INFLUENCE OF MOLECULAR ENVIRONMENT ON LIPID OXIDATION OF STRUCTURED LIPID-BASED MODEL EMULSIONS

by

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(Under the Direction of Casimir C. Akoh)

ABSTRACT

A structured lipid (SL) was enzymatically synthesized from canola oil and caprylic acid. The reaction mechanism and factors that influence oxidation of SL-based oil-in-water model emulsions were evaluated. Peroxide values, anisidine values, and TOTOX values were utilized to determine the oxidative stability of emulsions stored at 50°C.

Emulsion samples were prepared by admixing emulsifiers such as sucrose fatty acid ester (SFE) or whey protein isolate (WPI) with 10 or 30% oil, and then homogenized at 1000 or 10,000 psi to form different particle sizes. Oil concentration and emulsifier type significantly affected lipid oxidation rates in the emulsions, whereas particle size had no effect.

Oxidation properties of SL emulsions containing copper were evaluated at pH 3.0 and 7.0. Increased lipid oxidation occurred in the pH 7.0 emulsions. The addition of 0.5 M NaCl increased oxidation in the acidic emulsions. Both α-tocopherol and citric acid performed as antioxidants in the pH 3.0 emulsions.

The effects of pH and natural antioxidants (α-tocopherol, gallic acid, and quercetin) on iron-catalyzed oxidation of SL emulsions stabilized by WPI were evaluated for 15 days of storage. Greater iron-catalyzed lipid oxidation occurred in the pH 3.0 emulsions compared to their pH 7.0 counterparts. Quercetin and gallic acids had prooxidant effects on total oxidation in the pH 3.0 emulsions. The prooxidant effect of the phenolic compounds was only observed at pH 7.0 when higher concentrations of iron (100 μM) were added to the emulsions. α-Tocopherol did not affect total oxidation in the pH 3.0 emulsions. At pH 7.0, α-tocopherol had a prooxidant effect in the presence of higher concentrations of iron (100 μM).

The antioxidant efficacies of α-tocopherol, β-carotene, genistein, and daidzein in SL emulsions with no added transition metals were evaluated over a 30-day period. Less total oxidation occurred in the WPI emulsions compared to their SFE counterparts. β-carotene and soy isoflavones exhibited prooxidant activities in the SFE emulsions. Soy isoflavones also increased oxidation in WPI emulsions, but β-carotene did not exhibit prooxidant activity in these emulsions. α-Tocopherol did not affect the final TOTOX value of either WPI or SFE emulsions.

INDEX WORDS: Oil-in-water emulsions, Lipid oxidation, Natural antioxidants, Structured lipids, Sucrose fatty acid esters, Transition metals, Whey protein isolate

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DEDICATION

To my Dad and Mom for their unfailing belief in my abilities, continued support and involvement in all my endeavors, words of encouragement, and prayers.

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Overall, I am very proud to be a part of the Department of Food Science and Technology at The University of Georgia. Many of the professors have acted as friends, as well as educators, and my fellow students have been great to work/socialize with.

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CHAPTER 1

INTRODUCTION

Structured lipids (SLs) are tailor-made fats and oils with improved nutritional or physical properties because of modifications to incorporate new fatty acids, or to change the position of existing fatty acids on the glycerol backbone (Osborn and Akoh 2002). SLs may provide the most effective means of delivering desired fatty acids for nutritive or therapeutic purposes and for targeting specific diseases and metabolic conditions (Lee and Akoh 1998), making them ideal targets for functional food formulations. Although the food industry would like to incorporate structured lipids into their product formulations, a better understanding of their oxidation properties in food matrices is first needed.

Lipid oxidation in foods is a serious problem that leads to loss of shelf life, palatability, functionality, and nutritional quality. Most current knowledge on the mechanisms of lipid oxidation was obtained from the study of bulk oils for both unmodified lipids and SLs. However, the oxidation theories that apply to bulk oils may not be suitable for predicting reactions in more complex systems. The organization of lipid molecules within food systems, as well as their interactions with other types of molecules in their immediate vicinity influences their susceptibility to oxidation (McClements and Decker 2000). Therefore, oxidation of SLs must be evaluated in various model emulsion systems in order to better understand the complexity of SL oxidation in real food emulsions.

The hypothesis of this dissertation is that the molecular environment within and surrounding emulsion droplets significantly impacts oxidative stability of SL-based oil-in-water emulsions.

The overall objective of this research was to gain a better understanding of the reaction mechanism and factors that influence lipid oxidation of SL-based model oil-in-water emulsions in an attempt to expedite the incorporation of SLs into foods. Specific objectives were:

- To determine the effect of emulsifier type, droplet size, and oil concentration on lipid oxidation in model emulsions formulated with a canola oil/caprylic acid SL.
- To determine the effects of salt and pH on copper-catalyzed lipid oxidation in structured lipid-based model emulsions. α-Tocopherol, citric acid, and a combination of the two compounds will be incorporated into whey protein isolate-stabilized emulsions to determine their effects on lipid oxidation and how changes in pH or the addition of NaCl affect their antioxidant activity.
- To evaluate the effects of iron, pH, and natural antioxidants (α-tocopherol, gallic acid, and quercetin) on lipid oxidation of canola oil/caprylic acid SL-based emulsions stabilized by whey protein isolate.
- To illustrate the potential beneficial or deleterious effects of α-tocopherol, β-carotene, and soy isoflavones in structured lipid-based model emulsions stabilized by whey protein isolate or sucrose fatty acid esters.

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CHAPTER 2

LITERATURE REVIEW

Many natural and processed foods exist either partly or wholly as emulsions, or have been in an emulsified state sometime during their existence (McClements 2002). Examples include milk, cream, mayonnaise, soups, sauces, butter, salad dressings, powdered coffee whiteners, and many desserts. Emulsions are stabilized by emulsifiers that populate the oil-water interface and lower the interfacial tension (Rampon et al. 2001). The physicochemical properties of food emulsions depend on the type of molecules the food contains and their interactions with one another. Emulsified foods may contain a variety of ingredients, including: water, lipids, proteins, carbohydrates, minerals, sugars, small-molecule surfactants, transition metals, and antioxidants. Food emulsions undergo diverse treatments, including storage at various conditions, before they reach the consumer's plate. These treatments induce emulsion modifications including chemical reactions such as lipid oxidation, which can produce undesirable off-flavors and potentially toxic reaction compounds that make the products no longer acceptable for human consumption. Therefore, lipid oxidation is of great concern to the food industry and manufacturers must develop methods of preventing or retarding oxidation in foods (Coupland and McClements 1996). To effectively design such strategies, it is necessary to have a thorough understanding of the mechanisms of lipid oxidation and how these are affected by the molecular environment of the emulsified lipids.

FOOD EMULSIONS

An emulsion consists of two immiscible liquids (usually oil and water), one dispersed in the other in the form of small spherical droplets. The diameter of droplets in food emulsions are typically within the range of 0.1-100 μm (McClements and Decker 2000). A system that consists of oil droplets dispersed in an aqueous phase is known as an oil-in-water (o/w) emulsion, whereas a system that consists of water droplets dispersed in an oil phase is known as a water-in-oil (w/o) emulsion. This review will focus exclusively on o/w emulsions because the oxidation properties of oils in this type of emulsion are significantly different than in the bulk phase. Whereas, it has been suggested that lipid oxidation in w/o emulsions occurs at a rate similar to that in bulk oils (Coupland and McClements 1996).

Emulsions are thermodynamically unstable systems because of the positive free energy required to increase the surface area between the oil and water phases (McClements 2002). With time, emulsions tend to separate into a system that consists of a layer of oil on the top of a layer of water. To form emulsions that are kinetically stable for a reasonable period of time, emulsifiers are required. Emulsifiers are surface-active molecules that adsorb to the surface of freshly formed droplets during homogenization, forming a protective 'membrane' that prevents the droplets from aggregating (Coupland and McClements 1996). Food emulsifiers are often amphiphilic molecules that have both polar and nonpolar regions on the same molecule. Common emulsifiers in the food industry include amphiphilic proteins (from casein, whey, soy, or egg), phospholipids (egg or soy lecithin), and small-molecule surfactants (Spans, Tweens, partial acylglycerols, sugar fatty acid esters, or fatty acids). Once emulsifiers have been added

to the oil and water, forming drops is easy, but breaking them up into small droplets requires additional energy. Homogenizers provide the inertial forces needed to deform and break up emulsion droplets, which are produced by the rapid, intensive pressure fluctuations occurring in turbulent flow (Walstra 1996).

Emulsions can be divided into three distinct regions: the interior of the droplet (oil), the continuous phase (water), and the interfacial region (emulsifier). However, most food emulsions are more complex than this simple three-component (oil, water, and emulsifier) system. The aqueous phase may contain water-soluble ingredients of many different kinds, including sugars, salts, acids, bases, surfactants, proteins, and polysaccharides. The oil phase may contain a variety of lipid-soluble components, such as triacylglycerols, diacylglcyerols, monoacylglycerols, fatty acids, vitamins, and cholesterol. The interface consists of a narrow region surrounding each emulsion droplet that is comprised of surface-active molecules, including: small-molecule surfactants, phospholipids, and proteins. Additionally, some food ingredients are not located exclusively in one phase, but are distributed between the oil, water, and interfacial phases according to their partition coefficients (McClements 2002).

LIPID OXIDATION

Reaction Mechanisms

Lipid oxidation has been extensively studied in bulk fats and oils. However, o/w emulsions behave differently than bulk oils on oxidation. More unsaturated fatty acids have been observed to oxidize more slowly than less unsaturated fatty acids in o/w emulsions, whereas in bulk oils, the more unsaturated oils oxidize faster (Ponginebbi et

al. 1999). At present the reason for this observation is unknown, although it has been suggested that it is due to differences in the molecular arrangements of the fatty acids within the micelles. The more unsaturated fatty acids may be buried more deeply within the hydrophobic interior of the micelles and therefore may be less susceptible to attack by aqueous-phase prooxidants (McClements and Decker 2000).

A recent comparison of the oxidation properties of bulk and emulsified corn oil indicated hexanal formation was increased in the o/w emulsion. The interactions of emulsifiers with water were mainly responsible for the increased breakdown of hydroperoxides into secondary oxidation products in the corn oil-in-water emulsions compared to the bulk oil (Schwarz et al. 2000).

Food emulsions are susceptible to spoilage through the autoxidation of their unsaturated and polyunsaturated oil components (Depree and Savage 2001). In this process, oxygen from the atmosphere is added to certain fatty acids, creating unstable intermediates that eventually break down to form unpleasant flavor and aroma compounds. Although enzymatic and photogenic oxidation may play a role, the most common and important process by which unsaturated fatty acids and oxygen interact is via a complex, radical chain reaction characterized by three main phases (Erickson 2002):

Initiation: $In\bullet + RH \rightarrow InH + R\bullet$

Propagation: $R\bullet + O_2 \rightarrow ROO\bullet$

$ROO\bullet + RH \rightarrow R\bullet + ROOH$

Termination: $ROO\bullet + ROO\bullet \rightarrow O_2 + ROOR$

$ROO\bullet + R\bullet \rightarrow ROOR$

Initiation is frequently attributed in most foods to the reaction of fatty acids with active oxygen species (In•). Initiation occurs as hydrogen is abstracted from an unsaturated fatty acid (RH), resulting in a lipid free radical (R•), which in turn reacts with molecular oxygen (O_2) to form a lipid peroxyl radical (ROO•). The propagation phase of oxidation is fostered by lipid-lipid interactions, whereby the lipid peroxyl radical abstracts hydrogen from an adjacent molecule, resulting in a lipid hydroperoxide (ROOH) and a new lipid free radical. Such interactions continue 10 to 100 times before two radical species (R• or ROO•) combine to terminate the process by forming a nonreactive molecule (ROOR) (Erickson 2002). Most food oils naturally contain enough lipid peroxides to promote lipid oxidation, even if other sources of radical generators are rigorously eliminated (Coupland and McClements 1996). Additional magnification of lipid oxidation in food emulsions occurs through branching or secondary initiation reactions promoted by transition metals or other prooxidants as shown below (McClements and Decker 2000):

$$Fe^{3+} + ROOH \rightarrow Fe^{2+} + ROO\bullet + H^{+}$$

$$Fe^{2+} + ROOH \rightarrow Fe^{3+} + RO\bullet + OH^{-}$$

Lipid hydroperoxides are not considered harmful to food quality; however, they are further degraded into compounds that are responsible for off-flavors (Erickson 2002). Formation of the alkoxyl radical (RO•) leads to β-scission reactions that result in the generation of a wide variety of different molecules, including aldehydes, ketones, alcohols, and hydrocarbons, which are responsible for the characteristic physicochemical and sensory properties of oxidized oils (McClements and Decker 2000).

The above reaction mechanisms do not illustrate the importance of the physical location of the various reactive species within the emulsion system. Often, hydroperoxides accumulate at the surface of the emulsion droplets, whereas many of the molecular species responsible for accelerating lipid oxidation originate in the aqueous phase (i.e. transition metals or enzymes). Therefore, the inability of prooxidants to come into close contact with lipids at the droplet surface would decrease their ability to accelerate lipid oxidation in food emulsions. Additionally, the rate of lipid oxidation may be limited by the speed that free radicals, hydroperoxides, or lipids can diffuse from one region to another with a droplet (McClements and Decker 2000).

Experimental Techniques

A wide variety of analytical techniques have been developed to study lipid oxidation in bulk fats and oils. Many of these techniques can also be used to monitor lipid oxidation in emulsions. Changes in chemical, physical, or organoleptic properties of fats and oils during oxidation may be monitored to assess the extent of lipid oxidation (Shahidi and Wanasundara 2002). The available methods to monitor lipid oxidation in foods may be divided into two groups – those that measure primary oxidative changes, and those that measure secondary changes.

Primary oxidation. Primary changes can be measured by monitoring changes in reactants, weight gain, hydroperoxides, and conjugated dienes. The classical method for quantification of hydroperoxides is the determination of the peroxide value (PV). The PV can be determined with iodometric or colorimetric methods. The Active Oxygen Method (AOM) is a common accelerated method used for assessing oxidative stability of fats and oils. In this method air is bubbled through a heated oil at 98-100°C for different

time intervals and the PV are determined (Shahidi and Wanasundara 2002). A freshly refined fat should have a PV of less than 1 unit (Rossell 1986).

Secondary oxidation. The primary oxidation products (hydroperoxides) of fats and oils are transitory intermediates that decompose into various secondary products. Measurement of secondary oxidation products as indices of lipid oxidation is more appropriate since secondary products of oxidation are generally odor-active, whereas primary oxidation products are colorless and flavorless (Shahidi and Wanasundara 2002). The following are common techniques used to monitor secondary oxidation in foods: 2-thiobarbituric acid reactive substances (TBARS) values, oxirane values, and p-anisidine values (AnV). Carbonyls, hydrocarbons, and fluorescent products can also be monitored as secondary oxidation markers. The p-anisidine method determines the amount of aldehydes present in the oil sample. Highly significant correlations between AnV and flavor acceptability scores were found for salad oils (List et al. 1974). The anisidine is often used in conjunction with the PV to calculate the total oxidation (TOTOX) value according to the following equation (Rossell 1986):

$$\text{TOTOX value} = 2\text{PV} + \text{AnV}$$

The TOTOX value is often considered useful in that it combines evidence about the past history of an oil with the present state of the oil. TOTOX values over 10 indicate the onset of progressive oxidative deterioration in oil samples (Rossell 1986).

FACTORS THAT INFLUENCE LIPID OXIDATION

Chemical Structure of Lipids

The chemical structure of a lipid is the ultimate determinant of its susceptibility to oxidation. The number and location of the double bonds are of particular importance. As mentioned previously, the oxidative stability of emulsified oils often increases as their degree of unsaturation increases (McClements and Decker 2000).

Structured lipids. Structured lipids (SLs) are a new generation of fats and oils with improved nutritional or physical properties because of modifications to incorporate new fatty acids, or to change the position of existing fatty acids on the glycerol backbone by chemically- and/or enzymatically-catalyzed reactions and/or genetic engineering. Comprehensive reviews on the rationale, production, analysis, application, commercial outlook, and future prospects of SLs are available, and therefore, will not be covered in great detail here (Akoh 2002; Osborn and Akoh 2002; Lee and Akoh 1998).

In addition to altering the nutritional and physical properties, changing the fatty acids on a triacylglycerol will also affect its oxidation properties. SLs often contain both long-chain polyunsaturated fatty acids and saturated medium-chain fatty acids on the same glycerol backbone. Because of the inherent structural differences between SLs and unmodified oils, predicting oxidation properties of SL-based emulsions based on current knowledge of emulsified lipids is difficult. Additionally, enzymatically synthesized SLs require downstream processing to remove free fatty acids, which also results in a loss of natural tocopherols. Akoh and Moussata (2001) reported that the total tocopherol content of unmodified canola oil decreased from 45.34 mg/100 g oil to 0.77 mg/100 g oil after the acidolysis reaction with caprylic acid and subsequent purification. The loss of natural

antioxidant translated into a lower oxidative stability index for the SL compared to the unmodified canola oil (3.5 and 9.65 h at 110°C, respectively). This study was carried out on bulk oils and no information is currently available on the oxidation properties of canola oil-based SLs in emulsions. Recently lipid oxidation in model emulsions containing a SL synthesized from menhaden oil and caprylic acid was evaluated. The effects of the emulsifiers lecithin, Tween 20, whey protein isolate, mono-/diacylglcyerols, and sucrose fatty acid ester at concentrations of 0.25 and 1% were evaluated (Fomuso et al. 2002a). The lower concentrations of emulsifier generally resulted in a higher oxidation rate. The lowest levels of hydroperoxides were measured in emulsions containing whey protein isolate. In a separate study, these authors also evaluated the effects of temperature, time, metal, citric acid, and tocopherol on oxidation of fish oil-based SL emulsions (Fomuso et al. 2002b). Both metals increased lipid oxidation in the SL emulsions. Citric acid was an effective antioxidant in the iron-catalyzed emulsions, while tocopherol was more effective in reducing peroxide values in the copper-catalyzed systems. Extensive kinetics studies on canola oil-based SLs are now required to fully understand their oxidation properties in o/w emulsions.

Droplet Characteristics

Oil concentration. Understanding the kinetics of oxidation in emulsions of varying oil droplet concentrations is critical in the food industry, where emulsified systems range from fruit beverages (< 1% oil) to mayonnaise (> 80% oil). Sims et al. (1979) investigated the effect of lipid concentration on oxidation in safflower oil-in-water emulsions and found that decreasing the oil fraction in an emulsion resulted in increased oxidation rates. One explanation for this increase is that the number of radicals generated

per droplet may increase as the droplet concentration decreases (McClements and Decker 2000). Roozen et al. (1994a,b) also reported that increasing the lipid concentration resulted in a decrease of hexanal formation in model systems of linoleic acid in emulsified hexadecane or corn oil.

Droplet size. Little is known about the correlation between oil droplet size and oxidation rate in emulsion systems. On one hand, small droplet size signifies a large surface area, implying a high potential for contact between diffusing oxygen, water-soluble free radicals and antioxidants, and the interface. It also implies a high ratio of oxidizable fatty acids located near the interface to fatty acids embedded in the hydrophobic core of the droplets. According to this, decreasing the size of the oil droplets is therefore expected to favor development of oxidation. Data on the influence of droplet size on lipid oxidation are often contradictory. Fish oil-enriched mayonnaise with small droplets had higher free radical concentrations and more fishy off-flavor than their large droplet counterparts (Jacobsen et al. 2000). Gohtani et al. (1999) also reported higher PV in samples with small droplets compared to those with large droplets for docosahexaenoic acid-in-water emulsions. When oxygen is not the limiting factor, the formation of primary oxidation products in protein-stabilized emulsions is favored by small droplet sizes (Lethuaut et al. 2002). On the other hand, Roozen et al. (1994a) found no effect of droplet size on lipid oxidation rates of linoleic acid in hexadecane emulsions. Based on the results of a study on emulsified ethyl linoleate, Coupland et al. (1996) predicted that changes in particle size would only affect oxidation kinetics when the change allowed a higher proportion of the oxidizable material to accumulate at the interface. Meanwhile, Jacobsen et al. (1999) found that the addition of propyl gallate to

the fish oil-enriched mayonnaise increased the size of the oil droplets and promoted oxidation and flavor deterioration. Taken together these data indicate that the relationship between droplet size and the oxidation rate depends, at least in part, on the emulsion system in question.

Physical state. The physical state of the droplets in an oil-in-water emulsion would also be expected to influence the rate of lipid oxidation. The droplets in most foods are liquid at room temperature, but may become partially or totally solidified at refrigerated temperatures. Studies on bulk fats indicate that lipid oxidation occurs more slowly when the fat is crystalline than when it is liquid. However, similar studies are needed to determine if a similar phenomenon occurs in emulsified lipids (McClements and Decker 2000).

Interfacial Characteristics

The interfacial region is potentially very important in lipid oxidation since it represents the region where lipid- and water-soluble components interact and it is where surface-active compounds such as peroxides and chain breaking antioxidants concentrate (Mancuso et al. 1999).

Electrical charge. The interfacial membrane surrounding the oil droplets may have an electrical charge with a magnitude and sign that is determined by the type and concentration of charged surface-active species present. An electrically charged surface attracts oppositely charged ions (i.e. mineral ions, metal ions, and ionic antioxidants) in the surrounding aqueous phases and subsequently influences the rate of lipid oxidation in emulsions. Several studies have confirmed the importance of droplet charge in controlling the rate of lipid oxidation in o/w emulsions. Mei et al. (1998a) reported that

Fe^{2+} and Fe^{3+} associated with negatively charged sodium dodecyl sulfate (SDS)-stabilized emulsion droplets, but not with nonionic Brij (polyoxyethylene 10 lauryl ether)- and cationic DTAB (dodecyltrimethylammonium bromide)-stabilized emulsions. In this study, the increased ability of iron to associate with *n*-hexadecane emulsion droplets corresponded to the promotion of lipid oxidation in salmon oil-based emulsions. Similarly, the surface charge of the emulsion droplet influenced the rate of iron-catalyzed lipid oxidation of corn oil-based emulsions. Both primary and secondary oxidation increased at pHs < 5.0 in the anionic SDS emulsions, while the oxidation rates were unaffected by pH for Brij and DTAB emulsions (Mei et al. 1998b). Mancuso et al. (2000) also found that peroxide decomposition by iron redox cycling occurs when iron emulsion droplet interactions are high in hexadecane and trilaurin emulsions. While Fe^{2+} was capable of decomposing lipid peroxides in nonionic, cationic, anionic emulsion droplets, Fe^{3+} was only effective in anionic emulsion droplets at pH 3.0.

In the absence of added metals, the oxidative stability of salmon oil-in-water emulsions was influenced by surfactant type, with oxidation rates being greatest in emulsions stabilized by anionic SDS followed by nonionic Tween 20 and cationic DTAB (Mancuso et al. 1999). Additionally, attractive/repulsive electrostatic interactions between charged emulsion droplets and charged antioxidants were shown to affect the location and activity of galloyl derivative antioxidants in salmon oil emulsions (Mei et al. 1999).

Cationic and anionic emulsifiers such as DTAB and SDS are not commonly used by the food industry, but proteins above or below their isoelectric point (pI) could produce negatively or positively charged emulsion droplets. At pH 3.0, whey protein

isolate (WPI)-stabilized emulsion droplets have a net positive charge because they are below the pI of ~5.0 for WPI (Demetriades and McClements 2000). The positive charge decreases the likelihood of any interaction with positively charged prooxidant ions because of repulsive electrostatic forces. Low oxidation rates were reported for fish oil-based emulsions at pH 3.0 because of the electrostatic repulsion between iron and droplet surfaces (Donnelly et al. 1998).

Physical and chemical barriers. Less is known about how the emulsion droplet interfacial membrane properties impact oxidation rates. Silvestre et al. (2000) evaluated the stability of hydroperoxides and antioxidants in emulsion stabilized by surfactants that differed only in their hydrophilic headgroup size. Their data suggested that the thickness of the emulsion droplet interfacial membrane could be an important determinant in the ability of lipid peroxides to oxidize fatty acids. The ability of large surfactants to protect lipid hydroperoxides is likely due to the ability of these surfactants to alter the surface activity of lipid hydroperoxides or to provide a protective barrier around the emulsion droplet that would decrease lipid hydroperoxide-continuous phase prooxidant interactions.

Differences in the size of the hydrophobic tail group of Brij emulsifiers also resulted in different rates of methyl linoleate oxidation in hexadecane emulsions. Based on hydroperoxide and headspace propanal measurements, greater lipid oxidation occurred in salmon oil emulsion droplets stabilized by Brij-lauryl compared to those stabilized by Brij-stearyl. The ability of increasing hydrophobic tail group length to decrease the oxidation of emulsified salmon oil suggests that the longer tail group size decreases the

ability of free radicals originating from hydroperoxides in the aqueous phase to reach the polyunsaturated fatty acids (Chaiyasit et al. 2000).

Recently, the oxidative stabilities of soybean oil dispersed with sucrose esters or polyglycerol esters in emulsions were evaluated (Kubouchi et al. 2002). The stability of sucrose ester emulsions was always less than those stabilized with polyglycerol esters, and differences in droplet size were thought to be responsible for this occurrence. However, droplet sizes did not differ among emulsions stabilized with various sucrose esters or polyglycerol esters, but oxidation rates were still influenced. These findings were attributed to the different effectiveness of the packing of the emulsifier. The authors proposed that a higher hydrophile-lipophile balance (HLB) or a longer acyl chain of sugar ester caused tighter packing at the oil-water interface, which allowed the membrane to become a more efficient barrier against diffusion of lipid oxidation initiators (Kubouchi et al. 2002).

Duh et al. (1999) reported that Tweens and sucrose esters stabilized arachidonic acid (AA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA) in soybean oil-in-water model systems. The micelles composed of emulsifiers and AA, EPA, or DHA took on a more stable conformation against the attack by free radicals and/or oxygen, because the long-chain fatty acids become more tightly packed in emulsified systems.

Certain types of emulsifier molecules may also be able to act as a chemical barrier to lipid oxidation. Many emulsifier molecules contain either sugar or amino acid moieties that can act as radical scavengers. Adsorbed emulsifiers are likely to be particularly effective at retarding lipid oxidation because of their high local concentration

and close proximity to the oxidation substrate (McClements and Decker 2000). Whey protein isolate has been reported to inhibit lipid oxidation in structured lipid- and salmon oil-based emulsions (Fomuso et al. 2002a; Tong et al. 2000a). This emulsifier is thought to inhibit oxidation by chelation of iron and copper and by inactivation of peroxyl radicals (Donnelly et al. 1998).

Alternatively, some surfactant molecules used by the food industry are sources of hydroperoxides. Tweens and phospholipids have been found to contain 4-35 μmol hydroperoxides/g surfactant. Increasing Tween 20 concentrations also increased lipid oxidation in salmon oil-in-water emulsions (Nuchi et al. 2001). Additionally, these data indicated that in the presence of high surfactant-hydroperoxide concentrations, less iron was needed to promote oxidation. Therefore, food manufacturers need to closely monitor the hydroperoxide concentration in their surfactants during storage.

Interactions with Aqueous Phase Components

Most food emulsions contain a number of ingredients in addition to oil, water, and emulsifiers that may be of importance to the lipid oxidation processes. The hydrophilic components act as either prooxidants or antioxidants depending on their chemical properties, the prevailing environmental conditions, and their interaction with the other molecular species involved in the lipid oxidation reaction.

Salts, sugars, polysaccharides, and proteins. In corn oil emulsions stabilized by SDS, NaCl acted as a prooxidant in the presence of iron, but an antioxidant in the absence of iron. However, salts had no effect on lipid oxidation of Brij and DTAB stabilized emulsions (Mei et al. 1998b). Based on these data, the authors proposed that NaCl inhibition of oxidation was due to decreased iron-lipid interactions through the ability of

sodium to decrease iron binding at the droplet surface or by the formation of iron-chloride complexes, which would also decrease iron binding. Depree and Savage (2001) reviewed the oxidation properties of mayonnaise emulsions, and concluded that NaCl may promote oxidation in the absence of antioxidants, but the effect is neutralized in the presence of antioxidants. These reviewers believe that the oxidation-promoting properties of salts are unlikely to be a major problem in the food industry as they are easily overcome and because of the important contributions of salts to flavor and emulsion stability.

Sucrose significantly retarded oxidation in linoleic acid emulsions, however the effect was not concentration dependent (Ponginebbi et al. 1999). Addition of sugar to safflower oil emulsions has likewise been found to enhance the oxidative stability of the emulsion (Sims et al. 1979). The inhibitory mechanism of sugars has not been fully determined at this point. Sugar's ability to influence lipid oxidation may stem from its ability to increase viscosity and thus reduce the mobility of the reactants and reaction products or other mechanisms including quenching metals and scavenging radicals.

Polysaccharides are often added to o/w food emulsions to enhance the viscosity of the aqueous phase, which imparts desirable textural attributes and stabilizes the droplets against creaming (McClements and Decker 2000). Xanthan inhibited hydroperoxide formation in two DHA-based emulsions of different particle sizes (3.4 and 6.4 μm) for 20 days at 25°C (Gohtani et al. 1999). Polysaccarides may inhibit oxidation through metal chelation and hydrogen donation (McClements and Decker 2000).

In many food emulsions, there are appreciable quantities of nonadsorbed proteins dispersed in the aqueous phase, which may increase or decrease oxidative stability of

emulsions through enzymatic or nonenzymatic mechanisms (McClements and Decker 2000). The antioxidant capabilities of whey protein in a Tween 20-stabilized salmon oil emulsion were determined by Tong et al. (2000b). High-molecular-weight (HMW) fractions of whey had greater antioxidant activity than low-molecular-weight (LMW) fractions in this emulsion system. These authors also determined that the sulfhydryl groups were the primary antioxidants in whey proteins.

Bovine lactoferrin inhibited hydroperoxide and hexanal formation in corn oil emulsified with soybean lecithin (Huang et al. 1999). The inhibition of hydroperoxide formation by lactoferrin increased with increasing concentration, while the reverse effect was observed on hexanal formation. Additionally, lactoferrin decreased iron-catalyzed but not copper-catalyzed autoxidation in emulsions. Apparently, lactoferrin decreased the iron-catalyzed autoxidation by chelating ferrous ions. Donnelly et al. (1998) also reported that whey proteins exhibit antioxidant activity due to iron chelation by lactoferrin and copper chelation by β-lactoglobulin.

Surfactants. After a surfactant concentration is reached that saturates the droplet surface, excess surfactant molecules can form micelles in the continuous phase. It is well-known that micelles have the ability to solubilize certain compounds out of the lipid droplets into the continuous phase of the emulsion (Nuchi et al. 2002).

In general, nonpolar antioxidants that are retained in emulsion droplets are more effective inhibitors of lipid oxidation than polar antioxidants that have significant partitioning into the continuous phase of an o/w emulsion (Frankel et al. 1994). Partitioning of antioxidants in emulsions is mainly influenced by their molecular characteristics. However, surfactants could also play an important role in antioxidant

partitioning since they can aid in the solubilization of nonpolar compounds out of the lipid into the water phase. The presence of excess surfactant in the continuous phase of olive oil, salmon oil, and hexadecane emulsions affected the physical location of polar phenolic antioxidants (propyl gallate), but not nonpolar antioxidants such as butylated hydroxyltoluene (BHT). However, the solubilization of antioxidants did not alter the oxidative stability of salmon oil emulsions, suggesting that surfactant micelles influenced oxidation rates by mechanisms other than antioxidant solubilization (Richards et al. 2002). Nuchi et al. (2002) demonstrated that Brij micelles inhibited lipid oxidation in corn oil emulsions. These authors found that the surfactant micelles solubilized lipid hydroperoxides out of the emulsion droplets, which may better explain the ability of surfactants to inhibit lipid oxidation in o/w emulsions.

Acids, bases, and buffers. Acids, bases, and buffers are used to control the pH of food emulsions. The pH of an aqueous phase can impact the oxidative stability of o/w emulsions in a variety of ways and may account for the apparent contradictory results found for the effects of pH on lipid oxidation of emulsions. Hydroperoxide and hexanal formation increased in corn oil emulsions with increasing pH (Huang et al. 1996). Similarly, conjugated diene, hydroperoxide, and hexanal concentrations increased in pH 6.0 emulsions compared to pH 3.0 sunflower o/w emulsions (van Ruth et al. 1999). In salmon o/w emulsions, hydroperoxides increased at low pH, but propanal concentrations were similar at pH 3.0 and 7.0 (Mancuso et al. 1999). These authors proposed that the lower peroxide concentrations at low pH were not necessarily due to lower lipid oxidation rates, but instead were caused by iron's increased ability to decompose lipid peroxides, thus preventing peroxide accumulation.

Alternatively, Jacobsen and Timm (2001) suggested that low pH promoted oxidation based on the results of their study on oxidation of fish oil-enriched mayonnaise. Peroxide value and total volatiles increased with decreasing pH values due to the increased interactions between iron and lipid hydroperoxides at the o/w interface. When vinegar and lemon juice were added to fish oil-enriched mayonnaise, the pH of the emulsion was subsequently lowered and radical formation increased based on electron spin resonance spectroscopy measurements (Thomsen et al. 2000). In this study, ethylenediaminetetraacetic acid (EDTA) efficiently inhibited any radical formation, confirming that metal ions are involved in initiation of lipid oxidation and activated at low pH in mayonnaise systems. Obviously, the effect of pH on lipid oxidation of o/w emulsions depends on a variety of factors, including the nature of the oil and surfactant, the oxidation conditions, the absence or presence of added prooxidants (i.e. transition metals), and the analytical methods used to monitor oxidation.

Transition metals. Transition metals that can undergo electron redox cycling are a major food prooxidant. These transition metals decrease the oxidative stability of foods through their ability to decompose hydroperoxides into free radicals. Decomposition of hydroperoxides by transition metals may be the most important cause of oxidation in many foods because both metals and hydroperoxides are ubiquitous to lipid-containing foods (Decker and McClements 2001).

Transition metals are common constituents of raw food materials, water, ingredients, and packaging materials. However, not all transition metals in foods are equally reactive. The rate of hydroperoxide decomposition is dependent on the concentration, chemical state, and type of metal present in the emulsion. The most

common transition metals in foods capable of promoting lipid oxidation are copper and iron, with the latter generally being in the greater concentration (Decker and McClements 2001).

The ferrous (Fe^{2+}) ion is recognized as the biologically active form of iron due to its potent oxidizing activity. In the presence of lipids, Fe^{2+} reacts with hydrogen peroxide to produce hydroxyl radicals via the Fenton reaction. The ferric (Fe^{3+}) ion is the less biologically active iron. Ferric ions produce radicals from peroxides at a rate ~10-fold less than that of ferrous ions (Mei et al. 1998a). However, Fe^{3+} can be reduced to the Fe^{2+} form by reducing agents. One example of this reaction is the Haber-Weiss Cycle, where Fe^{3+} is reduced to Fe^{2+} by superoxide anions, ascorbic acid, tocopherols, dopamine, catechols, or other plant phenolics. Reduction of the Fe^{3+} ion allows it to participate in the Fenton reaction and produce hydroxyl radicals (Wong and Kitts 2001). Cuprous ions are capable of decomposing hydroperoxides to hydroxyl radicals over 50-fold faster than Fe^{2+}. Most foods contain 0.2 to 2 ppm Cu^{2+} (3.1 to 31 μM) (Mahoney and Graf, 1986).

RETARDING LIPID OXIDATION

One of the most effective means for retarding lipid oxidation in fatty foods is to incorporate antioxidants. In foods containing lipids, antioxidants delay the onset of oxidation or slow the rate at which it proceeds. Antioxidants can be broadly classified by their mechanism of action as primary antioxidants and secondary antioxidants (Reische et al. 2002). In some cases, an antioxidant will exhibit both primary and secondary inhibition mechanisms and these are referred to as multiple-function antioxidants. Many

"antioxidant" substances retard lipid oxidation under certain conditions, but actually promote it under other conditions. Antioxidant activity varies widely depending on the composition of the emulsions system (Schwarz et al. 2000). The efficacy of an antioxidant is affected by many of the colloidal properties discussed early for their influence on lipid oxidation of emulsions. Therefore, the term "antioxidant" must be used carefully, and a compound's anti- and prooxidant properties must both be carefully evaluated before it is added to a food emulsion.

Primary antioxidants. Primary antioxidants are free radical acceptors that delay or inhibit the initiation step or interrupt the propagation step of autoxidation. Primary antioxidants react with lipid and peroxy radicals and convert them to more stable, nonradical products. Primary antioxidants (AH) donate hydrogen atoms to lipid radicals and produce lipid derivatives and antioxidant radicals (A•) according to the following equations (Reische et al. 2002):

$$ROO\bullet + AH \rightarrow ROOH + A\bullet$$

$$RO\bullet + AH \rightarrow ROH + A\bullet$$

$$R\bullet + AH \rightarrow RH + A\bullet$$

The antioxidant radicals produced by these processes are much less reactive than lipid and peroxyl radicals, and therefore, reduce the rate of propagation. Chain-breaking antioxidants are also capable of terminating the lipid oxidation reaction by reacting with peroxyl radicals, alkoxyl radicals and other antioxidants (Reische et al. 2002):

$$ROO\bullet + A\bullet \rightarrow ROOA$$

$$RO\bullet + A\bullet \rightarrow ROA$$

$$A\bullet + A\bullet \rightarrow AA$$

The most commonly used primary antioxidants in foods are synthetic compounds, such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), propyl gallate, and tertiary butylhydroquinone (TBHQ). Tocopherols and carotenoids are natural compounds that are also frequently added to foods for their primary antioxidant mechanisms.

Secondary antioxidants. Secondary antioxidants can retard lipid oxidation through a variety of mechanisms, but do not convert free radicals to more stable products. Secondary antioxidants can chelate prooxidant metals and deactivate them, replenish hydrogen to primary antioxidants, decompose hydroperoxides to nonradical species, deactivate singlet oxygen, absorb ultraviolet radiation, or act as oxygen scavengers (Reische et al. 2002). The most important secondary antioxidants for food emulsions are those that chelate transition metal ions (McClements and Decker 2000). Chelation of metals by certain compounds decreases their prooxidant effect by reducing their redox potentials and stabilizing the oxidized form or the metal. Chelators may also sterically hinder formation of the metal-hydroperoxide complex that is known to decompose and produce free radicals (Reische et al. 2002). At present, most of the chelating agents used by the food industry are synthetic, including: EDTA, phosphoric acid and polyphosphates. However, any aqueous component that chelates transition metals and removes them from the vicinity of the emulsion droplet surface would be expected to retard lipid oxidation.

Natural antioxidants in o/w emulsions. There is a growing interest for natural antioxidants as replacements for the synthetic compounds that are currently used in the food industry for food emulsions because of safety concerns and the worldwide trend toward the use of natural additives in foods (Sanchez-Moreno et al. 2000). Extensive research efforts have been dedicated to the identification of natural antioxidants from various sources.

Tocopherols and ascorbic acid are the most important natural antioxidants in the food industry. The well-understood antioxidant mechanism of α-tocopherol involves the donation of hydrogen to a peroxy radical. In foods, ascorbic acid is a secondary antioxidant with multiple functions, including: scavenging oxygen, shifting the redox potential of food systems to the reducing range, acting synergistically with chelators, and regenerating primary antioxidants (Reische et al. 2002). Frankel et al. (1994) compared the effectiveness of lipophilic antioxidants, α-tocopherol and ascorbyl palmitate, to their hydrophilic counterparts, Trolox and ascorbic acid, on oxidation of corn oil emulsions. The lipophilic antioxidants were more effective in the o/w emulsion system than the hydrophilic antioxidants. These authors proposed that the lipophilic antioxidants were more surface-active and better able to orient in the oil-water interface and protect against lipid oxidation. Therefore, the efficacy of an antioxidant may be dependent on its ability to concentrate at the oil-water interface in o/w emulsions. Further studies of Trolox and α-tocopherol in corn oil emulsions indicated that their overall antioxidant activity depends on their hydrogen-donating ability, relative stability, and distribution in emulsions (Huang et al. 1996). Somewhat conflicting results were obtained when both water- and oil-soluble forms of tocopherol (Grindox 1032 and Toco 70, respectively)

were added to fish oil-enriched mayonnaise. Neither form of tocopherol was a suitable antioxidant in this emulsion system, because they could not prevent the metal-catalyzed decomposition of peroxides (Jacobsen et al. 2001).

Numerous naturally occurring phenolic antioxidants have been identified in plant sources (Reische et al. 2002). However, their effectiveness in emulsions is often difficult to predict because there are several different mechanisms by which phenolic compounds influence lipid oxidation rates (Mei et al. 1999). Gallic acid (100 μM) showed no antioxidant activity in corn oil emulsions, because it partitioned into the aqueous phase due to its polar nature (Schwarz et al. 2000). On the other hand, Sanchez-Moreno et al. (2000) reported that gallic acid and catechin inhibited hydroperoxide and hexanal formation at 20 μM in corn oil emulsions. However, when the concentration of antioxidant was decreased to 10 μM, both gallic acid and catechin had low activity.

Virgin olive oil contains a large number of phenolic compounds, which are responsible for its reported oxidative stability. Gordon et al. (2001) demonstrated that hydroxytyrosol acetate was a slightly more effective antioxidant in o/w emulsions compared to hydroxytyrosol because it is less polar. More recently, the effects of phenolic compounds occurring naturally in virgin olive oil, namely, hydroxytyrosol, oleuropein, 3,4-dihydroxyphenylethanol-elenolic acid, and 3,4-dihydroxyphenylethanol-elenolic acid dialdehyde, on lipid oxidation of stripped olive oil-in-water emulsions were evaluated (Paiva-Martins and Gordon 2002). Their work indicated that the behavior of phenolic compounds in emulsions may be explained by radical scavenging capacity, pH, the presence of metals, and the reducing capacity, in addition to the partition coefficients for partitioning between the oil and water phases. Phenolic extracts from olive oil also

showed synergistic properties in reinforcing the antioxidant activity of lactoferrin in o/w emulsions containing iron (Medina et al. 2002).

Rosemary extracts provide a major source of commercial natural antioxidants used in foods. Frankel et al. (1996) evaluated the effectiveness of components of rosemary extract on lipid oxidation of corn oil emulsions. Carnosic acid and carnosol were more active than rosmarinic acid in the emulsions. These differences can be explained by the same interfacial phenomenon as observed with tocopherol and Trolox (Frankel et al. 1994). Polar hydrophilic rosemary compounds may be less active in the emulsion system because they partition into the water phase and become less protective of the emulsion droplets where the oxidation reactions are occurring (Frankel et al. 1996). When carnosic acid and carnosol were added to emulsions in combination with α-tocopherol, carnosol decreased the antioxidant efficacy of tocopherol, while carnosic acid increased its efficacy (Hopia et al. 1996). In o/w emulsion dressings, the addition of dried rosemary leaves resulted in significantly better antioxidant protection than the addition of standard concentrations of propyl gallate (80 ppm). The addition of a methanol extract of rosemary had less antioxidant effect, but was comparable to the effect of propyl gallate (Madsen et al. 1998).

Phenolics were extracted from fruit cuticles of several varieties of apples and added to linoleate emulsions to determine their antioxidant activity. Diphenylamine (lipophilic) displayed higher antioxidant activity than methanol-extracted cuticular phenolics (hydrophilic) in the o/w emulsions. The antioxidant activity of quercetin was higher than that of gallic acids and free phenolics displayed higher activity than bound phenolics released by chemical methods. The lipid soluble antioxidants from the cuticle

displayed higher activity in the linoleate emulsion system than in bulk oil (Ju and Bramlage 1999). Similar to many of the other studies discussed in this section, the ability of the phenolics to orient in the oil-water interface determined their effectiveness as antioxidants in o/w emulsions.

Sesame (*Sesamum indicum* L.) is one of the most important oilseed crops cultivated in India, Sudan, China, and Burma. The ethanolic extracts of sesame coat (EESC) were evaluated for antioxidant activity in linoleic acid emulsions. EESC had antioxidant activity equal to tocopherol on peroxidation of linoleic acid. EESC also had an inhibitory effect on secondary oxidation of this model emulsion. Termination of free radical reactions, metal-binding abilities, and quenching of reactive oxygen were suggested to be responsible for the antioxidant activities observed in this study (Chang et al. 2002).

Water extract of Harng Jyur (*Chrysanthemum morifolium* Ramat) was evaluated as an antioxidant in soybean oil emulsions. Harng Jyur extracts decreased lipid oxidation to a greater extent than BHA and tocopherol in the emulsions. This effect may be due the presence of several antioxidant components in the extract which gave a range of solubilities, thus allowing some compounds to be more soluble at the oil-water interface and some to be more soluble in the aqueous portion of the emulsion (Duh 1999).

The list of natural antioxidants is growing as a result of the amount of research that is being conducted to isolate and identify these compounds (Reische et al. 2002). However, much research is still needed to determine the ability of these compounds to inhibit oxidation in o/w emulsions at levels that are nontoxic and economically feasible for food manufacturers. Potential natural antioxidants that merit further investigation in

o/w emulsions include: quercetin, soybean isoflavones, a wide range of herb/spice extracts, and flavonoids isolated from chrysantheumums, rice, buckwheat, barley, and malt.

Prooxidant activity. Compounds with antioxidant activity may also exhibit prooxidant behavior under certain conditions (Fukumoto and Mazza 2000; Galati et al. 2002; Rietjens et al. 2002). The relative antioxidant activity of tocopherols in o/w emulsions depends on temperature, lipid composition, and tocopherol concentration (Huang et al. 1994). α-Tocopherol's ability to have an antioxidant, neutral, or prooxidant effect is related to its complex function and chemical behavior (Rietjens et al. 2002). Increased levels of α-tocopherol may result in increased levels of α-tocopherol radicals, which can initiate processes of lipid peroxidation by themselves (Rietjens et al. 2002).

Carotenoids are a group of natural compounds that have both primary and secondary antioxidant activity. It is well known that β-carotene is an effective singlet oxygen quencher. However, its function in food emulsions is not as well defined. In rapeseed oil emulsions, β-carotene was a prooxidant based on the formation of hydroperoxides, hexanal, or 2-heptenal (Heinonen et al. 1997). The activity of β-carotene depends on the concentration used, other antioxidants present, the oxidation model employed, and the oxygen tension (Haila et al. 1996). It is proposed that β-carotene traps peroxyl radicals under conditions of high oxygen tension or at atmospheric conditions by an addition mechanism (Decker 2002). The carbon-centered β-carotene radical that is subsequently formed is readily autoxidized into a mixture of products with epoxy, hydroxy, and carbonyl groups (Haila et al. 1996). The autoxidation reactions begin to

consume β-carotene without scavenging peroxyl radicals and may thus attenuate β-carotene antioxidant activity.

Huang and Frankel (1997) studied the antioxidant activity of tea catechins, gallic acid, and propyl gallate in corn oil-in-water emulsions stabilized by Tween 20. All compounds accelerated hydroperoxide and hexanal formation at 5 and 20 μM. These authors proposed that phenolic compounds oxidize rapidly in Tween 20; it traps air and the oxidized phenolics then catalyze oxidation at the oil-water interface. Another study on green tea extracts and catechins in sunflower oil emulsions demonstrated that the flavonoids exhibit prooxidant activity in the presence of ferric ions, which demonstrates that the prooxidant activity of tea catechins may stem from their ability to reduce transition metals, which then promotes lipid oxidation (Roedig-Penman and Gordon 1997).

Schwarz et al. (2000) reported some prooxidant activity for propyl gallate and gallic acid in corn oil emulsions. Gallic acid and propyl gallate radicals may promote the oxidation process because of their low resonance stability. Additionally, propyl gallate and gallic acid show a strong reducing activity in aqueous systems by converting trace metals into more active catalysts at the lower valence states.

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CHAPTER 3

EFFECT OF EMULSIFIER TYPE, DROPLET SIZE, AND OIL CONCENTRATION ON LIPID OXIDATION IN STRUCTURED LIPID-BASED EMULSIONS[1]

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ABSTRACT: The effect of emulsifier type, droplet size, and oil concentration on lipid oxidation was determined for caprylic acid/canola oil structured lipid-based emulsions. Emulsion samples were prepared with sucrose fatty acid ester or whey protein isolate and 10 or 30% oil, and then homogenized at 1000 or 10,000 psi to form different particle sizes. The peroxide values, anisidine values, and TOTOX values of emulsions stored at 50°C were measured over time. A separate creaming study was performed on the emulsion samples stored at room temperature. Oil concentration and emulsifier type significantly affected lipid oxidation rates in the emulsions; whereas, particle size had no effect. Whey protein isolate-stabilized emulsions began creaming after two days of storage. The sucrose fatty acid ester emulsions were stable to creaming throughout the entire 28-day storage study.

KEYWORDS: Anisidine Value, Creaming Stability, Emulsions, Lipid Oxidation, Particle Size, Peroxide Value, Structured Lipids, TOTOX Value, Whey Protein Isolate

INTRODUCTION

Structured lipids (SLs) are tailor-made fats and oils with improved nutritional or physical properties because of modifications to incorporate new fatty acids, or to change the position of existing fatty acids on the glycerol backbone. This ability to combine the beneficial characteristics of component fatty acids into one triacylglycerol (TAG) molecule enhances the role fats and oils play in food, nutrition, and health applications (Osborn & Akoh, 2002).

In the past, long chain triacylglycerols (LCTs), which are metabolized slowly in the body, were incorporated into emulsions for total parenteral nutrition (TPN) and enteral administration as a source of energy and essential fatty acids. Later a physical

blend of medium chain triacylglycerols (MCTs) and LCTs was proposed because the MCTs would be readily metabolized in the body for quick energy. More recently, SLs were designed to provide simultaneous delivery of beneficial long chain fatty acids (LCFAs) at a slower rate and medium chain fatty acids (MCFAs) at a quicker rate (Babayan, 1987; Akoh, 2002).

The combination of an increased absorption rate and beneficial component fatty acids in one TAG should make SLs very attractive to the medical community and functional food manufacturers. However, the food industry has been slow to incorporate SLs into their product formulations. Researchers have generated numerous articles on SLs and in many cases a potential food application is given for their product. However, few have taken the next step and studied how the SL actually behaves when used in a particular food application. The opportunity for more studies on the kinetics, physical properties, and functionality of food systems containing structured lipids definitely exists.

Lipid oxidation is a major cause of quality deterioration in many natural and processed foods (McClements & Decker, 2000). It can alter the flavor and nutritional quality of foods and produce toxic compounds (Min & Boff, 2002). Akoh and Moussata (2001) reported that unmodified canola oil, in the bulk phase, was more stable to oxidation than a canola oil/caprylic acid SL. However, lipids often exist as emulsifier-stabilized dispersions in foods. Therefore, it is important to examine the oxidation properties of SLs in this medium.

Previous studies have highlighted the importance of the molecular environment in determining the oxidative stability of an oil-in-water emulsion. Emulsifiers play a role in the oxidative stability of oil droplets (Fomuso, Corredig, & Akoh, 2002). Droplet charge,

which is determined by the surface-active components in foods, affects the rate of lipid oxidation in an emulsion (Mei, McClements, Wu, & Decker, 1998; Mancuso, McClements, & Decker, 1999; Mei, McClements, & Decker, 1999). The physical characteristics of the droplets may also affect the oxidation kinetics in emulsions and vary considerably in foods, depending on their concentration, size, and physical state. Understanding the kinetics of oxidation in emulsions of varying oil droplet concentrations is critical in the food industry, where emulsified systems range from fruit beverages (< 1% oil) to mayonnaise (> 80% oil). Typical food emulsions contain particle sizes ranging from 0.2 to 100 μm. A recent review of oil-in-water emulsions indicated the need for more basic studies on the influence of droplet characteristics on lipid oxidation (McClements & Decker, 2000). Therefore, the objective of this study was to determine the effect of emulsifier type, droplet size, and oil concentration on lipid oxidation in model emulsions formulated with a canola oil/caprylic acid SL.

MATERIALS AND METHODS

Materials. Canola oil was purchased from a local supermarket. Caprylic acid (purity > 98%) was purchased from Sigma Chemical Company (St. Louis, MO). A sn-1,3 specific immobilized lipase from *Rhizomucor miehei* (IM 60) was obtained from Novo Nordisk A/S (Bagsvaerd, Denmark). Whey protein isolate (#27361) was provided by Land O'Lakes (St. Paul, MN). Ryoto sugar ester (S-1670) was supplied by Mitsubishi-Kasei Food Corporation (Tokyo, Japan). The sucrose fatty acid ester contained mainly stearic acid and consisted of approximately 75% monoester and 25% di-, tri-, or polyester. All other reagents were purchased from Fisher Scientific (Fair Lawn, NJ).

Structured Lipid Production. The SL was produced according to the apparatus set-up and optimal conditions previously reported (Xu, Fomuso, & Akoh, 2000) for reacting canola oil and caprylic acid in a packed bed bioreactor: substrate flow rate, 1 mL/min; temperature, 60 °C; substrate mole ratio 1:5 (canola oil:caprylic acid); water content, 0.20% added. The product was purified by short-path distillation (UIC Inc., Joliet, IL). The oil was passed through the distillation apparatus three times under the following conditions: holding temperature, 25 °C; heating oil temperature, 185 °C; cooling water temperature, 15 °C; pressure, < 0.01 Torr. The purified SL product contained the following fatty acids (mol %): 37.3% C8:0, 1.8% C16:0, 1.7% C18:0, 47.3% C18:1, 8.9% C18:2, and 3.0% C18:3 as determined by gas chromatography of methyl esters (Fomuso & Akoh, 1996).

Emulsion Preparation. SL emulsions (10 and 30 % oil) were prepared in 10 mM phosphate buffer solutions (pH 7.0). Whey protein isolate (WPI) and Ryoto sugar ester (SFE) were used at a 0.5% (wt/wt) level to stabilize the emulsions. The emulsions were passed through a high-pressure valve homogenizer (Emulsiflex, C5, Avestin, CA) six times at 1000 psi or 10,000 psi in an attempt to create different particle sizes. In order to prepare emulsions with intermediate and/or mixed particle sizes, equal volumes of the two previously processed emulsions were combined and passed through the homogenizer one time without pressure to ensure adequate mixing. All samples were held on ice during processing. Sodium azide (1 mM) was added to the final emulsion volume to slow microbial growth.

Particle Size Distribution. Particle size distribution was measured by integrated light scattering (Mastersizer S, Malvern Instruments, Malvern, UK). Emulsion samples

(0.5 mL) were introduced into the small volume presentation unit, which already contained about 120 mL of deionized water. The sample was pumped through the optical cell using a stirrer rotating at 2000 rpm. Size distributions (volume fraction against particle size) were calculated and the weight-average sizes were expressed as $d_{3,2} = \Sigma_i n_i d_i^3 / \Sigma_i n_i d_i^2$, where n_i is the number of particles of dia d_i.

Oxidation Experiments. Emulsions were allowed to oxidize in a covered water bath held at 50ºC. Samples were taken periodically to determine the hydroperoxide and aldehyde levels present in the oil. Oil was extracted from the emulsions by adding isooctane/isopropanol (3:2, v/v), vortexing 3 times for 10 s each, and centrifuging for 5 min at 1000 rpm. The clear upper layer was collected and the solvent was evaporated under nitrogen. Peroxide values (PV) were determined using the International Dairy Federation method (Shantha & Decker, 1994). Anisidine values (AnV) were determined according to the AOCS Official Method Cd 18-90 (AOCS, 1998). The TOTOX value was calculated as: TOTOX value = 2(PV) + AnV (Shahidi & Wanasundara, 2002).

Creaming Studies. Creaming stability was evaluated by storing emulsions in 10-mL graduated test tubes at room temperature (25 ± 2ºC). The volume of cream separated out at the top was measured at 1, 2, 3, 4, 5, 6, 7, 14, 21, and 28 days.

Statistical Analysis. All experiments were performed on duplicate samples. Statistical analyses were conducted with the SAS (2001) software package. Analyses of variance were performed by ANOVA procedures. Significant differences ($P < 0.05$) were determined by least squares means comparisons.

RESULTS

Particle Size Analysis. The particle size of each emulsion prepared was evaluated by integrated light scattering (Figure 3.1). The 10% oil emulsions stabilized by SFE produced bimodal droplet size distributions (Figure 3.1A) under the 1,000 psi and mixed processing conditions. This finding is similar to that observed previously for emulsions prepared with 1% SFE and may be a result of the different ester sizes in the sucrose fatty acid ester (Fomuso et al., 2002). Processing the SFE emulsion (10% oil) at higher pressure (10,000 psi) resulted in a monomodal size distribution. The 30% oil stabilized by SFE and the emulsions stabilized by whey protein isolate at both 10 and 30% oil showed a monomodal size distribution. Average particle diameters ($D_{3,2}$) of the emulsions ranged from 0.26 to 2.69 μm and are listed in Table 3.1.

By varying the processing conditions, significantly ($P < 0.05$) different particles sizes were produced in the SFE stabilized emulsions at both oil concentration levels (10 and 30 %). However, WPI emulsions responded differently to processing. At 10% oil, no changes in particle size were observed across the three processing conditions ($P < 0.05$). The WPI emulsion containing 30% oil had significantly ($P < 0.05$) larger particle diameters when processed at 10,000 psi compared to its 1,000 psi and mixed counterparts (Table 3.1).

Primary Oxidation. Initiation of lipid oxidation depends on the ease at which the radicals generated in the aqueous phase can interact with the substrate molecules at the oil-water interface. Therefore, changes in particle size will only affect oxidation kinetics when the change allows a higher proportion of the oxidized material to accumulate at the interface (Coupland, Zhu, Wan, McClements, Nawar, & Chinachoti,

1996). The effect of particle size on lipid oxidation rates was explored in this study. Hydroperoxides were measured to determine the initial rate of oxidation because they are generally accepted as the first product formed by oxidation (Rossell, 1986). Particle size did not significantly affect the peroxide values of the emulsions at any point in the study (Figures 3.2 and 3.3). Roozen, Frankel, and Kinsella (1994) also found no dependence of the lipid oxidation rate on droplet size. Because limited amounts of hydroperoxides were available in the systems (Day 0 PV ~ 0.3 mEq/kg oil), they may have all been present at the droplet surface in every emulsion system studied. This may explain why changing the droplet size did not affect the oxidation rates (McClements & Decker, 2000).

Emulsifier type significantly affected hydroperoxide formation on days 1, 2, 3, and 10 ($P < 0.05$). SFE emulsions initially showed a rapid increase in peroxides that peaked by day 2 and generally remained constant for the rest of the study (Figure 3.2). However, the PV increase of WPI emulsions was more gradual throughout the study, with the exception of the 30% oil mixed system, which peaked on day 4 (Figure 3.3). This antioxidant effect of WPI has been previously reported (Fomuso et al., 2002). It was hypothesized that whey proteins inhibit lipid oxidation by inactivating peroxyl radicals (Tong, Sasaki, McClements, & Decker, 2000). On the final day of the study, there was no significant difference between the peroxide levels of the WPI and SFE emulsions ($P < 0.05$).

The peroxide values were significantly affected by oil concentration on days 1, 2, and 15 of the study. The 10% emulsions had significantly higher amounts of hydroperoxides on these days compared to the 30% emulsions (Figures 3.2 and 3.3). This finding is similar to that previously reported for safflower oil-in-water emulsions

(Sims, Fioriti, & Trumbetas, 1979). One explanation for this increase is that the number of radicals generated per droplet may increase as the droplet concentration decreases (McClements & Decker, 2000).

Secondary Oxidation. The peroxides in oxidized oil are transitory intermediates that decompose into various carbonyl and other compounds (Rossell, 1986). Measuring secondary oxidation products is important in the determination of lipid oxidation in food products for human consumption, because they are generally odor-active, whereas primary oxidation products are colorless and flavorless. The AnV test was utilized to determine the level of aldehydes, principally 2-alkenals and 2,4-alkadienals, present in the emulsified oil (Shahidi & Wanasundara 2002). Significant correlations between AnV and flavor acceptability scores of soybean oil have been reported in the past (List, Evans, Kwolek, Warner, & Boundy, 1974). Unlike hydroperoxides, aldehydes do not decompose rapidly, thus allowing the past history of an oil to be determined with the AnV (Shahidi & Wanasundara, 2002). Therefore, the low initial AnV for the emulsions (~ 1.9) indicates that the acidolysis reaction, distillation process, and homogenization did not cause oxidative damage to the SL.

Emulsifier type had a significant effect on the AnV on days 1, 2, 10, and 15. The SFE emulsions had significantly ($P < 0.05$) higher AnV on those days (Figure 3.4). This was expected because of the delayed development of hydroperoxides in the WPI emulsions. The hydroperoxides must first be present before they can decompose into secondary products. Again, this result demonstrates that WPI is functional in oil-in-water emulsions as an antioxidant. The AnV for WPI emulsions did not change significantly between 0 and 15 days at 50ºC (Figure 3.5). The ability of WPI to inactivate peroxyl

radicals in the emulsified oil and thus prevent the development of secondary oxidation products may be responsible for the negligible changes in aldehydes measured during this study.

The oil concentration effect was significant near the end of the study on days 10 and 15. The AnV were relatively stable for the WPI emulsions throughout the study (Figure 3.5). Oil concentration effect was more pronounced in the SFE emulsions (Figure 3.4). Similar to the hydroperoxide results, the 10% SFE emulsions were significantly more oxidized than their 30% counterparts by the end of the study. Particle size did not significantly affect the AnV at any time, which is in agreement with the hydroperoxide results obtained in this study.

Total Oxidation. The TOTOX value combines evidence about the past history and present state of an oil, and is used frequently in the food industry (Shahidi & Wanasundara, 2002). Figure 3.6 illustrates the effect of emulsifier type on the total oxidation of the canola oil SL-based emulsions. This effect was highly significant ($P < 0.01$) on all days of analysis after day 0. On day 15 of the study, the mean differences in total oxidation between SFE and WPI emulsions were 4.99 and 2.46 for 10 and 30% oil emulsions, respectively. Again, this result demonstrates the antioxidant properties of WPI. The concentration effect was also significant on days 2, 4, and 15. By day 15, the mean TOTOX values for the 10% SFE emulsions were nearly twice that of the 30% SFE emulsions (7.06 and 4.48, respectively). Data from a study on palm oil quality that included both sensory and chemical analyses showed that oil samples with a TOTOX value of 3.48 corresponded to an overall quality rating of 3.3 on a scale of 1 to 5 (1 = very poor; 5 = very good). However, a second oil with a TOTOX value of 8.68 was

given an overall quality rating of 1.3 (poor) by the same sensory panel (Idris, Abdullah, & Halim, 1992). Rossell (1986) deems oil samples with a TOTOX value above 10 unacceptable. Therefore, antioxidants and/or cold storage may be necessary to slow oxidative deterioration in food emulsions stabilized by SFE, especially when low concentrations of oil are used in the product formulation. Particle size did not significantly affect the TOTOX values throughout the study.

Creaming Stability. A separate creaming study was performed on the samples to determine emulsion stability. This portion of the experiment was carried out at room temperature (25 ± 2°C) in an attempt to imitate typical storage conditions for many food emulsions. All SFE emulsions were stable throughout the 28-day storage period, independent of oil concentration and particle size. However, the WPI-stabilized emulsions creamed during storage. Figure 3.7 illustrates that the WPI emulsions were stable to creaming for two days. On the third day of storage, the 10% oil emulsions began to cream more rapidly than their 30% counterparts. By day 21, creaming had reached similar levels of ~10% in all WPI emulsions (10 and 30% oil). Phase separation was observed in the 10% oil emulsions on days 21 and 28 (< 0.5 mL clear liquid at the bottom of the tubes). Processing conditions did not significantly affect the creaming stability of the emulsions. Previous studies have reported WPI emulsions to be relatively stable to creaming (Tirok, Scherze, & Muschiolik, 2001; Fomuso et al., 2002). However, these studies were conducted over a shorter storage period or at lower storage temperatures, which may explain the different results among the studies.

CONCLUSION

Lipid oxidation was significantly affected by oil concentration and emulsifier type. A decrease in the oil concentration led to an increase in total oxidation. WPI had a significant antioxidant effect on the oxidation rates compared to SFE. SFE emulsions were stable to creaming throughout the study; whereas, WPI emulsions creamed 10% after 28 days of storage, regardless of oil concentration and particle size. Clearly, lipid oxidation in SL-based emulsions is a highly complex area, in which oil concentration and emulsifier type play an important role. Further studies on the influence of ingredients and antioxidant types are needed to expedite the incorporation of structured lipids into product formulations by the food industry.

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Table 3.1

Average apparent particle diameter ($D_{3,2}$) of structured lipid-based emulsions after six homogenizer passes

Emulsifier	% Oil	Processing conditions		
		1,000 psi	1:1 mixture	10,000 psi
S-1670	10	1.47 ± 0.34^{a}	0.83 ± 0.21^{ab}	0.26 ± 0.01^{b}
	30	1.00 ± 0.01^{a}	0.64 ± 0.09^{b}	0.37 ± 0.03^{c}
WPI	10	1.09 ± 0.12^{a}	1.09 ± 0.02^{a}	1.07 ± 0.18^{a}
	30	1.98 ± 0.21^{a}	2.08 ± 0.04^{a}	2.69 ± 0.06^{b}

^{a,b,c} Means within the same row with different superscripts are significantly different ($P < 0.05$).

Figure 3.1 Particle size distribution of structured lipid-based emulsions stabilized by sucrose fatty acid ester (A = 10% oil; B = 30% oil) and whey protein isolate (C = 10% oil; D = 30% oil). Data shown are the average of duplicate samples.

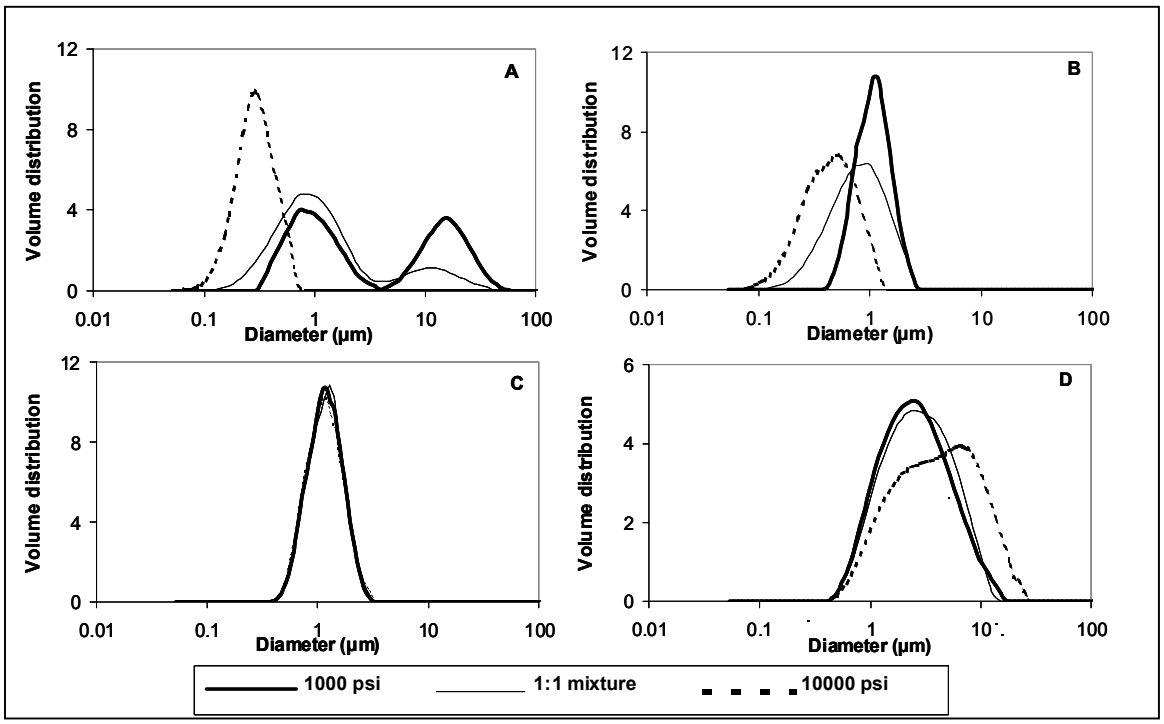

A
B
C
D
Volume distribution
Diameter (µm)
0.01
0.1
1
10
100
1000 psi
1:1 mixture
10000 psi

Figure 3.2 The effect of particle size on lipid peroxides over time in structured lipid-based emulsions stabilized by sucrose fatty acid ester (A = 10% oil; B = 30% oil). Data shown are the average of duplicate samples held at 50°C. Error bars on chart represent standard deviation.

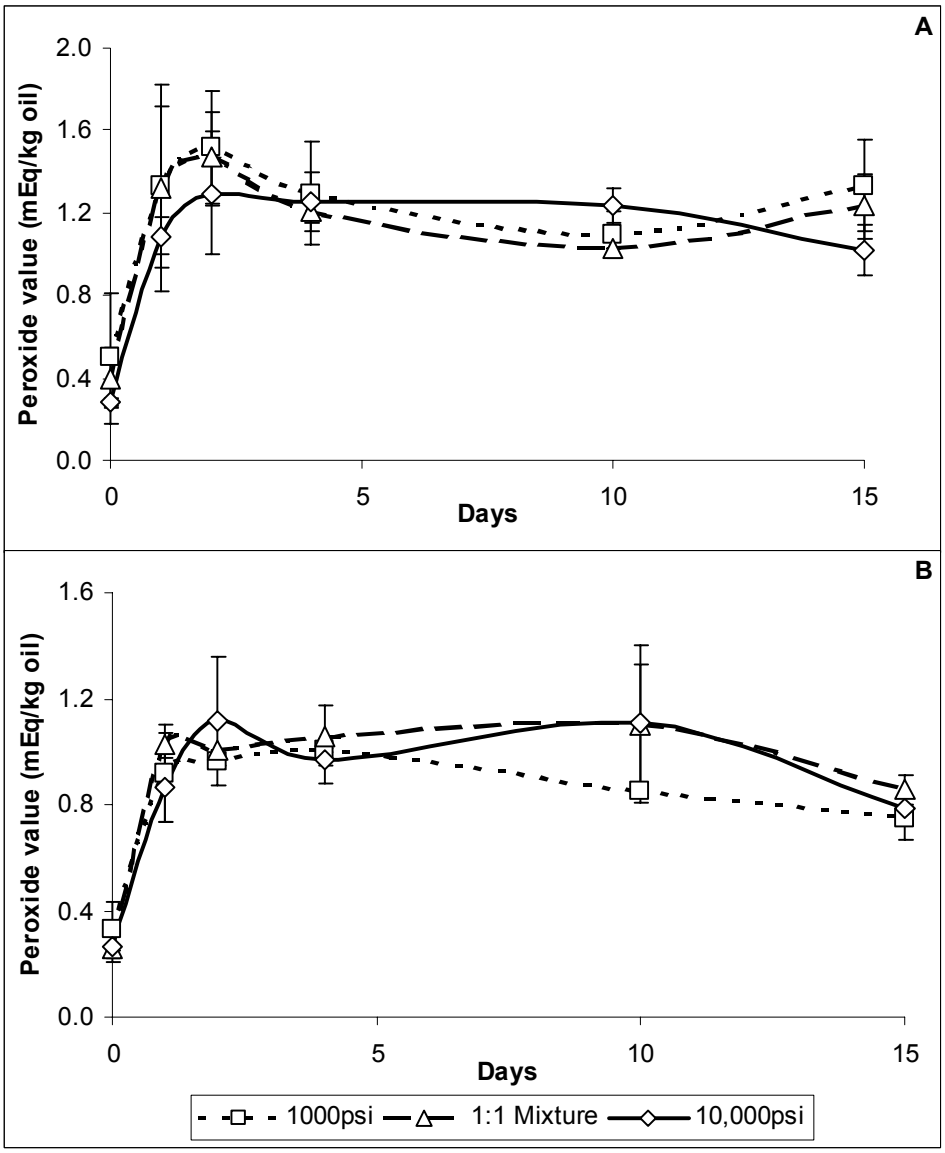

A
2.0
1.6
1.2
0.8
0.4
0.0
Peroxide value (mEq/kg oil)
0
5
10
15
Days
B
1.6
1.2
0.8
0.4
0.0
Peroxide value (mEq/kg oil)
0
5
10
15
Days
1000psi
1:1 Mixture
10,000psi

Figure 3.3 The effect of particle size on lipid peroxides over time in structured lipid-based emulsions stabilized by whey protein isolate (A = 10% oil; B = 30% oil). Data shown are the average of duplicate samples held at 50°C. Error bars on chart represent standard deviation.

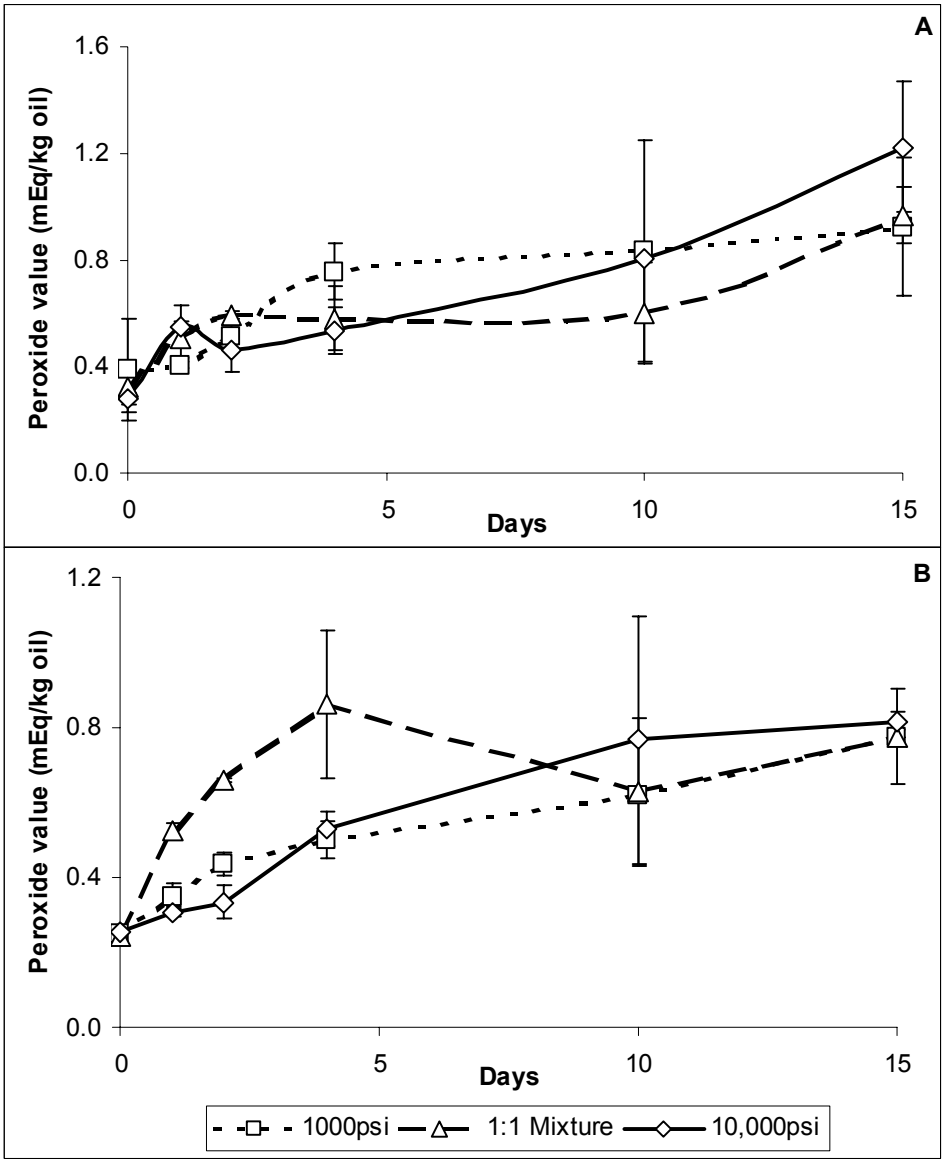

A
Peroxide value (mEq/kg oil)
1.6
1.2
0.8
0.4
0.0
0
5
10
15
Days
B
Peroxide value (mEq/kg oil)
1.2
0.8
0.4
0.0
0
5
10
15
Days
1000psi
1:1 Mixture
10,000psi

Figure 3.4 The effect of oil concentration on the anisidine value over time in structured lipid-based emulsions stabilized by sucrose fatty acid ester. Data shown are the average of duplicate samples held at 50°C. Error bars on chart represent standard deviation.

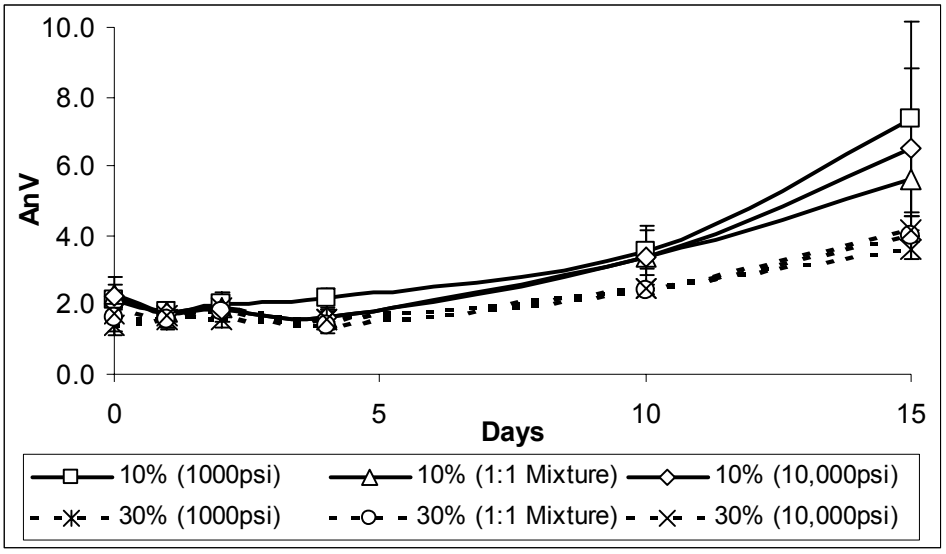

10.0
8.0
6.0
4.0
2.0
0.0
AnV
0
5
10
15
Days
10% (1000psi)
10% (1:1 Mixture)
10% (10,000psi)
30% (1000psi)
30% (1:1 Mixture)
30% (10,000psi)

Figure 3.5 The effect of oil concentration on the anisidine value over time in structured lipid-based emulsions stabilized by whey protein isolate. Data shown are the average of duplicate samples held at 50°C. Error bars on chart represent standard deviation.

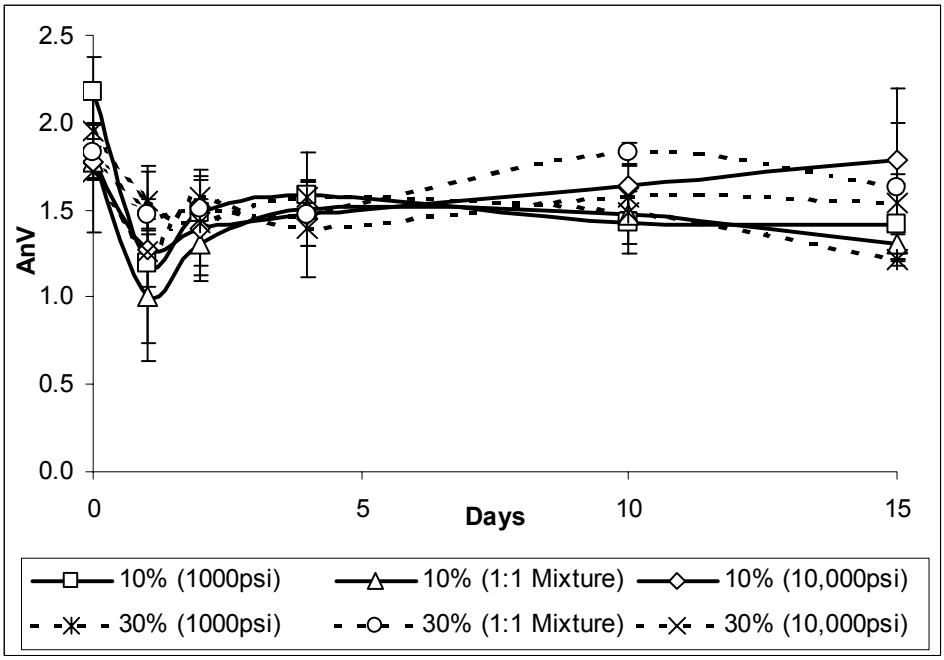

2.5
2.0
1.5
1.0
0.5
0.0
AnV
0
5
10
15
Days
10% (1000psi)
10% (1:1 Mixture)
10% (10,000psi)
30% (1000psi)
30% (1:1 Mixture)
30% (10,000psi)

Figure 3.6 The effect of emulsifier type (SFE = sucrose fatty acid ester; WPI = whey protein isolate) on total oxidation over time in structured lipid-based emulsions (A = 10% oil; B = 30% oil). Data shown are the average of duplicate samples held at 50°C. Error bars on chart represent standard deviation.

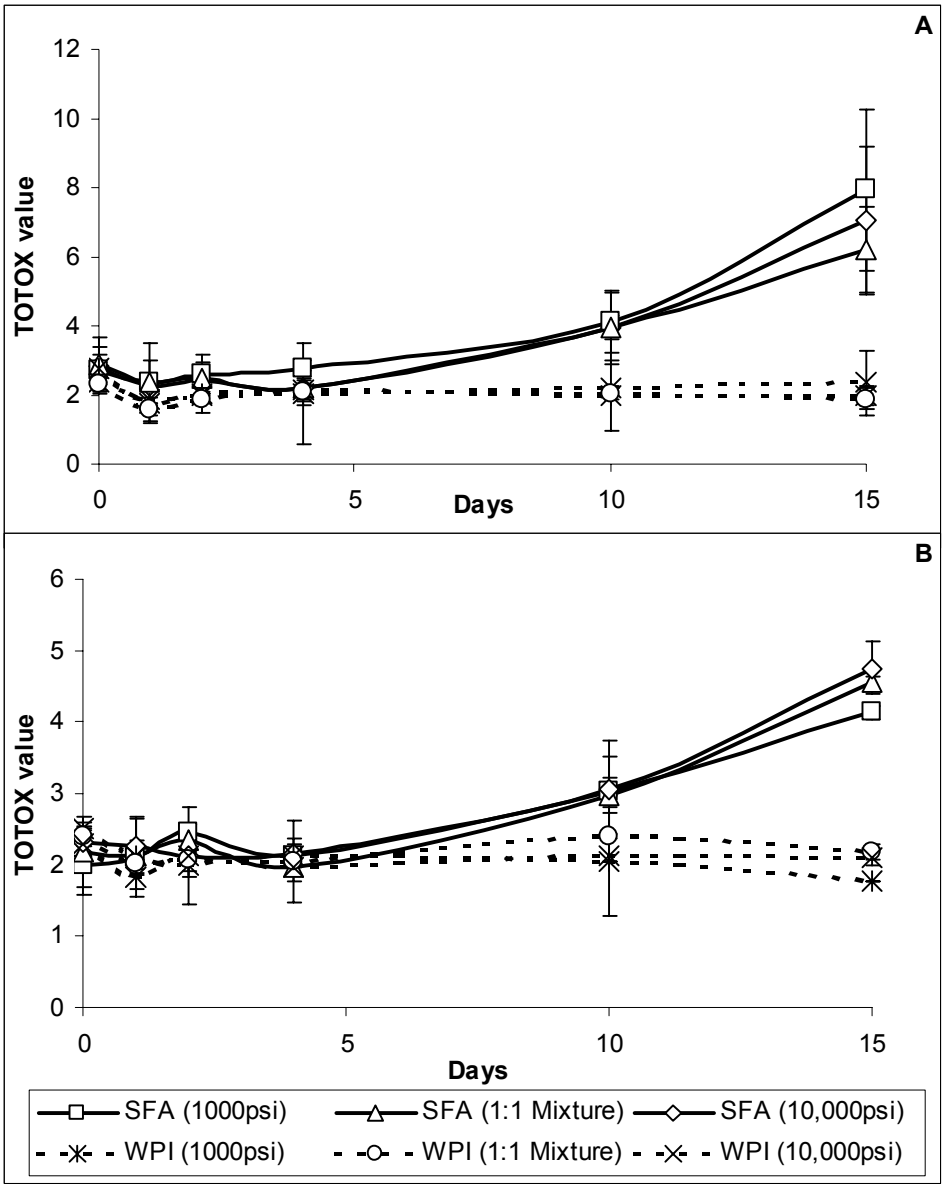
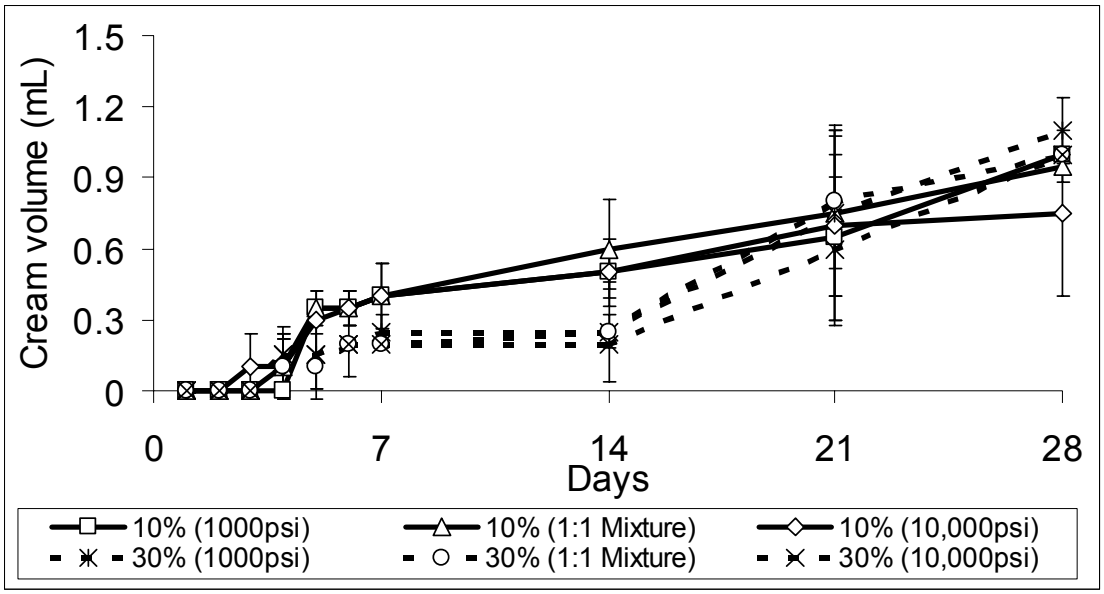

A
TOTOX value
Days
B
TOTOX value
Days
SFA (1000psi)
SFA (1:1 Mixture)
SFA (10,000psi)
WPI (1000psi)
WPI (1:1 Mixture)
WPI (10,000psi)

Figure 3.7 Cream volume separated out at top from structured lipid-based emulsions (10 mL) stabilized by whey protein isolate. Sucrose fatty acid ester-stabilized emulsions were stable to creaming throughout the study and are not shown. Data shown are the average of duplicate samples held at 25 ± 2°C. Error bars on chart represent standard deviation.


Cream volume (mL)
1.5
1.2
0.9
0.6
0.3
0
0
7
14
21
28
Days
10% (1000psi)
10% (1:1 Mixture)
10% (10,000psi)
30% (1000psi)
30% (1:1 Mixture)
30% (10,000psi)

CHAPTER 4

COPPER-CATALYZED OXIDATION OF A STRUCTURED LIPID-BASED EMULSION CONTAINING α-TOCOPHEROL AND CITRIC ACID: INFLUENCE OF pH AND NaCl[1]

[1] Osborn, H.T. and C.C. Akoh. Submitted to *Journal of Agricultural and Food Chemistry*, 11/22/2002.

ABSTRACT: The effects of salt and pH on copper-catalyzed lipid oxidation in structured lipid-based emulsions were evaluated. Ten percent oil-in-water emulsions were formulated with a canola oil/caprylic acid structured lipid and stabilized with 0.5% whey protein isolate. α-Tocopherol and citric acid were added to the emulsions to determine how changes in pH or the addition of NaCl affected their antioxidant activity. The peroxide values, anisidine values, and TOTOX values of emulsions stored at 50°C were measured over an eight-day period. Increased lipid oxidation occurred in the pH 7.0 emulsions and when 0.5 M NaCl was added to the pH 3.0 samples. Adding α-tocopherol, citric acid, or a combination of the two compounds slowed the formation of hydroperoxides and their subsequent decomposition products in pH 3.0 emulsions.

KEYWORDS: Anisidine Value, Citric Acid, Copper, Emulsions, Lipid Oxidation, Peroxide Value, Structured Lipids, α-Tocopherol, TOTOX Value, Whey Protein Isolate

INTRODUCTION

The ability of structured lipids (SLs) to combine the beneficial characteristics of component fatty acids into one triacylglycerol (TAG) molecule enhances the role fats and oils play in food, nutrition, and health applications (*1*). SLs may provide the most effective means of delivering desired fatty acids for nutritive or therapeutic purposes, and for targeting specific diseases and metabolic conditions (*2*). Structured TAGs that contain medium-chain fatty acids (MCFAs) may provide a vehicle for rapid hydrolysis and absorption due to their smaller molecular size and greater water solubility in comparison to long-chain TAGs (*3*). The combination of an increased absorption rate for MCFAs and beneficial long chain fatty acids (LCFAs) in one TAG should make SLs very attractive to the medical community and functional food manufacturers. However, the

food industry has been slow to incorporate SLs into their product formulations. Before SLs are accepted by the food industry, a better understanding of the physicochemical mechanisms of lipid oxidation is needed for SL-based products.

Lipid oxidation is of great concern to the food industry because it causes changes in the quality attributes of foods, such as taste, texture, shelf life, appearance, and nutritional profile (*4*). Although substantial amounts of fats are consumed as food emulsions, most oxidation studies to date have been carried out in bulk oils. Food emulsions contain an array of components such as salt, sugar, metals, and emulsifiers that affect the rate of oxidation and may interfere with added antioxidants (*5*). Salts act as pro-oxidants or antioxidants depending on the nature of the system involved (*4*), so their effect must be evaluated on a case-by-case basis. Contradictory results have been reported regarding the effects of pH on lipid oxidation. Mancuso et al. (*6*) reported that oxidation increases with increasing pH, whereas other researchers have found the opposite effect (*7,8*). Iron-catalyzed lipid oxidation has been the subject of many recent studies (*9-13*). However, the rate of iron-mediated oxidation is much slower than that with an equal concentration of copper, and most foods contain 3.1 to 31 μM Cu^{2+} (*14*). Therefore, more studies on copper-catalyzed lipid oxidation in various emulsion systems are needed.

The most commonly used method of retarding lipid oxidation in fatty foods is the addition of antioxidants (*15*). Antioxidants are classified according to their mechanisms of action as either primary or secondary antioxidants. Primary antioxidants are capable of accepting free radicals so that they can delay the initiation step or interrupt the propagation step of autoxidation (*4*). Tocopherols are highly potent primary antioxidants

that are widely employed in the food industry because they are able to react with the lipid hydroperoxyl and with alkoxyl radicals formed by the metal-catalyzed decomposition of hydroperoxides (*16*). Secondary antioxidants can retard lipid oxidation through a variety of mechanisms, including: chelating of metals, replenishing hydrogen to primary antioxidants, scavenging oxygen, and deactivating reactive species (*4*). Citric acid can chelate metal ions by forming bonds between the metal and the carboxyl or hydroxyl groups of the citric acid molecule. This secondary antioxidant is very effective in retarding the oxidative deterioration of lipids in foods and is commonly added to vegetable oils after deodorization (*17*). Combinations of chelators and radical scavengers often result in synergistic inhibition of lipid oxidation, because multicomponent antioxidant systems can inhibit oxidation at many different phases of oxidation. Citric acid can have a "sparing" effect on tocopherol. The chelator decreases the number of free radicals generated in a system by inhibiting metal-catalyzed oxidation (*18*). The physical state of lipid systems has been shown to affect antioxidant activities and further studies are needed to better understand the effect of pH on interfacial lipid oxidation (*19*).

The objective of this study was to determine the effect of salt and pH on copper-catalyzed lipid oxidation in structured lipid-based emulsions. α-Tocopherol, citric acid, and a combination of the two compounds were incorporated into the emulsions to determine their effect on lipid oxidation and how changes in pH or the addition of NaCl affected their antioxidant activity.

MATERIALS AND METHODS

Materials. Canola oil was purchased from a local supermarket. Caprylic acid (purity > 98%), citric acid, α-tocopherol, and cupric sulfate were purchased from Sigma

Chemical Company (St. Louis, MO). A sn-1,3 specific immobilized lipase from *Rhizomucor miehei* (IM 60) was obtained from Novo Nordisk A/S (Bagsvaerd, Denmark). Whey protein isolate (WPI) (#27361) was provided by Land O'Lakes (St. Paul, MN). All other reagents were purchased from Fisher Scientific (Fair Lawn, NJ).

Structured Lipid Production. The SL was produced in a packed bed bioreactor using optimal conditions previously reported (*20*) for reacting canola oil and caprylic acid. The product was purified using a KDL-4 short-path distillation unit (UIC Inc., Joliet, IL). The oil was passed through the distillation apparatus three times under the following conditions: holding temperature, 25°C; heating oil temperature, 185°C; cooling water temperature, 15°C; pressure, < 0.01 Torr. The purified SL product contained the following fatty acids (mol %): 37.3% C8:0, 1.8% C16:0, 1.7% C18:0, 47.3% C18:1, 8.9% C18:2, and 3.0% C18:3 as determined by gas chromatography of methyl esters (*21*).

Emulsion Preparation. Ten percent oil-in-water emulsions were prepared with the canola oil/caprylic acid SL, 10 mM sodium phosphate buffer, and 0.5% whey protein isolate (WPI). α-Tocopherol was premixed into the SL, while citric acid was added to the buffer. Total antioxidant addition levels were 0.02% of the oil weight in each emulsion. Mixed antioxidant systems contained equal parts of tocopherol and citric acid. NaCl (0.5 M) was added to the appropriate emulsion samples. The pH of the emulsions was adjusted to 3.0 or 7.0 by adding HCl or NaOH. The emulsions were passed through a high-pressure valve homogenizer (Emulsiflex, C5, Avestin, CA) six times at 10,000 psi. All samples were held on ice during processing. Sodium azide (1 mM) was added to the emulsions to slow microbial growth. Cupric sulfate (50 μM) was added to the emulsions

immediately prior to storage. Particle size distribution was measured by integrated light scattering (Mastersizer S, Malvern Instruments, Malvern, UK) using standard optical parameters to ensure that similar apparent particle diameters ($D_{3,2}$ values < 2.0 μm) were achieved in the emulsions during homogenization. This was done to eliminate the potential for droplet size differences to affect lipid oxidation rates.

Oxidation Experiments. Emulsion samples were allowed to oxidize in a 50°C covered water bath for eight days. The primary and secondary oxidation products were measured in the emulsion samples after 0, 1, 2, 4, and 8 days of storage. Oil was extracted from the emulsions by adding isooctane/isopropanol (3:2, v/v), vortexing 3 times for 10 s each, and centrifuging for 5 min at 1000 rpm. The clear upper layer was collected and the solvent was evaporated under nitrogen. Peroxide values (PV) were determined using the International Dairy Federation method described in detail by Shantha and Decker (*22*). Anisidine values (AV) were determined according to the AOCS Official Method Cd 18-90 (*23*). This method determines the amount of aldehyde (principally 2-alkenals and 2,4-alkadienals) present in the oil (*24*). The TOTOX value was calculated as: TOTOX value = 2(PV) + AV (*24*). The TOTOX value combines evidence about the past history of an oil with its present state and is useful for estimating oxidative deterioration of food lipids (*24*).

Statistical Analysis. All experiments were performed on duplicate samples. Statistical analyses were conducted with the SAS (*25*) software package. Analyses of variance were performed by ANOVA procedures. Significant differences ($p < 0.05$) were determined by the least squares difference method.

RESULTS AND DISCUSSION

Control (CON) samples that did not contain copper were included in this experiment to verify that the addition of 50 μM cupric sulfate affected the rate of lipid oxidation of the SL-based emulsions. Significantly ($p < 0.05$) greater PV, AV, and TOTOX values were observed in the copper-catalyzed control (Cu CON) samples compared to their CON counterparts on all days of storage (excluding day 0) for emulsions at pH 7.0 (**Figures 4.1, 4.3, and 4.5**). The addition of copper significantly ($p < 0.05$) increased the PV of the emulsions at pH 3.0 on days 4 and 8 of the study (**Figure 4.2**). The AV and TOTOX values were significantly ($p < 0.05$) increased by the addition of copper to the pH 3.0 emulsions on all days of storage (excluding day 0) (**Figures 4.4 and 4.6)**. The significant increase in lipid oxidation due to copper addition was expected because transition metals are known to accelerate lipid oxidation reactions by hydrogen abstraction and peroxide decomposition, which results in the formation of free radicals (*18*).

Effect of pH and NaCl on Oxidation. The pH of the emulsions had a significant ($p < 0.05$) effect on the primary (PV) and secondary oxidation (AV) products on days 1, 2, 4, and 8 of the study. On the final day of analysis, the hydroperoxide levels were greater in the copper-catalyzed emulsions at pH 3.0 compared to their pH 7.0 counterparts (**Figures 4.1 and 4.2**). However, the final anisidine values were higher in the pH 7.0 emulsions (**Figures 4.3 and 4.4**). Emulsion pH also significantly ($p < 0.05$) affected the total oxidation (TOTOX) on days 1, 2, and 4. On these days, TOTOX values for emulsions at pH 7.0 were higher than those at pH 3.0 (**Figures 4.5 and 4.6**). Similar pH effects on lipid oxidation have been reported for salmon oil-based emulsions. Lipid

peroxide formation in this system was greater at pH 3.0 compared to 7.0, while differences in thiobarbituric acid reactive substances (TBARS) formation were not detected between the two pHs (*26*).

When taken alone, the hydroperoxide data indicates that low pH further promotes copper-catalyzed lipid oxidation in emulsions. Hydroperoxides decompose rapidly in the presence of heat or transition metals via the Fenton reaction (*15*). Therefore, when the results of the primary and secondary oxidation studies are combined it seems more likely that the lower peroxide values at pH 7.0 were not due to decreased lipid oxidation rates, but rather to copper's increased ability to decompose the lipid hydroperoxides into secondary products at pH 7.0. The emulsion droplets carry a negative charge at pH 7.0, because the anionic whey proteins are above their isoelectric point (pI) of ~5.0 (*27*). When the surface charge of dispersed lipids as micelles is negative, metal-catalyzed oxidation rates are much higher than they are at positively charged interfaces (*18*). This effect presumably exists because of the electrostatic attraction between the positively charged metal and the negatively charged emulsion droplet membrane (*28*). Whey proteins are below their isoelectric point (pI) in the pH 3.0 systems (*27*), thereby producing positively charged emulsion droplets. Hence, at pHs < 5.0 the positively charged copper ions can no longer bind to the emulsion droplets, which explains the decreased secondary oxidation rates in the pH 3.0 emulsions compared to their pH 7.0 counterparts. Due to this phenomenon, food manufacturers may be able to lower the pH below the pI in protein-stabilized emulsions as a strategy for decreasing copper-catalyzed lipid oxidation. A similar strategy was proposed previously for iron-catalyzed lipid oxidation (*9*).

Sodium chloride did not significantly affect the hydroperoxide formation rate after eight days of storage at 50°C at either pH (**Figures 4.1 and 4.2**). However, NaCl significantly ($p < 0.05$) increased secondary and total oxidation at pH 3.0 (**Figures 4.4 and 4.6**). Final TOTOX value means for acidic emulsions with and without added NaCl are also compared in **Table 4.1**. Excess Na^+ is known to compete with transition metals for surface binding sites on negatively-charged emulsion droplets (*29*). In pH 3.0 emulsions containing added salt, the Cl^- are likely binding to the positively-charged surface of WPI-stabilized droplets, thus shielding the surface charge. Therefore, the repulsion forces between Cu^{2+} and the emulsion droplets would be reduced and may allow the copper to associate more closely with the emulsion droplet and increase lipid oxidation compared to the emulsions that do not contain added NaCl. When WPI is negatively charged at pH 7.0 strong repulsion forces between the emulsion droplets and Cu^{2+} do not exist in the absence or presence of NaCl. Because salt is an ubiquitous ingredient in foods, processors must be mindful of its ability to promote lipid oxidation in the presence of metals and incorporate antioxidants into their product formulations accordingly.

Effect of α-Tocopherol and Citric Acid on Oxidation. Radical scavengers and metal sequestrants were the antioxidant types utilized for this study. The former class, which includes tocopherols, does not block the initial generation of radicals, but merely reacts with them to form less reactive radicals. The second category consists of chelating agents, such as citric acid, that either precipitate the metal or suppress its reactivity by occupying all coordination sites (*14*).

The activity of the antioxidants in this study varied according to the pH of the emulsion, but was not influenced by the NaCl addition. At pH 3.0, the addition of tocopherol, citric acid, or a combination of the two compounds resulted in significantly ($p < 0.05$) lower PV and AV compared to the Cu CON samples (**Figure 4.2, 4.4, and 4.6**). Although antioxidant systems containing antioxidants with different mechanisms of action are currently hailed as the most effective strategy for inhibiting lipid oxidation (*18*), tocopherol and citric acid did not have synergistic effects on hydroperoxide or aldehyde formation in this study. Instead, all antioxidants (tocopherol, citric acid, and a combination of the two) had a similar effect on the rate of lipid oxidation (**Figures 4.2, 4.4, and 4.6**). At pH 7.0 none of these added antioxidants significantly affected the oxidation rates.

Huang et al. (*19*) reported that the oxidative stability of α-tocopherol was highest at pH 3.0 and lowest at pH 7.0 in Tween 20 micelle solutions. Their study showed that α-tocopherol was 90% depleted in the pH 7.0 systems after eight days of storage at 60°C, while only 20% depletion occurred in the pH 3.0 systems. Additionally, the hydrogen donating ability of α-tocopherol at the oil-water interface was reduced by protonation of its phenolic hydroxyl group at low pH. These authors concluded that the antioxidant activity of α-tocopherol depends on both its hydrogen-donating activity and its depletion rate (*19*). A similar rate of tocopherol depletion may have occurred in the pH 7.0 SL-based emulsions and led to the decreased antioxidant effect of α-tocopherol on day 8 in the pH 7.0 emulsions compared to the pH 3.0 emulsions.

The increased efficacy of citric acid at low pH may have resulted due to the increased solubility of transition metals in those environments (*27*). Additionally, the

activity of copper can be controlled by binding to proteins (*18*). Therefore, in the pH 7.0 systems the copper ions may have been bound to the negatively charged emulsion droplets and were, therefore, unable to complex with the citric acid. However, when the pH was lowered to 3.0, the copper was released from the proteins and became exposed to the citric acid. This may account for the increased antioxidant activity observed in the pH 3.0 emulsions that contained citric acid.

Copper-catalyzed lipid oxidation in SL-based emulsions appeared to be influenced by the system pH and the presence of NaCl. Emulsions at pH 7.0 had greater rates of lipid oxidation than their pH 3.0 counterparts. NaCl addition increased total oxidation of the acidic emulsions. Adding α-tocopherol, citric acid, or a combination of the two compounds to the pH 3.0 emulsions successfully slowed the formation of hydroperoxides and their subsequent decomposition into secondary carbonyl compounds. The results of this study will aid in understanding the complexity of lipid oxidation in real food emulsions prepared with canola oil/caprylic acid structured lipids and may expedite the incorporation of structured lipids into food products.

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Table 4.1 Mean TOTOX Values[a] for Structured Lipid-Based Emulsions at pH 3.0 and 7.0 After Eight Days of Storage at 50°C.

Emulsion[b]	pH 3.0		pH 7.0	
	No added NaCl	0.5 M NaCl	No added NaCl	0.5 M NaCl
CON	7.56 ± 0.04 a	18.94 ± 13.44 a	4.15 ± 1.68	4.46 ± 0.61
Cu CON	75.22 ± 4.35 a	84.15 ± 4.39 b	69.90 ± 8.29	60.03 ± 8.70
CIT	38.41 ± 1.00 a	53.32 ± 3.21 b	69.67 ± 5.97	74.16 ± 12.13
TOC	39.04 ± 4.41 a	48.46 ± 3.74 b	60.76 ± 8.51	75.24 ± 11.03
CIT + TOC	37.89 ± 2.89 a	48.20 ± 4.57 b	71.21 ± 12.28	84.87 ± 2.84

[a] Data shown are the average of duplicate samples. Means in the same row followed by different letters are significantly different at $p < 0.05$. Emulsions at pH 3.0 and pH 7.0 were analyzed separately.

[b] CON – contained no added cupric sulfate; Cu CON - contained 50 μM cupric sulfate; CIT - contained 50 μM cupric sulfate and citric acid (0.02% wt of oil); TOC - contained 50 μM cupric sulfate and α-tocopherol (0.02% wt of oil); CIT + TOC - contained 50 μM cupric sulfate, citric acid (0.01% wt of oil), and α-tocopherol (0.01% wt of oil).

Figure 4.1 Copper-catalyzed changes in peroxide values over time in structured lipid-based emulsions at pH 7.0. Cu CON - contained 50 μM cupric sulfate; CIT - contained 50 μM cupric sulfate and citric acid (0.02% wt of oil); TOC - contained 50 μM cupric sulfate and α-tocopherol (0.02% wt of oil); CIT + TOC - contained 50 μM cupric sulfate, citric acid (0.01% wt of oil), and α-tocopherol (0.01% wt of oil); CON – contained no added cupric sulfate. Data shown are the average of duplicate samples held at 50°C. Error bars on chart represent standard deviation.

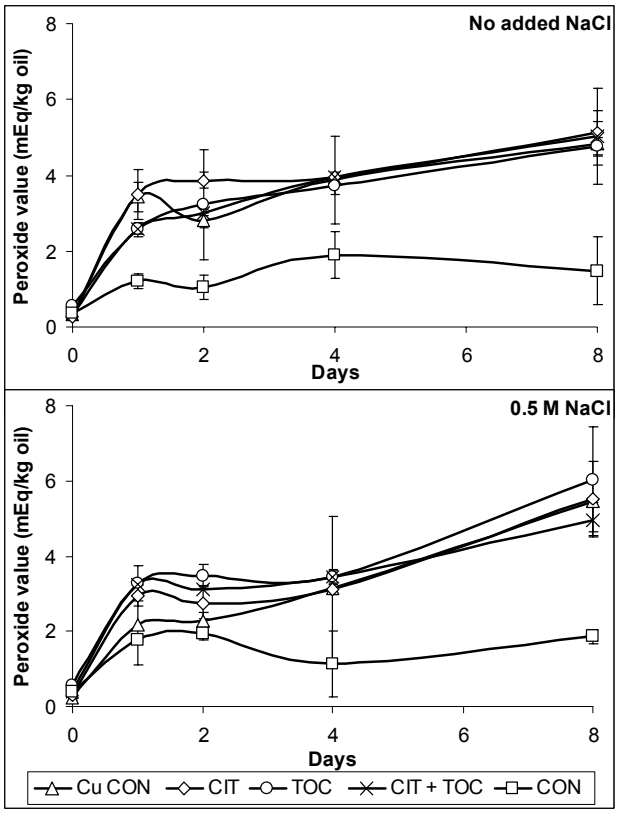

No added NaCl
Peroxide value (mEq/kg oil)
Days
0.5 M NaCl
Peroxide value (mEq/kg oil)
Days
Cu CON
CIT
TOC
CIT + TOC
CON

Figure 4.2 Copper-catalyzed changes in peroxide values over time in structured lipid-based emulsions at pH 3.0 (see **Figure 4.1** for sample abbreviations). Data shown are the average of duplicate samples held at 50°C. Error bars on chart represent standard deviation.

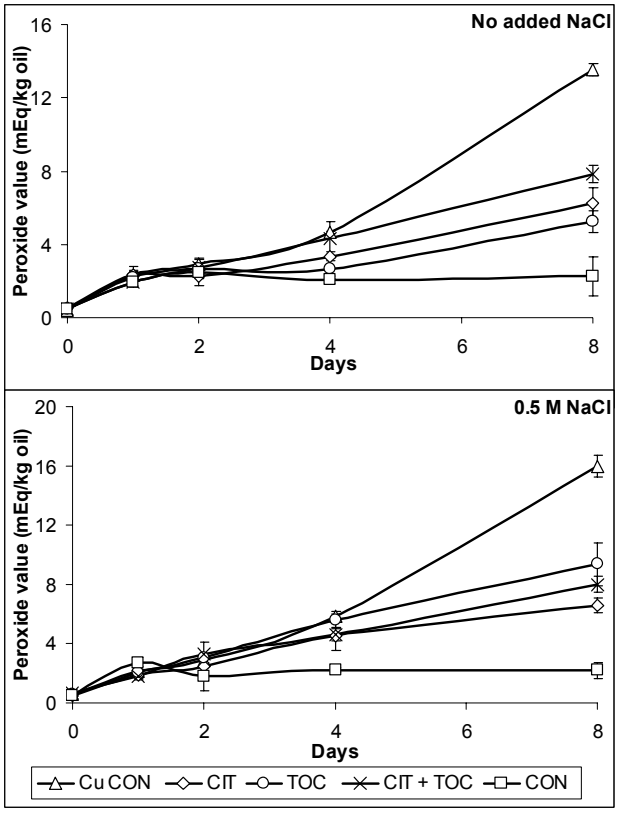

No added NaCl
Peroxide value (mEq/kg oil)
16
12
8
4
0
0
2
4
6
8
Days
0.5 M NaCl
Peroxide value (mEq/kg oil)
20
16
12
8
4
0
0
2
4
6
8
Days
Cu CON
CIT
TOC
CIT + TOC
CON

Figure 4.3 Copper-catalyzed changes in anisidine values (AV) over time in structured lipid-based emulsions at pH 7.0 (see **Figure 4.1** for sample abbreviations). Data shown are the average of duplicate samples held at 50°C. Error bars on chart represent standard deviation.

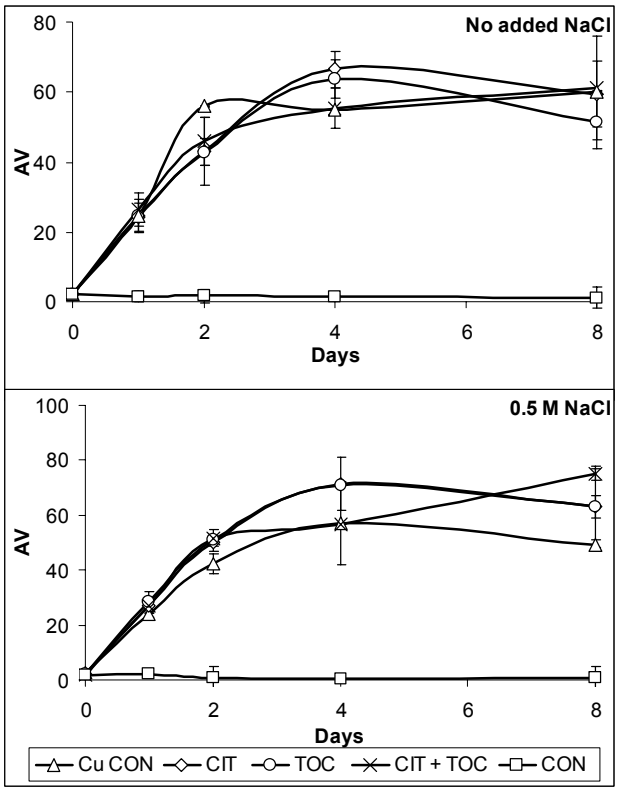

No added NaCl
AV
Days
0.5 M NaCl
AV
Days
Cu CON
CIT
TOC
CIT + TOC
CON

Figure 4.4 Copper-catalyzed changes in anisidine values (AV) with time in structured lipid-based emulsions at pH 3.0 (see **Figure 4.1** for sample abbreviations). Data shown are the average of duplicate samples held at 50°C. Error bars on chart represent standard deviation.

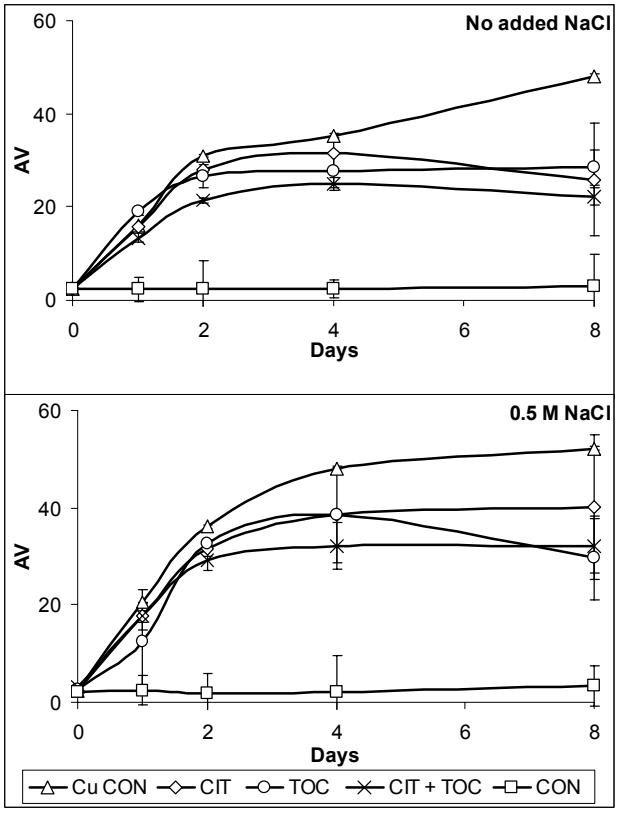

No added NaCl
0.5 M NaCl
AV
Days
0
20
40
60
0
2
4
6
8
Cu CON
CIT
TOC
CIT + TOC
CON

Figure 4.5 Copper-catalyzed changes in total oxidation (TOTOX) with time in structured lipid-based emulsions at pH 7.0 (see **Figure 4.1** for sample abbreviations). Data shown are the average of duplicate samples held at 50°C. Error bars on chart represent standard deviation.

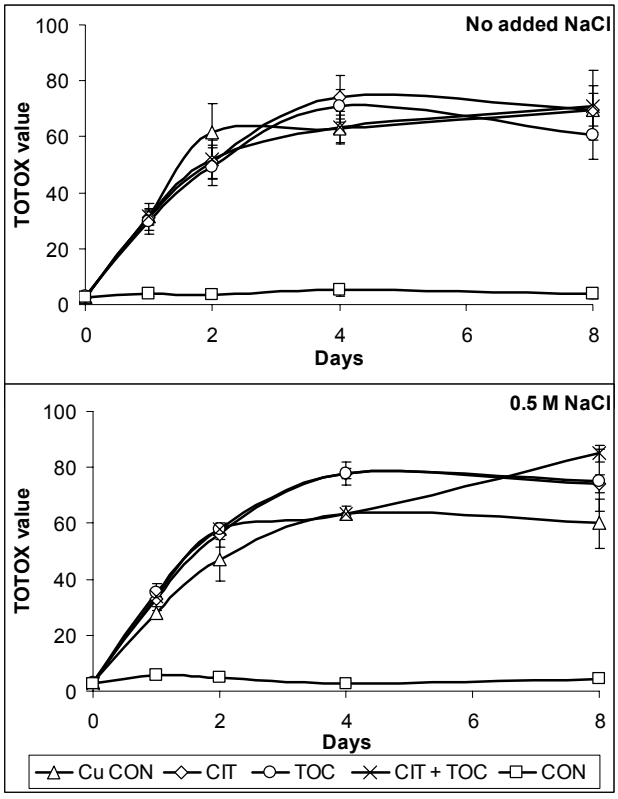
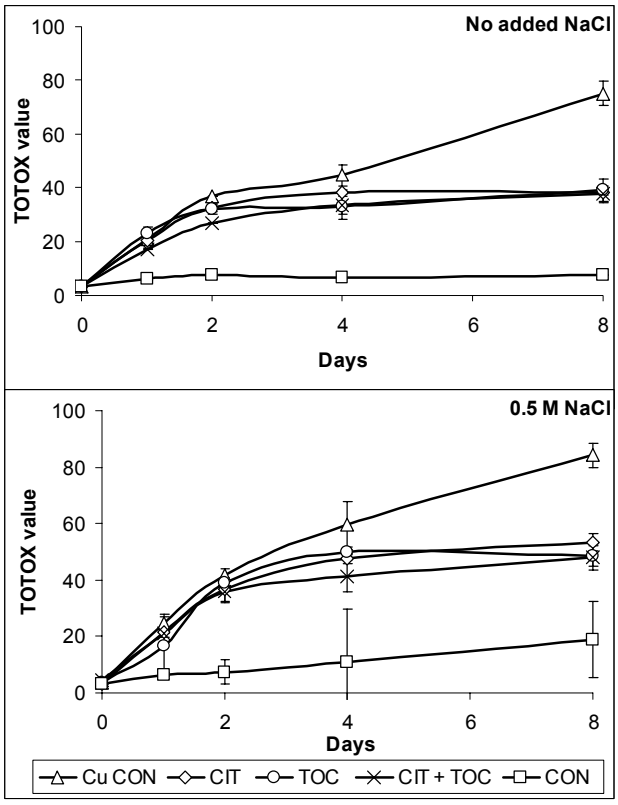

No added NaCl
TOTOX value
Days
0.5 M NaCl
TOTOX value
Days
Cu CON
CIT
TOC
CIT + TOC
CON

Figure 4.6 Copper-catalyzed changes in total oxidation (TOTOX) with time in structured lipid-based emulsions at pH 3.0 (see **Figure 4.1** for sample abbreviations). Data shown are the average of duplicate samples held at 50°C. Error bars on chart represent standard deviation.


No added NaCl
TOTOX value
100
80
60
40
20
0
0
2
4
6
8
Days
0.5 M NaCl
TOTOX value
100
80
60
40
20
0
0
2
4
6
8
Days
Cu CON
CIT
TOC
CIT + TOC
CON

CHAPTER 5

EFFECTS OF NATURAL ANTIOXIDANTS ON IRON-CATALYZED LIPID OXIDATION OF STRUCTURED LIPID-BASED EMULSIONS[1]

[1] Osborn, H.T. and C.C. Akoh. Submitted to *Journal of the American Oil Chemists' Society*, 12/12/2002.

ABSTRACT: The effects of iron, pH, and natural antioxidants (α-tocopherol, gallic acid, and quercetin) on oxidation of structured lipid-based emulsions were evaluated. Ten percent oil-in-water emulsions were formulated with a canola oil/caprylic acid structured lipid and stabilized with 0.5% whey protein isolate. The peroxide values, anisidine values, and TOTOX values of emulsions stored at 50°C were measured over a 15-day period. Iron significantly ($p < 0.05$) increased lipid oxidation rates compared to control emulsions. Greater iron-catalyzed lipid oxidation occurred in the pH 3.0 emulsions compared to their pH 7.0 counterparts. Quercetin and gallic acid exhibited significant ($p < 0.05$) prooxidant effects on total oxidation in the pH 3.0 emulsions. Emulsions at pH 7.0 were relatively stable to oxidation throughout the storage period. Due to the ability of some of these natural antioxidants to exhibit prooxidant activity, care should be exercised when adding them to food systems containing transition metals.

KEYWORDS: Anisidine Value, Emulsions, Gallic Acid, Iron-Catalyzed Lipid Oxidation, Peroxide Value, Quercetin, Structured Lipid, α-Tocopherol, TOTOX Value, Whey Protein Isolate

INTRODUCTION

Much research has been devoted to the production, functionality, and potential health benefits of structured lipids (SLs) in recent years. Among the fatty acids considered for SL synthesis are medium-chain fatty acids (MCFAs) and polyunsaturated fatty acids (PUFAs). Medium-chain fatty acids are readily metabolized in the body as a quick source of energy, while long-chain fatty acids are required as sources of essential fatty acids (1). Unfortunately, PUFAs are highly susceptible to oxidation in foods, which

is of great concern to the food industry because oxidation leads to the development of undesirable "off-flavors" and potentially toxic reaction products (2).

Iron is the most common transition metal found in foods. It promotes lipid oxidation by catalyzing the breakdown of peroxides into free radicals (3). In oil-in-water emulsion systems, the charge status of emulsion droplets greatly influences iron-catalyzed lipid oxidation. Attractive/repulsive electrostatic interactions between charged emulsion droplets and charged prooxidants greatly affects the location and hence the activity of transition metals. Mei et al. (4) showed that Fe^{3+} and Fe^{2+} are strongly bound to negatively charged sodium dodecyl sulfate (SDS)-stabilized emulsion droplets, but not to positively charged or uncharged emulsion droplets. Consequently, lipid oxidation occurred much faster in the negatively charged emulsions when positively charged transition metal ions were present. While SDS is not a common emulsifier in the food industry, proteins with pHs away from their isoelectric point (pI) will create charged emulsion droplets in food systems.

The incorporation of antioxidants into foods that contain fats and oils is effectively helpful in retarding the metal-catalyzed oxidation of lipids (5). Due to safety concerns, there is currently much interest in replacing synthetic antioxidants with natural antioxidants. Tocopherols are the most important natural antioxidants in the food industry and their antioxidant mechanism involves the donation of hydrogen to a peroxy radical (6).

Numerous naturally occurring phenolic antioxidants have also been identified from plant sources (6). However, their effectiveness is often difficult to predict because there are several different mechanisms by which phenolic compounds influence lipid

oxidation rates (7). Hydroxybenzoic acids, including gallic acid, are phenolic compounds that can form metal complexes (6). However, the antioxidant activity of these compounds varies greatly and is dependent on the food system (6). Quercetin has gained attention as a potent antioxidant because of its ability to scavenge hydroxyl radicals and superoxide anions (6). Unfortunately, quercetin is autoxidized under certain conditions, which leads to an increase in hydroxyl radicals (8). Additionally, both quercetin and gallic acid can reduce Fe^{3+} to the more active Fe^{2+}, thereby increasing lipid oxidation (7).

In this study, the effects of pH and natural antioxidants on iron-catalyzed lipid oxidation of canola oil/caprylic acid SL-based emulsions were evaluated. Primary, secondary, and total oxidation were determined in the whey protein isolate-stabilized emulsions containing α-tocopherol, gallic acid, and quercetin over a 15-day storage period.

EXPERIMENTAL PROCEDURES

Materials. Canola oil was purchased from a local supermarket. Caprylic acid (purity > 98%), gallic acid, quercetin, α-tocopherol, and ferrous sulfate were purchased from Sigma Chemical Company (St. Louis, MO). A sn-1,3 specific immobilized lipase from *Rhizomucor miehei* (IM 60) was obtained from Novo Nordisk A/S (Bagsvaerd, Denmark). Whey protein isolate (WPI) (#27361) was provided by Land O'Lakes (St. Paul, MN). All other reagents were purchased from Fisher Scientific (Fair Lawn, NJ).

Structured lipid production. The SL was produced in a packed bed bioreactor using optimal conditions previously reported (9) for reacting canola oil and caprylic acid. The product was purified using a KDL-4 short-path distillation unit (UIC Inc., Joliet, IL). The oil was passed through the distillation apparatus three times under the following

conditions: holding temperature, 25 °C; heating oil temperature, 185 °C; cooling water temperature, 15 °C; pressure, < 0.01 Torr. The purified SL product contained the following fatty acids (mol %): 37.3% C8:0, 1.8% C16:0, 1.7% C18:0, 47.3% C18:1, 8.9% C18:2, and 3.0% C18:3 as determined by gas chromatography of methyl esters according to a procedure previously described (10).

Emulsion preparation. Ten percent oil-in-water emulsions were prepared with the canola oil/caprylic acid SL, 10 mM phosphate buffer, and 0.5% whey protein isolate (WPI). α-Tocopherol was added directly to the SL. Quercetin was dissolved in ethanol and then added to the oil. The ethanol was then evaporated under N_2. Gallic acid was mixed into the phosphate buffer. Total antioxidant addition levels were 0.02% of the oil weight in each emulsion. The pH of the emulsions was adjusted to 3.0 and 7.0 by adding HCl (0.1 M) and NaOH (0.1 M), respectively. The emulsions were passed through a high-pressure valve homogenizer (Emulsiflex, C5, Avestin, CA) six times at 10,000 psi. All samples were held on ice during processing. Sodium azide (1 mM) was added to the emulsions to slow microbial growth. Ferrous sulfate (50 or 100 μM) was added to the emulsions immediately prior to storage. Particle size distribution was measured by integrated light scattering (Mastersizer S, Malvern Instruments, Malvern, UK) using standard optical parameters to ensure that similar droplet sizes were formed in the emulsions during homogenization. The mean apparent particle diameter ($D_{3,2}$ value) was 0.82 ± 0.09 μm for emulsions in this study.

Oxidation experiments. Emulsion samples were allowed to oxidize in a 50°C covered water bath for 15 days. The primary and secondary oxidation products were measured in the emulsion samples after 0, 1, 2, 4, 8, and 15 days of storage. Oil was

extracted from the emulsions by adding isooctane/isopropanol (3:2, v/v), vortexing 3 times for 10 s each, and centrifuging for 5 min at 1000 rpm. The clear upper layer was collected and the solvent was evaporated under nitrogen. Peroxide values (PV) were determined using the International Dairy Federation method described by Shantha and Decker (11). Anisidine values (AV) were determined according to the AOCS Official Method Cd 18-90 (12). This method determines the amount of aldehyde (principally 2-alkenals and 2,4-alkadienals) present in the oil (13). The TOTOX value was calculated as: TOTOX value = 2(PV) + AV (13). The TOTOX value combines evidence about the past history of an oil with its present state and is useful for estimating oxidative deterioration of food lipids (13).

Statistical analysis. All experiments were performed on duplicate samples. Statistical analyses were conducted with the SAS (14) software package. Analyses of variance were performed by ANOVA procedures. Significant differences ($p < 0.05$) were determined by the least squares difference method.

RESULTS AND DISCUSSION

Effect of iron. Control samples that did not contain added iron or antioxidants were included in this experiment. This was done to determine the effect of added iron on the rate of lipid oxidation of SL-based emulsions. Additionally, iron was added at two levels (50 and 100 μM) to determine the effect of concentration on the rate of oxidation.

Peroxide values were significantly ($p < 0.05$) increased in emulsions containing iron, compared to the corresponding control samples in all cases, except the pH 3.0 emulsion that contained 50 μM iron (Figure 5.1). Secondary oxidation was higher in the pH 3.0 emulsions containing iron than their control counterparts (Figure 5.2). However,

at pH 7.0 significant ($p < 0.05$) differences between the AV of the Fe control and control samples did not exist (Figure 5.2). Although the PV and AV results may seem contradictory, transition metals are known to both decompose peroxides and produce free radicals, and therefore, changes in peroxide concentrations in the presence of metals actually represents a balance between peroxide formation and decomposition (15). Thus, the similar PV between Fe control (50 μM) and control samples at pH 3.0 do not necessarily correspond to equal amounts of primary oxidation, because of the rapid decomposition that may also be occurring in iron-catalyzed emulsion. When PV and AV results were combined into TOTOX values, the effect of iron became significant ($p < 0.05$) in all emulsions. At both pHs, greater total oxidation occurred in SL-based emulsions containing iron compared to their control counterparts on the final day of the study (Figure 5.3). The prooxidant effect of ferrous sulfate was expected because iron is known to stimulate lipid peroxidation by the Fenton reaction, and also to accelerate peroxidation by decomposing lipid hydroperoxides into peroxyl and alkoxyl radicals. These radical species can then abstract hydrogen and further perpetuate the chain reaction of lipid peroxidation (5).

At pH 3.0, greater amounts of primary, secondary, and total oxidation occurred in the presence of higher concentrations of iron (100 versus 50 μM) on the final day of the study (Figures 5.1-5.3). However, at pH 7.0 no significant differences existed between the final PV, AV, or TOTOX values of emulsions containing 50 or 100 μM ferrous sulfate (Figures 5.1-5.3). Iron is more soluble, and therefore better able to promote lipid oxidation, at pH 3.0 compared to pH 7.0 (3), which may explain the increased prooxidant activity of iron at higher concentration in low pH systems.

Effect of pH. Emulsion pH significantly ($p < 0.05$) affected the PV on days 1, 4 and 8. However PV of emulsions at pH 3.0 and 7.0 were no longer different at day 15 (Figure 5.1). Emulsion pH affected secondary oxidation and total oxidation to a greater extent. The influence of pH on secondary oxidation was significant ($p < 0.05$) on days 2-15 and on total oxidation on all days of the study (excluding day 0). Secondary and total oxidation both increased in absolute values when the pH was decreased from 7.0 to 3.0 in emulsions containing either 50 or 100 μM ferrous sulfate (Figures 5.2 and 5.3). Similarly, low pH promoted oxidation in fish oil-enriched mayonnaise (16). Mei et al. (17) also reported that lipid oxidation increased with decreasing pH in corn oil emulsions prepared with the anionic surfactant sodium dodecyl sulfate (SDS). These authors proposed that the increases in oxidation were due to increased iron solubility at lower pHs.

Effect of natural antioxidants. The influence of natural antioxidants, α-tocopherol, gallic acid, and quercetin (Scheme 5.1), on iron-catalyzed lipid oxidation of SL-based emulsions at pH 3.0 and 7.0 were evaluated. The antioxidant variable had a significant ($p < 0.05$) effect on primary oxidation of the SL-based emulsions on days 1-8. In the pH 7.0 emulsions (50 μM Fe), α-tocopherol reduced the PV of the SL-based emulsion compared to the Fe control on day 15 (Figure 5.1). In the pH 3.0 emulsions containing 50 μM iron, all the natural compounds exhibited a prooxidant effect on primary oxidation at the final day of storage (Figure 5.1). In the emulsions containing higher concentrations of iron (100 μM), the PV for emulsions containing the natural compounds were not significantly different than the Fe control, except for gallic acid, which exhibited a prooxidant effect at pH 7.0 on day 15 (Figure 5.1). Decomposition of

hydroperoxides into secondary oxidation products is more closely related to flavor deterioration than hydroperoxide formation (18). Thus, when evaluating antioxidants, it is important to measure secondary and total oxidation before drawing conclusions on their efficacy and mechanisms of action.

Antioxidants significantly ($p < 0.05$) affected secondary oxidation on days 2-15 and total oxidation on all days (excluding day 0) of the study. Under all conditions, gallic acid and quercetin significantly ($p < 0.05$) increased AV compared to the Fe control samples on the final day of the study (Figure 5.2). Fukumoto and Mazza (19) also reported that phenolic compounds (including quercetin and gallic acid) exhibited prooxidant behavior at low concentrations (< 500 μM). The prooxidant activity of gallic acid may be related to the ability of flavonoids to undergo autoxidation catalyzed by transition metals to produce hydrogen peroxide and form hydroxyl radicals via Fenton chemistry (8). The prooxidant mechanism of flavonoids containing the catechol structural element, such as quercetin, has recently been demonstrated and described in detail by Rietjens et al. (20). The prooxidant activity is related to the formation of quinone type metabolites from the B-ring of catechol flavonoids. The oxidation of catechols to quinones generates potent electrophiles that promote oxidation (20). At pH 3.0, emulsions containing α-tocopherol had AV similar to the Fe controls. However, α-tocopherol was a prooxidant in the pH 7.0 emulsions in this study (Figure 5.2). α-Tocopherol is more stable at pH 3.0, less stable at pH 7.0 (21), and acts as an antioxidant or a prooxidant in different model conditions due to its ability to both scavenge oxy-radicals and reduce iron (22).

Total oxidation results (Figure 5.3) indicated that quercetin and gallic acid were both prooxidants at pH 3.0 in the presence of iron (50 or 100 μM), while α-tocopherol did not affect TOTOX values under acidic conditions. The physical location of phenolics will affect their ability to influence lipid oxidation. In oil-in-water emulsions, nonpolar phenolics are retained in the lipid droplets and are more effective antioxidants than their more polar counterparts (7). Additionally, the polar antioxidants (quercetin and gallic acid) are better able to interact with aqueous phase iron, which may explain their increased prooxidant activity, because the metal-reducing power of phenolics can increase oxidation reactions (7). In pH 7.0 emulsions, quercetin, gallic acid, and α-tocopherol did not significantly ($p < 0.05$) affect total oxidation compared to the Fe control sample in the presence of 50 μM iron. When higher concentrations of iron (100 μM) were incorporated into the emulsions, all three compounds exhibited prooxidant activity. The lack of full antioxidant activity exhibited by all three natural compounds in this study may be due to their inability to prevent the metal-catalyzed decomposition of peroxides. Jacobsen et al. (16) reported similar results for α-tocopherol in fish oil-enriched mayonnaise. However, it should be noted that all pH 7.0 emulsions were relatively stable to oxidation throughout storage at 50°C. TOTOX values of 10 correlate well with acceptable flavor scores for vegetable oils (23), and TOTOX values were less than 10 in all pH 7.0 emulsions at day 8 and remained below 15 on the final day of the current study.

Total oxidation was significantly ($p < 0.05$) higher in the pH 3.0 emulsions containing quercetin or gallic acid compared to their pH 7.0 counterparts. Phenolic compounds from olive oil also exhibited a marked prooxidant effect in the presence of

ferric ions at pH 3.5 (24). The increased prooxidant activity of quercetin and gallic acid at pH 3.0 may be due to their increased ability to reduce iron. Phenolic compounds can reduce up to 55-fold more iron per minute at pH 3.0 than at pH 7.0 (7). Therefore, food manufacturers must experiment carefully with gallic acid and quercetin before adding them to product formulations as antioxidants or functional ingredients due to their potential ability to exhibit prooxidant activity under certain conditions in the presence of transition metals.

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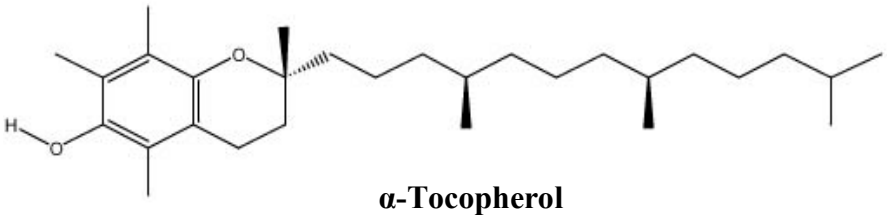
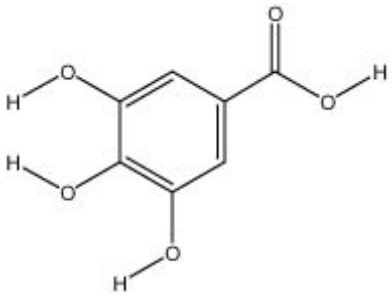
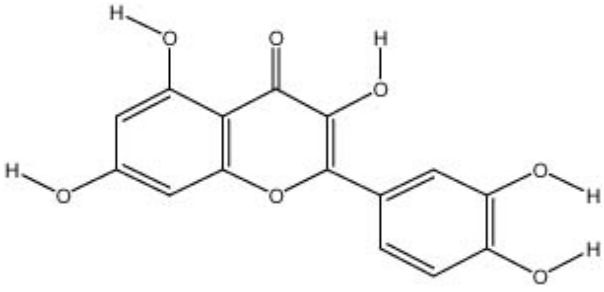
Scheme 5.1 Chemical structure of antioxidants added to the canola oil/caprylic acid structured lipid-based emulsions.



α-Tocopherol



Gallic acid



Quercetin

Figure 5.1 Effect of iron concentration (50 and 100 μM), pH (3.0 and 7.0), and natural antioxidants (α-tocopherol, gallic acid, and quercetin) on peroxide values over time in structured lipid-based emulsions stabilized by whey protein isolate. Control emulsions contained no added iron or antioxidants. Data shown are the average of duplicate samples held at 50°C. Error bars on chart represent standard deviation.

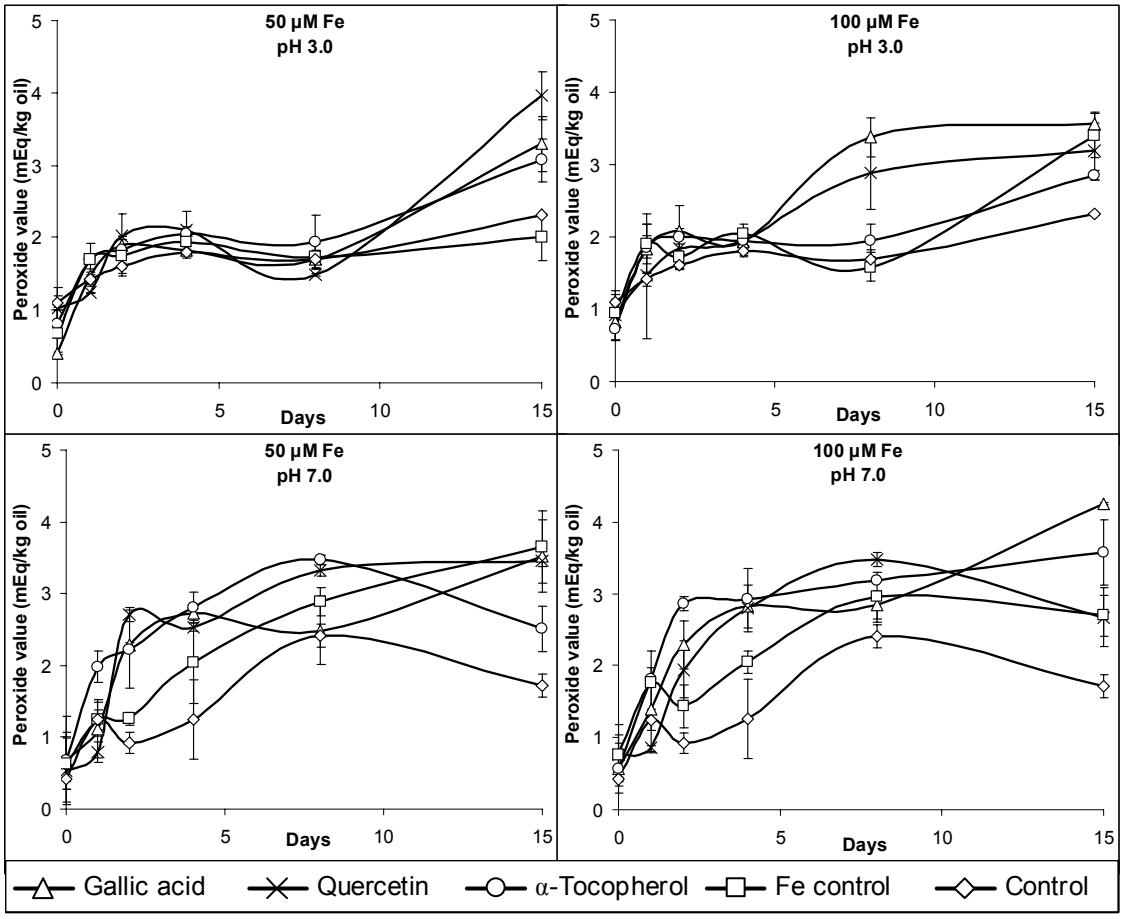

50 µM Fe
pH 3.0
100 µM Fe
pH 3.0
50 µM Fe
pH 7.0
100 µM Fe
pH 7.0
Peroxide value (mEq/kg oil)
Days
0
5
10
15
1
2
3
4
Gallic acid
Quercetin
α-Tocopherol
Fe control
Control

Figure 5.2 Effect of iron concentration (50 and 100 μM), pH (3.0 and 7.0), and natural antioxidants (α-tocopherol, gallic acid, and quercetin) on anisidine values (AV) over time in structured lipid-based emulsions stabilized by whey protein isolate. Control emulsions contained no added iron or antioxidants. Data shown are the average of duplicate samples held at 50°C. Error bars on chart represent standard deviation.

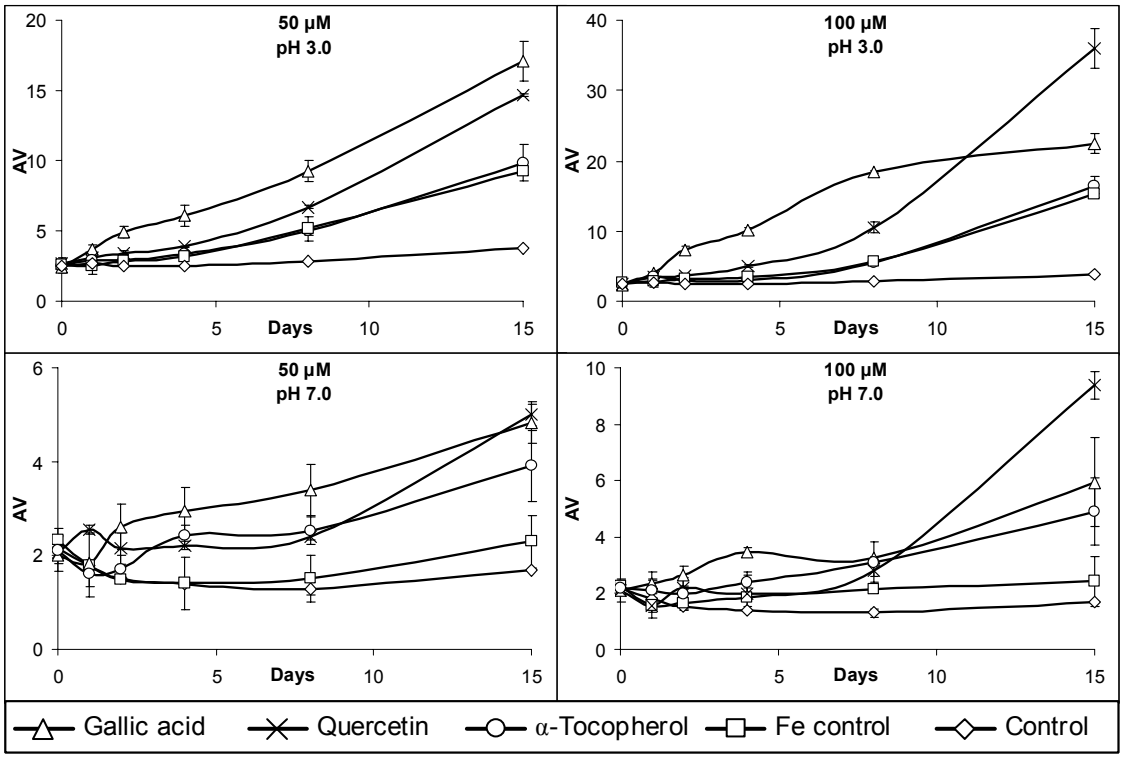
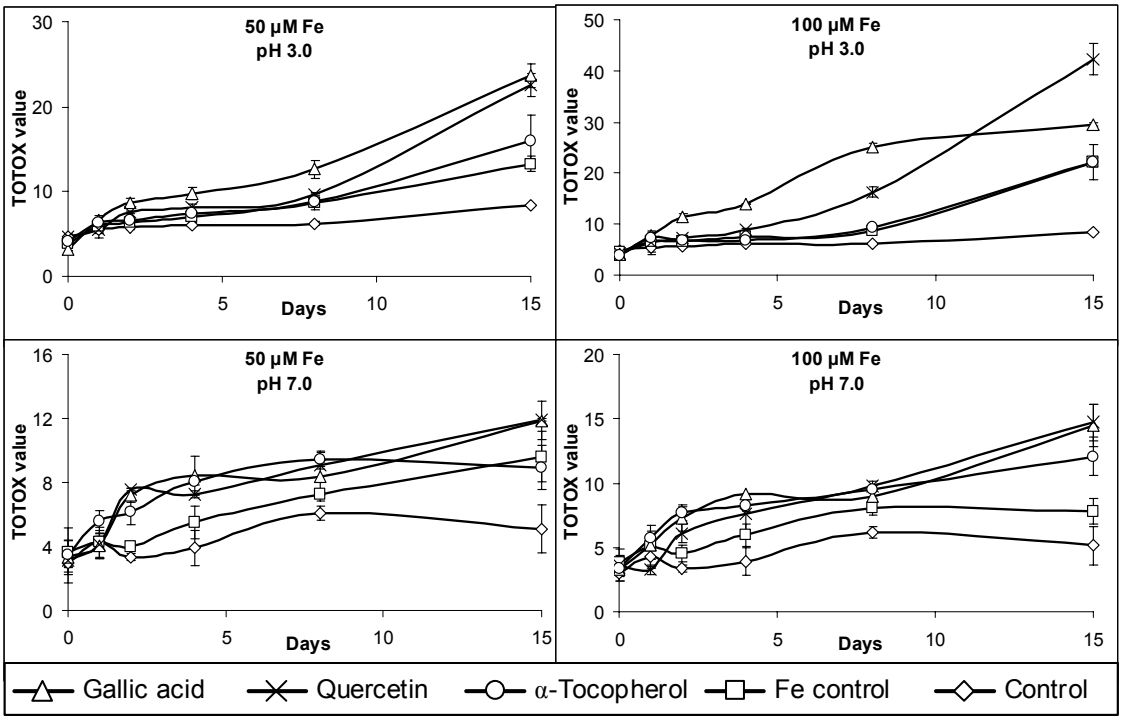

50 µM
pH 3.0
100 µM
pH 3.0
50 µM
pH 7.0
100 µM
pH 7.0
AV
Days
Gallic acid
Quercetin
α-Tocopherol
Fe control
Control

Figure 5.3 Effect of iron concentration (50 and 100 μM), pH (3.0 and 7.0), and natural antioxidants (α-tocopherol, gallic acid, and quercetin) on total oxidation (TOTOX) over time in structured lipid-based emulsions stabilized by whey protein isolate. Control emulsions contained no added iron or antioxidants. Data shown are the average of duplicate samples held at 50°C. Error bars on chart represent standard deviation.


50 µM Fe
pH 3.0
100 µM Fe
pH 3.0
50 µM Fe
pH 7.0
100 µM Fe
pH 7.0
TOTOX value
Days
Gallic acid
Quercetin
α-Tocopherol
Fe control
Control

CHAPTER 6

EFFECTS OF α-TOCOPHEROL, β-CAROTENE, AND SOY ISOFLAVONES ON LIPID OXIDATION OF STRUCTURED LIPID-BASED EMULSIONS[1]

[1] Osborn, H.T. and C.C. Akoh. Submitted to *Journal of Agricultural and Food Chemistry*, 12/11/2002.

ABSTRACT: Ten percent oil-in-water emulsions were formulated with a canola oil/caprylic acid structured lipid and stabilized with 0.5% whey protein isolate (WPI) or sucrose fatty acid ester (SFE). The antioxidant efficacies of α-tocopherol, β-carotene, genistein, and daidzein in these systems were evaluated over a 30-day period. Significantly ($p < 0.05$) less total oxidation occurred in the WPI emulsions compared to their SFE counterparts. β-carotene and soy isoflavones exhibited prooxidant activities in the SFE emulsions. β-carotene did not have prooxidant effects on oxidation of WPI emulsions, while the soy isoflavones significantly ($p < 0.05$) increased oxidation. α-Tocopherol did not affect total oxidation of both WPI and SFE emulsions on the final day of the study. Due to the ability of β-carotene, genistein, and daidzein to exhibit prooxidant activity in emulsions, manufacturers must experiment with them before adding them to SL-based products as antioxidants or functional ingredients.

KEYWORDS: Anisidine Value, β-Carotene, Daidzein, Emulsions, Genistein, Lipid Oxidation, Peroxide Value, Structured Lipids, Sucrose Fatty Acid Esters, α-Tocopherol, TOTOX Value, Whey Protein Isolate

INTRODUCTION

Lipid oxidation in foods is a serious problem that leads to loss of shelf life, palatability, functionality, and nutritional quality (*1*). Antioxidants can be added to foods to delay the onset of oxidation or to slow the rate at which it proceeds (*1*). Interest in natural antioxidants found in plants has grown recently because of the worldwide trend toward the use of "natural" additives in foods (*2*). Additionally, many natural antioxidants are highly desirable to consumers because of their purported health benefits.

Tocopherols and carotenoids are two important commercial natural antioxidants that have been the subject of many experimental and epidemiological studies. Several plant phenolic compounds have also been identified as natural antioxidants. Studies on the antioxidant activity of phenolic compounds are often based on the in vitro oxidation of LDL to evaluate their potential health effects (*2*). However, fewer studies have evaluated the ability of phenolic compounds to protect against oxidation in food-relevant model systems. Such is the case with the group of bisphenolic compounds found in soybeans, known as isoflavonoid phytoestrogens. These compounds (mainly genistein and daidzein) have been shown to significantly prolong the lag time for LDL oxidation in vitro and in vivo (*3,4*). Studies on their efficacy as antioxidants in food systems are now needed.

Compounds with antioxidant activity may also exhibit prooxidant behavior under certain conditions (*5*). Conflicting results have been reported for the effects of α-tocopherol on lipid oxidation. The relative antioxidant activity of tocopherols depends on temperature, lipid composition, physical state (bulk phase or emulsion), and tocopherol concentration (*6*). α-Tocopherol's ability to have an antioxidant, neutral, or prooxidant effect is related to its complex function and chemical behavior (*7*). Increased levels of α-tocopherol may result in increased levels of α-tocopherol radicals, which can initiate processes of lipid peroxidation by themselves (*7*).

β-Carotene has also demonstrated both antioxidant and prooxidant activity in previous studies in a wide variety of lipid systems (*8*). The activity of β-carotene depends on the concentration used, other antioxidants present, the oxidation model employed, and the oxygen tension (*9*). It is proposed that β-carotene traps peroxyl

radicals under conditions of high oxygen tension or at atmospheric conditions by an addition mechanism (*10*). The carbon-centered β-carotene radical that is subsequently formed is readily autoxidized into a mixture of products with epoxy, hydroxy, and carbonyl groups (*9*). The autoxidation reactions begin to consume β-carotene without scavenging peroxyl radicals and may thus attenuate β-carotene antioxidant activity.

Flavonoids with a phenol-type substitution in their B-ring, like apigenin and naringenin, have been reported to result in a 30-50 times increase in the formation of reactive oxidant species (*7*). Chen et al. (*11*) reported that apigenin accelerated lipid oxidation of canola oil. The basic structural difference between the flavones and the isoflavones is the location of the B ring at the 3-position of the C ring, which removes the 3-OH from the structure. However, the monophenolic B ring, which is thought to be responsible for the prooxidant activity of apigenin and naringenin, remains intact in the isoflavones daidzein and genistein (*12*). Therefore, soy isoflavones should also be examined for prooxidant activity in food systems.

Despite their well-documented prooxidant activity, tocopherols, carotenoids, and phenolics continue to be hailed as excellent antioxidants. The objective of this paper was to illustrate the potential deleterious effects of α-tocopherol, β-carotene, and soy isoflavones (**Figure 6.1**) in structured lipid-based model emulsions stabilized by whey protein isolate or sucrose fatty acid esters. Structured lipids (SLs) are a new generation of fats that may provide the most effective means for delivering desired fatty acids for nutritive or therapeutic purposes, and for targeting specific diseases and metabolic conditions (*13*). The potential health benefits of natural antioxidants and SLs make them ideal candidates for functional food formulations. Therefore, studies on the oxidation

properties of emulsion systems containing both natural antioxidants and SLs are merited because of their likeliness of being found together in functional foods. The emulsifier variable was incorporated into the experimental design in order to determine the efficacy of natural antioxidants in the presence of both anionic and nonionic emulsifiers. Emulsifying agents are in direct contact with lipids on the surface of the emulsion droplet and are known to affect the rate of lipid oxidation (*14-17*).

MATERIALS AND METHODS

Materials. Canola oil was purchased from a local supermarket. Caprylic acid (purity > 98%), citric acid, α-tocopherol, β-carotene, genistein, and daidzein were purchased from Sigma Chemical Company (St. Louis, MO). A sn-1,3 specific immobilized lipase from *Rhizomucor miehei* (IM 60) was obtained from Novo Nordisk A/S (Bagsvaerd, Denmark). Whey protein isolate (WPI) (#27361) was provided by Land O'Lakes (St. Paul, MN). Ryoto sugar ester (S-1670) was supplied by Mitsubishi-Kasei Food Corporation (Tokyo, Japan). The sucrose fatty acid ester contained mainly stearic acid and consisted of approximately 75% monoester and 25% di-, tri-, or polyester. All other reagents were purchased from Fisher Scientific (Fair Lawn, NJ).

Structured Lipid Production. The SL was produced in a packed bed bioreactor using optimal conditions previously reported (*18*) for reacting canola oil and caprylic acid. The product was purified using a KDL-4 short-path distillation unit (UIC Inc., Joliet, IL). The oil was passed through the distillation apparatus three times under the following conditions: holding temperature, 25 °C; heating oil temperature, 185 °C; cooling water temperature, 15 °C; pressure, < 0.01 Torr. The purified SL product contained the following fatty acids (mol %): 37.3% C8:0, 1.8% C16:0, 1.7% C18:0,

47.3% C18:1, 8.9% C18:2, and 3.0% C18:3 as determined by gas chromatography of methyl esters (*19*).

Emulsion Preparation. Antioxidants were added to the canola oil/caprylic acid SL at 0.02% weight of the oil. Mixed antioxidant systems contained equal amounts (0.01% weight of oil) of each antioxidant. α-Tocopherol was added directly to the SL. Genistein and daidzein were dissolved in methanol and then added to the oil. β-Carotene was dissolved in hexane before addition to the SL. Organic solvents were evaporated under N_2. Oil-in-water emulsions were then prepared with 10% SL, 0.5% whey protein isolate (WPI) or sucrose fatty acid ester (SFE), and distilled water. The emulsions were passed through a high-pressure valve homogenizer (Emulsiflex, C5, Avestin, CA) six times at 10,000 psi. All samples were held on ice during processing. Sodium azide (1 mM) was added to the emulsions to slow microbial growth. Particle size distribution was measured by integrated light scattering (Mastersizer S, Malvern Instruments, Malvern, UK) using standard optical parameters to ensure that similar droplet sizes were formed in the emulsions during homogenization. All samples had an apparent particle diameter ($D_{3,2}$ value) between 0.28 and 0.46 μm.

Oxidation Experiments. Emulsion samples were allowed to oxidize in a 50°C covered water bath for 30 days. The primary and secondary oxidation products were measured in the emulsion samples after 0, 1, 2, 4, 8, 15 and 30 days of storage. Oil was extracted from the emulsions by adding isooctane/isopropanol (3:2, v/v), vortexing 3 times for 10 s each, and centrifuging for 5 min at 1000 rpm. The clear upper layer was collected and the solvent was evaporated under nitrogen. Peroxide values (PV) were determined using the International Dairy Federation method described in detail by

Shantha and Decker (*20*). Anisidine values (AV) were determined according to the AOCS Official Method Cd 18-90 (*21*). This method determines the amount of aldehyde (principally 2-alkenals and 2,4-alkadienals) present in the oil (*22*). The TOTOX value was calculated as: TOTOX value = 2(PV) + AV (*22*). The TOTOX value combines evidence about the past history of an oil with its present state and is useful for estimating oxidative deterioration of food lipids (*22*).

Statistical Analysis. All experiments were performed on duplicate samples. Statistical analyses were conducted with the SAS (*23*) software package. Analyses of variance were performed by ANOVA procedures. Significant differences ($p < 0.05$) were determined by the least squares difference method.

RESULTS AND DISCUSSION

Effect of Emulsifier. The amount of hydroperoxides present in the SL-based emulsions was significantly ($p < 0.05$) affected on all days of storage (excluding day 0) by the emulsifier variable. In the SFE control emulsions, hydroperoxides increased rapidly on the first day of storage (**Figure 6.2A**). However, WPI control emulsions showed a four-day lag phase when little primary oxidation was occurring (**Figure 6.2A**). On the final day of the study, the WPI control emulsions had a higher PV, while the hydroperoxides in the SFE samples had already peaked (3.47 mEq/kg oil) at day 4 and were declining. The emulsifier variable also significantly ($p < 0.05$) affected the AV on all days of the study (excluding day 0). **Figure 6.2B** illustrates the large increase in AV after day 8 in the SFE control emulsions compared to the WPI controls. The rise in AV (**Figure 6.2B**) corresponds to the declining PV in the SFE-stabilized emulsions (**Figure 6.2A**) and demonstrates that the hydroperoxides are being decomposed into secondary

oxidation products. Similar to the AV results, TOTOX values were significantly ($p < 0.05$) lower in the WPI controls compared to the SFE control emulsions (**Figure 6.2C**).

The antioxidant effect of WPI has been previously reported in SL-based emulsions (*14*). It was hypothesized that whey proteins inhibit lipid oxidation by inactivating peroxyl radicals (*24*). Exposure to heat increases the antioxidant activity of whey proteins by exposing more free radical scavenging sulfhydryl groups (*24*). This may account for the antioxidant activity of WPI observed during this study, because the emulsions were stored at 50°C for 30 days.

Effect of Natural Antioxidants on Primary Oxidation. The primary oxidation results are presented for SFE and WPI emulsions in **Figures 6.3** and **6.4**, respectively. Primary oxidation was significantly ($p < 0.05$) influenced by the antioxidant variable on all days of storage (excluding day 0). On the final day of the study, the PV were declining in the SFE emulsions, which made the efficacy of the individual antioxidants difficult to determine. Emulsions containing β-carotene (**Figure 6.3A**), a combination of α-tocopherol and β-carotene (**Figure 6.3A**), or genistein (**Figure 6.3C**) had final PV significantly ($p < 0.05$) greater than the SFE controls. Day-30 PV for emulsions containing daidzein were statistically similar to those of the SFE controls (**Figure 6.3B**). α-Tocopherol, when added alone, exhibited antioxidant properties on days 2-8, but became ineffective later in the study (**Figure 6.3**). The oxidative stability of α-tocopherol has been shown to be depleted after 10 days of 60°C storage in emulsions with pHs > 3.0 (*25*), and may account for its lack of effectiveness in the SFE emulsions on day 15.

The antioxidants affected primary oxidation in different manners when added to the WPI-stabilized emulsions compared to the SFE-stabilized emulsions. α-Tocopherol

had a significant ($p < 0.05$) antioxidant effect on day 30 of the study, although it exhibited prooxidant activity in the WPI emulsions on all previous days of the study (**Figure 6.4**). α-Tocopherol's ability to increase PV in the WPI emulsions during the first 15 days of the study likely stems from its mechanism of action, which involves the donation of hydrogen to peroxyl radicals that results in the formation of lipid peroxides (*24*).

The final PV of emulsions containing β-carotene were not significantly different from the WPI controls (**Figure 6.4A**). Final hydroperoxide levels were also similar among the control, daidzein, and genistein WPI emulsions (**Figures 6.4B and 6.4C**). The genistein and tocopherol combination gave lower PV on days 8-15 after which a rapid increase in PV occurred (**Figure 6.4C**). However, final PV for emulsions containing mixtures of soy isoflavones with α-tocopherol were significantly ($p < 0.05$) higher than the control WPI emulsions (**Figure 6.4B and 6.4C**). Hydroperoxides are transitionary intermediates that decompose into various secondary products, including aldehydes, ketones, hydrocarbons, and alcohols. The PV quantifies the amount of hydroperoxides present in a sample at the time of measurement, which is affected by both formation and decomposition reactions. Therefore, it is difficult to determine an antioxidant's mechanism of action based on the results of primary oxidation studies alone.

Effect of Natural Antioxidants on Secondary Oxidation. α-Tocopherol had a final AV similar to the SFE controls (**Figure 6.5**). Fukumoto and Mazza (*25*) also reported that low concentrations (150 μM) of α-tocopherol had no effect on the formation of secondary oxidation products in linoleic acid emulsions.

Similar to the PV results, β-carotene and a combination of α-tocopherol and β-carotene had significant ($p < 0.05$) prooxidant effect on the secondary oxidation of SFE emulsions (**Figure 6.5A**). After β-carotene traps peroxyl radicals, a carbon-centered β-carotene radical is subsequently formed and is readily autoxidized into a mixture of products with epoxy, hydroxy, and carbonyl groups (*9*). The autoxidation reactions begin to consume β-carotene without scavenging peroxyl radicals and may thus attenuate β-carotene antioxidant activity and allow the prooxidant activity of β-carotene to predominate.

Daidzein, genistein, and their combinations with α-tocopherol significantly ($p < 0.05$) increased the model emulsion AV compared to the SFE controls (**Figures 6.5B and 6.5C**). The prooxidant chemistry of these isoflavones in model emulsions is not yet fully understood, but seems to partly correlate with the high one-electron oxidation potential of their corresponding phenoxyl radicals (*7*). Combinations of daidzein and α-tocopherol resulted in lower AV than those measured in SFE emulsions containing daidzein alone on the final day of the study (**Figure 6.5B**). However, significant differences were not observed between the secondary oxidation of SFE emulsions containing only genistein and those with a combination of genistein and α-tocopherol (**Figure 6.5C**).

α-Tocopherol significantly ($p < 0.05$) increased the AV of WPI emulsions compared to the control on the final day of the study (**Figure 6.6**). While α-tocopherol increased hydroperoxides during the first 15 days of storage (**Figure 6.4**), it inhibited the formation of secondary lipid oxidation products as can be seen by its ability to maintain AV similar to the control samples during those days (**Figure 6.6**). Hydroperoxides can be cleaved either to form peroxyl radicals or to produce the highly reactive alkoxyl

radicals. The alkoxyl radicals can then undergo a number of reactions, including β-cleavage to produce aldehydes. After α-tocopherol donates its hydrogen atom to a peroxyl radical, it becomes a tocopheroxyl radical. Tocopheroxyl radicals can trap alkoxyl radicals and, thus, inhibit the formation of secondary cleavage products (*6*). The increase in AV for the WPI emulsions containing α-tocopherol (after day 15) corresponds to the decrease in PV for the same samples, thus indicating peroxides have begun decomposing into secondary compounds because the tocopherol has been depleted (*25*).

β-Carotene did not significantly ($p < 0.05$) affect the AV of WPI-stabilized emulsions throughout the study (**Figure 6.6A**). WPI slowed oxidation, which in turn resulted in the formation of fewer free radicals to react with the β-carotene. Therefore, WPI may have retarded the formation of carotenoid radical adducts and their further degradation to autooxidation products, which would explain the lack of a prooxidant effect observed for β-carotene in the WPI-stabilized emulsions. However, combinations of α-tocopherol and β-carotene significantly ($p < 0.05$) increased secondary oxidation of WPI emulsions (**Figure 6.6A**), due to α-tocopherol's ability to promote the formation of hydroperoxides in the SL-based emulsion.

Both soy isoflavones significantly ($p < 0.05$) increased secondary oxidation compared to the control WPI emulsions on the final day of the study (**Figures 6.6B and 6.6C**). Flavonoids have been reported to exert prooxidant chemistry, including the formation instead of scavenging of radicals, especially at low concentration (< 500 μM) (*5*). Both genistein and daidzein were added to the model emulsions in our study at concentrations of 74 and 78 μM, respectively. The mean AV for emulsions containing daidzein was twice that of emulsions formulated with genistein (20.53 and 9.25,

respectively) at the conclusion of the study. The removal of the 5-hydroxyl group from the A ring of the genistein structure (as in daidzein) is associated with a 60% decrease in antioxidant activity (*12,26*), and may explain the greater prooxidant effect observed in this study for emulsions containing daidzein compared to their genistein counterparts. Combining the soy isoflavones with α-tocopherol further promoted secondary oxidation in WPI emulsions (**Figures 6.6B and 6.6C**). The final AV for the emulsions containing daidzein and α-tocopherol was 50.81 (**Figure 6.6B**), a five-fold increase over the final AV of 11.11 for WPI emulsions containing both genistein and α-tocopherol (**Figure 6.6C**).

Effect of Natural Antioxidants on Total Oxidation. The TOTOX value was significantly ($p < 0.05$) influenced by the antioxidant variable on all days of the study (excluding day 0). In SFE-stabilized emulsions, β-carotene, α-tocopherol, daidzein, genistein, and their combinations affected the final TOTOX values (**Figure 6.7**) similar to the AV. This was due to the fact that the PV were relatively low compared to the AV, and therefore, did not contribute substantially to the TOTOX value.

β-carotene did not significantly ($p < 0.05$) affect total oxidation in the WPI emulsions compared to the controls on the final day of storage (**Figure 6.8A**). The WPI emulsions containing β–carotene alone did not reach a TOTOX value above 10 during the 30-day study. This finding may be important for food manufacturers because sensory quality of vegetable oils is considered acceptable when the TOTOX values is below 10 (*27*). The addition of a mixture of α-tocopherol and β-carotene produced final TOTOX values similar to the WPI controls (**Figure 6.8A**). Zhang and Omaye (*28*) also observed

no synergistic antioxidant effects on lipid peroxidation or malondialdehyde formation when β-carotene and α-tocopherol were added in combination to microsomes.

Daidzein and genistein significantly ($p < 0.05$) increased total oxidation in the WPI emulsions on the final day of the study (**Figures 6.8B and 6.8C**). The hydrophobicity/hydrophilicity of an antioxidant influences its efficacy in emulsion systems (*11*). In emulsified oils, lipophilic antioxidants are more effective in inhibiting oxidation (*29*). Therefore, hydrophobicity may have been partially responsible for the occurrence of less total oxidation in the WPI emulsions containing α-tocopherol or β-carotene compared to those with hydrophilic daidzein and genistein. Daidzein mixed with α-tocopherol caused a two-fold increase in total oxidation, compared to genistein in combination with α-tocopherol (mean TOTOX values were 65.24 and 28.83, respectively).

The results of this study demonstrate that β-carotene, daidzein and genistein may exhibit prooxidant behavior on primary, secondary, and/or total oxidation of SL-based model emulsions stored at 50°C for 30 days. Many factors affect oxidation of emulsified oils and more studies are required to fully understand the prooxidant mechanisms behind these compounds. However, care should be exercised when adding some of these compounds (especially β-carotene and soy isoflavones) to foods as functional ingredients due to their ability to promote lipid oxidation in some emulsions under certain conditions.

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Figure 6.1 Chemical structure of antioxidants added to the canola oil/caprylic acid structured lipid-based emulsions.

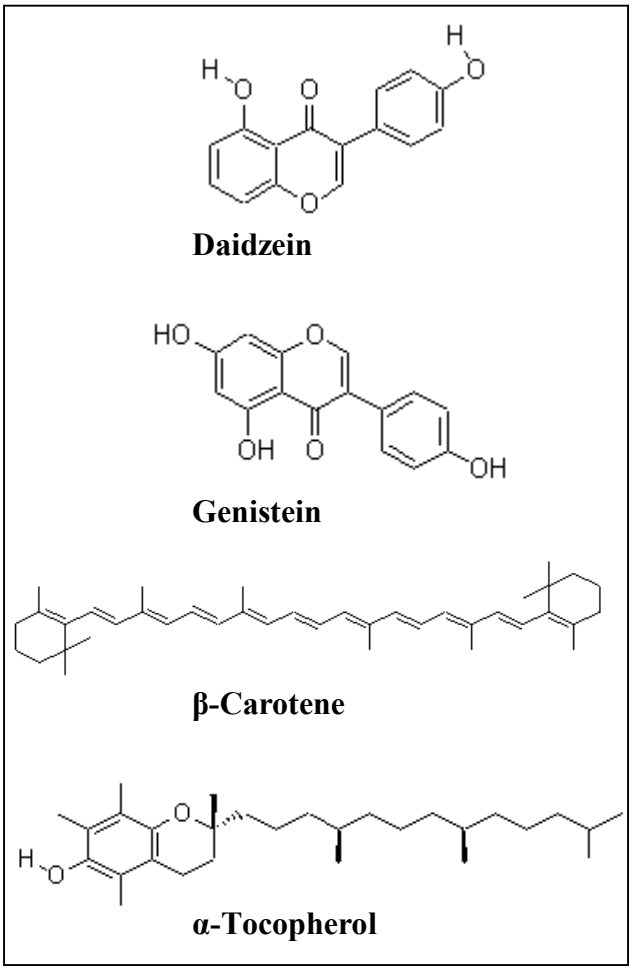

H
O
O
H
O
O
Daidzein
HO
O
OH
O
OH
Genistein
β-Carotene
O
H
O
α-Tocopherol

Figure 6.2 Effect of emulsifier (SFE – sucrose fatty acid ester; WPI – whey protein isolate) on (A) peroxide values; (B) anisidine values (AV); and (C) total oxidation (TOTOX) over time in structured lipid-based emulsions. Control emulsions contain no added antioxidants. Data shown are the average of duplicate samples held at 50°C. Error bars on chart represent standard deviation.

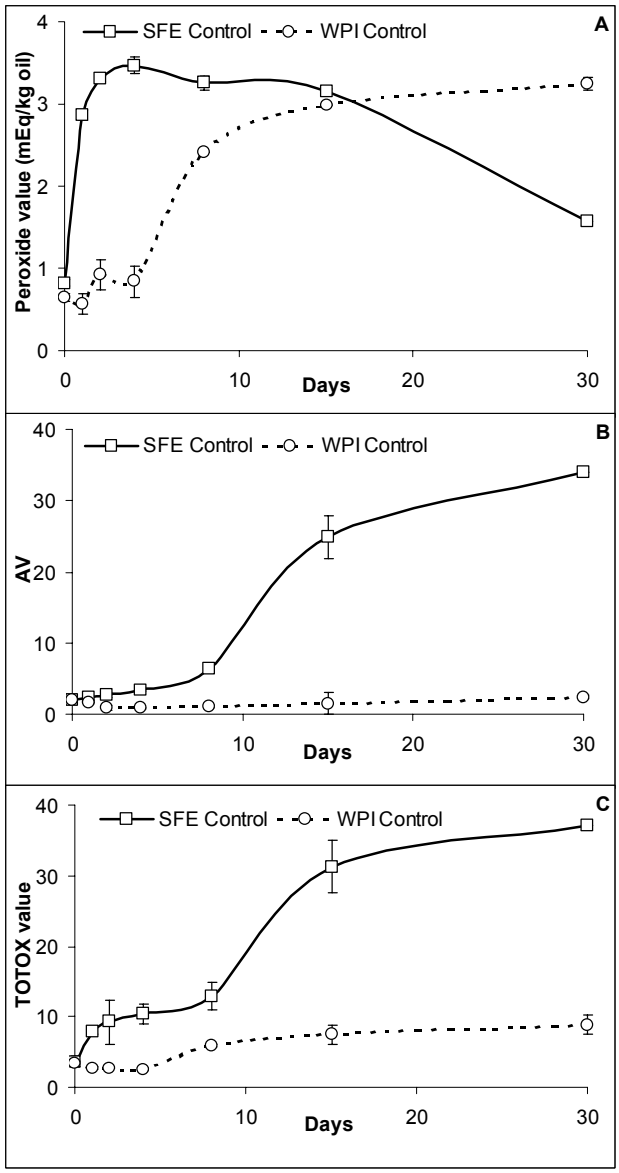

A
SFE Control
WPI Control
Peroxide value (mEq/kg oil)
Days
B
AV
C
TOTOX value

Figure 6.3 Effect of natural antioxidants (T – α-tocopherol; B – β-carotene; D – daidzein; G – genistein) on peroxide values over time in structured lipid-based emulsions stabilized by sucrose fatty acid esters (SFE). Data shown are the average of duplicate samples held at 50°C. Error bars on chart represent standard deviation.

SFE Emulsions

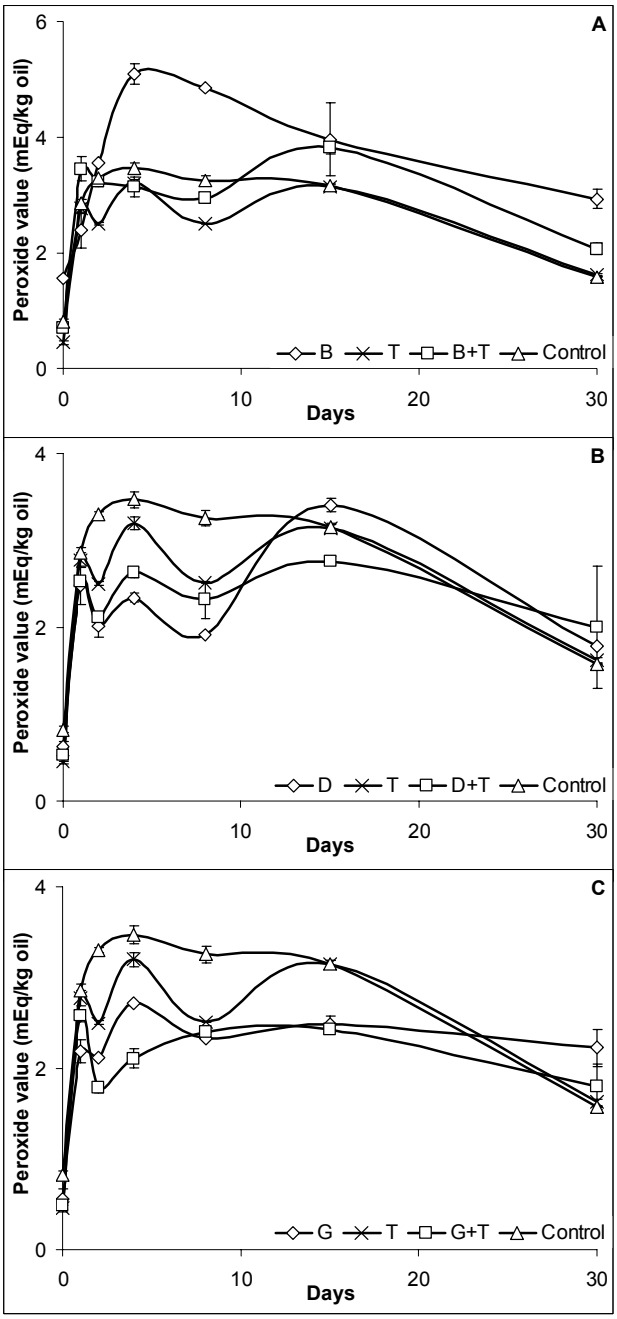


Figure 6.4 Effect of natural antioxidants (T – α-tocopherol; B – β-carotene; D – daidzein; G – genistein) on peroxide values over time in structured lipid-based emulsions stabilized by whey protein isolate (WPI). Data shown are the average of duplicate samples held at 50°C. Error bars on chart represent standard deviation.

WPI Emulsions

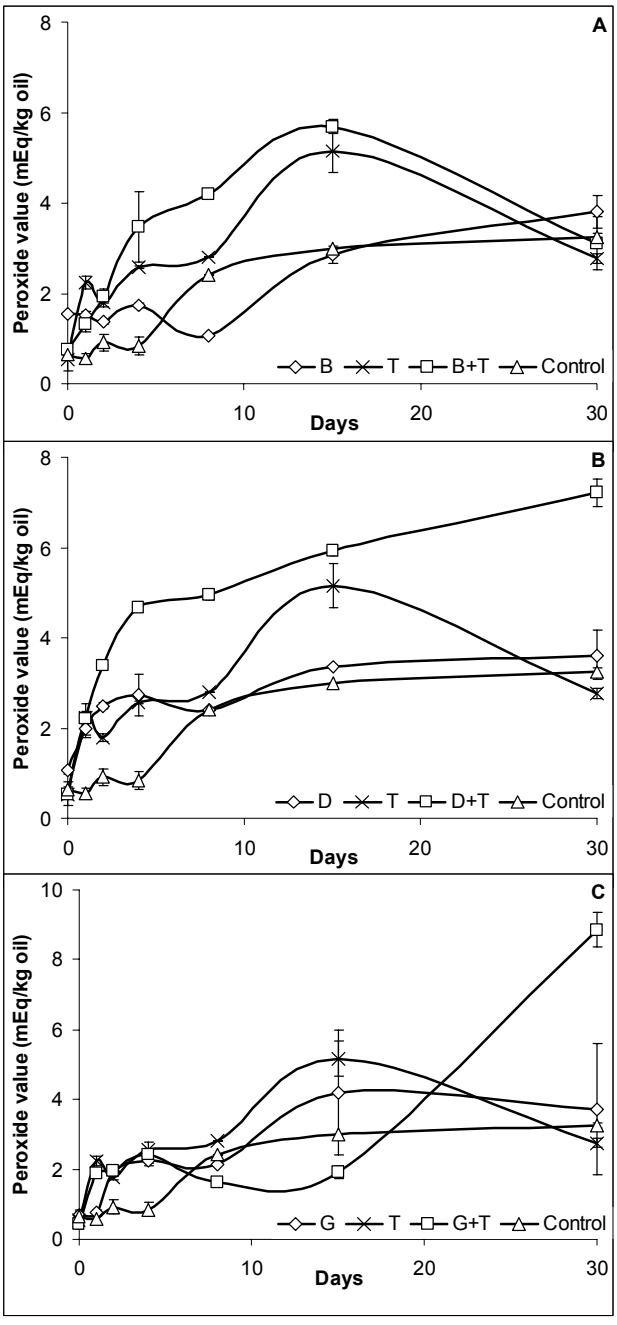


Figure 6.5 Effect of natural antioxidants (T – α-tocopherol; B – β-carotene; D – daidzein; G – genistein) on anisidine values (AV) over time in structured lipid-based emulsions stabilized by sucrose fatty acid esters (SFE). Data shown are the average of duplicate samples held at 50°C. Error bars on chart represent standard deviation.

SFE Emulsions

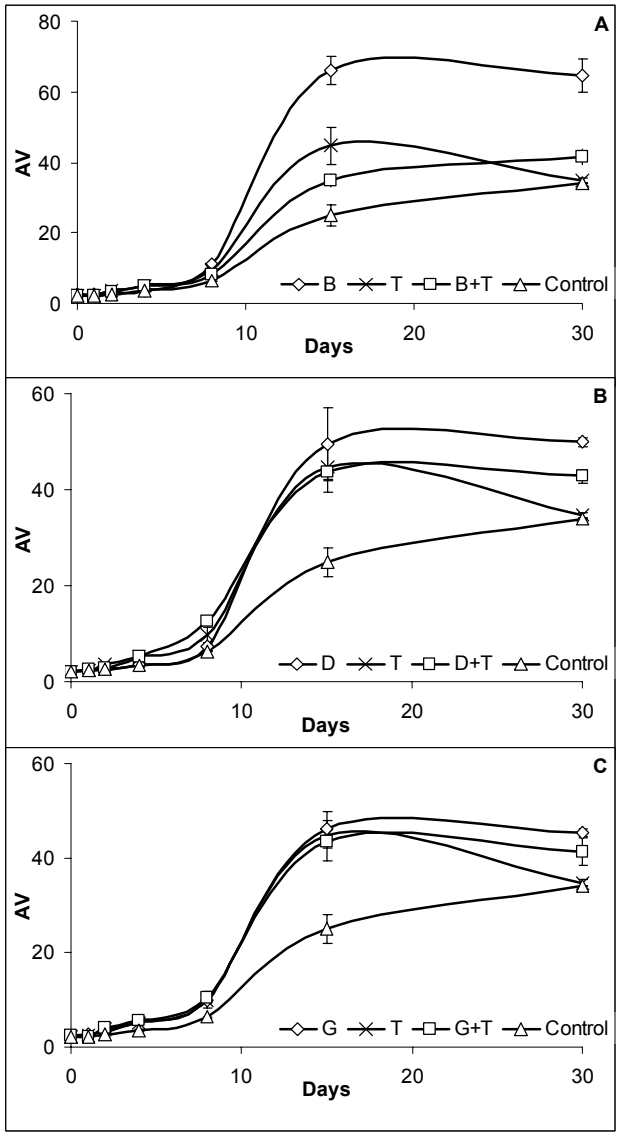


Figure 6.6 Effect of natural antioxidants (T – α-tocopherol; B – β-carotene; D – daidzein; G – genistein) on anisidine values (AV) over time in structured lipid-based emulsions stabilized by whey protein isolate (WPI). Data shown are the average of duplicate samples held at 50°C. Error bars on chart represent standard deviation.

WPI Emulsions

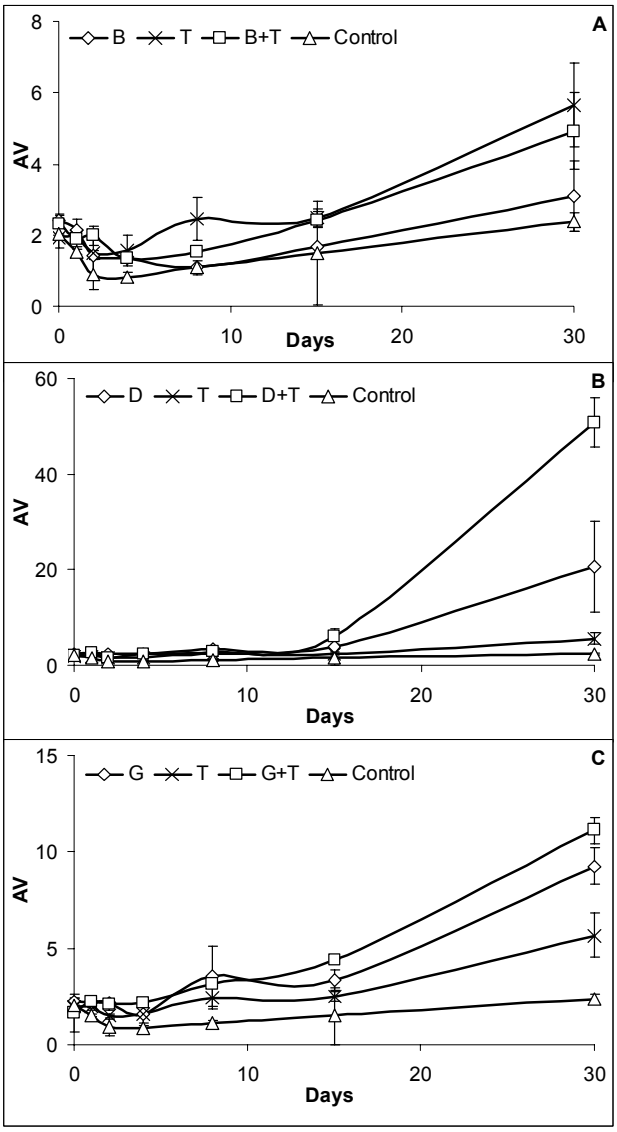


Figure 6.7 Effect of natural antioxidants (T – α-tocopherol; B – β-carotene; D – daidzein; G – genistein) on total oxidation (TOTOX) over time in structured lipid-based emulsions stabilized by sucrose fatty acid esters (SFE). Data shown are the average of duplicate samples held at 50°C. Error bars on chart represent standard deviation.

SFE Emulsions

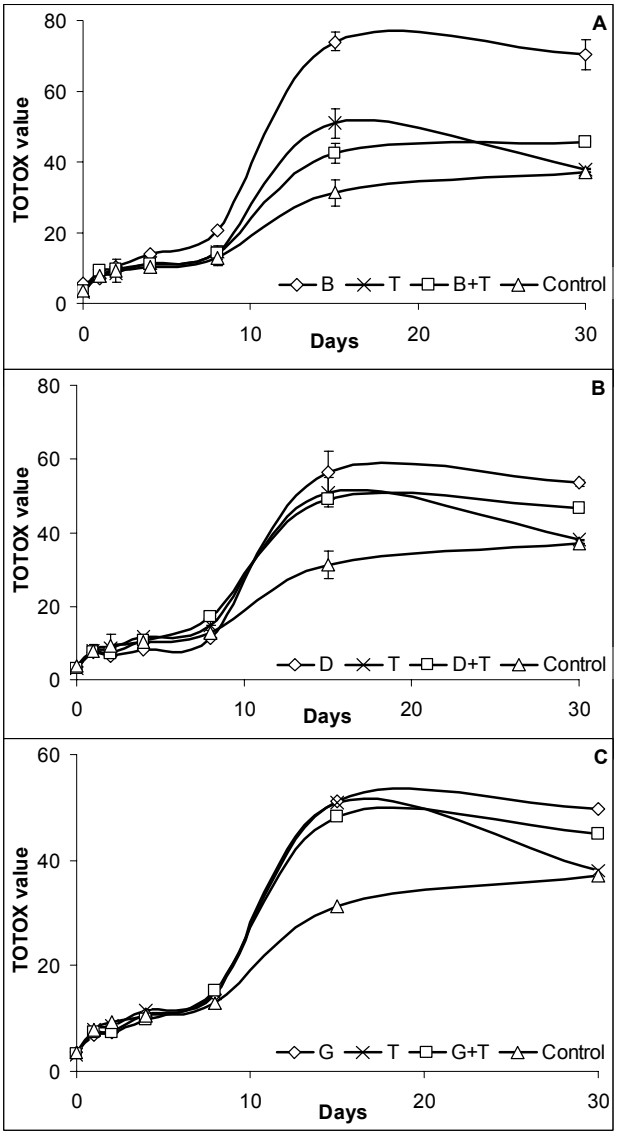
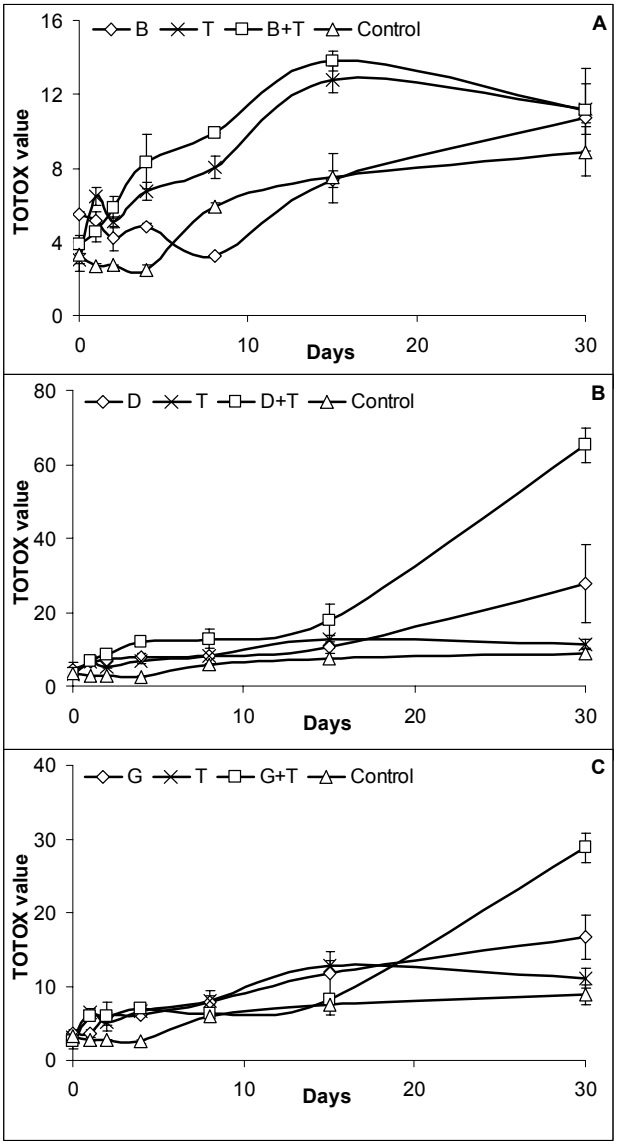


Figure 6.8 Effect of natural antioxidants (T – α-tocopherol; B – β-carotene; D – daidzein; G – genistein) on total oxidation (TOTOX) over time in structured lipid-based emulsions stabilized by whey protein isolate (WPI). Data shown are the average of duplicate samples held at 50°C. Error bars on chart represent standard deviation.

WPI Emulsions



CHAPTER 7

CONCLUSIONS

The ability of structured lipids (SLs) to combine an increased absorption rate for medium-chain fatty acids and beneficial long-chain fatty acids in one triacylglycerol molecule make SLs very attractive to the medical community and functional food manufacturers. Based on the results of this study, it can be concluded that the following factors influence lipid oxidation of SL-based oil-in-water model emulsions: droplet volume fraction, emulsifier type, transition metals, pH, NaCl, and natural compounds with anti- and prooxidant activity.

In our canola oil/caprylic acid SL-based model emulsions, decreasing the SL concentration led to an increased rate of oxidation. Whey protein isolate (WPI) possessed antioxidant properties in addition to its role as an emulsifier. Both transition metals, iron and copper, promoted lipid oxidation in SL-based model emulsions. Greater copper-catalyzed oxidation occurred at pH 7.0 compared to pH 3.0, while the reverse effect was observed for iron-catalyzed oxidation. The addition of NaCl increased total oxidation at acidic pH, while α-tocopherol and citric acid successfully retarded copper-catalyzed lipid oxidation at pH 3.0. Quercetin and gallic acids had a prooxidant effect on total oxidation in the pH 3.0 emulsions containing iron. In the absence of added transition metals, β-carotene and soy isoflavones exhibited prooxidant activities in the sucrose fatty acid ester (SFE)-stabilized emulsions.

Clearly, the molecular environment of SL-based emulsion droplets substantially impacts their oxidative stability, confirming our original hypothesis. The knowledge

gained from this research, in addition to further studies in this area, will enable food scientists to better "engineer" SL-based foods with enhanced oxidative stability by designing antioxidant strategies in a more systematic fashion.