Research Article

Fish oil sensory properties can be predicted using key oxidative volatiles

Jenna C. Sullivan and Suzanne M. Budge

Department of Process Engineering and Applied Science, Dalhousie University Halifax, NS, Canada

The high level of PUFA in fish oil, primarily eicosapentaenoic acid (EPA) and DHA result in rapid oxidation of the oil. Current methods used to assess oxidation have little correlation with sensory properties of fish oils. Here we describe an alternative method using solid phase microextraction (SPME) combined with GC-MS to monitor volatile oxidation products. Stepwise discriminant function analysis (DFA) was used to classify oils characterized as acceptable or unacceptable based on sensory analysis; a cross-validated success rate of 100% was achieved with the function. The classification function was also successfully validated with tasted samples that were not used to create the method. A total of 14 variables, primarily aldehydes and ketones, were identified as significant discriminators in the classification function. This method will be useful as a quality control method for fish oil manufacturers.

Practical applications: This paper describes an analytical method that can be used by fish oil manufacturers for quality control purposes. Solid phase microextraction and GC-MS were used to monitor volatile oxidation products in fish oil. These data, combined with results of analyses by a sensory panel, were used to create a function that classified fish oil samples as acceptable or unacceptable. The volatile oxidation products used to in the function were primarily aldehydes and ketones. This method can be used by fish oil manufacturers as an alternative to expensive sensory panels.

Keywords: Fish oil / GC-MS / Oxidation / Sensory analysis / SPME

Received: September 19, 2011 / Revised: December 19, 2011 / Accepted: January 18, 2012

DOI: 10.1002/ejlt.201100330

1 Introduction

Fish oil is a rich source of the long chain omega-3 PUFAs eicosapentaenoic acid (EPA) and DHA. These FA have been shown to have numerous health benefits, including lowering blood pressure and reducing the risk of cardiovascular disease [e.g., [1, 2]]. This has led to a surge in the popularity of fish oil products as dietary supplements. Unfortunately, the high levels of PUFA cause fish oil to be highly prone to oxidation, which produces negative off-flavors and odors. These fishy flavors that form over time often discourage people from consuming the oils.

The most common way to assess oxidation in fish oil is through the measurement of hydroperoxides, the primary products of lipid oxidation. Peroxide values (PV) are a measure of the level of hydroperoxides and the Global Organization for EPA and DHA (GOED) sets a PV limit of 5 meq/kg for fish oils in their Voluntary Monograph for Omega-3 [3]. Hydroperoxides themselves have very little impact on oil flavor, but are precursors to the volatile secondary oxidation products that negatively impact sensory properties of fish oil. Frankel [4] states that undesirable flavors can be detected in fish oils with PV of <1 meq/kg, as the unstable nature of hydroperoxides leads to rapid decomposition into secondary oxidation products. These secondary oxidation products are usually determined using anisidine values (AV), which reflect the content of aldehydes with α- and β-unsaturation. The AV limit set by GOED is 20. Unfortunately, this is not a sensitive method and there is some uncertainty that the specific components measured with this test are linked to oil flavor [4]. It is well documented

Correspondence: Jenna C. Sullivan, Department of Process Engineering and Applied Science Dalhousie University Halifax, NS, Canada B3J 2X4
E-mail: jcsulliv@dal.ca
Fax: +902 420 0219

Abbreviations: AV, anisidine value; **DF,** discriminant function; **DFA,** discriminant function analysis; **EE,** ethyl ester; **EPA,** eicospentaenoic acid; **GOED,** Global Organization for EPA and DHA; **PV,** peroxide value; **SPME,** solid phase microextraction

that both of these measures of oxidation have little relationship to the sensory properties of fish oil [4, 5]. The poor relationship between conventional oxidation testing and sensory parameters draws attention to the need for an alternative method to monitor oil quality.

The most accurate way to evaluate sensory qualities of oils is to use a taste panel, as humans can be trained to detect low levels of volatile components that traditional tests of oxidation cannot. Unfortunately, these panels are expensive to establish and maintain which makes their use unattractive. An alternative to a sensory panel is the monitoring of the amounts of volatile oxidation products in the headspace of samples, as these are the compounds most responsible for oil flavors. Solid phase microextraction (SPME), coupled with GC-MS, is often used to determine the levels of volatile components in fish oils [6–8]. The types and number of volatiles detected depends on experimental methods and materials, including oil type, SPME fiber type, extraction temperatures and times, etc.

The aim of this study, therefore, is to compare sensory panel assessment of quality with levels of oxidation products determined using PV, AV, and headspace analysis of fish oil. The goal was to identify the key oxidation products that are important in distinguishing between acceptable and poor quality oils in order to create a method to monitor fish oil oxidation that correlates well with the sensory characteristics of the oil.

2 Materials and methods

2.1 Materials

SPME fibers (divinylbenzene/Carboxen/polydimethylsiloxane, 50/30 μm coating), a SPME fiber holder for manual sampling, 22 mL glass vials, polytetrafluroethylene/silicone rubber septa, and phenolic screw caps were purchased from Supelco (Oakville, Canada). A custom-made heating block designed to accommodate 22 mL glass vials was used to control temperature. Fish oil containing a blend of mixed natural tocopherols was obtained from Ocean Nutrition Canada Ltd. (Dartmouth, Canada). Optima chloroform was obtained from VWR (Mississauga, Canada). Methyl tricosonate, methyl eicosapentaenoate, and methyl docosahexaenoate were purchased from Nu-Chek Prep (Elysian, USA). An Isotemp 100 Series Model 126G oven was used to incubate samples and was obtained from Fisher Scientific (Ottawa, Canada), along with all other chemicals and glassware.

2.2 Experimental design

Amber bottles (250 mL) were filled with 200 mL of fish oil each and placed, uncapped, in an oven held at 40°C. Oils were removed from the oven at varying time intervals, ranging from 2 to 15 h, over the course of 14 days, with a new bottle being used at each sampling point. Aliquots of 14.0 mL of each sample were placed in 22 mL glass vials and then capped with phenolic screw caps with PTFE/silicone septa and analyzed with SPME-GCMS. Approximately 15 mL of each sample was removed for PV and AV testing, and the remainder of each 200 mL sample was either stored at −80°C under nitrogen or evaluated by a sensory panel. One sample per 24 h time period underwent PV and AV analysis and was tasted by a sensory panel. All samples were analyzed by SPME-GCMS. The experiment was conducted in triplicate.

2.3 Measures of oil quality

The fatty acid profile of the fish oil was analyzed using the method described by Sullivan et al. [9]. The PV and AV of each sample was measured in triplicate following AOCS Official Method Cd 8-53 [10] and AOCS Official Method Cd-18-90 [11], respectively.

The SPME method was adapted from Lee et al. [6]. SPME samples were placed in a heating block set to 80°C and allowed to equilibrate for exactly 15.0 min. Meanwhile, the SPME fiber was placed in the injector port of the GC at 250°C to desorb any volatiles that had accumulated during storage or between samples. After the equilibration period, the SPME fiber was inserted into the vial at a depth of 2 cm and exposed to the sample headspace for exactly 45.0 min. Extracted volatiles were analyzed by ion trap GC-MS in electron ionization mode (200°C). The fiber was inserted into the injector of the GC to a depth of 5 cm (splitless mode, 250°C; 1 mm liner) and left for 5 min. Volatile analytes were separated on a free fatty acid phase column (30 m × 0.25 mm × 0.25 μm film thickness, Agilent, Mississauga, Canada). Helium was used as a carrier gas (1.0 mL/min). The oven temperature was initially held at 40°C for 5 min and increased at a rate of 10°C/min to 250°C and held for 5 min (total run time 29 min). Data were acquired as area counts and converted to area percent.

2.4 Sensory assessment of oil quality

An untrained, descriptive sensory panel was used to assess samples for quality [12]. Between 10 and 12 samples from each replicate were tasted by the sensory panel. Potential panelists were initially screened to eliminate those volunteers who could not distinguish fishy flavors by challenging them with a triangle test, consisting of acceptable, fresh fish oil with a low volatile content, and fish oil that had been incubated at elevated temperatures to force the formation of fishy volatiles. Those who could not distinguish between the fishy sample and the fresh sample were removed from the panel. The final panel consisted of 10 volunteers. Each panelist was asked to rank samples on a scale of 10, with 10 being excellent and 1 being terrible and to rank fishy flavors, using a fresh vegetable oil sample as a reference. These classifications were then used to group samples into acceptable and unacceptable categories. Each sample evaluated by the final panel was tasted by a

minimum of three people. Design and training of the sensory panel as well as the score sheets used to evaluate the samples were adapted from guidelines provided by the American Oil Chemists Society [12]. Of the 75 samples analyzed with SPME-GCMS, 31 were evaluated for sensory characteristics, leaving approximately 41% untasted.

2.5 Statistical analysis

Sensory panel results and peak area percent data from GCMS analysis of volatiles were analyzed using SPSS 11.0 (SPSS Inc., Chicago, Ill) statistical software to perform forward stepwise discriminant function analysis (DFA). This created a function to classify samples as acceptable or unacceptable and also identified the volatiles most useful for oil classification based on sensory characteristics. To improve normality, data were first transformed using a geometric mean function [13], following Eq. (1):

$$x_n = Ln\frac{a_n}{n\sqrt{a_1 a_2 \cdots a_n}} \quad (1)$$

where a_n is the proportion in area percent of each peak identified in the sample. To ensure that the dataset was concise, a filtering step was conducted where correlations between the three replicates were examined for each peak. Peaks that had strong negative correlations to each other were removed from the analysis, because they could not be responsible for differences between acceptable and unacceptable samples if they were not varying in a consistent manner among replicate experiments. These peaks were attributed to noise. The reduced dataset, made up of 78 oxidative volatiles, was combined with sensory classifications to create a discriminant function (DF), using an F-value for entry into the function of 3.84 and a value for removal of 2.71. Stepwise DFA was essential in this study as a function that uses all the 78 of oxidative volatiles present in the reduced data set for these fish oil samples would be difficult and impractical to apply. The classification method was created using the proportion of volatiles as the independent variable and sensory assessment (acceptable or unacceptable) as the grouping variables for 28 fish oil samples that underwent sensory testing. Leave-one-out cross-validation of the data was used to assess the accuracy of classification with the DF. As a further test of accuracy, the DF was applied to three tasted samples, one from each replicate, that were excluded from the data set when the DF was created. Last, the samples that were not tasted were classified using the DF. The variables selected by DFA as being useful to discriminate between acceptable and unacceptable samples were also identified.

2.6 Peak identification

The peaks recognized by DFA as being important discriminators between acceptable and unacceptable samples were identified using mass spectra library matches (National Institute for Standard Technologies), potential fragmentation patterns as predicted by HighChem Mass Frontier 4.0 (HighChem Ltd., Bratislava, Slovakia) and external standards, where possible.

3 Results and discussion

3.1 Fatty acid profile

The levels of EPA and DHA in the fish oil tested were found to be 166 ± 1.36 and 125 ± 0.50 mg/g, respectively. These results are typical of commercial fish oils.

3.2 Hydroperoxide and anisidine value testing

Peroxide value increased for the duration of the study (Fig. 1a). The initial PV for all three experiments was quite low (average 1.0 ± 0 meq/ kg), indicating that it was initially unoxidized. The GOED limit of 5 meq/kg was exceeded in ≤48 h for all experiments, with the highest PV of 28.1 meq/kg reached after ∼312 h. The AV for all three experiments started at an acceptable level (average 9.6 ± 0.4) and increased over time, though not as rapidly as PV (Fig. 1b). None of the three trials exceeded the GOED limit of 20 for AV during the study. The PV and AV for replicate II increased more rapidly than replicates I and III, though the general trend of all studies was the same. Despite the more rapid increase in PV and AV for replicate II, sensory ratings agreed with the other two replicates. It is likely that slight variations in incubation and ambient temperatures, or differences in the relative humidity during incubation resulted in differences between experiments. Interestingly, the samples were not rejected by the sensory panel until

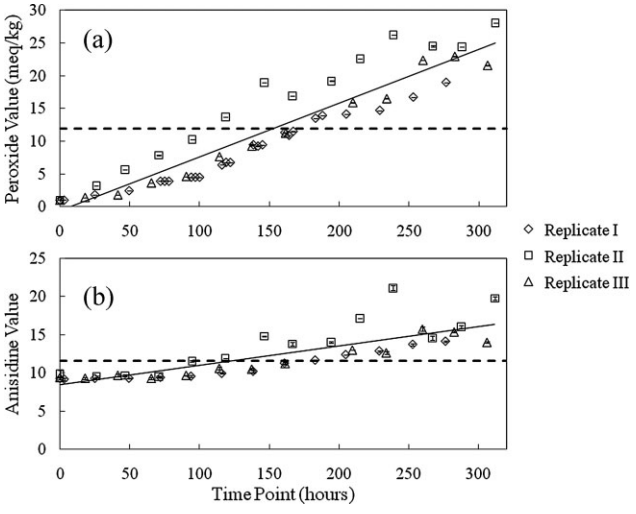

Figure 1. Variation in (a) peroxide and (b) anisidine (mean ± SD) values of fish oil over time. The level at which samples were rejected by the sensory panel are indicated by the dashed line.

the PV reached an average of 11.9 ± 3.5 meq/kg, more than double the 5 meq/kg maximum for PV set by the GOED [3]. The average AV when samples were rejected was 11.6 ± 1.4, which is lower than the GOED maximum of 20. Inconsistencies between PV and AV results and sensory scores have been found in other studies. For instances, MacFarlane et al. [14] found that oil samples with acceptable PV and AV results were deemed to be fishy and unacceptable. Similarly, little correlation was found between PV, AV, and sensory testing in fish oil-enriched mayonnaise and spreads [5], with samples with high AV often having high levels of acceptability in sensory testing. In the present study PV and AV values at the point of sensory rejection clearly indicate that current guidelines for fish oil quality do not correlate well with sensory properties.

3.3 Classification by DFA

Discriminant function analysis was chosen for statistical analysis because it classifies samples into different categories using descriptors selected by the researcher. This allowed tasted fish oil samples to be used to create a method that would identify the key differences in oxidative volatiles between acceptable and unacceptable samples. Because of the large number of volatile oxidation products, stepwise DFA was used, as this method uses only those variables which differ significantly between categories to create the DF, rather than using all variables in the classification scheme. This was an important consideration because the goals of this study were to develop a method where levels of only a small number of oxidation products must be determined to assess oxidation, and to identify what these key volatiles are. Stepwise DFA identifies the components important to classification and creates a function that can easily be applied for classification of future samples. The DFA method yielded 100% correct classification of all samples used to build the method. Similarly, leave-one-out cross-validation resulted in perfect classification of all samples. When applied to the three tasted samples excluded from the model, all were classified correctly, giving confidence that the method will hold for other, untasted samples.

The group centroids for the DF were found to be 11.86 for acceptable samples and −11.86 for unacceptable samples, with a sample classified as unacceptable when the DF score was <0 and acceptable when >0.

The distribution of DF scores for all samples were compared by calculating the squared Mahalonobis distance of each score from the group centroid of acceptable or unacceptable classifications. The smaller the distance to the centroid, the more likely a sample belonged to that group. When the DF was applied to the tasted samples it was clear from the small squared Mahalonobis distances (Fig. 2a) for most samples that the DF was likely successful at classifying samples. There were, however, some untasted samples that did not fit well with either the acceptable or unacceptable

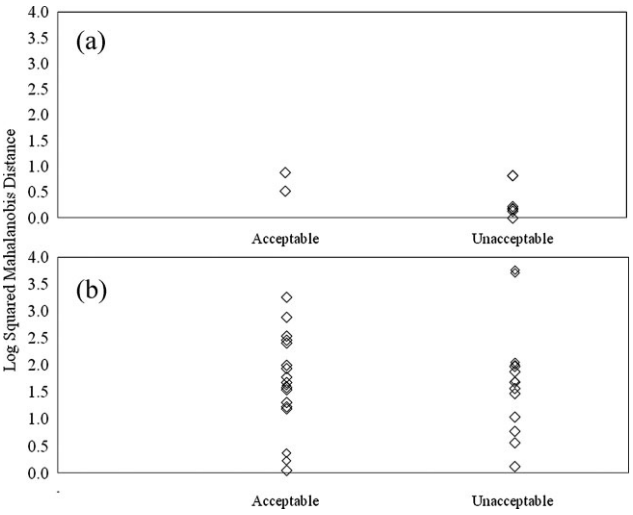

Figure 2. Log of the squared Mahalanobis distance from the centroid for (a) tasted and (b) untasted samples of fish oil.

classification, evident in their great distances from the group centroid (Fig. 2b). These samples were predominantly taken near the midpoint of the time courses, where the oils were experiencing a transition from relatively unoxidized to more rancid conditions and therefore would not necessarily fit into the simple classification scheme of acceptable and unacceptable.

3.4 Compounds important in classification

The function created using DFA identified the variables, in this case the volatile oxidation products, useful in differentiating between acceptable and unacceptable samples. A total of 14 oxidation products were identified as contributing to differences between acceptable and unacceptable samples (Table 1, Fig. 3). The majority of the compounds increased in proportion over time, but the direction of proportion change was not necessarily indicative of the type of effect the volatile had on the sensory properties of oil. The DF coefficients better indicated those effects, for instance, if a volatile is increasing in proportion over time, and has a coefficient >0, it drives the DF in a positive direction, closer to 11.86, and therefore results in an acceptable sensory classification. A volatile that increases in proportion but has a DF coefficient <0 would drive the DF score to more negative values, and closer to the group centroid for unacceptable classification. Table 1 lists the identities of these compounds, their DF coefficients and the direction of their proportion changes. The majority of the compounds identified were aldehydes, ketones, and alcohols. In addition, a hydrocarbon, acid, benzene derivative, and ethyl ester (EE) were also identified. Many of these structures have been previously reported in fish oil.

Table 1. Standardized canonical DF coefficients of oxidative volatiles used to differentiate between acceptable and unacceptable fish oils

Volatile	Standardized canonical DF coefficient	Direction of proportion change	Method of identification
2-butanone	14.47	Decrease	a, b
1,3,5-octatriene	−7.52	Increase	b, c
4,6,8-nonatrien-3-one	−10.38	Decrease	c
3-hexen-1-ol	−25.34	Decrease	a, b
3,6-nonadienal	13.74	Increase	a, c
(*E*,*E*)-2,4-heptadienal	−7.74	Increase	a, b
(*E*,*Z*) 2,4-octadienal	6.79	Increase	a, b
(*E*,*E*) 2,4-octadienal	12.34	Increase	a, b
Nonatrienone (undetermined isomer)	−4.14	Increase	c
2,4-Heptadien-1-ol	−3.10	Increase	a, b
2-Decanol	6.59	Increase	a, c
Ethyl benzaldehyde	21.92	Decrease	c
Tetradecanoic acid EE	2.26	Decrease	a
Octanoic acid	−7.89	Decrease	a, b

a, Retention time and MS fragmentation pattern compared with an external standard; b, NIST library match; c, probable ion fragmentation predicted using Mass Frontier 4.0.

Aldehydes are known to have an effect on the sensory quality of fish oil. All aliphatic aldehydes identified in this study as important indicators of sensory properties increased over time. (*E*,*E*)-2,4-heptadienal was one such compound and it has previously been found in oxidized fish oil [5, 6, 15]. Its isomer, (*E*,*Z*)-2,4-heptadienal, has been associated with oxidized fish oil flavors [8, 15] and was also identified in this study. However, here it was found to have little correlation with off-flavors, despite a dramatic increase in proportion over the course of the study, demonstrating that oxidation products present in high proportions may not necessarily be most useful in predicting sensory qualities of oil. Both isomers of 2,4-heptadienal have been linked to general rancid and painty flavor in oils [15, 16], though Hartvigsen et al. [17] found that (*E*,*Z*)-2,4-heptadienal had a distinctively fishy odor. (*E*,*E*) and (*E*,*Z*)-2,4-octadienal were also identified here as useful in classification of fish oil. 2,4-octadienal has been found in menhaden oil previously by Hseish et al. [18], though the geometric isomer was not specified. Hartvigsen et al. [17] identified both the (*E*,*E*) and (*E*,*Z*) isomers in fish oil-enriched mayonnaise using GC-MS combined with GC-Olfactory and found that the (*E*,*E*) isomer had a deep-fried odor while the (*E*,*Z*) isomer was associated with green odors. Interestingly, both isomers of 2,4-octadienal were found to increase over time and had a positive DF coefficient, suggesting that they had a positive impact on sensory qualities over the course of the experiment as their proportion increased. The final aliphatic aldehyde that was found to play a role in the sensory properties of fish oil, 3,6-nonadienal, is a decomposition product of the 11- and 12-hydroperoxide derivitatives of EPA and has been associated with a green flavor [4]. Like the octadienal isomers, its level increased with oxidation and it had a positive DF coefficient, suggesting a positive influence on sensory characterization. 3,6-nonadienal is a key oxidation product used by MacFarlane et al. [14] to predict the quality of fish oil samples, as it could be detected in oxidized oil. However, our results and the association with 3,6-nonadienal with a green flavor suggest that this compound may not be contributing to a fishy flavor.

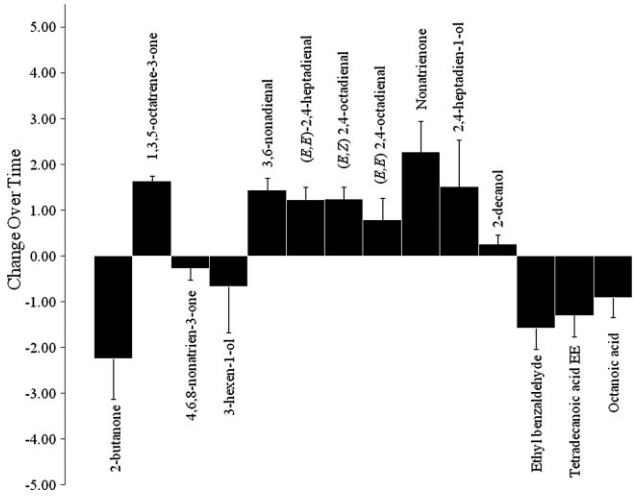

Figure 3. Change in levels of volatiles (mean ± SD) identified as significant in the classification function over the duration of the study. Data were transformed following $x_n = Ln(a_n/n\sqrt{a_1 a_2 \cdots a_n})$.

Alcohols are formed from primary oxidation products and also influence the sensory parameters of oxidized fish oils, though their taste and odor thresholds are so high that they are often disregarded [19]. 3-hexen-1-ol, identified in this study as being important in oil classification, had been identified previously by Karahadian and Lindsay [16], who believed that this compound contributed to a green flavor in the presence of nonadienal; it had no sensory impact on its own. We also found 2,4-heptadien-1-ol useful in differentiating between acceptable and unacceptable fish oil samples. This is the first report of it in oxidized fish oil. Similarly 2-decanol has not been found in other oxidized fish oils but was useful in oil classification. Other studies may have failed to identify this alcohol because of column and temperature program differences that resulted in either co-elution of compounds, or a temperature program that was insufficient to elute this alcohol. Both 2,4-heptadien-1-ol and 2-decanol had DF coefficients <0, suggesting that they have negative impacts on sensory characteristics.

An interesting alkene, 1,3,5-octatriene, was linked to oxidation and is described as smelling like solvent or plastic by Hailler et al. [20]. Unspecified isomers of octatriene were

identified by Kulås et al. [21] in purified fish oil and Guillén [7] in cod liver oil. 1,3,5-octatriene was also identified in fish oil-enriched mayonnaise by Hartvigsen et al. [17] and in various fish oils by Giogios et al. [22]. These authors, along with Karahadian and Lindsay [16] also identified another isomer, 1,3,6-ocatriene, in oxidized oil thought it was not detected in the present study.

Karahadian and Lindsay [16] identified octanoic acid as a fish oil oxidation product, and it was found in this study as well. In this case, the acid decreased in proportion over time, suggesting it was present in the oil at the start of the experiment. It is also possible that octanoic acid was a decomposition product of a fatty acid or another oxidation product that was initially present in small amounts. If that was the case, then as the precursor to this acid dissipated, the levels of octanoic acid would also decrease. Octanoic acid is generally associated with a goaty, musty smell, agreeing with its negative DF coefficient, and pointing to poor sensory qualities. Other studies have found shorter chain acids, including propanoic, butanoic, and hexanoic acid [18, 21]. These acids were also present in the oil used in this study but did not play a role in differentiating between acceptable and unacceptable samples.

Three ketones were recognized for influencing sensory quality of oils. 2-butanone, which has a buttery taste, was an early eluting ketone that decreased over time. This compound had a DF coefficient >0, indicating it positively influences sensory characteristics, so as this compound decreased in proportion, the sensory quality of the oil also decreased. It has been identified in a number of studies as a fish oil oxidation product [7, 15, 21, 22]. 4,6,8-nonatrien-3-one and an undetermined nonatrienone isomer were also deemed to be important in differentiating between acceptable and unacceptable oils. Neither of these ketones has been identified in fish oil studies previously but they are both probable lipid oxidation products.

Ethyl benzaldehyde was found to be useful for classification of fish oils, and decreased in proportion over time. Its DF coefficient was >0, suggesting that its positive impact on sensory characteristics decreased as oxidation progressed. It has not been previously identified as a fish oil oxidation product but other authors have found benzene derivatives, such as ethyl benzene [7, 17, 22]. The decrease in proportion of this compound with time, along with its chemical structure, suggests that it is not formed as a result of lipid oxidation but instead was present in the oil initially and degraded over the course of the experiment. Though benzene itself is unlikely to be formed during lipid oxidation, it is possible that ethyl benzaldehyde was generated through decomposition of a larger benzene derivative that was initially present in the oil. Giogios et al. [22] speculated that benzene compounds could result from degradation of sugars or amino acids, explaining their small proportions in fish oils. Though not important to sensory parameters, xylene, another benzene derivative, was found in this study and in studies of fish oil-enriched mayonnaise [17, 23, 24]. Jacobsen et al. [23, 24] attributed xylene formation to the presence of other ingredients, but the current study shows that benzene-containing compounds, including xylenes, exist in fish oil when only lipid ingredients are present.

The final compound of interest in distinguishing between acceptable and unacceptable oils was tetradecanoic acid EE. This compound decreased over time, likely because it is a component formed during fish oil manufacturing rather than via oxidation. Most commercially available fish oils undergo base-catalyzed transesterification, creating EE. These EE are fractionated to manipulate the fatty acid profile of the fish oil, then re-esterfied into triglycerides [25]. This final step often leaves traces of EE in the oil, and fish oil manufacturers usually specify a maximum of 3% EE in their triglyceride oils for this reason [9]. The unstable nature of EE results in rapid oxidation and may explain the decrease in this compound over time.

The majority of compounds identified by DFA as being important in the classification scheme were present only in low proportions, and did not vary greatly in proportion over time. There were six oxidation products that decreased over time: 3-hexen-1-ol, ethyl benzaldehyde, 2-butanone, 4,6,8-nonatrien-3-one, octanoic acid, and tetradecanoic acid EE. It is possible that these compounds were decomposing into other oxidation products that were then detected by sensory analysis. This is most likely with 3-hexen-1-ol and tetradecaonic acid EE as they had the largest proportions of the four volatiles and were therefore more likely to decompose into other oxidation products that might be present in sufficient proportions to influence the flavor profile of the fish oil. Even though decomposition products of other volatiles are often present at low levels, it is the compounds present in the smallest proportions that seem to be the most important variables in sensory quality. The volatiles present in larger amounts, including in this case (*E*,*Z*)-2,4 heptadienal, were not found to play an important role in oil quality. It is possible that some oxidation products have little contribution to sensory properties on their own, but when combined with other volatiles, have a large impact as a result of a synergistic relationship. The potential relationship between 3-hexen-1-ol and nonadienal is an example. These types of relationships highlight the importance of monitoring as many volatiles as possible when attempting to describe off-flavors in oils.

The individual volatiles identified in fish oil and fish oil-enriched foods are consistent from study to study, though those deemed to have the greatest impact on sensory characteristics vary. MacFarlane et al. [14] developed a method to monitor fish oil quality using dynamic headspace sampling, known as the Fatty Acid Smell and Taste (FAST) Index. This method monitors levels of 4-heptenal, 2,6-nonadienal, and 3,6-nonadienal, all of which are products of EPA oxidation, and uses a simple mathematical formula generated from principal component analysis of sensory data to give oil

samples a numerical rating of fishy taste. While this method can be very useful, it does not take into account the possibility that volatiles derived from fatty acids other than EPA are also likely to play a role in the formation of off-flavors in fish oil. By limiting the method to only three oxidation products, all of which come from EPA, this method may be missing other potent volatile oxidation products derived from other fatty acids that could be linked to oils with poor sensory properties. The authors also note that dynamic headspace method for the FAST Index test is time consuming and difficult to perform.

While studies relating fish oil oxidation products to sensory perceptions are rare, there are numerous studies that have attempted to associate sensory properties of fish oil-enriched food products with levels of volatile oxidation products. For instance, Jimenez-Alvarez et al. [26] attempted to correlate the proportions of volatiles in cod liver oil-enriched milk with sensory testing through the use of a triangle test where participants were asked to smell emulsion samples, some of which were spiked with (*Z*)-2-hexenal and (*E*)-4-heptenal, the oxidation products they had identified as having the largest increases in proportion over time. Only half of the panelists could differentiate between spiked and unspiked samples, and no panelists associated (*Z*)-2-hexenal or (*E*)-4-heptenal with lipid oxidation descriptors, highlighting the importance of screening sensory panelists for their ability to taste certain indicators. That study [26] also illustrates that it is not necessarily the oxidation products with the greatest increase in proportion over time that are important to sensory properties of oils. The sensory threshold of oxidation products varies amongst compounds [4], so it is also probable that some oxidation products that show large increases have very high sensory thresholds and thus cannot be tasted until levels are very high.

3.5 Applications

The method described here was originally designed as a tool in product development to test the efficacy of new antioxidants. Rather than using chemical tests that are not correlated with sensory properties of oil, this procedure will provide results that directly relate to the taste and odor of fish oil. Ideally, this method will eliminate pricey, subjective sensory panels but still provide the same type of information. In an industrial setting, this method could be used to monitor the quality of raw materials or finished products. However, this technique was developed using unflavored fish oil and has not been validated with flavored oil; our experience has shown that the compounds added to mask fishy flavors overload the SPME fiber, resulting in detection of only those compounds. This may limit its use as a method in evaluating finished products. Further, the oil used in the development and testing of this method had a specific fatty acid profile, roughly 30% EPA and DHA. Other oils such as fish oil concentrates with different fatty acid profiles, fish oil emulsions or complex fish oil products have not yet been tested. It is uncertain if the same volatiles will be linked to flavor degradation in fish oils with differing fatty acid profiles.

Currently, to use the technique developed here, data must be collected for all volatile peaks as area percent data from chromatograms. It was essential to monitor all volatiles during the construction of this method as there were uncertainties as to which volatiles might influence sensory properties of the oils. With the volatiles known to be important to classification selected, it may not be necessary to collect information on all oxidation products. Thus, this method may be easier to implement if an internal standard were used so that ratios of selected peaks relative to the internal standard could be compared, allowing only data for the significant peaks to be collected.

In conclusion, the technique described in this study identifies oxidative volatiles that can be used to distinguish between acceptable and unacceptable fish oils, as an alternative to sensory evaluation. DFA was used to classify fish oil samples and to identify volatile oxidation products that were important to classification. The DF scheme was validated on tasted samples that were not used to build the DF. The volatiles identified are primarily aldehydes and ketones, though other classes of compounds were also found to be important. The relationship between oxidation product proportion and sensory characteristics is not straight forward, and the DF coefficient of the compound must be taken into consideration when examining the relationship between a compound and sensory quality. Volatiles with DF coefficients >0 had a positive impact on sensory characteristics, while those with coefficients <0 negatively affected sensory perceptions. This method may be useful as a quality control method in industrial fish oil operations. Future research will attempt to apply this method to other fish oils and fish oil emulsions.

The authors have declared no conflict of interest.

References

[1] Morris, M. C., Sacks, F., Rosner, B., Does fish oil lower blood pressure? A meta-analysis of controlled trials. *Circulation* 1993, *88*, 523–533.

[2] Mozafarrian, D., Fish and *n*-3 fatty acids for the prevention of fatal coronary heart disease and sudden cardiac death. *Am. J. Clin. Nutr.* 2008, *87*, 1991S–1996S.

[3] Global Organization for EPA and DHA (Accessed May 2008) Voluntary monograph for omega-3. http://www.goedomega3.com/.

[4] Frankel, E. N., *Lipid Oxidation*, 2nd Edn., *The Oily Press*, Bridgwater (England) 2005.

[5] Jacobsen, C., Sensory impact of lipid oxidation in complex food systems. *Fett/Lipid* 1999, *101*, 484–492.

[6] Lee, H., Kizito, S. A., Weese, S. J., Craig-Schmidt, M. C. et al., Analysis of headspace volatile and oxidized volatile compounds in DHA-enriched fish oil on accelerated oxidative storage. *J. Food Sci.* 2003, *68*, 2169–2177.

[7] Guillén, M. D., Carton, I., Salmeron, J., Casas, C., Headspace composition of cod liver oil and its evolution in storage after opening. First evidence of the presence of toxic aldehydes. *Food Chem.* 2009, *114*, 1291–1300.

[8] Serfert, Y., Drusch, S., Schwarz, K., Sensory odour profiling and lipid oxidation status of fish oil and microencapsulated fish oil. *Food Chem.* 2010, *123*, 968–975.

[9] Sullivan, J. C., Budge, S. M., St-Onge, M., Determining ethyl esters in fish using with solid-phase microextraction and GC-MS. *J. Am. Oil Chem. Soc.* 2009, *86*, 743–748.

[10] Firestone, D. (Ed.), *Official Methods and Recommended Practices of the American Oil Chemists' Society*, 4th Edn., American Oil Chemists' Society, Champaign, IL 1997, Method Cd 8–53.

[11] Firestone, D. (Ed.), *Official Methods and Recommended Practices of the American Oil Chemists' Society*, 4th Edn., American Oil Chemists' Society, Champaign, IL 1997, Method Cd 18–90.

[12] Warner, K., in: Warner, K., Eskin, N. A. M. (Eds.), *Methods to Assess Quality and Stability of Oils and Fat-Containing Foods*, AOCS Press, Champaign, IL (USA) 1995, pp. 49–75.

[13] Aitchison, J., Principal component analysis of compositional data. *Biometrika* 1983, *70*, 57–65.

[14] Macfarlane, N., Salt, J., Birkin, R., The FAST Index-a fishy scale. *Inform* 2001, *12*, 244–249.

[15] Venkateshwarlu, G., Let, M. B., Meyer, A. S., Jacobsen, C., Chemical and olfactometric characterization of volatile flavour compounds in a fish oil-enriched milk emulsion. *J. Agric. Food Chem.* 2004, *52*, 311–317.

[16] Karahadian, C., Lindsay, R. C., Evaluation of compounds contributing characterizing fishy flavours in fish oils. *J. Am. Oil Chem. Soc.* 1989, *66*, 953–960.

[17] Hartvigsen, K., Lund, P., Hansen, K. F., Hølmer, 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.

[18] Hseih, T. C., Williams, S. S., Vejaphan, W., Meyer, S. P., Characterization of volatile components of menhaden fish (*Brevoortia tyrannus*) oil. *J. Am. Oil Chem. Soc.* 1989, *66*, 117.

[19] Ho, C. T., Chen, Q., Zhou, R., in: Hui, Y. H. (Ed.), *Bailey's Industrial Oil and Fat Products*, John Wiley and Sons Inc., New York, NY (USA) 1996, pp. 83–104.

[20] Hallier, A., Courcoux, P., Sérot, T., Prost, C., New gas chromatography–olfactometric investigative method, and its application to cooked *Silurus glanis* (European catfish) odor characterization. *J. Chromatogr. A* 2004, *1056*, 201–208.

[21] Kulås, E., Olsen, E., Ackman, R. G., Effect of α-, γ -, and δ-tocopherol on the distribution of volatile secondary oxidation products in fish oil. *Eur. J. Lipid Sci. Technol.* 2002, *104*, 520–529.

[22] Giogios, I., Grigorakis, K., Nengas, I., Papasolomontos, S. et al., Fatty acid composition and volatile compounds of selected marine oils and meals. *J. Sci. Food Agric.* 2009, *89*, 88–100.

[23] Jacobsen, C., Hartvigsen, K., Lund, P., Meyer, A. S. et al., Oxidation in fish-oil-enriched mayonnaise 1. Assessment of propyl gallate as an antioxidant by discriminant partial least squares regression analysis. *Eur. Food Res. Technol.* 1999, *210*, 13–30.

[24] Jacobsen, C., Hartvigsen, K., Lund, P., Adler-Nissen, J. et al., Oxidation in fish-oil-enriched mayonnaise. *Eur. Food Res. Technol.* 2000, *210*, 242–257.

[25] Breivik, H., in: Breivik, H. (Ed.), *Long Chain Omega-3 Specialty Oils*, Oily Press, Bridgwater (England) 2007, pp. 111–140.

[26] Jimenez-Alvarez, D., Giuffrida, F., Golay, P. A., Cotting, C. et al., Profiles of volatile compounds in milk containing fish oil analyzed by HS-SPME-GC/MS. *Eur. J. Lipid Sci. Technol.* 2008, *110*, 277–283.

