Formation of Headspace Volatiles by Thermal Decomposition of Oxidized Fish Oils *vs.* Oxidized Vegetable Oils[1]

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To understand the reasons for differences in oxidative stability among edible oils, the temperature dependence was investigated for the development of volatile lipid oxidation products in fish oils and in vegetable oils. A rapid headspace capillary gas chromatographic method was developed to determine volatile oxidation products of omega-6 (n-6) polyunsaturated fats (pentane and hexanal) and omega-3 (n-3) polyunsaturated fats (propanal) at different decomposition temperatures. Headspace gas chromatographic analyses of partially oxidized menhaden, bonita and sardine oils could be performed at 40°C, whereas soybean, canola, safflower, high-oleic sunflower and high-oleic safflower oils required temperatures greater than 100°C. Volatile formation by thermal decomposition of oxidized oils had lower apparent activation energies in fish oils than in vegetable oils, and significantly higher apparent activation energies in high-oleic oils than in polyunsaturated oils. The activation energy data on headspace volatiles provided another dimension toward a better understanding of the thermal stability of flavor precursors in unsaturated fish and vegetable oils.

KEY WORDS: Activation energy, fish oils, gas chromatography, headspace gas chromatography, hydroperoxide decomposition, hydroperoxides, linoleate, linolenate, n-3 PUFA, n-6 PUFA, vegetable oils, volatiles.

Many mechanisms have been proposed in the literature (1-3) for the genesis of the volatile oxidation breakdown products of polyunsaturated fatty acids (PUFA) in lipids and for their flavor and biological significance. The oxidative instability of linolenate in vegetable oils (4) and n-3 polyunsaturated lipids in fish oils (5) is well established. Polyunsaturated lipids in vegetable and fish oils produce a complex array of low- and high-molecular weight secondary products that provide rich sources of volatile compounds.

Several papers have been published on the identification of volatile products from vegetable (2,3) and fish oils (5), but there is a lack of information on the oxidation products relevant to flavor and oxidative stability. The n-3 eicosapentaenoic acid (EPA) (20:5) and docosahexaenoic acid (DHA) (22:6) found in fish oils are much more susceptible to oxidation than is the linolenic acid (18:3n-3) (6,7) found in unsaturated vegetable oils (soybean and canola oils), and DHA and EPA produce flavors and odors that are more objectionable (5,8).

To elucidate the reasons for differences in oxidative stability between unsaturated oils requires an understanding of the basic processes for the development of volatile lipid oxidation compounds that cause flavor deterioration. Different volatile decomposition products are formed according to the relative thermal stabilities of flavor precursors and the resulting carbonyl products. Knowledge about various interactions of flavors in complex foods is essential in controlling food acceptance and designing optimum food processing systems. Little information is available on the kinetics of formation of products contributing to the flavor impact of food lipids. The kinetics and thermodynamic parameters derived from the flavor and oxidative changes in unsaturated oils can be applied to improving process control, to better estimate shelf life and increase consumer acceptability of oil-bearing foods (9,10).

We recently described a rapid headspace gas chromatographic (GC) method for the determination of hexanal, an important volatile decomposition product as an indicator of n-6 PUFA oxidation in biological samples (11). No workup is necessary for this method and clean samples of volatiles can be taken from the headspace above biological and food specimen. This method was further improved for the determination of specific volatile oxidation products of n-6 PUFA (pentane and hexanal) and n-3 PUFA (propanal), and was applied to a study of the oxidative susceptibility of human red blood cell membranes (12) and human low-density lipoproteins (13). This paper reports a study of activation energies from headspace capillary GC analyses to compare the temperature dependence of volatile formation in oxidized n-3 polyunsaturated fish oils and n-6 polyunsaturated and high-oleic vegetable oils.

EXPERIMENTAL PROCEDURES

Materials. Refined, bleached and deodorized fish oils (menhaden, bonita and sardine oils) and vegetable oils (soybean, high-oleic sunflower, high-oleic safflower, canola and sunflower oils) with no additives and no citric acid were obtained commercially. Initial quality was checked by determining peroxide values (PV) colorimetrically (14), which ranged from 0.1 for soybean oil to 1.3 meq/kg for menhaden oil (Table 1). Fatty acid composition was determined by GC of the methyl esters prepared by base-catalyzed transesterification (15), and tocopherols were quantitated by normal-phase high-performance liquid chromatography (HPLC) with fluorescence detection (16) (Table 1).

Oxidation. Oil samples (5 g), weighed into screw-capped 25-mL Erlenmeyer flasks, were oxidized in duplicate at 40 and 50°C in a shaker oven (Lab-Line Instrument, Inc., Melrose Park, IL). Oxidative stability was evaluated by analyzing oil samples periodically for PV and for volatiles by static headspace GC.

Static headspace GC. Duplicate oil samples of 0.10 g were weighed into special 9-mL headspace vials (Tekmar Co., Cincinnati, OH), sealed with silicone rubber Teflon caps by using a crimper and equilibrated 10 min at 40-180°C, followed by pulsed mixing for 5 min with a Takmar 7000 headspace autosampler. After equilibration, an aliquot of the headspace was transferred with a six-port valve into a sample loop heated in an oven controlled at 50°C above the equilibration temperature, pressurized

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TABLE 1

Analyses of Fatty Acid Composition, Tocopherols and Peroxide Values (PV) of Fish and Vegetable Oils Used for the Oxidation Experiments

Analyses	Menhaden	Sardine	Bonita	Soybean	Canola	Safflower	High-oleic Sunflower	High-oleic Safflower
Fatty acids (%)								
14:0	9.8	7.6	4.3	0.1	0.1	0.1	0.1	0.1
16:0	21.6	18.0	23.3	10.7	3.8	6.5	4.0	5.0
16:1n9	12.9	9.1	5.3	0.1	0.2	0.1	0.1	0.1
18:0	3.8	2.6	6.4	4.6	1.8	2.5	4.3	2.2
18:1n9	9.9	11.4	15.3	23.8	58.3	13.4	79.4	77.3
18:2n-6	1.4	1.6	1.6	53.9	22.5	77.1	11.4	14.7
18:3n3	1.1	1.1	0.6	6.6	10.8	0.1	0.4	0.2
20:1n9	1.7	3.3	1.4	0.2	1.8	0.2	0.3	0.3
20:3n6	0.3	0.3	0.2	0.0	0.0	0.0	0.0	0.0
20:4n6	1.2	1.0	2.0	0.0	0.0	0.0	0.0	0.0
20:5n3	20.1	24.7	7.4	0.0	0.0	0.0	0.0	0.0
22:1n9	0.2	0.7	0.2	0.0	0.7	0.0	0.0	0.0
22:5n3	3.7	3.9	1.8	0.0	0.0	0.0	0.0	0.0
22:6n3	12.3	14.6	30.1	0.0	0.0	0.0	0.0	0.0
Tocopherols (μg/g)								
α	18	0	261	62	148	484	729	481
β	0	0	0	45	59	94	69	0
γ	0	0	0	774	474	0	0	0
δ	0	0	0	157	0	0	0	0
Total	18	0	261	1038	681	578	798	481
Initial PV	1.3	0.2	0.5	0.1	0.3	0.3	0.2	0.2

with carrier gas for 30 s and injected directly into the gas chromatograph through a stationary injection needle. Volatiles were determined by using a Hewlett-Packard 5890 gas chromatograph (Hewlett-Packard Co., Avondale, PA), equipped with a capillary DB-1701 column (14% cyanopropylphenylsilicone, 30 m × 0.32 mm, 1 μm thickness; J&W, Folsom, CA) and a flame-ionization detector heated at 180°C. The GC column was programmed with an initial hold of 2 min at 50°C and rise to 65°C at the rate of 5°C per min with a final hold of 5 min. The injector temperature was 180°C. Volatile compounds were identified by comparison of retention times with those of authentic reference compounds. Peak areas for individual volatiles and for total volatiles were integrated electronically to determine oxidative stabilities and to calculate activation energies.

Statistical analyses. Analysis of variance (ANOVA) was used to determine the least significant means between mean values (17) of duplicate oxidations and duplicate analyses of PV and of headspace volatiles. Values of P less than 0.05 were considered significant.

RESULTS

Oxidative stability of fish and vegetable oils. On the basis of PV determinations, menhaden and sardine oils oxidized rapidly at 40°C within one day with no induction period. In contrast, safflower and soybean oils oxidized slowly after induction periods of 3–4 d, while high-oleic sunflower oils did not oxidize significantly during 5 d at 40°C (Fig. 1a). At 50°C, bonita and sardine oils oxidized rapidly within 0.5 d with no induction period; safflower and soybean oils oxidized slowly with an inducation period of 3 d, but high-oleic sunflower oil did not oxidize much during 6 d (Fig. 1b).

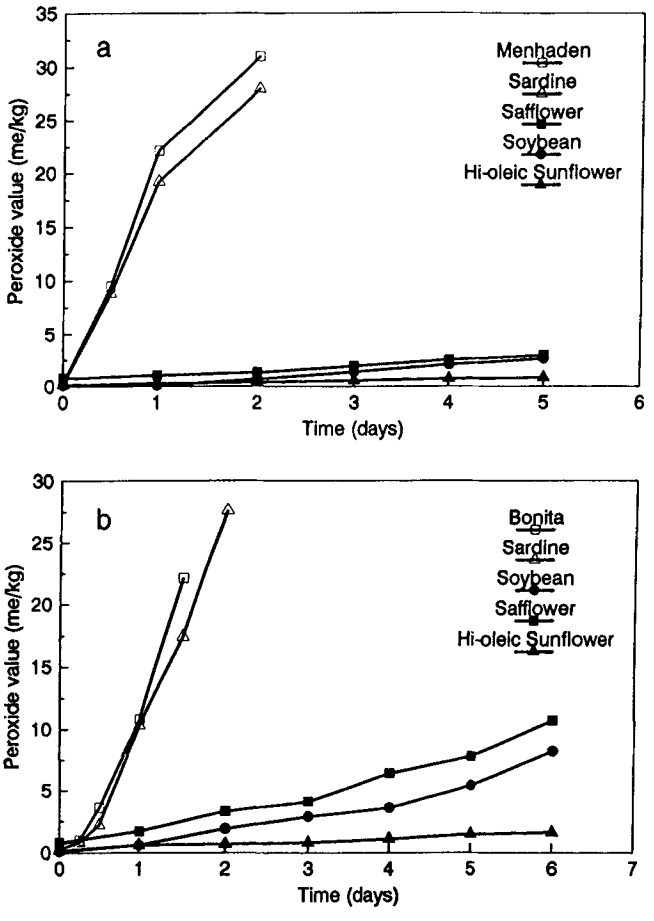

FIG. 1. Oxidative stability of fish and vegetable oils based on peroxide values. a, 40°C; b, 50°C.

Analysis of variance indicated that, at 40 and 50°C, the oxidative stability based on PV was not significantly different between the two fish oils and between the soybean and safflower oils ($P > 0.05$). However, the oxidative stability of high-oleic sunflower oil was significantly greater than that of safflower oil at 40°C and of both soybean and safflower oils at 50°C ($P < 0.05$).

The oxidative stabilities of fish and vegetable oils also were compared on the basis of GC headspace analyses of volatile products formed by decomposition. The main volatile products detected in menhaden oil oxidized at 40°C included pentane, propanal, pentenal and hexanal (Fig. 2a). The main volatile products in soybean oil oxidized at 50°C included pentane, propanal, pentanal and hexanal (Fig. 2b). Menhaden oil produced more propanal than did soybean oil, as expected from the relatively high levels of 20:5n3 and 22:6n3; and soybean oil produced more pentane, pentanal and hexanal, as expected from the greater levels of n-6 than of n-3 PUFA (Table 1). The formation of hexanal and pentane from linoleate hydroperoxides, as well as propanal from linolenate hydroperoxides, is well recognized (2,3). The formation of pentanal from n-6 PUFA may be explained by thermal decomposition of hexanal by loss of formaldehyde (2). Pentenal in oxidized menhaden oil may be speculated to come from further oxidation of 2,4-heptadienal, a major decomposition product of n-3 PUFA (2,3), by cleavage of the α-β double bond, as proposed for the autoxidation of 2,4-decadienal (18); or by retro-aldol degradation, as proposed for the conversion of *trans*-2,*cis*-4-nonadienal to yield *cis*-4-heptenal (19).

Evaluation of oxidative stability on the basis of GC headspace analyses at 40°C showed that formation of propanal in bonita and sardine oils oxidized at 40°C was appreciable after an induction period of about one day. On the other hand, corresponding GC headspace determinations of oxidized soybean oil showed no detectable amounts of volatiles at 40°C. GC headspace analyses at 100°C showed that oxidized soybean oil produced only small amounts of propanal after oxidation at 40°C for 5 d (Fig. 3a). Further GC headspace analyses at 40°C showed considerable propanal formation in menhaden and sardine oils oxidized at 50°C with no induction period (Fig. 3b).

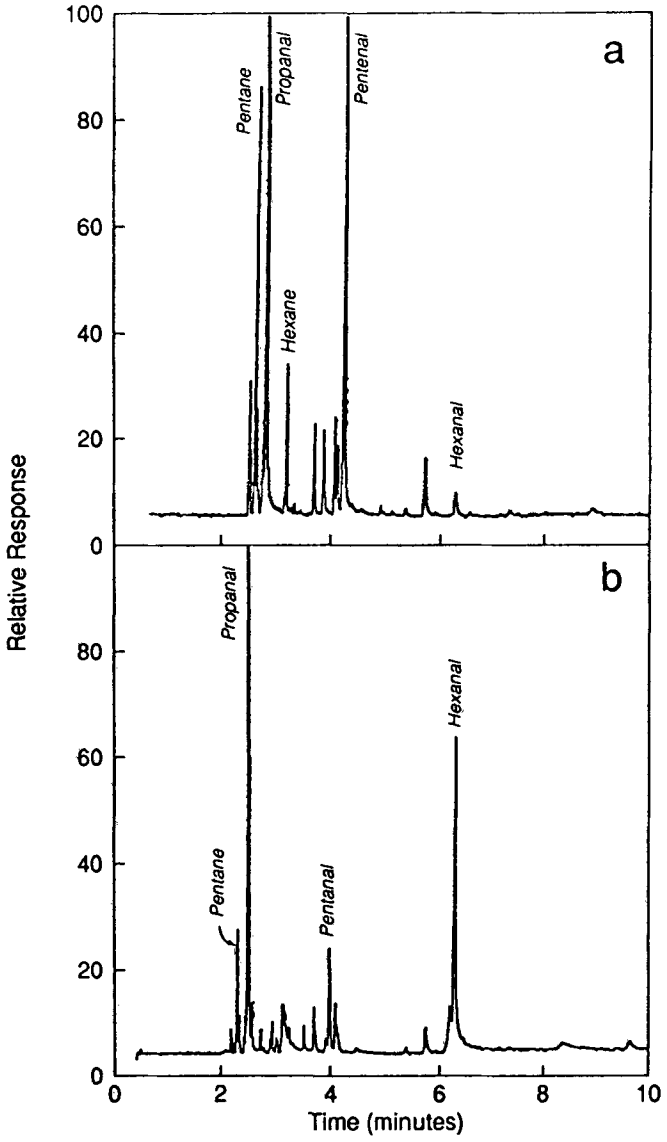

FIG. 2. Gas chromatographic headspace analyses. a, Menhaden oil oxidized at 40°C (peroxide vale 5.8); b, soybean oil oxidized at 50°C (peroxide value 4.9).

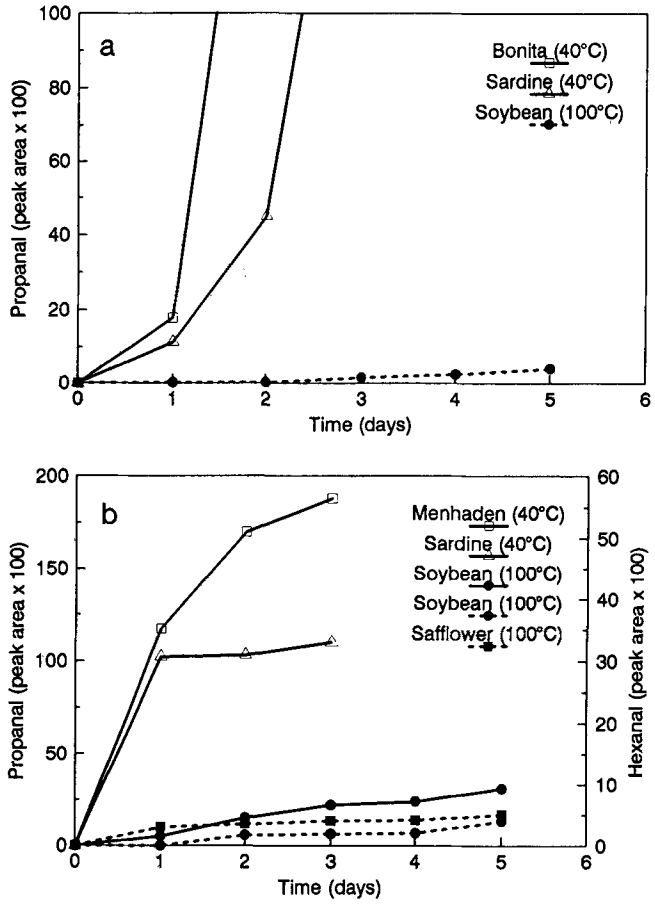

FIG. 3. Oxidative stability of fish and vegetable oils based on gas chromatographic headspace analyses. a, Oxidation at 40°C; propanal formation at 40°C (solid lines) and 100°C (dashed line). b, Oxidation at 50°C; propanal formation at 40°C (solid lines) and hexanal formation at 100°C (dashed lines).

In contrast, soybean oil oxidized at the same temperature showed much less propanal formation when analyzed by GC headspace at 100°C; hexanal also formed in much smaller amounts from soybean and safflower oils when analyzed by GC headspace at 100°C (Fig. 3b). Analysis of variance indicated that at 40 and 50°C, the oxidative stability based on propanal formation was significantly different between the two fish oils and soybean oil ($P < 0.05$). Similarly, at 50°C, the oxidative stability, based on hexanal formation, was significantly different between the two fish oils and the two vegetable oils ($P < 0.05$).

These GC headspace analyses for volatile decomposition products showed marked differences in temperature dependence between partially oxidized fish oils and vegetable oils. The GC headspace technique was further used as a valuable tool to determine activation energy in oxidized oils.

Activation energy of thermal decomposition of oxidized fish and vegetable oils. Fish and vegetable oils, oxidized at 50°C, were analyzed for total volatiles by GC headspace to determine activation energy values for volatile formation. Apparent activation energy values (E) were calculated from Arrhenius plots (Fig. 4) by the following relationship (20): E = slope/0.219; based on the Arrhenius equation:

$$\ln k = -E/R\ (1/T) + \text{constant} \quad [1]$$

where R = gas constant and T = absolute temperature.

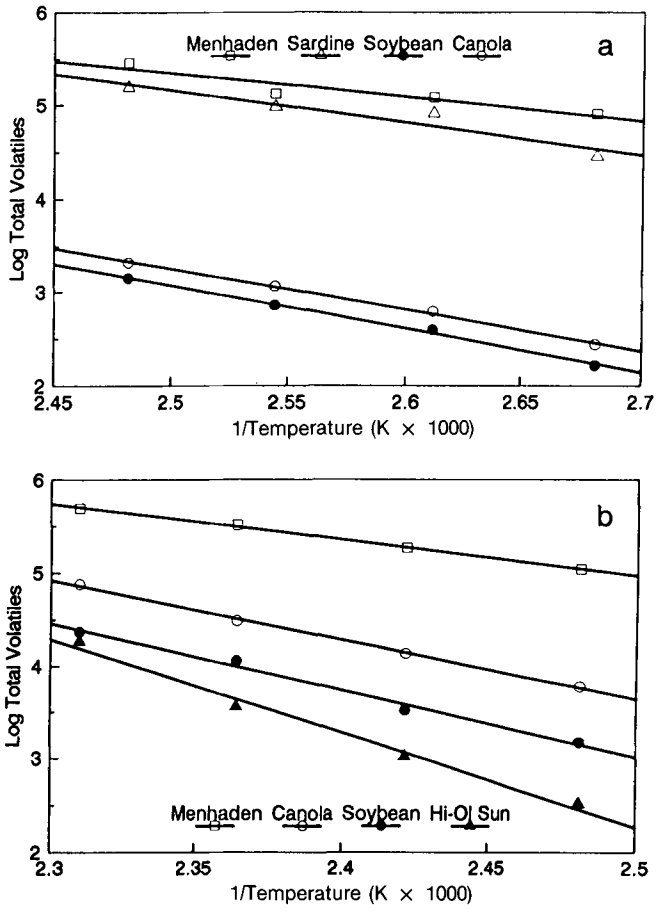

FIG. 4. Arrhenius plots for the formation of total volatiles in oxidized fish and vegetable oils based on gas chromatographic headspace analyses. a, 100–130°C; Correlation coefficient values *r:* menhaden, 0.945; sardine, 0.953; canola, 0.998; soybean, 0.998; b, 130–160°C; *r:* menhaden, 0.999; canola, 0.999; soybean, 0.995, Hi-Ol Sun, high-oleic sunflower, 0.995.

Apparent activation energy values, based on total volatile formation, increased with temperature of decomposition under the conditions of headspace GC analyses. Arrhenius plots were linear in ranges of 30°C. Between 100 and 130°C, volatile formation (measured at intervals of 10°C) was significantly higher and had a lower ($P < 0.05$) apparent activation energy in menhaden and sardine oils (18.4 kcal/mol) than in canola and soybean oils (21.2 kcal/mol) (Fig. 4a). Between 130–160°C, volatile formation (measured at intervals of 10°C) was highest in menhaden oil and had a significantly lower ($P < 0.05$) apparent activation energy (18.7 kcal/mol) than did canola and soybean oils (26.6 kcal/mol). High-oleic sunflower oil had the least volatile formation and the highest apparent activation energy (50.0 kcal/mol) (Fig. 4b). Excellent linear correlations of the slopes ($r = 0.945$ and 0.999) were obtained for the Arrhenius plots within each temperature range of 30°C.

In contrast to the effect of temperature, the PV had no significant effect on activation energy. Thus, oxidized soybean oils with a 2.4-fold difference in PV (runs 15 and 16), oxidized safflower oils with a 1.9-fold difference in PV (runs 17 and 18), oxidized canola oils with an 8-fold difference in PV (runs 23 and 24) and oxidized high-oleic oils with 1.8-fold difference in PV (runs 25 and 27) had activation energy values that were not significantly different than the respective standard deviations (Table 2).

Because the levels of oxidation had no significant effect, values for apparent activation energy were averaged for the oxidized fish oils, the polyunsaturated and the monounsaturated vegetable oils tested. In oxidized menhaden and sardine oils, the apparent activation energy values averaged 6.6 at 40–70°C, and increased to 11.7 at 70–100°C, to 18.4 at 100–130°C and to 18.7 at 130–160°C (Table 2, runs 1–11). In oxidized soybean, safflower and canola oils, the apparent activation energy values averaged 21.2 at 100–130°C, increasing to 26.6 at 130–160°C and to 47.2 at 150–180°C (Table 2, runs 12–24). High-oleic oils had significantly higher activation energy values at 130–160°C and 150–180°C (50.0 and 59.7, respectively) than did polyunsaturated oils (26.6 and 47.2, respectively) (Table 2, runs 15–29). These differences in activation energy values are consistent with previous observations that high-oleic sunflower oil is more resistant to total volatile formation than other polyunsaturated vegetable oils (Fig. 4).

Calculated values for the apparent activation energy of formation of individual volatiles were in the same range as total volatiles (Table 3). With fish and vegetable oils, a small increase was observed in the apparent activation energy values for volatile formation in the order: propanal < pentane < hexanal. Propanal may be more easily produced by thermal decomposition of the n-3 PUFA than pentane and hexanal from the corresponding n-6 PUFA. These thermodynamic determinations provide information on the relative ease of volatile formation, which is, in turn, related to the relative susceptibility of different oils to flavor deterioration.

TABLE 2

Apparent Activation Energy of Thermal Decomposition of Fish and Vegetable Oils Autoxidized at 50°C[a]

Run no.	Oils	PV (meq/kg)	Temperature (°C)	Activation energy (Kcal/mol)	Averaged activation energy[b]
1	Menhaden	3.1	40-70	6.7	
2	Menhaden	2.8	40-70	6.3	
3	Menhaden	4.6	40-70	6.5	
4	Sardine	57.0	40-70	6.7	6.6 ± 0.2
5	Menhaden	5.8	70-100	11.5	
6	Menhaden	5.4	70-100	11.7	
7	Sardine	7.3	70-100	11.9	11.7 ± 0.2
8	Menhaden	5.9	100-130	18.2	
9	Sardine	3.6	100-130	18.6	18.4 ± 0.2
10	Menhaden	2.0	130-160	18.4	
11	Sardine	19.2	130-160	18.9	18.7 ± 0.3
12	Soybean	4.9	100-130	21.4	
13	Safflower	19.4	100-130	22.1	
14	Canola	7.3	100-130	20.2	21.2 ± 0.8
15	Soybean	4.9	130-160	26.2	
16	Soybean	11.9	130-160	25.1	
17	Safflower	8.3	130-160	26.3	
18	Safflower	16.0	130-160	27.7	
19	Canola	7.3	130-160	27.9	
20	Canola	10.1	130-160	26.3	26.6 ± 1.0
21	Soybean	3.1	150-180	45.5	
22	Safflower	3.0	150-180	46.1	
23	Canola	1.3	150-180	48.6	
24	Canola	10.5	150-180	48.7	47.2 ± 1.4
High-oleic					
25	Sunflower	6.2	130-160	51.1	
26	Sunflower	7.1	130-160	45.3	
27	Safflower	11.2	130-160	53.6	50.0 ± 3.5
28	Sunflower	0.8	150-180	56.9	
29	Sunflower	5.1	150-180	62.4	59.7 ± 2.8

[a]Analysis of total volatiles with Tekmar equilibrium headspace instrument. Values for apparent activation energy were based on the Arrhenius plots (see Fig. 4) where slope of the line = $-0.219 \times$ activation energy (Ref. 20). Average SD of duplicate analyses is 7.1%. PV, peroxide value.
[b]Averages ± SD.

TABLE 3

Apparent Activation Energy of Formation of Individual Volatiles by Thermal Decomposition of Oils Autoxidized at 50°C[a]

Run no.	Oils	Activation energy (Kcal/mol)		
		Pentane	Propanal	Hexanal
5	Menhaden	11.8	11.5	—
6	Menhaden	12.0	11.7	—
8	Menhaden	18.5	18.2	19.2
9	Sardine	22.8	22.2	23.7
15	Soybean	26.1	25.0	27.2
18	Safflower	28.8	28.1	29.2
19	Canola	26.0	25.3	27.3
24	Canola	47.4	46.8	49.0

[a]See Table 2.

DISCUSSION

Only a few studies have reported data on activation energy of lipid oxidation, but no work has been reported on the temperature dependence of thermal decomposition of oxidized fats. Typical apparent activation energies range from 15 to 25 kcal/mole for lipid oxidation (21). Lea (22) studied the effect of temperature on oxidation of tocopherol-free fatty acid methyl esters. Lower apparent activation energies were found at 37 and 50°C for linseed (11 kcal/mole) and cod liver oil (13 kcal/mole) esters than for cottonseed (20 kcal/mole) esters, on the basis of induction periods measured at PV of 100 meq/kg. These values are in agreement with the lower apparent activation energies found in this study for fish as compared to vegetable oils. However, the endpoint of 100 meq/kg used in Lea's study (22) is too high to be relevant to flavor deterioration, which is detected in polyunsaturated oils at peroxide values below 10 (2,23).

In the current study, the differences observed in oxidative susceptibility among unsaturated oils (Fig. 1) can be accounted for by the greater oxidizability of n-3 EPA and DHA in fish oils than that of n-6 PUFA in vegetable oils (5). The thermal decomposition of oxidized oils could be effectively investigated by GC headspace analyses at different temperatures. By focusing on the low-molecular weight volatile compounds (24), the static GC headspace technique used in this work has the advantage over other GC volatile techniques (direct injection and dynamic headspace) in limiting the analysis to a few characteristic and diagnostic compounds that can be related to the origin of precursor hydroperoxides (2). Propanal was a specific volatile oxidation product that was readily formed in partially oxidized fish oils thermally decomposed at 40°C (Fig. 3). On the other hand, pentane and hexanal were less readily formed in partially oxidized vegetable oils thermally decomposed at 100°C. Thus, propanal characterized the oxidative decomposition of n-3 PUFA, and pentane and hexanal characterized the oxidative decomposition of n-6 PUFA in polyunsaturated oils.

Automated headspace GC was a useful tool to determine apparent activation energy of volatile formation, which is related to susceptibility of oils to flavor deterioration. Fish oils had lower apparent activation energies than did vegetable oils; high-oleic sunflower oils had a significantly higher apparent activation energy than did polyunsaturated vegetable oils (Table 2). Activation energies increased with decomposition temperatures, but PV of the oxidized oils had no significant effect.

The changes observed in apparent activation energy with temperature of decomposition indicate that mechanistic changes occurred. At elevated temperatures, hydroperoxides are expected to produce larger amounts of complex secondary products, including oxygenated cyclic, dimeric and polymeric compounds (23). Because activation energies increased with temperature, volatile formation is apparently more difficult by thermal decomposition of secondary products than from the corresponding hydroperoxide precursors.

The easier formation of propanal from n-3 PUFA than hexanal from n-6 PUFA (Fig. 3) is consistent with the lower apparent activation energies found for propanal formation than for pentane and hexanal formation (Table 3). Therefore, determination of activation energies by GC headspace provided an additional thermodynamic parameter to evaluate oxidative stabilities of unsaturated oils.

There is increased consumption of seafood and fish oils, and demand to improve food and nutritional quality from consumers and the food and drug industries, as well as

from regulatory agencies. The thermodynamic parameters derived from the oxidative changes and volatile formation in unsaturated oils can be applied to improving process control, to better estimate shelf life and ultimately to increase consumer acceptability of oil-bearing foods.

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