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Formation of Acrolein in the Autoxidation of Triacylglycerols with Different Fatty Acid Compositions

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Abstract: Fish, echium, linseed, and soybean oil triacyglycerols (TAGs) were oxidized at 50 or 60°C to determine the effect of the polyunsaturated fatty acid composition on the volatile product formation. The analysis of the oxygen consumption and total volatile formation demonstrated that the soybean oil TAG had the highest oxidative stability followed by linseed, echium, and fish oil TAGs. Our results were in agreement with the expected average number of bis-allylic positions of each TAG. Higher quantities of acrolein (2-propenal) and propanal were detected using the static headspace gas chromatography method at the early stages of oxidation of echium and fish oil TAGs; however, a considerable amount of propanal and only a small amount of acrolein were found in the oxidized linseed oil TAG. The peak area ratio of acrolein to propanal were 0.115, 0.569, and 2.554 after the 8 hr oxidation of linseed, echium, and fish oil TAG, respectively, suggesting the preferential formation of acrolein, especially during the fish oil TAG oxidation. The acrolein quickly increased during the first stage of oxidation, but afterward, it either did not change or slightly decreased during the fish oil oxidation. Because fish oil induces flavor deterioration from the very early stage of the oxidation, the acrolein formation observed in the present study may be important for fish oil deterioration.

Keywords: fish oil oxidation · flavor deterioration · volatiles · acrolein · oxidative stability

Introduction

Eicosapentaenoic acid (EPA; 20:5n-3) and docosahexaenoic acid (DHA; 22:6n-3) are

typical n-3 polyunsaturated fatty acids (PUFAs) found in fish oils. These two long chain PUFAs have been demonstrated to cause significant biochemical and physiological changes in the body that primarily exhibit a positive influence on human nutrition and health [1-5]. However, the development of fishy and metallic off-flavors that are often found in fish oils rich in EPA and DHA dissuades people from consuming them. These undesirable flavors mainly come from the oxidation of EPA and DHA in the fish oils. The first step of the lipid oxidation is the abstraction of the bis-allylic hydrogen radical from the substrate PUFA to form a lipid-free radical. The radical then reacts with oxygen to form hydroperoxides. Thus, the level of oxidative deterioration in fish oil is commonly assessed by measuring the hydroperoxides. However, EPA- and DHA-hydroperoxides are very easily decomposed into volatile secondary oxidation products that are responsible for the undesirable flavors in fish oils. Thus, it is possible to have fish oils with very low hydroperoxide levels that also have an undesirable taste. The rapid formation of volatile aldehydes is the most serious challenge that limits the addition of fish oil to general food products.

The determination of secondary products, such as polymers, using the anisidine value, the 2-thiobarbituric acid value, sensory analysis, and gas chromatographic (GC) methods for volatile compounds is more important in evaluating the oxidative deterioration of fish oil that contains EPA and DHA. Of the methods, the best technique for determination of the volatile oxidation products is using GC. Volatile compounds that are suitable for the GC determinations are primarily aldehydes and hydrocarbons. Jacobsen [6] conclusively showed that there was no correlation between the peroxide value, a typical value used to evaluate the oxidative stability of lipids, and the taste panel response for fish oil-enriched spreads. However, the data on volatile compounds obtained by the headspace methods using GC was correlated well with the sensory data. Many types of volatile compounds that contain hydrocarbons, such as vinyl alcohols, alkenals, alkadienals, alkatrienals, and vinyl ketones, have been identified in fish oil oxidation [7-13]. However, the sensory impact of individual or combinations of volatile oxidation compounds in food systems containing fish oil has yet to be established. Hence, further research is needed to use this method to evaluate fish oil deterioration.

Because of their high threshold, hydrocarbons and vinyl alcohols will be relatively insignificant in undesirable fishy flavors. However, different carbonyl compounds that contain alkenals, alkadienals, alkatrienals, and vinyl ketones with a low threshold have been demonstrated to be potent odorants that are responsible for the flavors, but none of the separated volatiles imparted a fishy off-flavor [8,13]. Typical GC analyses that are used to detect the volatile compounds from oxidized lipids include the direct injection

method, the dynamic headspace method and the static head space method, respectively. Recently, the dynamic headspace method with a solid-phase microextraction (SPME) fiber has been the most used [14-18]. Snyder *et al*. [19] have compared the three GC methods for the analysis of volatile compounds from oxidized soybean oil. They found that the proportions of 2,4-decadienal and 2,4-heptadienal in the direct injection and dynamic headspace techniques were much higher than those found in the static headspace method. However, the static head space method produced larger proportions of 2-heptenal, pentane, and propanal. Additionally, more low molecular compounds were found using the static head space method, including acrolein (2-propenal), at the early stage of the oxidation. Acrolein is approximately 100 times more reactive than 4-hydroxy-2-nonenal, a well-known toxic lipid oxidation compound [20-22]. Acrolein has also been known to produce undesirable and irritating odors with an odor threshold of 3.6 ppb [23].

There have been several possible pathways for acrolein formation: the enzymatic oxidation of spermine in biological systems [24], the oxidative homolytic fission of C-O bonds of glycerol after the hydrolysis of triacylglycerol (TAG) during oxidation [10], and cleavage of hydroperoxides of polyunsaturated fatty acids [23]. Endo *et al*. [23] have demonstrated that the acrolein formation levels in thermally oxidized vegetable oils increased with an increasing α-linolenate content in the oils, but the linoleate content did not correlate with the acrolein formation. Hirayama *et al*. [25] found that more acrolein was formed during the oxidation of methyl α-linolenate than during the oxidation of methyl linoleate. These results suggest that the acrolein formation may have a huge impact on the oxidative deterioration of lipids that have a high level of unsaturated fatty acids, such as α-linolenic acid (18:3n-3), EPA (20:5n-3), and DHA (22:6n-3). Thus, we report the volatile compounds formed during the autoxidation of four types of TAGs with different fatty acid compositions, giving specific attention to the acrolein formation.

Methods

Standards and Substrate Lipids

The silica gel (BW-60F) for the column chromatography was purchased from Fuji Sylysia Chem. Ltd., Kasugai, Aichi, Japan. The activated carbon and Celite (545 RVS) were products of Nacalai Tesque Inc., Kyoto Japan. The soybean oil and linseed oil were purchased from Wako Pure Chemical Ind., Osaka and Summit Oil Mill Co. Ltd.,

Chiba, Japan, respectively. The echium oil and fish oil was kindly donated by De Wit Special Oil Co., The Netherlands. Two kinds of fish oil, DHA concentrated oil (DHA-55) and EPA concentrated oil (EPA-28MN) were gifts from Maruha Nichiro Co., Tsukuba, Japan. Both oils were mixed in equal parts and used as fish oil. Triolein (purity>99%) was obtained from Wako Pure Chemical Ind. All the other chemicals and solvents were of analytical grade.

Lipid Substrate Purification

Each oil (*ca*. 25 g) was passed through a column (50 cm x 4 cm i.d.) packed with a *n*-hexane slurry mixture of activated carbon (100 g) and Celite (100 g) to remove the tocopherols and pigments by eluting with *n*-hexane (1200 mL). The obtained oil (*ca*. 10 g) was refined using a silicic acid column (50 cm x 4 cm i.d.) packed with a *n*-hexane slurry of Silica gel BW-60F (200 g) by eluting with *n*-hexane (200 mL) and a mixture of *n*-hexane-diethyl ether (98:2 (200 mL) and 90:10 (1200 mL), v/v). The fraction eluted with the *n*-hexane-diethyl ether (90:10) was composed of TAG and showed no other impurities, such as free fatty acids, monoacylglycerols, or diacylglycerols, via thin-layer chromatographic analysis using a 0.25 mm silica gel plate (Silica gel 60G; Merck) developed with *n*-hexane/diethyl ether/acetic acid (70:30:1, v/v/v). The TAG spot was detected with iodine vapor or 60% aqueous sulfuric acid charring. The identification of the spot was performed using a standard TAG (triolein). The peroxide value of the purified TAG was less than 1.0 meq/kg as determined by the AOCS Official Method [26]. Few peroxides and tocopherols were also detected in the TAG via HPLC analysis [27]. The fatty acid composition of the TAG was determined using gas chromatography (GC) after conversion of the fatty acyl groups in the lipid to their methyl esters by transesterification using sodium methoxide (CH_3ONa) as the catalyst [27]. 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 detector, injector, and column temperatures were 260, 250, and 200°C, respectively. The carrier gas was helium, and it had a flow rate of 50 kPa. The fatty acid content was expressed as a weight percentage of the total fatty acids.

Oxidation and Analysis

Each 300 mg TAG sample 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 60°C in the dark. Before the incubation, the level of oxygen in the headspace gas of the vial was estimated using a GC (Shimadzu GC-14B) [28]. The GC was equipped with a thermal conductivity detector and a stainless steel column (3 m x 3.0 mm i.d.) packed with a molecular Sieve 5A (GL Science). The temperatures at the injection port, detector port and column oven were 120°C, 120°C and 70°C, respectively. The helium flow was 50 kPa. More than three separate vials containing similar samples were prepared and incubated. A small portion (20 μL) of the headspace gas was taken from each vial using a microsyringe through the butyl gum septum at selected times during the oxidation. The decrease (%) in the oxygen was calculated from the changes in the oxygen to nitrogen ratio compared with the ratio before incubation. Each data value at different oxidation times of the different samples was expressed as the mean±SD (n=3).

The oxidation of the sample was also monitored via GC analysis of the volatile compounds. A TAG sample of 300 mg was sealed in a 20 mL clean vial and incubated at 50°C or 60°C in the dark. For the static headspace GC analysis, after a definite time of incubation, the sample vial was transferred into the HS-20 headspace auto-sampler (Shimadzu Corporation) of the GC apparatus. The headspace gas in the vial was automatically pressurized at 60°C for 2 min and then immediately injected through a loop into the GC (Shimadzu GC-2014AFSC) equipped with a HP-1 capillary column (50-m length, 0.32 mm i.d. and 1.05-μm film thickness; Agilent Technologies, CA, USA) and a flame ionization detector. An initial oven temperature of 40°C for 5 min was used, followed by a rate of 3°C/min to 70°C and then by a rate of 20°C/min to 200 °C, and finally, the temperature was held at 200°C for 4 min. Both the injection port and the flame ionization detector were set at 250°C. Three replicate measurements of each stored sample were performed, and the data were expressed as the mean±SD (n=3).

GC-MS Analysis

The identities of the volatile compounds were obtained using solid phase micro-extraction (SPME) and gas chromatography-mass spectrometry (GC-MS). The volatiles were collected from the sealed vials containing different TAG samples after incubation using a 50/30 μm DVB/CAR/PDMS SPME fiber (Supelco, Bellafonte, PA, USA). The SPME fiber was exposed to the headspace for 5 min at room temperature under the same conditions. The absorbed volatiles were then desorbed in the injection port of the gas chromatograph GC-2010 equipped with a Model GCMS-QP2010 Ultra

mass spectrometer (Shimadzu Corporation). The GC conditions were the same as described above. 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. Acrolein peak was identified from parent ion (m/z 56) and other target ions (m/z 55 and 27). In addition to GC-MS analysis, authentic sample of acrolein was used for the identification of the GC peak. GC was carried out using two different columns, namely HP-1 and DB-35.

Results and Discussion

Comparison of the oxidative stability of different TAGs

According to the traditional oxidation mechanism, the rate-limiting step in the reaction is abstraction of the hydrogen atom that occurs at the bis-allylic position (CH=CH-$\underline{CH_2}$-CH=CH) of the PUFA; therefore, the oxidative stability of polyunsaturated lipids increase with a decreasing number of bis-allylic positions [29]. The average number of bis-allylic positions per each TAG molecule was obtained from the mol concentration of the PUFA composing the TAG and the number of bis-allylic position(s) of the PUFA. The mol concentration of the PUFA was computed using the weight % of the PUFA (Table 1) and the molecular weight of each PUFA. Thus, the average number of bis-allylic positions per molecule of soybean, linseed, echium, and fish oil TAGs was calculated to be 0.613, 1.103, 1.287, and 2.187, respectively.

When the oxidative stability of the four types of TAGs at 50°C was compared by measuring the decrease in the oxygen concentration (Fig. 1A), the oxidative stability was found to be the highest for the soybean oil TAG, followed by the linseed and echium oil TAGs, respectively. This order was in agreement with that expected from the average number of bis-allylic positions. However, there was little difference in the rate decrease of the echium oil TAG and the fish oil TAG, even though the average number (2.187) of positions in the fish oil TAG was much higher than that (1.287) of the echium oil TAG. The highest oxidative stability of the soybean oil TAG was also confirmed using GC volatile analysis (Fig. 1B and 1C). Although the total peak area of the volatiles increased with the incubation time for all the TAG samples, the soybean oil TAG required several incubation times to provide a rapid increase in the total volatiles.

Volatile Compound Analyses Using the Static Headspace Method

Volatile oxidation products are directly responsible for or serve as markers for the flavor deterioration in oxidized lipids. Specifically, in the oxidation of n-3 PUFA-containing lipids, such as fish oil, the off-odors are formed at the very early stages of oxidation. Because volatile oxidation products sometimes have a strong impact on the flavor at extremely low concentrations, often below 1 ppm, much research has been performed on the key volatile compounds that are responsible for the fish oil flavor deterioration.

Although early studies recognized that trimethylamine and compounds from oxidizing PUFA were involved in the production of the distinct fishy flavors in marine foods, a trimethylamine-like fishy odor was only apparent at a high concentration of trimethylamine in the fish oils [8]. The major contributors to off-flavors in fish oil are short-chain saturated and unsaturated aldehydes and ketones that have a greasy, oily, green grassy or green plant-like odor [7]. 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 [11]. Additionally, 4-heptenal, 2,4-heptadienal, 2,6-nonadienal, 2,4,7-decatrienal, 1-pentene-3-one, 1-octen-3-one, and 1,5-octadien-3-one have also been demonstrated as major off-flavor contributors [8,12,13].

Some of these aldehydes and ketones were also detected in the oxidations of four types of TAGs that are studied here. The GC analysis of oxidized soybean, linseed, echium, and fish oil TAGs at 60°C are shown in Fig. 2A, 2B, 2C, and 2D, respectively. Although the same volatile compounds are found on each chromatogram (Fig. 2), the GC profiles are different because of the different fatty acid composition of each TAG (Table 1). For example, in the soybean oil TAG, the volatile compounds were relatively small before 90 hr of incubation. After 198 hr of oxidation, pentane (3) was found in the largest quantity followed by hexanal (5), propanal (2), and acrolein, respectively. However, even after 8 hr of incubation, acrolein (1) and propanal (2) were found in the oxidations of echium and fish oil TAGs (Fig. 2C and 2D, respectively), while the acrolein (1) level was found to be low in the oxidation of linseed oil TAG (Fig. 2B**)**. The formation of acrolein and propanal was also found in the early stages of oxidation of the echium and the fish oil TAGs at 50°C (data not shown). The peak area ratios of acrolein to propanal were 0.115, 0.569, and 2.554 for the 8 hr oxidation of linseed, echium, and fish oil TAG, respectively (Fig. 2), suggesting the preferential formation of acrolein, especially during the fish oil TAG oxidation.

Acrolein is well-known as a representative undesirable volatile aldehyde found in frying oils [30]. It provides undesirable and irritating odors [23]. Moreover, acrolein has

a harmful influence with a LD50 (oral) of 82 mg/kg [30]. It may cause eye, nasal, and respiratory tract irritations, membrane damage, mitochondrial dysfunction, myelin disruption in the nervous system, and it may induce epithelial cell injury [20-22]. Although acrolein can be formed in frying oils and in autoxidized lipids [19-22], a little attention has been given to the formation of acrolein in autoxidized unsaturated lipids. Based on the strong impact of acrolein on flavor deterioration and toxicity, more attention should be given to acrolein formed in the lipid autoxidation because it is one of the key volatile compounds that can be used to predict the sensory quality of unsaturated lipids that are susceptible to oxidative degradation, such as fish oil.

Changes in the Quantities of the Volatile Compounds During the TAG Oxidations

The quantitative analysis of the major volatile compounds using the static headspace GC method is shown in Fig. 3 and Fig. 4. The amounts of the volatile compounds are expressed as peak intensities. All of the volatile compounds except for acrolein increased with increasing incubation time. Acrolein was the most abundant volatile in the fish oil TAG (Fig. 3D). It quickly increased, but then it stayed constant or slightly decreased. The other main volatiles detected in the fish oil oxidation were propanal and 1-pentene-3-ol (Fig. 3D). The relative concentration of 1-pentene-3-ol was also significant in the oxidized linseed (Fig. 3B) and echium (Fig. 3C) oil TAGs. Acrolein was detected at a similar level to that of the 1-pentene-3-ol peak in the linseed and echium oil TAGs. The major volatiles found in the oxidations of both TAGs were pentane and propanal. These volatiles may be produced by the decomposition of the monohydroperoxides from the n-3 PUFAs, such as 18:3n-3 and 18:4n-3 (Table 1) [19]. The oxidized soybean oil produced only small amounts of volatiles at the early stage of the oxidation, but after 100 hr of incubation, the pentane rapidly increased (Fig. 3A). Hexanal, an indicator of n-6 PUFA oxidation, steadily increased. Pentane and hexanal are known to form from the 13-monohydroperoxide of linoleate that is abundant in soybean oil TAGs (Table 1) [31].

Fig. 4 displays the increase in the other major volatiles produced during the oxidation of the four types of TAGs. Although these volatile compound levels were relatively lower than those shown in Fig. 3, the characteristic difference was found in the rate increase of each volatile between the different TAGs. During the oxidation of the soybean oil TAG (Fig. 4A), the rate increases of heptane, octane, and pentanal were relatively higher than the other volatiles. However, during the oxidations of the linseed (Fig. 4B), echium (Fig. 4C), and fish oil (Fig. 4D) TAGs, 2-pentenal and 2-butenal were

found in much larger relative quantities than the other volatiles.

The relative volatile composition changes according to the GC method used. A relatively higher proportion of the low molecular weight volatiles may be found using the static headspace method than those found by other GC volatile analysis methods, including the SPME method [19]. The low molecular weight volatiles, such as acrolein, propanal, pentane, 1-pentene-3-ol and 2-pentenal, can be found in the greatest proportion at a high vapor pressure during the equilibration of the headspace gas in the static headspace method. However, there is a limitation in the analysis of several characteristic compounds, such as 2,4-decadienal and 2,4-heptadienal that originated from the n-6 and n-3 PUFA hydroperoxides, when using the static headspace method [9]. By operating at relatively lower temperatures (as low as 60°C), the dynamic headspace method may measure the actual oxidation products present in the oils without thermal decomposition of the flavor precursors (17). Another advantage of this method is that it permits component enhancement to reveal the minor components that have a significant flavor. However, the lower-boiling compounds may be lost during the purging cycle in the SPME method, while other components, such as heptadienal and decadienal, can be concentrated in the trap. Thus, using the dynamic headspace method, the low molecular weight or low boiling volatile compounds, such as acrolein, propanal and pentane, are found in much less relative quantities than those found using the static headspace method operated at 60°C.

Many types of volatiles have been reported from the fish oil oxidation using different types of analytical methods [8,13-18]. Each volatile detected from the oxidized fish oil may have some responsible role for the oxidative deterioration; however, the most notable volatiles are those formed during an early stage of the oxidation. This is because fish oil induces oxidative deterioration from a very early stage of the oxidation. Acrolein that was found in the present experiments may play an important role in fish oil deterioration. As shown in Fig. 2B, 2C, and 2D, acrolein was detected in linseed, echium, and fish oil TAGs, respectively, at the early stages of oxidation. Specifically, the highest relative quantity of acrolein was found in fish oil TAG, followed by the echium and linseed oil TAGs, respectively. Therefore, acrolein may have a large impact on fish oil deterioration.

Prior studies have found higher levels of acrolein from the oxidation of TAGs that are more abundant in α-linolenate than in linoleate or oleate [23]. When different types of oils were oxidized at 60°C for 15 days and the volatiles were analyzed via the SPME method, the highest level of acrolein was found in the cod liver oil, followed by the fish, flaxseed, walnut, soybean, the low α-linolenic soybean, and the partially hydrogenated

vegetable oils, respectively [18]. Acrolein was also found in the oxidation of methyl oleate, linoleate, and α-linolenate at 100°C [25] and 180°C [23]. Among the oxidation, the level of acrolein was greatest in methyl linolenate. Relatively smaller amount of acrolein was also found from methyl linoleate oxidation, but not or a little from methyl oleate. As shown in Fig. 2, acrolein was formed with propanal from the very early stage of oxidations of fish, echium, and linseed oil TAGs. In addition, the peak area ratio of acrolein to propanal on the GC was the much highest in fish oil TAG, being followed by echium oil TAG and linseed oil TAG, respectively. Therefore, more acrolein could be produced in the lipid oxidation when the lipid contains more unsaturated fatty acids, such as EPA and DHA.

Most probably, acrolein found in the early stage of fish oil oxidation is formed during the decomposition of the oxidation products from EPA and DHA. Endo *et al*. [23] have suggested the formation of acrolein by the cleavage of dihydroperoxides and hydroperoxy epidioxides, since these secondary oxidation products are known to be more readily produced from PUFAs having more double bonds [32]. Although the formation mechanism of acrolein from EPA and DHA has not yet been made clear, it might be quickly and continuously produced after propanal formation as shown in Fig. 5. Fig. 4 showed the different behavior of acrolein formation as compared with those of other volatile compounds. Acrolein increased in an early stage of oxidation, but thereafter, gradually decreased, while other volatile compounds did not decrease during the experimental period. This may be due to the higher reaction activity of acrolein (33,34). Acrolein would be quickly oxidized and/or decomposed during the incubation.

Conclusions

The present study revealed the preferential formation of acrolein (2-propenal) at the very early stage of fish oil oxidation. Acrolein was most abundant volatile formed during the fish oil oxidation, while other compounds such as propanal, pentane, and hexanal were detected as major volatiles in echium, linseed, and soybean oil TAGs. Thus, acrolein may act as a key compound having a strong impact on flavor deterioration of fish oil, especially at early stage of the oxidation.

GC methods are the best technique for determination of the volatile oxidation products formed during the lipid oxidation. Among them a dynamic headspace method with SPME fiber is often used for this purpose. The dynamic headspace method has the advantage of using lower temperatures and permitting enhancement of trace compounds in complex mixtures of a wide range of volatile compounds. However, the

lower-boiling compounds such as acrolein may be lost during the purging cycle in the SPME method. The present study indicated that static headspace method will be better to detect acrolein. A judicious use of several GC techniques is necessary to evaluate volatiles during the analysis of volatiles from lipid oxidation precursors.

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Abbreviations

DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; GC, gas chromatography; GC-MS, gas chromatography-mass spectrometry; HPLC, high-performance liquid chromatography; PUFA, polyunsaturated fatty acid; SPM, sphingomyelin; TAG, triacylglycerol;

References

1. Kinsella JE (1986) Food components with potential therapeutic benefits: the n-3 polyunsaturated fatty acids of fish oils. Food Technol 40:89-146
2. Narayan B, Miyashita K, Hosokawa M (2006) Physiological effects of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA)-a review. Food Rev Inter 22:291-307
3. Allayee H, Roth N, Hodis HN (2009) Polyunsaturated fatty acids and cardiovascular disease: implications for nutrigenetics. J Nutrigenet Nutrigenomics 2:140-148
4. Russ, GL (2010) Dietary n-6 and n-3 polyunsaturated fatty acids: from biochemistry to clinical implications in cardiovascular prevention. Biochem Pharmacol 235:937-946
5. Taneja A, Singh H (2012) Challenges for the delivery of long-chain n-3 fatty acids in functional foods. Annu Rev Food Sci Technol 3:105-123
6. Jacobsen C (1989) Sensory impact of lipid oxidation in complex food systems, Fett/Lipid 101:484-492
7. Hsieh TCY, Williams SS, Vejaphan W, Meyers SP (1989) Characterization of volatile components of menhaden fish (*Brevoortia tyrannus*) oil. J Am Oil Chem Soc 66:114-117
8. Karahadian C, Lindsay RC (1989) Evaluation of compounds contributing characterizing fishy flavors in fish oils. J Am Oil Chem Soc 66:953-960
9. Frankel EN (1993) Formation of headspace volatiles by thermal decomposition of

oxidized fish oils *vs.* oxidized vegetable oils. J Am Oil Chem Soc 70:767-772
10. Horiuchi M, Umano K, Shibamoto T (1998) Analysis of volatile compounds formed from fish oil heated with cysteine and trimethylamine oxide. J Agric Food Chem 46:5232-5237
11. Aidos I, Jacobsen C, Jensen B, Luten JB, van der Padt A, Boom RM (2002) Volatile oxidation products formed in crude herring oil under accelerated oxidative conditions. Eur J Lipid Sci Technol 104:808-818
12. Venkateshwarlu G, Let MB, Meyer AS, Jacobsen C (2004) Chemical and olfactometric characterization of volatile flavor compounds in a fish oil enriched milk emulsion. J Agric Food Chem 52:311-317
13. Venkateshwarlu G, Let MB, Meyer AS, Jacobsen C (2004) Modeling the sensory impact of defined combinations of volatile lipid oxidation products on fishy and metallic off-flavors. J Agric Food Chem 52:1635-1641
14. Dehaut A, Himber C, Mulak V, Grard T, Krzewinski F, Le Fur B, Duflos G (2014) Evolution of volatile compounds and biogenic amines throughout the shelf life of marinated and salted anchovies (*Engraulis encrasicolus*). J Agric Food Chem 62:8014-8022
15. Sae-leaw T, Benjakul S (2014) Fatty acid composition, lipid oxidation, and fishy odour development in seabass (Lates calcarifer) skin during iced storage. Eur J Lipid Sci Tech 116:885-894
16. Sae-leaw T, Benjakul S (2014) Oxidative stability of lipids from hepatopancreas of Pacific white shrimp (Litopenaeus vannamei) as affected by essential oils incorporation. Eur J Lipid Sci Tech 116:987-995
17. Serra A, Buccioni A, Rodriguez-Estrada MT, Conte G, Cappucci A, Mele M (2014) Fatty acid composition, oxidation status and volatile organic compounds in "Colonnata" lard from Large White or Cinta Senese pigs as affected by curing time. Meat Sci 97:504-512
18. Gómez-Cortés P, Sacks GL, Brenna JT (2015) Quantitative analysis of volatiles in edible oils following accelerated oxidation using broad spectrum isotope standards. Food Chem 174:310-318
19. Snyder JM, Frankel EN, Selke E, Warner K (1988) Comparison of gas chromatographic methods for volatile lipid oxidation compounds in soybean oil. J Am Oil Chem Soc 65:1617-1620
20. Shibamoto T (2006) Analytical methods for trace levels of reactive carbonyl compounds formed in lipid peroxidation systems. J Pharm Biomed Anal 41:12-25
21. Singh M, Nam DT, Arseneault M, Ramassamy C (2010) Role of by-products of

lipid oxidation in Alzheimer's disease brain: a focus on acrolein. J Alzheimers Dis 21:741-756
22. Moghe A, Ghare S, Lamoreau B, Mohammad M, Barve S, McClain C, Joshi-Barve S (2015) Molecular mechanisms of acrolein toxicity: relevance to human disease. Toxicol Sci 143:242-255
23. Endo Y, Hayashi C, Yamanaka T, Takayose K, Yamaoka M, Tsuno T, Nakajima S (2013) Linolenic acid as the main source of acrolein formed during heating of vegetable oils. J Am Oil Chem Soc 90:959-964
24. Pegg AE (2013) Toxicity of polyamines and their metabolic products. Chem Res Toxicol, 26:1782-1800
25. Hirayama T, Yamaguchi M, Nakata T, Okumura M, Yamasaki T, Watanabe T, Fukui S (1989) Formation of acrolein by the autoxidation of unsaturated fatty acid methyl esters. Eisei Kagaku 35:303-306 (Japanese)
26. American Oil Chemists' Society (AOCS) (1998) Official methods and recommended practices. Cd 8-53. AOCS, Champaign
27. Shimajiri J, Shiota M, Hosokawa M, Miyashita K (2013) Synergistic antioxidant activity of milk sphingomyeline and its sphingoid base with α-tocopherol on fish oil triacylglycerol. J Agric Food Chem 61:7969-7975
28. Cho S-Y, Miyashita K, Miyazawa T, Fujimoto K, Kaneda T (1987) Autoxidation of ethyl eicosapentaenoate and docosahexaenoate. J Am Oil Chem Soc 64:876-879
29. Miyashita K (2014) Paradox of omega-3 PUFA oxidation. Eur J Lipid Sci Tech 116:1268-1279
30. Takeoka G, Perrino C, Buttery R (1996) Volatile constituents of used frying oils. J Agric Food Chem 44:654-660
31. Frankel EN, Neff WE, Selke E (1981) Analysis of autoxidized fats by gas chromatography-mass spectrometry: VII. volatile thermal decomposition products of pure hydroperoxides from autoxidized and photosensitized oxidized methyl oleate, linoleate and linolenate. Lipids 16:279-285
32. Porter NA, Caldwell SE, Mills KA (1995) Mechanisms of free radical oxidation of unsaturated lipids. Lipids 30:277-290
33. Jan F. Stevens JF, Maier CS (2008) Acrolein: sources, metabolism, and biomolecular interactions relevant to human health and disease. Mol Nutr Food Res 52:7-25.
34. LoPachin RM, Terrence Gavin T (2014) Molecular mechanisms of aldehyde toxicity: a chemical perspective. Chem Res Toxicol 27:1081-1091.

Figure legends

Figure 1. Oxidative stability of soybean oil TAG (open circle), linseed oil TAG (open square), echium oil TAG (open triangle), and fish oil TAG (open diamond). The stability was analyzed by measuring the decrease in oxygen (A) and the increase in total volatiles (B) and (C) in a head space gas of the vial. Oxidation was done at 50°C (A) and (B) or 60°C (C) in the dark. The data value was expressed as the mean±SD of three separate experiments.

Figure 2. Representative GC of volatile compounds from oxidized soybean (A), linseed (B), echium (C), and fish (D) oil TAGs after 8, 16, 90, and 198 hr incubation at 60°C. Volatile compounds from the oxidized TAG was analyzed by static headspace GC method and identified by GC-MS method. Major volatiles: (1), acrolein; (2), propanal; (3), pentane; (4), 1-penten-3-ol; (5), hexanal.

Figure 3. Increase in major volatile compounds during the oxidation of soybean (A), linseed (B), echium (C), and fish (D) oil TAGs during the incubation at 60°C in the dark. Major volatiles: acrolein (open circle;), propanal (open triangle), pentane (open square), 1-penten-3-ol (open diamond), and hexanal (solid circle). The data value was expressed as the mean±SD of three separate experiments.

Figure 4. Increase in other major volatile compounds during the oxidation of soybean (A), linseed (B), echium (C), and fish (D) oil TAGs during the incubation at 60°C in the dark. Major volatiles: 2-butenal (open circle), pentanal (solid square), 2-pentenal (open triangle), heptane (open diamond), octane (solid circle, 2-heptenal (open square), and 2-octenal (solid triangle). The data value was expressed as the mean±SD of three separate experiments.

Figure 5. Proposed mechanism for the formation of acrolein from EPA and DHA.

Table 1. Composition (weight %) of Major Fatty Acids of Substrate TAG

Fatty acid	Soybean	Linseed	Echium	Fish
14:0	0.06±0.00	0.04±0.00	0.03±0.00	4.70±0.35
16:0	10.41±0.09	5.65±0.14	7.65±0.31	11.40±0.48
18:0	3.90±0.03	3.61±0.03	3.61±0.09	3.27±0.02
16:1n-7	0.10±0.00	0.08±0.00	0.15±0.00	3.51±0.16
18:1n-9	26.07±0.07	25.67±0.13	17.25±0.34	6.43±0.03
18:1n-7	1.41±0.01	0.84±0.03	0.59±0.01	1.79±0.02
20:1n-9	0.27±0.00	0.19±0.01	0.93±0.07	1.62±0.05
18:2n-6	51.41±0.01	16.92±0.02	16.15±0.18	0.54±0.00
18:3n-6	-	-	11.58±0.20	0.35±0.00
18:3n-3	4.58±0.01	43.92±0.13	29.28±0.11	0.24±0.00
18:4n-3	-	-	11.35±0.29	1.76±0.03
20:4n-6	-	-	-	2.67±0.05
20:5n-3	-	-	-	15.70±0.19
22:5n-3	-	-	-	2.90±0.09
22:6n-3	-	-	-	27.80±0.80
24:1n-9	-	-	-	0.55±0.02

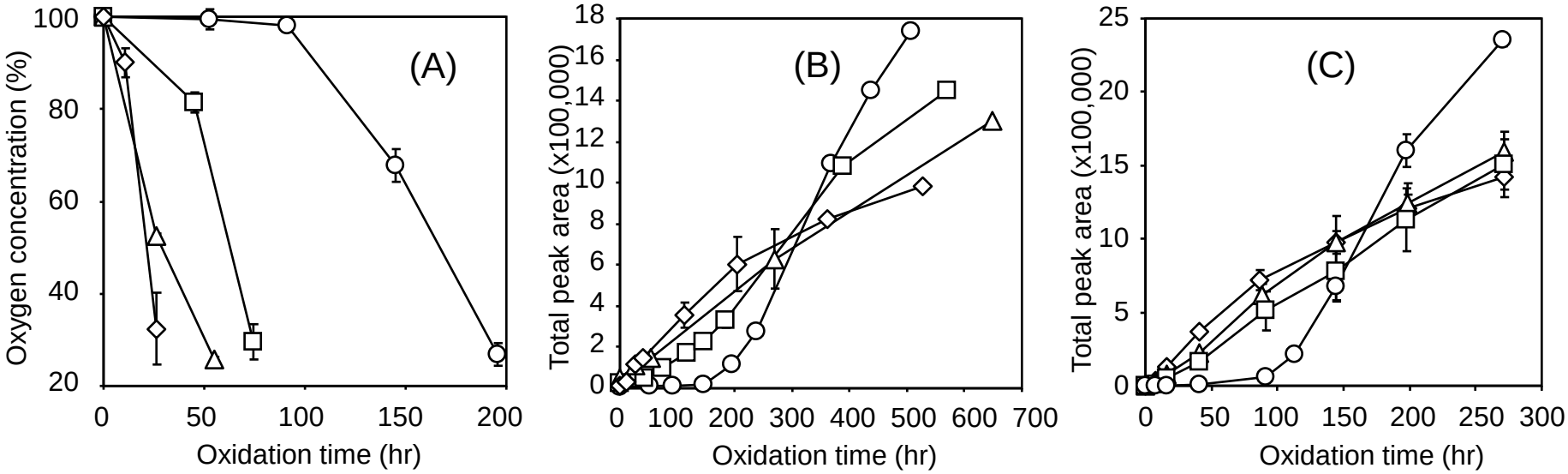
(A)
Oxygen concentration (%)
100
80
60
40
20
0
50
100
150
200
Oxidation time (hr)
(B)
Total peak area (x100,000)
18
16
14
12
10
8
6
4
2
0
0
100
200
300
400
500
600
700
Oxidation time (hr)
(C)
Total peak area (x100,000)
25
20
15
10
5
0
0
50
100
150
200
250
300
Oxidation time (hr)

Figure 1

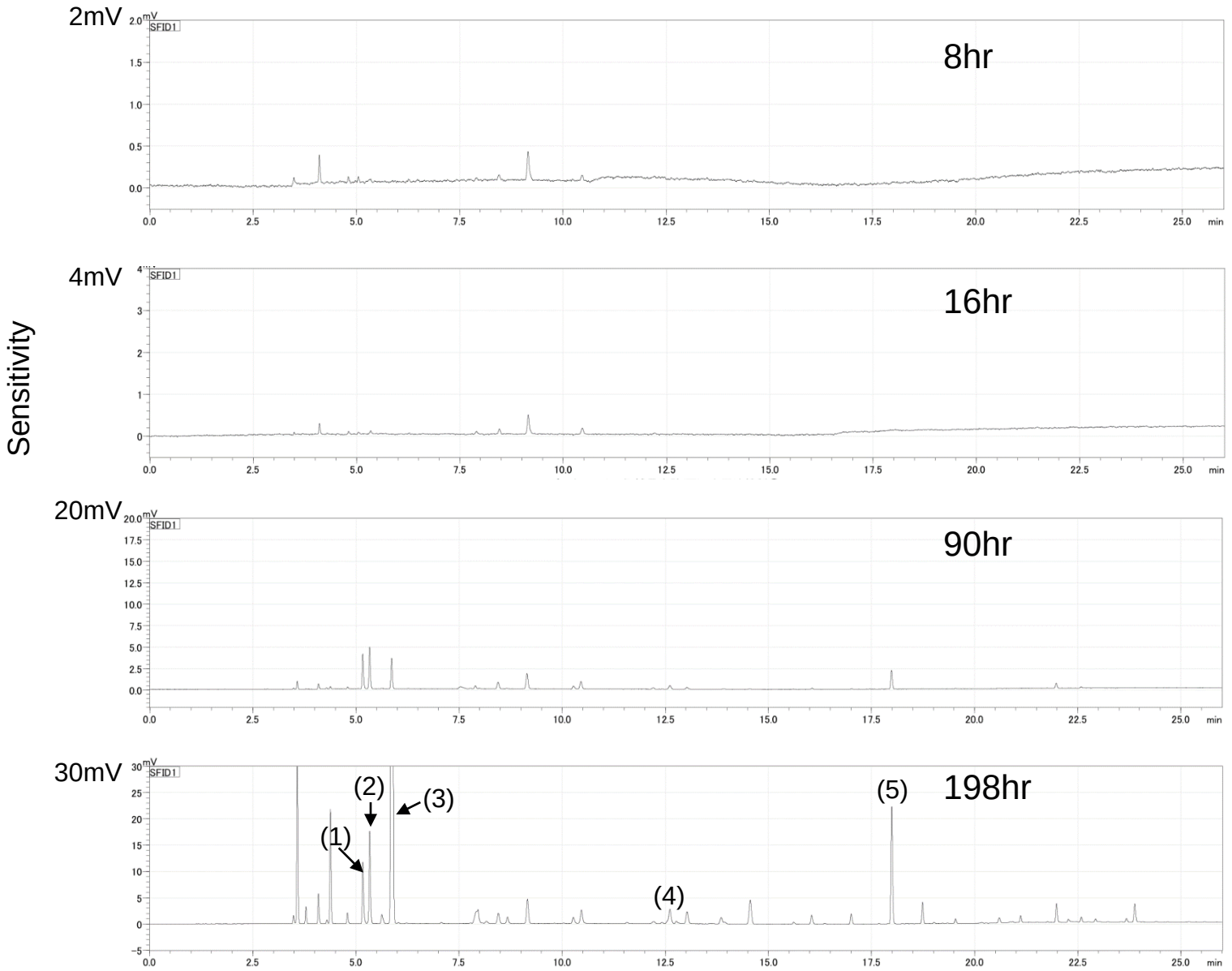
Sensitivity
2mV
8hr
4mV
16hr
20mV
90hr
30mV
198hr
(1)
(2)
(3)
(4)
(5)
SFID1
min

Figure 2 (A)

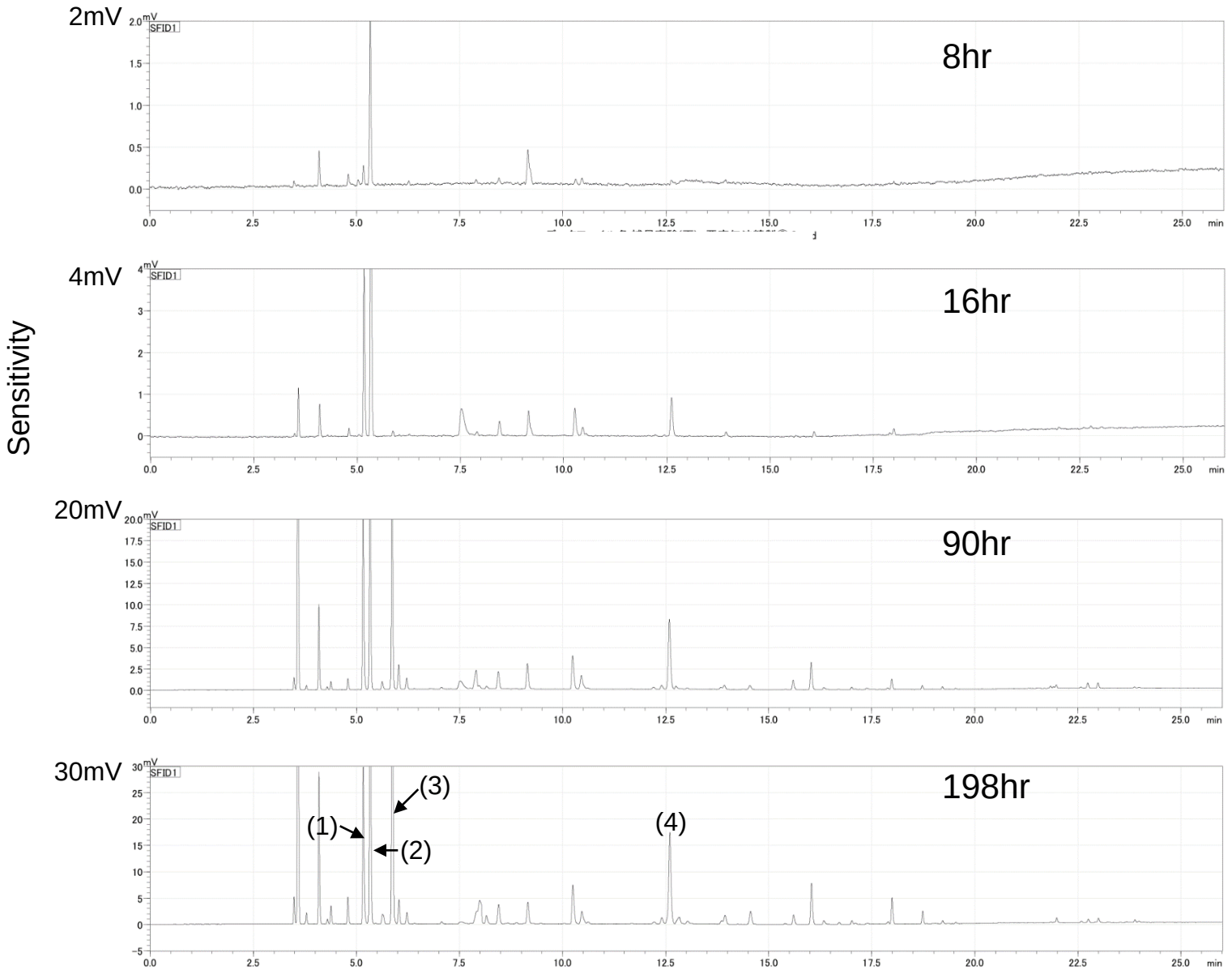

Figure 2 (B)

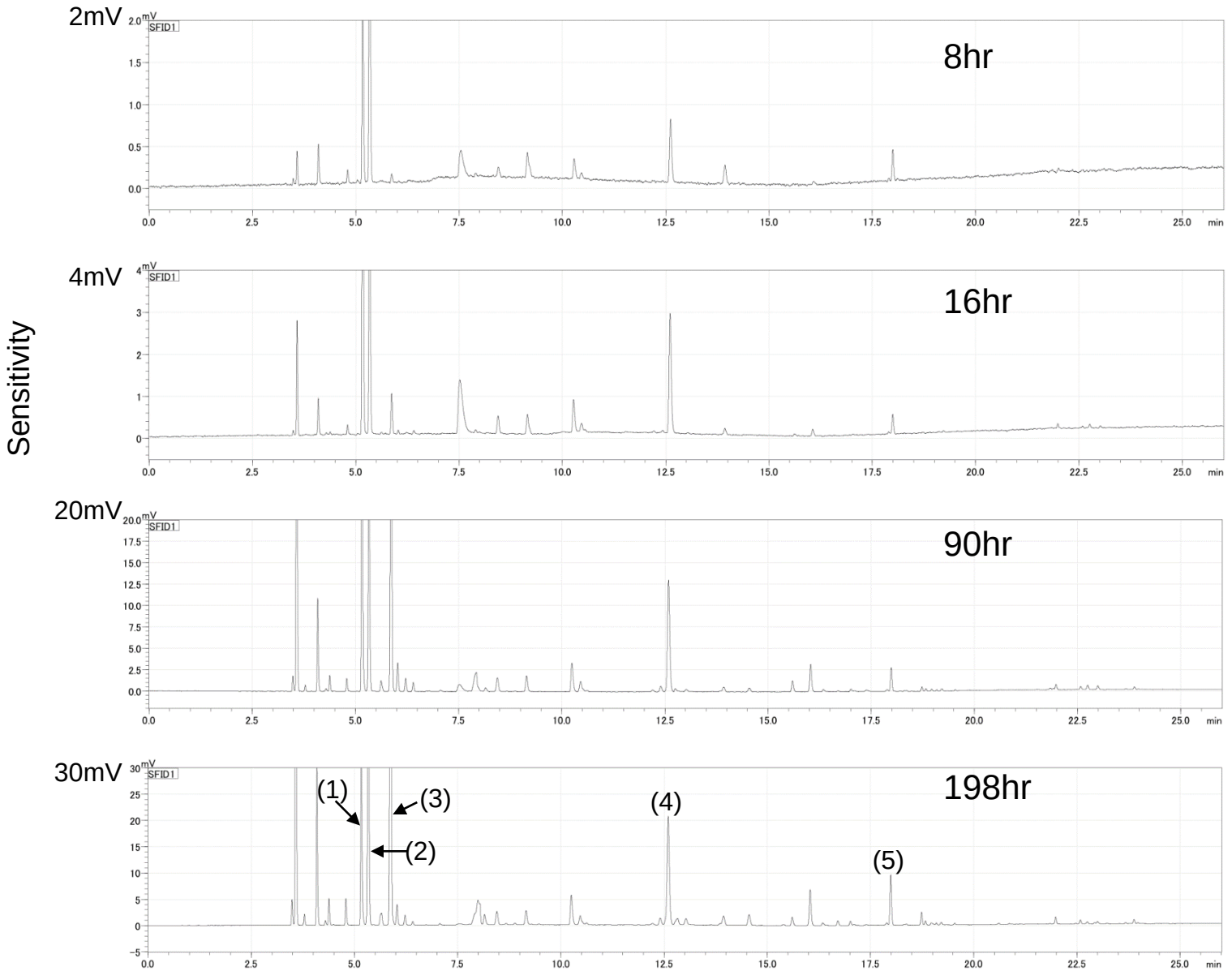

Figure 2 (C)

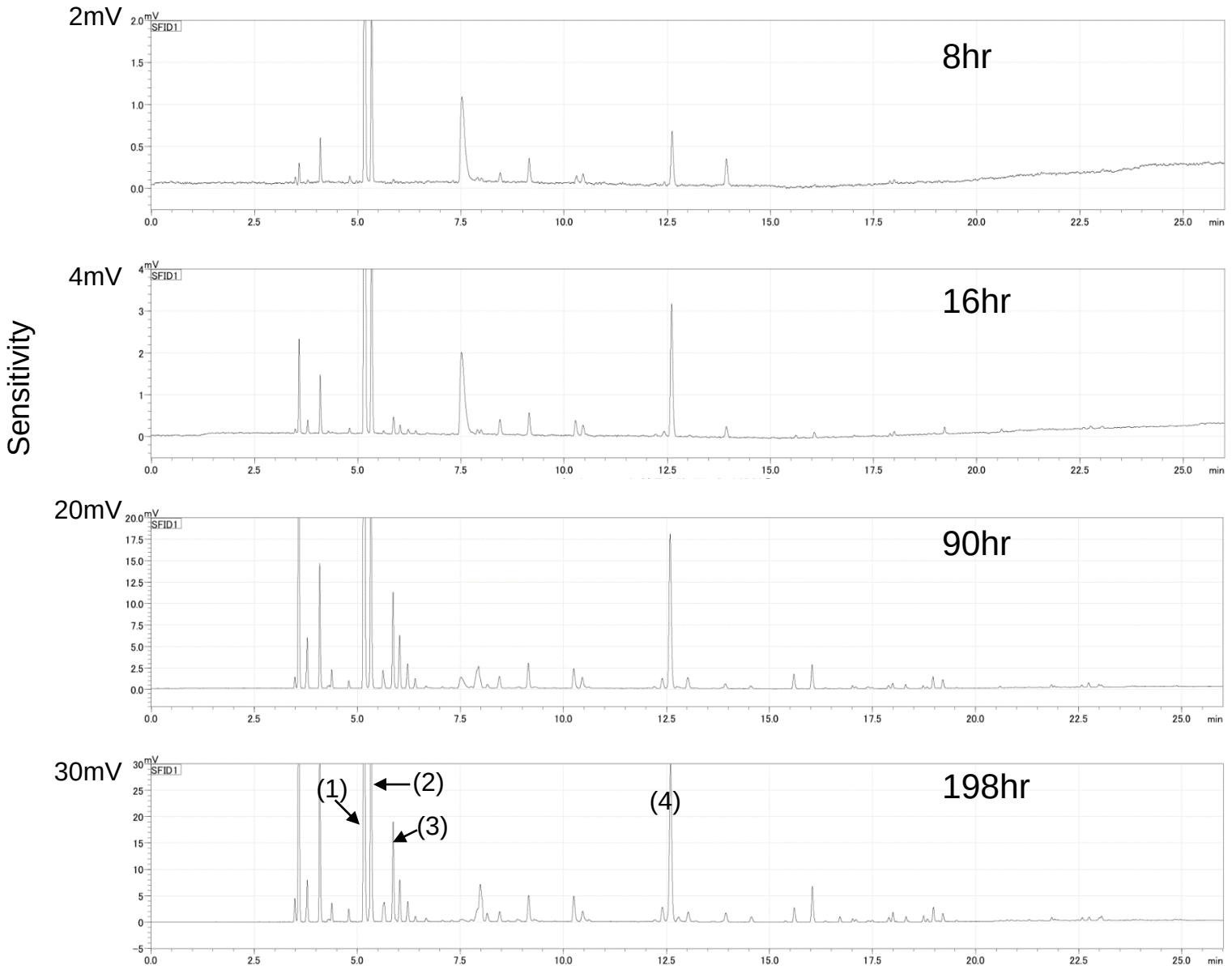
Sensitivity
2mV
8hr
4mV
16hr
20mV
90hr
30mV
198hr
(1)
(2)
(3)
(4)
SFID1
min

Figure 2 (D)

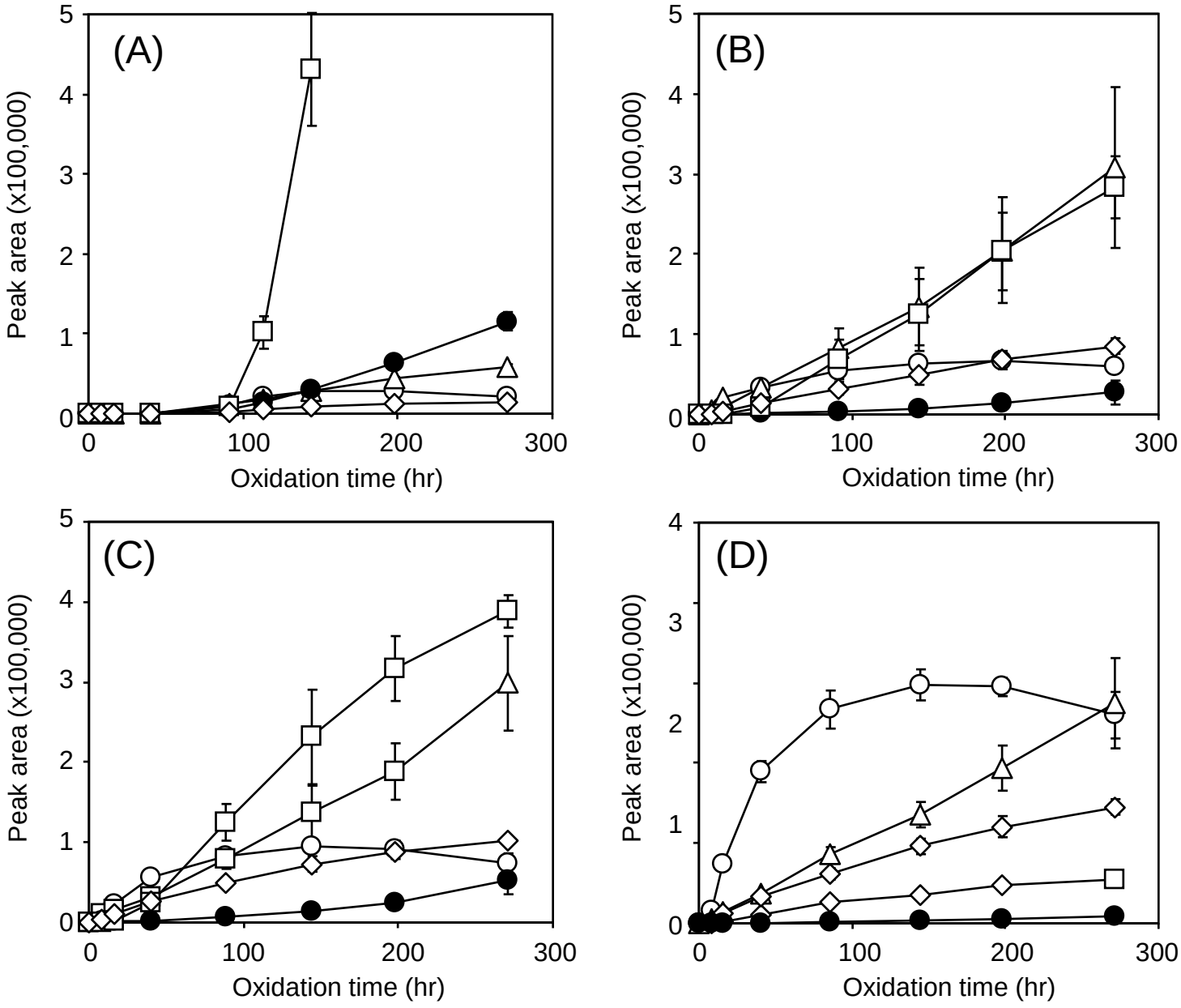

Figure 3

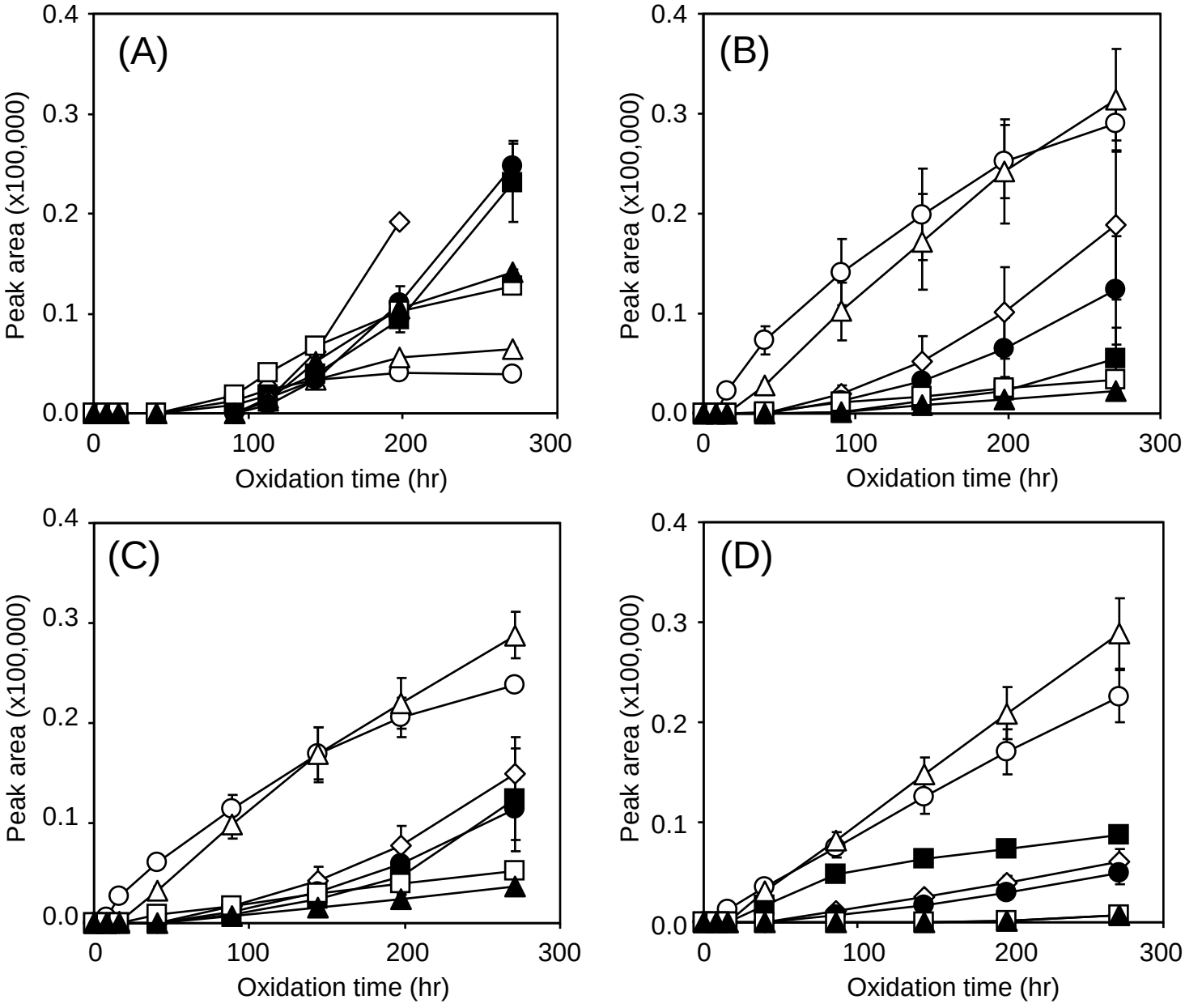
(A)
(B)
(C)
(D)
Peak area (x100,000)
Oxidation time (hr)
0.0
0.1
0.2
0.3
0.4
0
100
200
300

Figure 4

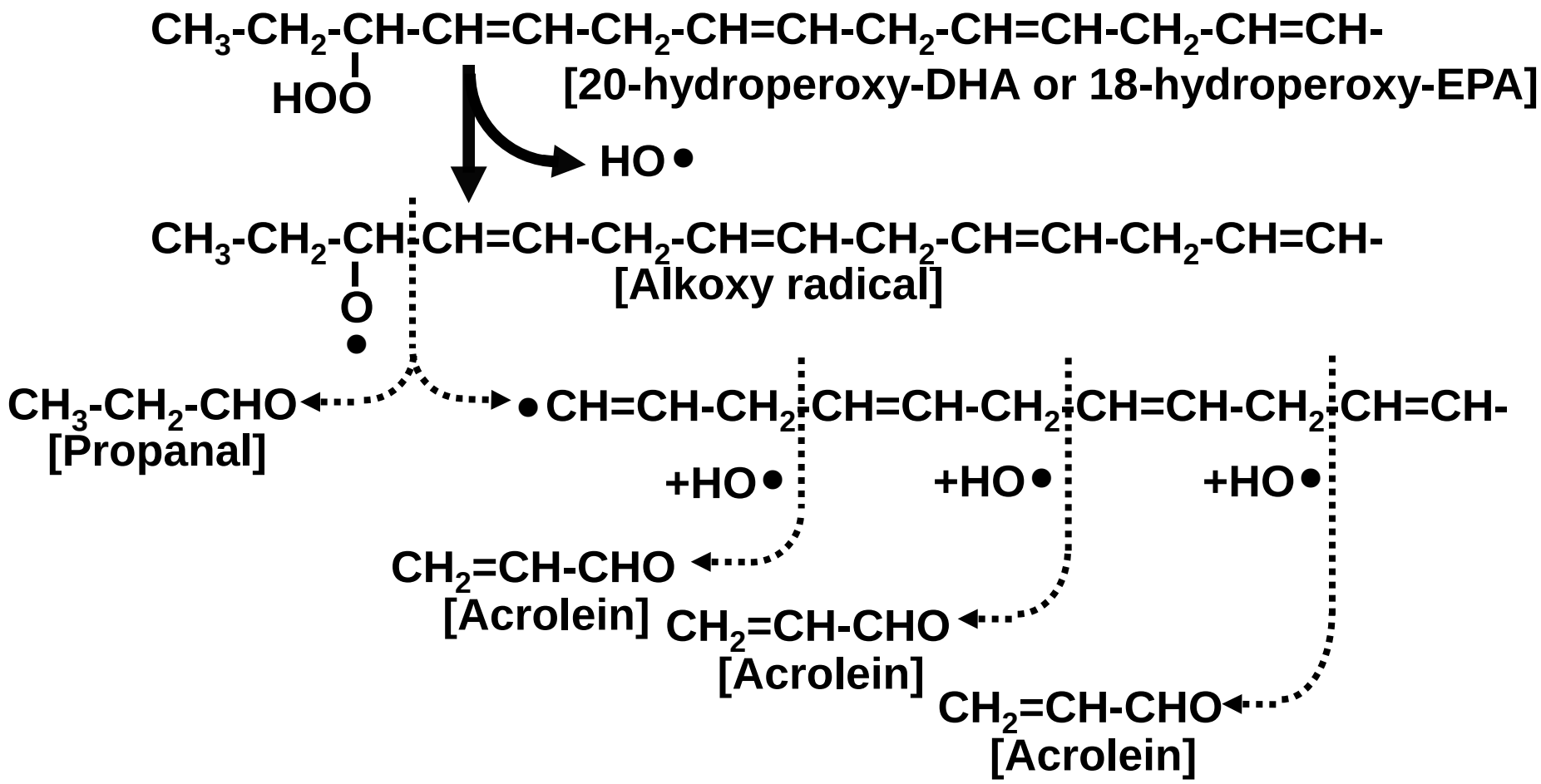
CH3-CH2-CH-CH=CH-CH2-CH=CH-CH2-CH=CH-CH2-CH=CH-
HOO
[20-hydroperoxy-DHA or 18-hydroperoxy-EPA]
HO•
CH3-CH2-CH-CH=CH-CH2-CH=CH-CH2-CH=CH-CH2-CH=CH-
O•
[Alkoxy radical]
CH3-CH2-CHO
[Propanal]
•CH=CH-CH2-CH=CH-CH2-CH=CH-CH2-CH=CH-
+HO•
+HO•
+HO•
CH2=CH-CHO
[Acrolein]
CH2=CH-CHO
[Acrolein]
CH2=CH-CHO
[Acrolein]

Figure 5