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ORIGINAL ARTICLE

Key Lipid Oxidation Products Can Be Used to Predict Sensory Quality of Fish Oils with Different Levels of EPA and DHA

Jenna C. Sullivan Ritter · Suzanne M. Budge

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Abstract Despite its many health benefits, many consumers avoid fish oil supplements due to fishy tastes and odors. Common chemical measures of oxidation have little correlation with sensory properties, making it difficult to determine the sensory quality of fish oil without the use of an expensive sensory panel. Here we investigate an alternative method to assess oxidation using solid phase microextraction and gas chromatography-mass spectrometry. Fish oils containing different amounts of eicosapentaenoic acid and docosahexaenoic acid were oxidized, and headspace volatiles were monitored over time and compared to sensory evaluations by a taste panel. Peroxide value and anisidine value were also measured. Sensory panel scores and headspace volatile data were analyzed using principal component analysis and linear regression to identify key volatiles responsible for changes in sensory degradation of oils over time. A total of eight compounds were identified, primarily aldehydes and ketones. By monitoring these volatiles, it may be possible to create a simple method to assess oxidation in fish oils that correlates well with sensory properties of the oil without the use of a sensory panel.

Keywords Fish oil · Oxidation · SPME · GCMS · Sensory · Principal component analysis

Abbreviations

AOCS	American Oil Chemists' Society
AV	Anisidine value
DAG	Diacylglycerol
DHA	Docosahexaenoic acid
EPA	Eicosapentaenoic acid
GOED	Global Organization for EPA and DHA
PV	Peroxide value
MAG	Monoacylglycerol
MANOVA	Multivariate analysis of variance
PC	Principal component
PCA	Principal component analysis
PUFA	Polyunsaturated fatty acid
SPME	Solid phase microextraction
TAG	Triacylglycerol
TBME	*tert*-Butyl methyl ether

J. C. Sullivan Ritter (✉) · S. M. Budge
Department of Process Engineering and Applied Science, Dalhousie University, Halifax, NS B3J 2X4, Canada
e-mail: Jenna.Ritter@dal.ca

Introduction

The strong link between the use of fish oil supplements and decreases in cardiovascular disease has resulted in a high demand for fish oil supplements. However, the fishy flavor often found in fish oil can dissuade people from consuming them. The long chain polyunsaturated fatty acids (PUFA) found in fish oil, specifically eicosapentaenoic acid (20:5n-3, EPA) and docosahexaenoic acid (22:6n-3, DHA), oxidize rapidly due to the large number of double bonds they contain producing negative off-flavors. It follows that oils with higher concentrations of these PUFA oxidize more rapidly than those with low concentrations.

Fish oil oxidation is commonly assessed by measuring levels of hydroperoxides, in oils. The Global Organization for EPA and DHA Omega-3 (GOED) specifies in their Voluntary Monograph that the peroxide values (PV) of fish oils should be <5 mequiv/kg [1]. Hydroperoxides are primary oxidation products that have little effect on the sensory properties of fish oil as they are inherently unstable and rapidly degrade into volatile secondary oxidation products

that are responsible for off-flavors in fish oils. Thus, it is possible to have oils with very low PV that taste very rancid. Secondary oxidation products are primarily assessed through the use of anisidine value (AV) for which GOED specifies a limit of 20 [1]. AV reflects the level of aldehydes with α- and β-unsaturation. There is very little evidence that AV and sensory properties are correlated; it may be that the sensitivity of this method is not high enough to detect minute changes in aldehyde concentrations that could affect sensory properties [2]. The poor relationship between PV, AV and sensory properties highlights the need for new methods to assess oil quality that correlate well with sensory parameters.

Sensory panels are the most accurate method for assessing fish oil quality as humans are capable of tasting certain compounds at concentrations much lower than chemical techniques are able to detect [2]. Unfortunately, sensory panels can be cost prohibitive due to the expenses associated with their set up and maintenance. An alternative to sensory panels is the use of solid phase microextraction (SPME), coupled with gas chromatography-mass spectrometry (GCMS), which can be used to monitor the presence of volatile secondary oxidation products in the headspace of fish oil. Volatile compounds are responsible for the off flavors and odors caused by oxidation.

The aim of this study is to compare the results of sensory panel assessment of three fish oils with different concentrations of EPA and DHA with PV, AV and proportions of volatile oxidation products. The goal is to create a robust method that can be used to predict sensory quality of fish oil and to identify key oxidative volatiles associated with those sensory properties.

Materials and Methods

Materials

SPME fibers (divinylbenzene/Carboxen/polydimethylsiloxane, 50/30 μm coating), a SPME fiber holder for manual sampling, 22-ml glass vials, polytetrafluoroethylene/silicone rubber septa and screw caps were purchased from Supelco (Oakville, ON). A custom-made heating block designed to accommodate 22-ml glass vials was used to control the temperature. Optima chloroform was obtained from VWR (Mississauga, ON). An Isotemp 100 Series Model 126G oven was used to incubate samples and was obtained from Fisher Scientific (Ottawa, ON) along with all other glassware. Methyl tricosanoate, methyl eicosapentaenoate, methyl docosahexaenoate and 1,2-dipalmitin were purchased from Nu-Chek Prep (Elysian, MN). 1,3-dipalmitin was purchased from Doosan Serdary Research Laboratories (Etobicoke, ON). Tripalmitin was purchased from Sigma Aldrich (Oakville, ON), along with all other chemicals. Two fish oils containing a blend of mixed natural tocopherols were obtained from Ocean Nutrition Canada Ltd. (Dartmouth, NS) and a third oil was obtained from EPAX (Oslo, Norway). Oil 1 contained approximately 30 % EPA and DHA, while Oil 2 contained roughly 50 %. Oil 3 contained approximately 65 % EPA and DHA. All oils were marketed as triacylglycerols and all were blends of anchovy and sardine oil sourced from Peru.

Experimental Design

Amber bottles (250 ml) were each filled with 200 ml of fish oil 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 10–14 days, with a new bottle being used at each sampling point. From each bottle, aliquots of 14.0 ml 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 used 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 for each of the three oil types.

Measures of Oil Quality

The fatty acid profile of the fish oil was analyzed using the method described by Sullivan Ritter et al. [3]. The PV and AV of each sample was measured in triplicate following American Oil Chemists' Society (AOCS) Official Method Cd 8-53 [4] and AOCS Official Method Cd-18-90 [5], respectively.

Lipid Class Analysis

Lipid classes were analyzed using HPLC. Aliquots of each oil were diluted with $CHCl_3$/MeOH (2:1 by vol) to achieve a final concentration of 0.25 mg/ml. Samples were filtered using 0.45-μm filters and transferred to autosampler vials. Analysis was done in triplicate using a Thermo Finnigan Surveyor HPLC with an autosampler (Thermo Fisher Scientific, Mississauga, ON) coupled to a Sedex 80 low temperature evaporative light scattering detector (Sedere, North York, ON) set to 30 °C with a gain of 8. The injection volume for each sample was 10 μl. The mobile phase was made up of hexane and *tert*-butyl methyl ether (TBME) (Table 1) at a flow rate of 2 ml/min and the total run time was 10 min. Samples were first analyzed on a YMC-Pack PVA-Sil column (5-μm particles,

Table 1 Composition of mobile phase for gradient elution with HPLC analysis

Time (min)	Hexane (%)	TBME (%)
1	98	2
5	100	0
7	100	0
8	98	2
10	98	2

100 × 30 mm I.D.) with matching guard cartridge (2.0 × 20 mm I.D.) to confirm that samples did not contain any monoglycerol (MAG). Because of inconsistent retention times when the YMC-Pack PVA-Sil column was used, a silica column (Waters Spherisorb, 5-μm particles, 250 mm × 4.6 mm) with matching guard column was used to quantify diacylglycerol (DAG) and triacylglycerol (TAG). The column was held at 20 ± 1 °C. Standard curves were prepared using tripalmitin, 1,2-dipalmitin and 1,3-dipalmitin with concentration ranges between 0.63 and 6.25 mg/ml depending on the standard.

SPME Analysis

The SPME method is described by Sullivan Ritter and Budge [6]. SPME samples were placed in a heating block held at 80 °C and allowed to equilibrate for exactly 15.0 min while the SPME fiber was placed in the injector port of the GC at 250 °C to desorb any volatiles that had accumulated during storage. The SPME fiber was then 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 GCMS 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 (Nitroterephthalic acid modified polyethylene glycol 30 m × 0.25 mm ID × 0.25 μm film thickness, Agilent, Mississauga, Canada). Helium was used as the 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 percentages. Retention indexes were calculated by comparing retention times of oxidative volatiles to those of the two closest eluting *n*-alkanes in a C8-C20 retention index (RI) standard. Volatile oxidation products were identified by comparison to pure external standards. When standards were not available, compounds were tentatively identified by matching them with mass spectral data in the NIST/EPA/NIH Mass Spectral Library (National Institute of Standards and Technology), through matching of fragmentation patterns generated by HighChem Mass Frontier 4.0 (HighChem Ltd., Bratislava, Slovakia) and through comparison to RI and fragmentation patterns available in the literature.

Sensory Assessment of Oil Quality

Between ten and twelve samples from each oil trial were assessed for quality using an untrained, descriptive sensory panel [7]. To assess tasting abilities of potential panelists, volunteers were screened to eliminate those who could not distinguish fishy flavors. This was accomplished 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 in an open container at 40 °C for 7 days 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 1–10, with 10 being "excellent" and 1 being "terrible", using a fresh vegetable oil sample as a reference. These classifications were then used to assign a scalar value to each tasted sample. Samples with sensory panel scores ≤7 were considered unacceptable, based on the descriptions of the scalar values. Each sample evaluated by the final panel was tasted by a minimum of three people. The design and screening of the sensory panel as well as the score sheets used to evaluate the samples were adapted from guidelines provided by the AOCS [7]. Of the 184 samples analyzed with SPME-GCMS, 93 were evaluated for sensory characteristics, leaving approximately half untasted.

Statistical Analysis

Because SPME-GCMS data was expressed as area percentages, data was transformed using a geometric mean function [8], following Eq. 1.

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

where a_n is the amount, in area percent, of each peak quantified in the sample. This was necessary because proportional data are constrained between zero and one, thereby violating the assumption of independence of observations inherent in most statistical techniques [9]. These data were then combined with sensory panel results. The next step in data analysis was to remove outliers, as multivariate techniques are highly influenced by outliers. Mahalanobis distances are commonly employed to identify outliers in multivariate data sets and were used here. This measure takes into account the variance of each variable and the covariance between variables. These were

calculated for all samples as described by Filzmoser and Hron [10] and those with χ^2 statistic greater than the critical value for $p \leq 0.001$ were eliminated. Following this elimination, 10 % of remaining tasted samples were removed from the dataset to be used for method validation.

Because variables were highly correlated with each other, principal component analysis (PCA) was then used to reduce the number of variables in the dataset into a smaller number of uncorrelated variables. The new variables, called principal components (PC), were linear combinations of the original variables and maximized the variability they represented. Each PC had a value, or score, which corresponded to a specific data point (e.g. Fig. 1). Since each PC represented a linear equation of the original variables, loadings were simply the coefficients associated with each original variable in the equation. For instance, if a variable was particularly important in calculating a PC score, its loading might be 10, while a less important variable might have a loading of 0.1. Each replicate of each of the three oils underwent PCA of the covariance matrices. In the present study, the original data set contained 106 volatile oxidation products that were highly correlated and PCA was used to reduce the number of variables into separate PC.

PC scores were then treated as the independent variables in multiple linear regression analysis in order to generate an equation that could be used to predict the sensory quality of fish oil. Multiple linear regression is analogous to simple linear regression, but rather than one explanatory variables, there are multiple ones. This technique also requires that independent variables are uncorrelated. In this case, backwards linear regression was used, which begins by entering all predictor variables, in this case PC, into a regression model. The weakest predictor is then removed and the regression is recalculated. If the removal of a variable significantly weakens the model to the point where it is no longer significant, then that variable is added back in. This is repeated until only significant variables remain in the model. Often, more than one significant model ($p < 0.05$) was generated. In these cases, the model that had the highest R^2 and adjusted R^2, and lowest standard error of the estimate was selected. To validate the function, a sensory score was calculated for the samples that were tasted but not used to create the regression by calculating the PCA scores for each sample that was not used in the original PCA and regression function and inputting them into the regression equation. The calculated sensory score was then compared to the actual sensory score assigned by the sensory panel. The final step in this analysis was to test in the same way the omitted samples in other trials of the same oil to check for robustness. SPSS 11.0 [11] statistical software was used for all statistical analysis.

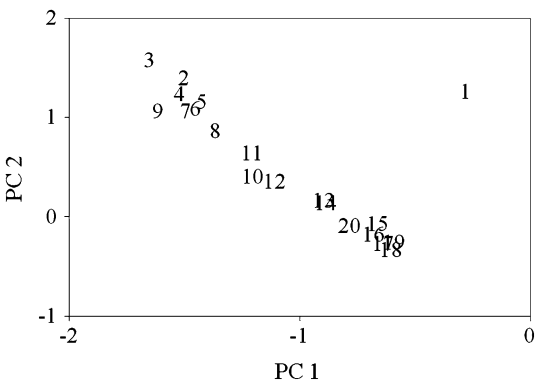

Fig. 1 Scores for principal components for Oil 2 Rep 1. Numbers correspond to the sequence in which samples were taken

Results

Fatty Acid Analysis

All three oils met suppliers' specifications for EPA and DHA (Table 2) and all three had different levels of these fatty acids.

Peroxide and Anisidine Values

As expected, PV and AV increased over time for all three oils. Initial PV was low (2.0 ± 1.3 mequiv/kg) for all samples, indicating that samples were only slightly oxidized at the beginning of the experiment. As the study progressed, the PV increased for all oils, far exceeding the 5 mequiv/kg limit specified by GOED (Fig. 2). Initial AV were also below the GOED limit of 20, with an average starting value of 9.4 ± 1.4. These values increased over time, but only one sample exceeded this limit at the end of the experiment (Fig. 3). Although the rate of increase of PV and AV differs from oil to oil, the sensory ratings for each oil are consistent within oils of the same type. The average PV at which samples were deemed unacceptable by a sensory panel was 14.9 ± 6.5 mequiv/kg which is almost 3 times the PV limit of 5 mequiv/kg specified by

Table 2 EPA and DHA content ($n = 3$, mean ± SD) of fish oils

Oil	EPA (mg/g)	DHA (mg/g)	Manufacturers specification (mg/g)
Oil 1	166 ± 1.4	125 ± 0.50	260
Oil 2	312 ± 5.5	225 ± 3.7	500
Oil 3	561 ± 3.3	157 ± 0.45	600

Manufacturer only guarantees an amount for combined EPA + DHA

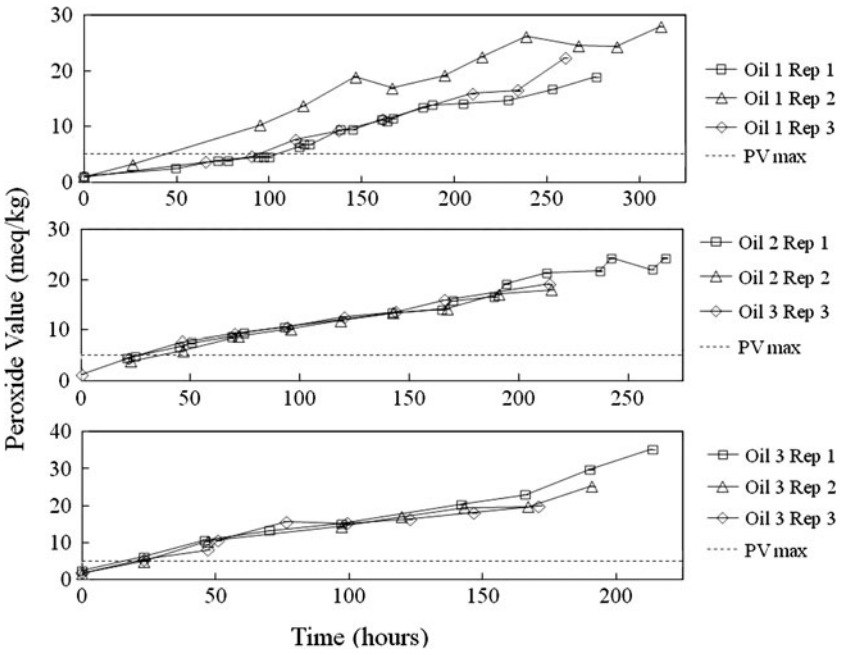

Fig. 2 Variation in peroxide value (PV; mean ± SD) in fish oil stored at 40 °C over time. The *dashed line* represents the PV limit set by GOED

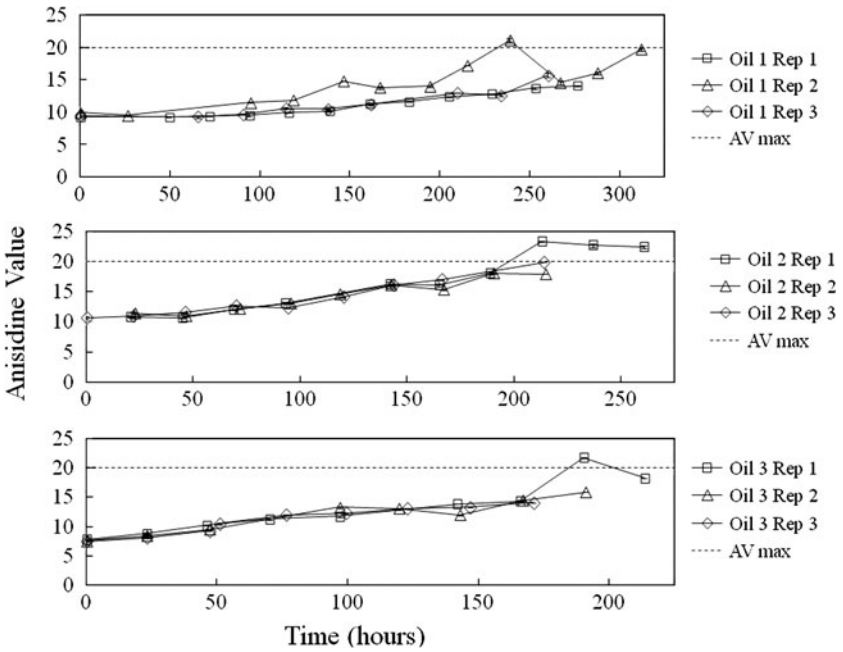

Fig. 3 Variation in anisidine value (AV; mean ± SD) in fish oil stored at 40 °C over time. The *dashed line* represents the AV limit set by GOED

GOED. The average AV at the sensory rejection point was 14.2 ± 3.7, almost 6 units below the GOED limit.

Lipid Class Analysis

TAG produced a linear standard curve while polynomial curves were the best fit for 1,2-DAG and 1,3-DAG. Oil 1 had higher TAG and lower DAG levels than specified by the manufacturer (Table 3), but Oil 2 and Oil 3 agreed with the manufacturers' specifications. In the case of Oil 1, the TAG value was likely higher because the oil did not undergo a concentration step during processing. The FA profile of Oil 1 with EPA and DHA levels of 16.6 and 12.5 %, respectively, was what one would expect to find naturally in a sardine-anchovy oil blend that has not undergone concentration during refining [12].

SPME and Sensory Analysis

A total of 106 oxidation products were detected using SPME-GCMS (Fig. 4). The most common structures were aldehydes and ketones, though alcohols, acids and

hydrocarbons were also detected. Typical compounds associated with oxidation were detected, including 3-hexen-1-ol, heptenal and multiple isomers of 2,4-heptadienal. Sensory scores for the samples ranged from 10 (excellent) to 4 (very poor). The scores of the test samples used to validate the regression models fell within the range of the scores used to build the models. There were no correlations between any of the oxidation products mentioned above and sensory scores.

Statistical Analysis

PCA of SPME-GCMS data generated from five to nine PC for each of the nine oil trials. When these PC were used to develop regression models, from two to five PC were needed to create a significant regression function, depending on the oil type and replicate (Table 4). Although the percent variance explained by the PC used in the regression models appear low, it was possible to achieve a high R^2 value (e.g. Oil 1 Trial 1). The reverse was also true (e.g. Oil 1 Trial 2). Regression of principal components gave well-fitting, significant ($p < 0.05$) models with for all but one oil replicate for Oil 3 (Table 4). Further statistical analyses on this trial were not carried out.

All samples that had not been used to create the regression functions were assigned sensory ratings using the appropriate regression function (Table 5). The calculated sensory scores for all three replicates of Oil 1 were quite close to the scores given by the sensory panel. All three samples had PV and AV levels below the average rejection values. The regression functions for two of the three replicates of Oil 2 also produced sensory scores that were very close to those given by the sensory panel. The sample with the poor result had a PV of 11.8 ± 0.0 mequiv/kg and an AV of 14.6 ± 0.1. The AV of the sample was very close to the average AV score at the sensory rejection point, while the PV was approaching the rejection point. The other two replicates of Oil 2 had PV and AV values well below the rejection thresholds. For Oil 3, the regression equation for the first replicate gave scores that were different from those assigned by the sensory panel. This replicate had a PV (23.0 ± 0.0 mequiv/kg) much higher than the average PV at the rejection point. The AV (14.4 ± 0.1) of this sample was also close to the rejection value for AV. In contrast, the regression equations for the second replicate of Oil 3 gave

Table 3 Lipid class composition for fish oil ($n = 3$, mean ± SD)

Oil	TAG (%)	Suppliers TAG specification	DAG (%)	Suppliers DAG specification
Oil 1	96.7 ± 12.0	70–75	3.35 ± 2.18	20–25
Oil 2	72.3 ± 13.1	70–75	27.7 ± 4.74	20–25
Oil 3	91.5 ± 10.0	90–91	8.55 ± 2.11	8

TAG Triacylglycerol, *DAG* Diacylglycerol

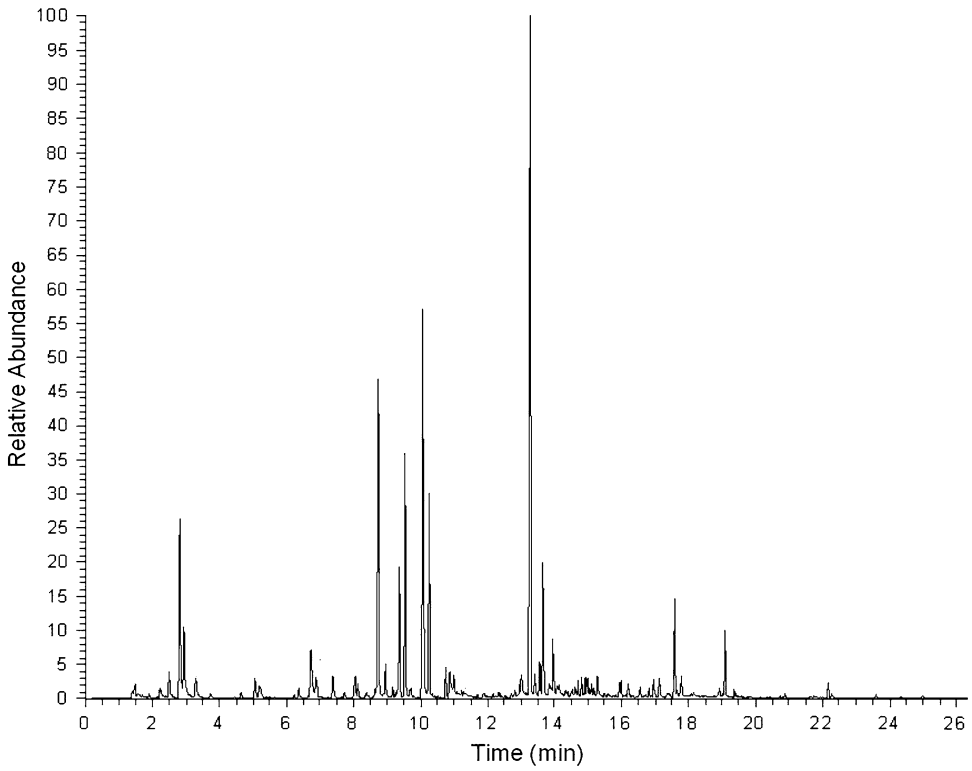

Fig. 4 Chromatogram of oxidation products for Oil 2 measured after 242 h of heating

Table 4 Regression models for fish oil samples

Oil	PC in regression model	% Variance explained by PC	p	R^2	Adjusted R^2	Std error of Est.
Oil 1 Trial 1	1, 3	56.2	0.001	1.00	1.00	0.05
Oil 1 Trial 2	1, 4	71.9	0.033	0.68	0.57	1.07
Oil 1 Trial 3	1, 2, 4	78.6	0.026	0.98	0.96	0.41
Oil 2 Trial 1	1, 2, 4, 5	87.7	0.037	0.88	0.77	0.76
Oil 2 Trial 2	1, 2, 3, 6	85.4	0.026	0.95	0.89	0.53
Oil 2 Trial 3	1, 3, 4	62.9	0.036	0.86	0.75	0.90
Oil 3 Trial 1	1, 3, 4, 5, 6	78.4	0.015	0.99	0.98	0.20
Oil 3 Trial 2	1, 2, 3, 5	92.7	0.040	0.98	0.94	0.40

very accurate scores when compared to those given by a sensory panel. This samples had PV (5.0 ± 0.0 mequiv/kg) and AV (8.44 ± 0.1) that were well below the average rejection values. Finally, when regression equations were tested with other replicates of the same oil type, the calculated sensory scores agreed with the actual scores for all but Oil 3 Replicate 2 (Table 5). When the sensory score for the omitted sample from Oil 3 Replicate 2 was calculated using the regression function for Oil 3 Replicate 1 a score of −1 was obtained, a value that is not valid. All other samples were within three sensory units of their taste panel score and most were within one unit.

Discussion

PCA and multiple linear regression were used instead of other multivariate techniques because of the characteristics of the SPME-GCMS data. The high number of volatile oxidation products (>100) resulted in a situation where there were many more variables than tasted samples, which eliminated the use of many common statistical methods such as multivariate analysis of variance (MANOVA). The levels of volatile oxidation products were highly correlated as most compounds were either increasing or decreasing over time making regression on raw data impossible. The

Table 5 Sensory scores given by a taste panel and calculated sensory scores, PV and AV ($n = 3$, mean ± SD) for select samples

Oil	Sensory panel score	Regression equation used to calculate sensory score	Calculated sensory score	Avg. PV (mequiv/kg)	Avg. AV
Oil 1 Trial 1	6	Oil 1 Trial 1	7	2.5 ± 0.0	9.3 ± 0.0
		Oil 1 Trial 2	4		
		Oil 1 Trial 3	5		
Oil 1 Trial 2	7	Oil 1 Trial 1	7	10.3 ± 0.0	11.5 ± 0.1
		Oil 1 Trial 2	6		
		Oil 1 Trial 3	7		
Oil 1 Trial 3	8	Oil 1 Trial 1	8	4.6 ± 0.0	9.7 ± 0.0
		Oil 1 Trial 2	5		
		Oil 1 Trial 3	7		
Oil 2 Trial 1	9	Oil 2 Trial 1	9	8.66 ± 0.0	12.0 ± 0.1
		Oil 2 Trial 2	9		
		Oil 2 Trial 3	9		
Oil 2 Trial 2	9	Oil 2 Trial 1	9	11.8 ± 0.0	14.6 ± 0.1
		Oil 2 Trial 2	8		
		Oil 2 Trial 3	9		
Oil 2 Trial 3	7	Oil 2 Trial 1	7	10.5 ± 0.0	12.3 ± 0.0
		Oil 2 Trial 2	4		
		Oil 2 Trial 3	5		
Oil 3 Trial 1	8	Oil 3 Trial 1	5	23.0 ± 0.0	14.4 ± 0.1
		Oil 3 Trial 2	8		
Oil 3 Trial 2	8	Oil 3 Trial 1	−1	5.0 ± 0.0	8.44 ± 0.1
		Oil 3 Trial 2	9		

changes in volatile levels occurred gradually so there was no clear divide between acceptable and unacceptable samples, instead there seemed to be an indistinct area where samples were neither good nor bad, making it difficult to obtain valid results using other classification techniques such as discriminant function analysis [6]. This was evident when scores of PC1 versus PC2 were plotted for each oil trial separately; samples showed a clear transition with time, with samples taken early in the time course in one quadrant and late samples in another quadrant (Fig. 3). Samples in the middle of the time course grouped near the center of the plot, emphasizing the lack of a distinct separation between new and old, or acceptable and unacceptable, indicating that the transition from acceptable to unacceptable happened gradually rather than suddenly. This gradual transition made it difficult to pinpoint the exact time when fish oil quality was considered unacceptable.

As an alternative, linear regression was considered. This is a useful technique for characterizing samples, but only if variables are uncorrelated. The volatile oxidation products studied here were highly correlated, so PCA was used to generate uncorrelated variables. PCA also greatly reduced the number of variables to <10. Linear regression of these variables generated a simple equation to assign sensory scores to fish oil samples. One tasted sample, selected at random, was left out of each trial with removal occurring before PCA analysis. Removal at this early point in data analysis allowed the samples to truly serve as a validation of the regression functions. One sample per oil trial corresponded to roughly 10 % of tasted samples being omitted during construction of the regression methods.

The combination of PCA and regression employed in this study allowed all data to be capitalized on as PCA transforms highly correlated variables, in this case volatile oxidation products, into uncorrelated linear combinations of the original variables, while still capturing all variability in the original data set. Initially, SPME-GCMS results of all replicates of all oils were combined and PCA was applied. When PC scores were examined, oils were clearly separated by oil type rather than state of oxidation as was expected due to differences in fatty acid and lipid class profiles between oils. It is well documented that PUFA, including EPA and DHA, oxidize more rapidly than other fatty acids [2, 13] so Oil 2 and Oil 3 which contained higher levels of these fatty acids were expected to oxidize at a different rate than Oil 1, with lower PUFA content. There is some evidence that DAG and MAG oxidize more rapidly than TAG [14, 15], so different levels of these structures in the oils may also be responsible for their clear separation based on volatile secondary oxidation products. Additionally, Oil 1 and Oil 2 were produced by the same manufacturer, while Oil 3 came from another, so slight differences in oil refining processes could affect the oxidation patterns and stability of the oils. For example, differences in the winterization process may result in oils having higher levels of shorter chain saturated fatty acids, which are more resistant to oxidation. Because PCA results were not useful when applied to all trials of all oils combined in a single group, data were separated by oil type and PCA was applied to Oil 1, 2, and 3 separately. PCA then detected the variance between oil replicates, rather than changes occurring in the same oil due to oxidation, resulting in three distinct groupings of samples according to replicate within a time course. Slight differences between replicates of the same oil were expected, as all trials were done sequentially rather than concurrently. This was done intentionally in order to develop a more robust method but unfortunately prevented all time courses of the same oil from being analyzed as one unit. These results demonstrate the sensitivity of PCA to small changes in volatile composition among replicates of the same oil. Thus, it was necessary to treat each replicate of each oil as an individual experiment.

When regression equations were tested with samples from other replicates of the same oil type, Oils 1 and 2, along with the first replicate of Oil 3 gave results consistent with the sensory panel. The results of the second replicate of Oil 3 were poor, giving an impossible score of -1. It is unclear what caused this odd result as no inconsistencies in the data were noted. With the exception of Oil 3 Trial 2, the consistency of the calculated scores compared to the sensory panel scores suggests that this classification method is robust and will hold true for oils of the same type, despite the need to develop separate regressions for each time replicate.

When PC and sensory panel scores were used to build regression equations, it became clear that the first PC (PC1) had a large affect on the reliability of the equation as all equations incorporated PC1. This is logical as PC1 is the component that describes the most variability in a given dataset. Because PC are linear combinations of the original variables, each PC is comprised of many oxidation products. For all trials, the compounds that had the highest correlations ($R \geq 0.80$) with each PC used in the regression equations were examined and compared. Since PC1 described such a large proportion of the change that occurred in the dataset and was incorporated into each regression equation, the replicates of the same oil type had many peaks in common, that were also highly correlated with PC1. When all replicates of all three oil types were compared there were eight peaks with high correlations with the PC used in the regression common to all trials (Table 6). These consisted of four aldehydes, one ketone, one benzene derivative and one hydrocarbon all of which had previously been identified in oxidized fish or fish oil. It

is interesting to note that not all of these compounds increased in proportion over time; three actually decreased.

It is well known that aldehydes play a role in the sensory properties of fish oil, and all four aldehydes identified here increase over time. (*E*,*Z*)-2,4-octadienal has been identified previously as a fish oil oxidation product and is associated with green odors [6, 16]. The second aldehyde, 3,6-nonadienal, is a degradation product of EPA and is associated with a green odor [2]. It has also been identified by MacFarlane et al. [17] as an oxidative volatile associated with sensory properties, and is a key component in the Fatty Acid Smell and Taste (FAST) method of predicting oil quality. The third aldehyde identified was (*E*,*E*)-2,4-decadienal, a compound with a deep-fried odor that is often identified in oxidized fish oil [17–19]. Jacobsen et al. [18] found that an increase in this oxidation product was correlated with an increase in rancid, fishy flavors in fish oil enriched mayonnaise. The final aldehyde associated with sensory properties of fish oil, (*E*,*E*,*Z*))-2,4,7-decatrienal, has been identified in oils and emulsions containing omega-3 fatty acids and is believed to have green, plant-like odors [16, 19, 20].

Only one ketone, 3,5-octadien-2-one, which increased over time, was shown to be strongly related to sensory parameters of all three oil types. The configuration of the double bonds could not be determined. Both the (*E*,*Z*) and (*E*,*E*) isomers of this compound have been identified in fish oil-enriched mayonnaise by Jacobsen et al. [18] and Hartvigsen et al. [16], who found that both isomers were strongly correlated with oxidation. Venkateshwarlu et al. [19] identified this ketone in fish oil-enriched milk. The (*E*,*Z*) isomer has been linked to green, fruity and fatty odors as well as plastic and synthetic odors, while the (*Z*,*Z*) isomer is associated with fresh, green and fruity odors [16, 19].

Table 6 Volatile oxidation products correlated with sensory characteristics of all 3 oils

Retention index	Compound	Direction of proportion change	Method of identification
963	2-Ethyl furan	Decrease	b, c
996	Octene	Decrease	b, c, d
1385	3,6-Nonadienal	Increase	b, c
1565	3,5-Octadien-2-one	Increase	b, c
1573	Benzaldehyde	Decrease	a
1608	(*E*,*Z*)-2,4-Octadienal	Increase	a
1820	(*E*,*E*) 2,4-Decadienal	Increase	a
1879	(*E*,*E*,*Z*) 2,4,7-Decatrienal	Increase	c, d

a: Retention index and MS fragmentation pattern compared with an external standard, b: NIST library match, c: probable ion fragmentation predicted using Mass Frontier 4.0, d: Comparison to retention index and spectral data from literature

The ether 2-ethylfuran was also identified as being a significant indicator of sensory characteristics, and decreased as oxidation progressed. This is a common oxidation product of omega-3 fatty acids and can be formed from the 12-hydroperoxide of EPA and the 16-hydroperoxide of DHA [21]. Venkateshwarlu et al. [19] identified this compound as having a sweet odor. Since sweet odors are commonly associated with positive sensory properties, a reduction in this compound could mean that positive sensory attributes are being eliminated as oxidation progresses, allowing negative attributes to become more noticeable.

An unknown isomer of decene had a strong relationship to sensory properties of oils and decreased over time. 1-decene can be formed during oxidation of oleic acid which is present in small amounts in fish oil [22]. Chung et al. [23] identified both 1-decene and 3-decene in mackerel although no relationship with sensory properties was mentioned. Although hydrocarbons are generally thought to be flavorless, the importance of this oxidative volatile to predicting sensory quality suggests that it plays a role in the flavor of fish oil.

Benzaldehyde was strongly associated with sensory properties of fish oil and decreased with time. This compound has a sweet odor and has been detected in fish oil-enriched mayonnaise [16], tuna oil [24] and cod liver oil [25]. It is unlikely that benzaldehyde is a direct product of fish oil oxidation, but it could be an oxidation product of a larger benzene derivative that was initially present in the oil. Giogios et al. [26] hypothesized that benzene compounds could be decomposition products of amino acid or sugars, explaining their low levels in fish oils.

PV and AV are convenient ways to monitor the formation of oxidation products in fish oil, but this work supports the well-known issue with these measures: limits, including those specified by GOED do not correlate with sensory properties of the oil. Hydroperoxides are primary oxidation products, which then degrade into volatile secondary oxidation products that are responsible for off-flavors and odors in oils. Frankel [2] has said that sensory panels can detect off-flavors in oils with PV < 1 mequiv/kg. This is supported by results collected by MacFarlane et al. [16] that showed freshly refined fish oil samples with PV of <1 mequiv/kg had strong fishy tastes. In some cases this might be because the hydroperoxides increased to their maximum and had now begun to degrade into secondary oxidation products. In that situation elevated AV would be expected. Conversely, the results of this study suggests that oils with high PV do not necessarily have poor sensory properties, likely because hydroperoxides have not yet

degraded to sufficient extent to form detectable levels of secondary oxidation products.

A number of samples in this study had PV >10 mequiv/kg and were classified as acceptable by the sensory panel and the PV at the point of rejection was 14.5 ± 6.5 mequiv/kg, suggesting that the commonly used limit of 5 mequiv/kg is not appropriate. Although there is little evidence that AV is related to sensory properties of fish oils [2], a value of 20 is commonly used as the rejection value when assessing fish oil quality. Because the regression model was created using data produced by monitoring proportions of volatile oxidation products produced over time, these results suggest that AV may be of more importance to sensory quality than was previously thought, with an average AV of 14.2 ± 3.7 at the sensory panel rejection point being lower than the limit of 20 specified by GOED. Interestingly, MacFarlane et al. [17] found that fish oil samples that had poor sensory properties with PV <1 mequiv/kg also had AV <20 indicating that hydroperoxides had likely not yet reached their maximum and begun to degrade. The regression models created from SPME-GCMS data classified samples with AV >14 as unacceptable, with predicted sensory scores ≤7, despite sometimes positive ratings given by the sensory panel. This also suggests that the current limit of 20 is too high. At least one study [27] found that AV was strongly correlated with sensory evaluation and headspace volatiles in partially hydrogenated soybean oil used for frying. The results of this study suggest that PV and AV may both be accurate and reliable methods to assess sensory quality but the limits that are currently used are not idea, with the PV limit too low and the AV limit too high.

Sensory panels have long been recognized as the best way to monitor fish oil quality; however, the expenses associated with them often prevent them from being implemented. It is costly to train and maintain panels. Often people do not want to participate in a panel that involves tasting foods with poor sensory characteristics, so compensation is essential. A proper sensory panel requires specially designed tasting booths, lighting and air flow systems. Despite the best training, sensory assessment is still highly subjective and even trained panelists can give inaccurate results on occasion. Our results offer support for this. When our regression model was used to assign a sensory score to samples, the majority of the results were within one unit of the score given by the sensory panel. There were three samples that were assigned a high score (8 or 9) by the sensory panel, but when classified using the regression model, received scores of 5 suggesting that the sample was of poor quality. On closer examination, it was found that all three were samples that were taken late in the time course, and all had AV above 14.2, the average AV at the point of sensory panel rejection. Two of the three samples also had PV >20 mequiv/kg indicating that oxidation had progressed considerably. The combination of these factors suggest that the regression models may actually be more accurate than the sensory panel in predicting the quality of highly oxidized oil samples; however, for the regression model to be accurate the sensory panel must also provide accurate initial assessments as this is the source of the data for the regression equations. Because of the cyclic relationship, it may be more logical to use PV and AV to evaluate the quality of very oxidized samples, or to present the taste panel with more samples to evaluate so that more data is available to build the regression model. This study was very small and limited samples were tasted by the sensory panel. Future research will focus on expanding the sensory panel and the number of oil samples evaluated to improve the robustness of this method.

The method presented here can be used by fish oil refiners and dietary supplement manufacturers to determine if fish oil has acceptable sensory parameters without the need to maintain an expensive sensory panel. Although a panel is required to develop the method, after the initial set up there should be no further need to maintain the sensory panel. Because the PV and AV limits currently used to determine oil quality seem to be of little use in predicting sensory quality, this method will provide more accurate results; however, in a commercial setting it should be possible to determine PV and AV limits that correlate with sensory parameters and then use these as an additional measure of quality. Based on results in this study, a maximum PV limit of 15 mequiv/kg and a maximum AV of 14 would appear to be appropriate for the oils used here.

In conclusion, PCA and linear regression can be used in conjunction with SPME-GCMS of oxidative volatiles to predict the sensory quality of fish oil. The method presented here suggests that the PV and AV values typically used to indicate fish oil quality have little relationship with sensory properties.

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