# Analysis of Headspace Volatile and Oxidized Volatile Compounds in DHA-enriched Fish Oil on Accelerated Oxidative Storage

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**ABSTRACT: Oxidative stability of docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) and volatile and oxidized volatile compounds in 2 types of DHA-enriched fish oil, triacylglycerol (TG) and ethyl ester (EE), were studied during storage at 80 °C with aeration. The rate of DHA autoxidation was higher than that of EPA. DHA in EE form was more susceptible to autoxidation than in TG form. Thirty-one volatile compounds were identified in EE and 23 volatile compounds in TG. (E)-2-pentenal, 2-(1-pentenyl) furan, and (E,E)-2,4-heptadienal were commonly detected as oxidized volatile compounds from TG and EE fish oil. These volatile oxidized compounds might be formed mainly from the oxidation of DHA and EPA, the main fatty acids of the oil.**

**Keywords: fish oil, triacylglycerol (TG), ethyl ester (EE), volatile oxidized compound, DHA, EPA**



## Introduction

Polyunsaturated fatty acids (PUFA) such as docosahexaenoic acid (DHA, 22:6ω-3) and eicosapentaenoic acid (EPA, 20:5ω-3) have attracted much attention for their beneficial health effects (Fischer 1989; Harris 1989; Harris and others 1990; Carlson 1994; Durrington and others 2001). DHA and EPA can prevent or ameliorate the effects of cardiovascular disorders such as arteriosclerosis and myocardial infarction (Leaf and Webber 1988; Sardesai 1992). A study on ω-3 polyunsaturated fatty acids has demonstrated that supplementation of ω-3 fatty acids during infancy doubled the membrane DHA concentration, improved visual activity, and decreased the accumulation of body fat (Carlson and others 1990; Jorgensen and others 2001). The ω-3 fatty acids are required for biochemical and functional growth of the normal central nervous system (Innis 1991). DHA is found at high levels in phospholipids of the brain and retina in animals, indicating its essential role in the development of optical nervous systems (Svennerholm 1968; Anderson 1970; Salem and others 2001).

Fish is an important dietary source of ω-3 fatty acids (Lands 1986; Passi and others 2002). The daily consumption of 30 g of fish was reported to have lowered the death rate associated with coronary heart diseases by 50% (Kromhout and others 1985). However, DHA and EPA in fish oil are more easily oxidized than the unsaturated fatty acids in vegetable oil (Miyashita and others 1982; Cho and others 1987). The off-flavors generated by oxidation have adversely affected acceptability and limited application of DHA in food products (Stansby 1971; Fujimoto 1989). To study the flavor change of fish oil, a number of investigations have been conducted using traditional extraction methods such as dynamic headspace purge-and-trap and steam distillation-solvent extraction methods (Crawford and others 1976; Hsieh and others 1989; Karahadian and Lindsay 1989). Karahadian and Lindsay (1989) used the dynamic headspace purge-and-trap method to extract the volatile compounds generated by the oxidation of fish oil. They reported that (E,Z)-2,6-nonadienal, (E)-2-hexenal, 1,5-octadien-3-one, (Z)-4-heptenal, (E,Z,Z)- and (E,E,Z)-2,4,7-decatrienal, hexanal, 2,4-heptadienal, and 2,4-decatrienal are the major volatile compounds associated with fishy flavor. In another study, using the dynamic headspace concentration procedure, (Z)-2-pentenal, (E)-2-hexenal, 2,4-heptadienal, 2,4-decadienal, and (E,Z,Z)- and (E,E,Z)-2,4,7-decatrienal were identified as the volatile compounds in menhaden fish oil produced from the oxidation of linolenic acid and other PUFAs (Hsieh and others 1989). (E,Z,Z)- and (E,E,Z)-2,4,7-decatrienal were also reported as fishy odor compounds derived from the autoxidation of oils containing ω-3 fatty acids (Bading 1973). (E)-2-hexenal, (Z)-4-heptenal, and 2,4-heptadienal were identified from tuna oil rich in DHA extracted by the steam distillation-solvent extraction method (Crawford and others 1976). Although widely used, dynamic headspace purge-and-trap and steam distillation-solvent extraction methods are expensive and time-consuming. They require additional concentration steps and may produce artifacts from thermal decomposition and reaction with solvents (Sugisawa 1981; Steffen and Pawliszyn 1996; Song and others 1997).

Solid-phase microextraction (SPME) was developed as an alternative sampling method to overcome these difficulties associated with the traditional flavor-extraction methods. SPME is simple to use, inexpensive, solvent-free, and relatively fast to conduct compared with dynamic headspace purge-and-trap and steam distillation-solvent extraction methods (Jia and others 1998; Ruiz and others 1998). The SPME can be inserted into the injector of the gas chromatograph to desorb and subsequently separate extracted volatile compounds (Jia and others 1998). This method has been applied to the flavor analysis of various foods and drinks, such as dry-cured ham, apple, tomato, strawberry, and fruit juice beverage (Song and others 1997, 1998; Ruiz and others 1998; Xiaogen and Peppard 1994).

Currently, DHA-enriched fish oil is commercially available in both triacylglycerol (TG) and ethyl ester (EE) form. EE oil is pro-

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duced by chemical transesterfication and concentration, whereas TG oil is produced by concentration only (ONC 2003). Although many studies have been conducted on oxidation of oil, no information is available on oxidative stability of DHA-enriched fish oil for food applications. Therefore, objectives of this study were to identify headspace volatile and oxidized volatile compounds in DHA-enriched fish and study degradation pathways to form oxidized volatile compounds on accelerated oxidative storage.

## Materials and Methods

### Materials

Two forms of fish oil, TG and EE, containing at least 48% (wt/wt) DHA, were obtained from Ocean Nutrition Canada Ltd. (Mulgrave, Nova Scotia, Canada) and stored at –80 °C until used. The fish oil was produced mainly from anchovy and sardines. SPME fibers with 65 μL polydimethylsiloxane/divinylbenzene, 50-mL serum-type vial, Teflon rubber septa, and aluminum caps were all purchased from Supelco Inc. (Bellefonte, Pa., U.S.A.). All chemicals including 14% boron trifluoride–methanol and heptadecanoic acid were purchased from Sigma Chemical Co. (St. Louis, Mo., U.S.A.).

### Sample treatment

Fish oil (20 g) was aliquoted in 50-mL serum-type reaction vials. The vials were sealed with Teflon rubber septa and aluminum caps. The vials containing the samples were incubated for up to 10 d in a gas chromatography oven at 80 °C with aeration at 20 mL/min. The vials were wrapped with aluminum foil to prevent exposure to light. A Teflon tube and needle were inserted into the sample vial through the septum. Air was blown through the Teflon tube. The high-performance liquid chromatography solvent filter was connected to the Teflon tube to distribute the air to the fish oil. Air escaped through the needle from the vial.

### Extraction of volatile compounds by solid-phase microextraction

The SPME method was optimized for best extraction efficiency. Extraction time (20 min, 40 min, and 60 min), extraction temperatures (40 °C, 60 °C, and 80 °C), and 3 different SPME fibers (polymethylsiloxane [PDMS; red fiber], polymethylsiloxane/carboxen [PDMS/carboxen; black fiber], and polymethylsiloxane/divinylbenzene [PDMS/DVB; blue fiber]) were tested for the optimal extraction condition. Volatile compounds were extracted using the optimized condition twice every 2 d during the 10-d storage. For the extraction, SPME fiber was inserted into a sample vial through the needle and then exposed to headspace (Figure 1).

### Analysis of volatile compounds on gas chromatography

Extracted volatile compounds were analyzed using gas chromatography (GC) in duplicate. A Hewlett-Packard (Palo Alto, Calif., U.S.A.) 6890 gas chromatograph equipped with a flame ionization detector (FID) was used for GC analysis. Extracted volatile compounds were desorbed by inserting the SPME fiber into the injector port (splitless mode, 220 °C) of a GC. The desorption time was 5 min. Before each use, SPME fibers were cleaned by preconditioning at 220 °C for 20 min. The desorbed volatile compounds were separated on a capillary column, HP-INNOWAX (30-m length, 0.32-mm inner dia, 0.2-μm film thickness). Nitrogen was used as the carrier gas at a flow rate of 1 mL/min. The oven temperature was initially set at 30 °C for 5 min, increased to 220 °C at a rate of 3 °C /min, and held at 220 °C for 20 min. Injector and FID temperatures were 220 °C and 250 °C, respectively.

### Identification of volatile compounds

Volatile compounds were identified using gas chromatography-mass spectrometry (GC-MS). Mass spectra were obtained with a Varian 3700 gas chromatograph interfaced to a VG 7070E high-resolution GC-MS (Varian, Palo Alto, Calif., U.S.A.) at an MS voltage of 70 eV. The mass range was 40 to 300 (m/z). Ion source and GC-MS interface temperatures were 250 °C and 200 °C, respectively. Column and oven conditions were identical to those of the separation procedures. Separated compounds were tentatively identified by comparing the mass spectral data with the reference spectra in a mass spectral library (Natl. Inst. for Standard Technology, Manchester, U.K.) as well as the retention indices with the reported values (Jennings and Shibamoto 1980; Kim and others 1994; Baek and Cadwallader 1996; Acree and Arn 1997; Cha and others 1998).

### Analysis of DHA content in fish oil

DHA content in fish oil was quantified by analyzing derivatized fatty acid methyl ester. The fatty acids were first derivatized to fatty acid methyl esters by using 14% boron trifluoride–methanol according to the method of Morrison and Smith (1964). An aliquot of 10 mg fish oil was put in a screw-cap test tube. One milliliter of 14% boron trifluoride–methanol was added to the 10-mg sample along with 100 μL of heptadecanoic acid (50 mg/mL hexane). Heptadecanoic acid was used as an internal standard. The test tubes were flushed with nitrogen and closed with the screw caps. The samples were heated in a heating block (110 °C) for 20 min. After heating, the test tubes were cooled in ice. One milliliter of distilled water and 2 mL hexane were added to the cooled test tubes. The test tubes were flushed with nitrogen, closed with the screw caps, and centrifuged for 5 min at 2000 rpm. After centrifugation, the upper phase, containing fatty acid methyl esters, was removed from the test tubes.

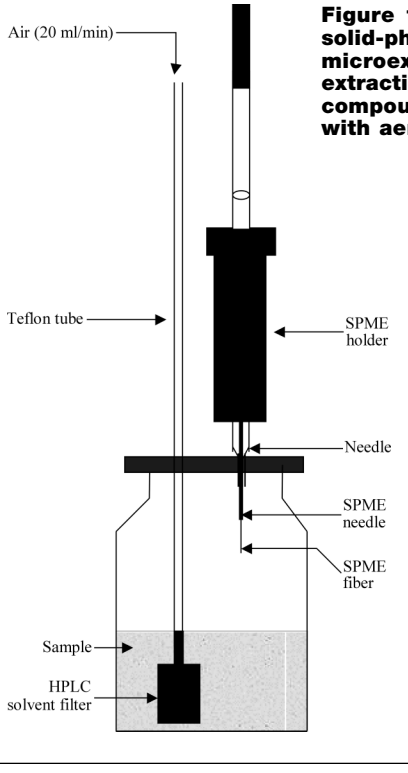


**Figure 1—Diagram for solid-phase microextraction (SPME) extraction of volatile compounds from fish oil with aeration**

The derivatized fatty acid methyl esters were injected into GC in splitless mode and separated on a capillary column, HP-INNOWAX (30-m length, 0.32-mm inner dia, 0.25-μm film thickness) in a Hewlett-Packard 6890 gas chromatograph. The injector and FID temperatures were set at 250 °C. The oven temperature was increased from 150 °C to 200 °C at a rate of 10 °C /min and then from 200 °C to 240 °C at a rate of 2 °C/min with the final holding time of 15 min. Nitrogen was used as the carrier gas and flow rate was 1.0 mL/min. The changes in DHA content of fish oil and headspace volatile compound generated were monitored by sampling daily.

## Results and Discussion

### The contents of DHA and EPA in TG and EE fish oil

The relative changes in DHA content of fish oil are shown Figure 2. During the 10-d accelerated storage with aeration at 20 mL/min, the relative contents of DHA in TG and EE forms decreased by 35.9% and 70.5% (wt/wt), respectively. Regarding the changes in EPA content, it was anticipated that they would be similar to those of DHA. However, the contents of EPA in TG and EE forms reduced only by 20.3% and 12.8% (wt/wt), respectively (Figure 2).

The higher rate of DHA autoxidation than that of EPA may be due to the more number of double bonds in DHA. DHA has 6 double bonds, whereas EPA has 5. Holman and Elmer (1947) reported that the relative rate of autoxidation of oleate:linoleate:linolenate was 1:40 to 50:100 with the order based on oxygen uptake, and that the increase in double bond in a fatty acid increased the oxidation rate of the fatty acid exponentially. Oxygen uptake of DHA was reported to be faster than oxygen uptake of EPA and linolenate. The relative rate of oxygen uptake of linolenate:EPA:DHA was reported as 1:5.2:8.5 (Cho and others 1987). We also postulate that there was competition for oxygen between DHA and EPA during the 10-d accelerated storage. In the fish oil, the content of DHA was initially higher than that of EPA. The initial DHA contents were 45.1% and 65.7% (wt/wt) in the TG and EE forms, respectively, whereas those of EPA in the TG and EE forms were 7.20% and 7.35% (wt/wt), respectively.

In general, EE fish oil is more susceptible to oxidation than TG. In this study, we also observed the faster degradation of EE oil than TG during the accelerated storage (Figure 2). It was reported that free fatty acid, particularly the carboxyl group, catalyzed the formation of free radicals by the decomposition of hydroperoxide causing a high oxidative rate (Miyashita and Takagi 1986; Yoshida and others 1992; Yoshida 1993; Aubourg 2001). Ethanol was detected at a high level in EE oil, even without storage. Free fatty acid and ethanol might be formed in the oil by the breakage of the ester bond in fatty acid or ethyl ester in EE fish oil. Free fatty acids formed in EE oil may have a catalytic effect on the formation of free radicals by the decomposition of hydroperoxide. Therefore, EE fish oil may be the more susceptible to oxidation than TG fish oil.

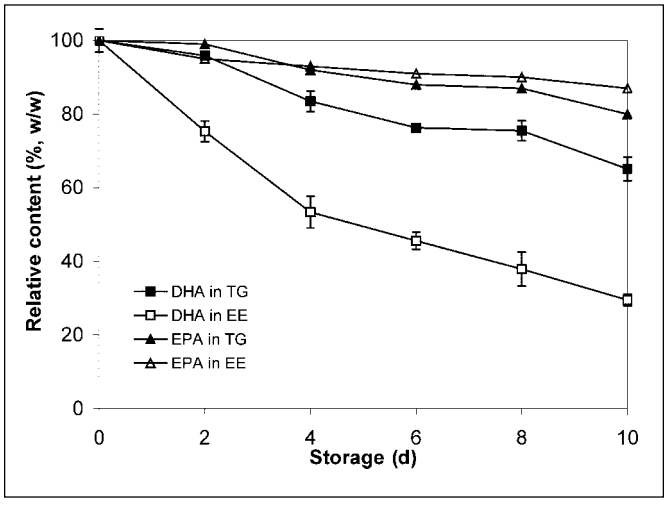


**Figure 2—Relative changes in docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) contents in fish oil (%, wt/wt) during the accelerated 10-d storage at 80 °C with 20 mL/min aeration. The expressed data are an average of 4 analyses. Standard deviation is shown as a bar.**

### Volatile compounds identified from fish oil

The optimal condition in which to extract the volatile compounds was 60 min at 80 °C using SPME fiber (PDMS/DVB, blue) based on the total peak area in electronic counts (Figure 3). On this condition, a total of 23 volatile flavor compounds in TG fish oil were identified by the GC-MS analysis and retention index (Table 1). More volatile compounds were isolated from EE than TG fish oil (31 compounds, Table 1). The total peak area in electronic counts representing total volatile compounds of EE fish oil was stronger than those from TG. The electronic counts of total volatile compounds from EE and TG was highest at Day 0 and Day 2, respectively, and decreased through the 10-d storage (Figures 4).

In the TG oil, acetic acid, (Z)-3-octen1-ol, (E,E)-2,4-heptadienal, (E,Z)-2,6-nonadienal, 4-octene, (E,Z)-3,6-nonadien-1-ol, and (Z,Z)-

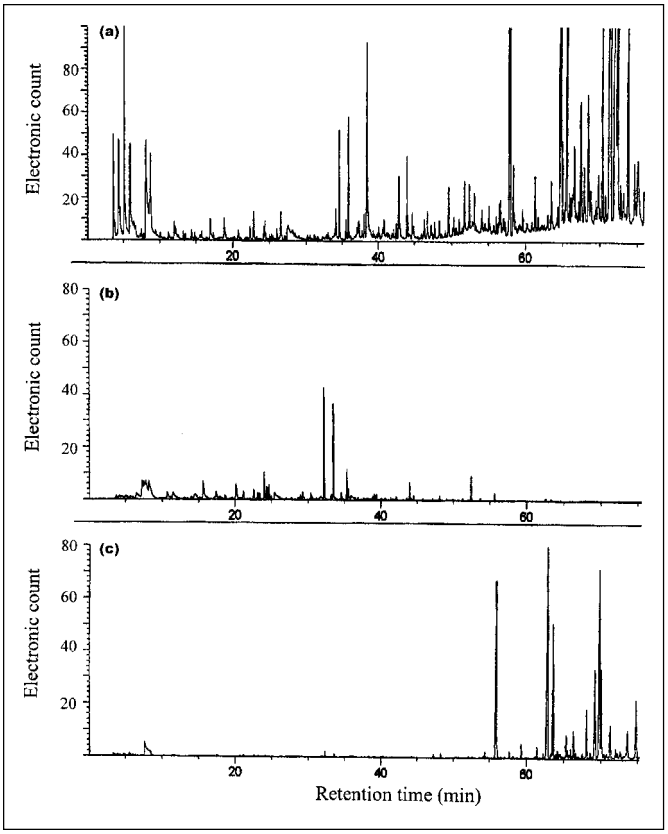


**Figure 3—Gas chromatograms of volatile compounds of ethyl ester (EE) fish oil extracted at 80 °C for 60 min by 3 different solid-phase microextraction (SPME) fibers: (a) polymethylsiloxane/divinylbenzene (PDMS/DVB, blue fiber), (b) polymethylsiloxane/carboxen (PDMS/carboxen, black fiber), and (c) polymethylsiloxane (PDMS, red fiber)**

**Table 1—Volatile compounds identified in docosahexaenoic acid (DHA)-enriched triacylglycerol (TG) and ethyl ester (EE) fish oil**

| Compounds[a] | Fish oil[b] TG | EE | Identification methods | Previous studies[c] (1)[d] | (2) | (3) | (4) | (5) | (6) |
|---|---|---|---|---|---|---|---|---|---|
| 2-Propenal | + | + | MS | | | | | | |
| Butadienal | | + | MS | | | | | | |
| (E)-2-Pentenal[e,f] | + | + | MS, RI | | + | + | + | | |
| (E,E)-2,4-Heptadienal[e,f,g] | + | + | MS, RI | + | | + | + | + | + |
| (E,Z)-2,6-Nonadienal[g] | + | | MS, RI | | + | | + | | |
| (E)-2-Decenal | | + | MS, RI | | | | | | |
| (Z)-9-Octadecenal | | + | MS | | | | | | |
| 1-Penten-3-one | + | | MS, RI | | + | + | + | | |
| 4-Hydroxy-hexanone | + | | MS | | | | | | |
| 2-Undecanone | | + | MS, RI | | | | | | |
| 2-Tridecanone | | + | MS | | | | | | |
| 4-Methyl-cyclohexanone | + | | MS | | | | | | |
| 2-Methyl-cyclohexanone | | + | MS | | | | | | |
| Ethanol[f] | | + | MS | | + | + | + | | |
| 1-Penten-3-ol[e,f] | + | + | MS, RI | | + | + | | + | + |
| (E)-2-Penten-1-ol[e,f] | + | | MS, RI | | + | + | | | |
| (Z)-3-Octen-1-ol | + | + | MS, RI | | | | | | |
| 2-Ethyl-3-hexen-1-ol | | + | MS | | | | | | |
| 2-Hepten-1-ol | + | | MS | | | | | | |
| 3-Noene-1-ol | | + | MS | | | | | | |
| 1,8-Nonadien-1-ol | + | | MS | | | | | | |
| (E,Z)-3,6-Nonadien-1-ol | + | | MS | | | | | | |
| 2,4-Decadien-1-ol | + | | MS | | | | | | |
| (Z,Z)-2,5-Pentadecadien-1-ol | + | + | MS | | | | | | |
| Hexadecanol | | + | MS | | | | | | |
| 2-Hexadecanol | | + | MS | | | | | | |
| 2-Methyl-1-hexadecanol | + | | MS | | | | | | |
| (Z,Z,Z)-9,12,15-Octadecatrien-1-ol | | + | MS | | | | | | |
| 6,9,12-Octadecatrien-1-ol | | + | MS | | | | | | |
| Acetic acid | + | + | MS, RI | | | | | | |
| Propanoic acid[e] | | + | MS, RI | + | | | + | | |
| 4-Hexenoic acid[e] | + | | MS, RI | + | | | | | |
| (E)-3-Hexenoic acid | | + | MS | | | | | | |
| 7-Oxo-octanoic acid | | + | MS | | | | | | |
| Decanoic acid, ethyl ester | | + | MS | | | | | | |
| 2-Furanpropanoic acid, ethyl ester | | + | MS | | | | | | |
| (E)-2-Heptenoic acid, ethyl ester | | + | MS | | | | | | |
| Undecanoic acid, ethyl ester | | + | MS | | | | | | |
| Dodecanoic acid, ethyl ester | | + | MS | | | | | | |
| Benzene | + | | MS, RI | | | | | | |
| 2-(1-Pentenyl) furan | + | + | MS, RI | | | + | | + | |
| (E)-2-Octene | | + | MS | | | | | | |
| 4-Octene | + | | MS | | | | | | |
| (E,E,E)-1,4,8-Dodecatriene | + | | MS | | | | | | |
| (Z,Z,Z)-4,6,9-Nonadecatriene | + | + | MS | | | | | | |

[a]Volatile compounds identified from fish oil in this study
[b]Volatile compounds identified in triacylglycerol (TG) and ethyl ester (EE) fish oil
[c]Volatile compounds identified in previous studies on flavor analysis of fish oil. (2) Karahadian and others (1989), (3) Horiuchi and others (1998), (4) Hsieh and others (1989), (5) Crawford and others (1976), and (6) Røbæk and Jensen (1997)
[d]Identified volatile oxidation compounds from docosahexaenoic acid (DHA), methyl ester (Noble and others 1971, 1975)
[e]Oxidized compound from DHA by authors
[f]Oxidized compound from eicosapentaenoic acid (EPA) by authors
[g]Major fishy flavor compound investigated by Karahadian and Lindsay (1989)

2,5-pentadien-1-ol showed a large change in peak areas in electronic counts during the 10-d accelerated storage with aeration (Table 2). These volatile compounds were also detected in the TG fish oil at Day 0 without storage (Table 2). Acetic acid, (Z)-3-octen1-ol, (E,E)-2,4-heptadienal, propanoic acid, 2-undecanone, (Z,Z,Z)-4,6,9-nonadecatriene, decanoic acid, ethyl ester, (E)-2-octene, dodecanoic acid, ethyl ester, (Z)-9- octadecenal, (Z,Z)-2,5-pentadien-1-ol, and 2 unidentified compounds showed great changes in the peak areas and were also detected in the EE oil during the storage (Table 3).

Unsaturated aldehydes, (E)-2-pentenal, and (E,E)-2,4-heptadienal were identified as volatile compounds from fish oil by Crawford and others (1976), Horiuchi and others (1998), and Hsieh and others (1989). Karshadian and Lindsay (1989) reported that (E,E)-2,4-heptadienal and (E,Z)-2,6-nonadienal were volatile compounds associated with general oxidized, painty, and green flavors in fish oil. Hartvigsen and others (2000) identified unsaturated aldehydes from fish oil–enriched mayonnaise and reported the odor characteristics of the aldehydes: (E)-2-pentenal as pungent, glue, green, and grassy; (E,E)-2,4-heptadienal as nasty, green, and fatty; (E,Z)-2,6-nonadienal as cucumber; and (E)-2-decenal as sweet and green. 2-Undecanone was also identified from fish oil–enriched mayonnaise, and its odor was characterized as sweet and fruity. 1-Penten-3-one was reported to be associated with pungent and rancid green odor. (E,Z)-2,6-Nonadienal and 1-penten-3-one have been known to be the major off-flavor characterized as fishy flavor from fish oil and fish meat (Karshadian and Lindsay 1989; Milo and Grosch 1995, 1996). 1-Penten-3-ol, (E)-2-penten-1-ol, and ethanol were identified from fish oil–enriched mayonnaise (Hartvigsen and other 2000). 1-Penten-3-ol and ethanol have butter and green odor characteristics, respectively (Acree and Arn 1997). (E)-2-Penten-1-ol is associated with green flavor (Hartvigsen and others 2000). The odor characteristic of 2-(1-pentenyl) furan was characterized as beany and grassy in soybean oil by organoleptic evaluation (Ho and others 1978). It was reported that volatile fatty acids with chain lengths of C2 to C6 gave intense sweaty odor (Hsieh and others 1989). Propanoic acid was identified as a short-chain volatile fatty acid with this flavor characteristic.

## Oxidized volatile compounds identified from fish oil

Of the 23 volatile compounds from TG fish oil, 6 were identified as oxidation products: 1-penten-3-ol, 2-(1-pentenyl) furan, (E)-2-

**Table 2—Changes in peak area of all major volatile compounds released from triacylglycerol (TG) fish oil during accelerated storage at 80 °C with 20 mL/min aeration**

| Compound | Storage time[a] 0 d | 2 d | 4 d | 6 d | 8 d | 10 d |
|---|---|---|---|---|---|---|
| 1-Penten-3-one | 13.0[b] | N.D.[c] | N.D. | N.D. | N.D. | N.D. |
| (E)-2-Pentenal | N.D. | 108 | 52.0 | 32.0 | 41.6 | 30.6 |
| 1-Penten-3-ol | 92.6 | 133 | 61.4 | 61.4 | 51.7 | N.D. |
| 2-(1-Pentenyl)· furan | N.D. | 47.8 | 24.5 | 13.9 | N.D. | N.D. |
| (E)-2-Penten-1-ol | N.D. | 27.7 | 29.1 | 19.2 | 14.5 | N.D. |
| Acetic acid | 17.3 | 233 | 174 | 112 | 144 | 118 |
| 4-Hexenoic acid | N.D. | 11.3 | 10.8 | N.D. | N.D. | N.D. |
| (Z)-3-Octen-1-ol | 47.5 | 378 | 218 | 139 | 92.4 | 63.0 |
| (E,E)-2,4-Heptadienal | 34.3 | 354 | 242 | 171 | 109 | 76.6 |
| (E,Z)-2,6-Nonadienal | 21.1 | 227 | 151 | 104 | 55.7 | 25.9 |
| (Z,Z,Z)-4,6,9-Nonadecatriene | 34.7 | 136 | 219 | 212 | 132 | 118 |
| 4-Octene | 12.3 | 714 | 1186 | 968 | 726 | 629 |
| (E,Z)-3,6-Nonadien-1-ol | 13.7 | 276 | 345 | 283 | 177 | 159 |
| (Z,Z)-2,5-Pentadien-1-ol | 43.8 | 477 | 635 | 506 | 346 | 298 |

[a]Headspace volatile compounds were monitored every 2 d during 10 d of accelerated storage at 80 °C with 20 mL/min aeration
[b]Peak area
[c]N.D. = not detected

**Table 3—Changes in peak area of all major volatile compounds released from ethyl ester (EE) fish oil during accelerated storage at 80 °C with 20 mL/min aeration**

| Compound | Storage time[a] 0 d | 2 d | 4 d | 6 d | 8 d | 10 d |
|---|---|---|---|---|---|---|
| Ethanol | 426[b] | 24.0 | 11.2 | 11.3 | 21.4 | N.D.[c] |
| (E)-2-Pentenal | 82.6 | 86.1 | 36.4 | 36.2 | 30.9 | N.D. |
| 1-Penten-3-ol | 9.27 | 13.4 | 6.28 | 6.57 | 4.87 | N.D. |
| 2-(1-Pentenyl) furan | 38.0 | 31.4 | N.D. | N.D. | N.D. | N.D. |
| Acetic acid | 91.9 | 391 | 169 | 172 | 175 | 91.9 |
| (Z)-3-Octen-1-ol | 260 | 186 | 95.4 | 36.0 | 38.3 | 18.1 |
| (E,E)-2,4-Heptadienal | 288 | 244 | 129 | 115 | 47.0 | 23.3 |
| Propanoic acid | 73.7 | 260 | 128 | 125 | 107 | 62.5 |
| 2-Undecanone | 719 | 942 | 539 | 466 | 195 | 116 |
| (E)-2-Decenal | 48.2 | 63.2 | 54.3 | 50.9 | N.D. | N.D. |
| (Z,Z,Z)-4,6,9-Nonadecatriene | 275 | 308 | 343 | 338 | 119 | 73.1 |
| Decanoic acid, ethyl ester | 202 | 221 | 245 | 245 | 98.4 | 59.8 |
| (E)-2-Octene | 94.9 | 1277 | 1391 | 1396 | 590 | 420 |
| Dodecanoic acid, ethyl ester | 195 | 311 | 335 | 276 | 102 | 84.0 |
| (Z)-9-Octadecenal | 114 | 333 | 529 | 544 | 213 | 176 |
| (Z,Z)-2,5-Pentadecadien-1-ol | 79.4 | 464 | 534 | 494 | 181 | 134 |
| Unidentified | 2380 | 1813 | 2399 | 2688 | 1164 | 1096 |
| Unidentified | 243 | 256 | 425 | 461 | 170 | 137 |

[a]Headspace volatile compounds were monitored every 2 d during 10 d of accelerated storage at 80 °C with 20 mL/min aeration
[b]Peak area
[c]N.D. = not detected

penten-1-ol, 4-hexenoic acid, (E,E)-2,4-heptadienal, and (E)-2-pentenal (Table 1). Similarly, the oxidized volatile compounds from EE fish oil were ethanol, 1-penten-3-ol, (E,E)-2,4-heptadienal, propanoic acid, 2-(1-pentenyl) furan, and (E)-2-pentenal (Table 1).

The intensity of oxidized volatile compounds from EE and TG fish oil was highest at Days 0 and 2 and decreased thereafter through the 10-d storage (Figure 5 and 6). Cho and others (1987) reported that DHA and EPA were oxidized faster after 3 to 4 d of storage at 5 °C. It was also reported that oxidation products were formed in the early stage of autoxidation (Cho and others 1987). The highest intensity of oxidized volatile compounds found on Days 0 to 2 may be explained by the formation of oxidized volatile compounds in the early stage of autoxidation and the rapid secondary reactions, which might have followed to degrade these intermediate compounds from the system. We also suspect that the open-air system used in this study also contributed to the removal of the oxidized volatile compounds, which could not be replaced because of the decreasing contents of intact fatty acid in the system over storage time.

Oxidized volatile compounds were more readily formed in EE than TG oil. Only 1 oxidized volatile compound, (E,E)-2,4-heptadi-

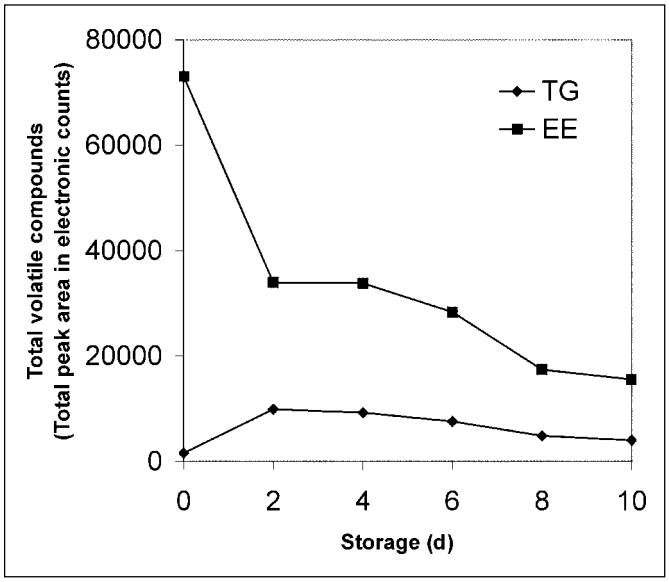


**Figure 4—Changes in total volatile compounds of triacylglycerol (TG) and ethyl ester (EE) fish oil enriched with docosahexaenoic acid (DHA) during the accelerated 10-d storage at 80 °C with 20 mL/min aeration**

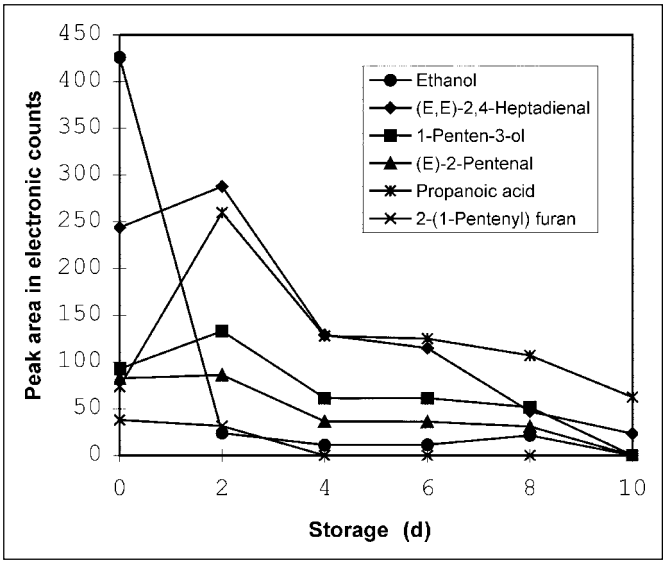


**Figure 5—Changes in peak area of oxidized volatile compounds released from ethyl ester (EE) fish oil during the accelerated 10-d storage at 80 °C with 20 mL/min aeration**

enal, was identified from TG oil at Day 0, whereas 5 oxidized volatile compounds, that is, (E)-2-pentenal, 1-penten-3-ol, 2-(1-pentenyl) furan, (E,E)-2,4-heptadienal, and propanoic acid, were detected in EE oil (Figure 5 and 6). This difference may reflect the difference in the degree of oxidation of the control groups rather than the effect of storage. It also may indirectly indicate the higher susceptibility of EE oil than TG to oxidation. After 2 d of storage, other oxidized volatile compounds, that is, (E)-2-pentenal, 1-penten-3-ol, 2-(1-pentenyl) furan, and (E)-2-penten-1-ol, were detected in TG oil (Figure 6) as more total volatile compounds were formed in EE than TG (Figure 4).

Noble and Nawar (1971, 1975) reported 2,4-heptadienal, 2-pentenyl furan, 4-hexenoic acid, and propanoic acid as oxidized volatile compounds from DHA methyl ester. Røbæk and Jensen (1997) identified 1-penten-3-ol and (E,E)-2,4-heptadienal as volatile compounds in oxidized fish oil. Lomano and Nawar (1987) reported that (E,E)-2,4-heptadienal and pentenyl furan were oxidized volatile compounds originating from linolenic acid by autoxidation. Callison and Min (2001) reported 2-(1-pentenyl) furan could be formed by linoleic acid. (E)-2-Pentenal was reported to be a volatile compound from thermally decomposed methyl linolenate hydroperoxide. Also, 1-penten-3-ol was reported to originate from soybean oil, which is rich in linoleic acid (Frankel 1985). However, oxidized volatile compounds identified from TG and EE fish oil in this study were considered to have mainly originated from DHA and EPA because the contents of linoleic acid and linolenic acid were very low in the sample (TG and EE). The contents of linoleic acid and linolenic acid in TG fish oil were 0.14% and 0.14% (wt/wt), and those in EE fish oil were 0.14% and 0.19% (wt/wt), respectively (Table 4).

**Table 4—Fatty acid compositions of triacylglycerol (TG) and ethyl ester (EE) fish oil enriched for docosahexaenoic acid (DHA)**

| | Fatty acid content[a] (%, wt/wt) | |
|---|---|---|
| Fatty acid | TG | EE |
| C14:0 | 0.12 ± 0.03 | 0.23 ± 0.11 |
| C16:0 | 0.37 ± 0.05 | 0.59 ± 0.28 |
| C16:1 | 0.88 ± 0.11 | 0.58 ± 0.32 |
| C18:0 | 0.32 ± 0.04 | 0.28 ± 0.07 |
| C18:1n-9 | 1.17 ± 0.06 | 1.12 ± 0.28 |
| C18:2n-6 | 0.14 ± 0.00 | 0.14 ± 0.05 |
| C18:3n-3 | 0.14 ± 0.05 | 0.19 ± 0.03 |
| C20:0 | 0.38 ± 0.02 | 0.28 ± 0.03 |
| C20:1n-9 | 1.80 ± 0.02 | 1.58 ± 0.07 |
| C20:2n-6 | 0.21 ± 0.01 | 0.32 ± 0.11 |
| C20:4n-6 | 0.45 ± 0.01 | 0.54 ± 0.03 |
| C20:5n-3 | 7.62 ± 0.00 | 8.54 ± 0.61 |
| C22:0 | 0.58 ± 0.00 | 0.49 ± 0.02 |
| C22:1n-9 | 12.50 ± 0.25 | 1.69 ± 0.04 |
| C22:4n-6 | 0.98 ± 0.01 | 0.98 ± 0.06 |
| C22:5n-3 | 8.76 ± 0.16 | 6.78 ± 0.16 |
| C22:6n-3 | 45.80 ± 0.46 | 58.50 ± 1.93 |
| C24:1 | 2.49 ± 0.08 | 2.96 ± 0.01 |
| Unidentified | 15.80 | 14.70 |

[a]Average ± standard deviation (n = 4).

## Mechanism of autoxidation to form oxidized volatile compounds

A variety of monohydroperoxide isomers (ROOH) can be formed by autoxidation. The homolysis of the hydroperoxide group yields an alkoxy (RO·) and a hydroxy radical (·OH). Volatile oxidation compounds are derived from β-scission of a carbon-carbon bond in alkoxy radical (RO·) (Grosch 1987). Figure 7 demonstrates the autoxidation mechanism to form (E,E)-2,4-heptadienal from 16-hydroperoxide (16-OOH) in DHA and 14-hydroperoxide (14-OOH) in EPA. Frankel (1982) reported the conversion of (Z,E)- to (E,E)-conjugated hydroperoxide in the autoxidation of linoleate. The authors postulated that (E,E)-conjugated hydroperoxide in DHA and EPA can be formed by the mechanism reported by Frankel (1982). Initial pentadienyl radical in DHA and EPA is converted to other conformations. Oxygen is added to this conformation to form a peroxy radical. This peroxy radical abstracts H· from RH to form (E,E)-conjugated hydroperoxide. The hydroperoxide undergoes loss of a hydroxy radical (·OH), β-scission of a carbon-carbon bond in alkoxy radical (RO·), and addition of ·H to generate (E,E)-2,4-heptadienal (Figure 7). By the scission of the C17-C18 bond of 17-O· in DHA and the C15-C16 bond of 15-O· in EPA, an alkenyl radical ($CH_3$-$CH_2$-CH = CH-$\dot{C}H_2$) can be formed. This alkenyl radical can undergo conversion to E-isomer postulated by Frankel (1982), and addition of the hydroxy radical (·OH) to form (E)-2-penten-1-ol. $CH_3$-$CH_2$-CH = CH-$\dot{C}H_2$ rearranges to $CH_3$-$CH_2$-$\dot{C}H$-CH = CH. By the addition of hydroxy radical (·OH) to the alkenyl radical ($CH_3$-$CH_2$-$\dot{C}H$-$CH_2$ = $CH_2$), 1-penten-3-ol is formed (Figure 8). 2-(1-Pentenyl) furan can be derived from 14-OOH in DHA. With the loss of the hydroxy radical (·OH), alkoxy radical (14-O·) is formed. This alkoxy radical undergoes cleavage of the C13-C14 bond to produce 3,6-nonadienal. This unsaturated aldehyde can be converted to a radical ($CH_3$-$CH_2$-CH = CH-$\dot{C}H$-CH = CH-$CH_2$-CHO) by thermal decomposition of peroxide or hydroperoxide (Frankel 1985). $CH_3$-$CH_2$-CH = CH-$\dot{C}H$- CH = CH-$CH_2$-CHO rearranges to $CH_3$-$CH_2$-$\dot{C}H$-CH = CH-CH = CH-$CH_2$-CHO. Frankel (1985) reported that alkoxy radical from hydroperoxide undergoes β-scission to generate an olefin and alkyl radicals and that these 2 radicals are converted to hydrocarbon by the addition of ·H. We postulate that addition of ·H to $CH_3$-$CH_2$-$\dot{C}H$-CH = CH-CH = CH-$CH_2$-CHO yields 3,5-nonadienal. Using the mechanism postulated by Ho and others (1978), 3,5-nonadienal can be converted to 2-(1-pentenyl) furan. Oxygen is added directly to 3,5-nonadienal to form a diradical. By the abstraction of 2H· from 2RH, hydroperoxide (5-OOH in 3,5-nonadienal) is formed.

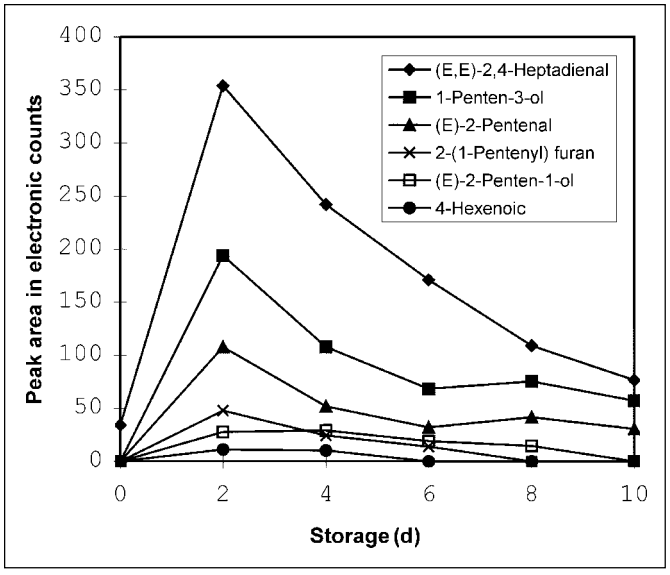


**Figure 6—Changes in peak area of oxidized volatile compounds released from triacylglycerol (TG) fish oil during the accelerated 10-d storage at 80 °C with 20 mL/min aeration**

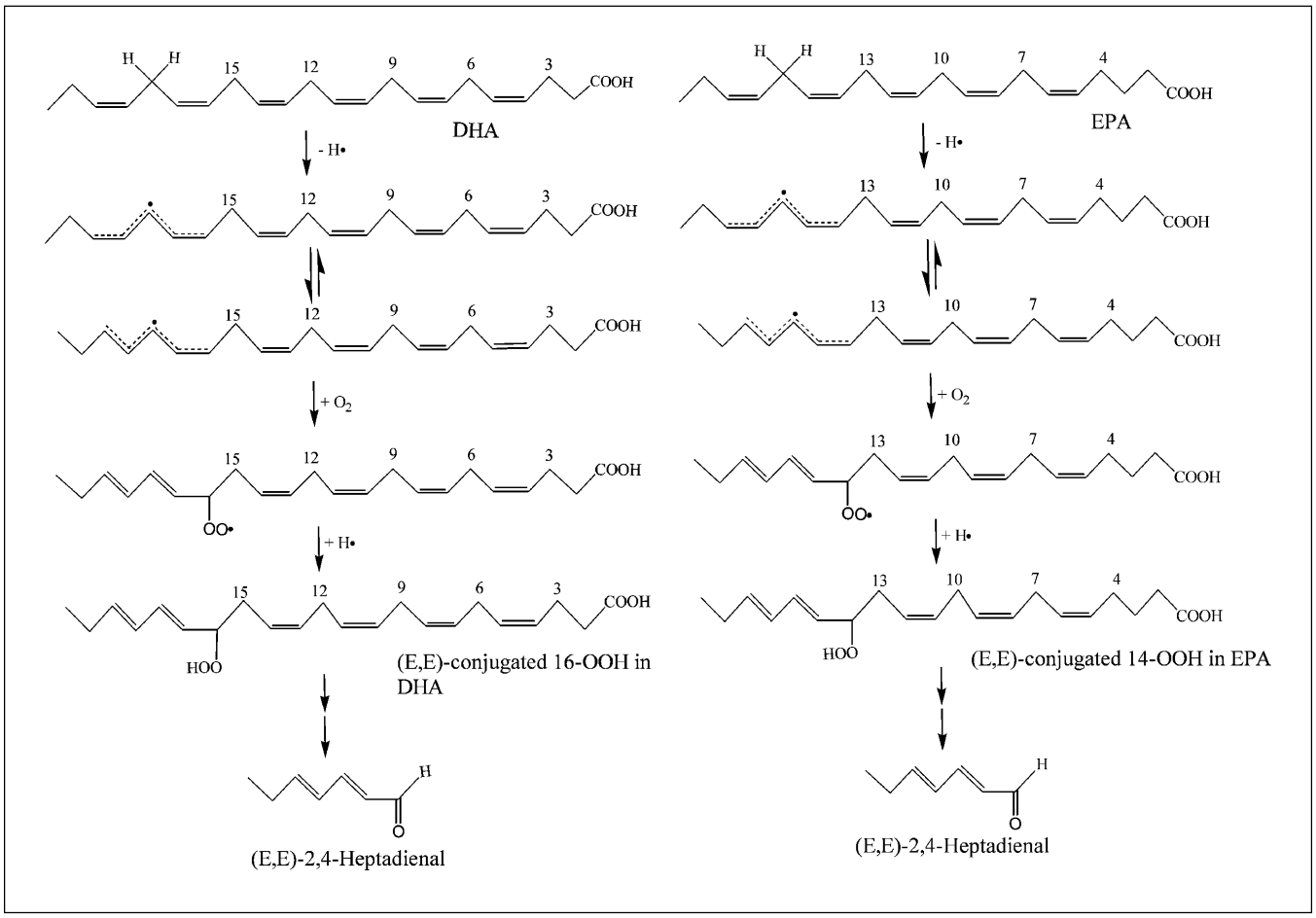


**Figure 7—The autoxidation mechanism to form (E,E)-2,4-heptadienal from 16-OOH in docosahexaenoic acid (DHA) and 14-OOH in eicosapentaenoic acid (EPA)**

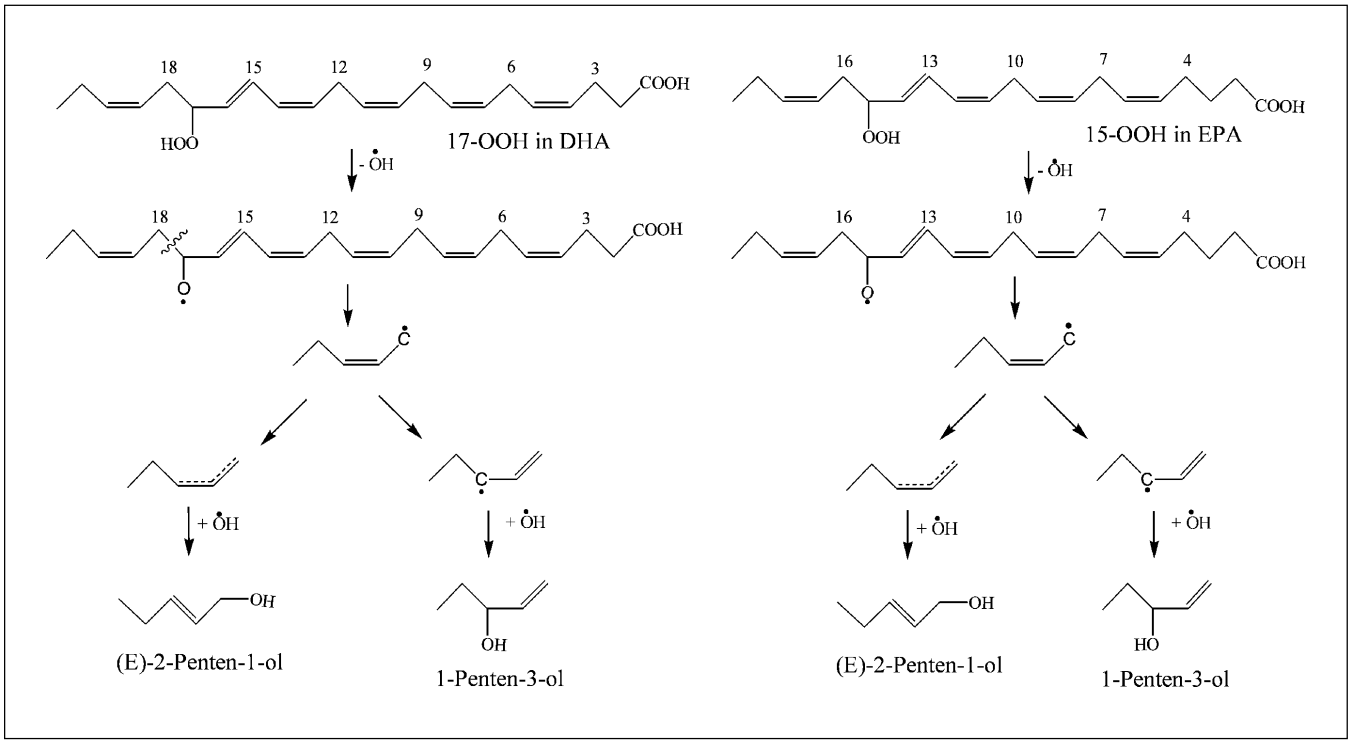


**Figure 8—The autoxidation mechanism to form (E)-2-penten-1-ol and 1-penten-3-ol from 17-OOH in docosahexaenoic acid (DHA) and 15-OOH in eicosapentaenoic acid (EPA).**

The hydroperoxide then undergoes loss of hydroxy radical (·OH) and abstraction of ·H to form 4-keto-non-5-enal. By enolization and dehydration of 4-keto-non-5-enal, 2-(1-pentenyl) furan is formed (Figure 9). Other oxidized volatile compounds also can be derived from hydroperoxide in DHA/EPA: 4-hexenoic acid from 7-OOH in DHA and propanoic acid from 4-OOH in DHA.

Chang and Kummerow (1953) and Johnson and others (1953) reported that polymeric compounds generated by the autoxidation of linoleate and linolenate undergo further oxidation to produce aldehydes and other volatile compounds. Decomposition oxidation products from hydroperoxide, such as unsaturated aldehydes and ketones, can undergo also the secondary oxidation to generate volatile compounds (Frankel 1982, 1985). Frankel (1962) postulated that 2-pentenal was derived from the secondary oxidation of 2,4,7-decatrienal and 3-hexenal. By loss of a hydroxy radical (·OH), β-scission of a carbon-carbon bond in alkoxy radical (RO·), 2,4,7-decatrienal is formed from 10-OOH in DHA and 8-OOH in EPA. This unsaturated aldehyde is converted to alkenyl radical by abstraction of H·. This alkenyl radical undergoes conversion to E-isomer, postulated by Frankel (1982), and addition of oxygen and ·H to form hydroperoxide in 2,4,7-decatrienal. By the autoxidation mechanism to generate volatile oxidation compounds from hydroperoxides in DHA and EPA, (E)-2-pentenal is formed from hydroperoxide in 2,4,7-decatrienal. 3-Hexenal can be derived from 10-OOH in DHA and 8-OOH in EPA.

By the same mechanism to form (E)-2-pentenal from 2,4,7-decatrienal, 3-hexenal decomposes to (E)-2-pentenal. Also, other decomposition oxidation compounds susceptible to secondary oxidation could be formed from hydroperoxides in DHA and EPA: 4,7,10,13-pentadecatetraenoic acid, 5,8,11,14-hexadecatetraenoic acid, 17-oxo-4,7,10,13,15-heptadecapentaenoic acid, 15-oxo-5,8,11,13-pentadecatetraenoic acid, 18-oxo-5,8,11,14,16-octadecapentaenoic acid, 2,4,7,10,13-hexadecapentaenal, 13-oxo-4,7,10-tridecatrienoic acid, 2,4,7,10,13,16,19-nonadecahexenal, 4,7-nonadienoic acid, 5-heptaenoic acid, 16-oxo-4,7,10,13-hexadecatetraenoic acid, and 14-oxo-5,8,11-tetradecatrienoic acid. We postulate that the oxidized volatile compounds identified in this study are derived from the polyunsaturated compounds by the autoxidation mechanism, resulting in oxidized volatile compounds from DHA and EPA. Polyunsaturated compounds can be converted to monohydroperoxide isomers. By homolysis of the hydroperoxide group followed by the β-scission of a carbon-carbon bond in alkoxy radical (RO·), volatile oxidation compounds are formed (Grosch 1987). 1-Penten-3-ol, 2-(1-pentenyl) furan, (E)-2-penten-1-ol, ethanol, and (E,E)-2,4-heptadienal can be derived from 2,4,7,10,13-hexadecapentaenal and 2,4,7,10,13,16,19-nonadecahexenal. From 4,7,10,13-pentadecatetraenoic acid, 17-oxo-4,7,10,13,15-heptadecapentaenoic acid, 13-oxo-4,7,10-tridecatrienoic acid, 4,7-nonadienoic acid, and 16-oxo-4,7,10,13-hexadecatetraenoic acid, 4-hexenoic acid and propanoic acid can be formed. 2-Propenal was not identified in other studies in the analysis of volatile compounds from oxidized DHA and fish oil (Noble and Nawar 1971, 1975; Crawford and others 1976; Hsieh and others 1989; Karshadian and Lindsay 1989; Røbæk and Jensen 1997; Horiuchi and others 1998). This aldehyde can be derived from 4,7,10,13-pentadecatetraenoic acid, 5,8,11,14-hexadecatetraenoic acid, 4,7-nonadienoic acid, and 5-heptaenoic acid. The unsaturated aldehydes whose chain lengths ranged from C6 to C10 were reported to originate from the oxidation of PUFAs (Hsieh and others 1989). In this study, (E,E)-2,4-heptadienal was identified as an oxidized volatile compound derived from the autoxidation of DHA and EPA. The odor characteristic of (E,E)-2,4-heptadienal has been described as having an oxidized, painty, nasty, green, and fatty flavor (Karshadian and Lindsay 1989; Hartvigsen and others 2000). Karshadian and Lindsay (1989) and Horiuchi and others (1998) analyzed 1-penten-3-ol and (E)-2-penten-1-ol as lipid oxidation products from fish oil. Ethanol and 2-(1-pentenyl) furan were identified from fish oil–enriched mayonnaise (Hartvigsen and others 2000). However, these 2 compounds and 4-hexenoic acid have not been reported in other studies on flavor analysis of fish oil (Crawford and others 1976; Hsieh and others 1989; Karshadian and Lindsay 1989; Røbæk and Jensen 1997; Horiuchi and others 1998). Ho and others (1978) described the odor characteristic of 2-(1-pentenyl) furan to be beany and grassy in soybean oil by sensory evaluation.

## Conclusions

DHA showed higher susceptibility to oxidation than EPA in both TG and EE fish oil enriched with DHA. EE oil was more susceptible to oxidation than TG as shown by the faster degrada-

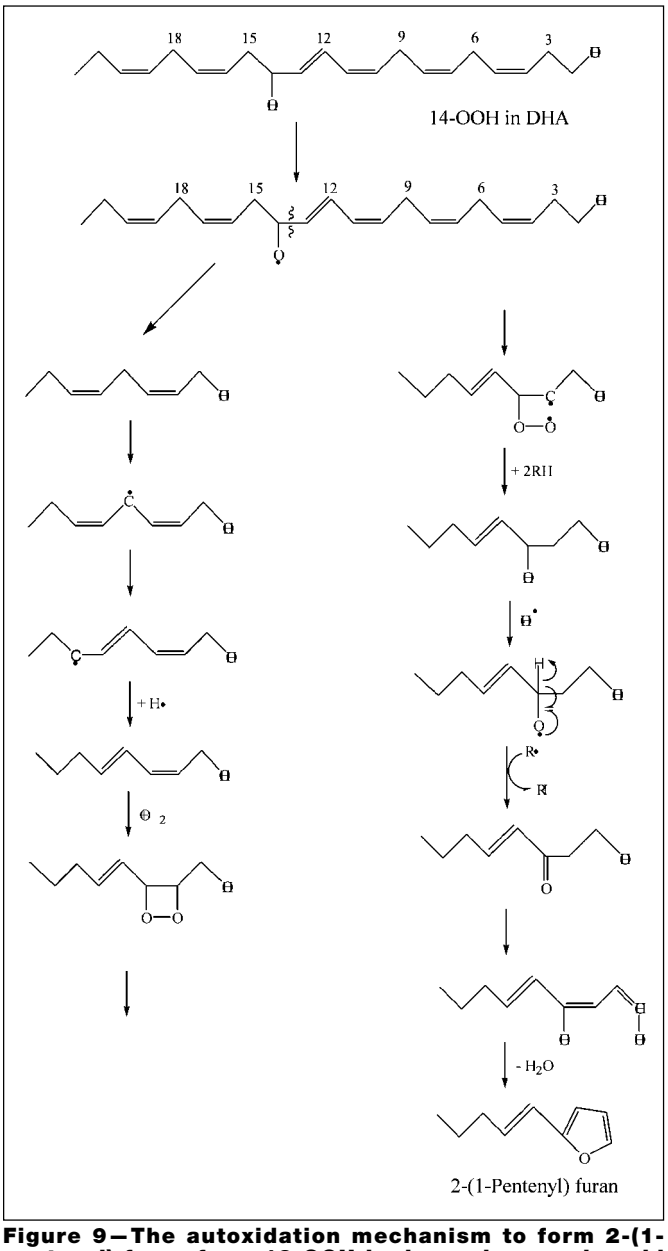


**Figure 9—The autoxidation mechanism to form 2-(1-pentenyl) furan from 13-OOH in docosahexaenoic acid (DHA).**

tion of fatty acids, formation of more volatile compounds, and higher intensity of the volatile compounds. From the proposed oxidation mechanisms, it was found that the oxidized volatile compounds were mainly generated from the oxidation of the 2 major fatty acids, DHA and EPA.

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