Characterization of Volatile Components of Menhaden Fish *(Brevoortia tyrannus)* Oil[1]

Thomas C.Y. Hsieh[*,a,b,c], **Stephen S. Williams**[b,2], **Warinda Vejaphan**[b,3] and **Samuel P. Meyers**[b,c]
[a]Louisiana Agricultural Experiment Station, LSU Agricultural Center; [b]Louisiana State University Department of Food Science, and [c]Louisiana Sea Grant College Program, Louisiana State University, Baton Rouge, Louisiana 70803

Volatile odor components of winterized and undeodorized menhaden (*Brevoortia tyrannus*) oil were characterized by dynamic headspace concentration on a porous polymer trap, thermal desorption, cryogenic focusing, high resolution gas chromatography, mass spectrometry and chromatography-coupled descriptive odor evaluation. Many volatile odor components derived from lipid oxidation in the fish oil were identified. These included, among others, short-chain saturated and unsaturated aldehydes and ketones, as well as short-chain carboxylic acids. The former imparted greasy, oily, oxidized oil and green grassy or green plant-like odors, and the latter gave rather objectionable sweaty odors. Normal alkanes were detected as major volatiles without significant odors. The odor-significant volatile components may be used as specific flavor quality markers to determine deodorization efficiency on crude fish oil and to monitor flavor stability of purified oils. This combined analytical approach can be readily applied to the study of volatile components in other oils.

The American fish oil industry produced an average of 125,000 tons of crude fish oil per year during the past decade. Menhaden oil constituted the majority of this production. Approximately 82% of this crude fish oil was exported to European countries where the oil was used in manufacturing margarine and shortening. Recently, long chain ω-3 polyunsaturated fatty acids (PUFA), which are abundant in fish oils, have been implicated in having an effect on reducing specific risk factors for cardiovascular diseases. The prospect of using ω-3 fatty acids as new and potentially beneficial ingredients in foods presents an enormous opportunity to the fish oil industry and the food industry in general (1,2). However, due to current harvesting and processing practices, as well as to the high concentration of PUFA and other contaminants, crude fish oils are susceptible to severe deteriorative changes in flavor quality. Off-odors and flavors in fish oils arise from metabolite contamination, from fish protein spoilage, or from oxidation products of the fish oil itself (3,4). The undesirable odors and volatile components should be removed during refining and deodorization to obtain food grade oil.

Numerous reports can be found in the literature concerning flavor or volatile components of oils from plants or animals. However, many of these studies used multiple steps for sample extraction and fractionation (5-10) or high temperature sampling of oil samples (8,11-28). The former methods were labor-intensive and time-consuming, while the latter approaches were subject to artifact formation due to thermal instability of oils. In addition, separation of volatile flavor components was often carried out by gas chromatography on low resolution packed columns (8,10,12-14, 18-29).

Recently, dynamic headspace capillary chromatographic analysis without solvent extraction was used to study volatiles in soybean oil (30). In this paper, we report a new, direct, sensitive and low-artifact dynamic headspace concentration procedure using high resolution gas chromatography-coupled odor evaluation and mass spectrometry for analyzing fish oil volatiles. This method can be applied readily to analysis of volatiles in other oils.

EXPERIMENTAL PROCEDURES

Materials. Winterized and undeodorized 1985 menhaden fish (*Brevoortia tyrannus*) oil samples were a gift from Zapata Haynie Corp. (Reedville, Virginia). Flavor standards were purchased from Aldrich Chemical Co. (Milwaukee, Wisconsin), Pfaltz & Bauer, Inc. (Waterbury, Connecticut) and Fluka Chemical Corp. (Hauppauge, New York).

Dynamic headspace sampling. A dynamic headspace sampler system (DHS) consisting of a Tekmar model 4200 Automatic Heated Sampler and a model 4000 Dynamic Headspace Concentrator (Cincinnati, Ohio) was used. Ultra high purity helium (99.999%, Linde Div., Union Carbide, Danbury, Connecticut) was used as a purge and desorption gas in this system and as a carrier gas for subsequent gas chromatography (GC). In each run, a 350-mg aliquot of the oil sample was pre-purged at ambient temperature for two min to remove any oxygen from inside the sample tube. The sample was then heated to 65 C, and the volatiles were purged from the oil sample onto a porous polymer Tenax TA (Chrompack, Raritan, New Jersey) sorbent cartridge at ambient temperature at a purge flow rate of 50 ml/min. After 60 min of purging and trapping, the helium flow was directed through the Tenax TA trap (bypassing the oil sample tube) for another 10 min to remove any trace of moisture that may have been transferred from the sample to the trap. The volatiles in the trap were then ready for desorption onto a stand-alone GC or a combined GC/mass selective detector (GC/MSD).

Gas chromatography. Chromatograms and aromagrams were obtained with a model 5793 GC (Hewlett-Packard Co., Palo Alto, California). During the 20-min desorption period, volatiles were flushed from the Tenax TA trap in the DHS by thermal desorption at 185 C and cryogenically focused with a dry ice/ethanol mixture into two parallel fused silica capillary columns (60 m × 0.32 mm i.d.; film thickness, 0.25 μm) bonded with polyethylene glycol stationary phase (Supelcowax 10, Supelco, Inc., Bellefonte, Pennsylvania) installed in the same injector using a two-hole ferrule (SGE, Austin, Texas). The column temperature was held at 40 C for five min and then programmed to 175 C at 1 C/min.

[1]Presented at the AOCS meeting in New Orleans, LA in May 1987.
[*]To whom correspondence should be addressed.
[2]Now with T.J. Lipton Inc., 800 Sylvan Ave., Englewood Cliffs, NJ 07632.
[3]Now with Lever Brothers, Bangkok, Thailand 10400.

TABLE 1

Volatile Components in Crude Menhaden Oil as Determined by DHS/GC/MS

Peak No.	compound Name	Retention index	Odor characteristics	Ion m/z	Conc. (ppb)	Peak No.	compound Name	Retention Index	Odor characteristics	Ion m/z	Conc. (ppb)
1	octane[a]	800		43	20	30	decanal[a]	1488	sweet, green fruity, fatty with citrus topnote	57	640
2	nonane[a]	900		43	2						
3	pentanal[b]	979		58	360						
4	decane[a]	1000		57	90	31	(E,E)-hepta-2,4-dienal[a, e]	1493		81	2180
5	2-methyl-5-ethyl-furan[c]	1014		110	10	32	pentadecane[a]	1500		57	520
6	1-penten-3-one[a]	1022		84	240	33	(Z,Z)-octa-3,5-dien-2-one[c, e]	1520		124	130
7	(E)-but-2-enal[a, e]	1040	painty	70	130	34	benzaldehyde[a]	1521	cherry, almond, sweet fruity	105	60
8	hexanal[a]	1085	cut grass, green	56	1380						
9	undecane[a]	1100		57	130						
10	(Z)-2-pentenal[b, e]	1110		84	70	35	nonenal[a]	1535	fatty, waxy, musty	70	200
11	(E)-pent-2-enal[a, e]	1130	greasy green, musty	84	930	36	propanoic acid[a]	1547	astringent, acidic	74	2410
						37	octa-2,4-dienal[a, d, f]	1563		81	10
12	heptan-3-one[a]	1162	sickly sweet, cooling	57	8530	38	(E,E)-octa-3,5-dien-2-one[c, e]	1570		124	40
13	heptanal[a]	1186	waxy green, grassy	70	410	39	isobutanoic acid[c]	1579	sweaty, dirty socks	73	100
14	dodecane[a]	1200		57	280	40	(E,Z)-nona-2,6-dienal[a, e]	1587		70	240
15	(E)-hex-2-enal[a, e]	1218	sharp green, oily	69	540						
16	2-pentylfuran[b]	1233		138	10	41	(E,E)-octa-2,4-dienal[c, e]	1590		81	40
17	1,3,5-trimethyl-benzene[a]	1242	pesticide-like	120	20	42	hexadecane[a]	1600		57	10
18	1,2,4-trimethyl-benzene[a]	1279	pesticide-like	120	40	43	butanoic acid[a]	1636	dirty socks	60	8110
19	octanal[a]	1290	citrus, fatty, orange	57	240	44	decenal[c]	1645		55	410
20	tridecane[a]	1300		57	370	45	heptadecane[a]	1700		85	80
21	(E)-hept-2-enal[a, e]	1323	sharp green, greasy	83	140	46	pentanoic acid[a]	1747	Parmesan cheese, dirty socks	60	330
22	1,2,3-trimethyl-bezene[a]	1332	pesticide-like	120	20						
23	nonan-2-one[a]	1390	musty with citrus topnote	58	620	47	5-ethyl-2(5H)-furanone[c]	1755		112	20
						48	deca-2,4-dienal[a, d]	1764	oxidized oil	81	150
24	nonanal[a]	1395	fatty floral	57	500	49	deca-2,4-dienal[a, d]	1806	oxidized oil	81	250
25	tetradecane[a]	1400		81	230	50	hexanoic acid[a]	1850	sweaty, dirty socks	60	810
26	(E,E)-hexa-2,4-dienal[c, e]	1402		81	100	51	nonatrienal[c, d, f]		oxidized oil	79	60
						52	nonatrienal[c, d, f]		oxidized oil	79	130
27	(E)-oct-2-enal[a, e]	1429	musty, waxy floral	70	280	53	decatrienal[c, d, f]		oxidized fish oil	79	70
28	acetic acid[a]	1471	irritating, vinegar-like	60	1380	54	decatrienal[c, d, f]		oxidized fish oil	79	8
29	hepta-2,4-dienal[c, d, f]	1467	vegetable green	81	1880	55	phenol[a]	2014	medicinal, disinfectant	94	110

[a]Identified by GC retention index and MS (standard and literature).
[b]Identified by GC retention index and MS (literature).
[c]Identified by MS (literature).
[d]Configuration of geometric isomers not determined.
[e]Prefix (E)- denotes a *trans*-isomer and prefix (Z)- denotes a *cis*-isomer.
[f]Calculation of MS response factors based on *trans, trans*-hepta-2,4,-dienal.

Effluent from one column was directed to a flame ionization detector. During the same chromatographic period, the effluent from the second column was directed to a sniffing port for descriptive odor evaluation on the components by three panel members who had experience in GC-coupled odor evaluation techniques and were able to recognize and describe odor characteristics of various flavor compounds as these compounds emerged from the end of the GC column. These panel members were also familiar with the fish oil's characteristic odor.

Gas chromatography/mass spectrometry (GC/MS). A Hewlett-Packard 5792 GC/5970B MSD system was used to analyze the fish oil volatiles transferred from the DHS. A similar column with a narrow bore (0.25 mm i.d.) was used. Additional conditions were as follows: ion source temperature, 250 C; ionization voltage, 70 eV; GC/MSD direct interface temperature, 200 C, and electron multiplier voltage, 1800 V. A series of standard hydrocarbons (PolyScience Corp., Niles, Illinois) were used as retention references to determine the retention indices (31) of the volatile components. The identification of the volatile components was based on chromatographic retention indices, odor characteristics and mass spectral pattern in comparison with those data obtained from reference standards under similar experimental conditions. When standards were not available, data in published literature (32) were used for comparison.

Quantification of volatile components. A 350-mg aliquot of the crude menhaden oil was purged and analyzed by GC/MSD under the conditions described above. At the end of the first chromatographic run, the spent sample was immediately purged and reanalyzed. Total area for each volatile component in the total ion chromatograms from a total of three consecutive runs on the same sample was calculated. The experiment was repeated twice with a new aliquot each time. External standard methods were used to determine the average concentrations of each component listed in Table 1. When incomplete chromatographic separation of components was observed in a segment of the total ion chromatogram, mass chromatography (33) of a characteristic ion was carried out to obtain a resolved peak profile in a mass chromatogram. The same ion was used in the mass chromatography on that particular peak from the external standard mixture. This approach for quantification of com-

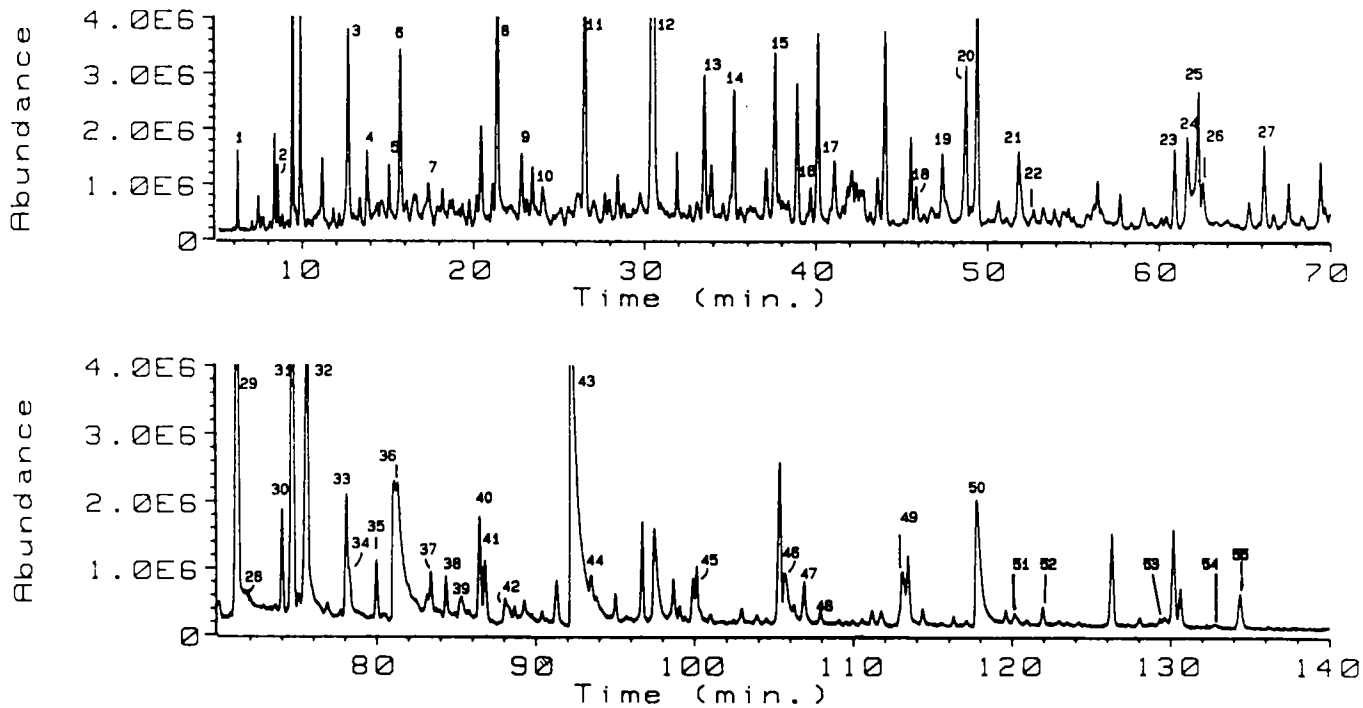

FIG. 1. Reconstructed total ion chromatogram of menhaden oil volatiles by DHS/GC/MS. Abundance indicates the total ion intensity. Peak numbers correspond to those in Table 1.

ponent concentrations was found to be more satisfactory than using total ion intensity because a characteristic ion minimized interference from partially overlapping or coeluting components.

RESULTS AND DISCUSSION

Figure 1 shows the total ion chromatogram of the volatile components of the menhaden fish oil sample used in this study. Table 1 lists the volatile components identified in the samples, retention indices, the odor characteristics, mass/charge (m/z) values of ions used in quantification, and the estimated concentrations of these components based on multiple purging analysis as described in the Experimental Procedures section. Most of the compounds were identified by comparing the mass spectra, GC retention indices and odor characteristics of the components with those of authentic standards and/or with literature data.

Most of these volatile components were derived from oxidative degradation of PUFA, which were abundant in fish oil. A series of alkenals and alkadienals constitute the major volatile components detected. Unsaturated aldehydes, such as (Z)-pent-2-enal and (E)-hex-2-enal, could come from oxidation of linolenic acid and other PUFA. Dienals were detected in series with chain lengths ranging from C6 through C10. These dienals, once formed, can be degraded quickly by further oxidation to form other smaller compounds. For example, decadienals can be degraded through bond cleavage between C4 and C5 to form hexanal, 2-butenal, hexane and but-2-en-1,4-dial (34).

Two compounds, (E,Z,Z)-deca-2,4,7-trienal and (E,E,Z)-deca-2,4,7-trienal, have been reported to contribute significantly to the fishy flavor of autoxidized linolenic acid (35,36). It has also been reported (36) that (E,Z,Z)-deca-2,4,7-trienal was the sole compound responsible for the fish odor in strongly autoxidized oils containing ω-3 fatty acids. Decatrienal and tridecatetraenal have been reported as tentatively identified in autoxidation of methyl docosa-4,7,10,13,16,19-hexaenoate as a model of oxidation of marine oils (10,41). In this study, two nonatrienals and two decatrienals were detected in the crude menhaden oil sample. Detail structures were not identified due to lack of standards. However, the oxidized oil odors given by the alkadienals and the alkatrienals were considered as important and characteristic of the odor of the crude menhaden oil.

Recently, the potential for artifact formation by heating oil samples at 90 C or higher was reported (30). The relative amounts of volatile thermal decomposition products of linolenate and linoleate hydroperoxides, such as hepta-2,4-dienal and deca-2,4-dienal, increased significantly when samples were heated at 90 C or higher (30). The alkadienals and alkatrienals detected in this study were most likely already present in the oil samples and not an artifact during analysis, because oxygen in the headspace above the sample was removed by pre-purging, and the sample purging temperature was set at 65 C.

Several saturated and unsaturated ketones were detected as shown in Table 1. A series of volatile fatty acids, with chain lengths ranging from C2 to C6, gave very intense and objectionable sweaty odors. Among these volatile acids, butanoic acid was most abundant in the fish oil samples. Short-chain fatty acids in oils have been reported before (16,38-40). These short-chain volatile acids were considered as important flavor quality markers for fish oil.

Low molecular weight alkanes (C1 to C7) were not detected,

possibly due to insufficient cooling of the cold trapping used (dry-ice/ethanol mixture at −76 C). Liquid nitrogen cold trapping will be used in future studies. However, higher molecular weight straight chain hydrocarbons (C8 to C17) that were detected in the fish oil did not contribute significantly to the flavor quality of the oil.

The odor-contributing volatile components identified in the crude fish oil (Table 1) may be used as flavor quality markers in the refining and deodorization processes leading to food grade oil. Application of mass chromatography (33) on the GC/MS data allowed determination of component concentrations based on compound-specific ion profiles in spite of possible chromatographic interference at the molecular level. The combined analytical approach used in this study can be applied readily to analysis of volatiles from other oils and many foods.

ACKNOWLEDGMENTS

This work was supported in part by research grants from the Louisiana Agricultural Experiment Station, LSU Agricultural Center; Louisiana Sea Grant College Program; and LSU College of Agriculture. A. Bimbo of Zapata Haynie Corp. provided encouragement and a generous gift of oil samples. R.G. Buttery of USDA provided reference spectra of nonatrienal and decatrienal. A. Pamidi and T.-Y. Chen provided technical assistance. This is Louisiana Agricultural Experiment Station manuscript No. 88-21-2090.

REFERENCES

1. Kinsella, J.E., *Food Technol. 40:*89 (1986).
2. Levy, R.I., *Am. J. Cardiol. 54:*1c (1984).
3. Stansby, M.E., *J. Am. Oil Chem. Soc. 48:*820 (1971).
4. Stansby, M.E., *Ibid. 50:*220A (1973).
5. Chipault, J.R., *Hormel Inst. Univ. Minn. Ann. Rpt.,* 1956, pp. 61-65.
6. Chipault, J.R., *Ibid.* 1959, pp. 7-8.
7. Chipault, J.R., *Ibid.* 1960, pp. 7-8.
8. Badings, H.T., *Neth. Milk Dairy J. 24:*177 (1970).
9. Crawford, L., and M.J. Kretsch, *J. Food Sci. 41:*1470 (1976).
10. Noble, A.C., and W.W. Nawar, *J. Am. Oil Chem. Soc. 48:*800 (1971).
11. Dupuy, H.P., E.T. Rayner and J.I. Wadsworth, *Ibid. 53:*628 (1976).
12. Dupuy, H.P., E.T. Rayner, J.I. Wadsworth and M.G. Legendre, *Ibid. 54:*445 (1977).
13. Warner, K., C.D. Evans, G.R. List, H.P. Dupuy, J.I. Wadsworth and G.E. Goheen, *Ibid. 55:*252 (1978).
14. Legendre, M.G., G.S. Fisher, W.H. Schuller, H.P. Dupuy and E.T. Rayner, *Ibid. 56:*552 (1979).
15. Dupuy, H.P., G.J. Flick, M.E. Bailey, A.J. St. Angelo, M.G. Legendre and G. Sumrell, *Ibid. 62:*1090 (1985).
16. Suzuki, J., and M.E. Bailey, *J. Agric. Food Chem. 33:*343 (1985).
17. Dupuy, H.P., G.J. Flick Jr., A.J. St. Angelo and G. Sumrell, *J. Am. Oil Chem. Soc. 63:*905 (1986).
18. Min, D.B., and J-G. Kim, in *Flavor Chemistry of Fats and Oils,* edited by D.B. Min and T.H. Smouse, American Oil Chemists' Society, Champaign, IL, 1985, p. 241.
19. Min, D.B., *J. Food Sci. 46:*1453 (1981).
20. Jackson, H.W., and D.J. Giacherio, *J. Am. Oil Chem. Soc. 54:*458 (1977).
21. Jackson, H.W., G.K. Mirmira and W.M. Wagner, *Ibid. 63:*117 (1986).
22. Waltking, A.E., and H. Zmachinski, *Ibid. 54:*454 (1977).
23. Waltking, A.E., and A.G. Goetz, *CRC Crit. Rev. Food Sci. Nutr. 19:*99 (1983).
24. Fore, S.P., L.A. Goldblatt and H.P. Dupuy, *J. Am. Peanut Res. Educ. Assoc. 4:*177 (1972).
25. Williams, J. L., and T.H. Applewhite, *J. Am. Oil Chem. Soc. 54:*461 (1977).
26. Selke, E., W.K. Rohweder and H.J. Dutton, *Ibid. 49:*636 (1972).
27. Mordret, F., O. Morin and J.L. Coustille, *Rev. Fr. Corp. Gras. 32:*193 (1985).
28. Jackson, H.W., *J. Am. Oil Chem. Soc. 58:*227 (1981).
29. Warner, K. and E.N. Frankel, *Ibid. 62:*100 (1985).
30. Selke, E., and E.N. Frankel, *Ibid. 64:*749 (1987).
31. Van den Dool, H., and P.D. Kratz, *J. Chromatogr. 11:*463 (1963).
32. Jennings, W., and T. Shibamoto, *Qualitative Analysis of Flavor and Fragrance Volatiles by Glass Capillary Gas Chromatography,* Academic Press, New York, 1980, p. 472.
33. Hites, R.A., and K. Biemann, *Anal. Chem. 42:*855 (1970).
34. Mathews, R.F., R.A. Scanlon and L.M. Libbey, *J. Am. Oil Chem. Soc. 48:*745 (1971).
35. Badings, H.T., *Neth. Milk Dairy J. 14:*236 (1970).
36. Badings, H.T., *J. Am. Oil Chem. Soc. 50:*334 (1973).
37. Meijboom, P.W., and J.B.A. Stroink, *Ibid. 49:*555 (1972).
38. Nakamura, K., I. Haruka and T. Tokunaga, *Bull. Japan. Soc. Sci. Fish. 46:*355 (1980).
39. Stern, D.J., R.A. Flath, T.R. Mon, R. Teranishi, R.E. Lundin and M.E. Benson, *J. Agric. Food Chem. 33:*180 (1985).
40. deMan, J.M., F. Tie and L. deMan, *J. Am. Oil Chem. Soc. 64:*993 (1987).
41. Nawar, W.W., in *Flavor Chemistry of Fats and Oils,* edited by D.B. Min and T.H. Smouse, American Oil Chemists' Society, Champaign, IL, 1985, p. 39.

[Received February 17, 1988; accepted August 3, 1988]