Acrolein as a Major Volatile in the Early Stages of Fish Oil TAG Oxidation

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Abstract: Fish oil rich in docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) is known to have an unpleasant smell, even at low oxidation levels. Therefore, it is highly important to know the major volatiles formed during the early stages of fish oil oxidation. Comparative study with solid-phase microextraction (SPME) and static headspace (SHS) methods showed that 2-propenal (acrolein) was formed as the major volatile from the beginning of fish oil triacylglycerol (TAG) oxidation. The effectiveness of SPME extraction on each volatile was different from each fiber. Among the three different SPME fibers used in the present study, carboxen/polydimethylsiloxane (CAR/PDMS) was determined to be a better fiber for measuring the volatiles, including acrolein. The present study also showed that the non-selective SHS method is useful for determining the characteristic volatile formation in the early stages of fish oil TAG oxidation.

Key words: fish oil oxidation, flavor deterioration, volatiles, acrolein, SPME, SHS

1 INTRODUCTION

Polyunsaturated fatty acids (PUFAs), containing more than two double bonds, are oxidized even at room temperature to form monohydroperoxides (LOOH) as a primary oxidation product. This oxidation proceeds through a free radical chain reaction, and the reaction begins with the abstraction of a hydrogen radical (H·) from the bis-allylic positions (CH = CH-CH₂-CH = CH) present in PUFA. The hydrogen abstraction forms lipid free radical (L·), which quickly reacts with molecular oxygen to form a lipid peroxyl radical (LOO·). LOO· abstracts an H· from another PUFA to form a LOOH and L·. Because the abstraction of an H· from PUFAs is the rate-limiting step of this reaction, the oxidative stability of PUFAs is inversely proportional to the number of bis-allylic positions on the molecule. Therefore, when the relative oxidative stabilities of typical PUFA are compared, docosahexaenoic acid (22:6n-3, DHA) is most rapidly oxidized followed by eicosapentaenoic acid (20:5n-3, EPA), arachidonic acid (20:4n-6), α-linolenic acid (18:3n-3), and linoleic acid (18:2n-6, LA).

The mechanism for lipid oxidation has been well-studied using simple lipid esters, such as methyl esters of PUFAs, mainly methyl linolate. Although any PUFA oxidation proceeds through a free radical chain reaction, and the primary oxidation product is LOOH, the characteristics of secondary oxidation product formation from EPA and DHA are very different from those from LA. When the oxidation products were compared between LA-, EPA-, and DHA-ethyl esters, a considerable amount of secondary oxidation products were formed even from the early stage of oxidation of EPA and DHA esters, while LOOH was detected mainly in LA oxidation. The development of fishy and metallic off-flavors can be often found in fish oils rich in EPA and DHA, even at very low oxidation levels, due to the extremely low stability of EPA- and DHA-OOH, which are easily decomposed into volatile oxidation products, even at low LOOH levels. This 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.

Volatile lipid oxidation products can be analyzed by gas chromatography (GC). The headspace technique with solid-phase microextraction (SPME) is a fast, sensitive, solventless and economical method for sample preparation before GC analysis. Therefore, the SPME method has mainly been used to measure volatile compounds in oxidized fish oils. However, a limitation of SPME has also been noted in the analysis of several volatile compounds. During SPME treatment, volatiles are pre-concentrated by absorption to the adsorbent of the SPME. Therefore, each volatile concentration is dependent on extraction conditions, such as adsorbent, sample volume, temperature,
salting-out effect and extraction or desorption time. Snyder et al. have demonstrated that lower-boiling compounds are lost during the purging cycle in the SPME procedure at high temperatures, whereas relatively higher-molecular-weight compounds, such as heptadecanil and decadecanal, can be concentrated in the trap. Iglesias et al. observed higher sensitivity for the carboxen/polydimethylsiloxane SPME adsorbent to detect volatiles from oxidized fish oil in emulsion, rather than polydimethylsiloxane/divinylbenzene and carbowax/divinylbenzene. These researchers also reported that a different volatile composition was observed with the use of different adsorbents.

Conversely, static headspace (SHS) GC analysis can measure the actual level of volatile compounds, as it is a non-selective method. The SHS technique is a simple, solventless, and economical method, as well as dynamic headspace technique, but SHS has lower sensitivity compared with other solventless methods. Despite SHS’s relatively low sensitivity, it has been used with satisfying results for differentiation between olive oils or detecting adulteration in virgin olive oils. Snyder et al. determined a higher proportion of low-molecular-weight volatiles, including propanal and acrolein, in highly oxidized soybean oil using the SHS method, but no or minimal acrolein was detected with the use of the SPME method.

The aim of this study was to compare SPME with different fibers and SHS in the analysis of volatiles formed in oxidized fish oil, specifically focusing on the early stages of oxidation.

2 EXPERIMENTAL PROCEDURES

2.1 Substrate lipids

Concentrated DHA oil (DHA-55) and concentrated EPA oil (EPA-28MN) were gifts from Maruha Nichiro Co., Tsukuba, Japan. Both oils were mixed in equal parts and used as fish oil. The oil was purified to remove impurities other than triacylglycerol (TAG) by active carbon-cellite and silica gel column chromatography. The fatty acid composition of the TAG was determined using GC after conversion of the fatty acid groups in the lipids to their methyl esters by transesterification using sodium methoxide (CH3ONa) as the catalyst. The GC analysis was performed on a Shimadzu GC-14B (Shimadzu Corporation, Kyoto, Japan) equipped with a flame-ionization detector and a capillary column (Omegawax-320; 30 m x 0.32 mm i.d.; Supelco, Bellefonte, PA). The major fatty acids in the fish oil TAG were DHA (27.80 ± 0.80 wt% of total fatty acids), EPA (15.70 ± 0.19 wt%), 16:0 (11.40 ± 0.48 wt%), 18:1n-9 (6.43 ± 0.03 wt%), and 14:0 (4.70 ± 0.35 wt%).

2.2 Analysis of substrate lipid purity

To confirm the removal of hydroperoxides, tocopherols, and other minor compounds in the above purification, high-performance liquid chromatography (HPLC) with fluorescence detector and thin layer chromatography (TLC) were performed. The hydroperoxide content in the TAG was determined using quantitative conversion of non-fluorescent diphenyl-1-pyrenylphosphine (DPPP) to fluorescent DPPP oxide by reaction with lipid hydroperoxides. Briefly, a small amount of TAG was weighed and dissolved in chloroform (containing 10 mg/mL butylhydroxytoluene (BHT))/methanol (2:1, v/v). To a test tube with a screw cap, 100 μL of the sample solution containing ca. 5 mg TAG and 50 μL of DPPP solution (1 mg/10 mL chloroform) were added and left for 60 min at 60°C in a water bath. Next, the solution was cooled in an ice bath, and 3 mL of 2-propanol was added. After the reaction mixture diluting to 100 times (v/v), 2 mL of the solution was injected to HPLC (Hitachi Seisakusho, Co., Tokyo, Japan). The HPLC analysis was performed at 40°C using a reversed-phase column (Develosil-ODS-UG-5, Nomura Chem. Co., Seto, Japan) protected with a guard column (10 x 4.0 mm i.d.) with the same stationary phase. The mobile phase was 1-butanol-methanol (10:90, v/v), and the flow rate was 1.0 mL/min. The fluorescence detector (Hitachi L-2485) was set at Ex. 352 nm and Em. 380 nm. The hydroperoxide concentration in the sample solution was calculated from the DPPP oxide detected using a DPPP oxide standard curve. The hydroperoxide content in the purified TAG was less than 0.1 μmol/mg sample.

HPLC for tocopherol analysis was also performed in the same conditions as described above, except for the column with a Hitachi HPLC system equipped with a pump (Hitachi L-2130) and a fluorescence detector (Hitachi L-2485). The analysis was conducted on a silica column (Si 60, 250 x 4.6 mm i.d.; Kanto Chem. Co., Tokyo, Japan) protected with a guard column (15 x 3.2 mm) with the same stationary phase. The mobile phase was n-hexane-2-propanol (99:2:0, v/v) with a flow rate of 1.0 mL/min. The fluorescence detector was set at Ex. 298 nm and Em. 325 nm. To check the lipid class purification of TAG, analytical TLC was performed on a 0.25 mm silica gel plate (Silica gel 60G; Merck, Darmstadt, Germany) developed with n-hexane-diethyl ether-acetic acid (70:30:1, v/v/v). Lipid spots were detected with iodine vapor or 60% aqueous sulfuric acid charring. Identification of the spot was performed using standard TAG (triolein). HPLC and TLC analysis showed the complete removal of tocopherols and minor lipid classes from the crude lipids.

2.3 Oxidation of TAG and volatile analysis

The TAG sample (300 mg) was placed in a 20 mL aluminum sealed vial with a butyl-gum septum (GL Science, Tokyo, Japan), and then incubated at 50°C or 15°C in the dark. Volatiles formed after incubation were extracted with the SPME and SHS methods. In the SPME method, the
Acrolein as a Major Volatile of Fish Oil Oxidation

J. Oleo Sci. 67, (5) 515-524 (2018)

fiber (Supelco, Bellafonte, PA, USA) was exposed to the headspace (HS) of the vial for 3 to 30 min at 50°C. The analysis was performed using three different fiber coatings: 85 μm carboxen/polydimethylsiloxane (CAR/PDMS), 50/30 μm divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS), and 65 μm polydimethylsiloxane/divinylbenzene (PDMS/DVB). Following the SPME extraction, volatiles were thermally desorbed from the fiber into GC. In the SHS method, the sealed sample vial was transferred into the HS-20 headspace auto-sampler (Shimadzu) of the GC apparatus. The same volume of HS gas in the vial was automatically pressurized at 60°C for 2 min and was immediately injected through a loop into the GC.

2.4 GC analysis

The GC analysis for the SPME method was performed on a Shimadzu GC-2010 equipped with a GCMS-QP2010 Ultra mass spectrometer (Shimadzu Co., Kyoto, Japan); while the Shimadzu GC-2014AFSC apparatus (Shimadzu Co.) was used for volatile analysis after SHS extraction. Both GCs were equipped with a flame-ionization detector (FID) and a capillary column (50-m length, 0.32 mm i.d. and 1.05-μm film thickness; Agilent Technologies, CA, USA). The GC oven temperature program was as follows: initial hold for 5 min at 40°C, followed by a 3°C/min ramp to 70°C, and then, 20°C/min ramp to 200°C, and finally, the temperature was held at 200°C for 4 min. Helium was the carrier gas at 50 kPa flow. Both the injection port and the flame ionization detector were set at 250°C. Three replicate measurements of each sample were performed, and the data were expressed as the mean ± SD (n = 3). The mass spectrometer was operated in the electron impact ionization mode (70 eV). The identification of the volatile compounds was performed by comparison with the mass spectra from the NIST Standard Reference Database and by injection of authentic standards. In addition to GC-MS analysis, authentic samples of major aldehydes were used for identification of the GC peak. Propanal and 2-propenal (acrolein) concentrations were determined in SHS method from peak areas using a standard curve made from authentic propanal and acrolein. Propanal and acrolein were obtained from Kanto Chem. Co. and Tokyo Chem. Ind. Co., Ltd, Tokyo, Japan, respectively.

3 RESULTS AND DISCUSSION

3.1 Effect of SPME fiber on volatile analysis

When volatiles formed in the fish oil TAG after 48 h incubation at 50°C, three SPME fibers were extracted: CAR/PDMS, DVB/CAR/PDMS, and PDMS/DVB. Many peaks were detected on the GC chromatogram (Fig. 1). However, each peak area (Fig. 2(A)) and the total peak area (Fig. 2(B)) were different among the different fiber coatings. CAR-PDMS showed a larger range of compounds with higher sensitivity than the other two fiber coatings. The higher sensitivity of CAR-PDMS compared to PDMS/DVB has also been reported in volatile analysis of oxidized fish oil in emulsion19 and in cooked turkey35. While the same volatile compounds are shown on each chromatogram (Fig. 1), the peak proportions were different among different fiber coatings (Fig. 2(C)).

In the sealed vials, each volatile is partitioned between the oil matrix, headspace (HS) phase, and the stationary phase of the SPME fiber. Volatiles distributed to the fiber phase were trapped by the fiber coating, pre-concentrated, and then desorbed inside the GC injector with a high temperature. Since GC and extraction conditions such as sample volume, temperature and extraction time were the same except for fiber coatings in the SPME analysis shown in Fig. 2, the effectiveness of the fiber in the pre-concentration of each volatile is mainly dependent on the type of

![Fig. 1 GC chromatogram of volatiles formed after incubation of fish oil TAG at 50°C for 48 h.](image-url)
fiber coating and its thickness (coating volume). In addition, relatively unstable volatiles may be decomposed during pre-concentration on the fiber. Therefore, differences in the major volatile compositions among the different SPME fibers shown in Fig. 2(C) can be attributed to the different absorption rate of each volatile on the fiber. In particular, a remarkable difference was found in acrolein (MW: 56.06), propanal (MW: 58.08), hexanal (MW: 100.16), 2,4-heptadienal (MW: 110.15), and 1-octen-3-ol (MW: 128.21) (Fig. 2(C)). Acrolein was found in the largest proportion (21.01 ± 0.47%) in CAR/PDMS fiber analysis, while the proportions in DVB/CAR/PDMS and PDMS/DVB analyses were 9.50 ± 0.46% and 2.23 ± 0.07%, respectively. CAR/PDMS analysis also showed a relatively high proportion (8.85 ± 0.65%) of propanal. However, the proportion was small in DVB/CAR/PDMS (3.75 ± 0.25%) and PDMS/DVB (1.17 ± 0.03%). On the other hand, PDMS/DVB analysis showed higher proportions of hexanal (7.49 ± 0.72%), 2,4-heptadienal (6.28 ± 0.48%), and 1-octen-3-ol (6.99 ± 0.79%), while these proportions in CAR/PDMS analysis were 4.47 ± 0.78%, 1.60 ± 0.31%, and 2.20 ± 0.36%, respectively. In this case, DVB/CAR/PDMS analysis showed intermediate proportions between PDMS/DVB and CAR/PDMS analysis. There were minimal differences in the proportions of 1-penten-3-ol and 2-pentenal among the different fibers.

Judging from the MMW of major volatiles and the results of each peak proportion (Fig. 2(C)), the CAR/PDMS fiber might have higher sensitivity to relatively lower-molecular-weight volatiles, such as acrolein and propanal, while the PDMS/DVB fiber might be effective at trapping relatively higher-molecular-weight volatiles, such as 2,4-heptadienal and 1-octen-3-ol. However, when each peak area is compared (Fig. 2(A)), minimal differences were found in 2,4-heptadienal and 1-octen-3-ol among the three SPME fibers, while the peak area of other major volatiles detected by the CAR/PDMS fiber was higher than those of the DVB/CAR/PDMS and PDMS/DVB analyses. The differences were especially remarkable for acrolein and propanal (Fig. 2(A)), although the exposure time and desorption time were the same. These differences in each peak proportion (Fig. 2(C)) and in each peak area (Fig. 2(A)) suggest a lower trapping capacity for PDMS/DVB, especially with relatively lower-molecular-weight volatiles such as acrolein and propanal. These volatiles might be unstable on PDMS/DVB fibers. The lower sensitivity of the PDMS/DVB fiber shown in Fig. 2(B) would be mainly due to the loss of these volatiles during pre-concentration. Therefore, among the three different fibers used in the present study, CAR/PDMS is a better fiber for measuring volatile formation in oxidized fish oil TAG.

Despite using the same fiber, the effectiveness of SPME extraction was affected by several factors, such as extraction time and temperature. Figure 3 shows the effect of extraction time on changes in major volatile proportion in fish oil TAG after incubation at 50°C for 28 hr. The volatile extraction was performed by exposing CAR/PDMS fibers to the HS phase of each vial for 1, 3, 5, 10, 20, and 30 min. Exposure time greatly influenced volatile proportion. With increasing exposure time, the proportion of acrolein and
Acrolein as a Major Volatile of Fish Oil Oxidation

J. Oleo Sci. 67, (5) 515-524 (2018)

Propanal decreased from 71.63 ± 0.48 to 33.52 ± 3.98 and from 4.56 ± 0.12 to 1.86 ± 0.12, respectively, but relatively less change was found in other major volatiles, such as 1-penten-3-ol (Fig. 3(A)). Other characteristic changes during the increase in exposure time were increases in the proportion of 2-pentenal, hexanal, 2,4-heptadienal, 1-octen-3-ol, and other volatiles (Fig. 3(B)). The relatively lower-molecular-weight compounds, such as acrolein and propanal, would be lost during the longer extraction time, whereas the relatively higher-molecular-weight compounds, such as 2-pentenal, hexanal, 2,4-heptadienal, and 1-octen-3-ol, would be concentrated.

3.2 Comparison of SPME and SHS

SPME is one of the most rapidly developing techniques for the extraction of HS volatiles. In SPME analysis, it is of paramount importance to choose the type of fiber according to the nature of the targeted compounds. For example, a polar fiber is better suited for analyzing polar compounds. By choosing an appropriate SPME fiber, researchers have reported short-chain saturated and unsaturated aldehydes and ketones with a low threshold as potent contributors to greasy, oily, green-grassy or green plant-like off-flavor odors formed during fish oil oxidation. Among the numerous carbonyl compounds identified in the oxidizing fish lipids, 1-penten-3-one and 2,4-heptadienal have been reported as the flavor deterioration indicators of oxidizing fish oil. Additionally, 4-heptenal, 2,4-heptadienal, 2,6-nonadienal, 2,4,7-decatrienal, 1-penten-3-one, 1-octen-3-one, and 1,5-octadien-3-one have also been demonstrated to be major off-flavor contributors. However, the most notable and key volatile compounds responsible for fish oil flavor deterioration have not been established to date.

The SPME method has many advantages in the rapid and sensitive analysis of volatiles. However, as this method is a selective sampling technique, its effectiveness at pre-concentration of each volatile is dependent on many factors, such as SPME fiber coatings, extraction time and temperature. Therefore, in SPME analysis, there is a possibility of obtaining higher or lower values than the true values for some volatiles. However, SHS analysis using a lower operating temperature as used in the present study is a non-selective method and can measure the actual

Fig. 3  Effect of trapping time by SPME fiber on peak ratio of major volatile peaks formed after the incubation of fish oil TAG at 50°C for 28 h. (A) Acrolein, propanal, 1-penten-3-ol, and others; (B) (E)-2-pentenal, hexanal, (E,E)-2,4-heptadienal, and 1-octen-3-ol. CAR/PDMS was used as the SPME fiber, and the fiber was exposed to the headspace for 1, 3, 5, 10, 20, and 30 min at 50°C. The data are expressed as the mean ± SD of three separate experiments.
level of volatile compounds. Figure 4 shows the proportion of major volatiles formed in fish oil TAG after 12 and 24 h incubation at 50°C. The analysis was performed using SPME and SHS methods. In the SPME method, volatiles were extracted by exposure to CAR/PDMS fiber for 20 min at 50°C. Both analyses have indicated acrolein as the most common major volatile formed in fish oil TAG oxidation. Both 1-Penten-3-ol and propanal were detected as major volatiles, but the effectiveness of propanal detection was lower in SPME than in SHS.

Propanal can be formed by the decomposition of LOOH of omega-3 PUFAs such as EPA and DHA, α-scission on the outer LOOH from EPA and DHA (18-OOH-EPA and 20-OOH-DHA, respectively) theoretically produces propanal\(^1\). Since these LOOH have been reported to show the largest contribution to the total amount of EPA-OOH and DHA-LOOH isomers\(^{36, 37}\), propanal has been used as an indicator of omega-3 PUFA deterioration\(^{38-41}\). Conversely, propanal level is not very high, and it is occasionally overlooked in the volatile analysis of oxidized fish oil\(^{8, 20, 21}\). In these studies, volatiles were analyzed by SPME. The low propanal level might be due to the low sensitivity of SPME. Therefore, to determine the key contributor to oxidative flavor deterioration in fish oil, SHS analysis will be useful despite its low sensitivity.

### 3.3 Acrolein as a major volatile from an early stage of fish oil TAG oxidation

Fish oil just after being refined by chromatographic separation has little to no flavor. The fish oil comes to have an unpleasant smell after leaving the chromatograph at room temperature for a very short time due to the high reactivity of EPA and DHA in fish oil and very low stability of the EPA- and DHA-LOOH. Therefore, the flavor deterioration of fish oil is still a problem even at low oxidation levels, and it is very important to know the major volatiles formed during early stages of fish oil oxidation. However, most of the volatiles reported to date were obtained after a long period of oxidation\(^{7, 8, 13, 14, 18, 20, 21, 38, 42, 43}\) and/or at high temperatures\(^{20, 25}\), while the most notable and practical problem of the volatiles formed during fish oil oxidation are those compounds formed during early stage and at lower temperatures.

We have previously demonstrated that acrolein would be a key volatile responsible for flavor deterioration in early stages of fish oil oxidation\(^{32}\). The study also showed that acrolein quickly increased during the first stage of TAG oxidation, but afterward, it either did not change or decreased slightly during fish oil TAG oxidation, while other volatiles, such as propanal, increased linearly. In the present study, SHS was applied to a much earlier stage of fish oil TAG oxidation by focusing on 0 h to 24 or 28 h incubation at 50°C. With this non-selective volatile extraction procedure, almost an equal amount of acrolein and propanal...
Acrolein as a Major Volatile of Fish Oil Oxidation

were found after only 4 h incubation, but other volatiles were small in quantity and could be ignored at this early stage of oxidation (Fig. 5). Another major volatile, 1-pentene-3-ol, appeared later. Although peak areas of these major volatiles increased with the progress of oxidation (Fig. 6(A)), the peak proportion of propanal decreased (Fig. 6(B)). The decrease in the peak proportion of propanal was also found in fish oil TAG oxidation at 15ºC (Fig. 7(B)). Due to the mild oxidation conditions at low temperatures, few volatiles were detected until 8 h incubation, and thereafter, acrolein, 1-pentene-3-ol, and propanal increased quickly (Fig. 7(A)). Propanal was detected as the most major volatile before 16 h incubation, and it subsequently decreased from 16 h to 20 h incubation, while acrolein drastically increased during this period.

Acrolein is well-known as a representative, undesirable volatile aldehyde with a low odor threshold. Moreover, acrolein has a harmful influence, and it is approximately 100 times more reactive than 4-hydroxy-2-nonenal, a well-known, toxic lipid oxidation compound. Concentrations of Acrolein and propanal during the early stage of fish oil oxidation was also analyzed by a different series of oxi-

Fig. 5 Representative GC of volatile compounds from oxidized fish oil TAG after 0, 4, 8, and 12 h incubation at 50ºC in a closed system.

Fig. 6 Formation of major volatiles during the oxidation of fish oil TAG at 50ºC in the dark. (A) Peak area of major volatiles; (B) peak proportion of major volatiles. Major volatiles: acrolein (open circle), 1-penten-3-ol (open triangle), and propanal (open square). The data are expressed as the mean ± SD of three separate experiments.
In this oxidation, fish oil TAG (600 mg) was placed in a 20 mL aluminum sealed vial with a butyl-gum septum (GL Science, Tokyo, Japan), and then incubated at 50°C in the dark for 8 h. Concentrations (ppm) of acrolein after 0, 4 and 8 h oxidation were 0, 4.92 ± 0.09, and 14.45 ± 0.38, respectively, while they were 6.02 ± 0.33, 13.25 ± 1.04, respectively, for propanal. Judging from the lower threshold value (3.6 ppb) of acrolein than that (60 ppb) of propanal, the acrolein formation would have the greater impact of flavor deterioration during the early stage of fish oil oxidation. Based on the low threshold and the strong toxicity, more attention should be given to acrolein formed during fish oil oxidation.

Several possible pathways have been demonstrated for acrolein formation: the enzymatic oxidation of spermine in biological systems, the oxidative homolytic fission of C-O bonds of glycerol after the hydrolysis of TAG during thermal oxidation in the presence of amine compounds, and cleavage of hydroperoxides of polyunsaturated fatty acids of thermally oxidized vegetable oils. However, the formation mechanism of acrolein from fish oil oxidation at room temperature has not yet been made clear. Prior studies have shown that acrolein was more easily formed from the oxidation of oils containing higher n-3 PUFA, especially EPA and DHA. Therefore, acrolein found in the present study would be primarily formed from the oxidation of EPA and DHA rich in fish oil TAG. By focusing on the changes in acrolein and propanal formed during the early stages of fish oil TAG oxidation, it is likely that acrolein might be quickly and continuously produced after propanal formation. In this pathway, DHA and EPA bonded to the TAG molecule are oxidized to produce 20-OOH-DHA and/or 18-OOH-EPA. These LOOHs are easily decomposed to form an alkoxyl radical (LO·) and a hydroxyl radical (HO·). The α-scission on the C-C bond of LO· results in a propanal and an alkyl radical (L·). The L· can successively react with HO· to produce acrolein (Shibata et al.). Usually, volatile compounds such as aldehydes are formed after lipid peroxide accumulation. However, acrolein and propanal were detected even in the first stage of fish oil TAG oxidation, while the lipid peroxide value (PV) was less than 30 meq/kg oil during the stage. This may show that the formation of acrolein and propanal by the hydroperoxides decomposition would be occurred at the same time with hydroperoxide production in fish oil TAG oxidation.

4 CONCLUSION

Acrolein has been demonstrated to be a major contributor to flavor deterioration in oxidized fish oil TAG. Recently, SPME has been most commonly used for volatile analysis of oxidized fish oil. However, the present study showed the loss of acrolein during the extraction of volatiles by SPME fibers. The effectiveness of acrolein extraction was dependent on each fiber, and CAR/PDMS was the best for the analysis.

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