-anisidine, free fatty acids, TOTOX values were monitored during storage. The parameters analyzed were statistically analyzed in order to determine whether there is significant differences.

4.3.1 Determination of Peroxide Value

Since the peroxide value (PV) is one of the first compounds formed during primary oxidation, it is considered as an indicator of oxidation. However, since it is an unstable compound and is converted very quickly into the secondary product, the peroxide alone does not represent a total degradation. In this study, when we look at the data

obtained at storage temperatures of 20°C, BHT antioxidant shows better protective properties than carvacrol (Figure 4.3). The most important reason for this may be attributed to the volatile property of carvacrol, a natural antioxidant. Similar results were observed at 30 and 40°C as shown in Figures B4 and B5 (Appendix B). It can be seen from Figure 4.3, during the storage period at 20°C, the increase in peroxide value of the carvacrol-containing sample is not stable and shows fluctuations. This can be explained by the fact that carvacrol is volatile at high temperatures (Tomaino et al., 2005).

The results of ANOVA and multiple comparison tests (Appendix, Table B.13-B.36) showed that the peroxide values of samples at 20, 30 and 40°C were significantly different from each other. ($p<0.05$)

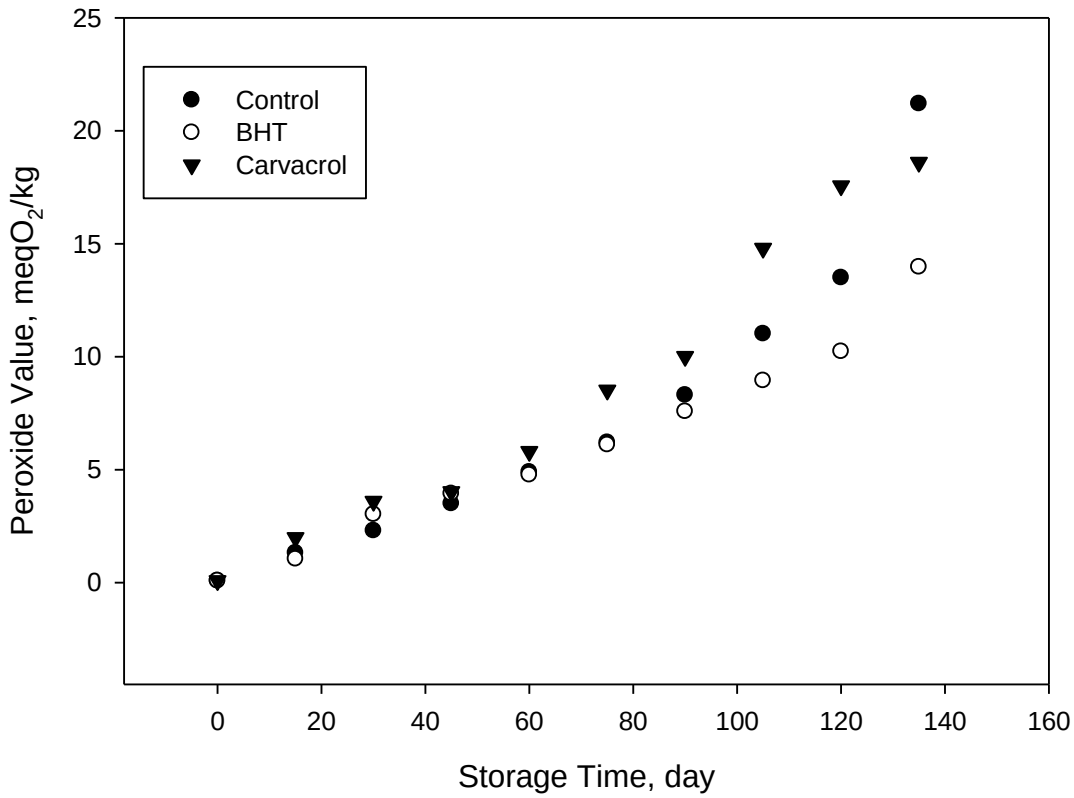


Figure 4.2 Peroxide value vs storage time of antioxidant added (Carvacrol, BHT) and control oils at 20°C

4.3.2 Determination of Free Fatty Acids

It has been clearly stated in many literature that free fatty acid values do not increase very much at normal storage temperatures. In this study, all three oil samples showed a slight increase in free fatty acid values over time as shown in the Figure 4.4. The results are consistent with each other. Treatment of oils with antioxidants decreased the rate of formation of FFA in the samples. Although carvacrol delayed formation of FFA, but BHT showed a strong effect on the formation of FFA in the oils during storage. Many studies showed similar results (Rehman et al., 2003). Also, it can be seen from Figure 4.4 that the FFA value during normal storage is not too high at 20°C. Change in FFA value vs storage time of antioxidant added (Carvacrol, BHT) and control oils at 30 and 40°C was shown in Figures B6 and B7.

At 20°C storage temperature, the results of ANOVA and multiple comparison tests (Appendix, Table B.37-B.44) showed that the free fatty acid values of samples were significantly different from each other. ($p<0.05$). However, at 30-40°C, there was no significant difference according to multiple comparison test results (Appendix, Table B.37-B.60)

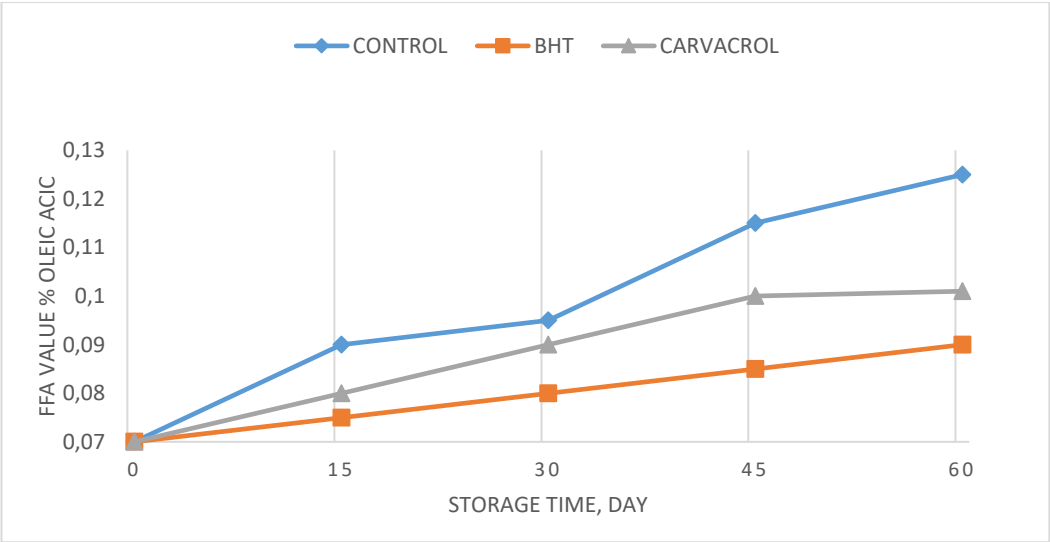


Figure 4.3 Change in FFA value vs storage time of antioxidant added (Carvacrol, BHT) and control oils at 20°C

It is observed that the FFA value does not increase too much at other storage temperatures and the antioxidant-containing samples have lower FFA values than non-containing samples (Figures B6 and B7). BHT appears to be more protective than

carvocrol at all storage temperatures. BHT antioxidant has been reported to be heat resistant in many literature (Yanishlieva and Marinova, 2001; Shahidi, 2005).

4.3.3 Determination of Para-Anisidine Value

Para-anisidine value (p-AV) known as an index of oxidative degradation in fats and oils. It is a secondary oxidation product. The changes in p-AV of sunflower oil samples during the storage processes at 20°C are presented in Figure 4.5. An increase in p-AV values of product was observed with time at both 30 and 40°C (Figures B8 and B9). Some studies show similar results (Lee et al., 2007). However, the increase in p-AV of BHT-containing oil sample was lower than that of carvacrol. A similar trend was observed in the formation of PV and FFA values during storage. This phenomenon shows that BHT has better protection against oxidation than carvacrol.

When the ANOVA and multiple comparison tests results (Table B.61-62) 15th storage day at 20 °C were examined, it was found that the values of para anisidine between the groups were different but the difference between them was not significant and all other data were different from each other ($p<0.05$) (Table B.63-84)

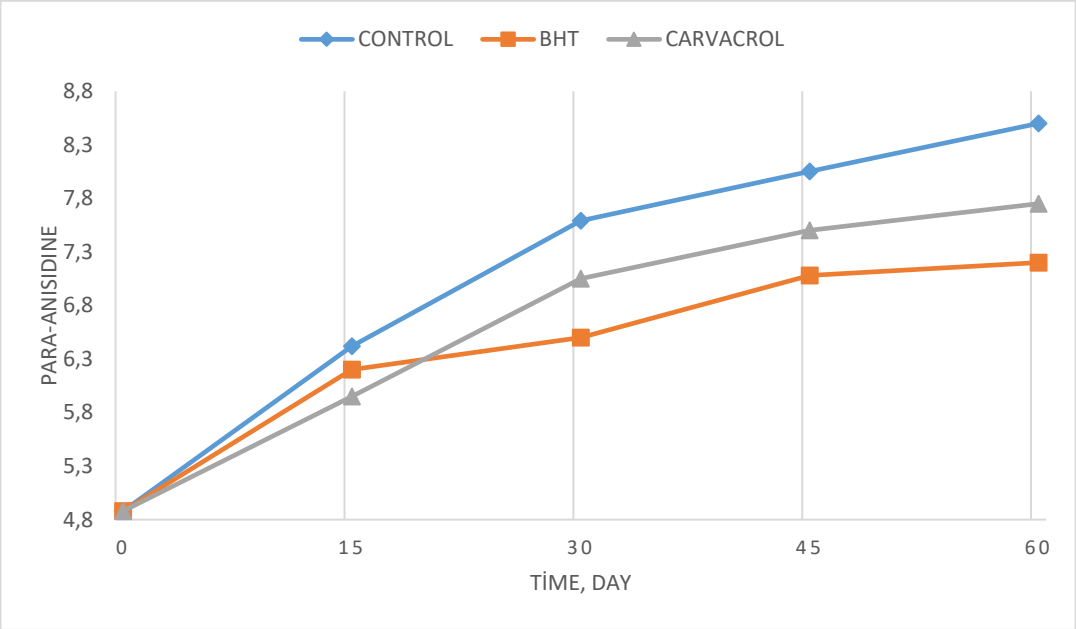


Figure 4. 4 Change in p-AV vs storage time of antioxidant added (Carvacrol, BHT) and control oils at 20°C

4.3.4 Determination of TOTOX value

The TOTOX value, which is an indicator of summary of oxidation in the oil, provides a good interpretation of the oxidation status and history of the oil. Effect of antioxidants on TOTOX value is not clear at 20°C as shown in Figure 4.6. However, as the storage temperature increased to 30 and 40°C (Figures 4.7 and B10, respectively), one can easily differentiate the effect of BHT and carvacrol on TOTOX value.When the results were examined at 30 and 40°C, it can be seen that BHT protected well and the carvacrol showed a moderate protection effect on the oil samples with respect to TOTOX value.

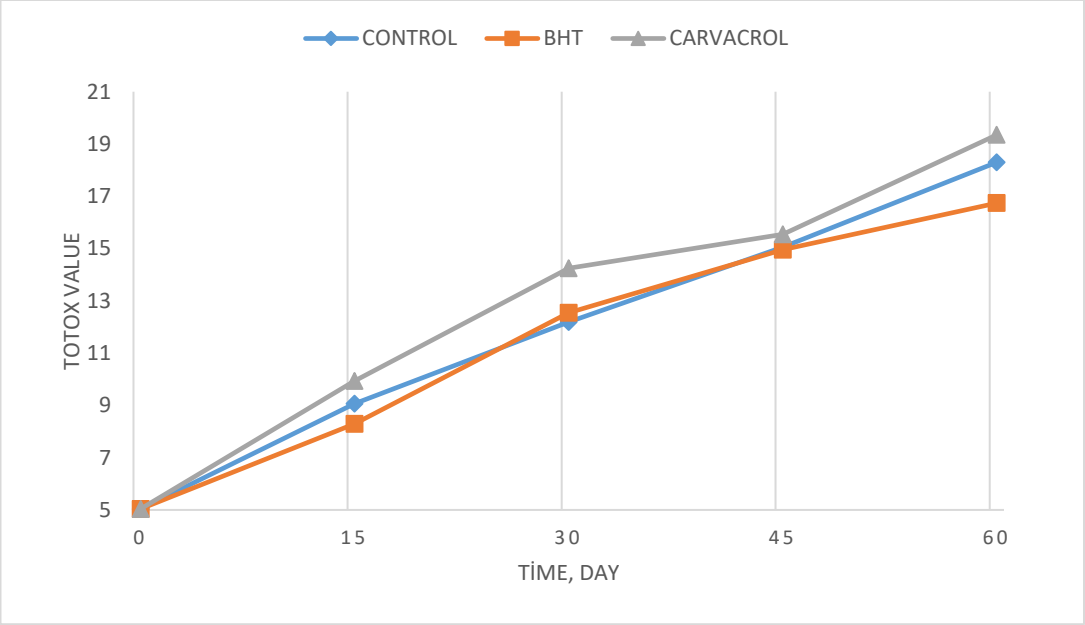


Figure 4.5 Change in TOTOX value vs storage time of antioxidant added (Carvacrol, BHT) and control oils at 20°C

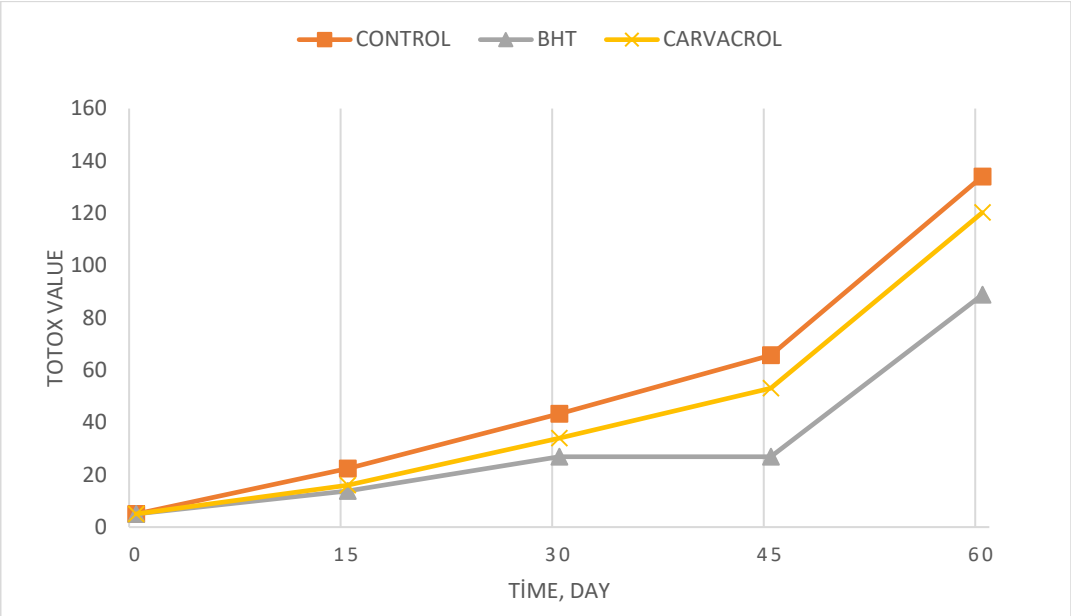


Figure 4.6 Change in TOTOX value vs storage time of antioxidant added (Carvacrol, BHT) and control oils at 30°C

4.4 Determination of Induction Period by Peroxide Value at Normal Storage Conditions

The peroxide value, which is the primary index of oxidation, is one of the compounds formed during lipid oxidation. In fact, a low PV value may not mean that it is not oxidized. As PV is the primary oxidation product, it is rapidly separated into secondary oxidation products, so that there is no overall degradation of the oil. Therefore, in many studies TOTOX value, total oxidation, the value is calculated. The degradation may occur faster than producing of new hydroperoxides due to definite storage circumstances.

A high peroxide value at the initial of the storage time has a unfavorable impact on the storage stability of product. For refined oils, manufacturers should aim for better 0.5 meq O_2 / kg oil with a peroxide value below 1, while peroxide value for virgin oils may be higher up to 3 meq O_2 / kg oil (Monzocco et al., 2010).

The kinetics of PV production during the oxidation of oil samples in normal storages (20, 30 and 40°C) are shown in Figure 4.3 at 20°C. The peroxide values vary over time were available in Tables A1-A3. The PV had a trend to rise over time and two linear lines were defined as shown in Figure 4.8 (Similarly, Figure B11and B12). In other

words, the tangent of these two lines was plotted and the peroxide value at the induction time was found. At 20°C storage temperature, the IP_{PV} and PV_{IP} for oil sample were estimated by using tangent method as shown in Figure 4.8 representatively. Both IP_{PV} and PV_{IP} values were determined by the same manner for other storage temperatures and given in Tables A4 and A5. IP_{PV} and PV_{IP} at 20°C were 117.6 days and 12.08 $meqO_2$/kg oil, respectively. In the same way, the induction time and the peroxide value of the induction time were determined for the storage temperatures of 30 and 40°C as 53.68 and 36 days, and 29.24 and 33.09 $meqO_2$/kg oil. Farhoosh (2013) also reported similar way to determine shelf-life of olive oil at 50°C storage temperature. Shim and Lee (2011) investigated the induction time measurements of Perilla oil at 25°C and found that the induction time at this temperature was 22.55 days and the peroxide value at the induction time was 13.99 $meqO_2$/kg oil with the same method.

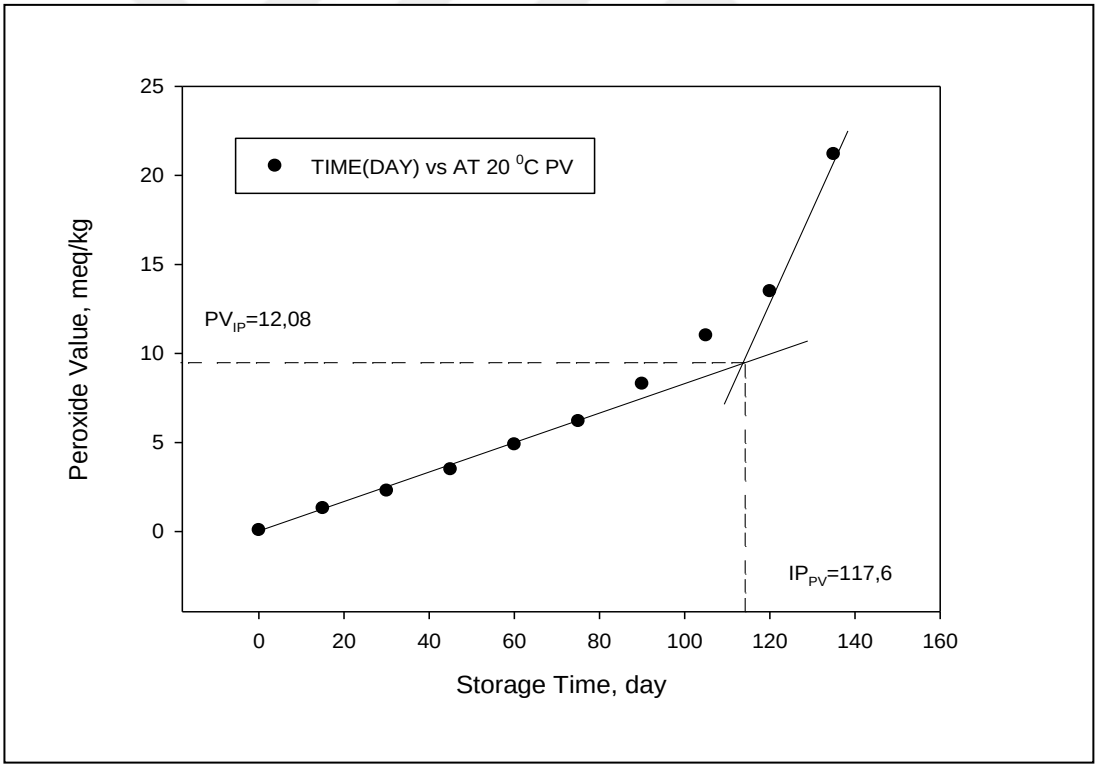


Figure 4.7 Representative determination of the induction time based on PV IP_{PV} and numerical value of peroxide PV_{IP} at that time

When the results are examined, it is seen that the induction time decreases and the peroxide value increased with temperature. It is also understood that high temperature storage reduces the shelf life or oxidative stability of the oil, and many studies illustrate this (Shim and Lee, 2011).

4.5 Prediction of Shelf-Life at Normal Storage and Correlation Between PetroOXY and Rancimat Results

The shelf-life of a food is not only a factor affecting the quality parameters of the food, it is also very important for human health. Hence, manufacturers want to know how long the products they produce are based on maximum quality and certain environmental factors. Likewise, consumers want to consume the product they have bought during this period. Therefore, researchers have developed several methods to make the most reliable way of estimating shelf life. In this study, in order to estimate shelf-life, induction times for each storage temperature were determined by plotting the change in peroxide amount over-time under normal storage conditions (20, 30, 40°C). Then, the natural logarithm of induction times obtained by normal storage and accelerated methods (Rancimat and PetroOXY) were plotted against temperature on the same graph as shown in Figures 4.9 and 4.10 for PetroOxy and Rancimat, respectively. Thus both the results of two accelerated methods and the results under normal storage conditions were compared. As it can be seen from both figures, the IP at either high or low temperatures are on the same linear direction. This means the results obtained from accelerated and normal storage conditions are correct, thus, shelf life prediction can be made safely. Since the reaction rate in lipid oxidation increases exponentially with temperature, the shelf life of sample is generally estimated in accelerated storage measurements performed at higher temperatures (Farhoosh, 2007). The shelf-life of thoroughly refined edible oils are 12–18 months at normal cases conditions but these, can be estimated in a short time with accelerated tests at high temperatures. The shelf life estimation can be made in a short time by using the Figures 4.9 and 4.10 or using the mathematical models derived from them.

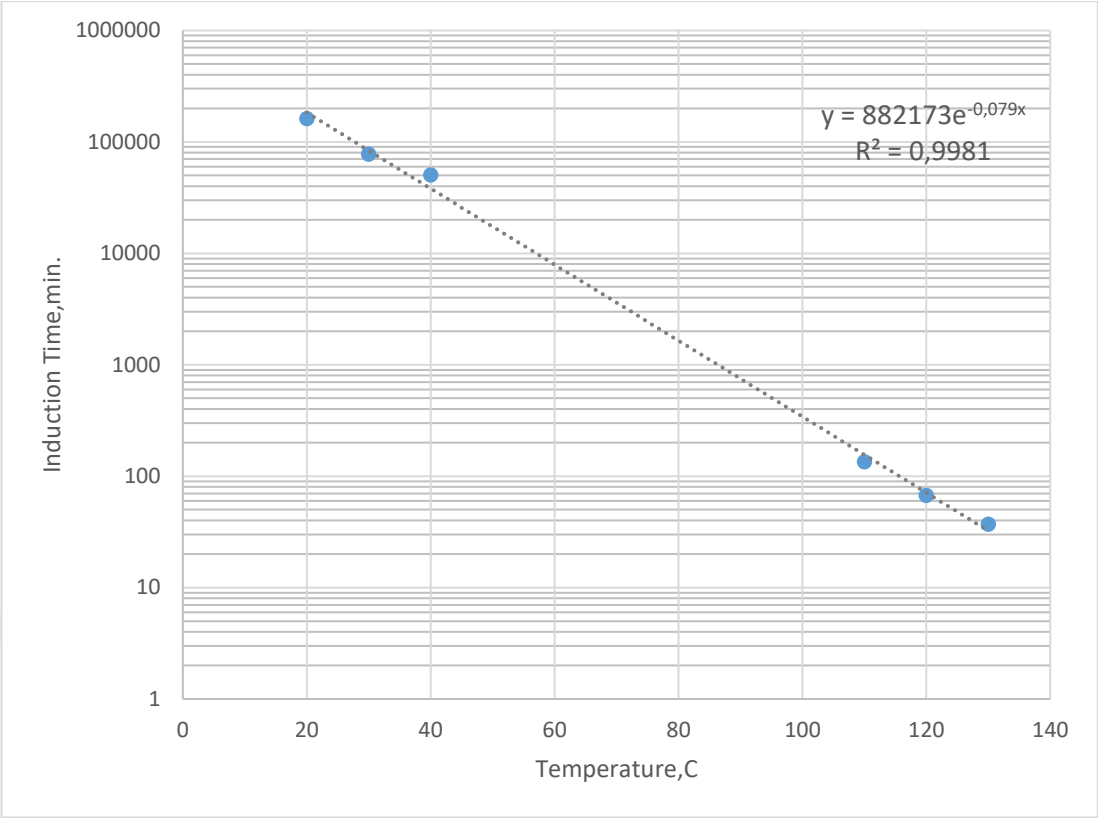


Figure 4.8 Extrapolation to normal storage with PetroOXY method

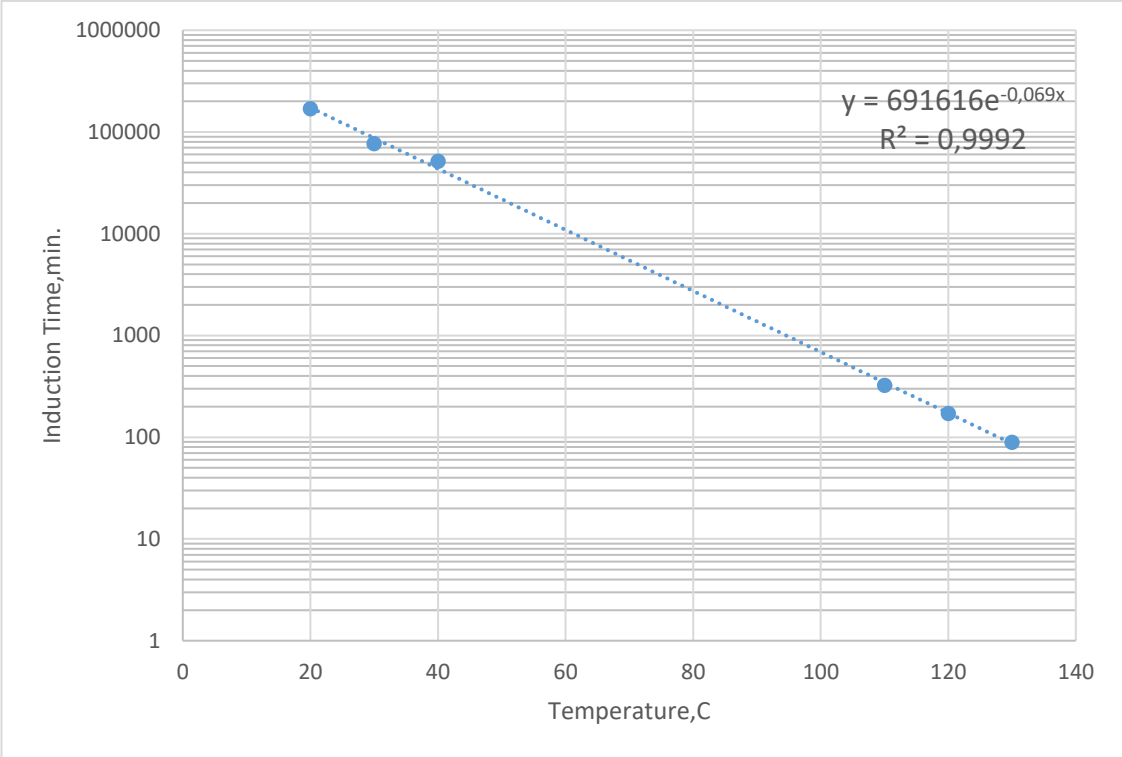


Figure 4.9 Extrapolation to normal storage with Rancimat method

In order to determine whether we can estimate the IP of oils at low temperatures, it would be better to estimate the IP values from the equations of log(IP) vs Temperature graphs and then compare with real IP values experimentally determined at 20, 30 and 40°C. By using the equation obtained from the Figure 4.1 (y = - 0.0279xTemperature + 3.4099) from PetroOXY, induction times estimated were 30.17, 15.91 and 8.38 days for 20, 30 and 40°C storage temperatures, respectively. According to Figure 4.2 from Rancimat, induction times or shelf-life of oil at 20, 30 and 40°C were calculated from equation of the graph (y = -0.028xTemperature +3.814). The results shows that the induction times at 20, 30 and 40°C were predicted as 88.71, 48.85 and 23.22 days, respectively. On the other hand, the experimental results obtained from storage of oils at 20, 30 and 40°C were 117.6, 53.68 and 36 days respectively. As it can be seen, the predicted results were different from the values obtained from normal storage conditions. The shelf-life results estimated by the Rancimat method were found to be closer to the shelf-life obtained by the normal storage method. However, the results obtained from PetroOXY does not match the real shelf life. There are many reasons for this . Primarily, lipid oxidation has a very complex mechanism and is significantly affected by temperature conditions. Farhoosh (2007) made similar interpretations in his study that Rancimat device which has different systems to accelerate the oxidation mechanism can give different results than the estimation of shelf-life under ambient conditions. Moreover, these shelf-life estimates may be above or below the estimate according to the type of sample used (Kaya et al., 1993), and sometimes give understandably admissible results (Presa et al., 1995). Another important reason of this results may be, the induction time measured by the Rancimat method is defined by a highly degradable oil sample containing aldehydes, ketones and carboxylic acids as well as peroxide and hydroperoxides, while the induction time in the PetroOXY method is said to represent partial oxidized oil (Duarte et al, 2018).

Duarte et.al, (2018) compared the thermal and oxidative stability of Moringa oil with olive and canola oils. In their study, the samples were analysed with Rancimat, PetroOXY and Schaal test. The IP data determined with Rancimat method exhibited that moringa oil had stability close to olive oil and that both moringa and olive oils had considerably high IP values than canola oil. But Moringa oil had the highest oxidative stability in the IP values obtained from the PetroOXY test.

Estimation of shelf-life is difficult because of the fact that PV is too low and, the rate of decomposition of peroxides and the producing of new peroxides may not be predicted. This information may explain why the estimate does not match the real shelf life (Duarte et al., 2018).

One of the benefits of PetroOXY techique with respect to Rancimat was that, although only one analysis was performed at a time with the PetroOXY equipment, there was less analysis time. The principle of the technique, it is assumed that the oxidative induction time determined by the PetroOXY test indicates the initial steps of the oxidation of the product (Duarte et al., 2018).

Souse et al. (2014) had studied the utilization of natural antioxidants in soybean biodiesel. Oil samples with added natural antioxidants were studied. Oxidation stabilities were determined using two accelerated methods, Rancimat and PetroOXY. The results determined from both techniques were correlated well with each other. In order to compare two accelerated oxidation methods, Rancimat and PetroOXY, the average induction periods based on these studies were plotted. Induction time with PetroOXY method was found to be faster than Rancimat test because the analysis was performed under pressure. Therefore, an average of 6 hours analysis time with Rancimat was performed in approximately 2-3 hours with PetroOXY technique.

According to the results obtained, there is a good correlation (R^2 = 0.998) between accelerated oxidation stability methods, as shown in Figure 4.11. In many oxidation stability studies, it is clear that there is a good relationship between these two methods (Souse et.al, 2014). However, the induction times obtained by the Rancimat method are almost twice than that of the PetroOXY method. One of the most important reasons for this is that the working mechanisms of the two methods are different, for example the Rancimat method determines both primary and secondary oxidation products, while the PetroOXY technique determines only primary oxidation products. Duarte (2018) determined that the induction time of virgin canola oil was found to be 7.58 hours at 110°C by Rancimat method and 3.08 hours by PetroOXY method at the same temperature.

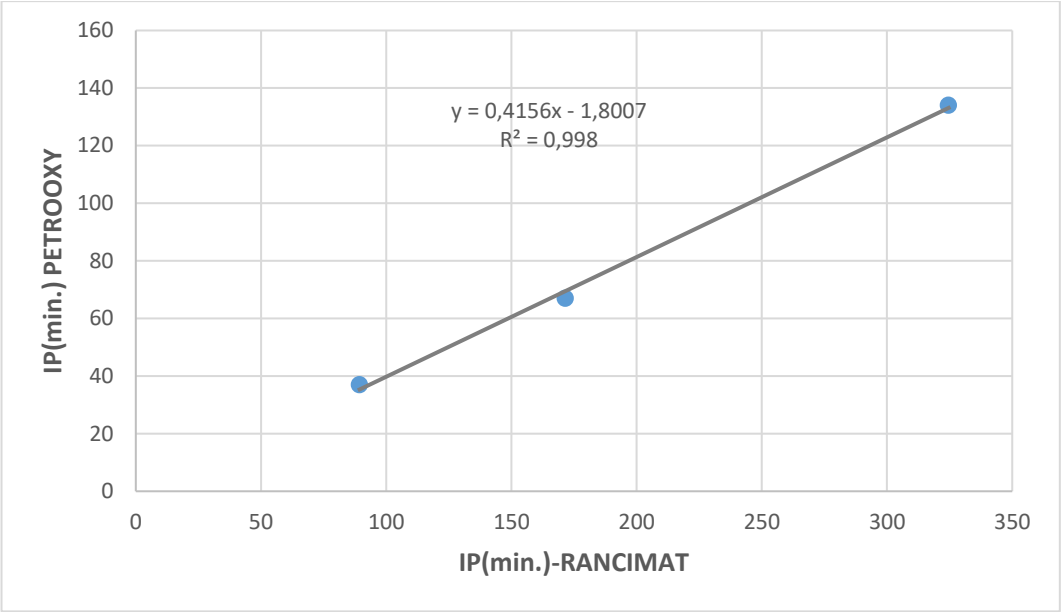


Figure 4.10 Correlation between Rancimat and PetroOXY methods

The shelf-life estimation with the PetroOXY method gave results far from the shelf - life values determined in the normal storage method. With this method, almost no research has been conducted to directly estimate or determine shelf life. Comparative oxidation stability has been studied in many studies and has been used mostly in the field of biodiesel (Souse et.al, 2014). This method is generally used in research to determine the antioxidant effect because it gives faster results than Rancimat method.

CHAPTER V

CONCLUSION

In this study, the results of accelerated oxidation experiments on mid-oleic sunflower oil were in agreement with normal storage results and showed that carvacrol natural antioxidant is not as protective as BHT. Therefore, this natural antioxidant cannot be used as an alternative to BHT for sunflower oil storage. Temperature had an important impact on the induction period of mid-oleic acid sunflower oil. Induction times obtained by normal storage method were compared with accelerated methods Rancimat and PetroOXY. The results showed that the shelf-life estimation obtained by the PetroOXY method was not reliable but gave consistent results in oxidation stability studies based on temperatures.

CHAPTER VI

RECOMMENDATIONS FOR FUTURE WORK

The new accelerated test method, PetroOXY, results in faster oxidation stability than other methods. Both the manufacturer and the consumer want to know the shelf life of the product in the most accurate and fast way. Because the concept of time is an important parameter of our lives, more detailed researches should be made on this method.

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APPENDIX A

Table A. 1 Peroxide values of sunflower oil during storage at 20 ° C

Time (day)	Peroxide Value (meq O_2/kg oil)
0	0.08
15	1.32
30	2.30
45	3.50
60	4.90
75	6.20
90	8.30
105	11.02
120	13.50
135	21.20

Table A. 2 Peroxide values of sunflower oil during storage at 30 ° C

Time (day)	Peroxide Value (meq O_2/kg oil)
0	0.08
3	1.09
6	1.99
9	2.90
12	4.30
15	7.72
18	10.01
21	12.90
24	13.75
27	15.20
30	17.60
33	19.20
36	21.85
39	23.10
42	25.42
45	28.60
48	31.25
51	35.23
54	40.00
57	50.20
60	62.50

Table A. 3 Peroxide values of sunflower oil during storage at 40 ° C

Time (day)	Peroxide Value (meq O_2/kg oil)
0	0
3	1.98
6	3.70
9	7.00
12	9.90
15	12.10
18	15.20
21	17.40
24	20.10
27	22.10
30	25.33
33	29.30
36	34.20
39	38.20
42	40.30
45	48.00
48	55.00
51	62.00
54	70.00
57	78.00
60	87.00

Table A.4 Summary table of the induction time of normal the storage, PetroOXY method and predicted shelf-life of oil by PetroOXY

	Temperature(° C)	Induction Time (Day)
Normal Storage	20	117.6
	30	53.68
	40	36.00
PetroOXY	110	0.09
	120	0.05
	130	0.03
Expected IP of Normal Storage from PetroOXY	20	30.17
	30	15.91
	40	8.38

Table A.5 Summary table of the induction time of normal the storage, Rancimat method and predicted shelf-life of oil by Rancimat

	Temperature(C)	Induction Time (Day)
Normal Storage	20	117.6
	30	53.68
	40	36.00
Rancimat	110	0.23
	120	0.12
	130	0.06
Expected IP of Normal Storage from Rancimat	20	88.71
	30	48.85
	40	23.22

APPENDIX B

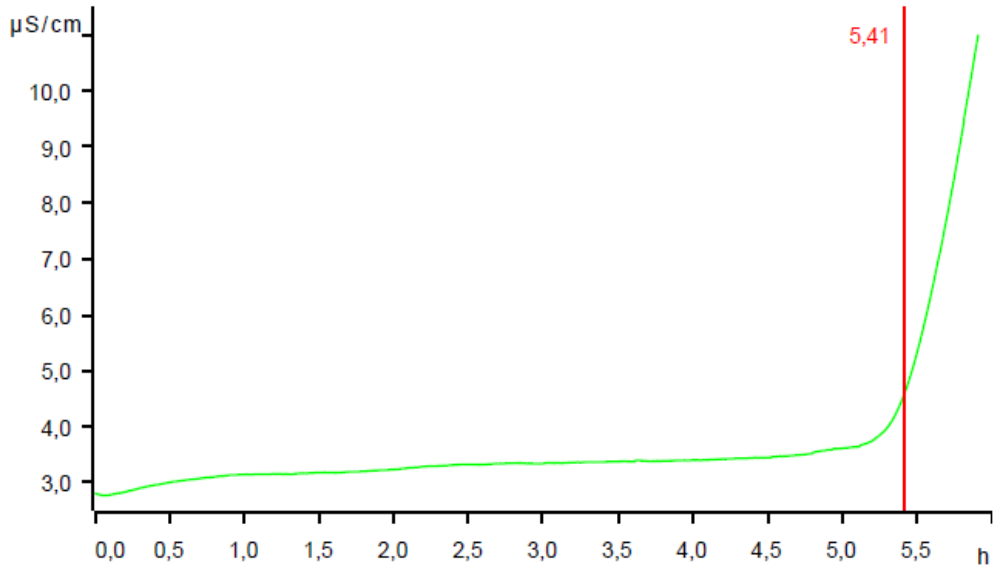

Figure B. 1 Result of induction time at 110 ^{O}C by Rancimat

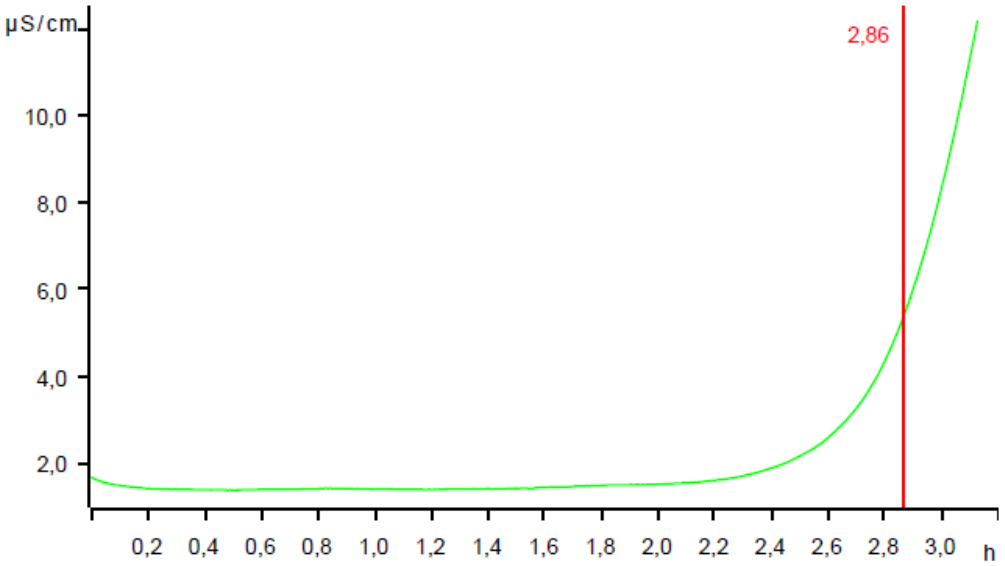

Figure B. 2 Result of ınduction time at 120 ^{O}C by Rancimat

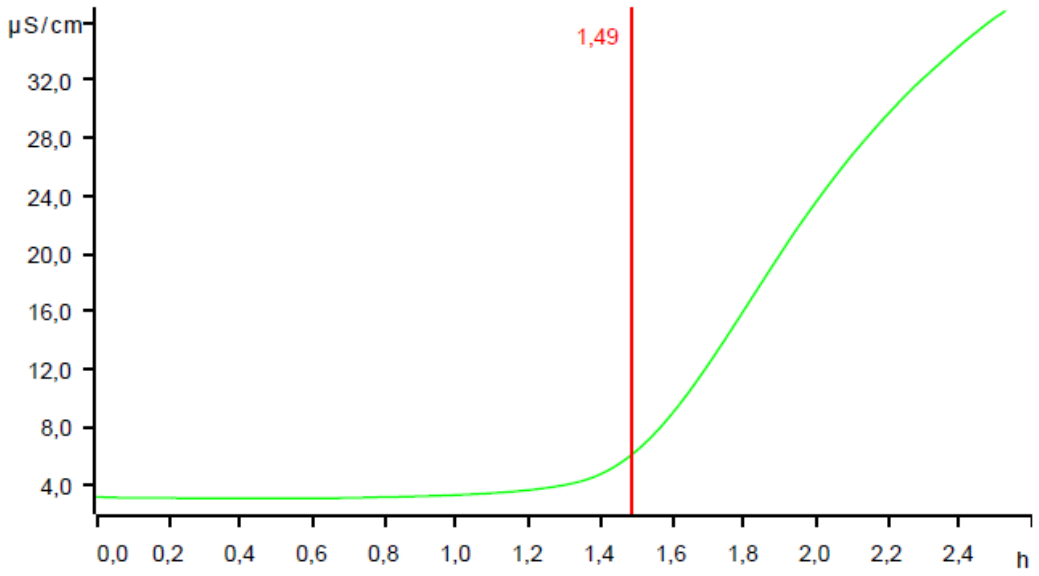

Figure B. 3 Result of ınduction time at 130 ^{O}C by Rancimat

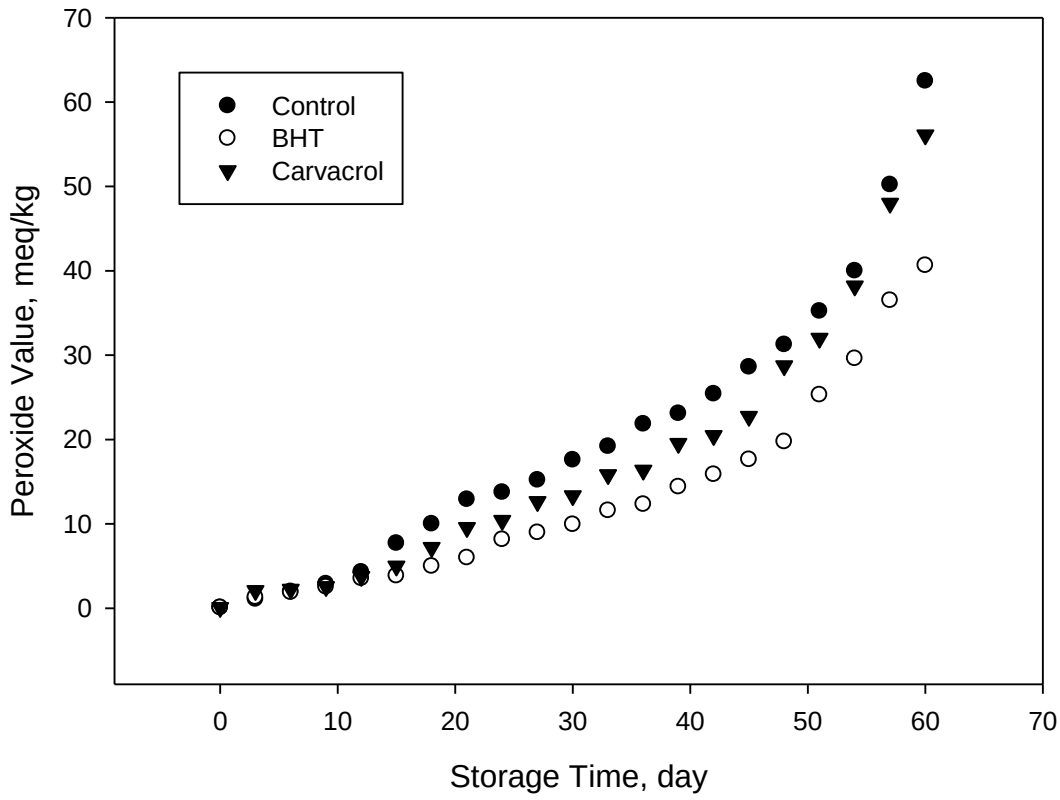

Figure B. 4 Peroxide value vs storage time of antioxidant added (Carvacrol, BHT) and control oils at 30°C

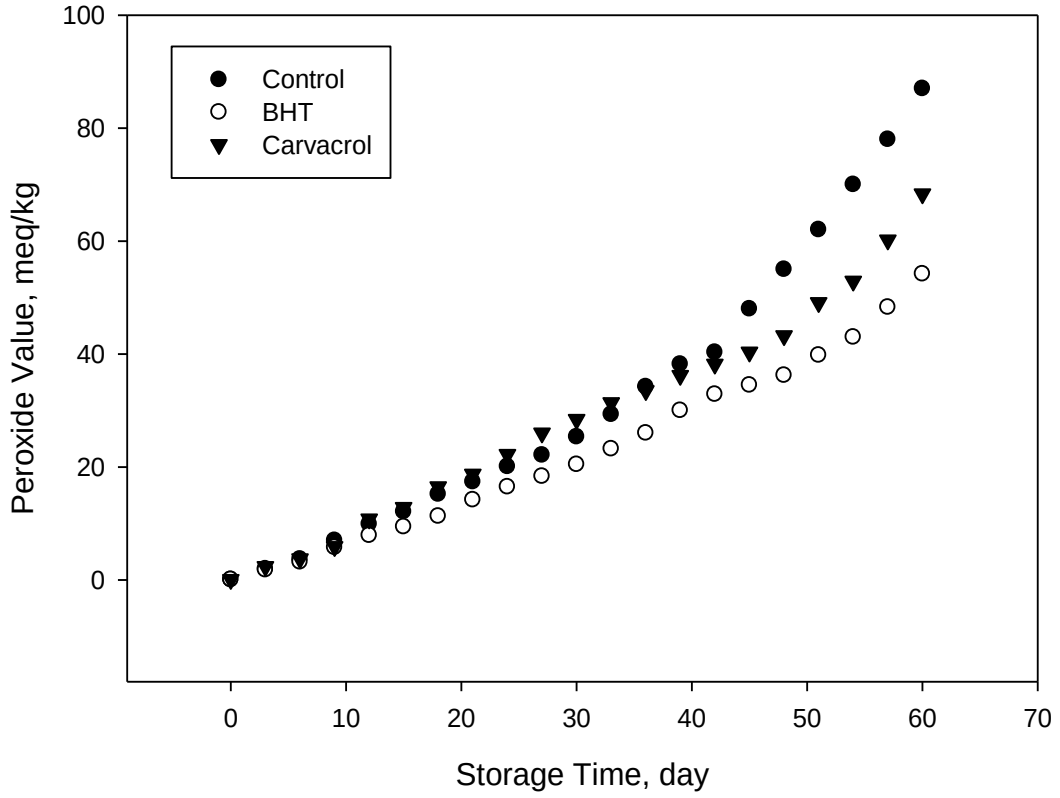

Figure B.5 Peroxide value vs storage time of antioxidant added (Carvacrol, BHT) and control oils at 40°C

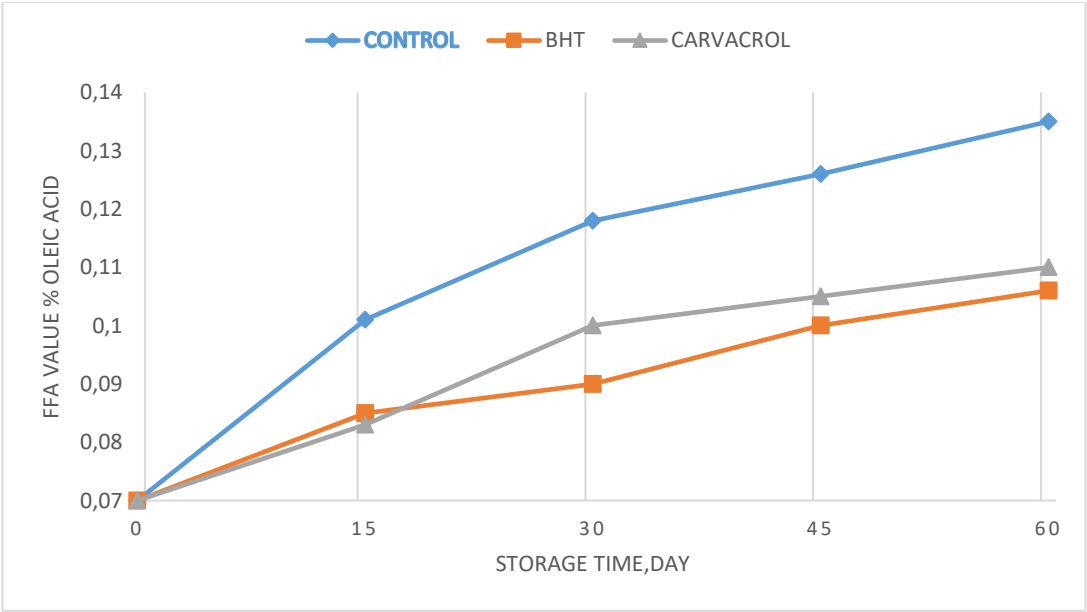

Figure B.6 Change of FFA value of samples with time at 30 °C

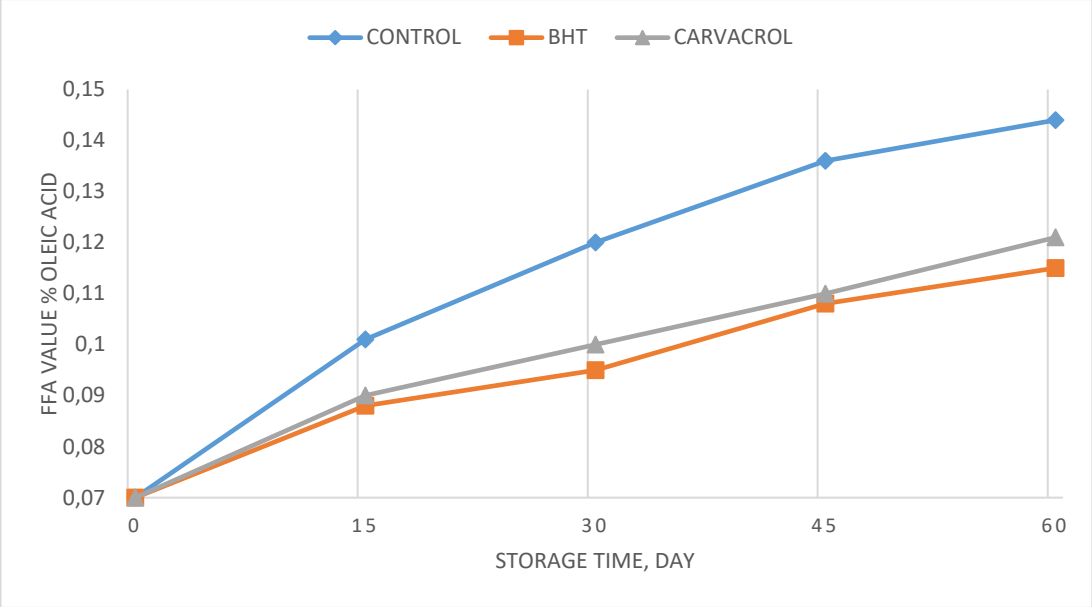

Figure B.7 Change of FFA value of samples with time at 40 °C

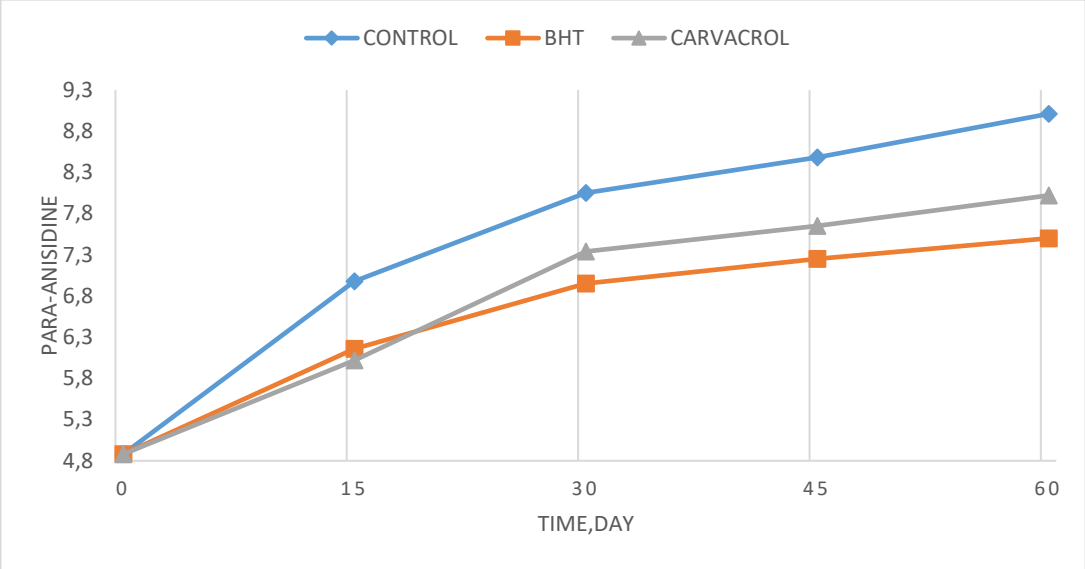

Figure B.8 Change of Para-Anisidine value of samples with time at 30 °C

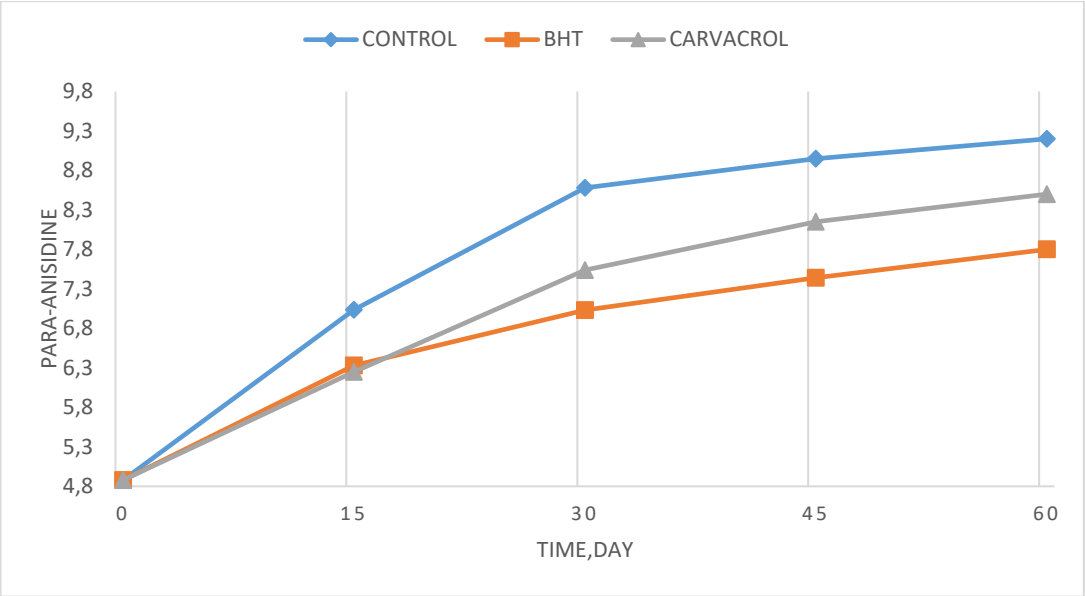

Figure B.9 Change of Para-Anisidine value of samples with time at 40 °C

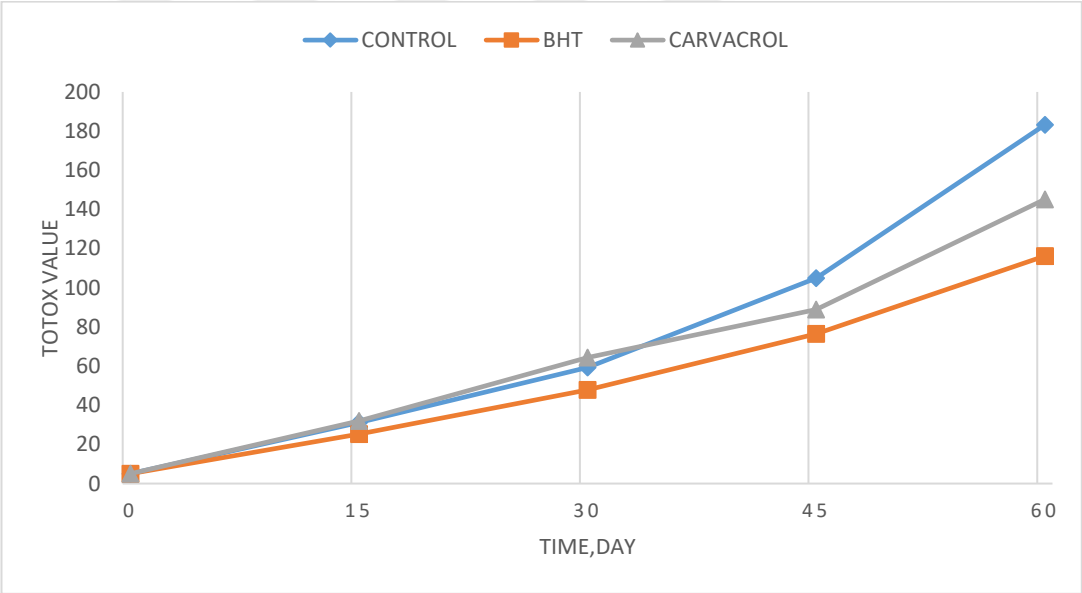

Figure B.10 Change in TOTOX value vs storage time of antioxidant added (Carvacrol, BHT) and control oils at 40°C

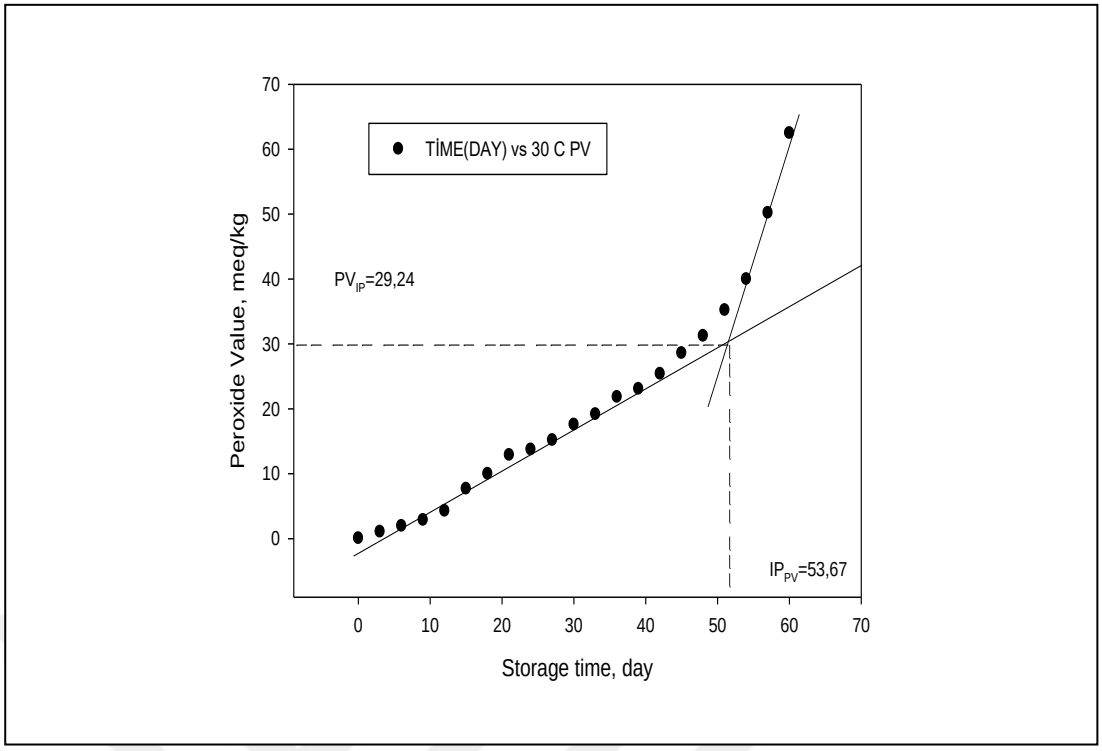

Figure B.11 Representative determination of the induction time based on PV, IP_{PV} and numerical value of peroxide PV_{IP} at that time (30° C)

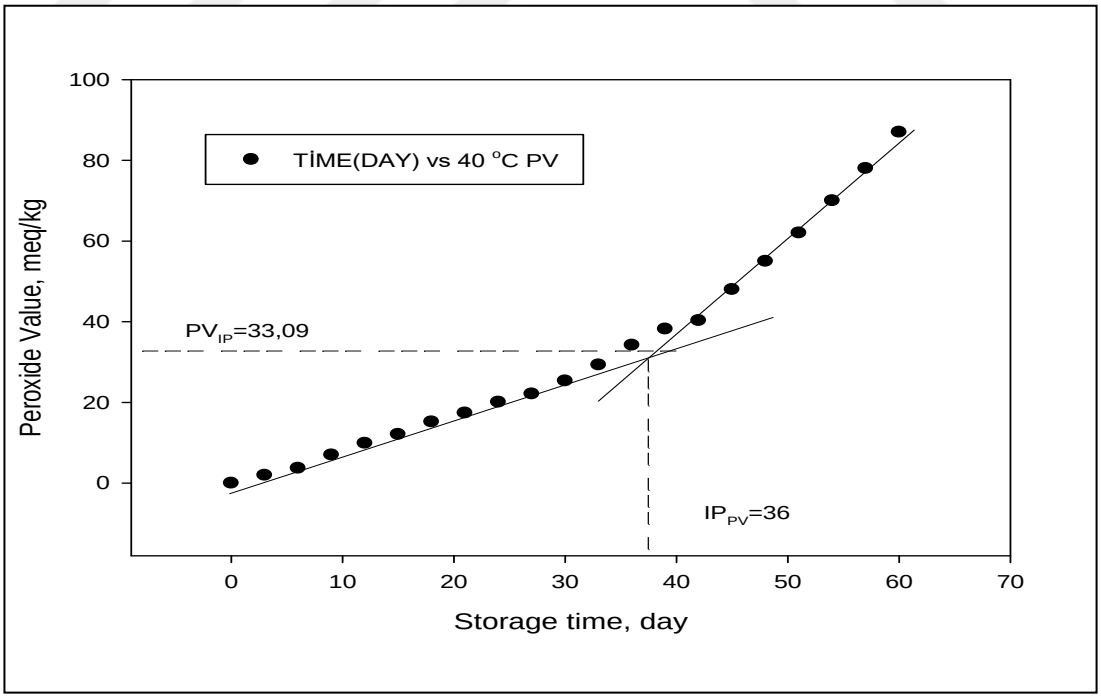

Figure B. 12 Representative determination of the induction time based on PV, IP_{PV} and numerical value of peroxide PV_{IP} at that time (40° C)

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	,937	2	,468	702,700	,000
Within Groups	,002	3	,001		
Total	,939	5			

Figure B.13 Analysis of variance for PV of oil samples at 20 ° C, 15th day

groups	N	Subset for alpha = 0.05		
		1	2	3
BHT	2	1,0500		
control	2		1,3200	
Carvacrol	2			1,9900
Sig.		1,000	1,000	1,000

Figure B.14 Duncan's Multiple Comparison Test for PV of oil samples at 20 ° C, 15th day

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1,697	2	,848	249,490	,000
Within Groups	,010	3	,003		
Total	1,707	5			

Figure B.15 Analysis of variance for PV of oil samples at 20 ° C, 30th day

groups	N	Subset for alpha = 0.05		
		1	2	3
control	2	2,3000		
BHT	2		3,0200	
Carvacrol	2			3,6000
Sig.		1,000	1,000	1,000

Figure B.16 Duncan's Multiple Comparison Test for PV of oil samples at 20 ° C, 30th day

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	,314	2	,157	392,000	,000
Within Groups	,001	3	,000		
Total	,315	5			

Figure B.17 Analysis of variance for PV of oil samples at 20 ° C, 45th day

groups	N	Subset for alpha = 0.05		
		1	2	3
control	2	3,5000		
BHT	2		3,9400	
Carvacrol	2			4,0200
Sig.		1,000	1,000	1,000

Figure B.18 Duncan's Multiple Comparison Test for PV of oil samples at 20 ° C, 30th day

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1,259	2	,629	1048,778	,000
Within Groups	,002	3	,001		
Total	1,260	5			

Figure B. 19 Analysis of variance for PV of oil samples at 20 ° C, 60th day

groups	N	Subset for alpha = 0.05		
		1	2	3
BHT	2	4,7700		
control	2		4,9000	
Carvacrol	2			5,8000
Sig.		1,000	1,000	1,000

Figure B.20 Duncan's Multiple Comparison Test for PV of oil samples at 20 ° C, 60th day

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	15,820	2	7,910	10786,091	,000
Within Groups	,002	3	,001		
Total	15,822	5			

Figure B.21 Analysis of variance for PV of oil samples at 30 ° C, 15th day

groups	N	Subset for alpha = 0.05		
		1	2	3
BHT	2	3,8500		
Carvacrol	2		4,9900	
control	2			7,7200
Sig.		1,000	1,000	1,000

Figure B.22 Duncan's Multiple Comparison Test for PV of oil samples at 30 ° C, 15th day

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	58,799	2	29,399	8646,843	,000
Within Groups	,010	3	,003		
Total	58,809	5			

Figure B.23 Analysis of variance for PV of oil samples at 30 ° C, 30th day

groups	N	Subset for alpha = 0.05		
		1	2	3
BHT	2	9,9500		
Carvacrol	2		13,3200	
control	2			17,6000
Sig.		1,000	1,000	1,000

Figure B.24 Duncan's Multiple Comparison Test for PV of oil samples at 30 ° C, 15th day

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	120,519	2	60,259	30129,633	,000
Within Groups	,006	3	,002		
Total	120,525	5			

Figure B.25 Analysis of variance for PV of oil samples at 30 ° C, 45th day

groups	N	Subset for alpha = 0.05		
		1	2	3
BHT	2	17,6300		
Carvacrol	2		22,7500	
control	2			28,6000
Sig.		1,000	1,000	1,000

Figure B.26 Duncan's Multiple Comparison Test for PV of oil samples at 30 ° C, 45th day

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	505,943	2	252,972	18878,483	,000
Within Groups	,040	3	,013		
Total	505,984	5			

Figure B.27 Analysis of variance for PV of oil samples at 30 ° C, 60th day

groups	N	Subset for alpha = 0.05		
		1	2	3
BHT	2	40,6500		
Carvacrol	2		56,2000	
control	2			62,5000
Sig.		1,000	1,000	1,000

Figure B.28 Duncan's Multiple Comparison Test for PV of oil samples at 30 ° C, 60th day

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	12,789	2	6,394	3552,444	,000
Within Groups	,005	3	,002		
Total	12,794	5			

Figure B.29 Analysis of variance for PV of oil samples at 40 ° C, 15th day

groups	N	Subset for alpha = 0.05		
		1	2	3
BHT	2	9,4400		
control	2		12,1000	
Carvacrol	2			12,8400
Sig.		1,000	1,000	1,000

Figure B.30 Duncan's Multiple Comparison Test for PV of oil samples at 30 ° C, 60th day

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	64,760	2	32,380	161901,000	,000
Within Groups	,001	3	,000		
Total	64,761	5			

Figure B.31 Analysis of variance for PV of oil samples at 40 ° C, 30th day

groups	N	Subset for alpha = 0.05		
		1	2	3
BHT	2	20,4400		
control	2		25,3300	
Carvacrol	2			28,4200
Sig.		1,000	1,000	1,000

Figure B.32 Duncan's Multiple Comparison Test for PV of oil samples at 40 ° C, 30th day

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	182,815	2	91,407	1371109,000	,000
Within Groups	,000	3	,000		
Total	182,815	5			

Figure B.33 Analysis of variance for PV of oil samples at 40 ° C, 45th day

groups	N	Subset for alpha = 0.05		
		1	2	3
BHT	2	34,5200		
Carvacrol	2		40,3500	
control	2			48,0000
Sig.		1,000	1,000	1,000

Figure B.34 Duncan's Multiple Comparison Test for PV of oil samples at 40 ° C, 45th day

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1082,711	2	541,355	78080,087	,000
Within Groups	,021	3	,007		
Total	1082,731	5			

Figure B.35 Analysis of variance for PV of oil samples at 40 ° C, 60th day

groups	N	Subset for alpha = 0.05		
		1	2	3
BHT	2	54,2000		
Carvacrol	2		68,3300	
control	2			87,0000
Sig.		1,000	1,000	1,000

Figure B.36 Duncan's Multiple Comparison Test for PV of oil samples at 40 ° C, 60th day

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	,000	2	,000	87,500	,002
Within Groups	,000	3	,000		
Total	,000	5			

Figure B.37 Analysis of variance for FFA of oil samples at 20 ° C, 15th day

groups	N	Subset for alpha = 0.05		
		1	2	3
BHT	2	,07500		
Carvacrol	2		,08000	
control	2			,09000
Sig.		1,000	1,000	1,000

Figure B.38 Duncan's Multiple Comparison Test for FFA of oil samples at 20 ° C, 15th day

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	,000	2	,000	58,333	,004
Within Groups	,000	3	,000		
Total	,000	5			

Figure B.39 Analysis of variance for FFA of oil samples at 20 ° C, 30th day

groups	N	Subset for alpha = 0.05		
		1	2	3
BHT	2	,08000		
Carvacrol	2		,09000	
control	2			,09500
Sig.		1,000	1,000	1,000

Figure B.40 Duncan's Multiple Comparison Test for FFA of oil samples at 20 ° C, 30th day

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	,001	2	,000	25,000	,013
Within Groups	,000	3	,000		
Total	,001	5			

Figure B.41 Analysis of variance for FFA of oil samples at 20 ° C, 45th day

groups	N	Subset for alpha = 0.05		
		1	2	3
BHT	2	,08500		
Carvacrol	2		,10000	
control	2			,11500
Sig.		1,000	1,000	1,000

Figure B.42 Duncan's Multiple Comparison Test for FFA of oil samples at 20 ° C, 45th day

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	,001	2	,001	320,333	,000
Within Groups	,000	3	,000		
Total	,001	5			

Figure B.43 Analysis of variance for FFA of oil samples at 20 ° C, 60th day

groups	N	Subset for alpha = 0.05		
		1	2	3
BHT	2	,09000		
Carvacrol	2		,10100	
control	2			,12500
Sig.		1,000	1,000	1,000

Figure B.44 Duncan's Multiple Comparison Test for FFA of oil samples at 20 ° C, 60th day

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	,000	2	,000	48,667	,005
Within Groups	,000	3	,000		
Total	,000	5			

Figure B.45 Analysis of variance for FFA of oil samples at 30 ° C, 15th day

groups	N	Subset for alpha = 0.05	
		1	2
Carvacrol	2	,08300	
BHT	2	,08500	
control	2		,10100
Sig.		,391	1,000

Figure B.46 Duncan's Multiple Comparison Test for FFA of oil samples at 30 ° C, 15th day

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	,001	2	,000	17,257	,023
Within Groups	,000	3	,000		
Total	,001	5			

Figure B.47 Analysis of variance for FFA of oil samples at 30 ° C, 30th day

groups	N	Subset for alpha = 0.05	
		1	2
BHT	2	,09000	
Carvacrol	2	,10000	
control	2		,11800
Sig.		,130	1,000

Figure B.48 Duncan's Multiple Comparison Test for FFA of oil samples at 30 ° C, 30th day

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	,001	2	,000	21,962	,016
Within Groups	,000	3	,000		
Total	,001	5			

Figure B.49 Analysis of variance for FFA of oil samples at 30 ° C, 45th day

groups	N	Subset for alpha = 0.05	
		1	2
BHT	2	,10000	
Carvacrol	2	,10500	
control	2		,12600
Sig.		,316	1,000

Figure B.50 Duncan's Multiple Comparison Test for FFA of oil samples at 30 ° C, 45th day

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	,001	2	,000	14,529	,029
Within Groups	,000	3	,000		
Total	,001	5			

Figure B.51 Analysis of variance for FFA of oil samples at 30 ° C, 60th day

groups	N	Subset for alpha = 0.05	
		1	2
BHT	2	,10600	
Carvacrol	2	,11000	
control	2		,13500
Sig.		,542	1,000

Figure B.52 Duncan's Multiple Comparison Test for FFA of oil samples at 30 ° C, 60th day

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	,000	2	,000	24,500	,014
Within Groups	,000	3	,000		
Total	,000	5			

Figure B.53 Analysis of variance for FFA of oil samples at 40 ° C, 15th day

groups	N	Subset for alpha = 0.05	
		1	2
BHT	2	,08800	
Carvacrol	2	,09000	
control	2		,10100
Sig.		,391	1,000

Figure B.54 Duncan's Multiple Comparison Test for FFA of oil samples at 40 ° C, 15th day

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	,001	2	,000	10,294	,045
Within Groups	,000	3	,000		
Total	,001	5			

Figure B.55 Analysis of variance for FFA of oil samples at 40 ° C, 30th day

groups	N	Subset for alpha = 0.05	
		1	2
BHT	2	,09500	
Carvacrol	2	,10000	
control	2		,12000
Sig.		,454	1,000

Figure B.56 Duncan's Multiple Comparison Test for FFA of oil samples at 40 ° C, 30th day

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	,001	2	,000	24,886	,014
Within Groups	,000	3	,000		
Total	,001	5			

Figure B.57 Analysis of variance for FFA of oil samples at 40 ° C, 45th day

groups	N	Subset for alpha = 0.05	
		1	2
BHT	2	,10800	
Carvacrol	2	,11000	
control	2		,13450
Sig.		,665	1,000

Figure B.58 Duncan's Multiple Comparison Test for FFA of oil samples at 40 ° C, 45th day

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	,001	2	,000	26,037	,013
Within Groups	,000	3	,000		
Total	,001	5			

Figure B.59 Analysis of variance for FFA of oil samples at 40 ° C, 60th day

groups	N	Subset for alpha = 0.05	
		1	2
BHT	2	,11500	
Carvacrol	2	,12100	
control	2		,14400
Sig.		,252	1,000

Figure B.60 Duncan's Multiple Comparison Test for FFA of oil samples at 40 ° C, 60th day

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	,221	2	,111	12,381	,036
Within Groups	,027	3	,009		
Total	,248	5			

Figure B.61 Analysis of variance for *p*-AnV of oil samples at 20 ° C, 15th day

groups	N	Subset for alpha = 0.05	
		1	2
Carvacrol	2	5,9500	
BHT	2	6,2000	6,2000
control	2		6,4200
Sig.		,077	,102

Figure B.62 Duncan's Multiple Comparison Test for *p*-AnV of oil samples at 20 ° C, 15th day

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1,188	2	,594	70,722	,003
Within Groups	,025	3	,008		
Total	1,213	5			

Figure B.63 Analysis of variance for *p*-AnV of oil samples at 20 ° C, 30th day

groups	N	Subset for alpha = 0.05		
		1	2	3
BHT	2	6,5000		
Carvacrol	2		7,0500	
control	2			7,5900
Sig.		1,000	1,000	1,000

Figure B.64 Duncan's Multiple Comparison Test for *p*-AnV of oil samples at 20 ° C, 30th day

	Sum of Squares	df	Mean Square	F	Sig.
Between roups	,947	2	,473	55,031	,004
Within Groups	,026	3	,009		
Total	,972	5			

Figure B.65 Analysis of variance for *p*-AnV of oil samples at 20 ° C, 45th day

groups	N	Subset for alpha = 0.05		
		1	2	3
BHT	2	7,0800		
Carvacrol	2		7,5000	
control	2			8,0500
Sig.		1,000	1,000	1,000

Figure B.66 Duncan's Multiple Comparison Test for *p*-AnV of oil samples at 20 ° C, 45th day

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1,703	2	,852	56,778	,004
Within Groups	,045	3	,015		
Total	1,748	5			

Figure B.67 Analysis of variance for *p*-AnV of oil samples at 20 ° C, 60th day

groups	N	Subset for alpha = 0.05		
		1	2	3
BHT	2	7,2000		
Carvacrol	2		7,7500	
control	2			8,5000
Sig.		1,000	1,000	1,000

Figure B.68 Duncan's Multiple Comparison Test for *p*-AnV of oil samples at 20 ° C, 60th day

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1,076	2	,538	896,444	,000
Within Groups	,002	3	,001		
Total	1,078	5			

Figure B.69 Analysis of variance for *p*-AnV of oil samples at 30 ° C, 15th day

groups	N	Subset for alpha = 0.05		
		1	2	3
Carvacrol	2	6,0200		
BHT	2		6,1600	
control	2			6,9800
Sig.		1,000	1,000	1,000

Figure B.70 Duncan's Multiple Comparison Test for *p*-AnV of oil samples at 30 ° C, 15th day

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1,244	2	,622	182,961	,001
Within Groups	,010	3	,003		
Total	1,254	5			

Figure B.71 Analysis of variance for *p*-AnV of oil samples at 30 ° C, 30th day

groups	N	Subset for alpha = 0.05		
		1	2	3
BHT	2	6,9500		
Carvacrol	2		7,3400	
control	2			8,0500
Sig.		1,000	1,000	1,000

Figure B.72 Duncan's Multiple Comparison Test for *p*-AnV of oil samples at 30 ° C, 30th day

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1,575	2	,787	112,467	,002
Within Groups	,021	3	,007		
Total	1,596	5			

Figure B.73 Analysis of variance for *p*-AnV of oil samples at 30 ° C, 45th day

groups	N	Subset for alpha = 0.05		
		1	2	3
BHT	2	7,2500		
Carvacrol	2		7,6500	
control	2			8,4800
Sig.		1,000	1,000	1,000

Figure B.74 Duncan's Multiple Comparison Test for *p*-AnV of oil samples at 30 ° C, 45th day

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	2,284	2	1,142	34,194	,009
Within Groups	,100	3	,033		
Total	2,384	5			

Figure B.75 Analysis of variance for *p*-AnV of oil samples at 30 ° C, 60th day

groups	N	Subset for alpha = 0.05		
		1	2	3
BHT	2	7,5000		
Carvacrol	2		8,2000	
control	2			9,0100
Sig.		1,000	1,000	1,000

Figure B.76 Duncan's Multiple Comparison Test for *p*-AnV of oil samples at 30 ° C, 60th day

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	,750	2	,375	54,111	,004
Within Groups	,021	3	,007		
Total	,771	5			

Figure B.77 Analysis of variance for *p*-AnV of oil samples at 40 ° C, 15th day

groups	N	Subset for alpha = 0.05	
		1	2
Carvacrol	2	6,2500	
BHT	2	6,3300	
control	2		7,0370
Sig.		,408	1,000

Figure B.78 Duncan's Multiple Comparison Test for *p*-AnV of oil samples at 40 ° C, 15th day

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	2,496	2	1,248	3120,167	,000
Within Groups	,001	3	,000		
Total	2,497	5			

Figure B.79 Analysis of variance for *p*-AnV of oil samples at 40 ° C, 30th day

groups	N	Subset for alpha = 0.05		
		1	2	3
BHT	2	7,0300		
Carvacrol	2		7,5400	
control	2			8,5800
Sig.		1,000	1,000	1,000

Figure B.80 Duncan's Multiple Comparison Test for *p*-AnV of oil samples at 40 ° C, 30th day

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	2,283	2	1,141	634,111	,000
Within Groups	,005	3	,002		
Total	2,288	5			

Figure B.81 Analysis of variance for *p*-AnV of oil samples at 40 ° C, 45th day

groups	N	Subset for alpha = 0.05		
		1	2	3
BHT	2	7,4400		
Carvacrol	2		8,1500	
control	2			8,9500
Sig.		1,000	1,000	1,000

Figure B.82 Duncan's Multiple Comparison Test for *p*-AnV of oil samples at 40 ° C, 45th day

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1,960	2	,980	49,000	,005
Within Groups	,060	3	,020		
Total	2,020	5			

Figure B.83 Analysis of variance for *p*-AnV of oil samples at 40 ° C, 60th day

groups	N	Subset for alpha = 0.05		
		1	2	3
BHT	2	7,8000		
Carvacrol	2		8,5000	
control	2			9,2000
Sig.		1,000	1,000	1,000

Figure B.84 Duncan's Multiple Comparison Test for *p*-AnV of oil samples at 40 ° C, 45th day