Chemical and Olfactometric Characterization of Volatile Flavor Compounds in a Fish Oil Enriched Milk Emulsion

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Development of objectionable fishy off-flavors is an obstacle in the development of fish oil enriched foods. Only little is known about the sensory impact of specific volatile fish oil oxidation products in food emulsions. This study examined the volatiles profiles of fish oil enriched milk during cold storage (2 °C) for 14 days by dynamic headspace sampling followed by gas chromatography-mass spectrometry analyses. Different volatiles ($n = 60$) comprising alkenals, alkadienals, alkatrienals, and vinyl ketones were identified in the fish oil enriched milk. The potent odorants identified by gas chromatography-olfactometry were 1-penten-3-one, (*Z*)-4-heptenal, 1-octen-3-one, (*Z*)-1,5-octadien-3-one, (*E,E*)-2,4-heptadienal, and (*E,Z*)-2,6-nonadienal, but despite their potency, none of the separated volatiles imparted a fishy or metallic odor. Two isomers, (*E,Z,Z*) and (*E,E,Z*) of 2,4,7-decatrienal were identified in fish oil enriched milk emulsions with peroxide values 0.8 and 3.4 meq/ kg, respectively. To our knowledge, this is the first report on appearance of these decatrienals in food emulsions having a relatively low peroxide value.

KEYWORDS: Volatile compounds; fish oil; milk emulsion; lipid oxidation; sensory analysis; olfactometry; 2,4,7-decatrienal

INTRODUCTION

A great deal of attention has been focused on the various health benefits apparently associated with consumption of fish oil (*1*). The health benefits appear to be related to the presence of high levels of the n-3 family of polyunsaturated fatty acids (*2*). Several attempts have been made to develop functional foods by incorporating fish oil (*3–5*). However, development of fishy and metallic off-flavors was encountered in various oil-bearing food emulsions due to the formation of volatiles resulting from the oxidation of fish oil (*6*). It is known that the relative oxidative stability of polyunsaturated lipids differs according to the composition and its form, such as bulk oil, water-in-oil emulsion or oil-in-water emulsion (*7*). The available literature also indicates that the flavor deterioration due to volatile components in emulsions is much more rapid than that in bulk oils (*8*).

Several volatile compounds have been identified in different fish species (*9–11*), bulk fish oils (*12–14*), and in foods tainted with fishy off-flavors such as butterfat (*15*) and soybeans (*16*). However, information about the volatile profiles and their exact sensory impact in fish oil based emulsions and real foods is

very limited. It is only in mayonnaise, among fish oil enriched food systems, that the profile of volatiles has been established (*17*).

Vacuum distillation followed by solvent extraction, static and dynamic headspace sampling (DHS), and solid phase microextraction (SPME) are the most widely applied techniques to isolate volatile flavor compounds. Though the DHS technique yields an extract with fewer components in comparison with the distillation technique, it has been shown to be a reliable method for the determination of the composition of volatile compounds (*18*). This method has also been used for investigations of the composition of volatile oxidation products in fish oil to identify the compounds responsible for off-flavors (*12–14*).

Gas chromatography−olfactometry (GC-O) has been extensively used in aroma research. Owing to the large differences between detection thresholds of volatile compounds, all compounds identified by instrumental techniques do not contribute equally to the overall aroma of the product. GC-O enables to determine the individual contribution of volatile compounds present in samples. In the literature, several techniques such as aroma extract dilution analysis, CharmAnalysis, etc. have been used to evaluate the data obtained by GC-O (*19–20*), one of which is based on detection frequency (*21*). In this method, only one dilution level is used, and olfactometry is repeated by several panelists under the same conditions. Responses of individuals

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are then combined to get an aromagram with peak heights corresponding to their detection frequencies (*22*).

In continuation of our research focus on "oxidation mechanisms in real foods enriched with fish oil" (*23*−*27*), we recently reported oxidative flavor deterioration of fish oil enriched milk (*28*). The study showed that incorporation of fish oil with a very low initial peroxide value of 0.1 meq/kg did not result in fishy off-flavor development upon storage, while the development of distinct fishy off-flavor was recorded when cod liver oil with initial peroxide value of 1.5 meq/kg was incorporated into milk. Considering the fact that the flavor perception depends on volatile components and also on the composition of the food matrix, the aim of the present study was to carry out a chemical and sensory characterization of the volatiles in fish oil enriched milk to understand the chemical nature of oxidized products to optimize the requirements for production of fish oil enriched milk with acceptable sensory attributes. In this paper, we report on the isolation and identification of the volatile compounds in fish oil enriched milk by DHS-gas chromatography-mass spectrometry (GC-MS) with special emphasis on the most potent odorants discerned by GC-O following detection frequency method.

MATERIALS AND METHODS

Milk and Preparation of Milk Emulsions. Pasteurized milk with fat contents of 0.5 and 1.5 wt % was purchased locally and subsequently mixed in a ratio of 1:1 to obtain milk of 1.0% fat content. This will hereafter be referred to as pure milk. Cod liver oil containing 368 ppm natural tocopherol, without any added antioxidants, was provided by Maritex A/S, Århus, Denmark. The oil was incorporated into milk to a final concentration of 0.5 wt % to give fish oil enriched milk with a total fat content of 1.5% as follows: The emulsion was prepared at a milk processing UHT pilot plant (Pasilac Therm, Kolding, Denmark) where process conditions were similar to commercial milk production. The plant was coupled to a two-valve Rannie homogenizer (APV, Albertslund, Denmark). The milk with 1.0% milk fat was continuously bubbled with nitrogen in the feeding tank. The milk (2 L/min) was heated to 50 °C, and the cod liver oil was added continuously to the milk through a vacuum chamber (10 g/min), followed by homogenization at a total pressure of 50 bar. The milk emulsion was pasteurized at 72 °C for 15 s, cooled to 13 °C, bottled sterile, and then immediately stored in the dark at 2 °C. Samples were taken after 1, 4, and 8 days for sensory analysis and after 1, 4, 8, 11, and 14 days for DHS-GC-MS analysis. The fish oil enriched milk stored for 14 days was chosen for olfactometric studies. The peroxide value of this sample was 5.4 meq/kg.

Analysis of Peroxide Value (PV) and Fatty Acid Composition. Lipids were extracted from the milk emulsions according to the method by Bligh and Dyer (*29*) using a 15 g sample, 30 mL of methanol, and 30 mL of chloroform. An aliquot (3 g) of the extract was taken to determine PV spectrophotometrically by the IDF method (*30*). The fatty acid composition of the milk was determined after Bligh and Dyer extraction, while the fatty acid composition of the cod liver oil was determined directly in the oil (*31*). The content of tocopherol in the cod liver oil was determined by HPLC (*32*).

Sensory Evaluation. The milk emulsions were evaluated by a sensory panel trained in descriptive analysis of fishy off-flavors. The descriptors used for flavor assessment were fishy odor, fishy taste, metallic odor, and metallic taste. Samples (5 °C) were served randomized in blind trials and evaluated on a continuous intensity scale ranging from 0 (no intensity) to 9 (maximal intensity). Data were recorded on hand-held PSION mini computers (PSION, UK). A more elaborate evaluation of the sensory data is reported elsewhere (*28*).

Dynamic Headspace Sampling of Volatiles. Volatiles were stripped from the sample by a stream of nitrogen (99.999% Hydrogas, Denmark) carrying the volatiles to an adsorbent trap with Tenax-GR (Chrompack, Netherlands) placed in 1/4 inch steel tubes (Perkin-Elmer, UK). The nitrogen flow (150 mL/min) was led to the milk emulsion (4 g) through a washing bottle head. The outlet from the bottle head was connected to the adsorbent trap (*33*). The sampling temperature was 45 °C, and the collection time was 30 min. At the end of the sampling period, water was removed from the trap by purging the tube in the opposite direction for 15 min (50 mL N2/min). Headspace collections were made in triplicate for each sample.

Gas Chromatography−Mass Spectrometry. The adsorbed volatiles were thermally desorbed by ATD-400 automatic thermal desorber and the analysis was done by 5890 IIA gas chromatograph (Hewlett-Packard, CA) equipped with an HP 5972 A mass selective detector. A DB 1701 column (30-m × 0.25-mm × 1.0-μm, J&W Scientific, CA) with a flow of 1.3 mL of helium/min, and the following temperature program was used: 45 °C for 5 min, 45−55 °C at 1.5 °C/min, 55−90 °C at 2.5 °C/min and 90−220 °C at 12 °C/min, and finally holding at 220 °C for 4 min. Identification of the volatiles was achieved by comparison of retention index (RI) and mass spectral data with authentic reference compounds of analytical grade analyzed under identical conditions. The gas chromatographic programmed-temperature retention index value was calculated for each peak by comparing its retention characteristics to those of the two closest eluting components in the retention index standards (*n*-alkanes, C8-C32, Sigma) (*34*).

The following standards were chromatographed under identical conditions: 1-penten-3-one (97%), pentanal (99%), pentanol (99%), 1-penten-3-ol (99%), (*E*)-2-pentenal (95%), 2,3-pentanedione (97%), (*E*)-2-hexenal (99%), heptanal (95%), (*E,E*)-2,4-hexadienal (95%), (*E*)-2-heptenal (97%), octanal (99%), (*E,E*)-2,4-heptadienal (90%), (*E*)-2-octenal (94%), 2-nonanone (99%), (*E*)-2-nonenal (97%), and (*E,Z*)-2,6-nonadienal (95%), (Aldrich-Chemie, Germany), hexanal (98%) (Riedel-de Haën AG, Germany) and (*Z*)-4-heptenal (TCI, Japan) propanal (97%) and decanal (99%) (Sigma), (*E*)-2-butenal and (*E*)-2-decenal. When standards were not available, compounds were tentatively identified by matching the mass spectral data using Wiley mass spectral library (Wiley138K, John Wiley and Sons, Hewlett-Packard) and by comparing the retention index value to the values available in the literature (*17*). The mass spectral data of alkatrienals was not available in the Wiley library. Hence, the reported mass spectral details of (*E,Z,Z*)-2,4,7-decatrienal (*35*), (*E,E,Z*)-2,4,7-decatrienal (*36*), 2,4,6-nonatrienal (*37*) were compared for identification of these compounds.

Extracted Ion Chromatograms. Data analysis was performed using HP G1034 MS ChemStation software. Extracted ion chromatograms were derived by extracting the characteristic mass ions of volatiles from the total ion chromatogram after GC/MS-SCAN analysis of the headspace volatiles isolated by the DHS. The mass range from −0.30 to +0.70 amu was selected to extract around each ion.

GC-O. The volatiles isolated by DHS were desorbed by the ATD. The transfer line of the ATD was connected to the gas chromatograph equipped with a flame-ionization detector (FID) and an olfactory outlet (SGE OD01 with a needle valve SGE OSS2). The effluent from the end of the capillary column was split 1:1 between the FID and the sniffing outlet. The same temperature program used in the GC-MS was followed in the GC-O. The separated compounds were evaluated by sniffing the effluent. A panel of 10 experienced judges who were involved in the sensory evaluation of fish oil enriched samples was selected. To get acquainted with the method, the panel members were first trained on GC-O by assessing fishy and metallic milk samples. Sniffing was divided into two parts of 20 min. Each person participated in the sniffing of both parts, but during two distinct sessions to remain alert. The panelists were asked to assign odor properties to each detected odorant. Detection of an odor at the sniffing port by fewer than three of the 10 assessors was not considered for construction of the aromagram. After the 10 judges had evaluated the samples and found that no odors from decadienals and decatrienals could be perceived, the analysis was repeated with three assessors using highly oxidized fish oil enriched milk samples.

RESULTS AND DISCUSSION

Identification of Volatile Compounds. The chromatograms obtained from fresh milk and fish oil enriched milk exhibiting distinct fishy and metallic off-flavors (**Figure 1**) illustrate the profiles of their headspace volatiles. A total of 14 volatiles were
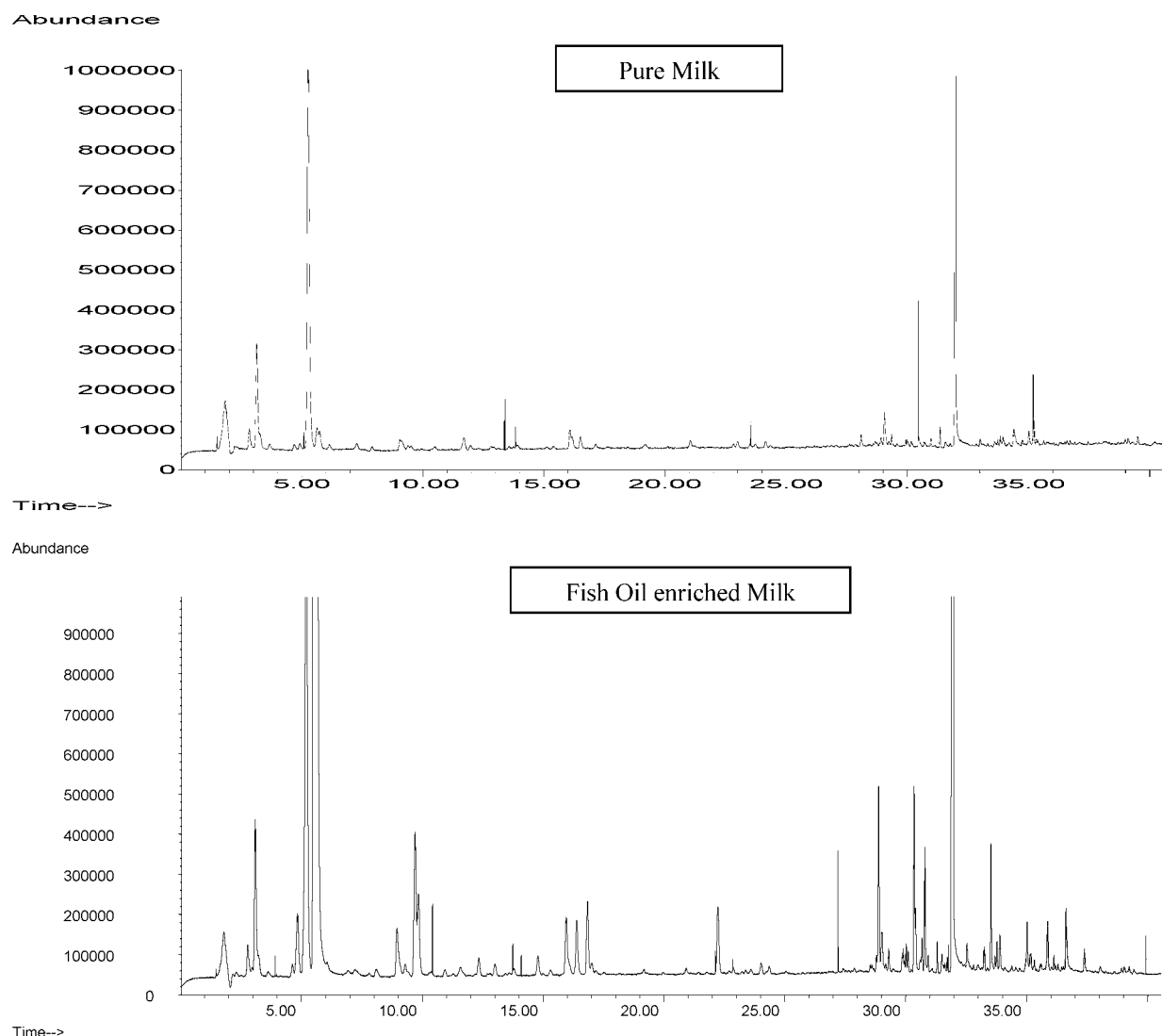
Figure 1. Total ion chromatograms of volatiles collected from pure milk and fish oil enriched milk both stored at 2 °C for 14 days.

identified in the pure milk, whereas 60 volatiles were identified in the fish oil enriched milk (**Table 1**). Most of the compounds isolated from pure milk belonged to the classes of ketones, especially methyl ketones, straight chain aldehydes, and *n*-alcohols. The methyl ketones, a characteristic feature of the volatile profile of pasteurized milk (*38*), which were identified in this study were 2-propanone, 2-butanone, 2-heptanone, and 2-nonanone.

The volatiles identified in fish oil enriched milk were mostly carbonyl compounds encompassing alkenals, alkadienals, alkatrienals, and vinyl ketones. It is obvious to mention that the volatiles that were identified in fish oil enriched milk but not in fresh milk samples were assumed to be the products originating from the oxidation of fish oil. Most of the identified volatiles in the study were observed in the samples stored for only a day. The volatiles (*E*)-2-butenal, (*E*)-2-pentenal, (*E*)-2-penten-1-ol, (*E*)-2-nonenal and (*E,E,Z*)-2,4,7-decatrienal were noticed in subsequent days of storage (**Table 1**).

Because of the structural similarity of the volatiles resulting from fish oil oxidation, many peaks appeared very closely in the chromatogram making the interpretation of chromatogram difficult (**Figure 1**). Further, the ions of adjacent peaks overlayed the chromatogram inhibiting the direct identification by MS library search. In view of these problems, the extracted ion chromatograms of the characteristic ions (**Table 2**), along with

the ions of adjacent peaks, were extensively studied. After subtraction of the alien ions, the identity of (*E*)-2-decenal, and the (*E,Z,Z*)- and (*E,E,Z*)-isomers of 2,4,7-decatrienal was subsequently confirmed by comparing with respective mass spectral data. The identification of these compounds in the fish oil enriched milk samples is discussed further below.

Alkenals. An array of (*E*)-2-alkenals ranging from butenal to undecenal was detected in the emulsion. As suggested by Grosch (*39*), these 2-alkenals can be viewed as indicators to determine the fatty acid precursors in the sample. The formation of these alkenals indicate that fish oil used in the emulsion contains n-3, n-6, and n-9 fatty acids, which is in agreement with the fatty acid profile listed in **Table 3**. Compared to the profile of volatiles identified in bulk cod liver oil (*12*), (*E*)-2-decenal, and (*E*)-2-undecenal were observed in the fish oil enriched milk. However, 4,5-epoxy-(*E*)-2-decenal, reported as a potent odorant responsible for metallic odor impressions in boiled cod (*10*), was not detected in the present study, even though the milk emulsions were described as metallic by the sensory panel.

Alkadienals. Among possible 2,4-alkadienals resulting from lipid oxidation, (*E,E*)-isomer of hexadienal, (*E,E*)- and (*E,Z*)-isomers of heptadienals and decadienals were detected in the present study (**Table 1**). Though various isomers of nonadienal (2,4; 2,6; and 3,6) were reported as secondary lipid oxidation products in the literature (*10*), only (*E,Z*)-2,6-nonadienal could

Table 1. Volatile Compounds Identified in Fish Oil Enriched Milk by DHS-GC-MS/GC-O

no.	cpd	retention index[a]	odor impression[b]	identification[c]	1	4	8	11	14
					days of storage at +2 °C[d]				
1	propanal	517	plastic	a					+
2	2-propanone	545	glue	b,f	+	+	+	+	+
3	2-propanol	585		b	+	+			+
4	propanol	658	fruity	b,f	+	+	+	+	+
5	butanal	667		b	+	+	+	+	+
6	2-butanone	683	cheese, butter	b,f	+	+	+	+	+
7	2-ethyl furan	735	sweet	b	+	+	+	+	+
8	(*E*)-2-butenal	751	plastic	a				+	+
9	1-penten-3-one	770	plastic, leather	a	+	+	+	+	+
10	pentanal	776	rubber	a	+	+	+	+	+
11	1-penten-3-ol	784	milky, butter	a	+	+	+	+	+
12	2,3-pentanedione	787	fresh	a,f	+	+	+	+	+
13	(*E*)-1,6-octadiene	807	synthetic, sulfur	b					+
14	(*E,E*)-2,4-octadiene	826	glue, warm	b					+
15	1,4-heptadiene, 3-methyl	835	green	b					+
16	(*E*)-2-pentenal	860	oily, soapy	a		+	+	+	+
17	3-hexanone	867	fresh	b,f	+	+	+	+	+
18	1-pentanol	876	fruity	a	+	+	+	+	+
19	hexanal	884	green, fresh	a,f	+	+	+	+	+
20	(*E*)-2-penten-1-ol	888	grass green	b			+	+	+
21	unknown	898	sweet, fruity						+
22	(*E,E*)-1,3,5-octatriene	920		b					+
23	1,2-butanediol	943	alcohol, butter	b,f	+	+	+	+	+
24	unknown	951	spicy						+
25	(*E*)-2-hexenal	961		a	+	+	+	+	+
26	3-heptanone	969	sweet	b,f	+	+	+	+	+
27	hexanol	976		b					+
28	2-heptanone	979		b,f	+	+	+	+	+
29	heptanal	985	chemical, burnt	a,f	+	+	+	+	+
30	(*Z*)−4-heptenal	989	sweet	a	+	+	+		+
31	unknown	1037	cooked and nutty						+
32	(*E,E*)-2,4-hexadienal	1050	green, vegetable	a					+
33	6-methyl-2-heptanone	1056	fresh, sweet	b					+
34	limonene	1065		b,f	+	+	+	+	+
35	1-octen-3-one	1075	mushroom	d	+	+	+	+	+
36	(*E*)-2-heptenal	1077	mushroom, earthy	a	+	+	+	+	+
37	2,3-octanedione	1082		b	+	+	+	+	+
38	(*Z*)-1,5-octadien-3-one	1088	green, leafy, geranium	b	+	+	+	+	+
39	benzaldehyde	1090	aromatic	b,f	+	+	+	+	+
40	octanal	1093	fragrant, citrus	a,f	+	+	+	+	+
41	1,5-cyclooctadiene	1111		b	+	+		+	+
42	(*E,Z*)-2,4-heptadienal	1132		b	+	+	+	+	+
43	(*E,E*)-2,4-heptadienal	1150	rancid, fatty	a	+	+	+	+	+
44	3-methylundecane	1171		b	+	+	+	+	+
45	(*E*)-2-octenal	1179	burnt, mushroom	a	+	+	+	+	+
46	(*E*)-2-nonen-1-ol	1185		b					+
47	2-nonanone	1189	fresh, sweet	a,f	+	+	+	+	+
48	(*E,Z*)-3,5-octadien-2-one	1199	synthetic, plastic	b					+
49	(*E,E*)-3,5-octadien-2-one	1231	fresh, sweet	b					+
50	(*E*)-2-nonenal	1279	cucumber	a				+	+
51	(*E,Z*)-2,6-nonadienal	1287	cucumber, green	a	+	+	+	+	+
52	decanal	1301	citrus	a	+	+	+	+	+
53	(*E*)-2-decenal	1396		a,e	+	+	+	+	+
54	undecanal	1409		b	+	+	+	+	+
55	(*E,Z*)-2,4-decadienal	1448		b,e					+
56	(*E,Z,Z*)-2,4,7-decatrienal	1452		c,e	+	+	+	+	+
57	2,4,6-nonatrienal	1464		c					+
58	(*E,E*)-2,4-decadienal	1478		b,e					+
59	(*E,E,Z*)-2,4,7-decatrienal	1485		c,e				+	+
60	(*E*)-2-undecenal	1503		b,e	+	+	+	+	+

[a] Retention index on DB-1701 column. [b] Odor impressions perceived by panelists during olfactometry. [c] a = identification on the basis of MS database, retention index and spiking with authentic standard; b = identification on the basis of MS database and retention index; c = identification on the basis of retention index and comparing spectral data from literature; d = identification on the basis of retention index and olfactometry; e = extracted ion chromatograms of the characteristic ions were studied; f = compounds identified in the pure milk. [d] + indicates the presence of the compound; blank indicates the absence of odor impression.

Table 2. Details of Characteristic Ions Used in the Study of Extracted Ion Chromatograms

class of compounds	characteristic ions (*m/z*)	fragmentation	references
2-alkenals	70 and 83	formation of α, β-unsaturated aldehydes due to McLafferty rearrangement involving H migration	*50*
2,4-alkadienals	81	generation of pyrelium ion	*51*
4,5-epoxy-2-alkenals	68	generation of furan ion due to cleavage across the epoxide ring with the loss of an aldehyde molecule	*52*
2,4,7-decatrienals	81	generation of pyrelium ion	*53*
	121	third double bond position at C7	

Table 3. Fatty Acid Profiles of Milk Fat and Cod Liver Oil (in % of Total Fatty Acids)

fatty acid	milk fat	cod liver oil
14:0	11.0	3.7
15:0	1.1	
16:0	36.5	10.2
17:0	0.5	
18:0	10.2	2.1
SAT	59.5	16.0
14:1(n-5)	1.1	
16:1(n-7)	2.0	6.4
18:1(n-9)	21.8	17.2
18:1(n-7)	2.4	3.9
20:1(n-9)		10.8
22:1(n-11)		7.3
22:1(n-9)		0.7
MUFA	27.4	46.3
18:2(n-6)	2.4	1.7
18:3(n-3)		0.9
18:4(n-3)	0.6	2.6
20:5(n-3)		8.4
22:5(n-3)		1.0
22:6(n-3)		11.7
other	10.2	11.3

by Milo and Grosch (*10*), was detected neither in the milk emulsion enriched with cod liver oil nor in a commercial cod liver oil (*12*).

Alkatrienals. Two isomers of 2,4,7-decatrienals have been reported as the volatile decomposition products of hydroperoxides of trilinolenoylglycerol (*40*). These isomers were also isolated from autoxidized methyl linolenate and characterized as *E,Z,Z*- and *E,E,Z*- decatrienals by Badings (*15*). However, only one of these isomers, *E,Z,Z*-decatrienal, was isolated from strongly autoxidized oils containing unsaturated fatty acids having an ω 3,6,9 double bond pattern (*41*). In the present study, both isomers of 2,4,7-decatrienals were identified in the milk emulsions. The *E,Z,Z*-isomer was detected in the samples stored for 1 day (PV 0.8 meq/kg), whereas the *E,E,Z*-isomer was noticed from the samples after storage for 11 days (PV 3.4 meq/kg). As per our knowledge, this is the first report on identification of decatrienals in an emulsion. The notable feature of this finding is that the isomers of 2,4,7-decatrienal, which were previously reported only in severely oxidized samples (PV above 70 meq/kg) (*42*), were detected in the milk emulsion with a relatively low peroxide value. Higher levels of metal ions may be present in milk emulsion compared to bulk oil. Moreover, lipid oxidation in emulsions is initiated at or near the oil−water interface, where the concentration of metal ions is higher than that in the oil phase. Therefore, decomposition of lipid hydro-

be detected in the fish oil enriched milk emulsion. This observation is in agreement with the volatile profile of cod liver bulk oil reported by Karahadian and Lindsay (*12*). The volatile (*Z,Z*)-3,6-nonadienal, reported as a potent odorant in boiled cod

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Table 4. Sensory Evaluation of Fish Oil Enriched Milk Emulsions[a]

	fishy odor			fishy taste		
	day 1	day 4	day 8	day 1	day 4	day 8
milk	0.4 ± 0.7	0.0 ± 0.1	0.2 ± 0.4	0.2 ± 0.5	0.1 ± 0.3	0.2 ± 0.3
fish oil enriched milk	0.4 ± 0.4	1.5 ± 1.4	1.4 ± 1.1	1.3 ± 1.0	2.1 ± 1.2	2.4 ± 1.4
	metallic odor			metallic taste		
	day 1	day 4	day 8	day 1	day 4	day 8
milk	0.1 ± 0.2	0.1 ± 0.2	0.1 ± 0.2	0.3 ± 0.8	0.1 ± 0.2	0.3 ± 0.6
fish oil enriched milk	0.5 ± 1.0	0.5 ± 0.6	0.7 ± 1.0	0.8 ± 0.7	0.6 ± 0.6	1.0 ± 1.0

[a] Values are averages of all 12 assessors' determinations ± standard deviation.

Figure 2. Aromagram of the selected volatiles determined by the GC-O. Retention index refers to compounds in **Table 1**, and detection frequency is number of assessors out of 10 recognizing the odor impression.

peroxides to volatile oxidation compounds may be faster in emulsions compared to that of bulk oils, and this may be the reason for the appearance of decatrienals in an emulsion with low PV value.

2,4,6-Nonatrienal is the other important member of this alkatrienal class of unsaturated aldehydes, which has rarely been reported as an oxidized product of the fatty acids. Hsieh et al. (*13*) and Triqui and Zouine (*43*) reported two isomers (the structural details were not identified) of nonatrienals in crude menhaden oil and anchovy, respectively. However, these compounds were not detected in the commercial cod liver oil (*12*). In the present study, one peak was identified as 2,4,6-nonatrienal on the basis of the mass spectrum reported by Buttery (*37*). Its absolute configuration was not ascertained due to the fact that the mass spectra of the three isomers reported in the literature were essentially identical.

Vinyl Ketones. The vinyl ketones have been reported to possess the lowest threshold values among the volatiles resulting from the oxidation of lipids (*44*). Three vinyl ketones, namely 1-penten-3-one, 1-octen-3-one and (*Z*)-1,5-octadien-3-one were identified in the fish oil enriched milk emulsions. The reported threshold values for these compounds in oils were 1-penten-3-one (0.003 mg/kg), 1-octen-3-one (0.0001 mg/kg), and (*Z*)-1,5-octadien-3-one (0.00003 mg/kg) (*15*, *45*).

Other Compounds. In addition to the above-mentioned classes of volatiles, several other volatiles belonging to aliphatic saturated aldehydes, aromatic compounds, aliphatic alcohols, and hydrocarbons were identified in the fish oil enriched milk samples (**Table 1**). To investigate whether some of these compounds were coming from sample or packaging material of Tenax tubes, empty Tenax tubes were run with every analysis. None of these volatiles were detected in the empty Tenax tubes.

Sensory Evaluation. The pure milk and the milk samples enriched with fish oil were evaluated by the sensory panel for fishy odor, fishy taste, metallic odor, and metallic taste. The samples containing fish oil had distinct fishy taste already at day one (**Table 4**). The intensity of both fishy odor and taste increased from day one to day four, and at day four and eight, the fishy odor and taste were significantly higher than the milk sample. The metallic attributes were only slightly higher than the milk sample, and no changes were observed during storage.

GC-O. The GC-O analysis of the isolated headspace of fish oil enriched milk stored for 14 days showed that none of the separated compounds gave a specific fishy or metallic odor, even though the sample was evaluated fishy and metallic by the sensory panel (**Tables 1** and **4**). Among the odor active compounds, seven were identified as the volatiles originating from the milk imparting milky and sweet odors. The volatiles resulting from the oxidation of fish oil were found to impart unpleasant odors. The most potent odorants, perceived at least
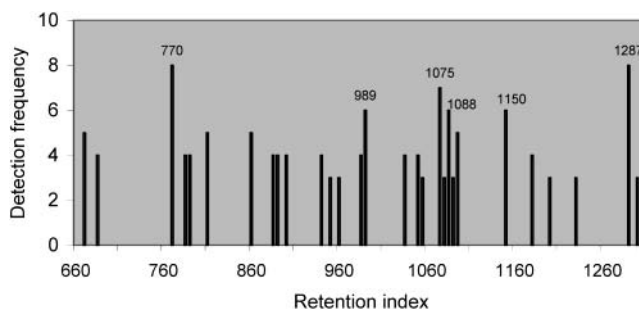
by six assessors, were 1-penten-3-one, (*Z*)-4-heptenal, 1-octen-3-one, (*Z*)-1,5-octadien-3-one, (*E,E*)-2,4-heptadienal, and (*E,Z*)-2,6-nonadienal (**Figure 2**).

The vinyl ketone, 1-penten-3-one, was found to impart strong plastic and leatherlike odor impressions (**Table 1**). It has been reported earlier as sharp and fishy in cold stored butter by Badings (*15*). It has also been indicated as the most noticeable volatile, with a very intense odor in rancid sardine oil (*46*). In a recent study on oxidative stability of crude herring oil, the concentrations of 1-penten-3-one showed a good correlation with peroxide value and anisidine value (*14*). The other two vinyl ketones identified in the samples, 1-octen-3-one and (*Z*)-1,5-octadien-3-one, were found to carry strong mushroom and green odors respectively, which is in agreement with previous observation in boiled cod (*9*).

The volatile (*Z*)-4-heptenal has been referred to as the cold-store cod compound (*47*). Nevertheless, it did not elicit a ready recognition in fishiness when evaluated alone in the studies by Karahadian and Lindsay (*12*). It was perceived as sweet and brown sugarlike odor in the present experiment (**Table 1**). On the basis of MS response, it was observed that (*Z*)-4-heptenal appeared in traces in early stages of storage period, and its concentration increased steadily in the milk emulsion. Considering its low threshold value 0.0005 mg/kg (*15*), it is believed to contribute significantly to the overall flavor of the fish oil enriched milk emulsion.

The odorant (*E,Z*)-2,6-nonadienal was encountered with cucumber smell by eight assessors (**Figure 2**). It was also found in cod liver oil and identified as the compound that provides the major contribution to the green and melon flavors encountered in fish oils (*12*). It has been established that oxidizing n-3 fish oils yield high amounts of 2,4-heptadienals. Though both isomers were detected in significant amounts during the entire storage period, only the *E,E-* isomer was noticed carrying rancid and fatty odor impressions.

Two isomers, (*E,Z,Z*)-2,4,7-decatrienal and (*E,E,Z*)-2,4,7-decatrienal, have been reported to contribute significantly to the fishy, whale oil, and cod liver oil-like flavors in oxidized oils containing n-3 fatty acids (*41*). The decatrienals exhibited distinct burnt/fishy flavors when they were added to bland canola oil (*12*). In a recent study, completely different from the lipid oxidation, "fishy-fish tank" odors were ascribed to the decatrienal produced by two chrysophytes, namely *Synura peternsii* and *Dinobryon cylindricum* (*48*). Contrary to these observations, Ke et al. (*42*) reported that they were unable to detect these isomers in oxidized mackerel oils exhibiting objectionable flavors. Furthermore, Badings (*49*) characterized the flavor of both isomers as being sliced beans. In our olfactometry experiments, no panel member could notice any odor in the

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region of these peaks. Even though the samples with double quantity were sniffed, the fishy odors were not perceived.

It is interesting to observe that the *E,Z-* isomer of 3,5-octadiene-2-one imparted synthetic and plastic odor notes, whereas its *E,E-* isomer smelled fruity and sweet (**Table 1**). Two compounds, identified as 1-penten-3-ol and 2,3-pentendi-one, appearing closely at retention indices 784 and 787 eluted almost together. The observed synthetic and milky odor notes could not be ascribed specifically to any of these volatiles. Moderate intensities of fruity, spicy, and nutty odor notes were noticed at retention index values 898, 951, and 1037, respectively (**Figure 2**). The compounds responsible for these odors could not be identified, as the concentrations of these volatiles were too low to observe full scan mass spectra.

It can be concluded that the major contributors to the odor profile of fish oil enriched milk were vinyl ketones, alkenals, and alkadienals. *E,Z,Z* and *E,E,Z* isomers of 2,4,7-decatrienal as oxidation products in a fish oil enriched emulsion that was only moderately oxidized were identified for the first time. Though decatrienals were not detected by GC-O, they may have sensory significance at higher concentration, and they may have impact in combination with other volatiles. Further, the sequential application of dynamic headspace sampling and gas chromatography in combination with olfactometry revealed that not a sole compound is responsible to the fishy and metallic off-flavors observed in the samples. We presume that the fishy and metallic off-flavors are the result of a combination of some of the potent odorants identified in the present study. This deserves further studies to identify and quantify the compounds responsible for off-flavors in fish oil enriched milk.

ACKNOWLEDGMENT

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