Evaluation of Compounds Contributing Characterizing Fishy Flavors in Fish Oils

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Vacuum steam-deodorized fish oils oxidized under flourescent light (950 lux) at 21°C initially developed green flavors which were caused principally by *t,c*-2,6-nonadienal, but some green-type flavor notes were contributed by *t*-2-hexenal and 1,*c*-5-octadien-3-one. Diminishment of green flavor notes resulted from the depletion of 2,6-nonadienal and the formation of *c*-4-heptenal (>1 ppm) which contributed oxidized, burnt flavor notes. Concomitant formation of *t,c,c*- and *t,t,c*-2,4,7-decatrienal (>1 ppm) yielded characterizing burnt/fishy or cod liver oil-like flavors in fish oils. Two unidentified compounds exhibiting minnow-bait-bucket and trout-like odor qualities, respectively, were encountered in oxidizing fish oils. Hexanal, 2,4-heptadienals and 2,4-decadienals contributed general oxidized, painty flavors to fish oils.

Recent nutritional claims about the potential health benefits from long-chain n-3 polyunsaturated fatty acids and seafoods (1-5) have stimulated interest in providing bland-flavored fish oils and n-3 fatty acid derivatives for a variety of uses (6-8). However, marine oils containing n-3 fatty acids (3,9) are noted for exhibiting distinct, unpleasant fishy odors and flavors (8,10,11). Fish and seafood often also exhibit objectional fishy flavors that deter many consumers (10,12), and fishy flavors may also extend into other foods when n-3 fatty acids are present. For example, excessive levels of fish oil or meal in diets of swine (13) and poultry (14,15) cause fishy flavors in meats and eggs, respectively.

Menhaden oil which is the most abundant domestic fish oil (8,16) currently is produced only with conventional refining (16-18) and is unsuitable for human consumption without further deodorization processing. Various deodorization processes have been investigated for menhaden oil (19,20) and other fish oils (21-24), but in spite of claims for improved flavors, fishy off-flavors are still viewed as a major problem in the utilization of fish oils.

Early studies on fishy flavors led to a recognition that trimethylamine as well as compounds from oxidizing polyunsaturated fatty acids were involved in the production of distinct fishy flavors in marine fish and seafoods (25-30). Trimethylamine and dimethylamine remain viewed as important aroma compounds in many marine fish and shellfish where they contribute to certain fishy, boiled-crab or fishouse-like aromas (29,31). Stansby (32) found that a trimethylamine-like fishy odor was apparent only when high concentrations of trimethylamine were added to oxidizing fish oils.

Numerous carbonyl compounds have been identified in oxidizing fish lipids (33-40). Certain fishy off-flavors in cold-store cod (41,42), cold-store butter (43) and rancid soybeans (44) have been reported to be caused by *c*-4-heptenal. However, Swoboda and Peers (45) concluded that *c*-4-heptenal was not involved in the flavor of a sample of fishy butterfat. Forss and coworkers (46,47) were unable to identify compounds with fishy aromas in a batch of fishy-flavored butterfat although they noted that 1-octen-3-one contributed some metallic notes also observed in the tainted butter. Subsequently, Swoboda and Peers (45,48,49) concluded that *t,c,c*-2,4,7-decatrienal contributed strongly to fishy flavors in tainted butter, but the contribution was modified by other characterizing odor compounds. They particularly associated 1,*c*-5-octadien-3-one with metallic/fishy flavored extracts, but indicated that when evaluated alone in butter, its flavor was predominantly metallic in nature.

In extensive studies of fresh fish aromas, Josephson *et al.* (50,51) more recently have found 1,*c*-5-octadien-3-one among other green aroma compounds in enzymatically-formed fresh-fish volatiles. When observed as an eluting GC peak, this potent aroma compound exhibited a heavy, green, geranium-leaf-like aroma quality which was believed to contribute characterizing notes to fresh fish aromas. Josephson *et al.* (51-54) also found that the aroma quality of fresh fish varied among species depending on the occurrence and concentration of contributing compounds. In addition to the C_6 and C_8 carbonyls, freshwater fish were found to typically produce C_9 compounds, such as 2,6-nonadienal, 3,6-nonadienal and 3,6-nonadienol, which impart fresh melon-like odors to these fish (51,53).

Meijboom and Stroink (55) first proposed that two 2,4,7-decatrienal isomers (*t,c,c*- and *t,t,c*-) were the most important contributors to the objectionable fishy flavors in oxidized fish oils and other oils containing linolenic acid or long-chain n-3 fatty acids. They described these fishy flavors as whale oil-like or trainy, and noted that the isomers exhibited slightly different fishy flavor qualities. Ke *et al.* (56), however, reported that they were unable to detect the decatrienal isomers with methods available at that time in mild to moderately oxidized mackerel oils exhibiting distinct trainy or whale oil-like flavors. As a result, they concluded that the flavors were caused by a complex mixture of carbonyl compounds rather than the decatrienals.

A progression of predictable characterizing aromas of oxidizing laboratory-deodorized menhaden oil has been described by Stansby and Jellinek (57), but these workers were unable to associate specific aroma compounds with the flavors observed. Recently, capillary GC methods for the analysis of volatile compounds have emerged (50, 58,59), and the purpose of this research was to apply some of these techniques in the investigation of the development of fishy flavors in oxidizing menhaden, cod liver and siscowet lake trout oils.

MATERIALS AND METHODS

Preparation of steam-deodorized fish oils. Refined, but not steam-deodorized, menhaden oil (Zapata Hayne, Corp., Reedville, VA) and pharmaceutical grade cod liver oil (McKesson Corp., Dublin, CA) were obtained commercially. Siscowet lake trout *(Salvilinus namaycush siscowets)* oil was prepared by heat-rendering (100°C for 10 min) freshly-caught commercial fish (4-6 kg, Bodin

Fisheries, Inc., Bayfield, WI) that had been skinned, deboned and minced. Heat-rendered oil was dried over excess anhydrous sodium sulfate (Amend Drug and Chemical Co., Irvington, NJ) and stored under vacuum (4 mm Hg) at 4°C until used.

Fish oils were vacuum steam-deodorized in a batch-type laboratory apparatus (60) which was used to process 250 ml batches of oil at 100°C ± 5°C and 4 mm Hg for 2 hr. Dilauryl thiodipropionate (200 ppm; DLTDP; Evans Chemetics, W.R. Grace, Lexington, MA) was added as a peracid inactivator prior to deodorization (61). Steam for the deodorization of the oil was generated from a solution of 0.01N acetic acid (J.T. Baker Chemical Co., Phillipsburg, NJ) (62).

Different concentrations (0, 100 or 1000) of butylated hydroxyanisole and tertiary butyl hydroquinone (BHA and TBHQ, respectively; Eastman Chemical Products, Kingsport, TN) were added to deodorized oils immediately upon cooling, using techniques that minimized air exposure. Twenty-ml samples of deodorized oils were placed into 60-ml clear glass bottles (85 mm × 30 mm) that were then placed uncapped in an aluminum tray at 21°C under fluorescent light (950 lux).

Flavor and odor assessments. Flavor and odor assessments of fish oils were made by the authors. For sampling, the large end of a disposable Pasteur pipette was dipped into an oil, and then a drop (ca. 0.25 ml) of oil was placed on the tip of the tongue thus minimizing flavor carryover through coating the lips with oil (57).

t,c-2,6-Nonadienal, *c*-4-heptenal (Bedokian Chemicals, Inc., Danbury, CT), *t,t*-2,4-heptadienal, hexanal, trimethyl amine, butyric, pentanoic, hexanoic, heptanoic and octanoic acids (Aldrich Chemical Co., Milwaukee, WI) were evaluated in a bland flavored vegetable oil (Wesson Oil, Beatrice, Fullerton, CA).

Additionally, various dilutions of condensates obtained from the first cold trap of the steam deodorizer and GC-collected 2,4,7-decatrienals were evaluated for fishy flavors in oil. Condensates and GC-collected 2,4,7-decatrienals were added to bland commercial canola oil (Puritan; Proctor and Gamble, Cincinnati, OH), as well as to mayonnaise (Kraft Corp., Glenview, IL), and assessed for odor and flavor quality. Authentic *t,c*-2,4-dodecadienal, *t,t*-2,4-dodecadienal, *t,c*-2,6-dodecadienal (Bedokian, CT) and a headspace extract of oxidized arachidonic acid (Sigma Chemical Co., St. Louis, MO) were assessed for odors as compounds eluted from Carbowax 20M packed GC columns.

Analysis of headspace volatiles in oils. Volatile compounds in fish oils were quantitatively measured using the dynamic headspace procedure described by Olafsdottir *et al.* (63) with modifications. Aliquots of oil (15 ml) were added to cylindrical glass 30-ml reservoirs (3 cm × 10 cm) constructed with 24/40 ST glass joints (female). Ethyl heptanoate (7.5 μl of a 4,160 ppm hexane solution) was added to the oil as an internal standard, and each reservoir was assembled with a purging head described in the initial report of the procedure. Headspace volatiles were purged from the oil by introducing a stream of nitrogen (270 ml/min for 3 hr at 75 ± 5°C) below the surface of the sample, and then were entrained onto Tenax GC® (60–80 mesh, ENKA N.V., Holland). After collection, volatile compounds were eluted from Tenax GC® traps with ca. 0.5 ml of redistilled diethyl ether (Fisher Scientific, Fairlawn, NJ), and extracts were then concentrated under a slow stream of nitrogen to about 30 μl at room temperature (21°C).

A Varian 3700 gas chromatograph (Varian Associates, Inc., Sunnyvale, CA) equipped with an on-column injector system, FID detector and a Carbowax 20M (60 m × 0.25 mm) fused silica capillary column (J&W Scientific, Inc., Rancho Cordova, CA) with helium as the carrier gas was employed. A program rate of 50°C (1 min hold) to 220°C at 4°C/min was used.

Mass spectra were obtained using a Finnigan 4500 mass spectrometer fitted with the same Carbowax 20M capillary column, and using a temperature program rate of 50°C (1 min hold) to 220°C at 4°C/min. Identification of peaks in chromatograms was achieved by matching electron impact (EI; 70/ev) mass spectral data to those published in "EPA/NIH Mass Spectral Data Base" (64,65) or those of authentic compounds. Coincidence of retention indices of unknown compounds (I_E; 66) with authentic compounds was also employed for identification of compounds.

For odor assessments, samples were separated with a glass column (3 m × 2 mm i.d.) packed with Carbowax 20M on Chromosorb W AW/DMCS which was operated at a temperature program rate of 50°C to 22°C at 4°C/min. The effluent from the GC column was split 90:1 in favor of the exit port (51).

Total fatty acid analysis of fish oils. Methyl esters prepared according to the procedure of Metcalf and Schmitz (67) using BF_3-methanol (Applied Science, State College, PA) were used to determine total fatty acids. Methyl esters were separated with a bonded polyethylene glycol fused silica capillary column (Supelcowax 10, 60 m × 0.32 mm; 0.25 μm coating; Supelco, Inc., Bellefonte, PA) in a Varian 3700 gas chromatograph. The detector was at 250°C, and the column oven was temperature-programmed at 50°C hold for 1 min followed by 50°C to 180°C at 30°C/min and finally 2°C/min from 180°C to 250°C. Methyl esters of fatty acids were identified by published retention times and positions of elution for methyl esters from a Supelcowax 10 column (68).

RESULTS AND DISCUSSION

The chromatogram in Figure 1 obtained from non-deodorized, commercial cod liver oil illustrates the volatile headspace profile obtained for fish oils with a Carbowax 20M fused silica capillary column. This sample of oil exhibited a very distinct fishy, cod liver oil-like character with heavy green, background notes. Identifications of compounds and their concentrations are reported in Table 1, and qualitatively similar patterns were obtained from menhaden and lake trout oils.

Initial green flavors and aromas in deodorized fish oils. The initial aroma observed by Stansby and Jellinek (57) in slightly oxidized menhaden oil was green and cucumber-like, and this flavor was followed by the development of fishy and rancid flavors. This progression of flavors was observed throughout the present study, and the earlier observations (57) are considered in agreement those presented here even though alternate terminology has been chosen to describe the rancid-type flavors that they observed in later stages of flavor development in oxidized fish oils.

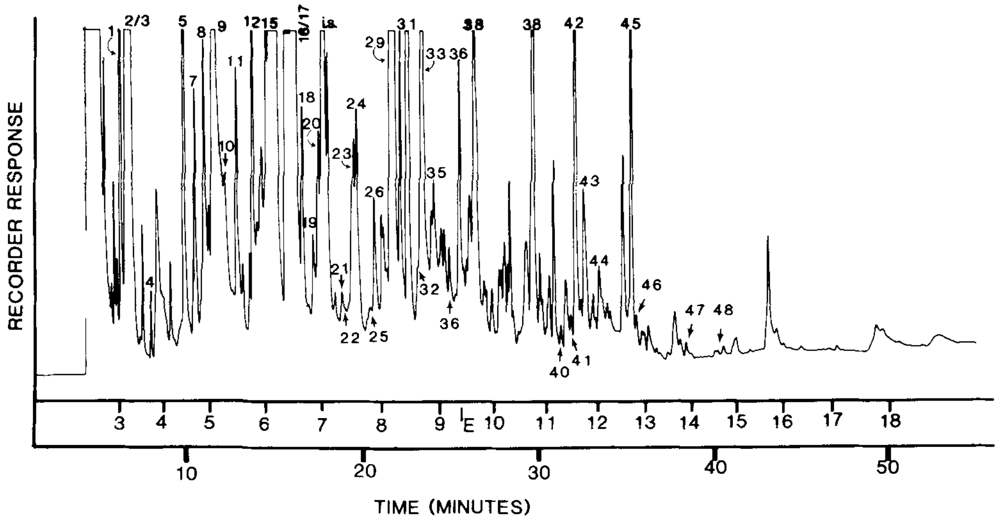

FIG. 1. Gas chromatogram of commercial cod liver oil headspace volatiles separated on a 60 m × .31 mm Carbowax 20M fused silica capillary column.

TABLE 1

Volatile Compounds Identified in the Headspace Analysis of a Commercial Cod Liver Oil

Peak no.	Compound	I_E Carbowax 20M	Concentration (ppb)	Peak no.	Compound	I_E Carbowax 20M	Concentration (ppb)
1	pentanal	3.31	1070	25	2,4,hexadienal	7.60	60
2	decane	3.46	5230	26	2-octenal	7.91	320
3	1-penten-3-one	3.67	2830	27	acetic acid	8.09	190
4	2-butenal	4.05	53	28	1-octen-3-ol	8.10	150
5	hexanal	4.49	1010	29	(E,Z)-2,4-heptadienal	8.24	12400
6	(Z)-2-pentenal	4.68	70	30	1,5-octadien-3-ol	8.48	370
7	1,3,6-octatriene	4.69	400	31	(E,E)2,4-heptadienal	8.52	2200
8	(E)-2-pentenal	4.90	660	32	decanal	8.60	70
9	cyclic compound	5.05	1700	33	(E,Z)-3,5-octadien-2-one	8.80	1700
10	1-penten-3-ol	5.27	310	34	benzaldehyde	8.89	195
11	heptanal	5.46	420	35	2-nonenal	8.95	380
12	(E)-2-hexenal	5.81	720	36	(E,E)-3,5-octadien-2-one	9.30	200
13	2-pentyl furan	5.88	210	37	(E,Z)-2,6-nonadienal	9.44	570
15	(Z)-4-heptenal	6.03	2000	38	butyric acid	9.83	1200
16	pentanol	6.20	4100	39	pentanoic acid	10.69	1400
17	octanal	6.52	2750	40	(E,Z)-2,4-decadienal	11.18	30
18	cyclopentanol	6.60	390	41	(E,E)-2,4-decadienal	11.62	50
19	2-penten-1-ol	6.66	150	42	(E,Z,Z)-2,4-7-decatrienal	11.72	930
20	(E)-2-heptenal	6.85	400	43	hexanoic acid	11.91	430
i.s.	ethyl heptanoate	7.00	i.s.	44	(E,E,Z)-2,4,7-decatrienal	12.23	170
21	c-3-hexen-1-ol	7.35	80	45	cyclic oxoacid	12.63	810
22	1,5-octadien-3-one	7.36	5	46	heptanoic acid	12.80	50
23	2-nonanone	7.51	530	47	octanoic acid	14.05	24
24	nonanal	7.55	520	48	meringue/minnow compd.	14.80	trace

Based on odor quality, the compounds identified in newly deodorized, as well as oxidized, fish oils which might be expected to contribute to overall green flavor quality were *c*-3-hexen-1-ol, *t*-2-hexenal, and *t,c*-2,6-nonadienal. In moderately oxidized samples, *t,t,c*-2,4,7-decatrienal may also contribute green flavor notes (55). Quantitative data for the formation of selected volatiles in low-temperature deodorized cod liver oil held through 28 d (21°C; 950 lux) are summarized in Figure 2. The concentrations of *t,c*-2,6-nonadienal found in deodorized fish oils exhibitng green flavors indicated that this compound provides the major character impact contribution to the green, melony flavors encountered in fish oils. The flavor threshold for *t,c*-2,6-nonadienal has been reported at 0.01 ppb (69), and distinct green cucumbery notes are produced at 10 ppb (51). The similarity of the green flavor in fish oils to that of *t,c*-2,6-nonadienal was verified by addition of 0.5–1.0 ppm of this compound to a bland vegetable oil.

Stansby and Jellinek (57) believed that the green flavors in freshly deodorized oils were caused by *c*-3-hexen-1-ol and *t*-2-hexenal, although they could not analytically

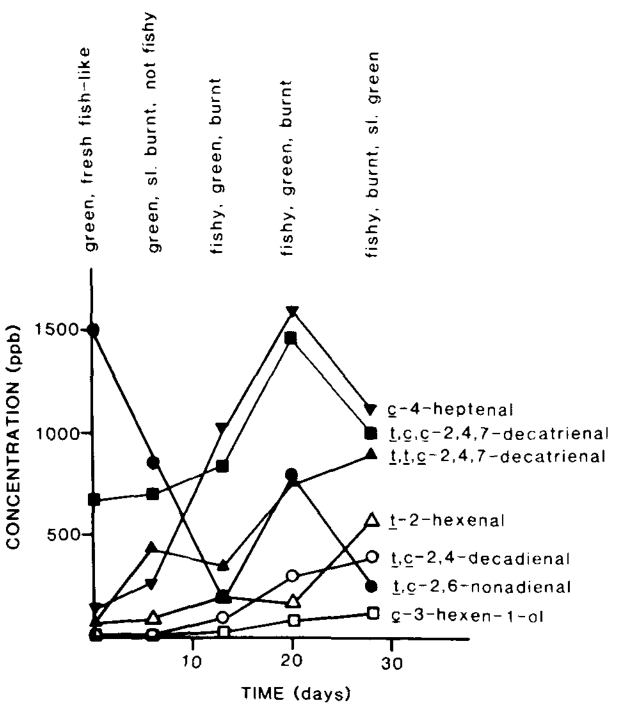

FIG. 2. Concentrations of compounds associated with fishy flavor development in oxidizing low-temperature (100°C for 2 hr) acid deodorized cod liver oil containing 200 ppm dilauryl thiodipropionate.

substantiate their presence. For the sample of cod liver oil indicated in Figure 2, the concentrations of *c*-3-hexen-1-ol were very low until after distinct fishy flavors developed. Even at this time (>20 d), the concentrations did not exceed 80 ppb. Commercially-refined menhaden oils in the current study did not contain detectable amounts of *c*-3-hexen-1-ol which is in agreement with the findings of Hsieh (58). Considering that the threshold of *c*-3-hexen-1-ol has been reported at 70 ppb in water (70), it is unlikely that this unsaturated alcohol is a character impact flavor compound in the earlier stages of oxidizing fish oils. Even in the latter stages of flavor development in oxidizing fish oils, it probably serves only a supporting role to the green flavors contributed by *t,c*-2,6-nonadienal.

Hoffmann (71) reported that the green flavor associated with oxidizing soybean oil was caused by c-3-hexenal, but this compound was not found in any of the oxidizing fish oils. If it was formed, it apparently was readily converted to *t*-2-hexenal. The concentration of t-2-hexenal in the deodorized cod liver oil (Fig. 2) initially was about 20 ppb, and increased to about 700 ppb after oxidizing for 28 days. Buttery (70) found that the threshold for *t*-2-hexenal was 110 ppb in paraffin oil, and the levels of this green aldehyde exceeded the threshold concentration after about 15 days. Concentrations of *t*-2-hexenal found in fish oils and flavors produced upon addition to bland vegetable oils indicated that it contributed only to the coarse, green notes observed later in oxidation.

The concentration of hexanal observed in oxidizing fish oils often exceeded 1 ppm, but additions of hexanal up to 10 ppm in vegetable oil did not provide characterizing green flavor notes found in fish oils. Hexanal has been reported to exhibit a flavor detection threshold of 150 ppb in paraffin oil (72), but in vegetable oil it exhibited heavy, slightly green aldehyde-like aromas only when evaluated at very high concentrations (>25 ppm). Thus, although hexanal might contribute green notes through subthreshold flavor interactions (73–75), it appears to contribute general, painty aromas instead of significant green-fishy notes.

The vinyl ketone, 1,5-*c*-octadien-3-one, contributes to fresh fish flavors (50,51), and it exhibits an intense heavy, green, geranium leaf-like aroma. The flavor threshold for 1,5-*c*-octadien-3-one has been reported at 0.001 ppb (69) and it has been found to cause metallic-like flavors in oxidized butter (45,48,49). While a defined characterizing role for 1,5-*c*-octadien-3-one in the fishiness flavors of fish oils cannot be made as yet, the concentrations observed in oxidizing oils (Table 1; to 5 ppb) suggest that it could be influential in contributing green-fishy aromas and flavors in oxidizing fish oils.

Oxoacids (aldo-acids) derived from the carboxyl-end of the long-chain polyunsaturated fatty acid molecules have not been found in oxidized fish oils although esterified oxoacids have been found in model systems of oxidizing methyl linoleate (76,77) and methyl docosahexaenoate (37). GC-MS analysis of volatiles from fish oil gave data that indicated peak 44 (Fig. 1) could be tentatively identified as 2-hydroxy-3-pentenalactone, the cyclized form of the five-carbon, unsaturated oxoacid (78), which could be derived from docosahexaenoic acid (DHA) (Fig. 3). The electron impact mass spectral fragment pattern for this compound was 57 (100); 85 (77); 58 (30); 39 (23); 41 (22); 56 (14); 114 (5); and the molecular weight was 114.

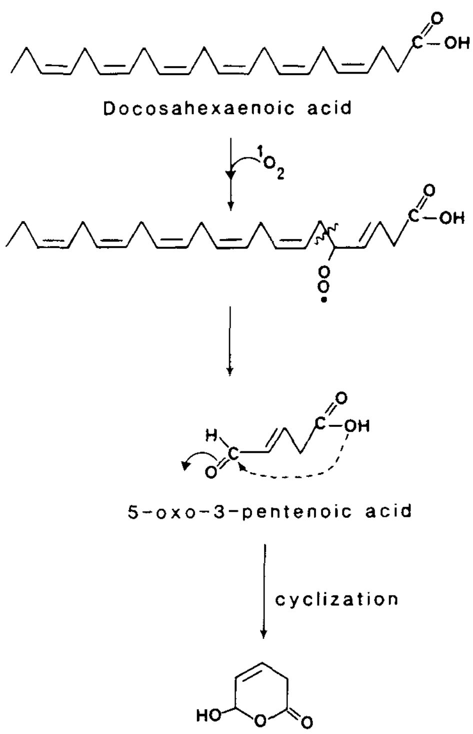

FIG. 3. Proposed singlet oxygen-mediated oxidation of docosahexaenoic acid to form 5-oxo-3-pentenoic acid and 2-hydroxy-3-pentenalactone.

In order for this 5-carbon oxoacid to be formed from the carboxyl-end of DHA, hydroperoxidation at the 5-carbon position must occur. Since this is an unfavored site for free radical hydroperoxidation (79–81), the attack would likely involve singlet oxygen. When high levels of BHA (1000 ppm) and TBHQ (1000 ppm) were incorporated into fish oil samples, the formation of this compound was substantially suppressed relative to other oxidation products. Although these phenolic antioxidants are primarily known as free radical terminators, they also have been reported to function as singlet oxygen scavengers (82) and thus, could have suppressed singlet oxygen-type oxidations that were needed to initiate the formation of the cyclic oxoacid.

The odor quality of the GC peak region for this compound exhibited vegetable- or cooked celery-like character, and it could contribute to the green notes of oxidized fish oils. Boldingh and Taylor (83) have reported that 2,3-dimethyl-4-keto-nonene-2-acid (bovolide), which is an enol-lactone that can be considered structurally-related to the cyclic oxoacid, exhibited an odor reminiscent of celery.

Fishy/burnt flavors and aromas in deodorized fish oils. Fishy, burnt, whale oil-like, cod liver oil-like flavors which develop in oxidized fish oils have been reported by Meijboom and Stroink (55) to be caused principally by the *t,c,c*- and the *t,t,c*-isomers of 2,4,7-decatrienals. In the present study, when distillates containing high concentrations of decatrienals or decatrienals obtained from collected GC peaks were added to bland canola oil (>400 ppb), distinct burnt/fishy aromas and flavors were observed. Therefore, because of a lack of familarity with whale oil-like or trainy flavor descriptors used by earlier investigators (55,84), the burnt/fishy terminology was adopted for this study. As the concentration of added decatrienals to oil was increased from about 4 ppb to 400 ppb, the flavor quality progressed from a mild green plant-like quality to a very distinct burnt/fishy quality. This progression was similar to that observed for oxidizing deodorized fish oils after the initial bland, melony and green stage.

In the oxidizing cod liver oil sample described earlier (Fig. 2), distinct burnt/fishy flavors were apparent after 14 days when a dramatic increase in *t,c,c*-2,4,7-decatrienal to 1.2 ppm was observed. An increase in the concentrations of both decatrienals combined with a reduction in the level of *t,c*-2,6-nonadienal (to 100 ppb) allowed fishy/burnt odor and flavor qualities to dominate over the melony, green notes. Similar observations were made for other fish oils, and therefore the importance of the 2,4,7-decatrienals in fishy off-flavors of oxidizing fish oils was confirmed.

Burnt/fishy flavors produced by high concentrations of decatrienals were suppressed when fish oils were incorporated into mayonnaise. The acidic medium (pH = 3.4) provided by acetic acid probably facilitated the hydration of the 2–3 double bond of some of the 2,4,7-decatrienals (85,86). Such a reaction would result in a loss of significant volatility as well as cause an alteration of the molecular features of the parent molecules.

Considerable attention has been given to *c*-4-heptenal in relation to fishy flavors (41–44,85). In the current study, when added to vegetable oils at levels of 0.2–0.5 ppm, *c*-4-heptenal gave stale, burnt flavors. Although *c*-4-heptenal has been referred to as the cold-store cod compound, it did not elicit a ready recognition of fishiness when evaluated alone by individuals in the laboratory which was in agreement with earlier observations (86). Instead, it contributed burnt aroma characteristics when high levels (1.0 ppm) were found in fish oil (Fig. 2). The threshold level of *c*-4-heptenal is 0.040 ppb (41), and it appears to complement the fishy/burnt flavor of the decatrienals when present at high concentrations. As fish oils oxidize, the concentrations of green and burnt/fishy compounds vary inversely (Fig. 2) because the retro-aldol conversion of 2,6-nonadienal to *c*-4-heptenal (85) diminishes the green fresh-fish quality while enhancing burnt notes.

Other fishy compounds in fish oil. During assessments of aromas of compounds eluting from GC separations of volatiles from fish oil condensates, a potent compound which was immediately suggestive of fishiness that could be described as fish-bowl-like or minnow-bait-bucket-like was observed at $I_E = 14.8$ (Fig. 1). Further assessments of the aroma of the compound led to the recognition that it also could be described as the very recognizable odor typical of a baked meringue prepared from sugar and egg whites.

The amount of the compound found in samples was very low, and like 1,5-octadien-3-one encountered in the analyses of fresh fish and fish oil, it seldom gave a discernible GC peak and was detectable mainly by aroma. Fresh oil extracted from siscowet lake trout contained the highest amount of this meringue/minnow-bait-bucket-like compound among the fish oils examined, and incidently this compound was also found in a sample of oxidizing authentic arachidonic acid. Since lake trout contains much higher amounts of arachidonic acid (C 20:4n-6) than menhaden or cod liver oils (Table 2), it suggests that arachidonic acid could possibly serve as the precursor of this potent aroma compound.

Repeated collections of the peak area in GC separations for this compound for mass spectral analysis provided a sample which gave an electron impact (EI) fragment pattern which suggested that the compound could possibly be either *t,t*-4,6-dodecadienal, 2-(oct-2-enyl)-3-oxolene or *t,c*-2,6-dodecadien-4-one. *t,t*-4,6-Dodecadienal is similar in structure to the long-chain unsaturated aldehydes described by Harkes and Begemann (87) which contribute to raw chicken flavors. However, although authentic *t,c*-2,6-dodecadienal ($I_E = 12.1$; Carbowax 20M), *t,c*-2,4-dodecadienal ($I_E = 12.9$; Carbowax 20M) and *t,t*-2,4-

TABLE 2

Selected Fatty Acid Profiles for Siscowet Lake Trout, Menhaden and Cod Liver Oils

	Sample description		
Fatty acid[a]	Siscowet lake trout oil	Menhaden oil	Cod liver oil
	Concentration (% total fatty acids)		
C 18:3n3	1.94	0.08	0.86
C 20:5n3	3.26	14.13	8.16
C 20:4n-6	2.04	0.64	0.36
C 22:6n3[b]	3.93	3.52	7.41

[a]Methyl esters analyzed with a 60-m Supelcowax-10 capillary column.

[b]Concentration may contain a small amount of C 24:0.

dodecadienal (I_E = 13.5; Carbowax 20M) exhibited aromas that could be associated with raw or partially-cooked chicken, and none had the odor or the retention index of the meringue/minnow-bait-bucket-like compound. The proposed cyclized oxolene compound seems to possess molecular features compatible with the site of elution of the unknown compound on a Carbowax 20M column (I_E = 14.8). Although various cyclized compounds readily form in oxidizing polyunsaturated fatty acids (90,91), it is doubtful that this compound accounts for the minnow-bait-bucket-like aroma.

Very recently, Kobayashi *et al.* (88,89) have identified *t,c,c-* and *c,c,c*-5,8,11-tetradecatrien-2-one in cooked shrimp, and have reported that these two compounds possess shrimp-like, shellfish-like, and sea cucumber-like aromas. The Carbowax 20M retention indices reported that these two ketone isomers (88) coincide with that of the minnow-bait-bucket-like compound (I_E = 14.8). Even though different descriptors have been used by each group of workers to describe the aroma, it seems reasonable that the same compounds are involved in shrimp and fish oil flavors. It also appears reasonable that the mass spectra obtained in the current study was incomplete for higher mass numbers, and led to the speculation noted earlier that the compound could possibly be *t,c*-2-6-dodecadien-4-one. However, the retention index for that 12-carbon ketone would be expected to be similar to those of the 12-carbon aldehydes, and therefore would be much too early an elution point to account for the compound. Thus, at this time it appears that the minnow-bait-bucket-like compound can be tentatively identified as a 5,8,11-tetradecatrien-2-one isomer.

A compound with a very low aroma threshold and a distinct fish-like aroma that is strongly reminiscent of trout (*Salmo sp.; Salvilinus sp.*) elutes at a retention index of 10.45 on a Carbowax 20M column. This compound occurs in fresh fish volatiles, especially *Salmo sp.*, and it was observed in siscowet trout oil. Attempts to identify this compound also have been unsuccessful because of its very low concentration, but it is believed also to have a threshold similar to that of 1,*c*-5-octadien-3-one (0.001 ppb; 69). The trout-like compound, however, is believed to be important in providing another distinct fishy flavor and aroma quality to some fish oils and fish products.

Compounds apparently not contributing to fishy flavors in oxidized fish oils. Traditionally, trimethylamine has been associated with fishy flavors because of its distinct crab-like, fishy or fishhouse odor quality (29,31). Trimethylamine and dimethylamine are significant in marine fish which have high concentrations of trimethylamine oxide (26,28,30,92), but they are generally absent from freshwater fish.

When sufficient quantities of trimethylamine are combined with oxidized fish oils, it has been reported to produce a distinct stale/fishy quality (32). The odor threshold of trimethylamine has been reported at 0.367 ppb (93). In the present study, when 0.5 to 1 ppm of trimethylamine was added to fresh steam-deodorized fish oils, an unusual fish oil aroma was noted that was reminiscent of fishy odors observed in canned sardines. When 0.5 ppb was added to vegetable oil, a distinct fishhouse-like odor was observed, but a fishy taste was not detected. At higher concentrations, the aroma of trimethylamine in vegetable oil became ammoniacal. Because refined fish oils contain very limited amounts of the short-chain alkyl amines and do not exhibit a fishhouse aroma, they cannot be considered as contributors to the characterizing fishy flavors and aromas of these oils. Nevertheless, trimethylamine contributes fishiness to seafood flavors and aromas when it is present, and its combined effects with certain lipid oxidation products is very important to characterizing certain fishy flavors that most consumers find objectionable in marine fish and shellfish.

t,c-2,4-Decadienal and *t,t*-2,4-decadienal have detection threshold concentrations of 20 ppb and 320 ppb in paraffin oil, respectively (72), and these concentrations indicate that the compounds could be influential in fish oil flavors. However, the decadienals contribute fatty, fried, aldehyde-like aromas at higher concentrations (>10 ppm). Furthermore, at low concentrations (ca. 1 ppm) they have been reported to suppress the aromas of green compounds (72) through subthreshold interactions (73). Therefore, even though the concentration of *t,c*-2,4-decadienal increased to 250 ppb (Fig. 2) and *t,t*-2,4-decadienal to 48 ppb after 20 days of oxidation, these compounds were observed to contribute only general oxidized flavor notes that developed in fish oils.

Oxidizing n-3 fish oils also yield high concentrations of *t,c*-2,4-heptadienal and *t,t*-2,4-heptadienal (94–96). Although the aromas of these aldehydes are distinct and pronounced at high concentrations (>1 ppm), additions of these compounds to vegetable oils and deodorized fish oils did not yield fishy flavor notes. Instead, distinct grainy, straw-like flavors were noted, and thus these aldehydes were observed to contribute only to the general, painty, oxidized flavor notes of fish oils.

Saturated short chain fatty acids (C_4–C_8) appear regularly in oxidizing fish oils (58) and arise from the peracid oxidation of aldehydes (61). Flavor thresholds for short n-chain fatty acids in oil systems (>.66 ppm; 75) indicate that they could contribute characterizing notes to oxidizing fish oils. However, when up to 20 ppm of several fatty acids (i.e., butyric, pentanoic, hexanoic, heptanoic and octanoic acids; Table 1) were added to vegetable or deodorized fish oils, only butyric acid gave a mild cheesey, buttery aroma. Therefore, it was concluded that n-chain free acids found in oxidizing fish oils were of insufficient concentration to express their flavor notes or contribute to the burnt notes found in oxidized fish oils.

In summary, *t,c*-2,6-nonadienal has been found to be primarily responsible for the green, fresh fish-like flavor observed soon after deodorization of fish oils, but *t*-2-hexenal, 1,5-octadien-3-one and low concentrations of *t,t,c*-2,4,7-decatrienal may add heavier green, plant-like notes. Compounds causing the distinct burnt/fishy flavors were verified as 2,4,7-*t,c,c*- and 2,4,7-*t,t,c*-decatrienal with *c*-4-heptenal acting as a modifier of overall burnt/fishy flavors. In addition to these carbonyl compounds, two unidentified compounds were observed to exhibit characterizing fishy aromas reminiscent of trout and minnow-bait-bucket odors, respectively. General oxidized, rancid and painty flavors in fish oils were caused by saturated and unsaturated aldehydes, including hexanal, *t,c*- and *t,t*-2,4-heptadienals and *t,t*- and *t,c*-2,4-decadienal. Future research should address means to selectively minimize the formation of influential fishy flavor compounds in fish oils.

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