

Model Studies on the Key Aroma Compounds Formed by an Oxidative Degradation of ω -3 Fatty Acids Initiated by either Copper(II) Ions or Lipoxygenase

Michaela Hammer and Peter Schieberle*

Deutsche Forschungsanstalt für Lebensmittelchemie, Lise-Meitner-Straße 34, 85354 Freising, Germany

S Supporting Information

ABSTRACT: Due to the high number of double bonds, ω -3-polyunsaturated fatty acids such as eicosapentaenoic acid (EPA) or docosahexaenoic acid (DHA) are prone to rapid oxidation, leading to the formation of intense taints often described as “fishy”. To clarify the compounds responsible for such off-flavors, EPA, DHA, and α -linolenic acid (ALA) were oxidized singly either in the presence of copper ions or in the presence of lipoxygenase. The autoxidation of EPA and DHA led to a mixture of odorants eliciting an overall fishy odor quality, whereas neither the oxidation of ALA by copper ions nor that by lipoxygenase led to an unpleasant odor. Application of aroma extract dilution analysis (AEDA) on the volatiles generated by autoxidation of EPA revealed *trans*-4,5-epoxy-(*E,Z*)-2,7-decadienal, identified for the first time as fatty acid degradation product, (*Z*)-1,5-octadien-3-one, (*Z*)-3-hexenal, (*Z,Z*)-2,5-octadienal, (*Z,Z*)-3,6-nonadienal, and (*E,E,Z*)-2,4,6-nonatrienal with the highest flavor dilution (FD) factors. The autoxidation as well as the enzymatic oxidation of all three acids led to the same odorants, but with different FD factors depending on the acid and/or the type of oxidation applied. Thus, the results suggested that a defined ratio of a few odorants is needed to generate a fishy off-flavor.

KEYWORDS: docosahexaenoic acid, eicosapentaenoic acid, α -linolenic acid, *trans*-4,5-epoxy-(*E,Z*)-2,7-decadienal, (*Z*)-1,5-octadien-3-one, 1-penten-3-one

INTRODUCTION

A sufficient consumption of omega-3 polyunsaturated fatty acids (ω -3 PUFAs), such as eicosapentaenoic acid (EPA; C-20:5) or docosahexaenoic acid (DHA; C-22:6), is reported to have beneficial effects on human health, for example, by lowering blood cholesterol, and was also found to reduce the risk of several diseases.^{1,2} Although humans are able to biosynthesize EPA and DHA from α -linolenic acid (ALA), the biosynthetic steps are reported to be quite slow.^{3,4} Therefore, it is recommended by nutritionists to enrich the human diet with fatty fish containing high amounts of ω -3 PUFAs.⁵ However, because the common Western diet does not contain enough fatty fish, the consumption of fish oils, for example, in industrially prepared convenience foods, could be an option to optimize the human diet. Unfortunately, unsaturated fatty acids, in particular those containing several bis-allylic hydrogen atoms, are prone to rapid oxidation, and the instability of an unsaturated fatty acid is well-known to increase with the number of double bonds. For example, it was shown in model experiments that esters of EPA or DHA are oxidized ~5–8 times more rapidly than ethyl linolenate.⁶ The rapid formation of hydroperoxides as primary oxidation products and their degradation into short-chain, volatile compounds is a big challenge for the food industry, as the degradation of the intermediate peroxides leads to the generation of numerous volatiles, some of which elicit unpleasant aromas for which the term “fishy” is quite often used.

The clarification of reactions involved in the degradation of PUFAs in model studies either with single fatty acid esters or with entire fish oils has been the topic of several

investigations.^{6–12} For example, to gain insight into the pattern of volatiles generated during oxidation of fish oil, Aidos et al.⁹ oxidized crude herring oil for 53 h at 50 °C in an atmosphere of oxygen. From the complex pattern of volatiles formed, a statistical data treatment proposed particularly 1-penten-3-one and (*E,E*)-2,4-heptadienal as indicators for the oxidative status of the oil. In many studies numerous volatiles were found; however, their contribution to the overall odor remained open. To shed light on the volatiles responsible for the overall unpleasant odor generated, Hsieh et al.¹¹ applied gas chromatography–olfactometry (GC-O) on the volatiles present in the headspace above crude menhaden fish oils and reported on the identification of 55 volatiles, 31 of which were odor-active. In particular, two isomers of decatrienal elicited a smell at the odor port reminiscent of “oxidized fish oil”. However, the results on odor qualities and identifications could not be confirmed by means of the respective reference compounds. In an investigation applying GC-O on the odorants present in the headspace above crude seal blubber oil, Cadwallader and Shahidi¹² found 27 odor-active volatiles. Their data suggested that, in particular, (*Z*)-1,5-octadien-3-one and (*E,E,Z*)-2,4,7-decatrienal should contribute to the taint of the investigated oil, because these compounds showed the highest odor intensity among the 22 odorants reported (lowest detection volumes in the GC-O headspace approach used). However, because no

Received: August 28, 2013

Revised: October 21, 2013

Accepted: October 23, 2013

Published: October 23, 2013

reference compounds were available, both odorants were only tentatively identified. Hartvigsen et al.¹³ also applied GC-O on the volatiles present in the headspace above a mayonnaise prepared with 80% crude sand eel oil. Among the 78 odor-active components detected, (*Z*)-4-heptenal and (*E,Z*)-2,4-heptadienal were reported to elicit a fishy odor. On the basis of available knowledge on the formation of volatile degradation products from ω -3-PUFAs, Venskaleshwarlu et al.¹⁴ used four compounds, namely, (*E,Z*)-2,6-nonadienal, 1-penten-3-one, (*Z*)-4-heptenal, and (*E,E*)-2,4-heptadienal, in a sensory model experiment. The four compounds were added in different concentrations and combinations to semifat milk, and the mixture was sensorially evaluated for the presence of a fishy or metallic taint. As a result, (*E,Z*)-2,6-nonadienal and 1-penten-3-one were suggested as the main contributors to fishy and/or metallic off-flavors.

It has to be considered, however, that entire fish oils or fish oil enriched foods such as milk or mayonnaise, respectively, do not only contain the ω -3 PUFAs and, thus, the pattern of degradation products formed cannot be reliably correlated to the degradation of these acids. Such data can, however, be obtained by systematic model studies on the oxidation of single ω -3 fatty acids under different conditions with a focus on odor-active volatiles. However, besides one study on the formation of odorants formed by a degradation of α -linolenic acid,¹⁵ no investigation was yet performed on the odor-active volatiles formed by an oxidation of EPA and DHA, when degraded singly. Furthermore, although the formation of volatiles from ω -3 fatty acids by enzymes is also known to be involved in the formation of the typical aroma of fish,^{16–18} no systematic study on aroma compounds formed by an enzymatic degradation of EPA or DHA was yet performed.

Therefore, the present study aimed at identifying the most odor-active compounds formed either by autoxidation or by lipoxygenase catalysis, respectively, from three ω -3 fatty acids, namely, eicosapentaenoic acid, docosahexaenoic acid, and α -linolenic acid.

MATERIALS AND METHODS

Chemicals. α -Linolenic acid, docosahexaenoic acid, and eicosapentaenoic acid were obtained from Fluka, Sigma-Aldrich Chemie, Taufkirchen, Germany.

The following reference aroma compounds were from Sigma-Aldrich Chemie (Taufkirchen, Germany): butanoic acid, (*Z*)-3-hexenal, 1-penten-3-one, 2,4-heptadienal, and (*E,Z*)-2,6-nonadienal. (*E*)-2-Decenal was from Lancaster (Mühlheim, Germany), and (*Z*)-4-heptenal was a gift from Symrise (Holzminden, Germany).

The following reference compounds were synthesized as reported previously: *trans*-4,5-epoxy-(*E*)-2-decenal,¹⁹ (*Z,Z*)-3,6-nonadienal,¹⁸ (*E,E,Z*)-2,4,6-nonatrienal,²⁰ and 1(*Z*)-5-octadien-3-one.¹⁵

Soy lipoxygenase type I-B was from Isocom (Landshut, Germany), Tween 80 and boric acid were from Serva (Heidelberg, Germany), and diethyl ether, *n*-pentane, Na₂SO₄, and silica were from Merck (Darmstadt, Germany). Diethyl ether and *n*-pentane were distilled prior to use. Copper(II) palmitate was produced by dissolving Cu(CH₃COO)₂ and palmitic acid in ethanol. After 20 h at room temperature, the precipitate was isolated and recrystallized from toluene.

Syntheses. *2,4,7-Decatrienal.* The isomers of the unsaturated aldehyde were synthesized by a Wittig reaction from (*Z*)-3-hexenal and activated methyl crotonate following closely a procedure described previously for 2,4,6-nonatrienal isomers from (*E*)-2-pentenal.²⁰

Methyl 2,4,7-Decatrienoate. Tris[2-(2-methoxyethoxy)ethyl]amine (TDA-1; 323 mg; 1 mmol), a saturated, aqueous Na₂CO₃ solution (80 mL), and methylene chloride (80 mL) were mixed and

stirred vigorously. Then, methyl-4-(triphenylphosphonium) crotonate bromide (662 mg; 1.5 mmol) and (*Z*)-3-hexenal (1 mmol) were added. The vessel was flushed with nitrogen, and the solution was stirred for 12 h in the dark at room temperature. The organic phase was separated, and the aqueous phase was extracted twice with methylene chloride (total volume = 100 mL). The combined organic phases were stirred with hydrochloric acid (75 mL, 10%) for 2 h in the dark. After separation of the organic layer, the aqueous phase was washed with methylene chloride (total volume = 100 mL), and the combined organic phase was concentrated to ~2 mL. The target compound was purified on a water-cooled glass column (30 × 1 cm) filled with purified silica²¹ in *n*-pentane.¹⁹ The column was flushed with *n*-pentane (50 mL), and the target compound (mixture of isomers) was eluted with *n*-pentane/diethyl ether (200 mL, 80:20 by vol).

MS-EI, *m/z* (%) 79 (100), 91 (90), 111 (65), 77 (43), 55 (39), 98 (37), 121 (36), 105 (30).

2,4,7-Decatrienal. The esters obtained were reduced to their corresponding alcohols using LiAlH₄.

MS-EI, *m/z* (%) 79 (100), 43 (81), 45 (62), 60 (43), 91 (37), 77 (20).

2,4,7-Decatrienal. Dess–Martin periodinane (1 mmol, 440 mg) was added to the solution of the alcohols, and the mixture was stirred for 12 h at room temperature in the dark. After filtration, the solution was concentrated by means of a Vigreux column, and two main isomers could be isolated by fractionation on a silica gel column in an *n*-pentane/diethyl ether gradient from 0 to 10% diethyl ether.

The isomers were characterized by their NMR data and mass spectra (MS-EI; MS-CI) as well as their retention indices (RI) on three stationary GC phases of different polarities:

(*E,E,Z*)-2,4,7-decatrienal: RI_{FFAP}, 1817; RI_{DB-5}, 1289; RI_{OV-1701}, 1439.

MS-EI, *m/z* (%) 79 (100), 81 (98), 77 (69), 91 (59), 67 (57), 41 (52), 39 (50), 92 (47), 121 (40).

(*E,E,E*)-2,4,7-decatrienal: RI_{FFAP}, 1864; RI_{DB-5}, 1320; RI_{OV-1701}, 1465.

MS-EI, *m/z* (%) 81 (100), 79 (73), 77 (53), 41 (51), 67 (50), 91 (47), 39 (43), 121 (42).

2,5-Octadienal. (*E,Z*)- and (*Z,Z*)-2,5-octadienal were synthesized from (*Z*)-3-hexenal in a similar approach as reported above for the 2,4,7-decatrienal isomers, but using (carbomethoxymethyl)-triphenylphosphonium bromide (662 mg; 1.5 mmol) as the Wittig reagent (Figure S1 in the Supporting Information).

(*E,Z*)-2,5-octadienal: RI_{FFAP}, 1469; RI_{DB-5}, 1056; RI_{OV-1701}, 1178.

MS-EI, *m/z* (%) 81 (100), 95 (90), 55 (57), 39 (55), 41 (50), 67 (50).

(*Z,Z*)-2,5-octadienal: RI_{FFAP}, 1435; RI_{DB-5}, 1038; RI_{OV-1701}, 1157.

MS-EI, *m/z* (%) 95 (100), 39 (83), 68 (78), 41 (75), 81 (74), 55 (55).

(E,E)-3,5-Octadien-2-one. The synthesis was performed by an aldol condensation of (*E*)-2-pentenal and acetone following a procedure described earlier²² with some modifications: (*E*)-2-Pentenal (130 mg; 1.5 mmol) and acetone (330 mg; 5 mmol) were heated in a small autoclave in the presence of zinc acetate for 6 h at 170 °C. After cooling, the target compound was isolated by column chromatography on silica gel in a pentane/diethyl ether gradient.

RI_{FFAP}, 1495; RI_{DB-5}, 1095.

MS-CI, *m/z* (%) 125 (100).

MS-EI, *m/z* (%) 95 (100), 81 (38), 79 (30), 55 (18), 124 (12), 109 (8).

Model Systems. *Copper-Initiated Autoxidation.* In a quartz glass vessel, the respective fatty acid (250 mg) and copper(II) palmitate (2 mg) were dissolved in freshly distilled diethyl ether (5 mL). The solvent was carefully removed under a stream of oxygen, the flask was closed, and oxidation was performed in a self-constructed light box at 25 °C for 48 h. Then, freshly distilled diethyl ether (100 mL) was added to dissolve the material.

Lipoxygenase-Initiated Oxidation. The respective fatty acid (250 mg) was dissolved in aqueous Tween 80 solution (8 mL; 0.1%) by addition of aqueous NaOH (1.5 mL; 1 mol/L). After addition of

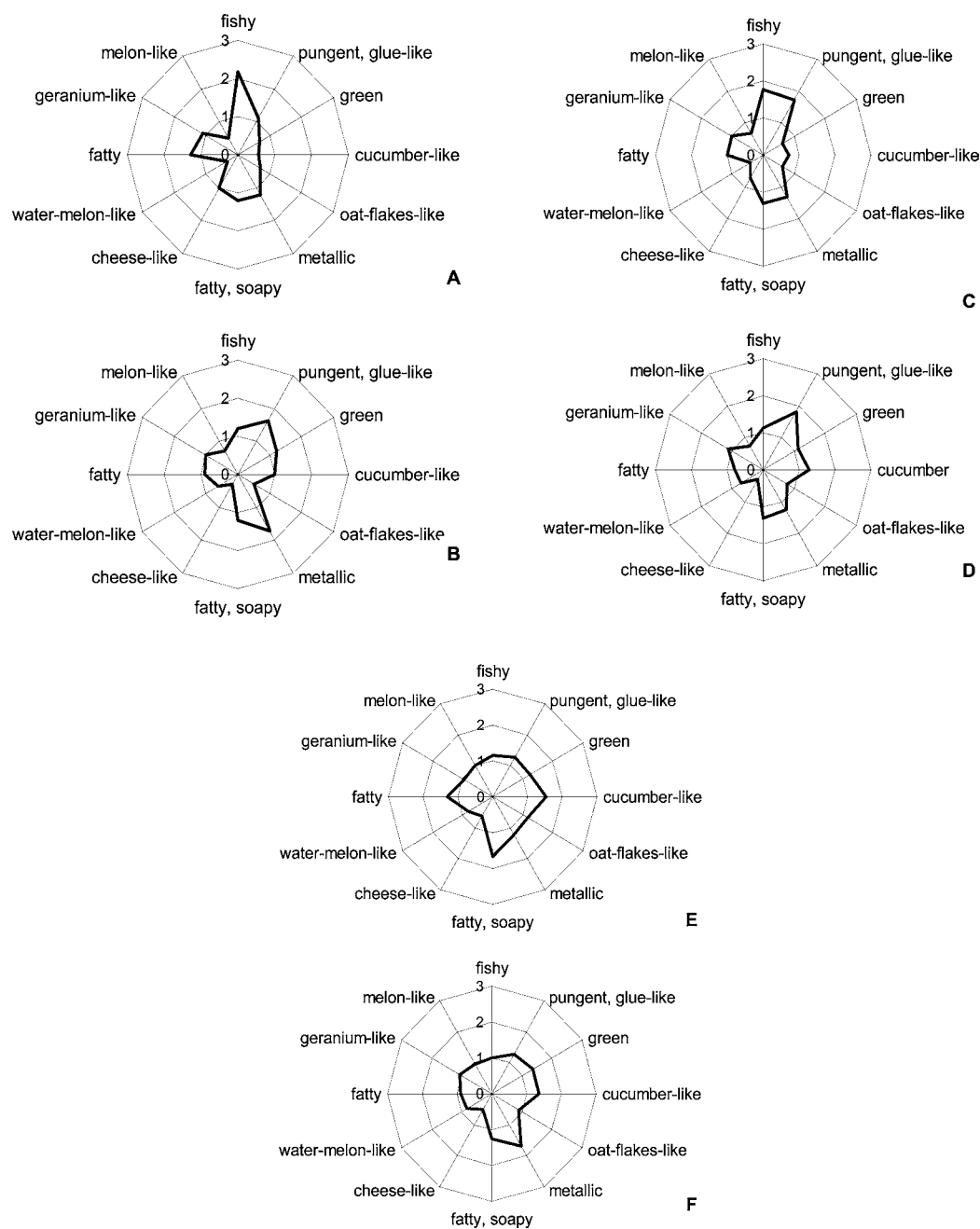


Figure 1. Results of the aroma profile analyses of the six distillates obtained after (A) autoxidation of eicosapentaenoic acid (EPA-AUT), (B) enzymatically oxidized eicosapentaenoic acid (EPA-LOX), (C) autoxidized docosahexaenoic acid (DHA-AUT), (D) enzymatically oxidized docosahexaenoic acid (DHA-LOX), (E) autoxidized α -linolenic acid (ALA-AUT), and (F) enzymatically oxidized α -linolenic acid (ALA-LOX).

borate buffer (200 mL; 0.1 mol/L; pH 9.0) and adjustment of the pH to 9.0, the solution was flushed with pure oxygen for 3 min. Then, lipoxygenase type I-B (10 mg) was added, and the flask was filled with oxygen and closed. After stirring for 60 min at 10 °C under an atmosphere of pure oxygen maintained by a balloon, the reaction was stopped by adjusting the pH to 3 with hydrochloric acid (1 mol/L). The mixture was extracted with diethyl ether (total volume = 450 mL), and the organic phase was washed with brine (total volume = 400 mL) and dried over Na_2SO_4 .

Isolation of the Volatiles. To remove the nonvolatile material, the extracts were concentrated to ~100 mL and distilled at 40 °C using solvent-assisted flavor evaporation (SAFE).²³ The distillates were concentrated to ~250 μL using a Vigreux column followed by microdistillation and then used for GC-O.

GC-O and Aroma Extract Dilution Analysis (AEDA). GC-O was performed by means of a Fisons Instruments gas chromatograph type 8000 (Mainz, Germany) using the following J&W capillary columns (Folsom, CA, USA): DB-FFAP (30 m \times 0.32 mm, film thickness = 0.25 μm), DB-5 (30 m \times 0.32 mm, film thickness = 0.25 μm), and OV-1701 (30 m \times 0.32 mm, film thickness = 0.25 μm). The samples were injected at 40 °C using the cold on-column technique, and the temperature was held for 2 min at 40 °C, then raised to 90 °C at 8 °C/min, followed by 3 °C/min to 130 °C, and finally at 8 °C/min to 230 °C and held for 5 min. Helium was used as carrier gas at a flow rate of 1.5 mL/min. Retention indices (RI) were calculated from the retention times of *n*-alkanes by linear interpolation.²⁰ The effluent was split evenly to a flame ionization detector (FID) and a heated sniffing port (200 °C). Flavor dilution factors (FD factors) were determined on the FFAP capillary column by sniffing the stepwise-

diluted solutions of the original distillate (1:1, by volume). The FD factors were calculated and plotted against the retention indices in an FD chromatogram.

GC–Mass Spectrometry (GC-MS). Mass spectra were generated by connecting the capillary columns described above to a Finnigan mass spectrometer MAT 95 S (Bremen, Germany) at 70 eV in the electronic ionization mode (MS-EI) and at 115 eV in the chemical ionization mode (MS-CI) with isobutane as the reagent gas.

Aroma Profile Analysis. For aroma profile analysis, an aliquot (125 μ L) of the concentrated distillate (20 mL) was added to distilled water (20 mL). Sensory evaluation was performed by 19 trained panelists (expert panel) in a sensory panel room. The following reference compounds in water were used to train the respective aroma descriptors (100 times above their odor threshold): fishy, (*Z*)-4-heptenal; pungent, 1-penten-3-one; green, (*Z*)-3-hexenal; cucumber-like, (*E,Z*)-2,6-nonadienal; oatflakes-like, (*E,E,E*)-2,4,6-nonatrienal; metallic, *trans*-4,5-epoxy-(*E*)-2-decenal; fatty-soapy, 2,4,7-decatrinal; cheesy, butanoic acid; fatty, (*E,E*)-2,4-heptadienal; geranium-like, (*Z*)-1,5-octadien-3-one; sweet/melon-like, 2,5-octadienal. The intensities were scaled using 0 (not perceivable) over 0.5, 1.0, 1.5, 2.0, 2.5, to 3.0 (strongly perceivable). In a second session, the hedonic acceptance of each mixture was scaled from 1 (acceptable) to 6 (strong off-taint). All results from the 19 evaluators were averaged.

Preparative Isolation of Compound 26. Distillates of the volatiles isolated from 10 batches of enzymatically oxidized eicosapentaenoic acid were combined and concentrated. The solution was separated by high-performance liquid chromatography (HPLC) on an NP-HPLC stainless steel column (30 \times 0.46 cm), filled with Nucleosil using *n*-pentane/*tert*-butyl methyl ether (95:5 by vol) as the mobile phase at a flow rate of 3 mL/min. The effluent was monitored at 254 nm, and 10 fractions were collected. The target compound was detected by GC-O in the fraction eluting between 80 and 85 mL. To obtain an isolate for NMR analysis, this fraction was purified again using the same HPLC system but a different mobile phase (*n*-pentane/*tert*-butyl methyl ether (80:20 by vol)). The target compound eluting between 30 and 36 mL was used for further analysis.

Compound 26: RI_{FFAP}, 2065; RI_{DB-S}, 1384; RI_{OV-1701}, 1571.

MS-EI, *m/z* (%) 68 (100), 67 (92), 41 (77), 39 (51), 69 (34), 81 (27).

Proton Magnetic Resonance Spectrometry. NMR spectra were recorded in ²H₆-benzene (compound 26) or CDCl₃ (all other compounds) by means of either a Bruker 360 MHz AMX (Rheinstetten, Germany) or a Bruker 500 MHz Avance III. Tetramethylsilane was used as the internal standard.

Odor Thresholds. The compound was added to either oil or water in a defined amount. This solution was diluted stepwise (1:1). The dilutions were presented in a triangle test using either odorless sunflower oil or water as the reference. Odor thresholds in air were determined as previously reported.²⁴

RESULTS AND DISCUSSION

In a first series of experiments, EPA, DHA, and ALA were either peroxidized for 48 h in the presence of oxygen and copper(II) ions or treated with lipoxygenase I in the presence of oxygen, respectively. The volatiles generated from the same amount of the respective acid in the six trials were isolated by high-vacuum distillation, and aliquots were evaluated by a sensory panel. The results of the aroma profile analyses (Figure 1) revealed that, in particular from the autoxidized eicosapentaenoic acid (EPA-AUT), a set of odorants was generated eliciting an intense fishy odor quality with metallic, fatty, and pungent bynotes (Figure 1A). On a scale from 1 (acceptable aroma) to 6 (strong off-taint) this mixture was judged with 5.1 (data not shown). In contrast, the distillate from the enzymatically oxidized EPA (EPA-LOX) had a weaker fishy aroma quality, but a more pronounced metallic smell and pungent, green, and geranium-like bynotes (Figure 1B). Also, the overall profile of the autoxidized docosahexaenoic acid

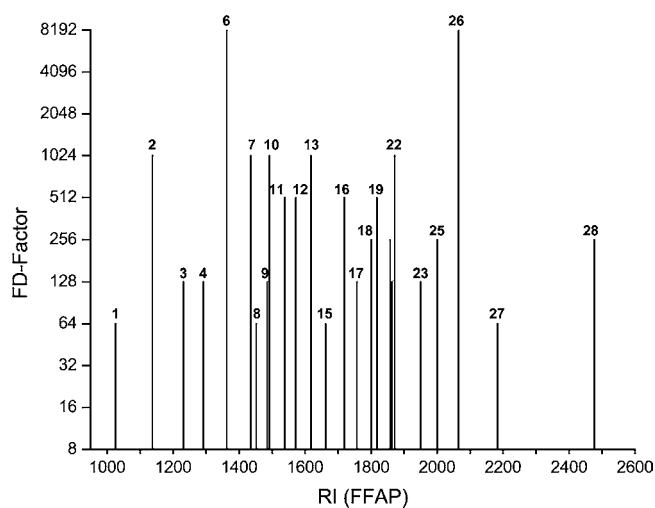


Figure 2. Flavor dilution (FD) chromatogram obtained by application of aroma extract dilution analysis on the volatiles isolated from autoxidized eicosapentaenoic acid (EPA-AUT).

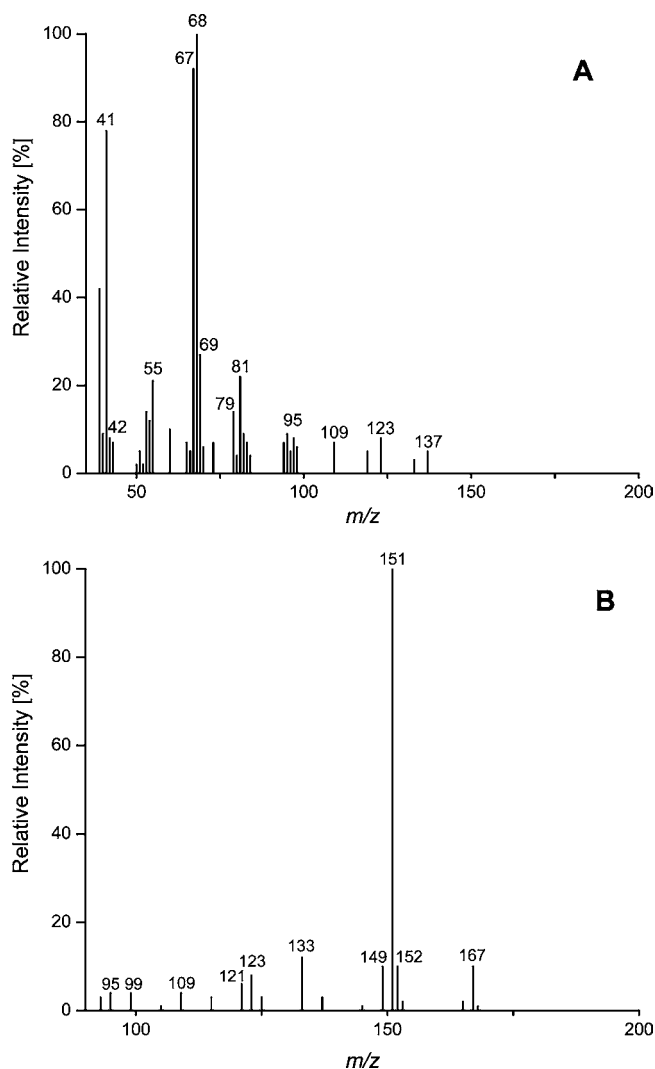
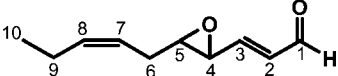


Figure 3. Mass spectra (A, MS-EI; B, MS-CI) obtained for compound 26.

(DHA-AUT; Figure 1C) was rated with a quite high fishy odor intensity, whereas the enzymatically oxidized DHA (DHA-

Table 1. ^1H NMR Data of Compound 26


H at carbon	δ (ppm)		J (Hz)	
1	9.28	d	nd ^a	nd
2	6.15	dd	7.75	15.80
3	5.78	dd	7.10	15.75
4	2.80	dd	7.02	1.57
5	2.53	dt	5.45	1.57
6	2.14	m	nd	nd
7	5.36	dt	7.9	9.8
8	5.55	dt	8.4	9.8
9	1.97	m	nd	nd
10	0.97	m	nd	nd

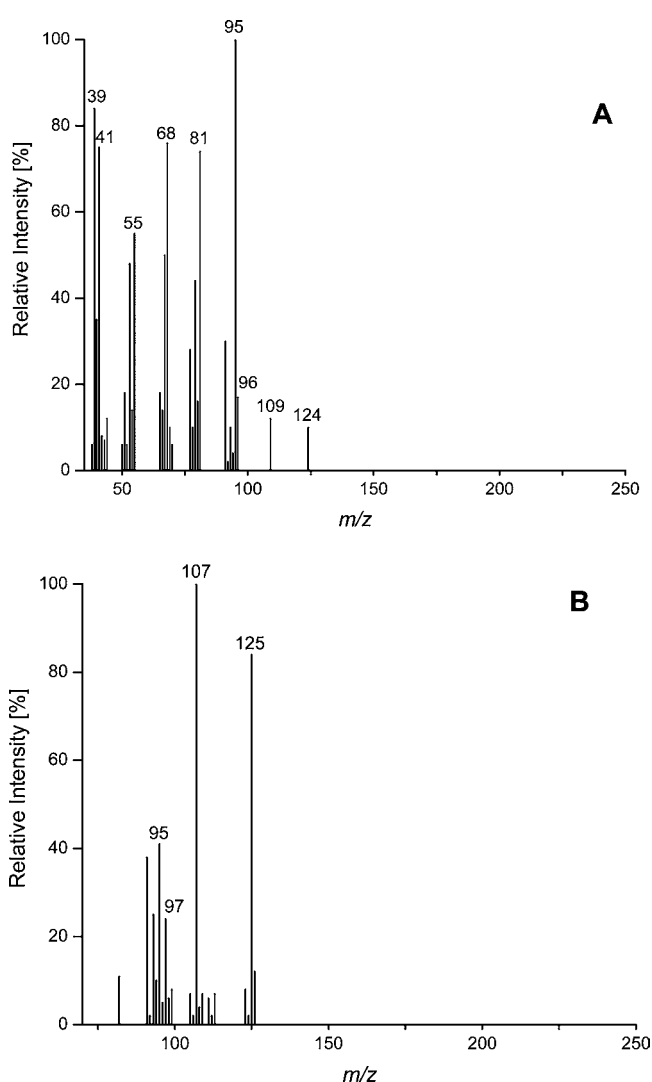
^and, not determined.

Figure 4. Mass spectra (A, MS-EI; B, MS-CI) obtained for (Z,Z)-2,5-octadienal.

LOX; Figure 1D) and both distillates generated from α -linolenic acid (ALA-AUT and ALA-LOX; Figure 1E,F) scarcely elicited an odor reminiscent of fish, and the hedonic evaluation of ALA-LOX even resulted in a 2.4 positive score.

On the basis of these results, the identification experiments were first focused on the distillate from EPA-AUT eliciting the most intense “fishy” odor. To characterize the odorants responsible for the overall odor, the aroma compounds were located by application of AEDA. In the FD factor range of 64–8192 a total of 28 odor-active areas were detected (Figure 2). Among them, compound 6 with a geranium-like odor and compound 26 with an intense metallic smell appeared with the highest FD factors. Five additional aroma compounds, 2 (green, leaf-like), 7 (sweet, melon-like), 10 (watermelon-like), 13 (cheese-like), and 22 (oatflakes-like), followed with somewhat lower FD factors. To identify the odorants causing the respective odors, first, the retention indices on two columns of different polarity as well as their odor qualities and odor intensities were compared to an in-house database containing ~800 odor-active compounds previously identified as food odorants. This procedure suggested a structure for some odorants, but to confirm their identity, a larger amount of eicosapentaenoic acid (2 g) was oxidized, and the analytes were enriched by column chromatography on silica gel in an *n*-pentane/diethyl ether gradient. The odor-active compounds in the fractions obtained were located again by GC-O and analyzed by GC-MS (MS-EI; MS-CI), and then the mass spectra obtained were compared to those of reference compounds. By this procedure, 6 was identified as (*Z*)-1,5-octadien-3-one, 2 as (*Z*)-3-hexanal, 13 as butanoic acid, and 22 as (*E,E,Z*)-2,4,6-nonatrienal.

However, compounds 26, 7, and 10 were not available as reference compounds. The mass spectrum of 26 obtained by MS-CI (Figure 3B) suggested a molecular weight of 166 (m/z 167), and analysis by high-resolution MS indicated that the base fragment (m/z 151) was generated by an elimination of 16, which points to oxygen elimination from an epoxide. The MS-EI was very similar to that of 4,5-epoxy-(*E*)-2-decenal,^{19,25} and high-resolution mass spectrometry indicated an elemental composition of $\text{C}_4\text{H}_4\text{O}$ for the molecular ion m/z 68 (Figure 3A). This result was also identical with the elemental composition measured in the main fragment m/z 68 of *trans*-4,5-epoxy-(*E*)-2-decenal (data not shown). Because the molecular mass of the latter compound (m/z 168) was 2 mass units higher than that of 26, a 4,5-epoxydecadienal structure was assumed. To prove this assumption, 26 was isolated by preparative HPLC for analysis by NMR. In Table 1, the ^1H NMR data measured for 26 are summarized. The chemical shifts of the H atoms at carbons 1–5 were nearly in the same range as those reported earlier for *trans*-4,5-epoxy-(*E*)-2-decenal.^{19,25} However, the signals at the olefinic carbons 7 and 8 were clearly shifted downfield compared to those of 4,5-epoxy-(*E*)-2-decenal, confirming an additional double bond at this position. The coupling constant J_{7-8} of 9.8 Hz indicated a (*Z*)-double bond. The data suggested the structure of 26 as *trans*-4,5-epoxy-(*E,Z*)-2,7-decadienal, which was finally proven by a COSY NMR spectrum (data not shown). Compound 25 showed a mass spectrum nearly identical to that of 26. Because the presence of *trans*-4,5-epoxy-(*E*)-2-decenal could be excluded, 25 was tentatively identified as *cis*-2,7-epoxy-(*E,Z*)-4,5-decadienal.

Also, the MS data for 7 were not available from a reference compound. Its mass spectrum (Figure 4), in particular the molecular mass at m/z 124, suggested the structure of an octadienal isomer. However, the retention indices of 7 (RI_{FFAP} , 1435; $\text{RI}_{\text{SE-54}}$, 1038) did neither agree with those of (*E,E*)-2,4-octadienal (RI_{FFAP} , 1596; $\text{RI}_{\text{SE-54}}$, 1115) nor with those of

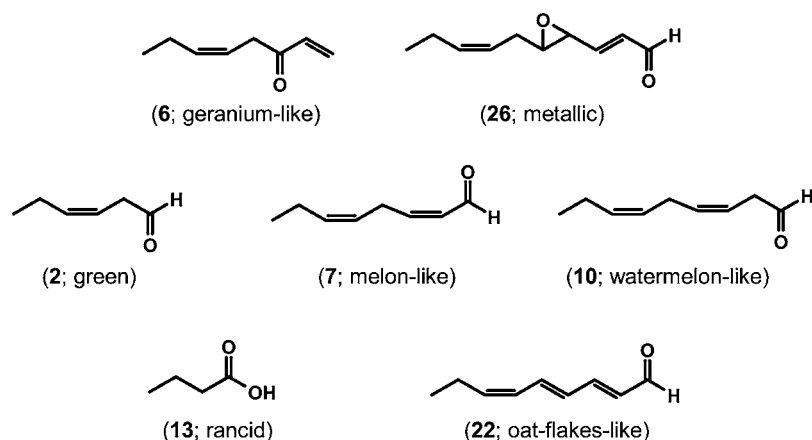


Figure 5. Key aroma compounds formed from autoxidized eicosapentaenoic acid (EPA-AUT).

Table 2. Important Aroma Compounds (FD Factor ≥ 64) Identified in a Distillate from Eicosapentaenoic Acid (EPA) Oxidized in the Presence of Copper(II) Ions (AUT) or in the Presence of Lipoxygenase (LOX)

no.	odorant	odor quality	RI		FD factors in EPA-	
			FFAP	DB 5	AUT	LOX
1	1-penten-3-one	pungent, glue-like	1025	693	64	≥ 8192
2	(Z)-3-hexenal	green	1137	802	1024	≥ 8192
3	(Z)-4-heptenal	fishy	1231	908	128	32
4	unknown	mushroom-like	1291	982	128	128
5	unknown	sweet, fresh	1320	996	32	128
6	(Z)-1,5-octadien-3-one	geranium-like	1363	987	≥ 8192	1024
7	(Z,Z)-2,5-octadienal	sweet, melon-like	1435	1038	1024	128
8	(E,Z)-2,4-heptadienal	fatty	1452	995	64	<1
9	(E,E)-2,4-heptadienal	fatty	1485	1017	128	8
10	(Z,Z)-3,6-nonadienal	fresh, watermelon-like	1492	1100	1024	512
11	unknown	cucumber-like, fatty	1538	1135	512	32
12	(E,Z)-2,6-nonadienal	cucumber-like	1572	1159	512	512
13	butanoic acid	cheesy	1618	800	1024	8
15	unknown	fatty	1662	nd ^a	64	256
16	unknown	sour	1718	nd	512	64
17	unknown	fatty, pungent	1757	nd	128	16
18	(E,Z,Z)- or (E,Z,E)-2,4,7-decatrinal ^b	fatty, pungent	1800	nd	256	128
19	(E,E,Z)-2,4,7-decatrinal	fatty	1817	1289	512	256
20	unknown	sour	1857	1362	256	32
21	(E,E,E)-2,4,7-decatrinal	fatty, cucumber-like	1864	1320	128	128
22	(E,E,Z)-2,4,6-nonatrienal	oatflakes-like	1871	1277	1024	4096
23	unknown	old	1950	nd	128	<1
24	unknown	metallic, pungent	1977	nd	16	128
25	cis-4,5-epoxy-(E,Z)-2,7-decadienal ^b	metallic, pungent	2000	nd	256	4096
26	trans-4,5-epoxy-(E,Z)-2,7-decadienal	metallic, pungent	2065	1384	≥ 8192	≥ 8192
27	unknown	fatty, metallic	2183	1518	64	128
28	unknown	metallic, pungent	2476	1667	256	32

^and, not determined. ^bTentative identification.

Table 3. Odor Thresholds Determined for Several Aldehydes

odorant	odor threshold (ng/L in air)
trans-4,5-epoxy-(E,Z)-2,7-decadienal	0.01
(Z,Z)-2,5-octadienal	0.08
(E,Z)-2,5-octadienal	0.70
(E,E,Z)-2,4,7-decatrinal	0.80
(E,E,E)-2,4,7-decatrinal	0.20

(E,Z)-2,4-octadienal (RI_{FFAP}, 1560; RI_{SE-54r}, 1100). Thus, on the basis of the structure of EPA as the ω -3 precursor fatty acid, it was assumed that 7 was a 2,5-octadienal isomer. To confirm

this structure, 2,5-octadienal was synthesized from (Z)-3-hexenal by a Wittig reaction (Figure S1; Supporting Information). As expected, the synthesis resulted in two isomers, and for ¹H NMR analysis these were separated by HPLC on silica gel, and the configuration of the double bonds was determined by ¹H NMR.

The mass spectra (Figure S2; Supporting Information) obtained for the quantitatively predominating isomer II (RI_{FFAP}, 1469; RI_{DB-5}, 1056; RI_{OV-1701}, 1156), in combination with the NMR analysis, suggested its structure as (E,Z)-2,5-octadienal with a coupling constant of $J_{2-3} = 15.6$ Hz, indicating an (E)-

Table 4. Important Aroma Compounds (FD Factor ≥ 64) Identified in a Distillate from Docosahexaenoic Acid (DHA) Oxidized either in the Presence of Copper(II) ions or (AUT) or in the Presence of Lipoxygenase I (LOX)

no.	odorant	odor quality	RI		FD factor in DHA-	
			FFAP	DB 5	AUT	LOX
1	1-penten-3-one	pungent	1025	693	64	2048
2	(Z)-3-hexenal	green	1137	802	1024	4096
3	(Z)-4-heptenal	fishy	1231	908	128	128
4	unknown	mushroom-like	1291	982	4	<1
5	unknown	sweet, fresh	1320	996	128	256
6	(Z)-1,5-octadien-3-one	geranium-like	1363	987	≥ 8192	1024
7	(Z,Z)-2,5-octadienal	sweet, melon-like	1435	1038	512	128
8	(E,Z)-2,4-heptadienal	fatty	1452	995	64	32
9	(E,E)-2,4-heptadienal	fatty	1485	1017	64	<1
10	(Z,Z)-3,6-nonadienal	fresh, watermelon-like	1492	1100	1024	1024
11	unknown	cucumber-like, fatty	1538	1135	512	8
12	(E,Z)-2,6-nonadienal	cucumber-like	1572	1159	512	1024
13	butanoic acid	cheesy	1618	800	<1	<1
14	unknown	fatty	1656	nd ^a	128	2
15	unknown	fatty	1662	nd	1024	256
16	unknown	sour	1718	nd	512	32
17	unknown	fatty, pungent	1757	nd	<1	8
18	(E,Z,Z)- or (E,Z,E)-2,4,7-decatrinal ^b	fatty, pungent	1800	nd	256	64
19	(E,E,Z)-2,4,7-decatrinal	fatty	1817	1289	512	256
20	unknown	sour	1857	1362	256	16
21	(E,E,E)-2,4,7-decatrinal	fatty, soapy	1864	1320	256	16
22	(E,E,Z)-2,4,6-nonatrienal	oatflakes-like	1871	1277	512	2048
23	unknown	old	1950	nd	256	<1
24	unknown	metallic, pungent	1977	nd	<1	<1
25	cis-4,5-epoxy-(E,Z)-2,7-decadienal ^b	metallic, pungent	2000	nd	<1	256
26	trans-4,5-epoxy-(E,Z)-2,7-decadienal	metallic, pungent	2065	1384	≥ 8192	≥ 8192
27	unknown	fatty, metallic	2183	1518	32	<1
28	unknown	metallic, pungent	2476	1667	512	64

^and, not determined. ^bTentative identification.

configuration at the double bond between carbons 2 and 3. A coupling constant of 10.9 Hz for the double bond at C-5/C-6 was in agreement with a (Z)-double bond. However, the retention indices and the mass spectra of this isomer did not agree with those of 7, whereas the retention indices of the earlier eluting isomer I (RI_{FFAP}, 1435; RI_{DB-5}, 1036; RI_{OV-1701}, 1157) were identical with those of compound 7. Because the mass spectra of this isomer (Figure S3; Supporting Information) showed a very similar fragmentation pattern compared to (E,Z)-2,5-octadienal, it was identified as (Z,Z)-2,5-octadienal, thus identifying also 7 as the (Z,Z)-isomer. A final confirmation of the geometry of the double bond by NMR was not possible due to the low yields obtained in the synthesis.

Compound 10, eliciting a watermelon-like odor, was detected by GC-O at RI_{FFAP} 1492 and RI_{DB-5} 1100. The mass spectra recorded in the MS-EI and MS-CI (data not shown) were in good agreement with those published earlier for (Z,Z)-3,6-nonadienal.¹⁸ However, Ulrich and Grosch¹⁵ had previously reported 3,5-octadien-2-one as a degradation product of α -linolenic acid with similar retention indices and also a melon-like odor. To unequivocally identify the structure of 10, (E,E)-3,5-octadien-2-one was synthesized. However, although its retention indices on FFAP (1495) and DB-5 (1100) were identical with those of 10, neither the mass spectrum (Figure S4, Supporting Information) nor the odor quality agreed with those of 10. Thus, 10 was identified as (Z,Z)-3,6-nonadienal.

The fatty-smelling 19 was detected at RI_{FFAP} 1817 and RI_{DB-5} 1289 and showed the mass spectra displayed in Figure S5

(Supporting Information). Because the MS-EI was in good agreement with data published for 2,4,7-decatrinal,¹⁵ the unsaturated aldehyde was synthesized by a Wittig reaction from (Z)-3-hexenal. The synthetic route resulted in a mixture of isomers, out of which two isomers could be isolated by HPLC on silica gel. On the basis of NMR measurements, the isomer eluting at the same retention index as compound 19 was identified as (E,E,Z)-2,4,7-decatrinal. The second isomer was identified as (E,E,E)-2,4,7-decatrinal. Because this isomer showed the same retention indices (RI_{FFAP}, 1864; RI_{DB-5}, 1320; RI_{OV-1701}, 1465) and the same mass spectra (Figure S6, Supporting Information) as compound 21, the latter was identified as (E,E,E)-2,4,7-decatrinal.

The mass spectra of compound 18 (data not shown) showed fragments very close to those of 19 and 21, suggesting the presence of another 2,4,7-decatrinal isomer. However, the amounts were too small to perform a reliable determination of the double-bond configuration by NMR. Thus, it can be assumed that 18 is either (E,Z,Z)- or (E,Z,E)-2,4,7-decatrinal, because the (E)-configuration of the double bond at C-2 is most likely.

In Figure 5, the structures of the most important odorants identified among the volatiles generated by autoxidation of EPA are displayed. Besides these odorants, also (E,Z)-2,6-nonadienal (12, cucumber-like), (E,E,Z)-2,4,7-decatrinal, and two unknowns (11, 16) were detected with comparably high FD factors of 512 (Table 2). The odor threshold of selected aldehydes was determined by GC-O in air and revealed that

Table 5. Important Aroma Compounds (FD Factor ≥ 64) Identified in Distillates of α -Linolenic Acid (ALA) Oxidized either in the Presence of Copper(II) Ions (AUT) or in the Presence of Lipoxygenase I (LOX)

no.	odorant	odor quality	RI		FD factors in ALA-	
			FFAP	DB 5	AUT	LOX
1	1-penten-3-one	pungent	1025	693	32	4096
2	(Z)-3-hexenal	green	1137	802	4096	≥ 8192
3	(Z)-4-heptenal	fishy	1231	908	32	128
4	unknown	mushroom-like	1291	982	16	64
5	unknown	sweet, fresh	1320	996	32	<1
6	(Z)-1,5-octadien-3-one	geranium-like	1363	987	1024	512
7	(Z,Z)-2,5-octadienal	sweet, melon-like	1435	1038	256	128
8	(E,Z)-2,4-heptadienal	fatty	1452	995	128	64
9	(E,E)-2,4-heptadienal	fatty	1485	1017	256	16
10	(Z,Z)-3,6-nonadienal	fresh, watermelon-like	1492	1100	512	512
11	unknown	cucumber-like, fatty	1538	1135	16	32
12	(E,Z)-2,6-nonadienal	cucumber-like	1572	1159	1024	1024
13	butanoic acid	cheesy	1618	800	<1	<1
15	unknown	fatty	1662	nd ^a	<1	64
16	unknown	sour	1718	nd	128	<1
17	unknown	fatty, pungent	1757	nd	8	<1
18	(E,Z,Z)- or (E,Z,E)-decatrienal ^b	fatty, pungent	1800	nd	128	64
19	(E,E,Z)-2,4,7-decatrienal	fatty	1817	1289	64	64
20	unknown	sour	1857	1362	16	16
21	(E,E,E)-2,4,7-decatrienal	fatty, cucumber-like	1864	1320	128	128
22	(E,E,Z)-2,4,6-nonatrienal	oatflakes-like	1871	1277	2048	2048
23	unknown	old	1950	nd	<1	8
24	unknown	metallic, pungent	1977	nd	16	32
25	<i>cis</i> -4,5-epoxy-(E,Z)-2,7-decadienal ^b	metallic, pungent	2000	nd	128	1024
26	<i>trans</i> -4,5-epoxy-(E,Z)-2,7-decadienal	metallic, pungent	2065	1384	≥ 8192	≥ 8192
27	unknown	fatty, metallic	2183	1518	8	2
28	unknown	metallic, pungent	2476	1667	16	32

^and, not determined. ^bTentative identification.

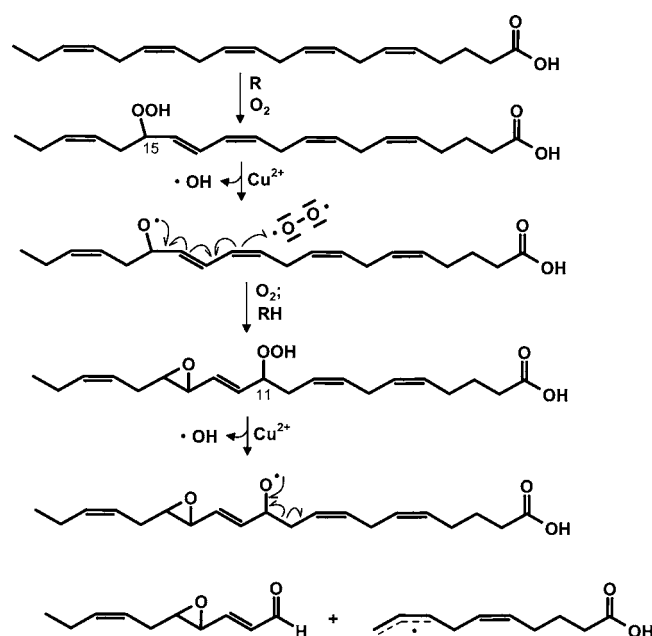


Figure 6. Proposed pathway for the formation of 4,5-epoxy-(E,Z)-2,7-decadienal from the 15-hydroperoxide of eicosapentaenoic acid.

trans-4,5-epoxy-(E,Z)-2,7-decadienal showed a very low odor threshold of ~ 10 pg/L in air (Table 3), thus explaining the high FD factor determined by AEDA.

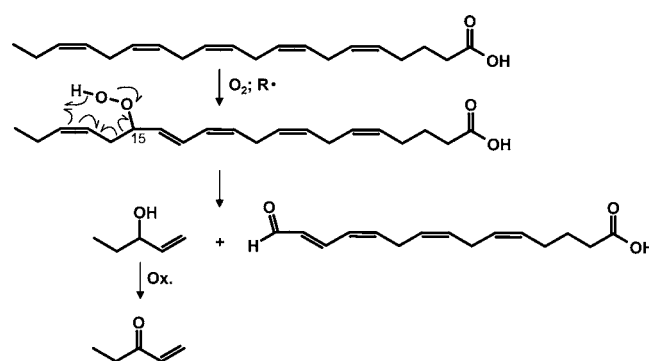


Figure 7. Pathway leading to the formation of 1-penten-3-one from the 15-hydroperoxide of eicosapentaenoic acid.

A comparison of panels A and B of Figure 1 indicated that the enzymatic oxidation of EPA with lipoxygenase led to a pattern of volatiles, which did not elicit the same overall odor profile as the volatiles generated by autoxidation of EPA. The identification experiments performed on the enzymatically generated aroma compounds in combination with the FD factors revealed 1-penten-3-one (pungent, glue-like), (Z)-3-hexenal (green), and *trans*-4,5-epoxy-(E,Z)-2,7-decadienal (metallic) with the highest FD factors (Table 2). Further important odorants were (E,E,Z)-2,4,6-nonatrienal (**22**) and *cis*-4,5-epoxy-2,7-decadienal (**25**) with a metallic odor followed by (Z)-1,5-octadien-3-one. Interestingly, with the exception of the unknown compounds **5** and **24** showing comparatively low

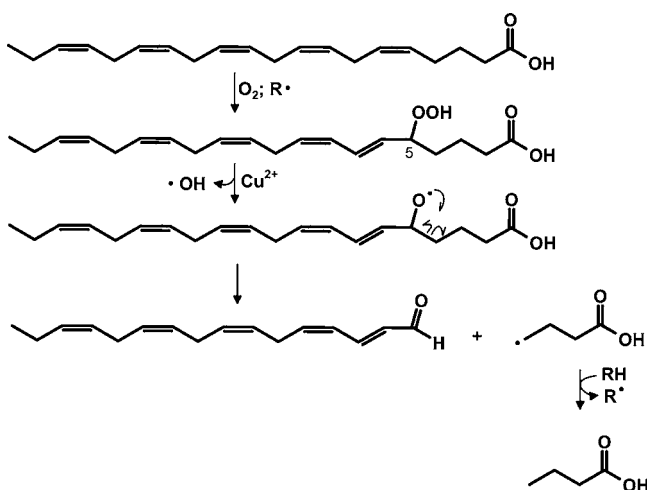


Figure 8. Generation of butanoic acid from the 5-hydroperoxide of eicosapentaenoic acid.

FD factors, all other odorants were identical with those identified in EPA-AUT (Table 2). However, differences in FD factors were observed, in particular for 1-penten-3-one (1), (*Z*)-3-hexenal (2), and the 4,5-epoxy-2,7-decadienal isomer (25), showing much higher FD factors in the enzymatically oxidized EPA, whereas especially (*Z*)-1,5-octadien-3-one (6) and (*Z,Z*)-2,5-octadienal (7) were lower compared to the volatiles from the autoxidized fatty acid.

Oxidation of DHA. In the distillate obtained from autoxidized DHA (DHA-AUT) nearly the same set of 28 odor-active volatiles was generated as in EPA-AUT, among which also *trans*-4,5-epoxy-(*E,Z*)-2,7-decadienal, (*Z*)-1,5-octadien-3-one, (*Z*)-3-hexenal, and (*Z,Z*)-3,6-nonadienal showed the highest FD factors (Table 4). In contrast, as also found for EPA, in the enzymatically oxidized DHA, 1-penten-3-one (1) and (*Z*)-3-hexenal showed higher FD factors compared to the volatiles from the autoxidized acid.

Oxidation of ALA. In the autoxidized α -linolenic acid (ALA-AUT), again *trans*-4,5-epoxy-(*E,Z*)-2,7-decadienal (26), (*Z*)-3-hexenal (2), (*E,E,Z*)-2,4,6-nonatrienal (22), (*Z*)-1,5-octadien-3-one (6), and (*E,Z*)-2,6-nonadienal (12) appeared with the highest FD factors (Table 5), and when the peroxidation was performed with lipoxygenase (Table 5), (*Z*)-3-hexenal and *trans*-4,5-epoxy-(*E,Z*)-2,7-decadienal (26) showed the highest odor activities, followed by 1-penten-3-one and (*E,E,Z*)-2,4,6-nonatrienal (Table 5).

Overall, the results revealed that qualitatively the set of odor-active volatiles generated by either peroxidation or an enzymatic oxidation from all three ω -3 fatty acids was nearly identical, but the odorants differed in their FD factors depending on the acid or on the type of peroxidation applied. Because differences in FD factors are caused by differences in the concentrations, it can be assumed that the differences observed in the overall odor profiles (Figure 1) are caused by the different quantitative compositions of the set of volatiles formed during peroxidation. Also in previous investigations on a fishy taint in anchovies²⁶ or dried spinach,²⁷ it has been observed that (*Z*)-1,5-octadien-3-one, although not eliciting a fish-like odor by itself, in mixture with other compounds can result in fishy off-odors. Therefore, a quantitative determination of the concentrations of the key odorants followed by aroma recombination experiments is a necessary further step in

clarifying the cause of the fishy odor generated especially from autoxidized EPA and DHA.

trans-4,5-Epoxy-(*E,Z*)-2,7-decadienal, identified here for the first time as a degradation product of ω -3 fatty acids, was formed with the highest FD factor from all three ω -3 acids either during autoxidation or during peroxidation by lipoxygenase, respectively. It can be assumed that the formation of the epoxydienal starts from the 15-hydroperoxide of eicosapentaenoic acid (Figure 6), which is then cleaved into the alkoxy radical. Addition of the radical to the double bond and a secondary oxidation at position 11 generates a 14,15-epoxy-11-hydroperoxy fatty acid, which, upon cleavage by a Fenton-type reaction, gives rise to either *cis*- or *trans*-4,5-epoxy-(*E,Z*)-2,7-decadienal. The same reaction is possible from docosahexaenoic acid and α -linolenic acid via the respective 17- or 13-hydroperoxide, respectively.

Another interesting aspect is the fact that 1-penten-3-one was predominantly formed by a LOX-catalyzed oxidation of the three fatty acids as compared to the autoxidation. Its formation may occur also from the 15-hydroperoxide of eicosapentaenoic acid as shown in Figure 7. Also, the 17-hydroperoxide of DHA or the 13-hydroperoxide of ALA may serve as the precursor of 1-penten-3-one in the same reaction. If the 12-hydroperoxide of EPA is formed, or the 9-hydroperoxide from α -linolenic acid, the same reaction will lead to (*Z*)-1,5-octadien-3-one. The ketone has, thus, been identified by several authors in either autoxidized α -linolenic acid,¹⁵ photoxidized ethyl eicosapentaenoic acid,²⁸ or autoxidized ethyl docosapentaenoic acid.^{29,30} Butanoic acid is, however, exclusively formed from autoxidized eicosapentaenoic acid and was also previously reported as a degradation product of ethyl eicosapentaenoate during photooxidation.^{29,30} The formation of butanoic acid can be assumed from the 5-hydroperoxide of EPA as shown in Figure 8, because this is the only PUFA among the three acids investigated with the first double bond in the 5-position.

The results suggest that in particular the ratio of positional hydroperoxides formed during oxidation of ω -3 PUFAs is an important factor in the generation of key aroma compounds. Thus, for example, minimizing the formation of the 15- and 12-hydroperoxides from eicosapentaenoic acid could be an appropriate way to reduce the formation of potent odorants such as (*Z*)-1,5-octadien-3-one involved in the formation of fishy odors.

■ ASSOCIATED CONTENT

Supporting Information

Additional figures. This material is available free of charge via the Internet at <http://pubs.acs.org>.

■ AUTHOR INFORMATION

Corresponding Author

*(P.S.) Phone: +49 8161 712932. Fax: +49 8161 71 2970. E-mail: peter.schieberle@ch.tum.de.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We thank Patrick Selmaier for his help with the interpretation of the NMR spectra.

■ REFERENCES

- (1) Schmidt, E. B.; Arnesen, H.; De Caterina, R.; Rasmussen, L. H.; Kristensen, S. D. Marine ω -3 polyunsaturated fatty acids and coronary heart disease part I: background, epidemiology, animal data, effects on risk factors and safety. *Thromb. Res.* **2005**, *115*, 163–170.
- (2) Schmidt, E. B.; Arnesen, H.; Christensen, J. H.; Rasmussen, L. H.; Kristensen, S. D.; De Caterina, R. Marine ω -3 polyunsaturated fatty acids and coronary heart disease part II: clinical trials and recommendations. *Thromb. Res.* **2005**, *115*, 257–262.
- (3) Burdge, G. C.; Calder, P. Conversion of α -linolenic acid to longer-chain polyunsaturated fatty acids in human adults. *Reprod. Nutr. Dev.* **2005**, *45*, 581–597.
- (4) Shahidi, F.; Cadwallader, K. R. Flavor and lipid chemistry of seafoods: an overview. In *Flavor and Lipid Chemistry of Seafoods*; Shadhi, F., Cadwallader, K. R., Eds.; ACS Symposium Series 674; American Chemical Society: Washington, DC, 1997; pp 1–8 (review).
- (5) Alasalvar, C.; Shahidi, F.; Wantick, P. Food and health: application of marine nutraceuticals: a review. In *Seafoods – Quality, Technology and Nutraceutical Applications*; Alasalvar, C., Taylor, T., Eds.; Springer Verlag: New York, 2002; pp 175–203.
- (6) Cho, S.-Y.; Miyashita, K.; Miyazawa, T.; Fujimoto, K.; Kaneda, T. Autoxidation of ethyl eicosapentaenoate and docosahexaenoate. *J. Am. Oil Chem. Soc.* **1987**, *64*, 876–879.
- (7) Yamauchi, R.; Yamada, T.; Kato, K.; Ueno, Y. Monohydroperoxides formed by autoxidation and photosensitized oxidation of methyl eicosapentaenoate. *Agric. Biol. Chem.* **1983**, *47*, 2897–2902.
- (8) Pan, X.; Kaneko, H.; Ushio, H.; Ohshima, T. Oxidation of all-*cis*-7,10,13,16,19-docosapentaenoic acid ethyl ester. Hydroperoxide distribution and volatile characterization. *Eur. J. Lipid Sci. Technol.* **2005**, *107*, 228–238.
- (9) Aidos, I.; Jacobsen, C.; Jensen, B.; Lutten, J. B.; van der Padt, A.; Boom, R. M. Volatile oxidation products formed in crude herring oil under accelerated oxidative conditions. *Eur. J. Lipid Sci. Technol.* **2002**, *104*, 808–818.
- (10) Pan, X.-Q.; Ushio, H.; Ohshima, T. Comparison of volatile compounds formed by autoxidation and photosensitized oxidation of cod liver oil in emulsion systems. *Fish. Sci.* **2005**, *71*, 639–647.
- (11) Hsieh, T. C. H.; et al. Characterization of volatile components of menhaden fish (*Brevoortia tyrannus*). *J. Am. Oil Chem. Soc.* **1989**, *66*, 114–117.
- (12) Cadwallader, K. R.; Shahidi, F. Identification of potent odorants in seal blubber oil by direct thermal desorption-gas chromatography-olfactometry. In *Omega-3-Fatty Acids. Chemistry, Nutrition and Health Effects*; Shahidi, F., Finley, J. W., Eds.; ACS Symposium Series 788; American Chemical Society: Washington, DC, 2001; pp 221–234.
- (13) Hartvigsen, K.; Lund, P.; Hansen, L. F.; Holmer, G. Dynamic headspace gas chromatography/mass spectrometry characterization of volatiles produced in fish oil enriched mayonnaise during storage. *J. Agric. Food Chem.* **2000**, *48*, 4858–4867.
- (14) Venkateshwarlu, G.; Let, M. B.; Meyer, A. S.; Jacobsen, C. Modeling the sensory impact of defined combinations of volatile lipid oxidation products on fishy and metallic off-flavors. *J. Agric. Food Chem.* **2004**, *52*, 1635–1641.
- (15) Ullrich, F.; Grosch, W. Identification of the most intense odor compounds formed during autoxidation of methyl linolenate at room temperature. *J. Am. Oil Chem.* **1988**, *65*, 1313–1317.
- (16) Hsieh, R. J.; Kinsella, J. E. Lipoxygenase generation of specific volatile flavor carbonyl compounds in fish tissues. *J. Agric. Food Chem.* **1989**, *37*, 279–286.
- (17) Josephson, D. B.; Lindsay, R. C. Enzymic generation of volatile aroma compounds from fresh fish. In *Biogenesis of Aromas*; Parliament, T. H., Croteau, R., Eds.; ACS Symposium Series 317; American Chemical Society: Washington, DC, 1986; pp 201–219.
- (18) Milo, C.; Grosch, W. Changes in the odorants of boiled trouts (*Salmo fario*) as affected by the storage of the raw material. *J. Agric. Food Chem.* **1993**, *41*, 2076–2081.
- (19) Schieberle, P.; Grosch, W. Potent odorants of the wheat bread crumb. *Z. Lebensm. Unters. Forsch.* **1991**, *192*, 130–135.
- (20) Schuh, C.; Schieberle, P. Characterization of (*E,E,Z*)-2,4,6-nonatrienal as a character impact aroma compound of oat flakes. *J. Agric. Food Chem.* **2005**, *53*, 8699–8705.
- (21) Esterbauer, H. On the autoxidation of linoleic acid in water: III. Chromatographic separation of water-soluble reaction products (in German). *Fette, Seifen, Anstrichm.* **1968**, *70*, 1–4.
- (22) Yoshida, T.; Hall, J. B. Flavoring with 5-methyl-3,5-octadien-2-one. U.S. Patent 4,301,184, 1981.
- (23) Engel, W.; Bahr, W.; Schieberle, P. Solvent assisted flavour evaporation – a new and versatile technique for the careful and direct isolation of aroma compounds from complex food matrices. *Eur. Food Res. Technol.* **1999**, *209*, 237–241.
- (24) Czerny, M.; Christlbauer, M.; Fischer, A.; Granvogl, M.; Hammer, M.; Hartl, C.; Moran Hernandez, N.; Schieberle, P. Re-investigation on odor thresholds of key food aroma compounds and development of an aroma language based on odor qualities of defined aqueous odorant solutions. *Eur. Food Res. Technol.* **2008**, *228*, 265–273.
- (25) Lin, J.; Fay, L. B.; Welti, D. H.; Blank, I. Synthesis of *trans*-4,5-epoxy-(*E*)-2-decenal and its deuterated analog used for the development of a sensitive and selective quantification method based on isotope dilution assay with negative chemical ionization. *Lipids* **1999**, *34*, 1117–1126.
- (26) Triqui, R.; Guth, H. Determination of potent odorants in ripened anchovy (*Engraulis encrasicolus* L.) by aroma extract dilution analysis and by gas chromatography-olfactometry of headspace samples. In *Flavor and Lipid Chemistry of Seafoods*; Shahidi, F., Cadwallader, K. R., Eds.; ACS Symposium Series 674; American Chemical Society: Washington DC, 1997; pp 31–38.
- (27) Masanetz, C.; Guth, H.; Grosch, W. Fishy and hay-like off-flavours of dry spinach. *Z. Lebensm. Unters. Forsch.* **1998**, *206*, 108–113.
- (28) Frankel, E. N.; Neff, W. E.; Selke, E. Analysis of autoxidized fats by gas chromatography-mass spectrometry: VII. Volatiles thermal decomposition products of pure hydroperoxides from autoxidized and photosensitized oxidized methyl oleate, methyl linoleate and linolenate. *Lipids* **1981**, *16*, 279–285.
- (29) Pan, X.; Ushio, H.; Ohshima, R. Photo-oxidation of lipids impregnated on the surface of dried seaweed (*Porphyr a yezoensis* Ueda). Characterization of volatiles. *J. Am. Oil Chem. Soc.* **2004**, *81*, 773–781.
- (30) Pan, X.; Kaneko, H.; Ushio, H.; Ohshima, R. Oxidation of all-*cis*-7,10,13,16,19-docosapentaenoic acid ethyl ester. Hydroperoxide distribution and volatile characterization. *Eur. J. Lipid Sci. Technol.* **2005**, *107*, 228–238.