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IMPACT OF SURFACE ACTIVE MINOR COMPONENTS ON PHYSICOCHEMICAL PROPERTIES OF ASSOCIATION COLLOIDS AND LIPID OXIDATION IN BULK OIL

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Food science

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A Dissertation Presented

by

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DEDICATION

To food lipid science

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ABSTRACT

IMPACT OF SURFACE ACTIVE MINOR COMPONENTS ON PHYSICOCHEMICAL PROPERTIES OF ASSOCIATION COLLOIDS AND LIPID OXIDATION IN BULK OIL

SEPTEMBER 2014

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Lipid oxidation is a great concern for food manufacturers and consumers as it negatively impacts not only food quality and nutritive values of food lipids, but also consumer health. Lipid oxidation in bulk oil is impacted by chemical factors, such as, prooxidants and antioxidants, and is also related to the existence of physical structures. Bulk oils contain a variety of surface active minor components which are able to form physical structures known as association colloids. These physical structures create oil-water interfaces which seem to be an important site where lipid oxidation occurs in bulk oil. Thus, this research focused on how the surface active minor components in bulk oil impact physical structure formation and oxidative stability in bulk oil.

In the first study, the influence of polar lipid oxidation products isolated from used frying oil on the oxidative stability of bulk oils and oil-in-water (O/W) emulsions was investigated. Polar compounds were added to bulk stripped corn oil (with and without reverse micelles formed by dioleoylphosphatidylcholine, DOPC) and O/W emulsion to evaluate their prooxidative activity by following the formation of lipid hydroperoxides and hexanal. Polar compounds increased lipid oxidation in bulk oil with and without DOPC. The presence of DOPC reverse micelles decreased the prooxidant activity of the polar oxidation products. On the other hand, there was no significant effect of the polar compounds on oxidation of O/W emulsions.

Besides phospholipids, other surface active minor components in commercial oils such as free fatty acids may impact lipid oxidation rates and the physical properties of association colloids. Thus, in the second study, the effects of free fatty acids on changes in the critical micelle concentration (CMC) of DOPC in stripped corn oil were determined by using the 7,7,8,8-tetracyanoquinodimethane (TCNQ) solubilization technique. Different free fatty acids including myristoleic, oleic, elaidic, linoleic and eicosenoic were added at 0.5% by wt along with the DOPC (1-2000 µmol/kg oil) into the bulk oils. There was no significant effect of free fatty acids with different chain length, configuration and number of double bonds on the CMC value for DOPC in bulk oil. However, increasing concentrations of oleic acid (0.5, 1, 3 and 5 % by wt) caused the CMC value for DOPC in bulk oils to increase from 400 to 1000 µmol/kg oil. Physical properties of DOPC reverse micelles in the presence of free fatty acids in bulk oils were also investigated by the small angle X-ray scattering technique. Results showed that free fatty acid could impact on the reverse micelle structure of DOPC in bulk oils. Moreover, free fatty acid decreased pH inside reverse micelle as confirmed by the NMR studies. The oxidation studies revealed that free fatty acids exhibited prooxidative activity in the presence and absence of DOPC. Different types of free fatty acids had similar prooxidative activity in bulk oil.

In the last experiment, multiple surface active minor components including DOPC, dioleoylphosphatidylethanolamine (DOPE), oleic acid, diacylglycerols (DAG) and stigmasterol were incorporated to form nanostructures in stripped corn oil. Individual component significantly decreased the oil-water interfacial tension on which the DOPC and DOPE exhibited the strongest impact. However, the CMC study shows that only DOPC and DOPE could form aggregates at the CMC of 40 and 200 µmol/kg oil. The CMC of the mixed components was as low as 20 µmol/kg oil. The absence of a component did not significantly change the CMC value.

However, in the absence of DOPC, we were not able to observe the CMC of the mixed components in bulk oil. The NBD-PE probe was used to study the interfacial activity of minor components. The addition of mixed components caused the emission fluorescence intensity increase, suggesting that these components were at the oil-water interface. Again, the absence of a component from the mixture did not significantly change the fluorescence intensity, except when lacking of the DOPC. This indicates that the DOPC plays an important role on association colloid formation. The oxidation study showed that the association colloids formed by adding 100 μmol/kg oil of mixed components decreased the oxidative stability of bulk oil. In addition, the impact of mixed minor components at below (10 μmol/kg oil) and above their CMC (100 μmol/kg oil) on antioxidant activity of α-tocopherol and Trolox (water soluble derivative of tocopherols) at 10 and 50 μmol/kg oil was investigated. The addition of α-tocopherol and Trolox at 10 μmol/kg oil already compensated the prooxidant activity of association colloids. Trolox exhibited stronger antioxidant activity than α-tocopherol. However, the association colloids did not influence the antioxidative effectiveness of either α-tocopherol or Trolox in this study.

In conclusion, the surface active minor components formed complex association colloids that decreased the oxidative stability of bulk oil. The presence of reverse micelle impacted the physical location of components such as polar lipid substrates, thus influenced their prooxidant activity. The physicochemical properties of association colloids could change according to the composition of minor components presenting at the oil-water interface. For example, the addition of free fatty acids extended the CMC and altered the pH of the water core of DOPC reverse micelles. The combination of multiple surface active components physically and chemically impacted the oxidative stability and activity of antioxidants in bulk oil. This research

demonstrates what happens in real commercial oils which are complicated and could provide an idea of how to protect the oil from lipid oxidation.

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CHAPTER 1

INTRODUCTION

Food oils are widely used as cooking medium and ingredients in a variety of foods such as salad dressing, mayonnaise, margarine, shortening, etc. Crude oils extracted from different sources contain triacylglycerol and also various kinds of other minor components including free fatty acids, monoacyglycerols, diacylglycerols, phospholipids, sterols, tocopherols, pigments, lipid oxidation products and water (1). These minor components can negatively impact physical and chemical stability of oils. Thus, further refining processes which include degumming, neutralization, bleaching and deodorization are performed to produce refined oil with acceptable flavor, appearance and stability (2).

The shelf life of oils, especially those containing high amounts of polyunsaturated fatty acids suffers from lipid oxidation which occurs over the time. Lipid oxidation is a free radical chain reaction involving the interaction between unsaturated fatty acyl radicals and oxygen leading to the formation of numerous free radical species, primary oxidation product like lipid hydroperoxides and various secondary oxidation products such as aldehydes, hydrocarbons, ketones and epoxides (2). Lipid oxidation is a great concern for food manufacturers and consumers as it negatively impacts not only food qualities parameters such as flavor, color and texture, but also the nutritive value. Moreover, lipid oxidation generates potentially toxic compounds that related to diseases such as atherosclerosis, asthma and cancer (3-6).

The oxidation rate of lipids is dependent on the chemical reactivity of numerous components including reactive oxygen species, prooxidants and antioxidants. In addition, research has shown that lipid oxidation rates are strongly affected by the physicochemical characteristics of interfaces as widely reported in heterogeneous systems such as oil-in-water

emulsions (7). Interfaces of oil and water play important role as the site of lipid oxidation according to the presence of both aqueous phase prooxidants such as free radicals, metals and surface active lipid substrates like lipid hydroperoxides (7). Controlling the physical properties of oil-water interface could provide a novel approach to retard lipid oxidation. For example, producing cationic interface in oil-in-water emulsion could electrostatically repel transition metals in the aqueous phase from lipid hydroperoxide at the emulsion droplet interface, thus decrease oxidation rate (8-10). Moreover, the interfacial thickness which is dependent on the size and conformation of the emulsifiers could physically influence the interaction between aqueous phase prooxidants and lipid substrates (11-12). In addition, partitioning of components into different physical location in heterogeneous systems greatly influences their activities as prooxidants and antioxidants. For instance, nonpolar antioxidants work effectively in oil-in-water emulsions as they tend to partition into the lipid core where oxidation occurs, while polar antioxidants are effective in bulk oil since they preferentially reside at the oil-water interface where is in close proximity to water soluble free radicals (13-15). In the presence of surfactant micelles in oil-in-water emulsions, water soluble components such as lipid hydroperoxides and metal ions could be solubilized out of emulsion droplets leading to decreased oxidation rate(16).

While extensive reports have shown the strong impact of the physicochemical properties on lipid oxidation in oil-in-water emulsions, there are only few studies looking at how physical structures influence oxidation rates in bulk oils (14, 17-19). Even though bulk oil is usually considered as homogeneous liquid, it contains numerous minor components that are not completely removed by the refining process (1). Most of these minor components are amphiphilic compounds that contain both hydrophilic and hydrophobic functional groups on the same structure. Therefore, in the presence of small amount of water in bulk oil these components tend to reside at the oil-water interface and form physical structures known as

association colloids. Recently, studies from our lab group confirmed the existence of association colloids such as reverse micelles in bulk oils by using small angle X-ray scattering (14, 18). Interest in the role of association colloids on lipid oxidation has been increasing. For example, the presence of association colloids formed by phospholipids in bulk oil enhanced the antioxidant activity of tocopherols by altering their partitioning into aqueous phase where they can effectively scavenge free radicals (14). On the other hand, reverse micelles formed in bulk oil could increase lipid oxidation rate by increasing the prooxidant activity of iron thus accelerating the decomposition of lipid hydroperoxides into free radicals (19). The complexity of association colloids structure in bulk oils would play important role in lipid oxidation rate as well. As mention earlier, bulk oils contain a variety of surface active minor components which would compete for the limited oil-water interface. The interaction between these components would be of great interest as they could impact on each other's chemical reactivity and also influence the physical properties of association colloids (e.g. size, charge and critical micelle concentration).

Therefore, this research aimed at gaining a better understanding of how the composition of surface active minor components in bulk oil impact the physical structure of association colloids and the oxidative stability of the oil. The knowledge gained from this study could provide critical information on the complex structures formed in real commercial oils which contain numerous kinds of minor, surface active compounds and could lead to a better understanding of lipid oxidation mechanisms and strategies to improve the oxidative stability of oils.

CHAPTER 2

LITERATURE REVIEW

2.1 Edible oil refining process

Edible oils are extracted from several plants (soybean, corn, sunflower, olive, etc.) and animal sources (fish, tallow, lard). Some oils are used without further purification such as extra virgin olive oil but most are subjected to refining process before use. Crude oils obtained from the extraction process contain not only triacylglycerols (TAG) but also several kinds of minor substances including phospholipids, sterols, polyphenols, tocopherols, tocotrienols, hydrocarbons, hydrolytic products, lipid oxidation products, pigments, metals (e.g. iron, sulfur, copper), waxes and pesticides (20-21). Thus, crude oils are generally processed through degumming, alkaline neutralization, bleaching and deodorization to improve quality and make them more stable. Each refining step has specific purpose for removing certain undesirable components which can negatively impact oil quality.

Degumming is performed to remove phosphatides since they could cause cloudiness and dark color. Moreover, the presence of phosphatides in frying oil can cause dangerous foaming due to the high surface activity of the phospholipids (2). Phosphatides also cause problems by increasing oil loss during neutralization as well as lowering the efficiency of bleaching and filtration (21). During degumming by water-washing process, the phosphatides become hydrated and then are removed from the oil by centrifugation. However, some phosphatides can form complex with divalent metal ions making them nonhydratable and unable to be removed by water washing alone. Thus, phosphoric acid or citric acid is usually

added to the oil to bind divalent cations prior to adding water so that the nonhydratable phosphatides become hydratable (1).

Neutralization is performed to remove free fatty acids since they can cause foaming and decrease the smoke point of the oil. Free fatty acids are chemically neutralized by caustic alkali added to the oils and form soapstock which is then mechanically removed. Bleaching is performed by mixing activated bleaching earths with the oils and heating to 100-110° C under vacuum. Pigments such as chlorophylls and other residual traces of phosphatides, soaps and metals are absorbed onto the bleaching materials which are removed by filtration. The final step of refining process is deodorization by steam distillation performed at relatively high temperature of 180-270° C under high vacuum to remove undesirable odor and flavor including residual free fatty acids. These conditions promote peroxide decomposition, thus decrease lipid hydroperoxide concentrations (2).

More recently, physical refining which uses superheated steam under low pressure and at temperatures higher than 220 °C has been widely used in some oil refineries(20). This process combines the neutralization and deodorization into one step. The physical refining process reduces the oil losses in soapstock and reduces the use of chemical additives compared to traditional alkali neutralization(20). However, a major concern of physical refining method is the formation of trans fatty acids and the loss of tocopherols according to high temperature use (22-25). Moreover, physical refining may need special degumming process for high phosphatide seed oils (2). The refining process should effectively remove undesirable components, while minimizing the loss of neutral oil and desirable components such as tocopherols and phytosterols. After passing through the refining process, the oils obtained are generally light colored, odorless, bland, and oxidatively stable which are acceptable to consumers.

2.2 Composition of edible oils

The refined oils consist predominantly of TAGs (>99%)(26). TAGs are composed of three variable fatty acids molecules esterified to a glycerol. The chain length and degree of unsaturation of fatty acids, the geometric configuration of double bond in fatty acids and the position of fatty acids on TAG are variable and constitute chemical signature for each type of vegetable oil. For example, soybean oil consists mainly of TAG containing linoleic and oleic acid. Palm oil contains mainly TAG with palmitic and oleic acid (27). The TAG profiles are correlated with the physical characteristic of oils such as crystal structure, solubility, viscosity and melting point which impact their suitability for various application (28). Moreover, the fatty acid compositions impact the oxidative stability of oil in which oils containing high ratio of unsaturated fatty acids are more susceptible to oxidative deterioration. However, in the nutritional viewpoint, oils containing long chain polyunsaturated fatty acids such as fish and algae oils have shown to be beneficial for human health especially for cardiovascular system(29). The fatty acid compositions and TAG profile are also used for detection of adulterated oils. For example, linolenic acid content could be used as a parameter to detect the adulteration of olive oil with vegetable oils such as soybean, canola, and walnut oil(30). Along with the TAG, there are trace amount of other components that are not completely removed from the oils by the refining process such as free fatty acids, monoacyglycerols, diacylglycerols, phospholipids, sterols, phenolic compounds, tocopherols, pigments, metals, lipid oxidation products as well as small amount of water (Table 2.1). Moreover, bulk oils also contain thermal oxidized and polymerized compounds arising during the refining process according to the harsh conditions used especially in the deodorization step. For example, the level of polymerized

Table 2.1 Typical minor components in refined vegetable oils (Adapted from Chaiyasit et al., 2007 and Chen et al., 2011)

Components	Amount
Triacylglycerols (%)	>99
Monoacylglycerols (%)	<0.2
Diacylglycerols (%)	0.8-5.8
Free fatty acids (%)	0.03-3.9
Phosphatides (%)	0.003-0.045
Phytosterols (%)	0.41-0.97
Free sterols (%)	0.2-0.4
Esterified sterols (%)	0.06-0.57
Tocopherols (%)	0.04-0.18
Chlorophylls (ppm)	0.06-0.10
Iron (ppm)	0.1-0.3
Copper (ppm)	0.02-0.06
Water (ppm)	200-2000
Oxidized triacylglycerols (%)	0.29-2.80
Polymeric triacylglycerols (%)	~1

glycerides increased 0.4-1 % during refining process (31). Some of these minor components have prooxidative impact on oil stability and others act as antioxidant.

2.3 Autooxidation of bulk oils

Edible oil is prone to lipid oxidation during processing and storage. Lipid oxidation is a significant problem in food oils as it leads to undesirable flavor, odor and toxic compounds formation as well as nutritional losses. Edible oil can undergo autooxidation, photosensitized oxidation, thermal oxidation and enzymatic oxidation under different conditions. Autooxidation is one of the most common pathways and is known as a free radical chain reaction. The mechanism of lipid autooxidation involves three stages including initiation, propagation and termination. A generalized sequence of reactions is shown in Figure 2.1.

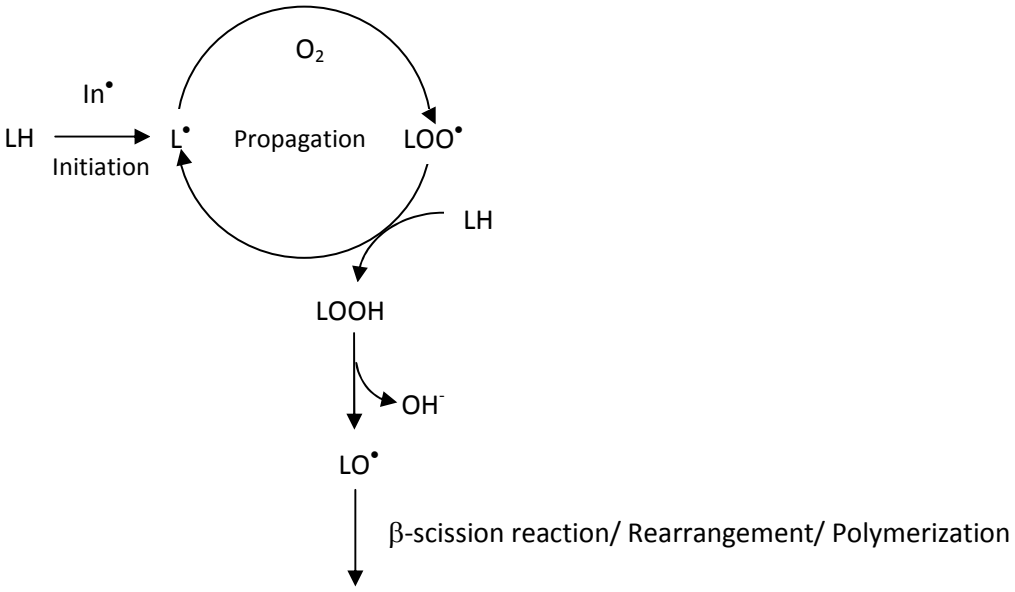

Figure 2.1 Schematic of lipid autooxidation

The reaction of lipid substrate (LH) with atmospheric oxygen (O_2) is thermodynamically difficult as it requires relatively high activation energy (about 35 kcal/mol)(32). Thus, the

formation of free radicals is necessary to start the reaction. In the initiation step, the initiators ($In^{\bullet}$) such as heat, light, metals and reactive oxygen species initiate the formation the alkyl free radicals ($L^{\bullet}$) by accelerating the abstraction of hydrogen from fatty acids or acylglycerols. The alkyl radical is not stable, so it reacts readily with oxygen resulting in the formation of peroxyl radical ($LOO^{\bullet}$) in the propagation step. The peroxyl radical is more energetic than alkyl radical. Thus, it could abstract hydrogen from another fatty acid and produce lipid hydroperoxide (LOOH) as a primary lipid oxidation product and another alkyl radical ($L^{\bullet}$) which continues the free radical chain reaction. The rate constant of the abstraction of hydrogen atom from fatty acid by peroxyl radical depends primarily on the strength of C-H bond being broken. The hydrogen atom attached to the carbon between two double bonds of fatty acid is easily removed due to the activating influence of the neighboring double bonds(32-33). This explains why increasing the polyunsaturated fatty acid composition of an oil enhances its susceptibility to lipid oxidation. The termination stage takes place when the radicals react with each other and form nonradical species, then the chain reaction stops. However, food oil usually gets rancid before significant termination reactions occur.

The rancidity of food oil occurs as a result of volatile oxidation products produced from the decomposition of fatty acid hydroperoxides. Lipid hydroperoxide produced in the propagation stage is odorless and relatively unstable. It is readily decomposed at high temperature and in the presence of metals. Lipid hydroperoxide commonly breaks down through the homolytic cleavage of the oxygen-oxygen bond, giving rise to an alkoxy ($LO^{\bullet}$) and hydroxy (OH^{-}) anion. The alkoxy radical is highly active and can enter into numerous reactions such as β-scission reaction, rearrangement and polymerization, yielding a variety of secondary lipid oxidation products of various molecular weights. The low molecular weights, volatile

compounds, especially aldehydes, are a major class of compounds responsible for the oxidative rancidity in food oils. The threshold flavor value for aldehydes is as low as 1 to 100 ppb (34). Therefore, the presence of minute level of these volatile compounds could greatly impact oil quality.

2.4 Influence of minor components on lipid oxidation in bulk oils

There are several factors that are responsible for the extent of lipid oxidation in food oils. Besides the environmental factors such as light, temperature and oxygen concentration, the endogenous composition of the oil can have a great impact on oxidation rate. Although, refined oil is processed through several steps of purification, bulk oil usually contains a variety of minor components that are not completely removed or are generated during the refining process. Several studies have investigated the influence of minor components including free fatty acids, mono-, di-acylglycerols, phospholipids, phytosterols, tocopherols/tocotrienols, pigments, water, trace metals and thermal decomposition products on lipid oxidation in bulk oil.

2.4.1 Free fatty acids

Free fatty acids (FFA) are produced from the hydrolysis of acylglycerols, phospholipids or sterol esters. The hydrolysis reaction could be accelerated by enzymes (e.g. lipases, phospholipases) or by exposure to high temperature and water during the refining process (2). The presence of FFA could cause quality problems. For instance, FFA are surface active compounds that can increase foaming tendency with the consequent risk of fire during frying process (35). Moreover, FFA have relatively high vapor pressure compared to triacylglycerols, thus oils containing high FFA contents usually have a lower smoke point (21). Most of FFA is removed from the crude oil by neutralization during the chemical refining process. For example, FFA in soybean oil are reduced from 0.3-0.7 % in crude oil to less than 0.05% in refined oil (26).

However, the presence of small amount of free fatty acids can catalyzes the further hydrolysis of TAG leading to increasing concentration of FFA in the oils during storage (1).

Several papers have been published on the prooxidant impact of FFA in oil. The prooxidant activity of FFA is exerted by its carboxylic group which accelerates the decomposition of lipid hydroperoxides into free radicals. This could explain why addition of FFA caused more rapid oxidation rates in oil compared to their corresponding hydrocarbons or methyl esters (36-38). The prooxidants activity of FFA could also be due to their ability to bind metals and make them more prooxidative or their ability to reduce surface tension and increase oxygen diffusion rate from headspace into the oil (36, 39). Several factors have been reported to impact the prooxidant activity of FFA such as FFA content, degree of unsaturation, acyl chain length, position and geometric configuration of double bonds, and type of oil substrate. It is well established that the prooxidative effect of FFA increases with increasing amount of FFA and their degree of unsaturation (38, 40-42). Some researchers studied the impact of saturated fatty acid with different chain length on lipid oxidation in bulk oil. They revealed that the shorter the chain length of saturated FFA, the more oxidation rates were accelerated (38, 40). The impact of the position of double bond on the prooxidant activity of FFA is controversial. For instance, many researchers reported that conjugated linoleic acid (CLA) oxidized faster than its nonconjugated linoleic acid (43-44), while others found that CLA has similar (45-46) or even higher oxidative stability than linoleic acid (47). On the other hand, some studies have shown that the geometric configuration has more impact on rate of oxidation than the location of double bonds. Myers and coworkers (1941) found that α- eleostearic acid (9*Z*,11*E*,13*E* isomer) and β- eleostearic acid (9*E*,11*E*,13*E* isomer) had remarkably different impact on rate of oxygen consumption, though both of them contain double bonds located in the 9,11,13 position (48). Yang and coworkers (2000) also reported variations of lipid oxidation rate among different CLA

isomers. The *trans, trans* CLA was the most stable isomer compared to *cis, trans* and *cis, cis* isomers (49). Moreover, the extent of prooxidative effect of FFA depends on the glyceride structure in the oil. The FFA exhibited lower prooxidative activity in the rapeseed oil than in the soybean oil or safflower oil during microwave heating (41).

2.4.2 Mono- and di-acylglycerols

Monoacylglycerols (MAG) and diacylglycerols (DAG) are esters of glycerol in which one or two hydroxyl groups are esterified with fatty acids. They are mainly residues from incomplete TAG biosynthesis or products of TAG hydrolysis (28). Many researchers have investigated the impact of MAG and DAG on lipid oxidation rate and found controversial results. Mistry and Min (1988) studied the effects of MAG and DAG on the oxidative stability of soybean oil, and found that the oil containing MAG and DAG had higher oxygen consumption rate in respect to the control oil (50). Colakoglu (2007) also reported that monoolein (1% by wt) exhibited prooxidant activity as seen by the oxygen consumption rate increasing in soybean oil incubated at 55° C under the light (51). The prooxidant activities of MAG and DAG could be attributed to their ability to reduce surface tension which causes oxygen dissolve more in the oil leading to the oxidation rate increase (51). Moreover, the presence of MAG and DAG has prooxidative impact in bulk oil by suppressing the efficiency of antioxidants in the systems. For instance, Aubourg (2001) reported that addition of MAG and DAG to hake liver oil showed an inhibitive effect on antioxidant activity of citric acid resulting in acceleration of lipid oxidation (52). Chen and coworkers (2014) also found that MAG (0.5 wt%), but not DAG, reduced the antioxidative activity of 40 μM α-tocopherol in stripped soybean oil incubated at 55 °C (53).

In contrast, many researchers have observed non-prooxidant or even antioxidant activity of MAG and DAG in bulk oil. For example, Nakatsugawa and coworkers (2001) found

that pure DAG oil produced from soybean oil had a longer lag time in terms of peroxide value compared to the pure soybean oil when incubated at 40° C (54). Shimizu and coworkers (2004) reported that DAG produced from soybean oil had similar or even slightly better thermal oxidative stability than that of commercial soybean oil (55). Gomes and coworkers (2010) found that the purified olive oil with added MAG (10-30 g kg^{-1}) had a significantly longer induction time than the control. They also indicated that the antioxidant activity of MAG increased with the amount of MAG (56). Lately, Chen and coworkers (2014) reported that addition of MAG and DAG (0–2.5 wt%) had no significant effect on the chemical stability of stripped soybean oil (53). The different influence of MAG and DAG on oxidative stability of bulk oil could attribute to the different fatty acid composition of the oil studied. For example, Caponio and coworkers (2013) investigated the influence of MAG (0.5 and 1%) on oxidative stability of three different purified oil including soybean, sunflower and peanut oil incubated at 60 °C. They found that MAG acted as antioxidant in sunflower and peanut oil. On the other hand, MAG increased rate of oxidation in soybean oil (57).

2.4.3 Phospholipids

Phospholipids are a group of fatty acyl containing lipid with a phosphoric residue. They are major components of cellular membrane in living organisms including the plant oilseeds (2). Generally, the most common phospholipids in oils are phosphatidylcholine, phosphatidylinosital, phosphatidylethanolamine, and phosphatidylserine (1). Phosphatidylcholine, as the most abundant class of phospholipids, is found at 57-68% of total phospholipids, while phosphatidylinosital and phosphatidylethanolamine account for 14.5-19.7 and 10.2-13.8 %, respectively in corn oil (58). Phospholipids also differ in their headgroup,

degree of unsaturation and chain length of their fatty acid. Fatty acyl residues in phospholipids are more oxidative stable than those in the TAG forms (59-60).

Numerous studies regarding the impact of phospholipids on lipid oxidation in bulk oils have been carried out. Phospholipids were found to be antioxidants in bulk oils as reported by several research groups (61-63). The observed antioxidant activities of phospholipids may attribute to different mechanisms including the metal chelating property of phosphate group, free radical scavenging ability of the amine group, the formation of Maillard reaction products between phospholipids and oxidation products (61, 64), the physical barrier effect at the oil-air interface, as well as, the synergism between phospholipids and tocopherols involving regeneration of tocopherols by amine groups of phospholipids by hydrogen transfer (65-68). Antioxidant activities of phospholipids have been reported to be effected by their headgroups and fatty acid compositions. For instance, Saito and Ishihara (1997) revealed that phospholipids containing amine and hydroxy groups in their side-chain, such as choline and ethanolamine, had effective antioxidant activity by acting as decomposer of peroxides, whereas, phosphatidic acid derivatives and glycerol did not have antioxidant activity (69). Similar results were found by Nwosu and coworkers (1997) reported that phospholipids containing choline group had stronger antioxidative activity compared to ethanolamine and phosphatide containing phospholipids. Moreover, they found that phospholipids with more saturated fatty acids and longer fatty acyl chain length exhibited higher antioxidant activity in salmon oil (70). While many of these experiments have shown a various degree of antioxidant activity of phospholipids, there are some reported disagreements. For example, Husain and coworkers (1986) noted that dipalmitoyl phosphatidylcholine and dipalmitoyl phosphatidylethanolamine showed no significant antioxidant activity in methyl linoleate incubated at 50° C, while, phosphatidylcholine and phosphatidylethanolamine from egg yolk exhibited prooxidants activity

(71). Nwoso and coworkers (1997) demonstrated that sphingomyelin, phosphatidylcholine and phosphatidylethanolamine added into menhaden oil showed little or no antioxidant effect (70). More recently, Takenaka and coworkers (2007) revealed that addition of unsaturated phosphatidylcholine and phosphatidylethanolamine promoted lipid oxidation in stripped bonito oil incubated at 40° C in the dark (72). The variation in the activity of phospholipids on lipid oxidation has not been fully resolved. Physical and structural differences of phospholipids may partly contribute to their different activities on oxidative stability of bulk oil.

2.4.4 Phytosterols

Phytosterols are major components of the unsaponifiable matter of vegetable oils. They are found in different forms either free or esterified with molecules such as fatty acids, ferulic acid, or glucosides (21). In canola oil and corn oil, sterols are presented as free and esterified sterols in about 1:1 ratio (21, 26). However, in soybean oil, most of sterols (94%) are in free form (26). Phytosterols is a collective name of plant sterols and stanols, the 5α-saturated forms of sterols. Sterols, such as campesterol, β-sitosterol, and stigmasterol occur widely in plants in variable amounts, while stanols are less abundant (73). Phytosterols have been reported to help decrease plasma cholesterol in humans and thus reduce the risk of cardiovascular disease (74). Nevertheless, due to their similar structure as cholesterols, phytosterols could undergo comparable oxidation processes and produce potentially toxic compounds (75-76).

Several factors contributing to the oxidative stability of phytosterols have been investigated including heating temperature, esterification and degree of saturation of phytosterols, as well as, the composition of lipid medium. Soupas and coworkers (2004) studied the oxidative stability of unsaturated stigmasterol and saturated sitostanol heated at different temperatures (100°C and 180°C) and found that stigmasterol was oxidized faster at high

temperature than at moderate temperature. However, sitostanol was relatively stable under all heating temperatures (77). Moreover, they found that the extent of oxidation has been shown to be matrix dependent. The phytosterols were oxidized more in the saturated lipid matrix than in the unsaturated matrix at high temperatures, while the reverse trend was found at low temperatures. The same researchers also investigated the impact of esterification on phytosterols oxidation by determining the formation and profile of secondary oxidation products. They revealed that phytosteryl esters were more reactive at moderate temperature (100°C) as respected to free phytosterols. On the other hand, free phytosterols were slightly more reactive than phytosteryl esters at 180°C (78).

Many studies have shown a potent antioxidant activity of phytosterols in bulk oils especially during thermal processing such as frying (79-81). For example, Gertz and Kochhar (2001) noted that phytosterols were more effective antioxidants compared to tocopherol, tocopheryl esters and BHA under frying conditions at 170° C (82). Antioxidant activities of phytosterols, specifically steryl ferulates, are attributed to their ability to donate hydrogen from ferulic acid hydroxyl group to free radicals, and also to their ability to decrease polymerization under high temperature (79-80).

2.4.5 Tocopherols and tocotrienols

Tocopherols and tocotrienols are natural antioxidants in bulk oils. Their structures consist of chromanol ring with 16 -carbon phytyl side chain. Tocopherols have a saturated phytyl side chain, whereas, tocotrienols contain side chain with three double bonds at position 3′, 7′ and 11′. Both tocopherols and tocotrienols have four isomers including α-, β-, α-, and δ- forms which differ in degree of methylation on the chromanol ring. The concentration of various homologs of tocopherols and tocotrienols vary in oils depending on the content of unsaturated

fatty acid, climate condition and genotype (21). Tocopherols are effective chain-breaking antioxidants by donating phenolic hydrogen to lipid peroxyl radicals and produce lipid hydroperoxides and tocopheroxyl radicals (2). Tocopheroxyl radicals contain resonance structure, thus they are relatively stable and do not react readily with unsaturated fatty acids. The reaction rate of tocopherols with lipid peroxyl radicals is 10^5-10^6 times faster than that of unsaturated lipid with lipid peroxyl radicals, therefore, tocopherols could protect unsaturated lipid from further free radicals chain reaction (83). However, antioxidant effectiveness of tocopherols depends on concentration of tocopherols. Several studies revealed that tocopherols at high concentration (e.g. at 250 ppm) exhibited prooxidants activity in bulk oils (84-86). The prooxidant mechanism of tocopherols could be due to their ability to convert trace metals into more prooxidant reduced forms which promote the decomposition of lipid hydroperoxides presented in bulk oil (86). Moreover, tocopherols could undergo oxidation during storage and produce oxidized tocopherols products such as peroxy radical and oxy radical of α-tocopherol, hydroxyl radical, and singlet oxygen formed from tocopherols. Oxidized tocopherols are surface active compounds thus they may reduce the surface tension of oil resulting in increasing the transfer of oxygen to oil with a consequence of lipid oxidation increase (87-88). In addition, the existence of high amount of tocopherol radicals could accelerate fatty acid oxidation (86). Thus, it is a great concern to prevent oxidation of tocopherols and to remove the oxidized tocopherols from refined oil.

2.4.6 Pigments

Vegetable oils contain pigments such as chlorophylls and carotenoids which are responsible for color characteristic of oil. Chlorophylls are pigments composting of porphyrin rings with magnesium ion at the center and a long phytol side chain. There are two classes of

chlorophylls including chlorophyll a and b. Chlorophyll a contains methyl group as a side chain at C-3 position, whereas, chlorophyll b contains aldehyde group instead. Chlorophylls could undergo chemically or enzymatically degradation which produces derivatives such as pheophytins (magnesium free derivatives) and pheophorbides (dephytyllated derivatives) (89). Chlorophylls in refined oil impacts not only color appearance, but also oxidative stability of oil. Light plays an important role in prooxidative activity of chlorophylls. In the dark, chlorophylls and derivatives act as antioxidants in bulk oils (90-92). Antioxidant activity of chlorophylls involves the ability of porphyrin ring to donate hydrogen to free radicals and break the chain reaction of lipid oxidation (93-94). On the other hand, chlorophylls present prooxidants activity in the oils under the light. This could be explained by the photosensitizing action of chlorophylls and their derivatives in which they could transfer energy from light to atmospheric triplet oxygen and produce singlet oxygen that can directly react with unsaturated fatty acid and accelerate photooxidation (93, 95). It has been reported that pheophytins have a greater prooxidant activity than chlorophylls but lower than pheophorbides (96-97). Among the chlorophylls, chlorophyll b shows stronger prooxidant activity compared to chlorophyll a. On the other hand, pheophytins and pheophorbides class b appear to have greater prooxidant effect than class a. The difference in the prooxidants activity of the chlorophylls and their derivatives could be related to the different stability of these pigments to photooxidation (96).

Carotenoids are conjugated tetraterprenoid pigments providing yellow/orange/red colors in food oils and particularly palm oil. Among other carotenoids, β-carotene is one of the most studied. β-carotene has been reported to serve as antioxidant in photosensitized bulk oil systems due to its ability to quench singlet oxygen (98-100). Energy could transfer from high energy singlet oxygen (93.6 kJ/mole) to β-carotene which has lower energy (88 kJ/mole) resulting in the formation of triplet oxygen (101). Moreover, β-carotene could retard lipid

oxidation by scavenging free radicals (102). β-carotene can donate hydrogen to hydroxyl radical and produce carotene radical which are more stable due to its delocalization of unpaired electron through the conjugated polyene system (2). In addition, the antioxidant activity of carotenoids involves a light filtering effect thanks to their conjugated double bond system (103). However, β-carotene has been revealed to be prooxidant under certain environments, such as high oxygen tension and high concentration of β-carotene in foods, as well as, in thermally oxidized bulk oil systems (100, 104). β-carotene could undergo electron transfer to free radicals and become carotene cation radical which may react with lipid peroxy radical at high oxygen concentration (e.g. > 500 mmHg) and produce carotene peroxy radical. Carotene peroxy radical reacts with triplet oxygen and then with lipid molecules and produce lipid radicals that accelerate the chain reaction of lipid oxidation (105).

2.4.7 Trace metals

Trace metals are found naturally in oilseeds and oils can be contaminated with metals during processing. The presence of metals in crude oil reduces efficiency of refining and hydrogenation process. Metal ions e.g. calcium and magnesium could form non hydratable complex with some phosphatides including phosphatidic and phosphatidylethanolamine requiring acid degumming (21, 106). Sodium soaps could inactivate adsorption sites on bleaching earth resulting in decreased bleaching efficiency (106). Sulfur can poison and reduce reactivity of the nickel catalyst in hydrogenation process (21).

Transition metals are well known as major prooxidants in refined oils. Refined oils normally contain trace amount metals e.g. iron and copper (21, 107). Metals facilitate lipid oxidation by reducing the activation energy of the initiation step in lipid autooxidation (108). Moreover, metals participate in producing lipid radicals and reactive oxygen species such as

singlet oxygen and hydroxy radicals. For instance, metals can directly react with lipids and produce lipid radicals ($L^{\bullet}$) which initiate lipid oxidation (reaction 1). In addition, transition metals can be involved in accelerating decomposition of lipid hydroperoxides (LOOH) into lipid peroxyl radicals ($LOO^{\bullet}$) or alkoxyl radicals ($LO^{\bullet}$) (reaction 2) (26, 51). Metals could also produce superoxide anion radicals ($O_2^{-\bullet}$) by the reaction with triplet oxygen ($^3O_2$) (reaction 3)(108). Metals could accelerate the decomposition of hydrogen peroxide (HOOH) to hydroxyl radicals (OH^-), so-called Fenton reaction (reaction 4). Moreover, metals are involved in the Haber-Weiss reaction which is the reaction between hydrogen peroxide (HOOH) and superoxide anion ($O_2^{-\bullet}$) producing hydroxyl radical (OH^-) and singlet oxygen ($^1O_2$)(reaction 5).

$LH + Fe^{2+} \longrightarrow L^{\bullet} + Fe^{3+}$ (reaction 1)

$LOOH + Fe^{3+} \longrightarrow LOO^{\bullet} + Fe^{2+} + H^+$ (reaction 2)

$LOOH + Fe^{2+} \longrightarrow LO^{\bullet} + Fe^{3+} + OH^-$

$^3O_2 + Fe^{2+} \longrightarrow O_2^{-\bullet} + Fe^{3+}$ (reaction 3)

$HOOH + Fe^{2+} \longrightarrow Fe^{3+} + OH^- + OH^{\bullet}$ (reaction 4)

$HOOH + O_2^{-\bullet} \longrightarrow OH^{\bullet} + OH^- + {}^1O_2$ (reaction 5)

The concentration, type, and chemical state of metals influence their prooxidants activity. Copper and iron could produce significant oxidative effects at concentrations as low as 0.005 and 0.03 ppm, respectively (109). Copper shows stronger prooxidants activity by accelerating decomposition of hydrogen peroxide 50 times faster than ferrous iron (108). However, iron is usually found at relatively higher concentration than copper in oils, for example, refined soybean oil contains 2.5 ppb of copper and 200 ppb of iron (26). Prooxidant activities of metals are also impacted by their oxidation states (110). Ferrous (Fe^{2+}), a reduced state of iron, accelerates decomposition of hydrogen peroxide 100 times faster than ferric (Fe^{3+}) (108). Ferric is generally more effective as a free radical initiator by undergoing one-electron

transfer. On the other hand, ferrous promotes the conversion of molecular oxygen ($^3O_2$) to give singlet oxygen ($^1O_2$) and perhydroxyl radical ($^{\bullet}OOH$) (51).

2.4.8 Water

Water is commonly used in degumming process and subsequently removed by centrifugation, adsorption, or vacuum drying. However, trace amount of water usually remains in refined oil. For quality standpoint, the water content of refined oils should be less than 1000 ppm and is often preferred at less than 500 ppm (111). Generally, water has limited solubility in oils, ranging from 0.05% to 0.3% (112). It is likely found as reverse micelles stabilized by surface active minor components in bulk oil (26). The presence of water in bulk oils not only negatively influences processing operations but also impacts the oxidative stability of oils during storage. Water is one of the required substrates for hydrolysis reaction which occurs under heating condition or in the existence of enzymes e.g. lipase, and produces free fatty acids leading to off-flavor, rancidity and reduced smoke point of oils (112). Moreover, water could be a solvent for hydrophilic or amphiphilic antioxidants and prooxidants such as ascorbic acid, citric acid, phenolic compounds, transition metals, free fatty acids, or lipid hydroperoxides. Park and coworkers (2013) found that water could migrate from the headspace and accelerate lipid oxidation in bulk oil. They presumed that the presence of water in bulk oil could facilitate association colloids formation with amphiphilic compounds. The prooxidants activity of water in bulk oil is greatly impacted by the temperature, which could be related to the vapor pressure of water in oils (113). However, Chen and coworkers (2011) noted that addition of water at 1000 ppm into stripped soybean oil did not have a significant impact on the lag phase of hexanal formation during storage at 55° C (1). On the other hand, several studies on oxidation in veiled

virgin olive oil have shown that water and dispersed particles in the oil have a positive antioxidant effect on the oil (114-116).

2.4.9 Thermal decomposition products

Oil refining is generally performed at high temperature which facilitates oxidation and polymerization of oil and produces numerous oxidized and polymeric compounds. Thermal decomposition products include oxygenated triacylglycerols, cyclic and noncyclic carbon-to-carbon-linked dimers and trimers, hydroxy dimers, and dimers and trimers joined through carbon-to-oxygen linkage (108). The amount and composition of these compounds greatly depend on fatty acid composition of oil and condition used during refining process. The formation of polymeric triacylglycerols has been reported to increase with degree of unsaturation of fatty acid (117). deGreyt and coworkers (1997) reported that oxidized triacylglycerols levels in refined oil increased during the bleaching step and ranged from 1.53-4.83%, while the level of polymeric triacylglycerols increased mainly during the refining steps with the highest temperatures such as deodorization and concentrations ranged from 0.32-2.01 % (117). Thermal degradation products have been reported to negatively impact on the oxidative stability of oil (118-119). Yoon and coworkers (1988) isolated the thermal oxidized compounds from soybean oil heated at 180° C for 96 hrs in the presence of air and then added them to refined and purified soybean oil at 0-2% . They found that thermal oxidized compounds increased lipid oxidation rate in both oils incubated at 55° C as determined by volatile compound formation and headspace oxygen consumption (120). Lately, Gomes and coworkers (2011) investigated the impact of oxidized and polymeric triacylglycerols on the oxidative stability of unpurified and purified olive oil under accelerated oxidation test at 105 and 85° C, respectively. These compounds were prooxidative in both oils, however, oxidized triacylglycerols were stronger prooxidants compared to triacylglycerol polymers (119).

Prooxidant activity of oxidized triacylglycerols is thought to be partly due to their amphiphilic structures which facilitate them to reduce surface tension of oil resulting in an increase oxygen diffusion to the oil and accelerate lipid oxidation (2).

2.5 Characteristics of association colloids formed by surface active minor components in bulk oil

Minor components in bulk oil including monoacylglycerols, diacylglycerols, free fatty acids, phospholipids, sterols, cholesterols, phenolic compounds, and polar lipid oxidation products (e.g. aldehydes and ketones), generally contain both hydrophilic and lipophilic functional groups on the same structures making them amphiphilic (26). These amphiphilic molecules preferentially reside at the oil/water interface to minimize the unfavorable free energy associated with the contact of hydrophilic with lipophilic groups. Amphiphilic groups have been observed to lower interfacial tension in oils stripped of their minor components indicating that these molecules are concentrating at the lipid-water interface (26). Considering the ratio of hydrophilic group to lipophilic group of compounds, known as the hydrophilic-lipophilic balance (HLB), most of minor components in bulk oil have a low HLB value, for instance, free fatty acids (HLB ≈1.0), diacylglycerols (≈1.8), and monacylglycerols (≈3.4–3.8)(121). Thus, these compounds will self-aggregate and form association when their concentration is above their critical micelle concentration (CMC) (2). For example, monoacylglycerols are able to form reverse micelles in triacylglycerol oils (122). However, phospholipids which have intermediate HLB values (≈8), are able to form lamellar structures as well as reverse micelles (123). The amphiphilic compounds in association colloids are generally hold together by the relatively weak forces such as the dipole-dipole interactions among

hydrophilic head groups (124), thus these physical structures are highly dynamic and sensitive to changes in surrounding condition. There are several factors influencing the structure of association colloid including the temperature, the concentration and molecular geometry of surface active components, the nature of oil (e.g. alkyl chain length), the water content in the system and the presence of co-surfactants (125-126).

It has been widely reported that increasing temperature can decrease the micellar size. Shrestha and coworkers (2009) found a decrease in the size of reverse rod-like micelles of diglycerol monolaurate and diglycerol monomyristate in olive oil with increasing temperatures (127). Moreover, in diglycerol oleic acid ester/decane system, the size of the micelle was decreased by 25% upon increasing temperature from 25 to 75°C (128). Besides, in diglycerol monolaurate /hydrocarbon oil systems, increasing temperature from 50 to 70° C decreased the length of cylindrical aggregates and induced a rod-sphere type of transition in the micellar shape. This phenomenon are attributed to the increased miscibility of the surfactant and oil and an increase of penetration of oil into the surfactant chain as well as the decrease in packing parameters at higher temperatures (125).

In general, increasing surfactant concentration could lead to the micellar size increase in both aqueous and nonaqueous systems. Shrestha and coworkers (2009) found that with increasing concentration of diglycerol monolaurate and diglycerol monomyristate from 5 to 15 wt % in olive oil, the size of reverse rod-like micelles increased (127). The same researchers also reported that increasing diglycerol monolaurate concentration increased the maximum length of the association colloid but it did not impact the cross-section diameter of the cylindrical reverse aggregates (125). However, in polyglycerol oleic acid ester/n-decane system, the concentration of polyglycerol oleic acid ester range from 5-25 wt % did not have impact on the structure of micelles. Nevertheless, increasing surfactant concentration reduces the

intermicellar distance, and a strong repulsive interaction peak was observed in the scattering curves at higher surfactant concentrations (128). In contrast, Chaiyasit and coworkers (2007) found that increasing concentration of anionic surfactant, sodium bis(2-ethylhexyl) sulfosuccinate (AOT) decreased reverse micelle size from 9.92 to 9.45 Å in hexadecane oil at constant water content (129).

Molecular geometry of surface active molecule could influence the shape and size of the aggregate that is formed in the system. For instance, phosphatidylethanolamine and phosphatidylcholine have similar structure except that the nitrogen atom in choline is surrounded by three methyl groups, whereas there are hydrogen atoms in ethanolamine. This causes a change of their molecular geometry in which phosphatidylethanolamine has a truncated cone shape, while phosphatidylcholine has a cylindrical shape. Therefore, phosphatidylethanolamine forms predominantly a hexagonal (H_{II}) mesophase and phosphatidylcholine forms lamellar (L_{α}) structure (130). This is supported by Shrestha and coworkers (2010) who investigated the structure of polyglycerol oleic acid ester micelles in n-decane at room temperature. They revealed that the size of the reverse micelles increased with increasing the head group size of the surfactant. While the monoglycerol oleic acid ester formed the globular type or micelles with maximum diameter of 6 nm in decane system at 25°C, the hexaglycerol oleic acid ester formed the elongated prolate type micelles with maximum diameter of 19.5 nm (128).

The impact of the chain length of oil on the structure of glycerol monooleate reverse micelle was investigated using small-angle X-ray scattering (SAXS). It was found that the glycerol monooleate spontaneously self-assemble into an ellipsoidal prolate type reverse micelle in alkane oil. The size of reverse micelle increased with the increasing chain length of oil e.g. from hexane to hexadecane. In addition, the curvature of the reverse micelle was affected by the

chain length of oil. The penetration of alkane oils in the hydrophilic/lipophilic interface of the micelles decreases with an increase in the chain length of the oil. As a result, the critical packing parameter decreases and reverse micelles with less curvature are formed (126).

It has been reported that the addition of water could impact the size of reverse micelle. In general, water is required to form association colloids in surfactant⁄oil systems. This is because the added water increases the dipole–dipole interactions among the head group of surfactants and leads to self-assembly in nonaqueous medium (127). Shrestha and coworkers (2007) reported that the significant growth of reverse micellar structure could be found upon the addition of 0.1 wt % water to a 5 wt % diglycerol monolaurate /decane system (125). The same researchers also found that addition of 1.2 % water into the 10 wt % diglycerol oleic acid ester/decane system could cause the size of the water swollen reverse micelles to be 40% bigger than the empty reverse micelle (128). Moreover, the addition of a small amount of water has shown to enhances the elongation of the reverse micelles in nonpolar oil (131). For instance, phospholipids generally forms spherical or ellipsoidal reverse micelles in bulk oil, however, in the presence of trace amount of water, phosphate group could form hydrogen bond with water resulting in reducing the interfacial curvature of the molecular assemblies which could induce the formation of reverse worm-like micelles (132-133). Chaiyasit and coworkers (2007) noted that added water was incorporated into reverse micelles formed by AOT surfactant in hexadecane oil leading to reverse micelle size increased from 9.64 to 15.59 Å at water-to-AOT molar ratios ranging from 0-4 (129).

The presence of co-surfactants has been reported to differently influence on the size of reverse micelles in bulk oil system. For instance, addition of cumene hydroperoxides at 0-100 mmol/kg lipid into hexadecane oil caused the size of AOT reverse micelles decrease. This could be due to the ability of cumene hydroperoxides to act as co-surfactant or by its ability to alter

the optimum curvature of the system. Addition of oleic acid also decreased the AOT reverse micelles size by either acting as co-surfactant or by altering the pH that could impact the charge of AOT leading to different packing properties at the lipid–water interface change. On the other hand, added phosphatidylcholine induced AOT reverse micelles increase in size. This could be attributed to the interaction between phosphatidylcholine (zwitterionic) and AOT (anionic) resulting in an alteration of the optimum curvature of the oil/water interface. Alternately, phosphatidylcholine contains larger polar head groups than AOT, thus it could produce a thicker oil/water interface region leading to the reverse micelle size increase (129).

2.6 Influence of association colloids on lipid oxidation

The presence of oil/water interface could be responsible for the observed differences in lipid oxidation mechanisms between bulk oils and the heterogeneous systems like O/W or W/O emulsions. Lipid oxidation in O/W emulsions has been reported to be greater than that in bulk oil according to the large interfacial surface area (26). Many of prooxidants and antioxidants are surface active, thus they could reside at the interface where lipid oxidation predominantly occurs. The impact of surface active components such as lipid hydroperoxides, free fatty acids, phospholipids, mono-, di-acylglycerols and phytosterols, on lipid oxidation in O/W emulsions has been extensively investigated. For example, Nuchi and coworkers (2002) reported that lipid hydroperoxides were able to partition away from metal ions into surfactant micelles and thus decreased lipid oxidation rate in O/W emulsions (16). Free fatty acids significantly impact on charge of the emulsion droplets as reported by Waraho and coworkers (2009) (10). The researchers found that oleic acid, but not methyl oleate, increased negative charges on emulsion droplets at pH values above its pK_a of 4.8-5.0. These negative charged droplets could

attract prooxidants metal ions to the surface where lipid hydroperoxides reside, thus inducing lipid hydroperoxides degradation thus increasing lipid oxidation rates.

Phospholipids have shown both prooxidant and antioxidant activity in O/W emulsion through different mechanisms. O/W emulsions droplets coated by phospholipids such as lecithin were susceptible to lipid oxidation as phospholipids produced negative surface charged droplets which can attract metal ions to the surface (11). The oxidative activity of phospholipids is pH dependent as reported by Cardenia (2011) who revealed that phosphatidylcholine, namely DOPC, inhibited lipid oxidation in O/W emulsion at pH 7 while they showed prooxidative effect at pH 3 (134). They attribute antioxidant activity of DOPC at pH 7 to the ability of phospholipids to form structures within the lipid phase of the emulsion droplets or to chelate metals. Antioxidant activity of mono- and di-acylglycerols in O/W emulsion has been reported by Waraho and coworkers (2012) (135). They demonstrated that both mono- and diacylglycerols inhibited lipid oxidation in stripped soybean oil O/W emulsion. Antioxidant properties of mono- and diacylglycerols could be partly due to their ability to increase the surface charge of the emulsion droplets. Diacylglycerols also are able to form a liquid crystal phase which could act as a physical barrier protecting unsaturated fatty acids in the emulsion droplet core from prooxidants in the aqueous phase of the emulsion. Phytosterols have been reported to be oxidized faster in O/W emulsion than in bulk oil as a result of their high surface activity which allow them to migrate to the oil/water interface where lipid oxidation preferentially occurs(136).

Traditionally, bulk oil has been thought of as a homogeneous liquid. However, there is strong evidence showing that surface active minor components could form association colloids such as reverse micelles in bulk oil in the presence of trace amount of water. Along with surface active lipid components, other prooxidants and antioxidants could be concentrated in formed

association colloids thus impacting the kinetics and mechanisms of lipid oxidation. Research on the impact of these physical structures on lipid oxidation in bulk oils has been increasingly investigated over the past 2 decades. Koga and Terao (1994 and 1995) were the first ones who revealed that phospholipids could enhance antioxidant effectiveness of α-tocopherol in bulk oil due to their ability to form association colloids which allow α-tocopherol to concentrate at oil-water interface where water soluble peroxyl radicals reside. The ability to enhance the effectiveness of α-tocopherol increased with increasing chain length of the fatty acids on the phospholipids. On the other hand, the phospholipids containing short chain fatty acids were unable to form aggregates, and thus did not have impact on the antioxidant activity of α-tocopherol (137-138).

Kasaikina and coworkers reported that lipid hydroperoxides and other polar lipid oxidation products were able to form physical structures in bulk oil. However, the structure and properties of reverse micelles were not stable as a result of changes in the concentrations and compositions of these components over the course of lipid oxidation (139). The same research group also observed that the ionic surface active components had different impact on lipid oxidation depending on the ionic charge of surfactants and also the nature of lipid substrate. For instance, reverse micelles formed by cationic surfactant could accelerate lipid hydroperoxides decomposition into free radicals thus increasing lipid oxidation in sunflower oil and alkylaromatic ethylbenzene. On the other hand, the anionic reverse micelles did not affect the lipid hydroperoxides decay in sunflower oil triacylglycerol but they showed strong antioxidant activity in alkylaromatic hydrocarbon by acting as catalyst for the heterolytic decay of hydroperoxides (140-141). In addition, these researchers reported that the presence of fatty alcohols such as 1-tetradecanaol, 1-octadecanol and 1-monopalmitoylglycerol increased lipid oxidation rate in sunflower oil. This could be due to the ability of these components to form

physical structures which allow the polar lipids such as hydroperoxides and peroxyl radicals to concentrate at oil-water interface, where lipid oxidation occurs.

Chaiyasit and coworkers (2007) investigated the impact of surface active compounds on iron catalyzed lipid oxidation in hexadecane containing methyl linoleate and AOT reverse micelles. They revealed that surface active compounds including cumene hydroperoxides and oleic acid increased lipid oxidation, while phosphatidylcholine decreased lipid hydroperoxides decomposition. Reverse micelles appear to play an important role in oxidative activity of these surface active components. For instance, prooxidant activity of oleic acid could be related to its ability to concentrate at oil-water interface and bind the aqueous phase metal ions thus bring them close to lipid substrates resulting in lipid oxidation increase. Moreover, antioxidant activity of phosphatidylcholine may be attributed to its ability to alter physical location of lipid hydroperoxides making them less accessible to metals in aqueous phase (129).

Recently, Chen and coworkers (2010) reported that dioleoyl phosphatidylcholine (DOPC) was able to form reverse micelles with a critical micelle concentration of 650 μM in stripped soybean oil. Reverse micelles formed by DOPC accelerated lipid oxidation rate as determined by following the lipid hydroperoxides and hexanal formation (18). Addition of ferric chelator, namely deferoxamine increased oxidative stability of bulk oil containing DOPC reverse micelles. This suggests that prooxidative effect of DOPC reverse micelles in bulk oil could be related to their ability to concentrate iron and lipid hydroperoxides at the oil/water interface, thus increasing the ability of iron to decompose lipid hydroperoxides into free radicals with a consequence of increasing lipid oxidation rates (19). DOPC reverse micelles also have impact on antioxidant effectiveness of tocopherols and Trolox. They enhanced antioxidant activity of both tocopherols and Trolox at low concentration (10 μM) but decreased their effectiveness at high concentration (100 μM). Water soluble Trolox was more effective than lipid soluble α-

tocopherols presumably because it was more concentrated in the DOPC reverse micelles as determined by using a surface active fluorescence probe (14). The existence of DOPC reverse micelles decreased iron-promoted α-tocopherol and Trolox decomposition and decreased the ability of α-tocopherol and Trolox to reduce ferric ions (19). This result may contribute to the varying role of reverse micelles on increasing and decreasing effectiveness of antioxidants.

Chen and coworkers (2014) also studied the impact of diacylglycerol (DAG) and monoacylglycerol (MAG) on the physical and chemical properties of stripped soybean oil. They revealed that MAG was more surface active than DAG as observed by their ability to decrease interfacial tension of oil. Wide angle X-ray scattering (WAXS) analysis showed that only MAG but not DAG was able to form physical structures in bulk oil. The addition of DAG or MAG to the SSO at a concentration 0.5 -2.5 % had no significant impact on oxidative stability of oil. The addition of DAG did not affect the antioxidant activity of α-tocopherol, whereas 0.5 wt% MAG suppressed the effectiveness of α-tocopherol in bulk oil. The authors attribute this result to the crystal structures formed by MAG in bulk oil. Alternatively, strong surface activity of MAG would drive the antioxidants to the water-oil interface resulting in accelerating the consumption of tocopherols as MAG is more susceptible to lipid oxidation than triacylglycerols (53).

CHAPTER 3

PROOXIDANT ACTIVITY OF POLAR LIPID OXIDATION PRODUCTS IN BULK OIL AND OIL-IN-WATER EMULSION

3.1 Introduction

In vegetable oils, lipid oxidation products arise during storage or when oils are subjected to heat processing as in some steps of refining or when cooking or frying foods. Many of lipid oxidation products contain oxygenated functional groups which make them have higher polarity than the original triacylglycerols. Under deep fat frying conditions, many of the volatile lipid oxidation products are removed from the oils due to steam distillation produced by water introduced into the oil from the food being cooked. This means that the polar compounds remaining in deep fat frying oils are comprised of polymers formed through thermal polymerization of triacylglycerols, diacylglycerols and monoacylglycerols as well as oxidation products still esterified to the glycerol such as the triacylglycerol-bound aldehydes or core aldehydes (142). In addition, oxidized and non-oxidized free fatty acids formed through hydrolytic cleavage of triacylglycerols that are not volatile under frying conditions can also remain in the frying oil (143-144).

In the fresh refined oils, polar compounds levels range from 3 to 5% (145). Their concentrations dramatically increase when the oils are exposed to heat, oxygen or moisture as a result of oxidation reactions and triacylglycerol hydrolysis, especially during frying (146). Polar lipid oxidation products have attracted much attention as they have been reported to increase the risk of diseases such as cancer, atherosclerosis, and chronic inflammatory diseases (5, 147-

148). According to this health concern, many countries have established a maximum allowable level of polar compounds in frying oil between 20 and 27% (149-150).

Moreover, the polar compounds have been reported to negatively impact on the oxidative stability of the oils. Free fatty acids accelerate the oxidation rate of bulk oils and O/W emulsions through the ability of the carboxyl acid group to accelerate the decomposition of hydroperoxides and form prooxidative complexes with metals (10, 151). In addition, Mistry and Min (1988) reported prooxidative actions of the diacylglycerols in soybean oil (50). The oxidized triglyceride monomers and the triglyceride polymers can also act as prooxidants in oils (118, 120). Gomes and coworkers (2011) found that the prooxidant activity of oxidized triacylglycerols was greater than that of polar triacylglycerol oligomers (119).

Most studies on the impact of polar compounds on lipid oxidation were carried out in bulk oils. However, the mechanism of lipid oxidation in bulk oils can be very different from those in O/W emulsions (152) and to date no studies have been conducted on the prooxidant activity of polar compounds in food emulsions. Moreover, edible oils contain numerous minor components that are surface active and are thus able to form physical structures in bulk oil in the presence of small quantities of water. These association colloids include structures such as reverse micelle, micro-emulsions, lamella structures and cylindrical aggregates (1). It is unknown if the polar oxidation products impact lipid oxidation in association colloids.

This study set out to investigate the impact of polar compounds on the oxidative stability of various systems including bulk oil, bulk oil with DOPC reverse micelle and oil-in-water emulsions. To gain better understanding of the polar compounds responsible for the prooxidant activity, linoleic acid and linoleic hydroperoxide, which are the hydrolysis and oxidation products respectively, were added to determine their impact on oxidative stability.

3.2 Materials and Methods

3.2.1 Materials

Corn oil and frozen French fries were purchased from a local retail store. French fries contained potaotes, soybean or corn oil and disodium dihydrogen pyrophosphate. The same lot of French fries was used for all experiments. 1,2-Dioleoyl-sn-glycero-3-phosphocholine (DOPC) was acquired from Avanti Polar Lipids, Inc. (Alabaster, AL). Silicic acid (100-200 mesh), activated charcoal (100-400 mesh) and silica gel (70-230 mesh), linoleic acid were purchased from Sigma-Aldrich Co. (St. Louis, MO). Medium-chain triacylglycerols (MCT, Miglyol) were obtained from Sasol North America Inc. (Houston, TX). All other reagents were of HPLC grade. Distilled and deionized water were used in all experiments. Glassware was incubated in 3 mM HCl overnight to remove metals, followed by rinsing with double-distilled water before use.

3.2.2 Methods

3.2.2.1 Frying Procedure

An electric 4 L stainless steel fryer (Presto, USA) was used for frying. Forty batches of 300 g frozen French fries were fried in 4 L of corn oil at a rate of ten fryings per day. Each batch of sample was fried for 8 min at an initial temperature of 177 °C with 20 min intervals between frying batches. There was no replenishment of oil. Total heating period was estimated as 19 h, which included an initial heating period (20 min) each day. After the fortieth frying operation, the used frying oil was stored at -20°C until further analyses.

3.2.2.2 Isolation and determination of polar compounds in fried oils

Polar compounds in fried oil were isolated and determined by the column chromatographic method of Arroyo et al. (153). Used oil samples were weighed (1 ± 0.01 g) and dissolved in 20 mL petroleum ether/diethyl ether (90:10, v/v). The solution was transferred to a silica gel (25 g) chromatographic column (3.0 cm internal diameter x 35 cm height) and eluted with 150 mL petroleum ether/diethyl ether (90:10, v/v) to remove the nonpolar fractions followed by 150 mL chloroform/methanol (1:1, v/v) for the isolation of polar fractions. The solvent in the polar fraction was removed with the rotary evaporator (RE 111 Buchi, Flawil, Switzerland) at 40 °C, and the traces of the remaining solvent were removed by flushing with nitrogen gas. The polar compounds were kept at -80 °C for subsequent studies. Amount of polar compounds were calculated indirectly by subtracting concentration of nonpolar components.

To determine that nonpolar compounds (triacylglycerols and sterol esters) were removed from the polar fraction, thin-layer chromatography (TLC) on 0.025-cm-thick (60Å) silica gel plate (20 × 20 cm) was used (Whatman, USA). Polar and nonpolar fractions were diluted in hexane/diethyl ether (87:13, v/v) 50 times (w/v). Plates were developed with hexane/diethyl ether/acetic acid (80:20:1, v/v/v) as the developing solvent and 10% molybdophosphoric acid as the visualizing agent.

3.2.2.3 Preparation of linoleic hydroperoxide

Linoleic hydroperoxide was prepared from linoleic acid which was heated at 55° C for 48 h. Separation of linoleic hydroperoxide from linoleic acid was achieved by passage through a 2 g silicic acid column, as described by Dix and Marnett (154) with slight modifications. Heated linoleic acid (0.5 g) was dissolved in 1 ml hexane/diethyl ether (95:5, v/v), then transferred to

the column (1.5 cm internal diameter x 8 cm height). Linoleic acid was eluted as the first fraction with 20 mL hexane/diethyl ether (95:5, v/v), followed by elution of hydroperoxides with 20 mL hexane/diethyl ether (70:30, v/v). The eluent was collected in 5 ml fractions which were analyzed by TLC to follow the progress of separation. Hydroperoxide positive spots were determined by spraying with $FeSO_4$ NH_4SCN reagent.

3.2.2.4 Preparation of stripped corn oil

Stripped corn oil (SCO) was prepared according to Boon et al. (9) and was used in all experiments. In short, silicic acid (100 g) was washed three times with a total volume of 3 L of distilled water and dried at 110 °C for 20 h. A chromatographic column (3.0 cm internal diameter x 35 cm height) was then packed sequentially with 22.5 g of silicic acid, followed by 5.63 g of activated charcoal and another 22.5 g of silicic acid. Thirty grams of corn oil was dissolved in 30 mL of n-hexane and passed through the column by eluting with 270 mL of n-hexane. To retard lipid oxidation during stripping, the container used to collect the triacylglycerols was held in an ice bath and covered with aluminum foil. The solvent present in the stripped oils was removed with a vacuum rotary evaporator (RE 111 Buchi, Flawil, Switzerland) at 37 °C and traces of the remaining solvent were evaporated under nitrogen stream. The stripped corn oil was flushed with nitrogen and kept at -80 °C for subsequent studies.

3.2.2.5 Sample preparation

3.2.2.5.1 Bulk oil

A mixture of 75% of MCT and 25% of SCO were used due to the high amount of samples needed and the difficulty in isolating large quantities of SCO. The formation of DOPC reverse

micelles in the bulk oil was done according to the method of Chen et al.(14). Briefly, DOPC in chloroform was pipetted into an empty beaker and then flushing with nitrogen to remove the chloroform. The oil mixtures were then mixed followed by addition of double distilled water. The samples were magnetically stirred at the speed of 1000 rpm in a 20 °C incubator room for 24 h. The final concentration of DOPC and water in bulk oils were 1000 μM and 200 ppm, respectively. Water content was determined by Karl Fisher method (155) (756 KF Coulometer, Metrohm, Herisau, Switzerland). Polar compounds were mixed with the oil samples at various concentrations (0.5, 1.0 and 2.0 %, w/w) and stirred for 6 h to obtain homogenous samples. Samples (1 mL) were aliquoted into 10 mL GC headspace vials (Supelco), capped with aluminum lids having PTFE/silicone septa and stored at 55 °C in the dark.

3.2.2.5.2 Emulsions

Oil-in-water (O/W) emulsions were made using 1.0%, w/w stripped corn oil and an aqueous phases consisting of 10 mM phosphate buffer solution at pH 7.0 containing 0.1% Tween 20. The aqueous phase was prepared and stirred overnight to ensure complete dispersion of the surfactant. The emulsion was prepared by adding polar compounds in chloroform into a beaker and flushing with nitrogen gas to remove the solvent. The final concentrations of polar compounds in emulsion were 0.5, 1.0 and 2.0 %, w/w. Stripped corn oil and aqueous phase were then added to the beaker at an emulsifier:oil ratio of 1:10 and a coarse emulsion was made by blending with a hand-held homogenizer (M133/1281-0, Biospec Products Inc., Bartlesville, OK) for 2 min. The coarse emulsion was then homogenized with a microfluidizer (Microfluidics, Newton, MA) at a pressure of 9 kbar for three passes. During homogenization, ice was used to cover the homogenizer chamber and coil, in order to maintain

the emulsion temperature at < 25 °C. One milliliter of each emulsion was transferred into the GC headspace vials (1 mL/vial) described above and stored in the dark at 37 °C.

3.2.2.6 Determination of free fatty acids

Free fatty acids content of polar compounds were determined according to method of Rukunudin et al.(156). Briefly, 2.8 g of polar compounds were dissolved in 5 mL of ethyl alcohol and then were titrated against 0.01 N sodium hydroxide using phenolphthalein as indicator. Free fatty acid concentrations in polar compounds were calculated as percentage of linoleic acid.

$$\%\ \text{Free fatty acids} = \frac{\text{NaOH volume (mL) x NaOH normality x 28.05}}{\text{sample weight (g)}}$$

3.2.2.7 Measurement of lipid oxidation

3.2.2.7.1 Lipid hydroperoxides

Lipid hydroperoxides were measured using a method adapted from Shanta and Decker (157). The bulk oil samples (20 µL) were weighed accurately and then 2.8 mL of methanol/butanol solution (2:1, v/v) was added followed by the addition of 15 µL of 3.94M ammonium thiocyanate and 15 µL of 0.072M ferrous iron solution (prepared by mixing 0.132 M $BaCl_2$ and 0.144 M $FeSO_4$). The absorbance of the samples was measured at 510 nm using a Genesys 20 spectrophotometer (ThermoSpectronic, Waltham, MA) 20 min after the addition of the iron. The concentration of hydroperoxides was calculated from a cumene hydroperoxide standard curve.

For emulsion samples, a mixture of 0.3 mL of sample and 1.5mL of isooctanol/isopropanol (3:1 v/v) solution were vortexed three times (10 s each). The samples

were then centrifuged for 2 min at 3400g (Centrific TM Centrifuge, Fisher Scientific) and 0.2 mL of the upper organic layer was mixed with 2.8 mL of methanol/butanol solution. In some cases the organic phase was diluted with additional methanol/butanol if the extent of lipid oxidation was high. Then the same procedures as those for bulk oil were followed.

3.2.2.7.2 Headspace Hexanal

Headspace hexanal was measured as described by Boon et al.(9) using a GC-17A Shimadzu gas chromatograph equipped with an AOC-5000 autosampler (Shimadzu, Kyoto, Japan). Samples (1 mL) in 10 mL glass vials capped with aluminum caps with PTFE/silicone septa were preheated at 55 °C for 15 min in an autosampler heating block. A 50/30 µm DVB/Carboxen/PDMS solid-phase microextraction (SPME) fiber needle from Supelco (Bellefonte, PA) was injected into the vial for 2 min to absorb volatiles and then was transferred to the injector port (250 °C) for 3 min. The injection port was operated in split mode, and the split ratio was set at 1:5. Volatiles were separated on a Supleco 30 m x 0.32 mm Equity DB-1 column with a 1 µm film thickness at 65 °C for 10 min. The carrier gas was helium at 15.0 mL/min. A flame ionization detector was used at a temperature of 250 °C. Hexanal concentrations were determined from peak areas using a standard curve prepared from authentic hexanal.

3.2.2.8 Measurement of emulsion droplet surface charge

Emulsions were diluted into 10 mM phosphate buffer (pH 7) at an emulsion:buffer ratio of 1:25 in order to prevent multiple scattering effect. The droplet surface charge was determined using ZetaSizer Nano-ZS (Malvern Instruments, Worcestershire, UK). Each measurement was determined in triplicate at room temperature.

3.2.2.9 Measurement of interfacial tension

The ability of polar compounds to concentrate at oil/water interface was determined using interfacial tensiometry (DSA 100, Kruss USA, Charlotte, NC) according to Chaiyasit et al. (2008) (17). Polar compounds were diluted in MCT at the concentration range of 0.5 to 2.0% (w/w). The tensiometer hypodermic needle was submerged in a 0.1% Tween 20 and 10 mM phosphate solution at room temperature. The sample was formed into a pendant drop at the inverted tip of the needle, which was positioned on an optical bench between a light source and a high speed charge couple device (CCD) camera. The CCD camera was connected to a video frame-grabber board to record the image at a speed of one frame per second. The drop shape analysis program supplied by the instrument manufacturer was used to determine interfacial tension values (158).

The methodology requires accurate determination of solution densities, which were measured using a digital density meter (DMA 35N, Anton Paar USA, Ashland, VA). All interfacial tension measurements were carried out in triplicate at room temperature.

3.3 Statistical analysis

All data shown represents the mean values $\pm$ standard deviation of triplicate measurements. Data results were analyzed by analysis of variance (ANOVA) using SPSS (SPSS Inc., Chicago, IL). The differences between mean values were compared using Duncan's multiple-range test with a level of significance of $p \leq 0.05$.

3.4 Results and Discussion

3.4.1 Effect of polar compounds on oxidative stability of bulk oil and oil- in- water emulsion

In this study, polar compounds were isolated from used frying corn oil after frying 40 batches of French fries. The final frying oil contained 19.3 $\pm$ 0.1 % (w/w) of polar compounds. This polar compounds level is lower than the regulatory limit of total polar compounds in many countries which is 20-27% (149-150). Concentrations of free fatty acids and lipid hydroperoxides in the isolated polar compounds were 1.3 $\pm$ 0.2 % (by wt as linoleic acid) and 15.0 $\pm$ 0.5 mmol/kg oil, respectively. Polar compounds were added to bulk oils at the concentrations of 0.5, 1, and 2 % (w/w). The lipid oxidation rates were evaluated by the formation of lipid hydroperoxides and hexanal as indicators of primary and secondary lipid oxidation products (Figure 3.1). Addition of polar compounds decreased lag phase of both lipid hydroperoxides and hexanal in bulk oil. At concentration of 0.5% (w/w), polar compounds were less prooxidative than higher concentrations. The prooxidant activity of the 1 and 2% (w/w) polar compounds were similar. Yoon and coworkers (1988) also reported that the addition of thermally oxidized triglycerides in a range between 0.5 and 2.0 % to soybean oil and purified soybean oil, resulted in an increase in lipid oxidation rates (120). Moreover, Gomes and coworkers (2011) reported that oxidized triacylglycerols and polar triacylglycerol oligopolymers (0.25-1%, w/w) accelerated oxidation in purified and unpurified olive oil (119).

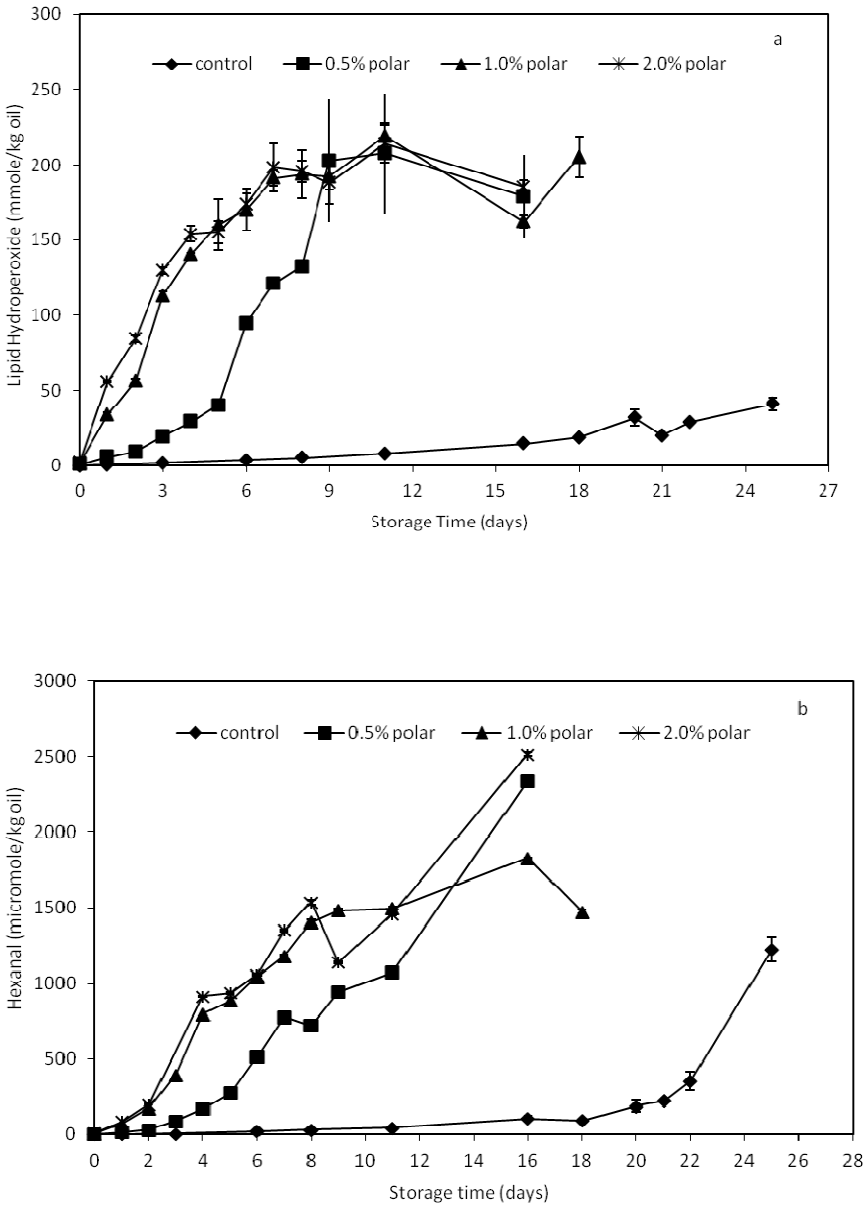

Figure 3.1 Formation of lipid hydroperoxide (a) and hexanal (b) in stripped corn oil/medium chained triacylglycerols without (control) or with addition of 0.5, 1.0, 2.0% of polar compounds during storage at 55°C in the dark. Data points represent means (n=3) ± standard deviations. Some error bars lie within data points.

The mechanism of lipid oxidation in O/W emulsions is different from bulk oils since emulsions contain an oil-water interface that impacts interactions between oil and water components (7). To determine the effect of polar compounds in O/W emulsions, the same concentration of polar compounds (0.5-2.0%) as those used in the bulk oil studies were tested. The emulsions were physically stable throughout storage as confirmed by no visual observation of creaming. The emulsion droplet size did not significant change under similar emulsions conditions as reported by Waraho and coworkers (2009) (10). The influence of polar compounds on the oxidative stability of O/W emulsions is shown in Figure 3.2. Unlike their prooxidant activity in bulk oils, polar compounds had no significant effect on oxidation rates in O/W emulsions at all concentrations ($p>0.05$).

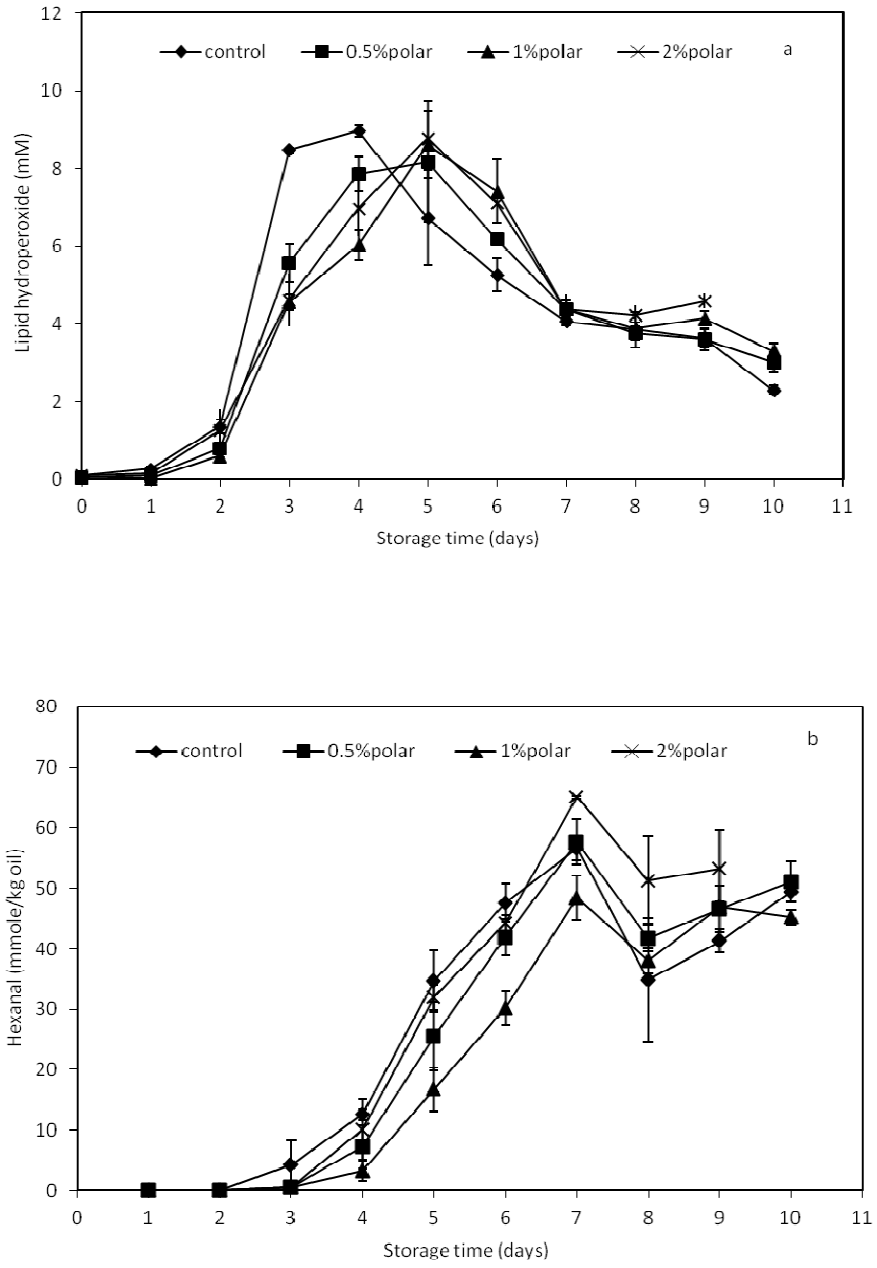

Figure 3.2 Formation of lipid hydroperoxide (a) and hexanal (b) in stripped corn oil in water emulsion without (control) or with addition of 0.5, 1.0, 2.0% of polar compounds during storage at 37°C. Data points represent means (n=3) ± standard deviations. Some error bars lie within data points.

The oxidative stability of O/W emulsions can be dependent on the surface charge of the emulsion droplet as a negatively charged droplet can attract prooxidative metals and accelerate oxidation rates (7, 10, 134). Table 3.1 shows the droplet surface charge of emulsions with varying concentrations of polar compounds. As seen in other studies, oil-in-water emulsions stabilized with Tween 20 were negatively charged (10, 134). Addition of polar compounds did not significantly change droplet surface charge of the emulsions ($p>0.05$). It was recently reported that oleic acid at concentrations $\geq$ 0.1% of the oil enhanced oxidation rate in stripped soybean O/W emulsions (10),(151). In our experiment, polar compounds contained only 1.25% free fatty acids, which means there were $\leq$ 0.025% added free fatty acids in the O/W emulsions used in this study. This suggests that amounts of free fatty acids added to the emulsions in this study would have no impact on emulsion droplet charge, thus would not affect the oxidative stability of the emulsions.

Table 3.1 Droplet surface charge of 1% stripped corn oil-in-water emulsion without (control) and with addition of 0.5, 1.0, 2.0% polar compounds at pH 7. Data represent means (n=3) $\pm$ standard deviations.

Sample	Droplet surface charge (mV)
Control	-8.84 $\pm$ 0.98
0.5% polar compounds	-7.41 $\pm$ 1.82
1.0% polar compounds	-7.25 $\pm$ 0.76
2.0% polar compounds	-8.52 $\pm$ 1.10

It is unclear why the polar compounds would be prooxidative in bulk oil but not in O/W emulsions. The polar compounds contain oxygen and thus are likely surface active. Therefore,

it is possible that the prooxidant activity of the polar compounds is at least partially due to their ability to concentrate at O/W interfaces. However, the ability of polar compounds to concentrate at the droplet surface in O/W emulsions also depends on their ability to compete with surface active agent using to stabilize emulsions. Thus, the impact of polar compounds on interfacial tension was determined in the presence of Tween 20, the emulsifier used in the O/W emulsions in this study. As can be seen in Figure 3.3, increasing concentration of polar compounds did not affect interfacial tension compared to the control. This suggested that the Tween 20 was more surface active than the polar compounds and thus the polar compounds were not able to concentrate at the oil-water interface. It could explain why polar compounds did not show prooxidant activity in O/W emulsions.

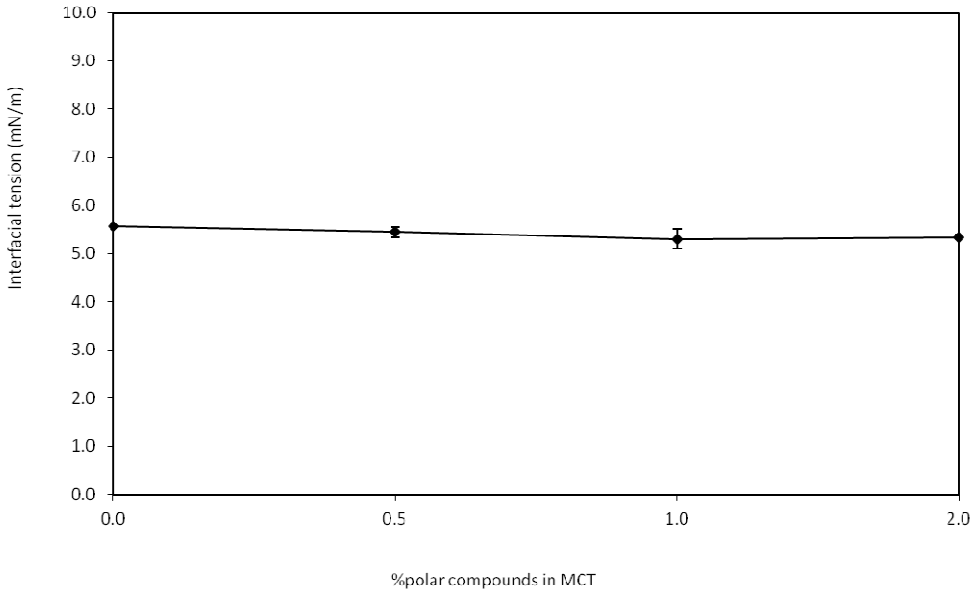

Figure 3.3 Effect of addition of 0.5, 1.0, 2.0% of polar compounds in medium chained triacylglycerols (MCT) on interfacial tension at ambient temperature. Data represents means (n=3) ± standard deviations. Some error bars lie within data points.

3.4.2 Effect of DOPC reverse micelles on prooxidative activity of polar compounds in bulk oil

In bulk oil, DOPC could form reverse micelle at concentration above its critical micelle concentration (CMC). The CMC of DOPC in stripped soy bean oils has been reported to be in the range of 650-950 μM (18). To determine the impact of these structures on prooxidant activity of polar compounds in bulk oil, DOPC (1000 μM) was added at concentrations above its CMC along with water (200 ppm) to form reverse micelles. Figure 3.4 confirms that DOPC was prooxidative as the lag phase of lipid hydroperoxides and hexanal formation was reduced compared to the control. Chen and coworkers (2011) reported that the prooxidant activity of DOPC was not due to its unsaturated fatty acids or its polar head group but instead was due to its ability to form reverse micelle (14).

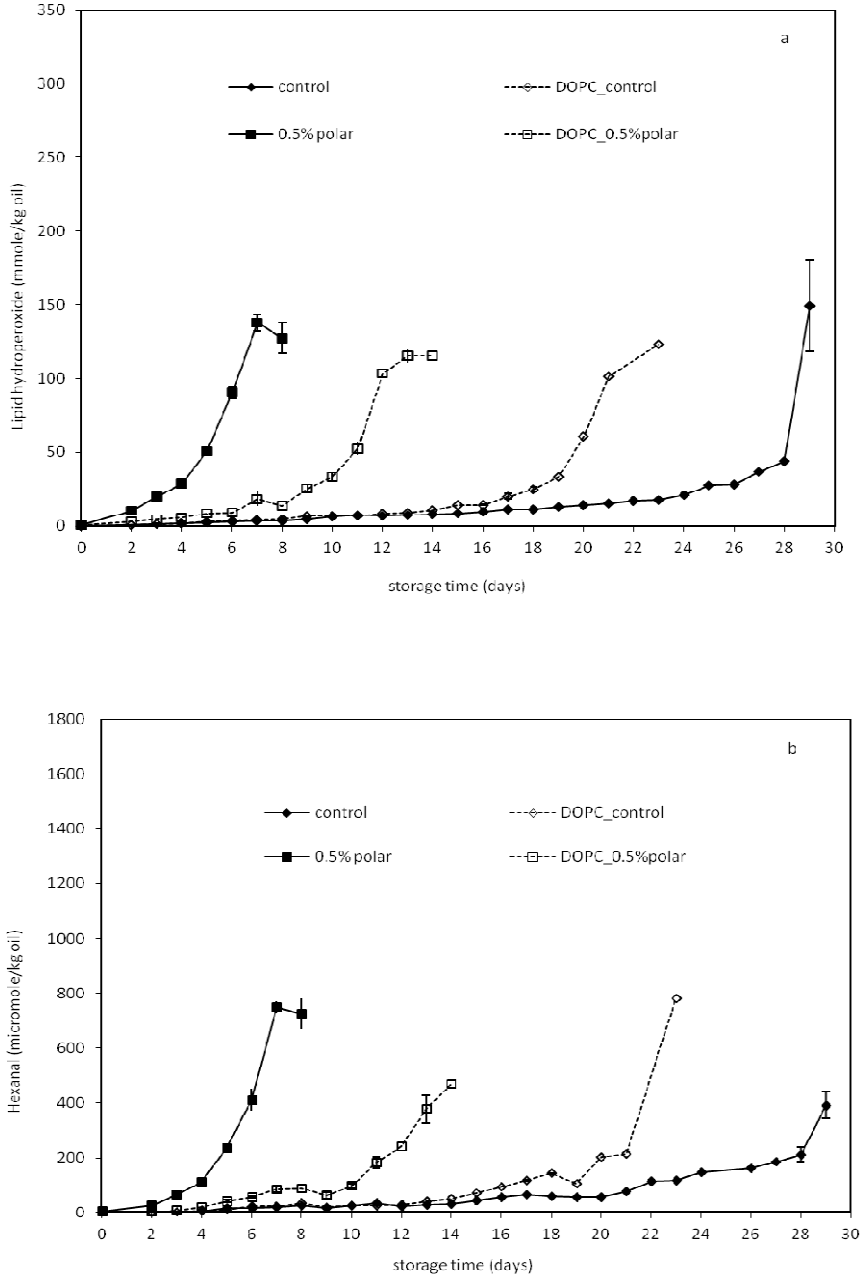

Figure 3.4 Formation of lipid hydroperoxide (a) and hexanal (b) in stripped corn oil/medium chained triacylglycerols in the absence/presence of 1000 μM DOPC without (control) or with addition of 0.5% of polar compounds during storage at 55°C. Data points represent means (n=3) ± standard deviations. Some error bars lie within data points.

Addition of the polar compounds (0.5%, w/w) in the absence of DOPC reverse micelles increased lipid hydroperoxide and hexanal formation more than the DOPC reverse micelles alone (Figure 3.4). However, in the presence of DOPC reverse micelles the polar compounds were less prooxidative in the bulk oil. In the absence of DOPC reverse micelles, it is possible that the polar compounds were able to form association colloids that could accelerate lipid oxidation rates. In the presence of DOPC it could be possible that the polar compounds could not form the same kind of physical structures or migrate to the oil-water interface at the same concentrations due to the presence of the surface active DOPC leading to a decrease in oxidation rates. This would be analogous to the observations in the O/W emulsions where Tween 20 could prevent the polar compounds from concentrating at the water-oil interface thus decreasing their prooxidative activity.

3.4.3 Effect of linoleic acid and linoleic hydroperoxide on oxidation rate of bulk oil with and without DOPC reverse micelle

Refined edible oils contain numerous components with higher polarity than the original triacylglycerol such as monoacylglycerols, diacylglycerols, phospholipids, sterols, free fatty acids, and products arising from lipid oxidation, such as lipid hydroperoxides, aldehydes, ketones, and epoxides (1). Of these compounds, free fatty acids and lipid hydroperoxides are known to be very strong prooxidants. Therefore, this study was performed to determine if the free fatty acid and lipid hydroperoxide concentrations in the polar compounds were responsible for the observed prooxidant activity. To test this possibility, linoleic acid and linoleic hydroperoxide were added in bulk oil with and without DOPC at the concentration levels corresponding to those in 0.5% polar compounds. Final concentrations of linoleic acid and linoleic hydroperoxide in oils were 0.006 and 0.002%, respectively. Figure 3.5 again shows that lipid hydroperoxide and

hexanal formation rates in the system with DOPC reverse micelle were higher than those of samples without DOPC. Neither linoleic acid nor linoleic hydroperoxides increased lipid hydroperoxides and hexanal formation in bulk oil with or without DOPC. In addition, the combination of linoleic acid and linoleic hydroperoxide was also not able to increased lipid hydroperoxide and hexanal formation.

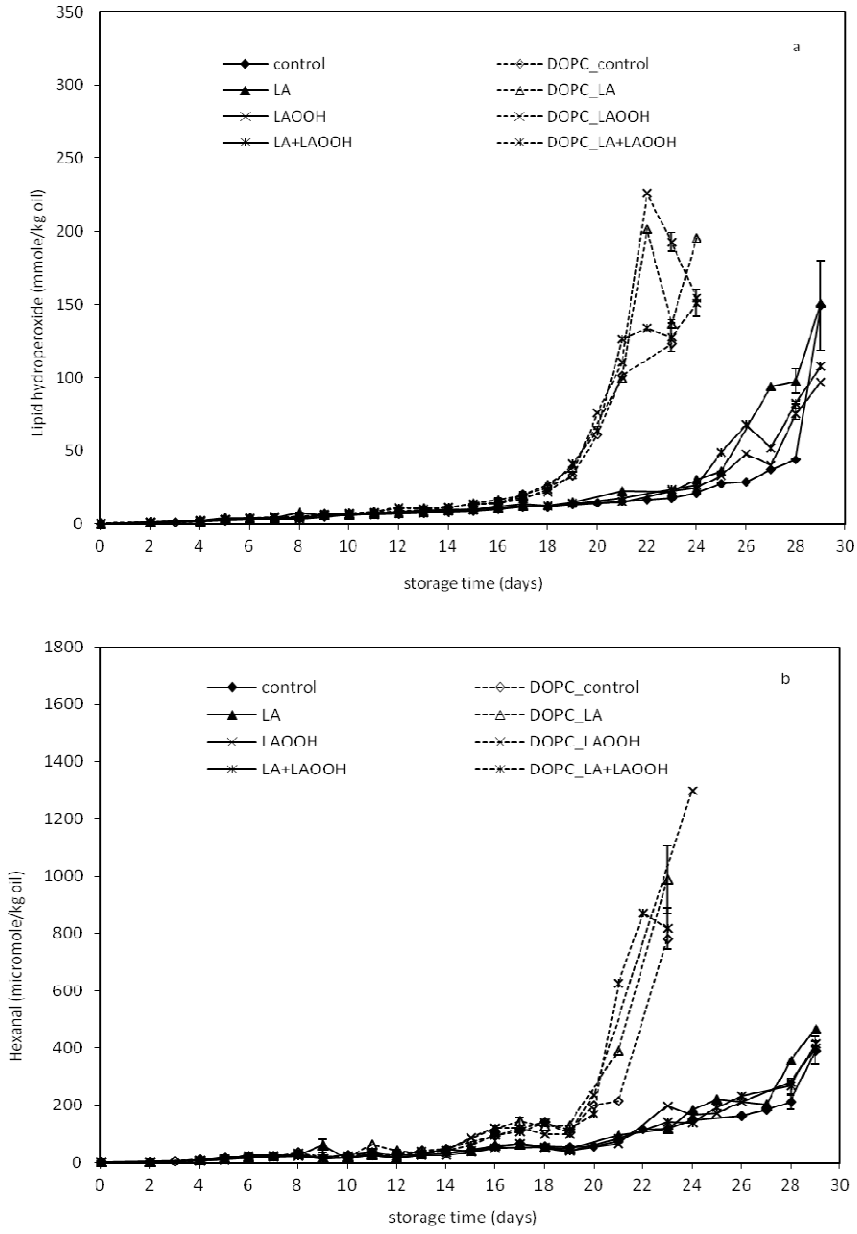

Figure 3.5 Formation of lipid hydroperoxide (a) and hexanal (b) in stripped corn oil/medium chained triacylglycerols in the absence/presence of 1000 μM DOPC without (control) or with addition of linoleic acid (LA), linoleic hydroperoxide (LAOOH), or LA+LAOOH during storage at 55°C. Data points represent means (n=3) ± standard deviations. Some error bars lie within data points.

Several papers have been published on the prooxidant action of free fatty acids (37, 42, 159-160), which is explained to be due to catalytic effect of the carboxyl groups on the formation of free radicals by the decomposition of lipid hydroperoxides (160) or the ability of free fatty acids to increase the prooxidant activity of metals. For example, Aubourg (2001) observed a prooxidant effect of myristic acid in cod liver oil at concentrations of 0.10-1.00% but there was no effect at 0.01 and 0.03% (161). Since the level of free fatty acids in 0.5% of the polar compounds was less than 0.01%, this could explain why they were not prooxidative. Lipid hydroperoxides are also well known to accelerate lipid oxidation as they can decompose into free radicals in the presence of light, metals, and high temperatures (162). Nevertheless, there was no prooxidant activity of linoleic hydroperoxide in our experiments suggesting that the concentration of these components in polar compounds could be too low to affect lipid oxidation rates. This suggests that either there are other components in the polar compounds responsible for the observed prooxidative activity or that the prooxidant activity of the polar compounds is due to their ability to form physical structures that promote oxidation.

3.5 Conclusions

Polar lipid oxidation products are formed in oils during refining, cooking or improper storage. There is growing interest in this class of compounds according to their potential toxicity and prooxidant activity. In these experiments, polar compounds produced during deep fat frying increased oxidation rates in bulk oil but did not affect the oxidative stability of O/W emulsions. Physical structures such as reverse micelles formed by DOPC in bulk oils decreased the prooxidant activity of polar compounds. Two of the major prooxidant components in the polar compounds, free fatty acids and lipid hydroperoxides, were not responsible for the observed prooxidant activity of the polar compounds when tested alone or in combination. Therefore, it

could be that the polar compounds contain other prooxidants such as triacylglycerols polymers that chemically destabilize oils (118). However, it is also possible that the prooxidant activity of the polar compounds is due to their ability to form association colloids and that the resulting water-oil interfaces in these association colloids promote lipid oxidation.

CHAPTER 4

Impact of Free Fatty Acids and Phospholipids on Reverse Micelles Formation and Lipid Oxidation in Bulk Oil

4.1 Introduction

Bulk oils contain various kinds of surface active components such as free fatty acids, monoacyglycerols, diacylglycerols, phospholipids and polar amphiphilic products arising from lipid oxidation, such as lipid hydroperoxides, aldehydes, ketones, and epoxides. Moreover, commercial oils contain amount of water that still remains after the refining process. This would provide an oil–water interface where the surface active components would self-aggregate into association colloids such as reverse micelles, which are thermodynamically more favorable than dispersed surfactant monomers in the oil or water (26).

Reverse micelles are nanometer-sized aggregates consisting of a water core surrounded by surfactants in nonaqueous media such as bulk food oils. Surfactant molecules arrange themselves in the way that polar head groups point inward to the water core and nonpolar tails point outward to bulk oil phase. Reverse micelles are in thermodynamic equilibrium with the surrounding medium. Thus changes of the composition of the lipid medium, or the concentration of surface active molecules could lead to exchange of surface active substances between the reverse micelles and the medium. This could alter the structure and characteristic of reverse micelles. Several researchers have observed that the reverse micelle droplet size increased with increasing the water-to-surfactant ratio (ω_0) (129, 163-164). Additionally, the presence of co-surfactants could affect the reverse micelle size and shape by altering the optimum curvature of the system. Chaiyasit and co-workers (2007) reported that cumene hydroperoxide and oleic acid caused the reverse micelle size to decrease in a sodium bis(2-

ethylhexyl) sulfosuccinate (AOT) reverse micelle model system while phospholipids caused the reverse micelle size to increase (129). Reverse micelles only could be formed when the critical micelle concentration (CMC) of surfactants is exceeded. The CMC value for phospholipids is affected by the phospholipid composition and solvent medium. The higher composition of phosphatidylcholine in mixed phospholipids lowered the CMC value (165). The dilution of the oil with hexane caused the CMC of phospholipids to decrease as compared to that of undiluted oil systems due to the greater hydrophobic repulsive forces between hexane and amphiphilic phospholipids (165).

Phospholipids are surface active components present in refined edible oils at concentration less than 0.03 mmol phosphorus/kg oil (26). Crude oil contains phospholipids such as phosphatidylethanolamine, phosphatidylcholine, phosphatidylinositol and phosphatidylserine even after the degumming process. Phospholipids were found to show antioxidant activity in bulk oils and their antioxidant activity was attributed to metal chelating, lipid hydroperoxide decompostion activity, and free radical scavenging ability (2). However, phospholipids at high concentration act as prooxidants by decreasing surface tension of oil leading to increased diffusion rate of oxygen into the oil (2). Phospholipids have been reported to form reverse micelles in bulk oils in the presence of small quantity of water (17-18, 166-168). The formation of phospholipid reverse micelles has been observed to involve in lipid oxidation in bulk oils in several aspects. A number of studies have suggested that reverse micelles formed by dioleoyl phosphatidylcholine (DOPC) accelerated oxidation rate in bulk oils (14, 18, 167). On the other hand, DOPC reverse micelles showed synergistic effect with some antioxidants in inhibiting oxidation. For example, phospholipids enhanced free radical scavenging activity of tocopherol in bulk oil as reverse micelles formed by phospholipids enhanced the accessibility of tocopherol into the aqueous microenvironment where the oxidation reactions can be

concentrated (137). This result was in agreement with Chen and coworkers (2011) who recently reported that antioxidant activity of α-tocopherol and Trolox at low concentration (10 μM) increased in the presence of DOPC reverse micelles in bulk oil (14). Moreover, the presence of reverse micelle structures in bulk oils also had impact on the antioxidant effectiveness of phenolics compounds with different hydrophobicity such as chlorogenic acid and hexadecyl chlorogenate as reported by Laguerre and coworkers (2011) (168). DOPC reverse micelles showed antagonistic effect with chlorogenic acid but did not impact the antioxidant activity of hexadecyl chlorogenate. The authors suggested that reverse micelle structures could promote the partitioning of chlorogenic acid into the water core, where chlorogenic acid could reduce the prooxidative effect of the metals. Antioxidant activity of hexadecyl chlorogenate was not affected by DOPC reverse micelle suggesting that the hydrophobicity of hexadecyl chlorogenate prevented it from partitioning into water phase and thus did not affect the prooxidant activity of metals.

Another surface active component that has an impact on lipid oxidation in bulk oil is free fatty acids. Since free fatty acids cause foaming and decrease the smoke point of the oil, they are removed from crude oil by neutralization using caustic alkali (1). However, refined edible oils still contain free fatty acids at concentrations ranging from 1.0 to 140 mmol free fatty acid/kg oil (26). Free fatty acids are well established as prooxidants in bulk oils by enhancing prooxidant activity of transition metals (169) and promoting the decomposition of lipid hydroperoxide (36). Moreover, free fatty acids could enhance lipid oxidation in oil by decreasing the surface tension of oil leading to increasing the diffusion rate of oxygen from the headspace into the oil (2). Chaiyasit and coworkers (2008) also reported that oleic acid was able to accumulate at oil/water interface in bulk oil and decrease the pH of the aqueous phase then promoting acid catalyzed lipid hydroperoxide decomposition (17). Free fatty acids could impact

the physicochemical properties of association colloids in bulk oils or even form reverse micelles in bulk oil as they are surface active with a hydrophilic lipophilic balance of around 1(170).

In this paper we attempt to add to the current knowledge by studying the combination effect of free fatty acids and phospholipids on reverse micelles formation and lipid oxidation in bulk oil. This system could imitate structures in real bulk oils which contain various kinds of surface active components providing a better understanding of the complexity of association colloids and their impact on lipid oxidation.

4.2 Materials and Methods

4.2.1 Materials

Corn oil was purchased from a local retail store and stored at 4°C. 1,2 -Dioleoyl-sn-glycero-3-phosphocholine (DOPC) and 1,2-Dibutyryl-sn-glycero-3-phosphocholine (DC_4PC) were acquired from Avanti Polar Lipids, Inc. (Alabaster, AL). Silicic acid (100–200 mesh), activated charcoal (100–400 mesh), 7,7,8,8-tetracyanoquinodimethane (TCNQ), barium chloride, ammonium thiocyanate, iron (II) sulphate heptahydrate, sodium metavanadate ($NaVO_3$), myristoleic acid, oleic acid, linoleic acid, elaidic acid, eicosenoic acid and methyl oleate were purchased from Sigma-Aldrich Co. (St. Louis, MO). Medium-chain triacylglycerols (MCT, Miglyol) were obtained from Sasol North America Inc. (Houston, TX). Chloroform and n-hexane (HPLC grade) were purchased from Fisher Scientific (Fair Lawn,NJ). Deionized water was used in all experiments. Glassware was submerged in 2 M HCl overnight to remove metals, followed by rinsing with deionized water before use.

4.2.2 Methods

The first part of the study is to determine the CMC of DOPC in bulk oil with addition of a) different free fatty acids including myristoleic acid (14:1), oleic acid (18:1, *cis*), elaidic acid (18:1,

trans), linoleic (18:2) and eicosenoic acid (20:1) at 0.5 % (by wt), then b) determine the effect of oleic acid at concentrations varied from 0.5 to 5.0 % (by wt) and c) determine the effect of methyl oleate (from 0.5 to 5.0 % by wt). The second part of the study determined the pH inside the reverse micelles of DOPC in the absence and presence of 3% (by wt) oleic acid by using ^{51}V NMR spectroscopy. The third part of the study is to confirm the reverse micelle structure of DOPC through SAXS. The last part of the study is to determine the lipid oxidation in bulk oil with addition of a) DOPC (at 1000 μmol/kg oil) in the absence or presence of different free fatty acids at 0.5 % by wt b) DOPC (at 200 and 1500 μmol/kg oil) in the absence or presence of oleic acid at 3% by wt c) DC_4PC at 1000 μmol/kg oil in the absence or presence of oleic acid at 0.5% by wt. The lipid oxidation lag time was determined by monitoring the lipid hydroperoxides and hexanal formation.

4.2.3 Preparation of Stripped Corn Oil

Stripped corn oil (SCO) was prepared as described by Boon et al.(9). Briefly, silicic acid (100 g) was washed three times with a total volume of 3 L of distilled water and dried at 110°C for 20 h. A chromatographic column (3.0 cm internal diameter x 35 cm height) was then packed sequentially with 22.5 g of silicic acid, followed by 5.63 g of activated charcoal and another 22.5 g of silicic acid. Thirty grams of corn oil was dissolved in 30 mL of n-hexane and passed through the column by eluting with 270 mL of n-hexane. The container used to collect the triacylglycerols was held in an ice bath and covered with aluminum foil to retard lipid oxidation during stripping. The solvent presented in the stripped oils was removed with a vacuum rotary evaporator (RE 111 Buchi, Flawil, Switzerland) at 37°C and traces of the remaining solvent were evaporated under a nitrogen stream. The water content of the oil was determined using the Karl Fisher Coulometer (756 KF Coulometer connected to 703 Ti Stand, Metrohm, Herisau, Switzerland). The stripped corn oil was kept at -80 °C for subsequent studies.

4.2.4 Determination of critical micelle concentrations in bulk oils

The critical micelle concentrations (CMC) of free fatty acids and those of DOPC in the presence and absence of free fatty acids in bulk oils were determined by using the TCNQ solubilization technique (171). Briefly, the bulk oil was prepared from a mixture of MCT and SCO (3:1, by wt; MCT was used as a non-oxidizable lipid). Various amounts of free fatty acids ranging from 0.1 to 5.0 % (by wt) were added into bulk oil, and then the mixture was magnetically stirred for 12 h. To study the effect of free fatty acids on the CMC of DOPC, the DOPC (1 to 2000 μmol/kg oil) was mixed with the oils for 12 h prior to adding free fatty acids at concentrations ranging from 0.5 to 5.0 % (by wt) followed by addition of 5 mg of TCNQ/5 g oil and mixing for 5 h. The excess TCNQ was removed by centrifugation at 2000g for 20 min and subsequent decanting. The absorbance was measured at 480 nm using a spectrophotometer (Shimadzu 2014, Tokyo, Japan). The CMC was determined as the inflexion point in the curve plotting absorbance as a function of DOPC concentration (semi-log plot) (168).

4.2.5 ^{51}V NMR spectroscopy

Sodium metavanadate solution at 200 mM was freshly prepared by dissolving $NaVO_3$ in deionized water. The solution was heated on the hotplate (approximate temperature of 100°C) and stirred magnetically until the $NaVO_3$ was completely dissolved. After cooling, 18 μL of sodium metavanadate solution was pipetted into 2 g of bulk oil containing DOPC (1000 μmol/kg oil) in the absence or presence of 3% (by wt) oleic acid. Then, the samples were sonicated on an ice bath using a 1/8" probe sonicator (Model FB505, Fisher Scientific, Pittsburgh, PA, USA) at 40% amplitude with 0.05 s/pulse for 1 min. To prepare standard pH solutions, the sodium metavanadate solution was mixed with 0.04 M Britton Robinson buffer (pH 1.5, 3.1, 5.0 and 6.6) at 18:1 ratio according to the volume ratio of sodium metavanadate solution: water in bulk oil. ^{51}V NMR spectra were recorded on Bruker Avance 400 at 105.2 MHz with the spectral window

of 64.9 kHz, a pulse angle of 30°, and acquisition time of 0.126 s with relaxation delay of 0.500 s. The pH of DOPC reverse micelle in bulk oil was determined by comparing the ^{51}V NMR spectra of vanadium in bulk oil with those of the standard pH solutions (172-174).

4.2.6 Small- angle X-ray scattering (SAXS) measurement

SAXS measurements were performed on the oil samples using a Rigaku Molecular Metrology SAXS instrument (Rigaku, Inc.) operating at 45kV and 0.67 mA. The instrument generates X-rays using microfocus Cu x-ray tube with point source (focal spot 30 × 30 μm^2) of which the CuK_α line at 0.1542 nm. Samples were placed into the 1mm outer diameter quartz capillary (Hampton Research, Aliso Viejo, CA) and were positioned inside the sample chamber. The whole system was evacuated by vacuum pump. After passing the samples, the scattered x-rays were collected by a 2D multiwire detector with a sample-to-detector distance of 1477 mm. The actual distance from the sample to the detector was calibrated using silver behenate. The 2D scattering patterns were recorded on the samples for 3 h and then integrated, using the program POLAR, to one-dimensional scattering function *I (q)*, where *q* is the length of the scattering vector defined by $q = (4\pi/\lambda) \sin(\theta/2)$, λ is the wavelength and θ is the scattering angle.

4.2.7 Samples preparation for oxidation study

Either DOPC or DC_4PC was added to the bulk oil (a mixture of MCT/SCO, 3:1, by wt) using chloroform as carrier which was removed by evaporation under nitrogen at room temperature. The samples were magnetically stirred at the speed of 1,000 rpm in a 20 °C incubator room for 12 h. Each of free fatty acids including myristoleic acid, oleic acid, linoleic acid, elaidic acid, and eicosenoic acid was added and stirred for 12 h to obtain homogenous samples. Samples (1 mL) were aliquoted into 10-mL GC headspace vials (Supelco), capped with aluminum lids having PTFE/silicone septa and stored at 55 °C in the dark.

4.2.8 Measurement of lipid oxidation

4.2.8.1 Lipid hydroperoxides

Lipid hydroperoxides were measured using a method adapted from Shanta and Decker (157). The bulk oil samples (20 µL) were weighed and dissolved in 2.8 mL of methanol/butanol solution (2:1, v/v). A mixture of 15 µL of 3.94 M ammonium thiocyanate and 15 µL of 0.072 M ferrous solution was used as an indicator. The ferrous solution was obtained from the supernatant of a mixture of one part of 0.144 M $FeSO_4$ and one part of 0.132 M $BaCl_2$ in 0.4 M HCl. After 20 min of incubation at room temperature, the absorbance of the samples was measured at 510 nm using a spectrophotometer (Genesys 20, Thermospectronic, Waltham, MA). The concentration of hydroperoxides was calculated from a cumene hydroperoxide standard curve.

4.2.8.2 Headspace hexanal

Headspace hexanal was measured using a GC-17A Shimadzu gas chromatograph equipped with an AOC-5000 autosampler (Shimadzu, Kyoto, Japan) (9). Samples (1 mL) in 10-mL glass vials capped with aluminum caps with PTFE/silicone septa were preheated at 55 ºC for 8 min in an autosampler heating block. A solid-phase microextraction (SPME) fiber needle (50/30 µm DVB/Carboxen/PDMS, Supelco, Bellefonte, PA) was injected into the vial for 2 min to absorb volatiles and then was transferred to the injector port (250 °C) for 3 min. The injection port was operated in split mode, and the split ratio was set at 1:5. Volatiles were separated on an Equity-1 column (30 m x 0.32 mm x 1 µm film thickness, Supelco, Bellefonte, PA) at 65 ºC for 10 min. The carrier gas was helium set at a flow rate of 15 mL/min. A flame ionization detector was used at a temperature of 250 °C. Hexanal concentrations were determined from peak areas using a hexanal standard curve.

4.3 Statistical Analysis

All experiments were conducted in triplicate samples. Data were presented as mean ± standard deviation. Data results were analyzed by analysis of variance (ANOVA) using SPSS 14.0 (SPSS Inc., Chicago, IL). The differences between mean values were compared using Duncan's multiple-range test with significance defined as $p \leq 0.05$.

4.4 Results and discussions

4.4.1 Effect of free fatty acids with and without DOPC on reverse micelle formation

The critical micelle concentration (CMC) is one important characteristic of surface active components. It is the concentration at which the surface active molecules begin to aggregate into association colloids. Due to the fact that physical properties of solutions such as surface tension, electrical conductivity, turbidity and osmotic pressure depend upon whether the surfactant molecules are dispersed as monomers or micellar aggregates, the CMC can be determined by monitoring the abrupt change of these physical properties when the CMC is exceeded (17). In this experiment, the CMC was determined by using TCNQ solubilization technique. The charge transfer interaction between DOPC and TCNQ occurred when the concentrations of DOPC exceed the CMC, leading to an increase in the solubility of TCNQ which can be observed by an increase in absorbance at 480 nm. The concentration where the absorbance abruptly changes was identified as the CMC. We initially investigated the ability of free fatty acids to form reverse micelles. The free fatty acids themselves did not show ability to form reverse micelle structure at concentration range from 0.1 to 5% (by wt) in bulk oil as determined by the TCNQ method (data not shown). From previous studies we know that DOPC forms reverse micelle in bulk oil in the presence of small amount of water (14, 18, 166-168). As DOPC is a zwitterion with head group containing negatively charged phosphate and positively charged amine. Changing the pH of the system could alter the net charge and surface activity of

DOPC thus free fatty acids could alter the ability of DOPC to form structures (175). Therefore, we investigated the effect of free fatty acids on the ability of DOPC to form reverse micelles in bulk oil by determining changes in the critical micelle concentration of DOPC. Figure 4.1 shows that the CMC of DOPC in stripped corn oil in the absence of added free fatty acids was at 400 µmol/kg oil. The CMC of DOPC has been reported to depend on oil type and water content. Chen and coworkers (2010) revealed that the CMC of DOPC in stripped soybean oil containing 200 ppm water was around 650 µM (18). Laguerre and co-workers (2011) reported that the CMC of DOPC in stripped corn oil equaled to 65 µM with endogeneous water content of < 40 ppm (168). In our system, the water content was 464.4±51.8 ppm which was in the range of the amount of water in commercial oil (200-865 ppm) (26).

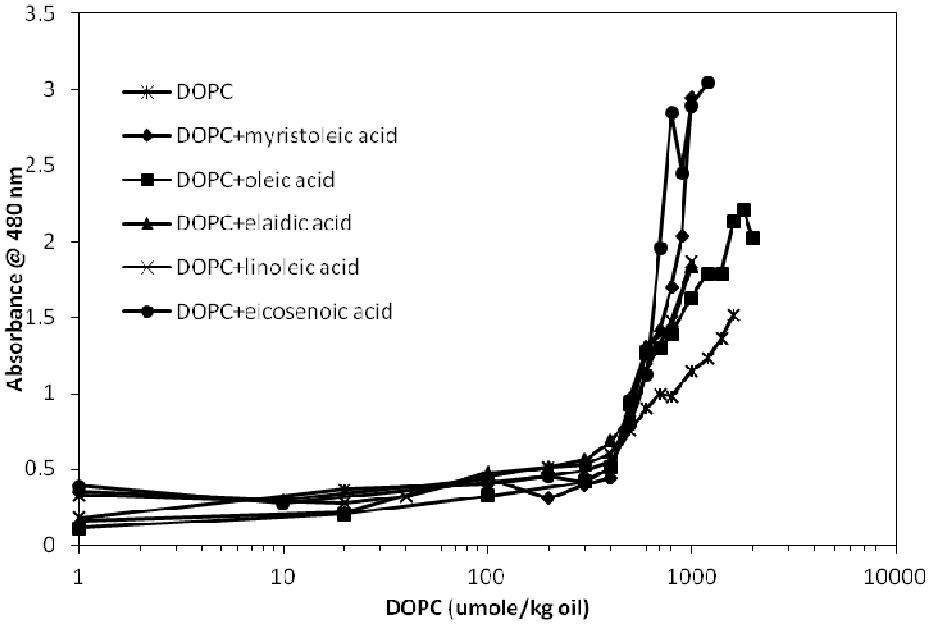

Figure 4.1 Determination of critical micelle concentration of DOPC in bulk oil with addition of different free fatty acids at 0.5% (by wt)

From Figure 4.1, we observed that all types of free fatty acids including myristoleic acid (14:1), oleic acid (18:1, cis), elaidic acid (18:1, trans), linoleic acid (18:2), and eicosenoic acid

(20:1) added at 0.5% (by wt) did not impact the CMC of DOPC. However, as shown in Figure 4.2, increasing fatty acid concentrations up to 5% could alter the CMC of DOPC. For example, the CMC of DOPC in oil containing oleic acid at 0.5 and 1% (by wt) was not different from that of the control which contained only DOPC. However, with the addition of 3 and 5% (by wt) of oleic acid, the CMC of DOPC increased to 800 and 1000 µmol/kg oil, respectively.

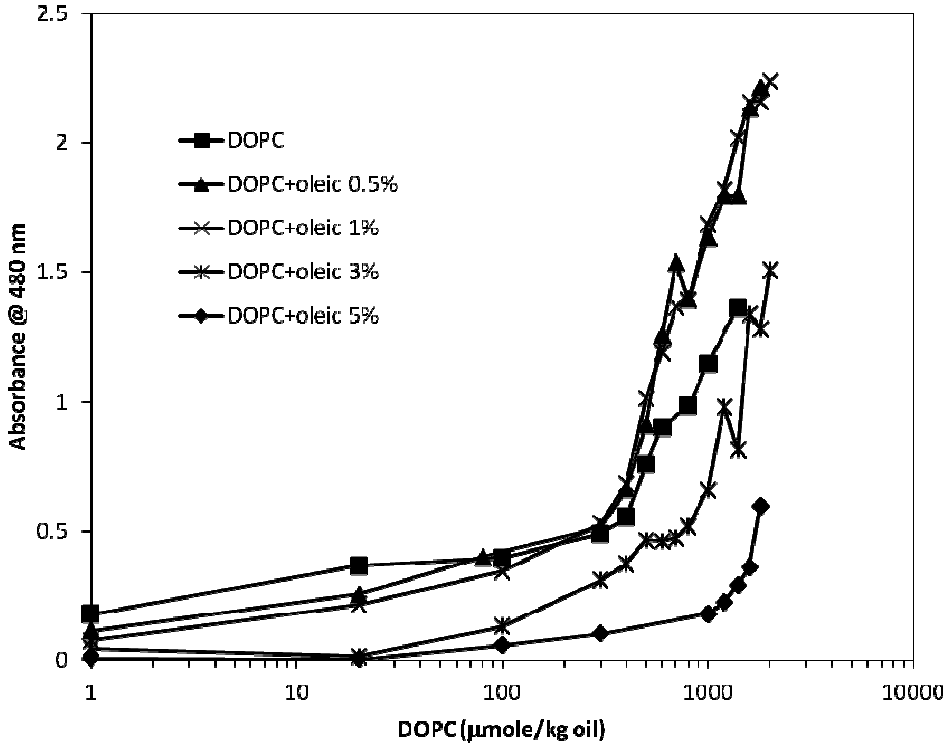

Figure 4.2 Determination of critical micelle concentration of DOPC in bulk oil with addition of oleic acid at 0.5, 1, 3 and 5% (by wt)

We presume that the effect of oleic acid on the CMC of DOPC could relate to the net charge on DOPC head group which is highly pH dependent (175). The addition of free fatty acids could alter the pH of the system then increase the positive charge on phospholipid head group. The protonation of the DOPC could increase repulsion between the head group of DOPC leading to an increase in the CMC. To test our hypothesis, oleic acid was substituted with methyl oleate

which is an ester form of oleic acid without carboxyl group. The result showed that methyl oleate at concentrations up to 5% did not influence the CMC of DOPC (Figure 4.3). This confirmed that the ability of oleic acid to impact the CMC of DOPC depending on the concentration of oleic acid and the presence of the free carboxylic group in the molecule.

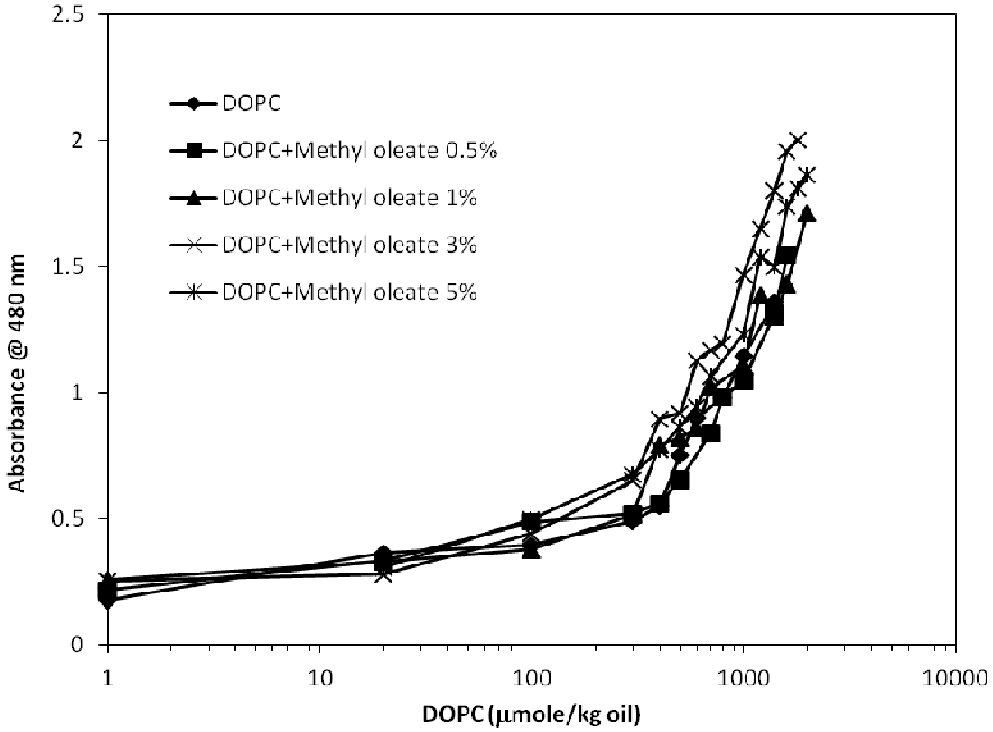

Figure 4.3 Determination of critical micelle concentration of DOPC in bulk oil with addition of methyl oleate at 0.5, 1, 3 and 5% (by wt)

To investigate the influence of free fatty acid on the pH inside reverse micelle, where the DOPC head group resides, we used sodium metavanadate ($NaVO_3$) as a probe that measures the proton concentration in the water pool of the reverse micelle. The speciation of vanadate is highly pH dependent and can be monitored using ^{51}V NMR spectroscopy (172-174). Changes in the signal chemical shift and intensities reflect the protonation state and the relative concentration of each vanadate species, respectively. Figure 4.4 shows the ^{51}V NMR spectra of vanadate species in aqueous solutions at different pH and in DOPC reverse micelle in bulk oil in

the absence and presence of 3% (by wt) of oleic acid. As the overall concentration of the vanadium nuclei probes in DOPC reverse micelle in bulk oil was much lower than that in the aqueous solutions, the signals from the reverse micelle samples were relatively weak. The ^{51}V NMR spectrum of vanadate species in DOPC reverse micelle in bulk oil was consistent with that of the aqueous solution of sodium metavanadate at pH above 5.0. In the presence of 3% (by wt) of oleic acid in combination with DOPC in bulk oil, the spectrum changed towards those observed in sodium metavanadate solutions at pH below 3.0.

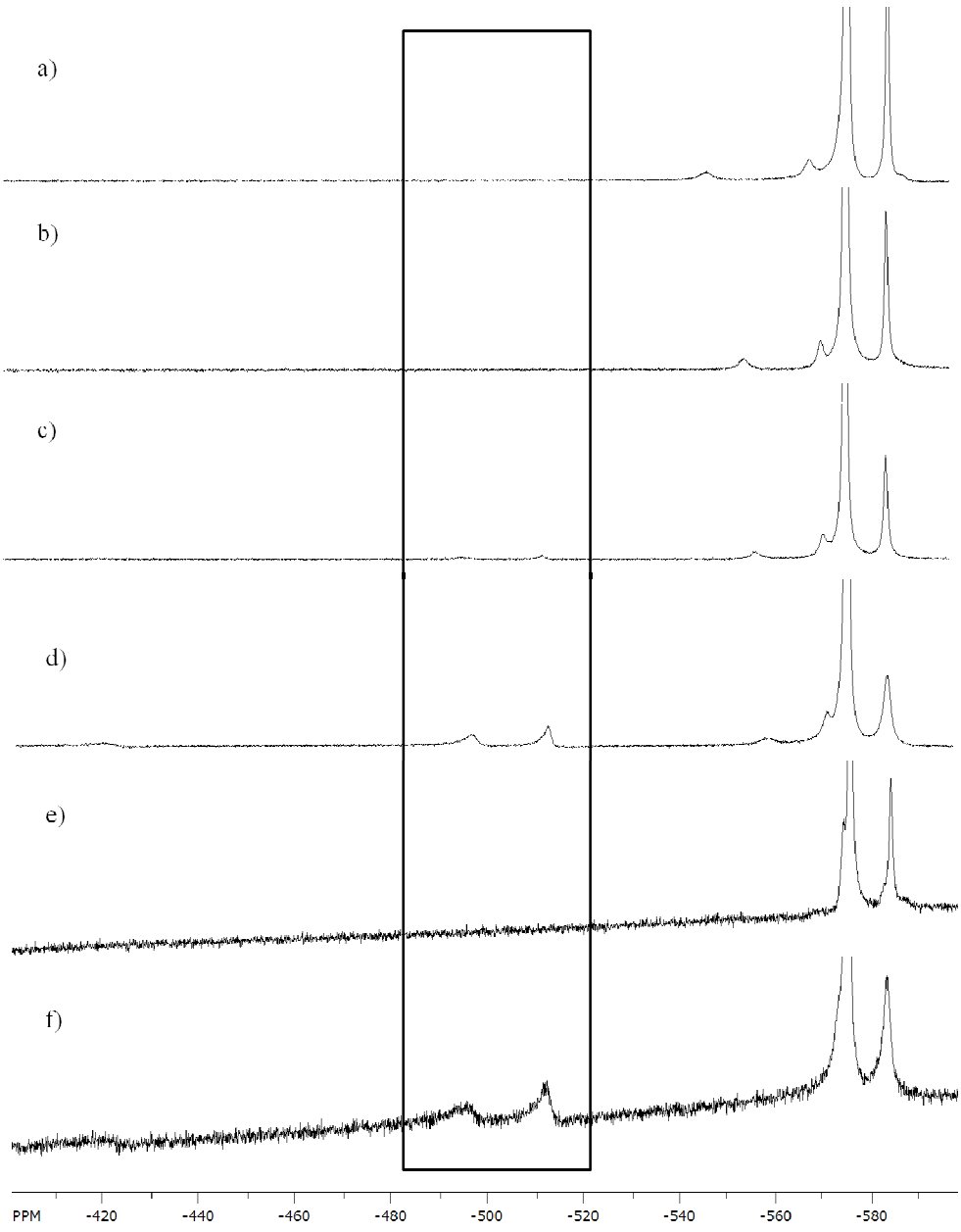

Figure 4.4 ^{51}V NMR spectra of 200 mM sodium metavanadate in Britton Robinson buffer pH: a) 6.6; b) 5.0; c) 3.1 and d) 1.5; e) DOPC (1000 μmol/kg oil) reverse micelle in stripped corn oil without oleic acid; f) DOPC reverse micelles in stripped corn oil with 3% (by wt) oleic acid in bulk oil

However, one should bear in mind that there are some limitations to quantitatively determine pH in reverse micelles in bulk oil by using vanadium probe. For example, the ^{51}V

chemical shift has been reported to change with the size of reverse micelle. The chemical shift in the larger reverse micelles is closer to that in bulk solution. This is attributed to different behavior of water molecules in small reverse micelle and in bulk solution (173, 176). Moreover, protonation and oligomerization of vanadium are also sensitive to factors such as ionic strength, concentration, temperature and solvent polarity, which could cause slight shifts in the speciation observed in reverse micelle (172). Nevertheless, this novel vanadium probe is a useful tool to investigate environments inside reverse micelles that are not readily accessible to direct measurements. The results from this study suggest that free fatty acids are able to accumulate at the oil/water interface and reduce the pH inside reverse micelle. It has been reported that the intrinsic pK_a of the phosphate group of phosphatidylcholine monolayer and bilayers were 0.8 and 2.6, respectively (177-178). Thus, it is possible that 3% oleic acid could alter pH and thus the charge of the DOPC head group thus altering repulsive and/or attractive forces among the surface active molecules, leading to increased CMC of DOPC. Also, as free fatty acids could reside at the oil/water interface, they could compete with DOPC for the interface and cause the CMC of DOPC to increase.

The formation of reverse micelle structure of DOPC in bulk oil was confirmed by Small Angle X-ray Scattering (SAXS) as shown in Figure 4.5a. The SAXS profile shows the Bragg peak at q = 1.4 nm^{-1}. This corresponds to a d-spacing of 4.49 nm, according to $d = 2\pi/q$. When the particles align themselves into a highly ordered arrangement, the Bragg peak indicates the distance between the aligned particles. The scattering pattern has equal intensities along concentric circle around the incident beam when the samples were randomly oriented (isotropic). The size and shape of reverse micelles can vary depending on the type of surfactants and surrounding medium. In the case of water/DOPC/bulk oil system, reverse micelles have been reported to form in spherical shape (18). However, when the 0.5% oleic acid was added

into the bulk oil without DOPC, no peak was observed (Figure 4.5b). This is in agreement with the CMC result which revealed that oleic acid at this concentration did not show the ability to form structure in bulk oil as determined by TCNQ technique. Moreover, no Bragg peak could be seen in the SAXS profile of DOPC in the presence of 0.5% (by wt) of oleic acid (Figure 4.5c). The TCNQ study (Figure 1) revealed that oleic acid at this concentration did not affect the CMC value of DOPC. As oleic acid is also surface active, it could reside at the oil/water interface. This made it plausible that oleic acid could act as co-surfactant and alter the physical structure of DOPC reverse micelle because of its difference in molecular geometry. For example, oleic acid could change the size of the reverse micelles. Chaiyasit and co-workers (2007) previously reported that the addition of oleic acid decreased AOT reverse micelle size in hexadecane system (129). In addition, when water content is constant, increasing concentration of surface active components in the system could also decrease the reverse micelle size (163). Moreover, oleic acid could impact the size of the reverse micelles by altering pH that could alter the charge of DOPC thus affecting packing properties at the oil–water interface. If the resulting size was out of the detection limits of SAXS, it is possible that a Bragg peak would not be detected. The accessible angular range for SAXS that we used corresponds to dimension between about 4 and 100 nm.

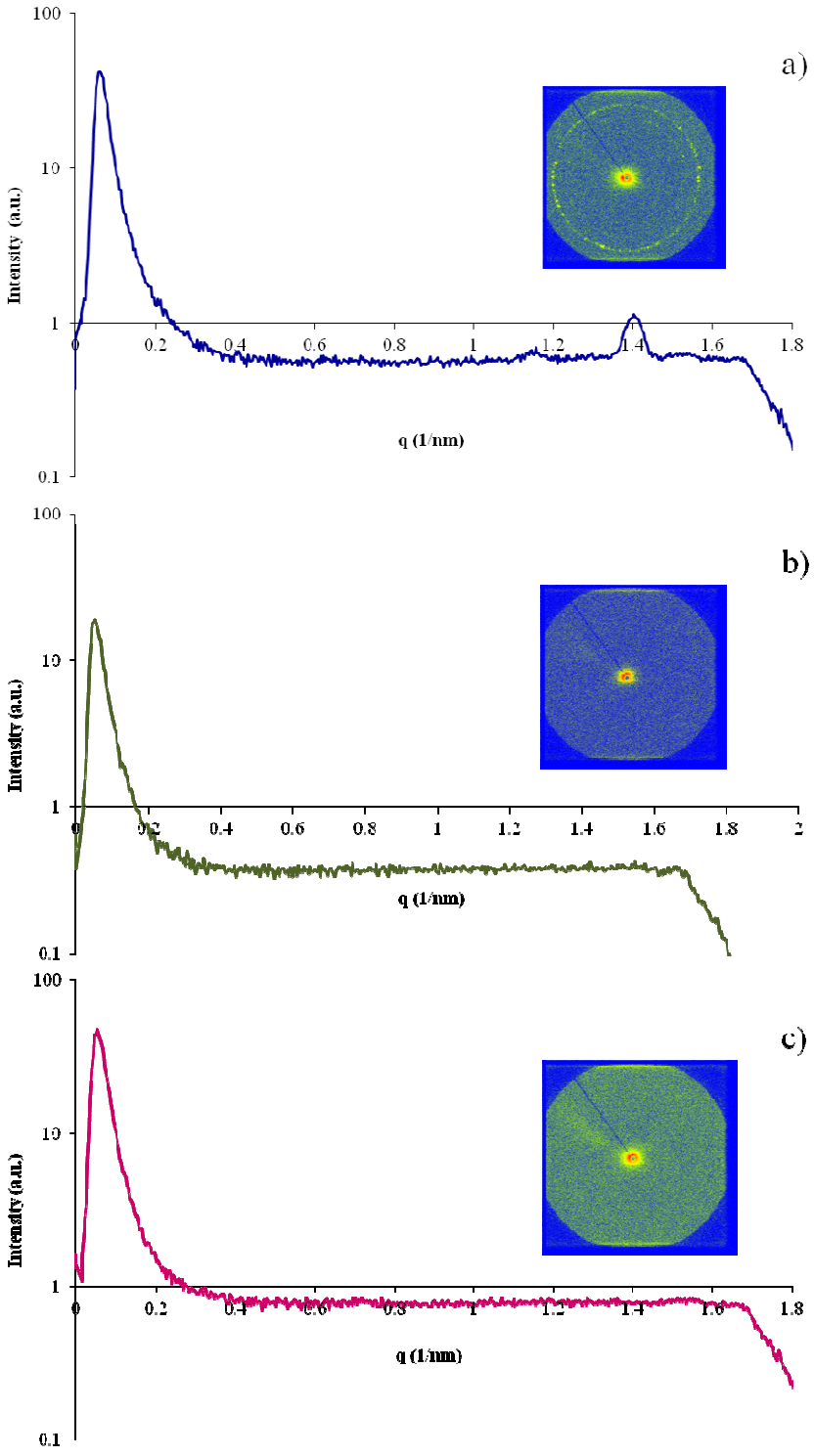

Figure 4.5 SAXS pattern of : a) DOPC (1000 μmol/kg oil) in bulk oil; b) oleic acid (0.5%,by wt) in bulk oil and; c) DOPC (1000 μmol/kg oil) in the presence of 0.5% (by wt) of oleic acid in bulk oil

4.4.2 Effect of free fatty acids and DOPC on the oxidation stability of bulk oil

Minor components in bulk oils have strong impact on lipid oxidation. Some of these components are surface active and could affect lipid oxidation through their ability to form physical structures in the presence of small amounts of water. Among those surface active components, free fatty acids are known as prooxidants, while phospholipids have been reported to be both prooxidative and antioxidative in bulk oils. In oil stripped of its minor components, phosphatidylcholine such as DOPC have been reported to form reverse micelles and exhibit prooxidant activity (14, 18, 167). In the presence of free fatty acids, it is possible that DOPC reverse micelles could negatively impact lipid oxidation by enhancing the prooxidant activity of free fatty acids in bulk oils. Therefore, the combination effect of free fatty acids and DOPC on lipid oxidation in bulk oil was investigated in this experiment. Different types of free fatty acids were added at 0.5% (by wt) in the presence and absence of DOPC in bulk oil. The concentration of 0.5% fatty acid was chosen since this concentration did not change the CMC of the DOPC reverse micelles and thus reverse micelles would be in the oil in both the presence and absence of fatty acids. Lipid oxidation was determined by following the lipid hydroperoxide and hexanal formation over the time. The length of lag phase of lipid hydroperoxide and hexanal indicate the lipid oxidation rate in which the shorter lag phase has the higher oxidation rate. As reported in Table 4.1, the lag phase for the control oil without DOPC was 20 days. The addition of DOPC at 1000 μmol/kg oil reduced the lag time to 13 days indicating that DOPC was prooxidative as previously reported (14, 18, 167). At concentrations above its CMC, DOPC could form reverse micelles which are able to increase lipid oxidation rates by attracting prooxidative factors such as lipid hydroperoxides and transition metals to the water-oil interface (18, 129). In addition, DOPC can accelerate lipid oxidation through its surface activity which can reduce the surface tension and increased the oxygen diffusion rate from the headspace to the oil (2). Free fatty

acids at 0.5% (by wt) exhibited strong prooxidant activities regardless of the type of free fatty acids by decreasing the lag phase to 9 to 10 days. Several mechanisms have been proposed to explain the prooxidative effects of free fatty acids. Miyashita and Takagi (1986) reported that free fatty acids could accelerate decomposition of lipid hydroperoxides and could bind metals to make them more prooxidative (36). Moreover, Mistry and Min reported that free fatty acids could reduce surface tension and increase oxygen diffusion rate from headspace into the oil (39). There was no significant difference in lag time observed for bulk oil containing free fatty acids in the absence or presence of DOPC. These data suggest that the presence of DOPC did not alter the prooxidant activity of free fatty acids at this concentration. Similar trends were also noticed for hexanal formation, which lag phases are shown in Table 4.1.

Table 4.1 Lag time of lipid hydroperoxide and hexanal formation in bulk oil containing different free fatty acids (0.5% by wt) without or with addition of DOPC (1000 μmol/kg oil) during storage at 55°C in the dark

Fatty acids	lag time (days)			
	Lipid hydroperoxide formation		Hexanal formation	
	Without DOPC	With DOPC	Without DOPC	With DOPC
Control	20[a]	13[b]	20[a]	14[b]
Myristoleic acid(14:1)	9[c]	9[c]	9[c]	9[c]
Oleic acid (18:1, cis)	10[c]	10[c]	10[c]	10[c]
Elaidic acid (18:1, trans)	10[c]	10[c]	10[c]	10[c]
Linoleic acid (18:2)	9[c]	9[c]	9[c]	9[c]
Eicosenoic acid (20:1)	9[c]	9[c]	9[c]	9[c]

a, b, c represent significantly different for mean value sharing different letter in each row
($p \leq 0.05$, n=3)

Our previous reverse micelle formation study revealed that increasing the concentration of oleic acid up to 3% (by wt) caused the CMC of DOPC to increase to 1000 μmol/kg oil. To investigate whether DOPC at concentrations below and above its CMC would impact the prooxidant activity of free fatty acids differently, DOPC at 200 and 1500 μmol/kg oil were added to the bulk oil in combination with oleic acid at 3% (by wt). Figure 4.6 shows that the duration of lag phase decreased with increasing the concentrations of DOPC compared to the control. The addition of DOPC at 200 μmol/kg oil and 1500 μmol/kg oil decreased the lag phase of both lipid

hydroperoxide and hexanal formation to 14 and 10 days, respectively. Oleic acid by itself at 3% (by wt) showed strong prooxidant activity by reducing the lag phase of lipid hydroperoxide and hexanal to 5 days. However, no significant differences in prooxidant activity were found between the bulk oil containing 3% (by wt) of oleic acid without and with DOPC at 200 and 1500 μmol/kg oil again suggesting that the presence of a DOPC reverse micelle had little impact on the prooxidant activity of free fatty acids. This could be attributed to the molar ratio of free fatty acids used in this model system where free fatty acids were over 70 times higher than that of DOPC (e.g. oleic acid (3% by wt) equal to 1.06×10^5 μmol/kg oil compared to DOPC at 1500 μmol/kg oil. In commercial refined oils free fatty acids would also be in large excess to phospholipids at a ratio of 10-50 (26). Thus, it is plausible that the majority of free fatty acids reside in the bulk oil phase and not in the reverse micelles and thus exert their prooxidative activity by catalyzing the decomposition of lipid hydroperoxides and forming prooxidative complexes with metals, regardless of the existence of DOPC (2, 36, 39).

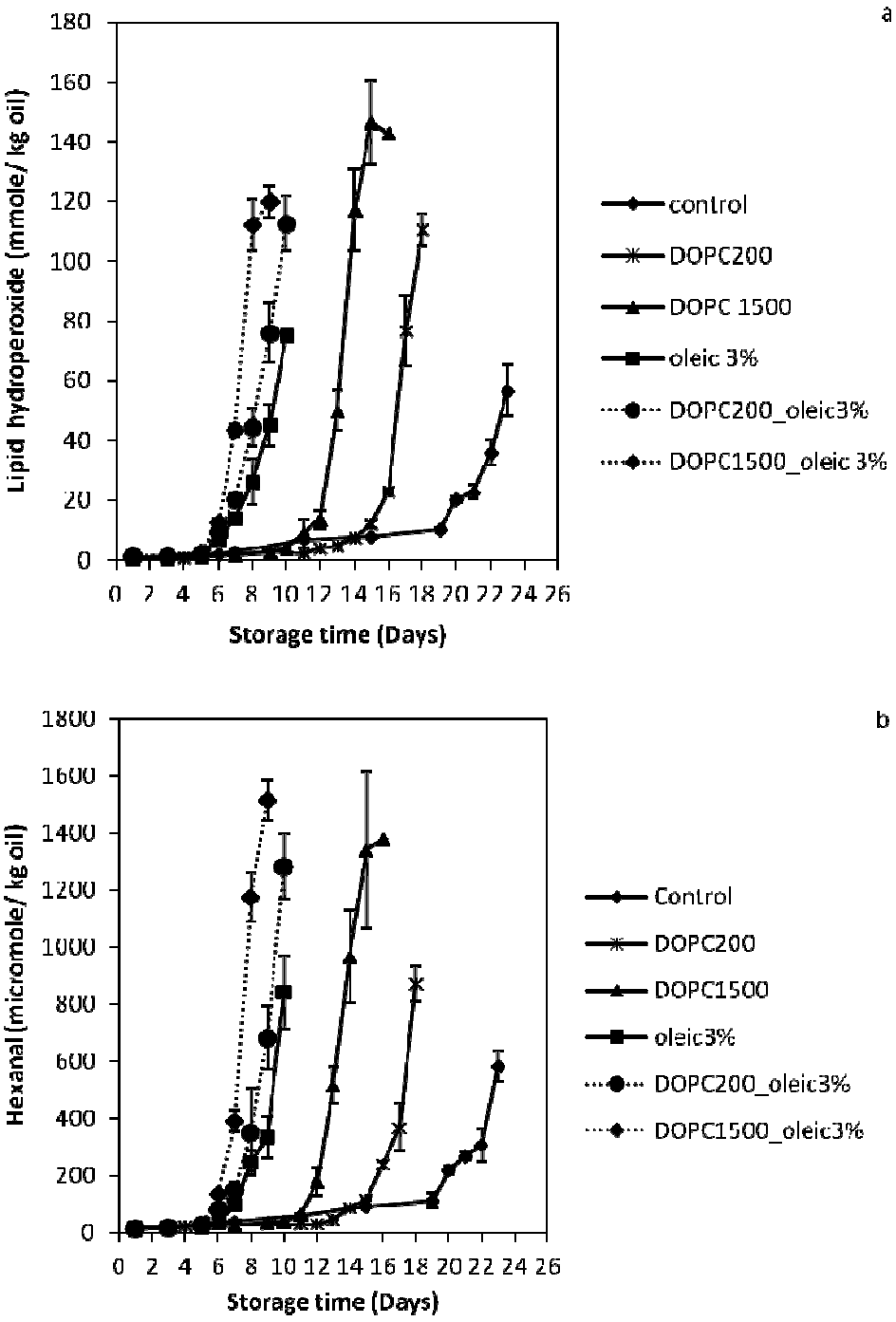

Figure 4.6 Formation of lipid hydroperoxide (a) and hexanal (b) in bulk oil containing oleic acid (3% by wt) without or with addition of DOPC at 200 and 1500 µmol/kg oil during storage at 55°C. Data points represent means (n=3) ± standard deviations. Some error bars lie within data points.

To confirm that the presence of reverse micelle structure had no impact on the prooxidant activity of free fatty acids, an experiment was conducted with and without addition of DC_4PC (at 1000 μmol/kg oil) to bulk oil containing 0.5% (by wt) of oleic acid. DC_4PC is a phosphatidylcholine containing an identical head group as DOPC but with different fatty acyl residues. By having butyric acid as the hydrophobic tails, DC_4PC does not form reverse micelles in bulk oil according to both CMC and the SAXS results (data not shown). This finding is in agreement with other reports that short chain phospholipids are likely present in the form of monomers (14, 179). Figure 4.7 shows that the presence of DC_4PC decreased the lag phase of lipid hydroperoxide and hexanal formation as compared to that of control. The possible explanation for prooxidant activity of DC_4PC is that the negative charge on the head group of DC_4PC could attract transition metals and increase their prooxidative activity (14). However, no significant differences in the lipid hydroperoxide and hexanal formation were observed between the oils containing oleic acid with and without added DC_4PC. These findings again suggest that free fatty acids accelerate lipid oxidation in the manner that independent on the existence of reverse micelle structures.

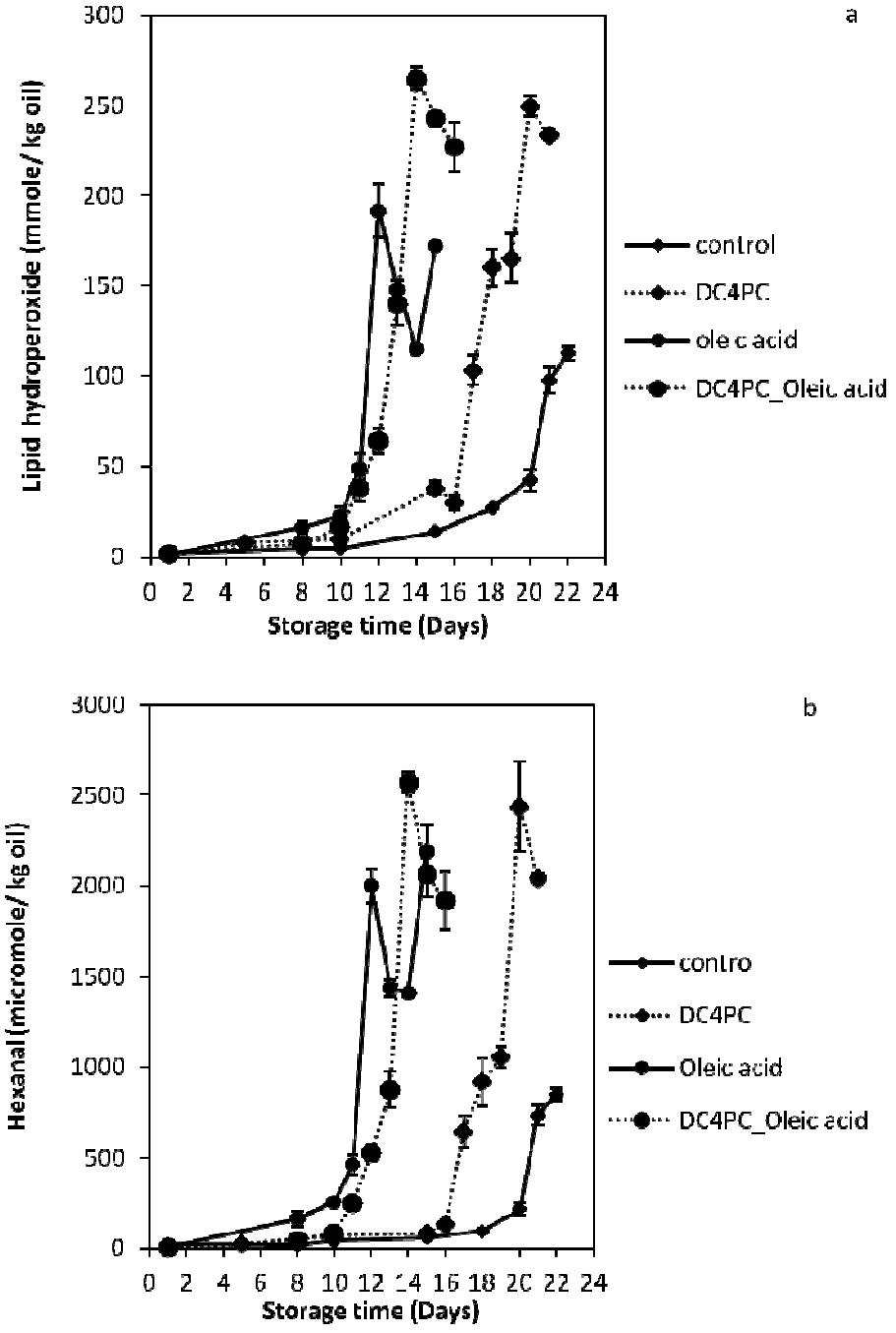

Figure 4.7 Formation of lipid hydroperoxide (a) and hexanal (b) in bulk oil in the presence/absence of oleic acid (0.5% by wt) with/without addition of DC_4PC (1000 µmol/kg oil) during storage at 55°C. Data points represent means (n=3) ± standard deviations. Some error bars lie within data points.

4.5 Conclusions

The rate and mechanism of lipid oxidation in bulk oils containing association colloids likely depends on the total amount and the ability of surfactants to form aggregates. Free fatty acids were shown to possess lower surface activity compared to DOPC as observed from the inability of free fatty acids to form aggregates according to the CMC and SAXS results. Free fatty acids were found to partition into DOPC reverse micelles as seen by the ability of free fatty acids to decrease the pH of the aqueous phase of the micelles and change the CMC of DOPC at high fatty acid concentrations. Reverse micelles formed by DOPC could increase lipid oxidation rate. However, the prooxidant activity of free fatty acids is independent of the presence or absence of reverse micelles.

CHAPTER 5

ASSOCIATION COLLOIDS OF MULTIPLE SURFACE ACTIVE MINOR COMPONENTS AND THEIR EFFECT ON LIPID OXIDATION IN BULK OIL

5.1 Introduction

Association colloids are physical structures formed by surface active molecules which self-aggregate in non-polar systems such as bulk oils in the presence of small amount of water (26). Bulk oils contain not only triacylglycerol, but also a variety of minor components such as free fatty acids, monoacylglycerols, diacylglycerols, phospholipids, sterols, and other polar lipids (1). These types of amphiphilic minor components have been reported to lower interfacial tension in bulk oils (26), suggesting that they are able to concentrate at oil-water interface and act as surfactants and co-surfactants. At concentration above their critical micelle concentrations (CMC), they will self-aggregate and form association colloids. For instance, diacylglycerol monolaurate and diacylglycerol monomyristate at concentration of 5-15 % (by wt) formed reverse rod-like micelles in olive oil (127). The formation of phospholipid reverse micelles in bulk oil was also studied by using small angle X-ray scattering technique (18).

These association colloids in bulk oil create oil-water interfaces which physically impact on lipid oxidation. The existence of oil-water interfaces could accelerate lipid oxidation since surface active lipid hydroperoxides and water soluble metal ions are able to migrate to the same location at the oil-water interfaces. This will promote metal promoted lipid hydroperoxides decomposition which leads to increasing lipid oxidation rates. Moreover, the association colloids could impact the effectiveness of antioxidants since their activities are greatly dependent on the physical locations in heterogeneous food oils (13, 180-181). For example, the polar paradox theory states that nonpolar antioxidants work well in O/W emulsions, whereas, polar

antioxidants work better in bulk oils (182). The existence of physical structures in bulk oil could explain why polar antioxidants have greater efficiency in bulk oil rather than in O/W emulsion as they could localize not in the bulk oil phase but toward the oil-water interface of association colloids where oxidation is supposed to primarily occur (14).

The characteristics of surface active molecules can impact the physical properties of reverse micelles. For example, the curvatures and sizes of the reverse micelles are correspondent to the molecular properties and geometry of the surfactants (128, 130). In addition, the interfacial properties such as charge and thickness will be dictated by the surface active molecules as has been widely reported in O/W emulsion systems (7). Bulk oils contain a diversity of surface active minor components which could form complex multi-component association colloids in bulk oils. The physical structures formed by multiple surface active components in bulk oil could greatly impact the activity of both prooxidants and antioxidants and thus the oxidative stability of oil. There are only few studies on the impact of association colloids on antioxidant activity in bulk oil. These studies only use one or two surface active components to form association colloids (14, 53).

Therefore, in this research, we aimed to study the ability of multiple surface active components found naturally in refined oil including free fatty acids, diacylglycerols, phospholipids, and sterols to form association colloids by determining their surface activities and critical micelle concentrations in bulk stripped corn oil. In addition, we investigated the influence of association colloids formed by multiple surface active components on antioxidative activity of α-tocopherol and Trolox (a water soluble derivative of tocopherols) in bulk oil. This study could lead to a better understanding of the mechanisms underlying their antioxidant activity in real bulk oil systems, thus could provide knowledge of how to improve oxidative stability of oil.

5.2 Materials and methods

5.2.1 Materials

Corn oil was purchased from a local retail store and stored at 4°C. 1,2 -Dioleoyl-sn-glycero-3-phosphocholine (DOPC) and 1,2 -Dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE) were acquired from Avanti Polar Lipids, Inc. (Alabaster, AL). N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)-1,2-dihexadecanoyl-sn-glycero-3-phosphoethanolamine, triethylammonium salt (NBD-PE, Cat. No. N-360) was acquired from Invitrogen. Silicic acid (100–200 mesh), activated charcoal (100–400 mesh), 7,7,8,8-tetracyanoquinodimethane (TCNQ), barium chloride, ammonium thiocyanate, iron (II) sulphate heptahydrate, oleic acid, 1,2-Dioleoyl-*sn*-glycerol (DAG), stigmasterol, α-tocopherol and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) were purchased from Sigma-Aldrich Co. (St. Louis, MO). Medium-chain triacylglycerols (MCT, Miglyol) was obtained from Sasol North America Inc. (Houston, TX). Chloroform and n-hexane (HPLC grade) were purchased from Fisher Scientific (Fair Lawn,NJ). Deionized water was used in all experiments. Glasswares were submerged in 2 M HCl overnight to remove metals, followed by rinsing with deionized water before use.

5.2.2 Methods

5.2.2.1 Stripped corn oil preparation

Stripped corn oil was prepared using column chromatography according to Boon and coworkers (2008) (9). Briefly, silicic acid (100 g) was washed three times with a total volume of 3 L of distilled water and dried at 110°C for 20 h. A chromatographic column (3.0 cm internal diameter x 35 cm height) then was packed sequentially with 22.5 g of silicic acid, followed by 5.63 g of activated charcoal and another 22.5 g of silicic acid. Thirty grams of corn oil dissolved

in 30 mL of n-hexane was passed through the column by eluting with 270 mL of n-hexane. The container used to collect the triacylglycerols was held in an ice bath and covered with aluminum foil to retard lipid oxidation during stripping. The solvent present in the stripped oils was removed with a vacuum rotary evaporator (RE 111 Buchi, Flawil, Switzerland) at 37°C and traces of the remaining solvent were evaporated under a nitrogen stream. The water content of the oil was determined using the Karl Fisher Coulometer (756 KF Coulometer connected to 703 Ti Stand, Metrohm, Herisau, Switzerland). The stripped corn oil was kept at -80 °C for subsequent studies.

5.2.2.2 Interfacial tension measurement of bulk oil containing multiple surface active components

The surface activity of minor components was determined by interfacial tensiometry using a drop shape analyzer (DSA100, Krüss GmbH, Hamburg, Germany). Minor components (1000 μmol/kg oil) were mixed in bulk oil (a mixture of stripped corn oil and MCT at 1:3 ratio), which then was loaded in a syringe. A pendant drop of oil was form at the inverted tip of a hypodermic needle (with a diameter of 1.5 mm) that was submerged in double distilled water at room temperature. The tip of the needle was positioned on an optical bench between a light source and a high speed charge couple device (CCD) camera. The CCD camera was connected to a video frame-grabber board to record the image onto the hard drive of a computer at a speed of one frame per 1 min. The shape of the pendent drops was determined through numerical analysis of the entire drop shape using the drop shape analysis program supplied by the instrument manufacturer. The interfacial tension was calculated from the drop shape using the Young–Laplace equation of capillarity (158).This methodology requires accurate determination of solution densities, which were measured using a digital density meter (DMA 35 N; Anton Paar

USA, Ashland, VA). The density of bulk oil was 0.9366 g/cm^3 at 20°C. All interfacial tension measurements were carried out after 10 minutes.

5.2.2.3 Determination of the critical micelle concentration of multiple surface active components in bulk oil

The critical micelle concentrations (CMC) of multiple surface active lipids in bulk oils were determined by using the TCNQ solubilization technique (171). Briefly, the bulk oil was prepared from a mixture of MCT and SCO (3:1, by wt; MCT was used as a non-oxidizable lipid). The CMC of DOPC, DOPE, stigmasterol, oleic acid, and DAG and their mixture at a molar ratio of 3.78:0.67:0.97:0.43:2.25, respectively was determined at concentrations ranging from 1 to 1000 μmol/kg oil in bulk oil. The ratios of the minor components are similar to those found in refined corn oil (26, 58). The oil containing surface active components was magnetically stirred for 12 h in a 55 °C incubator room prior to adding 5 mg of TCNQ/5 g oil and mixing for another 5 h. The excess TCNQ was removed by centrifugation at 2000g for 20 min. The absorbance was measured at 480 nm using a spectrophotometer (Shimadzu 2014, Tokyo, Japan). The CMC was determined as the inflexion point in the semi-log plot of absorbance versus surface active lipid concentration (168).

5.2.2.4 Fluorescence measurement of bulk oil containing reverse micelles and antioxidants

The surface active fluorescent probe, NBD-PE, is a phospholipid analogue comprised of a fluorescent functional group covalently attached to the choline head group. It was incorporated into bulk oil and used to study the surface activity of minor components. The mixture of DOPC, DOPE, stigmasterol, oleic acid, and DAG (molar ratio of 3.78:0.67:0.97:0.43:2.25) in chloroform was added to bulk oil at 100 μmol/kg oil. For antioxidants study, α-tocopherol and Trolox were added into the oil at the same time as mixed components. Chloroform was removed by

evaporation under nitrogen at room temperature. The samples were magnetically stirred at the speed of 1,000 rpm for 12 h. Then, NBD-PE was added at concentration of 0.95 μM, this concentration minimized self-quenching by the probe (19). The samples were stirred for another 5 h in the dark. Steady-state emission spectra of NBD-PE were collected at 22° C using a PTI spectrofluorometer (PTI, Ontario, Canada). To minimize any reflection of the excitation beam by the cell window and by the underlying liquid surface of the sample into the emission monochromator, measurements were conducted in triangular suprasil cuvettes. The emission was observed at 90° to the incident beam, that is, 22.5° with respect to the illuminated cell surface. A 2.0 nm spectral band width for both excitation and emission slits was employed for the NBD-PE excitation at 468 nm. The integration time was 1 s, and the wavelength increment during emission spectrum scanning was 1 nm. The intensity of the spectra were determined as the emission signal intensity (counts per second) measured by means of a photomultiplier.

5.2.2.5 Samples preparation for oxidation study

Mixtures of surface active minor components including DOPC, DOPE, stigmasterol, oleic acid, and DAG was added to the bulk oil as described above at concentrations below (10 μmol/kg oil) and above (100 μmol/kg oil) the CMC. The samples were magnetically stirred at the speed of 1,000 rpm in a 55 °C incubator room for 12 h. To study antioxidant activity, α-tocopherol and Trolox were added at 10 and 50 μmol/kg oil along with mixed surface active components. Samples (1 mL) were aliquoted into 10-mL GC headspace vials (Supelco), capped with aluminum lids having PTFE/silicone septa and stored at 55 °C in the dark.

5.2.2.6 Determination of lipid oxidation products in bulk oil

Lipid hydroperoxides and hexanal were determined as primary and secondary lipid oxidation products, respectively. The concentration of lipid hydroperoxides and hexanal formation were plotted against time in days. Lag phase which is defined as the time as the first

data points that were statistically greater than time 0 values were used to compare the oxidative stability of oils.

5.2.2.6.1 Lipid hydroperoxides measurement

Lipid hydroperoxides were measured using a method adapted from Shanta and Decker (20). The bulk oil samples (20 µL) were weighed and dissolved in 2.8 mL of methanol/butanol solution (2:1, v/v). A mixture of 15 µL of 3.94 M ammonium thiocyanate and 15 µL of 0.072 M ferrous solution was used as an indicator. The ferrous solution was obtained from the supernatant of a mixture of one part of 0.144 M $FeSO_4$ and one part of 0.132 M $BaCl_2$ in 0.4 M HCl. After 20 min of incubation at room temperature, the absorbance of the samples was measured at 510 nm using a spectrophotometer (Genesys 20, Thermospectronic, Waltham, MA). The concentration of hydroperoxides was calculated from a cumene hydroperoxide standard curve.

5.2.2.6.2 Headspace hexanal measurement

Headspace hexanal was measured using a GC-17A Shimadzu gas chromatograph equipped with an AOC-5000 autosampler (Shimadzu, Kyoto, Japan) (14). Samples (1 mL) in 10-mL glass vials capped with aluminum caps with PTFE/silicone septa were preheated at 55 °C for 8 min in an autosampler heating block. A solid-phase microextraction (SPME) fiber needle (50/30 µm DVB/Carboxen/PDMS, Supelco, Bellefonte, PA) was injected into the vial for 2 min to absorb volatiles and then was transferred to the injector port (250 °C) for 3 min. The injection port was operated in split mode, and the split ratio was set at 1:5. Volatiles were separated on an Equity-1 column (30 m x 0.32 mm x 1 µm film thickness, Supelco, Bellefonte, PA) at 65 °C for 10 min. The carrier gas was helium set at a flow rate of 15 mL/min. A flame ionization detector was used at a temperature of 250 °C. Hexanal concentrations were determined from peak areas using a hexanal standard curve.

5.3 Statistical analysis

All experiments were conducted in triplicate samples. Data was presented as mean ± standard deviation. Data results were analyzed by analysis of variance (ANOVA) using SPSS 14.0 (SPSS Inc., Chicago, IL). The differences between mean values were compared using Duncan's multiple-range test with significance defined as $p \leq 0.05$.

5.4 Results and discussions

5.4.1 The surface activity and critical micelle concentration of multiple surface active components in bulk stripped corn oil

Minor components are naturally present in bulk oils and also are generated during refining and storage due to the enzymatic and non-enzymatic hydrolysis, oxidation and thermal degradation reactions (2, 21-22). Unlike triacylglycerols, many of these components can act as amphiphilic surfactants as they contain both hydrophilic and hydrophobic functional groups on their structures. Surface activity of minor components at the oil-water interface can be investigated by measuring interfacial tension (water-oil interface). In this study, we determined interfacial tension of bulk oil containing each minor component including oleic acid, DAG, stigmasterol, DOPC or DOPE at concentration of 1000 μmol/kg oil. As shown in figure 5.1, all minor components significantly decreased the interfacial tension of bulk oil, suggesting that they were able to concentrate at the oil-water interface and reduce the interfacial free energy (23). Phospholipids including DOPC and DOPE show relatively strong surface activity compared to oleic acid, DAG, and stigmasterol, by reducing the interfacial tension of the control oil from 22.6 to 9.9 and 10.3 mN/m, respectively. The interfacial tensions of bulk oils containing oleic acid, DAG and stigmasterol were 18.2, 15.8, and 18.5 mN/m, respectively. As reported in several studies, the interfacial tension of the bulk commercial oil is lower than stripped oil due to the presence of surface active compounds and interfacial tension decreases during the frying

process due to the generation of surface active components by hydrolysis, oxidation and thermal degradation reactions (1, 24-25). Gil and Hendel (1995) found that phospholipid at concentration 0.2% w/w lowered the interfacial tension of the soybean oil/water by 42%, while the addition of DAG and free fatty acid up to 0.1% w/w did not have impact on the interfacial tension values (25). However, there are reports suggesting that the presence of DAG and free fatty acids significantly decreased the oil-water interfacial tension (24, 26). Cercaci and co-workers (2006) found that stigmasterol decreased the interfacial tension of hexadecane/water with increasing concentration of stigmasterol (27).

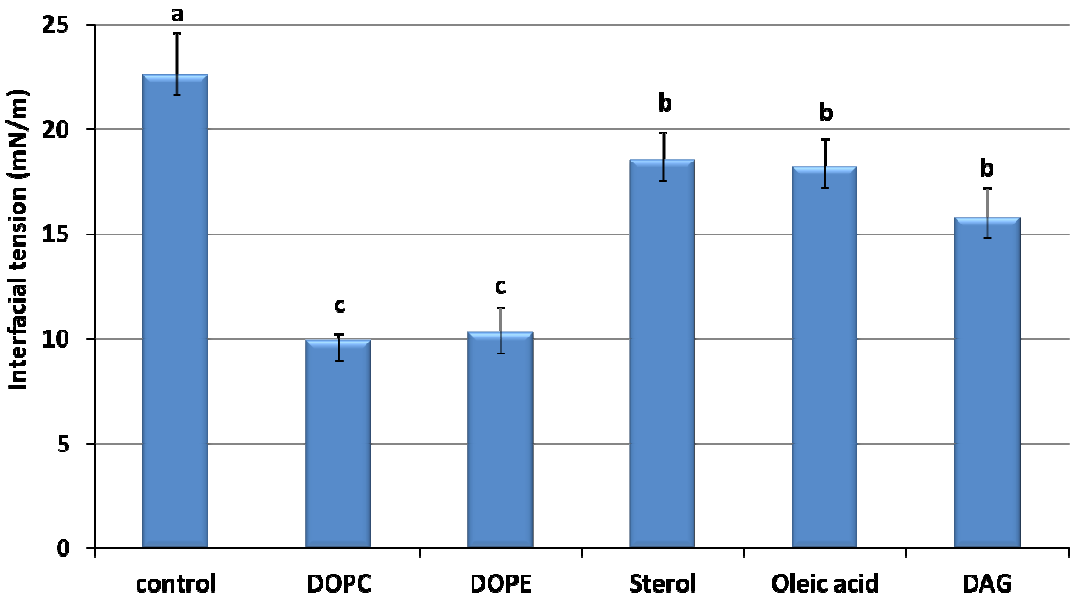

Figure 5.1 Interfacial tension of bulk oil containing DOPC, DOPE, stigmasterol, oleic acid, or DAG at 1000 μmol/kg oil. [a,b,c] Represent significantly different at $p \leq 0.05$

In the presence of trace amount of water in bulk oils, surface active components tend to aggregate so that the hydrophilic head groups orient toward the water core in order to minimize contact between the hydrophobic environment in bulk oil and the hydrophilic head groups. The

concentration at which the surface active molecules start aggregating is defined as a critical micelle concentration (CMC) (28). In this study, we determined the CMC of surface active minor components by using the TCNQ technique. The absorbance of TCNQ sharply increases upon formation of micelles due to the charge transfer of the TCNQ in the presence of aggregates in the system (16). The water content in the bulk oil that we used in this study was 383±2 ppm. The CMC of each minor component including DOPC, DOPE, stigmasterol, oleic acid, and DAG was determined by varying the concentration ranging from 1 to 1000 μmol/kg oil in bulk oil. Figure 5.2 shows that the CMCs of DOPC and DOPE at 55°C were 40 and 200 μmol/kg oil, respectively. Considering the chemical structures of DOPC and DOPE, it is not surprising that the DOPC had lower CMC than the DOPE since the choline head group of DOPC has higher polarity compared to the ethanolamine group of DOPE, thus this facilitates the DOPC to reside at the oil-water interface better than the DOPE (29). Moreover, the CMC of phospholipids depends on their degree of hydration. DOPC with higher hydration index of 100 was reported to have lower CMC than the DOPE which possesses the hydration index of 16 (30). We did not observe a typical CMC inflexion point in the absorbance plots of the oils containing oleic acid, DAG, and stigmasterol in the range of concentrations studied. Our results suggest that although oleic acid, DAG, and stigmasterol were able to concentrate at the oil-water interface according to the interfacial tension results (figure 5.1), they did not form aggregates at the concentrations used in this study.

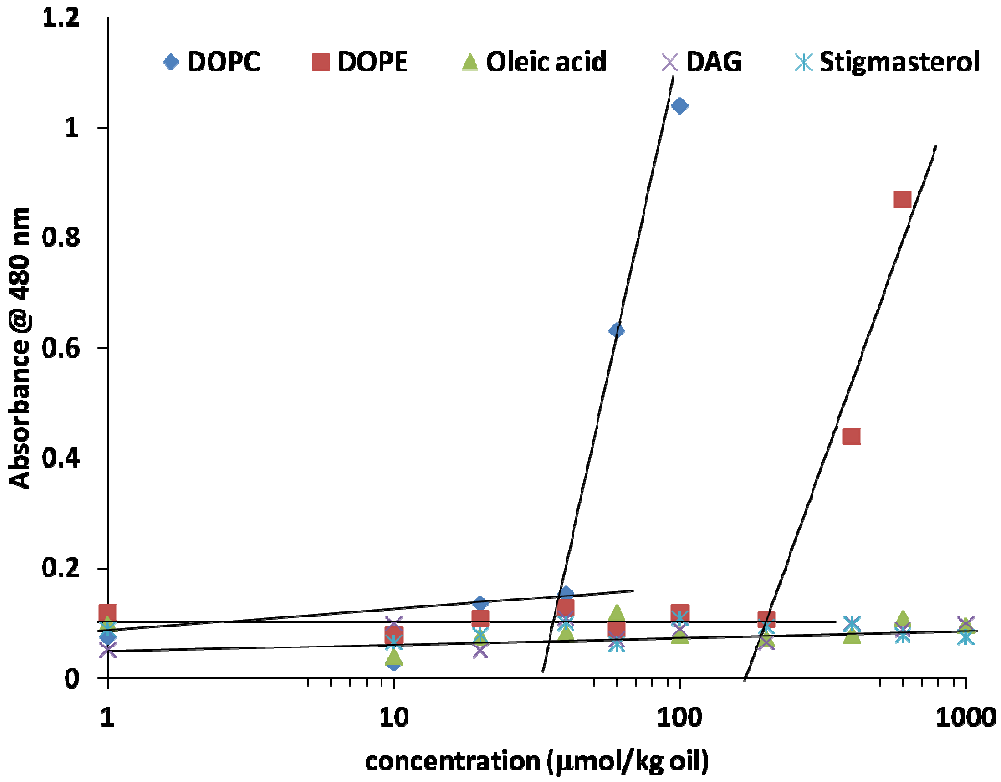

Figure 5.2 Critical micelle concentrations of DOPC, DOPE, stigmasterol, oleic acid, and DAG in bulk oil at 55° C

Furthermore, we investigated the CMC of the mixture of DOPC, DOPE, stigmasterol, oleic acid, and DAG at constant molar ratio of 3.78:0.67:0.97:0.43:2.25, respectively. This ratio was meant to imitate the diverse composition of the major surface active compounds in commercial refined bulk oil (1, 17). Moreover, each component was removed one at a time in order to understand how each component impacts the ability of other surface active molecules to form aggregates. As shown in figure 5.3, the mixed components were able to form association colloids at concentration of 20 µmol/kg oil at 55 °C which is lower than DOPC alone (figure 5.2). The removal of a component from the mixture did not significantly impact the CMC value with the exception of DOPC as its removal resulted in no formation of association colloids

as determined by the TCNQ method. Although, the mixed components without DOPC still contained the DOPE which can form association colloids, the concentration of DOPE in the mixed components was 155 μmol/kg oil which was lower than its CMC (200 μmol/kg oil), thus they did not form aggregates. This suggests that DOPC plays an important role as a major surfactant responsible for association colloid formation at the concentration of surface active compounds typically found in refined oil. The other components likely acted as co-surfactants since the CMC of the mixed systems was lower than DOPC alone.

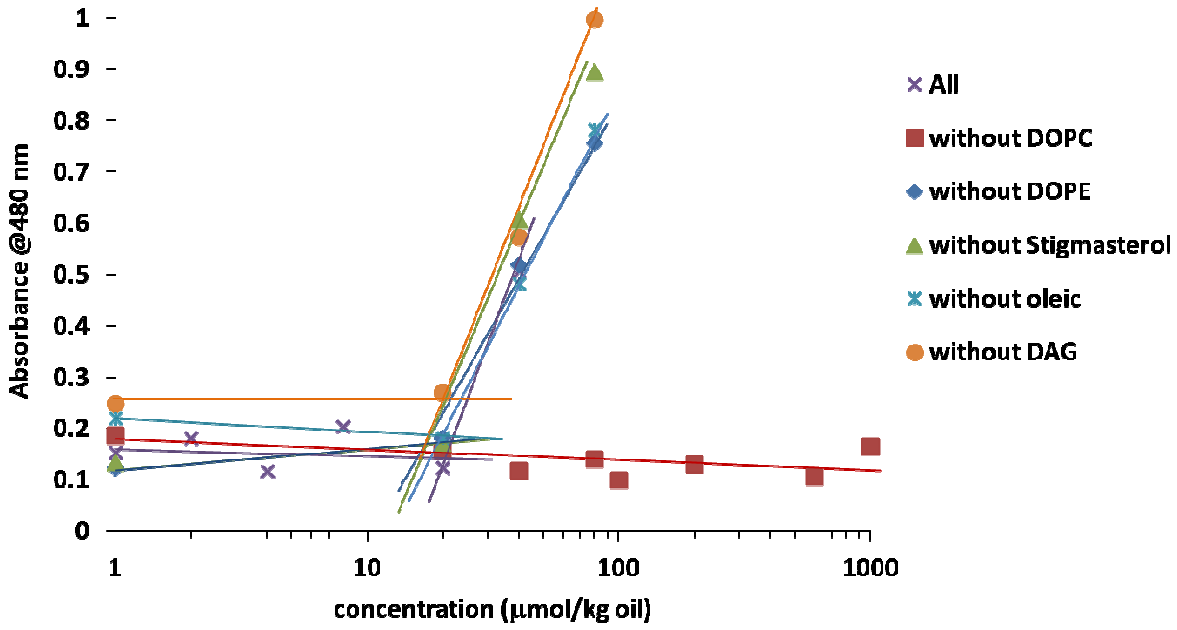

Figure 5.3 Critical micelle concentrations of mixed components of DOPC, DOPE, stigmasterol, oleic acid, and DAG (at molar ratio of 3.78:0.67:0.97:0.43:2.25, respectively) in bulk oil at 55° C

To further investigate the surface activity of multiple components at the oil-water interface, we used NBD-PE, which is a fluorescent phosphatidylethanolamine analog grafted with an NBD fluorophore on the head group. The emission fluorescence intensity of NBP-PE was determined and shown in figure 5.4. In the control oil containing only the NBD-PE, the emission

fluorescent intensity was relatively low. It has been reported that the exposure of the NBD group to the polar environment caused the fluorescence intensity to decrease (31-32). The NBD-PE, same as other phospholipids, preferentially resides at the oil-water interface and orients the hydrophilic head group toward the water core. The imino group and/or the oxygen molecule on the NBD could form H-bonds with water molecules, leading to a decrease in fluorescence intensity (33). Since the stripped oil contained 383±2 ppm water, it is possible that the probe associated with this water thus decreasing its fluorescence. The addition of 100 μmol/kg oil of mixed components (above the CMC so association colloids were present) caused the fluorescent intensity of NBP-PE to increase. This could be due to the ability of mixed surface active components to compete for the oil water-interface, thus decreasing the NBD-PE/water interaction, and thus leading to the fluorescence intensity increase. This result is in agreement with Chen and coworkers (2011) who observed that the emission fluorescence intensity of NBD-PE increased in the presence of DOPC in bulk soybean oil (9). In addition, Chattopadhyay and coworker (2002) found that the fluorescence emission intensity of the NBD-PE increased with decreasing [water]/[surfactant] molar ratio (19).

Compared to the presence of all mixed components, the absence of DOPE, stigmasterol, oleic acid, or DAG did not change the fluorescent intensity of NBP-PE. This is because all these mixtures would form association colloids (figure 5.3). However, in bulk oil containing mixed components without DOPC, the fluorescence intensity significantly decreased compared to the oil that contains all mixed components. Again, this supports the CMC results that DOPC is critical in the formation of the association colloids. However, in the absence of DOPC, the fluorescence intensity was still greater than the control even though there were no association colloids. In the absence of DOPC, the DOPE, stigmasterol, oleic acid and DAG could interact with the water without forming association colloids since they are all surface active. The interaction of these

compounds with water could prevent the NBD-PE probe from interacting with water thus increasing fluorescence intensity. The ability of the other surface active compounds to out compete NBE-PE for water could be due to their higher surface activity or the much higher concentration (0.95 μmol/kg oil of NBD-PE compared to 100 μmol/kg oil of mixed components).

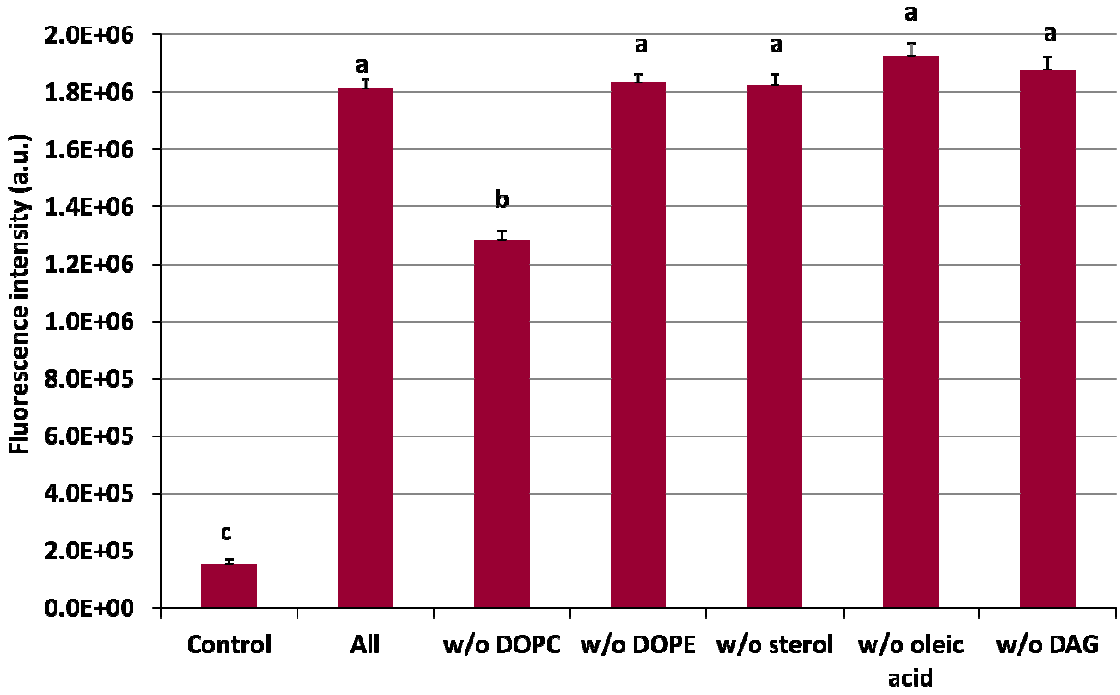

Figure 5.4 Fluorescence intensity of NBD-PE in bulk oil containing mixed components of DOPC, DOPE, stigmasterol, oleic acid, and DAG (at molar ratio of 3.78:0.67:0.97:0.43:2.25, respectively) at 100 μmol/kg oil. [a,b,c] Represent significantly different at $p \leq 0.05$.

5.4.2 The impact of the association colloids formed by multiple surface active components on oxidative stability of bulk oil

Lipid oxidation is a major factor causing undesirable flavors and aromas and reducing nutritional values as well as potential safety issues in food oils (29, 34-37). Minor components that have amphiphilic properties in bulk oil chemically and physically impact lipid oxidation

mechanisms (1-2). In this study, we investigated the effect of association colloids formed by multiple surface active components on oxidative stability of bulk corn oil. Moreover, the impact of association colloids on antioxidant activity of α-tocopherol (lipid soluble) and Trolox (water soluble analogue of tocopherol) was investigated by measuring the formation of lipid hydroperoxides and hexanal during storage at 55° C. Figure 5.5 shows that the addition of mixed components at concentration of 10 μmol/kg oil, which is below the CMC did not appreciably change the lag time of lipid hydroperoxides and hexanal formation compared to the control. However, once multiple components were added at 100 μmol/kg oil, which is above the CMC, the lag time of both lipid hydroperoxides and hexanal formation decreased from 15 to 11 days. This is in consistent with previous reports which revealed that the physical structures formed by surface active components decreased the oxidative stability of bulk oil (4, 38-39). Several mechanisms possibly involve in the prooxidative effect of physical structures in bulk oil. For example, the presence of aggregates could reduce the surface tension leading to increasing of oxygen transfer to the oil (29). Chen and coworkers (2012) proposed that the reverse micelles form by DOPC reduced the oxidative stability of oil by attracting the metal ions and lipid hydroperoxides to the oil-water interface resulting in increased lipid oxidation rates (39).

The addition of α-tocopherol at 10 and 50 μmol/kg oil extended the lag time of both lipid hydroperoxides and hexanal formation to 19 and 37 days, respectively, suggesting that α-tocopherol at these concentrations were able to overcome the prooxidant activity of association colloids in this study. We did not observe any impact of the physical structures formed by mixed components on the antioxidative effectiveness of α-tocopherol at both concentrations.

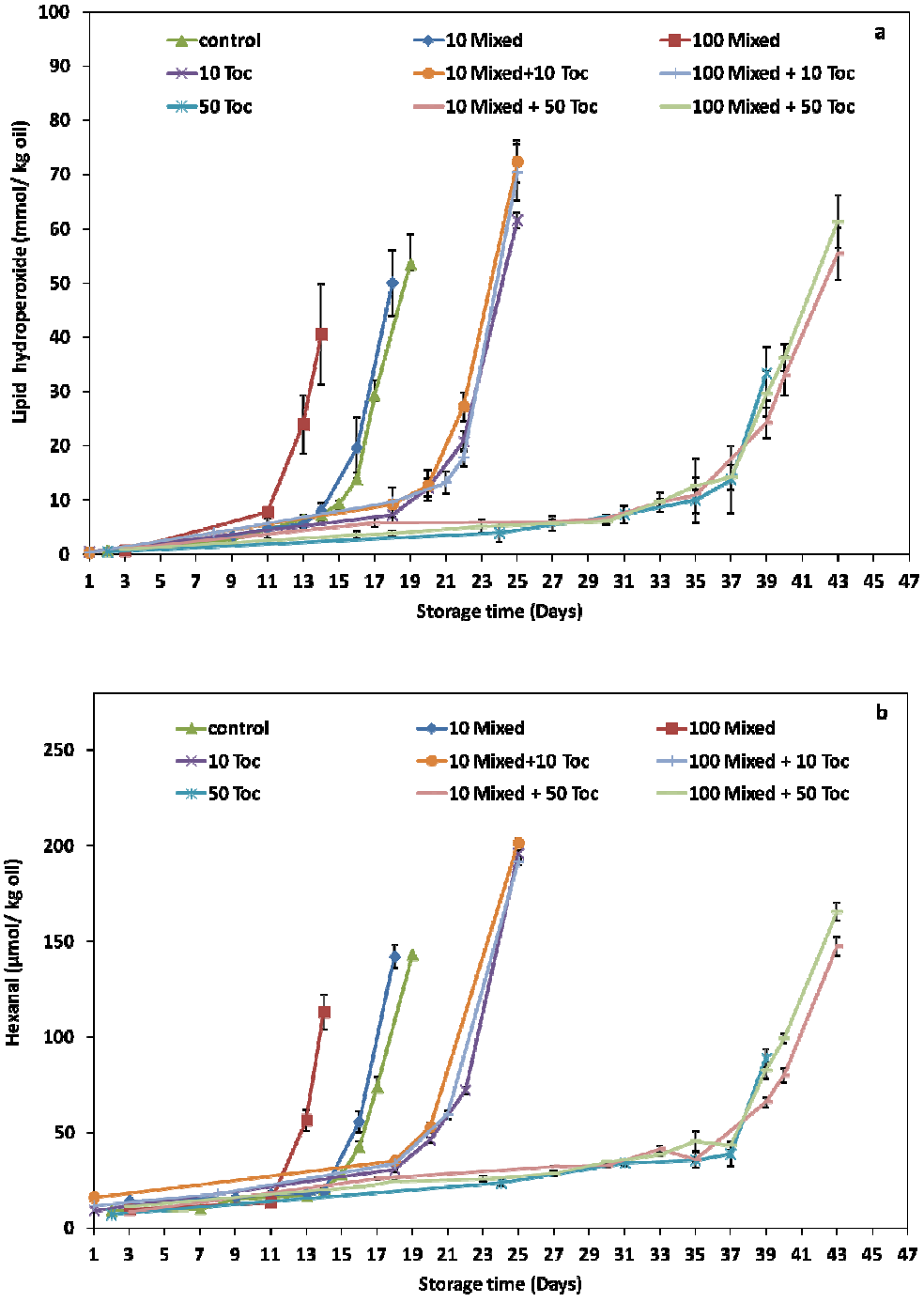

Figure 5.5 Formation of lipid hydroperoxides (a) and hexanal (b) in bulk oil containing mixed components of DOPC, DOPE, stigmasterol, oleic acid, and DAG (at molar ratio of 3.78:0.67:0.97:0.43:2.25, respectively) at 0, 10 and 100 µmol/kg oil in the presence of α-tocopherol at 0, 10 and 50 µmol/kg oil during storage at 55°C.

A similar experiment was done to investigate the impact of mixed components on the effectiveness of Trolox as shown in figure 5.6. The addition of Trolox at concentration of 10 and 50 μmol/kg oil extended the duration of lag phase of both hydroperoxide and hexanal formation from 15 to 55 and 105 days, respectively. The better antioxidant activity of Trolox compared to tocopherols is usually observed and is postulated to the higher polarity of Trolox which allows them to concentrate at the oil-water interface, thus to interact more efficiently with surface active lipid substrates (7, 40-41). Again, the oils containing the combination of Trolox (10 and 50 μmol/kg oil) and mixed components at below and above the CMC had similar lag time compared to the oil containing only Trolox at both concentrations.

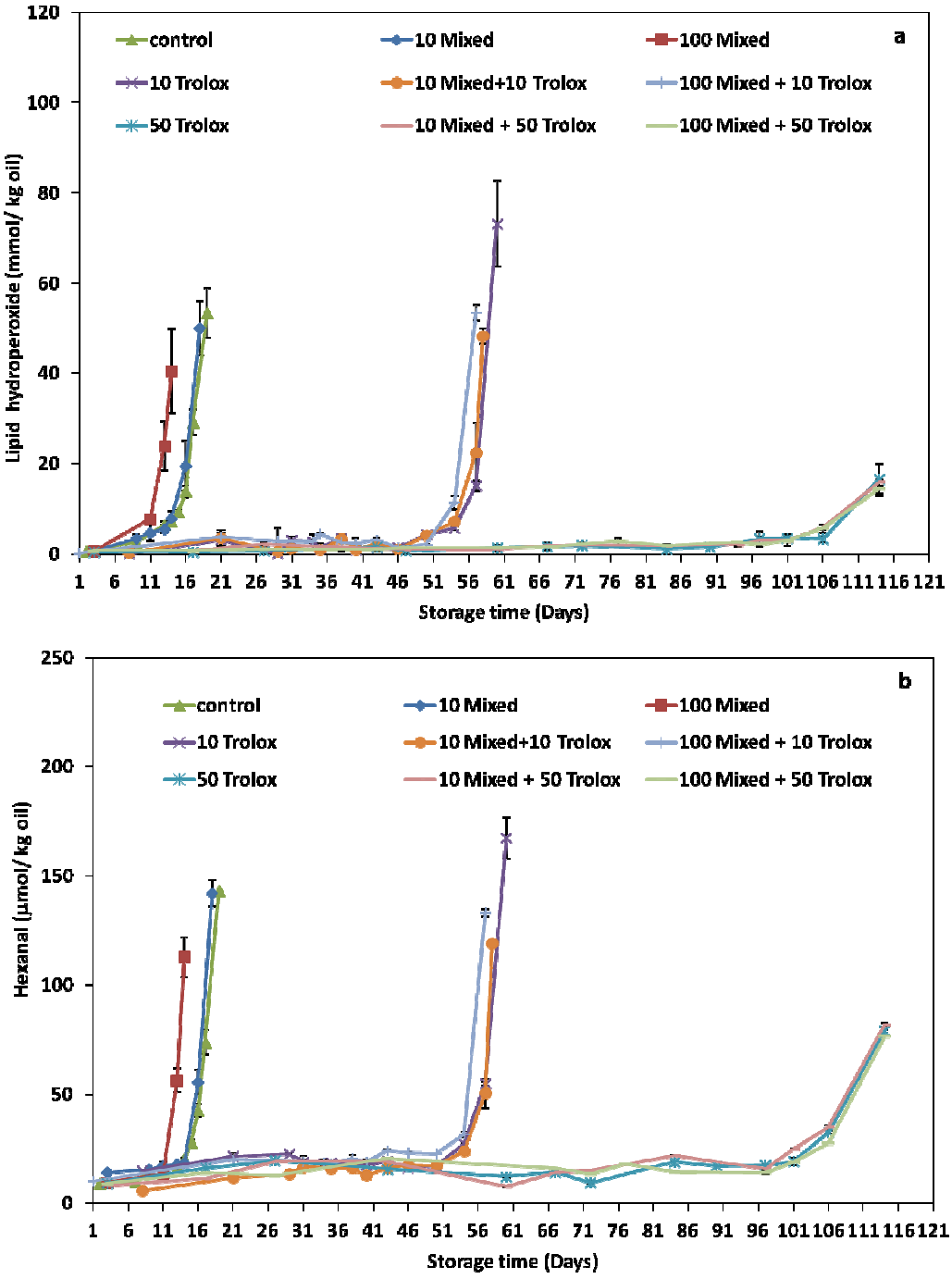

Figure 5.6 Formation of lipid hydroperoxides (a) and hexanal (b) in bulk oil containing mixed components of DOPC, DOPE, stigmasterol, oleic acid, and DAG (at molar ratio of 3.78:0.67:0.97:0.43:2.25, respectively) at 0, 10 and 100 µmol/kg oil in the presence of Trolox at 0, 10 and 50 µmol/kg oil during storage at 55°C.

To investigate if the association colloids impact the physical location of α-tocopherol and Trolox, we incorporated the NBD-PE probe to the bulk oil containing 100 μmol/kg oil of mixed components of DOPC, DOPE, stigmasterol, oleic acid, and DAG in the presence of either α-tocopherol or Trolox at various concentrations (0, 10, 50 and 100 μmol/kg oil). The emission fluorescence intensity of NBD-PE was measured and is shown in figure 5.7.

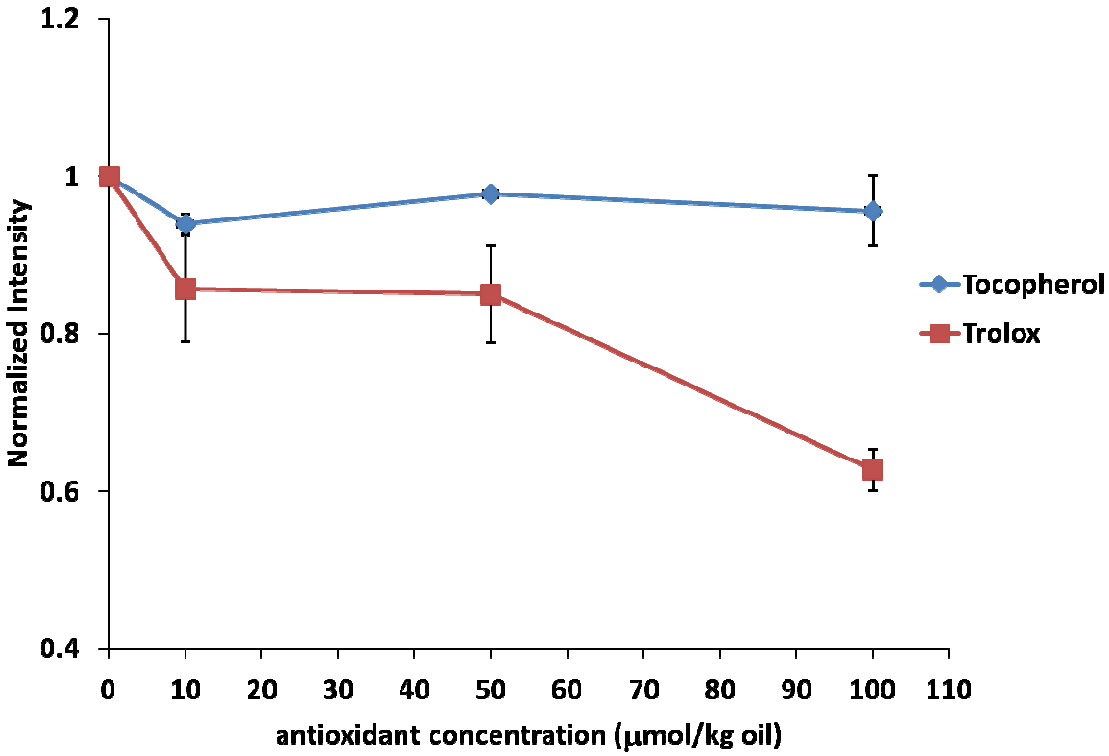

Figure 5.7 The normalized fluorescence intensity of NBD-PE in the bulk oil containing 100 μmol/kg oil of mixed components of DOPC, DOPE, stigmasterol, oleic acid, and DAG (at molar ratio of 3.78:0.67:0.97:0.43:2.25, respectively) in the presence of either α-tocopherol or Trolox at 0, 10, 50 and 100 μmol/kg oil.

Increasing the concentration of α-tocopherol did not impact the fluorescence intensity of NBD-PE, suggesting that α-tocopherol did not likely concentrate at the oil-water interface. On the other hand, Trolox caused the fluorescence intensity to decrease with increasing Trolox concentrations. Trolox was not as surface active compared to other surface active components (e.g. at 1000 μmol/kg oil, Trolox only decreased the interfacial tension of oil-water by 30%, while DOPC decreased the interfacial tension by 56 %). Therefore, instead of competing for the oil-water interface as other surface active compounds did, Trolox could partition into the same location as the NBD-PE at the oil-water interface of the association colloids, thus it could quench the NBD-PE leading to the observed decrease in fluorescence intensity. This is in agreement with Chen and coworkers (2011) who reported that the fluorescence intensity of NBD-PE decreased as increasing the concentration of Trolox in the bulk oil containing DOPC reverse micelles (9).

Surprisingly, we did not observe that the association colloids influenced the antioxidative activity of both α-tocopherol and Trolox. In contrast, several studies demonstrated that the physical structures formed by phospholipids enhanced the antioxidant activity of tocopherols and Trolox by facilitating the antioxidant molecules to accumulate at the oil-water where lipid oxidation mainly occurs (9, 42) or by decreasing the iron-promoted tocopherol and Trolox decomposition (39). Nevertheless, the impact of reverse micelles on the activity of antioxidants could be dependent on several factors including the concentration of antioxidant in the system and the physical and chemical properties of the reverse micelles. Chen and coworkers (2011) found that the effect of DOPC reverse micelles on the activity of antioxidants was varied depending on the antioxidant concentration. They revealed that DOPC reverse micelles enhanced the antioxidant effectiveness of low concentrations of α-tocopherol and Trolox (10 μM), while they decreased the antioxidant activity of 100 μM of α-tocopherol and Trolox (9).

Moreover, the composition of surface active molecules could affect the physical and chemical properties of the interface. The accessibility of antioxidant to the interface could be governed by the surfactant packing in the association colloid. For example, the if the surface active molecules closely packed in the association colloids this could limit the accessibility of antioxidant molecules to the interface which could occur with the mixed component system that did not occur with DOPC alone. In addition, the individual component of DOPC, DOPE, stigmasterol, oleic acid, and DAG have been reported to affect the oxidative stability of bulk oil differently. Phospholipids exhibit antioxidant activity which is attributed to different mechanisms including the metal chelating property of phosphate group, free radical scavenging ability of the amine group, the formation of Maillard reaction products between phospholipids and oxidation products (43-44). Free fatty acids are known as prooxidant which is attributed to their carboxylic groups that accelerate the decomposition of lipid hydroperoxides into free radicals or to their ability to bind metals and make them more prooxidative (45-47). Prooxidative, antioxidative and neutral effects of DAG have been observed in several studies (13, 48-49). Chen and coworkers (2014) reveal that the addition of 0-2.5% (by wt) of DAG did not significantly impact the oxidative stability of stripped soybean oil incubated at 55 °C and had no effect on the antioxidative activity of 40 μM α-tocopherol (13). Phytosterols such as stigmasterol exhibited antioxidant activity under high temperature. This is attributed to their ability to donate hydrogen to free radicals, and also to their ability to decrease polymerization under high temperature (50-52). Thus, the combination of these components not only physically but also chemically impact on the oxidative stability of bulk oil and all these factors together might negate enhancement of the activity of the antioxidants that were observed in other studies.

5.5 Conclusions

By determining the interfacial tension of minor components including DOPC, DOPE, stigmasterol, oleic acid, and DAG in bulk oil, we demonstrated that these surface active components were able to concentrate at the oil-water interface in bulk oil and decreased interfacial tension. Among other components, only DOPC and DOPE could form aggregates individually at the CMC of 40 and 200 μmol/kg oil, respectively. The combination of minor components formed association colloids at the CMC as low as 20 μmol/kg oil. The association colloids formed by the mixed components significantly decreased the oxidative stability of bulk stripped corn oil. However, these physical structures did not have an impact on the antioxidative effectiveness of tocopherols and Trolox at 10 and 50 μmol/kg oil. Understanding how these complex structures impact on lipid oxidation and on reactivity of antioxidants could provide a new perspective to improve oxidative stability in bulk oils.

CHAPTER 6

CONCLUSIONS

This research investigates the impact of association colloids formed by surface active minor components on oxidative stability of bulk oil. As a variety of minor components present in the bulk oil, the physicochemical properties of association colloids could be affected by these surface active components. In the first study, we isolated polar lipid oxidation products from used frying oil and found that the addition of polar lipid compounds (0.5-2.0% by wt) in bulk stripped corn oil significantly decreased the lag time of lipid hydroperoxides and hexanal formation. Interestingly, in the presence of other surface active components in the systems such as in the bulk oil containing DOPC reverse micelles and in O/W emulsion emulsified by Tween 20, the prooxidant activity of polar lipid compounds decreased. These results suggest that the prooxidative activities of polar compounds were greatly influenced by their physical location. In the presence of relatively strong surface active components such as DOPC and Tween 20, polar lipid compounds were not able to concentrate at the oil-water interface, thus less prooxidative activity was observed.

In the second research, we studied the impact of free fatty acids on the physicochemical properties of DOPC reverse micelles. We found that the combination of 3% (by wt) of oleic acid with DOPC caused the CMC to increase. Moreover, the corporation of oleic acid at 3% (by wt) significantly decreased the pH of water core of DOPC reverse micelle to below 3. This could alter the charge on the head group of DOPC and thus impacts the attractive and/or repulsive force between molecules leading to an increased CMC of the DOPC. In addition, by using SAXS technique, we were able to confirm the formation of DOPC reverse micelles in bulk oil with the

D-spacing of 4.49 nm. With this technique, we found that the addition of 0.5% of oleic acid could change the physical structure of DOPC reverse micelles as we did not observe the Bragg peak in the SAXS profile.

In our last experiment, we combined multiple surface active components including DOPC, DOPE, stigmasterol, oleic acid, and DAG and investigated their surface activities as well as the impact of association colloids formed by these mixed components on the oxidative stability of bulk oil. The surface activity of individual component was investigated by determining the oil-water interfacial tension. All of these components were able to reside at the oil-water interface and significantly decreased the interfacial tension. However, only DOPC and DOPE could form the association colloids with CMCs of 40 and 200 μmol/kg oil, respectively. The CMC of mixed components was as low as 20 μmol/kg oil. By using NBD-PE, a surface active fluorescence probe, we confirm that DOPC plays an important role on the association colloid formation while other components could act as co-surfactants. In lipid oxidation study, we found that association colloids decreased the oxidative stability of oil. However, we did not observe the ability of the association colloids to alter the antioxidant activity of α-tocopherol and Trolox. It is possible that not only the physical properties of association colloids but also the chemical properties of each components present at the interface have impact on antioxidant activity of α-tocopherol and Trolox.

BIBLIOGRAPHY

1. Chen BC, McClements DJ, Decker EA (2011) Minor components in food oils: A critical review of their roles on lipid oxidation chemistry in bulk oils and emulsions. Crit Rev Food Sci Nutr 51:901-916

2. Casimir CA, Min DB (2008) Food lipids: chemistry, nutrition, and biotechnology. CRC Press, Boca Raton

3. Holvoet P, Perez G, Zhao Z, Brouwers E, Bernar H, Collen D (1995) Malondialdehyde-modified low-density lipoproteins in patients with atherosclerotic disease. J Clin Invest 95:2611-2619

4. Wood L, Fitzgerald D, Gibson P, Cooper D, Garg M (2000) Lipid peroxidation as determined by plasma isoprostanes is related to disease severity in mild asthma. Lipids 35:967-974

5. Cohn JS (2002) Oxidized fat in the diet, postprandial lipaemia and cardiovascular disease. Curr Opin Lipidol 13:19-24

6. Bartsch H, Nair J (2004) Oxidative stress and lipid peroxidation-derived DNA-lesions in inflammation driven carcinogenesis. Cancer Detect Prev 28:385-391

7. Waraho T, McClements DJ, Decker EA (2011) Mechanisms of lipid oxidation in food dispersions. Trends Food Sci Technol 22:3-13

8. Mei L, McClements DJ, Wu J, Decker EA (1998) Iron-catalyzed lipid oxidation in emulsion as affected by surfactant, pH and NaCl. Food Chem 61:307-312

9. Boon CS, Xu Z, Yue X, McClements DJ, Weiss J, Decker EA (2008) Factors affecting lycopene oxidation in oil-in-water emulsions. J Agric Food Chem 56:1408-1414

10. Waraho T, Cardenia V, Rodriguez-Estrada MT, McClements DJ, Decker EA (2009) Prooxidant mechanisms of free fatty acids in stripped soybean oil-in-water emulsions. J Agric Food Chem 57:7112-7117

11. Klinkesorn U, Sophanodora P, Chinachoti P, McClements DJ, Decker EA (2005) Increasing the oxidative stability of liquid and dried tuna oil-in-water emulsions with electrostatic layer-by-layer deposition technology. J Agric Food Chem 53:4561-4566

12. Shaw LA, McClements DJ, Decker EA (2007) Spray-dried multilayered emulsions as a delivery method for omega-3 fatty acids into food systems. J Agric Food Chem 55:3112-3119

13. Frankel EN, Huang S-W, Kanner J, German JB (1994) Interfacial phenomena in the evaluation of antioxidants: bulk oils vs emulsions. J Agric Food Chem 42:1054-1059

14. Chen BC, Han A, Laguerre M, McClements DJ, Decker EA (2011) Role of reverse micelles on lipid oxidation in bulk oils: impact of phospholipids on antioxidant activity of alpha-tocopherol and trolox. Food Funct 2:302-309

15. Huang S-W, Hopia A, Schwarz K, Frankel EN, German JB (1996) Antioxidant activity of α-tocopherol and trolox in different lipid substrates: bulk oils vs oil-in-water emulsions. J Agric Food Chem 44:444-452

16. Nuchi CD, Hernandez P, McClements DJ, Decker EA (2002) Ability of lipid hydroperoxides to partition into surfactant micelles and alter lipid oxidation rates in emulsions. J Agric Food Chem 50:5445-5449

17. Chaiyasit W, McClements DJ, Weiss J, Decker EA (2008) Impact of surface-active compounds on physicochemical and oxidative properties of edible oil. J Agric Food Chem 56:550-556

18. Chen BC, Han A, McClements DJ, Decker EA (2010) Physical structures in soybean oil and their impact on lipid oxidation. J Agric Food Chem 58:11993-11999

19. Chen BC, Panya A, McClements DJ, Decker EA (2012) New insights into the role of iron in the promotion of lipid oxidation in bulk oils containing reverse micelles. J Agric Food Chem 60:3524-3532

20. Čmolík J, Pokorný J (2000) Physical refining of edible oils. Eur J Lipid Sci Technol 102:472-486

21. Ghazani S, Marangoni A (2013) Minor components in canola oil and effects of refining on these constituents: a review. J Am Oil Chem Soc 90:923-932

22. Maza A, Ormsbee RA, Strecker LR (1992) Effects of deodorization and steam-refining parameters on finished oil quality. J Am Oil Chem Soc 69:1003-1008

23. Pudel F, Denecke P (1997) Influences on the formation of trans fatty acids during deodorization of rapeseed oil. Ocl-Oleagineux Corps Gras Lipides 4:58-61

24. Naz S, Sherazi STH, Talpur FN (2011) Changes of total tocopherol and tocopherol species during sunflower oil processing. J Am Oil Chem Soc 88:127-132

25. Leon-Camacho M, Ruiz-Mendez MV, Graciani-Constante M, Graciani-Constante E (2001) Kinetics of the cis-trans isomerization of linoleic acid in the deodorization and/or physical refining of edible fats. Eur J Lipid Sci Technol 103:85-92

26. Chaiyasit W, Elias RJ, McClements DJ, Decker EA (2007) Role of physical structures in bulk oils on lipid oxidation. Crit Rev Food Sci Nutr 47:299-317

27. Endo Y, Ohta A, Kido H, Kuriyama M, Sakaguchi Y, Takebayashi S, Hirai H, Murakami C, Wada S (2011) Determination of triacylglycerol composition in vegetable oils using high-performance liquid chromatography: a collaborative study. J Oleo Sci 60:451-456

28. Andrikopoulos NK (2002) Triglyceride species compositions of common edible vegetable oils and methods used for their identification and quantification. Food Rev Int 18:71-102

29. Mozaffarian D, Wu JHY (2011) Omega-3 fatty acids and cardiovascular disease: effects on risk factors, molecular pathways, and clinical events. J Am Coll Cardiol 58:2047-2067

30. Christopoulou E, Lazaraki M, Komaitis M, Kaselimis K (2004) Effectiveness of determinations of fatty acids and triglycerides for the detection of adulteration of olive oils with vegetable oils. Food Chem 84:463-474

31. Ferrari RA, Schulte E, Esteves W, Brühl L, Mukherjee KD (1996) Minor constituents of vegetable oils during industrial processing. J Am Oil Chem Soc 73:587-592

32. Nawar WW (1996) Lipids. In Fennema OR (ed) Food Chemistry. Marcel Dekker, New York.

33. Porter N, Caldwell S, Mills K (1995) Mechanisms of free radical oxidation of unsaturated lipids. Lipids 30:277-290

34. Gunstone FD (1996) Fatty acid and lipid chemistry. Chapman & Hall, Glasgow

35. Dana D, Saguy IS (2006) Review: Mechanism of oil uptake during deep-fat frying and the surfactant effect-theory and myth. Adv Colloid Interface Sci 128–130:267-272

36. Miyashita K, Takagi T (1986) Study on the oxidative rate and prooxidant activity of free fatty acids. J Am Oil Chem Soc 63:1380-1384

37. Frega N, Mozzon M, Lercker G (1999) Effects of free fatty acids on oxidative stability of vegetable oil. J Am Oil Chem Soc 76:325-329

38. Yoshida H, Kondo I, Kajimoto G (1992) Participation of free fatty-acids in the oxidation of purified soybean oil during microwave-heating. J Am Oil Chem Soc 69:1136-1140

39. Mistry BS, Min DB (1987) Effects of fatty acids on the oxidative stability of soybean oil. J Food Sci 52:831-832

40. Aubourg SP (2001) Fluorescence study of the pro-oxidant effect of free fatty acids on marine lipids. J Sci Food Agric 81:385-390

41. Yoshida H (1993) Influence of fatty acids of different unsaturation in the oxidation of purified vegetable oils during microwave irradiation. J Sci Food Agric 62:41-47

42. Paradiso VM, Gomes T, Nasti R, Caponio F, Summo C (2010) Effects of free fatty acids on the oxidative processes in purified olive oil. Food Res Int 43:1389-1394

43. Zhang A, Chen ZY (1997) Oxidative stability of conjugated linoleic acids relative to other polyunsaturated fatty acids. J Am Oil Chem Soc 74:1611-1613

44. Yettella RR, Castrodale C, Proctor A (2012) Oxidative stability of conjugated linoleic acid rich soy oil. J Am Oil Chem Soc 89:685-693

45. Yurawecz M, Delmonte P, Vogel T, Kramer JK (2003) Oxidation of conjugated linoleic acid: initiators and simultaneous reactions: theory and practice. Advances in conjugated linoleic acid research 2:56-70

46. Suzuki R, Nakano K, Kobayashi M, Miyashita K (2001) Oxidative stability of conjugated polyunsaturated fatty acids and their esters in bulk phase. J Oleo Sci 50:491-495

47. Luna P, Fuente MA, Salvador D, Márquez-Ruiz G (2007) Differences in oxidation kinetics between conjugated and non-conjugated methyl linoleate. Lipids 42:1085-1092

48. Myers J, Kass J, Burr G (1941) Comparative rates of oxidation of isomeric linolenic acids and their esters. J Am Oil Chem Soc 18:107-109

49. Yang L, Leung LK, Huang Y, Chen Z-Y (2000) Oxidative stability of conjugated linoleic acid isomers. J Agric Food Chem 48:3072-3076

50. Mistry BS, Min DB (1988) Prooxidant effects of monoglycerides and diglycerides in soybean oil. J Food Sci 53:1896-1897

51. Colakoglu AS (2007) Oxidation kinetics of soybean oil in the presence of monoolein, stearic acid and iron. Food Chem 101:724-728

52. Aubourg SP (2001) Effect of partially hydrolysed lipids on inhibition of oxidation of marine lipids. Eur Food Res Technol 212:540-545

53. Chen B, McClements DJ, Decker EA (2014) Impact of diacylglycerol and monoacylglycerol on the physical and chemical properties of stripped soybean oil. Food Chem 142:365-372

54. Nakatsugawa K, Ohashi K, Shimada A (2001) Comparison of oxidative stability of diacylglycerol and triacylglycerol. Nippon Shokuhin Kagaku Kaishi 48:429-436

55. Shimizu M, Moriwaki J, Nishide T, Nakajima Y (2004) Thermal deterioration of diacylglycerol and triacylglycerol oils during deep-frying. J Am Oil Chem Soc 81:571-576

56. Gomes T, Caponio F, Bruno G, Summo C, Paradiso VM (2010) Effects of monoacylglycerols on the oxidative stability of olive oil. J Sci Food Agric 90:2228-2232

57. Caponio F, Bruno G, Paradiso VM, Summo C, Gomes T (2013) Monoacylglycerols and their influence on the evolution of the oxidation in purified vegetable oils. Prog Nutr 15:53-57

58. Harrabi S, Herchi W, Kallel H, Mayer PM, Boukhchina S (2009) Liquid chromatographic–mass spectrometric analysis of glycerophospholipids in corn oil. Food Chem 114:712-716

59. Le Grandois J, Marchioni E, Zhao M, Giuffrida F, Ennahar S, Bindler F (2010) Oxidative stability at high temperatures of oleyol and linoleoyl residues in the forms of phosphatidylcholines and triacylglycerols. J Agric Food Chem 58:2973-2979

60. Song J-H,Inoue Y, Miyazawa T (1997) Oxidative stability of docosahexaenoic acid-containing oils in the form of phospholipids, triacylglycerols, and ethyl esters. Biosci Biotechnol Biochem 61:2085-2088

61. King MF, Boyd LC, Sheldon BW (1992) Effects of phospholipids on lipid oxidation of a salmon oil model system. J Am Oil Chem Soc 69:237-242

62. Hara S, Okada N, Hibino H, Totani Y (1992) Antioxidative behavior of phospholipids for polyunsaturated fatty acids of fish oil. J Jpn Oil Chem Soc 41:130-135

63. Kashima M, Cha G-S, Isoda Y, Hirano J, Miyazawa T (1991) The antioxidant effects of phospholipids on perilla oil. J Am Oil Chem Soc 68:119-122

64. King MF, Boyd LC, Sheldon BW (1992) Antioxidant properties of individual phospholipids in a salmon oil model system. J Am Oil Chem Soc 69:545-551

65. Ohshima T, Fujita Y, Koizumi C (1993) Oxidative stability of sardine and mackerel lipids with reference to synergism between phospholipids and α-tocopherol. J Am Oil Chem Soc 70:269-276

66. Bandarra NM, Campos RM, Batista I, Nunes ML, Empis JM (1999) Antioxidant synergy of alpha-tocopherol and phospholipids. J Am Oil Chem Soc 76:905-913

67. Judde A, Villeneuve P, Rossignol-Castera A, Guillou A (2003) Antioxidant effect of soy lecithins on vegetable oil stability and their synergism with tocopherols. J Am Oil Chem Soc 80:1209-1215

68. Shimajiri J, Shiota M, Hosokawa M, Miyashita K (2013) Synergistic antioxidant activity of milk sphingomyeline and its sphingoid base with α-tocopherol on fish oil triacylglycerol. J Agric Food Chem 61:7969-7975

69. Saito H, Ishihara K (1997) Antioxidant activity and active sites of phospholipids as antioxidants. J Am Oil Chem Soc 74:1531-1536

70. Nwosu C, Boyd L, Sheldon B (1997) Effect of fatty acid composition of phospholipids on their antioxidant properties and activity index. J Am Oil Chem Soc 74:293-297

71. Husain SR, Terao J, Matsushita S (1986) Effect of browning reaction products of phospholipids on autoxidation of methyl linoleate. J Am Oil Chem Soc 63:1457-1460

72. Takenaka A, Hosokawa M, Miyashita K (2007) Unsaturated phosphatidylethanolamine as effective synergist in combination with alpha -tocopherol. J Oleo Sci 56:511-516

73. Hovenkamp E, Demonty I, Plat J, Lütjohann D, Mensink RP, Trautwein EA (2008) Biological effects of oxidized phytosterols: A review of the current knowledge. Prog Lipid Res 47:37-49

74. Kritchevsky D, Chen SC (2005) Phytosterols—health benefits and potential concerns: a review. Nutr Res 25:413-428

75. Maguire L, Konoplyannikov M, Ford A, Maguire AR, O'Brien NM (2003) Comparison of the cytotoxic effects of beta-sitosterol oxides and a cholesterol oxide, 7 beta-hydroxycholesterol, in cultured mammalian cells. Br J Nutr 90:767-775

76. Adcox C, Boyd L, Oehrl L, Allen J, Fenner G (2001) Comparative effects of phytosterol oxides and cholesterol oxides in cultured macrophage-derived cell lines. J Agric Food Chem 49:2090-2095

77. Soupas L, Juntunen L, Lampi A-M, Piironen V (2004) Effects of sterol structure, temperature, and lipid medium on phytosterol oxidation. J Agric Food Chem 52:6485-6491

78. Soupas L, Huikko L, Lampi A-M, Piironen V (2005) Esterification affects phytosterol oxidation. Eur J Lipid Sci Technol 107:107-118

79. Nyström L, Achrenius T, Lampi A-M, Moreau RA, Piironen V (2007) A comparison of the antioxidant properties of steryl ferulates with tocopherol at high temperatures. Food Chem 101:947-954

80. Wang T, Hicks KB, Moreau R (2002) Antioxidant activity of phytosterols, oryzanol, and other phytosterol conjugates. J Am Oil Chem Soc 79:1201-1206

81. Winkler JK, Warner K (2008) The effect of phytosterol concentration on oxidative stability and thermal polymerization of heated oils. Eur J Lipid Sci Technol 110:455-464

82. Gertz C, Kochhar SP (2001) A new method to determine oxidative stability of vegetable fats and oils at simulated frying temperature. Ocl Ol Corps Gras Li 8:82-88

83. Naumov VV, Vasil'ev RF (2003) Antioxidant and prooxidant effects of tocopherol. Kinet Catal 44:101-105

84. Huang S-W, Frankel EN, German JB (1994) Antioxidant activity of .alpha.- and .gamma.-tocopherols in bulk oils and in oil-in-water emulsions. J Agric Food Chem 42:2108-2114

85. Evans JC, Kodali DR, Addis PB (2002) Optimal tocopherol concentrations to inhibit soybean oil oxidation. J Am Oil Chem Soc 79:47-51

86. Yoshida Y, Niki E, Noguchi N (2003) Comparative study on the action of tocopherols and tocotrienols as antioxidant: chemical and physical effects. Chem Phys Lipids 123:63-75

87. Kim HJ, Lee HO, Min DB (2007) Effects and prooxidant mechanisms of oxidized alpha-tocopherol on the oxidative stability of soybean oil. J Food Sci 72:C223-C230

88. Chapman TM, Kim HJ, Min DB (2009) Prooxidant activity of oxidized alpha-tocopherol in vegetable oils. J Food Sci 74:C536-C542

89. Schelbert S, Aubry S, Burla B, Agne B, Kessler F, Krupinska K, Hortensteiner S (2009) Pheophytin pheophorbide hydrolase (pheophytinase) is involved in chlorophyll breakdown during leaf senescence in arabidopsis. Plant Cell 21:767-785

90. Gutierrezrosales F, Garridofernandez J, Gallardoguerrero L, Gandulrojas B, Minguezmosquera MI (1992) Action of chlorophylls on the stability of virgin olive oil. J Am Oil Chem Soc 69:866-871

91. Lanfer-Marquez UM, Barros RMC, Sinnecker P (2005) Antioxidant activity of chlorophylls and their derivatives. Food Res Int 38:885-891

92. Endo Y, Usuki R, Kaneda T (1985) Antioxidant effects of chlorophyll and pheophytin on the autoxidation of oils in the dark. I. Comparison of the inhibitory effects. J Am Oil Chem Soc 62:1375-1378

93. Min DB, Boff JM (2002) Chemistry and reaction of singlet oxygen in foods. Compr Rev Food Sci F 1:58-72

94. Endo Y, Usuki R, Kaneda T (1985) Antioxidant effects of chlorophyll and pheophytin on the autoxidation of oils in the dark. II. The mechanism of antioxidative action of chlorophyll. J Am Oil Chem Soc 62:1387-1390

95. Ghazani SM, Garcia-Llatas G, Marangoni AG (2013) Minor constituents in canola oil processed by traditional and minimal refining methods. J Am Oil Chem Soc 90:743-756

96. Endo Y, Usuki R, Kaneda T (1984) Prooxident activities of chlorophylls and their decomposition products on the photooxidation of methyl linoleate. J Am Oil Chem Soc 61:781-784

97. Rahmani M, Csallany AS (1998) Role of minor constituents in the photooxidation of virgin olive oil. J Am Oil Chem Soc 75:837-843

98. Lee EC, Min DB (1988) Quenching mechanism of β-carotene on the chlorophyll sensitized photooxidation of soybean oil. J Food Sci 53:1894-1895

99. Psomiadou E, Tsimidou M (2002) Stability of virgin olive oil. 2. Photo-oxidation studies. J Agric Food Chem 50:722-727

100. Ha D-O, Park C, Kim M-J, Lee J (2012) Antioxidant and prooxidant activities of β-carotene in accelerated autoxidation and photosensitized model systems. Food Sci Biotechnol 21:607-611

101. Beutner S, Bloedorn B, Frixel S, Hernández Blanco I, Hoffmann T, Martin H-D, Mayer B, Noack P, Ruck C, Schmidt M, Schülke I, Sell S, Ernst H, Haremza S, Seybold G, Sies H, Stahl W, Walsh R (2001) Quantitative assessment of antioxidant properties of natural colorants and phytochemicals: carotenoids, flavonoids, phenols and indigoids. The role of β-carotene in antioxidant functions. J Sci Food Agric 81:559-568

102. Lee JH, Ozcelik B, Min DB (2003) Electron donation mechanisms of β-carotene as a free radical scavenger. J Food Sci 68:861-865

103. Fakourelis N, Lee EC, Min DB (1987) Effects of chlorophyll and β-carotene on the oxidation stability of olive oil. J Food Sci 52:234-235

104. Haila K, Heinonen M (1994) Action of β-carotene on purified rapeseed oil during light storage. LWT - Food Sci Technol 27:573-577

105. Burton GW, Ingold KU (1984) Beta-carotene- an unusual type of lipid antioxidant. Science 224:569-573

106. Flider FJ, Orthoefer FT (1981) Metals in soybean oil. J Am Oil Chem Soc 58:270-272

107. Allen L, Siitonen P, Thompson H, Jr. (1998) Determination of copper, lead, and nickel in edible oils by plasma and furnace atomic spectroscopies. J Am Oil Chem Soc 75:477-481

108. Choe E, Min DB (2006) Mechanisms and factors for edible oil oxidation. Compr Rev Food Sci F 5:169-186

109. Anwar F, Kazi TG, Saleem R, Bhanger MI (2004) Rapid determination of some trace metals in several oils and fats. Grasas Y Aceites 55:160-168

110. Decker Eric A, Hultin Herbert O (1992) Lipid oxidation in muscle foods via redox iron. Lipid oxidation in food. Am Chem Soc 500:33-54

111. O'Brien RD (2004) Fats and oils: formulating and processing for applications. Taylor & Francis Group

112. Meng XH, Sedman J, van de Voort FR (2012) Improving the determination of moisture in edible oils by FTIR spectroscopy using acetonitrile extraction. Food Chem 135:722-729

113. Park J, Kim J, Kim M-J, Lee J (2013) Evaluation of oxygen-limitation on lipid oxidation and moisture content in corn oil at elevated temperature. J Am Oil Chem Soc: 1-6

114. Lercker G, Frega N, Bocci F, Servidio G (1994) “Veiled” extra-virgin olive oils: Dispersion response related to oil quality. J Am Oil Chem Soc 71:657-658

115. Ambrosone L, Angelico R, Cinelli G, Di Lorenzo V, Ceglie A (2002) The role of water in the oxidation process of extra virgin olive oils. J Am Oil Chem Soc 79:577-582

116. Ambrosone L, Cinelli G, Mosca M, Ceglie A (2006) Susceptibility of water-emulsified extra virgin olive oils to oxidation. J Am Oil Chem Soc 83:165-170

117. deGreyt WF, Kellens MJ, Huyghebaert AD (1997) Polymeric and oxidized triglyceride content of crude and refined vegetable oils - An overview. Fett-Lipid 99:287-290

118. Gomes T, Delcuratolo D, Paradiso VM (2008) Pro-oxidant action of polar triglyceride oligopolymers in edible vegetable oils. Eur Food Res Technol 226:1409-1414

119. Gomes T, Delcuratolo D, Paradiso VM, Summo C, Caponio F (2011) Pro-oxidant activity of oxidized triacylglycerols in olive oil and comparison with pro-oxidant action of polar triacylglycerol oligopolymers. Lwt-Food Sci Technol 44:1236-1239

120. Yoon SH, Jung MY, Min DB (1988) Effects of thermally oxidized triglycerides on the oxidative stability of soybean oil. J Am Oil Chem Soc 65:1652-1656

121. McClements DJ (2004) Food emulsions: Principles, practices and techniques, 2nd edn. CRC press, Boca Raton, Florida

122. Gulik-Krzywicki T, Larsson K (1984) An electron microscopy study of the L2-phase (microemulsion) in a ternary system: Triglyceride/monoglyceride/water. Chem Phys Lipids 35:127-132

123. Gupta R, Muralidhara HS, Davis HT (2001) Structure and phase behavior of phospholipid-based micelles in nonaqueous media. Langmuir 17:5176-5183

124. Shrestha LK, Sato T, Aramaki K (2007) Shape, size, and structural control of reverse micelles in diglycerol monomyristate nonionic surfactant system . J Phys Chem B 111:1664-1671

125. Shrestha LK, Sato T, Aramaki K (2007) Phase behavior and self-organized structures of diglycerol monolaurate in different nonpolar organic solvents. Langmuir 23:6606-6613

126. Shrestha LK, Shrestha RG, Abe M, Ariga K (2011) Reverse micelle microstructural transformations induced by oil and water. Soft Matter 7:10017-10024

127. Shrestha LK, Shrestha RG, Aramaki K (2009) Self-assembled structures of diglycerol monolaurate- and monomyristate in olive oil. J Disper Sci Technol 30:1525-1532

128. Shrestha LK, Dulle M, Glatter O, Aramaki K (2010) Structure of polyglycerol oleic acid ester nonionic surfactant reverse micelles in decane: growth control by headgroup size. Langmuir 26:7015-7024

129. Chaiyasit W, Stanley C, Strey H, McClements DJ, Decker E (2007) Impact of surface active compounds on iron catalyzed oxidation of methyl linolenate in AOT–water–hexadecane systems. Food Biophys 2:57-66

130. Seddon JM (1990) Structure of the inverted hexagonal (HII) phase, and non-lamellar phase-transitions of lipids. BBA 1031:1-69

131. Shrestha LK, Sato T, Acharya DP, Iwanaga T, Aramaki K, Kunieda H (2006) Phase behavior of monoglycerol fatty acid esters in nonpolar oils: reverse rodlike micelles at elevated temperatures. J Phys Chem B 110:12266-12273

132. Shchipunov YA (2001) Lecithin organogel: A micellar system with unique properties. Colloids Surfaces Physicochem Eng Aspects 183–185:541-554

133. Hashizaki K, Taguchi H, Saito Y (2009) A novel reverse worm-like micelle from a lecithin/sucrose fatty acid ester/oil system. Colloid Polymer Sci 287:1099-1105

134. Cardenia V, Waraho T, Rodriguez-Estrada MT, McClements DJ, Decker EA (2011) Antioxidant and prooxidant activity behavior of phospholipids in stripped soybean oil-in-water emulsions. J Am Oil Chem Soc 88:1409-1416

135. Waraho T, Cardenia V, Nishino Y, Seneviratne KN, Rodriguez-Estrada MT, McClements DJ, Decker EA (2012) Antioxidant effects of mono- and diacylglycerols in non-stripped and stripped soybean oil-in-water emulsions. Food Res Int 48:353-358

136. Cercaci L, Rodriguez-Estrada MT, Lercker G, Decker EA (2007) Phytosterol oxidation in oil-in-water emulsions and bulk oil. Food Chem 102:161-167

137. Koga T, Terao J (1995) Phospholipids increase radical-scavenging activity of vitamin E in a bulk oil model system. J Agric Food Chem 43:1450-1454

138. Koga T, Terao J (1994) Antioxidant activity of a novel phosphatidyl derivative of vitamin E in lard and its model system. J Agric Food Chem 42:1291-1294

139. Kasaikina OT, Golyavin AA, Krugovov DA, Kartasheva ZS, Pisarenko LM (2010) Micellar catalysis in the oxidation of lipids. Moscow Univ Chem Bull 65:206-209

140. Kasaikina OT, Kortenska VD, Kartasheva ZS, Kuznetsova GM, Maximova TV, Sirota TV, Yanishlieva NV (1999) Hydrocarbon and lipid oxidation in micro heterogeneous systems formed by surfactants or nanodispersed Al2O3, SiO2 and TiO2. Colloids Surfaces Physicochem Eng Aspects 149:29-38

141. Kasaikina OT, Kartasheva ZS, Pisarenko LM (2008) Effect of surfactants on liquid-phase oxidation of hydrocarbons and lipids. Russ J Gen Chem 78:1533-1544

142. Velasco J, Marmesat S, Marquez-Ruiz G, Dobarganes MC (2004) Formation of short-chain glycerol-bound oxidation products and oxidised monomeric triacylglycerols during deep-frying and occurrence in used frying. Eur J Lipid Sci Technol 106:728-735

143. Perezcamino MC, Garcia JM, Castellano JM (1992) Polar compound concentrations in virgin oils from stored cultivar Picual olive fruits. J Agric Food Chem 40:2260-2262

144. Houhoula DP, Oreopoulou V, Tzia C (2003) The effect of process time and temperature on the accumulation of polar compounds in cottonseed oil during deep-fat frying. J Sci Food Agric 83:314-319

145. Warner K, Knowlton S (1997) Frying quality and oxidative stability of high-oleic corn oils, J Am Oil Chem Soc 74:1317-1322

146. Dobarganes C, Marquez-Ruiz G, Velasco J (2000) Interactions between fat and food during deep-frying. Eur J Lipid Sci Technol 102:521-528

147. Ringseis R, Piwek N, Eder K (2007) Oxidized fat induces oxidative stress but has no effect on NF-κB-mediated proinflammatory gene transcription in porcine intestinal epithelial cells. Inflammation Res 56:118-125

148. Kanazawa A, Sawa T, Akaike T, Maeda H (2002) Dietary lipid peroxidation products and DNA damage in colon carcinogenesis. Eur J Lipid Sci Technol 104:439-447

149. Firestone D, Stier RF, Blumenthal MM (1991) Regulation of frying fats and oils. Food Technol 45:90-94

150. Gonzalez-Munoz MJ, Bastida S, Sanchez-Muniz FJ (2003) Short-term in vivo digestibility assessment of a highly oxidized and polymerized sunflower oil. J Sci Food Agric 83:413-418

151. Waraho T, McClements DJ, Decker EA (2011) Impact of free fatty acid concentration and structure on lipid oxidation in oil-in-water emulsions. Food Chem. 129:854-859

152. McClements DJ, Decker EA (2000) Lipid oxidation in oil-in-water emulsions: Impact of molecular environment on chemical reactions in heterogeneous food systems. J Food Sci 65:1270-1282

153. Arroyo R, Cuesta C, Garridopolonio C, Lopezvarela S, Sanchezmuniz FJ (1992) High performance size exclusion chromatographic studies on polar components formed in sunflower oil used for frying. J Am Oil Chem Soc 69:557-563

154. Dix TA, Marnett LJ (1985) Conversion of linoleic acid hydroperoxide to hydroxy, keto, epoxyhydroxy, and trihydroxy fatty acids by hematin. J Biol Chem 260:5351-5357

155. Felgner A, Schlink R, Kirschenbühler P, Faas B, Isengard H-D (2008) Automated Karl Fischer titration for liquid samples – Water determination in edible oils. Food Chem. 106:1379-1384

156. Rukunudin I, White P, Bern C, Bailey T (1998) A modified method for determining free fatty acids from small soybean oil sample sizes. J Am Oil Chem Soc 75:563-568

157. Shantha NC, Decker EA (1994) Rapid, sensitive, iron-based spectrophotometric methods for determination of peroxide values of food lipids. J AOAC 77:421-424

158. Song B, Springer J (1996) Determination of interfacial tension from the profile of a pendant drop using computer-aided image processing: 1. theoretical. J Colloid Interface Sci 184:64-76

159. Bruno G, Caponio F, Summo C, Paradiso VM, Gomes T (2011) The role of triacylglycerol hydrolysis products on evolution of oxidation in vegetable oil. Prog Nutr 13:99-103

160. Miyashita K, Takagi T (1986) Study on the oxidative rate and prooxidant activity of free fatty acids. J Am Oil Chem Soc 63:1380-1384

161. Aubourg SP (2001) Fluorescence study of the pro-oxidant effect of free fatty acids on marine lipids. J Sci Food Agric 81:385-390

162. Decker EA, McClements DJ (2008) Lipids, In : Fennema (ed) Food Chemistry. 4th edn, CRC Press, pp 155-216

163. Martinek K, Klyachko NL, Kabanov AV, Khmelnitsky YL, Levashov AV (1989) Micellar enzymology: its relation to membranology. BBA- Biomembranes 981:161-172

164. Vasquez VR, Williams BC, Graeve OA (2011) Stability and comparative analysis of AOT/water/isooctane reverse micelle system using dynamic light scattering and molecular dynamics. J Phys Chem B 115:2979-2987

165. Manjula S, Kobayashi I, Subramanian R (2011) Characterization of phospholipid reverse micelles in nonaqueous systems in relation to their rejection during membrane processing. Food Res Int 44:925-930

166. Subramanian R, Ichikawa S, Nakajima M, Kimura T, Maekawa T (2001) Characterization of phospholipid reverse micelles in relation to membrane processing of vegetable oils. Eur J Lipid Sci Technol 103:93-97

167. Kittipongpittaya K, Chen B, Panya A, McClements D, Decker E (2012) Prooxidant activity of polar lipid oxidation products in bulk oil and oil-in-water emulsion. J Am Oil Chem Soc 89:2187-2194

168. Laguerre M, Chen BC, Lecomte J, Villeneuve P, McClements DJ, Decker EA (2011) Antioxidant properties of chlorogenic acid and its alkyl esters in stripped corn oil in combination with phospholipids and/or water. J Agric Food Chem 59:10361-10366

169. Frankel EN (1998) Lipid Oxidation. The Oily Press, Dundee, UK

170. McClements DJ (2005) Food emulsions: principles, practices, and techniques. CRC Press, Boca Raton

171. Kanamoto R, Wada Y, Miyajima G, Kito M (1981) Phospholipid-phospholipid interaction in soybean oil. J Am Oil Chem Soc 58:1050-1053

172. Baruah B, Crans DC, Levinger NE (2007) Simple oxovanadates as multiparameter probes of reverse micelles. Langmuir 23:6510-6518

173. Baruah B, Roden JM, Sedgwick M, Correa NM, Crans DC, Levinger NE (2006) When is water not water? Exploring water confined in large reverse micelles using a highly charged inorganic molecular probe. J Am Chem Soc 128:12758-12765

174. Crans DC, Baruah B, Levinger NE (2006) Oxovanadates: a novel probe for studying lipid–water interfaces. Biomed Pharmacother 60:174-181

175. Lucero Caro A, Rodríguez Niño MR, Rodríguez Patino JM (2008) The effect of pH on surface dilatational and shear properties of phospholipid monolayers. Colloids Surfaces Physicochem Eng Aspects 327:79-89

176. Baruah B, Swafford LA, Crans DC, Levinger NE (2008) Do probe molecules influence water in confinement? J Phys Chem B 112:10158-10164

177. Moncelli MR, Becucci L, Guidelli R (1994) The intrinsic pKa values for phosphatidylcholine, phosphatidylethanolamine, and phosphatidylserine in monolayers deposited on mercury electrodes. Biophys J 66:1969-1980

178. Petelska AD, Figaszewski ZA (2000) Effect of pH on the interfacial tension of lipid bilayer membrane. Biophys J 78:812-817

179. Yoshimoto M, Miyazaki Y, Umemoto A, Walde P, Kuboi R, Nakao K (2007) Phosphatidylcholine vesicle-mediated decomposition of hydrogen peroxide. Langmuir 23:9416-9422

180. Frankel EN, Huang S-W, Aeschbach R, Prior E (1996) Antioxidant activity of a rosemary extract and its constituents, carnosic acid, carnosol, and rosmarinic acid, in bulk oil and oil-in-water emulsion. J Agric Food Chem 44:131-135

181. Frankel EN, Huang S-W, Prior E, Aeschbach R (1996) Evaluation of antioxidant activity of rosemary extracts, carnosol and carnosic acid in bulk vegetable oils and fish oil and their emulsions. J Sci Food Agric 72:201-208

182. Porter WL, Black ED, Drolet AM (1989) Use of polyamide oxidative fluorescence test on lipid emulsions: contrast in relative effectiveness of antioxidants in bulk versus dispersed systems. J Agric Food Chem 37:615-624

183. Chattopadhyay A, Mukherjee S, Raghuraman H (2002) Reverse micellar organization and dynamics: A wavelength-selective fluorescence approach. J Phys Chem B 106:13002-13009

184. Rosen MJ, Kunjappu JT (2012) Surfactants and interfacial phenomena. John Wiley & Sons, Hoboken, New Jersy

185. Kalogianni EP, Karapantsios TD, Miller R (2011) Effect of repeated frying on the viscosity, density and dynamic interfacial tension of palm and olive oil. J Food Eng 105:169-179

186. Gil B, Handel AP (1995) The effect of surfactants on the interfacial tension of frying fat. J Am Oil Chem Soc 72:951-955

187. Reis P, Miller R, Leser M, Watzke H, Fainerman VB, Holmberg K (2008) Adsorption of polar lipids at the water-oil interface. Langmuir 24:5781-5786

188. Haldar S, Chattopadhyay A (2013) Application of NBD-labeled lipids in membrane and cell biology. in Mély Y, Duportail G (eds) Fluorescent methods to study biological membranes. Springer Berlin Heidelberg, pp. 37-50.

189. Fery-Forgues S, Fayet J-P, Lopez A (1993) Drastic changes in the fluorescence properties of NBD probes with the polarity of the medium: involvement of a TICT state? J Photochem Photobiol A: Chem 70:229-243

190. Mukherjee S, Raghuraman H, Dasgupta S, Chattopadhyay A (2004) Organization and dynamics of N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)-labeled lipids: a fluorescence approach, Chem Phys Lipids 127:91-101

191. Kittipongpittaya K, Panya A, McClements D, Decker E (2014) Impact of free fatty acids and phospholipids on reverse micelles formation and lipid oxidation in bulk oil. J Am Oil Chem Soc 91:453-462

192. Huang S-W, Frankel EN, Aeschbach R, German JB (1997) Partition of selected antioxidants in corn oil–water model systems. J Agric Food Chem 45:1991-1994