Repressions of the Toxic α, β-Hydroxyaldehyde Homologues, HHE, HOE, HNE and HDE Formations by Synthetic and Natural Antioxidants in Commercial Corn Oil at Frying Temperature

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Dr. A. Saari. Csallany

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Abstract

Commercial corn oil, which contains high level of linoleic acid (~60%) is widely available and is often used for frying food products.

The α, β-unsaturated-4-hydroxyaldehydes, the toxic degradation products of lipid peroxidation is forming due to heat treatment at frying temperature in high PUFA vegetable oils containing high level of linoleic acid. The objective of present experiment was to find the suppressing effects of α, β-unsaturated-4-hydroxyaldehydes in the presence of synthetic and natural antioxidants.

In the present experiments corn oil was heated for 0, 1, 2, 4, and 6 hours at 185°C in the presence and absence of synthetic and natural antioxidants to measure the formation of the four α, β-unsaturated-4-hydroxyaldehydes, especially the 4-hydroxy-2-trans-nonenal (HNE) which is known to be the most toxic product of the unsaturated fatty acids peroxidation process. In addition to HNE, 4-hydroxy-2-trans-hexenal (HHE), 4-hydroxy-2-trans-octenal (HOE) and 4-hydroxy-2-trans-decenal (HDE) were also existed in commercial heat treated corn oil at 185°C. The formation of HHE, HOE, HNE and HDE increased with the length of heating time.

The combinations of 200ppm TBHQ+50ppm citric acid (CA)+100ppm ascorbyl palmitate (AP) and 50ppm or 100ppm rosemary extracts (RE) were selected to study the repressing effects on the oxidation retarding the α, β-unsaturated-4-hydroxyaldehydes formation in heat treated corn oil at 185°C.

It was found that the oxidation process and the formation of HHE, HOE, HNE and HDE in corn oil were suppressed by the addition of synthetic antioxidants and also by a natural antioxidant at frying temperature of 185°C. 200ppm TBHQ+50ppm CA+100ppm AP suppressed the oxidation of corn oil at 185°C after 6 hours by 27% for HNE, 88% for HHE, 70% for HOE and 96% for HDE formation. 50ppm or 100ppm Rosemary extracts (RE) suppressed 29% HNE, 40% HHE, 48% HOE and 74% HDE formation in corn oil.

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Part I: Literature Review

Introduction

This literature review is divided into five sections. In the first section, the basic knowledge about corn oil and reactions in deep-frying conditions will be discussed. The second section introduces the mechanism of lipid oxidation and basic chemical reactions. The third section will discuss the degradation of secondary oxidation products. The fourth section will focus on the α, β-unsaturated aldehydes, the toxic secondary oxidation products of unsaturated fatty acid oxidation — including the major toxic compound HNE. The final section investigates the repression of oxidation by synthetic and natural antioxidants in thermally oxidized fats and oils.

Part II contains a series of experiments which were conducted by using commercial corn oil. The average fatty acid composition of corn oil from three different sources is presented in Table 1 [1] and the structures of the primary fatty acids in corn oil are shown in Figure 1 [2].

Corn oil is rich in polyunsaturated fatty acids, especially high ω-6 linoleic acid, and the content is about 60%. Since corn oil is almost tasteless and it can withstand high temperatures without smoking, corn oil is widely used as frying oil in the food industry. Some of these polyunsaturated fatty acids may play a role in the prevention and possible treatment of arthritis, hypertension, coronary heart disease and other inflammatory disorders [3]. Since linoleic acid is sensitive to be easily oxidized due to the presence of two double bonds in its molecular structure and its high concentration in corn oil, corn oil is especially degraded at frying temperature (185°C).

Table 1. Fatty acid composition (%) of corn oil (From Ref. (1))

C16:0	Palmitic acid	10.47
C18:0	Stearic acid	2.02
C18:1	Oleic acid	24.23
C18:2	Linoleic acid	60.38
C18:3	Linolenic acid	0.99

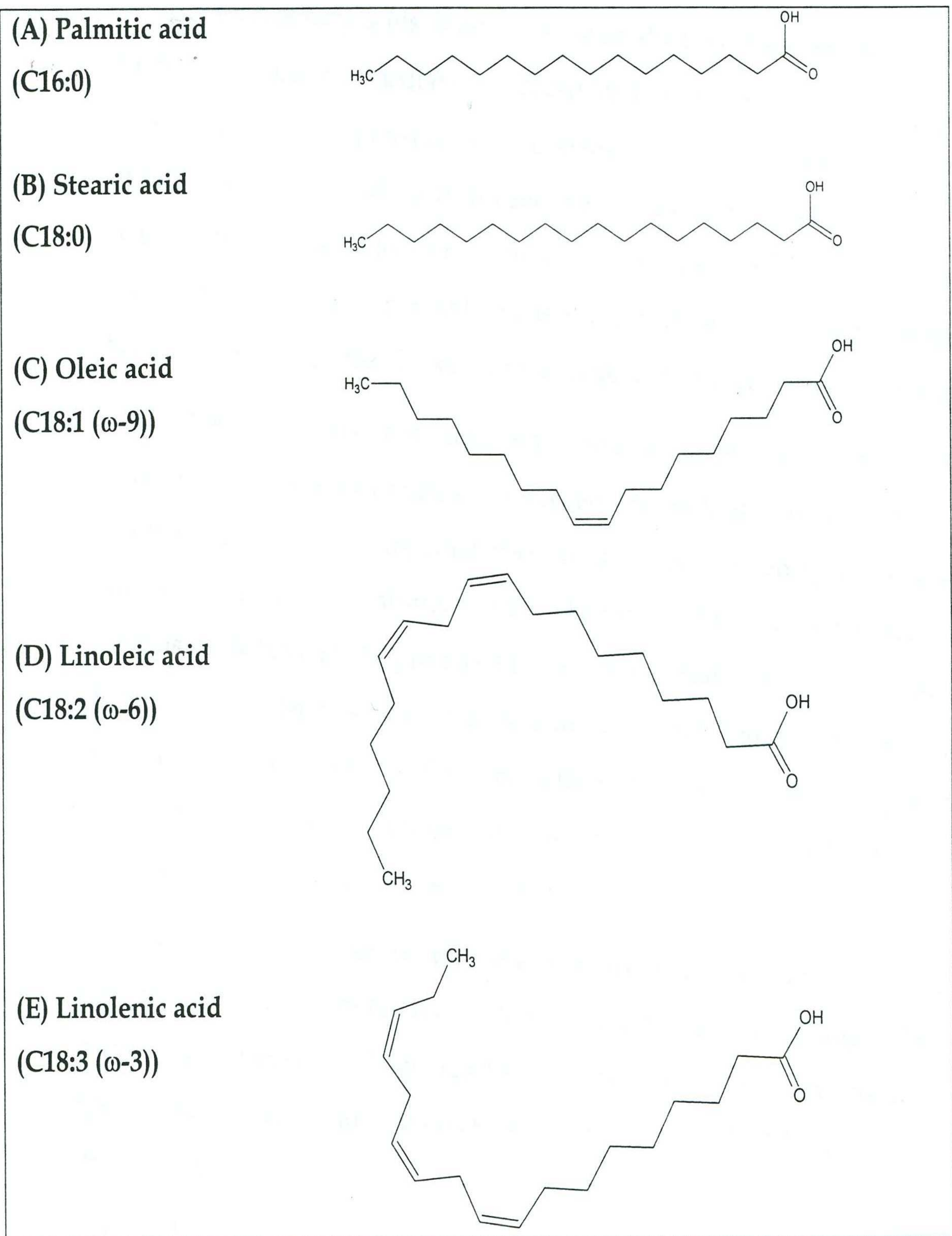


Figure 1. Primary fatty acids in corn oil (From Ref. (2))

Background Information of Corn Oil

Corn germ also called 'corn germ oil' has plenty of oil (>30%), and it is the source of all commercial corn oils. In general, a heating process is used to remove oil from wet milled germ, followed by prepress and hexane extraction. High quality and high yield crude corn oil is produced by the method of germ preparation for solvent extraction—extrusion [4].

The major component of crude corn germ oil is triacylglycerol (TAG), but other minor nonpolar and polar lipid components are also included in crude corn oil. Free fatty acids, pigments, volatile compounds, phospholipids, and waxes are the major undesirable components in crude corn oil and these compounds could be cleared by the refining procedures.

In the corn oil processing industry, free fatty acids can be clear of alkali refined by adding base, and then they could be subsequently neutralized into a byproduct. Moreover, free fatty acids could be removed by physical refining. The first step is removing phospholipids by a water degumming step [5]. After a subsequent bleaching step, steam distillation is the next step at high temperature and very low pressure, and free fatty acids could be volatized. Deodorization consists of treatment at high temperature (>200°C) and vacuum, and undesirable odors and flavor components could be clear [6]. Pigments are usually removed by the method that oils react with acid-activated bleaching clay [7]. Final refining step is winterization that makes sure physical stability of oils at low temperature. In the end corn oil is cooled to 5-10°C, and removing precipitates by filtration [8].

Corn oil is an abundant source of tocopherols, and γ-tocopherol is the most abundant tocopherol, followed by α-tocopherol and then δ-tocopherol. Among the four tocopherols, α-tocopherol has been paid more attention since its biological antioxidant activity - vitamin E. However the other isomers - tocotrienols are known to have significant antioxidant properties as well.

It is generally agreed that tocopherols and tocotrienols can protect against lipid oxidation in foods and in biological systems; α-tocopherol has the highest antioxidant activity in vivo [9]. The antioxidant activities of tocopherols and tocotrienols are due to

their abilities to donate their phenolic hydrogen to fatty acid free radicals and retard the autocatalytic lipid peroxidation processes [10].

Reactions in Deep-frying Condition

Deep-frying is widely used in commercial cooking, fast food and home cooking, and fats and oils are viewed as heat transfer medium in this deep-frying method.

Deep-frying could enhance the sensory properties of foods and it is a good food application of heating method. However, during deep-frying, fats and oils are repeatedly heated at elevated temperatures in the presence of atmospheric oxygen for long times. This process produces undesirable components that may cause health hazards [11, 12]. Heating in the presence of air causes partial conversion of fats and oils to dimeric, polymeric, nonvolatile oxidized derivatives and volatile chain-scission products [13, 14]. Some studies have reported that highly oxidized and heated lipids may have carcinogenic properties because of their potentially toxic compounds [15-21]. On the other hand, studies of commercial deep-frying have generally indicated that fats and oils have no detrimental effects on human health [22-28]. Besides the toxic effects, the nutritional value of frying fats and oils is influenced by loss of polyunsaturated fatty acids (PUFA), which supplement the requirement of the essential fatty acids in human metabolism [29, 30].

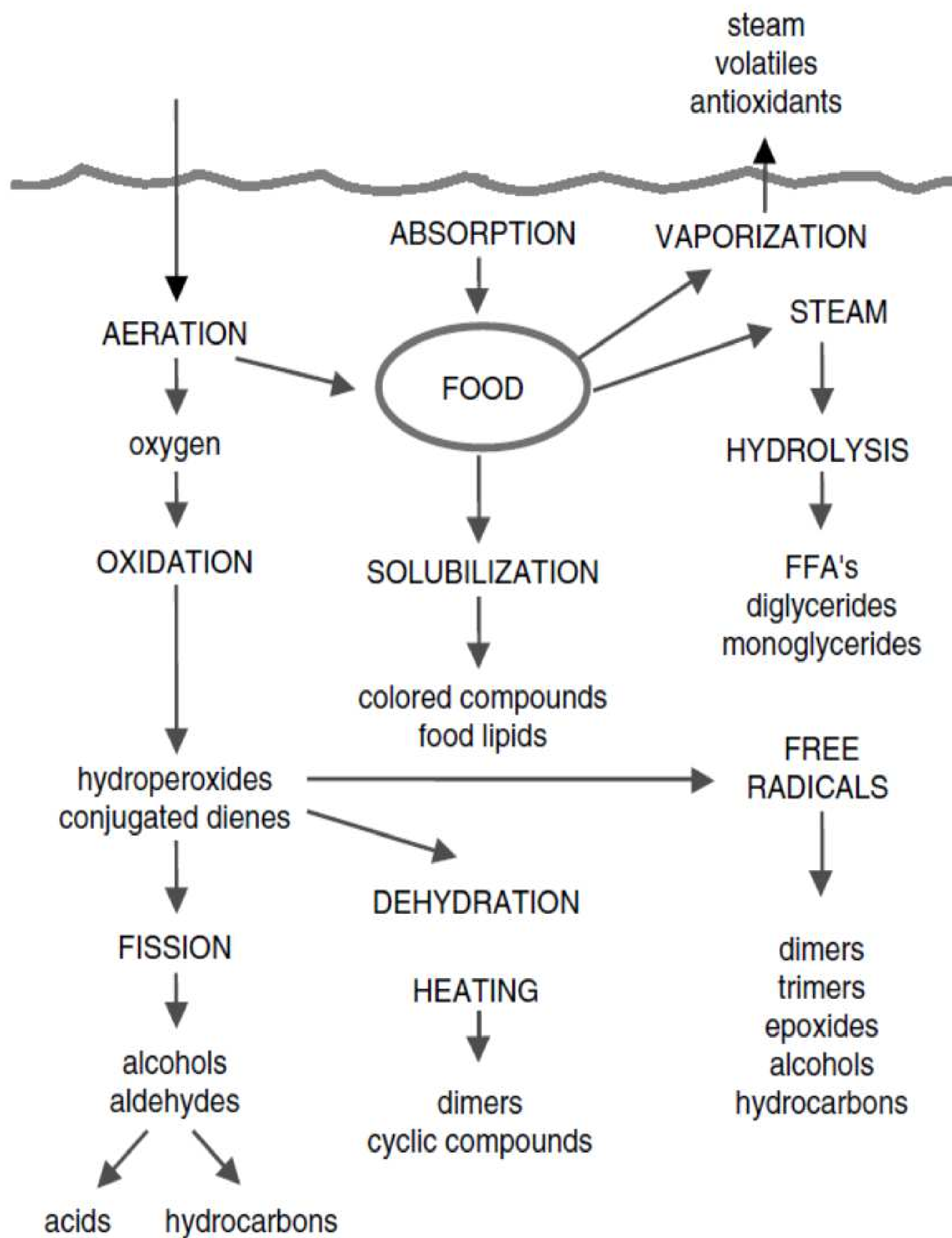


Figure 2. Reactions in oil during frying (From Ref. (31))

Lipid Oxidation

Lipid oxidation of fatty acids is an autocatalytic process caused by free radicals in the presence of oxygen. Free radicals have unpaired electrons that initiate hydrogen abstraction and additional chemical reactions.

Autoxidation is the oxidative deterioration of unsaturated fatty acids via an autocatalytic process which includes free radical chain mechanism. Autoxidation can break down the molecules as well as forming new molecules and causing huge changes in chemical and physical properties of the oxidizing substrate [33–35]. The process of lipid oxidation consists of three steps: initiation, propagation and termination.

Step1: Initiation

Loss of hydrogen radical ($H^•$) from unsaturated fatty acid (RH) reacts with oxygen (O_2) in presence of air, light, heat or trace metals to produce peroxyl radicals (ROO•)

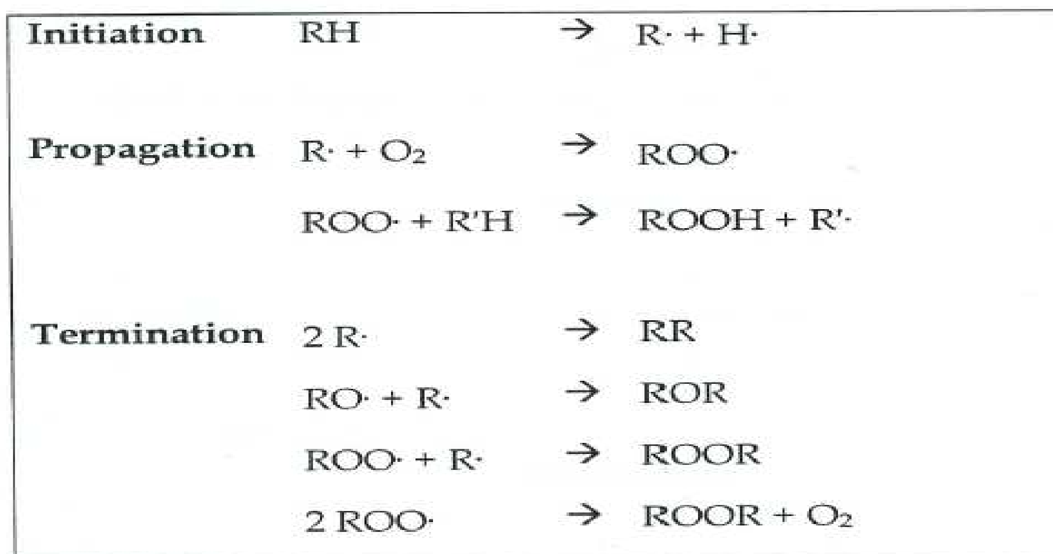
Step 2: Propagation

In this process, peroxyl radicals react with unsaturated fatty acids to form lipid hydroperoxides (ROOH).

Step 3: Termination

The oxidation chain process terminates when two peroxyl radical reacts together to yield non-radical species (ROOR or RR).

Table 2. Hypothetical free-radical induced autoxidation reaction (From Ref. (35))



Initiation	RH	→	R· + H·
Propagation	R· + O_2	→	ROO·
	ROO· + R'H	→	ROOH + R'·
Termination	2 R·	→	RR
	RO· + R·	→	ROR
	ROO· + R·	→	ROOR
	2 ROO·	→	ROOR + O_2

Thermal oxidation is also an autocatalytic process and it is viewed as metal-catalyzed since it is very difficult to eliminate trace metals as a catalyst. Redox metals of variable valency are likely to accelerate degradation of hydroperoxides as well [32, 33].

Photo oxidation happens because of the presence of molecules that absorb visible or near UV light to form electronically excited sensitizers. Pigments can initiate photosensitized oxidation in foods including chlorophylls, hemoproteins and riboflavin. The type I sensitizer works as photochemically activated free radical initiator, and type II sensitizers in the triplet state react with oxygen by energy transfer to form singlet oxygen ($^1O_2$) that interacts with unsaturated fatty acids further. Under photo-oxidation conditions, the reaction of unsaturated fatty acids with singlet oxygen ($^1O_2$) leads to rapid formation of peroxides and hydroperoxides [33, 35, 36].

Oxidative stability of vegetable oils can be evaluated by various compositional parameters. Peroxide value (PV) and conjugated diene hydroperoxide concentration are always used to evaluate the development of primary oxidation products. P-anisidine value (PAV) and thiobarbituric acid reactive substances (TBARS) are commonly used to evaluate the formation of secondary oxidation products [37].

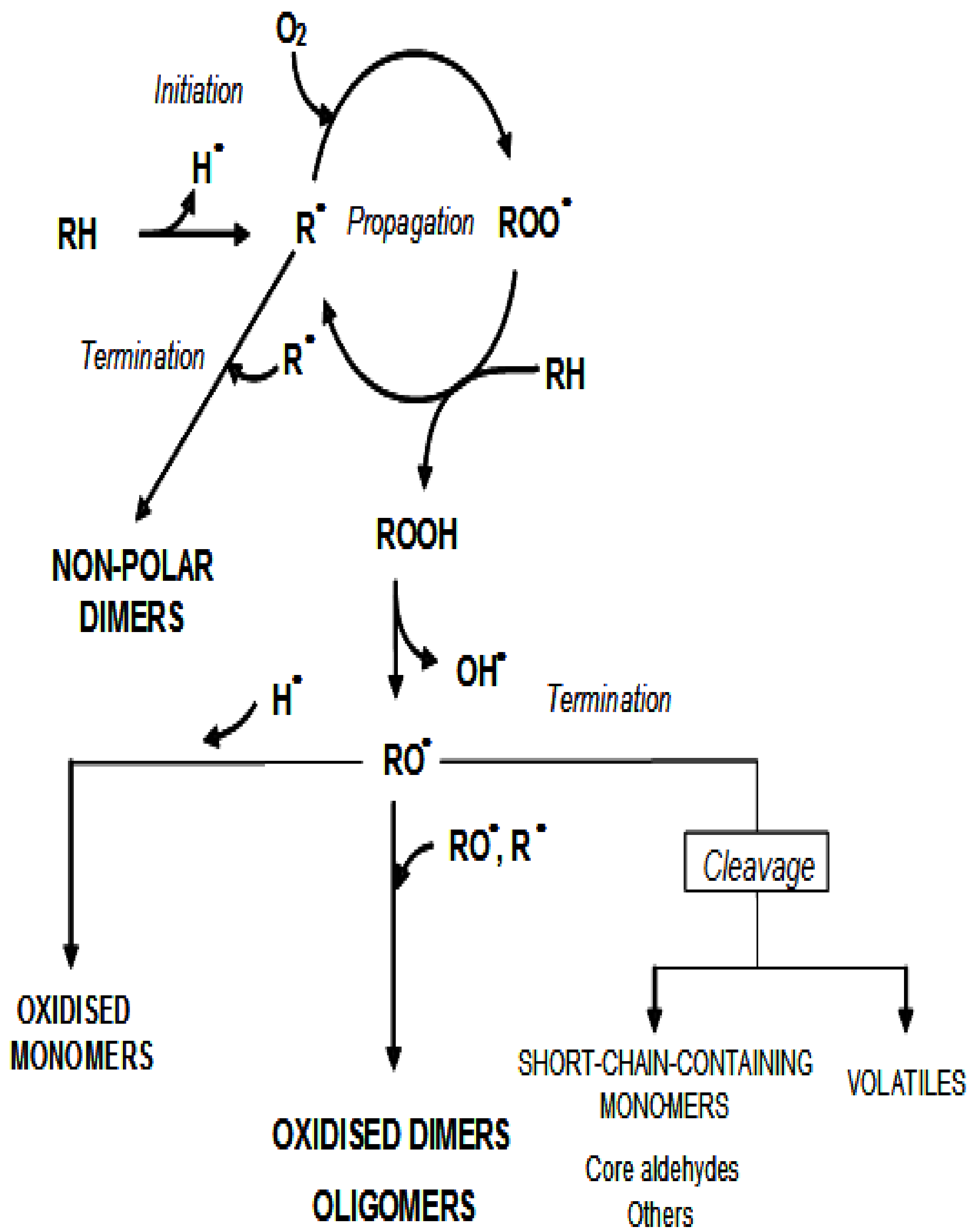


Figure 3. General scheme for lipid oxidation (From Ref. (38))

Formation of Hydroperoxides

The primary products of fatty acid peroxidation are fatty acid hydroperoxides. Hydroperoxides are formed when free radical abstraction of hydrogen from an unsaturated fatty acid is followed by molecular oxygen attack at the alkyl radical [39]. The methylene-interrupted double bond structure of linoleic acid makes it very susceptible to oxidation because the abstraction from the methylene is easy when the neighboring carbons contain double bonds [40].

Acyl lipids components, such as oleic, linoleic and linolenic acids, have one or more alkyl groups, so they are oxidized to unstable hydroperoxides easily. Subsequent degradation reactions yield a huge amount of other substances. Alternatively, the hydroperoxides condense into dimmers and polymers [41, 42].

Hydroperoxides are not stable and may decompose to radicals that accelerate propagation process. These are branching steps of lipid autoxidation process [31, 32].

The hydroperoxides generated by free radical-induced oxidation of fatty acids break down in several steps to yield a variety of decomposition products including a wide variety of hydrophilic and lipophilic aldehydic compounds. The initial products from hydroperoxides breakdown are the typical hydroperoxide products [43]. As the hydroperoxides degrade, more free radicals and hydroxyl free radicals are formed which in turn initiate more hydroperoxide products. Hydroperoxides begin to degrade as soon as they are formed. Scission of the oxygen-oxygen bond of the hydroperoxide forms a hydroxyl radical and an alkoxy radical. Further decomposition of oxy free radicals yields a wide variety of smaller molecular compounds including aldehydes, ketones, acids, esters, hydrocarbons, lactones and aromatic components.

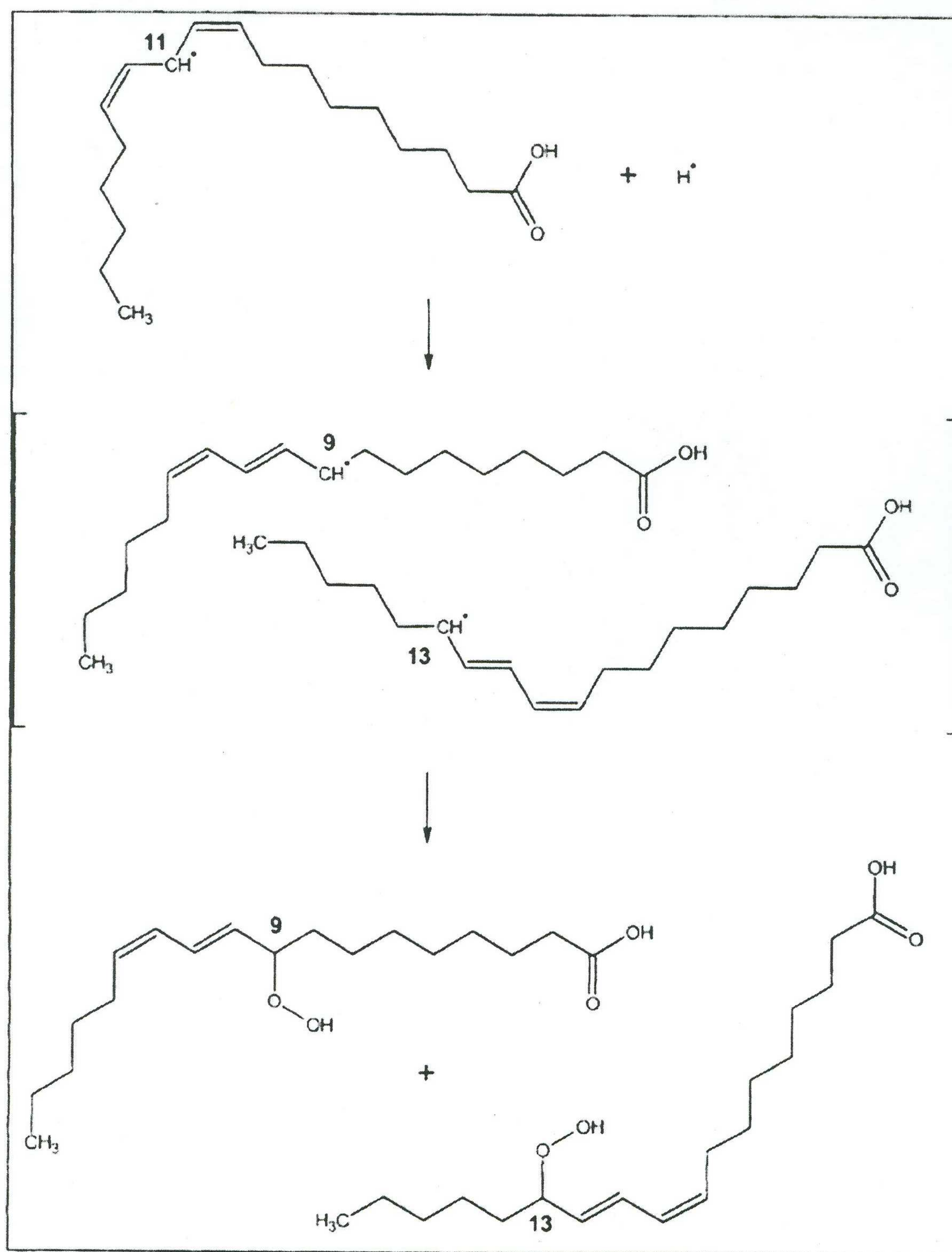


Figure 4. Formation of 9- and 13-hydroperoxides from linoleic acid (From Ref. (44))

Decomposition of Hydroperoxides by Homolytic and Heterolytic Cleavage

After the formation of alkoxyl radical, the carbon-carbon bond on either side of the alkoxyl group can be homolytically cleaved and oxidation proceeds farther. It is called β-cleavage or β-scission reaction.

If an alkenyl results from β-cleavage of hydroperoxides, it can react with either a hydroxyl radical or O_2 [45]. The reaction with molecular oxygen produces an alkenyl hydroperoxide which can further decompose to the saturated aldehydes.

If the cleavage of the methyl side results in an alkyl radical, it can enter into a variety of reactions: combination with hydroxyl radicals (which are produced by hydroperoxide decomposition) to produce an alcohol (which can rearrange to an aldehyde), abstraction of hydrogen to form 1-alkene, or peroxidation to form a terminal hydroperoxide. This hydroperoxide will decompose to the corresponding alkoxy radical and it forms an aldehyde or cleaves and yields an alkyl radical later.

Homolytic cleavage of hydroperoxides is the main reaction mechanism in the fats and oil phase of foods [40]. Heterolytic cleavage requires the presence of protons; therefore it is more easily to occur in aqueous solutions.

It is obvious that cleavage of hydroperoxides will result in the formation of a wide variety of secondary oxidation products, and most of them are volatile components due to the presence of carbonyl groups and short length of the chain. However, the longer chain aldehydes are only partially volatile.

Aldehyde products which have methylene-interrupted double bonds can undergo further oxidation. β-cleavage of hydroperoxy aldehydes leads to second generation of aldehydes and free radicals. Unsaturated aldehydes can undergo the same autoxidation which yield the aldehyde from the unsaturated fatty acids, leading to short-chain hydrocarbon, aldehydes and dialdehydes, such as malondialdehyde [46].

Some investigators have reported that the decomposition of these three unsaturated aldehydic oxidation products produced small amounts of α, β-unsaturated hydroxyaldehydes: 4-hydroxy-2-trans-hexenal (HHE), 4-hydroxy-2-trans-octenal (HOE), and 4-hydroxy-2-trans-decaenal (HDE) [47].

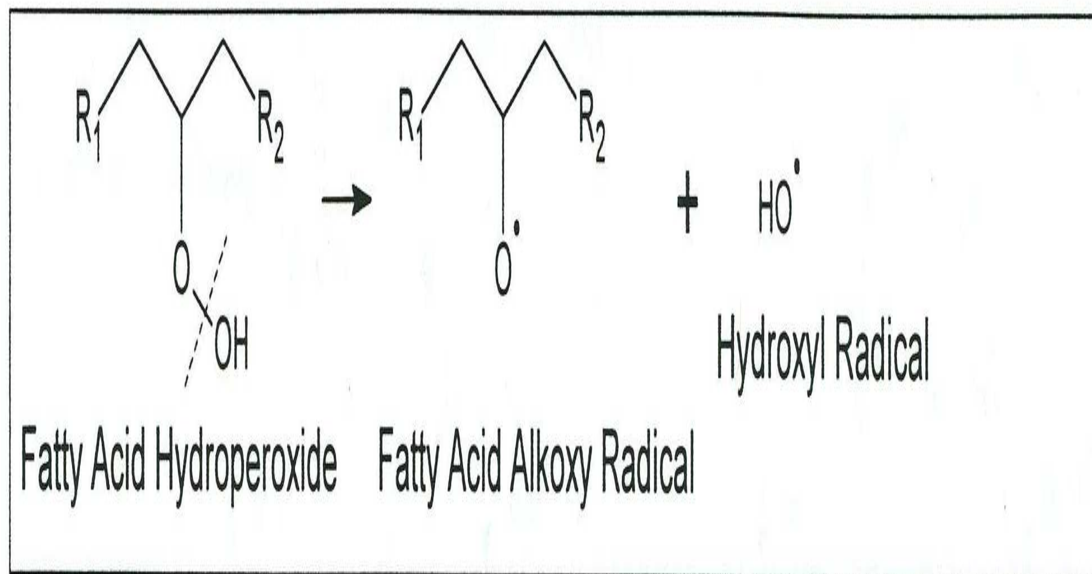


Figure 5. Decomposition of fatty acid hydroperoxides (From Ref. (43))

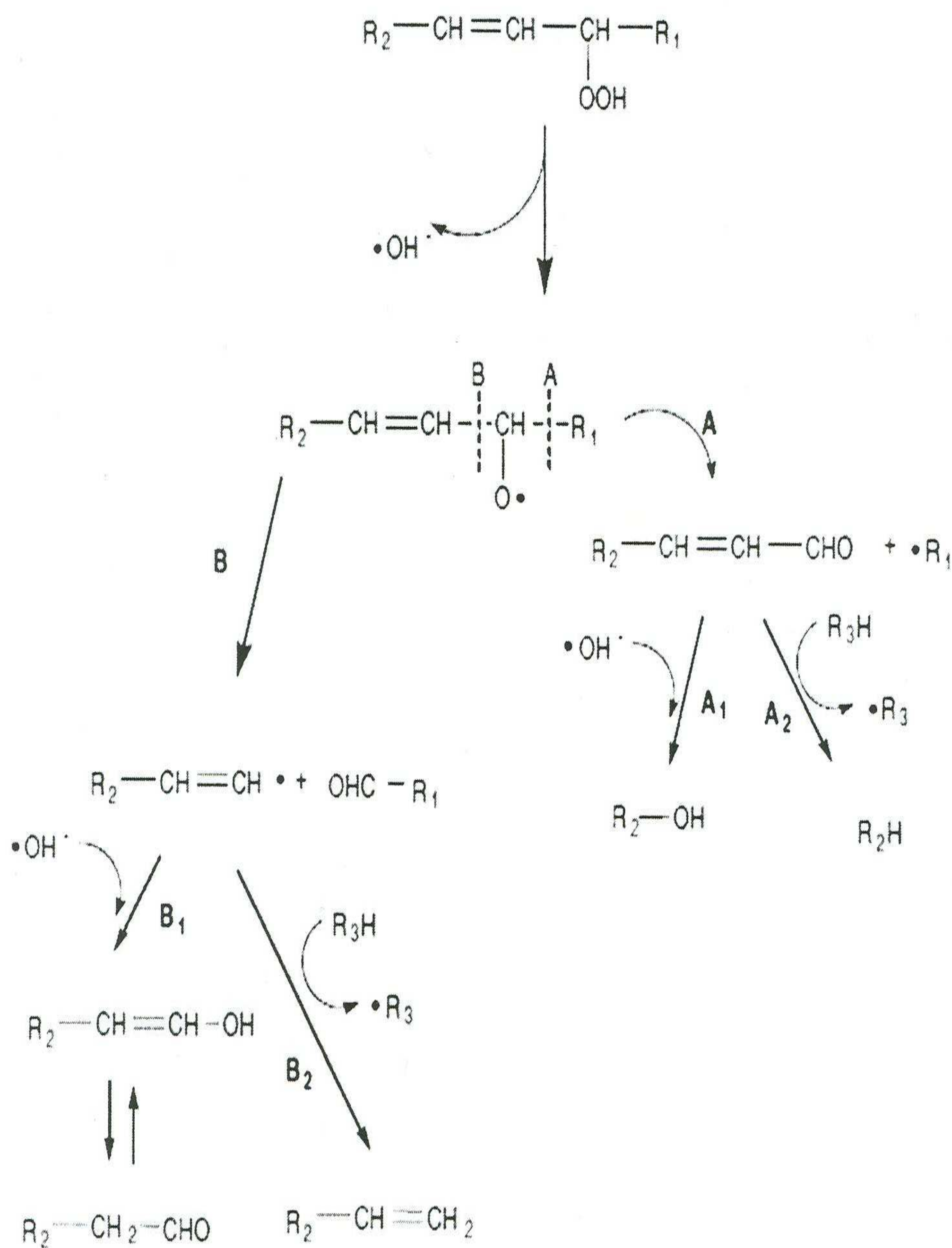
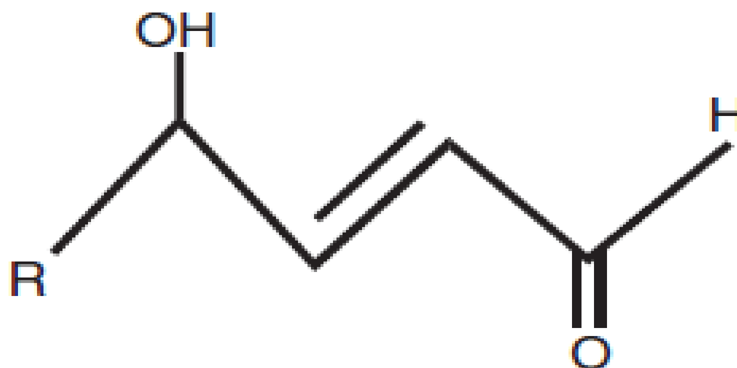


Figure 6. Mechanism of hydroperoxide decomposition to form secondary oxidation products (From Ref. (48))

Secondary Lipid Oxidation Products – HNE

Among the lipophilic aldehydes, α, β-unsaturated-hydroxyaldehydes including 4-hydroxy-2-trans-hexenal (HHE), 4-hydroxy-2-trans-octenal (HOE), 4-hydroxy-2-trans-nonenal (HNE) and 4-hydroxy-2-trans-decenal (HDE) are very important because of their reactivity with biomolecules and their toxicity [49].



R = C_2H_5: 4-hydroxy-2-hexenal, HHE
R = C_4H_9: 4-hydroxy-2-octenal, HOE
R = C_5H_{11}: 4-hydroxy-2-nonenal, HNE
R = C_6H_{13}: 4-hydroxy-2-decenal, HNE

Figure 7. Chemical structures of the four α, β-unsaturated-4-hydroxyaldehydes (From Ref. (49))

The length of these four compounds was different. It was found HHE came from linoleic acid but mainly from linolenic acid. HOE was from both linoleic acid and linolenic acid. Linoleic acid was the precursor for HNE formation [47].

It was suggested by Grein et al. [50] that HNE was from linoleic acid by the formation and decomposition of 2,4-decadienal. Han and Csallany in this laboratory did not find that decadienal was the precursor for HNE [47]. The present two pathways for HNE formation were published by Schneider et al. [51]. In this study, 9- and 13-hydroperoxides of linoleic acid were used as starting materials, and the results showed the formation of 9- and 13-hydroperoxides was from linoleic acid. Two different pathways yielded the formation of 4-hydroperoxy-2E-nonenal from the hydroperoxides of linoleic acid. The two pathways were shown in Figure 8.

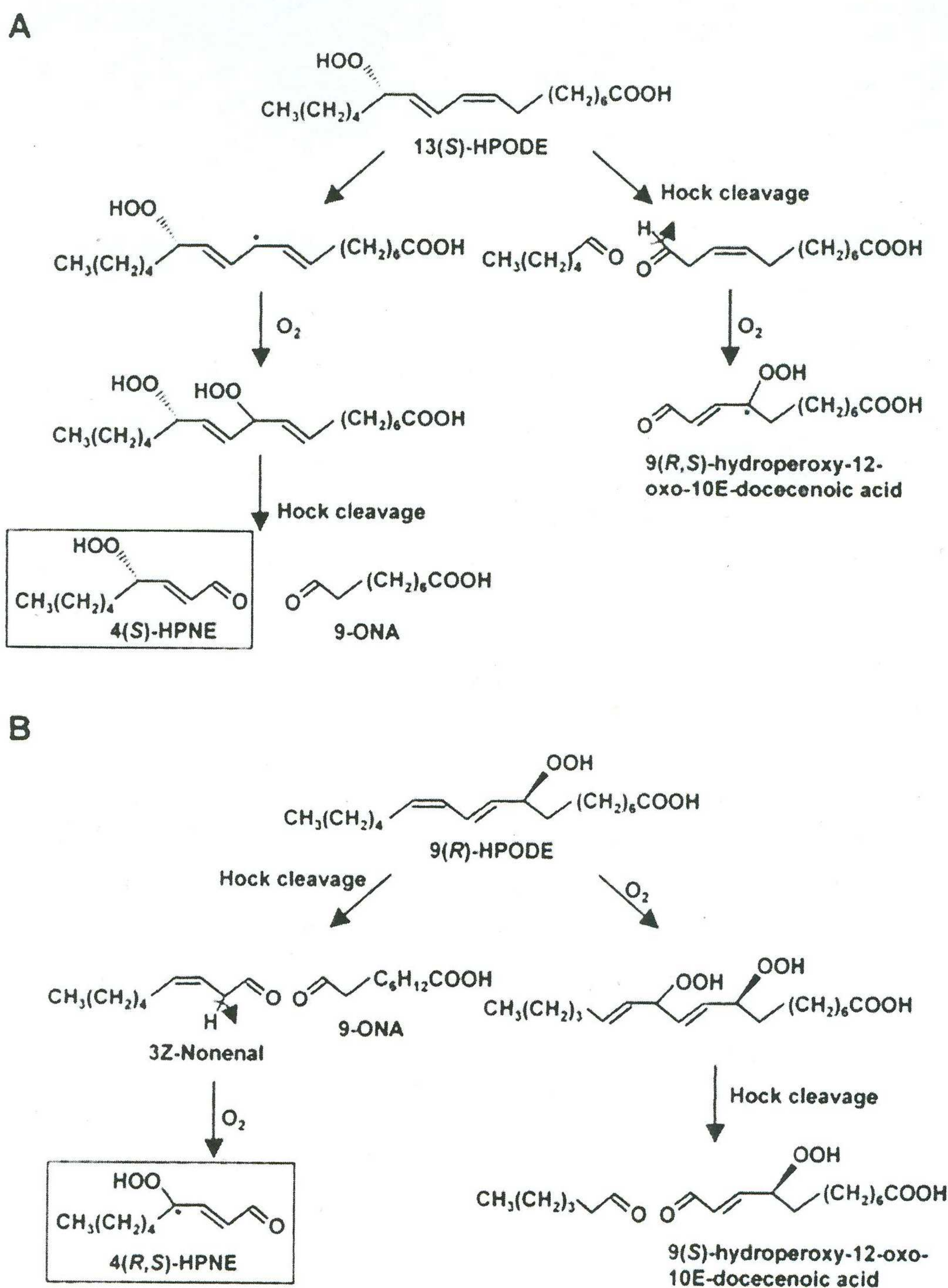


Figure 8. Mechanism of formation of HNE from 13(S)-HPODE(A) and 9(R)-HPODE(B) (From Ref. (51))

Previously Kim, Gallaher and Csallany have found that lipophilic aldehydes and related carbonyl compounds were detected in rat and human urine in *vivo*, indicating similarity between lipid peroxidation and lipophilic products formation in *vitro* [52]. It is questioning the worldwide dietary advice to substitute high ω-6 fatty acid, such as linoleic acid [53].

The α, β-unsaturated hydroxyaldehydes, especially HNE has been shown to be toxic to experimental animals. The lethal dose (LD_{50}) for HNE in mice is 68 mg/kg of body weight intraperitoneally [54]. HNE and HHE injected intravenously (13 to 18 mg/kg) produced severe liver damage. The toxicities of other hydroxyaldehydes such as HOE and HDE have not been fully investigated yet in the literature but they are very reactive as well.

The toxicity of these reactive α, β-unsaturated hydroxyaldehydes is based on their structures which provide high reactivity to other compounds. The C-2 to C-3 position of the double bond and a hydroxyl group in C-4 position easily form Michael adducts with thiol groups of protein residues [55, 56] and the aldehyde group at the C-1 position can react with the amino group of proteins or DNA [55]. The hydroxyl group attached to the C-4 position provides not only more reactivity but also more stability to Michael adducts than the non-hydroxylated alkenals [56]. The increase in chain length from six to nine carbons has been shown to increase apoptotic induction and the toxicity of alkenals [57].

Because of their high reactive activities, many studies of 4-hydroxyalkenals were conducted in biological systems and it has been proved that they are cytotoxic and mutagenic [58, 59]. HNE is known to be the major toxic compound produced during the oxidation of ω-6 PUFAs, such as linoleic acid and arachidonic acid. HNE can attach to proteins through the Michael adduction and form stable substances with cysteine, lysine and histidine amino acid residues. Therefore the structure of proteins is altered which is leading to the loss of protein function [60]. Additionally HNE can change the expression of several genes and inhibit growth [61, 62]. In the concentration range of 1 to 20μM, HNE can inhibit DNA and protein synthesis. Even at a very low concentration (less than 0.1μM), HNE can modify cellular metabolism [63]. HNE is used as a biomarker for pathophysiological processes as well [64].

Because of the increasing concern of the toxicity of HNE to human health and its readily absorption from the diet, more and more studies have been conducted to investigate HNE formation in fats and oils by heat treatment. Seppanen and Csallany [65] have proved that there was no HNE at very low concentration of polar lipophilic secondary oxidation products in unheated soybean oil. The concentration of both HNE and the sum of total lipophilic polar oxidation products were increasing with increased heating time at frying temperature. A considerable concentration of HNE had already formed after 2 hours of heat treatment and the concentration continued to increase after 4 and 6 hours of heating treatment in soybean oil which contains high levels of linoleic acid (45-52%).

The temperature dependence of the formation of HNE was further demonstrated in the high linoleic acid including soybean oil and the low linoleic acid including butter oil [66]. The oils were heated at both 190°C and 218°C up to 6 hours. The concentration of HNE at 218°C increased extremely for all of three oils compared to the lower temperature of 190°C under the same condition. It proved that HNE formation was dependent on the temperature.

The same authors also found that HNE could be incorporated into fried food from frying oil which had been heated at 185°C after 5 hours [67]. Similar concentrations of HNE were found in the oil prior to and after frying and in the oil extracted from the fried potato as well. This result showed that HNE was readily incorporated into food during frying from the thermally oxidized oil.

The effect of intermittent heating was compared with continuous heating on HNE formation in soybean oil [68]. Soybean oil samples were heated either 1h each day for five sequential days or 5 hours continuously at 185±5°C. The thermally oxidized samples were analyzed for the presence of HNE, HHE, HOE and HDE. It was found that the concentration of these four α, β-unsaturated-4-hydroxyaldehydes increased similarly in both intermittent and continuous heating conditions totally 5 hours. The results demonstrated that the formation of HNE and the other α, β-unsaturated-hydroxyaldehydes is a cumulative result of the peroxidation and degradation process of ω-6 PUFAs over time at frying temperature.

The formation of α, β-unsaturated-hydroxyaldyhydes was investigated in fatty acid methyl esters containing 0, 1, 2 and 3 double bonds [69]. Fatty acid methyl esters (FAMEs) of stearic, oleic, linoleic and linolenic acids were heated at 185°C for 0 to 6 hours. The results showed that methyl stearate (MS) and methyl oleate (MO) did not yield any of the α, β-unsaturated-hydroxyaldehydes after thermally induced lipid peroxidation. HHE formation was detected from both methyl linoleate (ML) and methyl linolenate (MLN), and the concentration was higher in MLN than in ML indicating that MLN is the major precursor. HOE formation was detected from both ML and MLN as well. HNE was found to form only from ML proving that ML is the precursor for HNE.

In general, HNE is the most well known compound among the four α, β-unsaturated-hydroxyaldehydes and its toxicity is well established in the literature. Its formation from high ω-6 PUFAs oils due to heat treatment is well established. HNE toxicity in *vivo* has been related to atherosclerosis [70, 71], stroke, low-density lipoprotein oxidation, Parkinson's, Alzheimer's, Huntington's [72–76], liver [77] and other diseases. HNE is able to modify proteins, nucleic acids and other biomolecules in *vivo* as well [78–81].

Background of Synthetic and Natural Antioxidants

Most antioxidants in dietary plants are primary antioxidants such as substituted phenols, which act as chain-breaking antioxidants because their hydrogen donating capacity of their –OH group scavenges reactive radicals such as peroxyl radicals ($RO_2^{\cdot}$) [82].

Some phenolic compounds have additional mechanisms of antioxidant capacity, for example, chelating transition metal ions [82].

Antioxidant activity (AH) refers to the inhibition of lipid oxidation or other molecules by inhibiting the initiation or propagation step of the oxidative chain reactions or forming stable radicals ($A^{\bullet}$) which are either unreactive or forming non-radical products [83].

Synthetic Antioxidants

Synthetic antioxidants added to fats or oils inhibit or retard the oxidation process by donating hydrogen to the lipid radical intermediates. Synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), or tert-butyl hydroquinone (TBHQ) are found to be effective as lipid oxidation inhibitors [84] and have been used until recently for preventing oxidation. However, health concerns over synthetic antioxidants such as BHA and TBHQ, which are potentially carcinogenic at high concentration [85], demand the use for more natural antioxidants. They are colorless, odorless and tasteless, however they have the best antioxidant abilities at lower temperatures.

TBHQ is used frequently in frying process for highly unsaturated vegetable oils. TBHQ is found to be more effective than BHA and BHT in vegetable oils. TBHQ is a primary antioxidant and either alone or in combination with other primary and secondary antioxidants. It has been shown as being effective in highly unsaturated vegetable oils and animal fats. TBHQ has also the advantage over some of the other antioxidants by being able to extend the storage stability of oils and fats for relatively long period of time. It has been found to be particularly effective when added during the frying process leading to a longer shelf life for certain products. It is stable to heat and is regarded as the

most effective antioxidant protecting the oxidation of frying oils. It is also used as a supplement to hydrogenation of oils for increasing their oxidative stability [86, 87]. It has been shown that the mixture of TBHQ, monoacylglycerol citrate (MGC), and ascorbyl palmitate (L-ascorbic acid, 6-hexadecanoate) exhibited the highest thermal stability and provided optimum protection from oxidation of oils during high temperature heat treatment [88].

It has also been found that TBHQ is especially an effective antioxidant in cottonseed, soybean and safflower oils. It is highly effective when combined with propyl gallate (PG) to protect in peanut oil and palm olein from oxidation as well. In margarine it has been combined with tocopherol and found to be very useful to increase shelf life in fried products such as chips, deep fried instant noodles, fish crackers and banana chips.

Ascorbyl palmitate (AP) is a fat-soluble ester of ascorbic acid and palmitic acid and used mostly in commercial vegetable oils shown to have antioxidant activity [89]. It was more effective than either BHT or BHA in preventing the oxidation of vegetable oils. It was recently proved to retard the deterioration of oils during frying applications [90]. AP was also shown to be a powerful synergist in combination with tocopherols during the storage of various oils [91]. Additionally AP in combination with PG has been reported to prolong the shelf life of sunflower, peanut and corn oils [92]. AP has been shown to be very effective in the protection of vegetable oils such as soybean, corn, peanut, safflower and sunflower, against oxidation [93]. Although AP is not as efficient in the protection of oxidation of animal fats as in vegetable oil, it potentiates the antioxidant activities of α- and γ -tocopherols. In safflower emulsions AP has been shown to synergize BHT, BHA, TBHQ and PG [94] and to synergize tocopherol and vitamin A in citrus oils [95]. AP was shown to extend the stability and increase the quality of frying fats and oils recently [96].

In Europe AP has been used widely for a long time. Klaui et al [97] demonstrated the protective activity against the oxidation of AP alone or in combination with α-tocopherol of butter fat, vegetable oils, ethyl linoleate, ethyl arachidonate vitamin A and beta-carotene. Pongracz [98] demonstrated the antioxidant properties of AP efficacy in butter, butter oil, salad dressings, biscuits, dried potatoes and dried milk products.

Although AP itself does not occur in nature, it is enzymatically broken down into

ascorbic acid and palmitic acid in many foods. Because of this characteristic unlike other synthetic antioxidants, its usage is not limited to 0.02% in fats or oils, and AP may be used at higher levels.

The mechanism of AP is not fully well known [99], although some investigators have found that it quenched singlet oxygen in photosensitized oil mixtures [100]. Coppen et al [101] suggested that AP achieved its effect by removing, or sequestering trace metals that catalyze the peroxidation process.

Natural Antioxidants

There are recently some concerns about the possible harmful effects of some synthetic antioxidants. Because of these concerns it is important to identify novel natural antioxidants for oils and fats to protect or retard their oxidative deteriorations. The addition of natural antioxidants using plant extracts as a way of increasing shelf life of various food products has become popular.

Major groups of chemicals present in plant extracts contain polyphenols, quinones, flavanols/ flavanoids, alkaloids, and lectins [101]. Phenolic extracts prepared from aromatic plants or many herbs, such as, sage, rosemary, thyme, hops, coriander, green tea, grape seed, cloves, and basil are known to have antimicrobial effects against foodborne pathogens and have pharmacological properties as well [102-109].

Rosemary extracts are regarded as a well-known antioxidant to stabilize lipid oxidation. Many investigators have reported that rosemary extracts have high potential protection in lipid oxidation [110-113] and antioxidant capacity in *vitro* [114, 115]. Rosemary (Rosmarinus officinalis) has been recognized as one of the herbs with great antioxidant activity. Substances related to the antioxidant activity are phenolic diterpenes such as carnosol, rosmanol, carnosic acid, methyl carnosate and phenolic acids namely rosmarinic and caffeic acids [116–120]. Commercially available rosemary extracts exhibited potential antioxidant activity in vegetable oils [121-123]. At least 12 diterpene phenols have been isolated from the rosemary plant and several of these, such as carnosol, carnosic acid, rosmanol, rosmaridiphenol, rosmadial, and miltirone, have been shown to display antioxidant capacity [124]. Carnosic acid has been found to be the

primary phenolic diterpene component in rosemary leaves [125]. Carnosic acid [126] is a diterpene found in the popular "Labiatae herbs" sage and rosemary [126, 127], and it is viewed as a precursor of other diterpenoid components [128]. Carnosic acid and related diterpenes such as carnosol and rosmanol are known to have antioxidant properties [129], and carnosic acid has the most effective antioxidant capacity among these diterpenes [130]. Carnosic acid has a typical ortho-phenol structure and is easily oxidized. Most diphenol compounds show potential chain-breaking antioxidant activity in food systems [131]. Wenkert et al. [132] suggested the oxidation and isomerization pathway to yield carnosol from carnosic acid via an ortho-quinone intermediate. The oxidative conversion pathways of other related diterpenes have been studies by Hall and Cuppett [133] as well.

Carnosol was isolated from sage the first time in 1940' and its chemical structure was established in 1960's by Brieskorn et al [134]. Rosemary and sage have been known to contain a variety of polyphenols such as carnosol, carnosic acid, rosmanol, rosmarinic acid as well as others [135]. It has been established that approximately 5% of the dry weight of rosemary leaves consist of carnosol and carnosic acid, and this fraction is estimated to account for over 90% of its antioxidant capacity [136]. Carnosol is an ortho-diphenolic-diterpene with an abietane carbon skeleton with hydroxyl groups at positions C-11 and C-12 and a lactone moiety across the B ring [137]. Carnosol is the product of oxidative degradation of carnosic acid (Figure 9) [138]. The most popular and expensive way to produce a highly purified form of carnosol is extraction and purification from natural sources such as rosemary. Recently, a semi-synthetic process has been described using pisiferic acid (Figure 9) extracted from Sawara leaves of cypress tree a native plant to Japan, and then synthesize carnosic acid and convert it to carnosol [139]. In detail carnosic acid was prepared through the oxidation of pisiferic acid using three different methods: a) m-chlorobenzoyl peroxide (mCBPO), b) chloroacetyl meta-chlorobenzoyl peroxide (CAMCBP) in Methylene Chloride, or c) 2-iodoxybenzoic acid (IBX) in Methylene Chloride-Methanol. The third semi-synthetic reaction had an overall yield of 72% while the first two had considerably lower yields ~10%. Carnosol was prepared from carnosic acid in the presence of silver oxide in methylene chloride with a purification yield of 67%. Alternatively, carnosic acid in the presence of methanol will

oxidize carnosic acid to carnosol at room temperature in one week [140, 141].

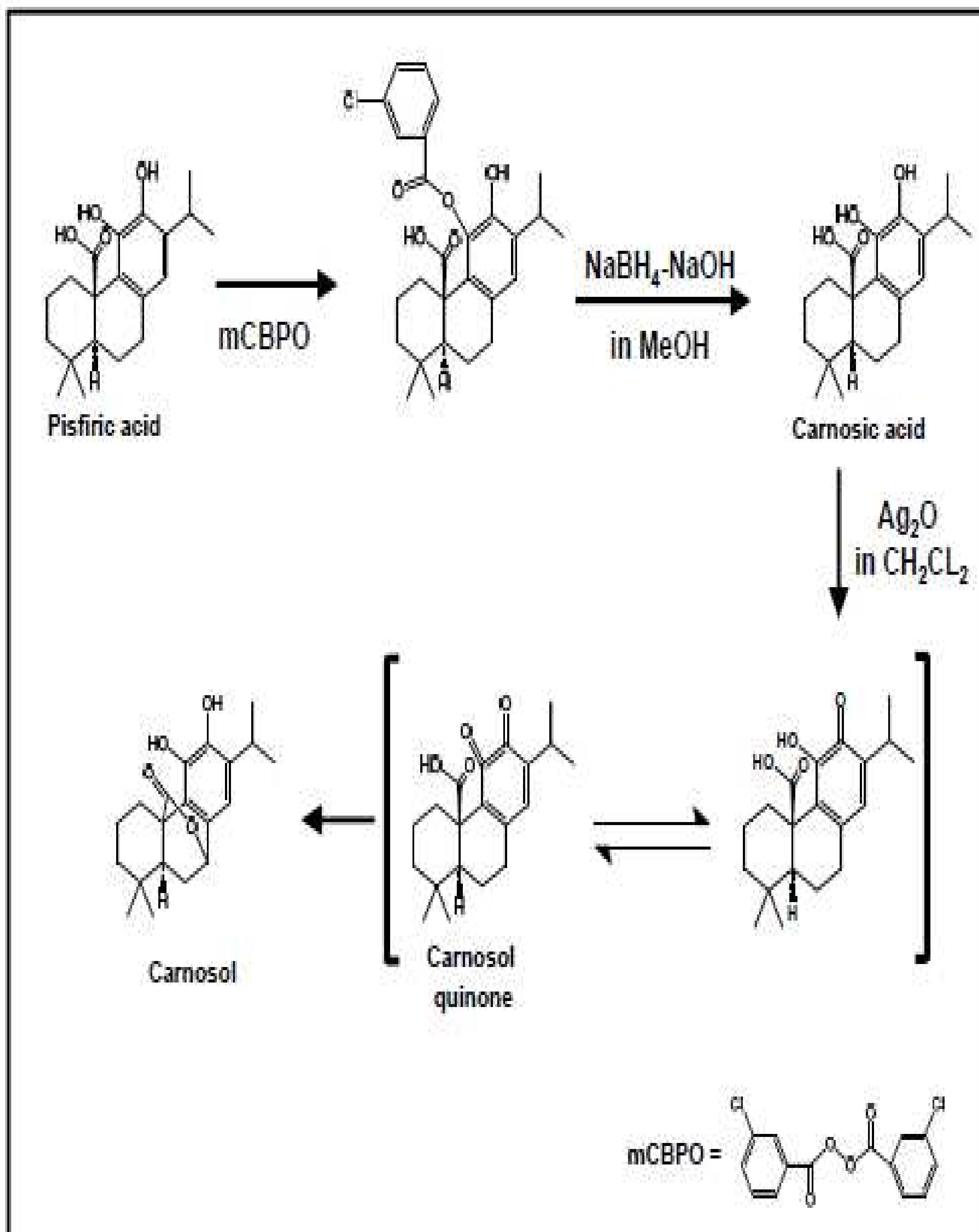


Figure 9. Formation of carnosol from pisiferic acid (From Ref. (142))

The health related properties of rosemary and sage have been attributed to the antioxidant capacity of polyphenols in these extracts [143-146]. Reactive oxygen species and depletion of antioxidant enzymes have been suggested to promote a variety of biological responses including neurodegenerative, inflammatory conditions, cardiovascular disease, and carcinogenesis in various tissues [147]. Rosemary extracts shown in the DPPH (2,2-diphenyl-1-pycrilhydrazil hydrate) radical scavenging activity assay to have a radical scavenging activity up to 95% [142] with approximately 90% of the antioxidant activity attributed to the diterpenes carnosol and carnosic acid [146]. Carnosol has been shown to inhibit Cu^{2+} induced LDL oxidation and lipid free radical formations in mouse liver microsomes [148] and it is a good scavenger of peroxyl radicals [136]. The antioxidant response is activated through the catechol-hydroxyl groups of carnosol and it is converted to a carnosol quinone [149]. This quinone derivative is the main oxidation product of carnosol essentially without any antioxidant activity [150]. Under the water-containing conditions the antioxidant activity can be recovered from carnosol quinone [151].

The greatest level of attention among all herbs and spices has been focused on rosemary. Many studies have been conducted to examine the antioxidant activities of crude rosemary and different rosemary extracts. Parallel with the evaluation of antioxidant activity of rosemary extracts, research was also focused on the isolation and identification of antioxidant compounds. As it is stated before components responsible for the antioxidant capacity of rosemary are mainly phenolic diterpenes such as carnosic acid, carnosol, rosmanol, epirosmanol, isorosmanol, methyl carnosate [152, 153] and other phenolic acids, such as rosmarinic acid [154]. Polyphenolic compounds (mainly flavonoids) present in rosemary extracts have also demonstrated potential antioxidant properties due to their redox potential which enable them to act as hydrogen donors, reducing agents, nascent oxygen quenchers and chelating metal ions in various food applications [155].

In various technologies the most important natural antioxidants are: ascorbic and citric acid and their salts, tocopherols and spice extracts in food systems [156]. Citric acid is found in many plant and animal species. It can chelate metal ions by forming bonds

between the metal and the carboxyl or hydroxyl groups of the citric acid molecule [157]. Citric acid is very effective in retarding the oxidative deterioration of lipids in food and is commonly added to vegetable oils after deodorization process [158].

The use of natural antioxidants has the advantage of being more acceptable by consumers because these are regarded as ‘‘non-chemical”. Additionally they do not require safety tests before being used. Their weaknesses are that they are more expensive and less effective than many synthetic antioxidants. A quality consistency issue may also arise by using plant extracts as antioxidants in food products and the change in antioxidant levels and compositions influence the harvesting [159] and the variety of plants [160].

Synergism and Synergists

Synergism is the cooperative effect of antioxidants or an antioxidant with other compounds to yield enhanced capacity over the sum of activities of the individual component when used separately [161].

In a combination of two or more different free radical scavengers, rapid reaction with free radicals occurs because of the differences in bond dissociation energies or steric hindrance of free radical scavenger and peroxyl free radical ($ROO^{\bullet}$) interactions [162]. These differences result in one scavenger being used faster than the other. The primary antioxidant is likely to be regenerated by transferring its radical to another scavenger.

All antioxidants have strengths and drawbacks. Therefore certain good factors, such as thermal stability, effective concentration, and synergism, should be taken into consideration when selecting antioxidants in particular food systems. Regulatory status is another factor that has to be considered, especially for some antioxidants that have been proved to have potential harmful health effects.

Objectives

The objective of the present study was to determine the suppressing effects of various antioxidants and their mixtures on the formation of the toxic α, β-unsaturated-4-hydroxyaldehydes such as 4-hydroxy-2-trans-hexenal (HHE), 4-hydroxy-2-trans-octenal (HOE), 4-hydroxy-2-trans-decenal (HDE) and especially the most toxic and most abundant compound 4-hydroxy-2-trans-nonenal (HNE) resulting from the oxidation of thermally treated commercial corn oil. The selection of commercial corn oil was based on the fact that it contains very high level of linoleic acid (~60% or more) which is a precursor for the homologues of the toxic α, β-unsaturated-hydroxyaldehydes and this oil is widely used by the public and the industry for heat treatments of various food items.

Part II: Experiments

Materials and Methods

A) Chemicals and Instruments

Mazola Corn Oil was purchased from a local store. (Roseville, MN); 2, 4-dinitrophenylhydazine was obtained from Eastman Kodak Co. (Rochester, NY). HPLC-grade methanol, HPLC-grade water, HPLC-grade dichloromethane, thichloroacetic acid, and boron trifluoride-methanol solution were purchased from Sigma Chemical Company (St. Louis, MO); sodium thiosulfate and glacial acetic acid came from Fisher Scientific (Fair Lawn, NJ); hydrochloric acid and potassium iodide were from Mallinckrodt Baker Inc. (Paris, KY). HPLC-grade hexane was obtained from EMD Chemicals, Inc. (Gibbstown, NJ). No. 1 filter paper and 0.45um syringe filters were from Whatman Ltd. (Kent, England). Thin layer chromatography (TLC) plates were obtained from EMD Millipore, Inc. (Billerica, MA). HNE was purchased from Cayman Chemical Co. (Ann Arbor, MI). Rosemary extracts was obtained from Kemin Industries, Inc. (Des Moines, IA); Carnosol and carnosic acid was purchased from LKT Laboratories, Inc. (Saint Paul, MN) and Santa Cruz Biotechnology, Inc. (Dallas TX) respectively, Citric acid and L-ascorbyl palmitate was purchased from MC/B Manufacturing Chemists (Norwood, OH) and MP Biomedicals, LLC (Solon, OH) respectively.

The gas chromatograph contained 18835B capillary inlet system (5830A Gas Chromatograph, Hewlett-Packard, Saginaw, MI). Supelco SP-2560 capillary column (100m×0.25mm, 0.20μm) with splitless injection and FID. The injection temerature was 230°C, and FID temperature 250°C.

The HPLC system contained a sample injector (712 WISP, Waters, Milford, MA), a solvent delivery system (9050, Varian, Walnut Greek, CA) and a UV-Vis detector (9010, Varian). The HPLC column was Ultrasphere ODS (5×4.6mm, 25cm, Hichrom, Berkshire, UK). Detailed operating parameters were provided later in the method section: General Outline of the Methods Used.

B) Methods

Various Antioxidants Preparations and Their Mixtures Used in the Experiments

1) 50ppm Rosemary extracts (RE): 5mg RE added to 10mL ethanol
2) 100ppm Rosemary extracts (RE): 10mg RE added to 10mL ethanol
3) 200ppm Tertiary Butylhydroquinone (TBHQ): 20mg TBHQ added to 10mL ethanol
4) 200ppm Tertiary Butylhydroquinone (TBHQ)+50ppm Citric acid (CA): 20mg TBHQ+5mg CA added to 10mL ethanol
5) 200ppm Tertiary Butylhydroquinone (TBHQ)+100ppm Ascorbyl palmitate (AP): 20mg TBHQ+10mg AP added to 10mL ethanol
6) 200ppm Tertiary Butylhydroquinone (TBHQ)+50ppm Citric acid (CA)+100ppm Ascorbyl palmitate (AP): 20mg TBHQ+5mg CA+10mg AP added to 10mL ethanol

Heating Condition of Commercial Corn Oil Samples

5g corn oil and 0.5mL natural or synthetic antioxidant samples were mixed using a shaker for 10 minutes before heating. All samples were heated in a 5cm diameter and 15cm height glass test tubes in a 185°C preheated sand bath. The test tubes were inserted down 10cm into the 200°C sand bath. Once the temperature reached 185°C (20 minutes), the temperature was held constant for the entire heating time of 1, 2, 4 and 6 hours. The unheated samples (0, heating time) used as reference for the start of the thermally induced oxidation process.

Peroxide Value Determination

The peroxide value in unheated corn oil was measured by the recommended method of the American Oil Chemists' Society to assess the oxidation process at the start of the experiments in the oil [163].

Detailed Methods of Peroxide Value Determination

In triplicate, 1g unheated corn oil samples were placed into a 125mL Erlenmeyer flask, then 15mL of a solution of 6 parts glacial acetic acid and 4 parts USP chloroform

was added. Then 1mL saturated aqueous potassium iodide (KI) solution and 15mL water were added and gently mixed for 1 minute. The solution was titrated with 0.002N sodium thiosulfate in the presence of starch indicator until the color disappeared. The peroxide value (PV) was expressed as milliequivalent PV per 1000g/oil.

Fatty Acid Methyl Ester (FAME) Formation and Gas Chromatography

Fatty acid distribution of unheated oils and fats was determined by gas chromatography. Trans esterification was carried out by the method of Metcalfe et al [164]. In this method 3mL of BF_3-Methanol (14% BF_3 in methanol) added with 2 drops of each unheated oils or fats in a 20mL test tube in duplicate. The test tubes were capped and shaken vigorously. Then they were placed in boiling water bath for 1h. After cooling, 3mL distilled water was added to 10mL hexane and the mixture was shaken for 10minutes. After separation into 2 layers, the top hexane layer was removed and saved. The hexane samples were dried with the addition of 1-2g of sodium sulfate. 5μL of the dry hexane samples were injected into GC and the fatty acid distribution was measured by comparison with the retention times of fatty acid standards. Corn oil FAME were sepated on a Supelco SP-2560 capillary column (100m×0.25mm, 0.20μm) with splitless injection and FID. The injection temerature was 230°C, and FID temperature 250°C.

Thiobarbituric Acid (TBA) Assay

TBARS is a common, simple, and sensitive colorometric method used to measure the general extent of lipid oxidation. The method is based on the measurement of the chromagen that is formed from condensation of two molecules of thiobarbituric acid with one molecule of malondialdehyde (MDA). However, secondary lipid peroxidation products other than MDA also react with thiobarbituric acid forming condensation products measured in the test. Although the TBA test measures all aldehydes and related carbonyl compounds, it is MDA which is used to standardize the test and the results are expressed as MDA equivalents.

The thiobarbituric acid reactive substances (TBARS) method of Buege and Aust was used to monitor the formation of secondary lipid peroxidation products in corn oil

after heating for 0, 1, 2, 4, 6h in the presence and absence of various antioxidants and their mixtures. The TBA reagent was prepared with equal volumes of 15% w/v trichloroethanoic acid (TCA), 0.375% w/v 2-thiobarbituric acid (TBA), and 0.25N hydrochloric acid. Duplicate of 200uL of oil were combined with 4mL of TBA- reagent, and the mixture was heated for 15min in a boiling water bath. Absorbance of the sample was measured at 535nm by an UV/Vis spectrophotometer. A calibration curve was prepared with pure malondialdehyde (MDA) standard, and the results are expressed as MDA equivalents [165,166].

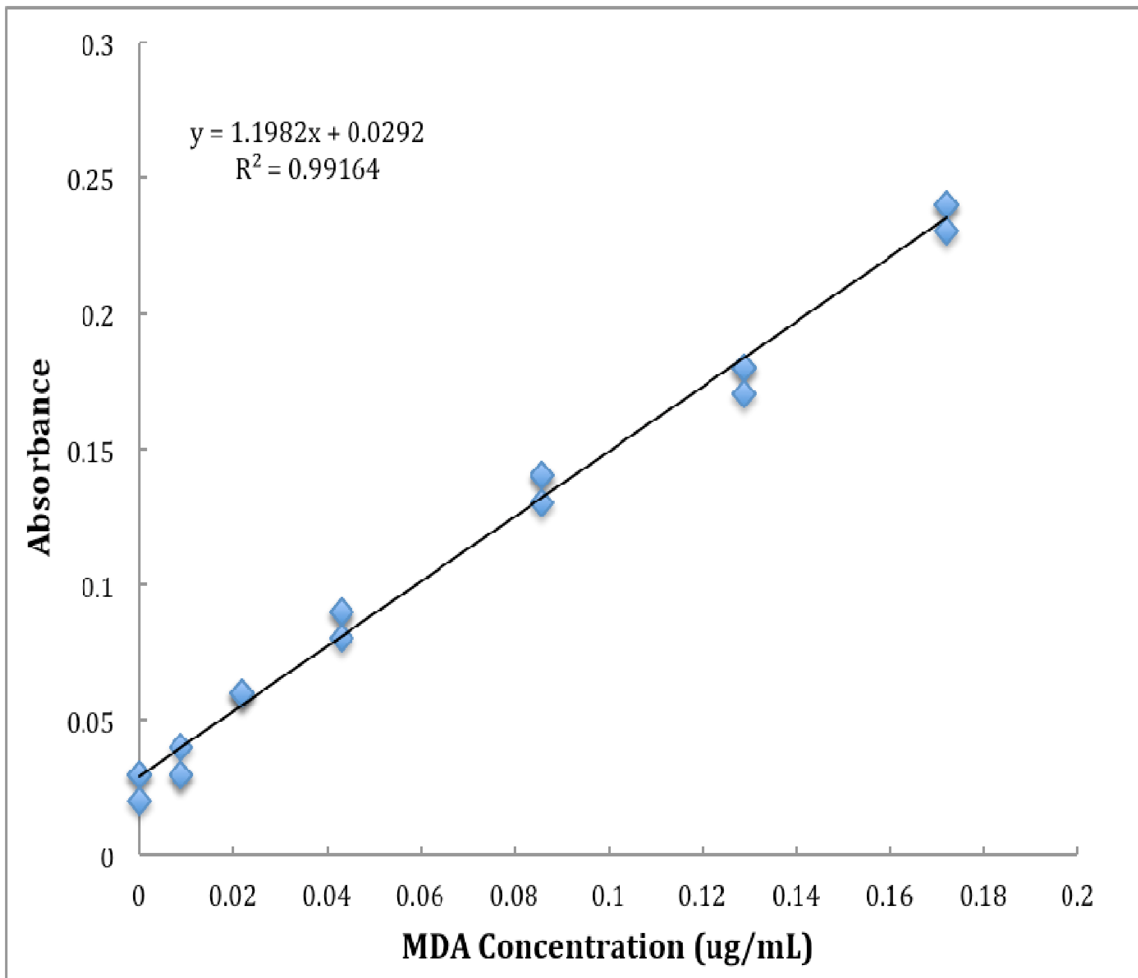


Figure 10. Standard Curve prepared for measuring the general oxidation process using the TBA assay for the expression of TBARS as MDA equivalents

The Method for Measuring the Polar and Nonpolar Lipophilic Aldehydes and Related Carbonyl Compounds Including the Toxic α, β-Unsaturated Hydroxyaldehydes such as 4-Hydroxy-2-*Trans*-Nonenal (HNE) and Its Three Homologues

In this method, the secondary lipid peroxidation products, aldehydes, acetones and related carbonyl compounds, were reacted with 2,4-dinitrophenylhydrazine (DNPH) to form 2,4-dinitropheylhydrazones. The DNPH derivatives were separated by thin-layer chromatography (TLC) into polar, nonpolar compounds and osazones. The polar and nonpolar secondary oxidation DNPH products were further separated on a C18 reverse phase HPLC column and were detected at 378nm.

Brief description of the method: Corn oil sample was incubated overnight with the DNPH reagent by shaking in a shaker at room temperature. The mixture was extracted with 75% methanol in water to separate the 2, 4-dinitrophenylhydrazones from the oil. Then it was followed by extraction with dichloromethane to separate the aqueous components from the lipophilic aldehydes and carbonyl compounds in the organic phase. After extraction, TLC and HPLC methods were used to separate and measure the DNPH derivatives quantitatively.

General Outline of the Methods Used:

1. Preparation of 2, 4-dinitrophenylhydrazine (2, 4-DNPH) reagent
2. Reaction of corn oil and oil antioxidant mixtures with DNPH reagent overnight
3. Extraction of 2, 4-DNPH derivatives from samples with 75% methanol in water three times to separate oil from sample
4. Extraction of lipophilic 2, 4-DNPH derivatives with dichloromethane three times
5. Preliminary separation of 2, 4-DNPH derivatives of oil-derived carbonyl compounds into polar and nonpolar compounds and osazones (sugar derivatives) by thin-layer chromatography (TLC) on silica gel plates developed with dichloromethane
6. Extraction of polar and nonpolar compounds from the TLC plates by methanol three times

7. Concentration of methanol by gas N_2 to 1mL

8. Analysis of 2, 4-dinitrophenylhydrazones of polar and nonpolar carbonyl compounds by high performance liquid chromatography (HPLC)

a) Polar compounds: Injection of 100uL aliquots onto a C18 reverse phase HPLC column using isocratic elution for 10min with 50% methanol followed by a linear gradient to 100% methanol for 30min at a flow rate of 0.8mL/min

b) Nonpolar compounds: Injection of 100uL aliquots onto a C18 reverse phase HPLC column using isocratic elution for 10min. with 75% methanol followed by a linear gradient to 100% methanol for 30min at a flow rate of 0.8mL/min

9. Identification of carbonyl compounds as DNPH derivatives of derived from the corn oil by comparison of retention times to pure standards and co-chromatography

Detailed Methods:

Preparation of DNPH Reagent

Fresh dinitrophenylhydrazine (DNPH) reagent was prepared by combining 2,4-dinitrophenylhydrazine (recrystallized from methanol four times) with 1N hydrochloric acid in a glass - stoppered Erlenmeyer flask. The mixture was heated in a 50°C water bath for 1 hour.

After cooling, DNPH reagent was extracted with HPLC-grade hexane to remove impurities four times. The volume of hexane used for each extraction was approximately 80% of the total volume of the reagent. The prepared liquid reagent was used immediately.

Preparation of DNPH Reagent Blank and the DNPH-Acetone Standard

The reagent blank and DNPH-acetone standard were prepared by combining 3mL HPLC-grade water or 3mL 1% acetone with 3mL freshly prepared DNPH reagent. The mixtures were incubated at room temperature overnight in the dark. After incubation, DNPH derivatives were extracted with 5mL CH_2Cl_2 three times, and the organic phase was separated from the aqueous phase by centrifugation 10 minutes at 2000r/min. The separated compounds were combined in conical graduated test tubes and evaporated

under N_2 gas to a volume of 500µL. The concentrated samples were stored overnight and tightly covered with parafilm at 0°C, if not used immediately.

Formation of 2,4-Dinitrophenylhydrazones of Lipophilic Aldehydes and Related Carbonyl Compounds from Unheated and Heated Corn Oil and Corn Oil Antioxidant Mixtures

1g corn oil was reacted with 5mL of freshly prepared DNPH reagent in a 25mL flask. The mixture was incubated at room temperature [164] overnight in the dark with shaking at ~140 oscillations/min in a shaking water bath. After incubation the hydrazones were extracted with 75% methanol in water three times and extracted with dichloromethane three times individually as follows:

1. 5mL 75% HPLC-grade methanol (MeOH) /25% HPLC-grade water was added to incubated mixture. The tube was mixed with a Vortex mixer, and the contents transferred to a 15mL test tube. The mixture was centrifuged to separate the aqueous and oil phases (10min at 2000rpm) and the oil layer removed to clean test tubes.
2. 5mL 75% MeOH was added to the original test tube to rinse any residue into the oil layer from the previous extraction. After mixing well, the aqueous layer was removed and added to the same layer from the previous step.
3. 5mL 75% MeOH was added to the remaining oil fraction, mixed and separated (10min, 2000rpm). The aqueous layer was placed in a clean 15mL test tube. The aqueous fractions from the previous step were added.
4. 5mL HPLC-grade dichloromethane (CH_2Cl_2) was added to the aqueous fractions from MeOH extraction. After mixing with a Vortex mixer, the mixture was divided evenly between two 15mL test tubes. The tubes were centrifuged (10min, 2000rpm) to separate the organic and aqueous phases, and the organic phase (CH_2Cl_2 layer) was removed and transferred to 15mL conical graduated test tubes.
5. The CH_2Cl_2 extraction was repeated twice more, using 5mL CH_2Cl_2 the first time, then 2.5mL CH_2Cl_2 the second time. The dichloromethane fractions were added to the conical test tubes.
6. The pooled extracts in CH_2Cl_2 were evaporated under nitrogen gas to 500µL.

7. The condensed samples were stored overnight at 0°C in test tubes tightly sealed with parafilm, if not immediately used for TLC separation.

Separation of Polar, Nonpolar Hydrazones and Osazones by Thin-Layer Chromatography (TLC)

The total amount of concentrated dichloromethane extract was applied onto two TLC (Silica gel 60, aluminum backed, 20cm × 20cm, 0.2mm thickness). The sample was applied in a very thin line across the plate with a 250uL micropipette attached to a Hamilton syringe with a piece of flexible rubber tubing.

Figure 11 shows a diagram of a typical separation on the TLC plate. DNPH reagent blank and DNPH acetone standard were spotted on the plate as references. Typical thin-layer chromatography plate was used for separation of polar and nonpolar DNPH derivatives and osazones from samples.

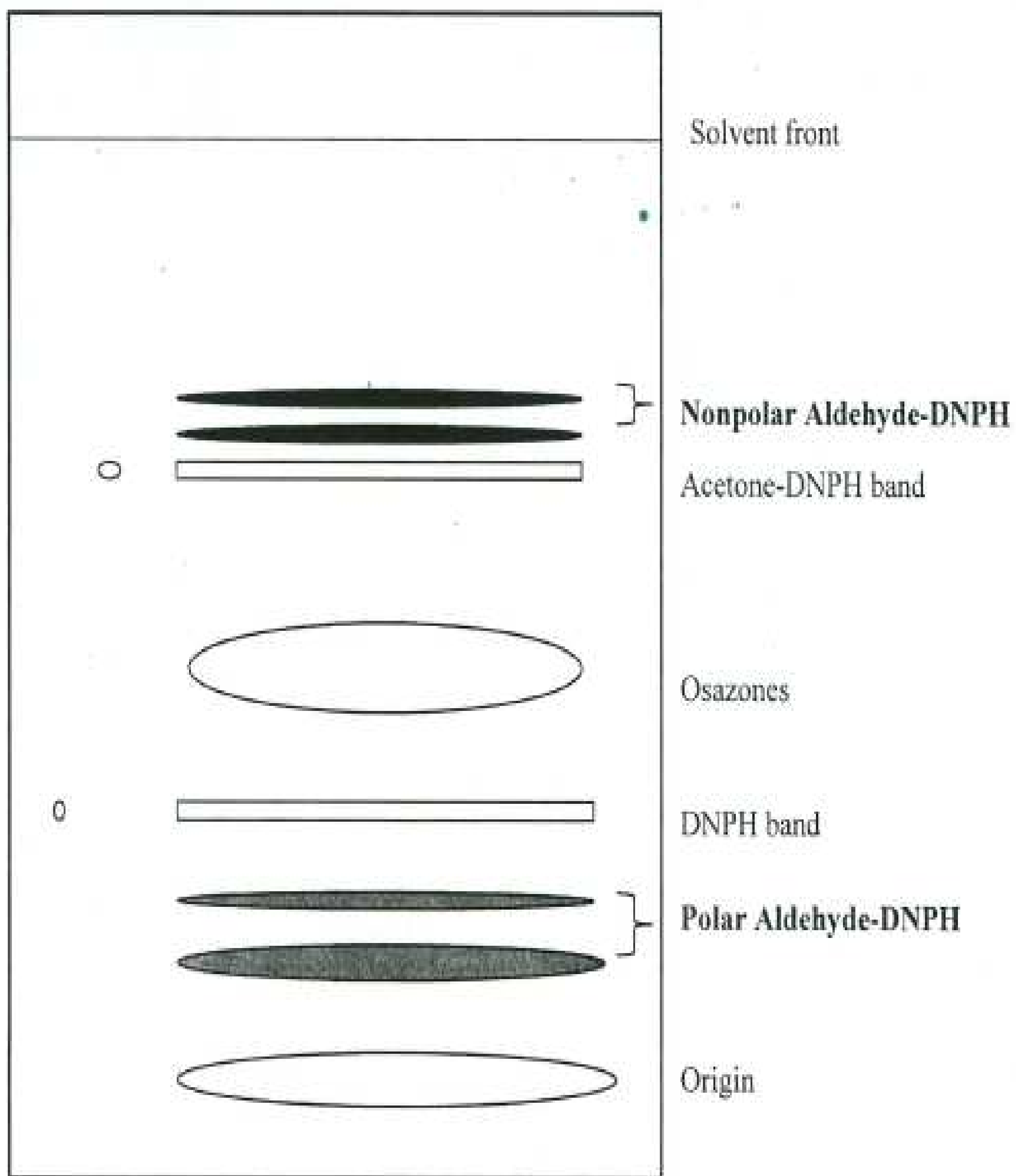


Figure 11. Typical diagram of thin-layer chromatography (TLC) plate

The TLC Plates were developed in dichloromethane in a glass tank. The polar and nonpolar aldehydes and related carbonyl compounds were separated by comparison to Rf values of acetone-DNPH (0.55) and purified DNPH reagent (0.23). Polar carbonyl compounds, including the hydroxyaldehydes, were located between the origin and Rf value 0.23 (just below the DNPH band). Nonpolar carbonyl compounds were located in the region above Rf value 0.55 (just above the DNPH-acetone band) and about 2cm below the solvent front. The polar and nonpolar regions were cut from the plate, then cut into small pieces and placed in small Erlenmeyer flasks. The compounds were eluted from TLC plate with 5mL of 100% MeOH three times. The polar and nonpolar extracts were collected separately in 15mL test tubes, and then reduced the volume under nitrogen gas to about 0.7mL and transferred to a volumetric flask and made it to 1mL exactly. The sample was stored at 0°C tightly sealed with parafilm until injection onto HPLC column.

Separation of Polar and Nonpolar DNPH Derivatives of Lipophilic Aldehydes and Related Carbonyl Compounds by High Performance Liquid Chromatography (HPLC)

100uL aliquots of polar or nonpolar derivatives were injected into a HPLC reverse-phase C18 column, equipped with a guard column.

a) For polar compounds, 10 min of isocratic elution with methanol/water (50:50, v/v) was followed by a linear gradient to 100% methanol for 20 min, then 100% methanol for an additional 10 min at a flow rate of 0.8mL/min. Absobance was measured at 378 nm.
b) For nonpolar compounds, 10 min of initial isocratic elution was methanol/water (75:25, v/v) was followed by a linear gradient to 100% methanol for 20 min, then 100% methaol for an additional 10 min at a flow rate of 0.8mL/min. Absorbance was also measured at 378 nm.

Pure HNE standards were injected onto HPLC column prior to the analysis of the samples.

Identification of compounds derived from corn oil samples was made by comparisons of the retention times of peaks to the known retention times of the standards and co-chromatography with pure standards.

Figure 12. Typical HPLC chromatography of the polar aldehydes in corn oil after 6 hours at 185°C

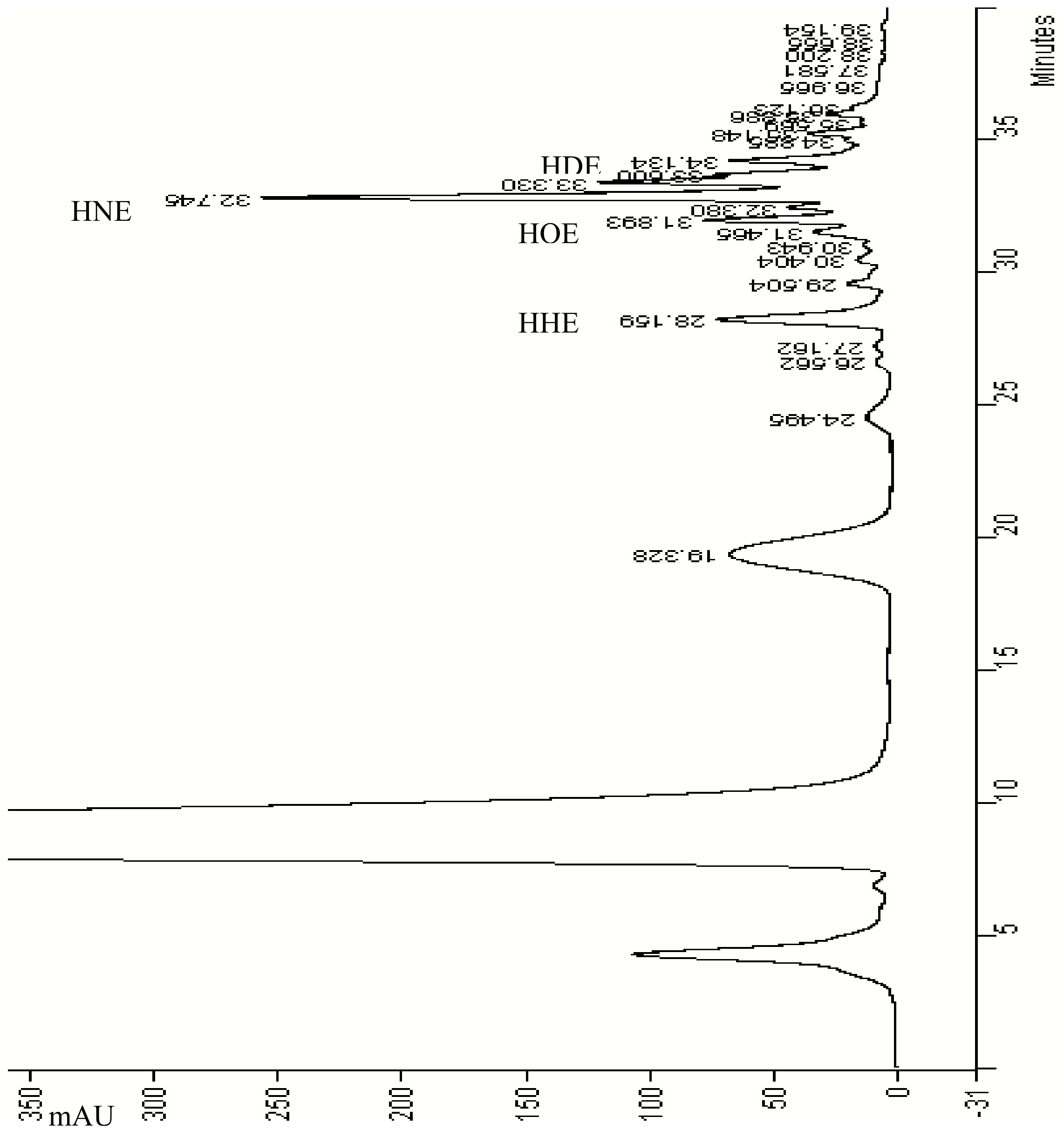


Figure 13. Typical HPLC chromatography of the non-polar aldehydes in corn oil after 6 hours at 185°C

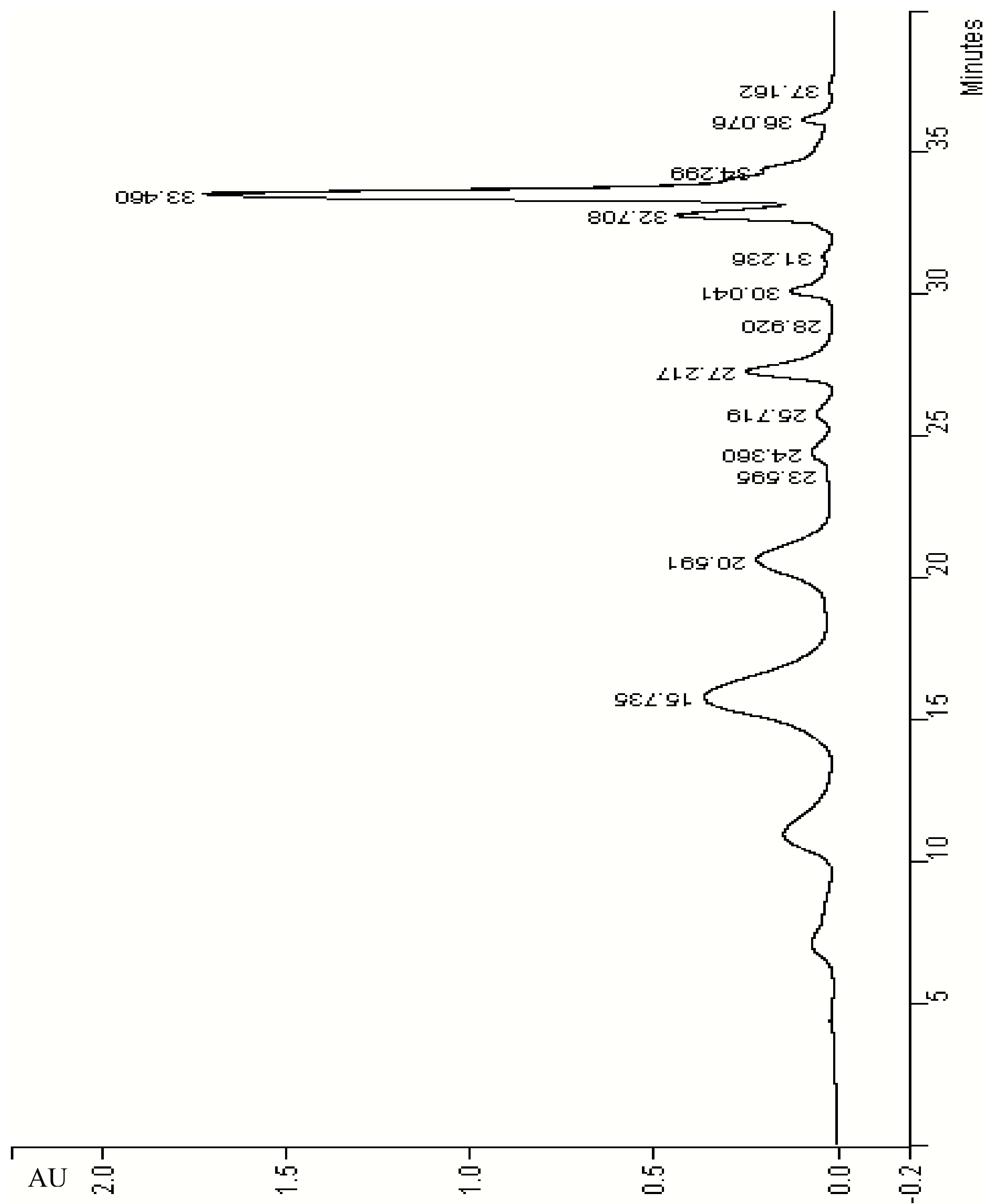
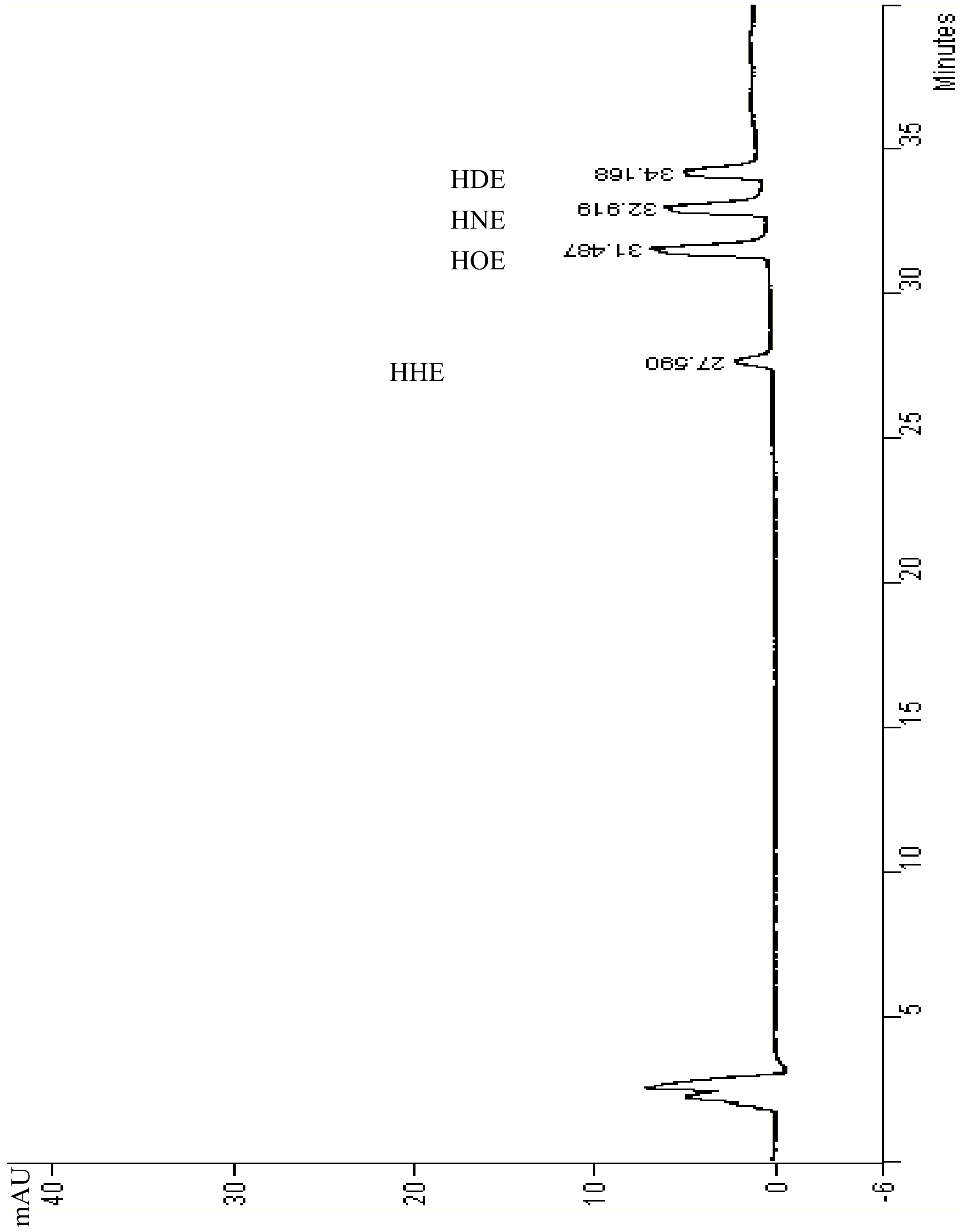


Figure 14. Retention times of the HPLC separation of the mixture of HHE, HOE, HNE and HDE standards



Statistical Analysis

All experiments were conducted in duplicates and the data are expressed as mean ± standard deviation. ANOVA two-way analysis was used to determine if there were significant differences between the groups. A Tukey's test was conducted to calculate the p values. Different letters indicate significant differences ($p<0.05$).

Results

The present experiments studied the differences of secondary oxidation product formations including the four homologues of α, β-unsaturated-hydroxyaldehydes, such as HHE, HOE, HNE and HDE formations in commercial corn oil in the presence and absence of synthetic antioxidant and their mixtures and natural antioxidant at 185°C.

Results of the preliminary experiments using the TBARS method was used to show the maximum amount of lipophilic aldehydes and related carbonyl compound formations in corn oil after 6 hours of heat treatment at 185°C in the presence and absence of synthetic and natural antioxidants.

A. Preliminary Experiments

Peroxide Value

Table 3 shows the peroxide values (PV) of unheated commercial corn oil using 4 identical samples using titrations in triplicate. The PV was in the range between 1.70 and 1.76 milliequivalents of peroxide per 1000g of oil. These values were relatively low which indicate that the corn oil was minimally oxidized before the beginning of its thermal oxidation process.

Table 3. Peroxide Value (PV) of Unheated Corn Oil Samples

Corn Oil Samples (n=3)	Average of triplicate of four identical samples of corn oil (PV=milliequivalents/1000g)
1 a, b, c	1.72±0.02
2 a, b, c	1.70±0.03
3 a, b, c	1.76±0.02
4 a, b, c	1.74±0.01
Average of 12 measurements	1.73±0.02

Fatty Acid Distribution

The fatty acid distribution of commercial corn oil was used mainly to measure the concentrations of linoleic and linolenic acids since these fatty acids were the precursors for the four α, β-unsaturated aldehyde homologues. Table 4 shows the retention times of palmitic, stearic, oleic and linoleic, linolenic and arachidonic acids standards used in the GC method.

Table 4. Retention Times of Fatty Acids by GC Method

Fatty acid	Retention time (min)
Palmitic acid	1.11
Stearic acid	1.69
Oleic acid	1.82
Linoleic acid	2.05
Linolenic acid	2.49
Arachidonic acid	2.77

Table 5 shows the percent fatty acid distribution measured by comparison to the retention times of the standards.

Table 5. Corn Oil Fatty Acid Distribution

Corn Oil Fatty Acid Distribution (%)		
Retention Times (min)	Fatty Acid	Average Area of triplicate measurement (%)
1.07	Palmitic Acid	19.3±0.04
1.65	Stearic Acid	2.8±0.08
1.78	Oleic Acid	34.2±0.12
2.05	Linoleic Acid	43.7±0.28

It was found that in corn oil linoleic acid (43.7%) was in the highest concentration which followed by oleic, palmitic and stearic acids. Linolenic and arachidonic acids were not found in the corn oil samples.

Thiobarbituric Acid Reactive Substances (TBARS)

Results of the TBA assay showed the formation of maximum amount of secondary oxidation products in corn oil after 6 hours of heat treatment compared to 0, 1, 2 and 4 hours at 185°C.

Duplicate samples of corn oil samples in the presence and absence of antioxidants were heated to the same time periods (0, 1, 2, 4 and 6 hours) at 185°C. The TBARS concentrations were shown for corn oil in the presence and absence of synthetic and natural antioxidants in Figures 15-23.

The figures clearly showed that the secondary oxidation products increased with the increasing of heating time. The maximum amount of TBARS concentration was found at 6 hours.

The TBARS concentration was used to determine the relation to heating time of the rate of oxidation needed to produce the maximum amount of lipophilic aldehydes and related carbonyl compounds.

Since the standard curve for TBARS was made with malondialdelyde (MDA) standard, the results were expressed by MDA equivalents.

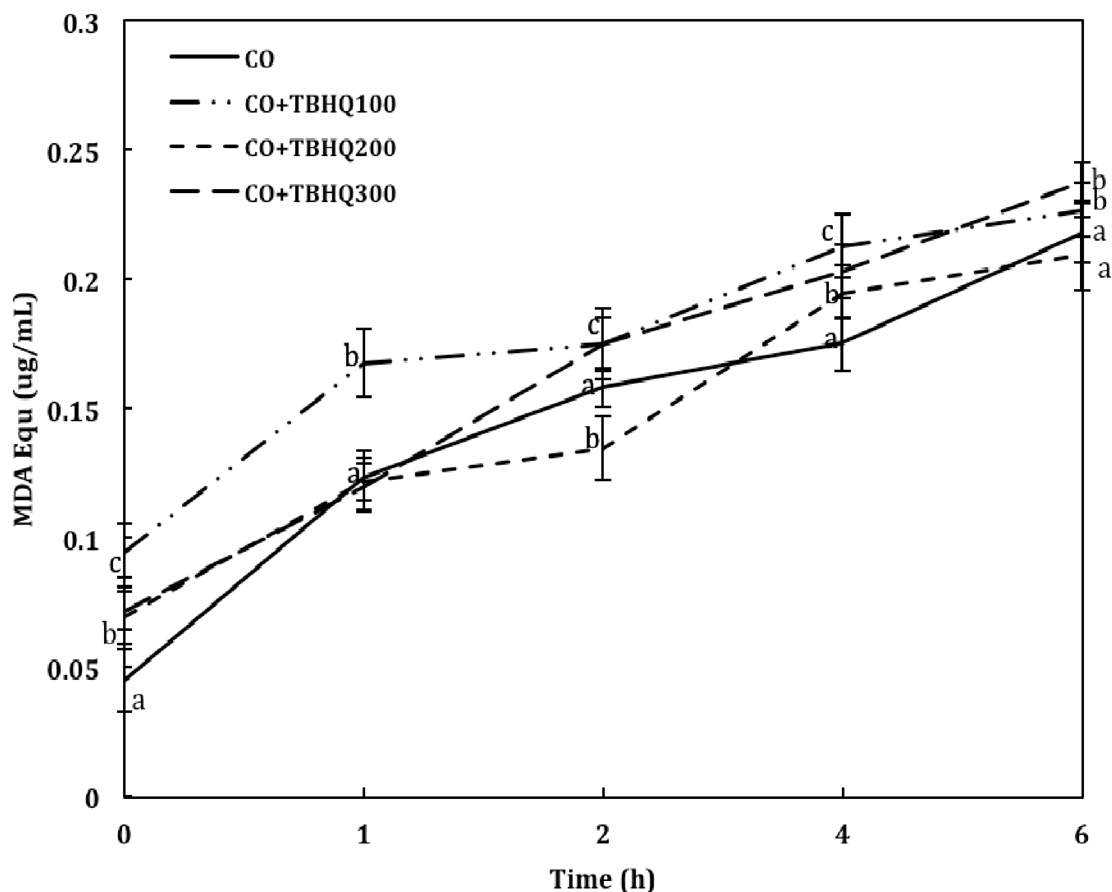


Figure 15. Comparison of TBARS expressed as MDA equivalents of heat treated corn oil for 0, 1, 2, 4 and 6 hours at 185°C in the presence and absence of 100ppm, 200ppm and 300ppm TBHQ

Result showed that by the addition of 200ppm TBHQ to the oil the oxidation process was reduced at 2 hours. However, 100ppm and 300ppm TBHQ seemed to have no effects on reducing the process of the oxidation. Therefore only 200ppm TBHQ was used in the further experiments.

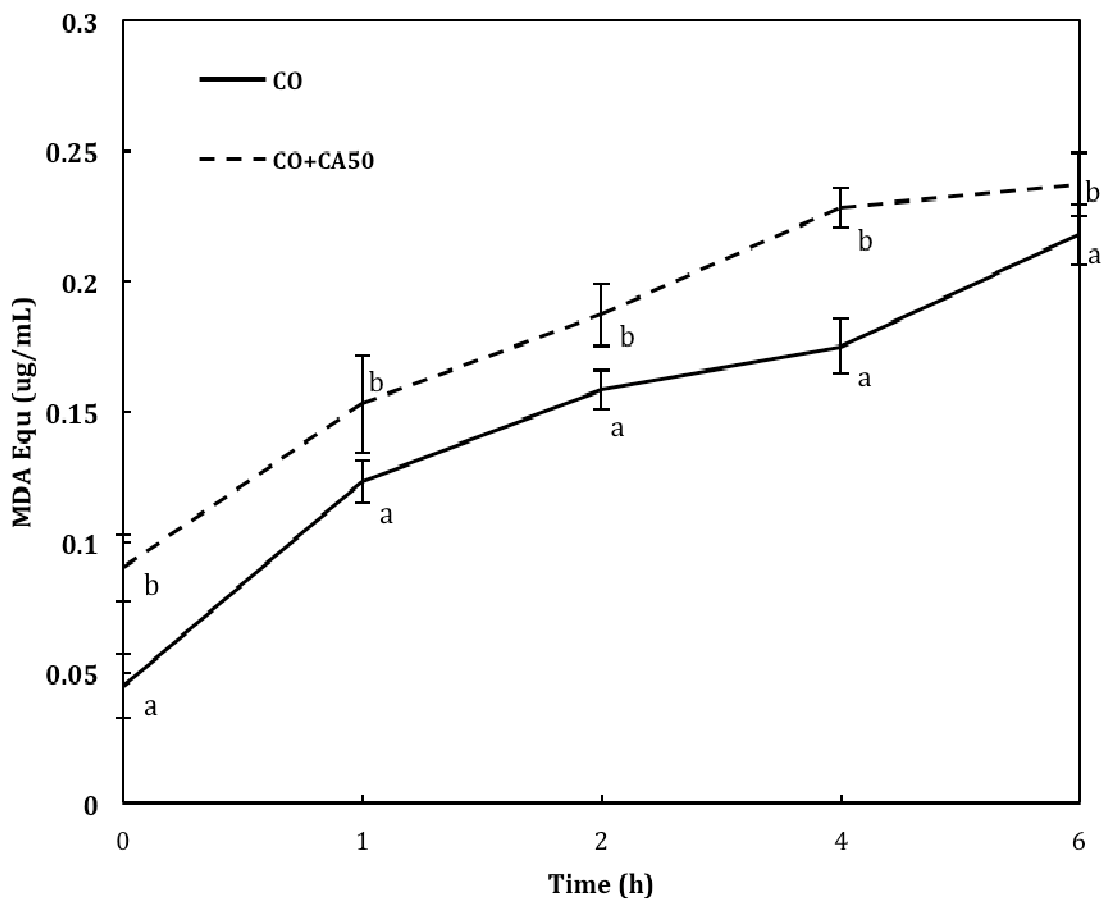


Figure 16. Comparison of TBARS expressed as MDA equivalents of heat treated corn oil for 0, 1, 2, 4 and 6 hours at 185°C in the presence and absence of 50ppm citric acid (CA)

Results showed that the addition of CA alone had no antioxidant effect, and it may even have pro-oxidation effect. Since CA was a secondary antioxidant and can be synergist together only with a primary antioxidant.

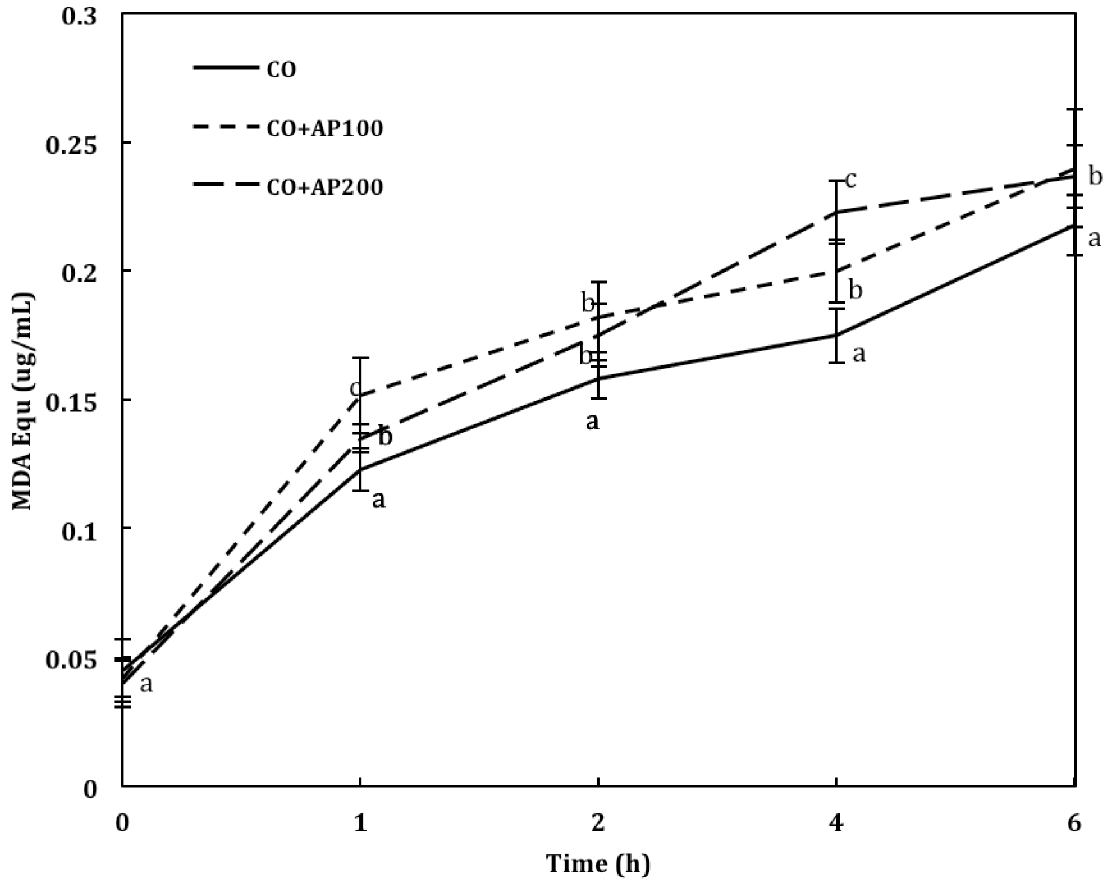


Figure 17. Comparison of TBARS expressed as MDA equivalents of heat treated corn oil for 0, 1, 2, 4 and 6 hours at 185°C in the presence and absence of 100ppm and 200ppm ascorbyl palmitate (AP)

The results indicated that there were no differences between 100ppm and 200ppm AP on reducing oxidative process. It showed that AP alone might even had a pro-oxidation effect. Since AP was a secondary antioxidant and can only be synergist together with a primary antioxidant.

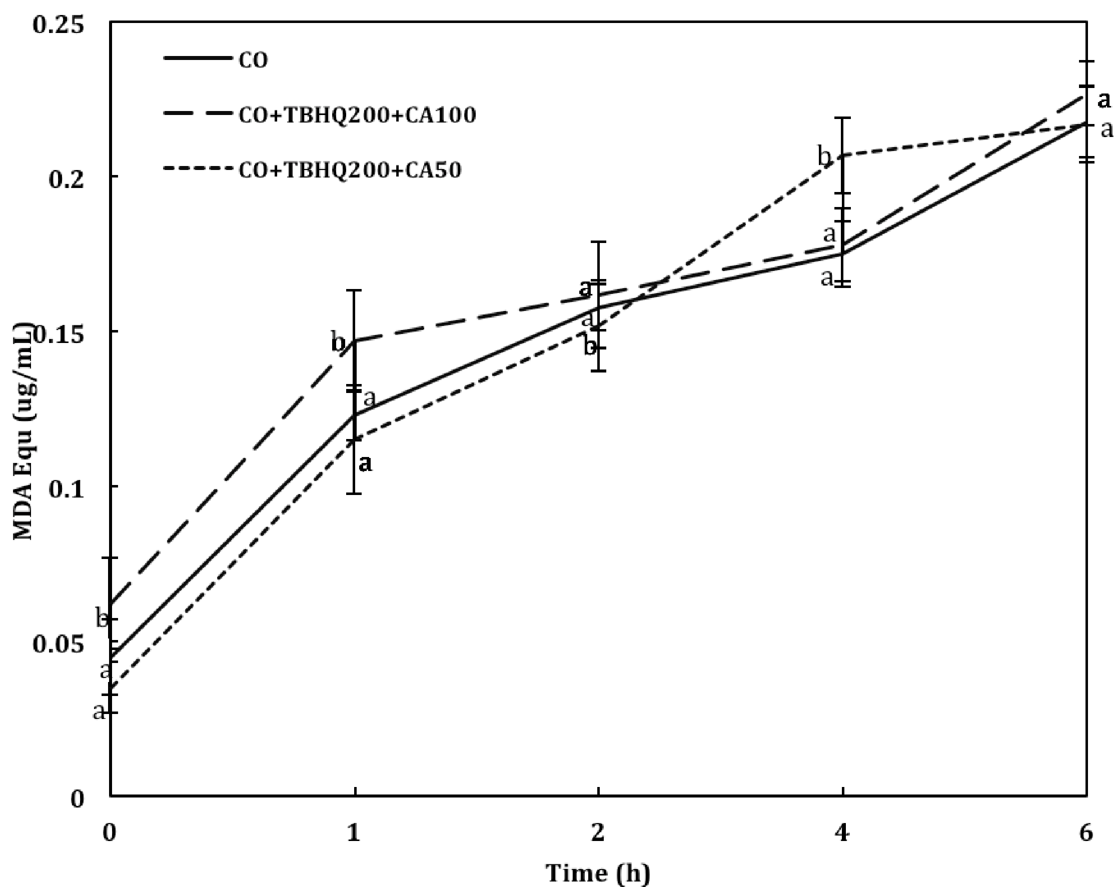


Figure 18. Comparison of TBARS expressed as MDA equivalents of heat treated corn oil for 0, 1, 2, 4 and 6 hours at 185°C in the presence and absence of 200ppm TBHQ and 50ppm or 100ppm citric acid (CA)

Results indicated that there was no effect by the addition of higher amount of CA to 200ppm TBHQ on the reduction of the oxidative process.

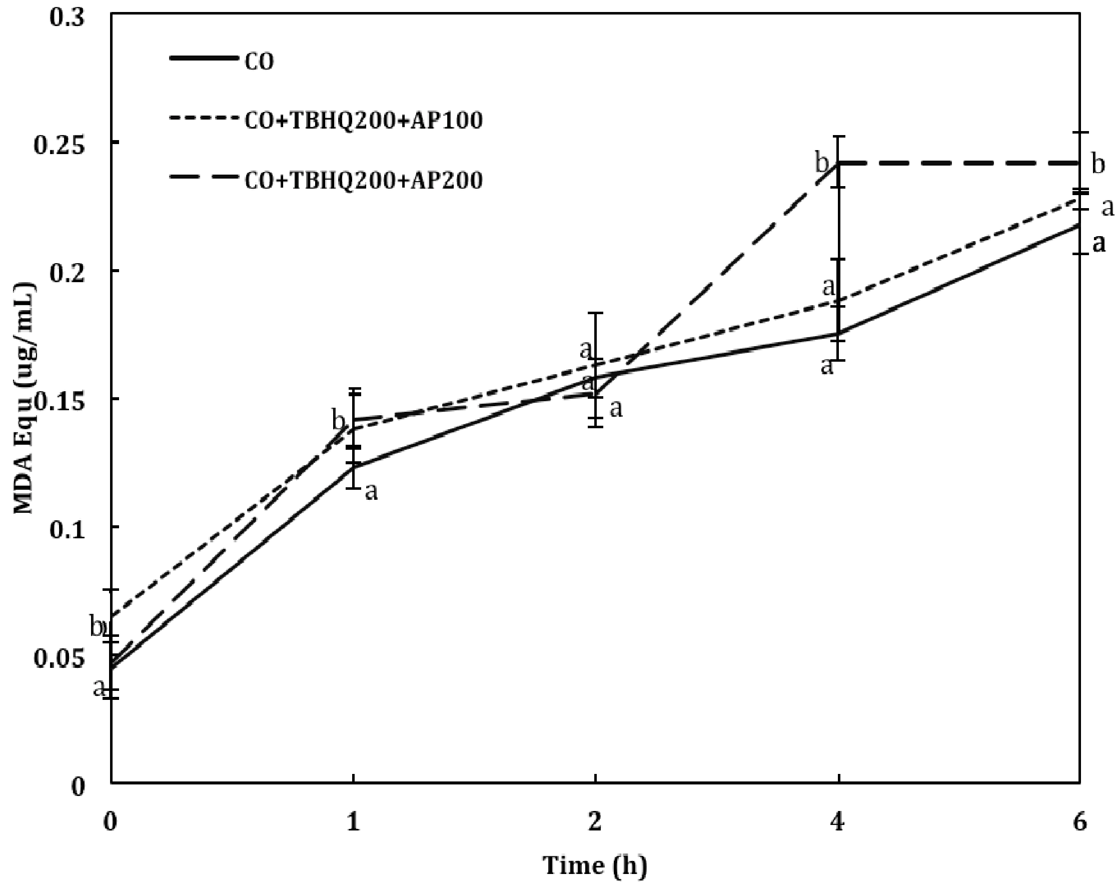


Figure 19. Comparison of TBARS expressed as MDA equivalents of heat treated corn oil for 0, 1, 2, 4 and 6 hours at 185°C in the presence and absence of 200ppm TBHQ and 100ppm or 200ppm ascorbyl palmitate (AP)

The results showed that the mixture of 100ppm or 200ppm AP with 200ppm TBHQ did not suppress the oxidation process measured up to 6 hours.

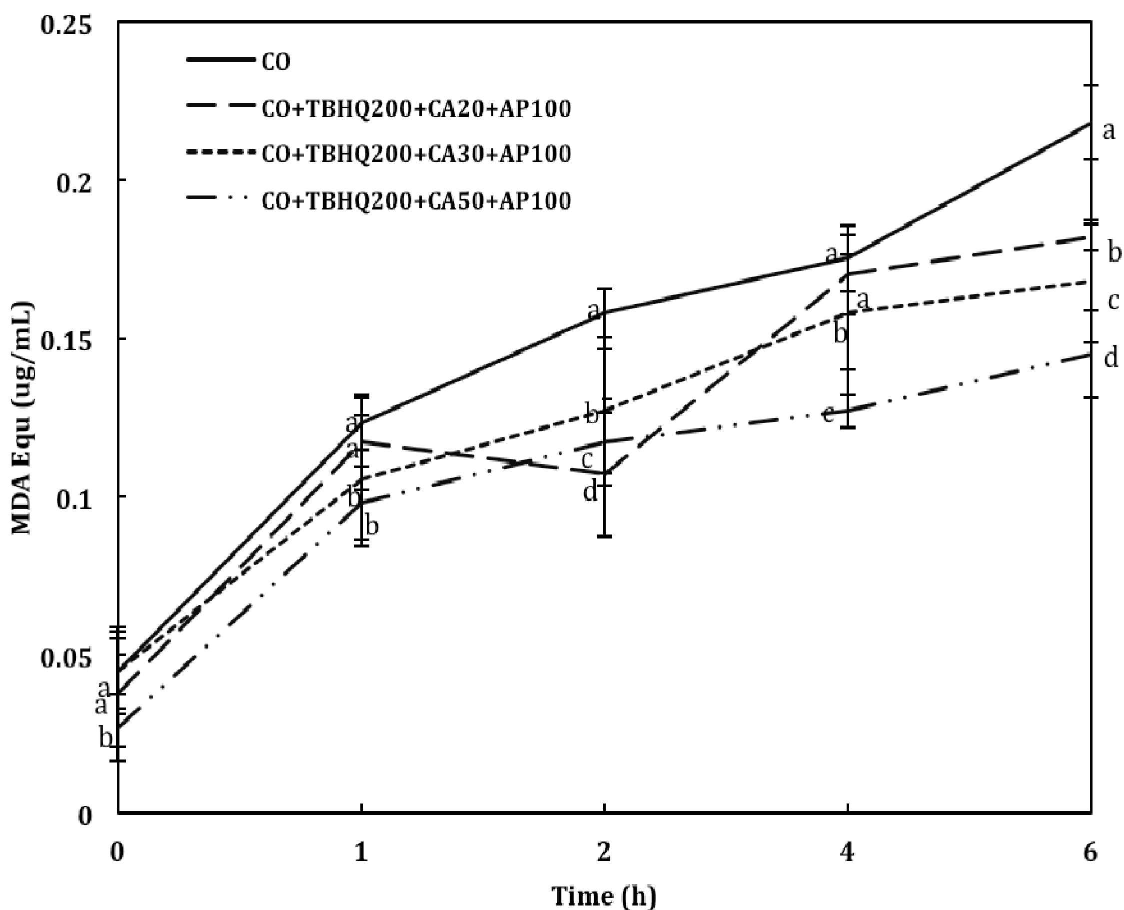


Figure 20. Comparison of TBARS expressed as MDA equivalents of heat treated corn oil for 0, 1, 2, 4 and 6 hours at 185°C in the presence and absence of a mixture of 200ppm TBHQ, 100ppm ascorbyl palmitate (AP) and 20ppm or 30ppm or 50ppm citric acid (CA)

Results showed the addition of 50ppm CA in the presence of constant 200ppm TBHQ and 100ppm AP produced the best suppression of the oxidation at 1, 2, 4 and 6 hours of heating the corn oil at 185°C. The oxidation was suppressed by 33% compared to corn oil after 6 hours of heating treatment.

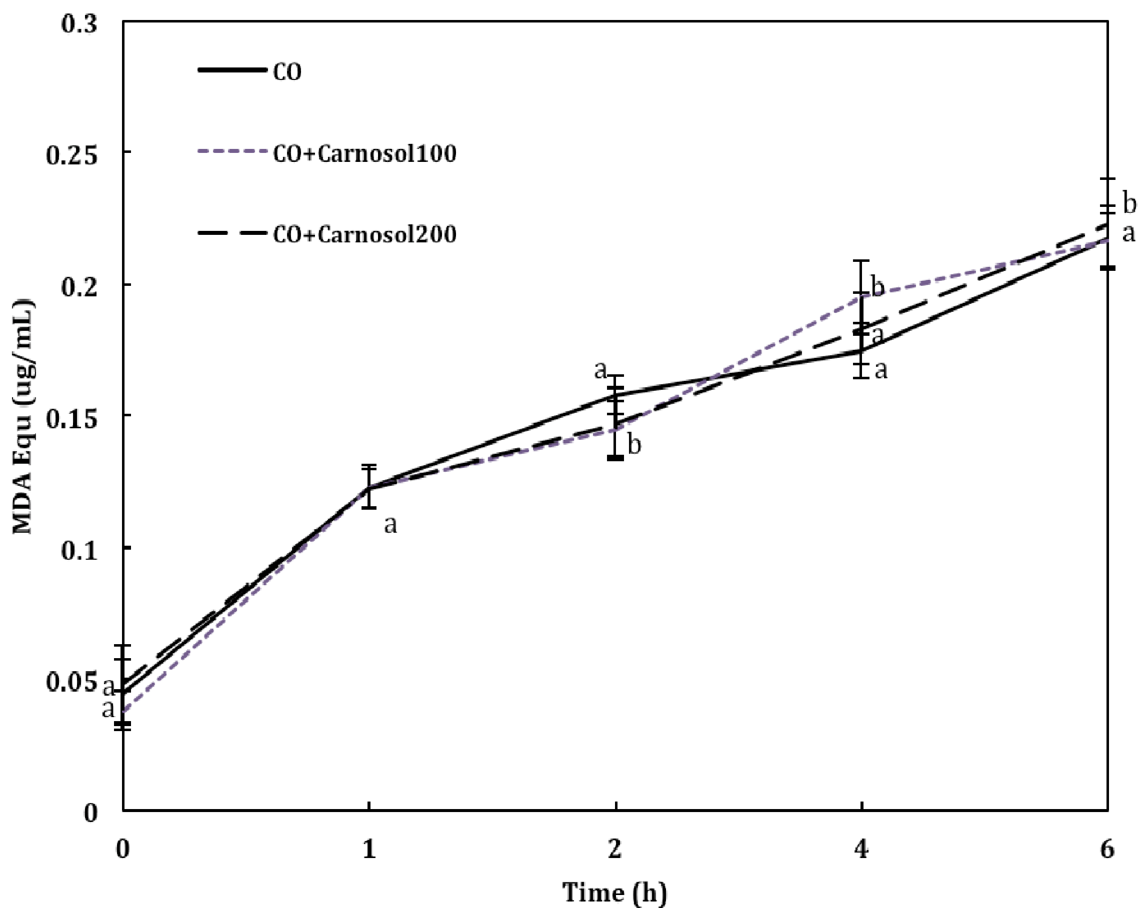


Figure 21. Comparison of TBARS expressed as MDA equivalents of heat treated corn oil for 0, 1, 2, 4 and 6 hours at 185°C in the presence and absence of 100ppm or 200ppm carnosol

The results showed that there was no effect on reducing the oxidation process by the addition of either 100ppm or 200ppm carnosol to the oil compared to the oxidation process without added carnosol to the oil. Therefore carnosol alone as one of the main components in rosemary extracts was not used in further experiments.

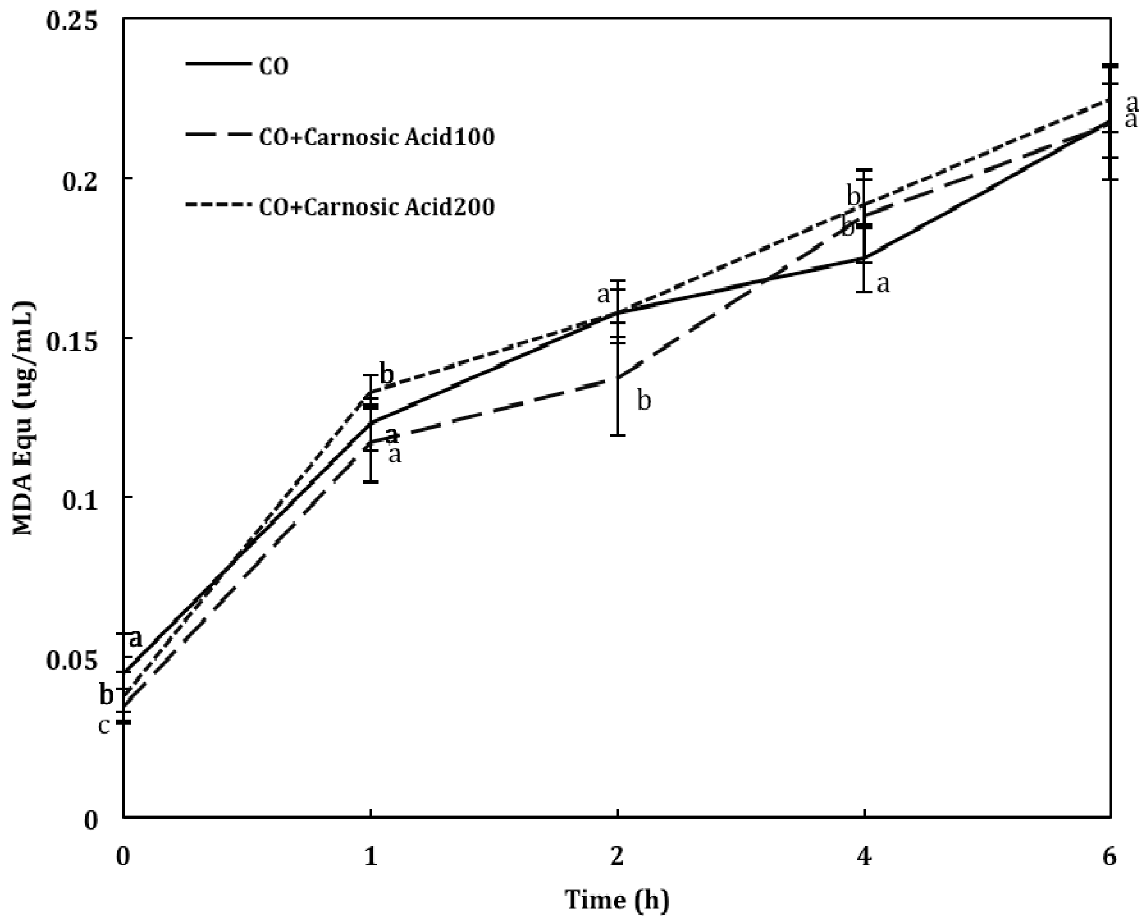


Figure 22. Comparison of TBARS expressed as MDA equivalents of heat treated corn oil for 0, 1, 2, 4 and 6 hours at 185°C in the presence and absence of 100ppm or 200ppm carnosic acid

Results indicated that there was no effect on reducing the oxidation process by the addition of either 100ppm or 200ppm carnosic acid to the oil compared to the oxidation process without added carnosic acid. Therefore carnosic acid alone as one of the main components in rosemary extracts was not used in further experiments.

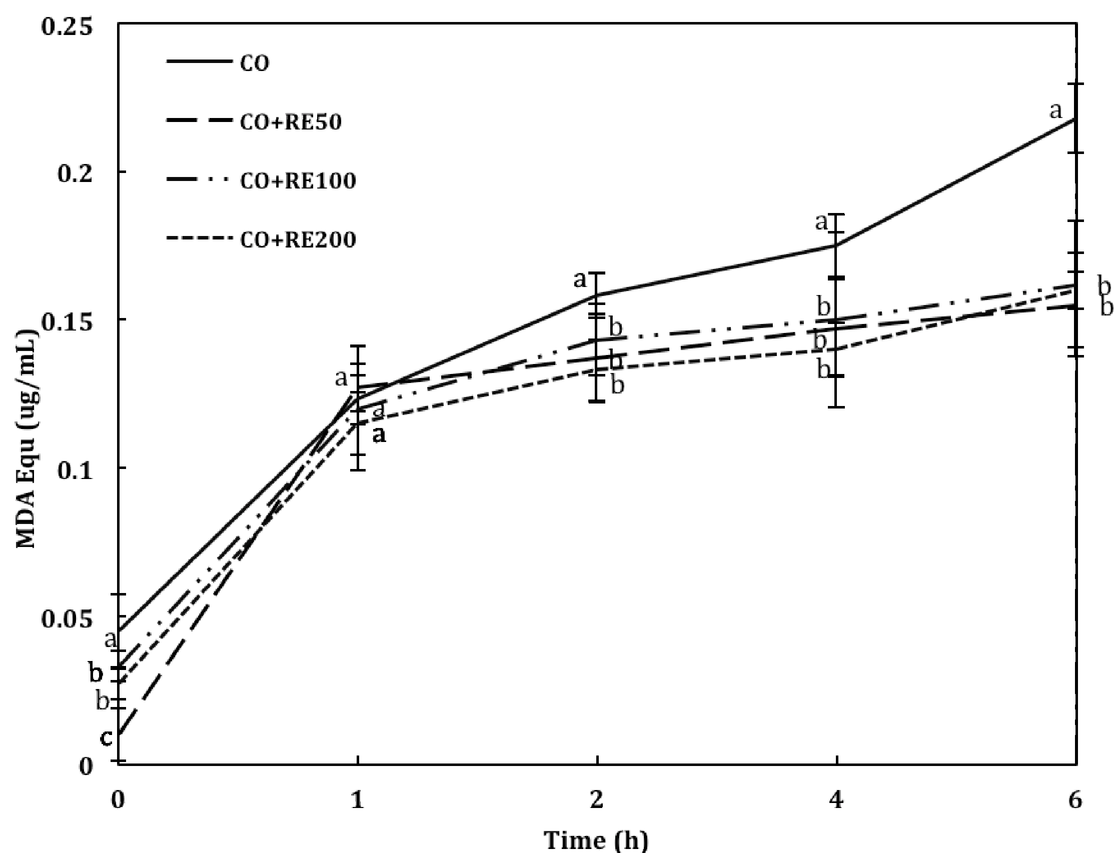


Figure 23. Comparison of TBARS expressed as MDA equivalents of heat treated corn oil for 0, 1, 2, 4 and 6 hours at 185°C in the presence and absence of 50ppm, 100ppm or 200ppm rosemary extracts (RE)

The results showed that there were no significant differences between 50ppm, 100ppm and 200ppm RE on the reduction of the oxidation process of the oil. Rosemary extracts definitely reduced the oxidation process. Therefore 50ppm and 100ppm RE were selected to use in further experiments. The oxidation was suppressed by 29% compared to corn oil after 6 hours of heat treatment.

B. Formation of Secondary Lipophilic Lipid Peroxidation Products in Heat Treated Commercial Corn Oil in the Presence and Absence of Synthetic and Natural Antioxidants

The production of lipophilic secondary oxidation products such as the four toxic α, β-unsaturated-hydroxyaldehydes (HHE, HOE, HNE and HDE) in corn oil in the presence and absence of synthetic and natural antioxidants, heated for 0, 1, 2, 4, and 6 hours at 185°C, was investigated.

The heating times and the concentrations of synthetic and natural antioxidants were selected from information of the results of the preliminary TBARS experiments.

Identification of HHE, HOE, HNE and HDE from the oil was made by comparison to the HPLC peak retention time of pure standards.

HNE was the main toxic compound of the four α, β-unsaturated-hydroxyaldehydes.

A) The Formation of the Major Toxic α, β-unsaturated-hydroxyaldehydes HNE in Corn Oil in the Presence and Absence of Synthetic and Natural Antioxidants Heat Treated for 0, 1, 2, 4 and 6 hours at 185°C

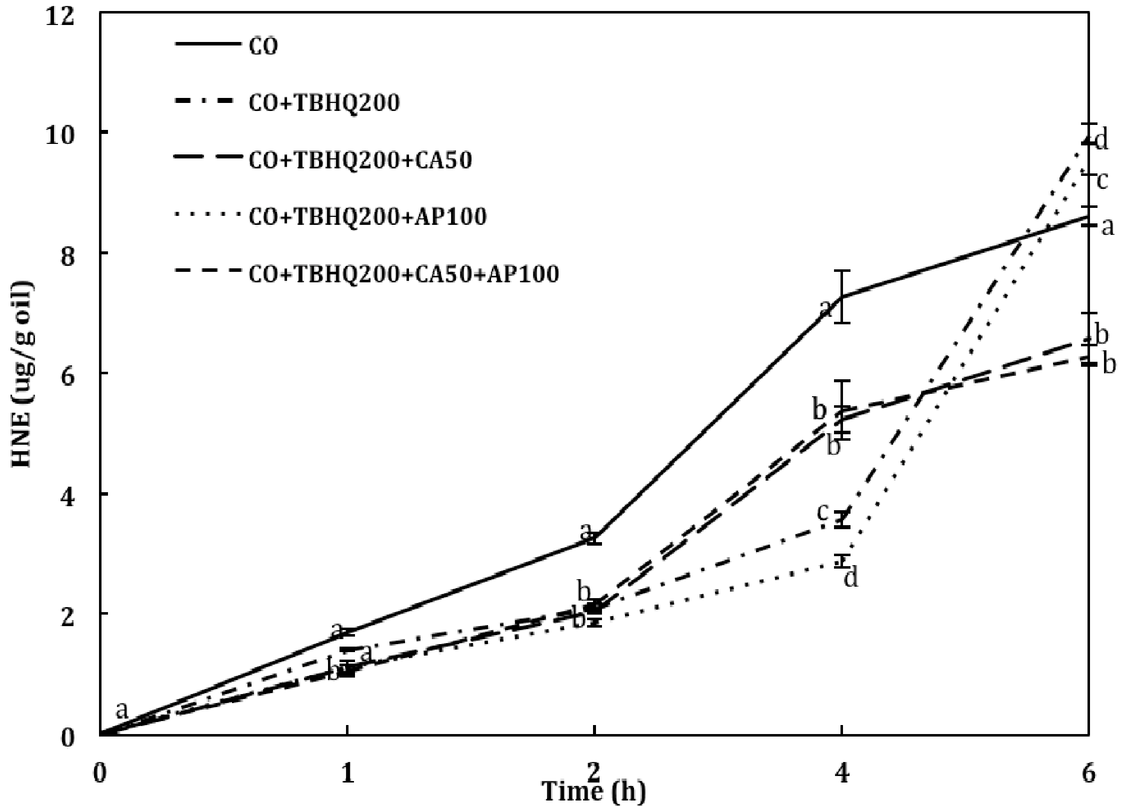


Figure 24. Formation of HNE in corn oil heat treated at 185°C for 0, 1, 2, 4 and 6 hours in the presence and absence of synthetic antioxidants and their mixtures

Results showed there were no significant differences in HNE formation between two combinations of antioxidant mixtures 200ppm TBHQ, and 200ppmTBHQ+100ppm AP. They did not have the effects on suppressing the formation of HNE.

There were no significant differences between the combination of 200ppm TBHQ+50ppmCA and the combination of 200ppmTBHQ+50ppmCA+100ppmAP over most of the heating periods they both similarly reduced HNE formation. Therefore the

added 100ppm AP had significant effect on suppressing HNE formation. The suppression of HNE formation was 27% by the addition of 200ppmTBHQ+100ppmAP+50ppmCA at 6 hours in corn oil at 185°C. HNE was not detected in the unheated corn oil in the presence and absence of synthetic antioxidants and their mixtures.

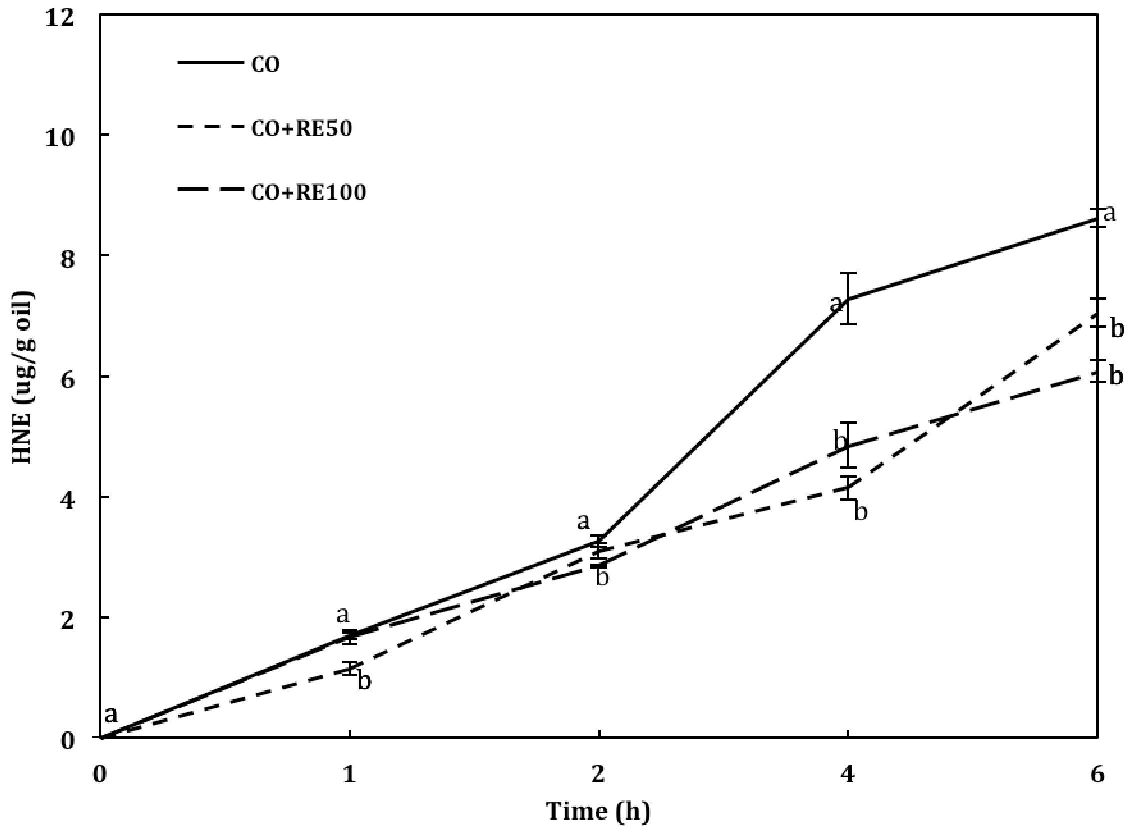


Figure 25. Formation of HNE in commercial corn oil heat treated at 185°C for 0, 1, 2, 4 and 6 hours in the presence and absence of 50ppm and 100ppm rosemary extracts (RE)

Results indicated there was no significant difference between 50ppm and 100ppm RE on the level of suppression of oxidation. 50ppm and 100ppm RE similarly suppressed the formation of HNE.

HNE formation was greatly influenced both by the addition of RE and the time of heating period. The suppression of HNE formation was 29% by the addition of 100ppm RE heat treated for 6 hours in corn oil at 185°C. HNE was not detected in the unheated corn oil in the absence and presence of natural antioxidants.

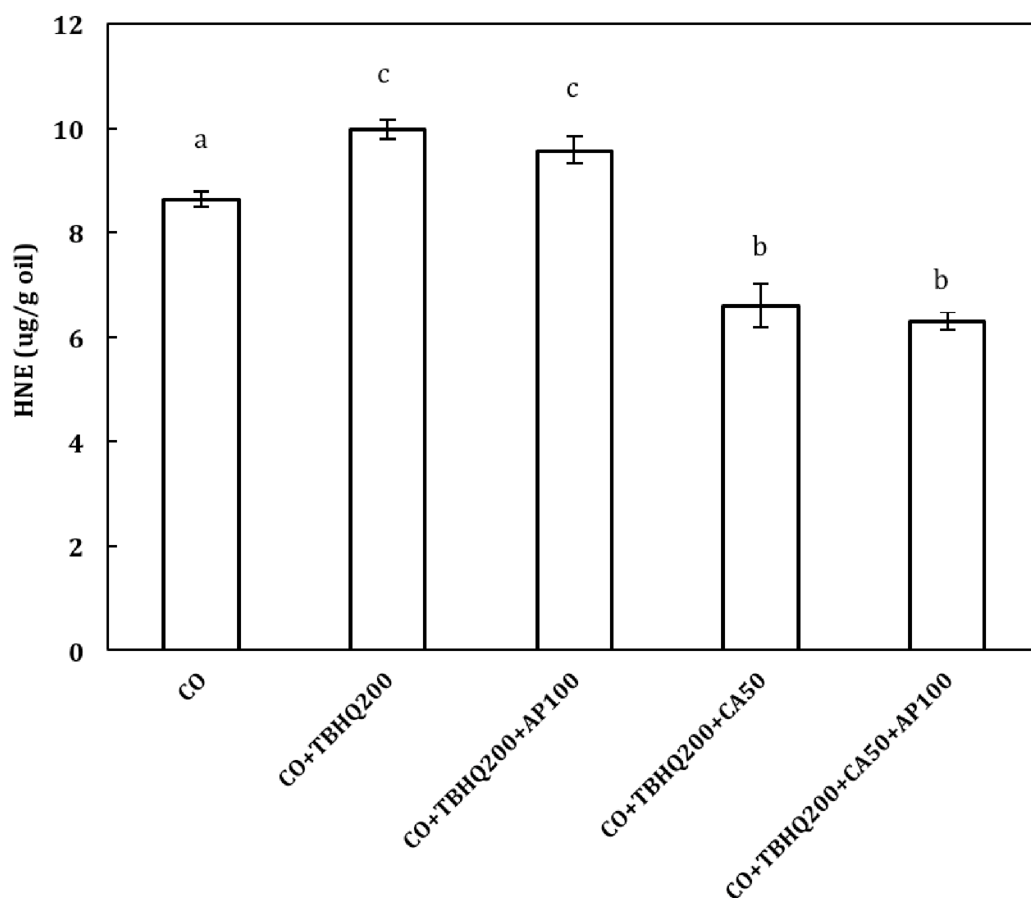


Figure 26. Formation of HNE in corn oil heat treated for 6 hours at 185°C in the presence and absence of synthetic antioxidants and their mixtures

Results indicated the best combination of synthetic antioxidant mixtures were 200ppmTBHQ+50ppmCA and 200ppmTBHQ+50ppmCA+100ppmAP to suppress HNE formation. TBHQ was a primary antioxidant and CA and AP were secondary antioxidant synergists. Interestingly 200ppm TBHQ or 200ppmTBHQ+100ppmAP did not reduce any suppression of HNE formation.

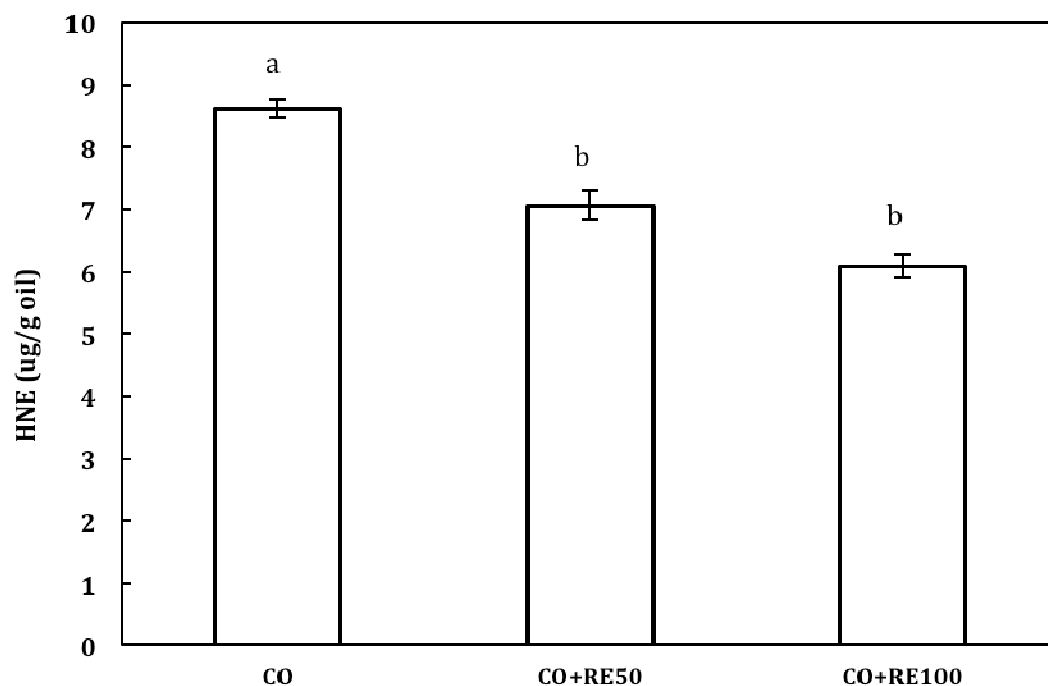


Figure 27. Formation of HNE in corn oil heat treated for 6 hours at 185°C in the presence and absence of 50ppm and 100ppm rosemary extracts (RE)

The results showed both 50ppm and 100ppm RE had similar significantly suppressing effects on the formation of HNE. The average suppression was found to be 24% by the addition of 50ppm or 100ppm RE.

B) The Formation of the Minor Toxic α, β-Unsaturated-Hydroxyaldehydes (HHE, HOE and HDE) in Corn Oil in the Presence and Absence of Synthetic and Natural Antioxidants Heat Treated for 6 hours at 185°C

1. Formation of HHE in corn oil in the presence and absence of synthetic and natural antioxidants measured after 6 hours of heat treatment at 185°C

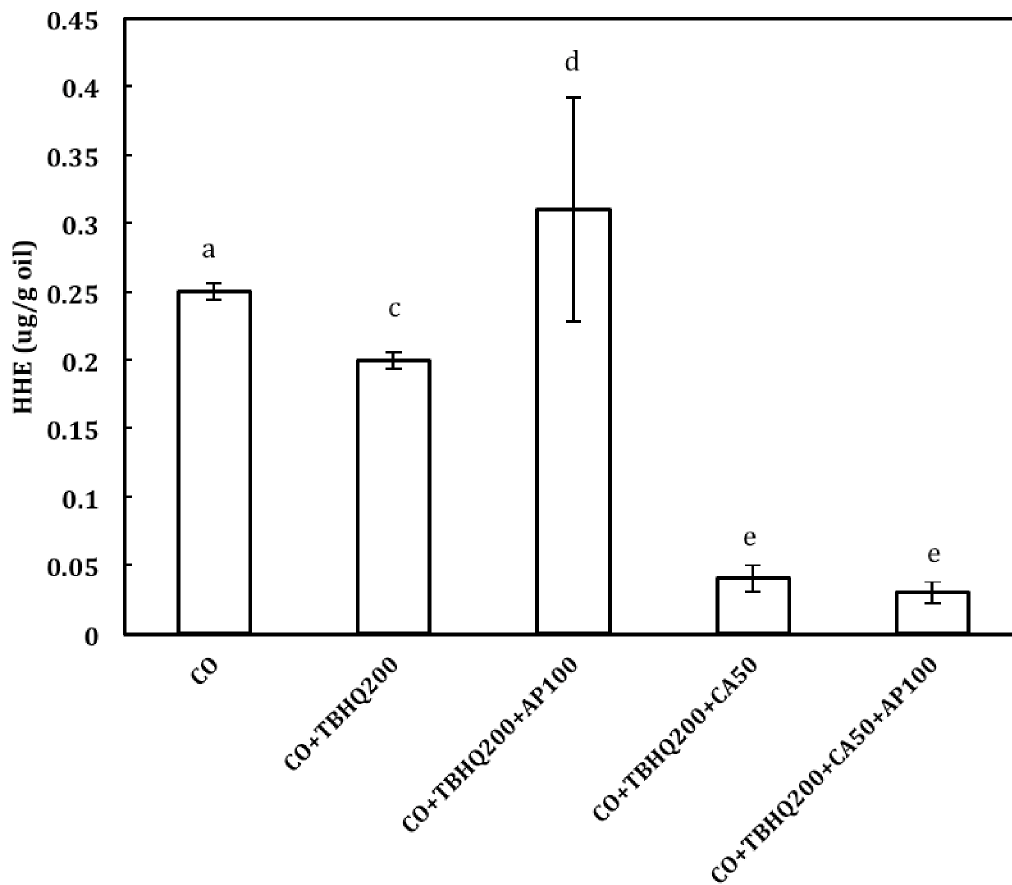


Figure 28. Formation of HHE in corn oil heat treated for 6 hours at 185°C in the presence and absence of synthetic antioxidants and their mixtures

The results showed that 200ppm TBHQ somewhat suppressed the formation of HHE, but 200ppmTBHQ+50ppmCA and 200ppmTBHQ+50ppmCA+100ppmAP reduced HHE formation very significantly. However, 200ppmTBHQ+100ppmAP had no

effect or seem to have pro-oxidation effect. The best suppression was found to be 88% by the addition of 200ppmTBHQ+100ppmAP+50ppmCA.

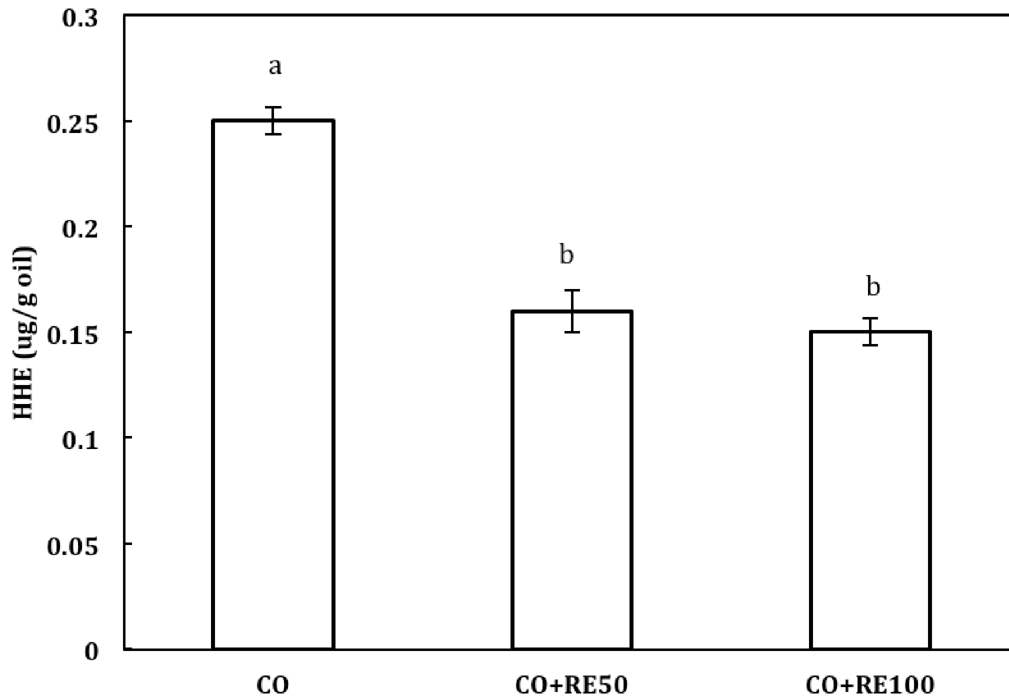


Figure 29. Formation of HHE in corn oil heat treated for 6 hours at 185°C in the presence and absence of 50ppm and 100ppm rosemary extracts (RE)

Results showed that 50ppm RE or 100ppm RE retarded the formation of HHE. There were no significant differences in the suppression of HHE formation between the addition of 50ppm or 100ppm RE. The average suppression of HHE formation was found to be 38% by the addition of RE.

2. Formation of HOE in corn oil in the presence and absence of synthetic and natural antioxidants measured after 6 hours of heat treatment at 185°C

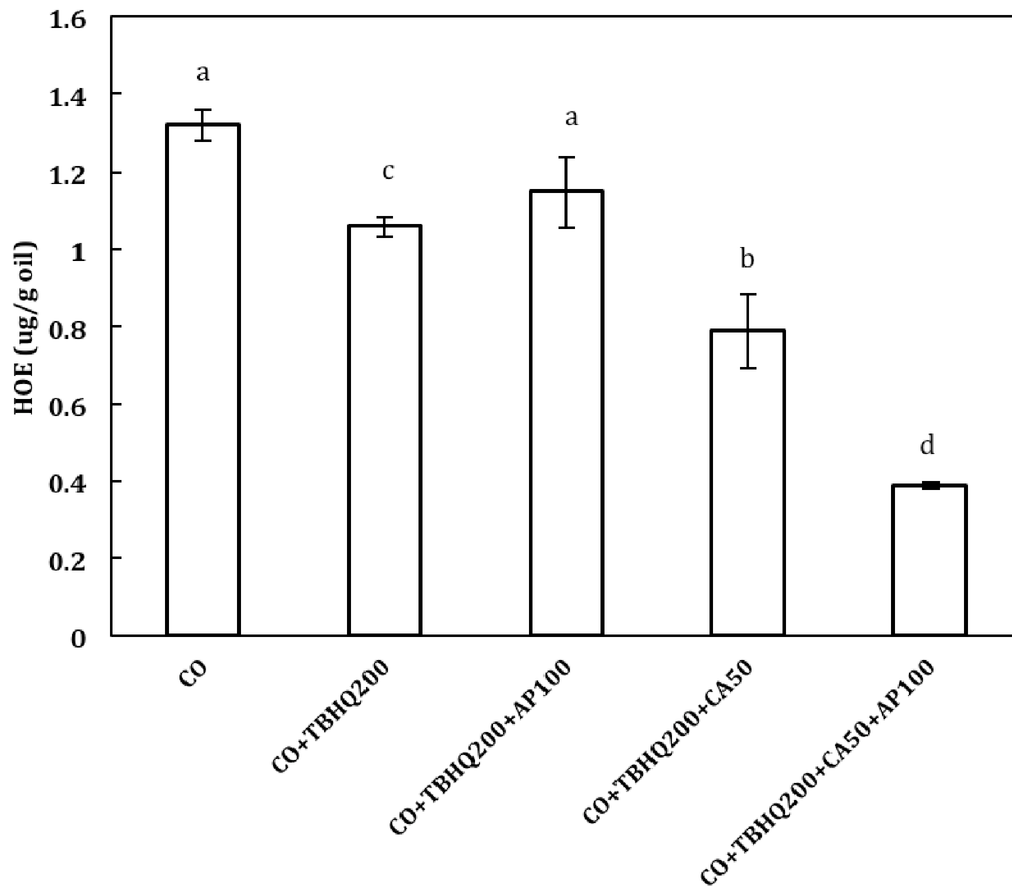


Figure 30. Formation of HOE in corn oil heat treated for 6 hours at 185°C in the presence and absence of synthetic antioxidants and their mixtures

Present results indicated the best antioxidant mixture was 200ppmTBHQ+50ppmCA+100ppmAP. This proportion had excellent antioxidative activity compared with the other antioxidant mixtures. The formation of HOE was suppressed by 70%. Both the mixtures of 200ppm TBHQ and 200ppmTBHQ+50ppmCA

produced significant suppressions but 200ppmTBHQ+100ppmAP produced no HOE suppression.

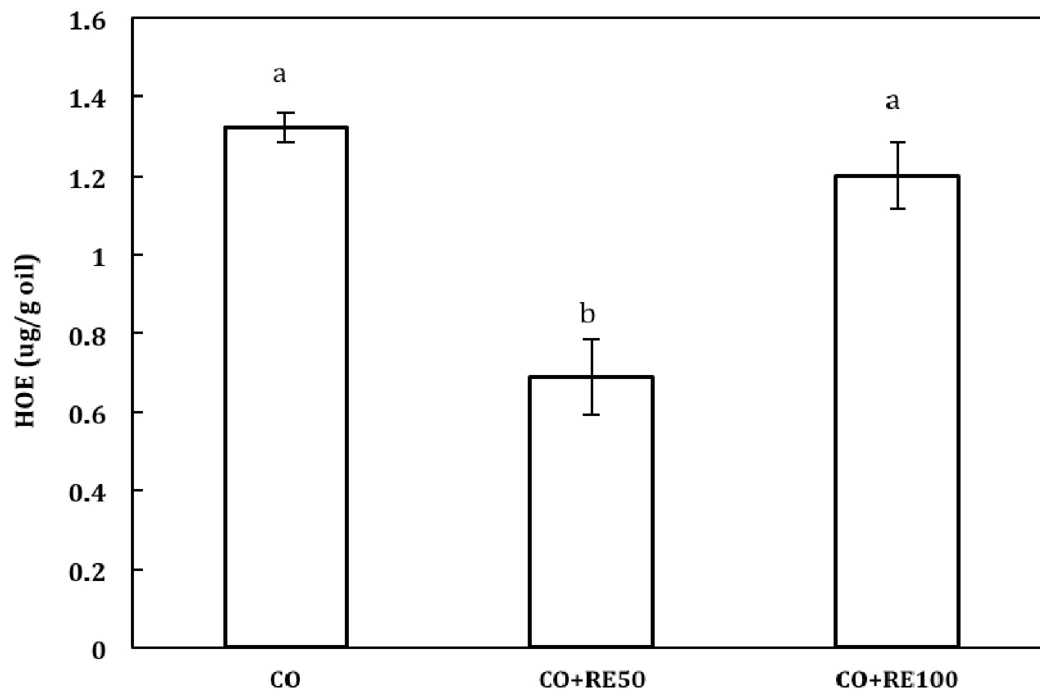


Figure 31. Formation of HOE in corn oil heat treated for 6 hours at 185°C in the presence and absence of 50ppm and 100ppm rosemary extracts (RE)

The results showed 50ppm RE suppressed the formation of HOE 48%. However, 100ppm RE had no effect on suppressing the formation of HOE.

3. Formation of HDE in corn oil in the presence and absence of synthetic and natural antioxidants measured after 6 hours of heat treatment at 185°C

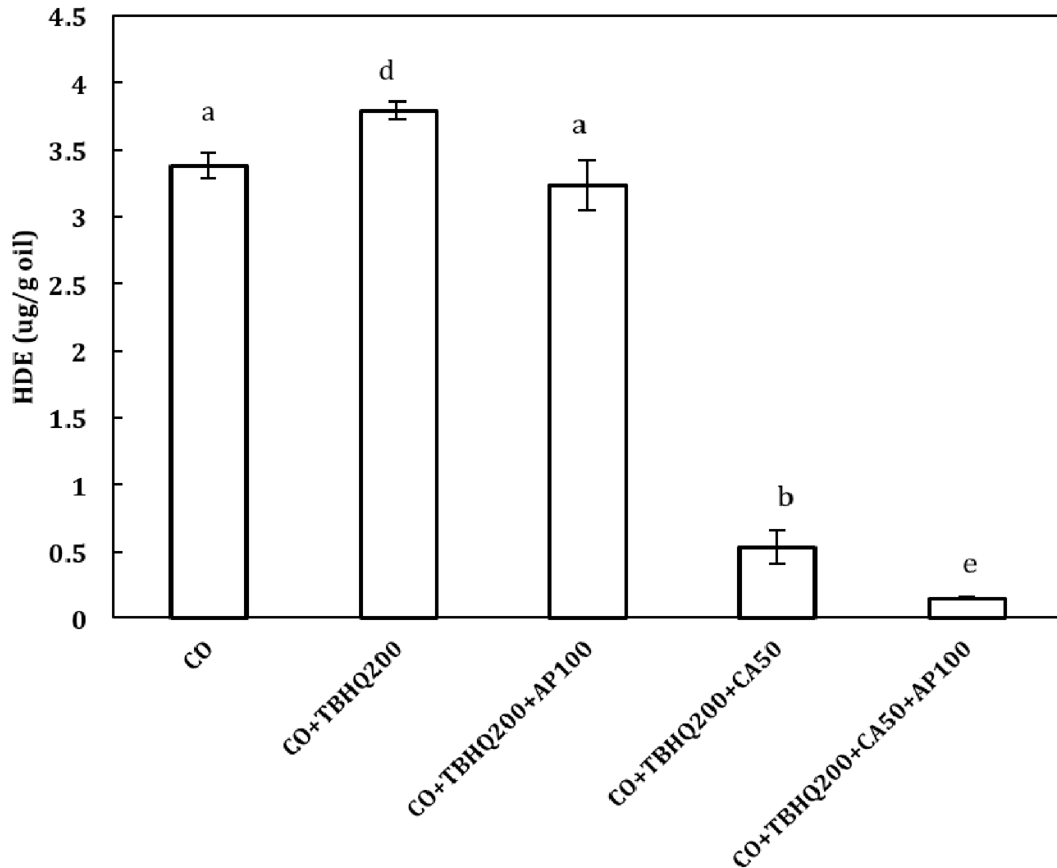


Figure 32. Formation of HDE in corn oil heat treated for 6 hours at 185°C in the presence and absence of synthetic antioxidants and their mixtures

Results showed that 200ppmTBHQ+50ppmCA produced great suppression of the formation of HDE, but the addition of 100ppm AP forming a mixture of 200ppmTBHQ+50ppmCA+100ppmAP produced 96% suppression of HDE formation.

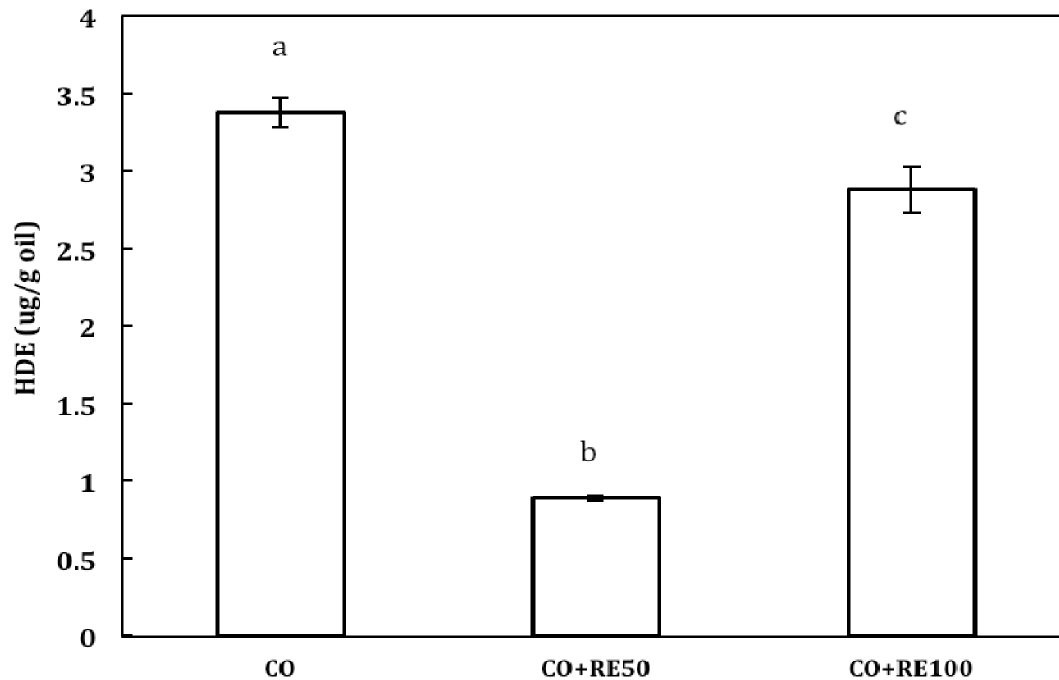


Figure 33. Formation of HDE in corn oil heat treated for 6 hours at 185°C in the presence and absence of 50ppm and 100ppm rosemary extracts (RE)

Results showed 50ppm RE produced the greatest suppression 79% of HDE formation. Interestingly 100ppm RE was not effective to reduce HDE formation. At higher concentration RE may have also some pro-oxidative effect.

4. Formation of the sum total of the four toxic α, β-unsaturated aldehydes in corn oil in the presence and absence of synthetic and natural antioxidants measured after 6 hours of heat treatment at 185°C

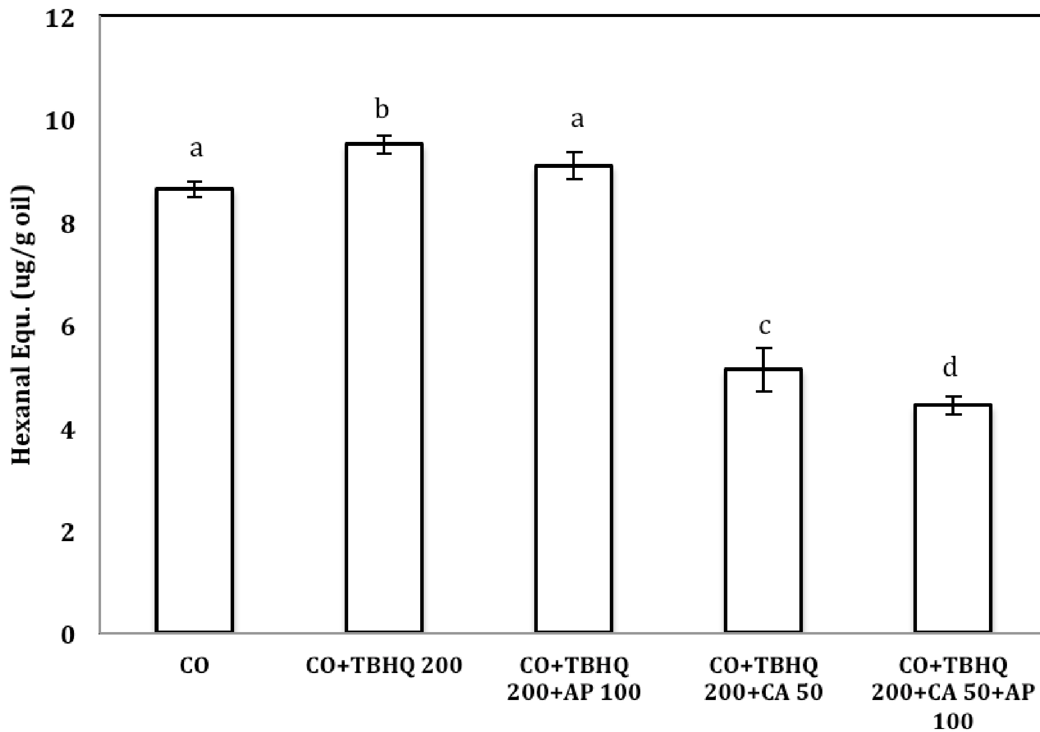


Figure 34. Formation of the sum total of the four α, β-unsaturated aldehydes (HHE, HOE, HNE and HDE) in corn oil heat treated for 6 hours at 185°C in the presence and absence of synthetic antioxidants and their mixtures

Results showed that 200ppmTBHQ+50ppmCA produced great suppression of the formation of the sum total of α, β-unsaturated aldehydes, but the addition of 100ppmAP forming a mixture of 200ppmTBHQ+50ppmCA+100ppmAP produced the best suppression.

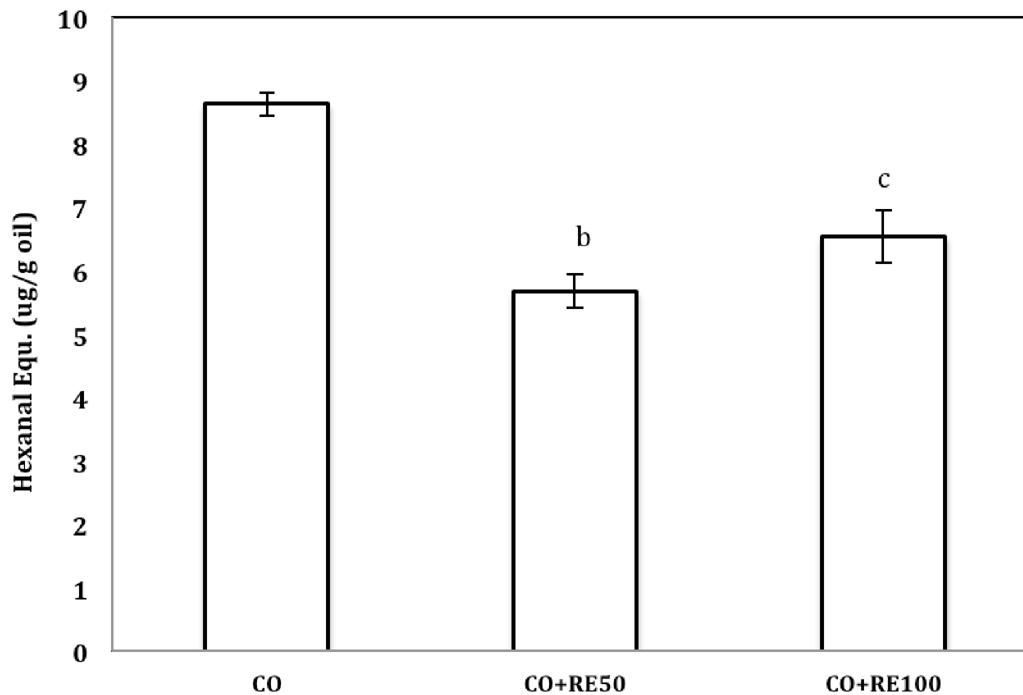


Figure 35. Formation of the sum total of the four α, β-unsaturated aldehydes in corn oil heat treated for 6 hours at 185°C in the presence and absence of 50ppm and 100ppm rosemary extracts (RE)

Results showed that 50ppm RE or 100ppm RE both retarded the formation of the sum total of the four α, β-unsaturated aldehydes. Interestingly 100ppm RE was not as effective as 50ppm RE. RE at higher concentrations may also have some pro-oxidative effect.

Table 6. Rates of HHE, HOE, HNE and HDE formation in commercial corn oil heat treated for 6 hours at 185°C (Unit: μg /g oil/h)

Calculation: (The amount of HHE, HOE, HNE and HDE at 6 hours – The amount of HHE, HOE, HNE and HDE at 0 hour) ÷ 6

	HHE	HOE	HNE	HDE
CO	0.042	0.220	1.437	0.563
CO+TBHQ200ppm	0.033	0.177	1.663	0.632
CO+TBHQ200ppm+AP100ppm	0.052	0.192	1.595	0.538
CO+TBHQ200ppm+CA50ppm	0.007	0.132	1.100	0.088
CO+TBHQ200ppm+AP100ppm+CA50ppm	0.005	0.065	1.048	0.025
CO+RE50ppm	0.027	0.115	1.177	0.148
CO+RE100ppm	0.025	0.200	1.015	0.480

C) The Sum Total of the Individually Measured Polar Aldehydes and Related Carbonyl Compounds in Corn Oil Heat Treated at 185°C for 0, 1, 2, 4 and 6 hours in the Presence and Absence of Synthetic and Natural Antioxidants

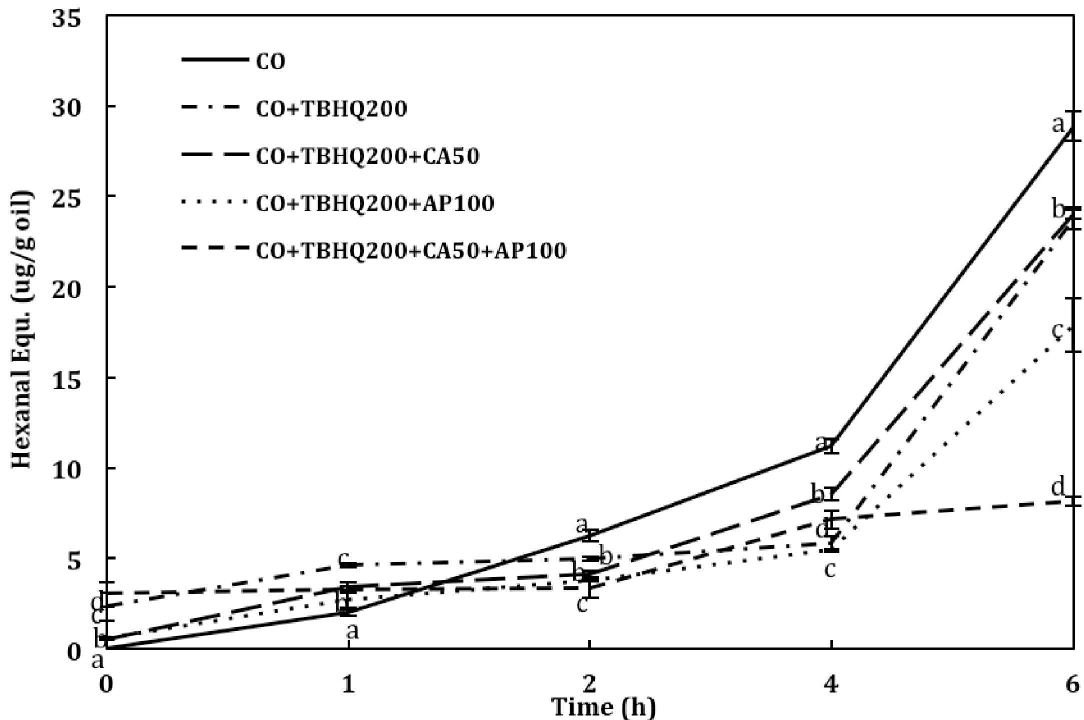


Figure 36. The sum total of polar aldehydes and related carbonyl compounds in corn oil heat treated at 185°C for 0, 1, 2, 4 and 6 hours in the presence and absence of synthetic antioxidants and their mixtures

The results showed the amount of the sum total of polar aldehydes and related carbonyl compounds was at very low level in the unheated corn oil in the presence of synthetic antioxidants. There were significant differences in the presence of synthetic antioxidants and their combinations over heating periods starting after already 1 hour of heat treatment at 185°C. By 4 hours the antioxidant had significantly suppressed the polar aldehydes formations. After 6 hours of heating time the combination of 200ppm TBHQ+50ppmCA+100ppmAP suppressed the formation of polar aldehydes by 72%.

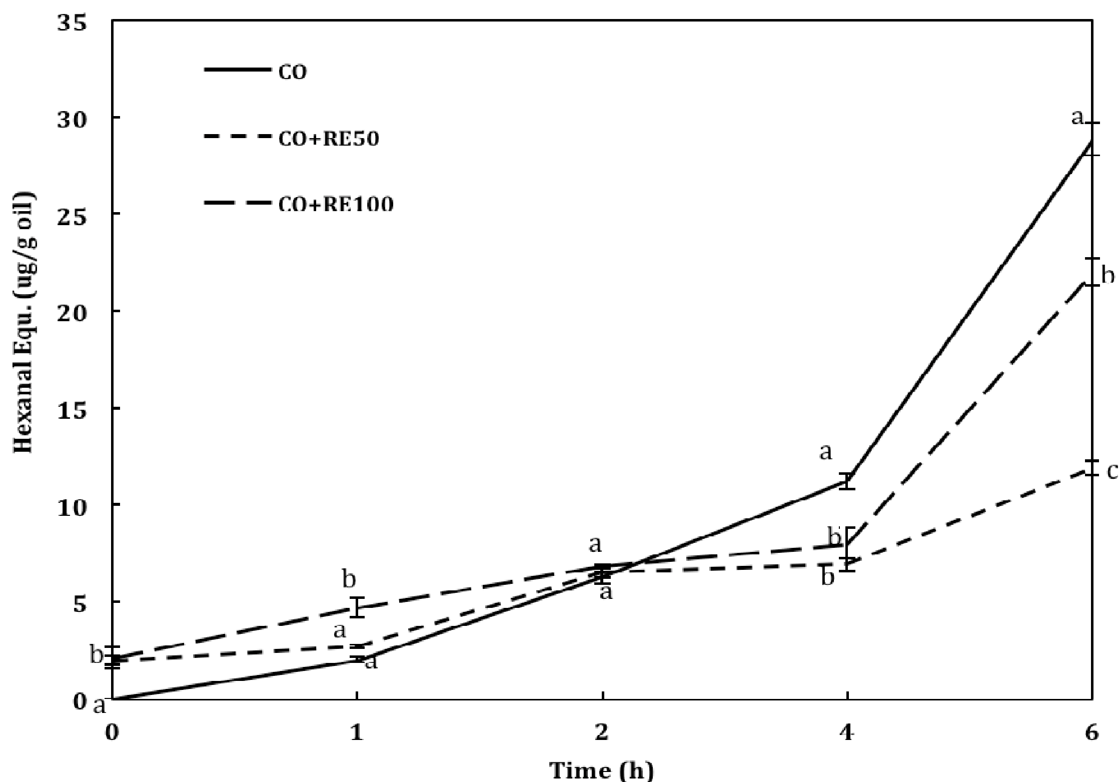


Figure 37. The sum total of polar aldehydes and related carbonyl compounds in corn oil heat treated at 185°C for 0, 1, 2, 4 and 6 hours in the presence and absence of 50ppm and 100ppm rosemary extracts (RE)

The results showed that up to 2 hours of heat treatment RE did not have much effect suppressing the formation of polar aldehydic degradation products. By 6 hours of heat treatment significant suppression could be seen both concentrations of RE. However, after 6 hours of heat treatment 50ppm RE was the most effective, it suppressed 59% the sum total of polar aldehyde and related carbonyl compounds formations.

Table 7. Rates of the sum total of polar aldehydes and related carbonyl compounds formations in commercial corn oil heat treated at 185°C for 6 hours in the presence and absence of synthetic and natural antioxidants (Unit: µg hexanal equivalent/g oil/h)

Calculation: (The amount of total Polar aldehydes at 6 hours – The amount of total Polar aldehydes at 0 hour) ÷ 6

	Temperature (185°C) Time (6h)
CO	4.805
CO+TBHQ200ppm	3.568
CO+TBHQ200ppm+AP100ppm	2.885
CO+TBHQ200ppm+CA50ppm	3.433
CO+TBHQ200ppm+AP100ppm+CA50ppm	0.860
CO+RE50ppm	1.657
CO+RE100ppm	3.317

D) The Sum Total of the Individually Measured Non-polar Aldehydes and Related Carbonyl Compounds in Corn Oil Heat Treated at 185°C for 0, 1, 2, 4 and 6 hours in the Presence and Absence of Synthetic and Natural Antioxidants

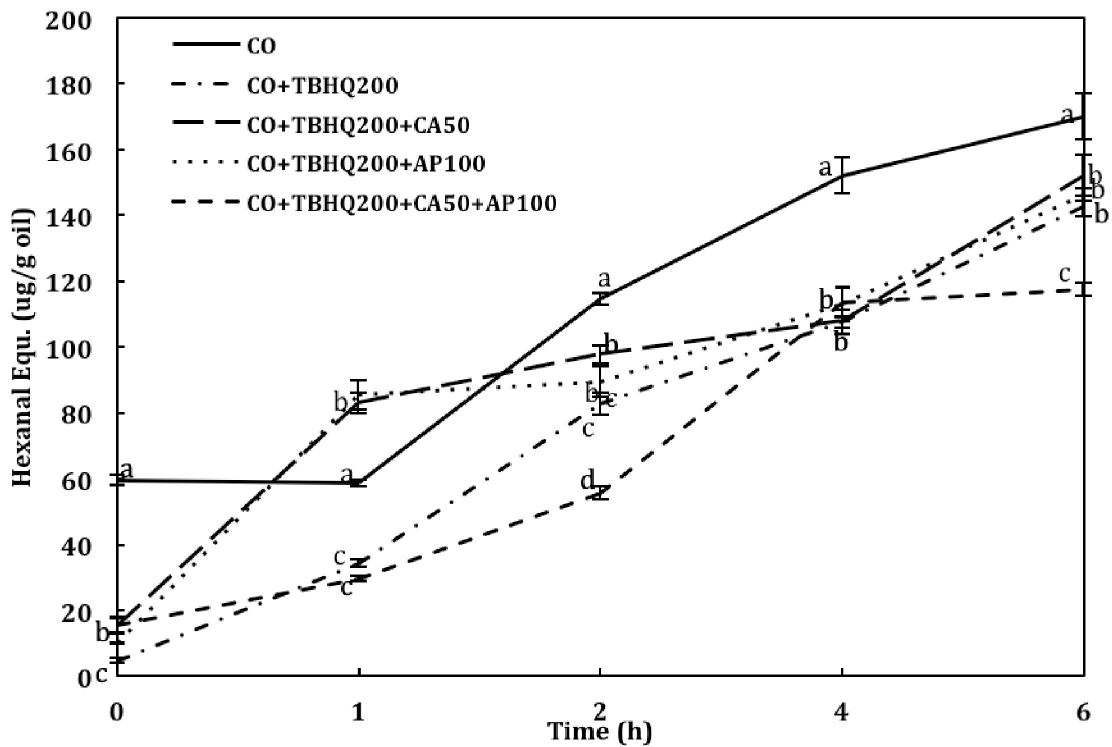


Figure 38. The sum total of non-polar aldehydes and related carbonyl compounds in corn oil heat treated at 185°C for 0, 1, 2, 4 and 6 hours in the presence and absence of synthetic antioxidants and their mixtures

Results showed the amount of the sum total of non-polar aldehydes and related carbonyl compounds was few but measurable amounts in the unheated corn oil in the absence and presence of synthetic antioxidants and their mixtures. After 2 hours of heat treatment at 185°C the combination of 200ppmTBHQ+50ppmCA+100ppmAP was more effective than the other antioxidant combinations suppressing the non-polar aldehydes and related carbonyl compounds. After 4 hours of heat treatment all combinations were

significantly lower than no antioxidants. After 6 hours of heat treatment at 185°C the mixture of 200ppmTBHQ+50ppmCA+100ppmAP lowered 31% the formation of non-polar aldehydic degradation products.

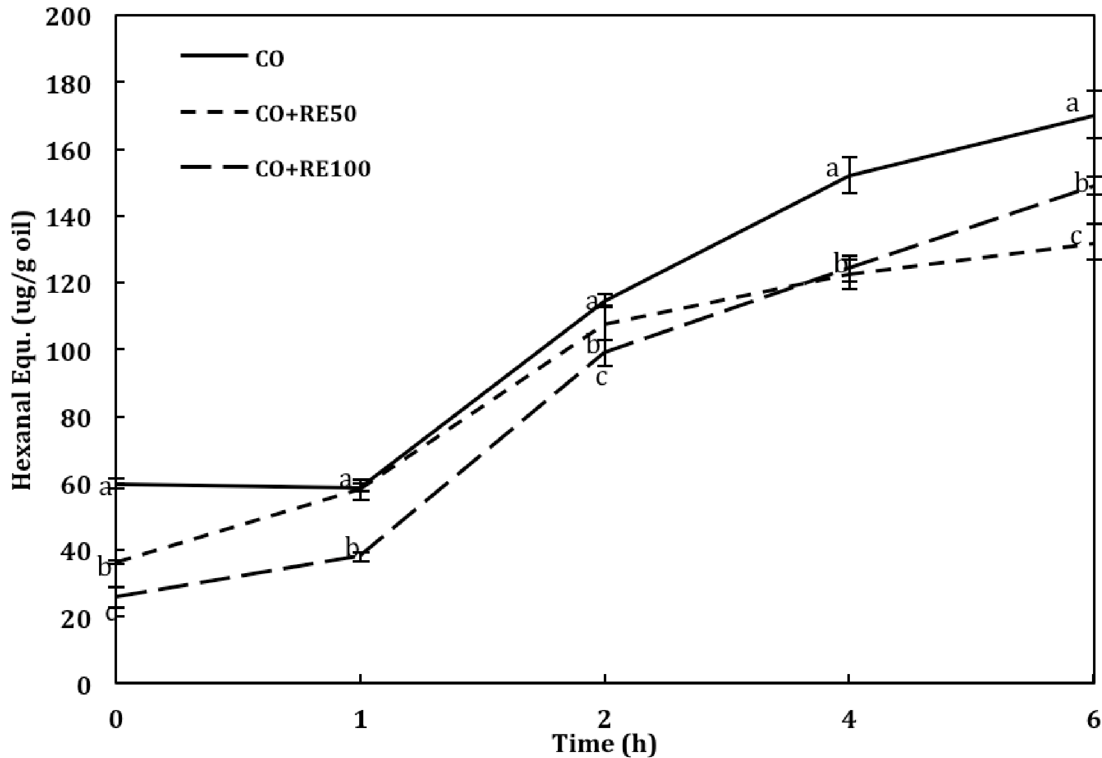


Figure 39. The sum total of non-polar aldehydes and related carbonyl compounds in corn oil heat treated at 185°C for 0, 1, 2, 4 and 6 hours in the presence and absence of 50ppm and 100ppm RE

The present results showed the amounts of the sum total non-polar aldehydes and related carbonyl compounds were found in the unheated corn oil in the presence and absence of natural antioxidants. Few but measurable amounts of non-polar aldehydic degradation products were found in the unheated corn oil. There were significant differences in the presence of RE over the different heating periods. After 1 hour of heat treatment 100ppm RE was significantly better suppressor than 50ppm RE. However, after 4 hours of heat treatment there were no significant differences between 50ppm and

100ppm RE. The best suppression of 23% was found by the addition of 50ppm RE after 6 hours of heat treatment corn oil at 185°C.

Table 8. Rates of the sum total of non-polar aldehydes and related carbonyl compounds formations in commercial corn oil heat treated at 185°C for 6 hours in the presence and absence of synthetic and natural antioxidant (Unit: µg hexanal equivalent/g oil/h)

Calculation: (The amount of total Non-polar aldehydes at 6 hours – The amount of total Non-polar aldehydes at 0 hour) ÷ 6

	Temperature (185°C) Time (6h)
CO	18.395
CO+TBHQ200ppm	23.002
CO+TBHQ200ppm+AP100ppm	22.723
CO+TBHQ200ppm+CA50ppm	22.755
CO+TBHQ200ppm+AP100ppm+CA50ppm	17.005
CO+RE50ppm	15.927
CO+RE100ppm	20.507

E) The Sum Total of Polar and Non-polar Aldehydes and Related Carbonyl Compounds in Corn Oil Heat Treated at 185°C for 0, 1, 2, 4 and 6 hours in the Presence and Absence of Antioxidants

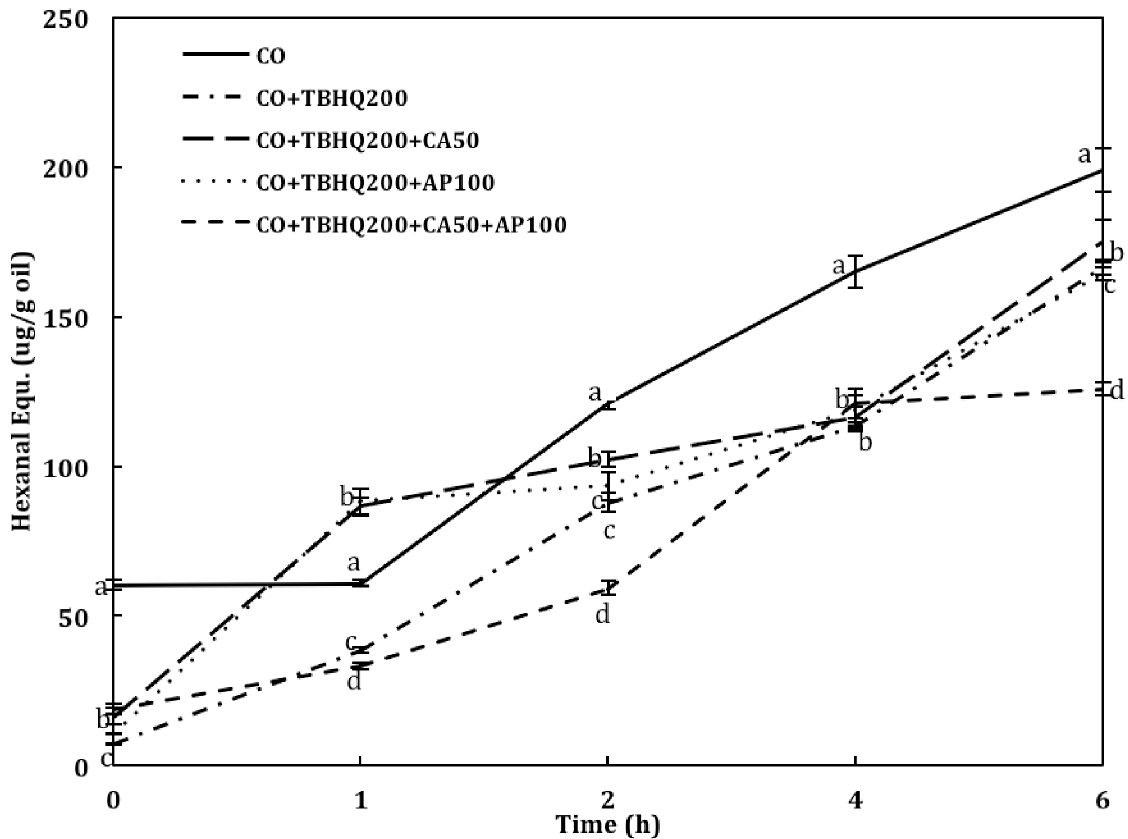


Figure 40. The sum total of polar and non-polar aldehydes and related carbonyl compounds in corn oil heat treated at 185°C for 0, 1, 2, 4 and 6 hours in the presence and absence of synthetic antioxidants and their mixtures

The results showed that the combination of 200ppm TBHQ+50ppm CA+100ppm AP was the best combination of synthetic antioxidants for suppressing the formation of the sum total of polar and non-polar aldehydes and related carbonyl compounds during heat treatment of corn oil for 1, 2, 4 and 6 hours at 185°C.The suppression of oxidative

degradation was 37% compared to commercial corn oil without containing added synthetic antioxidants after 6 hours of heat treatment at 185°C.

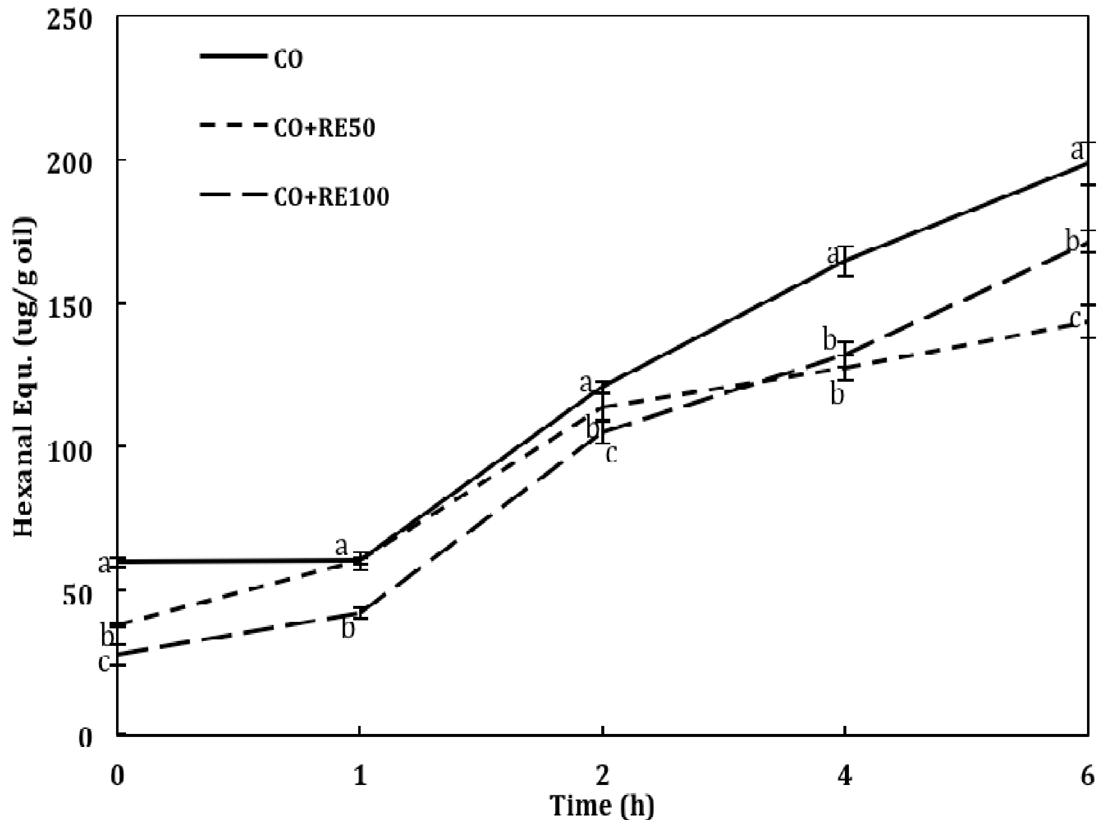


Figure 41. The sum total of polar and non-polar aldehydes and related carbonyl compounds in corn oil heat treated at 185°C for 0, 1, 2, 4 and 6 hours in the presence and absence of 50ppm and 100ppm rosemary extracts (RE)

The results showed the amount of total aldehydes and related carbonyl compounds was small in the unheated corn oil in the absence but somewhat higher than in the presence of RE. There was a significant suppression of oxidative degradation by 100ppm RE after 1, 2 and 4 hours of heat treatment. The suppression of the sum total of polar and non-polar compounds was 28% the largest using 50ppm RE after 6 hours of heat treatment at 185°C.

Table 9. Rates of the sum total of polar and non-polar aldehydes and related carbonyl compounds in corn oil heat treated at 185°C for 6 hours in the presence and absence of synthetic and their mixtures and natural antioxidant (Unit: µg hexanal equivalent/g oil/h)

Calculation: (The amount of total Polar and Non-polar aldehydes at 6 hours – The amount of total Polar and Non-polar aldehydes at 0 hour) ÷ 6

	Temperature (185°C) Time (6h)
CO	23.142
CO+TBHQ200ppm	26.555
CO+TBHQ200ppm+AP100ppm	25.618
CO+TBHQ200ppm+CA50ppm	26.462
CO+TBHQ200ppm+AP100ppm+CA50ppm	17.865
CO+RE50ppm	17.583
CO+RE100ppm	23.932

Discussion

The deterioration of frying fats and oils at high temperatures is complicated, because oxidative and thermolytic reactions are occurring simultaneously, and both saturated and unsaturated fatty acids undergo chemical degradations when exposed to oxygen and high temperatures [167]. The objective of the present study was to determine the suppressing effects of various antioxidants and their mixtures on the formation of the toxic α, β-unsaturated-4-hydroxyaldehydes such as 4-hydroxy-2-trans-hexenal (HHE), 4-hydroxy-2-trans-octenal (HOE), 4-hydroxy-2-trans-decenal (HDE) and especially the most toxic and most abundant compound 4-hydroxy-2-trans-nonenal (HNE) resulting from the oxidation of thermally treated commercial corn oil. Therefore, it is beneficial to study the factors, such as the usage of antioxidants, to reduce the deterioration of commercial corn oil in this experiment.

Previous studies have investigated that dietary PUFAs have potential health benefits on human beings. The biological effects of ω-3 and ω-6 fatty acids serve multiple functions. They play important roles on forming lipid rafts and acting on DNA. They failed to take into consideration the nature and concentration of any cytotoxic lipid ozonation products present in the fats and oils at high temperatures.

As the major unsaturated fatty acid in vegetable oils, oxidation of linoleic acid has been studied for years. Cis, trans- and trans, trans-conjugated diene 9- and 13-hydroperoxides are produced from autoxidation of free radicals [168-171]. The α, β-unsaturated 4-hydroxyaldehydes, HHE, HOE, HNE and HDE are formed from β-cleavage of 9- and 13-hydroperoxides. Previous investigations from this laboratory have reported the formation of the toxic α, β-unsaturated hydroxyaldehydes in heat treated corn and soybean oils, namely HHE, HOE, HNE and HDE, which are the most toxic compounds forming from linoleic acid due to heat treatment at 185°C.

The concentration of HNE, the most toxic compound of the four α, β-unsaturated 4-hydroxyaldehydes, was much higher than other three 4-hydroxyaldehydes in all commercial corn oil samples. Since corn oil contained higher concentration of linoleic

acid and linoleic acid is a precursor of HNE. The formation of HNE from ω-6 fatty acids including linoleic acid has been reported and proved in the literature before [172,173].

Antioxidants have been added to frying oils in an attempt to retard the rate of deterioration of the oils. However, one of the drawbacks of using phenolic antioxidants is the rapid loss of the antioxidants from the hot oils. It explains why TBHQ alone has no effects on oxidation process in this experiment by TBARS. TBHQ alone is used to compare antioxidative properties with the mixture of TBHQ and other antioxidants.

Studies have shown that ascorbyl palmitate (AP) has antioxidant capacity [89]. Very recently, it was reported that AP retarded deteriorative changes in fats and oils during frying treatment [90]. However, AP alone has no effects on reducing oxidation of corn oil in this experiment by TBARS. It may be explained heating hour is longer and heating temperature is higher, the oxidation process is more complicated.

The interaction between antioxidants could yield a positive or negative synergism during the high temperature treatment depending on the type and concentration of individual components in the combination. Since the decomposition and volatilization of antioxidants at high temperature may impair the stability of oils during thermal processing. Therefore, for some antioxidants mixture, there is no antioxidative activity in corn oil samples. The amount of α, β-unsaturated 4-hydroxyaldehydes is increased not decreased.

Merril et al. [174] confirmed that there was a synergistic effect between TBHQ and AP. The mixture resulted in the greatest increase in oxidative stability of oleic safflower and other high-oleic oils. And it has been proved in this experiment, since the best combination of synthetic antioxidants is TBHQ+AP+CA on suppression the α, β-unsaturated hydroxyaldehydes formation at frying temperature of 185°C.

The compounds responsible for rosemary extracts' antioxidant activities are mainly phenolic diterpenes, such as carnosic acid, carnosol, rosmanol, epirosmanol and isorosmanol [175]. Therefore, it explains rosemary extracts containing these effective compounds have excellent antioxidative properties in this experiments.

Several studies have recommended rosemary extracts can be used as an alternative antioxidant compound [174, 176]. In a previous study conducted by Ramalho

and Jorge [177], rosemary extracts were found to have a positive effect on the oxidative protection of soybean oil during heat treatment. Therefore, rosemary extracts were more efficient than TBHQ in preventing the decrease of oxidative stability, and it produced a protective effect. Rosemary extracts have excellent suppression on the α, β-unsaturated hydroxyaldehydes formation at frying temperature of 185°C in this experiment.

In this experiment, tocopherols naturally present in commercial corn oil were not taken into consideration. The amount of tocopherols is around 88mg/100g corn oil. However, they may influence the oxidative stability of corn oil, even as minor components. It is important to study the loss of tocopherols and their relationship with the deterioration of corn oil during heating at high temperatures.

The addition of antioxidants strongly influenced the retention of tocopherols present in vegetable oil due to heat treatment. The presence of rosemary extracts increased the retention of α-, γ-, δ-tocopherol after heating treatment. Some researchers reported that rosemary extracts have the ability to slow down the loss of tocopherols that are naturally present in vegetable oils [178]. So to some degree it could explain why the effects on retarding oxidation process are excellent when adding rosemary extracts to corn oil samples in this experiment.

In the future, the antioxidants naturally present in commercial corn oil could be taken into consideration. It is possible the mixture has prooxidative influence not antioxidative effect. Since it can reduce metal ions which lead to the generation of free radicals. Therefore, it may lead to unexpected results.

In conclusion the present experiments demonstrated that certain synthetic antioxidant mixtures, such as, TBHQ+CA+AP and a naturally occurring antioxidant-rosemary extracts at certain concentrations will retard well the oxidation process of heat treated commercial corn oil and therefore the formation of the toxic α, β-unsaturated aldehydes such as HHE, HOE, HNE and HDE due to heat treatment at frying temperature.

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Appendix: Statistical Analyses *

* significance level: p=0.05

TBARS, Figure15, 0 Hour Heat Treatment			
	CO	CO+TBHQ100ppm	CO+TBHQ200ppm
CO+TBHQ100ppm	**0.000**		
CO+TBHQ200ppm	0.781	**0.000**	
CO+TBHQ300ppm	0.516	**0.000**	0.788

TBARS, Figure15, 1 Hour Heat Treatment			
	CO	CO+TBHQ100ppm	CO+TBHQ200ppm
CO+TBHQ100ppm	**0.000**		
CO+TBHQ200ppm	**0.004**	**0.002**	
CO+TBHQ300ppm	**0.004**	**0.001**	0.766

TBARS, Figure15, 2 Hours Heat Treatment			
	CO	CO+TBHQ100ppm	CO+TBHQ200ppm
CO+TBHQ100ppm	**0.011**		
CO+TBHQ200ppm	**0.004**	**0.000**	
CO+TBHQ300ppm	**0.033**	1.000	**0.000**

TBARS, Figure15, 4 Hours Heat Treatment			
	CO	CO+TBHQ100ppm	CO+TBHQ200ppm
CO+TBHQ100ppm	**0.000**		
CO+TBHQ200ppm	**0.008**	**0.019**	
CO+TBHQ300ppm	**0.001**	0.156	0.196

TBARS, Figure15, 6 Hours Heat Treatment			
	CO	CO+TBHQ100ppm	CO+TBHQ200ppm
CO+TBHQ100ppm	0.220		
CO+TBHQ200ppm	0.293	**0.044**	
CO+TBHQ300ppm	**0.007**	0.052	**0.003**

TBARS, Figure17, 0 Hours Heat Treatment		
	CO	CO+AP100ppm
CO+AP100ppm	0.585	
CO+AP200ppm	0.440	0.734

TBARS, Figure17, 1 Hours Heat Treatment		
	CO	CO+AP100ppm
CO+AP100ppm	**0.004**	
CO+AP200ppm	**0.018**	**0.039**

TBARS, Figure17, 2 Hours Heat Treatment		
	CO	CO+AP100ppm
CO+AP100ppm	**0.005**	
CO+AP200ppm	**0.021**	0.292

TBARS, Figure17, 4 Hours Heat Treatment		
	CO	CO+AP100ppm
CO+AP100ppm	**0.008**	
CO+AP200ppm	**0.000**	**0.003**

TBARS, Figure17, 6 Hours Heat Treatment		
	CO	CO+AP100ppm
CO+AP100ppm	0.075	
CO+AP200ppm	**0.024**	0.760

TBARS, Figure19, 0 Hours Heat Treatment		
	CO	CO+TBHQ200ppm +AP100ppm
CO+ TBHQ200ppm+AP100ppm	**0.013**	
CO+ TBHQ200ppm+AP200ppm	0.804	**0.012**

TBARS, Figure19, 1 Hours Heat Treatment		
	CO	CO+TBHQ200ppm +AP100ppm
CO+ TBHQ200ppm+AP100ppm	**0.045**	
CO+ TBHQ200ppm+AP200ppm	**0.012**	0.655

TBARS, Figure19, 2 Hours Heat Treatment		
	CO	CO+TBHQ200ppm +AP100ppm
CO+ TBHQ200ppm+AP100ppm	0.597	
CO+ TBHQ200ppm+AP200ppm	0.317	0.276

TBARS, Figure19, 4 Hours Heat Treatment		
	CO	CO+TBHQ200ppm +AP100ppm
CO+ TBHQ200ppm+AP100ppm	0.124	
CO+ TBHQ200ppm+AP200ppm	**0.000**	**0.000**

TBARS, Figure19, 6 Hours Heat Treatment		
	CO	CO+TBHQ200ppm +AP100ppm
CO+ TBHQ200ppm+AP100ppm	0.094	
CO+ TBHQ200ppm+AP200ppm	**0.006**	**0.037**

TBARS, Figure18, 0 Hours Heat Treatment		
	CO	CO+TBHQ200ppm+CA50ppm
CO+TBHQ200ppm+CA50ppm	0.134	
CO+TBHQ200ppm+CA100ppm	0.060	**0.005**

TBARS, Figure18, 1 Hours Heat Treatment		
	CO	CO+TBHQ200ppm+CA50ppm
CO+TBHQ200ppm+CA50ppm	0.328	
CO+TBHQ200ppm+CA100ppm	**0.016**	**0.009**

TBARS, Figure18, 2 Hours Heat Treatment		
	CO	CO+TBHQ200ppm+CA50ppm
CO+TBHQ200ppm+CA50ppm	0.354	
CO+TBHQ200ppm+CA100ppm	0.677	0.306

TBARS, Figure18, 4 Hours Heat Treatment		
	CO	CO+TBHQ200ppm+CA50ppm
CO+TBHQ200ppm+CA50ppm	**0.001**	
CO+TBHQ200ppm+CA100ppm	0.615	**0.002**

TBARS, Figure18, 6 Hours Heat Treatment		
	CO	CO+TBHQ200ppm+CA50ppm
CO+TBHQ200ppm+CA50ppm	0.813	
CO+TBHQ200ppm+CA100ppm	0.220	0.156

TBARS, Figure20, 0 Hours Heat Treatment			
	CO	CO+ TBHQ200ppm+ AP100ppm+CA20ppm	CO+ TBHQ200ppm+ AP100ppm +CA20ppm
CO+ TBHQ200ppm +AP100ppm+CA20ppm	0.459		
CO+ TBHQ200ppm+AP100ppm +CA30ppm	1.000	0.477	
CO+ TBHQ200ppm+AP100ppm +CA50ppm	**0.019**	0.192	**0.028**

TBARS, Figure20, 1 Hours Heat Treatment			
	CO	CO+ TBHQ200ppm+ AP100ppm+CA20ppm	CO+ TBHQ200ppm+ AP100ppm +CA20ppm
CO+ TBHQ200ppm +AP100ppm+CA20ppm	0.369		
CO+ TBHQ200ppm+AP100ppm +CA30ppm	0.087	0.293	
CO+ TBHQ200ppm+AP100ppm +CA50ppm	**0.002**	**0.042**	0.512

TBARS, Figure20, 2 Hours Heat Treatment			
	CO	CO+ TBHQ200ppm+ AP100ppm+CA20ppm	CO+ TBHQ200ppm+ AP100ppm +CA20ppm
CO+ TBHQ200ppm +AP100ppm+CA20ppm	0.001		
CO+ TBHQ200ppm+AP100ppm +CA30ppm	0.009	0.109	
CO+ TBHQ200ppm+AP100ppm +CA50ppm	0.000	0.333	0.333

TBARS, Figure20, 4 Hours Heat Treatment			
	CO	CO+ TBHQ200ppm+ AP100ppm+CA20ppm	CO+ TBHQ200ppm+ AP100ppm +CA20ppm
CO+ TBHQ200ppm +AP100ppm+CA20ppm	0.474		
CO+ TBHQ200ppm+AP100ppm +CA30ppm	0.090	0.232	
CO+ TBHQ200ppm+AP100ppm +CA50ppm	**0.000**	**0.000**	**0.007**

TBARS, Figure20, 6 Hours Heat Treatment			
	CO	CO+ TBHQ200ppm+ AP100ppm+CA20ppm	CO+ TBHQ200ppm+ AP100ppm +CA20ppm
CO+ TBHQ200ppm +AP100ppm+CA20ppm	**0.000**		
CO+ TBHQ200ppm+AP100ppm +CA30ppm	**0.001**	0.156	
CO+ TBHQ200ppm+AP100ppm +CA50ppm	**0.000**	**0.001**	**0.040**

TBARS, Figure21, 0 Hours Heat Treatment		
	CO	CO+Carnosol100ppm
CO+Carnosol100ppm	0.288	
CO+Carnosol200ppm	0.679	0.179

TBARS, Figure21, 1 Hours Heat Treatment		
	CO	CO+Carnosol100ppm
CO+Carnosol100ppm	1.000	
CO+Carnosol200ppm	0.721	0.721

TBARS, Figure21, 2 Hours Heat Treatment		
	CO	CO+Carnosol100ppm
CO+Carnosol100ppm	0.032	
CO+Carnosol200ppm	0.105	0.818

TBARS, Figure21, 4 Hours Heat Treatment		
	CO	CO+Carnosol100ppm
CO+Carnosol100ppm	**0.019**	
CO+Carnosol200ppm	0.265	0.172

TBARS, Figure21, 6 Hours Heat Treatment		
	CO	CO+Carnosol100ppm
CO+Carnosol100ppm	0.799	
CO+Carnosol200ppm	**0.000**	**0.000**

TBARS, Figure22, 0 Hours Heat Treatment		
	CO	CO+ Carnosic acid100ppm
CO+Carnosic acid100ppm	0.111	
CO+ Carnosic acid200ppm	0.288	0.403

TBARS, Figure22, 1 Hours Heat Treatment		
	CO	CO+ Carnosic acid100ppm
CO+ Carnosic acid100ppm	0.293	
CO+ Carnosic acid200ppm	**0.034**	**0.018**

TBARS, Figure22, 2 Hours Heat Treatment		
	CO	CO+ Carnosic acid100ppm
CO+ Carnosic acid100ppm	**0.028**	
CO+ Carnosic acid200ppm	1.000	**0.030**

TBARS, Figure22, 4 Hours Heat Treatment		
	CO	CO+ Carnosic acid100ppm
CO+ Carnosic acid100ppm	0.104	
CO+ Carnosic acid200ppm	**0.011**	0.636

TBARS, Figure22, 6 Hours Heat Treatment		
	CO	CO+ Carnosic acid100ppm
CO+ Carnosic acid100ppm	0.851	
CO+ Carnosic acid200ppm	0.323	0.346

TBARS, Figure23, 0 Hour Heat Treatment			
	CO	CO+RE50ppm	CO+RE100ppm
CO+RE50ppm	**0.000**		
CO+RE100ppm	0.070	**0.001**	
CO+RE200ppm	**0.013**	**0.004**	**0.049**

TBARS, Figure23, 1 Hour Heat Treatment			
	CO	CO+RE50ppm	CO+RE100ppm
CO+RE50ppm	0.496		
CO+RE100ppm	0.728	0.494	
CO+RE200ppm	0.157	0.059	0.617

TBARS, Figure23, 2 Hour Heat Treatment			
	CO	CO+RE50ppm	CO+RE100ppm
CO+RE50ppm	**0.015**		
CO+RE100ppm	**0.032**	0.419	
CO+RE200ppm	**0.001**	0.665	0.156

TBARS, Figure23, 4 Hour Heat Treatment			
	CO	CO+RE50ppm	CO+RE100ppm
CO+RE50ppm	**0.007**		
CO+RE100ppm	0.098	0.816	
CO+RE200ppm	**0.000**	0.407	0.460

TBARS, Figure23, 6 Hour Heat Treatment			
	CO	CO+RE50ppm	CO+RE100ppm
CO+RE50ppm	**0.000**		
CO+RE100ppm	**0.001**	0.569	
CO+RE200ppm	**0.000**	0.536	0.861

HNE, Figure 24-25, 1 Hour Heat Treatment						
	CO	CO +RE50ppm	CO +RE100ppm	CO +TBHQ200ppm	CO +TBHQ200ppm +CA50ppm	CO +TBHQ200ppm +AP100ppm
CO+RE50ppm	**0.000**					
CO+RE100ppm	0.663	**0.000**				
CO+TBHQ200ppm	**0.005**	0.054	**0.007**			
CO+TBHQ200ppm+ CA50ppm	**0.000**	0.829	**0.009**	**0.044**		
CO+TBHQ200ppm+ AP100ppm	**0.000**	0.753	**0.005**	0.059	0.870	
CO+TBHQ200ppm+ AP100ppm+CA50ppm	**0.000**	0.082	**0.002**	**0.034**	0.275	0.381

HNE, Figure 24-25, 2 Hour Heat Treatment						
	CO	CO +RE50ppm	CO +RE100ppm	CO +TBHQ200ppm	CO +TBHQ200ppm +CA50ppm	CO +TBHQ200ppm +AP100ppm
CO+RE50ppm	0.100					
CO+RE100ppm	**0.003**	**0.036**				
CO+TBHQ200ppm	**0.000**	**0.000**	**0.000**			
CO+TBHQ200ppm+ CA50ppm	**0.000**	**0.000**	**0.000**	0.106		
CO+TBHQ200ppm+ AP100ppm	**0.000**	**0.000**	**0.000**	**0.001**	**0.004**	
CO+TBHQ200ppm+ AP100ppm+CA50ppm	**0.000**	**0.001**	**0.000**	0.175	**0.026**	**0.000**

HNE, Figure 24-25, 4 Hour Heat Treatment						
	CO	CO +RE50ppm	CO +RE100ppm	CO +TBHQ200ppm	CO +TBHQ200ppm +CA50ppm	CO +TBHQ200ppm +AP100ppm
CO+RE50ppm	**0.016**					
CO+RE100ppm	**0.006**	0.292				
CO+TBHQ200ppm	**0.011**	0.063	**0.012**			
CO+TBHQ200ppm+ CA50ppm	**0.015**	**0.039**	0.260	**0.000**		
CO+TBHQ200ppm+ AP100ppm	**0.008**	**0.002**	**0.001**	**0.000**	**0.000**	
CO+TBHQ200ppm+ AP100ppm+CA50ppm	**0.021**	0.116	0.257	0.006	0.619	**0.002**

HNE, Figure 24-25, 6 Hour Heat Treatment						
	CO	CO +RE50ppm	CO +RE100ppm	CO +TBHQ200ppm	CO +TBHQ200ppm +CA50ppm	CO +TBHQ200ppm +AP100ppm
CO+RE50ppm	**0.000**					
CO+RE100ppm	**0.007**	0.248				
CO+TBHQ200ppm	**0.000**	**0.001**	0.056			
CO+TBHQ200ppm+ CA50ppm	**0.005**	0.175	0.634	**0.001**		
CO+TBHQ200ppm+ AP100ppm	**0.002**	**0.001**	0.052	**0.048**	**0.000**	
CO+TBHQ200ppm+ AP100ppm+CA50ppm	**0.000**	**0.026**	0.934	**0.000**	0.448	**0.000**

HHE, Figure 28-29, 6 Hour Heat Treatment						
	CO	CO +RE50ppm	CO +RE100ppm	CO +TBHQ200ppm	CO +TBHQ200ppm +CA50ppm	CO +TBHQ200ppm +AP100ppm
CO+RE50ppm	0.426					
CO+RE100ppm	**0.000**	0.193				
CO+TBHQ200ppm	**0.000**	0.349	**0.001**			
CO+TBHQ200ppm+ CA50ppm	**0.000**	**0.060**	**0.000**	**0.000**		
CO+TBHQ200ppm+ AP100ppm	0.240	0.497	0.225	0.465	0.061	
CO+TBHQ200ppm+ AP100ppm+CA50ppm	**0.000**	**0.001**	**0.000**	**0.000**	**0.033**	0.068

HOE, Figure 30-31, 6 Hour Heat Treatment						
	CO	CO +RE50ppm	CO +RE100ppm	CO +TBHQ200ppm	CO +TBHQ200ppm +CA50ppm	CO +TBHQ200ppm +AP100ppm
CO+RE50ppm	**0.001**					
CO+RE100ppm	0.064	**0.001**				
CO+TBHQ200ppm	**0.000**	**0.012**	**0.049**			
CO+TBHQ200ppm+ CA50ppm	**0.000**	0.156	**0.004**	**0.006**		
CO+TBHQ200ppm+ AP100ppm	**0.013**	**0.001**	0.397	0.071	**0.001**	
CO+TBHQ200ppm+ AP100ppm+CA50ppm	**0.000**	**0.017**	**0.002**	**0.000**	**0.014**	**0.002**

HDE, Figure 32-33, 6 Hour Heat Treatment						
	CO	CO +RE50ppm	CO +RE100ppm	CO +TBHQ200ppm	CO +TBHQ200ppm +CA50ppm	CO +TBHQ200ppm +AP100ppm
CO+RE50ppm	**0.000**					
CO+RE100ppm	**0.010**	**0.001**				
CO+TBHQ200ppm	**0.000**	**0.000**	**0.001**			
CO+TBHQ200ppm+ CA50ppm	**0.000**	**0.025**	**0.000**	**0.000**		
CO+TBHQ200ppm+ AP100ppm	0.111	**0.001**	**0.018**	**0.002**	**0.000**	
CO+TBHQ200ppm+ AP100ppm+CA50ppm	**0.000**	**0.000**	**0.000**	**0.000**	**0.016**	**0.001**

The Sum of Total Polar Aldehydes, Figure 36-37, 1 Hour Heat Treatment						
	CO	CO +RE50ppm	CO +RE100ppm	CO +TBHQ200ppm	CO +TBHQ200ppm +CA50ppm	CO +TBHQ200ppm +AP100ppm
CO+RE50ppm	**0.002**					
CO+RE100ppm	**0.000**	**0.004**				
CO+TBHQ200ppm	**0.000**	**0.004**	0.060			
CO+TBHQ200ppm+ CA50ppm	**0.000**	**0.007**	**0.005**	**0.001**		
CO+TBHQ200ppm+ AP100ppm	**0.044**	0.932	**0.005**	**0.020**	**0.036**	
CO+TBHQ200ppm+ AP100ppm+CA50ppm	**0.000**	**0.016**	**0.020**	**0.002**	0.292	0.067

The Sum of Total Polar Aldehydes, Figure 36-37, 2 Hour Heat Treatment						
	CO	CO +RE50ppm	CO +RE100ppm	CO +TBHQ200ppm	CO +TBHQ200ppm +CA50ppm	CO +TBHQ200ppm +AP100ppm
CO+RE50ppm	0.273					
CO+RE100ppm	**0.027**	0.103				
CO+TBHQ200ppm	**0.001**	**0.000**	**0.000**			
CO+TBHQ200ppm+ CA50ppm	**0.000**	**0.000**	**0.000**	**0.000**		
CO+TBHQ200ppm+ AP100ppm	**0.000**	**0.000**	**0.000**	**0.000**	**0.011**	
CO+TBHQ200ppm+ AP100ppm+CA50ppm	**0.000**	**0.001**	**0.002**	**0.009**	**0.041**	0.295

The Sum of Total Polar Aldehydes, Figure 36-37, 4 Hour Heat Treatment						
	CO	CO +RE50ppm	CO +RE100ppm	CO +TBHQ200ppm	CO +TBHQ200ppm +CA50ppm	CO +TBHQ200ppm +AP100ppm
CO+RE50ppm	**0.000**					
CO+RE100ppm	**0.009**	0.182				
CO+TBHQ200ppm	**0.000**	0.102	**0.018**			
CO+TBHQ200ppm+ CA50ppm	**0.000**	**0.039**	0.221	**0.000**		
CO+TBHQ200ppm+ AP100ppm	**0.002**	**0.011**	**0.006**	0.123	**0.001**	
CO+TBHQ200ppm+ AP100ppm+CA50ppm	**0.000**	0.058	0.117	**0.005**	**0.004**	**0.006**

The Sum of Total Polar Aldehydes, Figure 36-37, 6 Hour Heat Treatment						
	CO	CO +RE50ppm	CO +RE100ppm	CO +TBHQ200ppm	CO +TBHQ200ppm +CA50ppm	CO +TBHQ200ppm +AP100ppm
CO+RE50ppm	**0.000**					
CO+RE100ppm	**0.027**	**0.004**				
CO+TBHQ200ppm	**0.000**	**0.000**	0.099			
CO+TBHQ200ppm+ CA50ppm	**0.003**	**0.001**	0.104	0.538		
CO+TBHQ200ppm+ AP100ppm	**0.000**	**0.003**	0.204	**0.002**	**0.020**	
CO+TBHQ200ppm+ AP100ppm+CA50ppm	**0.000**	**0.001**	**0.014**	**0.000**	**0.000**	**0.001**

The Sum of Total Non-Polar Aldehydes, Figure 38-39, 1 Hour Heat Treatment						
	CO	CO +RE50ppm	CO +RE100ppm	CO +TBHQ200ppm	CO +TBHQ200ppm +CA50ppm	CO +TBHQ200ppm +AP100ppm
CO+RE50ppm	0.669					
CO+RE100ppm	**0.000**	**0.000**				
CO+TBHQ200ppm	**0.000**	0.106	**0.031**			
CO+TBHQ200ppm+ CA50ppm	**0.000**	**0.000**	**0.000**	**0.000**		
CO+TBHQ200ppm+ AP100ppm	**0.001**	**0.000**	**0.000**	**0.000**	0.444	
CO+TBHQ200ppm+ AP100ppm+CA50ppm	**0.000**	**0.001**	**0.003**	**0.002**	**0.000**	**0.000**

The Sum of Total Non-Polar Aldehydes, Figure 38-39, 2 Hour Heat Treatment						
	CO	CO +RE50ppm	CO +RE100ppm	CO +TBHQ200ppm	CO +TBHQ200ppm +CA50ppm	CO +TBHQ200ppm +AP100ppm
CO+RE50ppm	0.054					
CO+RE100ppm	**0.002**	**0.031**				
CO+TBHQ200ppm	**0.000**	**0.008**	**0.000**			
CO+TBHQ200ppm+ CA50ppm	**0.000**	**0.035**	0.733	**0.000**		
CO+TBHQ200ppm+ AP100ppm	**0.000**	**0.006**	0.095	0.063	**0.025**	
CO+TBHQ200ppm+ AP100ppm+CA50ppm	**0.000**	**0.000**	**0.001**	**0.000**	**0.000**	**0.000**

The Sum of Total Non-Polar Aldehydes, Figure 38-39, 4 Hour Heat Treatment						
	CO	CO +RE50ppm	CO +RE100ppm	CO +TBHQ200ppm	CO +TBHQ200ppm +CA50ppm	CO +TBHQ200ppm +AP100ppm
CO+RE50ppm	**0.000**					
CO+RE100ppm	**0.000**	0.581				
CO+TBHQ200ppm	**0.000**	**0.003**	**0.004**			
CO+TBHQ200ppm+ CA50ppm	**0.000**	**0.011**	**0.019**	0.900		
CO+TBHQ200ppm+ AP100ppm	**0.000**	**0.004**	0.074	0.107	0.144	
CO+TBHQ200ppm+ AP100ppm+CA50ppm	**0.000**	0.086	**0.004**	0.069	0.097	0.875

The Sum of Total Non-Polar Aldehydes, Figure 38-39, 6 Hour Heat Treatment						
	CO	CO +RE50ppm	CO +RE100ppm	CO +TBHQ200ppm	CO +TBHQ200ppm +CA50ppm	CO +TBHQ200ppm +AP100ppm
CO+RE50ppm	**0.000**					
CO+RE100ppm	**0.005**	**0.005**				
CO+TBHQ200ppm	**0.002**	**0.006**	0.130			
CO+TBHQ200ppm+ CA50ppm	**0.008**	**0.018**	0.411	0.058		
CO+TBHQ200ppm+ AP100ppm	**0.007**	**0.020**	0.170	0.107	0.159	
CO+TBHQ200ppm+ AP100ppm+CA50ppm	**0.001**	**0.027**	**0.000**	**0.000**	**0.000**	**0.000**

The Sum of Total Polar and Non-Polar Aldehydes, Figure 40-41, 1 Hour Heat Treatment						
	CO	CO +RE50ppm	CO +RE100ppm	CO +TBHQ200ppm	CO +TBHQ200ppm +CA50ppm	CO +TBHQ200ppm +AP100ppm
CO+RE50ppm	0.979					
CO+RE100ppm	**0.000**	**0.000**				
CO+TBHQ200ppm	**0.000**	0.560	**0.014**			
CO+TBHQ200ppm+ CA50ppm	**0.000**	**0.000**	**0.000**	**0.002**		
CO+TBHQ200ppm+ AP100ppm	**0.001**	**0.000**	**0.001**	**0.000**	0.593	
CO+TBHQ200ppm+ AP100ppm+CA50ppm	**0.000**	**0.001**	**0.004**	**0.003**	**0.000**	**0.000**

The Sum of Total Polar and Non-Polar Aldehydes, Figure 40-41, 2 Hour Heat Treatment						
	CO	CO +RE50ppm	CO +RE100ppm	CO +TBHQ200ppm	CO +TBHQ200ppm +CA50ppm	CO +TBHQ200ppm +AP100ppm
CO+RE50ppm	0.062					
CO+RE100ppm	**0.002**	**0.037**				
CO+TBHQ200ppm	**0.000**	**0.007**	**0.000**			
CO+TBHQ200ppm+ CA50ppm	**0.000**	**0.019**	0.214	**0.001**		
CO+TBHQ200ppm+ AP100ppm	**0.000**	**0.004**	0.051	0.105	**0.021**	
CO+TBHQ200ppm+ AP100ppm+CA50ppm	**0.000**	**0.000**	**0.001**	**0.000**	**0.000**	**0.000**

The Sum of Total Polar and Non-Polar Aldehydes, Figure 40-41, 4 Hour Heat Treatment						
	CO	CO +RE50ppm	CO +RE100ppm	CO +TBHQ200ppm	CO +TBHQ200ppm +CA50ppm	CO +TBHQ200ppm +AP100ppm
CO+RE50ppm	**0.002**					
CO+RE100ppm	**0.004**	0.361				
CO+TBHQ200ppm	**0.006**	**0.013**	**0.004**			
CO+TBHQ200ppm+ CA50ppm	**0.007**	0.073	**0.024**	0.197		
CO+TBHQ200ppm+ AP100ppm	**0.002**	0.116	0.051	0.121	0.517	
CO+TBHQ200ppm+ AP100ppm+CA50ppm	**0.001**	**0.013**	**0.004**	**0.042**	0.189	0.535

The Sum of Total Polar and Non-Polar Aldehydes, Figure 40-41, 6 Hour Heat Treatment						
	CO	CO +RE50ppm	CO +RE100ppm	CO +TBHQ200ppm	CO +TBHQ200ppm +CA50ppm	CO +TBHQ200ppm +AP100ppm
CO+RE50ppm	**0.000**					
CO+RE100ppm	**0.017**	**0.001**				
CO+TBHQ200ppm	**0.001**	**0.001**	0.310			
CO+TBHQ200ppm+ CA50ppm	**0.024**	**0.001**	0.611	0.136		
CO+TBHQ200ppm+ AP100ppm	**0.003**	**0.008**	0.129	0.228	0.136	
CO+TBHQ200ppm+ AP100ppm+CA50ppm	**0.000**	**0.016**	**0.002**	**0.000**	**0.007**	**0.000**

Suppression of HNE formation				
Time (h)	1	2	4	6
RE50	32%		43%	18%
RE100		12%	33%	29%
TBHQ200	17%	36%	51%	
TBHQ200+CA50	34%	38%	28%	24%
TBHQ200+AP100	34%	43%	60%	
TBHQ200+CA50+AP100	38%	34%	26%	27%

Suppression of 4-hydroxyaldehydes formation (6h)				
	HHE	HOE	HNE	HDE
RE50	36%	48%	18%	74%
RE100	40%	9%	29%	15%
TBHQ200	20%	20%		
TBHQ200+CA50		13%	24%	4%
TBHQ200+AP100	83%	40%		84%
TBHQ200+CA50+AP100	88%	70%	27%	96%

Suppression of the sum of total polar aldehydes formation				
Time (h)	1	2	4	6
RE50			38%	59%
RE100			29%	24%
TBHQ200		21%	48%	18%
TBHQ200+CA50		34%	24%	17%
TBHQ200+AP100		40%	52%	38%
TBHQ200+CA50+AP100		47%	36%	72%

Suppression of the sum of total non-polar aldehydes formation				
Time (h)	1	2	4	6
RE50	38%		19%	23%
RE100	36%	14%	18%	13%
TBHQ200	42%	28%	29%	16%
TBHQ200+CA50		15%	29%	11%
TBHQ200+AP100		22%	26%	14%
TBHQ200+CA50+AP100	49%	51%	25%	31%

Suppression of the sum of total polar and non-polar aldehydes formation				
Time (h)	1	2	4	6
RE50	36%		22%	28%
RE100	30%	13%	20%	14%
TBHQ200	37%	28%	31%	16%
TBHQ200+CA50		16%	30%	12%
TBHQ200+AP100		23%	28%	17%
TBHQ200+CA50+AP100	46%	51%	27%	37%