

Critical Reviews in Food Science and Nutrition

Publication details, including instructions for authors and subscription information:

<http://www.tandfonline.com/loi/bfsn20>

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Published online: 11 Apr 2011.

To cite this article: Yue-E Sun, Wei-Dong Wang, Hong-Wei Chen & Chao Li (2011) Autoxidation of Unsaturated Lipids in Food Emulsion, Critical Reviews in Food Science and Nutrition, 51:5, 453-466, DOI: [10.1080/10408391003672086](https://doi.org/10.1080/10408391003672086)

To link to this article: <http://dx.doi.org/10.1080/10408391003672086>

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Autoxidation of Unsaturated Lipids in Food Emulsion

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Unsaturated lipids having various physiological roles are of significance in biochemistry, nutrition, medicine, and food. However, 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 and bulk lipids. This article gives a brief overview of the current knowledge on autoxidation of oil-in-water food emulsions, especially those that contain unsaturated lipids, which are important in the food industry. Autoxidation of unsaturated lipids in oil-in-water emulsion is discussed, and so also their oxidation mechanism, the major factors influencing oxidation, determination measures, research status, and the problems encountered in recent years. Some effective strategies for controlling lipid oxidation in food emulsion have been presented in this review.

Keywords lipids oxidation, emulsion, unsaturated fatty acid, influencing factors, determination, control methods

INTRODUCTION

Unsaturated lipids are one of the broad groups of naturally-occurring hydrophobic or amphiphilic molecules which contain one or more double bonds between the carbon atoms. Unsaturated lipids include fatty acids and their derivatives (including tri-, di-, and monoglycerides, and phospholipids), as well as other compounds such as sphingolipids, sterol lipids, and saccharolipids. Although the latter have a wide chemical nature and even in emulsion they may play a great negligible role in the oxidation of food containing lipids, they are a minor constituent of unsaturated lipids. Only autoxidation of fatty acids and their derivatives will be discussed in this review. Unsaturated fatty acids can be classified into omega-3 (ω -3), omega-6 (ω -6), and omega-9 (ω -9) type based on the chemical structure of the fatty acids. They refer to characteristics that are the first double bond of the fatty acid located at the third, sixth, and ninth bond from the methyl end of the fatty acid.

Unsaturated fatty acids, especially polyunsaturated fatty acids (PUFAs), are physiologically important because of their potential for treating and preventing disease (Hofmanová et al., 2008; Leitzmann et al., 2004; Pardini, 2006; Persaud, 2008). Due to the specific nutritional and beneficial effects of PUFAs, they

are being used in a variety of products for human consumption (Kolanowski and Laufenberg, 2006; Kris-Etherton et al., 2000). However, lipids with high PUFAs content are susceptible to oxidation and the development of undesirable flavors and toxic products during storage, making their use in foods a challenge for the food industry. Oxidation of lipids is closely related to some diseases, such as inflammation, autoimmune disease, cardiovascular disease and also to aging, etc. (Headlam and Davies, 2003; Valko et al., 2006). Some of those changes are desirable because they lead to the production of flavor, for example, in cheeses (Nawar, 1996). Most lipid oxidation is undesirable in foods because they lead to the generation of off-flavor and potentially toxic reaction products (Campo et al., 2006). Oxidation of lipids is one of the major causes of quality deterioration in food, which results in the changes of nutrition, taste, texture, and appearance, and may even shorten the shelf life of food and limit its use as beneficial lipids in functional food. It is contrary to current recommendations to advocate an increase in dietary unsaturated fatty acids as a therapeutic measure in preventive medicine (Forsythe et al., 2008; Persaud, 2008; Willemssen et al., 2008). So, research on lipid oxidation, especially on oxidation of unsaturated polyunsaturated fatty acid, has become a topic of significant importance for promoting the consumption of unsaturated lipids (Beltz et al., 2007; Binkoski et al., 2005; Sangiovanni and Chew, 2005).

Oxidation of unsaturated lipids is actually the oxidation of unsaturated fatty acids in their structure, and this could happen through photooxidation, autoxidation, and enzyme-catalyzed

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oxidation. Autoxidation is the main process among them. Oxygen interacting with unsaturated lipids is an important reaction, because it limits the application of unsaturated lipids in functional food. It is essential to clarify the mechanism, the influencing factors, and the determination methods of oxidation for setting forth a sound plan to control the deterioration of food containing lipid.

Lipid oxidation in a homogeneous system (bulk oil) has been under investigation by food scientists for several decades, and its mechanisms, dynamics, and products are now well established (Cercaci et al., 2007; Colakoglu, 2007; Frankel, 1998; McClements and Decker, 2000). Among researchers, Cercaci et al. (2007) focused on the factors that influence phytosterol oxidation in bulk and emulsified oil. However, emulsion is the important applicable system in food, and lipids commonly exist as an oil-in-water emulsion in food, and the oxidation of an unsaturated substrate in a heterogeneous system (micelle, emulsion, liposome) is different from that in bulk oil. Some previous studies arrived at completely opposite conclusions. In this study we have reviewed the influencing factors, determination methods of unsaturated lipids oxidation in emulsion, and systematically expounded the inconsistent reports and problems on unsaturated lipid oxidation in heterogeneous systems (oil-in-water emulsions) now aiming at giving a reference to perfect the oxidation theory of unsaturated lipid in aqueous solution and to highlight effective strategies for retarding oxidation.

AUTOXIDATION MECHANISM AND KINETICS OF UNSATURATED LIPID

Mechanism of Autoxidation

Lipids containing unsaturated fatty acids undergo spontaneous peroxidation. Peroxidation is important as it is responsible both for tissue deterioration in the living body and for the spoilage of food. Peroxidative modification of unsaturated lipids can occur in reactions triggered by free radical species such as peroxy radicals and nonradical species such as singlet oxygen. Lipid hydroperoxides are prominent nonradical intermediates of lipid peroxidation whose identification can provide valuable mechanistic information as to whether a primary reaction is mediated by singlet oxygen or oxyradicals.

Autoxidation is thoroughly spontaneous oxidation and it involves unsaturated lipid and oxygen without light and catalyst. Before oxygen and unsaturated fatty acids react nonenzymically, one of them must be activated. Reaction between unsaturated lipids and oxygen is a radical-chain process involving three stages of initiation, propagation, and termination (Gordon, 2001). Oxidation begins with even cleavage of α -carbon atom next to a double bond. Initiation can be introduced by free radicals, which are produced by breaking up of the preformed hydroperoxides that are present in badly stored food containing

unsaturated lipids. A free radical exists independently and it contains one or more unpaired electrons. The most common mechanism for the generation of radicals is bond cleavage. When metal ion exists in food, a metal-catalyzed decomposition of hydroperoxides is the main source of radicals. When radicals react with nonradicals, new radicals produce free-radical chain reactions in which several different radical species may take part. The rate of reaction and the detailed structure of the hydroperoxides depend on the structure of the resonance-stabilized allylic radical $R\cdot$ produced from the unsaturated acid. Gunstone (1984) determined the first-formed allylic hydroperoxides and the structure of further oxidation products including hydroperoxides with HPLC and IR.

As isomers of unsaturated fatty acid hydroperoxides readily undergo isomerization, the mixture of hydroperoxide isomers obtained from autoxidation of polyunsaturated fatty acid is complex. Hydroperoxide as the primary product is unstable, and it breaks up subsequently into secondary compounds through complex fragmentation and interaction. Moreover, it results in the production of volatile compounds such as aldehyde, ketone, organic acid, epoxide, or polymer taking on a strong and pungent taste. This accelerates the oxidation of coloring matter, flavor substance, and vitamins, which make foods containing unsaturated lipid thoroughly rancid. Frankel (1984) reviewed the formation and uneven distribution of fatty acid hydroperoxides in oxidized linolenate and elucidated the mechanism of autoxidation.

Decomposition of lipid hydroperoxides produces many materials that may cause flavor worsening in unsaturated lipid-containing foods. In addition, lipid hydroperoxides can react with oxygen to form secondary products such as epoxyhydroperoxides, ketohydroperoxides, dihydroperoxides, cyclic peroxides, and bicyclic endoperoxides, which in turn break up to form volatile compounds. Further, lipid hydroperoxides can condense into dimers and polymers that also can break down and produce volatile materials.

Antioxidants are of interest to the food industry, because they can prevent rancidity. Decomposition of hydroperoxides by heating or by transition metal ion catalysis can produce both peroxy and alkoxy radicals (Headlam and Davies, 2003), but peroxy radicals ($ROO\cdot$) can also form in nonlipid systems, such as proteins (Du and Gebicki, 2004). The formation of peroxy radical is the major chain-propagation step in lipid peroxidation (Niki et al., 2005). Antioxidants can break this chain reaction by reacting with peroxy radicals to form unreactive radicals which are more stable or form nonradical products and that cannot propagate the chain reaction. The stable oxidation products are usually epoxides or polyhydroxylated derivatives of fatty acids (Okieimen et al., 2005; Yamane, 2002; Harry-O'Kuru et al., 2002). A direct test of antioxidant ability towards lipids is to examine whether a substance inhibits peroxidation of artificial lipids (for example fatty acid or fatty acid ester emulsions), food systems, or biological systems.

The Kinetics of Oxidation

Autoxidation of lipids containing unsaturated fatty acid involved in rancidity has been researched considerably. Endo (1998) reported the number of acyl or hydroxyl groups of a molecule which affected the oxidation of the unsaturated fatty acid. Different kinetics have been brought forward and the parameters involved in the expressions have been experimentally determined for each of the oxidative stages (Brimberg and Kamal-Eldin, 2003). Adachi et al. (1995) and Cubillos et al. (2001) reported a simple kinetic expression that can express the entire process of the oxidation of oleoyl residue of its esters and oxidation of linoleic acid incorporated into dipalmitoylphosphatidylcholine vesicles initiated by the thermal decomposition of 2,2'-azobis (2-amidinopropane) dihydrochloride as a function of the initiator, particles, and intraparticle oxidable lipid concentrations, respectively. Farhoosh (2005) studied the antioxidative activity and mechanism of butein in linoleic acid.

FACTORS THAT INFLUENCE UNSATURATED LIPID OXIDATION IN FOOD EMULSION

Factors that affect the oxidation reaction of unsaturated lipid in oil phase, for example, the chemical structure of lipids (e.g., the number and location of the double bonds), oxygen concentration, antioxidants, temperature, surface area, and water content, etc., have been studied in detail. Emulsions represent a major group of colloidal systems relevant for foods, cosmetics, and pharmaceuticals. The behavior of oil-in-water emulsions in foods is determined by the three regions of the system—the oil that is in the interiors of the emulsion droplets, the interfacial material between this lipid material and the aqueous phase, and the aqueous phase itself. Each of these “phases” may be chemically complex. The lipid may be prone to oxidation. The interfacial material can be composed of proteins or of small emulsifiers or mixtures of a number of these components. The aqueous phase may contain ions, macromolecules (e.g., polysaccharides), salt, or amino acid, which may exert stabilizing or destabilizing effects. Therefore, besides the above factors influencing oxidation in a homogeneous system (bulk oil), it is necessary to understand the effect of these three parts of the emulsions on the oxidation of unsaturated lipid in emulsion.

Surface Characteristics

The Interfacial Membrane

The molecular environment of a molecule within an emulsion is known to have a significant impact on its chemical reactivity. An emulsion can be divided into three distinct regions: oil phase, aqueous phase, and the interfacial region. The properties of the interfacial layers depend not only on the quanti-

ties of materials adsorbed but also on their composition and structure, which in turn affect the detailed properties of an emulsion. The interfacial membrane formed by an emulsifier or protein or a combination of an emulsifier and protein located between the lipid substrate and the oxidative initiator originating in the aqueous phase, can enhance the stability of the emulsion as well as act as a physical barrier to prevent pro-oxidant penetrating and diffusing. Donnelly et al. (1998) and Kabalnov (1994) reported that the effect of physical barriers increased with the interfacial membrane thickening. Thus lipid oxidation is slowest in the emulsion containing droplets stabilized by the surfactant with the longest polar head group or hydrophobic tail group (Richards et al., 2002; Silvestre et al., 2000).

The Interfacial Charge

The role of droplet charge can be demonstrated by the measurement of the ζ potential of the emulsion droplets. Lipid oxidation rates were highest for negatively charged droplets, and were similar for positively charged and uncharged emulsion droplets which are far below the negatively charged droplets (Mancuso et al., 1999a; Mei et al., 1998).

Emulsifier Type, Concentration, and Charge Status

Because the oil phase in water-in-oil emulsion is directly exposed to air, the oxidative rate of lipid is similar to that in the bulk oil phase, while the oxidation of lipid in oil-in-water emulsion is different from the bulk oil phase. Frankel et al. (2002) found that fish and algal oils were initially much more stable to oxidation in bulk systems than in the matching oil-in-water emulsions.

An emulsifier is usually used to stabilize the emulsion. However, many unabsorptive emulsifier molecules exist in aqueous solution following homogenization, which can form micelles above their critical micelle concentration and can dissolve the lipid, the antioxidant, and the prooxidant, thereby increasing the emulsion stability.

The effect of the emulsifier type and amount on the oxidation of unsaturated lipid in oil-in-water emulsions has been studied in recent years. Fomuso and Akoh (2002) found the oxidative stability of oil-in-water emulsions containing different kinds of emulsifiers increased in the order: whey protein isolate > lecithin > mono-/diacylglycerols > Tween 20 > sucrose fatty acid ester. It was consistent with the conclusion of Osborn and Akoh (2004) who reported that the effect of whey protein isolate on caprylic acid/canola oil structured lipid-based oil-in-water emulsions was higher than that of sucrose fatty acid esters.

Donnelly et al. (1998) reported that Tween 20 or whey protein isolate (WPI) were pro-oxidative yet their combination was antioxidative in aqueous solution. They thought it could be due to the ability of surfactants to alter protein

conformation thereby increasing accessibility of free radical scavenging.

Cho et al. (2002) reported that the oxidative stability of emulsion containing unsaturated lipid increased with increasing continuous phase polyoxyethylene 10-lauryl ether (Brij) concentrations. Surfactant micelles could impact the physical location of iron in oil-in-water emulsions, and thus could impact lipid oxidation rates. Mei et al. (1998) and Mancuso et al. (1999a) determined the effects of charge status of oil droplets on lipid oxidation rate in oil-in-water emulsion and reported that anionic SDS-stabilized emulsions were less oxidatively stable than nonionic Brij-stabilized emulsion.

Hu et al. (2003) reported that proteins could be used to produce cationic oil-in-water emulsion droplets at pH 3.0 and oxidative stability increased when protein concentration increased at the boundary of emulsion droplets. Faraji et al. (2004) suggested that continuous phase proteins (e.g., whey protein isolate, soy protein isolate) were also antioxidative and the oxidative stability of emulsions increased with increasing protein concentration.

Droplet Characteristics

The effect of droplet concentration and particle size on the autoxidation of unsaturated lipid in emulsion has been investigated. Jo and Ahn (1999) reported that an increase in the oil concentration led to a decrease in total oxidation and the concentration of volatiles in the headspace of an emulsion. It attributed to higher resistance to mass transfer in oil than in water because flavor compounds must be released from the lipid phase to the aqueous phase before they are released from the aqueous phase to headspace in food emulsion. This point of view was similar to that of Osborn and Akoh (2004), who reported that a decrease in the oil concentration led to an increase in total oxidation in caprylic acid/canola oil structured lipid-based emulsions.

However, Coupland et al. (1996) studied the effect of droplet composition on the rate of oxidation of emulsified ethyl linoleate and arrived at a conclusion that with oil concentration increasing, the rate of initiation was relatively slow but the rate of the propagation step was fast, which accounted for the fact that the rate of lipid oxidation was slow in the initial stage but increased at longer time in food emulsion. At the same time, they thought that particle size would affect oxidation kinetics only when the surface activity of substrate molecules was high and thus could largely accumulate at the boundary.

For a fixed droplet concentration, the rate of lipid oxidation increases as the mean diameter of the droplet decreases because the droplet surface is increasing, which increase the touch among the interface, oxygen, and radicals in solution, and thus enable the unsaturated lipid located on the interface to oxidize easily (Coupland et al., 1996). However, if there are only a limited amount of reactants which scatter at the droplet surface, changing the droplet size may have an effect on the oxidation rate (Roozen et al., 1994).

Diffusion of Oxygen

Oxidation of lipid is the reaction between unsaturated lipid and oxygen. The rate-limiting step is the diffusion of oxygen in water phase when the oxygen concentration is low. However, the diffusion of oxygen is no more a rate-limiting step when oxygen concentration is high and the diffusion rate of oxygen is faster than the oxidation rate (Marcuse and Fredriksson, 1968; Lü et al., 2008). So the effective method to retard oxidation is to decrease the concentration of oxygen.

Purity of Materials

The hydroperoxide concentration in oil and emulsifier is rather high in fact and they readily break into radicals to induce oxidation when they exist in transition metal ion, light, or heat. This would greatly decrease the oxidation rate of unsaturated lipid in emulsion and increase the oxidation stability of emulsion as long as we remove the hydroperoxide in oil and emulsifier or add EDTA, inositol hexaphosphate, etc., to chelate transition metal ion (Yoshida and Niki, 1992).

Interactions with Aqueous Phase Components

Many food emulsions contain ingredients in the aqueous phase that may directly impact lipid oxidation or interact with the other molecular species involved in the lipid oxidation reaction. Some sugars and phenolic compounds in aqueous solution are capable of scavenging free radicals, thus retarding lipid oxidation (Chatterjee et al., 2007; Drusch et al., 2006; Yilmaz and Toledo, 2005). Some proteins are more readily oxidized than lipids (Tong et al., 2000). Mei et al. (1998) reported that the addition of NaCl to corn oil-in-water emulsions stabilized by a negatively-charged surfactant (SDS) slightly increased the rate of lipid oxidation in the presence of iron. Faraji et al. (2004) reported that proteins in the continuous phase of menhaden oil-in-water emulsions could inhibit lipid oxidation at pH 7.0 through a combination of free radical scavenging and metal chelation. Unsaturated fatty acid encapsulated with polysaccharide could retard its oxidation (Ishido et al., 2003; Paraskevopoulou et al., 2007).

Others

Endogenous antioxidant, exogenous antioxidant, and various components in continuous phase can affect the stability of oil-in-water emulsion in all kinds of ways.

Totally, factors influencing unsaturated lipid oxidation in emulsion are very complicated and controversial. The theory of lipid oxidation in aqueous solution should be perfected.

DETERMINATION OF AUTOXIDATION

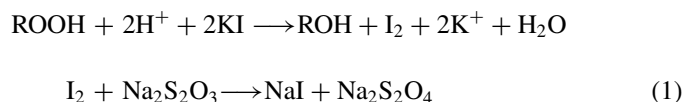
Many properties such as peroxide value, oxygen absorption, the content of volatile compound, color, and luster changed during primary oxidation and secondary oxidation. Various tests for lipid oxidation have been developed based on either products of the initiation and propagation stage, or products of the termination stage, or depletion of oxygen or substrate. The extent of oxidation can be inflected by one of the characteristic properties.

Over the past few years some new methods have been generated to detect lipid peroxidation. These methods based on recent technological developments, improved insights into the complexity of oxidation reactions. The major initial reaction products for lipid peroxidation are hydroperoxides, which are labile species that can undergo both enzymatic and nonenzymatic degradation to produce a complex array of secondary products, including volatile hydrocarbons, malondialdehyde precursor, malondialdehyde itself, olefins, and carbonyl compounds. The assay for hydroperoxides offers the most direct measure of lipid peroxidation. The assay for these secondary products forms the basis for several tests of peroxidation.

Determination of Primary Product

Determination of Peroxide Value (PV)

Hydroperoxide is the primary product of unsaturated lipid autoxidation. Peroxide value directly measures the concentration of hydroperoxides formed in the initial stage of lipid oxidation. The American Oil Chemists' Society has several official methods to determine the oxidative status of lipid, which the most widely used is to determine peroxide value. Iodometric titration method (Hamm et al., 1965) is commonly used to monitor the hydroperoxides. The principle of this method is sodium thiosulfate titrate iodine produced from a reaction of lipid peroxide and potassium iodine. Thus the content of peroxide can be calculated directly by titration of iodine produced. The equation can be expressed as:



A disadvantage of this classical method for liberated iodine determination is its susceptibility toward background reactions. Absorption of iodine by unsaturated fatty acids is a common disadvantage encountered in this determination that would lead to underestimation of the peroxide value (Gray, 1978). Moreover, the liberation of iodine from potassium iodide by oxygen present in the sample solution, which is accelerated in the presence of light and peroxides, is often referred to as the oxygen error and leads to high PV levels (Crowe and White, 2001). Additionally, the presence of water in this method is very detrimental; thus

in case of use in emulsion analysis, a previous quantitative and non-altering extraction of lipids is required.

Lipids with a greater number of double bonds provide more sites for oxidation. Because the iodometric titration lack sensitivity and need large amounts of lipid and cannot be applied into the determination of microanalysis of lipid peroxide, many new methods were developed. When measuring the lower levels of hydroperoxide formation, a sensitive method was developed based on spectrophotometric triiodide determination at 357 nm, a method which is specific for the hydroperoxide group (Chacón et al., 2000).

Iron Thiocyanate Method

Iron thiocyanate method described by Shantha and Decker (1994), is a spectrophotometric method that is based on the ability of peroxides to oxidize ferrous ions to ferric ion with the reaction equation: $\text{Fe}^{2+} + 2\text{H}^+ + \text{O} \rightarrow \text{Fe}^{3+} + \text{H}_2\text{O}$. After that, ammonium thiocyanate solution is added which forms flammulated Iron (III) thiocyanate. The content of hydroperoxidation can be obtained with colorimetry. This method is simple and easy to master, its principal disadvantage being that it is sensitive to oxygen in the solution, which will disturb the measure.

A modified ferrous oxidation-xylenol orange method was adopted to determine lipid hydroperoxides (Navas et al., 2004). Its mechanism is that ferrous ions can be oxidized to ferric ion by hydroperoxides in acidic medium in the presence of xylenol orange, and then ferric ion form blue-purple complex with xylenol orange, which has a UV maximum around 550-600 nm. The method is convenient, fast, and sensitive, whereas it is needed to know the characteristic of lipid peroxide and to strictly control the test condition which is not the same in a different situation (Dobarganes and Velasco, 2002). In view of the method, two versions of the assay were developed (Wolff, 1994), the first version being suitable for the determination of very low levels of hydrogen peroxide in aqueous buffers, and the second version being appropriate for the measurement of lipid hydroperoxides and can be used for the measurement of hydroperoxides accumulation during peroxidation of low-density lipoproteins present in plasma, and in edible vegetable oil.

Conjugated Dienes Value

Ultraviolet spectrophotometry is a commonly used method to detect lipid oxidation containing or generating conjugated unsaturation. Conjugated dienes can be produced during the refining process of oil or autoxidation, which has a UV maximum around 230-240 nm, with a strong characteristic absorption at 234 nm, and can be determined with UV spectroscopy. Iqbal et al. (2005) determined conjugated dienes content by measuring absorbance spectrophotometrically at 234 nm with a Perkin-Elmer lambda-2 spectrophotometer using *n*-hexane as a blank. The sample needs to be diluted before determining its big molar extinction coefficient. The method is applied to the study of pure lipid autoxidation because unoxidized lipid component and peroxidization of material other than lipid will disturb the

determination. In addition, conjugated dienes are often measured as indicators of free radical production; however, free radicals are very unstable and to gain stability would react quickly with other compounds as soon as they are produced. So, conjugated dienes reflect only the extent of lipid autoxidation at an early stage, and the change of absorption value has little relation with the extent of lipid autoxidation except for the early stage of lipid oxidation. The method is unsuitable for determination of lipid containing high proportion of saturated fatty acid.

Corongiu and Milia (1983) provided a second derivative of UV spectra method measurement to calculate the conjugated dienes formation. The author measured the extent of fatty acid autoxidation by the height of the peak at 233 nm, and then confirmed the conjugated double-bond structure by $^1\text{H-NMR}$ spectrometric determinations. The height of the peak increased linearly with an increasing amount of the autoxidized PUFA. However, the application of the method as a quantitative assay would depend upon the availability of pure hydroperoxides of unsaturated fatty acids.

Active Oxygen Method (AOM)

This method predicts the stability of lipid by bubbling air through a solution of the lipid using specific conditions of flow rate, temperature, and concentration (Markus and Peter, 1986).

At intervals, peroxides and hydroperoxides produced by this treatment are determined by titration with iodine. The AOM value is defined as the number of hours required for the peroxide concentration to reach 100 meq/kg of lipid. The more stable the lipid, the longer it will take to reach that level. For products other than lipid and oils, the lipids must first be gently extracted with solvents. Though it is a classical method in determining the stability of lipid, the determination of the parameter with AOM is both costly and time-consuming since a stable lipid may require 48 h or more before reaching the required peroxide concentration.

High Performance Liquid Chromatograph (HPLC)

High-performance liquid chromatograph is simple and highly sensitive, and it is applied to determine lipid peroxide in recent years. Hopia et al. (1996) determined the hydroperoxide content and the distribution of *cis*, *trans*, and *trans, trans* isomers using HPLC. Hydroperoxide with different volatility, different molecular weight, or different polarity, all can be determined with HPLC compared with gas chromatography. Furthermore, HPLC with a different detector, such as an ultraviolet detector, evaporative light-scattering detector, electrochemical detection, and a diode array detector can be applied to determine a different sample. Giuffrida et al. (2004) analyzed epoxy- and hydroperoxy-TAGs which originated from the oxidation of triacylglycerols (TAGs) containing oleic acid by reverse phase liquid chromatography-electrospray ionization-mass spectrometry (LC-ESI-MS) using a triple quadrupole mass analyzer, and this showed a valuable approach to investigate other oxidized molecules.

Infrared Spectroscopy Method

Infrared spectroscopy is a sensitive technique that can detect the major products of lipid peroxidation such as lipid hydroxyl and hydroperoxyl groups. There are some previous reports using near infrared spectroscopy (NIR) and middle infrared spectroscopy to measure the peroxide value in oxidized lipid (Guillén and Goicoechea, 2007; Sinelli et al., 2008). NIR is applied to the routine analysis of specific functional groups which have absorbance peaks in the NIR region. These functional groups are $-\text{OH}$, $-\text{CH}$, $-\text{NH}$, and other chemical bonds containing hydrogen and the functional groups are responsible for most of the major absorption bands observed in the NIR.

NIR spectroscopy is a rapid method of measurement giving quantitative and qualitative information. As a nondestructive testing technology, NIR has been used in many areas such as agriculture, food, chemistry, and medicine. The method can assay a sample with peroxide value from 0 to 100 meq/Kg. Mid-infrared spectroscopy and it has also been used to measure oxidation in lipid. Guillén and Cabo (1999) evaluated the oxidative stability of edible oils with information of FT-IR transmission spectroscopy.

Determination of Secondary Products

The primary products of lipid oxidation are pretty unstable and readily decompose into various secondary products. The methods of measurement are based on some secondary oxidative products which are taken as the effective methods to reflect the real extent of oxidation.

Thiobarbituric Acid Reactive Substance (TBARS) Test

The 2-thiobarbituric acid (TBA) method is an old and most frequently used test for assessing the extent of lipid oxidation in foods and other biological systems. The TBA value expresses lipid oxidation in milligrams of malonaldehyde (MDA) equivalents per kilogram sample or as micromoles MDA equivalents per gram sample (Pikul et al., 1989; Salih et al., 1987). Two molecular TBAs react with malondialdehyde (MDA) originated from oxidation of polyunsaturated fatty acids result in red color which can be measured using a spectrophotometer. There are several procedures for the determination of TBA values such as the distillation method and the extraction method (Ulu, 2004).

Other products of lipid oxidation, such as 2-alkenals and 2,4-alkadienals, also react with the TBA reagent. The solution takes on yellow when it reacts with the other aldehyde under acid condition. The maximal absorption of red and yellow product is 532 nm and 450 nm, respectively (Sun et al., 2001). Modifications of the original TBA test have been reported by Marcuse and Johansson (1973) and Hodges et al. (1999).

Because TBA can form an adduct not only with MDA but also with other oxidized products, the method is also called a

thioharbituric acid reactive substances (TRARS) test. Generally speaking, only fatty acid containing three or more double bonds can produce enough substrate to react with TBA.

Frankel and Neff (1983) investigated the formation of malonaldehyde from a wide assortment of primary and secondary lipid oxidation products by an acetalation-acid decomposition procedure. They pointed out that the TBA color reaction is not specific for malonaldehyde and many lipid oxidation products and their interaction products with other biological materials gave positive reactions. No correlation was found between the thiobarbituric acid values of the lipid oxidation products and analyses of malonaldehyde by the acetalation-acid decomposition procedure. Moreover, they thought that the specific methodology developed in their study would permit a more reliable evaluation of the potential of lipid oxidation products to form malonaldehyde.

Anisidine Value (AnV) Test

When hydroperoxides break down, they produce volatile aldehydes like hexanal, leaving behind a nonvolatile portion of the fatty acid that remains a part of the lipid molecule. This nonvolatile reaction product can be measured by reaction with anisidine. High anisidine values may be a hint that lipid has oxidized even when TBA and other aldehyde tests give low results because volatile aldehydes may incidentally or intentionally get removed during processing.

The anisidine value is defined as 100 times the absorbance (at 350 nm) of a solution resulting from reaction of 1 g of fat in 100 mL of solvent. The anisidine value is determined according to the AOCS Official Method Cd 18-90 (AOCS, 1998). Shahidi and Wanasundara (2002) used the AnV test to determine the aldehydes, principally 2-alkenals and 2, 4-alkadienals, present in the emulsified oil. Unlike hydroperoxides, aldehydes do not break up rapidly, thus allowing the history of an oil to be determined with the AnV.

The anisidine value is a combined measurement of mostly 2-alkenals, 2, 4-dienals, and less saturated aldehydes. The method involves a color reaction between carbonyl compounds and p-anisidine. The pink-red color is measured at 350 nm. The technique is convenient when applied to oils and edible fats; however, when lipids are extracted from a product, a correction for the product's own absorbance may be a source of error in AnV determination.

PV and AnV allow one to calculate total oxidation, the TOX value is determined as $\text{Totox} = 2 \times \text{PV} + \text{AsV}$. The TOTOX value combines evidence about the past history and present state of an oil, and at present is used frequently to estimate the extent of oxidation in the food industry.

Oxidative Stability Index (OSI) Method Rancimat Method

The main cause of deterioration of lipids and lipid-containing foodstuffs is lipid oxidation (Firestone, 1993). The degree of lipid oxidation can be measured by chemical and physical meth-

ods as well as stability tests, which measure the stability of oil under conditions that try to quicken the normal oxidation. Autoxidation includes the induction period and the oxidation period. Induction time indicates the stability of the lipid. The longer the induction time, the more stable is the lipid.

The oxidative stability index (OSI) is similar in principle to the active-oxygen method (AOM), but it is faster and more automated. It determines the oxidative stability of oil by passing air through a sample under stiff temperature control, which aids in the rapid degradation of the lipid into volatile organic acids. The airstream flushes the volatile acids from the oil into a reservoir of deionized water where the acids are dissolved and disassociated into ions, thus changing the conductivity of the water. The OSI value is defined as the hours needed for the rate of conductivity change to reach a predetermined value.

Rancimat is an automated instrument that measures the conductivity of low molecular weight fatty acids produced during autoxidation of lipids at 100°C or above. The principle of the conductivity determination in the Rancimat test is based on measuring the resistance of the solution of the recovered volatile acids. Rancimat apparatus can determine both the induction time of the lipid and the antioxidative effect of different antioxidants. Multiple samples can be tested simultaneously and software controls instrument parameters and data collection, so the Rancimat test is a promising analysis method with regard to timesaving and automatization. However, we should bear in mind that the Rancimat test is a method to evaluate the stability of the lipid by measuring forced oxidative stability at high temperature and in the presence of a huge amount of oxygen. It would be considered a valid method for comparing the oxidative behavior of lipid matrices (better when only one parameter is changed).

Romeu-Nadal et al. (2007) predicted an infant formula using the Rancimat method. Luther et al. (2007) applied the Rancimat method to assess the relative stability of fish oils. Nevertheless, several authors do not support the use of OSI for the evaluation of the oxidative stability of oils. Frankel (1993) considered high-temperature tests including AOM and the OSI method as unreliable because the mechanism of lipid oxidation changes significantly at elevated temperatures. The rates of oxidation become dependent on oxygen concentration because the solubility of oxygen decreases at elevated temperatures. The analyses of oxidation, including the determination of peroxide values, conjugated dienes, or carbonyl values of oils is questionable. Also, side reactions become important and may not be relevant to normal storage temperatures.

Läubli and Bruttel (1986) compared the Rancimat method and the active oxygen method in determination of the oxidative stability of fats and oils. They arrived at the conclusion that the Rancimat method yields results equivalent to the active oxygen method but offers a real alternative for the determination of oxidative stabilities owing to the noticeable saving in labor. Because of its ease of use and reproducibility, the Rancimat test is mainly used to determine the stability of oils (Mendez et al., 1996).

Acid Value

Free fatty acid is a mark of hydrolytic rancidity, but other lipid oxidation can also produce acids. In addition, free polyunsaturated fatty acid can easily break up into small molecular substances, thus degrading the quality of food. It may be useful to know the composition of the free fatty acids present in a sample in order to understand the cause of their formation or decomposition.

The acid value is an indicator of quality, which is defined as the number of milligrams of potassium hydroxide required to neutralize the free fatty acids present in one gram of fat. It is a relative measure of rancidity as free fatty acids are normally formed during the decomposition of oil. The acid value is determined by directly titrating the oil/fat in an alcoholic medium against standard potassium hydroxide/sodium hydroxide solution. The value is a measure of the amount of fatty acids which have been liberated by hydrolysis from the glycerides due to the action of moisture, temperature, and/or lipolytic enzyme.

Pelser et al. (2007) reported the effect of unsaturated fatty acid content on the quality of fermented sausages. The variational rate of fatty acid composition suggests the antioxidative ability. Gas chromatography (GC) has been an applicable tool in microscale analytical work in many research areas of fatty acids. Seppanen-Laakso et al. (2002) focused on optimization of the GC analysis, a methodological variation of fatty acids in dietary fat. Muller et al. (2006) detected conjugated fatty acid methyl esters using atmospheric pressure ionization in combination with silver-ion (Ag^+)-HPLC. Antonelli et al. (2002) gave a potentiometric quantitative analysis of free fatty acid and lipase lipolytic activity in different kinds of milk to control the good taste of milk during storage as well as gave a clear idea of the beginning of rancidity in milk.

Fluorescence Spectroscopy Method

Carbonyl compounds such as malonyl dialdehyde produced during lipid oxidation can react with protein or amino acid and create fluorescent schiff base containing $\text{N}=\text{C}=\text{C}=\text{N}$ structure (Iio and Yoden, 1987). Schiff base is of characteristic fluorescence excitation and emission spectra, so a fluorescence spectrophotometer can assay oxidation of lipid through determination of relative fluorescent intensity of lipid. The method has high sensitivity and important biological significance, and can reflect the interaction between malonyl dialdehyde and protein. Aldehyde can polymerize to form macromolecules therefore there is fluorescence for themselves even if no carbonyl compounds exist. Although the mechanism of the fluorescence spectroscopy method is very complex, its sensitivity is high. The extent of lipid oxidation is usually expressed as relative fluorescent intensity compared with a standard fluorescent substance.

Gas Chromatograph Method

Gas chromatograph is an instrumental method for separation and identification of chemical compounds that are widely

used for trace analysis of organic compounds. Lipid oxidation produces various volatile compounds including hydrocarbons, aldehydes, enals, dienals, ketones, and organic acids. As oxidation increases, these volatiles increase and can be measured by injecting a portion of the headspace into a gas chromatograph. Both the mono-component and the total content of the volatile compound can be selected for measurement. Jensen and Risbo (2007) monitored the hexanal content originated from lipid oxidation of snack and cereal products during storage by static headspace GC. Gaspardo et al. (2008) used a dynamic headspace gas chromatography-mass spectrometry (DHS-GC-MS) technique to detect the volatile fraction of Italian San Daniele ham. Kalua et al. (2006) used the headspace solid phase microextraction-gas chromatography (SPME-GC) method to quantify the changes in headspace volatile compounds of olive oil stored in the light. Formation of volatile lipid oxidation products closely relates to the deterioration of flavor (Garcia-Llatas et al., 2007). The higher the content of aldehyde with small molecular weight, the greater is the extent of oxidation. The method is of high sensitivity and can reflect the change of lipid flavor.

Determination of Oxygen Absorption

Taking static headspace oxygen, for example, the faster the oxygen content decreases, the worse is the stability of the lipid. Nostro et al. (2000) determined the oxygen uptake by monitoring $[\text{O}_2]$ as a function of time with an electrode. Pedrielli et al. (2001) monitored the rate of oxygen depletion by recording the electron spin resonance (ESR) spectra of substrate as a function of time.

Determination of Unoxidative Substrate

Autoxidation of lipids has been the subject of much research. Some research (Minemoto et al., 2002; 2006; Watanabe et al., 2004) measured unoxidative oleoyl residue, linoleic acid, and arachidonic acid using GC, respectively. Adachi et al. (1995) predicted the autoxidation kinetics for n-3 and n-6 polyunsaturated fatty acids and their ester by monitoring an unreacted substrate.

RESEARCH STATUS AND THE PROBLEMS OF UNSATURATED LIPID OXIDATION

Emulsion is the most common application system in food. The simplest emulsion is composed of oil, water, and emulsifier. A great deal of research has been carried out to elucidate the factors that affect lipid oxidation in emulsion. The previous study indicated that the oxidation of unsaturated substrate in aqueous solution is different from that in bulk oil. Besides several common influencing factors, some additional factors, including the structure of the emulsions, the physicochemical properties of

the aqueous phase, the properties of interfacial membrane, are important in emulsions to affect the stability of lipid containing unsaturated lipid. There exist many inconsistent standpoints in data about lipid oxidation in aqueous solution such as the effect of the structure and amount of emulsifier, characteristics of oil and other components, the preparation method of emulsion, etc.

Some studies suggested that the oxidative stability of PUFAs increased with increasing degree of unsaturation in aqueous solution (Bruna et al., 1989; Miyashita et al., 1993;1994), although it is well known that lipid oxidation can accelerate with more double bonds in oil phase. Some researchers explained that it resulted from the different arrangement of fatty acid molecule in micelle, and the fatty acid molecule located deeply in the micelle with increasing unsaturation, the conclusion is yet to be confirmed. Kato et al. (1992) have reported that DHA and EPA is more stable in aqueous solution than in oil. They demonstrated that this high selectivity is due to the coiled configurations of DHA and EPA in an aqueous medium. Miyashita et al. (1994) studied the stability of emulsion composed of unsaturated lipid, which is emulsified with a nonionic surfactant, tween 20. They found that the location of a double bond at unsaturated lipid would affect the oxidation, and the oxidation stability increased when the double bond nears the methyl end.

There is a difference in the effect of oil concentration, droplet size, and physical properties on the oxidative stability of unsaturated lipid. Sims (1994), Osborn and Akoh (2004) reported that decreasing oil concentrations led to an increase in total oxidation. However, the results of the study conducted by Coupland et al. (1996) indicated that the progress of lipid oxidation in the emulsions increased with the concentration of ethyl linoleate in the emulsion droplets increasing. Based on the theory that surface reaction can accelerate lipid oxidation, droplet size is an important factor for influencing the oxidative stability of oil-in-water emulsion. Fritsch et al. (1994) arrived at the conclusion that small droplets enhanced the oxidation rate as they implied a large oil/water interfacial area. However, Osborn and Akoh (2004) gave the inconsistent result that the size of particles had no effect on lipid oxidation in structured lipid-based oil-in-water emulsions. Additionally, Nakaya et al. (2005) investigated the effects of droplet sizes and emulsifiers on the oxidative stability of cod liver oil and soybean oil triacylglycerol (TAG) in oil-in-water emulsions prepared by an oxidative stress-free technique. The results showed that the oxidative stability of polyunsaturated TAG emulsion became worse by increasing the diameter of the oil droplets.

The literature on lipid oxidation published in the past years has been extensive; however, some of them that compared the effect of various pH on lipid oxidation were conflicting. This phenomenon might result from the different experimental plan adopted and the type of surfactant used. Mancuso et al. (1999a; 1999b) reported that low pH decreased lipid oxidation rates with pH from 3.0 to 7.0. This low pH can increase the solubility of iron and more iron can be partitioned into the continuous phase, whereas at high pH, insoluble iron precipitates onto the emulsion droplet surface, and may increase lipid oxidation rates

because of the close proximity of the iron and lipid substrate. However, Mei et al. (1998) found that as pH decreased from 8 to 3, oxidation of SDS-stabilized emulsions increased, while oxidation of Brij and DTAB emulsions are unaffected. Donnelly et al. (1998) reported that oxidation of a WPI-stabilized emulsion decreased with decreasing pH (3-7) but in a Tween 20 stabilized emulsion, oxidation increased with decreasing pH.

A great deal of research was carried out about the effect of the surfactant on lipid oxidative stability in oil-in-water emulsion and a controversial conclusion was arrived at. Kasaikina et al. (1998) found that the anionic surfactant SDS could inhibit oxidation while the cationic surfactant CTAB could accelerate oxidation. The conclusion was far from Mei's report (Mei et al., 1999).

Cho et al. (2002) found that oxidative stability increased with the emulsifier concentration increasing. It was similar to the result of Fomuso et al. (2002) that lipids in emulsion with 0.25% emulsifier concentration generally have a higher oxidation rate than 1% emulsifier concentration. However, Hu et al. (2003) reported that emulsifier concentration had no effect on lipid oxidation in oil-in-water emulsions. Chaityasit et al. (2000) studied the impact of surfactant hydrophobic tail group size on lipid oxidation in oil-in-water emulsions stabilized by polyoxyethylene 10-lauryl ether (Brij-lauryl) or polyoxyethylene 10-stearyl ether (Brij-stearyl). They found that the surfactant hydrophobic tail group size played a minor role in lipid oxidation in oil-in-water emulsions. Silvestre et al. (2000) prepared emulsions with polyoxyethylene 10-stearyl ether (Brij 76) or polyoxyethylene 100-stearyl ether (Brij 700), which are structurally identical except for their hydrophilic headgroups, and evaluated the effect of the surfactant headgroup size on lipid oxidation rates. They found that the characteristics of the surfactant polar headgroups can be an important factor in the oxidative stability of oil-in-water emulsions. Lipid oxidation decreased in the emulsion stabilized by thicker droplet interfacial membrane thickness, a thickness which originated from larger surfactant polar headgroups. It is now clear that the effect of the emulsifier whether it accelerates or inhibits lipid oxidation in emulsion is very complex, so there is much work to be done.

Adding antioxidants is a common method to inhibit lipid oxidation. The effectiveness of antioxidants depends on several factors such as the polarity of the antioxidants, lipid substrate, pH, temperature, concentration of antioxidants, and the physical properties of the food (Huang et al., 1997). In several studies a comparison has been made among the ability of various antioxidants to inhibit lipid oxidation in bulk oil and in oil-in-water emulsions. Some researchers have reported that polar antioxidants have properties described as the "antioxidant polar paradox," a paradox which is based on the hypothesis that polar antioxidants are more effective in bulk oil and nonpolar antioxidants are more effective in oil-in-water emulsions (Fang et al., 2002; Pekkarinen et al., 1999). The antioxidant polar paradox is due to retention of nonpolar antioxidants in the lipid phase of oil-in-water emulsions, or the ability of polar antioxidants to have a higher affinity toward the air-oil interface or

reverse micelles, and thus they are able to concentrate on oil/air or oil/water interfaces where oxidative reactions would be the greatest. The antioxidant polar paradox has been confirmed by studies carried out with tocopherol and vitamin C as antioxidant (Huang et al., 1996; Rietjens et al., 2002), whereas research performed with antioxidants such as caffeic acid and its related hydroxycinnamic acid compounds arrived at a different conclusion (Chen and Ho, 1997). Chaiyasit et al. (2005) found that the more nonpolar antioxidants were more effective in the bulk oil and the polar tocopherol and the nonpolar BHT derivative is more effective compared to other antioxidants in the oil-in-water emulsions, which disagreed with the antioxidant polar paradox. These results indicated that the influencing factors of antioxidant activity are manifold and antioxidant polarity is not the sole criteria.

In addition, the coexisting soluble components such as protein, sugar, acid, base, buffer solution, salt, and surfactant would have an effect on lipid oxidation in food emulsion. Mei et al. (1998) reported that the effect of NaCl, whether it accelerated or inhibited oxidation, depends on the reaction system. They found that NaCl decreased oxidation of the SDS emulsions but had little effect on Brij and DTAB emulsion and ascribed the inhibition effect to decreased iron-lipid interactions through the ability of sodium to decrease iron binding at the droplet surface or by the formation of iron-chloride complexes, which decreased iron binding. Ponginebbi et al. (1999) reported that sucrose could retard the oxidation involved in many mechanisms, e.g., quenching metals, scavenging radicals, and hydroperoxides besides retarding oxidation by increasing viscosity. Sakanaka et al. (2004) found that egg-yolk protein hydrolysates could inhibit the oxidation in the linoleic acid oxidation system.

METHODS TO CONTROL LIPID OXIDATION

Due to the specific nutritional and beneficial effects of unsaturated lipids, especially ω -3 polyunsaturated fatty acids, they are being used in a variety of products for human consumption (Kolanowski et al., 1999; Kris-Etherton et al., 2000). However, unsaturated lipids are susceptible to oxidation and develop undesirable flavors and toxic products during storage, which poses a challenge to the food industry. Lipid oxidation can be controlled by a variety of technologies such as processing characteristics, packaging, and storage conditions (Mortensen et al., 2004).

The oxidative stability of emulsion containing unsaturated lipid can be greatly increased by excluding oxygen from the system, for instance, food containing unsaturated lipid can be packed under vacuum or nitrogen. The quality of the ingredients used to make food emulsions can have a pronounced impact on their oxidative stability. Prooxidants include metals such as copper and iron and sensitizers. The ingredients used in food manufacture should be low in hydroperoxides, transition metals, or other pro-oxidants because the presence of even small amounts of transition metals in an emulsion can accelerate

lipid oxidation through their ability to promote the breakdown of hydroperoxides. The impurity in materials can retard lipid oxidation by preventing hydroperoxides from contacting to pro-oxidants or by inactivating pro-oxidants. It can be achieved in a number of ways. McClements and Decker (2000) reviewed in detail the impact of molecular environment on lipid oxidation in oil-in-water emulsions. Hydroperoxides may be segregated from prooxidants by using an emulsifier that forms a thick interfacial layer. Moreover, an emulsifier giving the droplets a positive charge can electrostatically repel the transition metals. Additionally, EDTA, phytate, proteins, or polysaccharides can chelate transition metals and thereby make them ineffective.

Controlling the initial gas composition and the product to headspace volume ratio in packaging is also important. Different packaging material categories offer varying degrees of protection against light-induced changes due to differences in reflectance, transmittance, and oxygen permeability. So, packaging materials with good oxygen transmission and light transmission is essential. The oxygen transmission rate of the packaging material depends not only on the materials used but also on the relative humidity, and the storage temperature (Holm et al., 2006; Kistrup et al., 2006). Storage conditions should be taken into account. Temperature is not expected to have a significant impact on photooxidation since photochemical processes have low energies of activation. On the contrary, autoxidation which proceeds once the initial free radicals are formed, are affected by temperature (Andersen et al., 2006).

The most important method used in the food industry to inhibit autoxidation is to add antioxidants, which mainly come from plants in the form of phenolic compounds (flavonoids, phenolic acids and alcohols, stilbenes, tocopherols, tocotrienols). It is extremely important to understand the antioxidant and pro-oxidant behaviors of bioactive substances according to their structure, chemical environment, and test-experimental conditions involved. It is now well established that flavonoids can have a pro-oxidant effect (Cao et al., 1997) in the presence of Cu^{2+} .

Antioxidants counteract oxidation in two different ways, by protecting target lipids from oxidation initiators or by stalling the propagation phase. Antioxidants can be broadly classified by the mechanism of action as primary antioxidants (chain-breaking antioxidants) and secondary antioxidants (preventive antioxidants). Primary antioxidants or chain-breaking antioxidants are free-radical acceptors that delay or inhibit the initiation step or interrupt the propagation step of autoxidation through reaction with lipid and peroxy radicals and convert them to more stable, non-radical products. Primary antioxidants are most effective if they are added during the induction and initiation stages of oxidation when the propagation steps have not occurred. The most commonly used primary antioxidants in foods are synthetic compounds such as phenolic antioxidants including butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), propyl gallate (PG), and tertiary butylhydroquinone (TBHQ). However, a few natural primary antioxidants such as tocopherols, flavonoids, as well as carotenoids are commonly

added to foods. The activity of phenolic antioxidants is often lost at high concentrations; therefore, they may become pro-oxidative. This is due to their involvement in initiation (Gordon, 1990). Carotenoids belong to the most common auxiliary antioxidants, although they are thought to be both singlet oxygen quenchers and they also react with chain-carrying peroxy radicals or alkyl-radical intermediates (Matsushita et al., 2000).

Secondary antioxidants or preventive antioxidants act through numerous possible mechanisms other than converting free radicals to more stable products to slow the rate of oxidation. They can hinder reactive oxygen species (ROS) formation or scavenge species responsible for oxidation initiation (O_2^- , 1O_2 , etc.). There are many different preventive antioxidation pathways including chelation of transition metals, singlet oxygen deactivation, enzymatic ROS detoxification, UV filtration, inhibition of prooxidant enzymes, antioxidant enzyme cofactors, etc. Moreover, they can replenish hydrogen to primary antioxidants, decompose hydroperoxides to nonradical species and act as oxygen scavengers, and also act as reducing agent, and so on. Because these secondary antioxidants can enhance the antioxidant activity of primary antioxidants, they are also called synergists. The commonly used secondary antioxidants are citric acid, ascorbic acid, ascorbyl palmitate, lecithin, and tartaric acid. Laguerre et al. (2007) reviewed the ability of antioxidants to prevent lipid from oxidation in detail. It should be kept in mind that antioxidants often act via mixed mechanisms that combine different types of antioxidation.

An antioxidant added to food must be effective at low concentrations. A combination of antioxidants is used to obtain a synergistic effect. In a food product, a synergy is obtained by combining added and inherent antioxidants. However, the behavior of antioxidants in food and their antioxidative ability can vary markedly, depending on the lipid-containing systems. Food products are predominantly multiphase systems. The effectiveness of antioxidants depends on several factors such as the polarity of the antioxidants, lipid substrate, non-lipid constituents, pH, ionic strength and temperature, concentration of antioxidants, the presence of metal ions, and the physical properties of the food (Huang et al., 1997). For this reason, paradoxically, polar antioxidants may be more effective in bulk lipids, whereas nonpolar antioxidants may be more active in emulsified media.

ACKNOWLEDGMENTS

This work has been funded by Xu Zhou Institute of Technology through the project XKY2009216.

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