Lipids and their Oxidation in Seafood

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Summary: Oxidative rancidity leads to the qualitative deterioration of seafood and other muscle food, resulting in the quality loss, there by shortening shelf life. The oxidation of unsaturated fatty acids or triglycerides in marine food involves the formation of free radicals and hydroperoxides. After polymerization, hydroperoxides form dark colored organic polymers. Other compounds such as ketones, aldehydes, alcohols, hydrocarbon, acids, and epoxides. Lipid oxidation takes place in fresh and frozen marine food and can be catalyzed by metal ion. Oxidized unsaturated lipids bind to protein and form insoluble lipid-protein complexes. This accounts for the poor flavour, toughened texture and unappealing odor of poorly stored marine food and thus control of oxidation has become increasingly important. Antioxidants are the most effective inhibitors of lipid oxidation.

Introduction

Marine lipids are important nutritional seafood particularly due to their concentration of poly-unsaturated fatty acids (PUFA) [1]. But, this is high content of unsaturated lipids, make fish products very susceptible to loss of quality by development of lipid oxidation. Rancidity is specially faster in species like mackerel (*Scomber scambrus*) or horse mackerel (*Trauchurus trauchurus*), in which muscle coexist large amounts of hemoglobin, a well-known activater of lipid oxidation, and lipids [2].

The storage stability and quality of fresh frozen and processed fish and other muscle food depends essentially on the composition of lipids and especially on their degree of unsaturation. The quality of fish may be effected by various biological factors such as feeding, genetics, temperature, light moisture, season etc. These biological factors affecting quality mainly concerns chemical and physical changes in the fish muscle. The quality parameters to be effected are muscle texture, color, taste, odor and flavor [3-6].

Lipid oxidation is one of the major causes of quality deterioration in muscle foods following storage at refrigerated or frozen temperatures. The off-odors and off-flavors that result from lipid oxidation lower the quality and thus shorten the shelf-life of the muscle. Hemoglobin is a likely catalyst of lipid oxidation in fish muscle. A better understanding of how hemoglobin promotes lipid oxidation in fish muscle could lead to quality improvements. Often seen in later stages of storage, quality losses are manifested through a variety of mechanisms [7-16].

Oxidative rancidity in fish and other muscle foods is one of the major cause of lipid oxidation responsible for losses in quality. It results from the chemical deterioration of fats whereas other deteriorative reactions such as microbial or enzyme attacks can be largely controlled by lowering the temperature, this is not particularly helpful in preventing oxidation since low energy threshold are involved, nor is exclusion of oxygen always practically possible [17].

Rancidity is develop in many fatty fishes during storage or handling, oxidation can occur in either the stored triglycerides or the tissue phospholipid since the oil in such fishes are rich in highly unsaturated fatty acid particularly polyunsaturated fatty acid majority of which belong to n-3 or n-6 families [6-19]. These highly unsaturated constituent in seafood are susceptible to oxidation and result in the formation of free radicals and hydroperoxides which cause oxidation of pigments, flavors and vitamins and thus decreases the nutritional value of the fish. Apart from this lipid oxidation can be catalyzed by metal ions. The oxidized unsaturated lipids binds to the proteins and form insoluble lipid protein complexes. This accounts for toughened texture, poor flavor and unappealing odor of poorly stored frozen sea food [19-20].

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The aim of this discussion is to review the mechanism, of the oxidation of unsaturated fatty components of seafood, the nature of oxidative reactions, reaction of oxidized fish lipid with proteins and amino acid, metal-catalyzed lipid oxidation and effects of different antioxidants. This review describe the fundamental mechanisms of lipid oxidation as they apply to muscle foods.

Mechanism of Lipid Oxidation

The two major components involved in lipid oxidation are unsaturated fatty acids and oxygen. In this process, oxygen from the atmosphere is added to certain fatty acids, particularly oleate linoleate and linolenate, creating unstable intermediates that eventually break down to form unpleasant flavor and aroma compounds. This type of oxidation is called autoxidation which involve primary autoxidative reaction which are further accompanied by various secondary reaction having oxidative and nonoxidative character. Although enzymatic and photogenic oxidation may play a role, the most common and important process by which unsaturated fatty acids and oxygen interact is a free radical mechanism characterized by three main phases, initiation, propagation and termination. The free radical chain mechanism has been generally accepted as the only process involved in autoxidation [21-24].

Initiation: $RH \longrightarrow R^{\cdot} + H$

$RH + O_2 \longrightarrow ROO^{\cdot} + H^{\cdot}$

Propagation : $R^{\cdot} + O_2 \longrightarrow ROO^{\cdot}$

$ROO^{\cdot} + RH \longrightarrow R^{\cdot} + ROOH$

Termination : $2RO_2^{\cdot} \longrightarrow O_2 + RO_2R$

$RO_2^{\cdot} + R^{\cdot} \longrightarrow RO_2R$

Where ROO· is a lipid peroxy radical, R is a lipid radical, and RH is an unsaturated lipid. Once the reaction has been initiated, the hydroperoxides (ROOH) which are formed are converted to free radicals, which in turn can accelerate the rate of lipid oxidation. Fig.1 shows the overall mechanism of lipid oxidation in addition to the formation of hydroperoxides (ROOH), generally called peroxides or primary products of oxidation [17], other types of reaction may occur. The peroxides may break down to carbonyls, form polymers, or react with protein, vitamins, pigments etc. [17-25].

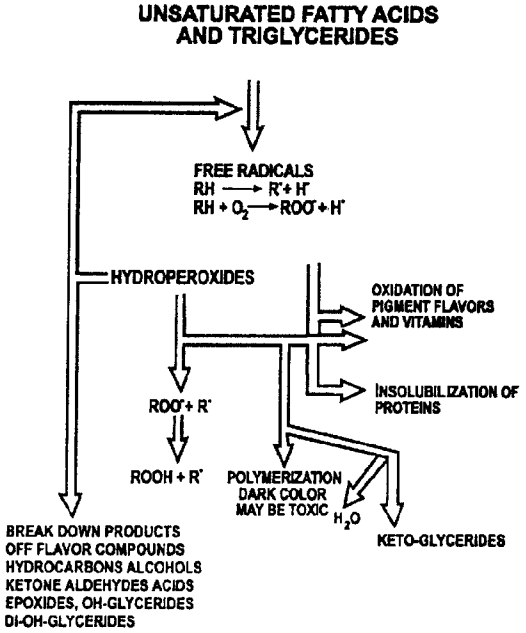


Fig. 1: Mechanisms of Lipid Oxidation.

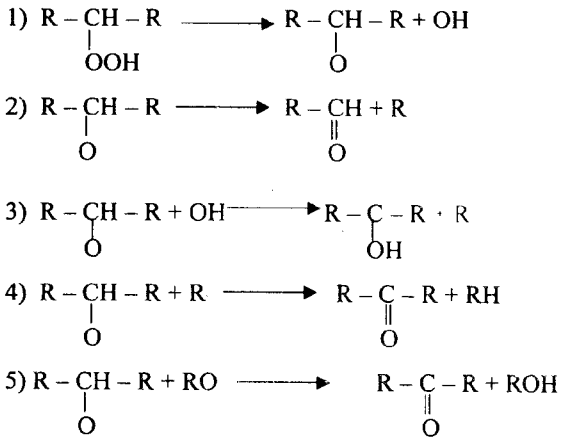
Initiation occurs as hydrogen is abstracted from an unsaturated fatty acid, resulting in a lipid free radical, which in turn reacts with molecular oxygen to form a lipid peroxyl radical. While irradiation can directly abstract this hydrogen from lipids, initiation is frequently attributed in most foods, including muscle foods, to reaction of the fatty acids with active oxygen species. The propagation phase of oxidation is fostered by lipid-lipid interactions, whereby the lipid peroxyl radical abstracts hydrogen from an adjacent molecule, resulting in a lipid hydroperoxide and a new lipid free radical. Interactions of this type continue 10 [26] to 100 times [27] before two free radicals combine to terminate the process.

Lipid hydroperoxides by themselves, are not considered harmful to food quality; however, they are further degraded into compounds that are responsible for off-flavors. The main mechanism for the formation of aldehydes from lipid hydroperoxides is homolytic scission (β cleavage) of the two C-C bonds on either side of the hydroperoxy group [28]. This reaction proceeds via the lipid alkoxyl radical, with the two odd electrons produced on neighboring atoms forming a carbonyl double bond. Two types of aldehydes are formed from the cleavage of the carbon bond: aliphatic aldehydes derived from the methyl

terminus of the fatty acid chain and aldehydes still bound to the parent lipid molecule. Since unsaturated aldehydes can be oxidized further, additional-volatile products may be formed [28].

Secondary Reaction Products

The lipid peroxide are very unstable and break down to produce different types of secondary reaction such products contribute to the oxidized flavour of food lipids. Hydroperoxide decomposition proceeds by a free radical mechanism and can be illustrated by the following steps:



The hydroperoxide is cleaved to alkoxy and hydroxyl free radicals. Reaction 2-4 indicate the reaction of alkoxy free radical with other free radicals or molecules to form secondary products. The oxidation products includes carbonyl compounds alchohols, acids, hydrocarbon, lactones and esters [6]. It is the first systematic study of the oxidation breakdown products. The rate of other oxidation reaction were found to be dependent upon the class of carbonyl compounds being oxidized. When N-nonanol was oxidized in oxygen atmosphere, the only oxidative product which was formed was N-nonanoic acid [29]. This work was confirmed by radio tracer techniques to follow the oxidation of similar classes of carbonyl compounds is in oxidizing soybean oil [30]. It was suggested, the following free radical lipid oxidation mechanism in oxidation of arachidonic and linolenic acid (Fig. 2 & 3). The abstractable hydrogen in polyunsaturated fatty acids are those attached to bis-allylic carbon (Fig. 2). *cis-trans* and *trans- trans* Conjugated diene-hydroperoxides are the primary products in linoleate autoxidation [31].

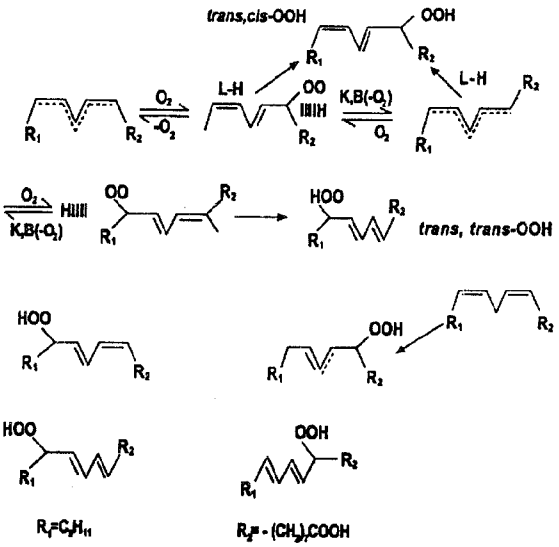


Fig. 2: Primary products formed in linoleate autoxidation (cit – trans and trans – trans conjugated diene hydroperoxides).

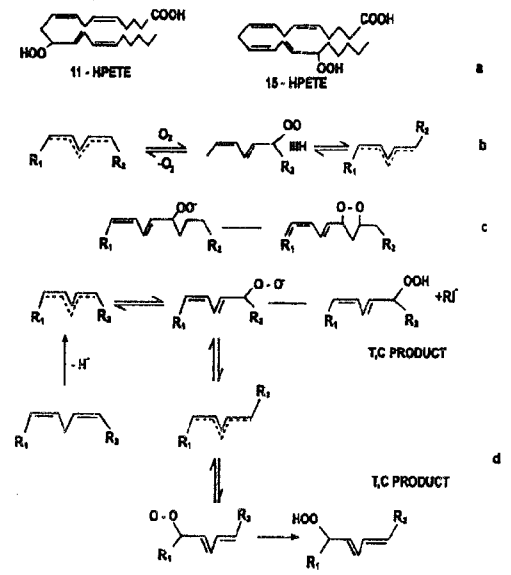


Fig. 3: Mechanism for (aq) formation of hydroperoxyeicosateraenoic acids (11-and 15-hpete) from arachidonic acid autoxidations (b) oxygen addition to pentadienyl radical, (c) radical cyclization through peroxy radical substrate, and (d) hydroperoxides derived from linoleic acid.

In arachidonic acid autoxidation, six major conjugated hydroperoxides have been isolated. 11-Hydroperoxy eicosatetraenoic acid (11-HPETE) & 15 HPETE derived from H abstraction at carbon -13 of arachidonic acid (Fig. 3a), oxygen addition to pentadienyl radicals is reversible (Fig. 3b).

Radical cyclization may occur if a remote double bond is present in the peroxy radical substrate, and monocyclic peroxides, bicyclic peroxides, and epoxy alcohols are prducts formed by cyclization (Fig. 3c). Hydrogen peroxides derived from linoleic acid are suggested to be formed as shown in (Fig. 3d).

The oxidation of polyunsaturated fatty components in mackerel oil was studied and. reported the formation of "fishy" off-flavor components, especially 2,4,7–decatrienals, in various rancid mackerel oil. The concentration of this component is related to the ratio of total polyenoic acids to total saturated acids. (C14:0 + C16:0 + C18:0). Where as all fish oil polyenoic fatty acids are sensitive to oxidation [32]. A decrease in the ratio of C22:6 to C16:0 has been used as an index of oxidative rancidity of lipid in fish [33].

The rate of the oxidation of mackerel lipids during frozen storage is dependent upon the temperature of storage. The rate of formation of peroxides in the dark muscle and skin of mackerel was significantly lower at – 40°C storage than at –15°C. At 60°C, the rate of the lipid oxidation of mackerel skin lipids was significantly higher than the rate of oxidation of mackerel meat lipids. The studies have shown that the faster oxidation of a skin lipid is probably not due to a greater surface / mass ratio of the skin and that there may be some fat soluble substance in the mackerel skin lipids which catalyzes their unusual oxidation. The effect is temperature dependent in the frozen condition, but the catalytic lipid oxidation activity can be inhibited by lowering the frozen storage temperature to – 40°C [34]. Lipid oxidation in raw and cooked oil sardine during refrigerated storage was studied. The result showed that raw sardine stored at refrigerated temperature become rancid in 2-3 days; whereas cooked sardine become rancid in 6 days. These additional days for storage and handling of fish without any development of rancidity is of great practical significance [35].

Lipid autoxidative changes in cold-stored condition. The analysis of lipid fractions indicated that the neutral lipid tended to remain unchanged, but the phospholipids fraction was hydrolyzed, and free fatty acids were generated. The proportion of the C_{22} acids increased with the expanse of the C_{18} component. The changes in the composition of free fatty acid appears to be closely related to the liberation of phosphotidic acid moieties [36].

Changes in the lipids of skipjack tuna during frozen storage were reported [37]. According to the study the triglycerides decreased rapidly in the early period of storage and free fatty acids increase. The increase in the free fatty acid content was due to hydrolysis of phospholipids and triglycerides [37].

The changes in fatty acids and sensory quality of fresh water prawn stored at –18°C were studied, and it was observed that, the fatty acids, especially the unsaturated ones, decreased during frozen storage for 6 months. No objectionable rancid flavor was detected during the frozen storage [38].

Reaction with Protein and Amino Acid

Lipid oxidation products can react with proteins and amino acids to cause damage in food and other biological systems. The reaction of an autoxidized lipid with proteins was studied, it was reported that both insulin and gelatin were readily soluble in the aqueous solvent but quiet insoluble when reacted with autoxidized methyl linoleate. Accordingly, when these proteins were subjected to trypsin hydrolysis, the gelatin system under went less hydrolysis then insulin system, it was concluded that gelatin was involved in cross-linking reaction, in the autoxidation of methyl linoleate, reactive intermediates arise which insolublize proteins via the cross linking reaction [39].

Interaction between lipid and protein during frozen storage was studied [40]. The effect of non polar and polar lipids on rainbow trout myofibrils was also studied. Salt soluble proteins rapidly decreased during frozen storage and the patterns of lipid insolubility were almost the same in each model system: untreated myofibrils, myofibrils treated with non polar lipids and myofibrils treated with polar lipids [40], the browning reactions of oxidized fish lipids with protein were studied, the discoloration mechanism of white fish muscle in the model systems consisting of methyl esters of polyunsaturated fish oil, fatty acids and fish muscle (Cod, Carp, and Mackerel) homogenates, fish myosin or pure

proteins of animal origin. Both the soluble and insoluble brown pigments were produced by the interaction of lipid peroxide and carbonylic peroxide decomposition products with primary and secondary amino groups of protein [41].

It was postulated that the browning lipids in a mixture of protein components proceeds in three steps:

(1) Formation of lipid peroxides.

(2) Formation of colorless or slightly colored precursors of brown pigments by interaction of peroxides with active groups of protein and by interaction of carbonylic peroxide decomposition products with active groups of protein.

(3) The transformation of the colorless or light – colored precursors into brown pigments.

It has also shown that the extent of browning was much smaller in Potassium-Iodide treated samples, where the content of peroxides was greatly reduced while the carbonyle group content remained nearly the same. Autoxidation in food containing significant quantities of polyunsaturated fatty acids can be very extensive and may result in the impairment of quality particularly in the case of seafood and fishery products. The reaction of myosin with malonaldehyde was reported [42], in frozen solution malonaldehyde reacted with lysine, tyrosine, methionine and argininé, in decreasing order of intensity. Myosin, a structural protein of muscle, was reacted at pH 6.8 and an ionic strength of 0.5 with malonaldehyde. The rate of reaction with the free amino groups of myosin was greater at – 20°C than at 0°C and was almost as great as at +20°C. The same relationship was observed when the decreasing malonaldehyde concentration was measured in the protein – malonaldehyde reaction mixture [42].

Since the discovery of malonaldehyde induced fluorescence, many studies have been carried out to ascertain whether similar fluorescent compounds are produced in biological systems in which autoxidized lipids and amino compounds are both present [43]. Investigators were also interested in whether fluorescence due to these compounds could be used to monitor the degree to which lipid autoxidation had taken place.

The compounds formed by the reaction of leucine, valine or glycine with malonaldehyde were characterized, and reported that two amino acid molecules reacted with one molecule of malonaldehyde to form a conjugated Schiff base by the mechanism shown in Figure 4 [44]. Ribonuclease was reacted with malonaldehyde and observed the development of florescence compounds. The fluorescence was attributed to the formation of a 1-amino-3-iminopropene structure [45]. The extent of lipid peroxidation occurring in a biological system was measured by measuring fluorescence. Phos-phatidyl ethanolamine (PE) which contain poly-unsaturated fatty acids and a free amino group is a natural component of biological membranes. It was found that by mixing varying amounts of oxidized PE with biological tissue the fluorescence intensity increased directly with increased PE content [46]. The measurement of lipid oxidative fluorescent product in aqueous and organic phases extracted muscle and skin lipid is very helpful in evaluating the crosslinking of malonaldheyde with amino acid, peptide, protein phospholipids and itself [47].

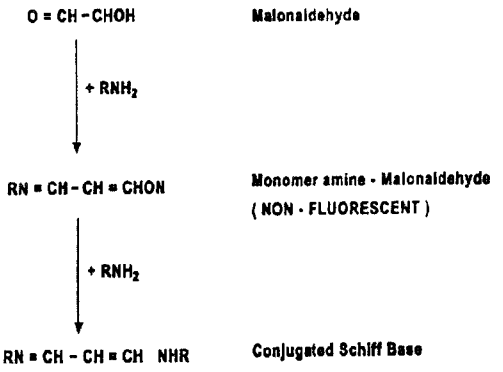


Fig. 4: Production of fluorescent chromophores by reaction of oxidation products and amines.

Metal Catalysis of Fish Lipid Oxidation

It has been generally known that lipid oxidation is affected by metal ions much effort has been devoted for identification of catalysts responsible for the oxidation of lipid. A brief look at some of the earlier studies shows that lipid oxidation could be readily induced in the flesh of both lean fish and fat fish, as well as crustaceans and shellfish, by the addition of trace amounts of certain metal ion [48]. In general Cu^{++}, Fe^{++} and V^{++} were the most active

catalysts. Cd^{++}, Co^{++} and Zn^{++} produced rancidity in fat fish and not in lean fish. The activity of individual heavy metal ions in producing lipid oxidation was not the same for all species of fish. Crustaceae and to a lesser extent the flesh of the shellfish, were extremely resistant to the Cu^{++} induced rancidity [49]. It was found that in nonaqueous system, Fe^{++} and Cu^{++} accelerated the oxidation of lipids prepared from mackerel skin and meat. The kinetic effects of added copper, zinc and iron compounds in oxidation of lipids, in the skin and meat of mackerel at 60°C were investigated. Inorganic Fe^{++} Cu^{++} were found to be strong catalysts in mackerel lipid oxidation. It was also found that the rate of free fatty acid formation in frozen fish was approximately proportional to the fat contained in the various parts except in lipids of the skin were hydrolyzed more slowly [50].

The effect of copper, iron and hemin on lipid oxidation of fish flesh homogenate was studied and found that lipid oxidation is accelerated by Cu^{++}, Fe^{++} and hemin [51]. Thus various biochemical substances, such as amino acids, organic acid, pigments, heme compounds in various fish tissues have been shown to catalyze the lipid oxidation reaction alone or in association with certain trace metal.

Control of Lipid Oxidation

Lipid oxidation is a major cause of quality deterioration in fish muscle. The off-odors and off-flavors that result from lipid oxidation lower the quality and thus shorten the shelf-life of the muscle.

By far the most important defense mechanism for lipid oxidation is the presence of antioxidants, which can delay or slow the rate of oxidation of autoxidizable materials. Inhibition may take two forms: a reduction in the rate at which the maximal level of oxidation is approached or a reduction in the maximal level of oxidation.

Most of the investigation showed that the deterioration of oil in fish muscle may be retarded by application of certain anti-oxidants. The use of various anti-oxidant in controlling lipid oxidation in fish systems has been reported by several workers [52-55].

Comparative studies on the effect of various anti-oxidant on fish oils, fish tissue homogenates, and fish meat have also been investigated by several researchers. Several antioxidants were used to study their effect on lipid oxidation in ground fish. On storage at (4°C) for 14 days, all of the antioxidants, except rutin (200 and 30 ppm) and α-tocopherol (30 ppm), were effective in inhibiting lipid oxidation in raw fish.

L-Ascorbic acid acted as a pro-oxidant in steam and microwave-cooked fish, as well as in one week – stored (at either 4 or 20°C) steam cooked fish. The polyphenols querceting (200 ppm), myricetin (200 ppm), tannic acid (30 and 200 ppm) and ellagic acid (30 and 200 ppm) were potent antioxidants under the same conditions [56].

The antioxidative potency of TBHQ and other antioxidant compounds on oxidation of mackerel skin lipids were studied and found the order of effectiveness for inhibiting the oxidation in mackerel skin lipid to be TBHQ > α– tocopherol > tempeh oil > BHA > BHT, at concentrations of 0.02% for all synthetic compounds and 0.1% and 5% for α-tocopherol and tempeh oil, respectively. In addition, it was reported that TBHQ not only was the most powerful antioxidant for the unsaturated marine lipids but also retarded formation of carbonyls from lipid hydrolysis and secondary oxidation reactions [54]. It was evaluated that the effects of different types of antioxidants including sodium chloride, polyphosphate, BHA, Tenox 11, Tenox A, EDTA, citric acid, ascorbic acid and tocopherol, the meat and antioxidant were mixed under N_2 and vacuum packaged, all samples were kept at 4°C for 1 week. Sodium chloride showed a linear pro-oxidant effect, dark meat had generally higher TBA values than light meat; and all TBA values were generally very low. The low TBA values are believed to be related to the extreme care taken in mixing under N_2 and vacuum packing of the products. Products stored for 6 months at -18 °C had increased TBA values, but all TBA values were again quiet low. Cooking meat after storage increased oxidation. Free-radical-terminator antioxidants inhibited oxidation more than metal cheaters, and sodium chloride acted as a pro-oxidant in this meat system [57-58]. The pro and anti-oxidant effects of eight amino acid on the oxidation of fish oil. L-Leucine and glycine had pro-oxidant activity on fish oil, but DL-valine, DL-methionine, DL-proline and L-cysteine had anti-oxidant activity. DL-phenylalanine and DL-tryptophan had no effect on oxidation of fish oil. Proline had a relatively strong anti-oxidant effect while tryptophan has no such

effect. Amino acids had per-oxidizing activity in alkaline medium [59]. These findings led to an understanding of the complex nature of the action of the protein decomposition products on oxidation of fat in the tissue.

In view of the importance of the oxidative stability of foods, efforts to find acceptable ways of limiting lipid oxidation are of great importance. Though significant research efforts have been directed towards better definition and control of the lipid oxidative processes, and a great deal is known about the complex series of reactions involved. However oxidative stability of foods is still a problem and continued research in this area is still needed.

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