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Lipid Oxidation in Oil-in-Water Emulsions: Impact of Molecular Environment on Chemical Reactions in Heterogeneous Food Systems

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ABSTRACT: The susceptibility of lipids to oxidation is a major cause of quality deterioration in food emulsions. The reaction mechanism and factors that influence oxidation are appreciably different for emulsified lipids than for bulk lipids. This article reviews the current understanding of the lipid oxidation mechanism in oil-in-water emulsions. It also discusses the major factors that influence the rate of lipid oxidation in emulsions, such as antioxidants, chelating agents, ingredient purity, ingredient partitioning, interfacial characteristics, droplet characteristics, and ingredient interactions. This knowledge is then used to define effective strategies for controlling lipid oxidation in food emulsions.

KEYWORDS: emulsions, lipid oxidation, microstructure

Introduction

The susceptibility of lipids to oxidation is a major cause of quality deterioration in many types of natural and processed foods (Nawar 1996; Frankel 1998). Lipid oxidation causes changes in the type and concentration of molecular species present in a food (Akoh and Min 1998). Each type of molecular species has unique physicochemical characteristics; for example, chemical reactivity, dimensions, polarity, interactions, surface activity, partition coefficient, volatility, and thermal stability. As a result, lipid oxidation causes changes in the quality attributes of foods, such as taste, texture, shelf life, appearance, and nutritional profile. In some foods, a limited amount of lipid oxidation is desirable because it leads to the generation of molecular species that have a desirable characteristic taste or smell; for example, cheeses (Nawar 1996). On the other hand, lipid oxidation is undesirable in most foods because it leads to the development of undesirable off-flavors ("rancidity") and potentially toxic reaction products (Halliwell and others 1995). The high susceptibility of polyunsaturated lipids to lipid oxidation has restricted their incorporation into many food products, which is unfortunate because greater consumption of polyunsaturated lipids is beneficial to health (Watkins and German 1998) and is recommended in dietary guidelines (Kritchevsky 1998). Progress in the development of fatty foods with desirable nutritional and physical attributes depends on the availability of improved methods of controlling their oxidative stability, which in turn relies on a thorough understanding of the mechanisms of lipid oxidation.

A great deal of research has been carried out to elucidate lipid oxidation mechanisms in bulk fats and oils (Fritsch 1994; Halliwell and others 1995; Frankel 1998). This research has provided important insights into the factors that influence lipid oxidation and strategies to control it. Nevertheless, the application of this knowledge to actual food products is often limited because the lipids are disseminated as discrete phases dispersed in structurally and compositionally heterogeneous matrices; for example, muscle or plant tissue, porous solids, powders, or emulsions (Fritsch 1994; Erickson and Sista 1997; Decker 1998a). In these foods, the organization of the lipid molecules within the system, as well as their interactions with other types of molecules in their immediate vicinity, has a pronounced influence on their susceptibility to lipid oxidation (Coupland and McClements 1996a). The purpose of this article is to review the current understanding of lipid oxidation in oil-in-water emulsions and to highlight effective strategies for retarding it. Previous studies suggest that transition metal-based catalysis is the dominant mechanism of lipid oxidation in most food emulsions, therefore we have mainly focused on this topic. Nevertheless, other pro-oxidant mechanisms have also been reviewed where relevant; for example, catalysis of lipid oxidation by enzymes dispersed in the aqueous phase or by impurities associated with food ingredients.

The information presented in this review is directly applicable to food emulsions such as milk, cream, salad dressings, mayonnaise, soups, sauces, cream liqueurs, fruit beverages, nutritional beverages, and infant formula (Dickinson 1992; McClements 1999). It should also facilitate an understanding of lipid oxidation in structurally more complex food materials, such as biological tissues.

Food Emulsions

An emulsion consists of two immiscible liquids (usually oil and water), with one being dispersed in the other in the form of small spherical droplets (Figure 1). Most foods contain a range of different sized droplets, with the mean diameter lying somewhere between 0.1 and 100 μm (Dickinson and Stainsby 1982; Dickinson 1992). Emulsions are normally categorized in terms of the relative location of the oil and water phases within the system. A system that consists of oil droplets dispersed in an aqueous phase is called an "oil-in-water" (or O/W) emulsion. A system that consists of water droplets dispersed in an oil phase is called a "water-in-oil" (or W/O) emulsion. This review focuses on lipid oxidation in O/W emulsions. It has been suggested that lipid oxidation in W/O emulsions will occur at a rate similar to that in bulk oils because the surface of the lipid phase is exposed directly to air (Fritsch 1994). Nevertheless, it is possible that pro-oxidants or antioxidants located within the water droplets or at the water-in-oil interface may influence lipid oxidation in W/O emulsions, and so many of the factors discussed in this review may also be relevant to these systems.

Emulsions are thermodynamically unstable systems because of the positive free energy needed to increase the surface area

between the oil and water phases and because oil and water have different densities (Dickinson 1992; McClements 1999). For this reason, emulsions tend to separate into a system that consists of a layer of oil (lower density) on top of a layer of water (higher density) so as to minimize the contact area between oil and water. To form emulsions that are kinetically stable for a reasonable period of time (a few weeks, months or years), chemical substances known as emulsifiers must be added prior to homogenization. Emulsifiers are surface-active molecules that adsorb to the surface of freshly formed droplets during homogenization. Once present at the droplet surface, they facilitate the further breakdown of droplets and form a protective membrane that prevents the droplets from aggregating (Dickinson 1992; McClements 1999). The most common emulsifiers used in the food industry are amphiphilic proteins, phospholipids, and small molecule surfactants. The nature of the interfacial membrane formed by these emulsifiers can have a large impact on the rate of lipid oxidation in emulsions. Consequently, food scientists can enhance the oxidative stability of emulsions by manipulating interfacial characteristics using different emulsifiers (see later).

Conceptually, it is convenient to divide an emulsion into three distinct regions: the interior of the droplets, the continuous phase, and the interfacial region (Figure 2). The interface consists of a narrow region surrounding each emulsion droplet that is comprised mainly of surface-active molecules, but that may also contain some oil and water molecules as well as any other type of molecules that are attracted towards it; for example, counter-ions attracted to a charged interface. The characteristics of the interface depend on the type and concentration of the molecules present. Typically, it is only a few nanometers thick, and its properties vary as one moves outwards from the hydrophobic to hydrophilic parts of the emulsifier molecules (Dickinson and McClements 1995). The fraction of space occupied by the interfacial region within an emulsion depends on the droplet size (Table 1). For relatively small droplets, the interfacial region comprises a significant volume of the total droplet, especially for relatively thick interfacial layers. A significant fraction of certain types of molecules within an emulsion may therefore partition into this interfacial region, particularly if they are present at relatively low concentrations. Many hydroperoxides, pro-oxidants, and antioxidants are present in emulsions at concentrations of a few μM to a few mM. For example, a typical antioxidant concentration of 0.2 mM is equivalent to less than 0.02% of the total volume of an emulsion, and therefore it would certainly be possible for it all to be localized within the interfacial region. When surfactants are used to stabilize emulsions, only a fraction of them actually surround the droplets, with the rest remaining in the aqueous phase, usually as surfactant micelles. Amphiphilic and nonpolar molecules may associate with surfactant molecules surrounding the emulsion droplets as well as those present in surfactant micelles. Thus, the total concentration of surfactant added, as well as its distribution between the droplets and micelles, may have a significant impact on the distribution of molecules within an emulsion.

The various molecules in an emulsion partition themselves between the three different regions according to their polarity and surface activity. Nonpolar molecules are located predominantly in the oil phase, polar molecules in the aqueous phase, and amphiphillic molecules at the interface. The molecular environment of a molecule within an emulsion is known to have a significant impact on its chemical reactivity (Wedzicha 1988; Wang and others 1999). Another factor that may also be important for lipid oxidation in food emulsions is the orientation of the lipid

Table 1—Dependence of emulsion characteristics on droplet radius (r) for monodisperse oil-in-water emulsions: A_s = droplet surface area per gram of oil; n = number of droplets per gram of oil; ϕ_l = volume percentage of droplet occupied by surfactant layer. Calculations of ϕ_l were made for surfactants of varying lengths (δ) using the relation: $\phi_l = 100[V_l/(V_{droplet} + V_l)] = 100[1 - r^3/(r + \delta)^3]$. The volume percentage of interfacial region in the whole emulsion is given by $\phi_l\phi$, where ϕ is the droplet volume fraction.

r (μm)	A_S ($m^2\ g^{-1}$)	n (g^{-1})	ϕ_l (%) δ = 2 nm	ϕ_l (%) δ = 5 nm	ϕ_l (%) δ = 10 nm
0.1	33	2.6×10^{14}	5.77	13.62	24.87
0.2	16	3.3×10^{13}	2.94	7.14	13.62
0.5	6.5	2.1×10^{12}	1.19	2.94	5.77
1	3.3	2.6×10^{11}	0.60	1.49	2.94
2	1.6	3.3×10^{10}	0.30	0.75	1.49
5	0.65	2.1×10^{9}	0.12	0.30	0.60
10	0.33	2.6×10^{8}	0.06	0.15	0.30
20	0.16	3.3×10^{7}	0.03	0.07	0.15
50	0.065	2.1×10^{6}	0.01	0.03	0.06

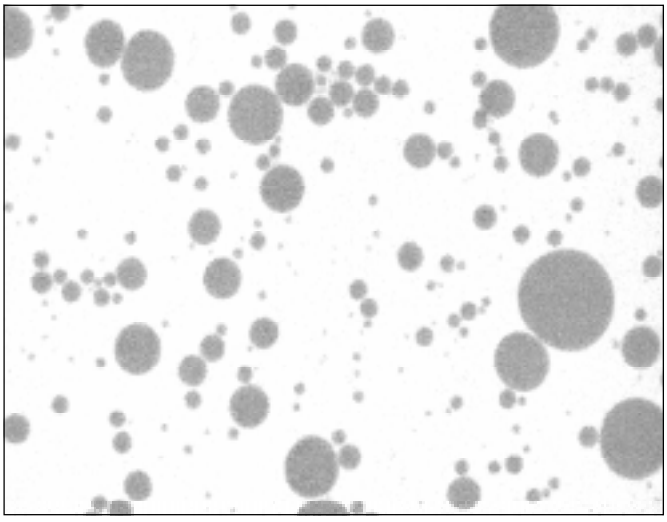

Figure 1—Photograph of hydrocarbon oil-in-water emulsion. The dark regions are emulsion droplets and the light regions are the aqueous phase.

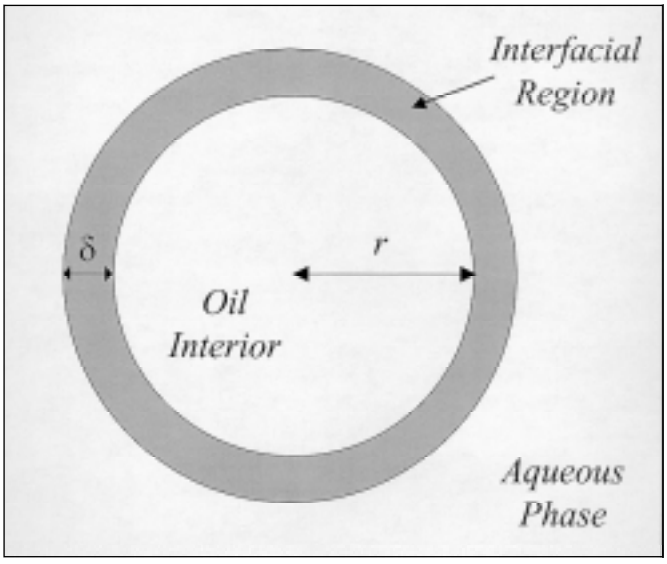

Figure 2—An oil-in-water emulsion can be considered to consist of three phases: the droplet interior, the interfacial region and the aqueous phase.

molecules at the interfacial region; for example, whether they are parallel or perpendicular to the interface (Hiemenz and Rajagopalan 1997), because this will affect their accessibility to water-soluble pro-oxidants or antioxidants. As oxidation proceeds, a variety of different reaction products are produced which may alter their location and orientation within the emulsion. As a consequence, they may become more or less susceptible to lipid oxidation depending on the other types of molecules present in their new environment.

Information about the physical characteristics of emulsion systems is required to understand the factors that influence lipid oxidation in food emulsions; for example, the size distribution, concentration and physical state of the emulsion droplets, the characteristics of the interfacial membrane, and the range and magnitude of any droplet-droplet interactions. Critical assessments of the various analytical techniques available to characterize emulsions properties are given elsewhere (Dickinson and Stainsby 1982; Dickinson 1992; McClements 1999).

Lipid Oxidation

"Lipid oxidation" is a general term that is used to describe a complex sequence of chemical changes that result from the interaction of lipids with oxygen-active species (Nawar 1996; Min 1998; Frankel 1998). The precise mechanism of lipid oxidation in a particular food depends on the nature of the reactive species present and their physicochemical environment (Fritsch 1994; Coupland and McClements 1996a; Erickson and Sista 1997; Decker 1998a). Lipid oxidation can be conveniently divided into three distinct stages: initiation, propagation, and termination (Nawar 1996; Frankel 1998; Min 1998). Studies of lipid oxidation in oil-in-water emulsions and aqueous colloidal systems suggest that the interaction between lipid hydroperoxides located at the droplet surface and transition metals originating in the aqueous phase is the most common cause of oxidative instability (Yoshida and Niki 1992; Mei and others 1998a, 1998b). Transition metals are capable of directly breaking down unsaturated lipids into alkyl radicals (for example, $Fe^{2+} + RH \rightarrow Fe^{3+} + R^{-} + H^{+}$), but this reaction occurs extremely slowly and is therefore not believed to be important in promoting lipid oxidation (Reische and others 1998). The most likely mechanism for the acceleration of lipid oxidation in emulsions is the decomposition of lipid hydroperoxides (ROOH) into highly reactive peroxyl ($ROO^{\cdot}$) and alkoxyl ($RO^{\cdot}$) radicals by transition metals (Eq. 1 and 2) or other pro-oxidants. These radicals react with unsaturated lipids (LH) within the droplets or at the oil-water interface, which leads to the formation of lipid radicals ($L^{\cdot}$ and $LOO^{\cdot}$) (Eq. 3 to 5). The lipid oxidation chain reaction propagates as these lipid radicals react with other lipids in their immediate vicinity (Eq. 6). Some of the lipid radicals formed may be terminated when they react with other radicals (Eq. 7).

$$Fe^{3+} + ROOH \rightarrow Fe^{2+} + ROO^{\cdot} + H^{+} \quad (1)$$

$$Fe^{2+} + ROOH \rightarrow Fe^{3+} + RO^{\cdot} + OH^{-} \quad (2)$$

$$ROO^{\cdot} + LH \rightarrow ROOH + L^{\cdot} \quad (3)$$

$$RO^{\cdot} + LH \rightarrow ROH + L^{\cdot} \quad (4)$$

$$L^{\cdot} + O_2 \rightarrow LOO^{\cdot} \quad (5)$$

$$LOO^{\cdot} + LH \rightarrow LOOH + L^{\cdot} \quad (6)$$

$$LOO^{\cdot} + LOO^{\cdot} \rightarrow \text{nonradical products} \quad [7]$$

Table 2—Influence of droplet size on the time taken for small molecules to diffuse out of emulsion droplets (adapted from McClements 1999).

Radius (μm)	Half-time (s)
0.1	6.6×10^{-7}
0.5	1.6×10^{-5}
1	6.6×10^{-5}
5	1.6×10^{-3}
10	6.6×10^{-3}
50	1.6×10^{-1}

Formation of alkoxyl radicals (Eq. 2) also leads to b-scission reactions, that in turn 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.

The above reaction mechanism does not illustrate the importance of the physical location of the various reactive species within the system. Hydroperoxides in emulsion droplets are often surface-active and therefore accumulate at the surface of the droplets, whereas many of the molecular species responsible for accelerating lipid oxidation originate in the aqueous phase; for example, transition metals or enzymes. Accelerated lipid oxidation may therefore require that the pro-oxidants come into close contact with the lipids at the droplet surface, which depends on the molecular characteristics of the various reactive species involved. Once free radicals have been formed at the droplet surface, they are able to interact with lipids in their immediate vicinity or within the droplet interior. The rate of lipid oxidation in emulsions may therefore be limited by the speed that free radicals, hydroperoxides, or lipids can diffuse from one region to another within a droplet. A rough estimate of the time-scale involved for the movement of molecules within a droplet can be obtained by estimating the time taken for half the molecules of a certain type to completely diffuse out of the droplet (McClements 1999). For the droplet sizes typically found in food emulsions, these time scales are very short (Table 2) compared to the time scales normally observed for lipid oxidation processes. This suggests that diffusion of molecules from one region to another within a droplet is not rate limiting, unless there is some substantial kinetic energy barrier restricting their motion. Nevertheless, more detailed theoretical and experimental studies are required to confirm this hypothesis.

Knowledge of the mechanism by which lipid oxidation proceeds enables food manufacturers to develop effective strategies for retarding its progress. Ideally, one would like to know the details of all the chemical reactions involved and their dependence on environmental conditions. This would require knowledge of the precise location, movement, and physicochemical properties of each of the reactants and products present in the system during the course of the entire reaction. In practice, lipid oxidation leads to the formation of such a huge number of different oxidation products that it is not possible to precisely establish the reaction mechanism. Nevertheless, it is often possible to identify one step (or at least a small number of steps) in the reaction sequence that determines the overall reaction rate; for example, the initiation step or one of the early propagation steps. By controlling this step, it is possible to effectively retard the progress of lipid oxidation. A number of potential strategies for controlling lipid oxidation in oil-in-water emulsions are discussed in a later section.

Our understanding of the factors that influence the oxidative stability of food emulsions relies on the availability of analytical techniques for monitoring lipid oxidation. A wide variety of analytical techniques have been developed to study lipid oxidation

in bulk fats and oils (Pike 1994; Nawar 1996; Shahidi and Wanasundara 1998b). Many of these techniques can also be used to monitor lipid oxidation in emulsions, although it is often necessary to extract the oil phase prior to analysis. These techniques measure changes in the concentration of molecular species that are indicative of the progress of lipid oxidation. Some techniques measure loss of initial reactants (such as oxygen, lipid, hydroperoxides and antioxidants), others the formation of intermediary products (such as hydroperoxides and conjugated dienes), and others the formation of end products (such as alcohols, aldehydes, hydrocarbons, and ketones). The chemical complexity of lipid oxidation means that one must be careful when selecting an appropriate technique to characterize its progress (Fritsch 1994; Huang and others 1994; Sims 1994). It is always advisable to use at least two, if not more, different analytical techniques to obtain an adequate description of the process.

Factors That Influence Lipid Oxidation in Emulsions

Chemical Structure of Lipids. Ultimately, it is the chemical structure of a lipid molecule that determines its susceptibility to oxidation, particularly the number and location of the double bonds. Saturated lipids are considerably more stable to lipid oxidation than unsaturated lipids. The most straightforward means of retarding lipid oxidation in food emulsions would therefore be to use a lipid source that contained little or no unsaturated fats. In practice, this is unlikely to be a feasible strategy because unsaturated fats have physical and sensory characteristics that cannot be obtained using saturated fats alone; for example, saturated lipids are more likely to be crystalline than unsaturated lipids of the same chain length. In addition, saturated fats have been associated with various types of health problems, and so increasing their content in foods would be contrary to dietary recommendations (Kritchevsky 1998). Food manufacturers must therefore find alternative means of controlling lipid oxidation in foods.

In bulk lipids, the rate of oxidation of fatty acids increases as their degree of unsaturation increases (Nawar 1996; Shahidi and Wanasundara 1998b). Thus, fats that contain high concentrations of polyunsaturated fatty acids are particularly prone to lipid oxidation. Perhaps surprisingly, a number of studies have found that fatty acids dispersed in aqueous colloidal dispersions show the opposite trend, with their oxidative stability increasing as their degree of unsaturation increases (Miyashita and others 1993, 1994). These experiments were carried out using unsaturated fatty acids solubilized in nonionic surfactant micelles (Tween 20). At present, the reason for this observation is unknown, although it has been suggested that it be due to differences in the molecular arrangements of the fatty acids within the micelles. It is possible that the more unsaturated fatty acids are buried more deeply within the hydrophobic interior of the micelles and are therefore less susceptible to attack by aqueous-phase pro-oxidants. Nevertheless, there is currently no direct experimental evidence to support this hypothesis. To the authors' knowledge, equivalent experiments have not been carried out using oil-in-water emulsions, and so we cannot say whether the oxidative stability of lipids in these systems increases or decreases with unsaturation.

The position of the double bond on an unsaturated fatty acid has also been shown to influence its susceptibility to lipid oxidation in colloidal dispersions (Miyashita and others 1995). Various geometrical and positional isomers of unsaturated fatty acids were solubilized in nonionic surfactant micelles (Tween 20) at pH 7.4, and oxidation was catalyzed by an Fe^{2+}/ascorbic acid system. These measurements showed that the closer the double bond was to the methyl end of a fatty acid, the greater its stability to oxidation. This was probably because the fatty acids are orientated within the micelles so that the carboxyl group protrudes into the aqueous phase, while the hydrocarbon tail is located in the hydrophobic interior. The Fe^{2+}/ascorbic acid system operates in the aqueous phase, and therefore the more deeply buried the double bond of an unsaturated fatty acid is in the micelle interior, the more stable it is to oxidation. This type of effect would also be expected to be important in oil-in-water emulsions, although the experiments needed to establish this have not yet been conducted.

The polarity of lipid molecules determines their location within an emulsion droplet, which in turn determines their susceptibility to oxidation. Experiments with oil-in-water emulsions containing surface-active ethyl linoleate show that this lipid is more susceptible to oxidation when it is located at the droplet surface than when it is in the droplet interior (Coupland and others 1995; Coupland and McClements 1996b). We would therefore expect that highly nonpolar lipids or antioxidants would be less susceptible to lipid oxidation than more polar or amphipilic ones, although studies need to be carried out to determine this.

Oxygen Concentration

Lipid oxidation involves the reaction between unsaturated lipids and oxygen. Oxygen is about three times more soluble in food oils than in water (Ke and Ackman 1973) and so there is always likely to be sufficient oxygen present in the oil phase to fuel lipid oxidation, unless specific measures are taken to exclude it. The effect of oxygen concentration on the kinetics of lipid oxidation in linoleic acid oil-in-water emulsions stabilized by Tween 20 has been studied (Marcuse and Fredricksson 1968, 1969). At low oxygen concentrations, it was observed that the rate-limiting step for lipid oxidation was the diffusion of oxygen through the aqueous phase. Under these oxygen limiting conditions, the rate of lipid oxidation increased with mechanical agitation and cooling because these processes increased the oxygen concentration. At high oxygen concentrations, oxygen diffusion was much faster than the rate of lipid oxidation, and so it was not limiting. An effective means of retarding lipid oxidation is therefore to reduce the concentration of oxygen present; for example, by packing the food under vacuum or nitrogen. Nevertheless, exclusion of oxygen from food products during processing and storage is often not practical, and once a product is opened to the atmosphere it will become susceptible to lipid oxidation.

Antioxidants

One of the most effective means of retarding lipid oxidation in fatty foods is to incorporate antioxidants (for reviews, see Nawar 1996; Decker 1998a,b; Frankel 1998; Reische and others 1998). Antioxidants work by a variety of different methods, including control of oxidation substrates (for example, oxygen and lipids), control of pro-oxidants (for example, reactive oxygen species and pro-oxidant metals), and inactivation of free radicals (Decker, 1998b; Frankel, 1996, 1998, 1999). The term "antioxidant" must be carefully applied because some substances that retard lipid oxidation under one set of conditions actually promote it under a different set (Decker 1998b; Frankel 1998; Reische and others 1998). It has proved useful to classify antioxidants according to the mechanism of their action as either primary or secondary antioxidants (Reische and others 1998). Nevertheless, it should be noted that certain substances have more than one mechanism of antioxidant activity.

Primary Antioxidants. A primary antioxidant, also known as a "chain-breaking" antioxidant, is a substance that is capable of accepting free radicals so that it can delay the initiation step or interrupt the propagation step of autoxidation (Reische and others 1998). Chain-breaking antioxidants react with lipid and peroxyl radicals and convert them to more stable, radical, or nonrad-

ical products (Eq. 8 to 10). The antioxidant radicals (A) produced by this process are much less reactive than lipid and peroxyl radicals, and therefore are less effective at promoting oxidation. Chain-breaking antioxidants have a higher affinity for peroxyl radicals than lipids, and therefore they tend to scavenge the free radicals produced during the initiation and propagation steps.

$$ROO^{\cdot} + AH \mapsto ROOH + A^{\cdot} \quad (8)$$

$$RO^{\cdot} + AH \mapsto ROH + A^{\cdot} \quad (9)$$

$$R^{\cdot} + AH \mapsto RH + A^{\cdot} \quad (10)$$

Chain-breaking antioxidants are also capable of terminating the lipid oxidation reaction by reacting with peroxyl radicals, alkoxyl radicals and other antioxidants (Eq. 11 to 13).

$$ROO^{\cdot} + A^{\cdot} \mapsto ROOA \quad (11)$$

$$RO^{\cdot} + A^{\cdot} \mapsto ROA \quad (12)$$

$$A^{\cdot} + A^{\cdot} \mapsto AA \quad (13)$$

Chain-breaking antioxidants differ in their effectiveness in inhibiting lipid oxidation, partly because of their chemical properties, but also because of their physical location within a system (Frankel 1998). Antioxidants that are effective at retarding lipid oxidation in bulk oils may not be as effective in emulsions (Porter 1980; Porter 1993; Frankel 1998). For example, hydrophilic antioxidants are less effective in oil-in-water emulsions than lipophilic antioxidants, whereas lipophilic antioxidants are less effective in bulk oils than hydrophilic antioxidants. This observation has been supported by more recent studies of the effectiveness of nonpolar and polar antioxidants in suppressing the oxidation of corn oil in bulk and in oil-in-water emulsions (Frankel and others 1994, 1996a,b; Hopia and others 1996; Huang and others 1996a,b; Pekkarinen and others 1999). Predominantly nonpolar antioxidants (α-tocophorol, ascorbyl palmitate, carnosol) were found to be more effective in oil-in-water emulsions than in bulk oil, while the opposite was observed for predominantly polar antioxidants (Trolox, ascorbic acid, carnosic acid, and rosmarinic acid). Differences in the effectiveness of antioxidants in bulk oils and emulsions are mainly due to their different affinities for the air-oil or oil-water interfaces in the two systems. Polar antioxidants are more effective in bulk oils because they accumulate at the air-oil interface. Oxygen is usually distributed throughout the whole volume of the oil, but it may become depleted in the interior of the oil as oxidation proceeds. However, at the oil's surface a relatively high oxygen concentration can be maintained so that lipid oxidation occurs more rapidly. In addition, hydroperoxides responsible for promoting the initial stages of lipid oxidation are relatively polar and are therefore likely to accumulate at an oil-air interface. Consequently, lipid oxidation occurs more rapidly at an oil-air interface than in bulk oil, so that antioxidants that are preferentially located at the interface are more effective at retarding oxidation than those that are evenly distributed throughout the oil. In contrast to bulk oils, predominantly nonpolar antioxidants are more effective in emulsions because they are retained in the oil droplets and/or accumulate at the oil-water interface, the location where interactions between hydroperoxides at the droplet surface and pro-oxidants originating in the aqueous phase occur. For the same reason, the effectiveness of chain-breaking antioxidants at retarding lipid oxidation in oil-in-water emulsions increases as their polarity decreases or their surface activity increases, because they are then more likely to be localized at the oil-water interface where oxidation occurs (Huang and others 1996a,b). These studies suggest that amphiphilic antioxidants should be the most effective at retarding lipid oxidation in emulsions because they tend to be located at the interfacial boundaries where oxidation reactions are promoted. Nevertheless, more research needs to be carried out in this area using well-characterized emulsion-pro-oxidant-antioxidant systems. In particular, quantitative information about the precise location of the different types of molecules within an emulsion is required to better understand the influence of physical location on lipid oxidation.

The electrical charge of chain-breaking antioxidants relative to that of the droplet surface is also important in determining their location within emulsions, and therefore their effectiveness at retarding lipid oxidation. The rate of lipid oxidation in salmon oil-in-water emulsions containing either negatively charged droplets (SDS) or uncharged droplets (Brij 35) was measured (Mei and others 1999). The influence of adding negatively charged (gallic acid, G^{-}), uncharged (methyl gallate, G^{0}) and positively charged (gallamide, G^{+}) phenolic antioxidants to the emulsions was examined. At pH 7, the effectiveness of the antioxidants in inhibiting lipid oxidation in the negatively charged (SDS) droplets was: G^{+} , $G^{0} > G^{-}$, which suggests that antioxidants that are electrostatically attracted to the surface of the emulsion droplets are more effective at retarding lipid oxidation than those that are electrostatically repelled. The uncharged antioxidant was also effective because it has the lowest water-solubility, and is therefore more likely to be present at the interface than highly polar-charged antioxidants (Schwarz and others 1996). On the other hand, at pH 3, the effectiveness of the antioxidants in inhibiting lipid oxidation in the negatively charged droplets was: $G^{0} > G^{-} > G^{+}$ with pro-oxidant activity being observed in some cases. The most likely reason for this apparent discrepancy is that the water-solubility of Fe^{3+} is much higher at lower pH (Graf and others 1984), and the antioxidant is capable of regenerating the iron into its most active form (for example $Fe^{3+} \mapsto Fe^{2+}$). At pH 3, there are appreciable quantities of both Fe^{3+} and G^{+} localized at the droplet surface near to the hydroperoxides, and so the antioxidant is able to regenerate the iron as it is used up in the oxidation reaction. It should also be mentioned that at the lower pH the G^{-} may lose some of its electrical charge (the pK for carboxyl groups is normally around pH 4-5), and therefore electrostatic repulsion between this antioxidant and the droplet surface will be less.

This study highlights the importance of considering the relative location of the different types of molecular species involved in the reactions; for example substrate, pro-oxidants, and antioxidants. In uncharged (Brij) droplets, the relative effectiveness of the antioxidants at both pH 3 and 7 was: $G^{0} > G^{+} > G^{-}$ (Mei and others 1999). The uncharged antioxidant is probably the most effective because it has the lowest water-solubility and is therefore more likely to be present at the oil-water interface than the charged antioxidants (Schwarz and others 1996).

The importance of antioxidant location on their effectiveness in inhibiting lipid oxidation has led researchers to measure the partitioning of a variety of antioxidants in water-oil bilayers, surfactant-oil bilayers, surfactant micelle solutions, and oil-in-water emulsions (Castle and Perkins 1986; Schwarz and others 1996; Huang and others 1997). The type, number, and position of the chemical groups that an antioxidant contains determines its polarity, which in turn determines its partitioning between oil, water, and interfacial regions. A direct correlation has been established between the polarity of antioxidants and their effectiveness in inhibiting oxidation in aqueous solutions of oxidizable lecithin membranes—the higher the polarity of the antioxidant, the lower its efficiency in inhibiting lipid oxidation (Schwarz and others 1996). The reason for this correlation is that highly polar

antioxidants are located predominantly in the aqueous phase, away from the place where lipid oxidation occurs. Nevertheless, it should be stressed that hydrophilic antioxidants can partition substantially into the polar head groups of surfactants, particularly nonionic surfactants with large polar head groups or ionic surfactants with an opposite charge to the antioxidant (Schwarz and others 1996; Huang and others 1997). The molecules in a system tend to adopt a configuration that maximizes favorable interactions and minimizes unfavorable interactions. Amphiphilic antioxidants may accumulate at an interface because this enables their polar parts to form dipole-dipole, ion-dipole, or ion-ion bonds with water or the polar head groups of surfactants, while the nonpolar parts can interact with the hydrophobic tail of the surfactants.

Many antioxidants have ionizable groups, and so there polarity is strongly influenced by the pH of the surrounding aqueous phase. The ionized form of an antioxidant is much more polar than the nonionized form and therefore has a greater affinity for aqueous solutions (Schwarz and others 1996). The influence of ionization on partitioning depends on the polarity of the rest of the antioxidant molecule. If the rest of the molecule is polar, then both the ionized and nonionized forms will be predominantly water-soluble. This type of behavior is exhibited by gallic acid in oil-in-water emulsions, which partitions about 94% into the aqueous phase at pH 7 when the acid group is fully ionized and about 86% at pH 3 when the acid is partially ionized (Schwarz and others 1996). On the other hand, if the rest of the antioxidant molecule is nonpolar, then the ionized form may be predominantly water-soluble but the nonionized form may have a strong tendency to partition into the oil or interfacial regions. This type of behavior is exhibited by Trolox in oil-in-water emulsions, which partitions about 90% into the aqueous phase at pH 7 when the acid group is fully ionized, and only about 30% at pH 3 when the acid group is partially ionized. The effectiveness of this type of antioxidant at retarding lipid oxidation should therefore depend strongly on pH.

The partitioning of hydrophilic and lipophilic antioxidants has recently been measured in mayonnaise (Jacobsen and others 1998, 1999a,b). Predominantly polar antioxidants were mainly located in the aqueous phase and the interfacial layer, with the fraction in the aqueous phase increasing as antioxidant polarity increased. Predominantly nonpolar antioxidants were mainly located in the oil phase and the interfacial layer, with the fraction in the oil phase increasing as antioxidant polarity decreased. This study showed that antioxidants vary widely in their location within a food emulsion depending on their molecular structure, which has important implications for their effectiveness at retarding lipid oxidation.

Synthetic food additives, such as BHA, BHT, or TBHQ, are common chain-breaking antioxidants used in food systems (Decker 1998b; Reische and others 1998). These synthetic antioxidants are often highly effective at controlling lipid oxidation; however, consumer demand for all-natural foods has prompted the food industry to look for more "label friendly" alternatives. For this reason, a number of studies have been carried out to assess the effectiveness of natural chain-breaking antioxidants in bulk oils and emulsions, including tocopherols, fruit extracts (blackberries, blueberries, cherries, grapes, raspberries, strawberries), and plant extracts (catnip, chrysanthemum, hyssop, lemon balm, oregano, rosemary, sage, tea, thyme) (Kanner and others 1994; Frankel and others 1995, 1996a,b, 1997; Hopia and others 1996; Huang and others 1994, 1996a,b,c; Huang and Frankel 1997; Heinonen and others 1997, 1998; Satue-Gracia and others 1997; Abdalla and Roozen 1999; Duh 1999; Frankel 1997, 1999). A number of these natural antioxidants have been shown to be highly effective at retarding lipid oxidation in food emulsions; however, others have shown only limited effectiveness. The natural extracts that are most effective in oil-in-water emulsions are those that contain antioxidant components that accumulate either in the droplets or at the oil-water interface; for example, those that are relatively nonpolar. It should be noted that, even though natural antioxidants have been identified that can retard lipid oxidation in emulsions, their utilization may still be limited because of their relatively high price and associated flavors and colors (Giese 1996). In addition, chain-breaking antioxidants are consumed during oxidation, which means that rancidity will eventually occur. For this reason, alternative strategies of retarding lipid oxidation in emulsions are being investigated.

Secondary Antioxidants. Secondary antioxidants can retard lipid oxidation through a variety of mechanisms, including chelation of transition metals, replenishing of hydrogen to primary antioxidants, oxygen scavenging, and deactivation of reactive species (Reische and others 1998). It should be noted that none of these mechanisms involves conversion of free radical species to more stable products. From the standpoint of oil-in-water emulsions, the most important type of secondary antioxidants are those that chelate transition metal ions. The presence of transition metals, such as iron or copper, in the aqueous phase of oil-in-water emulsions has been shown to be a major factor in the promotion of lipid oxidation. The effectiveness of transition metals at promoting lipid oxidation increases dramatically when they are located near droplet surfaces, because they are then in closer proximity to the lipid substrate. Consequently, any aqueous phase component that chelates transition metals and removes them from the vicinity of the droplet surface would be expected to retard lipid oxidation.

EDTA and phytate, both transition metal chelators, have been shown to dramatically retard lipid oxidation in salmon oil-in-water emulsions stabilized by an anionic surfactant because they removed iron from the droplet surface (Mei and others 1998b). EDTA and apo-transferrin, a protein that specifically binds iron, have been shown to greatly reduce the rate of lipid oxidation in salmon oil-in-water emulsions stabilized by anionic and nonionic surfactants (Mancuso and others 1999a). The ability of EDTA to chelate iron has also been shown to retard autoxidation of soybean oil-in-water emulsions stabilized by cyclodextrin (Shimada and others 1992) and the oxidation of phospholipid vesicles (Fukuzawa and others 1995). These studies indicate that trace metals naturally occurring as impurities in food emulsions are largely responsible for promoting lipid oxidation.

Chelators that act as antioxidants can inhibit metal-catalyzed reactions by a variety of different mechanisms, including prevention of metal redox cycling, formation of insoluble metal complexes, occupation of metal coordination sites, and steric hindrance of interactions between metals and lipid substrates (Decker 1998b; Reische and others 1998). It should be noted that some chelating agents can increase the pro-oxidant activity of transition metals, either by increasing their solubility in the oil or water phases or by altering their redox potential (Decker 1998b).

At present, most of the chelating agents used as additives to prevent lipid oxidation in foods are synthetic; for example, EDTA, phosphoric acid and polyphosphates (Reishche and others 1998). There is some concern about the use of synthetic chelating agents because they are believed to bind minerals so strongly that they may not be bioavailable, and because consumers do not regard them as "label friendly." Natural chelating agents, such as citric acid, can be used but they tend to be less effective at chelating transition metals and have limited use in many foods because of their flavor, solubility, and/or requirement for acid environments. Research is therefore being carried out to identify alternative natural chelating agents that can be used in a wider range of food applications. A variety of proteins, protein

hydrolysates, and polysaccharides have been shown to be effective at chelating transition metals and reducing lipid oxidation in emulsions (Allen and Wrieden 1982a,b; Shimada and others 1992, 1994; Gohtani and others 1999; Mancuso and others 1999a). Some of these components are already used for other purposes in food emulsions and may therefore play a dual function; for example, polysaccharides that are used as thickening agents may also have antioxidant activity.

Interfacial Characteristics

Electrical charge. Many of the surface-active components in foods are electrically charged; for example, proteins, phospholipids, and surfactants (McClements 1999). Consequently, the interfacial membrane surrounding the droplets may also have an electrical charge with a magnitude and sign that is determined by the type and concentration of charged surface-active species present. The electrical properties of a surface are characterized in terms of the *surface charge density* (σ) and the *surface potential* (φ_0) (Hunter 1993; Heimenz and Rajagopalan 1997). The surface charge density is the number of charges per unit surface area, whereas the surface potential is the energy required to charge the surface (Hunter 1993). An electrically charged surface attracts oppositely charged ions in the surrounding aqueous phase. These "counter-ions" may be simple mineral ions (for example Na^+, Ca^{2+}, Cl^-), or they may be ions capable of promoting (for example Cu^+, Cu^{2+}, Fe^{2+}, Fe^{3+}) or retarding (for example ionic antioxidants) lipid oxidation. The greater the surface charge density on an emulsion droplet, the greater its ability to attract oppositely charged counter-ions. It is therefore possible to control the rate of lipid oxidation in emulsions by manipulating the sign and magnitude of the charge on the droplet surfaces.

The importance of droplet charge in controlling the rate of lipid oxidation in oil-in-water emulsions has been demonstrated in a number of studies. The rate of lipid oxidation in corn oil-in-water emulsions containing negatively charged, positively charged, or uncharged droplets has been measured (Mei and others 1998a). Droplets with different charges were created by using anionic (SDS), cationic (DTAB), or nonionic (Brij 35) surfactants to stabilize the emulsions. Lipid oxidation was catalyzed by adding iron to the aqueous phase of the emulsions. Lipid oxidation rates were highest for negatively charged droplets, and were fairly similar for uncharged droplets and positively charged droplets (Figure 3). Similar results were found for the oxidation of salmon oil-in-water emulsions stabilized by anionic (SDS), cationic (DTAB), or nonionic (Tween 20) surfactants in the absence of added metals (Mancuso and others 1999a). These results were attributed to the fact that lipid oxidation was promoted when positively charged iron ions were electrostatically attracted to the surface of the negatively charged emulsion droplets, because then the lipid substrate and pro-oxidant were in close proximity. One would assume that lipid oxidation would be retarded when iron ions were electrostatically repelled from the surface of positively charged droplets, and therefore that a cationic surfactant would give lower oxidation rates than a nonionic surfactant. The fact that little difference was observed in the measured oxidation rates for the cationic and nonionic surfactant stabilized emulsions shown in Figure 3 may have been because the study was not carried out long enough for a significant amount of oxidation to have occurred.

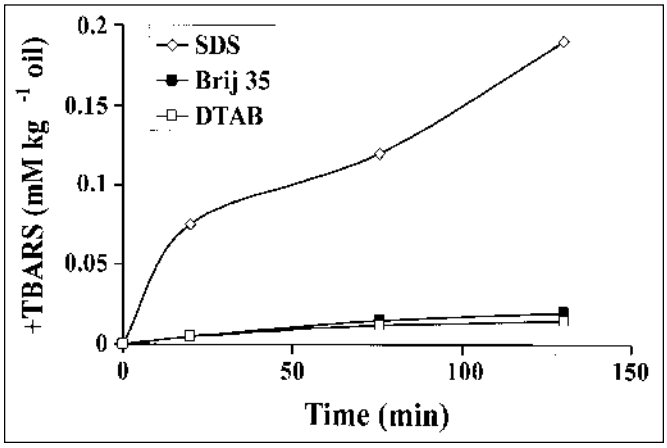

Figure 3—Influence of droplet charge on iron catalyzed oxidation of salmon oil-in-water emulsions (adapted from Mei and others 1998a)

The role of surface charge on lipid oxidation has been supported by measurements of the ϵ-potential of hexadecane emulsion droplets dispersed in aqueous solutions containing iron ions (Mei and others 1998b). Both ferrous and ferric ions are associated with negatively charged droplets, but not with positively charged or uncharged droplets. Further support for this hypothesis was obtained from experiments that showed factors that decrease electrostatic interactions between negatively charged droplets and iron ions decreased lipid oxidation rates; for example the addition of iron chelators (EDTA and phytate) or NaCl (Mei and others 1998a, b). As well as influencing the effectiveness of pro-oxidants at promoting oxidation, electrostatic interactions also play an important role in determining the effectiveness of antioxidants at retarding lipid oxidation (see Antioxidants section).

The role of droplet charge has also been demonstrated in studies of lipid oxidation in protein stabilized emulsions. The rate of lipid oxidation was measured in Menhaden oil-in-water emulsions stabilized by either Tween 20 or whey protein isolate (Donnelly and others 1998). Measurements were made at pH values above (pH 7) and below (pH 3) the isoelectric point of the protein (IEP pH 5). In the emulsions stabilized by the nonionic surfactant, the rate of lipid oxidation was faster at pH 3 than at pH 7. As the electrical charge of the droplets stabilized by nonionic surfactants did not change appreciably with pH, the observed difference in oxidation rates was attributed to the fact that iron is more water-soluble at the lower pH. The opposite trend was observed for emulsions stabilized by whey protein, the lipid oxidation rate being faster at the higher pH despite the fact that the solubility of the iron was less at this pH (Figure 4). At pH 3, the emulsion droplets were positively charged and therefore repelled the iron ions, but at pH 7 they were negatively charged and therefore attracted the iron ions (Donnelly and others 1998). When Tween 20 was added to the emulsions containing positively charged whey proteins (pH 3), their stability to lipid oxidation decreased because the surfactant displaced the proteins from the droplet surface (Donnelly and others 1998). This study clearly shows the important role that pH plays in determining the stability of protein-stabilized emulsions to lipid oxidation.

Physical barrier. Recent studies suggest that the interfacial membrane may be able to act as a physical barrier that separates lipid substrates from pro-oxidants in the aqueous phase (Silvestre and others 2000). Lipid oxidation was monitored in salmon oil-in-water emulsions stabilized by two different nonionic surfactants (Brij 76 and Brij 700) that had the same nonpolar tail group length ($CH_3(CH_2)_{17}$), but different polar head group lengths (either 10 or 100 oxyethylene groups). The emulsions all had similar droplet size distributions, so that the surface areas of lipids exposed to the aqueous phase were similar. Lipid oxidation was slowest in the emulsion containing droplets stabilized by the surfactant with the longest polar head group, which suggests that the thicker interfacial layer was able to act as a physical bar-

rier that separated the lipid substrate from catalysts originating in the aqueous phase. A number of researchers have suggested that the ability of proteins to form relatively thick and viscoelastic membranes around lipid droplets is at least partly responsible for their ability to retard lipid oxidation in emulsions (Donnelly and others 1998).

Chemical barrier. Certain types of emulsifier molecules may be able to act as a chemical barrier to lipid oxidation. Aqueous solutions of certain sugars and amino acids are capable of scavenging free radicals, therefore retarding lipid oxidation (Marcuse 1962; Sims and others 1979; Chen and Nawar 1991). Many emulsifier molecules contain either sugar or amino acid moieties (for example, gum arabic, modified starch, and proteins), and may therefore act as radical scavengers. Proteins also contain amino acids such as cysteine that may be oxidized more readily than lipids (Taylor and Richardson 1981; Moller and others 1998; Tong and others 2000a,b). 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.

Droplet Characteristics

The physical characteristics of the droplets in food emulsions may vary considerably; for example concentration, size, and physical state. The droplet concentration can vary from a fraction of a percent (for example fruit beverages) to more than 80% (for example mayonnaise). The effect of droplet concentration on lipid oxidation in safflower oil-in-water emulsions containing sucrose has been investigated (Sims and others 1979; Sims 1994). An increase in the fraction of oil that oxidized was observed as the concentration of oil droplets in the emulsion was decreased from 43.75 to 6.25%. One possible cause of this increase is that the number of radicals generated per droplet increased as the droplet concentration decreased. However, the overall sucrose concentration in the emulsions was kept constant (37.5%), and so the sucrose concentration in the aqueous phase increased as the droplet concentration increased. The observed decrease in the fraction of oxidized oil with increasing droplet concentration may therefore have been due to the ability of sucrose to retard oxidation.

The effect of droplet concentration on the autoxidation and enzymic oxidation of linoleic acid in aqueous colloidal dispersions has also been investigated (Roozen and others 1994a,b). Oxidation was monitored in model systems that consisted of lipid substrate (linoleic acid) solubilized in nonionic surfactant micelles (Tween 20), in the presence and absence of low concentrations of inert oil droplets (0–3 wt% *n*-hexadecane or triolein). The rate of linoleic acid oxidation decreased as the concentration of inert oil droplets increased. This was probably because some of the linoleic acid moved from the surfactant micelles into the interior of the emulsion droplets, and therefore became inaccessible to direct interaction with the pro-oxidants in the aqueous phase. The model system used in these studies is not a particularly good representation of lipid oxidation in real food emulsions because the concentration of unsaturated lipids was kept constant as the droplet concentration was varied. In practice, unsaturated lipids will normally be present in the oil contained within the droplets; therefore, increasing the droplet concentration would also increase the unsaturated lipid concentration. Nevertheless, these results do suggest that incorporation of unsaturated lipids into emulsion droplets is more effective at protecting them against oxidation than solubilizing them in micelles. This may have important implications in food emulsions stabilized by surfactants in which a significant fraction of the surfactant is present in the aqueous phase as micelles.

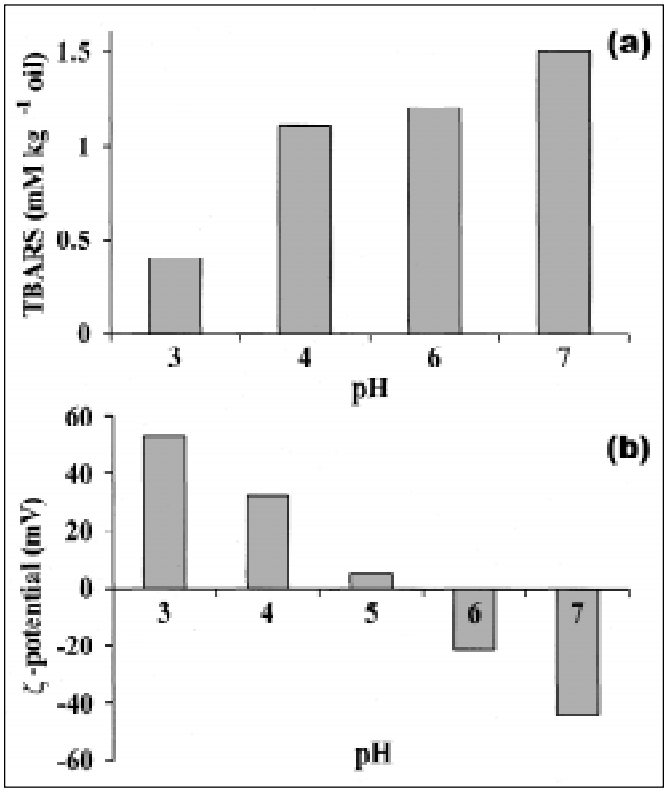

Figure 4—Influence of pH on the oxidative stability and ϵ-potential of whey protein stabilized Menhedan oil-in-water emulsions (adapted from Donnelly and others 1998).

The partitioning of lipid breakdown products between the oil phase, aqueous phase, and headspace may affect the sensory perception of food emulsions (McClements 1999; Jo and Ahn 1999). For example, flavor components are perceived much more strongly in water than in oil (McNulty 1987). In addition, the aroma of an emulsion is determined by the type and concentration of volatile molecules in the headspace (McClements 1999). Many of the reaction products from lipid oxidation tend to be more soluble in the oil phase than in the water phase. This means that for a fixed concentration of volatile components, the concentration of volatiles in the headspace of an emulsion decreases as the droplet concentration increases (Roozen and others 1994a,b; Jacobsen and others 1998, 1999a; Jo and Ahn 1999). As a consequence, a low-fat food may be perceived as being more oxidized than a high-fat food, even though they both contain the same overall concentration of volatile reaction products. This may pose a problem for the development of reduced-fat foods that contain unsaturated lipids.

The mean diameter of the droplets in food emulsions can vary from less than 0.2 μm (for cream liqueurs) to greater than 100 μm (for salad dressings), depending on the product. There have been few systematic studies of the influence of droplet size on the oxidative stability of oil-in-water emulsions. Nevertheless, it is well established that lipid oxidation is accelerated by reactions that take place at the surface of the droplets. The surface area of droplets exposed to the aqueous phase per unit volume of emulsion is given by the following expression: $A_S = 6\phi / d_{VS}$, where ϕ is the droplet volume fraction and d_{VS} is the volume-surface mean diameter (McClements 1999). For a fixed droplet concentration, the droplet surface area therefore increases as the droplet size decreases. One would therefore expect the rate of lipid oxidation to increase as the droplet size decreases, because

a greater amount of lipid would be exposed to the aqueous phase. A recent study of the oxidation of emulsified docosahexaenoic acid did find an increase in oxidation rate as the droplet size was decreased (Gohtani and others 1999). Nevertheless, the dependence of lipid oxidation on droplet size could also depend on the relative concentrations of the reactive species present. If there is an excess of reactants in a system, then doubling the surface area may double the concentration of reactants at the droplet surface, which would lead to the expected increase of lipid oxidation with decreasing droplet size. On the other hand, if there is only a limited amount of reactants (for example hydroperoxides) in the system, they may all be present at the droplet surface anyway and changing the droplet size may not have an effect on the oxidation rate. This might explain the fact that one study found no dependence of the lipid oxidation rate on droplet size (Roozen and others 1994a).

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 food emulsions are liquid around room temperature; however, they may become partially or totally solidified at refrigerated temperatures. Studies with bulk fats suggest that lipid oxidation occurs much more slowly when the fat is crystalline than when it is liquid, but similar studies have not yet been carried out with oil-in-water emulsions.

Interactions with Aqueous Phase Components

Many food emulsions contain ingredients in the aqueous phase that may impact lipid oxidation, such as proteins, sugars, acids, bases, buffers, salts, surfactants, and polysaccharides. These ingredients may act as either pro-oxidants 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. It is therefore important to determine the role of the ingredients commonly found in food emulsions on their susceptibility to lipid oxidation.

Salts. The addition of NaCl (0–173 mM) to corn oil-in-water emulsions stabilized by a negatively charged surfactant (SDS) was shown to slightly reduce the rate of lipid oxidation in the absence of added iron, but to slightly increase it in the presence of added iron (Mei and others 1998a,b). The antioxidant effect of NaCl is probably because of its ability to screen electrostatic interactions between charged species (Hunter 1993), and therefore reduce the tendency for iron ions to accumulate at the droplet interface. The pro-oxidant effect of NaCl is probably because of its ability to increase the catalytic activity of iron (Osinchak and others 1992). These observations suggest that salts may either act as pro-oxidants or antioxidants depending on the nature of the system involved.

Sugars. Nonreducing sugars, such as sucrose, have been shown to inhibit lipid oxidation. The addition of sucrose (0– 67%) and various other sugar alcohols to the aqueous phase of safflower oil-in-water emulsions stabilized by an anionic surfactant has been shown to reduce the rate of lipid oxidation (Sims and others 1979; Sims 1994). Sucrose has also been shown to reduce lipid oxidation of linoleic acid in oil-in-water emulsions stabilized by a nonionic surfactant (Tween 20) (Ponginebbi and others 1999). A number of mechanisms were proposed to account for the ability of sucrose to retard lipid oxidation in emulsions. Sucrose decreases the concentration of oxygen dissolved in the aqueous phase, increases the viscosity of the aqueous phase (thereby decreasing the diffusion of reactive species to the droplet surface), and acts as a free radical scavenger. Reducing sugars have been shown to promote lipid oxidation in aqueous colloidal dispersions (Yamauchi and others 1982, 1984; Shimada and others 1992). The origin of this pro-oxidative effect is the ability of reducing sugars to reduce transition metal ions to their most active state (for example Fe^{3+} Fe^{2+}) (Yamauchi and others 1984). Any other type of food component that can act as a reducing agent for transition metals may also be effective at promoting lipid oxidation; for example glutathione, ascorbate, or tocopherol (Yoshida and Niki 1992).

Polysaccharides. Polysaccharides are often added to oil-in-water food emulsions to enhance the viscosity of the aqueous phase, which imparts desirable textural attributes and stabilizes the droplets against creaming (Dickinson 1992; McClements 1999). Studies have shown that polysaccharides are also capable of retarding lipid oxidation in oil-in-water emulsions (Shimada and others 1992, 1994; Gohtani and others 1999). A number of mechanisms have been proposed to account for the ability of polysaccharides to inhibit lipid oxidation. Intuitively, one might expect that polysaccharides retard lipid oxidation because they increase the viscosity of the aqueous phase and therefore slow down the movement of reactants. In reality, this is not true because at the molecular level small molecules are able to move relatively unhindered through networks of polysaccharide molecules used as thickening agents (Basaran and others 1999). Two other mechanisms that have been proposed are metal ion chelation and hydrogen donation. Xanthan has been shown to be extremely effective at inhibiting lipid oxidation in oil-in-water emulsions due to its ability to chelate metal ions at negatively charged pyruvate sites (Shimada and others 1992, 1994). Tragacanth and methylcellulose were less effective antioxidants because they lack the ability to chelate transition metals (Shimada and others 1992). Nevertheless, tragacanth had some antioxidant activity because of its ability to donate hydrogen and therefore act as a radical chain breaker. These experiments suggest that polysaccharides added to stabilize oil-in-water emulsions against creaming or to impart desirable textural characteristics might also be able to stabilize lipids against oxidation.

Amino acids. A number of amino acids have been shown to have antioxidant activity in emulsions and micellar systems; for example histidine, phenylalanine, tryptophan, cysteine, proline, and lysine (Marcuse 1962; Karel and others 1975; Sims and Fioriti 1980; Decker 1998b). The primary mechanism for this antioxidant activity is believed to be inactivation of free radicals and metal chelation (Decker 1998b). Some amino acids have been shown to act as pro-oxidants when they are present at relatively high concentrations (Marcuse 1962). The above studies show that the effectiveness of amino acids at retarding lipid oxidation depends on their chemical structure and concentration.

Proteins. Proteins are often used in food emulsions to stabilize droplets against flocculation or coalescence (McClements 1999). The influence of these adsorbed proteins on lipid oxidation in emulsions was considered in the section on interfacial characteristics. In many food emulsions, there are appreciable quantities of nonadsorbed proteins dispersed in the aqueous phase; for example milk, nutritional beverages, infant formulations, and drinks for athletes. These nonadsorbed proteins may increase or decrease the oxidative stability of emulsions through enzymatic or nonenzymatic mechanisms. A variety of physicochemical mechanisms have been identified as contributing to the pro-oxidant or antioxidant activity of proteins, including catalysis of specific reactions, chelation of transition metals or other reactive species, preferential oxidation, and free radical scavenging (Decker 1998b).

Enzymes may either inhibit lipid oxidation (for example by inactivating pro-oxidants and oxidation substrates) or promote lipid oxidation (for example by catalyzing reactions that generate pro-oxidants). For example, the enzyme system glucose oxidase/catalase has been shown to retard lipid oxidation in mayonnaise containing fish oils, where it was proposed that glucose oxidation used up oxygen that would otherwise have taken part in the lipid

oxidation reaction (Isaksen and Adler-Nissen 1997a,b). Superoxide dimutase has been shown to be an antioxidant in trilinolein oil-in-water emulsions stabilized by phospholipids due to its ability to scavenge the superoxide anion (Allen and Wrieden 1982b). In the same study, it was shown that lactoperoxidase and xanthine oxidase were capable of promoting lipid oxidation due to their ability to catalyze the generation of reactive oxygen species. It should be noted that the catalytic activity of enzymes can be reduced by heating the system to a temperature above the protein denaturation temperature (Allen and Wrieden 1982b; Kristensen and Andersen 1997). Thus, thermal processing can be used to control the pro-oxidant or antioxidant activity of enzymes in emulsions.

Proteins may also inhibit or promote lipid oxidation through nonenzymatic mechanisms. A variety of dairy proteins have been shown to have significant antioxidant properties when added to oil-in-water emulsions, including casein, whey, and lactoferrin (Allen and Wrieden 1982a,b). Casein and lactoferrin were shown to be strongly antioxidative because of their ability to chelate iron. The origin of the antioxidant activity of whey proteins was probably due to free radical scavenging by sulfhydryl and nonsulfhydryl amino acids, plus some limited transition metal chelation (Allen and Wrieden 1982a; Tong and others 2000a,b). The ability of whey proteins to retard lipid oxidation in salmon oil-in-water emulsions stabilized by a nonionic surfactant (Tween 20) was found to increase when they were heated above a temperature where the protein molecules unfolded, which was attributed to exposure of reduced sulfhydryl groups (Tong and others 2000a,b). Nevertheless, a significant amount of antioxidant activity remained in the proteins when the sulfhydryl groups were blocked by NEM, which indicates that other antioxidant mechanisms must also be operating; for example free radical scavenging by nonsulfhydryl groups or chelation of transition metals. Similar results were found in a study of the effect of heating on the antioxidant activity of skim milk in methyl linoleate emulsions stabilized by a nonionic surfactant (Taylor and Richardson 1980). Under certain circumstances, noncatalytic proteins have been shown to have pro-oxidative characteristics. For example, the addition of a powdered whey protein isolate (0–1 wt%) to Menhaden oil-in-water emulsions stabilized by Tween 20 significantly increased lipid oxidation, probably because of pro-oxidant impurities (for example hydroperoxides or transition metals) in the protein ingredient (Donnelly and others 1998).

Surfactants. Surfactants are normally used to stabilize oil-in-water emulsions against flocculation and coalescence by forming a protective membrane around the droplets. Nevertheless, after homogenization there are often significant quantities of nonadsorbed surfactant molecules present in the aqueous phase of emulsions. Above a certain concentration, known as the critical micelle concentration (CMC), the nonadsorbed surfactant forms micelles. Micelles are aggregates of surfactant molecules in which the nonpolar tails form the interior and the polar headgroups form the exterior (Dickinson and McClements 1995). Surfactant micelles are capable of incorporating nonpolar molecules within their hydrophobic core and polar or amphiphilic molecules within the palisade layer formed by the surfactant head groups. Micelles may therefore be able to solubilize lipids, antioxidants, or pro-oxidants, which may alter the oxidative stability of a system.

In a study of the hemoglobin-catalyzed oxidation of emulsified safflower oil (Sims and others 1979), it was observed that the presence of excess anionic surfactant in the aqueous phase of the emulsion increased the oxidative stability. This may be because the anionic surfactant formed negatively charged micelles that were capable of attracting transition metals to their surface, thereby decreasing the concentration of pro-oxidants at the droplet surface. Alternatively, it could have been that, at higher surfactant concentrations, the packing of the surfactant molecules at the oil-water interface was tighter and therefore the interfacial layer was able to act as a more efficient physical barrier or because the surfactant denatured the protein, thereby reducing its enzymatic activity.

The impact of aqueous phase surfactants on lipid oxidation in emulsions may also be effected by interactions with other components in the aqueous phase. The influence of adding nonionic surfactants (Tween 20), whey protein (WPI), or combinations of both on the oxidation in Menhaden oil-in-water emulsions stabilized by Tween 20 has been studied (Donnelly and others 1998). When only Tween 20 (0–2 wt%) or only whey protein (0–1 wt%) was added to the emulsions, there was an appreciable increase in lipid oxidation which was probably due to the presence of pro-oxidant impurities in the surfactant or protein. However, when Tween 20 and whey protein were added in combination, there was a significant antioxidant effect. The origin of this effect is currently not understood, but it may be due to the ability of surfactants to alter the conformation of proteins so that they expose more free radical scavenging or chelating amino acids (Donnelly and others 1998). The above discussion indicates that the role of surfactants in inhibiting or promoting lipid oxidation in emulsions is complex and requires further study.

Acids, bases, and buffers. Acids, bases, and buffers are used to control the pH of food emulsions, which in turn determines the stability, rheology, and flavor of the product. The pH of an aqueous phase can impact the oxidative stability of oil-in-water emulsions in a variety of ways, due to its effect on the reactivity, solubility, and partitioning of the reactive species involved. This may account for the apparent contradictory results that have been found for the effects of pH on lipid oxidation in oil-in-water emulsions. Some workers have found that the rate of lipid oxidation increases with increasing pH (Huang and others 1996a; Mei and others 1999; Mancuso and others 1999a; van Ruth and others 1999a,b), whereas others have found the opposite (Shimada and others 1994; Donnelly and others 1998) on seemingly similar systems. All of these studies were carried out using food oil-in-water emulsions stabilized by nonionic surfactants; however, there were variations in the type of buffers used, the nature of the oil and surfactants, the oxidation conditions, the absence or presence of added pro-oxidants, and the analytical methods used to monitor oxidation.

Ingredient Quality

The quality of the ingredients used to manufacture food emulsions can have a pronounced impact on their oxidative stability. The presence of even small amounts of transition metals in an emulsion can greatly accelerate lipid oxidation through their ability to promote the breakdown of hydroperoxides. The importance of transition metal impurities has been demonstrated in studies that have shown the rate of lipid oxidation in oil-in-water emulsions is greatly reduced by adding substances that specifically chelate transition metal ions, such as EDTA, phytate, or transferrin (Shimada and others 1992; Mei and others 1998a,b; Mancuso and others 1999a). It is therefore extremely important to use ingredients (for example oils, water, emulsifiers, flavors, colors, thickening agents) that have low transition metal concentrations or to add substances that chelate and inactivate transition metals.

Many oils and emulsifiers that can be purchased commercially contain relatively high concentrations of lipid peroxides, or may develop them during storage (Yoshida and Niki 1992; Bergh

and others 1997; Mancuso and others 1999b). These peroxides are highly susceptible to breakdown into free radicals that can promote lipid oxidation, especially in the presence of transition metals, UV light, or heat (Decker 1998b). Studies have shown that removal of hydroperoxide impurities present in lipids or emulsifiers dramatically improved the oxidative stability of colloidal systems (Yoshida and Niki 1992). It therefore seems that a certain concentration of peroxides must be present in a system before lipid oxidation will occur at a significant rate (Fukuzawa and others 1995; Girotti 1998). Recent experiments in our laboratory showed that the rate of α-tocopherol oxidation in Tween20 micelles increased as the concentration of peroxide impurities present in the surfactant increased (Mancuso and others 1999b). This highlights the importance of using surfactants and oils with low peroxide contents.

Strategies For Retarding Lipid Oxidation

The above discussion has highlighted a variety of ways to retard lipid oxidation in oil-in-water emulsions. In this section, we use this knowledge to identify a number of practical strategies for retarding lipid oxidation in food emulsions.

The oxidative stability of emulsions can be greatly increased by excluding oxygen from the system; for example by packing under vacuum or nitrogen. This technique is used commercially to minimize lipid oxidation in mayonnaise and salad dressings during storage. Nevertheless, once the product is opened and oxygen enters, lipid oxidation will begin, thus reducing the time the product can be stored before consumption.

The susceptibility of food emulsions to lipid oxidation could be greatly improved by ensuring that the ingredients used in their manufacture were low in hydroperoxides, transition metals, or other pro-oxidants. This could be achieved by purchasing high-purity ingredients or by using a processing step that purifies the ingredients before use. Once pure ingredients have been obtained, it may be necessary to store them under carefully controlled conditions to avoid formation of hydroperoxides (for example refrigerated temperatures, reduced oxygen, low light) or contamination from pro-oxidants (such as pure ingredients and clean containers). From a practical standpoint, it may not be economically feasible to purchase highly purified ingredients or to use extensive clean up procedures prior to using them. Food manufacturers may therefore have to find alternative methods of dealing with the fact that most food ingredients contain significant amounts of impurities that can promote lipid oxidation.

Taken that there are likely to be impurities present in the ingredients used to formulate food emulsions, lipid oxidation can still be effectively retarded by preventing hydroperoxides from coming into close proximity to pro-oxidants or by inactivating pro-oxidants. This can be achieved in a number of ways. Firstly, hydroperoxides could be protected from oxidation by adding ingredients to the aqueous phase of the emulsion that chelate transition metals and thereby make them ineffective, such as EDTA, phytate, proteins, or polysaccharides. Secondly, the transition metals could be prevented from coming into close contact with the hydroperoxides at the interface by using an emulsifier that gives the droplets a positive charge so that the transition metals are electrostatically repelled. Thirdly, the use of ingredients that can reduce transition metals back to their most active form should be avoided. Fourthly, hydroperoxides may be segregated from pro-oxidants by using an emulsifier that forms a thick interfacial layer that keeps the hydroperoxides on one side and the pro-oxidants on the other. Fifthly, pro-oxidative enzymes may be deactivated; for example by heating, adding denaturants, or adjusting pH.

Additional protection against lipid oxidation can be achieved by using free radical chain-breaking antioxidants. It is important that these antioxidants are located in the region where they are most effective at chain breaking. This normally means that they should be located in the vicinity of the oil-water interface where the lipid hydroperoxides may be broken down to free radicals.

In practice, the most effective means of retarding lipid oxidation would be to use a number of these strategies in combination. Each type of food emulsion has its own unique composition and structure; therefore it is likely that the precise details of an antioxidant strategy will have to be tailored for each particular system.

Conclusions

THIS REVIEW HAS HIGHLIGHTED THE IMPORTANCE OF MOLECULAR environment in determining the oxidative stability of oil-in-water emulsions. Our understanding of this subject has progressed considerably during the past decade; however, it is clear that this is an extremely complex area and that much more basic research is required. Further studies of the relationship between the molecular structure of lipids, antioxidants, and pro-oxidants; their partitioning between oil, aqueous; and interfacial regions; and the oxidative stability of emulsions should be particularly fruitful. The influence of droplet characteristics, such as size, concentration, and physical state, on lipid oxidation has not been the subject of many previous studies and would certainly benefit from further research. The importance of interfacial characteristics in determining the oxidative stability of emulsions has already been demonstrated, but further research on the influence of interfacial thickness, packing, and composition could lead to new strategies for retarding lipid oxidation. Real food emulsions contain a wide variety of different components, including polysaccharides, proteins, sugars, salts, surfactants, and buffers. Further studies of the influence of these components on the oxidative stability of emulsions are required before we can form a more complete picture of the importance of ingredient interactions. The knowledge gained from these studies would enable food scientists to "engineer" foods with enhanced oxidative stability by designing antioxidant strategies in a more systematic fashion.

References

Abdalla AE, Roozen JP. 1999. Effect of plant extracts on the oxidative stability of sunflower oil and emulsion. Food Chem. 64:323–329.

Akoh CC, Min DB. 1998. Food lipids: chemistry, nutrition and biotechnology. Marcel Dekker:New York. 816 p.

Allen JC, Wrieden WL. 1982a. Influence of milk proteins on lipid oxidation in aqueous emulsion. I. Casein, whey protein and a-lactalbumin. J Dairy Res., 49:239–248.

Allen JC, Wrieden WL. 1982b. Influence of milk proteins on lipid oxidation in aqueous emulsion. I. Lactoperoxidase, lactoferrin, superoxidase dismutase and xanthine oxidase. J Dairy Res., 49:249–263.

Basaran TK, Coupland JN, McClements DJ. 1999. Monitoring molecular diffusion of sucrose in xanthan solutions using ultrasonic velocity measurements. J Food Sci. 64:125–130.

Bergh M, Magnusson K, Nilsson JLG, Karlberg AT. 1997. Contact allergenic activity of Tween(R) 80 before and after air exposure. Contact Dermatitis. 37:9–18.

Castle L, Perkins MJ. 1986. Inhibition kinetics of chain-breaking phenolic antioxidants in SDS micelles. Evidence that intermolecular diffusion rates may be rate limiting for hydrophobic inhibitors such as a-tocopherol. J Am Chem Soc. 108:6381–6382.

Chen ZY, Nawar WW. 1991. The role of amino acids in the autoxidation of milk fat. J Am Oil Chem Soc. 68:47–50.

Coupland JN, McClements DJ. 1996a. Lipid oxidation in food emulsions. Trends Food Sci. Tech. 7:83–91.

Coupland JN, McClements DJ. 1996b. Droplet composition affects the rate of oxidation of emulsified ethyl linoleate: Supporting evidence. J Am. Oil. Chem. Soc. 73:1207.

Coupland JN, Zhu Z, Wan H, McClements DJ, Nawar WW, Chinachoti P. 1996. Droplet composition affects the rate of oxidation of emulsified ethyl linoleate. J Am. Oil. Chem. Soc. 73:795–801.

Decker EA. 1998a. Strategies for manipulating the pro-oxidative/antioxidative balance of foods to maximize oxidative stability. Trends Food Sci. Tech. 9:241–248.

Decker EA. 1998b. Antioxidant mechanisms. In: Akoh, CC, Min DB, editors. Food lipids: chemistry, nutrition and biotechnology. New York: Marcel Dekker. p 397–421.

Dickinson E. 1992. An introduction to food colloids. Oxford:Oxford University Press. 216 p.

Dickinson E, Stainsby G. 1982. Colloids in foods. London: Applied Science Publishers. 750 p.

Dickinson E, McClements DJ. 1995. Advances in food colloids. London: Chapman and Hall. 333 p.

Donnelly JL, Decker EA, McClements DJ. 1998. Iron-catalyzed oxidation of Menhaden oil as affected by emulsifiers. J Food Sci. 63:997–1000.
Duh PD. 1999. Antioxidant activity of water extract of four Harng Jyur (Chrysanthemum morifolium Ramat) varieties in soybean oil emulsion. Food Chem. 66:471–476.
Erickson MC, Sista RV. 1997. Influence of microenvironment on oxidative susceptibility of seafood lipids. Flavor Lipid Chem. Seafoods. 674:175–185.
Frankel EN. 1996. Antioxidants in lipid foods and their impact on food quality. Food Chem. 57:51–55.
Frankel EN. 1997. Activity of wine and grape phenolic antioxidants in human LDL. Biofactors 6:433–435.
Frankel EN. 1998. Lipid oxidation. Dundee, Scotland;The Oil Press. 300 p.
Frankel EN. 1999. Natural phenolic antioxidants and their impact on health. In: Antioxidant food supplements in human health. London: Academic Press. p 385–392.
Frankel EN, Huang SW, Kanner J, German JB. 1994. Interfacial phenomena in the evaluation of antioxidants - bulk oils vs emulsions. J Ag Food Chem. 42:1054–1059.
Frankel EN, Waterhouse AL, Teissedre PL. 1995. Principal phenolic phytochemicals in selected California wines and their antioxidant activity in inhibiting oxidation of human low-density lipoproteins. J Ag Food Chem. 43:890–894.
Frankel EN, Huang SW, Aeschbach R, Prior E. 1996a. Antioxidant activity of a rosemary extract and its constituents, carnosic acid, carnosol and rosmarinic acid, in bulk oil and oil-in-water emulsion. J Ag Food Chem. 44:131–135.
Frankel EN, Huang SW, Prior E, Aeschbach R. 1996b. Evaluation of antioxidant activity of rosemary extracts, carnosol and carnosic acid in bulk vegetable oils and fish oil and their emulsions. J Sci Food Ag. 72:201–208.
Frankel EN, Huang SW, Aeschbach R. 1997. Antioxidant activity of green teas in different lipid systems. J Am Oil Chem Soc. 74:1309–1315.
Fritsch CW. 1994. Lipid oxidation – the other dimensions. Inform 5:423–436.
Fukuzawa K, Soumi K, Iemura M, Goto S, Tokumura A.1995. Dynamics of xanthine oxidase- and Fe^{3+}-ADP- dependent lipid peroxidation in negatively charged phospholipid vesicles. Arch. Biochem. Biophys. 316:83–91.
Giese, J. 1996. Antioxidants: Tools for Preventing Lipid Oxidation. Food Technol. 50(11) 73–77.
Girotti AW. 1998. Lipid hydroperoxide generation, turnover and effector action in biological systems. J Lipid Res. 39:1529–1542.
Gohtani S, Sirendi M, Yamamoto N, Kajikawa K, Yamano Y. 1999. Effect of droplet size on oxidation of docosahexaenoic acid in emulsion system. J Disp Sci. Tech. 20:1319–1325.
Graf E, Mahoney JR, Bryant RG, Eaton JW. 1984. Iron catalyzed hydroxyl radical formation: Stringent requirement for free iron coordination site. J Biol. Chem. 259:3620–3624.
Halliwell B, Murcia MA, Chirico S, Aruoma OI. 1995. Free radicals and antioxidants in food and in vivo: What they do and how they work. Crit. Rev. Food. Sci. Nutr. 35:7–20.
Hiemenz PC, Rajagopalan R. 1997. Principles of colloid and surface chemistry, 3rd ed. New York: Marcel Dekker. 650 p.
Heinonen MI, Haila K, Lampi AM, Piironen V. 1997. Inhibition of oxidation in 10% oil-in-water emulsions by beta-carotene with alpha- and gamma-tocopherols. J Am Oil Chem Soc. 74:1047–1052.
Heinonen MI, Meyer AS, Frankel EN. 1998. Antioxidant activity of berry phenolics on human low-density lipoprotein and liposome oxidation. J Ag Food Chem.46:4107–4112.
Hopia AI, Huang SW, Schwarz K, German JB, Frankel EN. 1996. Effect of different lipid systems on antioxidant activity of rosemary constituents carnosol and carnosic acid with and without alpha-tocopherol. J Ag Food Chem. 44:2030–2036.
Huang S-W, Frankel EN, German JB. 1994. Antioxidant activity of a- and g- tocopherols in bulk oils and oil-in-water emulsions. J Ag Food Chem. 42:2108–2114.
Huang S-W, Hopia A, Schwarz K, Frankel EN, German JB. 1996a. Antioxidant activity of a-tocopherol and Trolox in different lipid substrates. J Ag Food Chem. 44:444–452.
Huang S-W, Frankel, EN, Schwarz K, Aeschbach R, German JB. 1996b. Antioxidant activity of carnosic acid and methyl carnosate in bulk oils and oil-in-water emulsions. J Ag. Food Chem. 44:2951–2956.
Huang S-W, Frankel EN, Schwarz K, German JB. 1996c. Effect of pH on antioxidant activity of alpha-tocopherol and Trolox in oil-in-water emulsions. J Ag Food Chem. 44:2496–2502.
Huang S-W, Frankel EN. 1997. Antioxidant activity of tea catechins in different lipid systems. J Ag Food Chem. 45:3033–3038.
Huang S-W, Frankel E.N, Aeschbach R, German JB. 1997. Partition of selected antioxidants in corn oil-water model systems. J Ag Food Chem. 45:1991–1994.
Hunter RJ. 1993. Introduction to modern colloid science. Oxford:Oxford Science Publications.
Isaksen A, Adler-Nissen J. 1997a. Antioxidative effect of glucose oxidase and catalase in mayonnaises of different oxidative susceptibility .1. Product trials. Food Sci. Tech. – Leben. Wissen. Tech. 30:841–846.
Isaksen A, Adler-Nissen J. 1997b. Antioxidative effect of glucose oxidase and catalase in mayonnaises of different oxidative susceptibility. 2. Mathematical modelling. Food Sci. Tech. – Leben. Wissen. Tech. 30:847–852.
Jacobsen C, Meyer AS, Adler-Nissen J. 1998. Oxidation mechanisms in real food emulsions: Method for separation of mayonnaise by ultracentrifugation. J Food Lipids. 5:87–101.
Jacobsen C, Meyer AS, Adler-Nissen J. 1999a. Oxidation mechanisms in real food emulsions: oil-water partition coefficients of selected volatile off-flavor compounds in mayonnaise. Food Res. Tech. 208:317–327.
Jacobsen C, Schwarz K, Stockmann H, Meyer AS, Adler-Nissen J. 1999b. Partitioning of selected antioxidants in mayonnaise. J Ag Food Chem. 47:3601–3610.
Jo C, Ahn DU. 1999. Fat reduces volatiles production in oil emulsion system analyzed by purge-and-trap dynamic headspace/gas chromatography. J Food Sci. 64:(4)641–643.
Kanner J, Frankel E, Granit R, German JB, Kinsella JE. 1994. Natural antioxidants in grapes and wines. J Ag Food Chem. 42:64–69.
Karel M, Schaich K, Roy RB. 1975. Interaction of peroxidizing methyl linoleate with some proteins and amino acids. J Ag Food Chem. 23:159–165.
Ke PJ, Ackman RG. 1973. Bunsen coefficient for oxygen in marine oils at various temperatures determined by exponential dilution method with a polarographic oxygenelectrode. J Am Oil Chem Soc. 50:429–435.
Kristensen L, Andersen HJ. 1997. Effect of heat denaturation on the pro-oxidative activity of metmyoglobin in linoleic acid emulsions. J Ag Food Chem. 45:7–13.
Kritchevsky D. 1998. Fats and oils in human health. In: Akoh CC, Min, DB, editors. Food Lipids. New York: Marcel Dekker. p 449-462.
Mancuso JR, McClements DJ, Decker EA. 1999a. The effects of surfactant type, pH, and chelators on the oxidation of salmon oil-in-water emulsions. J Ag Food Chem. 47:4112–4116.
Mancuso JR, McClements DJ, Decker EA. 1999b. Ability of iron to promote surfactant peroxide decomposition and oxidize alpha-tocopherol. J Ag Food Chem. 47:4146–4149.
Marcuse, R. 1962. The effect of some amino acids on the oxidation of linoleic acid and its methyl ester. J Am Oil Chem Soc. 39: 97-103.
Marcuse R, Fredriksson PO. 1968. Fat oxidation at low oxygen pressure. I. Kinetic studies on the rate of fat oxidation in emulsions. J Am Oil Chem Soc. 45:400–407.
Marcuse R, Fredriksson PO. 1969. Fat oxidation at low oxygen pressure. II. Kinetic studies on linoleic acid oxidation in emulsions in the presence of antioxidants. J Am Oil Chem Soc. 46:262–268.
McNulty PB. 1987. Flavor release - elusive and dynamic. In: Blanshard JMV, Lillford P, eds. Food structure and behaviour. London:Academic Press. p 245-275.
McClements DJ. 1999. Food emulsions: principles, practice and techniques. Boca Raton:CRC Press. 392 p.
Mei LY, McClements DJ, Wu JN, Decker EA. 1998a. Iron-catalyzed lipid oxidation in emulsion as affected by surfactant, pH and NaCl. Food Chem. 61:307–312.
Mei LY, Decker EA, McClements DJ. 1998b. Evidence of iron association with emulsion droplets and its impact on lipid oxidation. J Ag Food Chem. 46:5072–5077.
Mei LY, McClements DJ, Decker EA. 1999. Lipid oxidation in emulsions as affected by charge status of antioxidants and emulsion droplets. J Ag Food Chem. 47:2267–2273.
Min DB. 1998. Lipid oxidation of edible oil. In: Akoh CC, Min DB, editors. Food lipids: chemistry, nutrition and biotechnology. New York: Marcel Dekker. p 283–296.
Miyashita K, Nara E, Ota T. 1993. Oxidative stability of polyunsaturated fatty acids in aqueous solution. Biosci. Biotech. Biochem. 57:1638–1640.
Miyashita K, Tateda T, Ota T. 1994. Oxidative stability of free fatty acids mixtures from soybean, linseed and sardine oils in an aqueous solution. Biosci Biotech Biochem 60:315–318.
Miyashita K, Azuma G, Ota T. 1995. Oxidative stability of geometric and positional isomers of unsaturated fatty acids an aqueous solution. J Jap Oil Chem Soc. 44:425–430.
Moller RE, Stapelfeldt H, Skibsted LH. 1998. Thiol reactivity in pressure-unfolded β-lactoglobulin. Antioxidative properties and thermal unfolding. J Ag Food Chem. 46:425–430.
Nawar WW. 1996. Lipids. In: Fennema OR, editor. Food chemistry, 3rd ed. New York: Marcel Dekker. p 225-319.
Osinchak JE, Hultin HO, Zajicek OT, Kelleher SD, Huang C. 1992. Effect of NaCl on catalysis of lipid oxidation by the soluble fraction of fish muscle. Free Radical Biol Med. 12:35–41.
Pekkarinen SS, Stockmann H, Schwarz K, Heinonen IM, Hopia AI. 1999. Antioxidant activity and partitioning of phenolic acids in bulk and emulsified methyl linoleate. J Ag Food Chem. 47:3036–3043.
Pike OA. 1994. Fat characterization. In: Nielsen SS, editor. Introduction to the chemical analysis of foods. Sudbury, MA: Jones and Bartlett. Chap 13.
Ponginebbi L, Nawar WW, Chinachoti P. 1999. Oxidation of linoleic acid in emulsions: Effect of substrate, emulsifier, and sugar concentration. J Am Oil Chem Soc. 76:131–138.
Porter NA. 1980. Recent trends in food applications of antioxidants. In: Simic MG, Karek M, editors. Autoxidation in food and biological systems. New York: Plenum Press. p 295–365.
Porter NA, Weber BA, Weenen, H, Khan JA. 1982. Autoxidation of polyunsaturated lipids: Factors controlling the stereochemistry of product hydroperoxides. J Am Chem Soc. 102:5597–5601.
Porter WL. 1993. Paradoxical behavior of antioxidants in food and biological systems. In: William GM, editor. Antioxidants: chemical, physiological, nutritional and toxicological aspects. Princeton:Princeton Scientifc. p 93–122.
Reische DW, Lilliard DA, Eitenmiller RR. 1998. Antioxidants. In: Akoh CC, Min DB, editors. Food lipids: chemistry, nutrition and biotechnology. New York: Marcel Dekker. p 423–448.
Roozen JP, Frankel, EN, Kinsella JE. 1994a. Enzymic and autoxidation of lipids in low fat foods: model of linoleic acid in emulsified hexadecane. Food Chem. 50:33–38.
Roozen JP, Frankel EN, Kinsella JE. 1994b. Enzymic and autoxidation of lipids in low fat foods: model of linoleic acid in emulsified triolein and vegetable oils. Food Chem. 50:39–43.
Satue-Gracia MT, Heinonen M., Frankel EN. 1997. Anthocyanins as antioxidants on human low-density lipoprotein and lecithin-liposome systems. J Ag Food Chem. 45:3362–3367.
Schwarz K, Frankel EN, German JB. 1996. Partition behavior of antioxidant phenolic compounds in heterophasic systems. Fett/Lipid 98:115–121.
Shahidi F, Wanasundara JPD. 1998a. Extraction and analysis of lipids. In: Akoh CC, Min DB, editors. Food lipids: chemistry, nutrition and biotechnology. New York: Marcel Dekker. p 115–136.
Shahidi F, Wanasundara UN.1998b. Methods of measuring oxidative rancidity of fats and oils. In: Akoh CC, Min DB, editors. Food lipids: chemistry, nutrition and biotechnology. New York: Marcel Dekker. p 377–396.
Shimada K, Fujikawa K, Yahara K, Nakamura T. 1992. Antioxidative properties of xanthan on the autoxidation of soybean oil in cyclodextrin emulsion. J Ag Food Chem. 40:945–948.
Shimada K, Muta H, Nakamura Y, Okada H, Matsuo K, Yoshioka S, Matsudaira T, Nakamura T. 1994. Iron-binding property and antioxidative activity of xanthan on the autoxidation of soybean oil in emulsion. J Ag Food Chem. 40:945–948.
Silvestre MPC, Chaiyasit W, Brannan RG, McClements DJ, Decker EA. 2000. Ability of surfactant head group size to alter lipid and antioxidant oxidation in oil-in-water emulsions. J Ag Food Chem. 48:2057–2061.
Sims RJ, Fioriti JA, Trumbetas J. 1979. Effect of sugars and sugar alcohols on autoxidation of safflower oil in emulsions. J Am Oil Chem Soc. 56:742–745.
Sims RJ, Fioriti JA. 1980. Effect of amino acids on autoxidation of safflower oil in emulsions. J Am Oil Chem Soc. 57:354–359.
Sims RJ. 1994. Oxidation of fats in food products. Inform 5:1020–1028.
Taylor JM, Richardson T. 1980. Antioxidant activity of skim milk: Effect of heat and resultant sulfhydryl groups. J Dairy Sci. 63:1783–1795.
Tong LM, Sasaki S, McClements DJ, Decker EA. 2000a. Antioxidant activity of whey in a salmon oil emulsion. J Food Sci. Submitted.
Tong LM, Sasaki S, McClements DJ, Decker EA. 2000b. Mechanisms of antioxidant activity of a high molecular weight fraction of whey. J Ag Food Chem. Submitted.
van Ruth SM, Roozen JP, Posthumus MA, Jansen FJHM. 1999a. Volatile composition of sunflower oil-in-water emulsions during initial lipid oxidation: Influence of pH. J Ag

Food Chem. 47:4365–4369.
van Ruth SM, Roozen JP, Posthumus MA, Jansen FJHM. 1999b. Influence of ascorbic acid and ascorbyl palmitate on the aroma composition of an oxidized vegetable oil and its emulsion. J Am Oil Chem Soc. 76:1375–1381.
Wang XH, Ohshima T, Ushio H, Koizumi C. 1999. Proportion of geometrical hydroperoxide isomers generated by radical oxidation of methyl linoleate in homogeneous solution and in aqueous emulsion. Lipids 34:675–679.
Watkins SM, German JB. 1998. Omega fatty acids. In: Akoh CC, Min DB, editors. Food lipids: chemistry, nutrition and biotechnology. New York: Marcel Dekker. p 463–494.
Wedzicha BL. 1988. Distribution of low molecular weight food additives in food systems. In: Dickinson E, Stainsby G, editors. Advances in food emulsions. London:Elsevier. Chap 10.
Yamauchi R, Aoki Y, Sugiura T, Kato K, Ueno Y. 1982. Effect of sugars and sugar analogs on autoxidation of methyl linoleate and safflower oil. Agric. Biol. Chem.46:2997–3002.
Yoshida Y, Niki E. 1992. Oxidation of methyl linoleate in aqueous dispersions induced by copper and iron. Arch. Biochem. Biophys. 295:107–114.
Yamauchi R, Goto Y, Kato K, Ueno Y. 1984. Pro-oxidant effect of dihyroxyacetone and reducing sugars on autoxidation of methyl linoleate in emulsions. Agric. Biol. Chem. 48:843–848.
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