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ANTIOXIDANT ACTIVITY OF CORN FIBER OIL

By

Vareemon Tuntivanich

A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE

Department of Food Science and Human Nutrition

2003

ABSTRACT

ANTIOXIDANT ACTIVITY OF CORN FIBER OIL

By

Vareemon Tuntivanich

The objective of this study was to evaluate the antioxidant activity of corn fiber oil (CFO). CFO was tested in two lipid systems, bulk oil and cooked ground meat, using peroxide value and 2-thiobarbituric acid tests, respectively. After characterizing the concentration dependence of CFO in cooked ground beef and turkey, subsequent studies were conducted in the meat systems to identify the relative contribution of CFO components (oryzanol, β-sitosterol, or linoleic acid) to the antioxidant activity. CFO and oryzanol were also tested for synergy with propyl gallate.

Concentration-dependent decreases in lipid oxidation were observed with increasing CFO concentration, both in bulk oil and in the meat systems. The greatest inhibition of peroxide formation was observed at 5% CFO containing linoleic acid in bulk oil system. However, the degree of inhibition in bulk oil was not sufficient to be of practical value. In the meat system, the highest concentration tested, 0.75% CFO, showed the highest inhibition of lipid oxidation. The combination of three principal compounds matched the antioxidant activity of CFO. Synergy was observed in the combination of 0.25% CFO with 0.01% (w/w) propyl gallate. We conclude that CFO can play an important role not only as a modulator of cholesterol absorption in human health but also as a natural antioxidant in processed food products.

To my beloved family, especially my sister,

for all their support and encouragement

ACKNOWLEDGMENTS

I would like to express gratitude to my major advisor, Dr. Gale Strasburg, for his guidance, encouragement, and support in my degree program, and his patience to understand my communication.

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I would like to thank Dr. Enayat Gomma for her assistance with training PV and TBARS analysis. I also would like to thank Dr. Wesley Osburn for allowing me to work in his lab and Christine Quinlan for assistance with distillation instrument. Special acknowledgment is also expressed to Dr. Rob Tempelman for his assistance and advice on the statistical analysis of my data. Jennifer Dominguez's assistance on meat processing lab is greatly appreciated. I am also appreciated for John Rodgers's training in HAAs analysis; even though, HAAs project is not completely done and included in this thesis and also for his encouragement. I wish to thank my laboratory colleagues, Andrea Molengraft, Hyo-Jung Yoon, Jin-Shan Shie, and Dr. Wen Chiang for all their support and understanding.

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INTRODUCTION

Lipid oxidation causes rancidity, which leads to the development of off flavors and odors, undesirable taste and appearance, reduction in shelf-life, and loss of quality (Frankel, 1998). Lipid oxidation is characterized by three stages: initiation, propagation, and termination. The end products include aldehydes, ketones, alcohols, alkenes, and/or hydroxy acids. The primary factors that promote lipid oxidation during processing and storage include oxygen, light, temperature, chelating agents, irradiation, and homogenization.

Antioxidants are free radical scavengers, which can delay the oxidation reactions. Antioxidants can be derived from synthetic or natural sources. Although synthetic antioxidants are efficient and relatively cheap, recent work has focused on identification of natural antioxidants that are safe, nontoxic, and may also promote health benefits (Mukhopadhyay, 2000).

Corn fiber oil, a by-product from wet-milling grain, is one natural source that potentially has antioxidant properties and may serve as an important ingredient in functional foods. There are no reports in the literature on antioxidant effects of corn fiber oil in food system. Corn fiber oil contains several compounds such as triacylglycerols, sterol esters, free fatty acids, phytosterols, and tocopherols. Among these, there are three important bioactive compounds: oryzanol, β-sitosterol, and linoleic acid. Oryzanol and β-sitosterol have been shown to lower serum cholesterol levels in laboratory animals and human (Wilson et al., 2000; Xu et al., 2001). Studies on oryzanols suggest that they acted as antioxidants via the ferulic acid moiety (Graf, 1992), but little has been done to analyze the antioxidant activity of β-

sitosterol. Finotti et al. (2000) showed that β-sitosterol is a weak pro-oxidant. However, in combination with α-tocopherol and/or squalene, there was significant positive synergy for inhibition of lipid oxidation. These studies were done using model systems, which may not be representative of its behavior in a food system. Based on the structural features of β-sitosterol and oryzanol in corn fiber oil, we hypothesize that corn fiber oil has antioxidant activity in food systems. However, the application of antioxidants into food depends on the type of food being stabilized because antioxidants exhibit differential activity in different food systems e.g. bulk oil, micelles, and emulsions (Frankel, 1993). Therefore, we investigated the antioxidant activity of CFO in bulk oil and cooked ground meats (beef and turkey).

The objectives of these studies were to: 1) determine antioxidant activity of CFO in two lipid systems (bulk corn oil and meat), 2) evaluate antioxidant activities of the three principal components (oryzanol, β-sitosterol, and linoleic acid) present in CFO, individually and in combination, and 3) assess the synergistic effect of CFO, oryzanol, and propyl gallate.

CHAPTER ONE

LITERATURE REVIEW

Lipid Oxidation

Oxidation of food products is a major factor in the development of off-flavors due to chemical changes of lipids. The free-radical-based oxidative reaction, referred to as autoxidation, is the direct reaction of atmospheric oxygen with lipids to produce hydroperoxides. The hydroperoxides are unstable and break down to form a variety of volatile flavor compounds associated with rancidity. Free radicals generated by the breakdown of hydroperxides generate new lipid radicals. Thus, once the reaction starts, it is difficult to stop (Schmidt, 2000).

Although autoxidation can proceed in any lipid-containing food product during storage, it is a particular problem in animal tissues such as muscle. Rapid oxidation referred to as "warmed-over flavor," is promoted by cooking and attributed mainly to autoxidation of the phospholipids, which are the principal lipid components of cellular membranes.

Mechanism of Lipid Oxidation

Swern (1961) showed that autoxidation, a free radical chain process involving unsaturated organic substances, follows the basic sequences of initiation, propagation, and termination as described below:

Initiation:

$$RH \longrightarrow R^{\bullet} + H^{\bullet} \qquad \text{(Eq. 1)}$$

Propagation:

$R^{\bullet} + O_2 \longrightarrow ROO^{\bullet}$ (Eq. 2)

$ROO^{\bullet} + R'H \longrightarrow R'^{\bullet} + ROOH$ (Eq. 3)

$ROOH \longrightarrow RO^{\bullet} + {}^{\bullet}OH$ (Eq. 4)

Termination:

$R^{\bullet} + R^{\bullet} \longrightarrow RR$ (Eq. 5)

$R^{\bullet} + ROO^{\bullet} \longrightarrow ROOR$ (Eq. 6)

$ROO^{\bullet} + ROO^{\bullet} \longrightarrow ROOR + O_2$ (Eq. 7)

The induction period or initiation step of lipid oxidation is a slow process that increases in rate with time (Chan, 1987). In the initiation step, a hydrogen atom (H•) is abstracted from an acylglycerol molecule (RH) resulting in a formation of an alkyl radical (R•) (Equation 1). This alkyl radical can react with diatomic oxygen (O_2) to form a peroxyl radical (ROO•) (Equation 2), which can initiate another acylglycerol molecule into the oxidation cycle by abstraction of a hydrogen atom to form a hydroperoxide (ROOH) and a new free radical species ($R'^{\bullet}$) (Equation 3). The resulting hydroperoxide is unstable and cleaves to form an alkoxyl radical (RO•) and hydroxyl radical (•OH) (Equation 4). The decomposition of hydroperoxides leads to formation of secondary oxidation products including aldehydes, hydrocarbons, and esters. This is referred to as the chain propagation step. Formation and breakdown of hydroperoxides are also involved in reactions that cause oxidation of pigments, flavor components, and vitamins (Dugan, 1996). Termination reactions involve the combination of radicals to form stable, non-radical

products (Equations 5, 6, and 7).

Factors Affecting Lipid Oxidation

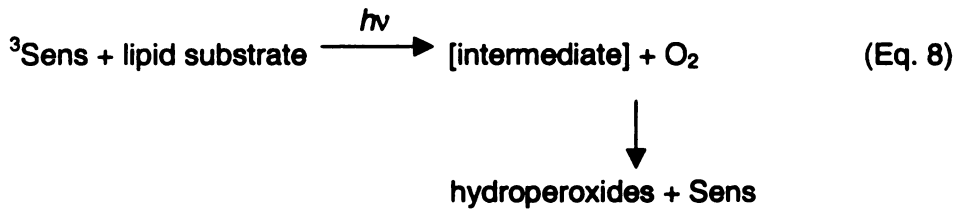
The oxidative mechanism and rate of lipid oxidation in food products are influenced by several factors such as oxygen partial pressure, light, temperature, water activity (Aw), trace metals, and enzymes.

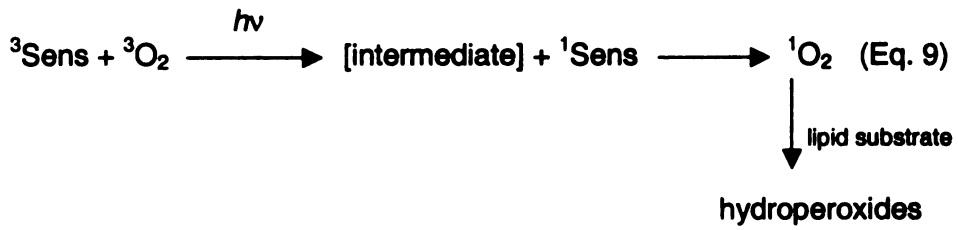
Temperature is the most important acceleration factor in the lipid oxidation process. Elevated temperatures increase diffusion and thus accelerate rate of lipid oxidation. Barends (1993) determined the rate constants for lipid oxidation of a model food system at three temperatures (23, to 40, and to 66 °C). The results indicated that at a constant oxygen concentration, greater amounts of hexanal were produced as the temperature increased.

Cooked meat is more susceptible to lipid oxidation than uncooked meat (Igene and Pearson, 1979; Pearson and Gray, 1983). Heating facilitates the interaction between membrane lipids and pro-oxidant ferrous/ferric ions released from denatured myoglobin and thus heating accelerates the oxidation rate. The rate of oxidation increases in direct proportion to the surface area of the lipid exposed to air (Nawar, 1996).

The rate of heating and final temperature of sample both can influence the release of iron from meat pigments (Schricker and Miller, 1983; Chen et. al., 1984). However, oxidative changes in lipids may occur in uncooked meat when it is subjected to freeze-thawing, size reduction, temperature changes in handling/distribution, and/or prolonged storage.

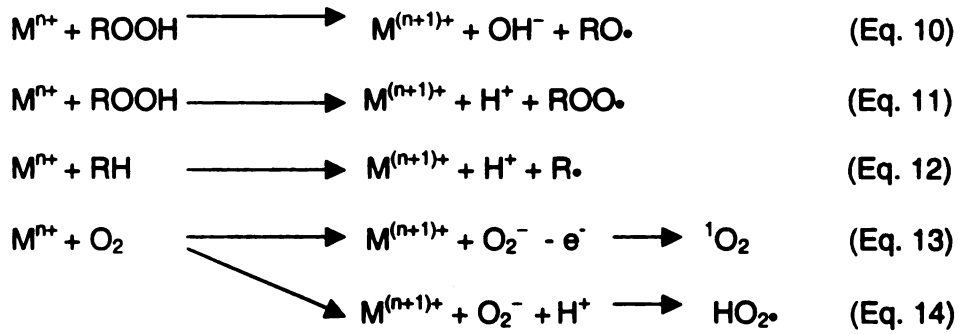
Light is a catalyst for oxidative rancidity in food products during storage. Photosensitized oxidation involves the production of singlet oxygen that can attack lipid double bonds directly or can react with free radicals. Photosensitized oxidation is classified as Type I or Type II according to the initial interaction of the excited sensitizer. Type I is the direct interaction of the sensitizer with another molecule and is associated with the production of free radicals (Equation 8). Type II is the transfer of energy from the excited triplet state of the sensitizer to produce singlet oxygen (Equation 9). Photosensitisers include riboflavin and chlorophyll. Quast and Karel (1972) demonstrated that artificial room light or sunlight would increase oxygen uptake by potato chips indicating that the chips are undergoing photosensitized lipid oxidation.





Transition metals including Co, Cu, Fe, and Mn can reduce the length of the induction period and increase the rate of lipid oxidation. Trace amounts of heavy metals are found in most edible oils. These metals are picked up

from the soil during plant growth or from equipment during processing (Nawar, 1996). Metals act as pro-oxidants by accelerating hydroperoxide decomposition (Equations 10 and 11), attacking an unoxidized substrate to remove a hydrogen and form a free radical (Equation 12), or by activating oxygen molecules to the singlet oxygen state and peroxy radical (Equations 13 and 14).



Oxygen is an essential factor required to initiate formation of hydroperoxides in the lipid oxidation reaction. Quast and Karel (1972) demonstrated that the rate of oxygen uptake of potato chips is a function of oxygen concentration when the oxygen partial pressure is lower than 0.1 atm. Thomas (1994) studied the effect of varying oxygen concentration levels, using oxygen absorbers, on the shelf life of packaged potato chips. An initial oxygen concentration of 0.2% (v/v) in the package headspace showed significantly less hexanal formation than that found at an oxygen concentration of 2%, after 22 weeks without oxygen absorber. The effect of oxygen concentration on rate is also influenced by other factors, such as temperature and surface area. The oxygen concentration can be controlled by the use of vacuum packaging, or modified atmosphere packaging with inert

gas or nitrogen to control the headspace composition within the package system.

Lipid oxidation is affected in complex fashion by water activity (Aw). In dried foods with Aw values of less than about 0.1, oxidation proceeds very rapidly due to the removal of water that shields pro-oxidant metal ions and lipid peroxides. Increasing the Aw from 0.1 to about 0.3 retards lipid oxidation; typically the minimum rate of oxidation is at Aw of about 0.3. This small amount of water is believed to reduce the catalytic activity of metal catalysts resulting in lower activity of the pro-oxidant. At higher water activities approximately 0.55-0.85, the rate of oxidation increases again. The increase in oxidation rate with increasing Aw is believed to be due to an increased mobility of pro-oxidant catalysts (Labuza, 1971).

Prevention of Lipid Oxidation

Antioxidants

Antioxidants comprise a major class of compounds (U.S. Food and Drug Administration: 21 CFR (Code of Federal Regulations) 170.3) that prolong the onset of deterioration, rancidity, or discoloration of food products which occur due to oxidation, as defined by the United States Food and Drug Administration (FDA) (Dziezak, 1986). Antioxidants may retard lipid oxidation, either by direct addition to the food products or by incorporating them into the packaging materials (Coulter, 1988).

Addition of antioxidants to food products is regulated by law in the United States. Newly discovered antioxidants that could be used for human consumption must be approved either by the Food and Drug Administration

(FDA) or the United States Department of Agriculture (USDA) depending on the food to which it is added. For example, the FDA allows the addition of butylated hydroxyanisole (BHA) or butylated hydroxytoluene (BHT) either alone or in combination with other antioxidants up to 200 ppm when used in emulsion stabilizers for shortenings (U.S. Food and Drug Administration: 21 CFR 172.115 and 21 CFR 172.110). The USDA allows the use of BHA and BHT as ingredients in fresh pork, sausage, and pregrilled beef patties at the concentration of 0.01% w/w based on fat content and 0.02% w/w in combination with other antioxidants (U.S. Department of Agriculture: 9 CFR 424.21).

Antioxidants can be classified into three categories according to their mechanism: (1) free radical terminators, (2) oxygen scavengers, and (3) chelating agents.

Free Radical Terminators

Typically, free radical scavenging antioxidants deactivate or terminate active free radicals by donating a hydrogen atom to peroxyl radicals, alkoxy radicals or alkyl radicals. The antioxidant free radical formed is comparatively stable and does not easily react to initiate autoxidation; phenolic antioxidant radicals may donate an additional hydrogen atom to yield quinones (Everson et al., 1957). Therefore, the radical chain is broken and lipid peroxidation is slowed. The basic mechanism is competition between the inhibitor reaction and the chain propagation reaction (Nawar, 1996). Antioxidants (AH) inhibit free radical chain propagation in the following manner:

$R\bullet + AH \longrightarrow RH + A\bullet$ (Eq. 15)

$RO\bullet + AH \longrightarrow ROH + A\bullet$ (Eq. 16)

$R\bullet + A\bullet \longrightarrow RA$ (Eq. 17)

$RO\bullet + A\bullet \longrightarrow ROA$ (Eq. 18)

$AH\bullet + AH\bullet \longrightarrow A + AH_2$ (Eq. 19)

Many of the most effective free radical scavenging antioxidants are phenolic compounds. The hydrogen in the hydroxyl group of phenolic compounds is relatively easily abstracted. Phenolic radicals are stable because of resonance delocalization of the unpaired electron around the aromatic ring (Figure 1.1).

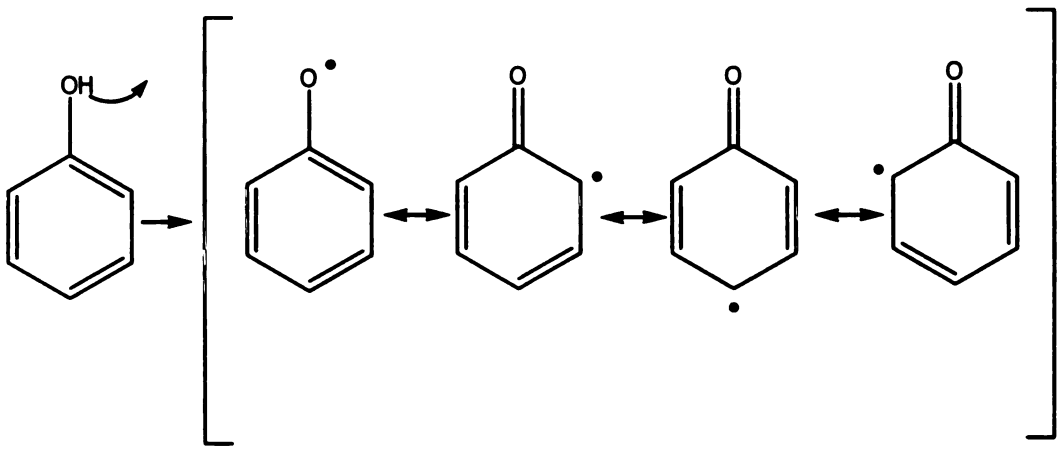


Figure 1.1. Resonance stabilization of a phenolic radical

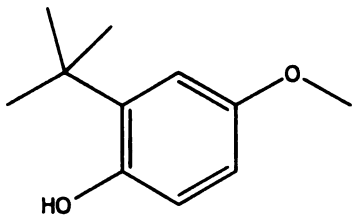
Numerous studies have evaluated synthetic antioxidants either alone or in combination with other antioxidants. In multi-component systems, antioxidant compounds can reinforce each other by cooperative effects known as synergism, a phenomenon in which the presence of one antioxidant enhances the effect of the second producing the greater activity than the sum of the individual antioxidant activity (Nawar, 1996). An example of antioxidant synergism is between BHA and BHT. Steric hindrance of BHT due to the tertiary groups results in the slower in reaction with ROO$^{\bullet}$ compared to BHA. The resulting BHA is the primary antioxidant reacting with peroxy radical (Equation 20). Consequently the resulting BHA free radical abstracts a hydrogen atom from BHT to regenerate itself (Equation 21). The following reaction thus occurs:

$$ROO^{\bullet}. + AH \longrightarrow ROOH + A^{\bullet}. \qquad \text{(Eq. 20)}$$

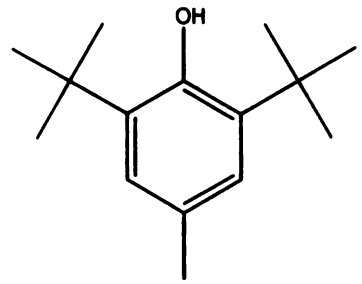
$$A^{\bullet}. + BH \longrightarrow B^{\bullet} + AH \qquad \text{(Eq. 21)}$$

where: AH = BHA and BH = BHT

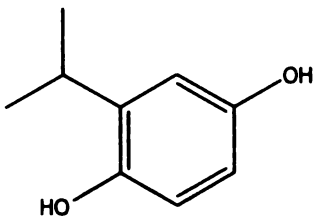
Some examples of free radical terminators include butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), tertiary butylhydroquinone (TBHQ), propyl gallate (PG), and tocopherols (Figure 1.2).



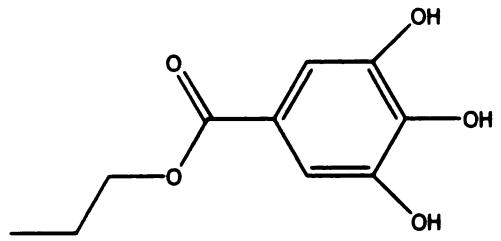
Butylated Hydroxyanisole (BHA)



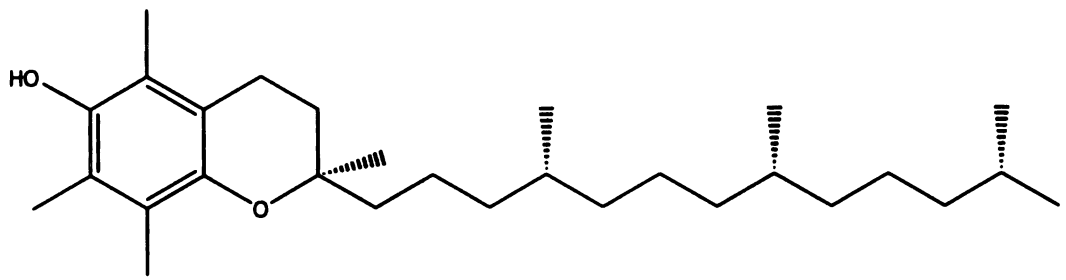
Butylated Hydroxytoluene (BHT)



2-tert-butylhydroquinone (TBHQ)



Propyl Gallate (PG)

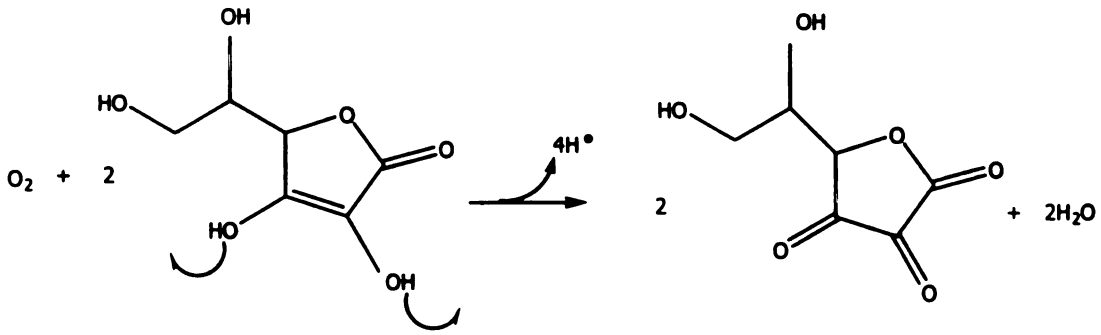


alpha-tocopherol

Figure 1.2. Structures of common antioxidants

Oxygen Scavengers

Oxygen scavengers reduce the presence of oxygen in packaged food products by transferring hydrogen atoms to an oxygen molecule and producing water. Generally, oxygen scavengers are reducing agents such as ascorbic acid, ascorbyl palmitate, sulfites, and erythorbic acid. An example of oxygen scavenging activity by ascorbic acid is shown in Figure 1.3. Xanthine oxidase, enzyme found in milk fat globule membranes generates superoxide radicals ($O_2^{-\bullet}$). This reactive oxygen species is formed when oxygen takes up one electron consequently generating enzymatic oxidation. Ascorbic acid can also act as an antioxidant by donating electrons and hydrogen ions, and reacting with reactive oxygen species such as the reaction with a superoxide to generate hydrogen peroxide (Nawar, 1996) (Figure 1.4).



Ascorbic Acid Dehydroascorbic Acid

Figure 1.3. Mechanism of oxygen scavenging by ascorbic acid

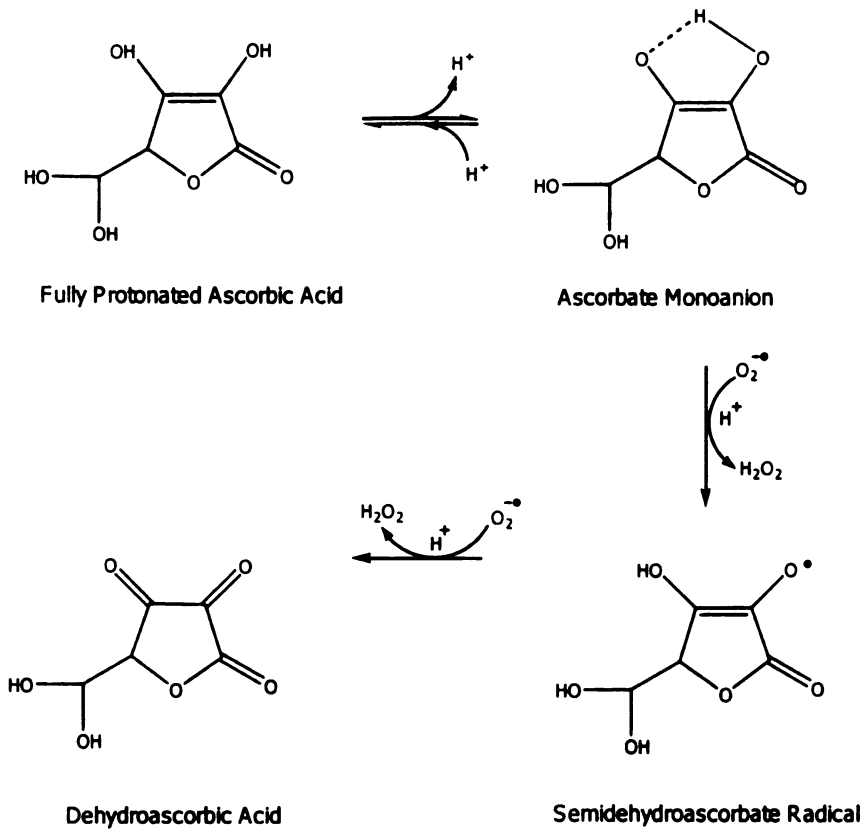
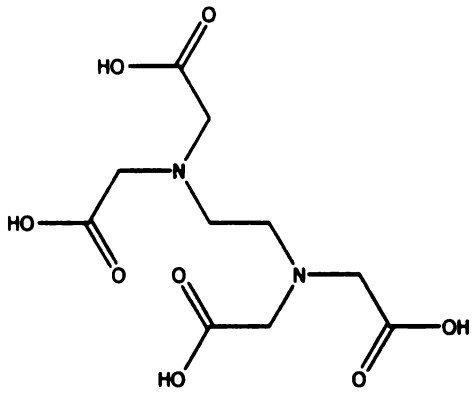


Figure 1.4. Mechanism of superoxide scavenging by ascorbic acid

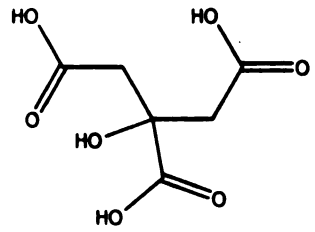
Chelating Agents

Metals, such as iron and copper, act as pro-oxidants. Chelators form complexes with metals because they contain unshared pairs of electrons in their molecular structure. These resulting complexes are stable and reduce the opportunity for metal-catalyzed oxidation. Examples of chelating agents (Figure 1.5) include citric acid, ethylenediaminetetraacetic acid (EDTA) and various polyphosphates. Timms and Watts (1958) reported that pyro-, tripoly-, and hexametaphosphates protect cooked meats against lipid oxidation by chelating heavy metal ions. Igene et al. (1979) showed that

EDTA chelated most of the free iron in cooked meat and therefore reduced the extent of lipid oxidation. However, some studies demonstrate a pro-oxidant effect of metal cheaters such as EDTA. Yoshida et al. (1993) showed that EDTA binds to both copper and iron at a molar ratio of 1 to 1. However, as the molar ratio of EDTA to metal increases, EDTA suppresses the copper-induced oxidation, whereas the iron-induced oxidation is enhanced. The acceleration effect of lipid oxidation could be described by the reduction of the higher valence states of cupric (Cu^{2+}) and ferric ions (Fe^{3+}) to lower the valence state of cuprous (Cu^{+}) and ferrous ions (Fe^{2+}), which react with hydroperoxide more rapidly. Therefore, the redox potential plays an important role on the oxidation rate.



Ethylenediaminetetraacetic Acid (EDTA)



Citric Acid

Figure 1.5. Structures of chelating agents

It is not practical to remove metal ions from foods because several transition ions that catalyze the formation of free radicals, such as iron and copper, are both essential nutrients. Moreover, iron is used to fortify many foods to ensure adequate intake by the general population. Therefore,

chelating agents capable of complexing metals in foods are often added under the regulation to reduce the pro-oxidant effects of metals (Nawar, 1996).

Synthetic Antioxidants

Synthetic phenolic antioxidants are the most widely used in food products because they are relatively inexpensive, highly effective, readily available, non-toxic, and readily available. Examples of synthetic antioxidants include BHA, BHT, PG, and TBHQ (Figure 1.2) (King, et al., 1993). These antioxidants act as free-radical terminators. BHT is one of the most extensively used antioxidants in the food industry. BHT is also widely used in combination with other antioxidants like BHA, PG, and citric acid, for the stabilization of oils and high fat foods.

Natural Antioxidants

The consumer is becoming increasingly concerned about the use of traditional synthetic antioxidants because of a perception that these compounds may be carcinogenic. Therefore, research has focused on the development and utilization of antioxidants from natural sources such as plants. The list of natural antioxidants includes tocopherols, ascorbic acid, carotenoids, flavonoids, lecithin, gum guaiac, and many others. A number of spices such as rosemary, sage, and paprika also possess excellent antioxidant activity (Loliger, 1983).

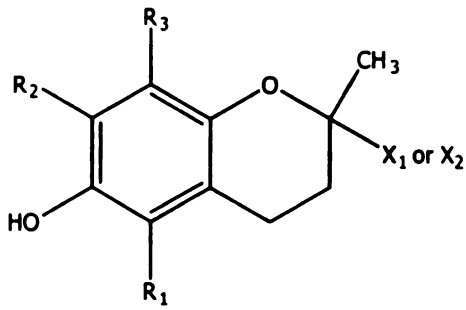
Tocopherols, which act as a free radical terminator, have the greatest popularity of the natural products typically found in vegetable oil. Vitamin E

consists of eight different compounds, four tocopherols and four tocotrienols (designated as α, β, γ, and δ) (Figure 1.6). Each tocotrienol has an identical chroman ring as the corresponding tocopherol by which carrying the active antioxidant group. The α, β, γ, and δ forms of tocopherol and tocotrienol differ according to the number and position of the methyl groups and thus differ significantly in vitamin E activity. The common commercial sources of natural vitamin E are soybeans, corn, cottonseed, canola, and sunflower oil.

The vitamin activity and antioxidant activity of the tocopherols tend to vary inversely. The order of reactivity toward singlet oxygen is $\alpha > \beta > \gamma > \delta$, and antioxidative potency is the reverse order. Alpha-tocopherol is recognized as the most effective form of vitamin E. However, in terms of antioxidant potency, α-tocopherol exhibits less activity than γ- and δ-tocopherols (Giese, 1996).

Recent studies have shown that increasing dietary tocopherols results in increased tissue levels of antioxidant. Mitsumoto (2000) showed that the average of α-tocopherol concentration in muscle is increased in crossbred beef steers that were supplemented with 1200 IU α-tocopheryl acetate per animal daily for 67 days prior to slaughter. Moreover, dietary vitamin E supplementation to cattle greatly suppressed lipid oxidation by reducing TBARS values in beef cuts.

Tocopherol activity is concentration dependent (Dugan, 1980). Kovats and Berndorfer-Kraszner (1968) and Dewdney et al. (1977) reported the most effective concentrations of tocopherol to be between 0.01% and 0.02% in a food product.



$X_1 = C_{16}H_{33} = (\text{-}CH_2\text{-}CH_2\text{-}CH_2\text{-}CH)_3\text{-}CH_3$ = Tocopherols
CH_3

$X_2 = C_{16}H_{30} = (\text{-}CH_2\text{-}CH_2\text{-}CH = CH)_3\text{-}CH_3$ = Tocotrienols
CH_3

Substance	R_1	R_2	R_3
α-Tocopherol	$-CH_3$	$-CH_3$	$-CH_3$
β-Tocopherol	$-CH_3$	-H	$-CH_3$
γ-Tocopherol	-H	$-CH_3$	$-CH_3$
δ-Tocopherol	-H	-H	$-CH_3$

Figure 1.6. Structures of tocopherols and tocotrienols

One of the most widely investigated natural antioxidants is rosemary extract. Barbut et al. (1985) found that the antioxidant effect of 20 mg/kg rosemary oleoresin incorporated in turkey sausage was comparable to that of a commercial BHA, BHT, citric acid mix. Houlihan et al. (1984, 1985) demonstrated that rosmaridiphenol androsmariquinone isolated from rosemary was more effective than BHA in preventing oxidation in lard. These compounds are thought to be capable of terminating free radical reactions and quenching reactive oxygen species.

Natural antioxidants potentially can be used in food processing if the production cost is not excessive, if the flavor and color are favorable, if the compounds are free of pathogenic or toxic activity and if they are bioavailable (Dugan, 1980). The interest of the food industry in phenolic antioxidants is related primarily to their antioxidant activity resulting in increased shelf life of food products. However, many of these compounds also show important biological activity *in vivo* and may be beneficial in reducing the incidence of chronic diseases such as artherosclerosis, aging, and some cancers. Antioxidants may also act as anti-mutagenic compounds because reactive oxygen species, such as $OH^{\bullet}$, are known to be involved in cancer development (Offord et. al., 1997). In a recent study, Balogh (1995) demonstrated that formation of carcinogenic heterocyclic aromatic amines could be inhibited by adding vitamin E (1% based on fat content) to ground beef before frying. The flavonoid constituent in olive oil appears to be responsible for the protection against coronary artery disease. The susceptibility of LDL to oxidation suggests that consumption of olive oil as a primary fat is responsible for the decreased incidence of cardiovascular disease reported in the Mediterranean countries (Kandaswami and Middleton, 1997)

Antioxidant Efficacy in Food Systems

There are many factors that must be taken into account when considering and selecting antioxidants or antioxidant-containing extracts for food application. The effectiveness of antioxidants depends not only on their chemical composition, structure or chemical interactions with components of

the oxidation pathway, but also on their location and orientation in a structured system (Coupland and McClements, 1996). Hydrophilicity and lipophilicity of the active components dictate appropriateness of antioxidants in systems. Porter (1980) showed that antioxidants that are polar or are amphiphilic with high hydrophile-lipophile balance (HLB), such as TBHQ and ascorbic acid tend to be more effective in a nonpolar medium (low surface to volume ratio; LSV), whereas antioxidants that are nonpolar or are amphiphilic with low HLB such as BHT, BHA, and ascorbyl palmitate tend to be relatively more effective in polar emulsions (high surface to volume ratio; HSV). Therefore this evidence leads to "polar paradox" terminology, which generally states that polar antioxidants function better in stabilizing nonpolar food matrices, while nonpolar antioxidants are more effective in polar lipid systems (e.g. oil-in-water emulsions and membranes) (Porter et al., 1989).

Polar antioxidants are more effective in bulk oils because they are more likely to concentrate at the air-oil interface forming a protective shield, which apparently reduces the accessibility of oxygen to the lipid substrate. On the other hand, nonpolar antioxidants are more effective in emulsions at oil-water interface because they partition into the lipid droplets forming a protective shield to reduce pro-oxidant effects of species in the aqueous medium (Figure 1.7) (Frankel et al., 1994).

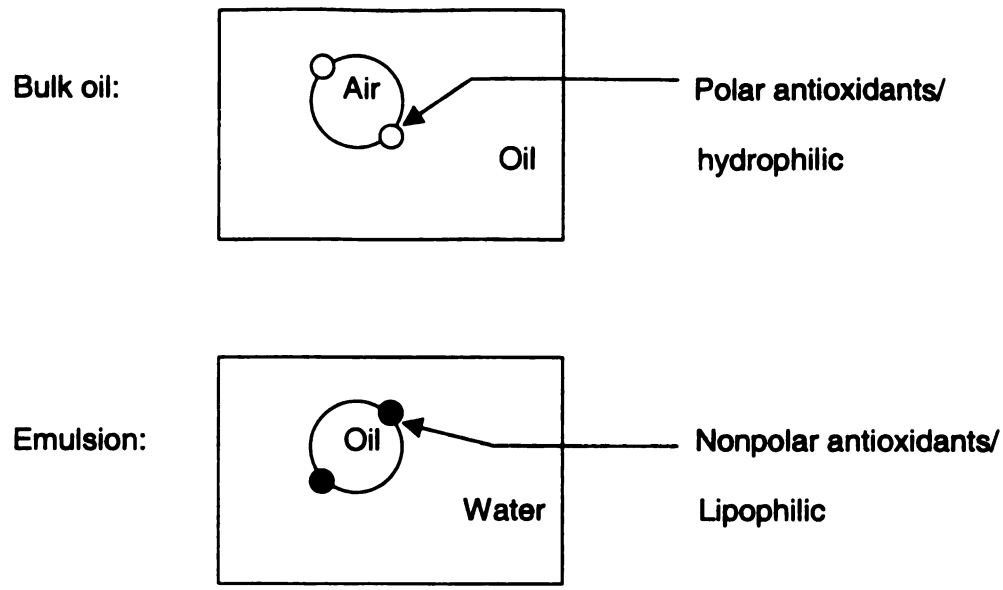


Figure 1.7. Explanation of interfacial phenomena: the action of antioxidants in bulk oil and oil-in-water emulsion systems (adapted from Frankel et al., 1994)

Measurements of Lipid Oxidation

Lipid oxidation can be measured by assessing the primary changes that can be described as the loss of reactants such as unsaturated fatty acids or oxygen. Another way to assess oxidation is the measurement of the formation of primary lipid oxidation products, such as hydroperoxides. Primary products decompose to form stable secondary products including carbonyls, aldehydes, and hydrocarbons (Gray and Monahan, 1992).

Chemical and physical tests for measuring lipid oxidation are generally based on the determination of hydroperoxides or their decomposition products. Chemical methods are more objective, although interferences and poor reproducibility are frequent problems. Physical tests, such as sensory evaluation (flavor assessment), are more practical for a judging flavor quality;

however, training of panelists is a time-consuming and the results are often variable because of the different training practices of the panel (Downey 1967).

Since lipid oxidation products are varied, it is necessary to use several sophisticated techniques to analyze them reliably. Among the many methods available, the peroxide value and thiobarbituric acid tests were used to assess the progress lipid oxidation in our studies and they are discussed in detail below.

Peroxide Value Test (PV Test)

PV test is based on an iodometric titration procedure and is a common measurement that has been used to determine the concentration of peroxide or hydroperoxide, primary autoxidation products, in bulk lipid substrate (Frankel 1993).

Peroxides can be quantified based on their ability to react with potassium iodide (iodometry) to produce iodine (I_2) and alcohol (ROH):

$$ROOH + KI \longrightarrow I_2 + KOH + ROH \qquad \text{(Eq. 22)}$$

Starch introduced into the system complexes with iodine to form a purple-black color. The starch-iodine complex is titrated with sodium thiosulfate ($Na_2S_2O_3$), which reduces iodine to iodide resulting in the disappearance of the purple-black color in the solution:

$$I_2 + 2\ Na_2S_2O_3 \longrightarrow 2\ NaI + Na_2SO_4 \qquad \text{(Eq. 23)}$$

The peroxide value is usually expressed in terms of milliequivalents of oxygen per kilogram of fat (Nawar, 1996). Since peroxides are the primary products of lipid oxidation, they are a good measure of oxidation in its initial stage. However, these hydroperoxides are unstable and decompose to secondary oxidation products (especially at temperatures above 60°C); therefore, one may underestimate the true extent of rancidity (Frankel 1993). The results from PV test vary with the incubation temperature of oil and with specific procedures used. Interferences may arise from the oxidation of iodide by dissolved oxygen from the air, exposure to light, absorption of iodine by the unsaturated bonds of fatty acid and variations in reactivity of different peroxides (Antolovich et al., 2002). Moreover, the determination of the endpoint of titration may vary from individual to individual and laboratory to laboratory.

The determination of peroxide value is useful for bulk oils because oil can be analyzed directly. However, this test is not well suited for analysis of multiphase complex food systems because lipid from emulsions or tissues must be extracted with mixtures of solvents that must be carefully removed without decomposition of hydroperoxides. Moreover, solvents might contain endogenous antioxidants that could interfere with peroxide value determination (Frankel, 1998).

Various attempts have been made to correlate peroxide values with development of oxidative off flavors. Lee et al. (2002) studied on the shelf-life of precooked frozen pork meat patties at various temperatures. They indicated that the sensory scores and peroxide value of the stored patties were related to each other. Abdalla (1999) determined the effect of garlic

supplementation on lipid oxidation in chicken breast and thigh meat after cooking and storage. He correlated the improvement of oxidative stability with sensory evaluation confirming the results of peroxide value.

Thiobarbituric Acid Test (TBA Test)

The TBA test is one of the most widely used methods for determining changes in oxidative deterioration in muscle foods (Gray and Monahan, 1992). It is based on the determination of the formation of malonaldehyde (MDA), an important lipid oxidation product in foods and biological systems. The radicals with a double bond β to the carbon carrying the peroxy groups (arising from fatty acids that contain more than two double bonds) cyclize to form peroxides with five-membered rings (hydroperoxy epidioxides), which then decompose to form MDA (Figure 1.8). 2-thiobarbuturic acid (TBA) reacts with MDA to produce a red chromogen and is useful for monitoring the initial steps of lipid oxidation (Figure 1.9). The intensity of the color complex is used to determine the concentration of MDA (Crackel et al., 1988). This absorbance is measured spectrophotometrically at its wavelength maximum at 532- 535 nm.

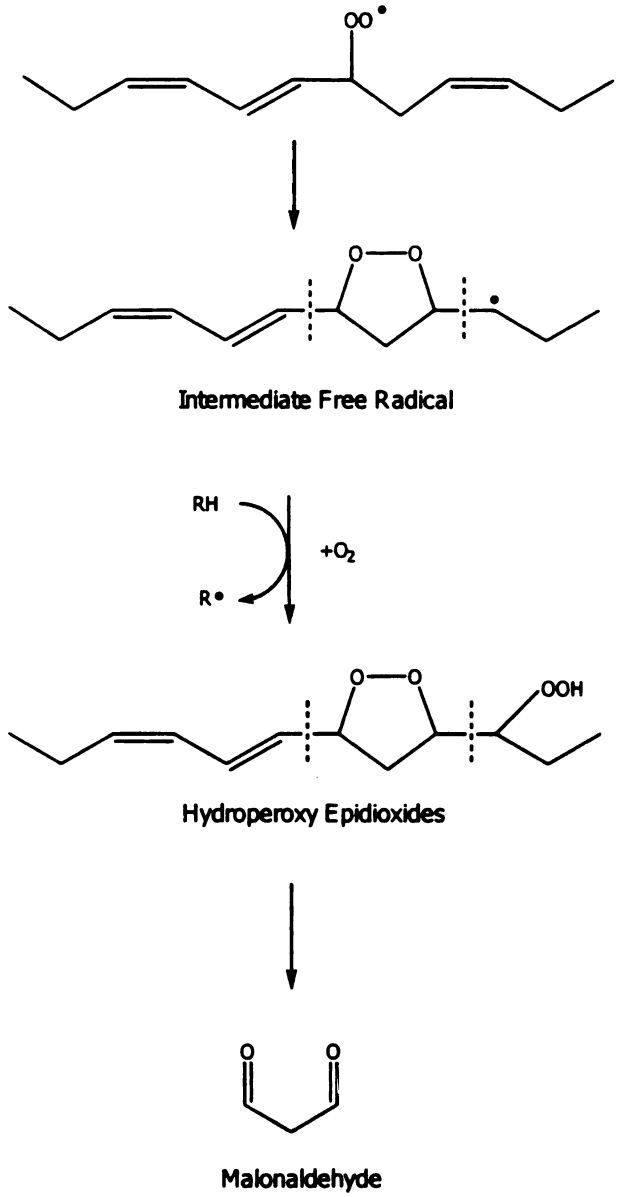


Fig 1.8. Formation of malonaldehyde

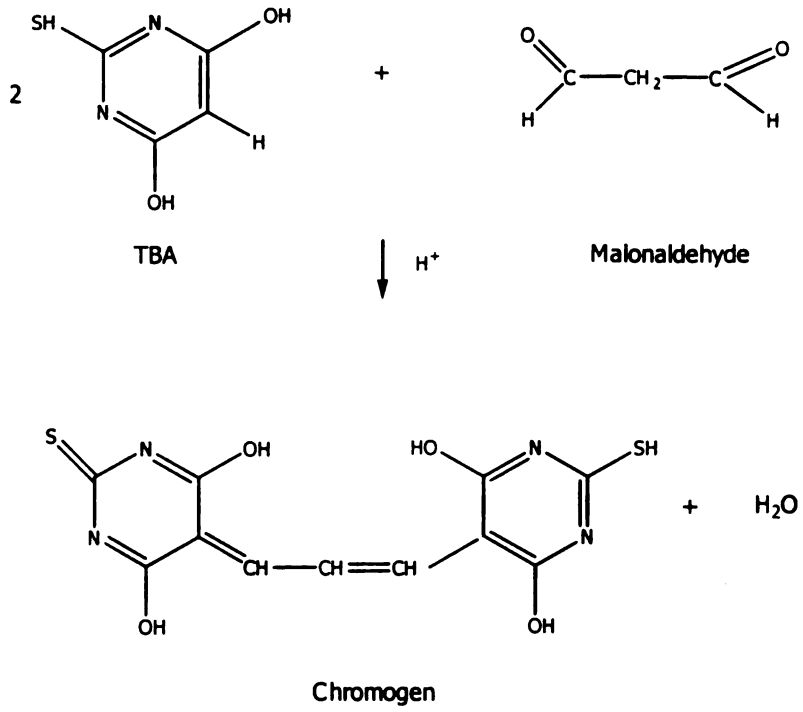


Figure 1.9. Condensation of malonaldehyde with thiobarbituric acid

The TBA test was originally meant to measure the level of MDA in a product. However, more substances than just MDA react with TBA. Therefore, thiobarbituric acid reactive substances (TBARS) is a more descriptive term that represents the reaction.

Various compounds, other than those found in oxidized lipids, have been found to react with TBA to yield the characteristic red color. Fructose can react with TBA and then create interference with the TBA-MDA peak (Guillen-Sans and Guzman-Chozas, 1998). Sometimes compounds indigenous to the test sample, such as red anthocyanins, also make it difficult to come up with a precise TBA measurement (Hodges et al., 1999). In these situations the observed results must be corrected for the presence of these

interfering compounds. In addition, flavor scores for different systems cannot be consistently estimated from TBA values because the amount of TBA products obtained from a given amount of oxidation varies from product to product due to the endogenous polyunsaturated fatty acids esterified with phospholipids in meats.

Thiobarbituric acid method is expected to measure oxidation products of tissue lipids not extracted with common fat-solvent (Tarladgis et al., 1960). These tissue lipids refer to protein-bound lipids and phospholipids characterized by a high degree of unsaturation. The oxidation of this fraction is responsible for types of odor and flavor deterioration in meat. Moreover, the procedure involved heating the sample with a strong acid is essential for the liberation of malonaldehyde. This method is widely used in measuring lipid oxidation in meat.

Corn Fiber Oil (CFO)

Corn fiber is a low-value by-product of wet milling, the industrial process that produces starch, sweeteners, fuel grade ethanol, and other products from corn. The corn milling industry produces about 4 million tons of corn fiber each year (Hicks et al., 1997). This low-cost residue is sold as livestock feed.

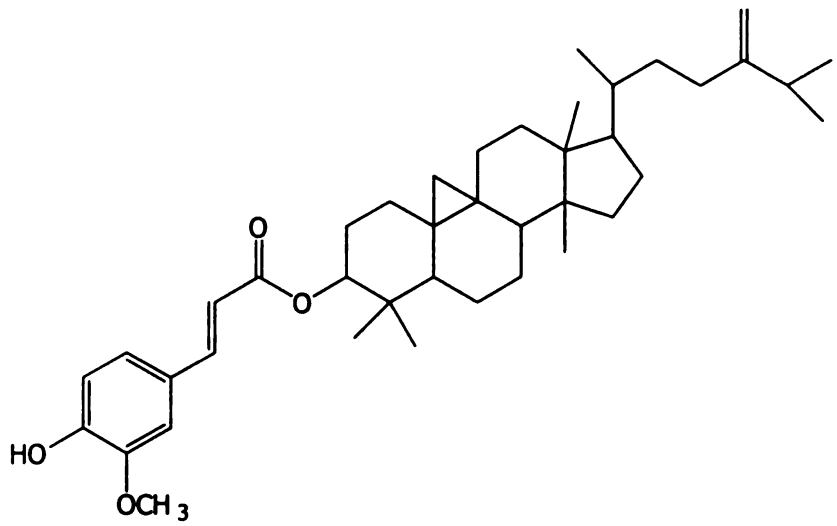
Norton (1994, 1995) and Seitz (1989) have reported that a hexane extract from corn bran yielded an oil containing high levels of ferulate esters, similar in composition to oryzanols, compounds found in rice bran and rice bran oil. Moreau et al. (1996) reported that extraction of commercial corn fiber with hexane or supercritical CO_2 yielded an oil that comprised from 0.54 to

3.68 wt% of the fiber and also rich in ferulate esters (up to 6.75 wt%). Moreau et al. (1996) also showed that corn fiber oil contained triacylglycerols, the most abundant lipid class as well as sterol esters, free fatty acids, phytosterols and very low levels of tocopherols.

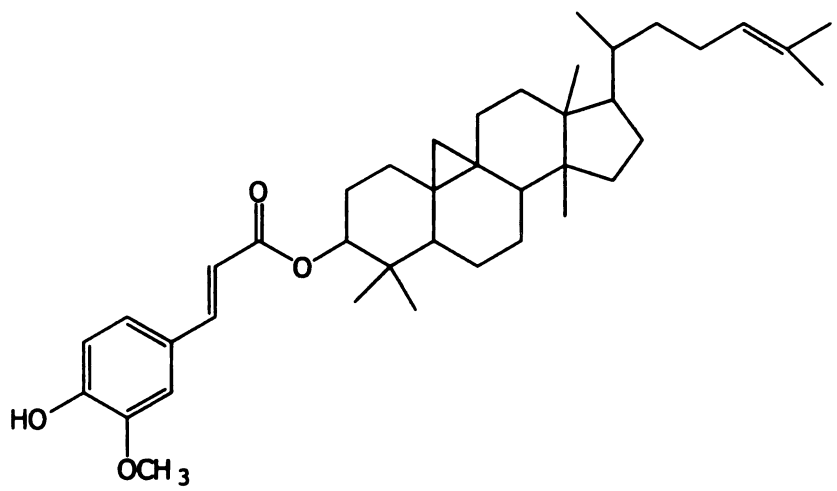
There are many factors that could affect corn fiber and corn fiber oil yields, and phytosterol composition in corn fiber oil. These factors include corn hybrids, heat pretreatment of corn fiber, harvest moisture content, and acids and use of sulfites during steeping process. Singh et al. (2000) demonstrated that there is a significant variation in the commercial corn hybrids for the amount of oil and the amount of individual and total phytosterol compounds in the oil. Moreover, the hybrids that have high fiber yield do not necessarily have a large amount of oil in the fiber fractions. Heat treatment over the range of 100-175 °C of corn fiber in either a convection oven or a vacuum oven caused only a modest reduction in the levels of the phytosterol components. However, the same heat pretreatments caused a considerable increase in the levels and yields of γ-tocopherol in corn fiber oil (Moreau et al., 1999). As harvest moisture content goes down, the amount of oil extracted from the corn fiber decreases but the concentration of the phytosterols in the corn fiber oil increases proportionally (Singh et al., 2001). Different acids and sulfite compounds were tested in the conventional corn wet-milling steeping process. Weak acids have a positive effect on increasing the individual phytosterol compounds in the corn fiber. When comparing the effect of acids and sulfites, acids have a more positive effect than sulfites in increasing the yield of phytosterol compounds in corn fiber oil (Singh et al., 2000).

Oryzanol

Oryzanol (Figure 1.10) is a mixture of sterol esters of ferulic acid. High-oryzanol rice bran oil contains tocopherols and tocotrienols as part of unsaponifiable matter, and has well-studied antioxidative properties (Graf, 1992). The antioxidant function of oryzanol may depend on the hydroxyl group on the phenolic ring in the ferulate (Figure 1.11) portion of their structure. Nanua et al. (2000) studied on the oxidation of fortified whole milk powder with 0.1 and 0.2% rice bran oil in which contained 76.4 and 180.1 μg of oryzanol/g of whole milk powder, respectively. The oxidation was reduced by addition of 0.1% rice bran oil, which was approximately the same level of inhibition achieved by adding 0.2% rice bran oil. However, with increasing oryzanol concentration beyond approximately 191 μg/g, the oxidation of the whole milk powder increased with oryzanol content. This was likely caused by an increase in polyunsaturated fatty acid content associated with the rice bran oil. Ferulic acid, a component of oryzanol, efficiently protected phosphatidylcholine liposomes from UV radiation-induced peroxidation and reaction with nitrogen oxides (radicals involved in oxidative reactions) (Saija et al., 1999).



24-methylenecycloartanyl ferulate



Cycloartenyl ferulate

Figure 1.10. Structures of oryzanols

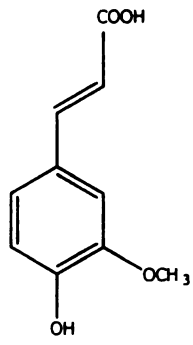


Figure 1.11. Structure of ferulic acid

Xu and Godber (2001) demonstrated that the antioxidant activity of γ-oryzanol components from rice bran reduces the production of toxic cholesterol oxidation products in which may support the potential hypocholesterolemic property of rice bran. Moreover, oryzanol has been shown to lower the level of serum cholesterol in laboratory animals and humans (Nicolosi et al., 1991). This cholesterol reduction might be caused by the sterol moiety of oryzanol, which is split off from the ferulic acid part in the small intestine by cholesterol esterase causing cholesterol to precipitate and resulting in non-absorbable state (de Deckere and Korver, 1996).

Beta-sitosterol

In plants, more than 40 sterols have been identified, of which β-sitosterol, stigmasterol, and campesterol are the most abundant. In a typical Western diet, individuals consume an average of 250 mg of phytosterols per day, largely derived from vegetable oils, cereals, fruits, and vegetables (Hicks and Moreau, 2001).

Plant sterols significantly reduce cholesterol levels in mildly hypercholesterolemic and hypercholesterolemic humans (Moreau et al., 2002). The exact mechanism by which phytosterol decrease serum cholesterol levels in not completely understood. The serum cholesterol-lowering effects of phytosterol and phytostanols are believed to be associated with an inhibition of cholesterol absorption causing a decrease in serum cholesterol levels (Moreau et al., 2002). The FDA suggested that more than twice the amount of stanyl esters (3.4 g/day) than steryl esters (1.3 g/day) were needed to ensure a significant reduction in LDL-cholesterol lowering.

Most of the phytosterols in corn fiber oil are naturally esterified with either fatty acids or phenolic acids, such as ferulic acid, a powerful antioxidant (Moreau et al., 1998). Beta-sitosterol (Figure 1.12), another major component in corn fiber oil, possesses a wide spectrum of biological activities, namely antibacterial, antifungal and anti-inflammatory properties (Sharma et al., 1975). Wilson (2000) has reported that corn fiber oil, sterol, and stanol diets inhibit the elevation of plasma low-density lipoprotein cholesterol. The efficacy of β-sitosterol has been shown to inhibit cholesterol synthesis in animals. Beta-sitosterol is thought to be agent that prevents hypercholesterolemia and dietary β-sitosterol has been reported to reduce serum cholesterol level in animals and mans (Moreau et al., 2002).

The antioxidant activity of β-sitosterol is not well studied. Beta-sitosterol isolated from *Salvia plebeia*, a herb grown in China and India, was studied for an antioxidant activity in lard (Weng and Wang, 2000). The result showed that the antioxidant activity of β-sitosterol was superior to BHT and α-tocopherol. In contrast, the study by Finotti et al. (2000) showed that β-sitosterol is a weak

pro-oxidant when tested alone. However, in combination with α-tocopherol and/or squalene, there was significant positive synergy for inhibition of lipid oxidation.

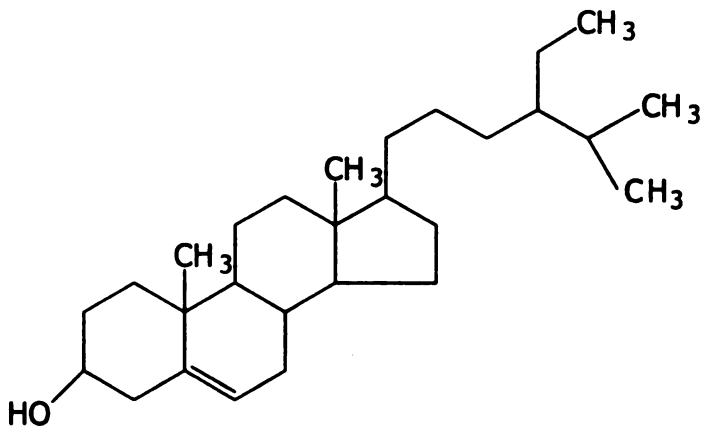


Figure 1.12. Structure of β-sitosterol

Linoleic Acid

Linoleic acid (Figure 1.13) is an essential fatty acid that has 18 carbons and 2 double bonds at the 9 and 12 positions. Linoleic acid is required for good health because it cannot be synthesized by animals. As an essential fatty acid, linoleic acid is required in the diet at a level of about 1% of dietary energy. Linoleic acid has been shown to lower plasma cholesterol and low density lipoprotein (LDL). The replacement of saturated fatty acids with polyunsaturated fatty acids is generally accompanied by a lowering of plasma total cholesterol and LDL cholesterol. Although polyunsaturated fatty acids in

the diet lowers LDL cholesterol, they increase the susceptibility of LDL to oxidation due to pentadiene structure in linoleic acid that contains an active methylene group, which can be easily oxidized.

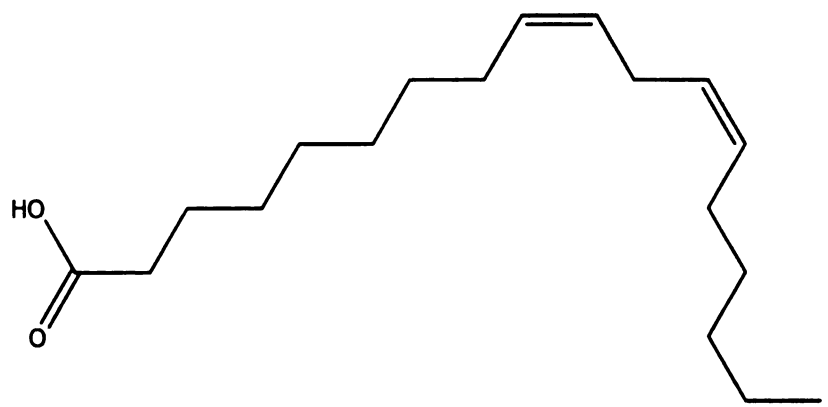


Figure 1.13. Linoleic acid structure

A unique oil discovered in corn fiber, a fiber-rich by-product from corn wet-milling, with health promoting from reducing serum cholesterol would be an excellent natural nutraceutical ingredient for food products. While the antioxidant activities of the individual compounds of CFO have been studied, there is nothing in the literature on the antioxidant activity of CFO as a whole added to food system.

CHAPTER TWO

ANTIOXIDANT ACTIVITY OF CORN FIBER OIL

Introduction

Oxidative rancidity is one of the primary causes of food deterioration and decline in acceptability of food products. Lipid oxidation end products including aldehydes, esters, and other compounds are responsible for the changes in flavor and color (Gray et al., 1996), and aroma associated with rancidity (Melton, 1983). In addition, there is increasing concern with respect to toxicity, loss of nutritive value, and the role that oxidized lipids play in chronic disease such as atherosclerosis (Ladikos and Lougovois, 1990).

Antioxidants are widely used to delay the onset of perceptible rancidity associated with peroxidation of lipid. Antioxidants inhibit lipid peroxidation by scavenging free radicals, chelating metal ions, or reducing oxygen in packaged products. Antioxidants can be derived from synthetic or natural sources. Synthetic antioxidants are preferred by industry because of their low cost, reliability of supply, and effectiveness. However, rising consumer concerns with the safety of synthetic ingredients have generated industrial interest in use of antioxidants from natural products. Moreover, many of the naturally occurring compounds with antioxidant activity afford additional health benefits, such as anti-cancer, anti-arthritic, and anti-atherosclerotic activities (Shahidi, 1997). In some cases these compounds may be derived from enriched extracts of low value by-products of food processing.

Corn fiber is a pericarp-rich fraction obtained as a processing by-product via wet-milling of the grain (Moreau et al., 1998). Extraction of corn

fiber with hexane yields an oil, comprising about 1.2 wt% of the fiber, which is termed "corn fiber oil or CFO" (Moreau et al., 1997). This oil contains high levels of ferulate esters, similar in composition to oryzanols found in rice bran and rice bran oil (Moreau et al., 1996). The oil also contains sterols and stanols such as β-sitosterol and β-sitostanol.

The components of CFO show significant bioactivity that could justify its use for nutraceutical application. Dietary oryzanols have been shown to lower the levels of serum cholesterol in laboratory animals and humans (Kahlon et al., 1992, Nicolosi et al., 1991) and thus may enhance cardiovascular health. Xu et al. (2001) demonstrated that three major components of γ-oryzanol from rice bran, cycloartenyl ferulate, 24-methylene cycloartanyl ferulate, and campesteryl ferulate, show significant antioxidant activity. The phenolic nucleus of ferulic acid and an extended conjugated side chain readily form a resonance-stabilized phenoxy radical, which accounts for its antioxidant potential (Graf 1992). Likewise, β-sitosterol, one of the phytosterol compounds, has anti-cholesterolemic effects. Consumption of approximately 1.3 g of phytosterol significantly reduces serum cholesterol (Moreau et. al., 2002). Few studies have been done to analyze the antioxidant activity of β-sitosterol. Finotti et. al. (2000) showed a weak pro-oxidant effect of β-sitosterol at a level of 0.33 μM when tested alone in a model system; however, a synergistic antioxidant effect was observed when tested in combination with alpha-tocopherol and/or squalene.

There are no reports in the literature evaluating the antioxidant activity of CFO in a food system. Since two of the principal components of corn fiber oil, oryzanol and β-sitosterol, may act as antioxidants, we hypothesized that

addition of CFO to food products would inhibit lipid oxidation. The application of antioxidants into food depends on the type of food being stabilized because antioxidants exhibit differential activity in different food systems (e.g. bulk oil, micelles, and emulsions) (Frankel 1993). Therefore, the first objective of this study was to evaluate the antioxidative activity of highly purified CFO in a bulk oil system consisting of corn oil stripped of endogenous antioxidants. The CFO used in these experiments consisted of approximately equal amounts of oryzanol, β-sitosterol, and linoleic acid. The second objective of this study was to evaluate the ability of CFO and of its principal components to inhibit lipid oxidation in meat system and to determine whether there was antioxidant synergy of CFO with the synthetic antioxidant propyl gallate.

Materials and Methods

Objective 1: Evaluation of CFO in a Bulk Lipid System

Materials

Propyl gallate and 1% starch indicator solution were purchased from Sigma Chemical Company (St. Louis, MO), sodium thiosulfate from Aldrich Chemical Company (Milwaukee, WI), chloroform and glacial acetic acid from EM Science (Gibbstown, NJ), dimethly sulfoxide (DMSO) from J.T. Baker Co. (Philipsburg, NJ), and corn oil stripped of antioxidants from Fisher Scientific (Pittsburgh, PA). Corn fiber oil, CFO, and corn fiber oil stripped of linoleic acid, CFO(-LA), were obtained from Dr. Muraleedharan G. Nair, National Food Safety and Toxicology Center, Michigan State University (East Lansing, MI).

Methods

Preparation of Samples

Antioxidant-free corn oil (stripped corn oil) was used as the bulk lipid substrate for the oxidation studies; a constant volume was used for all treatments. For treatment group one, CFO was tested at final concentrations of 0.01% and 0.1% (w/v) by adding CFO directly to stripped corn oil. For treatment group two, CFO was added to stripped corn oil to achieve final concentrations of 1%, 2%, and 5% (w/v). Stripped corn oil with nothing added was used as a negative control for both groups. In treatment group three, CFO(-LA) was added to stripped corn oil at the concentrations of 0.01, 0.1, and 1% (w/v). Twenty mL of hexane was added to the mixture of CFO (-LA) and stripped corn oil to improve CFO(-LA) solubility. For the 1% CFO(-LA) treatment, an additional 20 mL of chloroform was added also to the mixture of

CFO(-LA) with stripped corn oil to increase the dispersion of CFO(-LA). All solutions including the negative control (stripped corn oil plus hexane) were placed under high vacuum to evaporate the hexane or chloroform. The synthetic antioxidant, propyl gallate, was used as a positive control. DMSO was added at 0.5 mL to dissolve propyl gallate and then this mixture was added to stripped corn oil to obtain the final concentration of 0.01% (wt/v). The propyl gallate treatment was compared to the treatment consisting of 0.5 mL of DMSO in stripped corn oil.

Lipid Oxidation Assessment (PV Test)

All treatments were incubated in an oven (Blue M Eletric Company, Blue Island, Illinois) at 60 °C, and aliquots were withdrawn in triplicate for analysis every other day for over a total period of three weeks. The mixtures were stirred manually before sampling to ensure that any precipitate was resuspended. In order to measure peroxide value, 5.00 ± 0.05 g samples were mixed with 30 mL of glacial acetic acid-chloroform solution (3:2 v/v). The mixture was swirled until completely dissolved. A saturated solution of potassium iodide, 0.5 mL, was added, and the mixture was occasional shaken for 1 min period before adding 30 mL of distilled water. A 1% starch indicator solution, 1 mL, was added into the mixture followed by a titration either with 0.01 N or 0.1 N sodium thiosulfate. The mixture was shaken vigorously and continuously until the purple-black color disappears. A blank determination was daily conducted with 5 mL of distilled water (A.O.C.S. Official method Cd 8-53).

Note: Saturated potassium iodide solution was freshly prepared before use in recently boiled distilled water and stored in the dark. Sodium thiosulfate at the concentration of 0.01 N was used if the titration with 0.1 N was less than 0.5 mL.

Peroxide Value Calculation

Peroxide value was determined using the following equation:

$$\text{Peroxide value} = \frac{(S-B)(N)(1000\ g/kg)}{W}$$

where: B = Titration of blank (mL),

S = Titration of sample (mL),

N = Normality of sodium thiosulfate solution (either 0.01 N or 0.1 N),

and W = weight of sample (g).

Peroxide value was expressed as milliequivalents of peroxide per 1000 g of sample.

Statistical Analysis

The data were analyzed by ANOVA using the Proc GLM generalized linear model (SAS system version 8, SAS Institute Inc., Cary, NC). Also LSMEANS (from Fisher LSD test) was used in the GLM procedure to obtain pairwise comparison of the treatments. Three replicates were carried out for each treatment.

Objective 2: Evaluation of CFO in Meat Systems

Materials

Boneless turkey breast muscle was purchased from Michigan Turkey Producers (Wyoming, MI) on the day of slaughter. The muscle was ground, vacuum-packaged, and stored at -30 °C within one hour. For all but the synergism experiments, ground beef was obtained from a commercial supplier (Lansing, MI), vacuum-packaged, and stored at -30 °C until needed. For the synergism experiments, three different lots of ground beef were obtained from the same commercial supplier, and the beef was stored at 3.3 °C prior to use on the same day. Propyl gallate, thiobarbituric acid (TBA), β-sitosterol, and linoleic acid were purchased from Sigma Chemical Company (St. Louis, MO), hydrochloric acid from EM Science (Gibbstown, NJ), γ-oryzanol from Wako Chemicals USA (Richmond, VA), antifoam from Thomas Scientific (Swedesboro, NJ), boiling beads and corn oil stripped of antioxidants from Fisher Scientific (Pittsburgh, PA). Corn fiber oil (CFO) was obtained from Dr. Muraleedharan G. Nair, National Food Safety and Toxicology Center, Michigan State University (East Lansing, MI).

Methods

Three studies were performed as described below. All procedures described below apply to each study unless otherwise stated.

Preparation of Beef and/or Turkey Samples

The fat contents of ground turkey and beef, determined by the Soxlet Fat Extraction Method (AOCS 960.39.16A), were 1.1% and 15%, respectively.

Prior to mixing and cooking, ground meat was thawed overnight at 4 °C. The mixed ground meat and antioxidants were mixed manually with a mortar and pestle. Mixed ground meat was formed into 100-g patties using a Petri dish (9 cm dia. x 1.5 cm thickness); three replications for each treatment were performed. Patties were refrigerated at 4 °C for two days before frying in a Teflon-coated electric frying pan until they reached an internal temperature of 71 ± 3 °C. The cooked patties were refrigerated in the dark at 4 °C, and 10-15 g samples were taken at 0, 24, 48, 72, and 96 hours for analysis of lipid oxidation using the TBA method (Crackel et. al., 1988). The three studies were designed as follows:

Study #1: Concentration-dependent Antioxidant Activity of CFO

Treatment Preparation

Turkey and beef were tested separately. The individual treatments included 0.05, 0.25, 0.5, and 0.75% CFO (w/w), a negative control that had no CFO added, and two positive controls [0.01% propyl gallate based on fat content (F) and 0.01% propyl gallate based on total weight of sample (W)]. Ethanol (1 mL/100 g meat) was applied to every treatment since it was used to dissolve propyl gallate.

Study #2: Antioxidant Activity of Three CFO Components Individually and in Combination

Treatment Preparation

Treatments consisted of the individual CFO components, a combination of the three components, and CFO. Based on the previous study,

0.75% CFO (w/w) was used as the reference concentration against which the activity of the components was compared. Based on the fact that oryzanol, β-sitosterol, and linolic acid were present at approximately equal weight percentages (Dr. M. Nair personal communication), each individual treatment consisted of 0.225 g of each compound. One treatment consisted of a combination of 0.225 g of each of oryzanol, β-sitosterol, and linoleic acid. Corn oil stripped of antioxidants (2 mL/100 g meat) was used to dissolve/disperse oryzanol and β-sitosterol and was therefore added to all treatments.

Study #3: Synergistic Antioxidant Effect between CFO, Oryzanol, and Propyl Gallate

Treatment Preparation

This study was designed as a 2 x 2 x 2 factorial design with 8 different treatment groups. Each treatment group had either CFO, oryzanol, or propyl gallate or a combination. CFO was applied at a concentration of 0.25% (w/w), oryzanol was added based on its content in CFO, and propyl gallate was added at 0.01% (w/w) based on fat content. Stripped corn oil (2 mL/100 g meat) was also added to all treatments.

Lipid Oxidation Assessment (TBA Test)

Ten-gram meat samples were homogenized with 10 mL antioxidant solution (0.5% propyl gallate and 0.5% EDTA) and 10 mL deionized water using a Polytron homogenizer (PCU-2-110, Brinkmann Instruments, Westbury, NY) to prevent further lipid oxidation. The homogenate was

transferred to 500 mL Kjeldahl flasks and the sample residue was rinsed from the flask with distilled water to obtain a total volume of 100 mL. Then, 2.5 mL of 4 M HCl, 5 pumps of antifoam, and several glass boiling beads were added. The mixtured was boiled, and 50 mL of distillate was collected in a 50 mL screw-capped test tube.

A 5-mL aliquot of distillate was added to 5 mL of TBA reagent (0.72 g TBA in 250 mL double distilled water) in a test tube and capped. The mixture was shaken vigorously and placed into a boiling water bath for 30 min, then cooled at room temperature for 15 min. The absorbance of the solution was measured at 532 nm with a Cary |3E| UV-Visible spectrophotometer (Varian Analytical Instruments, Walnut Creek, CA). Absorbance was converted to mg malonaldehyde/kg tissue sample (TBARS value) using the following equation:

$$\text{TBARS} = A_{532nm} \times K \text{ (mg MDA/kg sample)}$$

$$\text{where: } K = \frac{\text{conc. in moles/5 mL of distillate} \times \text{M.W. MDA} \times 10^7 \times 100}{\text{Absorbance} \times \text{wt. of sample} \times \% \text{ recovery}}$$

Molecular weight of MDA is equal to 72.

K value or distillation constant used in this experiment was earlier determined in our lab, which equal to 6.8.

Statistical Analysis

The data were analyzed by ANOVA using the Mixed Procedure (SAS system version 8, SAS Institute Inc., Cary, NC) to obtain the effect of source of beef, day of experiment, and treatment. Also LAMEANS (from Fisher LSD test) was use to obtain pairwise comparison of treatments. Three replicates were carried out using different batches of meat in the last two studies.

Results

The structural features of oryzanols and β-sitosterol suggested these compounds could scavenge free radicals. There is limited supporting experimental data in the literature suggesting that these two compounds function as antioxidants; therefore, we hypothesized that addition of CFO to food products will retard lipid oxidation. Two experimental systems were used to analyze the antioxidant activity of CFO: bulk oil and cooked ground meat systems.

Evaluation of CFO in a Bulk Lipid System

Preliminary experiments were conducted to determine antioxidant activity of CFO in bulk oil using the peroxide value test. Corn oil stripped of endogenous antioxidants was used as the lipid substrate. In the first study, CFO was tested at concentrations of 0.01% and 0.1% (w/v) and results were compared with the antioxidant activity of 0.01% (w/v) propyl gallate. (Figure 2.1). After an induction period of four days, both CFO treatments and the control showed near-exponential increase in peroxide value until reaching a plateau at ten days. These results indicated that neither of the CFO treatments showed the inhibition in lipid oxidation comparable to propyl gallate, which was still effective after 12 days. The reason may be due to the low level of antioxidant component of CFO added into stripped corn oil. Therefore, CFO was tested at higher concentrations (1%, 2%, and 5% (w/v)) and antioxidant activity was again evaluated by determining peroxide value as a function of time (Figure 2.2). Peroxide values for the CFO concentrations at levels of 2% and 5% were significantly different ($P \leq 0.05$) from the

corresponding control and remained lower throughout the experiment. Taken together, these experiments indicate that there is concentration-dependent inhibition of lipid oxidation with increasing levels of CFO. However, despite the fact that there was inhibition of lipid oxidation by CFO, the degree of oxidation was still unacceptably high, even at 5% CFO.

One possible explanation for the weak antioxidant activity of CFO is that linoleic acid, one of the principal components of CFO, could be oxidized during the experiment period and serve as a source of free radicals to propagate lipid oxidation. We hypothesized that removal of linoleic acid from CFO may result in higher antioxidant activity. Therefore, linoleic acid was removed from CFO [CFO(-LA)] and the latter was tested at the concentrations of (0.01%, 0.1%, and 1% (w/v)) (Figure 2.3). One percent CFO showed the highest inhibition among concentrations tested. The 0.1% and 1% CFO concentrations also showed significant inhibition ($P \leq 0.05$) compared to the corresponding controls. The treatment comparison between 1% CFO and CFO(-LA) demonstrated that corn fiber oil containing linoleic acid showed the significantly higher peroxide value($P \leq 0.05$) than the sample that did not contain linoleic acid beginning with day two of the experiment. However, a complicating factor in our experiments was that CFO(-LA) was difficult to disperse into oil phase. Therefore, CFO with linoleic acid was used for the rest of the experiments.

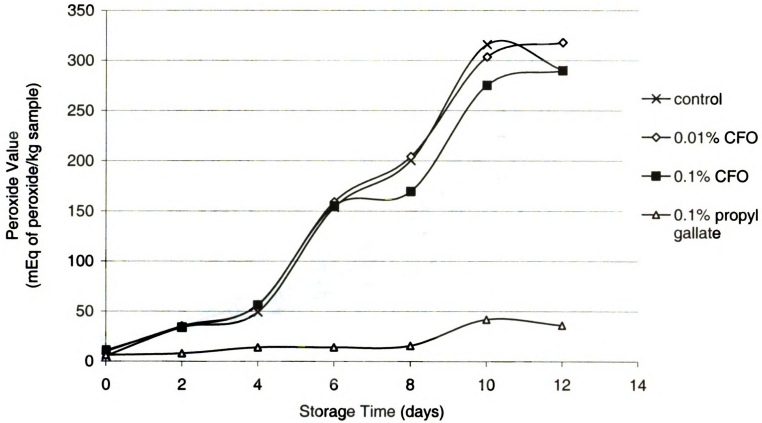


Figure 2.1. Antioxidant activity of corn fiber oil at 0.01% and 0.1% (w/v) levels in stripped corn oil stored at 60 °C

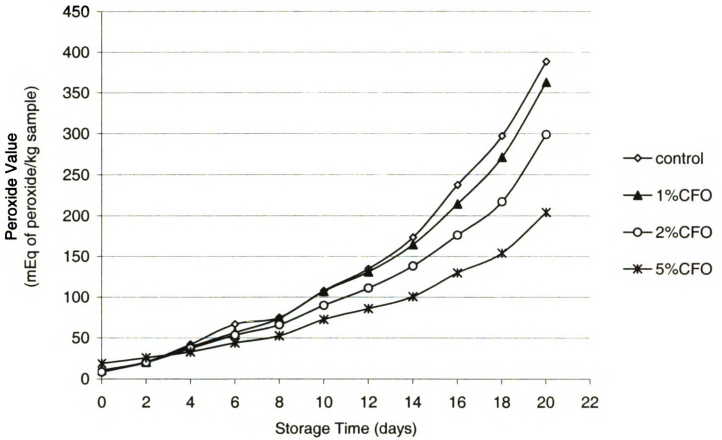


Figure 2.2. Antioxidant activity of corn fiber oil at 1%, 2%, and 5% (w/v) levels in stripped corn oil stored at 60 °C

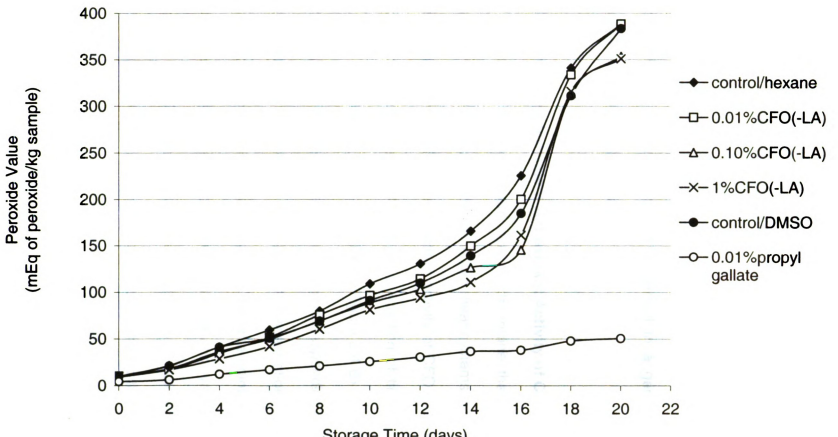


Figure 2.3. Antioxidant activity of corn fiber oil (without linoleic acid) in stripped corn oil stored at 60 °C

Evaluation of CFO in Meat Systems

The polar paradox theory of Porter (1989) suggests that an antioxidant will vary in effectiveness, depending on the food matrix. Since CFO was relatively ineffective in bulk oil, another food system was used to evaluate antioxidant activity of CFO. Cooked ground meat (beef and turkey) was chosen because the phospholipid membrane fraction, which is dispersed in a generally aqueous matrix, is the primary site for lipid oxidation.

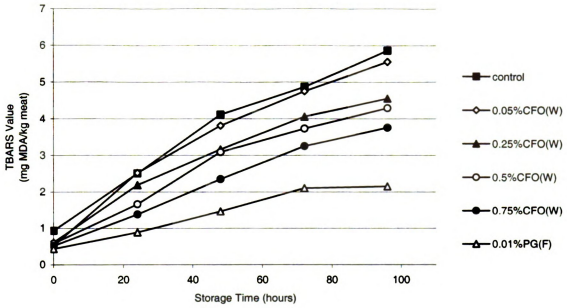
Study #1: Concentration-dependent Antioxidant Activity of CFO

The purpose of this experiment was to determine the effect of CFO concentration on inhibition of lipid oxidation in two meat systems, cooked ground turkey and cooked ground beef. The antioxidant activity of CFO was determined by the 2-thiobarbituric acid test (TBA test) as a function of time (Figure 2.4). TBARS values for beef (Figure 2.4 A) and turkey (Figure 2.4 B) with CFO added were significantly ($P \leq 0.05$) lower than those of the corresponding controls, with the exception of 0.05% CFO. At the highest CFO concentration, the degree of inhibition was ranged from 40-60% depending on the time point. Moreover, it is evident that a plateau of concentration dependence has not yet been reached. Figure 2.4 shows that CFO concentration and inhibition of lipid oxidation were positively correlated. For example, in the beef system during the 96-hr storage time, R^2 is equal to 0.92. The data strongly indicated that CFO had an antioxidant effect in both systems.

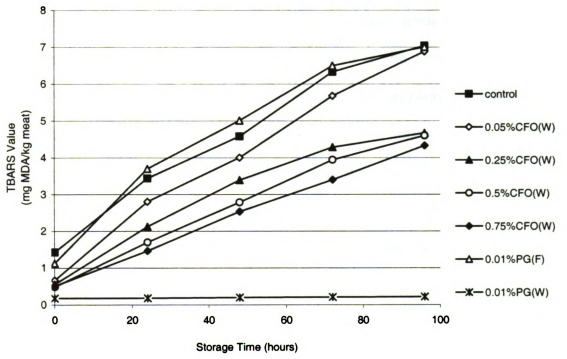
Propyl gallate 0.01% (F) was effective in the beef system, but was little different from the control in the turkey experiment (Figure 2.4 B). However, increasing the propyl gallate concentration to 0.01% based on the total weight of sample resulted in complete inhibition of lipid oxidation over the time course of the experiment.

Based on these results, 0.75% CFO (the highest concentration) was chosen for subsequent studies to determine which component or components of CFO — oryzanol, β-sitosterol, linoleic acid or the combination of those three compounds — was responsible for the antioxidant activity.

Figure 2.4. Concentration-dependence of CFO antioxidant activities in cooked ground beef (A) and cooked ground turkey (B). CFO additions were based on total sample weight (W) whereas propyl gallate (PG) was added based on fat content (F) or total weight of sample (W).


TBARS Value
(mg MDA/kg meat)
Storage Time (hours)
control
0.05%CFO(W)
0.25%CFO(W)
0.5%CFO(W)
0.75%CFO(W)
0.01%PG(F)

(A)


TBARS Value
(mg MDA/kg meat)
Storage Time (hours)
control
0.05%CFO(W)
0.25%CFO(W)
0.5%CFO(W)
0.75%CFO(W)
0.01%PG(F)
0.01%PG(W)

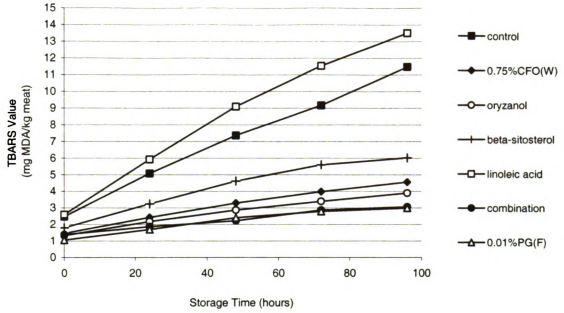
(B)

Figure 2.4.

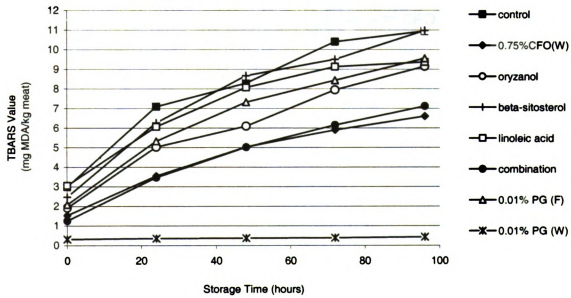
Study #2: Antioxidant Activity of Three CFO Components Individually and in Combination

The antioxidant activities of the three most abundant individual components of CFO, and a combination of the three were compared with CFO in cooked ground beef and cooked ground turkey (Figure 2.5). Antioxidant activity was determined by 2-thiobarbituric acid test (TBA test) as a function of time. Of the individual components tested, oryzanol showed the greatest antioxidant activity in both systems compared to the corresponding controls. About 50% TBARS inhibition was observed when applied β-sitosterol was tested in beef; however, its activity was lower in turkey (Figure 2.5 B). Linoleic acid exhibited different activity in the two systems. In turkey, there was the same level of TBARS compared to control; however, in beef patties, linoleic acid acted as a prooxidant with increasing lipid oxidation relative to the control after zero-day storage time. TBARS values for the combination of oryzanol, β-sitosterol, and linoleic acid in beef system were approximately the same as the 0.01% PG treatment and significantly ($P \leq 0.05$) lower than that of 0.75% CFO. However, in the turkey system, this combination showed comparable inhibition to CFO and lower than PG (F). In ground beef, lipid oxidation was always inhibited to a greater degree than in ground turkey except for the linoleic acid treatment.

Figure 2.5. Antioxidant activities of individual and combined CFO components in cooked ground beef (A) and cooked ground turkey (B). Propyl gallate was added at 0.01% based on fat content (F) or total weight of sample (W).


TBARS Value
(mg MDA/kg meat)
Storage Time (hours)
control
0.75%CFO(W)
oryzanol
beta-sitosterol
linoleic acid
combination
0.01%PG(F)

(A)


TBARS Value
(mg MDA/kg meat)
Storage Time (hours)
control
0.75%CFO(W)
oryzanol
beta-sitosterol
linoleic acid
combination
0.01% PG (F)
0.01% PG (W)

(B)

Figure 2.5.

Study #3: Synergistic Antioxidant Effect between CFO, Oryzanol, and Propyl Gallate

To determine whether there was synergism between CFO and propyl gallate (synthetic antioxidant widely used in the industry), individual compounds (CFO, oryzanol, and propyl gallate) and combinations were tested in ground beef (Figure 2.6). Antioxidant activity was also determined by 2-thiobarbituric acid test (TBA test) as a function of time. CFO concentration at 0.25% was chosen because a significant antioxidant activity started to observed at this level (in study #1). Each treatment was significantly different from the corresponding negative control ($P \leq 0.05$) at every time point after 0 hour. The mixtures of propyl gallate with oryzanol showed additive antioxidant effects in this system. However, a small but significant ($P \leq 0.05$) synergistic effect was observed on 24, 72, and 96 hours storage time when propyl gallate was combined with CFO.

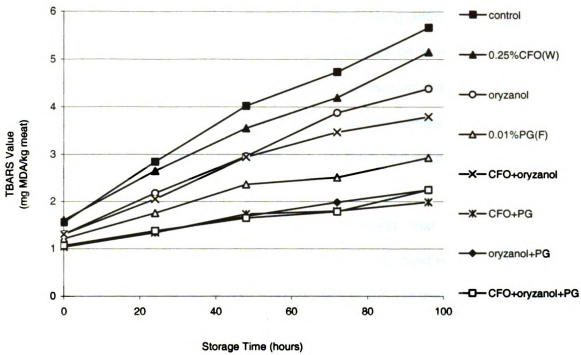


Figure 2.6. Synergistic antioxidant activity of CFO and propyl gallate (PG) in cooked ground beef. CFO was added at 0.25% based on total weight of sample. PG was added at 0.01% based on fat content.

Discussion

Previous studies suggest that oryzanol is an efficient antioxidant due to the presence of a ferulic acid moiety, which is a strong antioxidant (Xu and Godber, 2001 and Xu et al., 2001). The limited studies on antioxidant activity of β-sitosterol were done in a model system and showed mixed results (Finotti et al., 2000; Weng and Wang, 2000). Since these two compounds are major components in CFO, we hypothesized that CFO may act as a potent natural antioxidant in a food system.

Our preliminary results in bulk oil suggest that CFO at low concentrations display little or no antioxidant activity. A possible reason is that there is a very low level of the active component of CFO at the concentrations of CFO used. Frankel (1993) indicated that the efficiency of plant extracts containing antioxidants is based on the amount of active components. The 0.01% CFO treatment comprises about 33% each of oryanol and β-sitosterol. If only one of these components is active, the effective concentrations of antioxidant at 0.01% CFO would be 0.003%. Therefore, CFO was tested at higher concentrations. CFO at 2% and 5% inhibited lipid oxidation about 20% and 40%, respectively. This experiment indicated a concentration-dependent inhibition in lipid oxidation with increasing CFO concentration.

Although, the higher concentrations of CFO did increase inhibition of lipid oxidation, the efficiency was still low compared to propyl gallate and level of oxidation and likely the rancidity was still unacceptably high. One possible explanation for the low inhibition is that CFO contains linoleic acid as one of the major components (about 30%) and this polyunsaturated fatty acid is more likely to be oxidized and thus may enhance lipid oxidation. Therefore,

linoleic acid was removed from CFO and this product was tested for antioxidant activity. The results suggest that the presence in CFO of linoleic acid resulted in somewhat higher lipid oxidation compared to that of CFO without linoleic acid. The limited effect of removing linoleic acid may have been compounded by problem of dispersion of CFO(-LA) because linoleic acid seems to be a solvent for the other components of CFO. For this reason, CFO with linoleic acid was used for the rest of the experiments.

Propyl gallate in the bulk corn oil system maintained the peroxide value at less than 50 mEq/kg through out the study period, which means this sample did not become rancid during the course of the experiment. Fritsch (1994) indicated that oil showing a peroxide value higher than 50 mEq/kg will be detected as the rancid due to the presence of decomposition products of peroxides. Propyl gallate is an efficient antioxidant in tocopherol-stripped corn oil system (Schwarz et al., 2000) and has long been known as an effective antioxidant in bulk oil. The hydrophilic property of propyl gallate make it a good antioxidant in bulk oil due to its formation of a protective membrane at the air-oil interface, which results in reduced reaction of lipid substrate with oxygen or other reactive oxygen species (Coupland and McClements, 1996).

The distribution of an antioxidant in a food matrix, in part, determines its efficacy. Thus, Frankel (1993) suggested using more than one type of food system in order to evaluate activity of antioxidant compounds. In our next set of experiments, we tested CFO in cooked meat (beef and turkey). The initial study was a test of concentration-dependence of antioxidant activity of CFO. Our results suggest a decrease in lipid oxidation with increasing CFO levels.

There was approximately 50% inhibition in TBARS associated with 0.75% CFO concentration (Figure 2.4), the highest tested level in our study.

From our results with two different lipid systems, CFO (a lipophilic antioxidant due to the very nonpolar character of its components) is a better antioxidant in meat than in stripped corn oil. This can be explained by the "polar paradox". Porter et al. (1989) first described the differential effectiveness of antioxidants in different systems. He observed that polar or hydrophilic antioxidants are more effective in non-polar lipid such as bulk oil, whereas non-polar or lipophilic antioxidants are more potent in polar lipid emulsion and membrane. For example, α-tocopherol (a lipophilic antioxidant) is less active in bulk oil than in emulsion, on the other hand, propyl gallate (polar antioxidant) has higher activity in bulk oil than in the emulsions (Schwarz et al., 2000).

In the cooked ground turkey system, 0.01% PG (F) treatment did not lower lipid oxidation when compared to the negative control; however, it did lower lipid oxidation in the beef system. The reason could be that the percentage of fat in turkey is far lower than that of in beef, and the maximum concentration of PG allowed by the USDA is only 0.01% of fat content. Therefore, the absolute amount of PG incorporated in turkey system was far less than in beef system.

Since CFO was an effective antioxidant at the levels tested as described earlier, the next experiment was designed to identify active component(s) in CFO responsible for antioxidant property. The studies were done in both beef and turkey with a CFO concentration of 0.75% (the most potent tested level from previous study). Oryzanol, β-sitosterol, and linoleic

acid were tested individually and in combination at levels equivalent to their presence in CFO.

The results indicated oryzanol as an effective antioxidant in both beef and turkey. The class of oryzanols comprises a variety of ferulic acid esters called α-, β-, and γ-oryzanol (Graf, 1992). Ferulic acid is a powerful hydrophilic antioxidant due to the hydroxy group on the phenolic ring. The radical formed upon donation of a hydrogen atom is highly stable because the unpaired electron can delocalize across the entire molecule (Figure 2.7). Studies confirmed that γ-oryzanol exhibits significant antioxidant activity in a linoleic acid model system (Xu and Godber, 2001) and it inhibits cholesterol oxidation product in a model system (Xu et al. 2001).

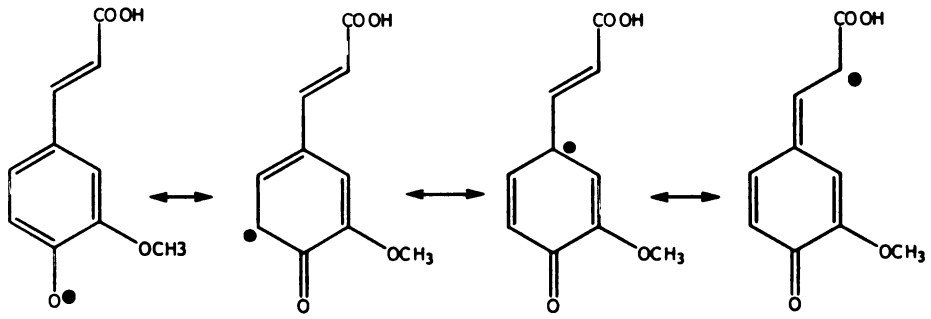


Figure 2.7. Resonance stabilization of a ferulic acid radical

About 50% TBARS inhibition was observed when β-sitosterol was tested in beef; however, its activity was lower in turkey. Two possible explanations of β-sitosterol as an antioxidant are proposed. One is based on its ability to donate a hydrogen atom to a lipid free radical, thereby retarding lipid oxidation. However, the free radical scavenging activity is likely to be low because β-sitosterol lacks a phenolic nucleus to delocalize an electron. It is possible that β-sitosterol could donate a hydrogen atom from a carbon α to a double bond. The proposed free radical scavenging of β-sitosterol is shown in Figure 2.8.

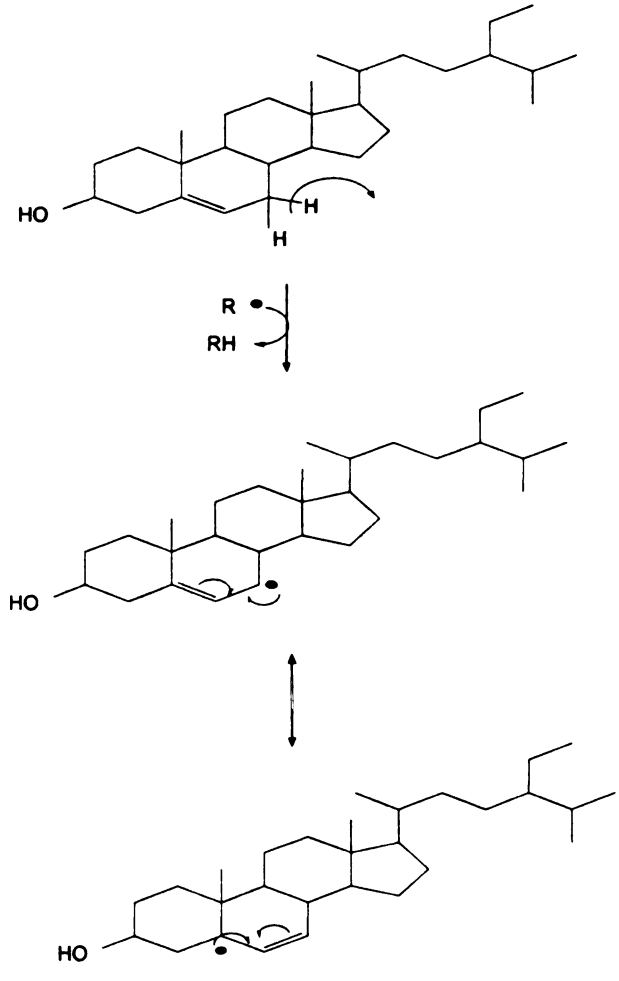
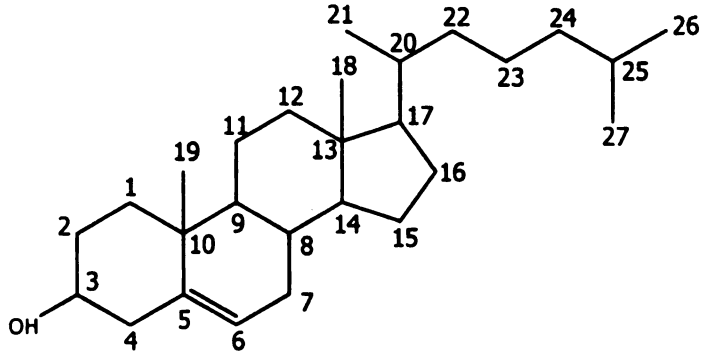
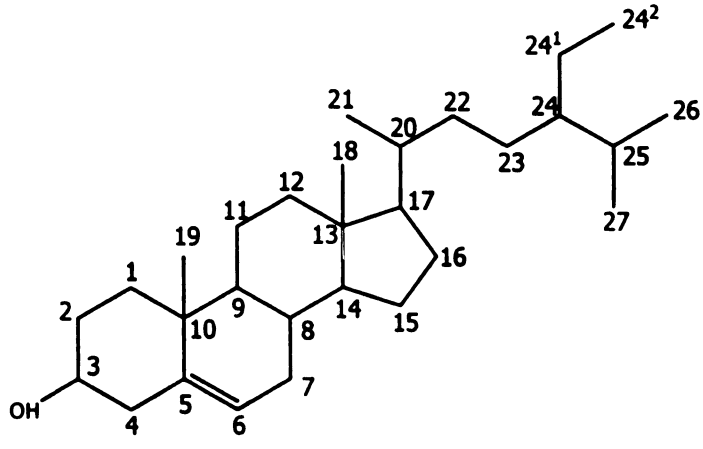


Figure 2.8. Predicted hydrogen abstractions from β-sitosterol

Another possible explanation of β-sitosterol as an antioxidant is based on its structural similarity to cholesterol; both share the same polycyclic ring structure (Figure 2.9). Cholesterol alters the fluidity of membranes, which may make it difficult for free radicals and pro-oxidants to diffuse the membrane (Moreau et al., 2002). Beta-sitosterol could partition into the membrane and physically alter the properties of the membrane, thus making it less susceptible to oxidation.



Cholesterol



Beta-sitosterol

Figure 2.9. Structures of cholesterol and β-sitosterol

From a practical standpoint in industry, antioxidants are sometimes combined in order to achieve a synergistic effect. An antioxidant that can reinforce another antioxidant and generate overall activity greater than the sum of the individual effect will be beneficial. Therefore, in our next study, individual compounds (CFO, oryzanol, and propyl gallate) and combinations (CFO with propyl gallate and oryzanol with propyl gallate) were tested in cooked ground beef for synergism. The mixtures of propyl gallate with oryzanol showed additive antioxidant effects, whereas, a small but significant ($P \leq 0.05$) synergistic effect was observed when propyl gallate was combined with CFO. These results suggest that even greater stabilization of food products could be achieved if the natural antioxidant CFO is used in combination with a powerful synthetic antioxidant. This could be of particular value in low fat products like turkey or chicken in which use of synthetic antioxidants is more limiting. However, the mechanism by which CFO and propyl gallate reinforced each other is not known and should be investigated in future research.

In our study, we have demonstrated the potential application of CFO, which is a product recovered from low value corn fiber, as an antioxidant for food processing. While the levels of CFO used in these experiments would suggest that its antioxidant activity is much less efficient than popular synthetic compounds when normalized to a molar basis, CFO indeed displays good inhibition of lipid oxidation over the concentration range that it would likely be used. Consumption of approximately 1.3 g of phytosterol significantly reduces serum cholesterol (Moreau et. al., 2002). Based on the β-sitosterol content of CFO, this translates into consumption of approximately 3.9 g of

CFO per day (about 1 g of CFO per 100 g hamburger for one serving). Therefore we will overall not only benefit from the hypocholesterolemic effect of CFO but also from the lipid-stabilizing effect in food products.

CHAPTER THREE

SUMMARY AND CONCLUSIONS

CFO, obtained from a low value by-product of corn processing, is rich in oryzanol and β-sitosterol. These compounds are potentially active antioxidant components, which could donate a hydrogen atom to a lipid free radical and/or physically stabilize membranes against diffusion of pro-oxidants and consequently retard the oxidation reaction. Thus, in this study, we hypothesized that the addition of CFO into food products would inhibit lipid oxidation.

The distribution of an antioxidant in a food matrix affects the activity of an antioxidant. Therefore, CFO was evaluated for antioxidant activity in two different experimental systems: bulk corn oil and meat (ground beef and ground turkey). CFO displayed little antioxidant activity in the bulk oil system. The linoleic acid component of CFO tended to enhance lipid oxidation but its removal led to little improvement in antioxidant activity. In contrast to bulk oil, CFO showed good antioxidant activity in cooked ground beef and ground turkey systems, at relatively high concentrations (0.25-0.75%). Oryzanol was identified as an important active component contributing to CFO antioxidant activity. However, the contribution of β-sitosterol is unclear, showing significant activity in beef, but little activity in turkey. The combination of three principal components to a level comparable to 0.75% CFO reproduced the antioxidant activity of CFO itself, and was comparable to the antioxidant activity of 0.01% propyl gallate in beef, and

superior to 0.01% propyl gallate in turkey. Moreover, when CFO was combined with propyl gallate in ground beef, a synergistic effect was observed.

CFO has been previously shown to be an effective nutraceutical ingredient because of its cholesterol-lowering activity. Our results suggest that an additional benefit of using CFO—at levels needed to achieve cholesterol-lowering activity—is stabilization of the product against lipid oxidation. Thus CFO is attractive as a multi-functional food ingredient, which is readily obtained from food processing.

FUTURE RESEARCH

1. To understand more about the antioxidant activity of CFO, different food products or different systems including emulsions and processed meats should be performed. Moreover the degree of oxidation should be determined by more than one method such as the measurement of conjugated diene hydroperoxides produced from polyunsaturated lipids. The absorbance of sample diluted in methanol or in isooctane is spectrophotometrically measured at 234 nm. This method is generally used to follow the early stages of lipid oxidation before the hydroperoxides decomposed. An alternative measurement of secondary oxidation product such as an analysis of carbonyl compounds by using gas chromatography (GC) is also applicable. The gas phase of volatile carbonyl compounds responsible for off-flavor is collected from product headspace and quantified by injecting into a GC column.

2. Because γ-oryzanol from rice bran inhibits cholesterol oxidation, the antioxidant activity of CFO should be investigated in a cholesterol oxidation system. The antioxidant function of CFO against cholesterol oxidation may contribute to potential anti-artherosclerosis activity of CFO.

3. More research should be done to investigate β-sitosterol compound as an antioxidant. Generally, β-sitosterol should be tested at different

concentration levels and also in different systems to determine/confirm antioxidant activity. A more in depth study using HPLC/MS, for example, to identify oxidation products would consequently help to understand the mechanism for inhibition of oxidation by β-sitosterol.

4. Higher CFO concentrations should be studied for the antioxidant activity in the concentration-dependent study to determine the optimum concentration for inhibition of lipid oxidation and the point, if any, at which CFO will act as a pro-oxidant.

5. Higher levels of CFO should be tested for synergy with propyl gallate and with the other synthetic antioxidants including BHA and BHT or natural antioxidants including tocopherols and ascorbic acid. The use of a synergistic combination will result in a greater stability than can be obtained by using the equivalent quantity of either antioxidant alone.

6. A study should be conducted to determine the effect of CFO on formation of heterocyclic aromatic amines (HAAs), mutagenic/carcinogenic compounds formed during frying of meat. A free radical-based mechanism has been proposed as a pathway by which HAAs are formed. Therefore, the addition of compounds that possess antioxidative activity such as CFO might reduce the formation of these HAAs. The Ames test would be a useful preliminary screening test to address this question.

7. From a consumer perspective, taste panel studies should be done to investigate the acceptability of CFO-added meat products prior to moving to an industrial scale. Moreover, sensory analyses provide a useful approach to identify off-flavors and off-odors of lipid oxidation in processed foods that cannot be detected by other objective chemical or instrumental analyses.

APPENDIX A

Table 1. Peroxide values[1] as milliequivalents of peroxide per 100g of sample in corn fiber oil

Treatments	Storage Time (days)						
	0	2	4	6	8	10	12
Control	5.35[a] ±0.15	33.95[a] ±14.44	49.17[a] ±1.14	153.73[a] ±9.57	200.37[a] ±2.01	315.88[a] ±5.40	290.21[a] ±8.92
0.01% CFO	9.75[a] ±2.24	35.13[a] ±2.94	56.54[a] ±8.36	159.09[a] ±8.58	204.17[a] ±13.44	303.55[a] ±3.28	318.03[b] ±20.44
0.1% CFO	10.94[a] ±1.64	34.01[a] ±4.22	56.17[a] ±4.75	155.17[a] ±8.12	169.57[b] ±2.02	275.22[b] ±3.32	289.89[a] ±5.90
0.01% PG	6.22[a] ±2.71	8.04[b] ±0.65	13.95[b] ±0.21	14.12[b] ±0.22	15.69[c] ±0.15	41.83[c] ±3.25	36.06[c] ±7.30

[1] Mean ± standard deviation of 3 determinations for each treatment.

[a-c] Mean values in the same column with different superscript differ significantly ($p \leq 0.05$).

Table 2. Peroxide values[1] as milliequivalents of peroxide per 100g of sample in corn fiber oil with linoleic acid

Treatments	Storage Time (days)					
	0	2	4	6	8	10
Control	10.07[a] ±0.13	20.73[a] ±0.11	42.14[a] ±0.18	66.72[a] ±3.30	76.45[a] ±1.18	107.72[a] ±3.02
1%CFO	10.56[a] ±0.01	20.53[a] ±0.18	39.08[b] ±0.01	56.41[b] ±1.04	73.70[a] ±2.03	107.08[a] ±1.39
2%CFO	8.38[a] ±0.21	20.04[a] ±0.18	37.25[b] ±0.07	53.27[c] ±3.05	65.83[b] ±2.06	89.77[b] ±2.07
5%CFO	18.79[b] ±0.28	25.79[b] ±0.19	32.97[c] ±0.02	43.81[d] ±2.00	52.54[c] ±1.25	72.38[c] ±2.38

Treatments	Storage Time (days)				
	12	14	16	18	20
Control	134.65[a] ±2.86	173.38[a] ±1.04	237.52[a] ±0.12	297.08[a] ±2.62	388.37[a] ±2.06
1%CFO	131.06[a] ±3.08	164.56[b] ±0.97	214.59[b] ±2.16	271.17[b] ±0.12	362.93[b] ±1.85
2%CFO	110.94[b] ±1.05	138.27[c] ±0.94	176.16[c] ±0.94	217.07[c] ±1.20	298.82[c] ±1.33
5%CFO	85.80[c] ±0.02	100.43[d] ±1.13	129.78[d] ±0.13	154.23[d] ±0.99	204.06[d] ±1.25

[1] Mean ± standard deviation of 3 determinations for each treatment.

[a-d] Mean values in the same column with different superscript differ significantly ($p \leq 0.05$).

Table 3. Peroxide values[1] as milliequivalents of peroxide per 100g of sample in corn fiber oil without linoleic acid

Treatments	Storage Time (days)					
	0	2	4	6	8	10
Control (for CFO(-LA))	8.89[a] ±0.11	21.44[a] ±0.09	41.16[a] ±1.26	59.80[a] ±1.05	79.82[a] ±2.36	107.85[a] ±1.17
0.01%CFO(-LA)	10.21[a] ±0.22	17.01[a] ±0.11	34.67[ac] ±0.38	51.83[ab] ±0.05	75.73[ab] ±0.12	96.48[b] ±2.35
0.1%CFO(-LA)	9.84[a] ±0.09	17.95[a] ±0.02	36.29[ab] ±0.33	49.82[bc] ±3.47	69.22[b] ±1.24	88.52[bc] ±1.16
1%CFO(-LA)	9.17[a] ±0.00	6.69[a] ±0.13	28.62[bc] ±0.11	41.87[c] ±0.05	60.48[c] ±1.15	81.07[c] ±1.09
Control(for PG)	9.83[x] ±0.1	20.83[x] ±0.11	41.12[x] ±0.32	51.08[x] ±1.13	69.04[x] ±4.87	91.01[x] ±2.12
0.01%PG	4.25[x] ±0.12	6.04[y] ±0.11	12.11[y] ±0.10	17.14[y] ±0.16	21.07[y] ±0.20	25.67[y] ±0.08

Treatments	Storage Time (days)				
	12	14	16	18	20
Control (for CFO(-LA))	130.54[a] ±1.11	165.65[a] ±0.22	225.53[a] ±2.51	340.98[a] ±7.08	388.41[a] ±1.98
0.01% CFO(-LA)	114.21[b] ±1.41	149.60[b] ±1.85	200.04[b] ±0.79	333.64[a] ±9.61	388.35[a] ±0.92
0.1% CFO(-LA)	103.03[c] ±1.09	126.36[c] ±1.11	145.59[c] ±36.45	313.62[b] ±1.17	353.59[b] ±2.08
1% CFO(-LA)	93.74[d] ±0.12	110.53[d] ±1.16	161.19[d] ±1.06	315.68[b] ±6.77	350.97[b] ±2.54
Control (for PG)	109.67[x] ±0.08	139.06[x] ±1.33	184.88[x] ±1.05	311.3[x] ±1.00	383.51[x] ±1.8
0.01% PG	30.55[y] ±1.18	36.56[y] ±2.33	37.93[y] ±0.06	47.87[y] ±0.09	50.51[y] ±1.16

[1] Mean ± standard deviation of 3 determinations for each treatment.
[a-d] Mean values for CFO(-LA) treatments in the same column with different superscripts differ significantly ($p \leq 0.05$).
[x-y] Mean values for propyl gallate (PG) treatments in the same column with different superscripts differ significantly ($p \leq 0.05$).

Table 4. TBARS values[1] form the study of concentration-dependent antioxidant activity of CFO in beef system

Treatments	Storage Time (hr)				
	0	24	48	72	96
Control	0.93^{a} ±0.10	2.50^{ab} ±0.13	4.11^{a} ±0.35	4.87^{a} ±0.37	5.86^{a} ±0.38
0.05% CFO(W)	0.53^{bc} ±0.25	2.51^{a} ±0.34	3.81^{a} ±0.09	4.75^{a} ±0.30	5.56^{a} ±0.11
0.25% CFO(W)	0.62^{ac} ±0.08	2.19^{b} ±0.14	3.16^{b} ±0.25	4.05^{b} ±0.22	4.56^{b} ±0.40
0.50% CFO(W)	0.58^{bc} ±0.04	1.66^{c} ±0.16	3.09^{b} ±0.41	3.73^{c} ±0.49	4.29^{b} ±0.08
0.75% CFO(W)	0.52^{ac} ±0.03	1.38^{c} ±0.06	2.35^{c} ±0.13	3.25^{d} ±0.36	3.76^{c} ±0.09
0.01% PG(F)	0.44^{bc} ±0.16	0.89^{d} ±0.07	1.47^{d} ±0.30	2.10^{e} ±0.50	2.15^{d} ±0.59

[1] Mean ± standard deviation of 3 different patties from each treatment.

[a-e] Mean values in the same column with different superscript differ significantly ($p \leq 0.05$).

Table 5. TBARS values[1] form the study of concentration-dependent antioxidant activity of CFO in turkey system

Treatments	Storage Time (hr)				
	0	24	48	72	96
Control	1.43^{a} ±0.18	3.43^{a} ±0.29	4.58^{a} ±0.45	6.33^{a} ±0.31	7.04^{a} ±0.26
0.05% CFO(W)	0.66^{b} ±0.13	2.80^{b} ±0.24	4.00^{b} ±0.36	5.68^{b} ±0.36	6.88^{a} ±1.23
0.25% CFO(W)	0.56^{bc} ±0.04	2.12^{c} ±0.17	3.39^{c} ±0.45	4.29^{c} ±0.53	4.67^{b} ±0.48
0.50% CFO(W)	0.48^{bd} ±0.02	1.70^{ce} ±0.07	2.78^{d} ±0.12	3.94^{c} ±0.16	4.60^{b} ±0.95
0.75% CFO(W)	0.51^{be} ±0.04	1.46^{de} ±0.10	2.53^{d} ±0.22	3.40^{d} ±0.58	4.32^{b} ±0.36
0.01% PG(F)	1.13^{a} ±0.26	3.70^{a} ±0.45	5.01^{a} ±0.49	6.50^{a} ±0.22	7.01^{a} ±0.52
0.01% PG(W)	0.18^{cde} ±0.02	0.18^{f} ±0.02	0.20^{e} ±0.03	0.21^{e} ±0.03	0.22^{c} ±0.03

[1] Mean ± standard deviation of 3 different patties from each treatment.

[a-f] Mean values in the same column with different superscript differ significantly ($p \leq 0.05$).

Table 6. TBARS values[1] form the study of antioxidant activity of three CFO components individually and in combination in beef system

Treatments	Storage Time (hr)				
	0	24	48	72	96
Control	2.48^{a} ±0.88	5.08^{a} ±1.34	7.36^{a} ±1.41	9.16^{a} ±1.42	11.48^{a} ±2.19
0.75% CFO(W)	1.46^{bd} ±0.63	2.42^{bd} ±0.88	3.28^{b} ±0.90	3.98^{bd} ±0.73	4.55^{bd} ±0.89
Oryzanol	1.32^{b} ±0.59	2.17^{bc} ±0.96	2.86^{bd} ±1.19	3.39^{bc} ±1.10	3.89^{bc} ±1.51
Sitosterol	1.08^{d} ±0.65	3.24^{d} ±1.11	4.61^{c} ±1.54	5.59^{d} ±1.82	6.02^{d} ±1.86
Linoleic acid	2.60^{a} ±0.78	5.92^{a} ±1.32	9.10^{a} ±1.76	11.53^{a} ±2.19	13.50^{a} ±2.27
Combination	1.38^{b} ±0.73	1.86^{be} ±0.77	2.22^{df} ±0.95	2.86^{c} ±1.23	3.05^{c} ±1.20
0.01% PG(F)	1.06^{c} ±0.62	1.69^{ce} ±0.60	2.40^{de} ±0.69	2.79^{c} ±0.70	2.99^{c} ±0.46

[1] Mean ± standard deviation of 3 replications from 3 different sources of beef for each treatment.
[a-f] Mean values in the same column with different superscript differ significantly ($p \leq 0.05$).

Table 7. TBARS values[1] form the study of antioxidant activity of three CFO components individually and in combination in turkey system

Treatments	Storage Time (hr)				
	0	24	48	72	96
Control	2.99[a] ±0.73	7.09[a] ±1.76	8.29[a] ±1.34	10.40[a] ±2.40	10.94[a] ±1.59
0.75% CFO(W)	1.53[b] ±0.31	3.55[b] ±0.99	5.04[b] ±0.83	5.90[b] ±1.30	6.60[b] ±1.30
Oryzanol	1.92[c] ±0.69	5.02[c] ±1.56	6.09[c] ±1.30	7.94[c] ±2.13	9.15[c] ±2.15
Sitosterol	2.47[d] ±0.51	6.27[ad] ±2.20	8.67[a] ±2.51	9.51[ae] ±2.76	10.97[a] ±2.35
Linoleic acid	3.05[a] ±0.64	6.06[ad] ±1.47	8.06[a] ±1.06	9.13[ac] ±1.45	9.36[ac] ±1.09
Combination	1.25[e] ±0.35	3.46[b] ±0.72	5.02[b] ±1.04	6.15[b] ±1.34	7.11[b] ±1.02
0.01% PG(F)	2.08[c] ±0.65	5.33[cd] ±1.86	7.32[a] ±1.78	8.44[ce] ±2.32	9.55[ac] ±2.36
0.01% PG(W)	0.31[f] ±0.03	0.36[e] ±0.04	0.38[d] ±0.08	0.39[d] ±0.12	0.43[d] ±0.13

[1] Mean ± standard deviation of 3 replications from 3 different sources of turkey for each treatment.
[a-f] Mean values in the same column with different superscript differ significantly ($p \leq 0.05$).

Table 8. TBARS values[1] form the study of synergistic antioxidant effect between CFO, oryzanol, and propyl gallate in beef system

Treatments	Storage Time (hr)				
	0	24	48	72	96
Control	1.56 ±0.37	2.84 ±0.29	4.02 ±0.61	4.73 ±0.36	5.67 ±0.87
0.25% CFO(W)	1.61 ±0.42	2.65 ±0.33	3.55 ±0.37	4.19 ±0.43	5.15 ±0.46
Oryzanol	1.31 ±0.39	2.17 ±0.40	2.96 ±0.44	3.87 ±0.54	4.38 ±0.82
0.01% PG(F)	1.21 ±0.42	1.75 ±0.31	2.36 ±0.32	2.51 ±0.35	2.93 ±0.43
CFO + Oryzanol	1.31 ±0.42	2.05 ±0.42	2.94 ±0.45	3.47 ±0.52	3.79 ±0.56
CFO + PG	1.04 ±0.37	1.34* ±0.47	1.74 ±0.39	1.80* ±0.61	1.99* ±0.47
Oryzanol 0.01% PG(F)	1.07 ±0.33	1.35 ±0.41	1.69 ±0.58	1.98 ±0.59	2.25 ±0.84
CFO + Oryzanol + 0.01% PG(F)	1.07 ±0.35	1.38 ±0.34	1.65 ±0.43	1.78 ±0.64	2.25 ±0.51

[1] Mean ± standard deviation of 3 replications from 3 different sources of beef for each treatment.
* means significantly synergistic effect ($P \leq 0.05$).

APPENDIX B

Additional study #1: Effect of holding time prior to cooking on lipid oxidation in ground turkey

This study was designed to evaluate the effect of holding times after mixing meat with CFO on lipid oxidation in cooked ground turkey system. Ground turkey patties were mixed with antioxidant and incubated for 0, 1, 2, or 3 days at 4 °C before frying. Lipid oxidation was determined by the TBA test (as described in Chapter 2 on page 43) at 0, 24, and 72 hours. Treatments used in this study were control (without CFO added) and 0.25% CFO (w/w).

There were no significant differences ($P > 0.05$) in TBARS among the controls at the four different holding times. However, there were significant differences ($P < 0.05$) in TBARS at 24 and 72 hours. Likewise, the 0.25% CFO samples showed no differences in TBARS at the different times. However at 72 hours, there were significant differences among the holding times for the CFO treatments.

Differences in holding time could affect the extent to which partitioning of the CFO components occurs and thus may affect lipid oxidation. The overall results showed that lipid oxidation was reduced when mixed samples were kept at 4 °C for the longer period of time before frying. Two-days holding time was chosen for subsequent studies since this would represent a typical time period between mixing and sale.

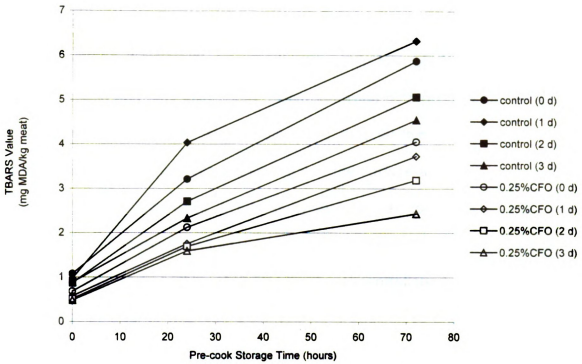


Figure 2.10. Effect of pre-cook incubation time on lipid oxidation in ground turkey samples. Treatments included control and 0.25% CFO (w/w). Patties were prepared by mixing and were subsequently incubated at 4 °C for 0,1, 2, or 3 days prior to cooking. Lipid oxidation was determined by the TBA test as described in Materials and Methods.

Additional study #2: The effect of the emulsifying agent, lecithin (phosphatidyl choline), on antioxidant activity of CFO in turkey patties

This study was done to determine whether dispersion of CFO in meat could be improved by addition by lecithin. Lecithin is a phospholipid (ester of phosphoric acid and fatty acid and/or glycerol) having both positive and negative charges on the molecule. We hypothesized that lecithin would assist CFO dispersion and partitioning into the lipid due to the surface-active property of this compound. Treatments used in this study were either control or 0.25% CFO (w/w) added with corn oil, lecithin, or corn oil and lecithin. Corn oil was used at 1 mL/100 g-patty and lecithin at 0.3% (w/w) concentration. Lecithin was first dissolved in ethanol (1 mL/ 100 g-patty) before addition to ground turkey. The mixtures were then kept for 2 days holding time. Lipid oxidation was determined by TBA test (as described in Chapter 2 on page 43) at 0, 24, and 72 hours.

The result from this study suggested that there were no significant differences between the treatments of CFO with and without lecithin. Similarly, the treatments of CFO with and without corn oil together with lecithin did not show differenced in TBARS values. In fact, the overall result suggested that the samples having lecithin tended to enhance lipid oxidation by increasing TBARS formation. Therefore, we did not use lecithin to disperse CFO in our consequent studies.

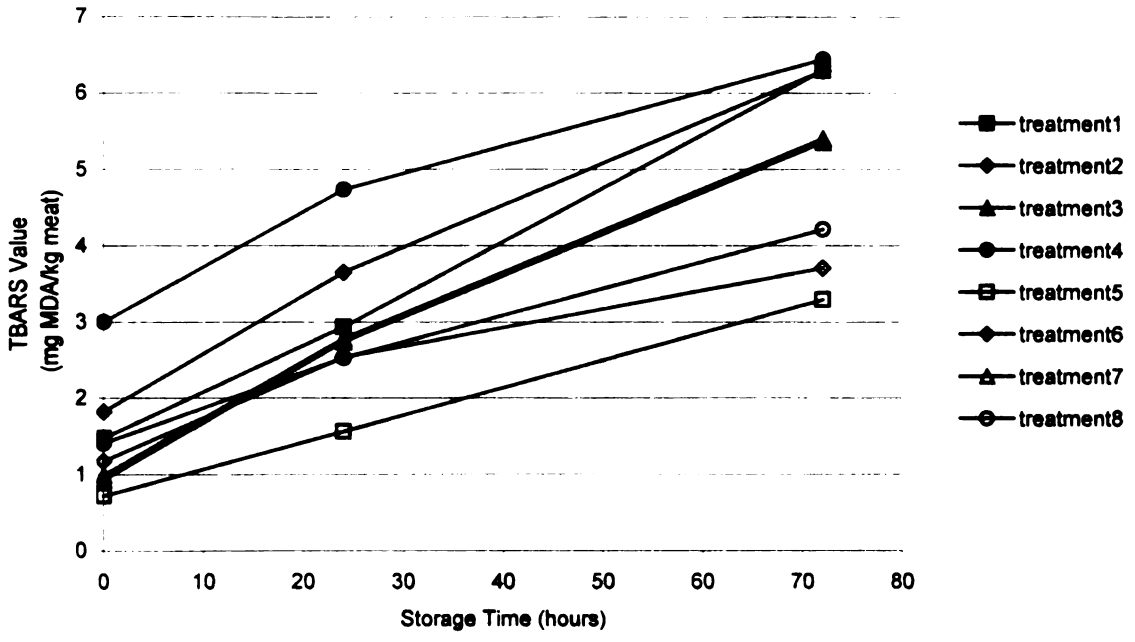


Figure 2.11. The effect of emulsifying agent, lecithin, on antioxidant activity of CFO in turkey patties. Treatments 1 though 8 represent control, control + corn oil, control + lecithin, control + corn oil + lecithin, 0.25% CFO (w/w), 0.25% CFO (w/w) + corn oil, 0.25% CFO (w/w) + lecithin, and 0.25% CFO (w/w) + corn oil + lecithin, respectively. Samples were cooked and lipid oxidation was determined by the TBA test as described in Materials and Methods.

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