Characterization of Forced Oxidation of Sardine Oil: Physicochemical Data and Mathematical Modeling

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ABSTRACT: It is of major interest to the food industry to understand the mechanisms and kinetics underlying spontaneous oxidation of marine oils because these polyunsaturated fatty acid (PUFA)-rich oils, the object of several health claims, have been repeatedly recommended for dietary intake. The present study attempts to characterize forced oxidation and hydrolytic breakdown of glycerides and fatty acids in sardine oil. A simple, first-order mathematical model was postulated and successfully fitted to the experimental data. This model confirmed that the rate of decrease in concentration of intact fatty acid moieties is almost directly proportional to the number of double bonds present. Therefore, as expected, the rate of oxidative decay was virtually independent of chain length, with an overall activation energy of *ca.* 22 kJ mol^{-1}. Additionally, the rate of hydrolysis was correlated with the rate of oxidative decay. With the exception of fatty acids possessing more than four double bonds, PUFA proved to be relatively stable to oxidation for up to 10 h at 50–70°C, and the qualitatively richest pattern of volatiles was obtained when the reaction was performed at the highest temperature (80°C).

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KEY WORDS: Fish oil, hydrolysis, lipid decay.

Sardine is a pelagic marine fatty fish species that accounts for *ca.* 40% of the total annual catch by Portuguese trawlers. Since most of the sardine harvest is used by the fish canning industry, an impetus exists for research efforts directed toward its oil, normally a waste by-product characterized by a high oxidative instability. Sardine oil contains a high concentration of polyunsaturated fatty acids (PUFA), which have been shown to have positive effects on cardiovascular diseases (1).

The present study attempts to experimentally describe changes in the fatty acid pattern of sardine oil, including formation of oxidation products. It further tries to postulate mechanistic models and eventually fit them to experimental data obtained *via* forced oxidation of bulk oil. Such models might be useful for long-term prediction of oil behavior with regard to oxidation. However, they should not be blindly extrapolated because whole fish undergoes seasonal changes in lipid composition (2), and natural antioxidants that compete for available molecular oxygen often are present in the fish body (3). Beltrán and Moral (4) monitored oxidative deterioration in sardines during frozen storage and found that results were highly dependent on the total fat content and the degree of protein-lipid bonding. Thus, in sardines caught in June, which were characterized by a high lipid content (10.9% by weight), the relative level of free and oxidized PUFA in the fat fraction increased faster throughout storage time than in sardines caught in March, which were characterized by a low lipid content (5.1% by weight).

Fish oils are oxidized *via* the same general mechanism that plant oils follow, but the breakpoint in the induction curve of the former is not as distinct as that of the latter. Therefore, antioxidants usually play a minor role in extending such an induction period (5). An artificial antioxidant that is effective in preventing rancidity in fish oils is tertiary-butylhydroquinone (6). Marine oils contain 15–30% fatty acids bearing between four and six double bonds, whereas in plant oils they do not usually exceed 1%. Such high contents of PUFA determine the major susceptibility of marine oils to oxidation. The amounts of products formed by oxidation vary widely, as does the pattern of volatiles produced *via* oxidation of marine oils. A good example is the array of oxidation products of purified menhaden oil (7). Use of modern biotechnological methods could improve the stability of marine oils, e.g., *via* enzymatic acidolysis or transesterification of the triglyceride structure, thereby retarding autoxidation. Incorporation of oleic (18:1, *cis*-9) and elaidic (18:1, *trans*-9) acids has also been shown to have a rather marked lowering effect upon the rate of oxidation of sardine oil (8).

The major goal of the present study was to shed light on the mechanism and kinetics of oxidative decay of lipids in sardine oil. This knowledge may aid in tailoring storage conditions or mild processing techniques designed to extend its shelf life rather than deliberately modifying it at the level of triglyceride structure.

MATERIALS AND METHODS

Feedstock. Antioxidant-free, semirefined fish oil consisting of at least 90% sardine oil (characterized by a volumetric mass of 0.90 g/cm^3) was a gift by Gomes & Severino (Cama-

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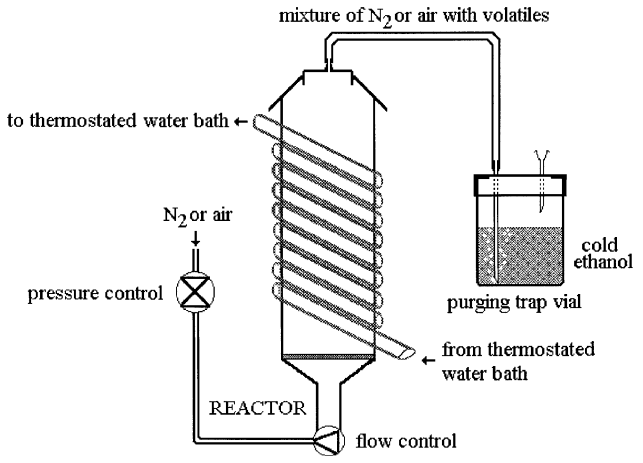
rate, Portugal). The acid value and the peroxide indices of the oil were sufficiently low so that it could be considered as a high-quality feedstock. The oil was stored at 4°C in brown 2.5-L glass bottles, leaving as little headspace as possible and flushing it with nitrogen before stoppering.

Setup. Forced oxidation of oil was carried out using the experimental setup represented in Scheme 1; this setup consisted basically of an isothermally operated batch reactor and was selected owing to its high efficiency of heat and mass transfer brought about by purge-induced mixing. Gas-carrier tubing and connections were made of silicone to ensure full inertness. In order to promote intimate gas/oil mixing, the inlet at the bottom of the reactor was through a sintered glass plate and the flow rate of gas was adjusted to 46 ± 2 mL/min. Isothermal operation was ensured *via* covering the outer surface of the reactor with a heating coil connected to an external thermostated water bath; efficiency of heat transfer was double-checked in preliminary trials, where it could be shown that heating was fast (reactor temperature reached desired set values by 5–10 min after startup), accurate (variability was within 0.5°C of set value), and sustained (set temperature could be maintained throughout a 10-h span). Air (or nitrogen) was made available in compressed form from high-pressure bottles after reduction using a pressure regulator coupled in line with a flow regulator.

Finally, the gaseous outlet from the reactor (containing volatiles released as lipid oxidation products) was bubbled through an ice-cooled purging trap, using ethanol as solvent owing to its amphiphilic properties. Such trap used vials that, after purging, were tightly screwcapped for subsequent analysis by gas chromatography (GC).

Experimental design and sampling. Each experiment was started with loading of the reactor with 25 mL of fish oil, warming it until the desired experimental temperature was reached and then starting the stopwatch. After a few preliminary trials, we concluded that, for a feasible maximum of eight experimental runs, the most informative sampling times were 0, 1, 2, 3, 4, 6, 8 and 10 h; at each such time, a sample of oil was taken as well as a sample of the ethanol-based purging trap. This protocol was repeated for the operating temperatures 50, 60, 70, and 80°C. The process using nitrogen as flushing gas at the highest temperature (i.e., 80°C) was taken as control. The experimental program was thus laid out as a classical two-factor, four-level factorial design with an additional blank experiment (9).



SCHEME 1

Prior to analysis, the samples were transferred to 10-mL screw-cap bottles, the headspace volume was flushed with nitrogen before stoppering, and the bottles were wrapped in aluminum foil to protect them from decay brought about by incident light during storage. Assays for the acid value were performed on the same day as sampling, whereas derivatization and chromatographic analyses were not delayed more than 24 h. Vials containing the ethanol extracts of volatiles after purging for 20 min were analyzed directly on the same day.

Analytical methodology. The chemical analyses were selected to give as clear a picture as possible of forced oxidation in terms of decay pattern of the major lipid groups in the oil and of mapping of the volatile oxidation products generated.

The ethanol extracts were analyzed using a gas chromatograph AutoSystem XL from Perkin-Elmer (Norwalk CT). Separation was achieved in a column DB-Wax (length 30 m, internal diameter 0.25 mm narrow bore, and film thickness 0.25 μm) from J&W Scientific (Folsom CA). The temperature program followed was: 40°C for 5 min, a linear ramp from 40 to 150°C at a rate of 4°C/min, a linear ramp from 150 to 250°C at a rate of 20°C/min, and 250°C for 5 min. The injector temperature was 200°C, and the flame-ionization detector was operated at 220°C. Injections were performed by taking advantage of the autosampler capacity under a split ratio of 1:20 using helium as carrier gas at a flow rate of 2.0 mL/min. Data acquisition and analysis used the Turbochrome™ software from Perkin-Elmer.

The acid value, which was calculated based on the acid/base titration of the free fatty acids originating from the glycerides that constitute the bulk oil, is often used and referred to when trying to describe the quality of edible oils with respect to rancidity. The standard method by AOAC (10) was adopted for this purpose. The sample size was *ca.* 0.5 g (with a weight accuracy of no less than four decimal places), and the sample was dissolved in 10 mL of an ice-cooled mixture of 1:1 (vol/vol) ethanol/diethyl ether. Titer concentrations (ethanolic KOH) were 4 or 30 mM, depending on the expected degree of hydrolysis of the sample, and titration was carried out in an ice-bath (in order to avoid further breakdown of glycerides) to phenolphthalein endpoint. The titer, expressed in mg of KOH per g of oil, is by definition the acid value. The assays were replicated several times with typical reproducibilities of ± 0.11 mg of KOH per gram of oil sample.

The fatty acids (in either free or residue form) were fully converted to their corresponding methyl esters (FAME) using anhydrous methanol (as derivatization agent) mixed with acetyl chloride (as catalyst). The original method (11) was slightly modified for this purpose in that pure oil was sampled rather than a dilute aliquot. Use of tristearin [a triglyc-

eride (TAG): 18:0] as internal standard together with margaric acid [a free fatty acid (FFA): 17:0] confirmed that the derivatization extent was not affected by such modification. In a typical assay, *ca.* 100 mg of oil was dissolved in 10 mL of a mixture of acetyl chloride/methanol at a ratio of 5:100 (vol/vol), and reaction was allowed to proceed for 1 h at 80°C in a water bath in complete darkness under an atmosphere of pure nitrogen. After quenching *via* cooling to room temperature (speeded up by immersion in ice), the pretreated samples were extracted *via* sequentially adding 5 mL of hexane and 5 mL of water. The compounds responsible for the dark yellowish color of the extracts (especially in the case of oxidized samples) were soluble in the hexane phase, from which 0.5-mL aliquots were spectrophotometrically assayed. Besides the expected strong absorption between 200 and 350 nm, the spectrophotometric scan within the range 200–800 nm revealed two distinct absorption peaks at 374 and 400 nm, but the latter was much weaker than the former and was accordingly neglected. Strong absorption at *ca.* 374 nm is a characteristic of conjugated dienes and trienes, which build up during oxidation. Measurement of absorbance of the hexane phase was done right after preparation of the samples for chromatographic analysis using a spectrophotometer UV-1203 from Shimadzu (Duisburg, Germany); the cuvette width was 10 mm. Dilutions of 10- or 20-fold using hexane as solvent were done when absorbance exceeded 1.2 units.

The hexane phase also contained FAME, so 1.5-mL aliquots of the remaining volume were transferred to vials and duly stoppered. The hexane extracts were analyzed directly using the same gas chromatograph and column described above. The temperature program followed was: a linear ramp from 170 to 225°C at the rate of 1°C/min and 225°C for 5 min. The injector temperature was 220°C, and the flame-ionization detector was operated at 250°C. Injections were performed with the autosampler at a split ratio of 20:1 using helium as carrier gas at the flow rate of 2.0 mL/min. Data acquisition and analysis also used the Turbochrome™ software. Calibrations using TAG18:0 and FFA17:0 as standards were previously done in both external and internal mode. Identification of individual FAME was done by comparing the chromatogram retention time patterns with reference chromatograms (12–14). The molar mass responses of the FAME were not determined; however, previous experience suggested that the deviation in the response factors should be negligible and well below the level of experimental error (for the 14–22 range of carbon chain lengths, the factors ranked 1.28–1.34).

Another derivatization method was employed (15) specifically to obtain fatty acid methylesters only from acyl residues in the glyceride structure; this method does not quantify free fatty acids present in the oil as a result of hydrolysis. In practice, *ca.* 0.5 g of oil was accurately (with no less than four decimal places) weighed into a screw-capped vial, and a mixture of 5 mL hexane and 0.5 mL sodium methoxide (5.4 mol/L in anhydrous methanol) was added. After vigorous homogenization for 1 min in a vortex and centrifugation at *ca.* $800 \times g$ for 10 min, the supernatant (organic phase) was transferred to GC sample vials and analysis of FAME proceeded as described above. Without further concentration or dilution steps, 1 μL was injected into the gas chromatograph using its autosampler facility.

The data from the various analytical measurements were statistically assessed using the multivariate method of partial least squares (PLS) correlation in latent variables. The data obtained from the chromatographic analyses of the various fatty acids were also modeled with respect to chain length, number of double bonds, reaction time, and reaction temperature as seen below. Statistical significance was set to $P < 0.05$.

MATHEMATICAL METHODOLOGY

The overall chemical process of lipid oxidation proceeds *via* several stages, which in their simplest form may be represented by the following set of elementary steps:

$$A \xrightarrow{k_1} B \xrightarrow{k_2} C \qquad [1]$$

where k_1 and k_2 are first-order kinetic constants. A mass balance to A then reads

$$\frac{dC_A}{dt} = -k_1 C_A \qquad [2]$$

where t denotes processing time, and where the mole concentration C_A ($t = 0$) is given by $C_{A,0}$ and C_B ($t = 0$) is zero. Integration of Equation 2 using the former initial condition then leads to

$$C_A = C_{A,0} \exp\{-k_1 t\} \qquad [3]$$

This formula can be further developed so as to become valid for all types of fatty acid moieties using chain length and degree of unsaturation (i.e., number of double bonds) as regressors. The above-postulated form was inspired by realization that the experimental data with respect to the evolution of the concentration of original fatty acid residues with processing time was approximately linear provided that a log-linear scale was selected and was characterized by an intrinsic kinetic parameter k. When a plot of k vs. number of double bonds was in turn prepared (now using a linear-linear scale), one again observed an approximately linear shape. Finally, one found that the Arrhenius relationship essentially holds for the multitemperature data set generated. Therefore, the tentative overall model to be fitted to the experimental data on lipid oxidative decay (corresponding to consumption of A) can be written as

$$\ln (C_{i,n}/C_{i,n,0}) = -n\, k_{i,0}\, t \exp\{-E_{a,i}/RT\} \qquad [4]$$

where $C_{i,n}$ is the mole concentration, at time t, of a fatty acid moiety containing i carbon atoms and n double bonds, $C_{i,n,0}$ is the initial counterpart of $C_{i,n}$, $k_{i,0}$ is a pre-exponential kinetic parameter, $E_{a,i}$ is an activation energy, R is the universal gas constant, and T is the processing absolute temperature.

Recalling Equation 1, intermediate *B* and final product(s) *C* are also formed, which can be accounted for by volatiles and chromophores, although the reaction mechanisms are yet to be completely understood. The material balance to *B* reads

$$\frac{dC_B}{dt} = k_1 C_A - k_2 C_B \quad [5]$$

which, subject to integration using the aforementioned latter initial condition and Equation 3 *via* the integrating factor method, yields

$$C_B = \frac{k_1 C_{A0}}{k_2 - k_1} \cdot (e^{-k_1 t} - e^{-k_2 t}) \quad [6]$$

RESULTS AND DISCUSSION

As expected, hydrolysis increased as reaction time elapsed, as shown in Figure 1; this increase was simultaneous with development of primary oxidation products, as apparent from the increasing absorbance values, shown in Figure 2 from accumulation of secondary oxidation products (released as volatiles) as shown in Figure 3; and from the decreases in concentration of PUFA as shown in Figure 4.

At the lower experimental temperatures tested, a relatively stable foam tended to build up and disturbed to some extent the analytical assaying. On the other hand, a remarkable increase in viscosity was observed in oil samples that had undergone extensive oxidation, which eventually led to a sticky appearance and color fading away toward light yellow.

The acid value data, depicted in Figure 1, indicated clearly the presence of a putative induction period for hydrolysis within *ca.* 4 h after startup. It could be statistically confirmed that the variation in the resulting acid values during that period was not significantly higher than the overall standard deviation in the values obtained (±0.59 vs. ± 0.47 mg/g, respectively; $P < 0.05$). However, immediately after 4 h, the curves of the acid values increased rapidly, especially at temperatures above 70°C. The acid value results correlated well with the chromatographic assays for the FAME ($r^2 = 0.90$), which suggested that the rate of oxidative decay was related to the rate of hydrolysis. This result is usually found regardless of oil source, whereas the naturally occurring amounts of free fatty acids do not likely affect the onset of lipid oxidation and do not appear to be important in storage stability (16).

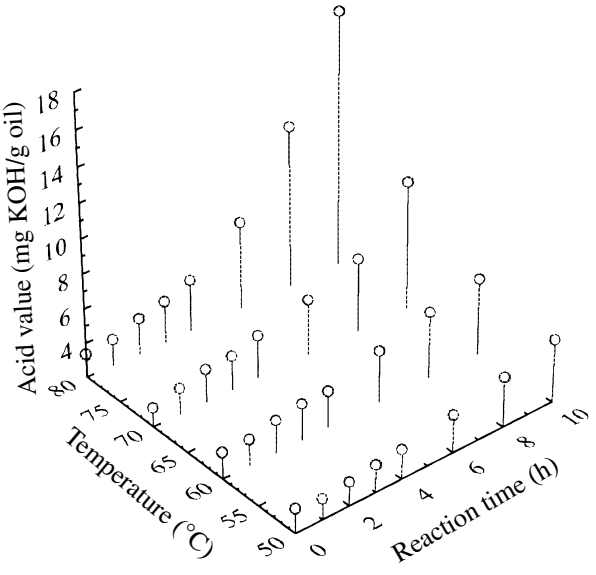


FIG. 1. Acid value vs. time of reaction under bubbling with air at various temperatures in forced oxidation of sardine oil.

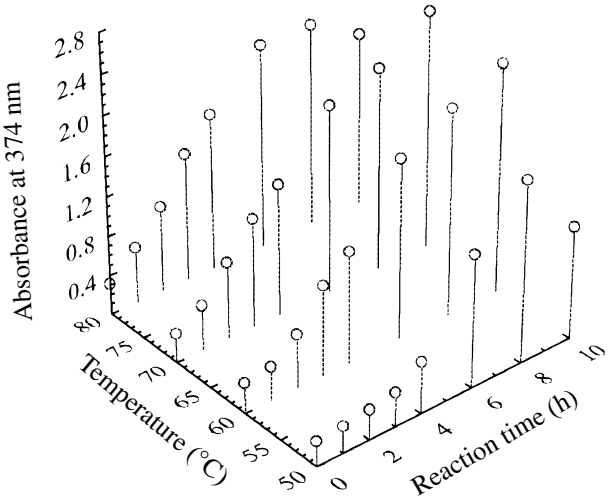


FIG. 2. Absorbance at 374 nm vs. time of reaction under bubbling with air at various temperatures in oxidized sardine oil.

In one experiment utilizing sardine oil, the two methods available for assessment of FAME were combined; derivatization using acetyl chloride in anhydrous methanol (thus yielding total fatty acids) will hereafter be referred to as method A, whereas derivatization using sodium methanolate (thus yielding fatty acid residues only) will hereafter be denoted as method B. By subtracting the results obtained using method B from those using method A, it could be concluded that the total concentration of FFA was 80 ± 24 mg/g. Furthermore, when said oil was titrated with ethanolic KOH, the average value found was 262 ± 10 $\mu mol_{KOH}/g_{oil}$. The average molecular weight of FFA in sardine oil was thus estimated as 284 g/mol. This piece of information was useful when attempting to compare the acid value with the overall results of the chromatographic assays. By using this calculation method and information on fish oil compositions available in the literature, Table 1 could be prepared. The aforementioned values combined yielded an acid value of 74 ± 3 mg/g.

Data pertaining to the FFA profiles of sardine oil at the longest processing time (10 h) and at the highest temperature (80°C) after bubbling with nitrogen and air are shown in Figure 4. As expected, PUFA were far more affected by oxidation than mono- or unsaturated fatty acids. The results of the most aggressive conditions used under nitrogen bubbling are tabulated in Table 2. Inspection of this table indicates that there

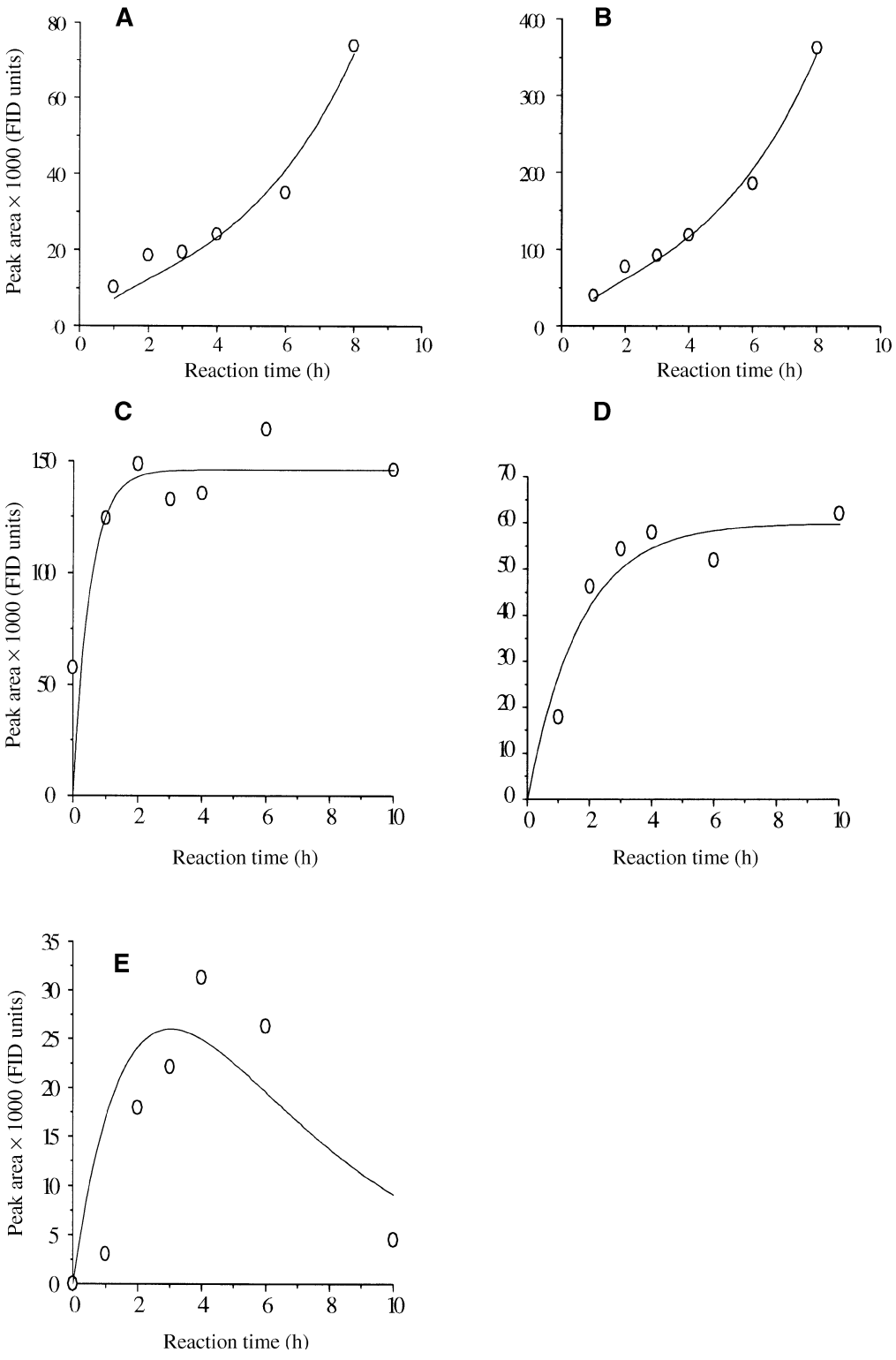


FIG. 3. Experimental data (○) and theoretical fits (—) for typical volatiles produced by forced oxidation of sardine oil at 80°C. (A) *cis*- and *trans*-2-Methyl-3,5-heptadiene (retention time 2.07 min); (B) 2,3-butanedione (retention time 2.39 min); (C) propanal (retention time 2.72 min); (D) 4-*cis*-heptenal (retention time 8.28 min); and (*E*) 2,4,7-decatrienal (retention time 20.08 min). FID, flame-ionization detector.

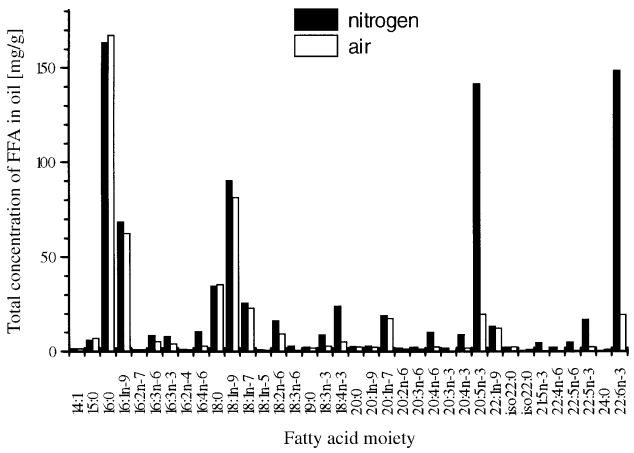


FIG. 4. Profile of free fatty acids (FFA) after bubbling nitrogen and air for 10 h through sardine oil at 80°C.

were virtually no differences between sardine oil at startup and after 10 h at 80°C under pure nitrogen, so the decay observed when air was bubbled could be safely attributed to the oxygen present and not to the temperature itself. The effect of air bubbling for 10 h at 80°C is also depicted in Table 2, and again it is apparent that the higher the degree of unsaturation of the carbon backbone the higher the oxidative loss.

An additional result obtained after combination of the aforementioned methods (A and B) to assess slightly oxidized sardine oil was the domination of PUFA moieties in the FFA inventory. As can be seen in Figure 5, concentrations of saturated fatty acids (especially 16:0 and 18:0) were rather low, *ca.* 85 mg/g, which corresponded to 11% of the total. Studies regarding storage stabilities of plant fats (16) confirm that during oxidation PUFA are liberated to a greater extent than saturated fatty acids.

Estimates for $k_{i,0}$ and $E_{a,i}$ (and associated 95% confidence intervals) were 20.6 ± 0.8 h^{-1} and 20.8 ± 0.4 kJ/mol, respectively. Selected experimental data overlaid on the best model fits can be seen in Figure 6. The same level of oxidative loss reached by 10 h at 60°C had already been reached by 6 h at 80°C. The oxidation of oil (a typical case of a bimolecular reaction) should kinetically be first order in the concentration of molecular oxygen and first order in the concentration of substrate if it corresponds to an elementary molecular event. However, although our data indicated that there was good agreement with this reasoning with respect to the substrate, a zero-order process in oxygen resulted apparently because the concentration of this compound had been kept essentially constant (and well in excess) *via* continuous bubbling of air.

It is known that primary oxidation products lead, in turn, to generation of such volatiles as aldehydes, ketones, and hydrocarbons of short chain lengths (17). As expected, the qualitatively richest pattern was found for samples held at the highest temperature (80°C). Three of the most abundant components found had relatively short retention times, so they could be claimed as being nonpolar since separation was performed on a medium-polar column (DB-Wax), and consequently as pos-

TABLE 1
Average Molecular Weights of Fatty Acids in Fish Fats and Ratios with Nutritional Significance[a]

Fish species	Reference number	Mw	n-3 FA / n-6 FA	(PUFA + MUFA) / Saturated FA
Sardine (oil)	Our work	284	9.4	2.3
Sardine (raw)	28	284	8.1	2.1
Sardine (raw)	2	296	12.0	2.7
Capelin (oil)	6	282	13.6	3.8
Rainbow trout (raw)	13	265	5.6	3.3
Atlantic herring (oil)	29	290	5.8	3.8
Atlantic herring (raw)	29	274	8.3	3.9
Pacific herring (oil)	29	270	13.1	2.9
Menhaden (raw)	29	256	11.4	1.5

*Mw, molecular weight; FA, fatty acids; PUFA, polyunsaturated fatty acid; MUFA, monounsaturated fatty acid.

TABLE 2
Concentration (mg/g) of Various Fatty Acid Moieties in Fresh Sardine Oil, Control, and Oil Subject to Aggressive Oxidation (by air bubbled for 10 h at 80°C)

Fatty acid moiety[a]	Fresh oil	Nitrogen, 10 h, 80°C (control)	Air, 10 h, 80°C
14:1	1.55	1.65	1.52
15:0	6.12	6.24	7.16
16:0	162	164	167
16:1n-9	68.2	68.6	62.7
16:2n-7	1.26	1.13	1.06
16:3n-6	9.58	8.54	5.33
17:0	6.17	5.63	6.30
16:3n-3	7.91	7.96	4.32
16:2n-4	1.47	1.29	1.10
16:4n-6	10.9	10.7	3.00
18:0	34.6	34.9	35.6
18:1n-9	90.3	90.5	81.6
18:1n-7	25.2	25.7	23.2
18:1n-5	1.21	1.19	0.99
18:2n-6	16.3	16.5	9.58
18:3n-6	3.08	3.07	1.01
19:0	2.74	2.65	2.16
18:3n-3	8.92	8.92	3.02
18:4n-3	24.3	24.0	5.21
20:0	2.80	2.82	2.63
20:1n-9	2.78	3.00	2.36
20:1n-7	18.1	19.0	17.7
20:2n-6	1.91	1.86	1.45
20:3n-6	2.19	2.31	1.37
20:4n-6	9.82	10.2	2.56
20:3n-3	1.61	1.78	0.55
20:4n-3	9.00	9.00	1.95
20:5n-3 (EPA)	142	142	19.8
22:1n-11	12.7	13.4	12.3
22:0, branched	2.15	2.39	2.49
22:0, branched	0.91	0.82	1.12
21:5n-3	4.77	4.65	0.64
22:4n-6	2.23	2.23	0.62
22:5n-6	4.75	5.04	0.82
22:5n-3 (DPA)	16.7	17.0	2.49
24:0	0.93	0.88	1.04
22:6n-3 (DHA)	148	149	19.6

[a]Fatty acid moieties are listed by order of increasing retention times, EPA, eicosapentaenoic acid; docosapentaenoic acid; DHA, docosahexaenoic acid.

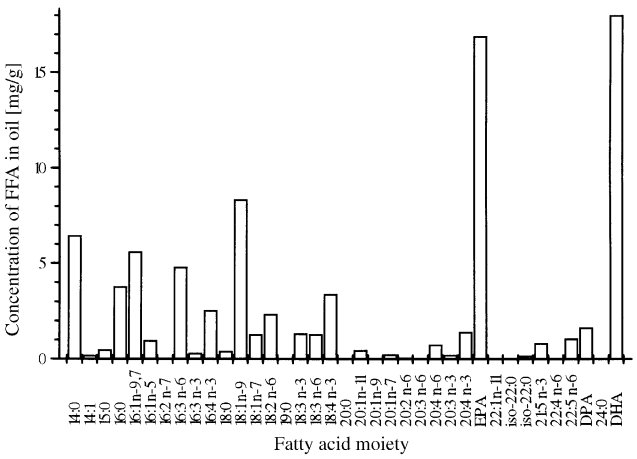


FIG. 5. Profile of free fatty acids (FFA) in rancid sardine oil. Fatty acids in residue form: 690 mg/g, corresponding to 89% (w/w); fatty acids in free form: 85 mg/g, corresponding to 11% (w/w). EPA, eicosapentaenoic acid; DPA, docosapentaenoic acid; DHA, docosahexaenoic acid.

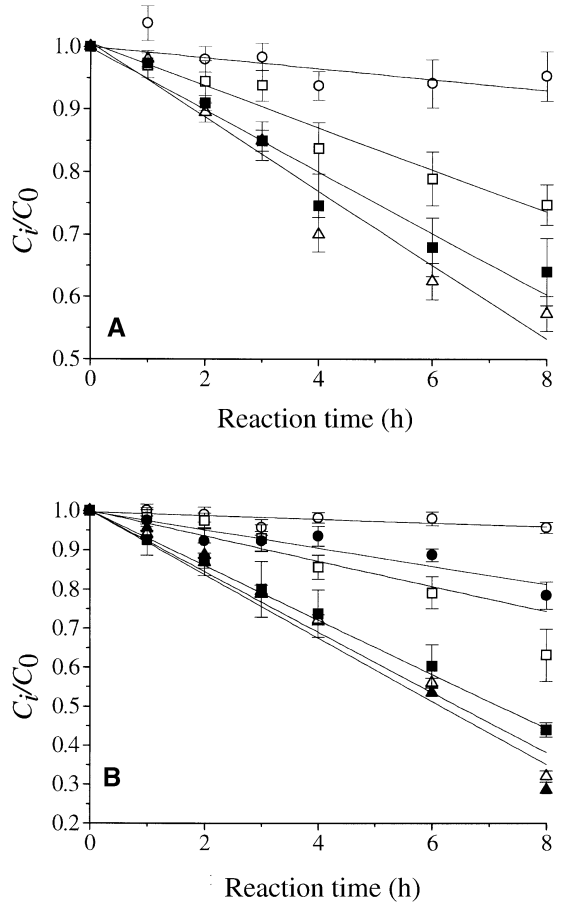


FIG. 6. Experimental data (and corresponding error bars) and theoretical fits (—) for oxidized sardine oil. (A) Reaction performed at 60°C, and (B) reaction performed at 80°C: (○) one double bond, (●) two double bonds, (□) three double bonds, (■) four double bonds, (△) five double bonds, and (▲) six double bonds. C_i denotes concentration of the *i*th fatty acid at time *t* and subscript 0 refers to startup of reaction.

sessing low boiling points. As most PUFA of marine oils belong to the n-3 family, appearance of propanal together with three stereoisomers of 2-methyl-3,5-heptadiene could be explained in essentially the same way as appearance of hexanal and pentane were explained as major products of oxidation of plant oils rich in PUFA of the n-6 type (18–20). The volatiles analyzed and their characteristics are given in Table 3. Experimental data and best model fits can be seen in Figure 3. Some volatiles built up and reached maximum values at later stages of reaction, whereas other volatiles appeared in the reactor headspace in as soon as 30 min of reaction time and then attained steady-state. Thus, three different situations were identified in attempts to describe mathematically the patterns in concentration of volatiles, i.e., $k_2 < 0$ (see Fig. 3A,B); $k_2 = 0$ (see Fig. 3C,D) and $k_2 > 0$ (see Fig. 3E). The pattern of release of the volatile for which $k_2 = 0.50 \pm 0.10\ h^{-1}$ (i.e., 3,4,7-decatrienal) was one of a kind, and this unsaturated aldehyde reacted further, likely *via* polymerization.

Although it resembled paint or varnish, the overall odor perceived as oxidation time elapsed could not be described as unpleasant. In the beginning, the smell accounted for by diacetyl (2,3-butanedione) dominated and was comparable to those potent odorants that appear in boiled trout (21). However, Meijboom and Stroink (22) claimed that 4-*cis*-heptenal at low levels can produce a similar, butterscotch-like flavor that is also associated with diacetyl. The odor at later times acquired sticky, pungent notes, which were likely the result of increasing levels of propanal and decatrienals (23). One plausible reason is that humans perceive a mixture of several carbonyl compounds as rancid, cod liver oil-like, and "painty." Potent odorants derived *via* lipid oxidation in fish tissues, their origin, concentration, thresholds, and flavor notes (24) are tabulated in

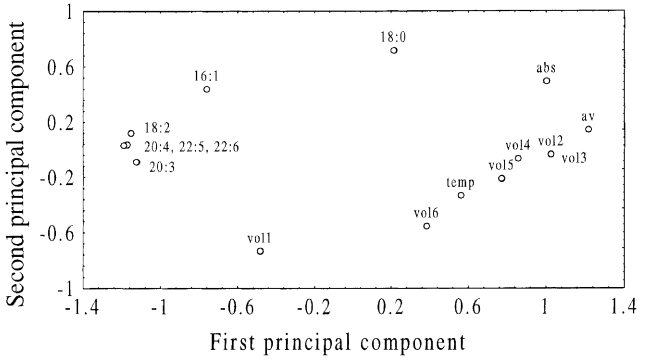


FIG. 7. Multivariate analysis plot as the second principal component vs. the first principal component, using as independent variable the reaction time, and using 16 dependent variables: the concentrations of 7 fatty acids [18:0, octadecanoic acid (stearic acid); 16:1, hexadecenoic acid (palmitoleic acid); 18:2, octadecadienoic acid (linoleic acid); 20:3, eicosatrienoic acid; 20:4, eicosatetraenoic acid; 22:5, DPA; 22:6, DHA]; av, acid value; abs, absorbance; vol 1, concentration of unidentified component eluting at 1.89 min; vol 2, concentration of two isomers of 2-methyl-3,5-heptadiene; vol 3, concentration of 2,3-butanedione; vol 4, concentration of propanal; vol 5, concentration of 4-*cis*-heptenal; vol 6, concentration of 2,4,7-decatrienal; and temp., temperature.

TABLE 3
Volatile Compounds from Oxidized Sardine Oil and Their Characteristic Retention Times and Kinetic Constants

Identification	Retention time (min)	C_{A0}	k_1 (h^{-1})	k_2 (h^{-1})
2-Methyl-3,5-heptadiene	2.07	10,000 ± 500	0.95 ± 0.19	–0.28 ± 0.008
2,3-Butanedione	2.39	50,000 ± 1,400	0.95 ± 0.11	–0.28 ± 0.004
Propanal	2.72	146,000 ± 5,000	1.90 ± 0.60	0
4-*cis*-Heptenal	8.28	60,000 ± 3,000	0.60 ± 0.09	0
3,4,7-Decatrienal	20.08	94,000 ± 15,000	0.25 ± 0.07	0.50 ± 0.10

TABLE 4
Carbonyl Volatile Compounds Produced by Oxidation in Fish[a] (24)

Compounds	Origin	Concentration (ppb)	Detection threshold (ppb)	Flavor note
4-Heptenal	n-3 PUFA	1–10	1	Creamy
2,4-Heptadienal	n-3 PUFA	1–10	10	Rancid hazelnuts
2-Hexenal	n-3 PUFA	1–10	17	Green grass
2,4,7-Decatrienal	n-3 PUFA	1–10	150	Oxidized fish oil
1-Octen-3-ol	n-6 PUFA	10–100	10	Mushroom, melon-like
1,5-Octadien-3-ol	n-3 PUFA	10–100	10	Mushroom, seaweed
2,5-Octadien-1-ol	n-3 PUFA	1–10	—	Mushroom, seaweed
1,5-Octadien-3-one	n-3 PUFA	0.1–5	0.001	Mushroom
2-Nonenal	n-6 PUFA	0–25	0.08	Cucumber-like
2,6-Nonadienal	n-3 PUFA	0–35	0.01	Cucumber-like

[a]For abbreviation see Table 1.

Table 4. With the exception of the first volatile that eluted from the GC column (at 1.89 min), resolution of all other volatiles correlated well with results published by a number of researchers (25–27). The predominating pleasant base flavor may have resulted from esters of shorter chain lengths; nevertheless, more sophisticated analytical techniques would be required for a full explanation.

A plot of the multivariate analysis applied to our data is presented in Figure 7, and it shows correlations between all concentration-type variables considered. The concentrations of FFA moieties correlated very well with one another, except for the saturated acids (i.e., 18:0) and monounsaturated acids (i.e., 16:1), which were essentially unaffected by oxidation. The acid value, absorbance, and concentration of most volatiles generated also correlated well with one another, except the volatile which eluted at 1.89 min (and mentioned earlier). The broader clustering of volatiles along the x axis was explained by the typical pattern of variation in concentration of these compounds with time (see Fig. 3).

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