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ANTIOXIDATIVE EFFECT OF ESSENTIAL OILS OF *Thymbra spicata* ON PALM AND CORN OILS

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Antioxidative Effect of Essential Oils of *Thymbra spicata* on Palm and Corn Oils

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Supervisor
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ABSTRACT

ANTIOXIDATIVE EFFECT OF ESSENTIAL OILS OF *Thymbra spicata* ON PALM AND CORN OILS

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In this study, Rancimat Method, which is one of the Accelerated Shelf – Life Test (ASLT) method, was used to determine the effect of *Thymbra spicata* extract on the oxidative stability of corn oil and palm oil. For the comparison of the effect of *Thymbra spicata* extract butylated hydroxyltoluene (BHT) was used as a standard antioxidant. During the ASLT, the conductivity was followed at three temperatures, 90, 100 and 120^{o}C, with the addition of *Thymbra spicata* essential oils and BHT to corn and palm oils. The concentrations of *Thymbra spicata* essential oils and BHT used were in the range of 1.39 – 5.49 mg ml^{-1} and 0.014 – 2.4 mg ml^{-1} respectively. The same concentrations of both antioxidants used were also added into corn and palm oils to observe the chenge in peroxide value under storage temperatures of 15, 25 and 35^{o}C.

2,2-Diphenyl-1-picrylhydrazyl (DPPH) free radical-scavenging test was used for the determination of scavenging effect of *Thymbra spicata* extract. BHT was used as the standard antioxidant. The scavenging effect of *Thymbra spicata* extract was found to be 75.7 % while scavenging effect of BHT was found to be 73.7 %. Scavenging effect of *Thymbra spicata* was found to be significant compared to BHT.

It was observed that the corn oil was less stable to oxidation than palm oil. The addition of *Thymbra spicata* and BHT increased the induction times of corn oil and palm oil at each studied temperature. As the temperature decreased the induction time increased. *Thymbra spicata* extract was more effective than BHT.

Key Words: antioxidant, *Thymbra spicata*, BHT, lipid oxidation

ÖZET

Thymbra spicata UÇUCU YAĞLARININ HURMA VE MISIR YAĞLARI ÜZERİNDEKİ ANTİOKSİDAN ETKİSİ

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Bu çalışmada, Hızlandırılmış Raf-Ömrü Test (HRÖT) metodlarından biri olan Rancimat Metodu, *Thymbra spicata* uçucu yağlarının, mısır ve hurma yağlarının oksidatif stabilitesi üzerine etkisini belirlemek için kullanıldı. *Thymbra spicata* uçucu yağlarının etkisini kıyaslayabilmek amacıyla bütillendirilmiş hidroksitoluene (BHT) standart antioksidant olarak kullanıldı. HRÖT sırasında, mısır ve hurma yağlarına *Thymbra spicata* uçucu yağları ve BHT eklenerek, üç sıcaklıkta, 90, 100 and 120^{o}C, iletkenlik ölçümleri takip edildi. *Thymbra spicata* uçucu yağlarının ve BHT'nin kullanılan konsantrasyonları sırasıyla 1.39 – 5.49 mg ml^{-1} ve 0.014 – 2.4 mg ml^{-1} aralığındaydı. Aynı konsantrasyonlar mısır ve hurma yağlarına, peroksit değerindeki değişimi gözlemlemek için, saklama şartlarında, 15, 25 ve 35^{o}C, eklenmiştir.

Thymbra spicata uçucu yağlarının serbest radikal-giderim etkisinin belirlenmesi için 2,2-Diphenyl-1-picrylhydrazyl (DPPH) serbest radikal-giderim testi kıllanıldı. Standart antioksidant olarak BHT kullanıldı. *Thymbra spicata* uçucu yağlarının giderim etkisi % 75.7 olarak bulunurken, BHT'nin giderim etkisi % 73.7 olarak bulundu. *Thymbra spicata* uçucu yağlarının giderim etkisinin BHT ile kıyaslandığında önemli olduğu bulundu.

Mısır yağının hurma yağına göre daha dayanıksız olduğu gözlemlendi. *Thymbra spicata* uçucu yağları ve BHT ilavesi mısır ve hurma yağlarının indüksiyon sürelerini çalışılan her sıcaklıkta arttırdı. Sıcaklık düştükçe indüksiyon süresi arttı. *Thymbra spicata* uçucu yağları, BHT'den daha etkiliydi .

Anahtar Kelimeler: antioksidant, *Thymbra spicata*, BHT, yağ oksidasyonu

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INTRODUCTION

Lipid oxidation is one of the major factors that cause deterioration during the storage and processing of foods (Gu and Weng, 2001). Lipid oxidation may reduce the flavor and nutritive value of fats, oils and lipid containing products. Unsaturated fatty acids are sensitive to oxidation because of their chemical structure. Nutritional quality losses are related to losses in essential fatty acids, amino acids and vitamins. To overcome this problem, synthetic antioxidants such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) are incorporated into fats and oils (Basaga *et al*, 1997).

Nowadays, consumers all over the world are becoming more conscious of the nutritional value and safety of their foods and food ingredients. At the same time, there is a preference for natural foods and food ingredients that are believed to be safer, healthier and less subject to hazards than their artificial counterparts (Azizah *et al*, 1999). However, the use of synthetic antioxidants in food products has been falling off due to their instability as well as due to a suspected action as promoters of carcinogenesis. For this reason there is a growing interest in the studies of natural additives as potential antioxidants (Abdala and Roozen *et al*, 1999).

It has been known for some time that addition of certain aromatic herbs or spices to lipid containing materials will delay the oxidation process (Basaga *et al*, 1997). The antioxidant properties of many herbs and spices are reported to be effective in retarding the process of lipid oxidation in oils and fatty foods. There are many studies reported for the antioxidant activity of herbs (Koleva *et al*, 2002).

1.1. Lipid Oxidation

Lipid oxidation is a major cause of food quality deterioration. Oxidation of lipids initiates other changes in food, which affect its nutritional quality, wholesomeness and safety, color, flavor and texture. Antioxidants are principal ingredients which protect food quality by preventing oxidative deterioration of lipids (Shahidi and Naczk, 1995, Nawar, 1985).

General mechanism of lipid oxidation is explained with free-radical chain reaction involving three stages. Free radicals are atoms or a group of atoms with an odd (unpaired) number of electrons on the outer shell and can be formed when oxygen interacts with certain molecules. Free radicals are very unstable and react quickly with other compounds trying to capture the needed electron to gain stability. A chain reaction is started when free radical attacks the nearest stable molecule ‘stealing’ its electron, and the ‘attacked one‘, then becomes a free radical itself.

The classical route of autoxidation includes;
1. Initiation - formation of free radicals
2. Propagation - free-radical chain reaction
3. Termination - formation of non-radical products

$$\begin{array}{llll}
\text{Initiation} & \mathrm{RH} \rightarrow \mathrm{R}^{\bullet} + \mathrm{H}^{\bullet} & & (1.1) \\
\text{Propagation} & \mathrm{R}^{\bullet} + \mathrm{O}_2 \rightarrow \mathrm{ROO}^{\bullet} & & (1.2) \\
 & \mathrm{ROO}^{\bullet} + \mathrm{RH} \rightarrow \mathrm{R}^{\bullet} + \mathrm{ROOH} & & (1.3) \\
\text{Termination} & \mathrm{R}^{\bullet} + \mathrm{R}^{\bullet} & & \\
 & \mathrm{R}^{\bullet} + \mathrm{ROO}^{\bullet} & \Rightarrow \text{non - radical products} & (1.4) \\
 & \mathrm{ROO}^{\bullet} + \mathrm{ROO}^{\bullet} & &
\end{array}$$

Considering the rancidity in food lipids, the most important problem is that of initiation. Since direct attack of oxygen on unsaturated fatty acids is thermodynamically improbable. So, something must occur to help the formation of first few peroxides.

As implied in the reaction (1.1), (1.2) and (1.3)

$$\mathrm{RH} + \mathrm{O}_2 \rightarrow \text{free radicals or ROOH} \qquad (1.5)$$

has a very high activation energy (about 35 to 65 kcal/mol), so something must assist this reaction. It has been proposed that the initiation step may take place by hydroperoxide decomposition, by metal catalysis, or by exposure to light. Hydroperoxide formation requires a change in total electron spin since both the substrate and product are in singlet states, while oxygen is a triplet. It was postulated that if singlet oxygen was the species involved, then the reaction is possible for formation of original hydroperoxides (Nawar, 1985).

Singlet state oxygen is more electrophilic than triplet state oxygen. It can thus react rapidly (~1500 times faster than $^3O_2$) with moieties of high electron density, such as C=C bonds. The resulting hydroperoxides can then cleave to initiate a conventional free radical chain reaction

The most important ways for the formation of singlet O_2 is through photo-chemical reactions in the presence of a sensitizer:

$$S + h\nu \rightarrow {}^1S^* \rightarrow {}^3S^* \tag{1.6}$$

$${}^3S^* + {}^3O_2 \rightarrow {}^1O_2^* + {}^1S \tag{1.7}$$

$${}^1O_2^* + RH \rightarrow ROOH \tag{1.8}$$

$$ROOH \rightarrow \text{free radicals} \tag{1.9}$$

${}^1S$ = Singlet state sensitizer
${}^1S^*$ = Excited singlet state sensitizer
${}^3S^*$ = Excited triplet state sensitizer
${}^3O_2$ = Normal triplet oxygens
${}^1O_2^*$ = Excited singlet state oxygen
$h\nu$ = UV light energy in photons

The normal plant and tissue pigments such as chlorophyll, pheophytin and myoglobin can act as sensitizer. The peroxide formed was non-conjugated. These non-conjugated products are produced in small quantities and once oxidation starts they disappear rapidly, so only conjugated fatty acids are found, even when the oxygen reacted is unmeasurable (Nawar, 1985).

It has been postulated that trace metals are responsible for primary initiation (1.10). Metals that are oxidized by one electron transfer are the most active. This fits very well with singlet oxygen theory in that most of the sensitizers are bound metal complexes which can undergo this oxidation and may be more active than free metals due to geometrical considerations.

$$RH + M^{+n} \rightarrow R \bullet + H^{+} + M^{+(n-1)} \qquad (1.10)$$

In addition, it has been postulated that increased temperature can be responsible for direct attract of oxygen. Perhaps at these higher temperatures the singlet oxygen formation increases.

If the proper sensitizers are present, ultraviolet light would be a good initiator under the explanations above. It was found that under UV light activation energy decreased from 35 to 65 kcal/mole predicted for oxygen interaction to value of about 4 kcal/mole. This result supports the singlet oxygen initiation (Nawar, 1985).

It can be assumed that once the first hydroperoxides are produced, the chain reactions take over. However, by the time in the presence of peroxides initiation proceeds by a process which requires less energy, hydroperoxide decomposition (1.11) and (1.12).

$$ROOH \rightarrow RO \bullet + \bullet OH \qquad (1.11)$$

or

$$ROOH \rightarrow R \bullet + HO_2 \qquad (1.12)$$

The reaction is usually written as if it were metal catalyzed. Thus,

$$ROOH + M^{+n} \rightarrow ROO \bullet + H^{+} + M^{+(n-1)} \qquad (1.13)$$

or

$$ROOH + M^{+(n-1)} \rightarrow RO \bullet + OH^{-} + M^{+n} \qquad (1.14)$$

It can be seen that by the latter scheme the metal in the oxidized state can always be regenerated and show true catalysis.

Upon the formation sufficient free radicals, the chain reaction is propagated by the abstraction of hydrogen atoms at the positions α to double bonds. Oxygen attack then occurs at these locations, resulting in the production of peroxy radicals ROO·, and there in turn abstract hydrogen from α-methylenic groups, RH of other molecules to yield hydroperoxides ROOH and R· groups. Due to the resonance stabilization of the R· species, the reaction sequence is usually accompanied by a shift in the position of double bonds, resulting in the formation of isomeric hydroperoxides often containing conjugated diene groups. The new R· groups react with oxygen, and the sequence of reactions is repeated.

Hydroperoxides, the primary initial products of lipid autoxidation, are relatively unstable. They enter into numerous and complex breakdown and interaction mechanisms responsible for the production of myriad compounds of various molecular weights, flavor thresholds, and biological significance Figure 1.1.

Polyunsaturated components of fats are oxidized much more rapidly than are monounsaturated and saturated components. In the required for fats to become rancid it is likely that only the polyunsaturated components undergo autoxidation; thus it is the polyunsaturated components that are the focal points in autoxidation of fats. Since linoleic acid is the main polyunsaturated component of our principal edible fats, the mechanism of autoxidation of this acid is of major importance in oxidative rancidity. The more highly unsaturated acids, although they autoxidize more rapidly than linoleic acid does, are present in only minor proportions (Mattil, 1964).

However, the readiness with which any pure fatty acid oxidizes is not measured simply by its overall degree of unsaturation. On the contrary, ease of oxidation is determined by the distribution and geometry, as well as the number of double bonds. In particular, a single methylene group ($-CH_2-$) between double bonds constitutes a very active center for oxidation. Linolenic acid having two such activated methylene groups oxidizes twice as lonoleic acid. Conjugated double bonds are highly reactive toward oxygen. *Cis*-isomers generally undergo addition at the double bond more readily than *trans*-isomers. Geometrical isomerism generally has a considerable effect on the rate of oxidation, with resistance to oxidation being conferred by isomerization to *trans*-forms.

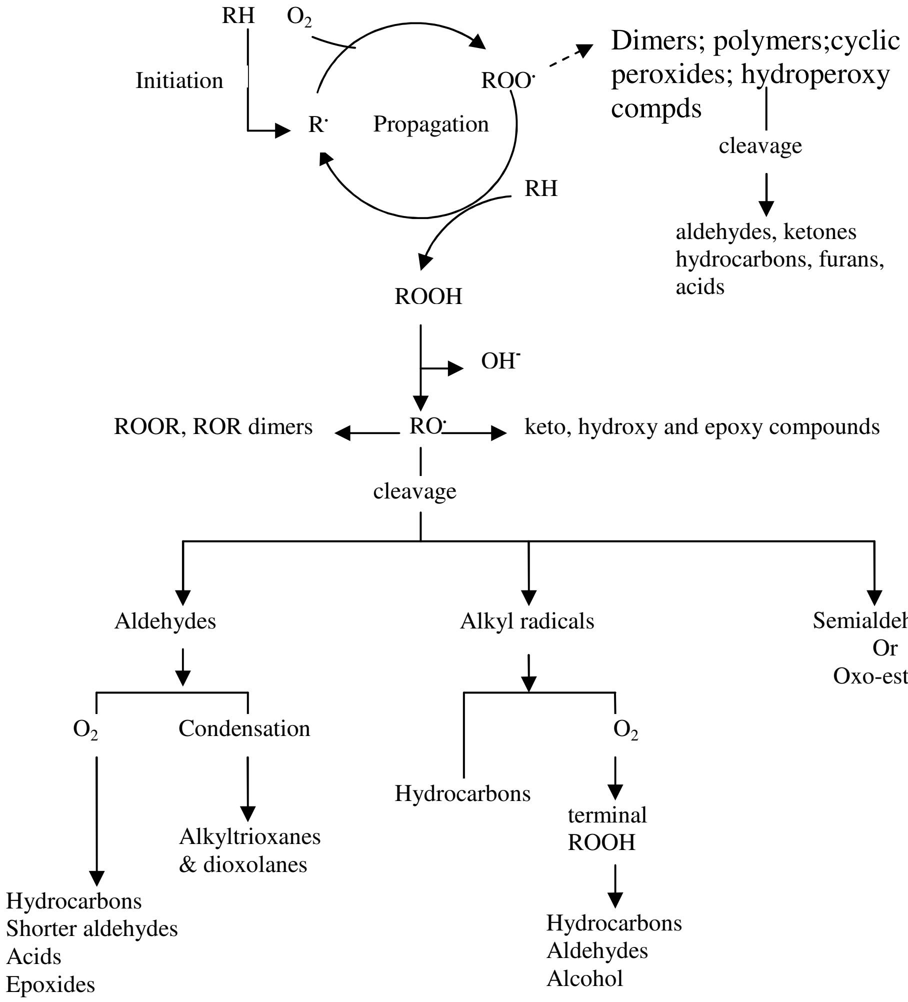


Figure 1.1. General scheme for lipid oxidation (Nawar, 1985).

As oxidation continues, further oxidation of the conjugated hydroperoxides occurs, and decomposition products, such as low- and medium-molecular weight saturated and unsaturated aldehydes, ketones and acids are formed. These have strong unpleasant odors and account for the typical rancid odors and flavors of autoxidized fats. As the aldehydes themselves are oxidized, tertiary oxidation products including free fatty acids of low molecular weight are formed (Nawar, 1985).

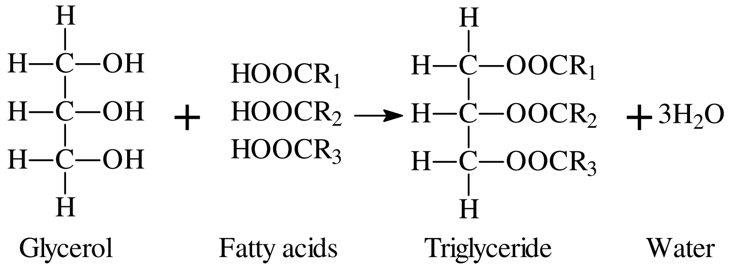
1.1.1. Lipid Structure

Lipids are defined as the heterogeneous groups of naturally occurring substances which are insoluble in polar solvents e.g. water, but soluble in organic solvents. Lipids contain carbon, oxygen, while some lipids also contain phosphorus and nitrogen. Lipids consist predominantly of glyceryl esters of fatty acids, or triglycerides. The word ‘fat’ is ordinarily used to refer to triglycerides that are solid or semisolid at ordinary temperatures, whereas the word ‘oil’ is used for triglycerides that are liquid under the same conditions. (Mattil, 1964)

Edible fats are complex mixtures of triglycerides and small amounts of other substances occurring naturally or are derived through processing and storage of the fats. The natural fats are made up of mixed triglycerides with only trace amounts of the mono- and di-glycerides and little or no free fatty acids.

1.1.1.1. Mono-, Di- and Triglycerides

Structurally, a triglyceride is the condensation product of one molecule of glycerol with three molecules of fatty acids to yield three molecules of water and one molecule of a triglyceride (1.15). (Mattil, 1964)



$$\begin{array}{c}\mathrm{H}\\ |\\ \mathrm{H{-}C{-}OH}\\ |\\ \mathrm{H{-}C{-}OH}\\ |\\ \mathrm{H{-}C{-}OH}\\ |\\ \mathrm{H}\end{array} + \begin{array}{c}\mathrm{HOOCR_1}\\ \mathrm{HOOCR_2}\\ \mathrm{HOOCR_3}\end{array} \longrightarrow \begin{array}{c}\mathrm{H}\\ |\\ \mathrm{H{-}C{-}OOCR_1}\\ |\\ \mathrm{H{-}C{-}OOCR_2}\\ |\\ \mathrm{H{-}C{-}OOCR_3}\\ |\\ \mathrm{H}\end{array} + 3H_2O \qquad (1.15)$$

Naturally occurring fats are always mixtures of different triglycerides and the individual triglyceride may have three different fatty acids, called mixed triglycerides.

Monoglycerides and diglycerides contain only one or two fatty acids, respectively and consequently have two or one free hydroxyl groups. They do not occur naturally in appreciable quantities except in fats that have undergone partial hydrolysis. The extent of the hydrolysis depends on the presence of water, heat and hydrogen or hydroxide ions. Mixtures of mono- and diglycerides are readily prepared from fatty acids or fats and glycerol, and they have special industrial uses (Mattil, 1964).

1.1.1.2. Fatty acids

Most fatty acids are unbranched monocarboxylic acids which vary with length and degree of saturation and unsaturation. Fatty acid is a long-chain aliphatic carboxylic acid. (Nawar, 1985) Although there are a large number and variety of naturally occurring fatty acids, we can simplify matters with generalizations concerning those acids that occur most frequently in nature:

1. Most are monocarboxylic acids containing linear hydrocarbon chains with an even number of carbon atoms, generally in the range of C_{12}-C_{20}. Shorter- and longer-chain acids, branched- and cyclic-chain acids, and acids of odd-number carbon content do occur but at much lower frequency.

2. Unsaturation is common but largely confine to the C_{18} and C_{20} acids. When two or more double bonds exist they are almost always separated by a single methylene group, that is;

$$\mathrm{-CH{=}CH-CH_2-CH{=}CH-}$$

and $$\mathrm{-CH{=}CH-CH_2-CH{=}CH-CH_2-CH{=}CH-}$$

3. In the unsaturated fatty acids the double bonds are nearly always in the cis configuration.

The many C-C and C-H nonpolar bonds in the hydrocarbon chain confer considerable nonpolar character to the entire molecule even though there is one polar COOH group. Obviously, any substance composed in part of one or more fatty acids will also be largely nonpolar (Bohinski, 1987).

Those fatty acids in which all carbon atoms in the chain contain 2 hydrogen atoms and thus contain no double bonds are termed saturated. The fatty acids which contain double bonds are termed unsaturated (Figure 1.2). The degree of unsaturation of oil depends upon the average number of double bonds in its fatty acids (Mattil, 1964).

$—CH_2—CH_2—CH_2—CH_2—$ $—CH_2—CH=CH—CH_2—$

a) Carbon chain of a
saturated acid

b) Carbon chain of an
unsaturated acid

Figure 1.2. Structure of a) carbon chain of a saturated acid b) carbon chain of an unsaturated acid.

These saturated fatty acids range from C_4 toC_{30}, but fatty acids of greater than C_{20} comparatively rare. The most common saturated fatty acids found in animal fats are palmitic (C_{16}) and stearic acid (C_{18}). Saturated acids of less than 16 carbon chain length are frequently found in plant fats. In animal fats, fatty acids containing less than 16 carbons occur only in small quantities. Higher fatty acids such as arachidic acid (C_{20}) may also occur in animal fats but branched-chain acids are uncommon.

Lauric (C_{12}) acid is one of the three most widely distributed naturally occurring fatty acids, the others being palmitic (C_{16}) and stearic (C_{18}). Palmitic acid is the most widely distributed of the saturated fatty acids. It occurs in practically all animal, vegetable and aquatic fats. Stearic acid is also widely distributed. It is an important component of the body fats of most animals (Mattil, 1964).

The melting points of the saturated acids exhibit a progressive increase as the carbon chain is lengthened. Differences in the melting points of the acids are reflected in the melting points of the simple glycerides. Thus milk fats and vegetable fats of the coconut oil type, which contain large proportions of C_6 toC_{12} fatty acids, have lower melting points than fats with an equivalent degree of unsaturation that are composed substantially of glycerides of C_{16} and C_{18} fatty acids.

A large number of unsaturated fatty acids occur naturally and are more difficult to isolate, purify and characterize than the saturated acids. Their study is further complicated by the fact that they are less stable and are readily converted to position (difference in the position of the double bond) and geometric (cis, trans) isomers. The naturally occurring acids usually contain an even number of carbon atoms (most frequently 18). In most cases the double bonds have the cis-configuration, and preferred position for a double bond is between the ninth and tenth carbon atoms in the fatty acid chain.

Unsaturated fatty acids are found in the fats of animals, plant and marine animals. Those occurring in animal fats are principally oleic and palmitic acid because animal cells are unable to synthesize unsaturated fatty acids containing more than one double bond. In contrast linoleic and linolenic acid are found primarily in plant fats and oil while polyunsaturated acid (arachidonic) are found in fish oils.

Unsaturated fatty acids are more chemically reactive than the saturated fatty acids. The presence of double bond permits the addition of hydrogen atoms to the double bond, in the presence of a suitable catalyst (nickel, copper) to give the corresponding saturated fatty acids. Thus, oleic, linoleic and linolenic acids give stearic acid upon hydrogenation. In addition to hydrogenation, unsaturated fatty acids are susceptible to oxidation because of the double bond. Exposure to oxygen of the air causes a fat containing polyunsaturated fatty acids to undergo gradual formation of peroxides together with a mixture of volatile aldehydes, ketones and acids (Nawar, 1985).

1.1.1.3. Effect of saturation on oxidation

Most of fats are in the form triglyceride, many of which are of importance to oxidation. The triglyceride is a chemical structure made up of glycerol to which on each hydroxyl group is attached via an ester linkage, a fatty acid. The fatty acids are primary importance to the rate of development of oxidation, since they can be very susceptible to attack of oxygen. It has been well known that the unsaturated fatty acids cause the development of off flavors in foods. The mechanism of the breakdown reaction has been reviewed extensively for oleate, linoleate and linolenate. For the susceptibility of oxidation of food products it should be noted that the total fat content is not important, but the amount of unsaturated fatty acid content is (Mattil, 1964).

a) Corn Oil

Crude corn oil has dark reddish amber color and even after refining is somewhat darker in color than most other vegetable oils. It contains relatively large amounts (1-3%) of phosphatides and other non-oil substances, mainly sterols (often 1 % or more), and its free fatty acid content (usually 1.5 %) is higher than that of other common vegetable seed oils. Tocopherol is also an important component (0.1 %) of the unsaponifiable fraction, thus accounting for its stability. The low cloud point and melting point of the final product and its good keeping qualities make it desirable edible oil.

A number of samples of refined corn oil were found to have the following average characteristics (Table 1.1): iodine number,126; saponification number, 190.6; specific gravity, 25^o/25^{o}C, 0.9198; refractive index at 20 ^{o}C, 1.4748, unsaponifiable matter, 1.25 %, saturated fatty acids, 9.4 %; unsaturated fatty acids, 85.6 %. Oleic and linoleic acids usually comprise over 80 % of the fatty acids in a ratio of 1:2-1:3. Linolenic acid is either absent or present in traces. Palmitic acid is the main saturated fatty acid (10%). The triglycerides are mainly di- and triunsaturated.

Table 1.1. Characteristics and composition of Corn oil and Palm oil (Mattil, 1964)

Analysis	Corn Oil	Palm Oil
Iodine number	103-133	44-60
Saponification number	187-193	195-205
Refractive index	1.470-1.474 at 25^{o}C	1.453-1.456 at 40^{o}C
Specific gravity	0.916-0.921 at 25^{o}C	0.898-0.901 at 37.8^{o}C
Unsponifiable matter, %	0.8-2.9	Not over 0.8
Myristic, wt %	trace-1.7	0.5-6
Palmitic, wt %	8-12	32-45
Stearic, wt %	2-5	2-7
Total Saturated, wt %	12-18	39-50
Hexadeceneoic, wt %	0.2-1.6	0.8-1.8
Oleic, wt %	19-49	38-52
Linoleic, wt %	34-62	5-11
Total Unsaturated, wt %	82-88	50-61

b) Palm Oil

Palm oil is colored a deep orange red by the large amount of carotene it contains. The color is not much affected by alkali refining, but palm oil is bleached to a yellow color similar to that of other vegetable oils by hydrogenation. It has a pleasant, characteristic odor, is very stable toward oxidation, and has no drying properties. At ordinary temperatures it is semisolid. Its consistency and melting point depend to a large degree upon its content of free fatty acids, as the free fatty acids higher in melting point than the glycerides. Palm oil’s saturated and unsaturated fatty acids are present in approximately equal amounts (Table 1.1). Palmitic and oleic acids are the main component acids.

1.2. Antioxidants

Antioxidants are substances that can delay the onset or slow the rate of oxidation of autoxidizable materials. They do not improve the quality of the product, but rather, maintain it by preventing oxidation of labile lipid components (Labuza, 1971). Antioxidants cannot reverse the oxidation of rancid oils, nor are they effective in suppressing hydrolytic rancidity which is an enzymatically catalyzed hydrolysis of fats.

Literally hundreds of products have been proposed for retarding oxidative deterioration of easily autoxidised substances: Paints and surface coatings (anti-skimming agents), lubricating oils and greases, anti-gum and anti-sludge compounds, fats and oils, rubber, essential oils, vitamin preparations, and pharmaceuticals. Many of these antioxidants are effective in fat containing foods, but health and aesthetic reasons, only a few can be incorporated into products destined for human consumption (Lundberg, 1961).

According to mode of reaction antioxidants broadly can be divided into two groups (Figure 1.3);

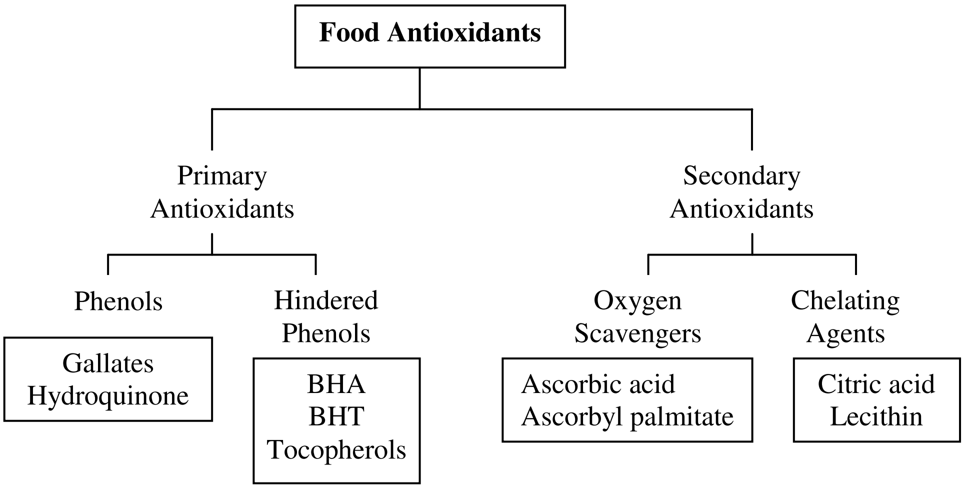


Figure 1.3. Classification of antioxidants

1.2.1. Primary Antioxidants

These are the antioxidants that operate most directly from a mechanistic standpoint, might well be called antioxygens. In other words they interrupt the free-radical chain of oxidative reactions by contributing hydrogen from the phenolic hydroxyl groups, themselves forming stable free radicals which do not initiate or propagate further oxidation of lipids. Primary antioxidants react with high energy lipid radicals to convert them to thermodynamically more stable products (Shahidi and Naczk, 1995). The reaction mechanism of primary antioxidants can be described by the reactions (1.16), (1.17), (1.18), (1.19) and (1.20):

$$ROO^{\bullet} + AH \rightarrow ROOH + A^{\bullet} \quad (1.16)$$

$$RO^{\bullet} + AH \rightarrow ROH + A^{\bullet} \quad (1.17)$$

$$ROO^{\bullet} + A^{\bullet} \rightarrow ROOA \quad (1.18)$$

$$RO^{\bullet} + A^{\bullet} \rightarrow ROA \quad (1.19)$$

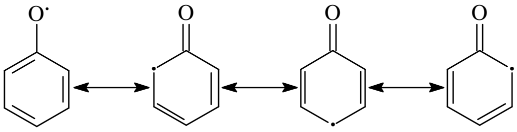
$$RO^{\bullet} + RH \rightarrow ROOH + R^{\bullet} \quad (1.20)$$

Those compounds most commonly used in foods are called phenolic antioxidants because the best known and most effective substances are polyphenols. Antioxidant activity, however, is not restricted to the phenols, and a number of other compounds, usually containing nitrogen or sulfur functional groups with electronic configuration similar to that of the polyphenols, are also powerful antioxidants (Lundberg, 1961).

The chemical activities of polyphenols in terms of their reducing properties as hydrogen or electron-donating agents, predicts their potential for action as free-radical scavengers (antioxidants). The activity of antioxidant is determined by:

- Its reactivity as a hydrogen or electron-donating agent
- The fate of the resulting antioxidant-derived radical, which is governed by its ability to stabilize and delocalize the unpaired electron
- Its reactivity with other antioxidants
- The transition metal-chelating potential (Rice-Evans *et al*, 1997)

Phenolic antioxidants are excellent hydrogen or electron donors and, in addition, their radical intermediates are relatively stable due to resonance delocalization and lack of suitable sites for attack by molecular oxygen.



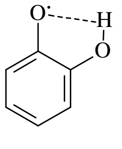
(1.21)

The phenoxy radical formed by reaction of a phenol with a lipid radical is stabilized by delocalization of unpaired electrons around the aromatic ring as indicated by the valence bond isomers (1.21).

The more effective the resonance forms, the better the antioxidant. However, phenol itself is inactive as an antioxidant. Substitution of the hydrogen atoms in the ortho and para positions with alkyl groups increases the electron density of the OH moiety by an inductive effect and thus enhances its reactivity towards lipid radicals (Shahidi and Naczk, 1995). The introduction of alkyl groups, particularly of branched groups such as tertiary butyl radicals, in positions next to the hydroxyl groups decreases considerably their activity; at the same time, it retards their destruction by higher temperatures and other conditions employed during the preparation of baked and fried foods. These compounds, known as 'carry-through' or 'carry-over' antioxidants, remain effective in foods prepared from fats in which they are present (Lundberg, 1961).

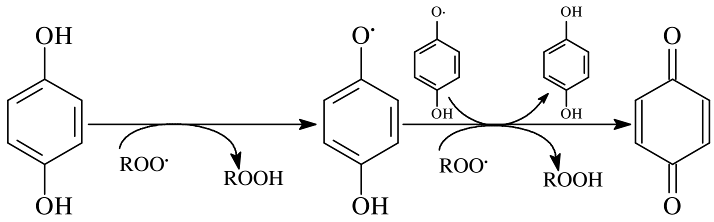
The stability of the phenoxy radical is increased by bulky groups at the ortho positions. Since this these substituents increase the stearic hindrance in the region of the radicals, they further reduce the rate of possible propagation reactions that may occur involving antioxidant free radicals (Shahidi and Naczk, 1995).

The introduction of second hydroxyl group at the ortho or para position of the hydroxyl group of a phenol increases its antioxidant activity. The effectiveness of a 1,2-dihydroxybenzene derivative is increased by the stabilization of the phenoxy radical through intramolecular hydrogen delocalization (1.22).



(1.22)

The antioxidant activity of dihydroxybenzene derivatives is partly due to the fact that the semiquinoid radical, produced initially, can be further oxidized to a quinone by reaction with another lipid radical. It can also form into a quinone or hydroquinone molecule (1.23) (Shahidi and Naczk, 1995).



(1.23)

The activity of 2-methoxyphenol is much lower than that of catechol possessing two free hydroxyl groups. This is due to the fact that 2-methoxyphenols are unable to stabilize the phenoxy radical by hydrogen bonding as in reaction (1.22) (Shahidi and Naczk, 1995).

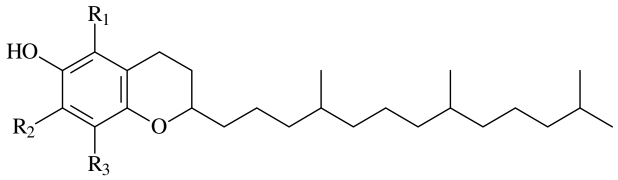
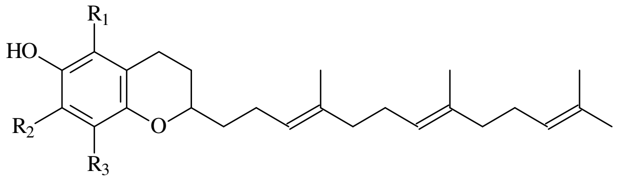
1.2.1.1. Natural antioxidants

Antioxidants in food may be naturally occurring ones or produced during processing of food material. Natural antioxidants are primarily plant polyphenolics occurring in all parts of the plants. Plant phenolics are multifunctional compounds and can as reducing agents (free radical terminators), metal chelators, and singlet oxygen quenchers. Examples of common plant phenolic antioxidants include flavonoid compounds, cinnamic acid derivatives, comarins, tocopherols and polyfunctional organic acids (Shahidi and Naczk, 1995).

a) Tocopherols

Tocopherols occur widely in nature and are monophenolic antioxidants that help to stabilize most vegetable oils. Tocopherols are composed of eight different compounds belonging to two families, namely tocols and tocotrienols, reffered to as α, β, γ or δ, depending on the number and position of methyl groups attached to the chromane rings (Table 1.2). In tocols, the side chain is saturated while in tocotrienols it is unsaturated (Shahidi and Naczk, 1995).

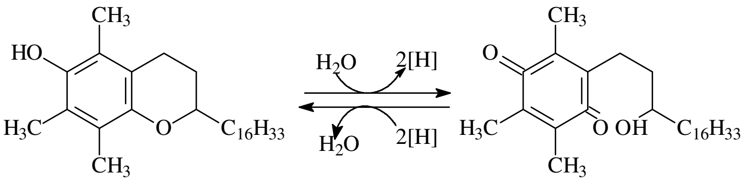
Table 1.2. Structures of Vitamin E and related compounds

Compound	R_1	R_2	R_3
Tocols			
			
5,7,8-Trimethyl tocol (α-tocopherol)	CH_3	CH_3	CH_3
7,8-Dimethyl tocol(β-tocopherol)	H	CH_3	CH_3
5,8-Dimethyl tocol (γ-tocopherol)	CH_3	H	CH_3
8-Methyl tocol (δ-tocopherol)	H	H	CH_3
Tocotrienols			
			
5,7,8-Trimethyl tocotrienol (α-tocotrienol)	CH_3	CH_3	CH_3
7,8-Dimethyl tocotrienol(β-tocotrienol)	H	CH_3	CH_3
5,8-Dimethyl tocotrienol (γ-tocotrienol)	CH_3	H	CH_3
8-Methyl tocotrienol (δ-tocotrienol)	H	H	CH_3

Tocopherols also possess vitamin E activity. With regard to vitamin E activity, α-tocopherol is the most potent member of this family. The discrepancies often observed between the antioxidant potency and biological vitamin E activity of various natural tocopherol or antioxidant preparations may be accounted for satisfactorily by the following considerations: (a) the tocopherols with high vitamin E activity are relatively poor antioxidants, while those with low biological potency have a high antioxidant activity. (b) The esters of the tocopherols are biologically active but, except for these allophanates, have no antioxidant activity. (c) Certain quinoid substances which may be associated with the tocopherols have no vitamin E activity but possess some antioxidant activity (Lundberg, 1961).

Tocopherols are important biological antioxidants. α-Tocopherol or vitamin E prevents oxidation of body lipids including polyunsaturated fatty acids and lipid components of cells and organelle membranes.

The antioxidant activity of tocopherol is based on the tocopherol-tocopheryl quinone redox system (1.24) (Shahidi and Naczk, 1995).



(1.24)

α-tocopherol α-tocopherylquinone (stable)

Tocopherols (AH_2) are radical scavengers and quench lipid radicals ($R^{\bullet}$), thus regenerating RH molecules as well as producing a tocopheryl semiquinone radical. Two tocopheryl semiquinone radicals may form a molecule of tocopheryl quinone and are generated molecule of tocopherol (1.25) and (1.26) (Shahidi and Naczk, 1995).

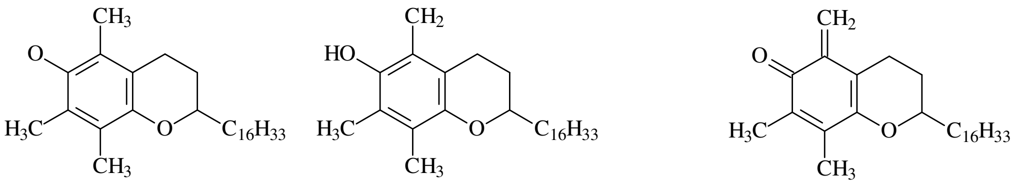
$$R^{\bullet} + AH_2 \rightarrow RH + AH^{\bullet} \qquad (1.25)$$

$$2AH^{\bullet} \rightarrow A^{\bullet} + AH_2 \qquad (1.26)$$

After releasing one H atom, the formed α-tocopheryl radical releases another H atom to produce methyl tocopheryl quinone (Figure 1.4). Methyl tocopheryl quinone is unstable and gives rise to α-tocopheryl quinone as its main product (1.24). The reaction between two semiquinoid radicals may also lead to the formation α-tocopherol dimer which possesses antioxidant properties.

b) Gallic Acid and the Gallates

Gallic acid (Figure 1.5), the common name for 3,4,5-trihydroxybenzoic acid is widely distributed in the vegetable kingdom, mostly as a component of the vegetable tannins, although free gallic acid has been reported to occur in tea (Lundberg, 1961).

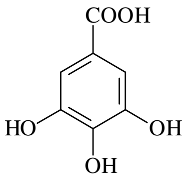


α-tocopheryl semiquinone
(a)

Methyl-tocopheryl quinone
(b)

Figure 1.4. Structure of α-tocopheryl semiquinone (a) and methyl-tocopheryl quinone (b)

Gallic acid is soluble in water but nearly insoluble in fats. Esterification of the carboxyl group with fatty alcohols gives esters which become progressively less soluble in water and more soluble in fats as the molecular weight of the alcohol increases. The lower gallates such as ethyl, propyl and butyl gallate remain slightly soluble in both water and fats, but the higher octyl, decyl, and dodecyl esters are practically insoluble in water but easily dissolve in fats and oils.



Gallic acid

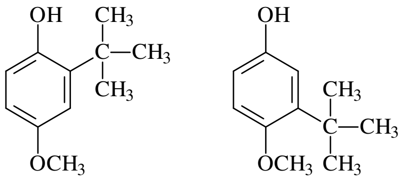
Figure 1.5. Structure of Gallic acid

The antioxidant properties of gallic acid were found to be peculiar in that it behaved as a primary phenolic antioxidant and as a synergist. The antioxidant properties of propyl gallate were described to be less effective than pyrogallol but more effective than gallic acid.

1.2.1.2. Synthetic phenolic antioxidants

Synthetic food antioxidants currently permitted for use in foods are butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), propyl gallate (PG), tertiary-butylhydroquinone (TBHQ) (Shahidi and Naczk, 1995).

BHA (Figure 1.6) is monohydric phenolic antioxidant. Chemically BHA is a mixture of 3-tertiary-butyl-4-hydroxyanisole (90 %) and 2-tertiary-butyl-4-hydroxyanisole (10 %) (Shahidi and Naczk, 1995). This compound does not occur naturally, but are synthesized by butylation of paramethoxyphenol. (Lundberg, 1961)



Butylated hudroxyanisoles

(3-BHA) (2-BHA)

Figure 1.6. Structure of BHA

The antioxidant activity of 3-BHA is greater than that of 2-BHA, and there is some evidence of a slight synergism when the isomers are used together, so that mixtures containing a high proportion of the 3-isomer are nearly as effective as the pure 3-c compound (Lundberg, 1961).

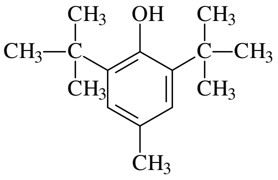
BHA, like most phenolic antioxidants, is inactive in vegetable oils containing appreciable amounts of naturally-occurring antioxidants. It is somewhat more effective in hydrogenated vegetable oils but is best with animal fats (Lundberg, 1961).

The most important property of BHA, which accounts for its great popularity as a food antioxidant, is its ability to remain active in baked and fried foods.

The stability of fats containing BHA decreases when they are heated to high temperatures, such as deep-fat frying temperatures, and the loss of stability is due to partly vaporization of the antioxidant, but the destruction of BHA is slow and enough antioxidant remains in the fat to protect for some time the foods prepared from it. BHA is particularly effective the flavor and color of essential oil and is considered as the most effective of all food-approved antioxidants for this application. BHA is particularly effective in controlling the oxidation of short-chain fatty acids (Lundberg, 1961, Shahidi and Naczk, 1995).

Due to the volatile nature of BHA, also BHT (Figure 1.7), it is important additive in packaging materials because they can migrate into foods. For this purpose, these antioxidants are either added directly to the wax used in making innerliners or applied to the packaging boards an emulsion. A synergistic effect has been shown to exist when BHA and BHT were used in combination. The oxidative reactions of nut and nut products are very responsive to the combination of these two antioxidants (Shahidi and Naczk, 1995).

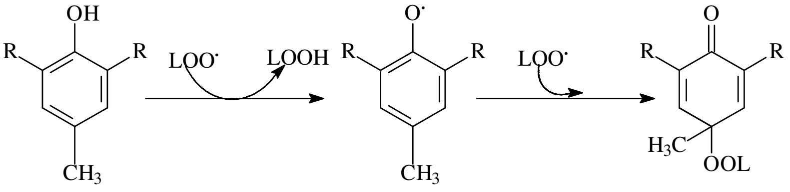
BHT is also monohydric phenolic antioxidant. Chemically BHT is 3,5-di-*tert*-butyl-4-hydroxytoluene (Williams *et al,* 1999). BHT like BHA belongs to a group of compounds known as 'hindered phenols', in which the reactivity of the phenolic group is decreased by the ortho or para substituents in their aromatic ring (Lundberg, 1961).



Butylated hudroxytoluene (BHT)

Figure 1.7. Structure of BHT

As a monophenol, BHT can produce radical intermediates with moderate resonance delocalization. The tertiary butyl groups of BHT do not generally allow the involvement of the radical formed from it after hydrogen abstraction in other reactions. Thus, a lipid peroxy radical may join the molecule of BHT as in (1.27) (Shahidi and Naczk, 1995).



R= tertiary butyl

(1.27)

The antioxidant property of BHT is transferred to baked goods also, but its carry-through is not as great as that of BHA. BHT does not have an optimum concentration, and the stability of fats to which it is added continues to increase with concentration, although the rate of increase is less at higher levels (Lundberg, 1961).

Tertiary-butyl hydroquinone (Figure 1.8) is regarded as the best antioxidant for protecting frying oils against oxidation. It provides a good carry-through protection to fried products similar to those of BHA and BHT. It has been stated that TBHQ may be considered as an alternative to hydrogenation for increasing oxidative stability. TBHQ is adequately soluble in fats and does not complex with iron or copper. Therefore, it does not discolor the treated products (Shahidi and Naczk, 1995).

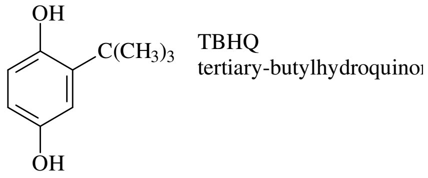


Figure 1.8. Structure of TBHQ

Chelating agents such as citric acid and monoglyceride citrate can further enhance lipid-stabilizing properties of TBHQ. This combination is primarily used in vegetable oils and shortenings but not extensively for animal fats. Confectioneries, including nuts and candies, also benefit from the use of TBHQ or its mixture. As a diphenolic antioxidant, TBHQ reacts with peroxy radicals to form a semiquinone resonance hybrid. The semiquinone radical intermediates may undergo different reactions to form more stable products. They can also react with one another to produce dimmers, dismutate, regeneration of the semiquinone, as well as reacting with another peroxy radical as summarized in reactions (1.28), (1.29) and (1.30) (Shahidi and Naczk, 1995).

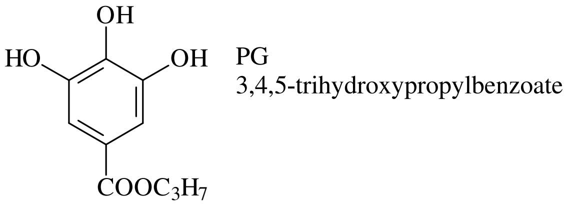
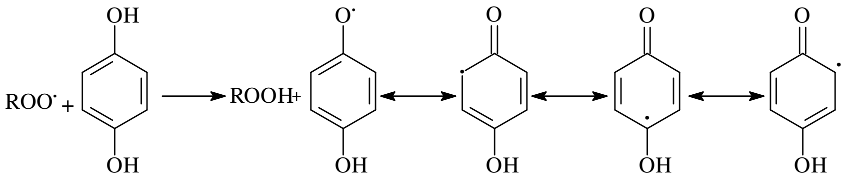
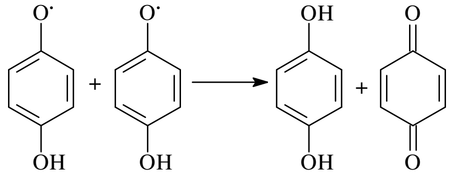


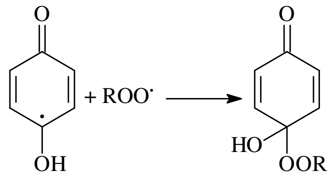
Figure 1.9. Structure of PG



(1.28)



(1.29)

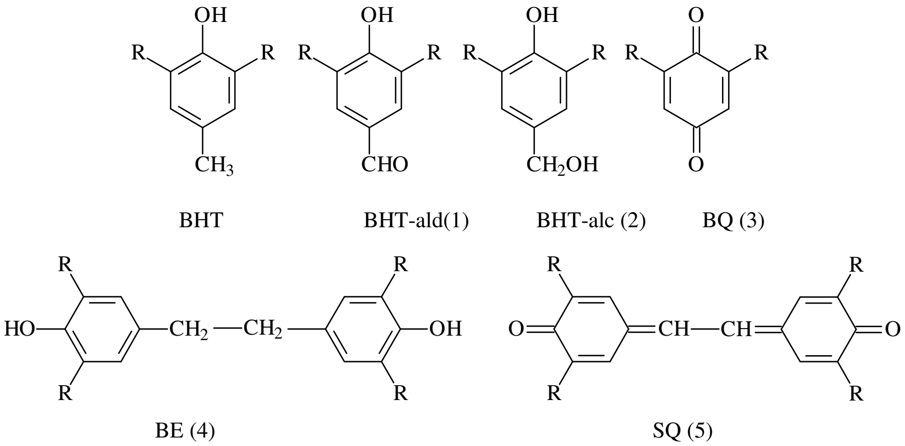


(1.30)

Propyl gallate (Figure 1.9) is commercially produced by esterification of gallic acid with propyl alcohol followed by distillation to remove the excess alcohol. It functions particularly well in stabilizing animal fats and vegetable oils. With a melting point of 148^{0}C, PG loses its effectiveness during heat processing and is therefore not suitable in frying applications which involve temperatures exceeding 190^{0}C. PG chelate iron ions and forms an unappealing blue-black complex. Hence, PG is always used with chelators such as citric acid to eliminate the pro-oxidative iron and copper catalysts (Shahidi and Naczk, 1995). It is used, or has been proposed for use, in all types of fats and fat containing foods (Lundberg, 1961).

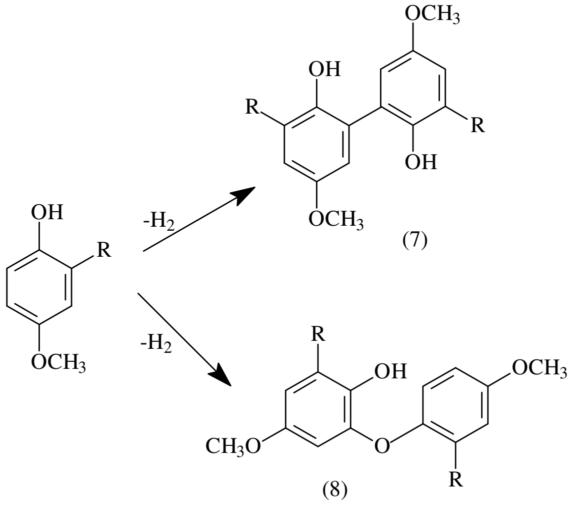
Phenolic antioxidants undergo degradation during the course of oxidation of fats and oils and produce a range of products, especially dimers of the antioxidants. Most oxidation products of antioxidants retain some antioxidant activity. Among the breakdown products of BHT (Figure 1.10) products (2)-(4) possessed antioxidant properties while products (1) and (5) were not effective as antioxidants. Meanwhile, degradation products of BHA (Figure 1.11) were less effective than BHA itself, in the order of BHA>8>7.

To control the oxidation process, in addition to free radical terminators (primary antioxidants), also there are agents which affect initiation rate, i.e. control the source of the production of free radicals before the propagation step. After free radicals were formed, the propagation step must be inhibited. Antioxidants that inhibit the initiation step are classified as ‘secondary antioxidants’.



R= tertiary butyl
(1) 3,5-ditert-butyl-4-hydroxybenzaldehyde
(2) 3,5-ditert-butyl-4-hydroxybenzylalcohol
(3) 2,6-ditert-butyl-benzoquinone
(4) 3,5,3’,5’-tetra-tert-butyl-4,4’-dihydroxy-1,2-diphenylethane
(5) 3,5,3’,5’-tetra-tert-butyl-stilbenequinone

Figure 1.10. Degradation products of BHT



R= tertiary butyl

(7) 2,2’-dihydroxy-5,5’-dimethoxy-3,3’-di-tert-butyl biphenyl
(8) 2’,3-ditert-butyl-2-hydroxy-4’,5,-dimethoxy-biphenyl ethe

Figure 1.11. Degradation products of BHA

1.2.2. Secondary Antioxidants

Secondary antioxidants are known as preventive antioxidants, function by retarding the rate of chain initiation by breaking down hydroperoxides (Shahidi and Naczk, 1995). In other words, agents which affect the initiation rate, i.e., control the source of the production of free radicals before the propagation step (Labuza, 1971). These are compounds which by themselves in the absence of primary antioxidants have very little effect on the oxidation of fats. They enhance or greatly prolong the anti-oxygenic action of primary antioxidants. These compounds may be organic or inorganic compounds and usually are acidic in character. These are the polyfunctional compounds containing several –OH or –COOH groups, or both, or other similar groups where oxygen is replaced by sulfur or nitrogen (Lundberg, 1961).

The major secondary antioxidants for food use are the chelating agents which coordinate with trace metals making them less reactive. In addition to the chelating agents there are also some other antioxidants which are named as the oxygen scavengers, reducing agents which function by transferring hydrogen atoms also retard rancidity.

Chelating agents are secondary antioxidants, yet they play a valuable role in stabilizing foods. Classified as synergists, chelating agent's complex with pro-oxidative metal ions such as iron and copper. An unshared pair of electrons in their molecular structures promotes the complexing action.

Trace metals present in food or lipid systems reduce the activation energy for initiation. This tends to increase the rate at which oxidation occurs and is of importance to the food industry in the removal or inactivation of these metals (Labuza, 1971).

The overall catalytic activity of a metallic ion is controlled by the slower step of the redox cycle (1.31).

$$M^{+2} \underset{slow}{\overset{fast}{\rightleftharpoons}} M^{+3} + e^{-} \qquad (1.31)$$

It not only depends on the electronic structure of the metals but on the solvent system and ligant (coordinated) groups attached to the metal. Electron donor ligants such as amines stabilize the oxidized state thereby increasing the reaction rate of direct radical formation. The reverse is generally found with acceptor ligands such as the heterocyclic bases. These compounds, such as hemes, stabilize the M^{+2} state, but also increase the rate of peroxyl-chelate complex formation, thus also increase the rate of radical formation (Labuza, 1971).

1.2.2.1. Flavonoids

The flavonoids are a large class of compounds, ubiquitous in plants, and usually occurring as glycosides. In plants these compounds afford protection against ultraviolet radiation, pathogens, and herbivores. The anthocyanin copigments in flowers attract pollinating insects and are responsible for the characteristic red and blue colors of berries, wines and certain vegetables-major sources of flavonoids in the human diet (Heim *et al*, 2002).

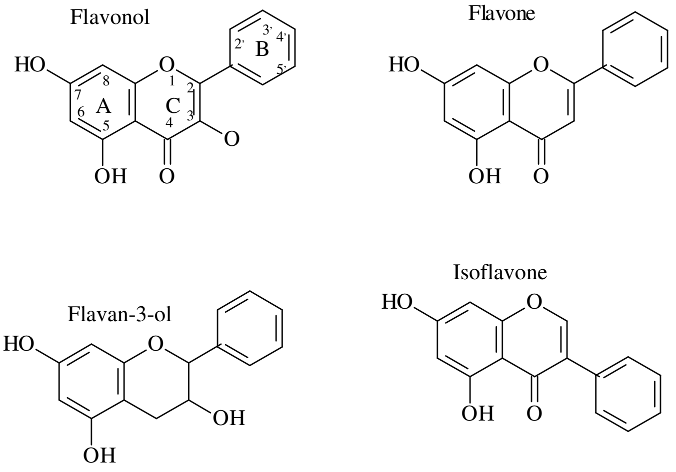


Figure 1.12. Structure of flavonoids

They contain several phenolic hydroxyl functions attached to ring structures (Figure 1.12), designated A, B and C (Rice-Evans *et al*, 1997, Heim *et al*, 2002). Structural variations within the rings subdivide the flavonoids into several families:

- **Flavonols** (e.g. quercetin and kaempferol), with the 3-hydroxypyran-4-one C ring
- **Flavones** (e.g. luteolin, apigenin and chrysin), lacking the 3-hydroxyl group.

- **Flavanols** (e.g. catechin), lacking the 2,3-double bond and the 4-one structure.
- **Isoflavones** (e.g. genistein), in which the B ring is located in the 3 position on the C ring (Rice-Evans *et al*, 1997, Shahidi and Naczk, 1995).

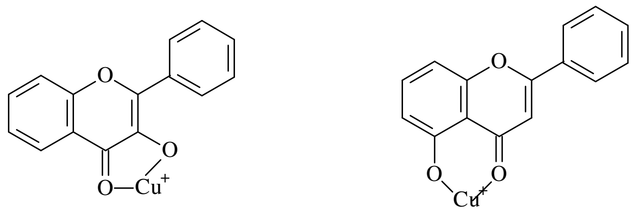
The antioxidant properties of flavonoids appear to be accounted largely by the entailing the transfer of radical character with formation of a reactive antioxidant-derived radical and secondarily by their ability to chelate metals. The antioxidant properties of flavonoids can be accounted for partly in terms of the formation of complexes with iron or copper (1.32), thereby being an important factor of their inhibitory reaction of free radical-mediated processes.

$$F - OH + Fe \rightarrow F - OH \cdots\cdots Fe \qquad (1.32)$$

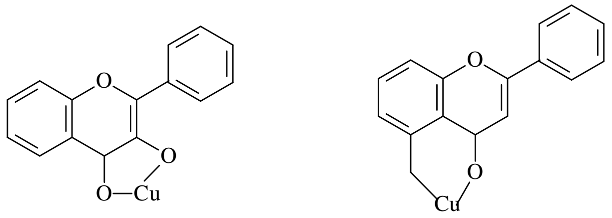
It has been established that the position and the degree of hydroxylation is of primary importance in determining antioxidant activity of flavonoids. The o-dihydroxylation of B-ring contributes to the antioxidant activity. Hydroxylation of the B-ring is the major consideration for antioxidant activity. since, hydroxyl groups on the B-ring donate hydrogen and an electron to hydroxyl, peroxyl and peroxynitirte radicals, stabilizing them and giving rise to a relatively stable flavonoid radical (Heim *et al*, 2002). Attributed to high reactivities of hydroxyl groups, they participate in the following reaction (1.33).

$$F - OH + R^{\bullet} \rightarrow F - O^{\bullet} + RH \qquad (1.33)$$

For the chelation, the two points of attachment of transition metal ions to the flavonoid molecule are the 0-diphenolic groups in the $3^{'}$,$4^{'}$-dihydroxy position in the B ring, and the ketol structures 4-*keto*, 3-hydroxy or 4-keto and 5-hydroxy in the C ring of the flavonols. However, it is likely that metals differ with regard to chelation by polyphenols (Rice-Evans *et al*, 1997). The ability of flavonoids to form complexes with cupric ion has also been demonstrated (Figure 1.13). Such complexation may contribute to the antioxidative action of flavonoids. Chelation of metal ions renders them catalytically inactive.



3-hydroxyflavone 5-hydroxyflavone



3-hydroxyflavanone 5-hydroxyflavanone

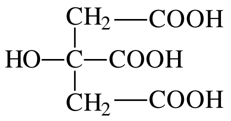
Figure 1.13. Forms of copper complexes wit flavones and flavonones.

It has been mentioned that at least two, and even better three, neighboring phenolic hydroxyl groups and a carbonyl group in the form of an aromatic ester, flavonone or flavone are the essential molecular features required to achieve a high level of antioxidant activity.

Although the flavonoids, secondary antioxidants, are the most abundant compounds present in human diet, there are also additional compounds present. These compounds act as secondary antioxidants classified as oxygen scavengers and chelating agents. These compounds are citric acid, ascorbic acid ascorbyl palmitate, EDTA, lecithin, β-carotene, vitamin A, tea extracts and nitrites classified as miscellaneous antioxidants.

1.2.2.2. Citric acid

Citric acid (Figure 1.14) in various forms such as isopropyl citrate is one of the popular chelating agents, especially for oils and intermediate moisture foods. The mono-esters and mono-sodium salts of citric acid are active while the di- and tri-citrates are completely ineffective, indicating that at least two carboxylic groups are necessary for antioxidative potency. Citric acid is readily decomposed by heat, but its thermal decomposition products are also good synergists. Its greatest disadvantage as an antioxidant is its insolubility in fats, but its esters are more soluble, and isopropyl citrate and stearyl citrate have been approved by use in foods.

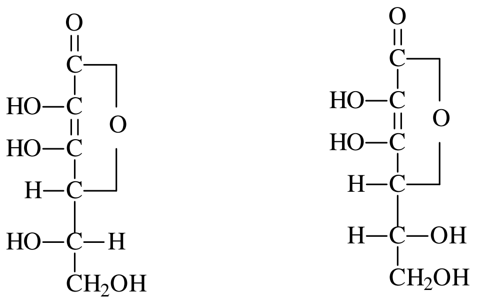


Citric acid

Figure 1.14. Structure of Citric acid

1.2.2.3. Ascorbic acid

The antioxidant effect of ascorbic acid and its derivatives can be ascribed pertly to their capacity to bind metal ions and it had been found that ascorbic acid and ascorbyl palmitate were effective against copper but not against iron. Its synergistic activity is believed to reside in the ene-diol portion of the molecule and an isomer, isoascorbic acid (Figure 1.15) is as effective a synergist as vitamin C although it has very little biological activity (Lundberg, 1961).



L-ascorbic acid

D-araboascorbic acid
(Isoascorbic acid)

Figure 1.15. Structure of Ascorbic acid

1.2.3. Evaluation of Antioxidant Activity

1.2.3.1. DPPH method

Free radicals are involved in the propagation of lipid oxidation, and many radical species of different reactivity are formed. Relatively stable molecules ($DPPH^{\cdot}$, $ABTS^{\cdot+}$) are often preferred in the assessment of radical scavenging effect. In the $DPPH^{\cdot}$ free radical method antioxidant efficiency is measured at ambient temperature and thus eliminates the risk of thermal degradation of the molecules tested. The method mainly is based on the reduction of alcoholic $DPPH^{\cdot}$ solutions at 515-520 nm in the presence of a hydrogen donating antioxidant (AH) due to formation of the non-radical form DPPH-H by the reaction (1.34).

$$\mathrm{DPPH}^{\bullet} + \mathrm{AH} \rightarrow \mathrm{DPPH}-\mathrm{H} + \mathrm{A}^{\bullet} \qquad (1.34)$$

The remaining $\mathrm{DPPH}^{\cdot}$, measured after a certain time, corresponding inversely to the radical scavenging activity of antioxidant. The method is rapid and requires little manpower, no expensive reagents or sophisticated instrumentation is needed (Koleva *et al, 2001*, Yepez *et al*, 2001, Bondet *et al*, 1997).

1.2.3.2. B-carotene Bleaching Test (BCBT)

The method is based on the loss of the yellow color of the β-carotene due to its reaction with radicals formed by linoleic acid oxidation. β-carotene is measured by decrease in the initial absorbance by the addition of antioxidants. The method is sensitive due to strong absorption of β-carotene, but it is slower than the DPPH method. Common reagents are used and no special treatment was necessary. However, careful solvent evaporation and preparation of an emulsion with reproducible composition and droplet size was essential for achieving reproducible results.

BCBT method employs an emulsified lipid which introduces an enhanced number of variables influencing oxidation in comparison with bulk lipids. An interesting phenomenon formulated as polar paradox has been reported: apolar antioxidants exhibit stronger antioxidative properties in emulsions because they concentrate at the lipid: air surface, thus ensuring high protection of the emulsion itself. On the other hand, polar antioxidants remaining in the aqueous phase are more diluted and are thus less effective in protecting the lipid. The opposite is observed in bulk lipids (Koleva *et al, 2001*, Tepe *et al*, 2005).

1.2.3.3. Thiobarbituric acid-reactive substances (TBARS) assay

This assay is used to measure the lipid peroxide formed. Malondialdehyde, a secondary end product of the oxidation of polyunsaturated fatty acids, reacts with two molecules of thiobarbituric acid (TBA) yielding a pinkish red pigment. However, malonaldehyde is not always found in all oxidized systems. Many alkanals, alkenals and diaenals produce yellow pigment in conjunction with TBA, but only dienals produce red pigment. Thiobarbituric acid-reactive substances are determined colorometrically. The results are expressed as percent inhibition of lipoperoxidation with respect the control. However the TBARS assay test is applicable for comparison of samples of a single material at different states of oxidation. Various compounds other than those found in oxidized systems, have been found to interfere with the TBARS assay by producing the characteristic red pigment upon reaction (Nawar, 1985, Banerjee *et al*, 2005).

1.2.3.4. Free radical scavenging capacity against $ABTS^{\cdot+}$

This test is also known as the ‘trolox equivalent antioxidant capacity assay’. It is based on the oxidation of ABTS (2,2’azino-bis-3-ethyl-benzothiazoline-6-sulphonic acid) in the presence of H_2O_2 and metmyoglobin to the radical cation $ABTS^{\cdot+}$ (blue-green color), which is photometrically measured. Dependent on the concentration of radical trapping substances oxidation is delayed. Absorbance is recorded continuously. After formation of the radical cation $ABTS^{\cdot+}$, an increase of absorbance is registered. Results are expressed in terms of molar Trolox equivalent, a water soluble vitamin E analogue (Mantle *et al*, 1998, Schlesier *et al*, 2002).

1.3. Shelf-Life Concept for Food Products

The shelf life of a food can be defined as the time period within which the food is safe to consume and/or has an acceptable quality to consumers. In order to make a useful prediction about shelf-life, some information is needed;

- Major modes of deterioration for loss of quality of the products,
- Conditions that food product will be exposed to,
- How the food product is handled, packaged or not? If food product is packaged, properties of the packaging material,
- The kinetics of the reactions leading to loss of quality or nutritional value.

Major modes of deterioration which lead to the quality loss of food products are as follows;

- Microbiological decay of foods
- Senescence
- Enzymatic chemical deterioration
- Non-enzymatic chemical deterioration
- Lipid oxidation
- Vitamin loss

- Protein loss
- Color changes
- Sensory changes
- Physical deterioration

Useful model to test the shelf-life is to accelerate the test conditions. This is the most often used methodology. The objective is to store the finished product under some test abuse condition, examine the product periodically until the end of shelf life occurs and then use these results to project the product shelf life under true distribution conditions.

The most common method is to select single abuse condition, expose the food to it, test it two or three times during some specific period, generally sensory methods, and then extrapolate the results to normal conditions. Another approach is to assume that certain principles of chemical kinetics apply with respect to temperature dependency such as the Arrhenius and the simple shelf-life plot approach.

1.3.1. Accelerated Shelf-Life Testing (ASLT) Methods

In an ASLT study, shelf life of the product mainly as an induction time is measured in a short time, usually in hours. Firstly suitable method must be selected to study the shelf life of the food product in under consideration. The most difficult step in shelf life test is to translate the data (induction time) obtained to actual shelf life of the product in months of storage.

Some parameters are manipulated in ASLT procedure to speed up the deterioration of the food product. These parameters are selected according to major modes of the deterioration of selected food product. For the fats and oils, major mode of deterioration is rancidity. So, the parameters selected for ASLT of fats and oils must be rancidity increasing parameters. These parameters are temperature, oxygen, added metals and reactants contact. Among these increased temperature is the most common and effective means of accelerating the rancidity. Some commonly used ASLT methods for rancidity (oxidation) of fats or oils are explained below.

1.3.1.1. The Schaal Oven Test

The test is especially convenient for determining the stability of fats where aeration is not feasible. The experimental procedure of this test, samples are incubated in a loosely closed container in an oven at 60-63^{o}C until they develop a specific peroxide number or rancid odor and flavor. No standards for this method have been adopted; hence no strict comparisons can be made among results of different laboratories (Mattil, 1964, Nawar, 1985).

1.3.1.2. Oxygen Absorption Methods (OAM)

The sample is kept at atmospheric pressure in oxygen at 100^{o}C. The amount of oxygen absorbed by the sample, as determined by the time to produce a specific pressure decline in a closed chamber or the time to absorb a pre-established quantity of oxygen under specific oxidizing conditions, is taken as a measure of stability (Nawar, 1985).

1.3.1.3. Active Oxygen Methods (AOM)

Active Oxygen Method or The Swift Stability Test is widely used test to determine the oxidation stability of edible oils and fats. It involves continuously aerating the sample and determining the time required to attain a pre-determined peroxide value under specific conditions (Mattil, 1964). Disadvantage of this method is being time consuming, labor intensive and wasteful. The most successful version of this method is based on the observation of volatile acids formed as a result of oxidation of oil. The effluent air from the sample in the AOM test can be led into water ant the acids are determined potentiometrically or conductometrically.

1.4. The Use of Herbs as Antioxidants

A number of compounds occurring naturally in plants are good antioxidants for fats. These include the flavonoids, such as quecitin and related compounds, and pomiferin obtained from Osage orange, and the tannins. Epicatechol gallate and epigallocatechol gallate, found in tea, are nearly as effective as propyl gallate (Lundberg, 1961).

Spices, condiments, and flavorings have been found to possess antioxygenic properties. The use of antioxidant properties of several spices in ground meats were reported (Labuza, 1971). One method used to preserve meat is smoking. Smoking of meat and fish deposits phenolic compounds on the surface and retards the development of rancidity in these products.

There are many studies done with herb extracts for their antioxidant activity all over the world. Simandi et al. (2001) have studied the antioxidant activity of ethanolic extracts and SCF (supercritical fluid) extracts of leaves and flowering tops of thyme (Thymus vulgaris L). BHT was used as a reference for the comparison of the antioxidant activity of extracts. Taking different concentrations of both thyme extracts and BHT, it was found that 0.6 % levels of extracts were equal to 0.1 % of BHT. Antioxidant activity of thyme extract was evaluated in sunflower oil in the dark at 60^{o}C. Thyme extract inhibited the formation of hydroperoxides and generation of pentanal and hexanal in sunflower oil during the incubation period (Abdala and Roozen, 1999).

Milos et al. (2000) examined the content and chemical composition of the glycosidically bound volatiles from oregano as well as antioxidative properties. Antioxidant activity was measured by measuring peroxide values of lard stored at 60^{o}C. Glycosidically bound volatiles in the spice oregano were found to be potent antioxidants, comparable in activity with its essential oil as well as to widely used natural antioxidant α-tocopherol. Volatiles of oregano spice inhibited the formation of hydroperoxides more than α-tocopherol. (Milos *et al*, 2000) The antioxidative effect of oregano extract was evaluated also in sunflower oil in the dark at 60^{o}C. Formation of hydroperoxides and generation of hexanal and pentanal were inhibited by oregano extract in sunflower oil (Abdala and Roozen, 1999).

It was reported that aoutoxidation of corn and soybean oil was prevented in the presence of 0.1 g/kg rosemary (Rosemarinus officinalis L.) extract and BHT. Oils with extract and BHT were stored for four days at 30^{o}C. At the 4th day corn oil samples containing rosemary extract and BHT reached 13.0 and 8.2 meq/kg of peroxide value (POV), respectively, whereas control was 16.9. Soybean oil samples containing 0.1 g/kg of extract and BHT, at the end of 4th day, had POV of 9.9 and 6.6, respectively, whereas the control was 18.2 (Basaga *et al*, 1997).

In another investigation on the oxidation stability of sunflower oil, extracts of *Melissa officinalis L., Mentha spicata L., Mentha piperita L., Ocimum basilicum L., Origanum vulgare L.* and S*aturejae hortensis L.* have been studied. It has been established that the extracts from *Ocimum basilicum L.* and *Origanum vulgare L.* do not improve the oxidation stability of sunflower oil. The ethanol extracts obtained from other four species have proved to be the most active in retarding the autoxidation process. The stabilization factor F (Induction time in the presence of extract / Induction time in the absence of extract) for the ethanol extracts (0.1-0.5 %) from S*aturejae hortensis L.* was 1.8-2.3. It was higher than F for 0.02 % BHT (F= 1.2). F factor calculated from the ratio of induction time in the presence of extract to induction time in the absence of extract (Marinova and Yanishlieva, 1996).

In another study on the effects of tocopherols and their mixture on the oxidative stability of olive oil and linseed oil, it was reported that the tested tocopherols (γ, δ, α) had showed antioxidative activity at all levels of addition to the monounsaturated olive oil. The effects increased as a function of concentrations. In the highly unsaturated linseed oil containing 58 mg/100 g initial concentration of γ-tocopherol, γ-tocopherol showed antioxidative behavior up to the addition of 100 mg/100 g oil. Additions of more than 100 mg/100 g affected the oil, resulting in a faster oxidation (Wagner and Elmadfa, 2000). The study of antioxidant activity of old tea leave extracts using Schaal oven test with rapeseed oil showed that all samples of rapeseed oil containing old tea leave extracts (0.02-0.25 %) were more stable on heating at 60°C than the control. Ascorbyl palmitate (0.02 %) chosen as a reference antioxidant had been very effective antioxidant for 13 days, but then the oil deteriorated rapidly so this sample had been significantly less stable than the old tea leave extract (0.25 %) sample after 16 days (Zandi and Gordon, 1999). Peanut oil stability was studied in another study using several antioxidants including phospholipids, ascorbyl plamitate, rosemary, tocoherol and catechin. Among these antioxidants, catechin alone and composites of catechin with other antioxidants showed significant increases in oil stability as compared to control oil (Chu and Hsu, 1999).

The antioxidant activities of four *Helichrysum* species were studied by using two complementary test systems, namely DPPH free radical scavenging and β-carotene/linoleic acid systems. When compared with the synthetic antioxidant BHT, polar sub-fractions of all extracts showed better radical scavenging activity. In the β-carotene/linoleic acid system, inhibition rates of extracts ranged from 50 to 60 %, while inhibition rate of BHT was 96 % (Tepe *et al*, 2005).

Table 1.3. Composition of *Thymbra spicata* extracted at different temperatures (Ozel *et al*, 2003).

Thymbra spicata	100°C	125°C	150°C	175°C
Essential Oil Content	2.0	2.5	3.4	3.2
α-Thujene	0.19	0.18	0.22	0.24
(-)α-Pinene	0.37	0.43	0.44	0.42
Camphene	Nd*	0.19	0.25	0.36
(+)-α-Pinene	0.50	0.69	0.62	0.62
Sabinen	Nd*	Nd*	Nd*	0.84
α –Terpinene	Nd*	Nd*	Nd*	0.22
p-Cymene	0.64	2.52	2.93	2.69
Limonene	Nd*	Nd*	Nd*	0.18
o-Cymene	Nd*	Nd*	Nd*	0.49
γ-Terpinen	1.90	0.38	0.58	0.37
E-3-caren-2-ol	3.08	6.72	7.71	7.16
Terpinen-4-ol	0.41	0.37	0.39	0.39
l-carvone	0.26	0.22	0.21	0.20
Thymol	3.67	1.92	1.24	0.97
Carvacrol	86.2	83.5	82.5	79.5
Caryophyllene	0.29	0.48	0.86	1.94
Spathulenol	Nd*	Nd*	Nd*	0.28
Caryophyllene oxide	Nd*	Nd*	Nd*	0.68
Unknown	2.52	2.41	2.10	2.46

* Nd, not detected

Extracts of herbs contain many products some of which are effective on oxidation of lipid systems but some are not. Because of this there were some studies done directly to see the effect of isolated compounds. Yanishleva *et al*, 1999, studied 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. The results obtained with inhibited lipid systems showed that thymol had the highest antioxidant effectiveness and activity during TGSO oxidation. Carvacrol molecules did not participate in side reactions during TGSO oxidation. Thymol and carvacrol contributed to chain inhibition to higher degree during TGSO oxidation than during TGL oxidation. Both antioxidants differ in the mechanism of their inhibiting action which depends on the character of the lipid medium.

There are many herbs containing compounds having antioxidative effect. Herbs containing thymol and carvacrol in their composition are Origanum species, Thymus species and Thymbra species etc. *Thymbra spicata* known as black thyme in Turkey grows mainly mountainous areas of Mediterranean parts of Turkey. The chemical composition of essential oils depends on climatic, seasonal, and geographic conditions, harvest period, and distillation technique. The composition of *Thymbra spicata* at different temperatures was tabulated in Table 1.3.

1.5. The Aim of Present Study

The oxidative stability of oils and fats may be influenced by many factors such as light, metal ions, oxygen, temperature and enzymes. These factors may reduce the flavor and nutritive value of fats, oils and lipid containing foods. The addition of antioxidants to the oil helps to prevent or decrease oil oxidation. In order to prevent or decrease oil oxidation traditionally synthetic antioxidants such as butylated hydroxy toluene (BHT) and butylated hydroxyl anisole (BHA) are used. However, their use in food products has been falling off due to their instability, as well as due to a suspected action as promoters of carcinogenesis. For this reason there is a big interest on natural antioxidants. The antioxidant properties of spices and many herbs were reported in literature to have strong antioxidative effect.

The main aim of this study was to compare the antioxidant activity of T*hymbra spicata* essential oils with that of BHT.

It was also aimed to observe the effect of concentration of essential oils of *Thymbra spicata* and temperature on the oxidative stability of both unsaturated corn oil and saturated palm oil.

CHAPTER 2

MATERIAL AND METHOD

2.1. Material

2.1.1. Samples

Corn oil used in the present study was bought from the local market. Palm oil was obtained from the Oil – Company (Marsa A.Ş.) after rafination before hydrogenation and addition of any chemicals.

Enough part of the oil samples was used firstly for storage in incubator at three selected temperatures, 15°C, 25°C and 35°C to observe shelf–lives at normal storage conditions. Also antioxidants BHT and *Thymbra spicata* extract was added at concentrations of 0.014–2.4 mg ml^{-1} and 1.39–5.49 mg ml^{-1} respectively. The remaining part of the oil was used for ASLT method.

The separation of the essential oils from air dried *Thymbra spicata* was conducted by steam distillation in a Clavenger apparatus for 3 hours. The essential oils were dried over anhydrous sodium sulphate, stored in a dark glass bottle until use.

2.1.2. Reagents

Pure 2,2-Diphenyl-1-picrylhydrazyl radical (DPPH) was purchased from Sigma (Steinhein, Germany). Acetic acid, sodium sulphate (Na_2SO_4), potassium bichromate ($K_2Cr_2O_7$), sulphuric acid (H_2SO_4) were purchased from Riedel De-Haen (Riedel De-Haen, Germany). HPLC grade methanol, chloroform, sodium thiocyanate ($Na_2S_2O_3$), potassium iodine (KI), butylated hydroxytoluene (BHT) were purchased from Merck (Darmstad, Germany) and starch was purchased from Pancreac (Pancreac, Spain).

2.2. Methods

2.2.1 DPPH assay

The DPPH assay measured hydrogen atom donating activity and hence provided an evaluation of antioxidant activity due to free radical scavenging. 2,2-Diphenyl-1-picrylhydrazyl radical (DPPH) a purple-colored stable free radical is reduced into the yellow-colored diphenylpicryl hydrazine. The method for the determination of DPPH assay was carried out by the following method: 1 ml of 0.2 mM of DPPH solution in methanol was added to 4 ml of methanolic solutions of *Thymbra spicata*. Absorbance at 515 nm was measured using Novaspech II Spectrophotometer (Pharmacia Biotech., England) after waiting for 30 min and the percentage of activity was calculated. BHT was used as reference compound (Mau *et al.*, 2003).

2.2.2. ASLT Measurements

2.2.2.1. Apparatus

The ASLT used in the present study was based on the principles of both the AOM and Rancimat method. It can be named as ASLT apparatus or Rancimat apparatus (Figure 2.1). This apparatus setup was designed according to study of Maskan, (1992).

In designed ASLT apparatus, the air was supplied by an air pump (manufactured by Thomas Industries Inc.) and passed through a series of washing columns. The columns consist of triple distilled water and 2 % $K_2Cr_2O_7$ in 1 % H_2SO_4 respectively. Also a condenser was mounted on the second column. Then the air was dried and filtered in a column containing glass wool and Na_2SO_4. Washed, dried and filtered air was compressed into reaction vessel containing oil sample. The reaction vessel was jacketed and heating oil was supplied from the heating bath (J.P. Selecta, s.a.) by the pump (J.P. Selecta, s.a.). The volatile compounds which were formed in the reaction vessel passed through a glass pipe into the absorption vessel and trapped by triple distilled water in the absorption vessel. A second condenser was mounted on absorption vessel to prevent loss of volatile compounds. The greater part of these compounds is formic acid, probably produced by the oxidative decomposition of aldehydes and ketones or organic peroxides, which can easily change the conductivity of the triple distilled water. Measurements were controlled conductometrically with an electrode by using a Conductivity-Meter, (Oakton Portable).

2.2.2.2. Method of measurement

The ASLT measurements were carried out for each oil sample by the following procedure. Approximately 50 ml of oil samples were measured accurately into the reaction vessel. In the case of addition of antioxidants, BHT and *Thymbra spicata*, firstly they are weighed according to previously defined concentration and then oil samples was added. Then heating oil in the heating bath was pumped to jacket of the vessel. The absorption vessel containing an electrode of the Conductivity-Meter was filled with 400 ml of triple distilled water. When all the connections between the washing columns, reaction vessel and absorption vessel were performed, the reaction vessel was allowed to reach thermal equilibrium with the heating oil at selected temperature (90, 100 and 120^{o}C) for a few minutes. The air flow rate was adjusted to 18 lt/h. Conductivities of triple distilled water in which formic acid had dissolved were measured at every fifteen minutes time intervals. A steeply rise in conductivity showed the reaction had completed. Measurements were performed by Datalog Assist PC Software for Oakton Portable meters (Oakton Portable Ver 1.0.0).

2.2.3. Peroxide value determination

5 gr of oil samples from each sample stored were weighed and 1 ml of saturated KI was added. It was stirred vigorously for about 1 min. Then 25 ml of acetic acid-chloroform mixture (3:2) was added. After the addition of acetic acid-chloroform mixture solution was left to stay for 5 min in dark place. After that time 75 ml of water was added and by addition of 1 ml 1 % starch solution was titrated with 0.01 N $Na_2S_2O_3$. Results were expressed as milliequivalents of peroxide per kilogram of sample.

2.3. Analysis of Obtained Data

Statistical analysis of obtained data was made by using SPSS (version 11.0) package programme at 95 % confidence interval. Bivariate correlation's of Pearson's two tailed tests was used to compare experimental data.

Figure 2.1. Modified Rancimat Method Apparatus

CHAPTER 3

RESULTS AND DISCUSSION

3.1. DPPH assay

The free radical scavenging activity, one of the mechanisms known to inhibit lipid oxidation (Cheung *et al.*, 2002), of *Thymbra spicata* (TSO) and BHT as reference was tested by their ability to bleach the stable free radical 1,1-diphenyl-2-picryl-hydrazyl (DPPH). This assay provided information on the reactivity of test compounds with a stable free radical. Because of its odd electron DPPH gives a strong absorption at 517 nm in visible spectroscopy. As this electron becomes paired off in the presence of a free radical scavenger, the absorption vanishes, and the resulting decolorization is stoichiometric with respect to the number of electrons taken up (Russo *et al.*, 2002). However, the reactional mechanism between DPPH and antioxidant depends on the structural conformation of the antioxidant. Some compounds react very quickly with DPPH, reducing a number of DPPH molecules equal to their number of available hydroxyl groups (Bondet *et al.*, 1997).

In Figure 3.1, the scavenging activity of the DPPH radical due to its reduction by *Thymbra spicata* and BHT with different concentrations is illustrated. The scavenging effect of the *Thymbra spicata* on DPPH radical linearly increased up to 71 % as concentration increased to 0.89 mg ml^{-1}. As the concentration increased from 0.89 to 2.5 mg ml^{-1} scavenging effect increased to 75.65 %. Increase in the concentration of the *Thymbra spicata* above 2.5 mg ml^{-1} did not increase the scavenging effect. The scavenging effect of the BHT linearly increased up to 72.31 % as the concentration was 0.07 mg ml^{-1}. Increase in the concentration of BHT above 0.07 mg ml^{-1} caused slight increase in; at 2.4 mg ml^{-1} scavenging effect is 73.7 %.

The scavenging activity of the *Thymbra spicata* is higher than that of BHT; *Thymbra spicata* having concentration 2.5 mg ml^{-1} with scavenging activity 75.65 % and BHT having concentration 2.4 mg ml^{-1} with scavenging activity 73.7 %. However, BHT showed higher scavenging activity at low concentration compared to *Thymbra spicata*. As the concentration of BHT increases, increase in scavenging effect of BHT does not increase. Higher scavenging activity of BHT at lower concentrations may be due to the mechanism of BHT / DPPH reaction (Bondet *et al.*, 1997) in which 1 mol of BHT reduces about 3 mol of DPPH.

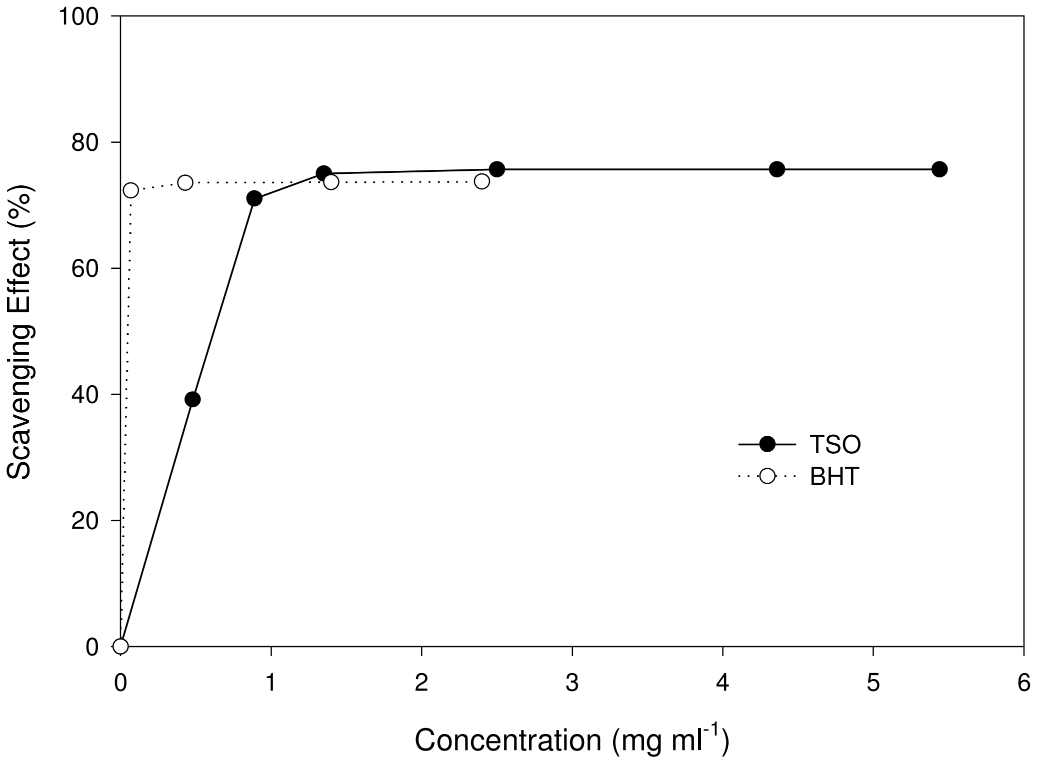


Figure 3.1. Scavenging effects of *Thymbra spicata* and BHT.

Scavenging effect of the *Thymbra spicata* is due to its composition including about 83 % carvacrol and 2 % thymol. Both carvacrol and thymol, isomers of each other, are monophenolic compounds as BHT is. Scavenging effect of *Thymbra spicata* is mainly due to carvacrol (Yanishleva *et al*, 1999).

3.2. Accelerated Shelf Life Test (ASLT)

3.2.1. Determination of Induction Time

The induction times were determined by plotting the conductivities against time. Then, the induction time was found by regression line method. In this method, regression lines were drawn on both the vertical and horizontal portions of the curve

(Figure 3.2.). The regression line's equation of both curves was equalized to each other and by solving the equality the intersection point of both curves was found. The result obtained from the equality gave the induction time. This can be done also by drawing the tangent lines (tangent method) on both portions of the line. The extent of intersection of these two lines to x-axis gave the induction time (end of shelf-life).

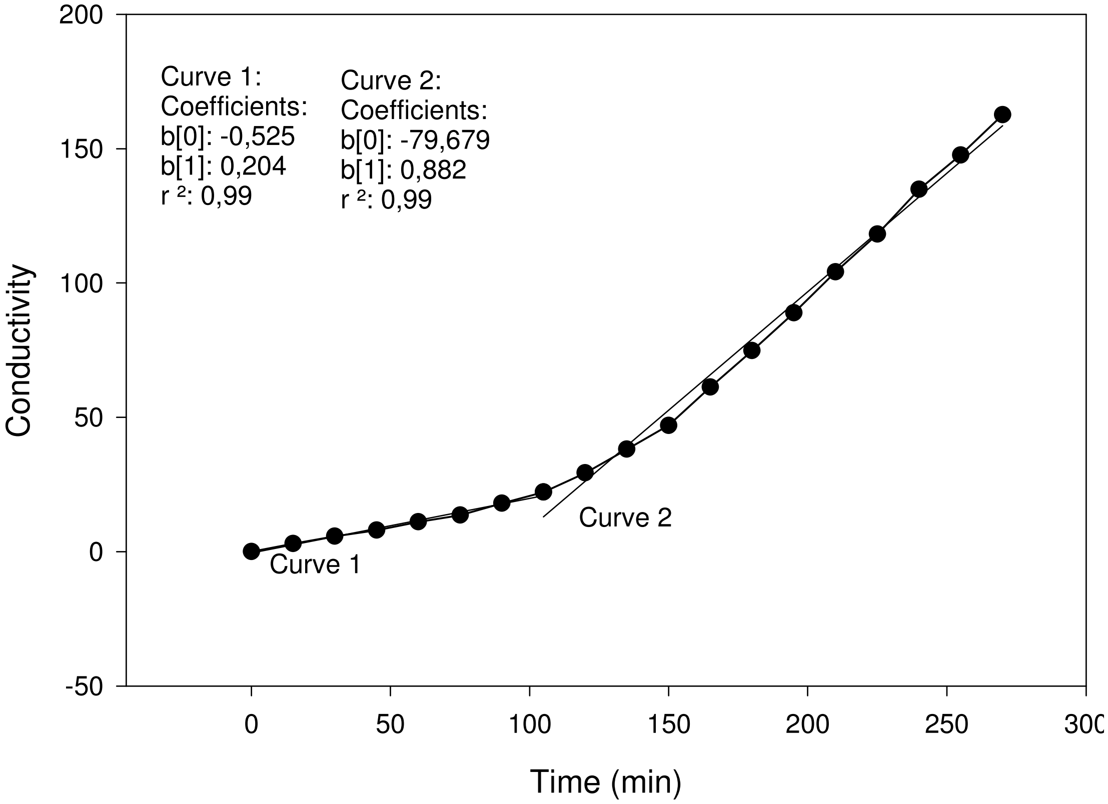


Figure 3.2. Graphical representation of determination of induction time

From the coefficients of the curve;

$$0.204x - 0.525 = 0.882x - 79.679$$
$$x = 116.7 \text{ min}$$

3.3. ASLT Results

3.3.1. Corn Oil

The conductivity values were plotted against the time and the induction times of corn oil with different concentrations of *Thymbra spicata* and BHT studied at different temperatures were determined according to method described in part 3.2.1. The temperatures studied were 90, 100 and 120^{o}C. At each temperature different concentrations of *Thymbra spicata* (1.39 to 5.49 mg ml^{-1}) and BHT (0.014 to 2.4 mg ml^{-1}) were studied as well as corn oil without the addition of antioxidant. Plots of conductivity versus time (min) for corn oil with *Thymbra spicata* and BHT as antioxidant are shown in Figure 3.3, 3.4, 3.5, 3.6, 3.7 and 3.8.

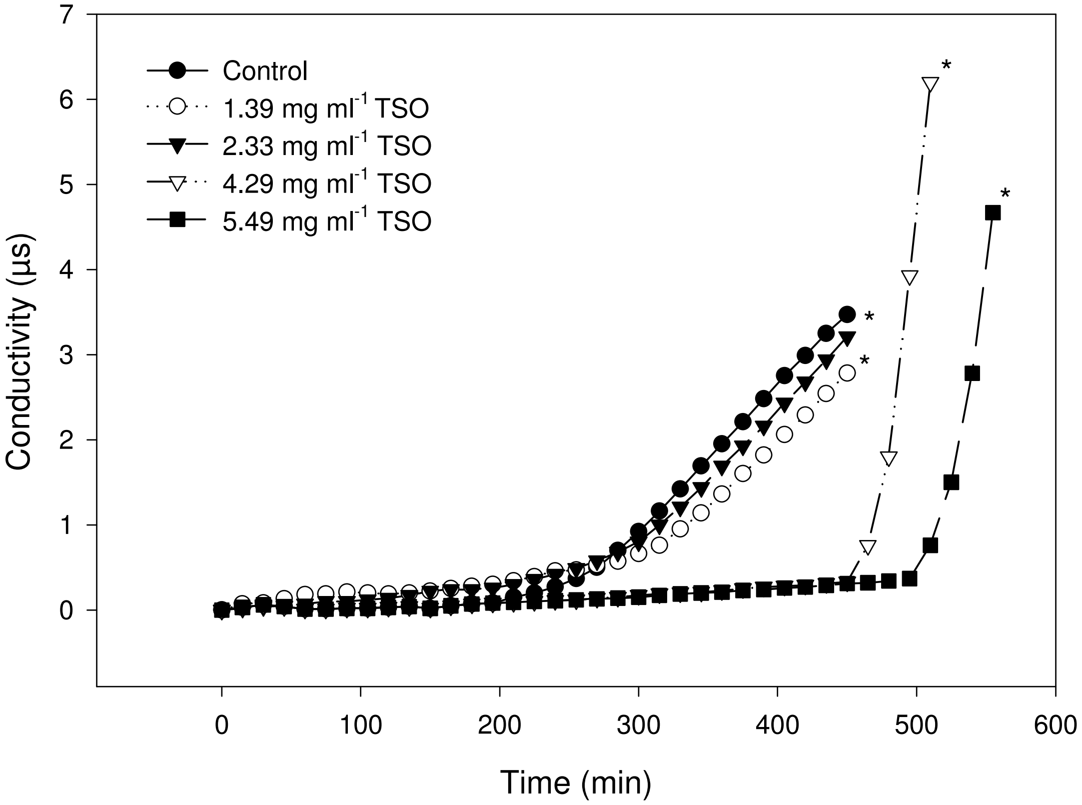


Figure 3.3. Plot of conductivity against time for corn oil at 90^{o}C with *Thymbra spicata* as antioxidant (* $P< 0.05$, significantly different from the control).

According the method defined, induction times of all the plots were determined. The induction times of corn oil at various temperatures were tabulated in Table 3.1 and 3.2.

Table 3.1. Induction times of corn oil at various temperatures with *Thymbra spicata*.

Temperature (^{o}C)	Induction Times (min)				
	Control	1.39 TSO	2.33 TSO	4.29 TSO	5.49 TSO
90	246	248	282	485	519
100	204	236	267	288	353
120	116	124	128	125	125

TSO: Concentration of *Thymbra spicata* in mg ml^{-1}.

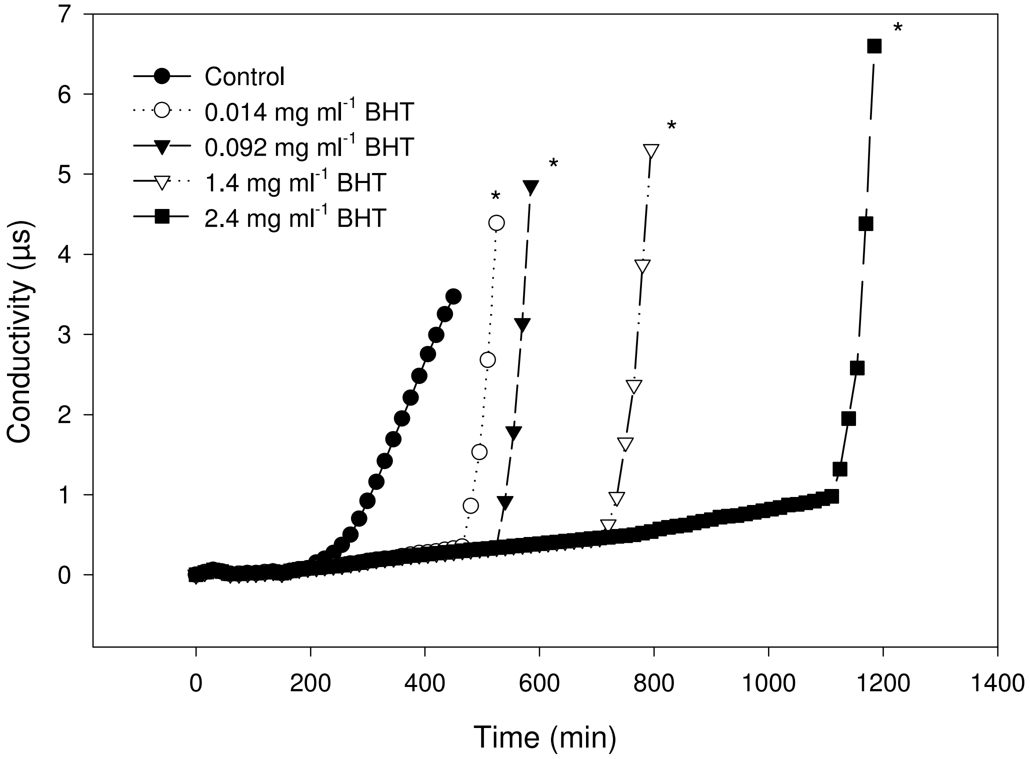


Figure 3.4. Plot of conductivity against time for corn oil at 90°C with BHT as antioxidant (* $P < 0.05$, significantly different from the control).

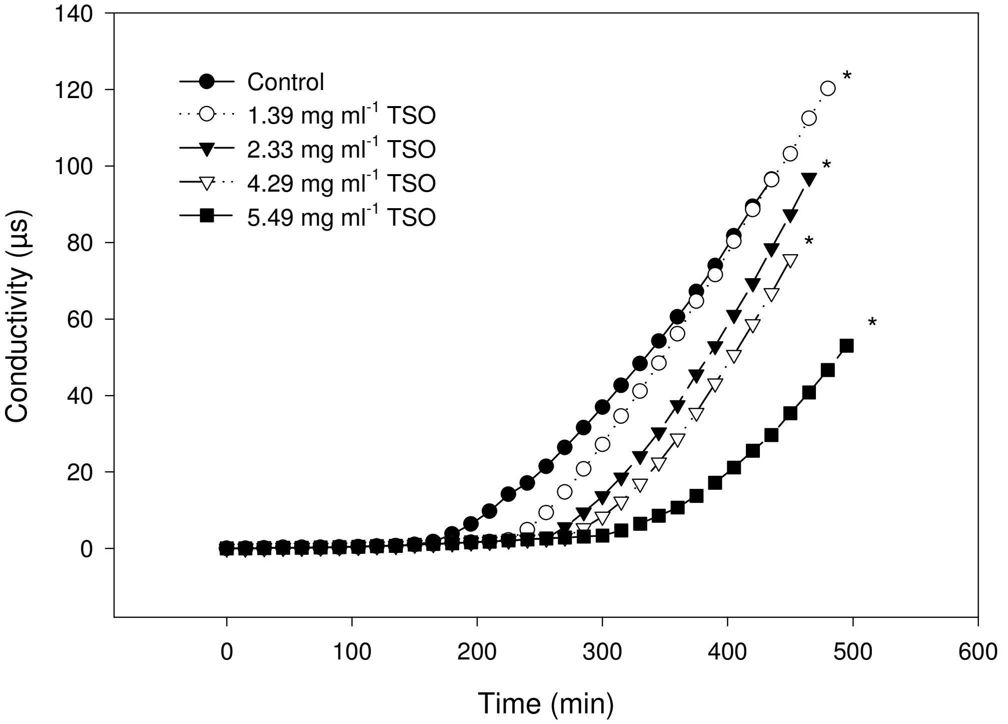


Figure 3.5. Plot of conductivity against time for corn oil at 100°C with *Thymbra spicata* as antioxidant (* $P < 0.05$, significantly different from the control).

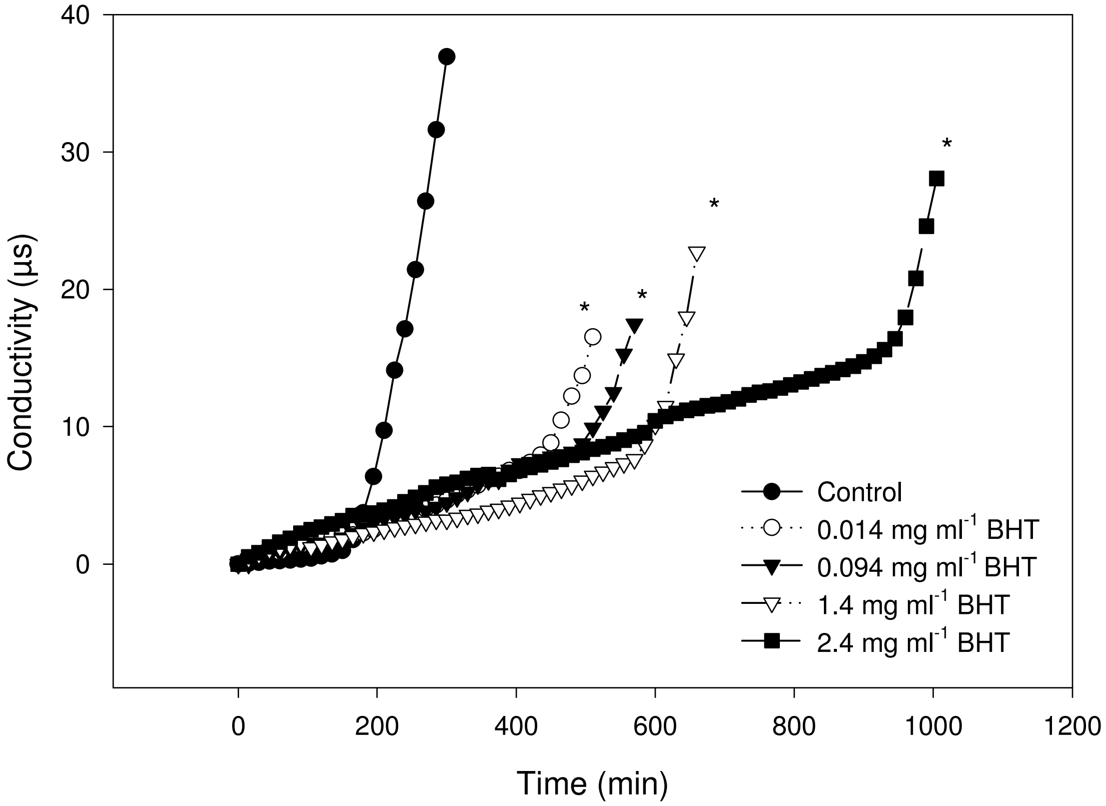


Figure 3.6. Plot of conductivity against time for corn oil at 100°C with BHT as antioxidant (* $P< 0.05$, significantly different from the control).

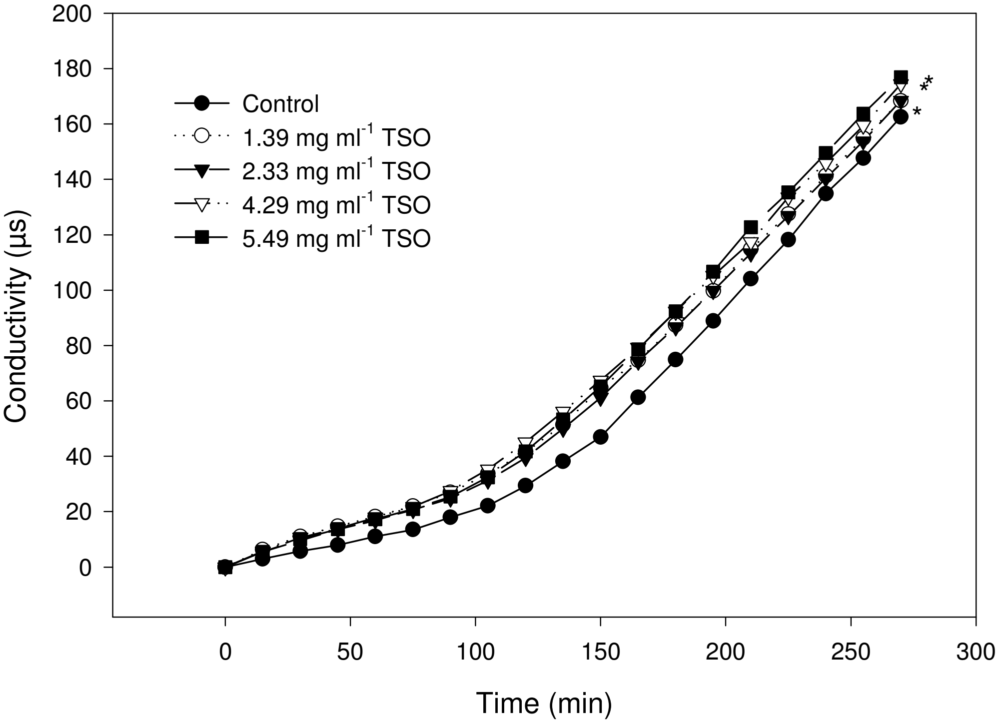


Figure 3.7. Plot of conductivity against time for corn oil at 120°C with *Thymbra spicata* as antioxidant (* $P< 0.05$, significantly different from the control).

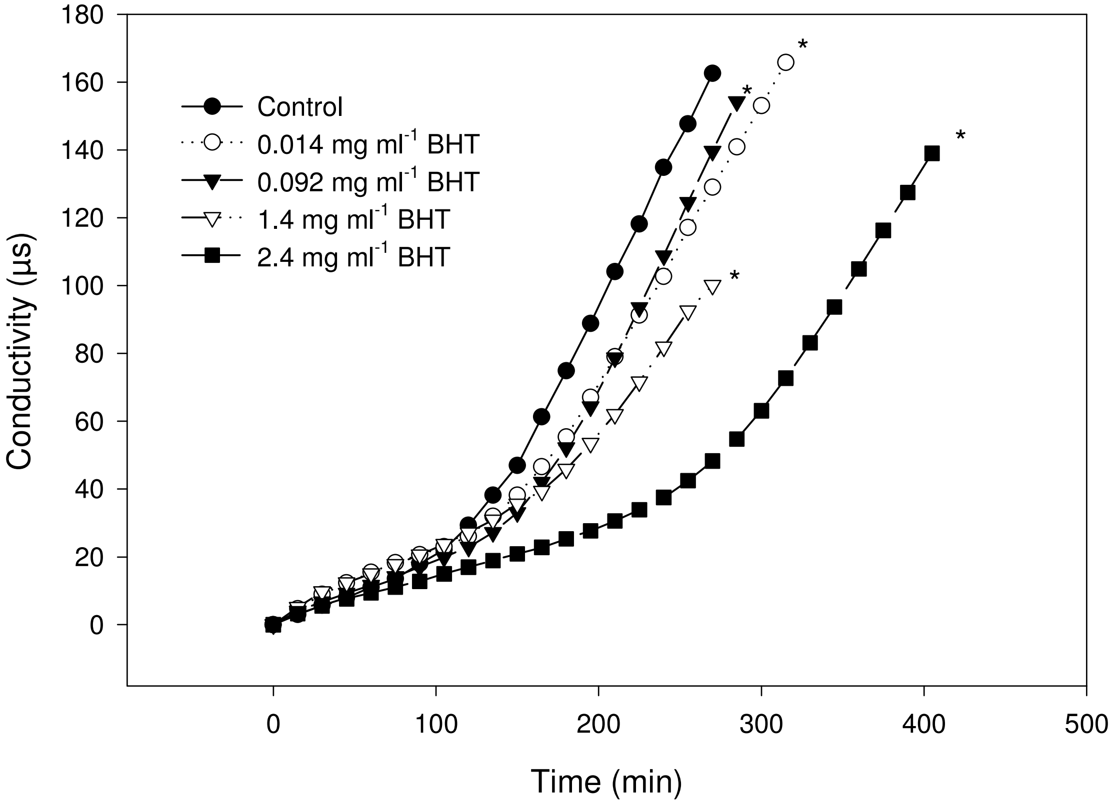


Figure 3.8. Plot of conductivity against time for corn oil at 120°C with BHT as antioxidant (* $P < 0.05$, significantly different from the control).

Table 3.2. Induction times of corn oil at various temperatures with BHT.

Temperature (°C)	Induction Times (min)				
	Control	0.014 BHT	0.092 BHT	1.4 BHT	2.4 BHT
90	246	472	551	731	1141
100	204	428	513	596	950
120	116	138	152	168	253

BHT: Concentration of BHT in mg ml^{-1}.

At 90°C induction time of corn oil without any antioxidant is 246 min. With addition of *Thymbra spicata* essential oils induction time increased to 248 min (1.39 mg ml^{-1}), 282 min (2.33 mg ml^{-1}), 485 min (4.29 mg ml^{-1}) and 519 min (5.49 mg ml^{-1}). There was up to 2.11 fold increase in induction time of corn oil at 90°C depending on the concentration of the *Thymbra spicata*. It can be easily seen from the Tables (3.1 and 3.2) that the effect of BHT is higher than *Thymbra spicata*. At 90°C addition of BHT as antioxidant increased the induction time of corn oil to 472 min (0.014 mg ml^{-1}), 551 min (0.092 mg ml^{-1}), 731 min (1.4 mg ml^{-1}) and 1141 min (2.4 mg ml^{-1}). The increase caused by BHT is up to 4.64 times depending on concentration of BHT added. The highest concentration of BHT added in the study caused 2.2 times more increase in the induction time of corn oil compared to the highest concentration of *Thymbra spicata* added in the study.

At 100^{o}C induction time of corn oil without any antioxidant is 204 min. With addition of *Thymbra spicata* essential oils induction time increased to 236 min (1.39 mg ml^{-1}), 267 min (2.33 mg ml^{-1}), 288 min (4.29 mg ml^{-1}), 353 min (5.49 mg ml^{-1}). There was up to 1.73 fold increase in induction time of corn oil at 100^{o}C depending on the concentration (1.39-5.49 mg ml^{-1}) of the *Thymbra spicata*. It can be easily seen from the Tables (3.1 and 3.2) that the effect of BHT is higher than *Thymbra spicata*. At 100^{o}C addition of BHT as antioxidant increased the induction time of corn oil to 428 min (0.014 mg ml^{-1}), 513 min (0.092 mg ml^{-1}), 596 min (1.4 mg ml^{-1}) and 950 min (2.4 mg ml^{-1}). The increase caused by BHT is up to 4.65 times depending on concentration (0.014-2.4 mg ml^{-1}) of BHT added. The highest concentration of BHT added in the study caused 2.69 times more increase in the induction time of corn oil compared to the highest concentration of *Thymbra spicata* added in the study.

At 120^{o}C induction time of corn oil without any antioxidant is 116 min. With addition of *Thymbra spicata* as antioxidant induction time increased to 124 min (1.39 mg ml^{-1}), 128 min (2.33 mg ml^{-1}), 125 min (4.29 mg ml^{-1}), 125 min (5.49 mg ml^{-1}). There was up to 1.1 fold increase in induction time of corn oil at 120^{o}C depending on the concentration of the *Thymbra spicata*. It can be easily seen from the Tables (3.1 and 3.2) that the effect of BHT is higher than *Thymbra spicata*. At 100^{o}C addition of BHT as antioxidant increased the induction time of corn oil to 138 min (0.014 mg ml^{-1}), 152 min (0.092 mg ml^{-1}), 168 min (1.4 mg ml^{-1}) and 253 min (2.4 mg ml^{-1}). The increase caused by BHT is up to 2.18 times depending on concentration of BHT added. The highest concentration of BHT added in the study caused 1.98 times more increase in the induction time of corn oil compared to the highest concentration of *Thymbra spicata* added in the study.

Induction times of corn oil, no antioxidant added, obtained in the study provided information that the increase in the temperature caused decrease in the induction time. Increase in temperature by 10°C (90 to 100°C) caused 17 % decrease in induction time of corn oil while 20°C (100 to 120°C) increase in temperature caused 43 % decrease in induction time of the corn oil. From accelerated tests on many samples of commercial animal and fat shortenings, it has been established that the average rate of oxidation at 110°C is about 2.5 times that at 97.8°C; this corresponds to a doubling interval of 9°C (Mattil, 1964). As the temperature increases, the increase in rate of oxidation with increasing oxygen concentration become less evident, since oxygen becomes less soluble as the temperature is raised (Nawar, 1985).

The oxidation of the corn oil is mainly due to its composition having high amount of linoleic acid up to 62 % and total unsaturated fatty acid composition of up to 88 %. Polyunsaturated components of fats are oxidized much more rapidly than are monounsaturated and saturated components. In the time required to become rancid it is likely that only the polyunsaturated components undergo autoxidation; thus it is the polyunsaturated components that are the focal points in autoxidation of fats (Mattil, 1964). So the induction time of the corn oil mainly was related with the linoleic acid composition and then with the total unsaturated fatty acid composition.

Addition of antioxidant increased the induction time of corn oil almost in all concentrations of added antioxidants and at studied temperatures. Only at 120°C the highest two concentrations of *Thymbra spicata* induction time is very close to the induction time of the lowest concentration of *Thymbra spicata* added. At the highest two concentrations of *Thymbra spicata* induction times are the same. It shows us that at 120°C increase in the concentration of *Thymbra spicata* above a level does not cause any change in induction of corn oil. However, the increase in induction time of corn oil at 120°C was not so much compared to other temperatures. The effect of BHT on induction time of corn oil at 120°C was not same as the *Thymbra spicata*. Increase in the concentration of BHT increased the induction time of corn oil. BHT does not have an optimum concentration, and the stability of fats to which it is added continues to increase with concentration (Lundberg, 1961).

Increase in induction times of corn oil at 90 and 100°C was related with the concentration of antioxidant added. As the concentration of *Thymbra spicata* increased the induction time of corn oil increased according to concentration and BHT did the same. Increase in the induction time of corn with the addition of *Thymbra spicata* with increase in concentration is not so high compared to BHT. The stability of fat to which BHT is added is less at higher levels (Lundberg, 1961). As for the *Thymbra spicata*, induction time increases with relation to increase in concentration.

The increase in the induction time of corn oil with the addition of *Thymbra spicata* was due to its composition having main products carvacrol and thymol which are isomers of each other. Carvacrol content is higher than thymol in composition. Relatively low stabilizing effect of carvacrol and thymol is due to the volatilities of them at high temperatures (Yanishleva *et al*, 1999).

3.3.2. Palm Oil

The conductivity values were plotted against the time and the induction times of palm oil with different concentrations of *Thymbra spicata* and BHT studied at different temperatures were determined according to method described in part 3.2.1. The temperatures studied were 90, 100 and 120°C. At each temperature different concentrations of *Thymbra spicata* (1.39 to 5.49 mg ml^{-1}) and BHT (0.014 to 2.4 mg ml^{-1}) were studied as well as palm oil without the addition of antioxidant (control). Plots of conductivity versus time (min) for palm oil with *Thymbra spicata* and BHT as antioxidant are shown in Figure 3.9 - 3.14. The induction times, determined according to method defined, of palm oil at various temperatures were tabulated in Tables 3.3 and 3.4.

Table 3.3. Induction times of palm oil at various temperatures with *Thymbra spicata*.

Temperature (°C)	Induction Times (min)				
	Control	1.39 TSO	2.33 TSO	4.29 TSO	5.49 TSO
90	611	725	736	974	1126
100	457	518	586	656	805
120	254	279	287	291	296

TSO: Concentration of *Thymbra spicata* mg ml^{-1}.

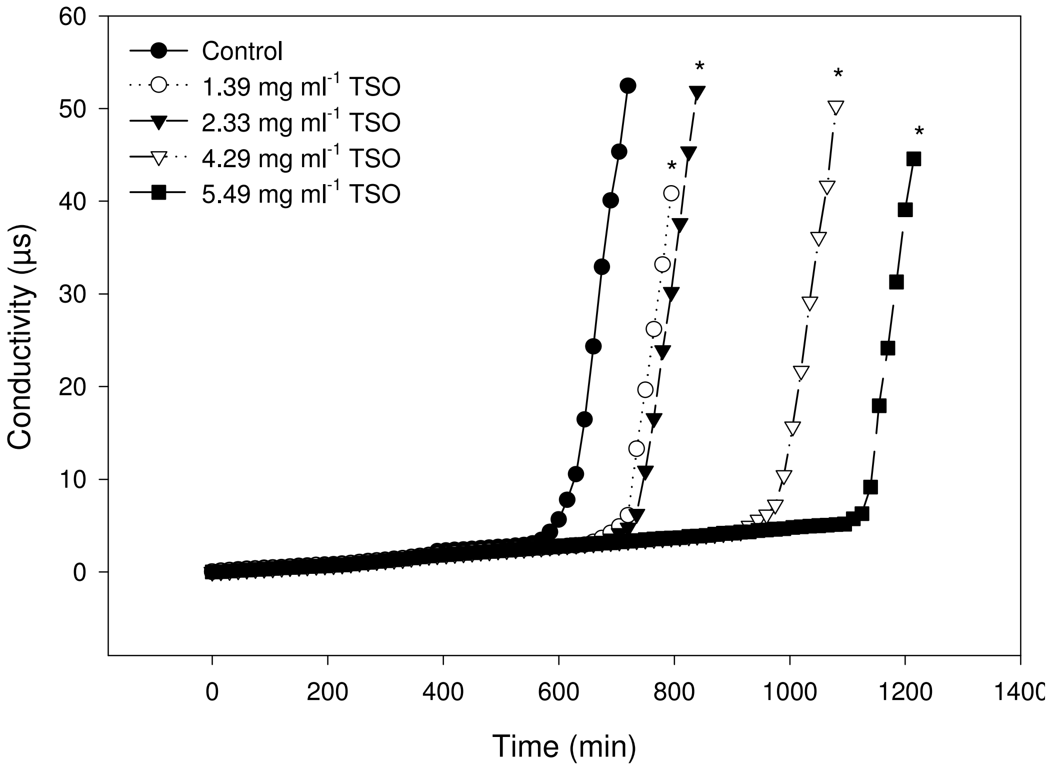


Figure 3.9. Plot of conductivity against time for palm oil at 90°C with *Thymbra spicata* as antioxidant (* $P < 0.05$, significantly different from the control).

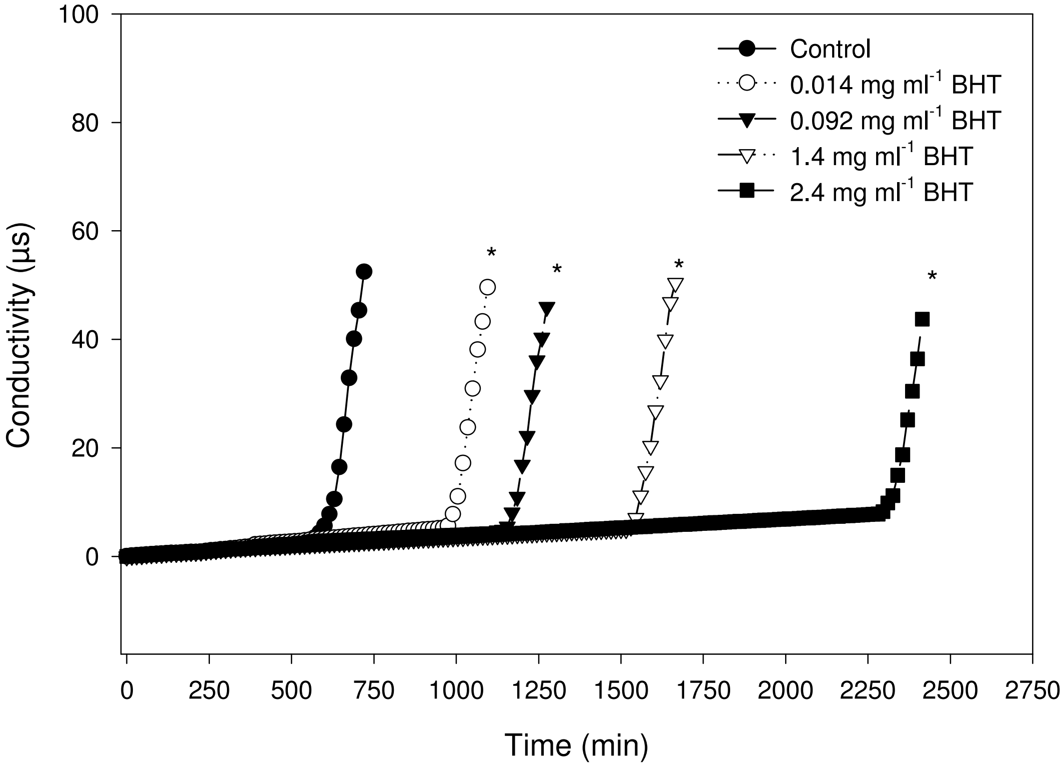


Figure 3.10. Plot of conductivity against time for palm oil at 90°C with BHT as antioxidant (* $P < 0.05$, significantly different from the control).

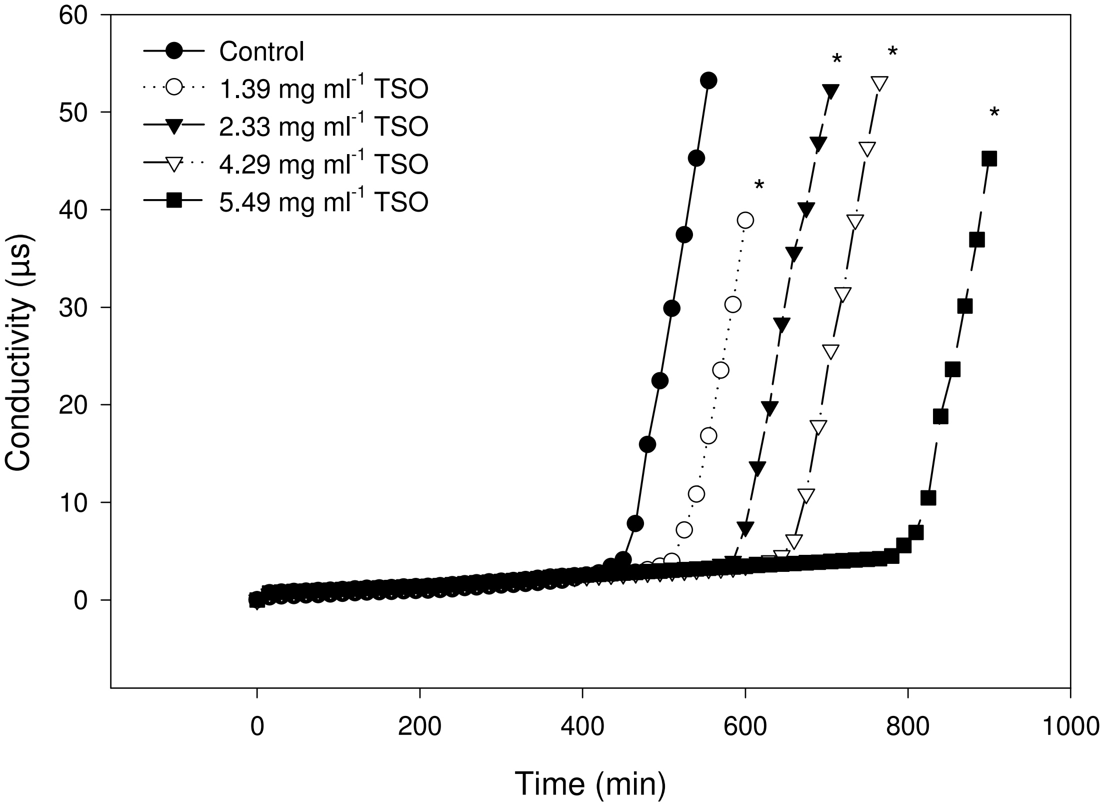


Figure 3.11. Plot of conductivity against time for palm oil at 100°C with *Thymbra spicata* as antioxidant (* $P< 0.05$, significantly different from the control).

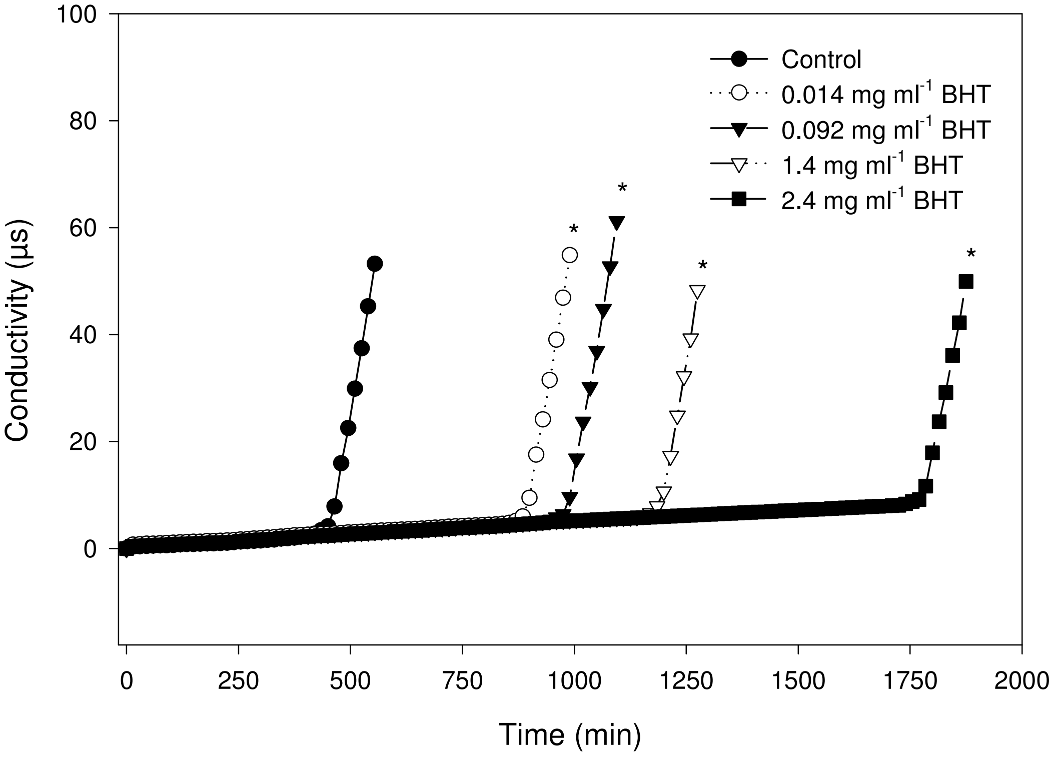


Figure 3.12. Plot of conductivity against time for palm oil at 100°C with BHT as antioxidant (* $P< 0.05$, significantly different from the control).

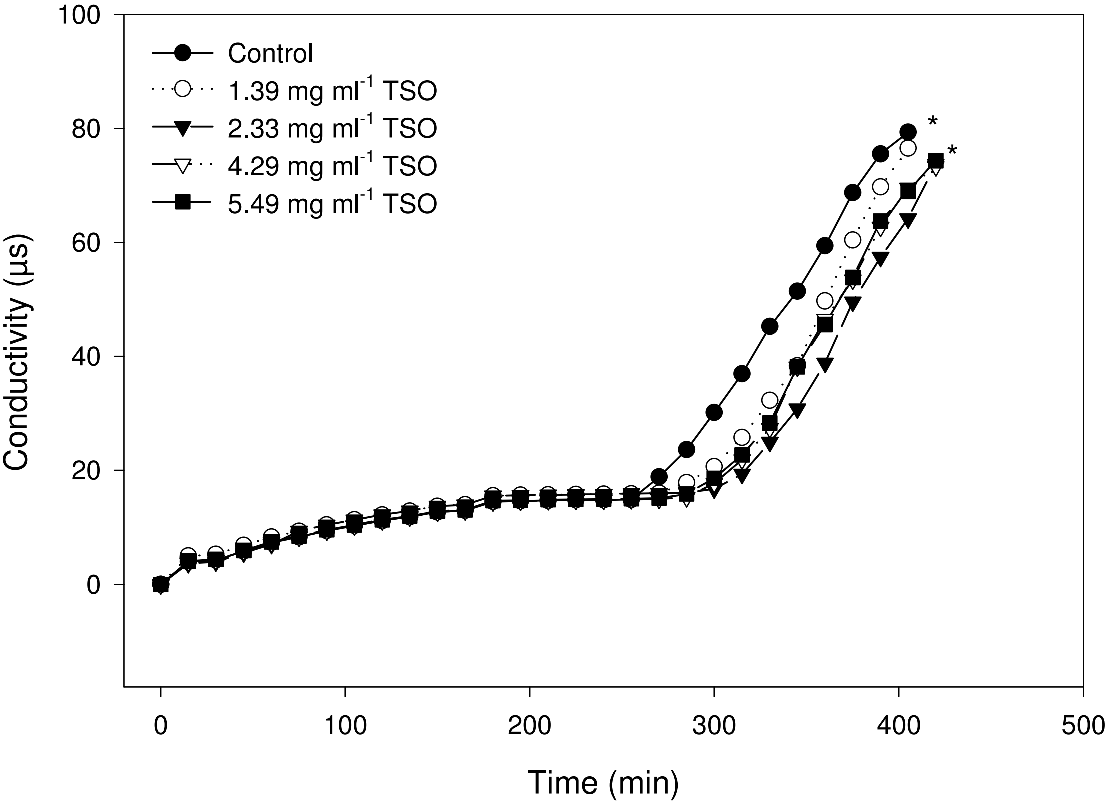


Figure 3.13. Plot of conductivity against time for palm oil at 120°C with *Thymbra spicata* as antioxidant (* $P < 0.05$, significantly different from the control).

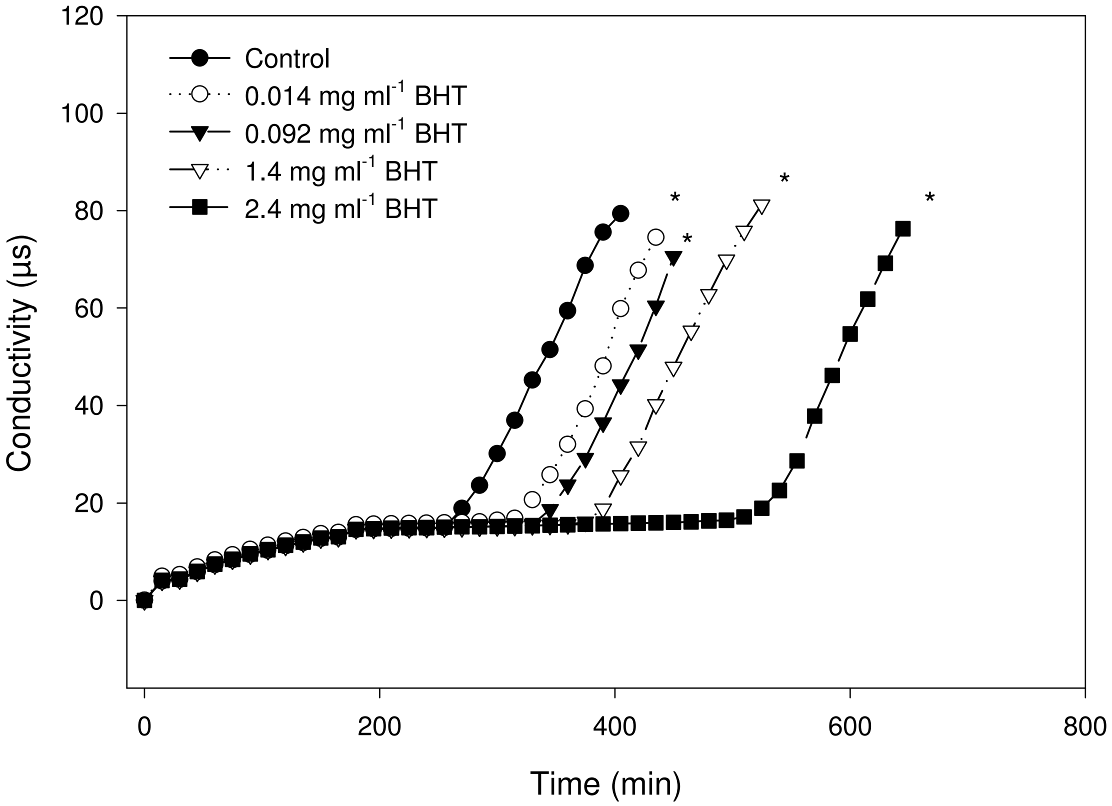


Figure 3.14. Plot of conductivity against time for palm oil at 120°C with BHT as antioxidant (* $P < 0.05$, significantly different from the control).

At 90°C induction time of palm oil without any antioxidant was 611 min. With addition of *Thymbra spicata* essential oils induction time increased to 725 min (1.39 mg ml^{-1}), 736 min (2.33 mg ml^{-1}), 974 min (4.29 mg ml^{-1}) and 1126 min (5.49 mg ml^{-1}). There was up to 1.84 fold increase in induction time of palm oil at 90°C depending on the concentration of the *Thymbra spicata*. At 90°C addition of BHT as antioxidant increased the induction time of palm oil to 984 min (0.014 mg ml^{-1}), 1167 min (0.092 mg ml^{-1}), 1539 min (1.4 mg ml^{-1}) and 2318 min (2.4 mg ml^{-1}). The increase caused by BHT is up to 3.79 times depending on concentration of BHT added. The highest concentration of BHT added in the study caused 2.05 times more increase in the induction time of palm oil compared to the highest concentration of *Thymbra spicata* added in the study. The results obtained for induction times of palm oil showed that BHT is more effective than *Thymbra spicata*.

Table 3.4. Induction times of palm oil at various temperatures with BHT.

Temperature (°C)	Induction Times (min)				
	Control	0.014 BHT	0.092 BHT	1.4 BHT	2.4 BHT
90	611	984	1167	1539	2318
100	457	889	980	1191	1779
120	254	302	339	361	476

BHT: Concentration BHT in mg ml^{-1}.

At 100°C induction time of palm oil without any antioxidant was 457 min. With addition of *Thymbra spicata* as antioxidant induction time increased to 518 min (1.39 mg ml^{-1}), 586 min (2.33 mg ml^{-1}), 656 min (4.29 mg ml^{-1}), 805 min (5.49 mg ml^{-1}). There was up to 1.76 fold increase in induction time of palm oil at 100°C depending on the concentration of the *Thymbra spicata*. At 100°C addition of BHT as antioxidant increased the induction time of palm oil to 889 min (0.014 mg ml^{-1}), 980 min (0.092 mg ml^{-1}), 1191 min (1.4 mg ml^{-1}) and 1779 min (2.4 mg ml^{-1}). The increase caused by BHT is up to 3.89 times depending on concentration of BHT added. The highest concentration of BHT added in the study caused 2.21 times more increase in the induction time of palm oil compared to the highest concentration of *Thymbra spicata* added in the study.

At 120^{o}C induction time of palm oil without any antioxidant was 254 min. With addition of *Thymbra spicata* as antioxidant induction time increased to 279 min (1.39 mg ml^{-1}), 287 min (2.33 mg ml^{-1}), 291 min (4.29 mg ml^{-1}), 296 min (5.49 mg ml^{-1}). There was up to 1.17 fold increase in induction time of palm oil at 120^{o}C depending on the concentration of the *Thymbra spicata*. At 100^{o}C addition of BHT as antioxidant increased the induction time of palm oil to 302 min (0.014 mg ml^{-1}), 339 min (0.092 mg ml^{-1}), 361 min (1.4 mg ml^{-1}) and 476 min (2.4 mg ml^{-1}). The increase caused by BHT is up to 1.58 times depending on concentration of BHT added. The highest concentration of BHT added in the study caused 1.61 times more increase in the induction time of palm oil compared to the highest concentration of *Thymbra spicata* added in the study.

Induction time of palm oil used in the study decreased with increase in temperature. The same conditions exposed to palm oil caused about 2.41 fold decrease in induction time of palm oil at 30^{o}C temperature range. The effect of temperature can be easily explained with the solubility decrease of gases with temperature increase. As the temperature decreases, effect of oxygen air increases.

The effect of composition on oxidation of palm oil was due to the oleic acid of up to 52 %. Palm oil contains up to 61 % total unsaturated fatty acid composition and highest amount is oleic acid which is mono-unsaturated fatty acid. Oleic acid compared to linoleic acid having two double bonds is more resistant to oxidation. Because a single methylene group between double bonds constitutes a very active center for oxidation. The high reactivity of the isolated methylene group in normal 9,12-linoleic acid responsible for the fact that this acid and its esters oxidize approximately fifteen times as rapidly as those of oleic acid, which lack such a group (Mattil, 1964).

Addition of antioxidants *Thymbra spicata* and BHT to palm oil caused increase in induction time. Effect of antioxidants on oxidation of palm oil was not the same in all temperatures. At 120^{o}C effect of *Thymbra spicata* was not so high ($P<0.05$). There was an increase in induction time of up to about 6 % increase with highest concentration used. At highest concentration of BHT added, the increase in induction time of palm oil was 87 %. As the temperature decreased induction times obtained increased related with the temperature. Effect of *Thymbra spicata* was not high compared to BHT, but its effect could not be neglected. At 100°C highest concentration of *Thymbra spicata* (5.49 mg ml^{-1}) was relatively close to the lowest concentration of BHT (0.014 mg ml^{-1}) 805 and 889 min respectively. That effect of *Thymbra spicata* on induction time of palm oil increased with decrease in temperature. At 90°C the induction time at 4.29 mg ml^{-1} concentration of *Thymbra spicata* was very close to the 0.014 mg ml^{-1} concentration of BHT having induction times of 974 and 984 min respectively. The induction time obtained at highest concentration of *Thymbra spicata* was very close to 0.092 mg ml^{-1} concentration of BHT having induction times of 1126 and 1167 min respectively. The results for 120°C was the same as 100°C.

From these results it could be said that as the temperature decreased the effect of *Thymbra spicata* increased. That might be due to volatility of the *Thymbra spicata*.

3.3.3. Effect of *Thymbra spicata* and BHT on corn oil and palm oil under ASLT conditions

Two samples of oils studied for their oxidative stability with addition of antioxidants having different fatty acid composition. One of the oils, corn oil, had high amount of unsaturated fatty acid mainly linoleic acid of up to about 62 %. In addition to linoleic acid corn oil had in its composition oleic acid completing the unsaturated fatty acid composition of corn oil up to 82 – 88 %. The remaining part was saturated fatty acids. The composition of corn oil was mainly constitute of from polyunsaturated and monounsaturated fatty acids. The other oil, palm oil, studied had about 50 % to 50 % saturated and unsaturated fatty acid composition (Mattil, 1964). Unsaturated fatty acid composition of palm oil was mainly due to the oleic acid of about up to 50 %. In addition to oleic acid palm oil had also linoleic acid of up to about 11 %. The remaining part of the palm oil composition was consisted of saturated fatty acids.

Oxidation of fats and oils is due to the reaction of oxygen with fatty acids. Then, for oxidation reaction fatty acid must be reactive with oxygen. In other words it must have reactive sides for oxygen. So, for the oxidation or stability of any oil firstly fatty acid composition must be considered with environmental factors and any catalyst that can present. Generally it is accepted that the higher the degree of unsaturation of an oil, the more susceptible it is to oxidative deterioration (Yanishleva and Marinova, 2001). The same temperatures and the same concentrations of antioxidants were studied for both oil samples. The results obtained for both oil samples shows that the oxidation characteristic of oils mainly is due to their composition.

Since the air flow rate was constant during whole study we can only consider the effect of temperature and antioxidant added with different compositions. Effect of temperature was higher for corn oil compared to effect of palm oil. Corn oil sample without any antioxidant has induction time at 90^{o}C nearly the same induction time with palm oil without any antioxidant at 120^{o}C, 246 min and 254 min respectively. When the induction times of corn oil and palm oil at the same temperatures were compared it can be easily seen that the induction times of palm oil without any antioxidant were greater two fold than the induction time of corn oil. This was mainly due to the unsaturated fatty acid composition of palm oil having of about 25 % less unsaturated fatty acid than corn oil.

As for the induction times of corn oil and palm oil with addition of *Thymbra spicata*, at 120 ^{o}C effect of temperature on corn oil was very high compared to effect on palm oil. Since *Thymbra spicata* caused about 10 % increase on induction time of corn oil. At 120^{o}C *Thymbra spicata* caused about 16 % increase on induction time of palm oil. Difference between the induction times of corn oil and palm oil was not so high but, increase in concentration of *Thymbra spicata* did not cause increase on induction time of corn oil. Increase in concentration of *Thymbra spicata* increased the induction time of palm oil in all added concentrations but increase in concentration of *Thymbra spicata* increased the induction time of corn oil up to some point (2.33 mg ml^{-1}) and then it showed small decrease on induction time and the induction time got constant. The addition of BHT caused up to 2.18 fold increase on induction time of corn oil at 120^{o}C while the increase in induction time of palm oil was up to 1.8 fold. The increase in concentration of BHT caused increase in all concentrations. Effect of BHT was higher than the effect of *Thymbra spicata* on both corn oil and palm oil but, the effect of BHT on corn oil was higher than the effect of BHT on palm oil. At 120^{o}C 2.33 mg ml^{-1} *Thymbra spicata* corresponds to 0.013 mg ml^{-1} of BHT (found by interpolation).

At 100°C effect of *Thymbra spicata* on corn oil was not so high compared to BHT. Since at 100°C *Thymbra spicata* increased induction time of corn oil up to 1.73 times while, BHT increased up to 4.56 times the induction time. *Thymbra spicata* showed highest increases in induction time at higher concentrations while BHT showed high increases in all concentrations. At 100°C 5.49 mg ml^{-1} corresponds to 0.012 mg ml^{-1} concentration of BHT. Palm oil showed very high resistance to oxidation at 100°C compared to corn oil. The induction times obtained for palm oil with *Thymbra spicata* was 2.3 times the induction time of corn oil with the same concentration. This rate is 1.9 times the induction time of corn oil with BHT. Increase rate of induction time of palm oil with BHT was so high compared to induction times of palm oil with *Thymbra spicata*. Induction time of palm oil with BHT was 2.21 times the induction time of palm oil with *Thymbra spicata*.

Both corn oil and palm oil showed highest resistance to oxidation at 90°C. Since corn oil showed highest induction times both with *Thymbra spicata* and BHT, 519 and 1141 min respectively. These results are 4.1 times the highest induction time at 120°C for corn oil with *Thymbra spicata* and 4.5 times for that of corn oil with BHT. Palm oil again showed higher resistance to oxidation compared to corn oil, but the increase rates of induction times compared to induction times at 120°C are almost the same as corn oil. Corresponding BHT concentrations for 5.49 mg ml $^{-1}$ concentration of *Thymbra spicata* are nearly the same (around 0.010 – 0.015 mg ml^{-1} BHT).

Results obtained from the accelerated testing of two oil samples showed that the antioxidants used are effective on oxidation of oils. It is well known that the heat, air, light and moisture are the main contributors to autoxidation. In our study the main two factors were studied with addition of antioxidants. It was stated that the corn oil supplemented with 0.20 % sesamol heated at 180°C for 3 h was significantly more stable than the control sample (Ivanov and Davcheva, 2001). Also the addition of 0.02% 2-acetyl-1,8-dihydroxy-3-methyl naphthalene, antioxidant isolated form Rumex japonicus Houtt., to corn oil increased its oxidative stability determined at 60°C 1.8-fold (Nishina *et al*, 1991). These results showed that the effectiveness of antioxidants used in this were significantly effective on corn oil. The sunflower oil examined under 100°C with the addition of 0.02 % BHT had stabilization factor of 1.2 (Yanishleva and Marinova, 1996). Corn oil with 0.014 mg ml^{-1} BHT had stabilization factor of about 2.1. As it can be seen the stabilization factor for corn oil was higher than the stabilization factor of sunflower oil. This is mainly due to difference in the fatty acid composition of sunflower oil and corn oil.

The effectiveness of antioxidant on oxidation of palm oil was also significant compared to results obtained with corn oil. Palm oil containing more saturated fatty acids in it composition compared to corn oil showed higher resistance to oxidation. Conditions for oxidation in this study was hard to compare to normal storage conditions. The results obtained must have been bigger, if mild conditions had been applied. In the study in addition to heat also air was bubbled in the system. Application of air directly to system reduced the induction times obtained. Lard stored at 60°C had induction time of about 8 days (Milos, *et al*, 2000). Addition of some antioxidants such as volatile aglycones to lard at this temperature increased the induction time up to 80 days. Heat being the one of the most important parameters for oxidation of oils itself is not so effective without the other parameters. Gu and Weng (2001) studied the antioxidative effect of *Salvia plebia* R.Br. extracts in lard using oxidative stability instrument at 110°C. Control showed the induction time of about 2.1 hour and by the addition of isolates from the extract showed induction times of up to 4.3 hour. Addition of BHT and *Thymbra spicata* extract increased the induction time of palm oil at 120°C from 4.2 hour to 7.9 and 1.16 hour respectively. As the temperature decreased in our study effectiveness of antioxidants on palm oil at 90 and 100°C increased compared to effectiveness at 120°C. This showed that the antioxidants were also affected by the heat.

3.4. Normal Storage at 15, 25 and 35°C

The peroxide values were plotted against time. Induction times were determined for each graph by the way described in section 3.2.1.

3.4.1. Normal Storage Results of Corn Oil

The peroxide values obtained from the study were plotted against time and the induction times of corn oil with different concentrations of *Thymbra spicata* and BHT studied at different temperatures were determined. At each temperature different concentrations of *Thymbra spicata* (1.39 to 5.49 mg ml^{-1}) and BHT (0.014 to 2.4 mg ml^{-1}) were studied as well as corn oil without the addition of antioxidant (control). Plots of PV versus time (days) for corn oil with *Thymbra spicata* and BHT as antioxidant are shown in Figures 3.15-3.20.

According to the method defined, induction times of all the plots were determined. The induction times of corn oil at various temperatures were tabulated in Tables 3.5 and 3.6.

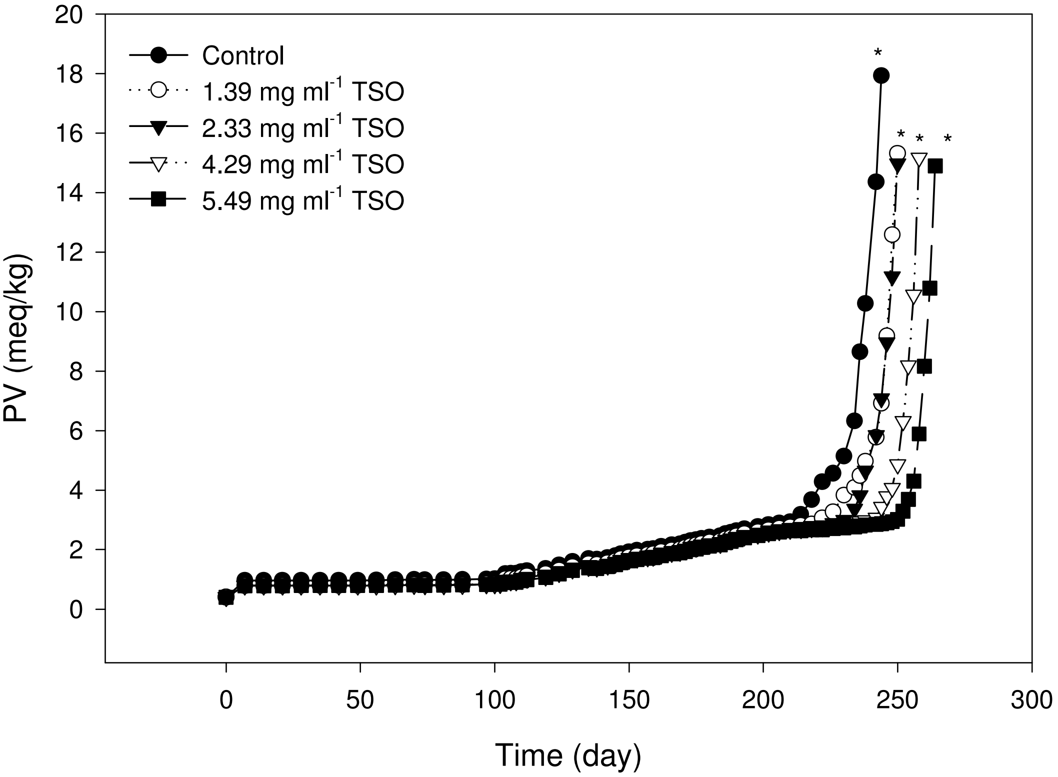


Figure 3.15. Plot of PV against time for corn oil at 15°C with *Thymbra spicata* as antioxidant (* $P < 0.05$, significantly different from the control).

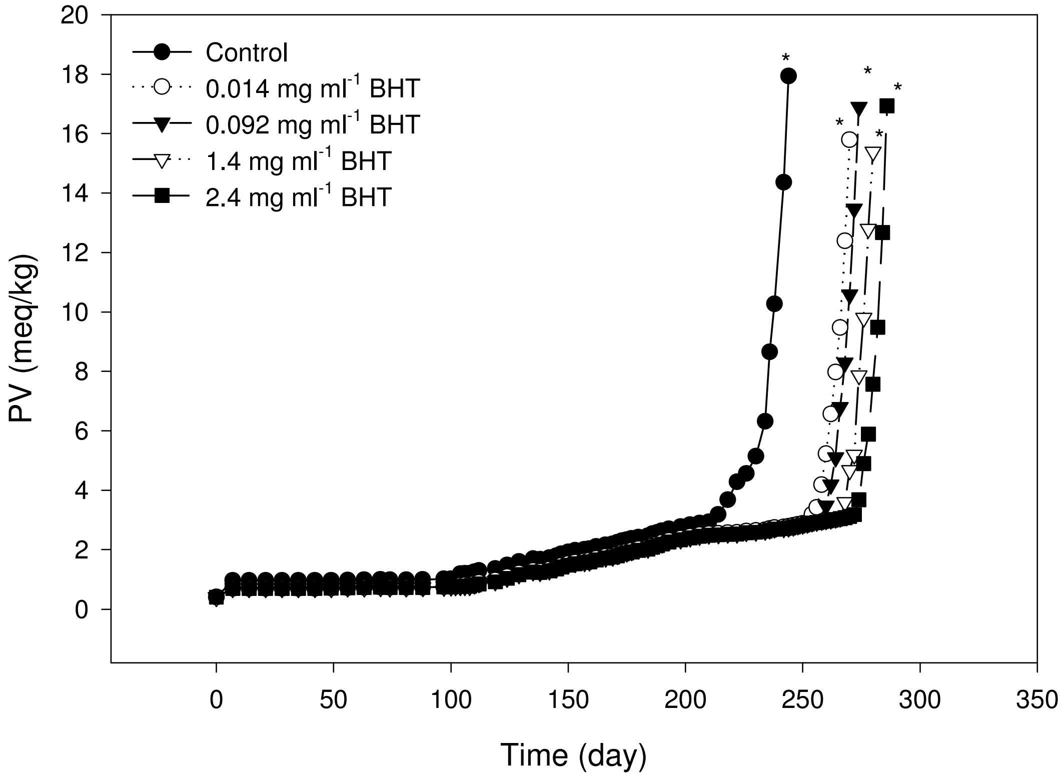


Figure 3.16. Plot of PV against time for corn oil at 15°C with BHT as antioxidant (* $P < 0.05$, significantly different from the control).

Table 3.5. Induction times of corn oil at 15, 25 and 35°C with *Thymbra spicata*.

Temperature (°C)	Induction Times (days)				
	Control	1.39 TSO	2.33 TSO	4.29 TSO	5.49 TSO
15	218	226	234	243	254
25	137	140	147	152	161
35	96	104	107.6	109.4	113

TSO: Concentration of *Thymbra spicata* mg ml^{-1}.

Table 3.6. Induction times of corn oil at 15, 25 and 35°C with BHT.

Temperature (°C)	Induction Times (days)				
	Control	0.014 BHT	0.092 BHT	1.4 BHT	2.4 BHT
15	218	256	262	269	275
25	137	167	171	180	186
35	96	115	120.3	124	129.3

BHT: Concentration BHT in mg ml^{-1}.

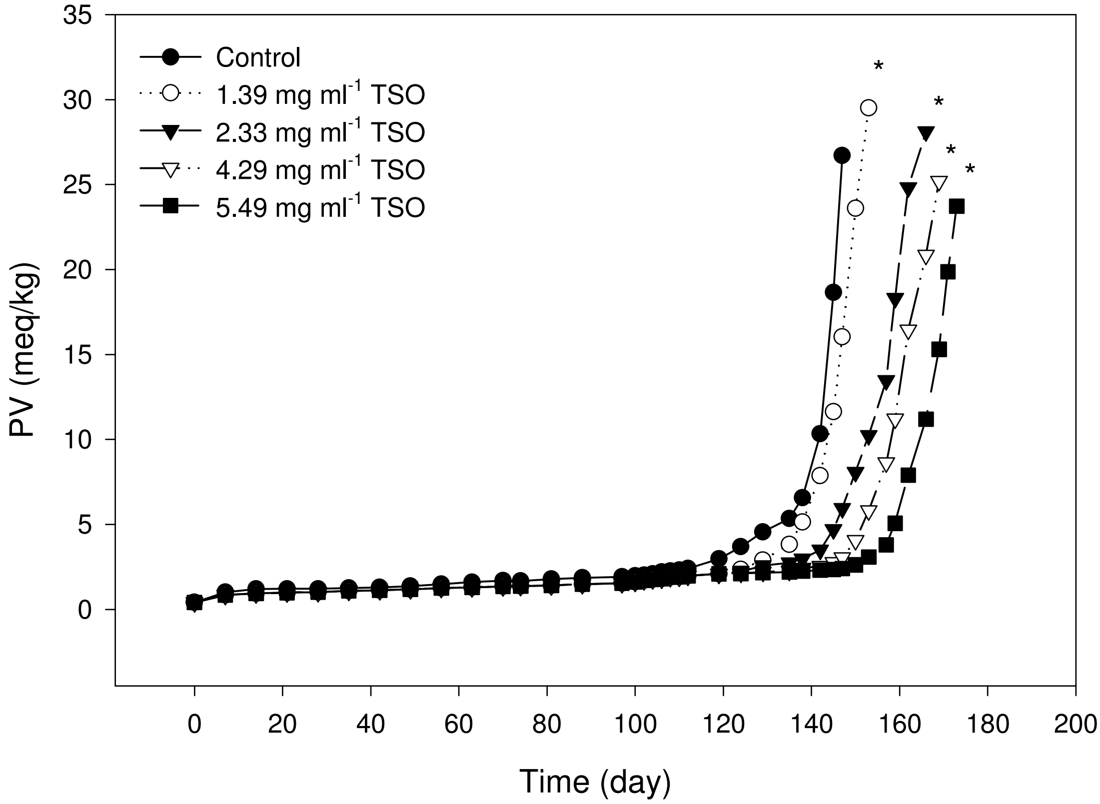


Figure 3.17. Plot of PV against time for corn oil at 25°C with *Thymbra spicata* as antioxidant (* $P< 0.05$, significantly different from the control).

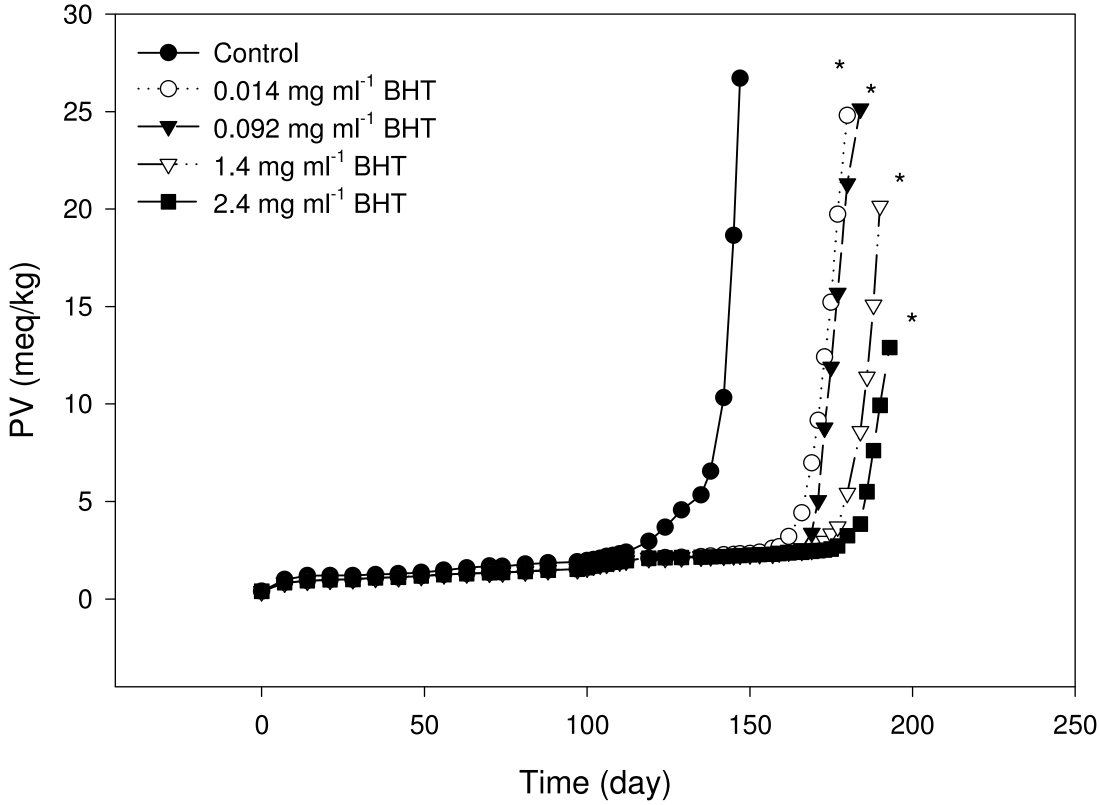


Figure 3.18. Plot of PV against time for corn oil at 25°C with BHT as antioxidant (* $P< 0.05$, significantly different from the control).

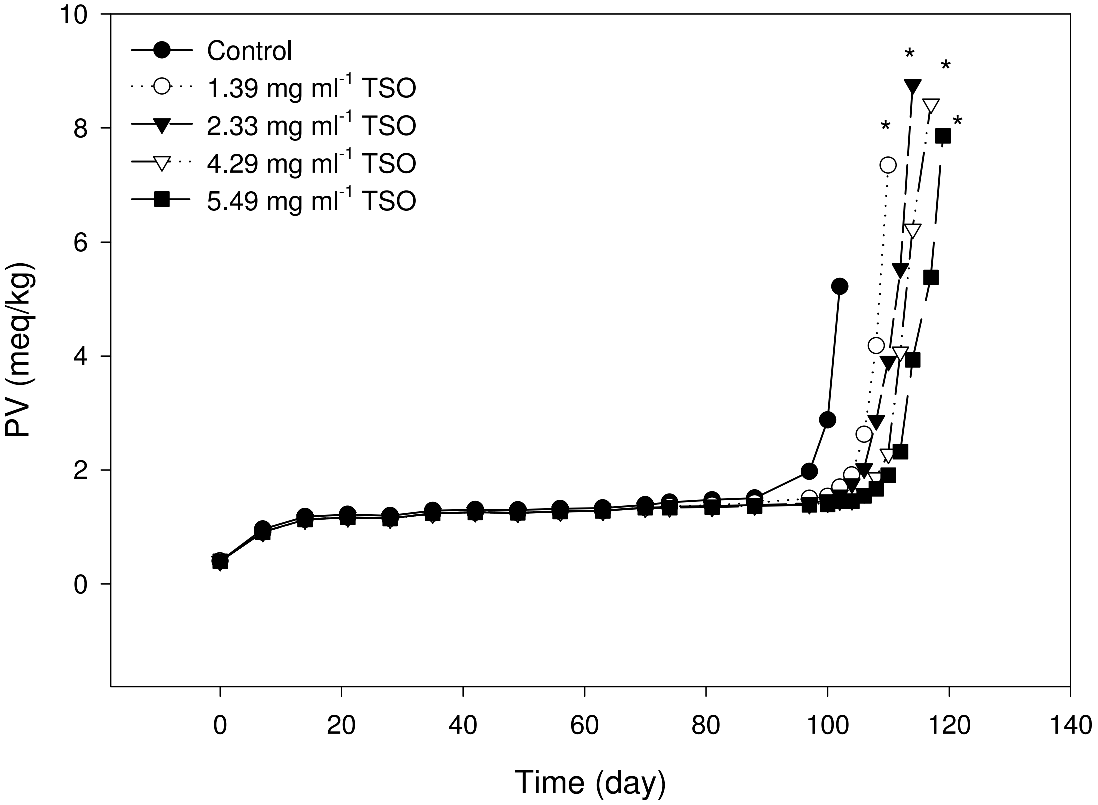


Figure 3.19. Plot of PV against time for corn oil at 35°C with *Thymbra spicata* as antioxidant (* $P < 0.05$, significantly different from the control).

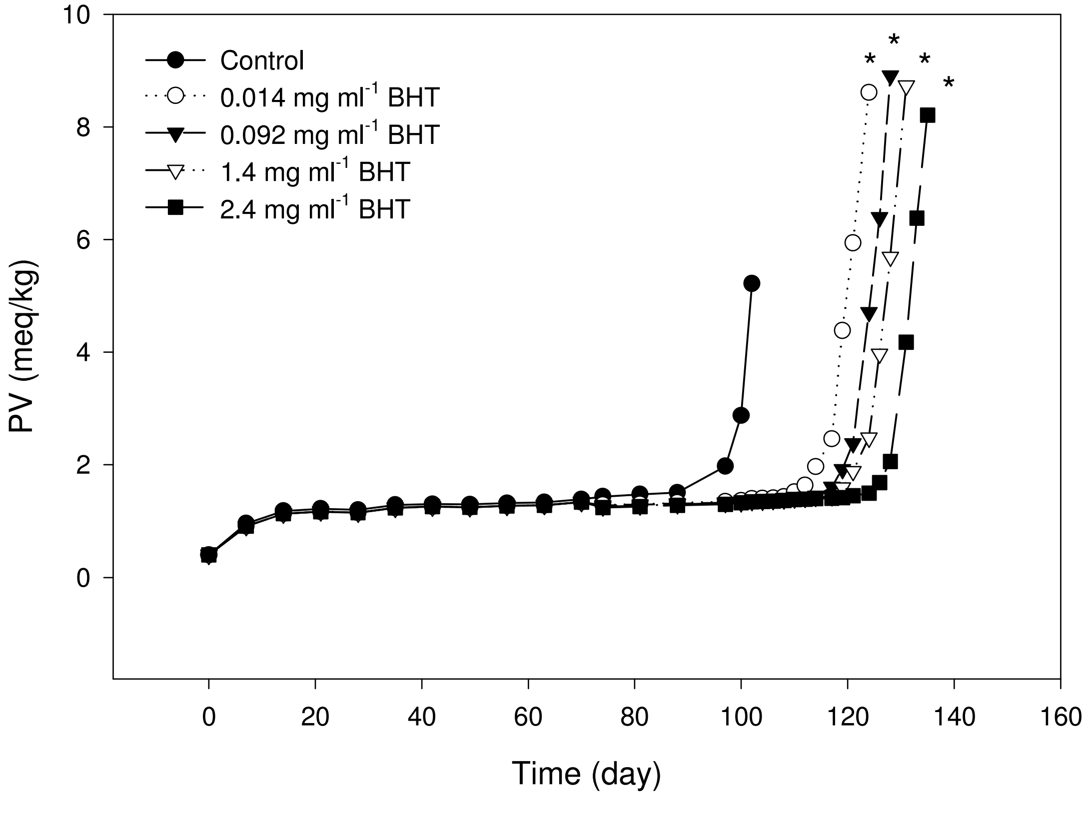


Figure 3.20. Plot of PV against time for corn oil at 35°C with BHT as antioxidant (* $P < 0.05$, significantly different from the control).

At 15^{o}C the induction time of control was 218 days. With the addition of *Thymbra spicata* as antioxidant, induction time increased to 226 days (1.39 mg ml^{-1}), 234 days (2.33 mg ml^{-1}), 243 days (4.29 mg ml^{-1}) and 254 days (5.49 mg ml^{-1}). There was up to 1.17 fold increase in induction time of corn oil at 15^{o}C depending on concentration of the *Thymbra spicata*. The addition of BHT caused more increase in induction time of corn oil compared to *Thymbra spicata*. At 15^{o}C addition of BHT as antioxidant increased the induction time of corn oil to 256 days (0.014 mg ml^{-1}), 262 days (0.092 mg ml^{-1}), 269 days (1.4 mg ml^{-1}) and 275 days (2.4 mg ml^{-1}). The increase in concentration of BHT caused up to 1.26 fold increase in induction time of corn oil.

At 25^{o}C the induction time of control was 137 days. With the addition of *Thymbra spicata* as antioxidant, induction time increased to 140 days (1.39 mg ml^{-1}), 147 days (2.33 mg ml^{-1}), 152 days (4.29 mg ml^{-1}) and 161 days (5.49 mg ml^{-1}). There was up to 1.17 fold increase in induction time of corn oil at 25^{o}C depending on concentration of the *Thymbra spicata*. The addition of BHT caused more increase in induction time of corn oil compared to *Thymbra spicata*. At 25^{o}C addition of BHT as antioxidant increased the induction time of corn oil to 167 days (0.014 mg ml^{-1}), 171 days (0.092 mg ml^{-1}), 180 days (1.4 mg ml^{-1}) and 186 days (2.4 mg ml^{-1}). The increase in concentration of BHT caused up to 1.36 fold increase in induction time of corn oil.

At 35^{o}C the induction time of control was 96 days. With the addition of *Thymbra spicata* as antioxidant, induction time increased to 104 days (1.39 mg ml^{-1}), 107.6 days (2.33 mg ml^{-1}), 109.4 days (4.29 mg ml^{-1}) and 113 days (5.49 mg ml^{-1}). There was up to 1.18 fold increase in induction time of corn oil at 35^{o}C depending on concentration of the *Thymbra spicata*. The addition of BHT caused more increase in induction time of corn oil compared to *Thymbra spicata*. At 35^{o}C addition of BHT as antioxidant increased the induction time of corn oil to 115 days (0.014 mg ml^{-1}), 120.3 days (0.092 mg ml^{-1}), 124 days (1.4 mg ml^{-1}) and 129.3 days (2.4 mg ml^{-1}). The increase in concentration of BHT caused up to 1.35 fold increase in induction time of corn oil.

3.4.2.Normal Storage Results of Palm Oil

The peroxide values obtained from the study were plotted against time and the induction times of corn oil with different concentrations of *Thymbra spicata* and BHT studied at different temperatures were determined. At each temperature different concentrations of *Thymbra spicata* (1.39 to 5.49 mg ml^{-1}) and BHT (0.014 to 2.4 mg ml^{-1}) were studied as well as corn oil without the addition of antioxidant (control). Plots of PV versus time (days) for palm oil with *Thymbra spicata* and BHT as antioxidant are shown in Figures 3.21-3.26.

According to the method defined, induction times of all the plots were determined. The induction times of corn oil at various temperatures were tabulated in Tables 3.7 and 3.8.

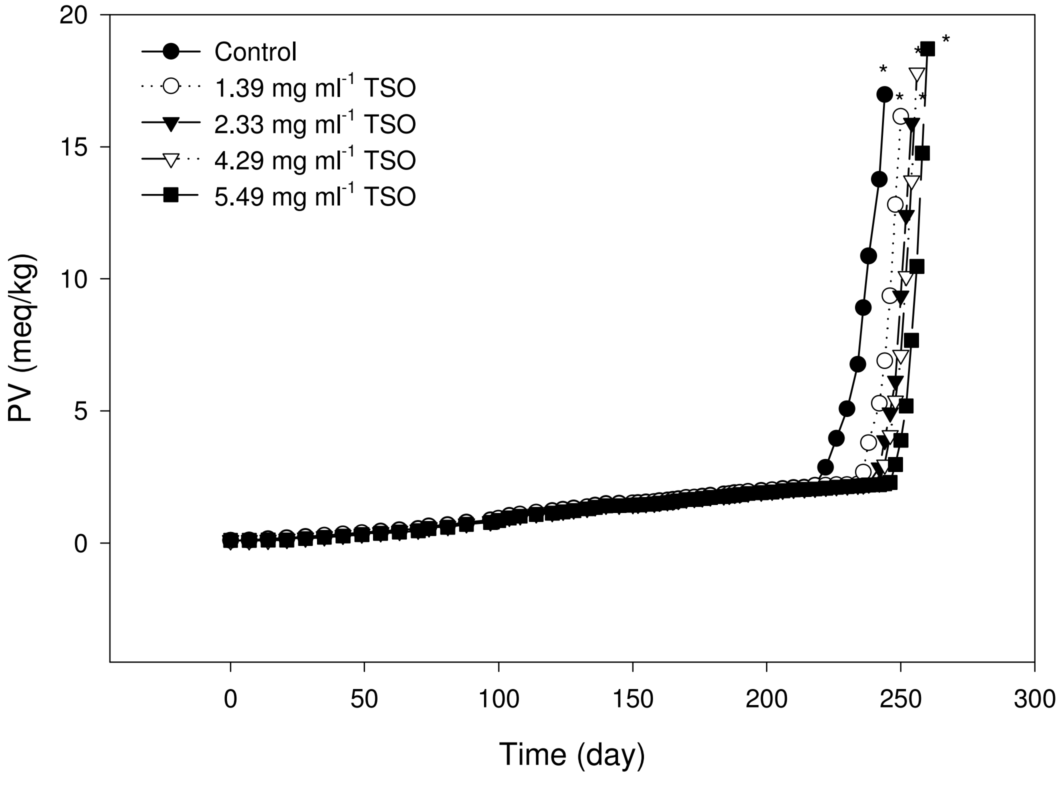


Figure 3.21. Plot of PV against time for palm oil at 15°C with *Thymbra spicata* as antioxidant (* P< 0.05, significantly different from the control).

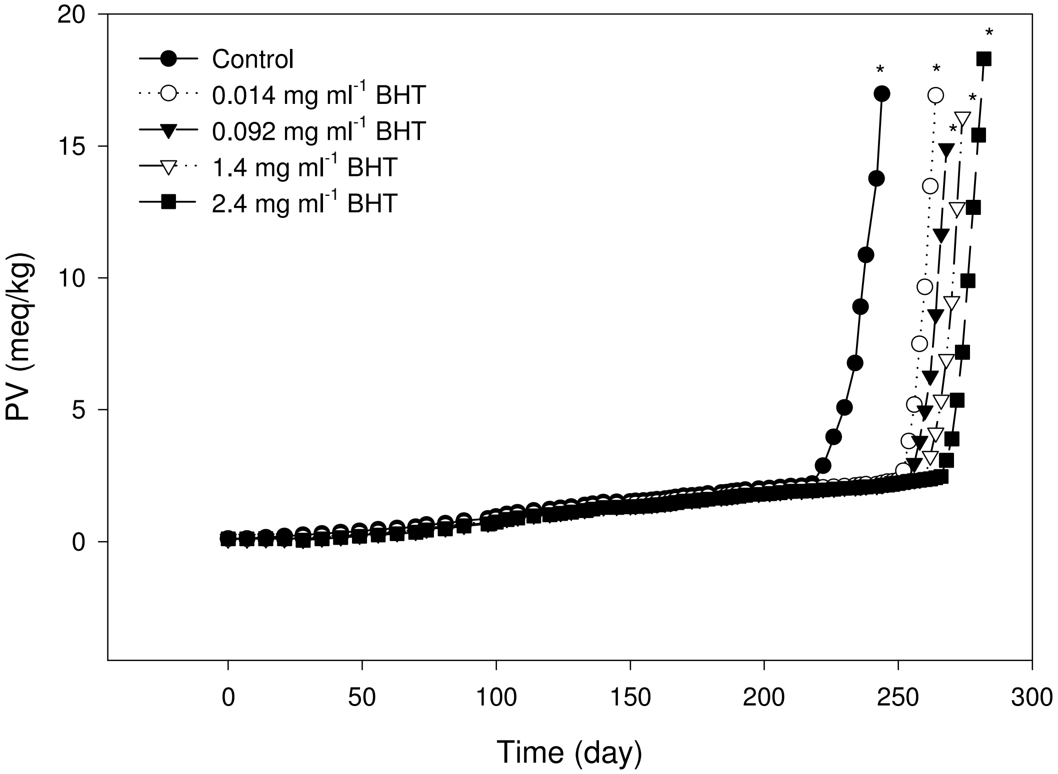


Figure 3.22. Plot of PV against time for palm oil at 15^{o}C with BHT as antioxidant (* $P< 0.05$, significantly different from the control).

Table 3.7. Induction times of palm oil at 15, 25 and 35^{o}C with *Thymbra spicata*

Temperature (^{o}C)	Induction Times (days)				
	Control	1.39 TSO	2.33 TSO	4.29 TSO	5.49 TSO
15	224	239	244	246	250
25	147	149	153.5	156	161
35	106	111	116	120	124

TSO: Concentration of *Thymbra spicata* mg ml^{-1}.

Table 3.8. Induction times of palm oil at 15, 25 and 35^{o}C with BHT.

Temperature (^{o}C)	Induction Times (days)				
	Control	0.014 BHT	0.092 BHT	1.4 BHT	2.4 BHT
15	224	254	258	264	270
25	147	163	169	175	183
35	106	126	130.5	135	143

BHT: Concentration BHT in mg ml^{-1}.

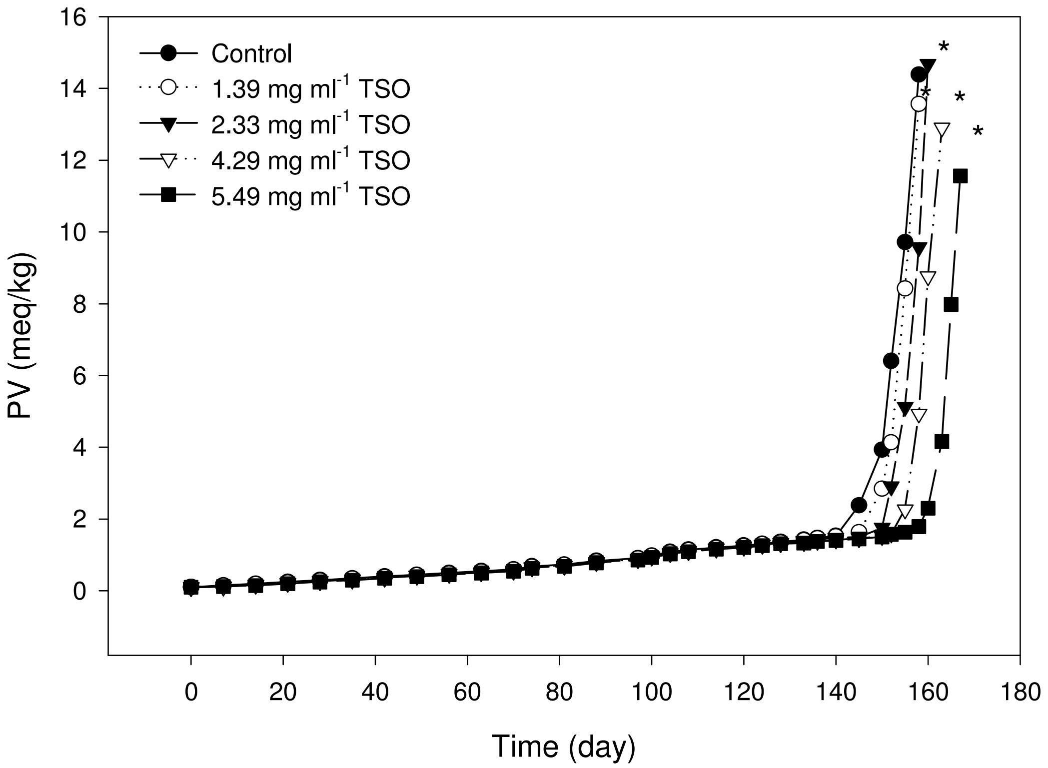


Figure 3.23. Plot of PV against time for palm oil at 25°C with *Thymbra spicata* as antioxidant (* $P< 0.05$, significantly different from the control).

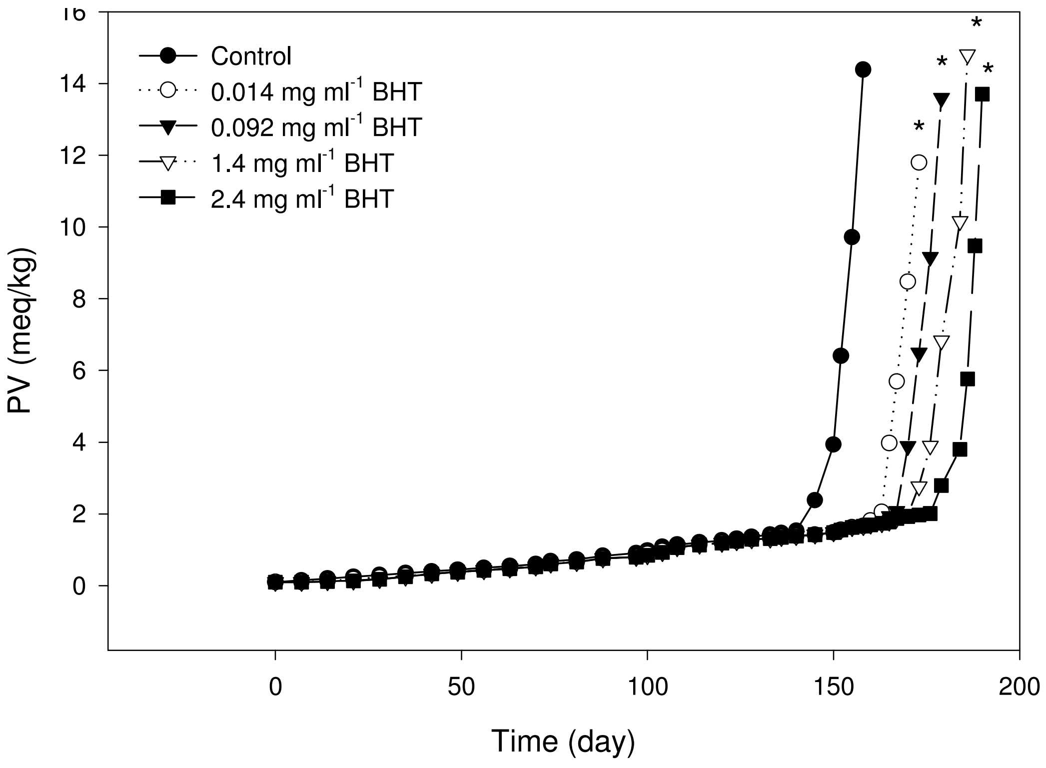


Figure 3.24. Plot of PV against time for palm oil at 25°C with BHT as antioxidant (* $P< 0.05$, significantly different from the control).

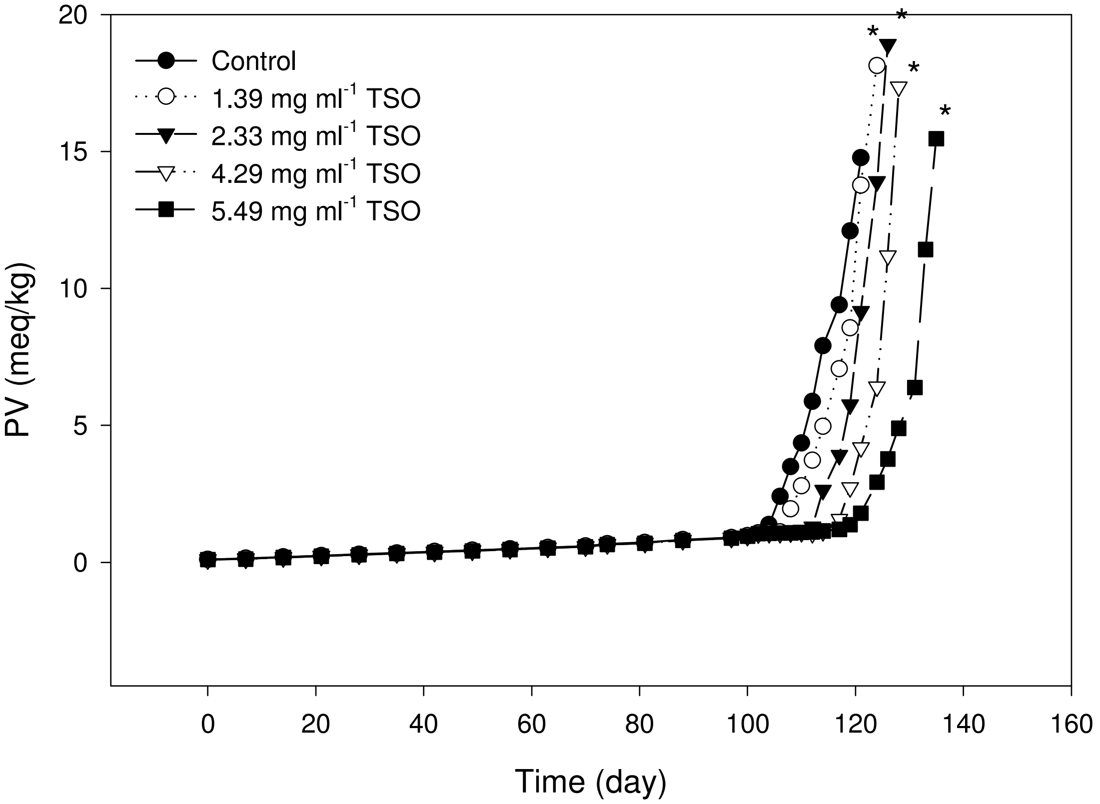


Figure 3.25. Plot of PV against time for palm oil at 35°C with *Thymbra spicata* as antioxidant (* $P < 0.05$, significantly different from the control).

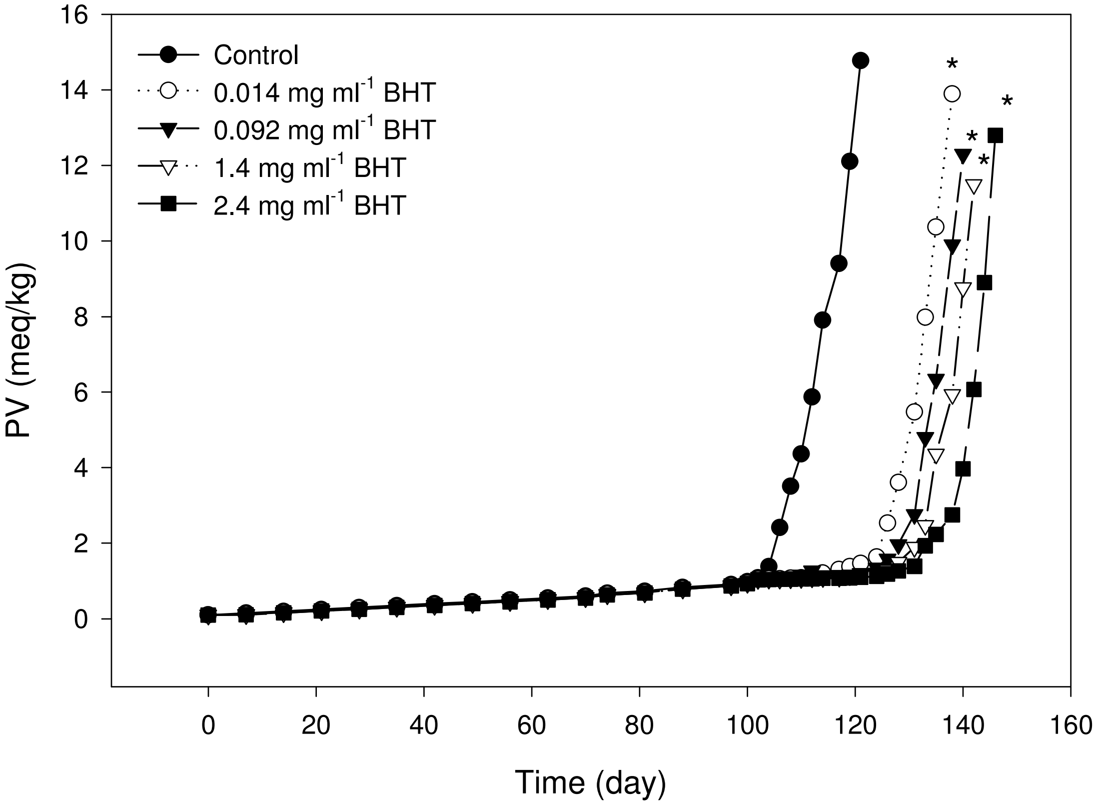


Figure 3.26. Plot of PV against time for palm oil at 35°C with BHT as antioxidant (* $P < 0.05$, significantly different from the control).

At 15°C the induction time of control palm oil was 224 days. With the addition of *Thymbra spicata* as antioxidant, induction time increased to 239 days (1.39 mg ml^{-1}), 244 days (2.33 mg ml^{-1}), 246 days (4.29 mg ml^{-1}) and 250 days (5.49 mg ml^{-1}). There was up to 1.12 fold increase in induction time of palm oil at 15°C depending on concentration of the *Thymbra spicata*. The addition of BHT caused more increase in induction time of palm oil compared to *Thymbra spicata*. At 15°C addition of BHT as antioxidant increased the induction time of palm oil to 254 days (0.014 mg ml^{-1}), 258 days (0.092 mg ml^{-1}), 264 days (1.4 mg ml^{-1}) and 270 days (2.4 mg ml^{-1}). The increase in concentration of BHT caused up to 1.21 fold increase in induction time of palm oil.

At 25°C the induction time of control palm oil was 147 days. With the addition of *Thymbra spicata* as antioxidant, induction time increased to 149 days (1.39 mg ml^{-1}), 153.5 days (2.33 mg ml^{-1}), 156 days (4.29 mg ml^{-1}) and 161 days (5.49 mg ml^{-1}). There was up to 1.1 fold increase in induction time of palm oil at 25°C depending on concentration of the *Thymbra spicata*. The addition of BHT caused more increase in induction time of palm oil compared to *Thymbra spicata*. At 25°C addition of BHT as antioxidant increased the induction time of palm oil to 163 days (0.014 mg ml^{-1}), 169 days (0.092 mg ml^{-1}), 175 days (1.4 mg ml^{-1}) and 183 days (2.4 mg ml^{-1}). The increase in concentration of BHT caused up to 1.24 fold increase in induction time of palm oil.

At 35°C the induction time of control palm oil was 106 days. With the addition of *Thymbra spicata* as antioxidant, induction time increased to 111 days (1.39 mg ml^{-1}), 116 days (2.33 mg ml^{-1}), 120 days (4.29 mg ml^{-1}) and 124 days (5.49 mg ml^{-1}). There was up to 1.17 fold increase in induction time of palm oil at 35°C depending on concentration of the *Thymbra spicata*. The addition of BHT caused more increase in induction time of palm oil compared to *Thymbra spicata*. At 35°C addition of BHT as antioxidant increased the induction time of palm oil to 126 days (0.014 mg ml^{-1}), 130.5 days (0.092 mg ml^{-1}), 135 days (1.4 mg ml^{-1}) and 143 days (2.4 mg ml^{-1}). The increase in concentration of BHT caused up to 1.35 fold increase in induction time of palm oil.

3.4.3. Effect of *Thymbra spicata* and BHT on Corn Oil and Palm Oil under Normal Storage Conditions

In order to discuss the storage stability of corn oil and palm oil, firstly it will be useful to mention about the composition and the physical properties of them. Compositional properties of both oils are present in Table 1.1. As a physical property, the important parameter affecting the stability of the oil is melting point. Corn oil under normal conditions (15°C and 25°C) is in liquid form (having melting point of about - 2°C). Palm oil having melting point of about 38°C under normal conditions is in solid form. So palm oil at15°C and 25°C is completely solid and at 35°C it is expected to be completely solid. At 35°C palm oil was not completely solid, but soft and having a little amount of liquid form on the top.

The storage stability of corn oil was shorter than the stability of palm oil. This was mainly due to the compositional difference between the corn oil and palm oil. The other interesting parameter affecting the oxidation is whether the lipid is in the solid or liquid state. Several studies have shown that the rate of oxidation of a lipid is much higher than expected when in the solid phase (Labuza, 1971). This is because in the solid phase the ROO· radical, diffuse slowly and reacts more readily within the substrate continuing the propagation, whereas the radical in the liquid fat can terminate more readily with other radicals (Nawar, 1985).

In the present study inductions times of palm oil were not so higher than the induction times of corn oil. The main problem was the solid state of the palm oil. The top layer was directly exposed to air present on the head space.

At all temperatures induction times obtained for corn oil were lower than that of palm oil. This is the expected result. Corn oil having higher amount of unsaturated fatty acid composition will naturally oxidize more readily than palm oil. Basaga *et al*, 1997, stated that the rosemary extract and BHT significantly reduced the formation of peroxides in corn oil stored at 30°C. Although the induction times that can be calculated were going to be very close to each other peroxide values were lower than the control. At 60°C, induction time obtained for lard was about 10 days. The addition of essential oil from *Origanum vulgare* L. ssp. *hirtum* increased the induction time of lard to about 85 days. In the same study the addition of thymol increased the induction time of the lard to about 15 days (Milos *et al*, 2000).

CONCLUSIONS

The oxidative stability of corn oil and palm oil was studied as a function of temperature and antioxidant. Mainly the effect of antioxidants *Thymbra spicata* and BHT was studied. It was concluded that palm oil is more stable than corn oil and BHT is more effective against oxidation of oil than *Thymbra spicata*.

The study of oxidative stability of corn oil and palm oil with addition of *Thymbra spicata* and BHT revealed the following facts:

1. DPPH free radical-scavenging test showed that the Thymbra spicata extract is effective antioxidant compared to BHT. Scavenging effect of Thymbra spicata and BHT is 75.65 % and 73.7 %, respectively.

2. The induction time obtained for control corn oil from the ASLT decreased 2.12 fold when the temperature increased from 90 to 120°C. This decrease was 2.41 fold for control palm oil. This because of the compositional changes of oil samples studied.

3. Addition of *Thymbra spicata* as antioxidant at 90, 100 and 120°C increased the induction time of corn oil up to 2.11 fold, 1.73 fold and 1.1 fold respectively compared to control corn oil. Addition of BHT as antioxidant into corn oil sample increased the induction time up to 4.64 fold, 4.66 and 2.18 fold respectively at the same temperatures. This shows the effectiveness of the BHT compared to *Thymbra spicata*. Although the results obtained for BHT are higher than the *Thymbra spicata*, the effect of *Thymbra spicata* on corn oil cannot be neglected. *Thymbra spicata* is also effective on the stability of corn oil.

4. Addition of *Thymbra spicata* as antioxidant at 90, 100 and 120°C increased the induction time of palm oil up to 1.84 fold, 1.76 fold and 1.16 fold respectively compared to control palm oil. Addition of BHT as antioxidant into palm oil sample increased the induction time up to 3.79 fold, 3.89 and 1.87 fold respectively at the same temperatures.

5. At normal storage conditions both *Thymbra spicata* and BHT are effective on oxidative stability of corn oil and palm oil. Effectiveness of antioxidants on palm oil was not as the expected one. Since palm oil is in solid form at the studied temperatures (15-35°C).

6. It is expected to calculate the shelf life of the corn oil and palm oil from ASLT results and compare them with the results obtained from the normal storage conditions. In ASLT two accelerating parameters (temperature and air) were used while in normal storage conditions only one parameter is used (temperature). Calculation of results from ASLT results are not in correlation with results obtained from normal storage conditions. For the comparison of these results during the normal storage air must have been supplied to samples at same rate used in ASLT.

7. *Thymbra spicata* as a natural antioxidant is not effective as BHT, but nowadays people prefer mainly natural food products. It can be used as an antioxidant, but its natural flavor will be the main problem in its use.

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