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Quantitative analysis of volatiles in edible oils following accelerated oxidation using broad spectrum isotope standards

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Abstract

Analysis of food volatiles generated by processing are widely reported but comparisons across studies is challenging in part because most reports are inherently semi-quantitative for most analytes due to limited availability of chemical standards. We recently introduced a novel strategy for creation of broad spectrum isotopic standards for accurate quantitative food chemical analysis. Here we apply the principle to quantification of 25 volatiles in seven thermally oxidized edible oils. After extended oxidation, total volatiles of high n-3 oils (flax, fish, cod liver) were 120-170 mg/kg while low n-3 vegetable oils were <50 mg/kg. Separate experiments on thermal degradation of d5-ethyl linolenate indicate that off-aroma volatiles originate throughout the n-3 molecule and not solely the n-3 terminal end. These data represent the first report using broad-spectrum isotopically labeled standards for quantitative characterization of processing-induced volatile generation across related foodstuffs, and verify the origin of specific volatiles from parent n-3 fatty acids.

Keywords

n-3 fatty acids; broad spectrum isotopic standards; terminal end; off-flavours; autoxidation; HS-SPME-GC/TOF-MS

1. INTRODUCTION

Oxidative stability is the major challenge in the incorporation of omega-3 fatty acids (n-3 FA) into food products (Decker, Akoh, & Wilkes, 2012). These lipids contain numerous 1,4-*cis*-pentadiene systems that are prone to hydrogen abstraction and subsequent free-radical

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APPENDIX

Supplementary data (Figure S1 and Table S1) associated with this article can be found, in the online version, at ScienceDirect: <http://www.sciencedirect.com>.

oxidation reactions. Thus, their presence significantly shortens the shelf-life of the supplemented food and can even give rise to uncontrolled oxidation problems (Kolanowski, Jaworska, Laufenberg, & Weissbrodt, 2007). Most vegetable oils, except the rarely used flax oil, contain a relatively low proportion of n-3 polyunsaturated fatty acids (n-3 PUFA). Conversely, marine oils contain high amounts of these fatty acids (FA), especially eicosapentaenoic acid (EPA, 20:5 n-3) and docosahexaenoic acid (DHA, 22:6 n-3) which can account for one third or more of total FA.

Lipid autoxidation also causes significant changes to the sensory properties and consumer acceptance of food products including odour, flavour, colour and texture (Jacobsen, 2010). While hydroperoxides, the primary products of lipid autoxidation, are odourless and tasteless, their degradation leads to the formation of complex mixtures of low-molecular-weight compounds with distinctive aromas. (Shahidi & Pegg, 1994). Principally, these include alkanes, alkenes, aldehydes, ketones, alcohols, esters, epoxides, and FA. Those of greatest importance to the aroma of oils rich in n-3 PUFA appear to be medium-chain unsaturated aldehydes and ketones (Ho & Chen, 1994; Genot, Meynier, & Riaublanc, 2003).

While a few comparative studies on volatile profiles of oils exist, these are compromised by the fact that analyses relied on a limited number of internal standards (usually, only one) to quantify a diverse range of compounds (Bendini, Barbieri, Valli, Buchecker, Canavari, & Toschi, 2011; Mildner-Szkudlarz, Jelen, Zawirska-Wojtasiak, & Wasowicz, 2003; Uriarte, Goicoechea, & Guillen, 2011). Quantitative analyses of volatiles by headspace solid phase microextraction (HS-SPME) GC-MS require the use of internal standards to correct matrix effects, losses of analytes during sample preparation and GC-MS response. Ideally, these standards are stable isotopically labeled analogues of the compounds of interest, as these possess near-identical physiochemical properties to the analytes (Grosch, 2001). Assembling an array of standards presents a daunting challenge to studies of complex foodstuffs such as oxidized oils which may possess hundreds of analytes of possible interest. Because of the cost associated with preparing labeled standards, a more typical approach for analysis of edible oils is to use a small number of non-native compounds as internal standards. This approach may result in poor accuracy both because of differences in MS response between the analyte and standard, but also because of compound-dependent variation in extraction efficiency across matrices. For example, we recently reported that matrix effects observed in HS-SPME-GC-MS analyses of soybean oil could result in errors of over an order of magnitude for some oil-derived volatiles when a single internal standard is employed, even if calibration curves were run with external standards (Gómez-Cortés, Brenna, & Sacks, 2012). As a result, volatile concentrations reported in previous profiling studies may be inaccurate.

We recently presented a method to overcome the limitations for quantitative analysis of oil autoxidation by creating a broad-spectrum mixture of isotopically labeled standard prepared from controlled oxidation of [U-¹³C]-linolenic acid (Gómez-Cortés, Brenna, & Sacks, 2012). Here, we quantify 25 volatiles derived from n-3 FA in oils with various initial FA compositions using headspace solid phase microextraction gas chromatography time-of-flight mass spectrometry (HS-SPME GC/TOF-MS) using our novel uniformly isotopically labeled [U-¹³C] standards, and apply the method for the first time using a familiar and

important food chemical problem. Additionally, we investigated whether these volatiles are derived solely from the n-3 terminal end or from interior portions of the fatty acid.

2. MATERIALS AND METHODS

2.1. Chemicals and Oils

Polyethylene glycol (PEG 400) and non-labeled ethyl linolenate standard (98%) were purchased from Sigma-Aldrich (St Louis, MO, USA). The isotopically labeled [17,17',18,18',18''-d5]-ethyl linolenate (98%) and [U-¹³C]-linolenic acid (99%) standards were obtained from Cambridge Isotope Laboratories (Andover, MA, USA). The seven oils used in the study are as follows. (1) Partially hydrogenated vegetable oil (PHVO), specifically soybean oil, and (2) low α -linolenic acid soybean oil (LSO) containing nominally 55% linoleic acid and 3% α -linolenic acid were gifts of Bunge Ltd (White Plains, NY, USA); (3) refined commodity soybean oil (CSO) with nominally 55% linoleic acid and 7% linolenic acid (Wegman's Food Markets, Inc, Rochester, NY, USA); (4) expeller pressed refined walnut oil (WO; Spectrum Naturals, Lake Success, NY, USA); (5) cold pressed unrefined, unfiltered flax oil (FO; Barlean's Ferndale, WA, USA); (6) cod liver oil (CLO; Solgar, Inc, Leonia, NJ, USA) containing 28 mg DHA and 28 mg EPA per 460 mg, and (7) refined fish oil (FO; Wegman's Food Markets, Inc, Rochester, NY, USA) were obtained in the form of capsules which were carefully opened and oil removed. The oil is a blend of anchovy, herring, and sardine oils. All oils were used as set forth below without further processing. Detailed fatty acid profiles of all oils prior to oxidation are provided in Supplementary Table S1, determined as described below.

2.2. Oil FA Composition Analysis

FA compositions of the seven oils prior to oxidation were determined by the one step extraction/methylation method described by previously (Zhou, Nijland, Miller, Ford, Nathanielsz, & Brenna, 2008). Analyses were performed in triplicate on a HP 5890 Series II gas chromatograph coupled to a flame ionization detector (GC-FID) (Hewlett Packard, Palo Alto, CA, USA) equipped with a BPX70 fused-silica capillary column (25m $\times$ 0.22 mm i.d. $\times$ 0.25 μ m film thickness; SGE Inc., Austin, TX, USA). The column temperature program was as follows: the initial temperature of 80°C was ramped up to 170°C at 30°C/min, 2 min hold, then increased to 240°C at 10°C/min, 14 min hold. The injector was at 250°C in splitless mode and hydrogen was used as carrier gas at a flow rate of 1mL/min.

2.3. Oil Oxidation

Oils were oxidized following the protocol described in the AOCS (1999)Oven Storage Test Cg 5-97. Ten millilitres of each oil were placed in an amber 20 mL solid-phase microextraction (SPME) vial, sparged with O₂ for 5 min, sealed with Teflon and vortexed. Oils were incubated at 60°C in the dark for 0, 3, 6, 9, 12 and 15 days. A separate SPME vial was used for each sampling time. After oxidation, the headspace (HS) of the container was sparged with N₂ and stored at -80°C until volatile analysis (AOCS, 1999).

2.4. HS-SPME-GC/TOF-MS Volatile Analyses

Volatiles were extracted by HS-SPME using a LEAP CombiPAL autosampler (Carrboro, NC, USA) as described previously (Gómez-Cortés, Brenna, & Sacks, 2012). Samples were incubated for 10 min at an agitation rate of 300 rpm and with an incubation temperature of 50°C prior to fibre insertion. A 2 cm 50/30 µm divinylbenzene/carboxen/poly(dimethylsiloxane) SPME fibre (Supelco, Bellefonte, PA, USA) was then introduced into the HS and the vial was agitated at 100 rpm for 20 min at 50°C.

Following HS-SPME, volatiles were thermally desorbed from the fiber into the injector of an Agilent 6890 gas chromatograph coupled to a time-of-flight mass spectrometer (GC/TOF-MS, Pegasus 4D, LECO Corp., St. Joseph, MI, USA). SPME injections were splitless with 5 min of desorption at 250°C. The GC column was a DB-FFAP capillary column (30 m × 0.25 mm i.d. × 0.25 µm film thickness; Agilent Technologies Inc., New Castle, DE, USA). The column temperature program was as follows: initial hold for 3 min at 40°C, followed by a 5°C/min ramp to 185°C and then, 8°C/min ramp to 240°C, 5 min hold. Helium was the carrier gas at a flow rate of 1 mL/min, and the detector temperature was 200°C. The TOF-MS was operated in electron impact mode with an ionization energy of 70 eV. The electron multiplier was set to 1500 V. MS data were stored at an effective acquisition rate of 5 spectra/s over a mass range of m/z 35–400, and data processing was carried out by the native LECO ChromaTOF software.

For quantitation purposes, a mixture of [U-¹³C] volatile standards was generated via [U-¹³C]-linolenic acid degradation as described in previously (Gómez-Cortés, Brenna, & Sacks, 2012). A 30 µL sub-sample of the [U-¹³C] mixture was added to each oxidized oil aliquot (220 µL) in a 20 mL amber SPME vial. All samples were analyzed in quadruplicate by HS-SPME-GC/TOF-MS to quantify the volatiles derived from n-3 FA oxidation.

Odour activity values (OAV) were calculated for seven selected volatiles (acetaldehyde, propanal, 2,3-pentanedione, hexanal, (E)-2-pentenal, acetic acid and (E,E)-2,4-heptadienal) based on the availability of their odours thresholds in oil in the literature. OAV was calculated as (odourant concentration) / (odourant threshold in oil).

2.5. Characterizing Provenance of n-3 Derived Volatiles using Deuterated Ethyl Linolenate

To characterize the volatiles arising from the terminal end of n-3 FA, 5 mg of either unlabeled ethyl linolenate or [17,17',18,18',18''-d5]-ethyl linolenate standards were added to an amber 20 mL SPME vial and dissolved into 500 mg of PEG 400. Both vials (i.e. unlabeled 18:3 and d5-18:3) were sparged with O₂ for 5 min, sealed with Teflon, vortexed and incubated at 60°C in the dark. After 72h of oxidation, vials were sparged with N₂ and directly analyzed under the conditions described in section 2.4.

2.6. Statistical Analysis

Statistical analysis was conducted with JMP Version 9 (SAS Institute, Cary, NC). Initial exploration of the data was performed by principal component analysis (PCA) on all the time points (0, 3, 6, 9, 12 and 15 days) of the 7 oils (PHVO, LSO, CSO, WO, Flax oil, FO and CLO). A direct visual scree test performed on the eigenvalue distribution showed two

factors were sufficient. Paired comparisons, using Tukey's HSD test, were then used to compare oils volatile composition. $P < 0.05$ was considered to be statistically significant.

3. RESULTS AND DISCUSSION

3.1. FA Composition and Total Volatile Production from Commercial Oils

Table S1 (see the Supplementary Data) shows the FA composition of the 7 oils selected for the autoxidation study. The oils were chosen based on differences in their n-3 content. The FA profiles coincide with the common composition of these oils and are in agreement with those reported previously (Moffat & McGill, 1993; Tompkins & Perkins, 2000; Uriarte, Goicoechea, & Guillen, 2011). As expected, the only n-3 FA detected in vegetable oils corresponds to α -linolenic acid (ALA, *cis*-9 *cis*-12 *cis*-15 18:3) which accounted for 0.5, 3, 7, 12 and 56% of the total fat in PHVO, LSO, CSO, WO and flax oil, respectively (Table S1, Supplementary Data). The low oxidative stability associated with ALA (i.e. in comparison to linoleic or oleic acids) is commonly related to its higher number of double bonds, as an increase in the number of double bonds increases the rate of autoxidation of the oils, a chemical reaction with very low activation energy (Zhu & Sevilla, 1990). For example, due to the sensitivity to oxidation of soybean oil, in order to increase the storage stability and improve its performance in frying applications, the FA profile of soybean oil is normally altered. By lowering the ALA levels to approximately 3%, low-ALA soybeans create a more stable oil and eliminate the need for partial hydrogenation, a process that creates *trans* fats, which have been linked to heart disease and other health concerns (Dhaka, Gulia, Ahlawat, & Khatkar, 2011). On the other hand, flax oil and in a lesser extent WO are expected to be particularly susceptible to oxidation as their concentrations of ALA were roughly eight-fold and two-fold greater than in CSO. The present study also included two marine oils (FO and CLO) that are well known for their high levels of n-3 PUFA and their susceptibility to autoxidation (De Leonardis & Macciola, 2006). Although total n-3 FA contents were lower than in flax oil, these marine oils were characterized by major levels of highly unsaturated EPA (20 and 10% of total FA methyl esters in FO and CLO, respectively) and DHA (11 and 8% of total FA methyl esters in FO and CLO, respectively).

Figure S1 (see *Supplementary Data*) presents the total concentration of volatile compounds derived from n-3 FA when these 7 oils were subjected to thermal oxidation. Increasing oxidation time was correlated with greater volatile production, and the highest volatile production was observed at 15 d for all oils. As expected, the total volatile concentration was much greater in oils with high levels of n-3 FA such as flax oil, FO or CLO than in vegetable oils with lower levels of n-3 FA. PHVO was the least susceptible to oxidation and generated the lowest levels of volatiles (Figure S1, Supplementary Data), followed by LSO. Although flax oil had the greatest levels of total n-3 FA, marine lipids yielded more volatiles and thus were more prone to degradation. Furthermore, a sharp increase between day 0 and day 3 was observed in total volatile concentration of FO and CLO (Figure S1, Supplementary Data).

The greater oxidizability of EPA- and DHA-containing marine oils as compared to vegetable oils can be rationalized by differences in the dissociation energy of the carbon-hydrogen (C-H) bonds and their susceptibility to proton abstraction. In general, the

dissociation energy of the C-H bond at *bis*-allylic methylene groups is lower than at allylic methylene groups (50 kcal/mole vs 75 kcal/mole), whereas the dissociation energy of the C-H bond at methylene groups without adjacent double bonds is markedly higher (approximately 100 kcal/mol) (Min & Boff, 2002). Hence, PUFA are more easily oxidized than monounsaturated and saturated FA (Eder & Ringseis, 2010). Among PUFA, increasing the number of *bis*-allylic methylene groups increases susceptibility to oxidation; for the FA series linoleic acid (18:2), ALA, arachidonic acid (20:4), EPA, DHA, each *bis*-allylic methylene group increases the relative rate of oxidation two-fold (Cosgrove, Church, & Pryor, 1987). Our work shows that the degree of volatile production is related to the presence of FA with greater number of *bis*-allylic positions, which is in turn critically related to the risk of autoxidative rancidity and spoilage.

3.2. Quantitative Distribution of n-3 Volatiles

Table 1 presents the quantitative distribution of volatile compounds derived from n-3 FA oxidation in the 7 oils after 15 days of autoxidation. These data is very valuable because most HS-SPME-GC/MS oil oxidation studies only provide semi-quantitations of the volatile components (Jelen, Mildner-Szkudlarz, Jasinska, & Wasowicz, 2007; Petersen, Kleeberg, Jahreis, & Fritsche, 2012; Uriarte, Goicoechea, & Guillen, 2011). Quantitative analyses in our current work were performed using a previously developed uniformly labeled [U-¹³C] volatile mixture, derived from thermal degradation of a [U-¹³C]-ALA standard (Gómez-Cortés, Brenna, & Sacks, 2012). In total, 25 compounds were identified and quantified (Table 1). The majority of the volatiles were aldehydes (four alkanals, three alkenals and one dienal) and ketones (three saturated, three unsaturated, three hydroxy ketones and one α -diketone). Two unsaturated alcohols, two carboxylic acids, two alkyl furanones and one alkyl furan were also found. These compounds represent characteristic groups of FA secondary oxidation products, resulting mainly from autoxidation of ALA (Gómez-Cortés, Brenna, & Sacks, 2012).

To obtain an overview of the similarities and differences among the autoxidized oils, a PCA was performed on the volatile profiles for each oil at every time point (0, 3, 6, 9, 12 and 15 days). The PCA scores plot is shown in Figure 1 (top). The first two principal components accounted for 82.3 and 8.28%. Oils separated based on a combination of storage time and oil type in the first dimension. Low n-3 oils segregated to the left, and higher n-3 oils (flax oil, FO or CLO) to the right along PC1, with further discrimination of the high n-3 oils by storage time. In the loadings plot (Figure 1, bottom), all volatiles were located on the right side of the plot and thus PC1 is separating based on total volatile production, similar to data in Figure S1 (Supplementary Data). PC2 differentiated the high ALA flax oil from the high DHA and EPA marine oils (FO and CLO). Based on the loadings plot, flax oil was characterized by higher concentrations of saturated, unsaturated and hydroxymethyl ketones (2-propanone, 2-pentanone, 2-hydroxy-3-pentanone, 1-hydroxy-2-butanone, (E)-3-penten-2-one and 3-hexen-2-one) while the marine oils were characterized by higher concentrations of a diverse range of compound, including aldehydes (1-penten-3-ol, 2-ethylfuran, butanal, 2-propenal and 2,3-pentanedione; Figure 1).

The differences between the volatile profiles of oils were further investigated by ANOVA of each volatile for the seven oils at the final time point, day 15 (Table 1). Compounds that discriminated marine and flax oils in the loading plot of PC2 in Figure 1 generally differed significantly. For example, butanal was 3-fold higher in the marine oils, while 2-pentanone was 50% higher in flax oil. To our knowledge, these differences in volatile profile have not been previously reported, although the reasons for their appearance are unclear.

From a quantitative point of view, the most prominent volatile degradation products from n-3 FA were (E,E)-2,4-heptadienal, acetaldehyde, acetic acid, propanal, propanoic acid and 2-propenal (Table 1). The high concentration of aldehydes could explain the off-flavour and limited frying life usually associated with n-3 oils. Aldehydes tend to be more potent, more stinky and can react with amino groups to produce imino Schiff bases, which themselves polymerize by aldol condensation to dimers and complex high-molecular-weight brown macromolecules known as melanoidins (Eder & Ringseis, 2010). This reaction significantly contributes to the development of the typical flavour of fried foods and causes significant losses of labile amino acids reducing the nutritive value of food proteins (Eder & Ringseis, 2010). Furthermore, aldehydes are longer lived than free radicals and its increased dietary intake may potentially adversely affect health (Dyall, 2011). On the other hand, 2-propenal is known for reacting with phenolic hydroxyl groups from other food products to yield bitter tasting compounds (Novo, Quirós, Morales, & González, 2012).

The volatile compounds quantified in the present study have a large range of sensory thresholds. To better evaluate the potential contribution of these compounds to aroma in oil samples, OAV were calculated (Table 2). In flax oil, FO and CLO, the seven odourants were present in concentrations above their odour thresholds. By far the highest OAV was calculated for the sweet burning-smelling acetaldehyde. Although (E,E)-2,4-heptadienal was the major volatile compound from n-3 autoxidized PUFA oils, it also has a high threshold (10000 ng/g of oil, Table 2) and is thus less likely to contribute to the aroma. (E)-2-pentenal and “sharp-irritating” propanal were below thresholds in PHVO, LSO and CSO (OAV < 1) with supra- or peri-threshold concentrations only observed in the high n-3 oils (Table 2). Surprisingly, the butter-like 2,3-pentanedione had a high OAV, due to its low odour threshold (0.3 ng/g of oil, Table 2). The OAV data should be interpreted with care as they ignore additive and masking effects that often occur. Moreover, these OAV were obtained using odour thresholds in oil and cannot be extrapolated to food systems with more complex matrices. Oxidized oils contain a complex mixture of different volatiles and the relationship between their concentration and the sensory impact is still poorly understood (Jacobsen, 2010). Additionally, the SPME-GC/TOF-MS method employed is less sensitive to volatiles with more than eight or more carbons due to their limited volatility, and several longer chain volatiles (e.g. 1-octen-3-one, 2,4,7-decatrinal) have been reported to be important to the aroma of oxidized n-3 oils (Genot, Meynier, & Riaublanc, 2003; Venkateshwarlu, Let, Meyer, & Jacobsen, 2004). Finally, the presence of other ingredients during cooking could significantly alter the odour impression by reason of dilution, masking, or the formation of new volatiles (Urbach & Gordon, 1995).

3.3. Origin of Volatiles from n-3 FA Oxidation

It is well known that ALA autoxidation occurs at a higher rate than linoleic (18:2 n-6) and oleic (18:1 n-9) acids degradation (Hsieh & Kinsella, 1989; Lea, 1952). Although the degree of unsaturation is a major determining factor in the rate of lipid oxidation, it is unclear how the n-3 terminal end contributes to volatile formation. To address the molecular origin of the volatile compounds quantified in the present study, unlabeled ethyl linolenate and [17, 17', 18, 18', 18''-d5]-ethyl linolenate standards were purposively degraded by heat treatment and the mass spectra (MS) of corresponding volatiles compared. As an example, Figure 2 shows the MS of four ALA degradation metabolites that derive from the n-3 terminal end. The d5-propanal MS has prominent ions at m/z 62 and 63 (M^+), 5 amu higher than the respective ions m/z 57 and 58 (M^+) from unlabeled propanal. A similar shift was also detected for M^+ in unlabeled (E)-2-pentenal and d5-(E)-2-pentenal (m/z 84 and 89, respectively), for unlabeled 2,3-pentanedione and d5-2,3-pentanedione (m/z 100 and 105, respectively), and for unlabeled 5-ethyl-2(5H)-furanone and d5-5-ethyl-2(5H)-furanone (m/z 112 and 117, respectively). After degradation of [17, 17', 18, 18', 18''-d5]-ethyl linolenate standard, these unlabeled volatiles (propanal, (E)-2-pentenal, 2,3-pentanedione, and 5-ethyl-2(5H)-furanone) were not detected in the chromatogram. Thus, we can assert that they derive almost exclusively from the n-3 terminal end of the molecule.

In contrast, Figure 3 presents the partial chromatogram and the MS of two volatiles whose origin is the terminal end and other parts of the n-3 molecule at different ratios (butanal and 2-butanone). After [17, 17', 18, 18', 18''-d5]-ethyl linolenate autoxidation, two peaks were detected for each compound (d5-labeled and unlabeled). Both d5-butanal and d5-2-butanone MS have the molecular ion at m/z 77, 5 amu higher than their respective unlabeled compounds ($M^+ = 72$). Unlabeled butanal is characterized by the McLafferty rearrangement (ML, m/z 44) and loss of the methyl group (m/z 57) while these ions are detected at m/z 45 and 59 in the deuterated form. On the other hand, unlabeled 2-butanone presents ions at m/z 43 (base peak, loss of ethyl group) and m/z 57 (loss of methyl) which were also observed in d5-2-butanone (m/z 43 and 62, respectively). The equivalents of terminal end contributing to each volatile were calculated by comparing the relative peak heights (unlabeled analyte / d5-analyte $\times 100\%$) measured by HS-SPME-GC/TOF-MS (Table 3). Accordingly, the amount of butanal and 2-butanone that derives from the ALA n-3 terminal end would be 65 and 45%, respectively (Figure 3 and Table 3).

The n-3 terminal end was the precursor of most of the volatiles quantified in the present study (at least 14 from the 25). Although volatiles with 5 carbon atoms derived almost exclusively from the ALA n-3 terminal end, most 3- and 4-carbon compounds contained <100%, indicating that these smaller molecules can be derived from interior regions of ALA (Table 3).

The formation of short chain aldehydes from interior sites of ALA can be rationalized based on the mechanism of unsaturated FA autoxidation (Ho & Chen, 1994). ALA autoxidation generates 9-, 12-, 13- and 16- conjugated diene-triene hydroperoxide isomers in various proportions (30, 12, 12 and 46%, respectively) (Frankel, 1984). Thermal decomposition of the hydroperoxide involves the homolytic cleavage of the O-O bond to produce alkoxyl and

hydroxyl radicals. The alkoxy radical undergo C-C bond scissions on either sides to produce an alkyl radical on one side and a vinyl radical on the other which lead to a large number of volatile products including alkanes, alkenes, aldehydes, ketones, alcohols, esters, and acids. Many aldehydes could be produced by scission of the lipid molecules on either side of the radical, which would explain why they were not derived entirely from the n-3 terminal end (e.g. acetaldehyde, 2-propenal, butanal and hexanal, Table 3). Regarding other aldehydes, propanal is well known to be specifically formed from the oxidation of n-3 PUFA (Liang, Wang, Simon, Shahidi, & Ho, 2007). (E)-2-butenal and (E)-2-pentenal were fully derived from the n-3 terminal end and their levels in all oils were closely related ($R^2 = 0.987$). These three compounds all share structural similarity to the n-3 terminal end.

Peroxy radical cyclization is believed to be the mechanism for formation of cyclic volatiles in the autoxidation of FA having 3 or more double bonds (Porter, Caldwell, & Mills, 1995). Adams et al. proposed a mechanism for the formation of 2-alkylfurans from the corresponding (E)-2-alkenals (Adams, Bouckaert, Van Lancker, De Meulenaer, & De Kimpe, 2011), e.g. 2-pentenal would be the precursor of 2-methylfuran and 2-hexenal would be the precursor of 2-ethylfuran. We observed that 2-pentenal and 2-ethylfuran originated exclusively from the ALA n-3 terminal end, most likely following the pathway hypothesized by Adams et al (Adams, Bouckaert, Van Lancker, De Meulenaer, & De Kimpe, 2011). 5-ethyl-2(5H)-furanone was also derived from the terminal end (Table 3). In this case, it would be generated via oxidation of (Z)-3-hexenal, although this compound was not quantified in our study. This mechanism involves the conversion of (Z)-3-hexenal to the peracid followed by the oxidation of the (Z)-3-double to form an epoxide. Then, in an intramolecular process, the peracid would open the epoxide ring to first form the hydroxy lactone and then dehydrate to yield 5-ethyl-2(5H)-furanone (Buttery & Takeoka, 2004). Although we could not determine the source of 5-methyl-2(5H)-furanone due to its low signal, it would also derive from the ALA n-3 terminal end due to its close relation detected with 5-ethyl-2(5H)-furanone ($R^2 = 0.921$).

Our results indicate that cyclic volatiles and volatiles with 5 carbon atoms derive almost exclusively from the terminal end of n-3 FA. However, the origin of lower molecular weight compounds (with 4 or 3 carbon atoms) is more variable, with compounds sharing structural similarity to the n-3 terminal end being more likely to derive from the terminal end. However, more FA autoxidation studies quantifying volatiles and high molecular weight compounds are needed to determine whether the susceptibility to oxidation of oils rich in n-3 PUFA is solely due to the terminal end or to the total number of *bis*-allylic groups in the molecules.

4. CONCLUSIONS

In this study, we provide quantitative data of 25 volatile compounds derived from the thermal degradation of seven oils with different n-3 FA content using a new methodology that relies on a range of [U-13C]-labeled compounds generated from the thermal oxidation of [U-13C] linolenic acid. In addition, autoxidation of [17,17',18,18',18''-d5]-ethyl linolenate revealed that the n-3 terminal end of PUFA is not the only responsible of volatiles commonly related with off-aromas.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- A broad spectrum isotopic standard facilitates quantitative analysis
- Twenty-five volatiles derived from n-3 polyunsaturated oil degradation were determined.
- High n-3 oils flax and marine oil generate volatiles at greater rates than low n-3 oils
- Volatiles originated throughout the n-3 molecule, not just the terminal end.

Figure 1.

Scores plot (top) and loadings plot (bottom) for principal component analysis carried out on the volatile data profile of all oils at all time points.

Figure 2.

Comparison of mass spectra of four volatile compounds obtained by thermal degradation of unlabeled ethyl linolenate and [17, 17', 18, 18', 18''-d5]-ethyl linolenate standards: (A) Propanal, (A') d5-Propanal, (B) (E)-2-Pentenal, (B') d5-(E)-2-Pentenal, (C) 2,3-Pentanedione, (C') d5-2,3-Pentanedione, (D) 5-Ethyl-2(5H)-Furanone and (D') d5-5-Ethyl-2(5H)-Furanone.

Figure 3.

Mass spectra and partial GC chromatogram displaying m/z 72 and m/z 77 illustrating separation of d5-Butanal and d5-2-Butanone from their parallel unlabeled compounds.

* ML, McLafferty rearrangement.

Table 1

Quantification of volatile compounds in oils with different n-3 fatty acid content after 15 days of oxidation. Data presented as mean $\pm$ SD; units are ng compound per gram oil.

Compound (ng/g oil)	PHVO ¹	LSO ²	CSO ³	WO ⁴	Flax Oil	FO ⁵	CLO ⁶
Acetaldehyde	-	3571 $\pm$ 131 ^c	5826 $\pm$ 702 ^{bc}	9063 $\pm$ 1362 ^b	13972 $\pm$ 4084 ^a	15354 $\pm$ 1688 ^a	18696 $\pm$ 2352 ^a
Propanal	289 $\pm$ 56 ^d	2109 $\pm$ 204 ^d	3008 $\pm$ 182 ^d	6444 $\pm$ 582 ^c	12729 $\pm$ 2973 ^b	14269 $\pm$ 1853 ^{ab}	16587 $\pm$ 1475 ^a
2-Propanone	273 $\pm$ 26 ^d	48 $\pm$ 4 ^d	121 $\pm$ 4 ^c	76 $\pm$ 4 ^d	438 $\pm$ 29 ^a	314 $\pm$ 27 ^b	293 $\pm$ 4 ^b
2-Propenal	-	416 $\pm$ 15 ^d	694 $\pm$ 40 ^d	954 $\pm$ 102 ^d	1773 $\pm$ 224 ^c	4071 $\pm$ 75 ^b	5847 $\pm$ 696 ^a
Butanal	70 $\pm$ 36 ^c	40 $\pm$ 4 ^c	81 $\pm$ 17 ^c	185 $\pm$ 24 ^c	584 $\pm$ 99 ^b	1870 $\pm$ 248 ^a	1674 $\pm$ 126 ^a
2-Butanone	6.0 $\pm$ 0.3 ^d	13.2 $\pm$ 0.4 ^d	24 $\pm$ 1 ^d	65 $\pm$ 4 ^c	227 $\pm$ 18 ^a	227 $\pm$ 16 ^a	194 $\pm$ 4 ^b
2-Ethylfuran	2.1 $\pm$ 0.3 ^e	5.1 $\pm$ 0.5 ^e	26 $\pm$ 2 ^{de}	99 $\pm$ 4 ^d	700 $\pm$ 17 ^c	2336 $\pm$ 48 ^b	4364 $\pm$ 76 ^a
2-Pentanone	19 $\pm$ 5 ^d	10.34 $\pm$ 0.04 ^d	21 $\pm$ 7 ^d	22 $\pm$ 14 ^d	174 $\pm$ 12 ^a	92 $\pm$ 13 ^b	60 $\pm$ 9 ^c
1-Penten-3-one	3.5 $\pm$ 0.3 ^e	25.5 $\pm$ 0.5 ^e	59 $\pm$ 2 ^{de}	124 $\pm$ 4 ^d	496 $\pm$ 30 ^c	933 $\pm$ 77 ^a	816 $\pm$ 61 ^b
(E)-2-Butenal	-	117.8 $\pm$ 0.7 ^f	323 $\pm$ 3 ^e	750 $\pm$ 3 ^d	2707 $\pm$ 29 ^a	2357 $\pm$ 71 ^b	2004 $\pm$ 73 ^c
2,3-Pentanedione	-	41 $\pm$ 6 ^d	39 $\pm$ 6 ^d	83 $\pm$ 4 ^c	78 $\pm$ 8 ^c	107 $\pm$ 6 ^b	192 $\pm$ 9 ^a
Hexanal	485 $\pm$ 83 ^d	1375 $\pm$ 95 ^b	734 $\pm$ 63 ^c	668 $\pm$ 60 ^c	1318 $\pm$ 62 ^b	1353 $\pm$ 79 ^b	1753 $\pm$ 57 ^a
(E)-3-Penten-2-one	-	3.7 $\pm$ 0.2 ^e	7.1 $\pm$ 0.7 ^e	37 $\pm$ 1 ^d	440 $\pm$ 13 ^a	322 $\pm$ 2 ^b	269 $\pm$ 2 ^c
(E)-2-Pentenal	38 $\pm$ 6 ^g	241 $\pm$ 4 ^f	520 $\pm$ 21 ^e	1062 $\pm$ 39 ^d	3649 $\pm$ 38 ^a	2782 $\pm$ 17 ^c	2841 $\pm$ 23 ^b
1-Penten-3-ol	-	-	-	-	-	55 $\pm$ 5 ^b	63 $\pm$ 3 ^a
3-Hexen-2-one	-	1.6 $\pm$ 0.2 ^e	7.2 $\pm$ 0.8 ^e	27 $\pm$ 1 ^d	219 $\pm$ 8 ^a	133 $\pm$ 2 ^b	101 $\pm$ 1 ^c
1-Hydroxy-2-propanone	-	41 $\pm$ 2 ^d	26.7 $\pm$ 0.9 ^e	48 $\pm$ 3 ^d	132 $\pm$ 8 ^c	197 $\pm$ 3 ^b	231 $\pm$ 3 ^a
(E)-2-Penten-1-ol	-	-	-	-	328 $\pm$ 43 ^b	466 $\pm$ 80 ^a	506 $\pm$ 45 ^a
2-Hydroxy-3-pentanone	-	28 $\pm$ 1 ^e	30 $\pm$ 1 ^e	95 $\pm$ 6 ^d	623 $\pm$ 32 ^a	296 $\pm$ 18 ^c	470 $\pm$ 7 ^b
1-Hydroxy-2-butanone	-	58 $\pm$ 4 ^e	160 $\pm$ 18 ^d	463 $\pm$ 24 ^c	1473 $\pm$ 58 ^a	726 $\pm$ 16 ^b	773 $\pm$ 40 ^b

Compound (ng/g oil)	PHVO ¹	LSO ²	CSO ³	WO ⁴	Flax Oil	FO ⁵	CLO ⁶
Acetic acid	2626 ± 811 ^e	2299 ± 243 ^e	2718 ± 19 ^e	4725 ± 153 ^d	8617 ± 508 ^c	16599 ± 578 ^b	20026 ± 596 ^a
(E,E)-2,4-Heptadienal	725 ± 194 ^f	10437 ± 225 ^e	18129 ± 2283 ^d	24108 ± 2175 ^c	61902 ± 1474 ^b	94749 ± 5127 ^a	56485 ± 2647 ^b
Propanoic acid	442 ± 89 ^e	540 ± 22 ^e	673 ± 12 ^e	1895 ± 35 ^d	5993 ± 401 ^b	5462 ± 197 ^c	7431 ± 242 ^a
5-Methyl-2(5H)-Furanone	-	129 ± 15 ^d	225 ± 46 ^{cd}	424 ± 136 ^c	1029 ± 80 ^b	1384 ± 135 ^a	1469 ± 106 ^a
5-Ethyl-2(5H)-Furanone	-	43 ± 3 ^d	80 ± 8 ^d	233 ± 21 ^c	822 ± 84 ^b	839 ± 92 ^b	1100 ± 64 ^a
Total Volatiles	4978 ± 974 ^f	21592 ± 519 ^e	33533 ± 2931 ^e	51652 ± 3565 ^d	120430 ± 8035 ^c	167294 ± 9616 ^a	144244 ± 6810 ^b

¹ PHVO, partially hydrogenated vegetable oil.

² LSO, low α-linolenic acid soybean oil.

³ CSO, commodity soybean oil.

⁴ WO, walnut oil.

⁵ FO, fish oil.

⁶ CLO, cod liver oil.

^a Means within a row with different superscripts differ significantly.

^b Means within a row with different superscripts differ significantly.

^c Means within a row with different superscripts differ significantly.

^d Means within a row with different superscripts differ significantly.

^e Means within a row with different superscripts differ significantly.

^f Means within a row with different superscripts differ significantly.

^g Means within a row with different superscripts differ significantly.

Table 2

Odour thresholds and odour activity values (OAV) of selected odour-active compounds in oil after 15 days of autooxidation; units are ng compound per gram oil.

Odourant	Odour threshold (ng/g oil)	OAV ¹						
		PHVO ²	LSO ³	CSO ⁴	WO ⁵	Flax Oil	FO ⁶	CLO ⁷
Acetaldehyde	0.22 ^a	-	16231	26482	41197	63535	69791	84980
Propanal	3600 ^b	0.1	0.6	0.8	1.8	3.5	4.0	4.6
2,3-Pentanedione	0.3 ^c	-	137	129	277	261	358	640
Hexanal	276 ^c	1.8	5.0	2.7	2.4	4.8	4.9	6.4
(E)-2-Pentenal	2300 ^d	0.0	0.1	0.2	0.5	1.6	1.2	1.2
Acetic acid	124 ^c	21	19	22	38	69	134	161
(E,E)-2,4-Heptadienal	10000 ^d	0.1	1.0	1.8	2.4	6.2	9.5	5.6

¹ OAV = (odourant concentration) / (odourant threshold in oil).

² PHVO, partially hydrogenated vegetable oil.

³ LSO, low α -linolenic acid soybean oil.

⁴ CSO, commodity soybean oil.

⁵ WO, walnut oil.

⁶ FO, fish oil.

⁷ CLO, cod liver oil.

^a (Curioni & Bosset, 2002).

^b (Genot, Meynier, & Riaublanc, 2003).

^c (Chetschik, Granvogl, & Schieberle, 2010).

^d (Jacobsen, 2010).

Table 3

Retention index (RI), aroma properties and molecular origin of volatile compounds arising from oxidation of n-3 fatty acids (FA). Units of Equivalents (rightmost column) are percent of compound originating from the terminal end.

Compound	RI ^I	Odour descriptor	Equivalents of terminal end in fragment (%)
Acetaldehyde	812	Apple skin, fruity, green leaves, sweet, sweet burning ^{a,b}	20
Propanal	833	Sharp-irritating ^c	97
2-Propanone	842	Pungent, irritating ^b	0
2-Propenal	857	Irritating ^b	0
Butanal	873	Burnt, irritating, green, penetrating pungent, varnish ^b	65
2-Butanone	890	-	45
2-Ethylfuran	940	Flower ^c	100
2-Pentanone	968	Acetone, sweet fruity ketone ^b	96
1-Penten-3-one	1018	Pungent, rancid green, glue, sharp fishy ^c	100
(E)-2-Butenal	1039	Old cheese ^c	100
2,3-Pentanedione	1059	Butter-like ^a	100
Hexanal	1080	Pungent, green, fresh grass, fatty ^{a,c}	48
(E)-3-Penten-2-one	1125	-	?
(E)-2-Pentenal	1128	Pungent, glue, green, grassy, apple ^c	100
1-Penten-3-ol	1156	Sweet ^c	100
3-Hexen-2-one	1214	-	91
1-Hydroxy-2-propanone	1300	-	0
(E)-2-Penten-1-ol	1308	Green ^c	100
2-Hydroxy-3-pentanone	1355	-	100
1-Hydroxy-2-butanone	1373	-	100
Acetic acid	1450	Vinegary ^d	2
(E,E)-2,4-Heptadienal	1492	Nasty, green, fatty, fatty-oily, rancid hazelnuts ^{a,c}	?
Propanoic acid	1538	Goaty, vinegar, cooked, potato-like ^{d,e}	100
5-Methyl-2(5H)-Furanone	1684	Caramel, fruity, phenolics ^e	?
5-Ethyl-2(5H)-Furanone	1765	-	100

- Data not found. ? No conclusive data were obtained from the present study.

^I Retention index on DB-FFAP.

^a (Triqui & Guth, 1997).

^b The LRI and Odour Database.

^c (Genot, Meynier, & Riaublanc, 2003).

^d(Rapp & Mandery, 1986).

^e(Vazquez-Cruz, Jimenez-Garcia, Torres-Pacheco, Guzman-Maldonado, Guevara-Gonzalez, & Miranda-Lopez, 2012).