Review

Methods for Evaluation of the Oxidative Stability of Lipid-Containing Foods

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Lipid oxidation and associated changes are major causes of quality deterioration of lipids and lipid-containing foods. Oxidative deterioration of lipids directly affects their flavor, color and nutritive value. In addition, free radicals formed during this process may also damage membranes, enzymes, vitamins and proteins that may be involved in the process of ageing. Thus, assessing the extent of oxidation in foods is necessary. This paper reviews the conventional methods as well as new procedures for determining oxidative stability of lipids. Criteria for selection of methods depend on a number of factors including the nature and history of sample, the type of information required, the time available for experimentation and test conditions.

Keywords: lipids, fats and oils, oxidative stability, methodologies

Autoxidation is a major deteriorative reaction affecting food lipids and is a primary concern for both the processing industries and consumers as it affects economics, nutrition, flavor, safety and storage of products (Labuza, 1971; Frankel, 1980). Other oxidative processes such as photooxidation and hydrolytic rancidity may also be encountered. Both of these latter processes may be controlled by cold storage, proper packaging or inactivation of enzymes. However, since autoxidation is a chemical reaction with a low activation energy (4-14 kcal·mol⁻¹), the rate of this reaction is not significantly diminished by lowering the temperature of food storage.

Extensive research has been carried out to elucidate the mechanism of autoxidation and to devise methods to control lipid oxidation in foods. Various oxidative products and their possible interaction with food components and biological molecules have been studied (Horton & Fairhurst, 1987; Kanner *et al.*, 1987). The oxidation of oils occurs at sites of unsaturation and proceeds via a free-radical chain mechanism (Labuza, 1971) consisting of initiation, propagation and termination steps.

Initiation starts with the abstraction of a hydrogen atom adjacent to a double bond in a fatty acid (RH) moiety and this may be catalyzed by light, heat or metal ions to form a free radical (Reaction 1). The resultant alkyl free radical (R[•]) reacts with atmospheric oxygen (triplet state) to form an unstable peroxy free radical (Reaction 2) which may in turn abstract a hydrogen atom from another unsaturated fatty acid to form a hydroperoxide (ROOH) and a new alkyl free radical (Reaction 3). The new alkyl free radical initiates further oxidation and contributes to the chain reaction. The chain reaction (or propagation) may be terminated by formation of non-radical products resulting from combination of two radicals species (Reactions 4 to 6).

Initiation:

Propagation:

Termination:

The mechanism of lipid autoxidation has been postulated by Farmer *et al.* (1942), Boland and Gee (1946) and Bateman *et al.* (1953). The propagation step in the autoxidation process includes an induction period when hydroperoxide formation is minimal (Labuza, 1971; Hawrysh, 1990). The rate of oxidation of fatty acids depends on the degree of unsaturation; the relative rate of autoxidation of oleate, linoleate and linolenate is in the order of 1 : 40-50 : 100, on the basis of oxygen uptake, and 1 : 12 : 25, on the basis of peroxide formation (Hsieh & Kinsella, 1989). Polyunsaturated fatty acids (PUFA) such as arachidonic acid, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), containing 4, 5 and 6 double bonds, respectively, are much less stable than linoleic and linolenic acids. Arachidonic acid is oxidized 2.9 times faster than linoleic acid (Porter *et al.*, 1981). Ethyl esters of EPA and DHA oxidized rapidly even at 5°C in the dark after an induction period of 3-4 days, whereas the induction period of linoleate and linolenate under similar conditions are 20 and 60 days, respectively. Therefore, oils that contain relatively high amounts of PUFA experience stability problems. The breakdown products of hydroperoxides such as alcohols, aldehydes, ketones and hydrocarbons generally possess offensive off-flavors. These compounds may also interact with other food components and bring about changes in their functional and nutritional properties (Sherwin, 1978). In addition, free radicals formed

Fig. 1. General scheme for autooxidation of food lipids and its possible products.

during oxidation of lipids may cause damage to membranes, enzymes, vitamins and proteins and may also be involved in the process of ageing (Hamilton, 1983). Autooxidation of food-lipids and its possible products are illustrated in Fig. 1.

Measurement of Oxidative Stability of Food Lipids

There are various methods available to measure lipid oxidation in foods. Changes in chemical, physical, or organoleptic properties of fats and oils during oxidation may be monitored in order to assess the extent of lipid oxidation. However, there is no uniform and standard method for detecting all oxidative changes in all food systems. The available methods to monitor lipid oxidation in foods and biological systems may be divided into two groups. The first group measures primary changes and the second group measures secondary changes that occur in each system. Primary changes are generally measured by monitoring (a)

loss of unsaturated fatty acids, (b) oxygen uptake by weight gain or other methods, (c) hydroperoxide values and (d) conjugated diene value. Secondary changes are followed by quantitation of (a) carbonyl compounds (as their dinitrophenylhydrazones or by gas chromatography), (b) malonaldehyde and other aldehydes (TBA test), (c) hydrocarbons (ethane or pentane content) and (d) fluorescence products (1-amino-3-iminopropene derivatives). The method of choice depends on a number of factors including the nature and the history of the oxidized sample, the type of information required, the time available, and the test conditions. Nonetheless, it is advisable to use a combination of methods to assess both primary and secondary oxidative changes in lipids. However, the results must be in agreement with sensory perception and acceptability standards for foods.

Primary changes

Changes in reactants Methods that measure primary

changes of lipids may be classified as those that quantify loss of reactants (unsaturated fatty acids), addition of oxygen or formation of primary lipid oxidation products (hydroperoxides). These methods are more suitable to measure low levels of oxidation in uncooked products at low temperatures (Coxon, 1987). Measurement of changes in fatty acid composition is not widely used in assessing lipid oxidation since it requires total lipid extraction and conversion into derivatives suitable for gas chromatographic analysis; separation of lipids into neutral, glycolipid, phospholipid and other classes may also be necessary. However, it has been proven that this method serves as a useful technique to identify which class of lipids and fatty acids is involved in the oxidative changes (Coxon, 1987; Gray & Monahan, 1992) and also to assess lipid oxidation simulated by different metal complexes that give different products (Gutteridge & Halliwell, 1990). On the other hand, changes of fatty acid composition can not be used in more saturated oils since this indicator reflects only the changes that occur in unsaturated fatty acids during oxidation. Therefore, oxidative changes in marine oils and highly unsaturated vegetable oils may be monitored using this indicator. Similarly, changes in iodine value (IV) due to loss of unsaturation may be employed to monitor oxidation of food lipids (Hudson, 1983).

During oxidation the oxygen above the lipid surface slowly reacts with lipids and changes in the oxygen pressure may be measured quantitatively. This is the principle used in the Sylvester test in which the oil is subjected to a temperatures of 100°C in a closed system. The drop in pressure of the system is quantified as oxidation proceeds.

Weight gain It is generally accepted that addition of oxygen to lipids and formation of hydroperoxides is reasonably quantitative during initial stages of autoxidation. Therefore, the measurement of induction period from weight gain data is theoretically sound. In this method oil samples (about 2.0 g) are weighed into Petri dishes; traces of water are removed by placing them overnight in a vacuum oven at 35°C and over a desiccant. The samples are then reweighed and stored in an oven at a set temperature. The weight gain of the samples may be recorded at different time intervals.

Olcott and Elnest (1958) reported that marine oils exhibit a fairly sharp increase in mass by the end of the induction period and are rancid by the time they gain 0.3–0.5% in weight (at 30–60°C). Ke & Ackman (1976) reported that this method is simple, has a satisfactory reproducibility and may be used to compare oxidation of lipids from different parts of fish. Recently, Wanasundara & Shahidi (1994) and Shahidi *et al.* (1994) used this method to monitor storage stability of both vegetable and marine oils as such, and as affected by added natural and synthetic antioxidants. Relative activity of different antioxidants may also be compared in this manner. However, surface exposure of the sample to air is an important variable in determining the rate of oxidation. Therefore, use of equal size containers to store samples is very important in carrying out such experiments.

The weight-gain method also suffers from certain disadvantages: (1) the weighing frequency hinders monitoring of fast kinetics (a higher frequency would involve nocturnal weighing), and low or moderate temperatures require long analysis

times for stable samples, (2) discontinuous heating of the sample (which must be cooled before weighing) may give rise to irreproducible results, so the heating and cooling intervals must be accurately controlled, (3) the method involves intensive human participation and (4) the working conditions (sample size, shape of the container and temperature) influence the results. Nevertheless, this method offers advantages such as low instrumentation cost and unlimited capacity for sample processing.

Hydroperoxides In the oxidation of fats and oils, the initial rate of formation of hydroperoxides exceeds their rate of decomposition, but this is reversed at later stages. Therefore, monitoring the amount of hydroperoxides as a function of time will indicate whether a lipid is in the growth or decay portion of the hydroperoxide concentration curve. This information can be used as a guide for considering the acceptability of a food product with respect to the extent of product deterioration. By monitoring the induction period before the appearance of hydroperoxides, one can assess the effectiveness of added antioxidants on the stability of a food lipid.

Peroxide value The classical method for quantitation of hydroperoxides is the determination of “peroxide value” (PV). The hydroperoxide content, generally referred to as peroxide content, is determined by an iodometric method. This is based on the titration of the iodine released from potassium iodide by the peroxides in a biphasic system with a thiosulfate solution as given below.

Potential drawbacks of this method are absorption of iodine at unsaturation sites of fatty acids and liberation of iodine from potassium iodide by oxygen present in the solution to be titrated (Gray, 1978). Results may also be affected by the structure and reactivity of peroxides as well as reaction temperature and time. The official iodometric method Cd 8-53 (AOCS, 1990) for determination of PV is applicable to all normal fats and oils, but is highly empirical and any variation in procedure may result in variation in the results. The official method also fails to adequately measure low PV because of difficulties for determination of the titration end point. Therefore, the official method has been modified in an attempt to increase sensitivity for determination of low PV. The modification involves the replacement of the titration step with an electrochemical technique in which the liberated iodine is reduced at a platinum electrode maintained at a constant potential with this method. PV ranging from 0.06 to 20 have been determined with this method, but it is essential to deaerate all solutions in order to prevent further formation of peroxides.

Several other chemical methods have also been suggested for monitoring PV. Colorimetric methods based on the oxidation of Fe^{2+} to Fe^{3+} and determination of Fe^{3+} as ferric thiocyanate, and a 2,6-dichlorophenol-indophenol procedure are reported in the literature (Lips *et al.* 1943). In studies on the oxidation of biological tissues and fluids, measurement of fatty acid hydroperoxides is more common than measuring their decomposition products. Fatty acid hydroperoxides can

be analyzed by high performance liquid chromatography (HPLC), or their corresponding hydroperoxy acid reduction products can be determined by gas chromatography-mass spectrometry (GC-MS) (Hughes *et al.*, 1983, 1986). Fluorescence methods have also been developed to determine hydroperoxides by allowing them to react with substances such as luminol and dichlorofluorescein which form fluorescent products (Gray, 1978). Although determination of peroxide value is common, its usefulness is generally limited to the initial stages of lipid oxidation.

Active oxygen and oil stability index/Rancimat/Oxidative Stability Instrument (OSI) methods The Active Oxygen Method (AOM), also referred to as the Swift test of the American Oil Chemist's Society, is a commonly used accelerated method for assessing oxidative stability of fats and oils. This method is based on the principle that aging and rancidification of a fat is greatly accelerated by aeration in a tube held at a constant elevated temperature. The peroxide value reached by the AOM at which a fat will be rancid by organoleptic evaluation varies with the nature of the fat (Dugan, 1955). Even though this method has been used extensively over the years, its inherent deficiencies and difficulties also have been identified; for example, (1) the end point is determined by the amount of peroxides in the oxidized oil and peroxides are unstable and decompose readily to more stable secondary products, (2) during the rapid oxidation phase, the reaction is extremely susceptible to variation in the oxygen supply.

Automated versions of the AOM apparatus, known as the Oil Stability Instrument (OSI) and Rancimat, are now available. The Rancimat method uses a commercial apparatus marketed by Metrohm, Ltd. The OSI, computer-assisted version developed by Archer-Daniels-Midland Co. (ADM) is now produced commercially by Omnion Inc. (Rockland, MA). These methods may be considered as automated AOM since both employ the principle of accelerated oxidation. However, the OSI and Rancimat tests measured the changes in conductivity caused by ionic volatile organic acids, mainly formic acid, automatically and continuously, whereas in the AOM, peroxide values are measured. These organic acids are stable oxidation reaction products which are produced when oil is oxidized by a stream of air bubbled through it. As in the AOM, the OSI or Rancimat tests proceed slowly at first because during the induction period, little acid is released. The end point is selected when the rapid rise in conductance begins. The Rancimat became available in the early 1980s and is capable of running only six samples at the same time, whereas the OSI is capable of running twenty-four samples at the same time.

Jebe *et al.* (1993) have reported that the newly developed OSI method is superior over the AOM in many respects: (a) in the AOM, the end point does not determine the induction period, but is merely related to it, whereas the OSI end point determines the induction period precisely, (b) the AOM provides a single data point, whereas OSI provides continual data points, (c) the AOM relies on analysis of unstable primary reaction products, while the OSI is based on analysis of stable secondary products, (d) in the AOM, the end point is determined during the oxidant dependent, highly variable

and difficult-to-reproduce rapid oxidation phase, whereas the OSI end point is determined at the end of the induction period, which is relatively insensitive to oxidant flow, (e) the AOM depends on a manually obtained end point, whereas OSI has an instrumental automatic end point and (f) the AOM is manual, and labor-intensive and time-consuming, but OSI is automated. Despite the high cost of these apparatus, OSI and Rancimat are currently regarded as the methods of choice for fat stability studies by the industry.

Conjugated dienes Oxidation of polyunsaturated fatty acids is accompanied by an increase in the ultraviolet absorption of the product. Lipids containing methylene-interrupted dienes or polyenes show a shift in their double bond position during oxidation due to isomerization and conjugation formation (Logani & Davies, 1980). The resulting conjugated dienes exhibit intense absorption at 234 nm; similarly, conjugated trienes absorb at 268 nm. Farmer and Sutton (1946) indicated that the absorption increase due to the formation of conjugated dienes and trienes is proportional to the uptake of oxygen and formation of peroxides during the early stages of oxidation. St. Angelo *et al.* (1972; 1975) studied the autooxidation of peanut butter by measuring the PV and absorption increase at 234 nm due to the formation of conjugated dienes. Shahidi *et al.* (1994) and Wanasundara *et al.* (1995a) have found that conjugated dienes (CD) correlate with peroxide values during the oxidation of marine (seal blubber and cod liver) and vegetable (canola and soybean) oils. These authors concluded that the CD method may be used as an index of stability of lipids in place of, or in addition to PV. However, carotenoid-containing oils may give high absorbance values at 234–236 nm due to the presence of double bonds in their conjugated structures and may therefore, give erroneous results. The CD method is faster than the PV determination, is much simpler, does not depend upon chemical reaction or color development, and requires a smaller sample size. However, existence of a linear relationship between PV and CD may not be expected for all oils and the latter method is especially suitable when extraction is not desirable.

Parr & Swoboda (1976) have described another spectroscopic method to determine lipid oxidation of stored oils. In this assay, hydroperoxides of polyenoic fatty acids as well as hydroxy and carbonyl compounds derived from them are converted to more conjugated chromophores by two chemical reaction steps, first reduction and then dehydration. These yield "conjugable oxidation products (COP)" which are measured and expressed as the COP value. The first step of the analytical procedure, reduction with sodium or sodium borohydride, results in the disappearance of the characteristic ultraviolet absorption of the carbonyl compounds of oxidized polyenoic fatty acids (oxodienes). The decrease in absorption at 275 nm is known as the oxodiene value. Another feature of this assay is that it distinguishes between the oxidation products derived from dienoic and polyenoic fatty acids as measured by the COP ratio.

Secondary changes The primary oxidation products (peroxides) of fats and oils are transitional intermediates that decompose into various secondary products. Measurement of secondary oxidation products as an index of lipid

oxidation is more appropriate since secondary products of oxidation are generally flavor-active, whereas primary oxidation products are colorless and flavorless. Secondary oxidation products include ketones, aldehydes, hydrocarbons and alcohols, among others.

2-Thiobarbituric acid (TBA) value One of the oldest and most frequently used tests for assessing lipid oxidation in foods and other biological systems is the 2-thiobarbituric acid (TBA) test. The extent of lipid oxidation is reported as the TBA value and is expressed as milligrams of malonaldehyde (MA) equivalents per kilogram sample or as μ mole MA equivalents/g sample. Malonaldehyde is a relatively minor product of oxidation of polyunsaturated fatty acids which reacts with the TBA reagent to produce a pink-colored complex with an absorption maximum at 530–532 nm (Tarladgis *et al.*, 1964). The adduct is formed by condensation of 2 mol of TBA with 1 mol of MA as given above.

Dahle *et al.* (1962) postulated a mechanism for the formation of MA and indicated that only peroxides which possess unsaturation β or γ to the peroxide group are capable of undergoing cyclization with the ultimate formation of MA. Such peroxides may be produced from fatty acids containing three or more double bonds (Dahle *et al.*, 1962). Other products of lipid oxidation, such as aldehydes, alkenals and other dienals (alka-2,4-dienals) may also react with the TBA reagent to form a pink-colored complex with the same absorption maximum as the MA-TBA complex (Marcuse & Johansson, 1973). Therefore, the term “thiobarbituric acid-reactive substances” (TBARS) is now commonly used in place of TBA value (Shahidi *et al.*, 1985; Ke *et al.*, 1984; Wanasundara & Shahidi, 1994).

There are certain limitations when using the TBA test for evaluation of the oxidative state of foods and biological systems because of the chemical complexity of these systems. Dugan (1955) has reported that sucrose and some compounds in woodsmoke react with the TBA reagent to give a red color interfering with the TBA test. Baumgartner *et al.* (1975) have also found that a mixture of acetaldehyde and sucrose when subjected to the TBA test produced a 532 nm absorbing pigment identical to that produced by MA and TBA. Modifications of the original TBA test have been reported by Marcuse & Johansson (1973), Ke & Woyewoda (1979), Robles-Martinez *et al.* (1982), Pokorny *et al.* (1985), Shahidi *et al.* (1987), Tomas & Funes (1987) and Schmedes & Holmer (1989). However, it has been suggested TBARS provide an excellent means for evaluating the relative oxidative status of a system as affected by storage or processing variables (Gray & Pearson, 1987). It is always preferable to quantitate the extent of lipid oxidation by a complementary and analytical procedure to verify the results.

Several attempts have been made to establish a relationship between TBA values and the development of undesirable

Fig. 2. Possible reactions between p-anisidine and malonaldehyde.

flavors in fats and oils. It has been shown that flavor threshold values correlate well with TBA values of vegetable oils such as those of soybean, cottonseed, corn, safflower (Gray, 1978) and canola (Hawrysh, 1990).

Oxirane value The oxirane oxygen groups, also known as the epoxide groups, are formed during autoxidation of fats and oils. The epoxide content is determined by titrating the oil sample with HBr in acetic acid, in the presence of crystal violet, to a bluish-green end point. This method has been standardized by the American Oil Chemists' Society in their tentative methods Cd 9-57 (1981). However, this test is not sensitive and lacks specificity. HBr also attacks α,β -unsaturated carbonyls and conjugated dienols and this reaction is not quantitative with some *trans*-epoxides. Picric acid was the best of several acidic chromophores in its reaction with epoxides. Despite a non-quantitative reaction, the product concentration followed Beer's Law. This method has been found to be particularly well suited to the determination of epoxides in heated fats where the oxirane content is often less than 0.1%.

p-Anisidine value p-Anisidine value is used to measure secondary products of lipid oxidation. This method determines the amount of aldehydes (principally 2-alkenals and alka-2,4-dienals) in animal fats and vegetable oils. Aldehydes in an oil and the p-anisidine reagent react under acidic conditions (IUPAC, 1987; method number 2,504). The proposed reaction of p-anisidine with aldehydes to form yellowish products is shown in Fig. 2. List *et al.* (1974) have reported a highly significant correlation between p-anisidine values of salad oils processed from undamaged soybeans and their flavor acceptability scores.

TOTOX value The p-anisidine value is often used in the industry in conjunction with PV to calculate the so-called total oxidation or TOTOX value:

$$\text{TOTOX value} = 2\text{PV} + p\text{-Anisidine value}$$

The TOTOX value is often considered to have the advantage of combining evidence about the past history of an oil (as reflected in the p-anisidine value) with its present state

(as evidenced in the PV). Therefore, determination of TOTOX value has been used extensively to estimate oxidative deterioration of food lipids (Rossell, 1983). However, despite its practical advantages, the TOTOX value does not have any sound scientific basis since it combines variables with different dimensions. Recently, Wanasundara and Shahidi (1995a) have defined $TOTOX_{TBA}$ as $2PV + TBARS$ since measuring of *p*-anisidine may not always be as feasible as that of TBARS.

Carbonyls An alternative approach for monitoring the extent of lipid oxidation in fats and oils is to measure the total or individual volatile carbonyl compounds formed from degradation of hydroperoxides (Shahidi *et al.*, 1986). One of the more reliable methods for total carbonyl analysis is based on the absorbance of the quinoidal ion, a derivative of aldehydes and ketones. This ion is formed from the reaction of 2,4-dinitrophenylhydrazine (2,4-DNPH) with an aldehyde or ketone, followed by the reaction of the resulting hydrazones with alkali as given above. Many variations of this spectroscopic method have been reported (Yukawa *et al.*, 1993; Meyer & Rebrovic, 1995). Each method offers an alternative solvent, wavelength, or workup in which to analyze the quinoidal ion.

Hexanal, one of the major secondary products formed during the oxidation of linoleic acid in fats and oils (Frankel *et al.*, 1981; Shahidi & Pegg, 1994), and other aldehydes have been used to follow lipid oxidation in meat products. Shahidi *et al.* (1987; 1991) reported a linear relationship between hexanal content, sensory scores and TBA numbers of cooked ground pork, while St. Angelo *et al.* (1987) established a similar correlation for cooked beef. Recent studies in our laboratories have shown that during oxidation of oils rich in polyunsaturated fatty acids of the omega-3 type, large amounts of propanal are formed (Frankel, 1993) and a good correlation existed between the propanal content and TBARS values (Wanasundara & Shahidi, 1995b). These studies suggested that compounds usually associated with lipid oxidation could be used as marker compounds to follow development of off-flavors in lipid-containing foods.

Hydrocarbons and fluorescent products Oxidative studies with methyl linoleate and soybean oil (Selke *et al.*, 1970) have revealed that saturated hydrocarbons could be detected when aldehydes are either absent or undetectable. Snyder *et al.* (1985) have reported that ethane, propane and pentane are the predominant short-chain hydrocarbons formed through thermal decomposition of soybean oil. Correlations between flavor acceptability scores and pentane content, as determined by gas chromatographic techniques,

have been used to assess rancidity of fats and oils (Jarvi *et al.*, 1971). Significant correlations existed between the amount of pentane produced and the number of rancid descriptions of aged vegetable oils (Warner *et al.*, 1974). Headspace pentane concentrations and sensory scores for rancidity development in stored freeze-dried pork samples were reported by Coxon (1987).

Another secondary change that occurs during autoxidation of biological systems is the formation of fluorescent products from the reaction of MA with amino compounds such as proteins and nucleotides (Bidlack & Tappel, 1973). This method has been used in determining the extent of lipid oxidation in biological tissues. Dillard & Tappel (1973) have established that fluorescent compounds with the general structure of a 1-amino-3-iminopropene may develop through the reaction of an amino group with carbonyl compounds, mainly malonaldehyde:

Gray (1978) reported that the development of fluorescent compounds depends not only on the formation of condensation products between malonaldehyde and free amino group but also on the nature of the substituents of the amino groups. Different excitation and emission maxima were observed for different condensation products. Advantages of the fluorescent compounds method as a means of measuring lipid oxidation have been reported by Dillard & Tappel (1971; 1973). This method can detect fluorescent compounds at a parts per billion level and was found to be 10 to 100 times more sensitive than the TBA assay.

Recent Developments for Quantitation of Lipid Oxidation

Lipid oxidation in foods and biological systems has conventionally been studied by monitoring either primary or secondary oxidation products. Over the last twenty years or so advances in pulse radiolysis (Simic, 1980) and electron spin resonance (ESR; Schnaich and Borgi, 1980) techniques have facilitated the detection and study of short-lived free radical intermediates. ESR spectroscopy selectively detects species with an unpaired electron, such as free radicals. The technique depends on the absorption of microwave energy (which arises from the promotion of an electron to a higher energy level) when a sample is placed in a variable magnetic field. A major limitation in the detection of free radicals by ESR is the requirement that radical concentrations remain higher than 10^{-8} M. Radical lifetimes in solution are very short (<1 ms) and steady state concentrations generally remain well below 10^{-7} M. Several approaches have been developed to overcome this problem, based on either enhancing the rate of radical production or diminishing the rate of its disappearance.

These techniques include rapid freezing, lyophilization or spin trapping (Davies, 1987). Although application of ESR as a precise method to study lipid oxidation in animal tissues and other biological model systems is commonplace, its application to food systems is relatively new.

Recently, Burkow *et al.* (1992) have reported that hypochlorite-activated chemiluminescence could provide a useful method for evaluation of antioxidants in edible oils. Due to high sensitivity and ability to detect small changes in the degree of oxidation, this method provides a useful means to evaluate effect of antioxidants on oils at low temperature (about 35°C) storage within 24 h. Chemiluminescence generally originates from electrically excited stages, such as singlet molecular oxygen in lipid peroxidation (Neeman *et al.*, 1985). The chemiluminescent method has now been tested for estimating the deterioration of edible oils containing antioxidants (Burkow *et al.*, 1995), as well as for shelf-life dating of fish and meats (Miyazawa *et al.*, 1991).

Infrared (IR) spectroscopy has also been used for the measurement of rancidity, and it is of particular value in the recognition of unusual functional groups and in the study of fatty acids with *trans* double bonds. Production of hydroperoxides during oxidation of lipids gives rise to a band at about 2.93 μm whereas the disappearance of a band at about 3.20 μm indicates the replacement of a hydrogen on a double bond, or polymerization. It is also suggested that the appearance of an additional band at 5.72 μm , due to C=O stretching, indicates the formation of aldehydes, ketones or acids. Furthermore, changes in the absorption bands in the 10 to 11 μm region indicates *cis*, *trans*-isomerizations and formation of conjugated ethylenic bonds. Recently, van de Voort *et al.* (1994) have reported that Fourier Transform Infrared (FTIR) spectroscopy could provide a rapid measurement of oxidative deterioration of lipids quantitatively by percent hydroperoxides, percent alcohols and total carbonyl content.

Determination of overall changes in the oxidative state of food lipids including both primary and secondary changes remains desirable (Shahidi, 1992). During oxidation of food lipids, changes occur to the protons in an oxidizing molecule. These changes may be monitored by employing proton Nuclear Magnetic Resonance (^1H NMR) spectroscopy as originally described by Saito & Udagawa (1992) and Wanasundara & Shahidi (1993). During oxidation of lipids with unsaturated fatty acid, the relative number of olefinic and diallylmethylene protons decreases and the total number of aliphatic protons increases. Diallylmethylene protons are first attacked by free radicals and intramolecular rearrangement of the olefinic protons would follow. Thus, the relative number of diallylmethylene and olefinic protons decreases. NMR absorption peaks of diallylmethylene, olefinic and aliphatic protons appear at δ 2.6–2.9, δ 5.1–5.6 and δ 0.6–2.5, respectively (Saito & Udagawa, 1992). Wanasundara & Shahidi (1993) have shown that the ratios of aliphatic to olefinic protons (R_{ao}) and aliphatic to diallylmethylene protons (R_{ad}) increase steadily during the oxidation of canola and soybean oils. Similar results were observed for both cod liver and seal blubber oils (Shahidi *et al.*, 1994). Furthermore, plotting of R_{ao} and R_{ad} against corresponding TOTOX values of these oils indicated significant ($p < 0.05$) linear

correlations. Therefore, the NMR methodology offers an alternative method to estimate the overall changes in the primary and secondary oxidation products of lipids. It also provides a rapid, nondestructive and simple procedure for evaluation of the oxidative changes during storage of lipids.

Methods of Assessing and Screening of Antioxidant Activity

Methods described above might also be used for scrutinizing the activity of antioxidants or comparing their effectiveness with other antioxidants. However, there are other methods such as Fenton reaction (Mason *et al.*, 1990), free radical scavenging determination (Brand-Williams *et al.*, 1995), β -carotene bleaching (Miller, 1971) and hemoglobin peroxidation test (Cort, 1974) which may also be used to compare oxidative stability of lipids or screening of new antioxidants.

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