Static Headspace Gas Chromatographic Analyses to Determine Oxidation of Fish Muscle Lipids During Thermal Processing

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ABSTRACT: Oxidation in fish during thermal processing was studied by determining volatile production with a static headspace gas chromatographic system. Different processing temperatures and periods were evaluated to simulate conditions of fish industrial treatments. The major volatiles formed included acetaldehyde, propanal, heptane, 2-ethylfuran, pentanal, and hexanal. Changes in volatile composition were studied for different processing times and temperatures. The method for volatile analyses to determine oxidation in fish muscle was tested by correlation with peroxide value, conjugated diene, and thiobarbituric acid indices. Significant single and multivariate regressions were found between the time of thermal treatment and volatiles produced, showing that the amount of 2-ethylfuran was the best predictor of oxidative stability in fish.

Paper no. J8854 in *JAOCS 76*, 231–236 (February 1999).

KEY WORDS: 2-Ethylfuran, fish oxidation, static headspace gas chromatography, thermal processing, volatiles.

Several papers have described the important role of lipid deterioration during food processing on the quality of the final products (1–3). Lipid oxidation is often related to a significant number of volatile compounds that can be produced from polyunsaturated fatty acids (PUFA) during thermal treatment of foods (3,4). The highly unsaturated fatty acid composition of fish muscle makes it extremely susceptible to oxidation and degradation during thermal treatments (5,6).

Different methods were employed to evaluate the mechanism of fish lipid oxidation and to assess its overall flavor quality (7,8). These methods were based mainly on the formation of specific compounds or their interaction with other products in foods. However, the formation of some of these compounds can be so rapid that these methods have not proved to be useful to evaluate the thermal processing of fish. Hydroperoxides, formed as primary products of lipid autoxidation, are rapidly decomposed to produce a variety of secondary volatile compounds of low molecular weight. Aldehydes are the main volatile secondary products responsible for off-flavors and odors during storage and treatments of

foods (8–11). Several methods have been employed to determine aldehydes in complex food systems (12–14). Recently, static headspace gas chromatographic (SHS-GC) methods were shown to provide a simple and rapid method to determine aldehydes in fish and vegetable oils formed during oxidation (15–17). Propanal, resulting from n-3 PUFA oxidation, and hexanal, from n-6 PUFA oxidation, were commonly used together with conjugated diene values to evaluate the peroxidation of PUFA in biological systems (18,19). This method has the advantage of not requiring sample workup and of permitting the injection of clean aliquots of volatile compounds from the headspace produced from foods or biological systems. The direct and accurate analysis of volatiles in fish muscle by SHS-GC requires careful standardization of instrumental parameters such as sample size, equilibration time and temperature, and instrumental conditions required for the separations of volatile compounds (20,21).

The current work was aimed at developing a simple SHS-GC method to determine the extent of oxidation during thermal treatment of fish muscle by analyzing the volatile compounds formed. Processing temperatures were those commonly employed in fish technology: 40 and 60ºC, used in processes such as smoking, and 100ºC, used for canning. The amounts of aldehydes produced were correlated with other established parameters such as peroxide value, conjugated dienes, and thiobarbituric acid-reactive substances (TBARS).

EXPERIMENTAL PROCEDURES

Materials. Three cans of tuna were purchased at a local market. After arrival at our laboratory, the fish was treated immediately to obtain samples for analysis. Portions of about 200 g of white muscle were minced, wrapped in filter paper, and well mixed to obtain homogeneous samples. Samples were then prepared by weighing 1 g of minced tuna muscle into 6- mL headspace vials (Perkin-Elmer, Norwalk, CT). All chemicals and solvents used were either analytical or high-performance liquid chromatography grade (Fisher Scientific, Pittsburgh, PA).

Incubation experiments. Vials containing 1 g of minced tuna muscle were immediately sealed with silicone rubber Teflon caps and oxidized in the dark in an oven at 40, 60, and

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100ºC. Sampling times ranged between 0 and 4 d for 40 and 60ºC, and 0 to 150 min for 100ºC. For each sampling time and temperature, three vials were analyzed. Each treatment was replicated three times using the three different fish subjected to the same handling conditions as described above.

Volatile analyses. SHS-GC analysis was performed with a Sigma 3B gas chromatograh equipped with an H-6 headspace sampler (Perkin-Elmer). Vials were equilibrated 5 to 15 min using temperatures ranging from 20 to 100ºC and pressurized with carrier gas for 30 s before injection into a capillary column DB-1701 (30 m \times 1 mm thickness; J&W, Folsom, CA). Injector and oven temperatures were set at 180 and 70ºC, respectively. Helium at 20 cm/s linear velocity was the carrier gas. The flame-ionization detector temperature was set at 200ºC. Volatile compounds were identified by comparison of retention times with those of authentic reference compounds: acetaldehyde, propanal, heptane, pentanal, hexanal, and 2 ethylfuran (Sigma, St. Louis, MO). Peak areas for individual and for total volatiles were integrated. Propanal and pentanal (Sigma) were used as external standards.

Gas chromatography–mass spectrometry (GC–MS). The identities of volatile compounds were confirmed with a Hewlett-Packard Model 5890 Plus GC coupled with a 5989 A Hewlett-Packard MS system and a SHS Hewlett-Packard Model 7694. The GC conditions were the same as those described above. The mass spectrometer was operated in the electron impact ionization mode (70 eV). The transfer line and ion source temperature were both set at 280ºC.

Lipid extraction. After analysis of volatiles, triplicate vials were carefully opened and lipids were extracted from each tuna muscle by the Bligh and Dyer method (22). Lipid content was determined in duplicate and expressed as percentage wet weight (23). Lipids were also extracted from the starting minced tuna muscle before thermal treatments to calculate the initial fatty acid composition.

Fatty acid analysis of the starting tuna muscle. Duplicate aliquots of oils were converted to methyl esters (24) and analyzed by GC as described by Christie (25).

Oxidation measurements. Peroxide value (mmol/kg lipids) was determined by the ferric thiocyanate method (26). Conjugated diene values (absolute absorbance/mg lipids) were determined according to AOCS Method Ti 1a-64 (27): aliquots of lipid extracts were evaporated to dryness under nitrogen, dissolved in 5 mL isooctane, and the absorbance was measured at 234 nm. TBARS (mg malonaldehyde/kg dry muscle) was determined according to the method of Vyncke (28). All the analyses were performed in duplicate.

Statistical analysis. Multiple regression was calculated by Forward Stepwise Regression using a statistical package (29). BETA values were calculated, which represented the independent contribution of each variable to the regression model. The data were subjected to one-way analysis of variance (ANOVA) according to Sokal and Rohlf (30). Comparisons of means by ANOVA test were determined using a least squares difference (LSD) method (29).

Precision of the SHS-GC method. Twelve vials containing

1 g of minced tuna muscle from the same fish were sealed and kept at 0ºC until incubation. They were divided in three groups of four samples, and each group was incubated separately for 4 d at 40ºC and then analyzed by the SHS-GC method. The coefficient of variance was then compared with the coefficient of variance between groups.

RESULTS AND DISCUSSION

Fatty acid analyses of lipids of tuna muscle before oxidation showed that they were particularly rich in PUFA and especially in n-3 PUFA (Table 1). The lipid fraction contained 25% 22:6n-3 and 7.4% 20:5n-3, having a lower proportion of saturated and monounsaturated fatty acids in agreement with previous results (Table 1) (7,31). Due to the high degree of PUFA present in tuna and the lipid content of muscle before any incubation $(2.5 \pm 0.8\%$ expressed as wet weight), processes involving thermal treatments of fish are expected to promote significant degradation of hydroperoxides by thermal oxidation of PUFA (3,4).

SHS-GC. The time and temperature of equilibration are known to be important factors affecting volatile analyses by SHS-GC of fish muscle (20). Volatile analyses by SHS-GC were standardized by subjecting 1-g samples of tuna fish muscle to various equilibration temperatures and time periods. Peak intensities due to total volatiles and the number of peaks increased at equilibration temperatures above 60ºC (Fig. 1). The rate of volatile formation increased sharply above 80ºC resulting from further thermal decomposition of oxidized fish muscle lipids during equilibration. To minimize artifact formation during SHS-GC analyses, the equilibration conditions for tuna muscle were set at 60°C for 15 min.

Oxidative stability of fish muscle. Samples of fish muscle were oxidized at 40 and 60ºC and the extent of oxidation was followed by measuring peroxide values, conjugated dienes, TBARS and volatiles. The peroxide values of fish muscle increased during oxidation at 40 and 60ºC, reaching a maximum after 1 d with no apparent induction period, followed by a decrease between 1 and 4 d (Table 2). There was no significant difference in oxidative stability of fish samples based on per-

a Data are expressed as mean ± standard deviation of three different fish.

FIG. 1. Effects of incubation temperature and time on total volatiles.

oxide value between 40 and 60ºC. Peroxide values reflected significant decomposition of hydroperoxides during heating of fish muscle (31–33). In contrast to peroxide values, the formation of conjugated dienes increased significantly after one day at 40ºC and after day at 60ºC (Table 2). TBARS increased from day 0 to day 2, and reached a maximum at two days followed by a gradual decrease (Table 2). Decreasing values of TBARS were previously reported during thermal treatment of fish muscle (32). Values for TBARS measure interaction compounds formed during heating between aldehydes and other biological compounds (34).

Analyses of volatiles by SHS-GC in tuna muscle oxidized at 40ºC showed the formation of acetaldehyde, propanal, heptane, 2-ethylfuran, pentanal, and hexanal (Fig. 2, Table 2). With the exception of 2-ethylfuran, these volatile compounds were reported to arise from different oxidized oils containing n-3 PUFA (15,17). Propanal is derived from the decomposi-

tion of linolenate hydroperoxides, and hexanal and pentanal from the decomposition of linoleate hydroperoxides (10). Heptane also was detected in significant quantities in canned pink salmon (20). The presence of low quantities of 2-ethylfuran was detected in dried and smoked fish meal (35). Recently 2-ethylfuran also was reported in heated fish oils (36).

Propanal was found in much higher amounts than pentanal and hexanal, as expected by the higher content of n-3 (35.7%) than n-6 PUFA (3.3%) in the fish sample (Table 1). The formation of propanal showed no induction or lag period during oxidation for four days at 40ºC (Table 2). This result is in agreement with a previous report of high propanal formation in menhaden and sardine oils oxidized at 50ºC with no induction period (15). The easier formation of propanal by thermal decomposition of the n-3 PUFA, than hexanal by the decomposition of n-6 PUFA, was previously shown by the lower activation energy of propanal than hexanal formation (15). Both propanal and acetaldehyde showed a significant increase during the first two days of oxidation at 40 and 60ºC and reached a plateau during the later days (Table 2). The formation of 2 ethylfuran, pentanal, and hexanal at 40 and 60ºC showed an induction period of about one day followed by a slow but significant increase with oxidation time (Table 2). The trends in volatile formation observed during oxidation of fish muscle at 60ºC were similar to those at 40ºC. However, heptane formation at 60ºC showed no induction period and reached a plateau after the first day of oxidation with no significant increases until day 4 (Table 2). These differences in volatile formation may indicate that the decomposition of different hydroperoxides varies with temperature of oxidation.

The oxidative stability of tuna fish samples was also investigated at 100ºC because this temperature is commonly employed in canneries (fo, critical lethality, or time needed at 250ºF or 121ºC to reduce a population of *Clostridium botulinum* of $10^{12} = 4-6$ min). The peroxide values and conju-

TABLE 2

Analysis of Lipid Oxidation and Volatiles Produced After Incubation of Fish Muscle at 40, 60, and 100°C*^a*

	Peroxide	Conj. diene	TBARS	Acetaldehyde	Propanal	Heptane	2-Ethylfuran	Pentanal	Hexanal
40° C									
0 Day	0.64 ± 0.23 ^a	0.388 ± 0.018^a	1.01 ± 0.35 ^a	321 ± 43^a	$387 \pm 68^{\rm a}$	0 ± 0^a	0 ± 0^a	$0 \pm 0^{\rm a}$	0 ± 0^a
1 Day	$5.80 \pm 0.65^{\rm b}$	0.408 ± 0.021^a	2.15 ± 0.21^b	1125 ± 124^b	$2041 \pm 76^{\rm b}$	0 ± 0^a	0 ± 0^a	$101 \pm 9^{\rm b}$	0 ± 0^a
2 Day	$4.31 \pm 1.02^{b,c}$	$0.474 \pm 0.08^{\rm b}$	3.53 ± 0.43^c	$4405 \pm 302^{\circ}$	3778 ± 238 ^c	$208 \pm 16^{\rm b}$	32 ± 12^{b}	$340 \pm 32^{\circ}$	$22 \pm 7^{\rm b}$
3 Day	3.59 ± 0.32 ^{c,d}	0.545 ± 0.032 ^c	$3.06 \pm 0.17^{c,d}$	5561 \pm 718 ^{c,d}	3848 ± 179 ^c	938 ± 85 ^c	$69 \pm 9^{\circ}$	$584 \pm 66^{\rm d}$	$75 \pm 36^{\circ}$
4 Day	2.65 ± 0.71 ^d	0.594 ± 0.017 ^d	$2.56 \pm 0.07^{b,d}$	6779 ± 431 ^d	4480 ± 466 ^c	$1562 \pm 332^{\rm d}$	$136 \pm 7^{\rm d}$	987 ± 52^e	186 ± 27 ^d
60° C									
0 Day	0.64 ± 0.16^a	0.409 ± 0.025^a	1.00 ± 0.32 ^a	390 ± 11^a	493 ± 15^a	0 ± 0^a	0 ± 0^a	0 ± 0^a	0 ± 0^a
1 Day	$4.86 \pm 0.15^{\rm b}$	$0.437 \pm 0.048^{a,b}$	3.20 ± 0.10^b	$4618 \pm 545^{\rm b}$	5706 ± 652^b	$325 \pm 37^{\rm b}$	$975 \pm 51^{\rm b}$	$277 \pm 34^{\rm b}$	$60 \pm 5^{\rm b}$
2 Day	4.57 ± 0.22^b	0.488 ± 0.009^b	4.53 ± 0.18 ^c	$6812 \pm 921^{b,c}$	7328 ± 748 ^b	359 ± 3^{b}	$4210 \pm 320^{\circ}$	$482 \pm 5^{\circ}$	$148 \pm 21^{\circ}$
3 Day	3.23 ± 0.35 ^c	0.560 ± 0.037 ^c	3.95 ± 0.23^c	8124 ± 479 ^c	10447 ± 621 ^c	$428 \pm 54^{b,c}$	12234 ± 743 ^d	916 ± 150 ^d	293 ± 19^d
4 Day	1.65 ± 0.76 ^d	0.670 ± 0.033 ^d	$3.21 \pm 0.08^{\rm b}$	$8829 \pm 357^{\circ}$	$12706 \pm 901^{\circ}$	$545 \pm 66^{\circ}$	23427 ± 1407 ^e	1357 ± 148^e	442 ± 28^e
100° C									
0 min	0.64 ± 0.16^a	0.405 ± 0.030 ^a	$1.01 \pm 0.22^{\text{a}}$	127 ± 20^a	$368 \pm 68^{\rm a}$	$0 \pm 0^{\rm a}$	0 ± 0^a	0 ± 0^a	0 ± 0^a
30 min	0.87 ± 0.23 ^a	$1.058 \pm 215^{\rm b}$	1.55 ± 0.04^b	2973 ± 167^b	2580 ± 145^b	250 ± 22^b	154 ± 22^{b}	$172 \pm 15^{\rm b}$	$58 \pm 8^{\rm b}$
60 min	1.62 ± 0.13^b	2.283 ± 117^c	$1.29 \pm 0.18^{a,b}$	$11057 \pm 1047^{\circ}$	$7345 \pm 901^{\circ}$	$398 \pm 49c$	1826 ± 233 ^c	$353 \pm 32^{\circ}$	$184 \pm 62^{\circ}$
90 min	2.32 ± 0.31^c	$2.831 \pm 65^{\rm d}$	1.06 ± 0.09 ^a	15126 ± 2023^c	$9719 \pm 847^{c,d}$	$515 \pm 57^{\rm c,d}$	$4824 \pm 126^{\rm d}$	$575 \pm 47^{\rm d}$	214 ± 27^c
120 min	3.40 ± 0.56 ^d	2.717 ± 125 ^d	0.42 ± 0.08^c	22455 ± 1876 ^d	12837 ± 1103 ^d	$651 \pm 37^{\text{d,e}}$	10425 ± 133^e	$920 + 157^e$	329 ± 24^d
150 min	$1.90 \pm 0.42^{b,c}$	1.526 ± 89^e	$0.36 \pm 0.11^{\circ}$	26146 ± 3257 ^d	17101 ± 503^e	791 ± 91^e	22319 ± 1187 ^t	$1533 \pm 168^{\dagger}$	424 ± 12^e

a Results are expressed as mean ± standard deviation of three independent treatments. Peroxide is expressed as mmol/kg, conjugated diene (conj. diene) as absolute absorbance/mg lipid, thiobarbituric acid-reactive substances (TBARS) as mg malonaldehyde/kg dry muscle, and volatiles as peak areas of gas chromatography-headspace. Values within each column followed by the same letter are not significantly different $(P < 0.01)$.

FIG. 2. Static headspace-gas chromatogram of volatiles formed in a fish sample after four days of fish oxidation at 40ºC. Peaks are identified as follows: 1, acetaldehyde; 2, propanal; 3, heptane; 4, 2-ethylfuran; 5, pentanal; and 6, hexanal.

gated diene of fish muscle heated at 100ºC increased during oxidation, reaching a maximum between 90 and 120 min (Table 2). TBARS increased markedly up to 30 min at 100ºC, followed by a significant decrease between 30 and 150 min (Table 2). At elevated temperatures, hydroperoxides are expected to produce larger amounts of complex secondary products (9). The main volatiles formed at 100ºC were the same as observed at 40 and 60ºC, namely, acetaldehyde, propanal, heptane, 2-ethylfuran, pentanal, and hexanal (Table 2). The formation of acetaldehyde and propanal occurred rapidly in fish muscle oxidized at 100ºC without showing an induction period. There were significant differences between values of

TABLE 3 Analysis of Volatiles in Fish Muscle*^a*

Volatiles	Mean	S. D.	C. V.				
Acetaldehyde	5125.00	500.29	9.8				
Propanal	4378.00	466.21	10.6				
Heptane	2041.50	219.44	10.7				
2-Ethylfuran	201.25	7.36	3.7				
Pentanal	873.25	52.87	6.1				
Hexanal	149.25	14.12	9.5				

a Results are expressed as total peak area for each volatile. Mean, standard deviation (S.D.), and coefficient of variation (C.V.) of a group of four different samples corresponding to the same fish and incubated at 40ºC for 4 d.

these volatiles during the first 60 min (Table 2). After this period, there were no significant differences between sampling times. Formation of heptane increased from 0 min until 150 min. Formation of 2-ethylfuran, pentanal, and hexanal occurred more slowly than the other volatiles with significant differences between each sampling time. 2-Ethylfuran showed the greatest increase among the volatiles during oxidation at 100ºC.

Validation of the SHS-GC procedure. Good precision was achieved by the SHS-GC method for volatile analysis of fish muscle, as shown by the relatively low standard deviations for a group of four different samples corresponded to the same fish and incubated at 40ºC for 4 d (Table 3). Coefficients of variance between groups were similar to those of replicated samples of the same group.

Data obtained at 40, 60, and 100°C showed highly significant differences in the production of acetaldehyde and propanal during the first periods of heating and in pentanal, 2-ethylfuran, and hexanal formation during the last periods. This result suggests that the analysis of aldehydes, such as acetaldehyde and propanal, can be useful to determine oxidation of fish muscle during the early stages of heating; 2-ethylfuran and heptane, pentanal, or hexanal can be useful in later stages of thermal oxidation. The use of acetaldehyde may be limited, however, because it is difficult to separate from pentane and other early peaks formed during oxidation (15).

To test how volatile compounds formed during thermal treatment can be used to determine oxidative stability of fish muscle, regression analyses were carried out between each volatile and measurements of peroxide value, conjugated diene, and TBARS. Data from the three independent treatments using different fish at 40 and 100ºC were used for regression analysis. No significant correlation could be determined between peroxide value and volatiles, because peroxide values decreased while volatiles began increasing due to the decomposition of hydroperoxides (Table 2).

Significant multivariate regressions between conjugated diene and TBARS and selected volatiles were determined by calculating BETA values, which represent the independent contribution of each variable on the model. With fish muscle samples oxidized at 40ºC good correlations were found between conjugated diene and 2-ethylfuran and pentanal, with an R^2 of multivariate regressions equalling 0.724 (Table 4).

TABLE 4

a Data used were the total peak area for each volatile.

*^b*Coefficient of Multivariate Regressions. BETA: independent contribution of each variable to the regression model.

c Coefficient of Nonlinear Regressions. BETA: independent contribution of each variable to the regression model. Numerical superscripts are expressing functions. Significance was accepted at *P* < 0.01. For abbreviation see Table 2.

The amount of 2-ethylfuran gave the highest independent contribution in the multivariate regression (BETA = 1.44526). For TBARS, multivariate regressions with propanal and hexanal, gave significant parameters $(R^2 = 0.995)$, with propanal having the highest independent contribution, BETA = 1.39031. A multiple regression analysis showed a high correlation (R^2 = 0.934) between heating time at 40^oC and 2-ethylfuran, heptane, and propanal. Again, 2-ethylfuran was the best predictor of the effect of heating time (BETA = 0.51550).

For samples oxidized for 150 min at 100ºC, single and

CH₃ - CH₂ - CH = CH - CH = CH - CH (OOH) - R (Hydroperoxide)
\n
$$
\beta
$$
- cleavage
\nCH₃ - CH₂ - CH = CH - CH = CH' + 'CH (OOH) - R
\n+ O₂ Reaction with oxygen
\nCH₃ - CH₂ - CH = CH - CH = CH - OOH (Vinyl hydroperoxide)
\n- 'OH Loss of hydroxyl radical

 $CH - CH - CH = CH - CH = CHO$ (Alcoxyl radical)

2-Ethylfuran

FIG. 3. Mechanism of 2-ethylfuran formation.

multivariate regressions also were calculated between heating time and volatiles formed (Table 4). An excellent nonlinear regression was calculated with time of incubation at 100°C and 2-ethylfuran (R^2 = 0.999). Conjugated diene value was highly correlated with the amounts of acetaldehyde and 2-ethylfuran by multivariate regression $(R^2 = 0.969)$. The best results for TBARS values were obtained using a nonlinear function of the amounts of 2-ethylfuran formed $(R^2 = 0.993)$.

The formation of 2-ethylfuran had the highest independent contribution for the prediction of oxidative stability of fish muscle held 4 d at 40ºC and 150 min at 100ºC. The formation of 2-ethylfuran in oxidized n-3 PUFA in fish can be explained by the same mechanism postulated previously for 2-pentylfuran formed in oxidized n-6 PUFA (10) (Fig. 3). The 12-hydroperoxide of linolenate (18:3n-3), the 14-hydroperoxide of eicosapentaenoate (20:5n-3), and the 16-hydroperoxide of docosahexaenoate (22:6n-3) can undergo β-cleavage to produce a conjugated diene radical which can react with oxygen to produce a vinyl hydroperoxide. Cleavage of this vinyl hydroperoxide by loss of a hydroxyl radical forms an alkoxyl radical that undergoes cyclization to produce 2-ethylfuran.

The SHS-GC analysis of acetaldehyde, propanal, heptane, 2-ethylfuran, pentanal, and hexanal provided a rapid, nondestructive, and easy method to monitor oxidation during thermal oxidation of fish. This method can be useful to study effects of different variables in fish processes using thermal treatments. Regression models calculated for both 40 and 100ºC showed that 2-ethylfuran is a highly useful volatile to determine oxidative stability in fish muscle during thermal treatments. The possibility of using this volatile in assessing the quality of overheated foods may be considered. Determination of propanal may also be useful to follow early stages of fish thermal oxidation.

ACKNOWLEDGMENTS

We acknowledge financial support from the Spanish Local Goverment (Xunta de Galicia) (to IM) and from the Spanish Ministry of Education and Culture (to MTSG). The Centre of Scientific Technology for Research of University of Vigo and Dr Manolo Marcos are gratefully acknowledged for technical assistance with the mass spectrometry.

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[Received April 27, 1998; accepted October 20, 1998]