Dynamic Headspace Gas Chromatography/Mass Spectrometry Characterization of Volatiles Produced in Fish Oil Enriched Mayonnaise during Storage

Karsten Hartvigsen,* Pia Lund, Lotte F. Hansen, and Gunhild Hølmer

Department of Biochemistry and Nutrition, Centre for Advanced Food Studies, Technical University of Denmark, Building 224, DK-2800 Lyngby, Denmark

Protection against lipid oxidation and formation of unpleasant fishy and rancid off-flavors in oil-in-water food emulsions, such as fish oil enriched mayonnaise, is difficult to achieve. Volatile profiles from stored mayonnaises with different oil phase compositions were collected using a developed dynamic headspace sampling technique, in which interfering acetic acid was removed in situ with potassium hydroxide, and subsequently 148 volatiles were characterized and monitored by gas chromatography/mass spectrometry. Multivariate statistics showed correlation between the concentration of 62 volatiles and the fish oil and storage parameters, indicating the formation of lipid oxidation products, which impose fishy off-flavors. Further verification was obtained by gas chromatography/olfactometry, by which, among 78 odors, *cis*-4-heptenal and *trans*,*cis*-2,4-heptadienal were detected as distinct fishy notes. In total, 27 volatiles, including 1-penten-3-one, *cis*-2-penten-1-ol, *cis*-3-hexenal, *cis*-4-heptenal, 1-octen-3-one, 1,*cis*-5-octadien-3-one, 1-octen-3-ol, *trans*,*cis*-2,4-heptadienal, and *trans*,*cis*-2,6-nonadienal, were suggested to contribute to the developed unpleasant fishy and rancid off-flavors.

Keywords: *Selective dynamic headspace sampling; emulsions; odors; off-flavors; volatiles*

INTRODUCTION

Current health advisories recommend a higher intake of unsaturated fatty acids, especially the long-chain marine $n-3$ polyunsaturated fatty acids (PUFA) (Haumann, 1997; de Deckere et al., 1998). As the intake of fish and fish products in the Western world is low, efforts have been made to increase the consumption of marine lipids by incorporating fish oil into different functional food products such as bread, salad dressing, and mayonnaise (Nielsen, 1992; Schnepf et al., 1991; Jacobsen et al., 1999a). However, marine PUFA are highly susceptible to peroxidation, forming inter alia volatile secondary lipid oxidation products, which cause loss of food quality through flavor deterioration.

The mechanisms of lipid oxidation in complex food oil-in-water emulsions are not fully yet understood. Reviews by Coupland and McClements (1996) and Decker (1998) describe the current knowledge on lipid oxidation and its possible prevention in food emulsions. In previous studies, the efficacy of different antioxidants was tested in fish oil enriched mayonnaise, for example, propyl gallate, ascorbic acid, and different tocopherol systems (Jacobsen et al., 1999a,b, 2000); however, these antioxidant systems were either inefficient or prooxidative. In these investigations refined and deodorized fish oil was used, initially containing only traces of volatiles, but the mayonnaises soon developed a very unpleasant fishy off-flavor. In fish oil, meat, and foods, a number of potent odorants were suggested to impose a significant impact on fishy flavor (Karahadian and Lindsay, 1989; Milo and Grosch, 1996; Peralta et al., 1996). Nevertheless, flavor release is strongly affected by the food matrix (Druaux and Voilley, 1997), making prediction of potent odorants in mayonnaise difficult. In previous studies on fish oil enriched mayonnaise, the progress of oxidation was monitored by dynamic headspace sampling (DHS) and gas chromatography/mass spectrometry (GC/MS) analysis (Jacobsen et al., 1999a, 2000), but the presence of acetic acid in mayonnaise created an array of practical and analytical problems with loss of valuable information.

The present study describes a modified DHS method for the isolation and collection of volatiles from mayonnaise using a potassium hydroxide (KOH) trap for selective removal of volatile acids in situ. The sampling method was applied, in combination with GC/MS, to the identification and semiquantification of volatile compounds. The development of 148 volatiles was monitored during storage, and the potential fishy off-flavor compounds were identified by discriminant partial least-squares regression analysis (DPLSR). In a parallel study the olfactory profile of fish oil based mayonnaise was characterized.

MATERIALS AND METHODS

Oils. Raw fish oil (sand eel) (FO) was obtained from Esbjerg Fiskeindustri (Esbjerg, Denmark) and refined and deodorized at the pilot plant of the Department of Biotechnology (Technical University of Denmark). The following unsaturated fatty acids were present: 16:1$n-7$, 18:1$n-9$, 18:2$n-6$, 18:3$n-3$, 18:4$n-3$, 20:1$n-9$, 20:5$n-3$, 22:1$n-11$, and 22:6$n-3$ with contents of 5.3, 10.1, 2.0, 2.0, 4.6, 6.4, 10.2, 11.6, and 10.8%, respectively. Other analytical data were as follows: peroxide value, 0.1 mequiv/kg; anisidine value, 3.7; free fatty acids, 0.01%; iron, 0.1 μg/g; copper, <0.05 μg/g; α-tocopherol, 94 μg/g; and γ-tocopherol, <5 μg/g.

Refined and deodorized rapeseed oil (RO) was purchased from Aarhus Oil A/S (Aarhus, Denmark). The following unsaturated fatty acids were present: 18:1$n-9$, 18:2$n-6$, 18:3$n-3$, and 20:1$n-9$ with contents of 60.3, 20.6, 9.0, and

* Corresponding author (telephone +45 4525 2795; fax +45 4588 6307; e-mail karsten@mimer.be.dtu.dk).

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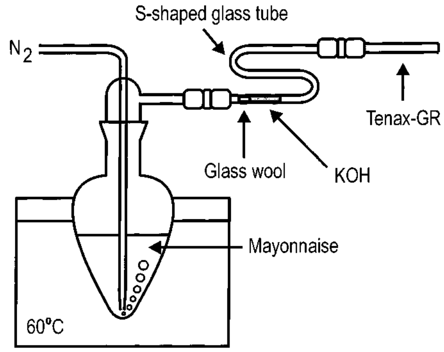

Figure 1. Schematic diagram of the DHS technique of volatiles in mayonnaise with a vertically inserted S-shaped glass tube containing the KOH trap.

1.5%, respectively. Other analytical data were as follows: peroxide value, 0.05 mequiv/kg; anisidine value, 1.8; free fatty acids, 0.04%; iron, 0.2 μg/g; copper, <0.05 μg/g; α-tocopherol, 251 μg/g; and γ-tocopherol, 459 μg/g.

Sample Preparation. Mayonnaises were prepared as previously described (Meyer and Jacobsen, 1996) and contained, by weight, 80.0% oil, 10.4% water, 4.0% vinegar, 0.3% salt (NaCl), 1.0% sugar, 0.1% potassium sorbate, 4.0% egg yolk, and 0.15% Grindsted FF DC (Danisco Ingredients, Brabrand, Denmark). Three mayonnaises with different oil phase compositions were prepared with either RO, FO, or a 4:1 mixture of the RO and FO (ROFO). Aliquots (300 g) of the mayonnaises, stored in nontransparent jars at 20 °C in darkness, were sampled after 0, 2, and 4 weeks and kept at −80 °C.

Internal Standard (IS) Solution. The IS solution consisted of *n*-dodecane (99%, final concentration = 21.5 ng/mg; BDH, Poole, U.K.) dissolved in volatile-free medium-chain triacylglycerol oil (Brøste A/S, Lyngby, Denmark).

Preparation of KOH Trap. KOH pellets (p.a.; Merck, Darmstadt, Germany) were cautiously crushed in an ∼50 °C hot mortar to form a dry, fine powder, prior to the transfer of ∼100 mg of KOH powder into an S-shaped glass tube, followed by glass wool (∼1 mg, Chrompack, Middelburg, The Netherlands) to prevent contamination of the mayonnaises with KOH, and the KOH traps were kept in a desiccator until use. The KOH was concentrated at the glass wool by gently tapping the S-shaped glass tube to give the best contact with the purge flow. The strong alkaline KOH must be handled cautiously, as it may cause severe burns.

Dynamic Headspace Sampling (DHS). Frozen mayonnaise (4.0 g) was weighed into a pear-shaped glass flask equipped with a purge head, thawed, and mixed thoroughly with IS solution (32.5 mg). The volatiles were collected using the DHS system, shown schematically in Figure 1, which is modified from that of Refsgaard et al. (1999). Three parallel samples were purged with nitrogen (99.995%, 150 mL/min, Hydrogas, Fredericia, Denmark) for 30 min at 60 °C, and the volatiles were passed through the KOH trap before being trapped onto Tenax GR (225 mg, Chrompack), packed in 0.25 in. stainless steel tubes (Perkin-Elmer, Norwalk, CT) (Figure 1). Adsorbed water was subsequently removed at ambient temperature by blowing N_2 through the Tenax tube for 20 min at 50 mL/min.

Thermal Desorption. Trapped volatiles were desorbed by heating the tube for 2 min at 200 °C in an automatic thermal desorber (ATD 400, Perkin-Elmer) by passing helium (99.9995%, Hydrogas) at 60 mL/min through the tube. The released volatiles were then cryofocused in a cold trap (−30 °C) packed with Tenax GR (37 mg, Chrompack) and flash heated to 250 °C to inject the volatiles onto the GC capillary column. Carrier gas (6.0 mL/min helium, 99.9995%, Hydrogas) and volatiles were split in the ratio 1.0 to 5.0 before entering the capillary column.

Gas Chromatography/Mass Spectrometry (GC/MS). The collected volatiles were resolved in a GC (model 8000, Fisons Instruments, Manchester, U.K.) on a 30 m DB-1701 fused silica capillary column, 0.25 mm i.d. and 0.25 μm film thickness (J&W Scientific, Folsom, CA). The column temperature was kept at 35 °C for 5 min, increased at 1.5 °C/min to 55 °C, at 2.5 °C/min to 90 °C, at 15 °C/min to 220 °C, and kept at 220 °C for 4 min. The on-line mass spectrometer (model MD800, Fisons Instruments) was directly interfaced to the GC and operated in electron ionization mode with continuous scanning of masses from 35 to 350. Electron ionization was 70 eV, and the ion source temperature was 200 °C. Volatile compounds were identified by comparison of retention index (RI) and mass spectral data with authentic reference compounds of analytical grade under identical analytical conditions. When standards were not available, the compounds were tentatively identified by mass spectrum matching using the NIST mass spectral library and/or by RI matching using an array of 125 reference compounds. Semiquantification was based on a characteristic ion for each compound, normalized to the IS. The average relative standard deviation (SD) of triplicate determinations of the 148 volatiles for the nine mayonnaises was 16 ± 15% (mean ± SD). Data were acquired and processed using Fisons data system (MassLab v 1.4).

GC/Olfactometry (GC/O). The collected volatiles were resolved in a Hewlett-Packard GC (5890 IIA, Palo Alto, CA) on a 30 m DB-1701 fused silica capillary column, 0.32 mm i.d. and 1.0 μm film thickness (J&W Scientific) using the temperature program described above. The GC effluent was split 1:1 between a flame ionization detector and a sniffing port, where a three-person sniffing panel evaluated the odors, as described by Refsgaard et al. (1998). Two members of the panel sniffed consecutively for 22 min, evaluating each part twice, and only odors detected at least three times are reported. Selected volatiles were identified by comparison of RI and perceived odor with authentic reference compounds under identical analytical conditions.

Data Analysis. Correlations between the volatile profiles and the design parameters were elucidated by employment of DPLSR analysis using the software program The Unscrambler v 7.01 (CAMO, Oslo, Norway). The *X* variables were defined as the levels of the 148 volatiles (and the IS), whereas the design (*Y*) variables were defined from the oil composition (RO, FO, and ROFO) and storage period (0, 2, and 4 weeks) parameters. *X* and *Y* variables were standardized by 1/SD. For each mayonnaise sample three volatile profiles were determined, and these replicates together with the corresponding mean value were used as samples. Full cross-validation was used to validate the calculated model.

RESULTS AND DISCUSSION

DHS of Volatiles through KOH Trap. A schematic diagram of the DHS technique with the included KOH trap is shown in Figure 1. The S-shaped glass tube was preferred to straight or angled glass tubes, as powdered KOH traps water during purging, forming a droplet, which may otherwise reach and destroy the Tenax adsorbent. The trapping of water is also beneficial, as water interferes with the GC analysis.

Figure 2 shows the total ion current (TIC) chromatograms of volatiles from the same mayonnaise sample (same amount of mayonnaise and IS solution) purged (A) directly and (B) through the KOH trap. Previously, a solvent delay of 8 min was employed to avoid damage of the mass spectrometer filament by acetic acid (Jacobsen et al., 1999a, 2000), resulting in loss of information about the low molecular weight volatile compounds. The vast amount of acetic acid also varied the RI, resulting in difficult and inaccurate data handling. Ions of acetic acid and 2,4-hexadienoic acid significantly raised the background, making identification laborious and misleading.

Introduction of the KOH trap solved these analytical problems by removing the two volatile nonoxidation products, acetic acid (retention time = 5.0−6.5 min) and 2,4-hexadienoic acid (sorbic acid, retention time =

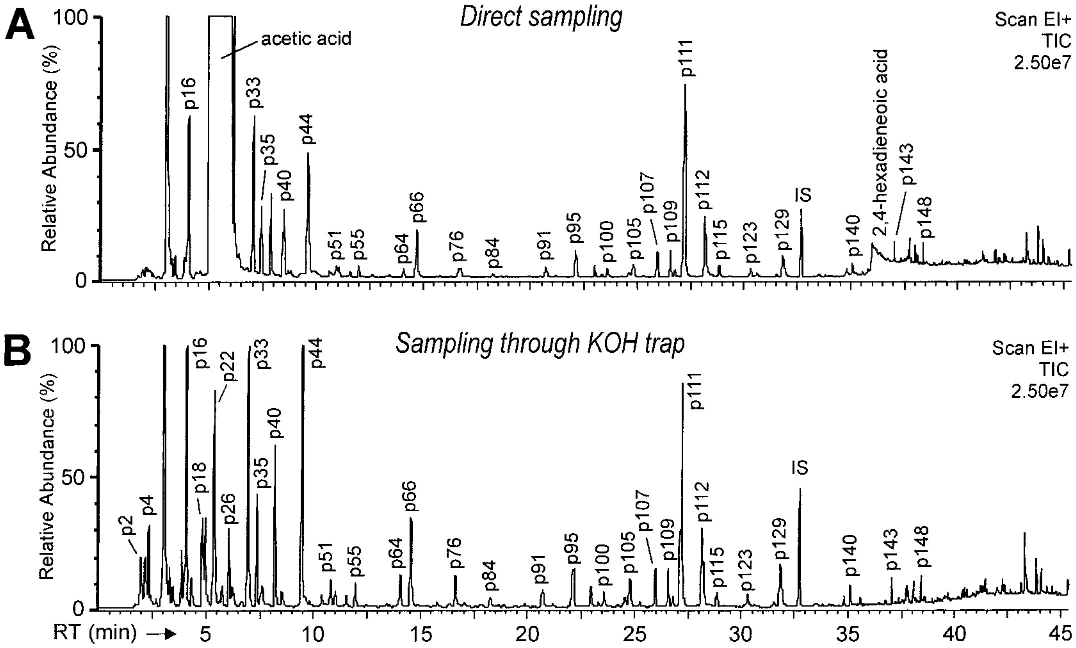

Figure 2. Reconstructed TIC chromatograms of volatile compounds from the same ROFO mayonnaise, purged (A) directly and (B) through the KOH trap. Peak numbers are as in Table 1.

36−38 min) (Figure 2, chromatogram A versus chromatogram B), yielding TIC chromatograms with low background and reproducible RI in addition to reproducible mass spectra of the volatiles during the whole time of analysis. Furthermore, neither molecular transformations nor loss of volatiles was observed during the KOH trap sampling. Parallel olfactory analysis revealed identical odor profiles for the two sampling methods, except for the long, pungent vinegar-like note of acetic acid (data not shown). Thus, introduction of the KOH trap clearly improved the applicability of DHS to mayonnaise.

Characterization of the Volatile Profile. Previously, we have shown that ROFO, in contrast to RO, mayonnaises quickly develop unpleasant fishy and rancid off-flavors (Jacobsen et al., 1999a), which are therefore expected to originate in the fish oil ingredient. Using the KOH trap sampling technique for stored mayonnaises, the TIC chromatograms revealed >200 chromatographic peaks (Figure 3), of which 148 volatiles, that is, 16 alcohols, 39 aldehydes, 7 furans, 20 ketones, 40 noncyclic and 15 cyclic hydrocarbons, and 11 so-called miscellaneous compounds, were identified and monitored by GC/MS. Most of these volatiles are well-known oxidation products of either vegetable oil (Frankel, 1982; Raghavan et al., 1994; Morales et al., 1997) or fish oil (Hsieh et al., 1989; Karahadian and Lindsay, 1989; Horiuchi et al., 1998). The identified volatiles are listed in Table 1 together with peak number, RI, degree of identification, and detected odor by GC/O.

Figure 3 shows the TIC chromatograms of the volatile profiles of the RO, ROFO, and FO mayonnaises after 4 weeks of storage at 20 °C. A number of volatiles developed to higher levels in the FO mayonnaise than in the RO mayonnaise, notably in the 0−12 and 25−30 min intervals, in contrast to the 13−24 min interval, where the RO mayonnaise had the highest levels of volatiles (Figure 3A versus Figure 3C). As expected, the ROFO mayonnaise (Figure 3B) was an intermediate between the RO and FO mayonnaises.

Olfactory Analysis. The FO mayonnaise was subjected to GC/O analysis after 0, 1, and 4 weeks of storage at 20 °C. Initially, only a few odors were detected, but number, intensity, and unpleasantness of the odors increased during storage. The majority of the detected odors were attributed to alcohols, aldehydes, and ketones (Table 1), but a number of odors were unassignable to identified volatiles, which is why suggestions based on RI, odors, and threshold values were made.

Interestingly, *cis*-4-heptenal (**79**) and *trans,cis*-2,4-heptadienal (**111**) were detected with distinct fishy odors (Table 1), suggesting roles as potent fishy odorants in fish oil enriched mayonnaise. The aldehydes listed in Table 1 have different odor qualities depending on the number of carbon atoms as well as the number, position, and configuration of the double bonds. The odor characteristics ranged from sour, cheese-like, and glue through grassy, fishy, burnt, fatty to cucumber and deep-fried notes, with the general aldehydic notes, pungent and green. Especially, the cucumber and fishy notes have been associated with unpleasant fishy off-flavor at certain intensities, whereas notes such as pungent, green, glue, burnt, fatty, and deep-fried normally are associated with rancid off-flavor. Likewise, the odor characteristics of the ketones ranged from buttery through rancid green, glue, earthy, mushroom-like to metallic and fruity notes. The tendencies were not as pronounced for the alcohols and the other molecular groups. Some additionally unpleasant notes were associated with the alcohols, for example, nauseating, compost-like, sweaty, and rotten, and the hydrocarbons, for example, nauseating, yeast-like, and rotten. Interestingly, many odors have been attributed to alcohols, proving that they may occur in concentrations high enough to be detected by GC/O, thus suggesting important roles as odorants in fish oil enriched mayonnaise.

Multivariate Data Analysis on Volatile Profiles from Mayonnaises with Various Oil Phase Compositions. The complete data set of mayonnaise volatiles was subjected to DPLSR analysis to categorize the

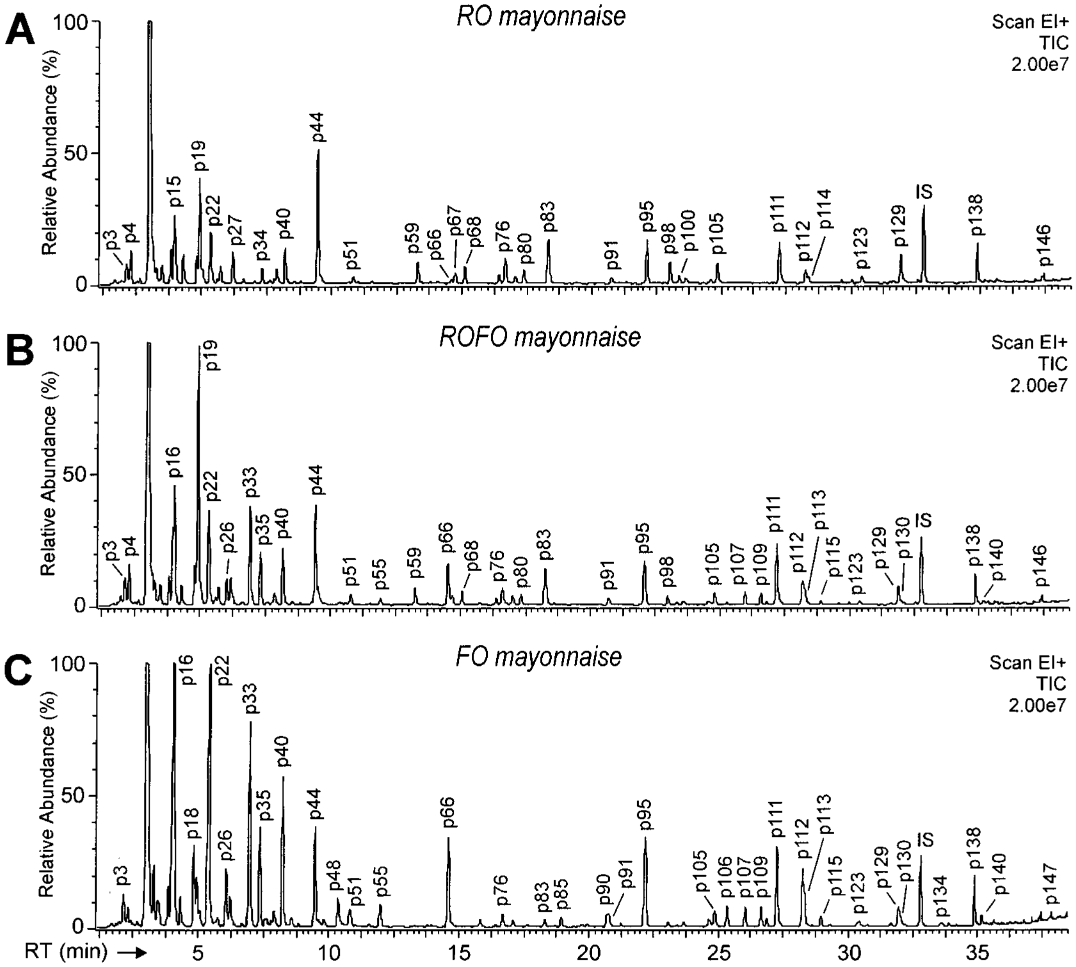

Figure 3. Reconstructed TIC chromatograms (1.3−38.8 min) of mayonnaises with different oil phase compositions after 4 weeks of storage at 20 °C: (A) RO; (B) ROFO; (C) FO. Peak numbers are as in Table 1.

characteristic volatiles, originating in the fish oil ingredient, and thus determine volatiles potentially responsible for the fishy off-flavor. In total, seven principal components (PCs) were validated, accounting for 88% of the calculated variance in *X* variables (volatiles) and for 97% in *Y* variables (design). This suggests a well-explained model, describing strong correlations between changes in the levels of volatiles, oil composition, and storage intervals. PC1 and PC2 explained 46 and 23%, respectively, of the variance in *X* and 22 and 24%, respectively, of the variance in *Y*. Only these two PCs will be discussed, as they describe the majority of correlations.

Scores Plot. As illustrated in the reconstructed scores plot for PC1 and PC2 (Figure 4), all of the fresh mayonnaises are located in the third quadrant, but already at this point differences in the volatile profiles exist. Both PCs explain this difference, as indicated by the ability to draw a straight line through the three fresh mayonnaises, a line that is almost diagonal to the axes of the two PCs. Similar lines may be drawn for samples stored for 2 and 4 weeks. Together, these diagonal lines describe differences in the volatile profiles caused by the oil phase compositions. The ROFO mayonnaise is always located between the RO and FO mayonnaises, close to RO mayonnaise in the fresh samples and moving closer toward FO mayonnaise during storage, indicating that the fish oil oxidizes more quickly than the rapeseed oil. During storage the samples move toward more positive values on the PC1 and PC2 axes. Again, diagonal lines, drawn through samples with identical oil phase compositions, describe differences in the volatile profiles caused by storage. The relative levels of volatiles increase more quickly during the first 2 weeks of storage, during which the off-flavors also seem to be developed, than during the last 2 weeks. The fact that the volatile profiles from RO and FO mayonnaises move in different directions indicates, as expected, differences in both the amount and formation of volatile molecular species.

Loadings Plot. Figure 5 shows the reconstructed loadings plot for PC1 and PC2. The design variables follow the same tendencies as explained for the scores plot. The location of IS near the origin of the loadings plot (Figure 5) indicates a fairly stable ratio between added amount IS and mayonnaise, which is important for comparison of samples analyzed over different days. The individual volatiles are categorized into four groups, as shown by the ellipses in Figure 5 and as listed in Table 1, where the volatiles in groups 1, 2, and 3 tend to correlate with the storage parameter in addition to the FO, the FO and RO, and the RO parameters, respectively, whereas volatiles in group 4 showed no correlation.

The discussion of the volatiles in the four groups is partially based on the following results and considerations: (1) During the first 2 weeks of storage the mayonnaises containing fish oil, that is, ROFO and FO, developed very unpleasant rancid and fishy off-flavors, whereas the RO mayonnaise only slowly developed a rancid off-flavor. This is in accordance with our previous study (Jacobsen et al., 1999a). (2) The majority of the

Table 1. Volatile Compounds Identified in Mayonnaises with Different Compositions of the Oil Phase by DHS-GC/MS and DHS-GC/O

peak[a]	Kovats RI[b]	compound	identification[c] RI[d]	identification[c] std[e]	odor	corr group
		alcohols				
3	556	ethanol	+	+		4
13	709	2-methyl-3-buten-2-ol	+	+	green, earthy	4
15	727	2-methyl-1-propanol	+			4
22	784	1-penten-3-ol	+	+	sweet[f]	1
38	841	3-methyl-1-butanol	+	+	nauseatingly sweet	2
39	843	2-methyl-1-butanol	+	+		4
46	881	*trans*-2-penten-1-ol	+		green	1
47	888	3-methyl-2-buten-1-ol	+	+		1
48	893	*cis*-2-penten-1-ol	+	+	musty, compost-like	1
72	967	4-ethylphenol	+		sweaty, nauseating	1
95	1049	1-methyl-4-(1-methylethyl)-3-cyclohexen-1-ol				4
96	1050	1-cyclohexyl-2-buten-1-ol			sour cheese, compost-like	1
104	1083	1-octen-3-ol	+	+	pungent, soil, fruity [f]	2
106	1090	1,*cis*-5-octadien-3-ol	+		citrus, green	1
132	1201	2-butyl-1-octanol				4
138	1269	4-methyl-1-(1-methylethyl)-3-cyclohexen-1-ol	+		nasty, rotten, burnt	3
		aldehydes				
1	487	ethanal	+	+		4
7	625	2-methylpropanal	+	+	miscellaneous[f]	1
9	661	butanal	+	+	sour[f]	1
14	723	3-methylbutanal	+	+	pungent, glue, green[f]	2
17	741	*trans*-2-butenal	+	+	old cheese[f]	1
19	767	pentanal	+	+	glue, green[f]	3
21	780	2-methyl-2-butenal	+	+		2
37	837	*cis*-2-pentenal	+		fruity	1
40	849	*trans*-2-pentenal	+	+	pungent, glue, green, grassy[f]	1
44	874	hexanal	+	+	pungent, green, grassy[f]	3
50	900	*cis*-3-hexenal	+		sour, old cheese	1
63	942	4-methyl-3-pentenal	+		synthetic, glue	1
65	948	2-methylpentadienal	+		sweet, green	1
66	950	*trans*-2-hexenal	+	+	sour, green[f]	1
69	959	2-furaldehyde	+			4
70	960	5-methylhexanal	+			2
76	978	heptanal	+	+		3
79	984	*cis*-4-heptenal	+	+	fishy, sweet[f]	1
88	1021	*cis*-2-heptenal	+			1
90	1030	*trans*,*trans*-2,4-hexadienal	+	+	green, burnt[f]	1
98	1060	*trans*-2-heptenal	+	+		3
100	1068	benzaldehyde	+	+	sweet	2
105	1084	octanal	+	+	fruity, green[f]	3
111	1117	*trans*,*cis*-2,4-heptadienal	+		fishy, fatty, burnt	1
112	1132	*trans*,*trans*-2,4-heptadienal	+	+	nasty, green, fatty[f]	1
118	1150	*cis*-2-octenal	+			4
123	1164	*trans*-2-octenal	+	+		3
125	1168	benzeneacetaldehyde	+	+	pungent, deep-fried	1
129	1187	nonanal	+	+	green plant-like, compost-like[f]	3
135	1228	*trans*,*cis*-2,4-octadienal	+		green	1
137	1246	*trans*,*trans*-2,4-octadienal	+	+	deep-fried[f]	1
139	1277	*trans*-2-nonenal	+	+	green, cucumber-like[f]	2
140	1278	*trans*,*cis*-2,6-nonadienal	+	+	cucumber[f]	1
141	1294	decanal	+	+	fruity, green[f]	2
-	1325	*trans*,*cis*-2,4-nonadienal[g]	+		deep-fried, fatty	-
142	1344	*trans*,*trans*-2,4-nonadienal	+	+	deep-fried, fatty[f]	4
143	1380	*trans*-2-decenal	+	+	sweet, green	4
145	1397	undecanal	+			4
-	1424	*trans*,*cis*-2,4-decadienal[g]	+		deep-fried	-
147	1457	*trans*,*trans*-2,4-decadienal	+	+	deep-fried[f]	3
148	1486	*trans*-2-undecenal	+	+	synthetic, green	4
		furans				
8	635	2-methylfuran	+	+	glue	2
16	731	2-ethylfuran	+	+	flower	1
41	856	3-methylfuran	+			1
49	897	2,3-dihydro-4-methylfuran	+			3
53	908	2-(2-propenyl)furan	+			1
58	927	2-butylfuran	+			2
91	1032	2-pentylfuran	+	+	green[f]	2
		noncyclic hydrocarbons				
2	508	2-pentene	+			1
4	600	hexane	+	+		4
5	610	1,*trans*-4-hexadiene	+			1

Table 1. (Continued)

peak[a]	Kovats RI[b]	compound	identification[c] RI[d]	std[e]	odor	corr group
		noncyclic hydrocarbons (continued)				
6	618	1,*cis*-4-hexadiene	+	+		1
11	700	heptane	+	+		4
24	800	octane	+	+		3
26	806	1,6-octadiene	+		mushroom-like, earthy	1
28	811	*trans*-4-octene	+			1
32	819	*trans*-2-octene	+			1
33	824	*trans*,*trans*-2,4-octadiene	+		fresh	1
35	832	3-methyl-1,*trans*-4-heptadiene	+			1
55	916	1,*trans*,*trans*-3,6-octatriene	+			1
57	924	1,*trans*,*trans*-3,5-octatriene	+			1
59	933	4-ethyl-3-octene				4
67	953	3,3-diethylpentane				4
68	957	5-methyl-*trans*-4-undecene				4
74	975	5-methyl-4-nonene				4
78	983	2,2-dimethyldecane				4
80	987	5-methyl-4-nonene				4
81	991	2-decyne			earthy, metallic	1
83	1000	1-decyne				2
84	1000	2,6-dimethyl-3-octene				4
85	1008	1,9-decadiene	+			1
86	1013	3-methyl-2-nonene				4
87	1018	*trans*,*trans*-2,8-decadiene	+		nasty, cheese-like, yeast-like	1
89	1028	*trans*,*cis*-2,8-decadiene	+			1
92	1038	4,5-dimethyl-2,6-octadiene			nauseous	1
113	1133	*trans*-3-undecen-5-yne				1
114	1133	6-dodecene				4
115	1142	*cis*-3-undecen-5-yne			miscellaneous	1
119	1153	4-methyl-1-undecene				4
120	1157	7-methyl-1-undecene				4
121	1159	5-methyl-1-undecene				4
124	1167	5-methyl-5-undecene				4
126	1181	7-methyl-5-undecene				4
128	1183	5-methyl-4-undecene				4
131	1197	6-dodecene				4
133	1220	3-methyl-2-undecene				4
136	1237	3,7-dimethyl-1,3,6-octatriene			fruity, celery, flowery	1
146	1400	*trans*-7-tetradecene			green	4
		cyclic hydrocarbons				
12	706	benzene	+	+	glue	4
27	809	toluene	+	+		4
30	816	5,5-dimethyl-1,3-cyclopentadiene				1
52	903	ethylbenzene	+	+		4
54	910	*p*-xylene	+	+		4
60	935	1-ethenyl-3-methylenecyclopentene				4
64	944	styrene	+	+		3
73	974	1,2-dimethyl-1,4-cyclohexadiene				1
93	1039	1,3,5-trimethylbenzene				3
94	1047	*d*-limonene	+	+		4
97	1057	1-ethyl-3-methylcyclopentane			pungent, synthetic	1
107	1100	3-ethenylcyclooctene			green plant-like	1
109	1108	2,4-diethenyl-1-methylcyclohexane			pungent, earthy, rotten	1
110	1112	3-(2-propenyl)cyclooctene			pungent, earthy, rotten	1
117	1147	1-methylcyclooctene				1
		ketones				
-	680	2,3-butanedione[g]	+	+	buttery[f]	-
18	762	1-penten-3-one	+	+	pungent, rancid green, glue[f]	1
36	836	*trans*-3-penten-2-one	+	+		1
43	871	2-hexanone	+	+	green	3
45	878	1-hexen-3-one	+		earthy, metallic	3
51	901	1-methoxy-3-methylene-2-pentanone			fruity, compost-like	2
56	923	3-hydroxy-2-pentanone	+			1
71	965	3-heptanone	+	+		1
75	976	2-heptanone	+	+		3
77	980	1-methoxy-3-methylene-2-pentanone			flowery	2
82	995	cyclohexanone	+	+		2
99	1065	1-octen-3-one	+	+	mushroom[f]	3
101	1077	2,3-octanedione	+		pungent, sour	3
102	1080	6-methyl-5-hepten-2-one	+	+	earthy, rancid green	3
103	1081	1,*cis*-5-octadien-3-one	+		geranium, metallic	1
116	1146	*trans*-3-octen-2-one	+	+		3
122	1162	1-nonen-3-one	+		mushroom-like	1

Table 1. (Continued)

peak[a]	Kovats RI[b]	compound	identification[c] RI[d]	std[e]	odor	corr group
		ketones (continued)				
127	1183	2-nonanone	+	+	pungent, nasty, sour	1
130	1187	*trans,cis*-3,5-octadien-2-one	+		citrus, fruity, green, fatty	1
134	1226	*trans,trans*-3,5-octadien-2-one	+		celery, fruity	1
144	1390	2-undecanone	+		sweet, fruity	4
		miscellaneous				
10	690	chloroform	+	+		4
25	802	dimethyl disulfide	+	+	rancid, glue	3
31	817	2-methylthiophene	+	+		3
-	675	ethyl acetate[g]	+	+	fruity, solvent-like[f]	-
20	772	propyl acetate	+			4
34	831	butyl acetate	+			4
42	861	pentyl acetate	+			4
61	937	3-methyl-1-butanol acetate	+			4
62	940	2-methyl-2-butanol acetate	+			4
-	776	acetic acid[g]	+	+	vinegar-like[f]	-
23	795	1-chloropentane	+		green plant-like	2
29	815	1,2-dichloropentane	+			2
108	1104	1-chloroheptane	+			3
-	1514	unknown[g]			nasty, fatty	-
-	1595	unknown[g]			damp-stained	-

[a] Compounds, monitored during storage, were numbered in succession. [b] Modified Kovats retention time index on DB-1701 according to van den Dool and Kratz (1963). [c] All compounds are tentatively identified by MS library (NIST). [d] Additional identification by RI of literature and molecular related external reference compounds. [e] Additional identification by RI and mass spectra of external reference compounds. [f] Identification by odor and RI of external reference compounds. [g] Compounds detected by only GC/O.

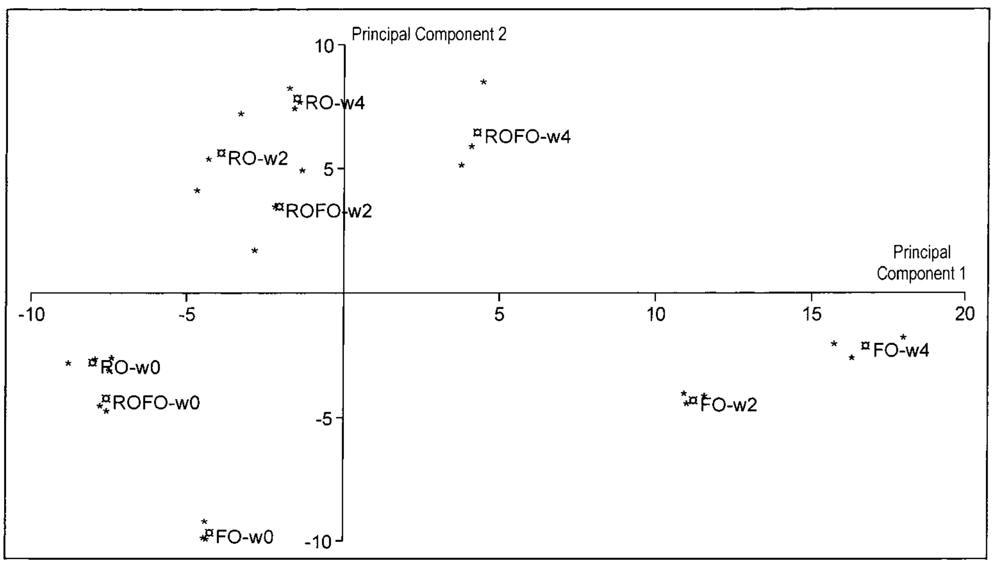

Figure 4. Reconstructed scores plot of PC1 versus PC2 from DPLSR analysis on the volatile profiles of the stored mayonnaises with different oil phase compositions. ROFO refers to the 4:1 mixture of RO and FO. The prefixes w0, w2, and w4 refer to the number of weeks the mayonnaise was stored at 20 °C. Each * represents one of the three replicates. The average values are represented by the symbol, ⌖.

detected odors have been attributed to alcohols, aldehydes, and ketones (Table 1), which correlate with storage (groups 1−3) and notably FO (group 1). Thus, GC/O analysis verified the DPLSR analysis on the volatile profiles. (3) In general, aldehydes and ketones have low threshold values and thus high flavor impact, in contrast to alcohols and alkynes, which have moderate flavor impact, whereas hydrocarbons and alkylfurans have low flavor impact due to high threshold values (Kochhar, 1996).

Group 1—Correlation of Volatiles with FO and Storage Parameters. The 62 volatiles in this group, identified as 7 alcohols, 18 aldehydes, 15 alkenes, 3 alkynes, 7 cyclic hydrocarbons, 3 furans, 1 hydrox ketone, and 8 ketones, originate primarily in fish oil and increase in concentration during storage (Table 1).

Hydrocarbons are usually regarded to be flavorless; however, the majority of the "hydrocarbons" are only tentatively identified and thus may be important odorants, as exemplified by the unpleasant notes of 1,6-octadiene (**26**) and *trans,trans*-2,8-decadiene (**87**) (Table 1). Alkynes have been reported as oxidation products of vegetable oils (Kochhar, 1996; Morales et al., 1997). Three alkynes were tentatively identified, that is, 2-decyne (**81**), *trans*-3-undecen-5-yne (**113**), and *cis*-3-undecen-5-yne (**115**), of which the former may be an important odorant (Table 1). The three furans, that is, 2-ethylfuran (**16**), 3-methylfuran (**41**), and 2-(2-propenyl)furan (**53**), have been identified in fish oil and fish sauce (Peralta et al., 1996; Horiuchi et al., 1998) but are unlikely to have important sensory impact.

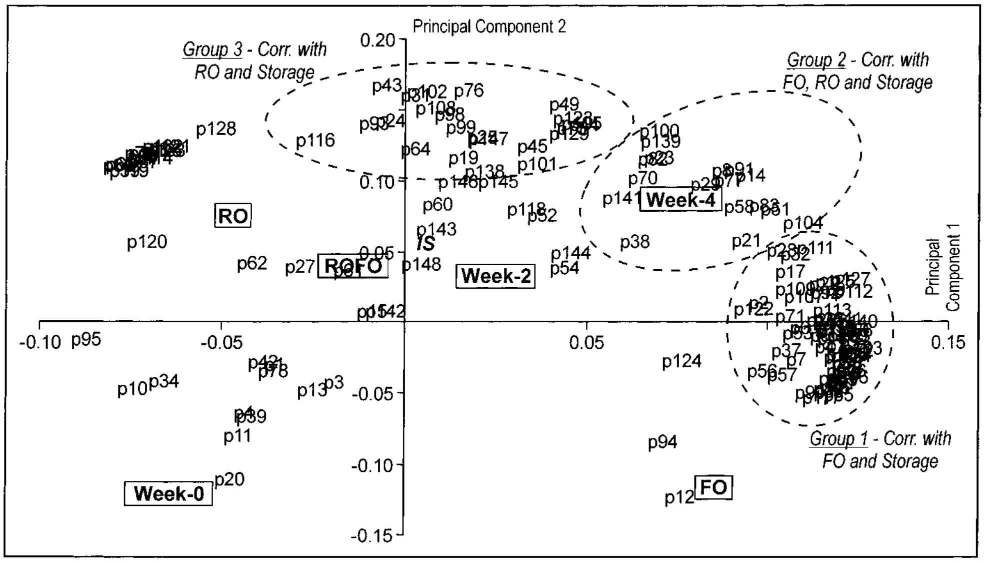

Figure 5. Reconstructed loadings plot of PC1 versus PC2 from DPLSR analysis on the volatile profiles of the stored mayonnaises with different oil phase compositions. ROFO refers to the 4:1 mixture of RO and FO. Week-0, Week-2, and Week-4 refer to the number of weeks the mayonnaise was stored at 20 °C. Each volatile is represented by its peak number, as in Table 1. IS refers to the internal standard (*n*-dodecane). The marked areas encircle volatiles that correlate well with one or more of the design variables.

Five of the seven alcohols are well identified and characterized as lipid oxidation products in fish oil (Karahadian and Lindsay, 1989; Horiuchi et al., 1998), that is, 1-penten-3-ol (**22**), *trans*-2-penten-1-ol (**46**), 3-methyl-2-buten-1-ol (**47**), *cis*-2-penten-1-ol (**48**), and 1,*cis*-5-octadien-3-ol (**106**). Notably, *cis*-2-penten-1-ol (**48**), increasing predominately in level in the FO mayonnaise (Figure 3C versus Figure 3A), revealed an unpleasant odor (Table 1), as did the two tentatively identified alcohols, 4-ethylphenol (**72**) and 1-cyclohexyl-2-buten-1-ol (**96**), which are structurally related to volatiles in crabmeat (Chung, 1999). Unless the concentration of the alcohols is high, their flavor impact in mayonnaise is believed to be low; however, current knowledge is limited and cautions should be taken in disregarding any volatile.

The eight ketones in group 1 have been identified as products of lipid oxidation in fish oil (Hsieh et al., 1989; Karahadian and Lindsay, 1989; Horiuchi et al., 1998), that is, 1-penten-3-one (**18**), *trans*-3-penten-2-one (**36**), 3-heptanone (**71**), 1,*cis*-5-octadien-3-one (**103**), 1-nonen-3-one (**122**), 2-nonanone (**127**), *trans,cis*-3,5-octadien-2-one (**130**), and *trans,trans*-3,5-octadien-2-one (**134**). Although these ketones probably all contribute to the unpleasant off-flavor, the vinyl ketone, 1,*cis*-5-octadien-3-one (**103**), is probably the most potent fishy odorant due to its extremely low threshold value and characteristic geranium-like odor (Table 1) (Karahadian and Lindsay, 1989; Milo and Grosch, 1995, 1996). Additionally important ketone odorants are 1-penten-3-one (**18**) and 2-nonanone (**127**), causing unpleasant pungent odors (Table 1), and the former has been associated with fishy off-flavor (Khiari et al., 1995). Hence, notably 1-penten-3-one (**18**) and 1,*cis*-5-octadien-3-one (**103**), both originating from $n-3$ PUFA, should receive special attention. A hydroxy ketone, 3-hydroxy-2-pentanone (**56**), was tentatively identified; however, its potential flavor impact remains to be established.

Most of the 18 identified aldehydes in group 1 are well-known oxidation products of mainly the $n-3$ PUFA but also the $n-6$ PUFA content (Kochhar, 1996) of fish oil (Hsieh et al., 1989; Karahadian and Lindsay, 1989; Horiuchi et al., 1998). Two alkanals, that is, 2-methylpropanal (**7**) and butanal (**9**), eight alkenals, that is, *trans*-2-butenal (**17**), *cis*-2-pentenal (**37**), *trans*-2-pentenal (**40**), *cis*-3-hexenal (**50**), 4-methyl-3-pentenal (**63**), *trans*-2-hexenal (**66**), *cis*-4-heptenal (**79**), and *cis*-2-heptenal (**88**), and one cyclic aldehyde, that is, benzeneacetaldehyde (**125**), were identified. Furthermore, seven alkadienals were identified, that is, 2-methylpentadienal (**65**), *trans,trans*-2,4-hexadienal (**90**), *trans,cis*-2,4-heptadienal (**111**), *trans,trans*-2,4-heptadienal (**112**), *trans,cis*-2,4-octadienal (**135**), *trans,trans*-2,4-octadienal (**137**), and *trans,cis*-2,6-nonadienal (**140**). Branched aldehydes, for example, 2-methylpropanal (**7**) and 2-methylpentadienal (**65**), originating in either lipid oxidation or deamination of amino acids, may cause unpleasant fishy off-flavors in foods (Milo and Grosch, 1995; Shimoda et al., 1996). Odor descriptors, usually related to rancid, for example, green, burnt, and deep-fried, and fishy, for example, fishy and cucumber, off-flavors, were attributed to 17 of the 18 aldehydes (Table 1) in agreement with Kochhar (1996).

Of the aforementioned aldehydes, 2-methylpropanal (**7**) has been described to partially cause a malty off-flavor in cod (Milo and Grosch, 1995), whereas especially the $n-3$ PUFA-derived potent odorants, *cis*-3-hexenal (**50**), *cis*-4-heptenal (**79**), and *trans,cis*-2,6-nonadienal (**140**), have been associated with fishy off-flavors in oxidized fish oil (Karahadian and Lindsay, 1989), drinking water supplies (Khiari et al., 1995), and the fish oil character of boiled salmon (Milo and Grosch, 1996). Furthermore, Khiari et al. (1995) reported a distinct fishy odor of *trans,trans*-2,4-heptadienal (**112**) in drinking water, which is in agreement with the present results for *trans,cis*-2,4-heptadienal (**111**) (Table 1). In contrast, Karahadian and Lindsay (1989) reported the two 2,4-heptadienals (**111** and **112**), originating from $n-3$ PUFA, to give generally oxidized, rancid, and painty, but not fishy, off-flavors in fish oils. The volatiles in group 1 are believed to be the most potent odorants, significantly contributing to the unpleasant rancid and fishy off-flavors that develop in fish oil enriched mayonnaise.

Group 2—Correlation of Volatiles with FO, RO, and Storage Parameters. The 17 volatiles in this group increase in concentration during storage, independently of the oil composition. Most of the volatiles have been characterized as oxidation products of the $n-3$, $n-6$, and $n-9$ fatty acid content of vegetable oils (Morales et al., 1997) and fish oil (Karahadian and Lindsay, 1989), for example, the aldehydes 3-methylbutanal (**14**), 2-methyl-2-butenal (**21**), 5-methylhexanal (**70**), benzaldehyde (**100**), *trans*-2-nonenal (**139**), and decanal (**141**); the alcohols 3-methyl-1-butanol (**38**) and 1-octen-3-ol (**104**); the ketones 1-methoxy-3-methylene-2-pentanone (**51** and **77**) and cyclohexanone (**82**); and the furans 2-methylfuran (**8**), 2-butylfuran (**58**), and 2-pentylfuran (**91**). The three branched aldehydes, notably 3-methylbutanal (**14**), may cause an unpleasant fishy off-flavor in foods (Milo and Grosch, 1995; Shimoda et al., 1996). The volatiles in group 2, detected with green, sweet, fruity, and cucumber odors (Table 1), will probably mainly contribute to the rancid off-flavor and only to a minor degree to the fishy off-flavor, which develops in fish oil enriched mayonnaise. As in group 1, some volatiles are only tentatively identified and cautions should be taken in disregarding the potential flavor impact of any volatile. Furthermore, if volatiles act synergistically, it may be hypothesized that volatiles from group 2 may modify and/or enhance the flavor impact of other volatiles.

Group 3—Correlation of Volatiles with RO and Storage Parameters. Most of the 23 volatiles in this group have been characterized previously as oxidation products from predominantly the $n-6$ PUFA content of vegetable oils (Frankel, 1982; Raghavan et al., 1994), dairy products (Christensen and Hølmer, 1996; Milo and Reineccius, 1997), and fish oil (Karahadian and Lindsay, 1989), for example, pentanal (**19**), 2-hexanone (**43**), hexanal (**44**), 1-hexen-3-one (**45**), 1-octen-3-one (**99**), 2,3-octanedione (**101**), 6-methyl-5-hepten-2-one (**102**), octanal (**105**), nonanal (**129**), and *trans*,*trans*-2,4-decadienal (**147**). The volatiles in group 3 gave typical odors of rancidity, for example, green and deep-fried notes (Table 1); therefore, they are not considered as important fishy odorants but rather as contributors of metallic, green, and rancid off-flavors in mayonnaises.

Group 4—"No Correlation". In total, 46 volatiles are categorized in this group, consisting of compounds not included in groups 1−3. The concentrations of these volatiles did not develop systematically and were therefore independent of storage and oil phase composition. The volatiles may be present in the original ingredients, for example, vinegar, RO and FO, or the plastic storage containers of the ingredients or from the air, etc. This group also included well-known oxidation products, for example, ethanal (**1**) and *trans*,*trans*-2,4-nonadienal (**142**). The group 4 compounds are not important lipid oxidation products of the mayonnaises.

Off-Flavors in Fish Oil Enriched Mayonnaise. On the basis of our results obtained by the KOH trap DHS technique coupled with GC/MS and GC/O detection, the following volatiles seem to be the most potent odorants of the unpleasant rancid and fishy off-flavor and off-aroma in FO and ROFO mayonnaises: *trans*-2-butenal (**17**), 1-penten-3-one (**18**), 1,6-octadiene (**26**), 3-methyl-1-butanol (**38**), 1-hexen-3-one (**45**), *cis*-2-penten-1-ol (**48**), *cis*-3-hexenal (**50**), 4-ethylphenol (**72**), *cis*-4-heptenal (**79**), 2-decyne (**81**), *trans*,*trans*-2,8-decadiene (**87**), *trans*,*trans*-2,4-hexadienal (**90**), 4,5-dimethyl-2,6-octadiene (**92**), 1-cyclohexyl-2-buten-1-ol (**96**), 1-octen-3-one (**99**), 6-methyl-5-hepten-2-one (**102**), 1,*cis*-5-octadien-3-one (**103**), 1-octen-3-ol (**104**), 2,4-diethenyl-1-methylcyclohexane (**109**), 3-(2-propenyl)cyclooctene (**110**), *trans*,*cis*-2,4-heptadienal (**111**), *trans*,*trans*-2,4-heptadienal (**112**), 1-nonen-3-one (**122**), 2-nonanone (**127**), 4-methyl-1-(1-methylethyl)-3-cyclohexen-1-ol (**138**), *trans*-2-nonenal (**139**), and *trans*,*cis*-2,6-nonadienal (**140**). These 27 volatiles were chosen from the following criteria; their concentration increased during storage and their odors were described by the panel as one of the following notes: burnt, compost-like, cucumber, earthy, fishy, metallic, mushroom, musty, nasty, nauseating, rancid green, rotten, soil, sour/old cheese, and sweaty. These notes are either unpleasant themselves or have been reported to contribute to the unpleasant off-flavor in fish oils and fish products, for example, cucumber, metallic, and mushroom. However, other notes such as deep-fried, fatty, glue, grassy, green, pungent, and synthetic cannot be excluded as they probably contribute to the rancid off-flavor.

Karahadian and Lindsay (1989) and Milo and Grosch (1995, 1996) have characterized the following volatiles as important odorants of fishy flavors/off-flavors in fish oil and fish meat: 2-methylpropanal (**7**, group 1, miscellaneous odor), 3-methylbutanal (**14**, group 2, pungent, green odor), 1-penten-3-one (**18**, group 1, pungent, rancid green odor), *cis*-3-hexenal (**50**, group 1, old cheese odor), *cis*-4-heptenal (**79**, group 1, fishy odor), 1-octen-3-one (**99**, group 3, mushroom odor), 1,*cis*-5-octadien-3-one (**103**, group 1, metallic odor), *trans*,*cis*-2,6-nonadienal (**140**, group 1, cucumber odor), *cis*,*cis*-3,6-nonadienal (not detected), *trans*,*trans*,*cis*-2,4,7-decatrienal (not detected), and *trans*,*cis*,*cis*-2,4,7-decatrienal (not detected). The aforementioned volatiles detected in this study increased in concentration during storage and correlated with the fish oil content of the mayonnaises, except 1-octen-3-one (**99**), as it represents an oxidation product of $n-6$ PUFA. Karahadian and Lindsay (1989) incorporated the two decatrienals into mayonnaise, which resulted in a suppression of the off-flavor, probably due to hydration of the 2−3 double bond caused by the acidic matrix. This may explain why the decatrienals were not identified in this study.

As mayonnaise consists of three phases, that is, oil phase, water phase, and oil−water interface, parameters other than concentration, partition coefficients, and threshold values may influence the flavor release and thereby the sensory impact of volatiles in the emulsion system (Druaux and Voilley, 1997). For instance, the current knowledge about the influence of the acidic milieu on the flavor impact of volatiles is very limited. Therefore, to identify volatiles with potent flavor impact, studies on correlations between sensory analysis and volatile profiles, obtained by the developed KOH trap DHS technique, in fish oil enriched mayonnaises are in progress.

In previous studies the levels of a number of volatile compounds, collected by DHS without the KOH trap, correlated with metallic, rancid, and fishy off-flavors when mayonnaises were supplemented with propyl gallate and different tocopherol systems (Jacobsen et al., 1999a, 2000). Cross-checking these volatiles with those in Table 1 reveals that all of the isolated volatiles from the previous studies also appear and increase during storage in the present study. However, it is obvious that the characterization of volatile profiles in mayonnaises has been significantly improved.

In this work a KOH trap DHS technique was developed and tested for its applicability to mayonnaises. The technique proved to be excellent in the characterization of the volatile profiles of RO- and/or FO-containing mayonnaises. Volatiles potentially responsible for fishy off-flavors in mayonnaise were isolated by DPLSR analysis and included 2-methylpropanal (**7**), 1-penten-3-one (**18**), *cis*-3-hexenal (**50**), *cis*-4-heptenal (**79**), 1,*cis*-5-octadien-3-one (**103**), and *trans*,*cis*-2,6-nonadienal (**140**), which previously have been identified as important odorants in bulk fish oils, fish meats, and fish foods. The olfactory analysis showed a large number of odorants; two of these had distinct fishy notes, that is, *cis*-4-heptenal (**79**) and *trans*,*cis*-2,4-heptadienal (**111**). At least 27 volatiles increased in concentration during storage and gave very unpleasant odors. We are currently investigating correlations between volatile profiles and sensory analysis to obtain better understanding of the flavor defects in fish oil enriched mayonnaises.

ABBREVIATIONS USED

DHS, dynamic headspace sampling; DPLSR, discriminant partial least-squares regression; FO, fish oil; GC, gas chromatograph/chromatography; IS, internal standard; KOH, potassium hydroxide; MS, mass spectrometry; O, olfactometry; PC, principal component; PUFA, polyunsaturated fatty acid; RI, retention index; RO, rapeseed oil; std, standard/reference compound; TIC, total ion current.

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