Prevention of Fish Oil Oxidation
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Abstract: The benefit of fish oil to health has been widely recognized because of the high contents of functional EPA (20:5n-3) and DHA (22:6n-3) in the oil; however, the application of fish oil has been limited by its high susceptibility to oxidation. This review reports the characteristics of EPA and DHA oxidation compared with those of other fatty acids such as linoleic acid (18:2n-6). In addition, effective approaches to protect against oxidation are discussed, focusing on the unique antioxidant potential of amine compounds. Finally, the exceptionally high oxidative stability of EPA and DHA in biological systems is discussed. Understanding the protective mechanism against EPA and DHA oxidation in such systems may be useful for the development of an effective antioxidant procedure for fish oil that is rich in EPA and DHA.

Key words: fish oil oxidation, EPA, DHA, antioxidants, amine compounds

1 INTRODUCTION
Fatty acids, a major constituent of lipids, play an important role in human nutrition and health by providing a concentrated source of energy and playing a key role in many metabolic processes. Most naturally occurring fatty acids that are found in lipids possess a saturated and unsaturated unbranched carbon chain containing an even number of carbon atoms, from 4 to 28. Among these fatty acids, linoleic acid (18:2n-6, LA) and α-linolenic acid (18:3n-3, LN) are known as essential fatty acids; their omega-6 and omega-3 metabolites, arachidonic acid (20:4n-6, AA), eicosapentaenoic acid (20:5n-3, EPA) and docosahexaenoic acid (22:6n-3, DHA), are known to exhibit significant biochemical and physiological effects in the body that primarily induces a positive influence on human nutrition and health. Thus, the functions of these omega-3 and omega-6 polyunsaturated fatty acids (PUFAs) have attracted consumer attention, and they are used in functional foods and nutraceuticals.

On the other hand, these PUFAs are easily oxidized, even at room temperature, causing undesirable flavors and lowering the nutritional quality and safety of lipid-containing foods. In foods, lipids have a significant effect on quality even when the lipid content is very small. They not only contribute to the flavor, odor, color and texture but also confer a feeling of satiety and palatability to foods. Thus, the oxidative deterioration of PUFAs in food lipids is a serious problem in many sectors of the food industry because of the increasing emphasis on the importance of these PUFAs on our health. For effective control methods against lipid oxidation, especially PUFAs oxidation, improved strategies have been demonstrated, including use of antioxidants, inactivation of pro-oxidant metals, and minimization of exposure to air and light. Significant progress has recently been achieved through micro- and nano-encapsulation to produce lipid emulsions and powders with improved oxidative stability. However, to date, it has been very difficult to completely inhibit the oxidative deterioration of PUFAs such as EPA and DHA that are abundant in fish oils. The commercialization of these PUFAs requires further improvements in the control of EPA and DHA oxidation.

The present review discusses the characteristics of fish oil oxidation and its prevention.

2 FREE RADICAL CHAIN REACTION
Although the direct oxidation of PUFAs via oxygen is spin forbidden, this spin barrier between PUFAs and oxygen can be readily overcome through a free-radical chain mechanism consisting of initiation, propagation and termination processes (Fig. 1). In the initiation process, the PUFAs substrate (LH) loses a hydrogen radical (H·) to form a lipid free radical (L·). This H· abstraction usually needs initiators; they are, in most cases, free radicals formed via the
decomposition of hydroperoxides (LOOH). The propagation stage begins with the addition of molecular oxygen to the L· to form peroxy radical (LOO·), which abstracts a hydrogen atom from another lipid to form a LOOH and a lipid free radical (L·). The latter reacts with molecular oxygen in a repetition of the first reaction. The initially formed LOOH may subsequently decompose to yield free radicals, such as alkoxyl (LO·) and hydroxyl (HO·) radicals after LOOH accumulation. These radicals serve as initiators for the above reactions. At the last stages of oxidation, non-radical products form via the reaction between LOO·, L·, and other free radicals (termination). However, the termination step is not generally important in food systems, because most foods are rancid before there is significant termination.

The abstraction of H· from LH is the first and rate-limiting step in lipid oxidation. This abstraction can occur at the bis-allylic positions (CH = CH-CH₂-CH = CH) of PUFA in the presence of initiators. Because the susceptibility of PUFA to oxidation depends on the availability of bis-allylic hydrogens, the oxidative stability of each PUFA is inversely proportional to the number of bis-allylic positions in the molecule. Therefore, when the relative oxidative stabilities of typical PUFAs are compared, DHA (22:6n-3) is most rapidly oxidized, followed by EPA (20:5n-3), AA (20:4n-6), LN (18:3n-3), and LA (18:2n-6).

LOOH is the first and a major oxidation product, and free radicals serve as initiators for the formation of LOOH, as described above. Therefore, the chain-breaking antioxidants are of considerable practical importance in preserving PUFA from oxidation. These antioxidants inhibit or retard LOOH formation from PUFA by readily donating hydrogen atoms to free radicals (Fig. 1). Phenolic compounds with bulky alkyl substituents such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), propyl gallate (PG), tert-butylhydroquinone (TBHQ), and tocopherols are effective chain-breaking antioxidants because they produce stable and relatively unreactive antioxidant radicals. On the other hand, during the development of oxidative rancidity, PUFA originates not directly from LOOH but from the formation of volatile secondary oxidation compounds that result from the decomposition of LOOH. In fish oil oxidation, LOOH has little effect on sensory properties because they are inherently unstable and rapidly degrade into volatile secondary oxidation products. As a result, fish oil sometimes tastes very rancid even at a low peroxide value (PV), which is an indicator of LOOH content. Jacobsen also conclusively showed that there was no correlation between the PV and the taste panel response to fish oil-enriched spreads. Therefore, it is practically more important to inhibit volatile formation to effectively prevent the oxidative deterioration of PUFA.

The mechanism for the formation of these secondary oxidation products such as volatile compounds is very complicated and varies with the PUFA substrate. Although a number of excellent studies have provided a general overview of PUFA oxidation and improved the current knowledge regarding PUFA oxidation kinetics, processes, mechanisms, and products, the prevention of volatile compounds formed during PUFA oxidation is still significantly challenging.
3 CHARACTERISTICS OF EPA AND DHA OXIDATION

It is true that any PUFA oxidation study should be based on the classical free-radical chain reaction. On the other hand, it should also be noted that basic research on the free radical chain mechanism has been performed by using simple lipid esters such as methyl esters of PUFA, mainly LA. LA is the best substrate to study the theoretical aspects of PUFA oxidation; however, the knowledge obtained through this approach is not always consistent with that obtained by studying other types of PUFAs and lipids. For example, the characteristics of the oxidation products from EPA and DHA are different from those obtained from LA. A considerable amount of secondary oxidation products including volatiles could be formed even from the early stage of oxidation of EPA and DHA, while a little amount of these products was detected in the first stage of LA oxidation. This observation is attributed to the much lower stability of EPA- and DHA-LOOH compared with that of LA-LOOH. Therefore, the development of fishy and metallic off-flavors can be often found in fish oils that are rich in EPA and DHA even at a very low oxidation level.

The volatiles are mainly formed through the decomposition of LOOH (Fig. 1). LOOH is not stable and is easily decomposed to form several radicals such as alkoxyl radical (LO·) and hydroxyl radical (HO·), which can serve as initiators of PUFA oxidation. Among them, LO· has been considered as the key radical to be a major intermediate for volatile formation (Fig. 1). The scission of the C-C bond on either side of the LO· forms other types of radical fragments with or without oxygen, which can produce aldehydes, saturated and unsaturated hydrocarbons, and alcohols. These secondary oxidation pathways become more complicated in the presence of oxygen and different kinds of free radicals such as HO· and H₂O₂.

EPA- and DHA-LOOH are more easily decomposed to form more free radicals than formed with other PUFAs such as LA and LN, which are abundant in vegetable oils. These radicals greatly accelerate EPA and DHA oxidation, complicate the reaction more than generally portrayed, and induce several branching reactions that amplify the propagation stage. In fact, the formation of some volatiles is often impossible to control or predict in the oxidation of EPA and DHA. The off-odors are formed at very early stages of oxidation, and many types of unexpected secondary oxidation compounds can be produced during the oxidation of these PUFAs. The rapid formation of volatile compounds is the most serious problem that limits the addition of fish oil to general food products; therefore, many attempts have been made to identify the volatile compounds responsible for flavor deterioration in fish oil oxidation. However, the sensory impact of individual compounds or combinations of volatile oxidation products has yet to be established in food systems that contain fish oil.

Many types of volatiles have been identified in fish oil that has been oxidized under different conditions. They are primarily hydrocarbons and aldehydes such as vinyl alcohols, alkenals, alkadienals, alkatrienals, and vinyl ketones. Among them, aldehydes and vinyl ketones are major contributors to the unpleasant fishy and rancid off-flavors because of their low threshold. Each volatile detected from oxidized fish oil may have some role in oxidative deterioration. However, most of the volatiles were obtained after a long time of oxidation and/or at high temperatures, while the most notable and practical problem is volatiles formed during the early stage of oxidation and at low oxidation levels.

Shibata et al. have observed the formation of higher quantities of acrolein (2-propenal) after only 4 hr of incubation of purified fish oil triacylglycerols (TAGs) at 50°C in the dark. Propanal was also detected at the early stage of oxidation, but other volatiles were small in quantity and were ignored. After an 8-40 hr incubation, 1-pentene-3-ol, 2-pentenal, 2-butenal, and pentane were formed in considerable amounts. The quantities of all of the volatile compounds except for acrolein increased with increasing incubation time, while acrolein quickly increased but then remained constant or decreased slightly. On the other hand, oxidation of vegetable oils that are rich in LA produced only small amounts of volatiles at the early stages of oxidation. Although volatiles could be detected after a long incubation, a little amount of acrolein was detected during the oxidation of these vegetable oils.

Acrolein could be detected in the oxidation of linseed and echium oil, which are rich in LN; however, the level was much lower than that found in fish oil that is rich in EPA and DHA. These results show that acrolein is more easily formed from the oxidation of oils containing higher n-3 PUFA, especially EPA and DHA. Although the formation mechanism of acrolein from EPA and DHA oxidation at room temperature has not been clarified yet, most likely, the acrolein might be quickly and continuously produced from EPA- and/or DHA-LOOH after propanal formation. Acrolein is known as an undesirable volatile aldehyde that shows toxicity and irritation odors with an odor threshold of 3.6 ppb. Therefore, more attention should be given to acrolein as a major contributor to flavor deterioration in fish oil oxidation.

4 GENERAL METHODS TO PREVENT FISH OIL OXIDATION

With a growing interest in the health benefits of EPA and DHA, much attention has been paid to the improvement of oxidative stability of fish oil, and technical innovations have rendered it easier to apply fish oil to supplements and functional foods. Now, fortification of commonly consumed food products with EPA and DHA has been con-
Antioxidants are essential to protect EPA and DHA from oxidative deterioration\(^1\)\(^2\). Because of consumer preferences for "natural" foods and their negative perceptions and safety concerns regarding "synthetic" foods, the food industry has been keenly interested in exploring more effective natural antioxidants\(^3\)\(^4\). Among them, tocopherols are the most well-known natural antioxidants that are used in foods and cosmetics. They can inhibit or retard PUFA oxidation via readily donating hydrogen atoms to free radicals. Some extracts from natural plants such as rosemary, oregano, tea, and others are also used as chain-breaking antioxidants. Furthermore, the combination of tocopherols and other antioxidants such as ascorbic acid derivatives and/or natural plant extracts is known to effectively retard the oxidation of EPA- and DHA-rich fish oil.

In addition to the essential concept of using effective antioxidants, the quality of fish oil is also an important factor to control oxidation after the application. Crude fish oils usually contain varying amounts of metals, oxidation products, and other substances. Most of them act as a promoter of lipid oxidation. Although a higher quality of fish oil can be obtained using recent technical progress, it is still very difficult or nearly impossible to eliminate traces of metals and oxidation products. Trace amounts of metals, such as iron and copper, have a marked accelerating effect on the oxidation of EPA- and DHA-rich fish oil. They catalyze the hemolytic EPA- and DHA-LOOH decomposition to form free radicals, which induce a radical-chain reaction as shown in Fig. 1. The rate of oxidation is proportional to the square root of the metal concentration. Therefore, it is sometimes necessary to inactivate these pro-oxidant metals using chelators.

Recently, oil-in-water emulsification and encapsulation has been of interest as oxidatively stable delivery systems for fish oil that is rich in EPA and DHA\(^1\)\(^2\)\(^3\)\(^4\). Fish oil can be protected against attack by free radicals, oxygen, and metal ions using a barrier in these systems. The barrier is composed of the interface of the emulsion and the encapsulating wall materials. In oil-in-water emulsions, the interface surrounding the oil droplets consist of surface-active substances such as emulsifiers together with oils and water. They can sometimes provide a protective barrier to the penetration and diffusion of metals or radicals that initiate lipid oxidation. Particularly, it is more protective to use emulsifiers with antioxidant properties such as a surface-active molecule conjugated with an antioxidant molecule\(^5\). In micro- and nanoencapsulation of fish oil, EPA and DHA can be protected by a wall material against the oxidation. The stability of the microencapsulated fish oil depends on how effectively the carrier matrix inhibits the penetration of free radicals and oxygen into the oil core from the outside\(^6\).

5 ANTIOXIDANT ACTIVITY OF PHOSPHOLIPIDS

The effective combination of phospholipids (PLs) and \(\alpha\)-tocopherol inhibits the oxidation of several marine oils that are rich in EPA and DHA\(^45\)\(^52\). On the other hand, PL alone promotes PUFA oxidation in most cases\(^51\)\(^53\). Relatively higher oxidative stability of EPA and DHA has also been reported in marine-animal tissue lipids compared with that of purified fish oils\(^49\)\(^50\). This increased stability is due to the presence of PL together with \(\alpha\)-tocopherol in the tissue lipids, while the purified fish oil contain little PL. The synergistic effect of PL with tocopherols could be confirmed through comparative study of the oxidation of fish oil with or without PL\(^54\)\(^55\)\(^56\). The antioxidant activity of PLs is only found in the presence of tocopherols; therefore, much attention has been focused on the antioxidant synergy between PLs and tocopherols\(^52\)\(^57\)\(^58\).

Although the details regarding the antioxidant mechanism of PL with tocopherols have not yet been elucidated, previous studies have demonstrated that the mechanism is derived from synergism between PL and tocopherols. Several researchers have demonstrated that the amine group of PL can act as a hydrogen or electron donor to regenerate and recycle the tocopheroxyl radical, which is intermediate to the parent phenol tocopherol\(^51\)\(^50\), while direct electron transfer between the PL and tocopherol is unlikely because the reduction potential of both compounds is similar. Other studies have reported that the primary amine group of PL could promote the conversion of \(\alpha\)-tocopherolquinone, an oxidation product of \(\alpha\)-tocopherol, to \(\alpha\)-tocopherol\(^7\)\(^60\). Furthermore, the surface activity property of PL may change the location of tocopherols to be more active\(^7\).

Another mechanism for the antioxidant activity of PL with tocopherols is the formation of Maillard reaction products during the oxidation of fish oil in the presence of \(\alpha\)-tocopherol\(^7\)\(^64\). Several studies have been conducted by a Spanish group on the antioxidant activity of Maillard reaction products in food systems\(^62\)\(^63\). They found that an amine group of PL can react with aldehydes that are formed during PUFA oxidation, and the reaction of the amine group of phosphatidylethanolamine occurred 10 times more readily than that of amino acids\(^64\)\(^66\). Maillard reaction products exhibit a scavenging effect on free radicals\(^57\)\(^60\) and synergistic effects on the antioxidant activity of tocopherols\(^7\)\(^61\), and they act as metal chelators\(^59\)\(^71\). The abovementioned...
Spanish group has demonstrated that the antioxidative activity of the Maillard reaction products was greatly increased with the addition of tocopherols. Bandarra et al. have also reported that the synergistic antioxidant activity between PL and \( \alpha \)-tocopherol found in sardine oil oxidation is possibly due to Maillard reaction products.

6 PREVENTION OF THE OXIDATIVE DETERIORATION OF FISH OIL BY USING A SPHINGOID BASE

EPA- and DHA-LOOH are very unstable, and they immediately decompose to form volatiles, which induces the formation of fishy and rancid odors, even at the very early stages of oxidation. Therefore, an effective approach to scavenge the volatile compounds may be necessary at the early stage of fish oil oxidation. Therefore, amines may be used as promising compounds to effectively prevent EPA- and DHA-oxidative deterioration.

As amine compounds are effective antioxidants for fish oil, many studies have focused on PLs. On the other hand, Shimajiri et al. compared the effect of other amine compounds on fish oil TAG oxidation including sphingolipids (SLs) and their backbone, a sphingoid base (SPG). It has been found that SPG had the highest antioxidant activity, followed by SPM and other amine-containing PLs and SLs such as phosphatidylcholine (PC), phosphatidylethanolamine, ceramides, and ganglioside. In contrast, all amine compounds exhibited little effect on TAG oxidation in the absence of \( \alpha \)-tocopherol. The higher activity of SPG and SPM could be partly explained by the higher number of amine groups per molecule for both compounds. However, it is difficult to explain the strong synergistic antioxidant activity of SPG with \( \alpha \)-tocopherol based on only the different number of amine groups.

A recent study has confirmed that the combination of SPG and \( \alpha \)-tocopherol could completely inhibit the formation of volatile compounds including acrolein and propanal during incubation of purified fish oil TAG for up to 380 hr at 50°C, whereas volatiles quickly increased at the first stage of oxidation upon addition of \( \alpha \)-tocopherol or SPG alone. The most likely mechanism for the antioxidant activity of SPG with \( \alpha \)-tocopherol is the formation of antioxidative Maillard reaction products between the amine group of SPG and the carbonyl group of aldehydes, mainly acrolein and propanal, which are formed at a very early stage of fish oil oxidation. In the presence of \( \alpha \)-tocopherol, SPG might effectively react with aldehydes, resulting in few aldehyde peaks detected on the chromatograms. However, abundant aldehydes were found with the addition of SPG without \( \alpha \)-tocopherol.

Several model experiments have suggested the quick reaction of SPG with aldehydes to form antioxidative Maillard reaction products in fish oil oxidation. In the presence of \( \alpha \)-tocopherol, SPG formed novel antioxidants with aldehydes that were formed during fish oil TAG oxidation; however, such antioxidants could not be found in nonoxidized tricaprylin that contained the same amount of \( \alpha \)-tocopherol and SPG as that found in the fish oil TAG. Therefore, only SPG could not produce any antioxidants to inhibit fish oil TAG oxidation.

The formation of antioxidative Maillard reaction compounds needs any oxidation products such as aldehydes. In fish oil TAG oxidation, acrolein is detected as a major oxidation product at a very early stage of oxidation. Acrolein and other aldehydes can react with SPG to form antioxidative Maillard reaction products, resulting in the removal of aldehydes that are responsible for flavor deterioration; this process occurs in synergy with \( \alpha \)-tocopherol. The extremely higher reaction rate of SPG with aldehydes, especially acrolein, compared with other amine compounds is due to the higher antioxidant activity of SPG.

7 UNEXPECTED OXIDATIVE STABILITY OF EPA AND DHA IN BIOLOGICAL SYSTEMS

Based on the common free-radical oxidation theory, EPA and DHA should be very quickly oxidized because of the higher number of bis-allylic hydrogens in the molecules. On the other hand, several experimental findings have demonstrated the exceptionally high oxidative stability of these PUFAs in biological systems and in some foods. In such systems, EPA and DHA are usually effectively protected against oxidative deterioration via different antioxidant mechanisms that involve the presence of free-radical scavengers, active-oxygen scavengers, metal binders, peroxide destroyers, and antioxidant enzymes. In addition, PUFAs can adopt a protective conformation against oxidative attack by free radicals and/or oxygen. If EPA and DHA can be more effectively protected by this conformation than LA, the oxidative stability of both PUFAs may become higher than that of LA.

Lipids are present with water in biological systems and in many food systems. In the presence of water, lipids tend to acquire an ordered arrangement to keep their hydrophobic portion away from the water molecules. These arrangement forms are known as micelles, as interface in an emulsion, and as lipid bilayers in liposomes and biological membrane, where each part of the lipid can interact with...
other biological and food components to form a physically protective conformation against oxidative attacks. Thus, evaluation of the oxidative stability of EPA and DHA in such heterogeneous systems is very important, because it may provide us a hint to develop novel methods to effectively protect EPA and DHA against oxidative deterioration. In this section, the characteristic oxidative stability of EPA and DHA in natural systems is discussed.

Animal experiments have shown that EPA and DHA ingestion did not increase the different parameters that indicated the lipid peroxide index in the tissues. Particularly, Kubo et al. reported that lipid peroxide levels in the brain and testis decreased when DHA was administered to animals, compared with the control. Human clinical studies have also reported no significant change in the lipid peroxidation or less oxidative stress following increased consumption of EPA and DHA. Araseki et al. demonstrated lesser degree of increase in the lipid peroxidation levels of human hepatoma cells that incorporated DHA compared with that in the cells that incorporated LA and arachidonic acid, when the cellular oxidation was promoted by the addition of H₂O₂. The exceptionally high oxidative stability of EPA and DHA are also found in some foods, especially marine foods.

In biological tissues and in most foods, lipids are present as an aqueous dispersion in complex, multicomponent, and heterogeneous systems with various other components. In such systems, there are important factors other than antioxidant systems to stabilize the lipids. They include the relative location of the lipid substrates, antioxidants, and pro-oxidants and the partitioning and affinity of these components to different aqueous phases. These factors are very important to fully understand the lipid oxidation in foods and in biological systems. Therefore, an effort has been undertaken to clarify the characteristic oxidative stability of EPA and DHA in such systems using model aqueous systems.

8 CHARACTERISTIC OXIDATIVE STABILITY OF EPA AND DHA IN AQUEOUS SYSTEMS

Miyashita et al. have found that DHA (22:6n-3) was the most stable against oxidation, followed by EPA (20:5n-3), AA (20:4n-6), LN (18:3n-3), and LA (18:2n-6) when they were dispersed as micelles using Tween 20. These relationships are the opposite of those found in the bulk phase or in organic solvents. The higher oxidative stability of EPA and DHA in micelles could be explained based on the physical and stereochemical characteristics of EPA and DHA molecules in micellar conformations. EPA and DHA form tightly coiled configurations in the micelles to protect the bis-allylic positions, especially the inner positions, from hydrogen abstractions. Furthermore, the oxidative stability of DHA was markedly increased by the addition of emulsifiers such as Tween 20 through the interaction between DHA and the emulsifier molecule.

Lipids are mainly dispersed as emulsions in aqueous food systems. The various components in food emulsions become distributed according to their polarity and surface activity between different phases, which include the lipid phase, water phase, and interfacial region (interface). In the interface composed of the lipid, emulsifier, and water, the emulsifier arranges itself with the lipid molecule so that the polar head-groups are located in the water phase, and the nonpolar tails are located in the oil phase. This structure is almost the same as that of micelles. Lipid oxidation in an emulsion usually initiates from PUF oxidations at the interface. Therefore, the characteristic oxidative stability of EPA and DHA in aqueous micelles is important to better understand the oxidation of these PUFAs in food emulsion systems. Several studies have reported the higher oxidative stability of DHA ethyl esters and acylglycerols in micro- and nanoemulsion systems. This stability is derived from the protective stereochemical structure of the interface against oxidative attack on the DHA molecule.

There is also a difference in the oxidative stability of EPA and DHA in the bulk phase and in the phospholipid bilayers that compose liposomes and cell membranes. Several papers have reported the high oxidative stability of DHA in liposomes as a model for biological membranes. In a study that used PC synthesized with LA, AA, or DHA at the sn-2 position, DHA-PC exhibited the highest oxidative stability in liposomes, followed by AA-PC and LA-PC, in that order; however, the opposite result was obtained for the oxidation of PC in the bulk phase and in organic solvents, such as chloroform and t-butyl alcohol. In another study that analyzed the oxidative stability of soybean and the salmon roe PC, the salmon roe PC exhibited a higher oxidative stability than the soybean PC; however, the major PUFAs in the salmon roe PC were EPA and DHA, while LA was the major PUF in the soybean PC.

The higher oxidative stability of EPA and DHA in liposomes could be derived from the protective conformation of the bis-allylic positions of these PUFAs against hydrogen abstraction. This type of structure has been suggested in a study that used DHA as the model membrane PUF. A molecular modeling approach and NMR analysis showed that DHA in diacylglycerol and in PC can form tight packing structures such as back-bended, helical and angle-iron conformations in aqueous systems. Molecular dynamics simulations also indicate a remarkable overlap of water molecules with the double-bond regions of the DHA chains in these packed conformations. The tight conformation of DHA molecules and the presence of water molecules near the DHA molecules are the major preventative mechanisms against hydrogen abstraction at the bis-allylic positions of DHA.
Steric hindrance toward hydrogen abstraction from DHA molecules in liposomes and in cellular membranes has been suggested via analysis of the composition of DHA-LOOH isomers (Fig. 2<sup>77,96</sup>). Because DHA has five bis-allylic methylene groups, there are several possible positions for hydrogen abstraction: carbon-6, carbon-9, carbon-12, carbon-15 and carbon-18 (Fig. 2<sup>a</sup>). Because oxygen can attack carbon at either end of the pentadienyl radical, the resulting LOOH isomers are mixtures with a LOOH substitution of carbon at the positions 4, 8, 7, 11, 10, 14, 13, 17, 16 and 20. In organic solvents, there is no steric hindrance against hydrogen abstraction; therefore, oxidation of DHA-PC in t-BuOH exhibited a similar amount of 4,8-, 7,11-, 10,14-, 13,17-, and 16,20-LOOH (Fig. 2<sup>b</sup>). In contrast, the characteristic distribution of LOOH isomers was found in liposomes and in cellular membranes. In the liposome oxidation, the distributions of LOOH derived from hydrogen abstraction at carbon-9 and those at carbon-15 were very low. Furthermore, the LOOH formed via the abstraction at carbon-9 and carbon-15 was not found. This uneven distribution of LOOH indicates steric protection against hydrogen abstraction at carbon-9 and carbon-15, and the oxygen attack favored the carboxyl terminal side of the pentadienyl radical.

**9 CONCLUSION**

Many attempts have been undertaken to stabilize fish oil against oxidation; however, rapid flavor deterioration can be observed at a very early stage of oxidation, even in the presence of antioxidants such as tocopherols. The major volatiles found in the early stage of fish oil oxidation are acrolein and propanal. Judging from the low threshold and high toxicity, acrolein formation is considered to be the biggest problem in the application of fish oil. For the effec-
tive protection of fish oil against oxidative flavor deterioration, considerable attention should be paid to amine compounds. Amine compounds can react with aldehydes that are formed during the very early stage of fish oil oxidation to form novel antioxidants. Through this amino-carbonyl reaction, volatile aldehydes responsible for flavor deterioration can also be scavenged. The amino-carbonyl reaction is commonly found in biological systems and in foods during processing and storage. Exceptionally high oxidative stability of EPA and DHA are sometimes observed in such systems due to the protective conformation EP

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