Changes in Headspace Volatiles and Peroxide Values of Undeodorized Menhaden Oil over 20 Weeks of Storage

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Undeodorized menhaden oil was stored in the dark at 30 °C under a headspace of air. Oil samples were removed at 0, 1, 2, 3, 4, 8, 12, 16, and 20 weeks of storage and analyzed for peroxide value and headspace volatiles by GC and GC−MS. Total volatiles increased in concentration during the first 2 weeks of storage, fell slightly at week 3, and then rose steadily reaching a second maximum at week 16, before declining again at week 20. Peroxide values exhibited three maxima, at weeks 1, 4, and 12. Several volatiles that are potentially derived from lipoxygenase activity in menhaden were identified in the oil including 1-penten-3-ol, (*E*)-2-hexenal, (*Z*)-3-hexen-1-ol, (*E*)-2-octenal, 1-octen-3-ol, 2,5-octadien-1-ol, 2-nonenal, and (*E*,*Z*)-2,6-nonadienal. Some volatiles derived from autoxidation are as follows: (*E*,*E*)-2,4-heptadienal, (*E*,*Z*)-2,4-hepadienal, (*E*,*E*)-2,4-decadienal, and (*E*,*E*)-3,5-octadien-2-one. Five of these volatiles reached maximum concentrations by the first or second week of storage. These findings suggest that pro-oxidant mechanisms at work prior to and/or during oil refining lead to rapid buildup of objectionable flavors and aromas in menhaden oil.

Keywords: *Menhaden oil; lipoxygenase; headspace volatiles*

INTRODUCTION

With the approval of hydrogenated and partially hydrogenated menhaden oil as GRAS (U.S. Government, 1989) and the expectation that unhydrogenated menhaden oil will receive GRAS status in the near future (Bimbo and Crowther, 1992), the fisheries industry is interested in developing menhaden oil into a major food ingredient. However, the incorporation of refined unhydrogenated menhaden oil into foods poses problems because of odor and flavor instability due to rapid oxidation of η-3-fatty acids (Park et al., 1989; Schnepf et al., 1991). One approach to improving the stability of the unhydrogenated oil is to identify the initiators of lipid oxidation and develop means of controlling them.

Lipid oxidation in foods may proceed via a free radical mechanism known as autoxidation. Autoxidation is initiated by abstraction of hydrogen from the α-carbon atom adjacent to a double bond of unsaturated fatty acids, and this reaction is considered to be the rate-limiting step in lipid oxidation (Nawar, 1985). The direct reaction of oxygen with unsaturated fatty acids is thermodynamically favored but requires energy for the reaction to occur spontaneously. This activation energy is reduced during enzymatic catalysis, and the initiation of the self-proliferating chain reaction of free radicals that results in the oxidative spoilage of oils is facilitated (German and Kinsella, 1985).

Enzymes, such as peroxidases, cyclooxygenases, and lipoxygenases, have been associated with the initiation of lipid oxidation. Peroxidases interact with H_2O_2, activating these ferric−heme enzymes to a higher redox state, which can then attack lipids. Cyclooxygenases and lipoxygenases carry out controlled lipid oxidation in prostaglandin and leukotriene synthesis. These enzymes are responsible for the introduction of oxygen into fatty acids such as arachidonic acid, resulting in the formation of lipid hydroperoxides. If disturbed, these hydroperoxides can cause uncontrolled lipid peroxidation (Kanner et al., 1987).

Several investigators found enzymatic systems to be important in the oxidative spoilage of fish (Karahadian and Lindsay, 1989a; Josephson et al., 1987; Hsieh et al., 1988; Slabyj and Hultin, 1984). Lipoxygenase, which has been identified in many fish species (Hsieh, 1988), is one of the enzymes that appears to play a key role in lipid oxidation in fish (German and Kinsella, 1985). Lipoxygenases catalyze the oxygenation of *cis*,*cis*-1,4-pentadiene systems by abstracting a methylene hydrogen from unsaturated fatty acids forming an allyl radical which is readily attacked by molecular oxygen, producing trans,cis-conjugated double-bond hydroperoxides (Maycock et al., 1989).

We have recently found evidence for the presence of one or more active lipoxygenases in the gill tissue of menhaden (*Brevoortia tyrannus*) (Gruen and Barbeau, 1995). Thus, the purpose of this investigation was (1) to attempt to identify volatile degradation products, potentially derived from lipoxygenase activity, in undeodorized menhaden oil and (2) to monitor changes in the quantities of these volatiles, as well as those derived from lipid autoxidation, over a 20 week storage period.

MATERIALS AND METHODS

Undeodorized menhaden oil was kindly donated by Zapata Haynie Corp., Reedville, VA. The oil had been extracted from freshly captured menhaden by a previously described wet reduction method (Bimbo and Crowther, 1992) and contained 200 ppm of the antioxidant *tert*-butylhydroquinone (TBHQ). The oil was transferred upon arrival in our laboratory to crimp-top vials. Each vial held 50 mL of oil and a 10 mL headspace of air. The vials were stored in the dark at 30 °C. Oil samples were removed from the vials at 0, 1, 2, 3, 4, 8, 12, 16, and 20

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weeks of storage and analyzed for peroxide value and headspace volatiles by gas chromatography (GC) and gas chromatography−mass spectrometry (GC−MS). Peroxide values were determined in triplicate by AOAC Method 28.026 (AOAC, 1984). Analysis of variance and Duncan's multiple range test were used to determine significant differences in peroxide values due to week of storage.

Dynamic Headspace Analysis of Oil Volatiles. Menhaden oil volatiles were collected by a purge and trap technique using a dynamic headspace sampling method adapted from Olafsdottir et al. (1985). Oil samples (15 mL) were added to a 50 mL round-bottom flask together with 10 μL of a 1% ethyl ether (Mallinkrodt, Paris, KY) solution of an internal standard, (*Z*)-2-decenal (Bedoukian, Danbury, CT). Nitrogen was bubbled through the oil mixture for 16 h at a flow rate of 100 mL/min, and volatiles were trapped in a collection tube filled with 100 mg of Tenax GR powder (2,6-diphenyl-*p*-phenylene oxide polymer, Alltech, Deerfield, IL). Volatiles were recovered from the Tenax by extraction into two 1 mL portions of ethyl ether. The combined ethyl ether solution was concentrated to 0.1 mL under a slow stream of nitrogen, and 0.8 μL was injected splitless into a Shimadzu (9A, GC instrument. A Supelcowax 10 (Supelco, Bellefonte, PA) capillary column (60 m, 0.25 mm i.d., 0.25 μm film thickness) was used for the separation of volatiles along with the following temperature-programing conditions: 50 °C initial temperature (1 min), 2 °C/min to 150 °C, then 4 °C/min to 220 °C, held at 220 °C for 21.5 min; 90 min total run time. Injector and flame ionization detector (FID) were both held at 240 °C. The helium gas carrier was maintained at a linear flow rate of 20 cm/s.

GC−MS Identification of Volatile Compounds. Undeodorized menhaden oil was also subjected at 0 and 3 weeks of storage to GC−MS analysis, and resulting mass spectra were computer matched with library spectra to identify various volatiles. The HP 5790-VG Analytical 7070 E GC−MS system was run under the same conditions used for GC analyses. The ionization energy of the mass spectrometer was set at 70 eV, the ion acceleration voltage at 2000 V, the ion source temperature was 240 °C, the transfer line temperature at 280 °C, and the analyzer source temperature at 270 °C. The National Institute of Standards and Technology mass spectra library with 42 000 entries was used for identifying volatiles (NIST, 1983). The library search was conducted in the "mixed search" mode after background subtraction of stray ions and the carrier gas. The mixed search mode was used because of its reported advantages in identifying individual components in a mixture (Heller et al., 1980). The library reference spectra had to match the mass spectra by at least 75% to be considered a correct match. Oil volatiles not identified in this way were declared as unknown compounds. Three additional compounds (decane, (*E*)-2-octenal, and 1,3,5-trimethylbenzene) with less than a 75% correspondence between a library mass spectrum and that of the unknown were tentatively identified on the basis of mass spectral data and retention times/indices of menhaden oil volatiles reported in the literature. Results of GC−MS analysis of oil volatiles at 0 and 3 weeks of storage were used to identify volatiles in other stored oil samples by carefully matching the retention time of each unknown volatile with the retention times of volatiles identified by GC−MS.

Estimation of Volatile Concentrations in ppm. The detector response rates of the various volatiles identified in this study were assumed to be equal to that of our internal standard. The concentration of each volatile was then estimated using the equation

$$CV = (AV \times CIS)/AIS$$

where CV = concentration of the individual volatile, AV = GC peak area of the volatile, CIS = concentration of the internal standard, which in this study was held constant at 6.67 ppm, and AIS = peak area of the internal standard. The concentration of individual volatiles was estimated from this equation after averaging the peak areas from duplicate GC runs on each of the oil samples.

RESULTS AND DISCUSSION

More than 68 volatiles were recovered from the Tenax powder after 16 h of continuous exposure to each of the undeodorized menhaden oil samples. Forty-eight of these volatiles were identified by GC−MS at 0 and 3 weeks of storage. Changes in the concentration of each volatile were then followed over 20 weeks of storage (see Table 1). Many of these volatiles are C5−C9 carbonyl compounds that likely impart green, grassy, plantlike odors to menhaden oil which are particularly detectable in the early stages of oil oxidation (Karahadian and Lindsay, 1989a; Hsieh et al., 1989). Quantitifiable levels of hexa-, hepta-, octa-, nona-, and decadienals were also present. These dienals are thought to contribute to the painty, oxidized aromas and flavors of rancid fish oils. Investigators have also found that objectionable burnt, fishy aromas are elicited by the presence of <1 ppm of geometric isomers of 2,4,7-decatrienal (Karahadian and Lindsay, 1989a). While a scan for the specific mass ions of 2,4,7-decatrienal revealed that most of them were found in a very small GC peak eluting at 62.5 min, it was impossible to confirm this peak as a decatrienal isomer because of coelution of another compound. 1,4-Dimethylbenzene 1,3,5-trimethylbenzene, and benzaldehyde were also detected. Since these compounds are not known to be derived from enzymatic or autoxidative degradation of fish oils, they are most likely environmental contaminants (Lin et al., 1990).

The concentration of total volatiles recovered from the undeodorized menhaden oil at any time during storage should be proportional to the total peak area of corresponding GC chromatograms. The total peak areas for duplicate GC runs were therefore averaged. These mean values are given at the bottom of Table 1. Total peak area increased during the first 2 weeks of storage, fell slightly at week 3, and then rose steadily reaching a second maxima at week 16 before declining again at week 20. Peroxide values for the undeodorized oil exhibited three maxima, at weeks 1, 4, and 12 (see Figure 1). Values at week 12 were significantly greater than values at all other weeks. Values at week 1 were significantly greater than those at weeks 0, 2, 3, 8, 16, and 20, while values at week 4 were significantly greater than those at weeks 0, 8, 16, and 20.

Other investigators have also observed multiple maxima for peroxide and total volatle levels of stored menhaden oil (Carlat, 1990; Schnepf et al., 1991) which suggests that more than one mechanism is at work with regard to volatile generation and oxidative deterioration of the oil. It should be pointed out that there were more than 3 times the number of menhaden oil peroxides present at week 12 than at week 1 and nearly 2 times the concentration of total volatiles present at week 16 as at week 2. It seems conceivable to us that autoxidatively derived volatiles accumulated in our oil samples throughout the 20 week storage period reaching a maxima at week 16 before undergoing polymerization to larger molecular weight components which were not volatile enough to be entrapped and recovered from the Tenax at week 20. The earlier, less pronounced peaks in oil peroxides and total volatiles are likely due, at least in part, to peroxidation initiated prior to and/or during oil processing and refining.

Lipoxygenase (LOX) activity has been detected in the gill and skin tissue of a growing number of fresh and salt-water fish (Josephson et al., 1984; German and Kinsella, 1985; German and Creveling, 1990; Mohri et

Table 1. Changes in Mean Concentration of Volatiles Identified in Undeodorized Menhaden Oil over 20 Weeks of Storage[a]

compound name	concentration (ppm) and week of storage								
	0	1	2	3	4	8	12	16	20
decane[b−d]	4.98	9.13	9.46	4.20	8.35	6.19	4.57	7.31	3.96
2,3-dimethyl-1,4-hexadiene	19.18	39.83	37.05	18.69	42.79	43.55	11.87	16.21	21.45
1-penten-3-one[b−d]	2.66	7.73	6.40	3.21	0.00	0.00	0.00	0.00	0.00
3-ethyl-1,4-hexadiene	0.98	5.03	4.22	2.38	0.00	0.00	0.00	0.00	0.00
5-ethyl-2-methyloctane	0.00	0.34	0.24	0.00	2.00	1.94	1.73	2.44	0.00
hexanal[b−e]	91.92	167.27	145.75	82.63	121.14	126.26	88.98	127.45	55.84
(*E*,*E*)- and (*Z*,*E*)-3,6-octatrienes	31.65	60.75	59.95	30.37	66.50	63.41	81.97	69.04	30.78
(*Z*)-2-pentenal[b−d]	27.39	44.62	34.26	33.55	44.35	22.71	42.22	22.95	6.93
1,4-dimethylbenzene	22.41	43.73	38.99	23.38	ns	16.56	41.73	72.65	10.04
1-penten-3-ol[c]	**21.39**	**44.23**	**35.83**	**27.33**	**ns**	**87.59**	**81.53**	**141.51**	**11.44**
2-methyl-2-pentenal	6.07	12.95	11.96	8.25	0.00	0.00	0.00	0.00	3.61
heptanal[b−e]	50.47	86.51	85.22	29.24	72.64	65.07	42.95	54.65	39.08
4,5-dimethyl-1-hexene	1.51	1.39	1.49	1.14	1.37	1.08	2.30	0.00	0.60
4-methyl-3-pentenal	3.12	6.65	5.39	2.44	85.02	95.40	99.01	116.21	5.97
2-hexen-3-one	6.11	11.38	10.72	5.39	10.89	10.09	8.27	8.22	5.11
(*E*)-2-hexenal[b−d]	**61.53**	**122.73**	**113.50**	**50.44**	**80.17**	**77.51**	**70.17**	**62.03**	**48.22**
(*Z*)-4-heptenal[c]	29.67	47.95	46.73	21.35	35.42	31.98	19.42	34.67	22.38
1,3,5-trimethylbenzene[b,d]	1.54	1.98	2.46	1.36	2.75	0.00	0.00	0.00	1.52
octanal[b,c]	9.60	14.03	14.62	8.17	42.67	48.56	51.18	65.02	7.95
(*E*)-2-penten-1-ol[c,d]	8.52	13.88	13.02	11.37	12.37	13.35	9.97	13.54	5.29
(*Z*)-2-penten-1-ol	5.11	8.53	7.71	7.08	10.93	12.22	8.63	10.99	2.89
(*Z*)-2-heptenal[b−e]	16.11	26.77	29.54	16.07	20.65	18.78	17.54	23.04	15.58
6-octen-2-one	0.00	0.00	0.24	0.78	3.16	2.61	2.19	1.06	0.10
1-hexanol[d]	3.02	7.33	7.08	3.74	7.24	7.19	7.28	8.83	3.55
2-methyl-2-cyclopenten-1-one	3.85	6.91	6.44	3.09	4.67	4.42	3.97	5.45	3.01
(*Z*)-3-hexen-1-ol	**2.43**	**3.97**	**3.73**	**2.11**	**3.08**	**2.78**	**2.23**	**2.85**	**1.96**
2-nonanone[b,c]	4.66	7.19	7.44	4.93	6.67	6.90	5.67	1.82	4.00
nonanal[b−e]	7.23	9.87	10.08	6.78	8.94	8.23	5.55	4.22	5.09
(*E*,*E*)-2,4-hexadienal[b,c]	8.03	14.31	14.33	7.27	11.68	11.26	9.96	9.71	7.20
(*E*)-2-octenal[b,c,e]	**17.17**	**27.47**	**28.73**	**17.67**	**24.58**	**23.38**	**20.70**	**22.43**	**16.04**
1-octen-3-ol[c,d]	**5.45**	**8.80**	**9.80**	**6.26**	**9.36**	**9.17**	**9.88**	**9.26**	**5.97**
(*E*,*Z*)-2,4-heptadienal[b−e]	93.69	141.90	146.33	82.24	122.90	115.39	109.15	105.87	85.21
pentadecane[b,d]	72.45	111.08	83.87	60.11	73.74	77.95	73.30	68.20	61.67
(*E*,*E*)-2,4-heptadienal[b−e]	39.12	60.16	28.92	39.98	72.81	53.84	42.21	36.80	32.95
(*E*,*Z*)-3,5-octadien-2-one[c,e]	3.23	4.85	5.44	2.78	3.68	11.31	9.49	6.70	4.24
benzaldehyde[b−d]	7.06	9.22	8.89	5.79	91.69	111.91	141.69	peaks not	5.57
2-nonenal[b−e]	**4.15**	**7.76**	**9.85**	**3.03**	**22.48**	**22.94**	**17.23**	**separated**	**2.48**
(*Z*,*Z*)-2,4-octadienal[b−d]	3.64	5.38	4.94	3.41	5.71	9.35	7.17	6.36	3.39
(*E*,*E*)-3,5-octadien-2-one[b,d]	2.31	3.46	3.14	2.50	3.81	3.87	3.64	3.43	3.81
(*E*,*Z*)-2,6-nonadienal[b,c]	**6.38**	**6.38**	**7.22**	**4.14**	**4.50**	**7.67**	**9.23**	**12.81**	**3.37**
(*E*,*E*)-2,4-octadienal[b,d]	3.85	7.43	7.19	4.26	6.19	7.28	5.70	4.81	3.08
(*Z*)-2-decenal (internal standard)	6.67	6.67	6.67	6.67	6.67	6.67	6.67	6.67	6.67
hexadecane[b,d]	2.14	3.43	3.35	2.10	2.95	2.86	2.85	3.21	2.13
2,5-octadien-1-ol	**1.71**	**2.75**	**2.50**	**1.66**	**1.73**	**1.76**	**1.74**	**1.79**	**1.06**
heptadecane[b,d]	2.21	3.71	2.77	2.58	3.10	2.70	2.12	2.45	1.89
5-ethyl-2(5*H*)-furanol[b]	2.14	2.97	2.64	1.65	4.44	2.65	3.14	2.59	1.14
(*E*,*E*)-2,4-decadienal	0.54	1.44	0.90	0.34	1.08	1.62	2.61	2.77	0.35
TBHQ	11.83	19.09	16.60	18.30	3.98	3.68	1.76	2.91	6.03
1-dodecanol[d]	4.14	5.80	8.66	6.08	8.32	5.72	4.61	2.22	0.62
total volatile area	4 326 903	5 055 734	5 407 352	4 803 161	5 898 202	7 494 378	9 288 356	9 680 695	5 075 052

[a] ns: compounds not separated. Compounds in bold lettering have been reported to be generated by lipoxygenase activity. [b] Hsieh et al., 1989 (undeodorized menhaden oil). [c] Karahadian and Lindsay, 1989a (deodorized menhaden oil). [d] Lin et al., 1990 (crude menhaden oil). [e] St. Angelo et al., 1987 (crude menhaden oil).

al., 1992; Zhang et al., 1992). The majority of well-characterized fish lipoxygenases seem to have site specific activity toward the *n*−9 carbon (ninth carbon atom from methylene terminus) of C20−C22 polyunsaturated fatty acids which after insertion of molecular oxygen and positional/geometric isomerization leads to the formation of 12-hydroperoxides (Winkler et al., 1991). Such enzymes are therefore commonly called 12-lipoxygenases. There is also evidence for the existence of 15-lipoxygenases in the gills of trout, *Salmo gairdneri*, and rockfish, *Sebastes flavidus*, that require double bonds at the *n*−6 and *n*−9 positions. These enzymes are referred to as 15-lipoxygenases because of their preferential formation of 15-hydroperoxides from arachidonic and eicosapentaenoic acids (German and Crevelling, 1990).

Catalysis of lipid oxidation by 12- and 15-lipoxygenases will, following hydroperoxide lyase activity, lead to the formation of a different set of carbonyls and alcohols. For example, the action of a 12-lipoxygenase on eicosapentaenoic acid should yield eight- and nine-carbon aldehydes, ketones, and alcohols, while 15-lipoxygenase should result in five- and six-carbon volatiles such as 1-penten-3-ol, (*Z*)-3-hexenal, (*E*)-2-hexenal, and (*Z*)-3-hexen-1-ol (Josephson and Lindsay, 1986). It is interesting to note therefore that relatively larger amounts of 1-penten-3-ol and (*E*)-2-hexenal and much smaller quantities of (*Z*)-3-hexen-1-ol, but not (*Z*)-3-hexenal, were found in our menhaden oil samples (see 1-penten-3-ol, (*E*)-2-hexenal, and (*Z*)-3-hexen-1-ol in bold lettering in Table 1). The concentration of 1-penten-3-ol doubled during the first week in storage, dropped to

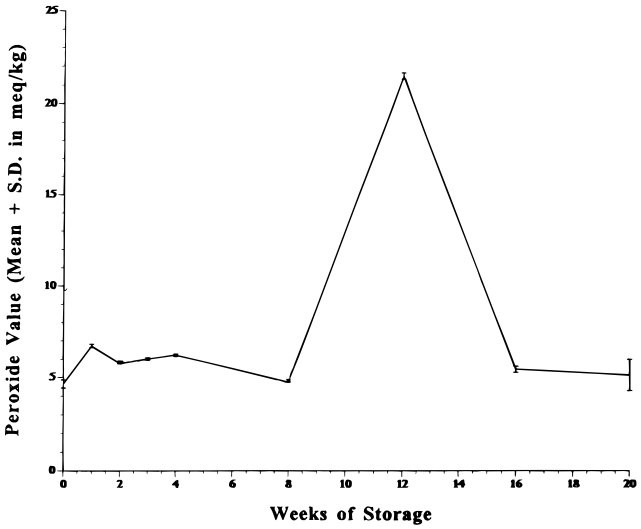

Figure 1. Peroxide values of undeodorized menhaden oil over 20 weeks of storage at 30 °C.

a minimum at week 3, and then increased nearly 5-fold by week 16 before declining again at week 20. The six-carbon volatiles, (*E*)-2-hexenal and (*Z*)-3-hexen-1-ol, reached maximum concentrations at week 2, with a second, smaller concentration maxima at week 4. To our knowledge this is the first time that (*Z*)-3-hexen-1-ol has been identified as a volatile component of menhaden oil, but like other investigators, we failed to detect (*Z*)-3-hexanal, believed to be an early product of 15-lipoxygenase/lyase activity (Josephson and Lindsay, 1986).

Hsieh and Kinsella (1989) reported that a group of eight-carbon volatiles, (*E*)-2-octenal, 1-octen-3-ol, and 2-nonenal, were formed following incubation of a trout gill 12-lipoxygenase with arachidonic acid, while (*E,Z*)-2,6-nonadienal, 1,5-octadien-3-ol, and 2,5-octadien-1-ol were derived from 12-lipoxygenase-catalyzed oxidation of eicosapentaenoic acid (EPA). All of these volatiles have been positively identified by others during headspace analysis of menhaden oil with the exception of 2,5-octadien-1-ol (Karahadian and Lindsay, 1989a; Hsieh et al., 1989; Lin et al., 1990). GC−MS analysis revealed that our menhaden oil samples contained an estimated 1−3 ppm of 2,5-octadien-1-ol but no detectable quantities of 1,5-octadien-3-ol. Changes occurring in the headspace concentrations of (*E*)-2-octenal, 1-octen-3-ol, 2,5-octadien-1-ol, 2-nonenal, and (*E,Z*)-2,6-nonadienal can be found in bold lettering in Table 1. (*E*)-2-Octenal concentration nearly doubled in the first week of storage before reaching a maximum at week 2 with a second, smaller concentration peak at week 8. Similar changes were noted in 1-octen-3-ol with two almost equal concentration peaks occurring during storage, one at week 2 and the second at week 12. 2,5-Octadien-1-ol, which is believed to be derived from EPA, reached its maximum concentration at week 1 with very little change in concentration after the third week of storage. Changes in the concentrations of 2-nonenal and (*E,Z*)-2,6-nonadienal more closely resembled those changes that occurred in total oil volatiles over 20 weeks of storage. Both compounds more than doubled in concentration during the first 2 weeks of storage; concentrations of 2-nonenal then increased more than 2-fold by week 4 before reaching a maximum at week 8, while (*E,Z*)-2,6-nonadienal nearly doubled in concentration from week 2 to week 16.

Detection of various C5, C6, C8, and C9 volatiles should not be construed as a prior evidence for the existence of 12- and 15-lipoxygenases in the gills and/or skin the menhaden, since these volatiles may also be generated autoxidatively (Karahadian and Lindsay, 1989b). (*E,E*)-2,4-Heptadienal, (*E,Z*)-2,4-heptadienal, and 3,5-octadien-2-one were detected in our menhaden oil samples at week 0. These three volatiles are believed to be derived from autoxidation of EPA (Karahadian and Lindsay, 1989b). One of the autoxidation derived decadienals (Josephson and Lindsay, 1986), (*E,E*)-2,4-decadienal, was also detected at week 0. Thus, it appears that the undeodorized menhaden oil had begun to autoxidize prior to its arrival in our laboratory. It is interesting to note that, like other oil volatiles, 2,4-heptadienal isomers and (*E,E*)-2,4-decadienal exhibited two concentration maxima, the first again occurring at week 1 or 2 of storage.

As Karahadian and Lindsay have pointed out (1989b), mechanisms leading to aromas and flavors in fish and fish oils are not necessarily restricted to autoxidative or lipoxygenase-initiated processes. There are other enzymes, including NADH-dependent microsomal peroxidases (Slabyj and Hultin, 1982) and myeloperoxidases (Kanner and Kinsella, 1983), that are endogenous to fish that may also play a role in oxidative deterioration of menhaden oil. Transfer of light energy from photosensitizers such as chlorophyll and heme pigment to molecular oxygen leads to the formation of singlet oxygen, a potent initiator of lipid oxidation in biological systems (Kanner et al., 1987; Karahadian and Lindsay, 1989b). Menhadens are phytoplankton feeders, and thus chlorophylls are likely to be in abundance along with heme iron during wet rendering of menhaden oil.

Peroxidation of unsaturated lipids by singlet oxygen does not proceed via a free radical mechanism. Singlet oxygen can add directly to either carbon of a double bond. Abstraction of hydrogen from an adjacent carbon atom and rearrangement lead to a different array of hydroperoxides than in autoxidation or lipoxygenase-catalyzed reactions (Kanner et al., 1987). Singlet oxygen-initiated oxidation of docosahexaenoic acid (DHA) leads to formation of 2-hydroxy-3-pentenalactone, a compound found to contribute green notes to oxidizing fish oils (Karahadian and Lindsay, 1989b). This compound was not one of the 48 volatiles identified in this study. It could, however, be among the more than 20 volatiles that remain unidentified.

Edible oils with a peroxide value of 7.5 mequiv/kg have been deemed as unacceptable from a sensory viewpoint by some investigators (Robards et al., 1989; Schnepf et al., 1991). The peroxide values of undeodorized menhaden oil came close to 7.5 as early as the first week of storage (mean peroxide value of 6.7) and easily exceeded this value by week 12 (mean value of 21.5). Lin et al. (1990) demonstrated that a sequential process of alkali refining, clay bleaching, and steam deodorization effectively removes many undesirable volatiles from crude menhaden oil and reduces peroxide values to ≤1.5. However, we have stored steam-deodorized and undeodorized menhaden oils under identical conditions and found that deodorization delays but does not completely prevent oil peroxidation (Grün, 1993).

The findings presented here strongly suggest that there are pro-oxidant mechanisms at work prior to and/or during oil refining that lead to early development of off-flavors and aromas in menhaden oil. These factors will need to be identified and controlled if manufacturers are interested in producing acceptable forms of the unhydrogenated oil.

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