Isolation and Quantification of Volatiles in Fish by Dynamic Headspace Sampling and Mass Spectrometry

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A dynamic headspace sampling method for isolation of volatiles in fish has been developed. The sample preparation involved freezing of fish tissue in liquid nitrogen, pulverizing the tissue, and sampling of volatiles from an aqueous slurry of the fish powder. Similar volatile patterns were determined by use of this sample preparation method and for samples chewed for 10 s. Effects of sampling time, temperature, and purge flow on level of volatiles were tested. Purging at 340 mL/min for 30 min at 45 °C was found to be optimal. Detection limits for a number of aldehydes were 0.2−2.7 μg/kg. Levels of volatiles are given for fresh salmon, cod, saithe, mackerel, and redfish.

Keywords: *Dynamic headspace sampling; volatiles; aldehydes; salmon; fresh fish*

INTRODUCTION

To gain a better understanding of food quality it is of interest to measure compounds contributing to the flavor and off-flavor. Important flavor components, characteristic of fresh fish, are lipid-derived volatiles formed through the action of lipoxygenase (Lindsay, 1990). During storage, autoxidatively produced volatiles are formed. In both fresh and frozen stored cod, trout, and salmon, lipid oxidation products, like aldehydes, are character impact odorants (Milo and Grosch, 1995; 1996). The concentrations of volatiles are very low, at the microgram per kilogram level, especially in fresh fish. Some of these volatile compounds have a very intense odor and are, even in small concentrations, able to affect the sensory quality. Moreover, volatiles have a polarity similar to the polarity of triacylglycerols, which make up the major part of lipids in fatty fish. Separation and quantification of volatiles in fish and other fat containing food are therefore hampered by this combination of very low concentration and similarity in polarity with the lipid matrix. Conventional extraction methodology is not usable, but by headspace techniques lipids and volatiles can be separated due to differences in volatility. Many methods for isolation of food volatiles by headspace sampling have been developed; for reviews see Jensen et al. (1997) and Marsili (1997). For most of these, a static headspace sampling technique has been used, e.g., for analysis of potent odorants in cod, trout, and salmon (Milo and Grosch, 1995, 1996). Dynamic headspace sampling gives a lowering of the detection limit for volatiles compared to static headspace (Overton and Manura, 1995).

Here we present a dynamic headspace sampling method for isolation and collection of volatiles in low concentration from fish tissue. The developed method is reasonably accurate and fast. To obtain high specificity and low limits of detection, identification and quantification of the volatiles were based on mass spectrometry.

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MATERIALS AND METHODS

Fish. For the majority of the investigations the fish meat used was from farmed salmon (*Salmo salar*), obtained from Sekkingstad A/S (Skogsvåg, Norway). The fish were stored for 3 days on ice during transport before being sampled for analysis. Other fish species used to test the methodology were cod (*Gadus morhua*), saithe (*Pollachius virens*), redfish (*Sebastes* spp.), and mackerel (*Scomber scombrus*). These were obtained from a local fish store and immediately prepared for analysis.

Materials. The standards used for quantification were obtained from different companies: pentanal (99%), 2-(*E*)-pentenal (95%), 2-(*E*)-hexenal (99%), 3-heptanone (98%), heptanal (95%), 2-(*E*)-heptenal (97%), 2,4-(*E*,*E*)-heptadienal (90%), octanal (99%), 2-(*E*)-octenal (94%), 2-nonanone (99%), nonanal (95%), 2,4-(*E*,*E*)-nonadienal (90%), and 2,6-(*E*,*Z*)-nonadienal (95%) were from Aldrich-Chemie (Steinheim, Germany). Hexanal (98%) was from Riedel-de Haën AG (Seelze, Germany). 1-Octen-3-ol (97%) was from Merck (Darmstadt, Germany). Decanal and methional were from Sigma (St. Louis, MO). 1-Octen-3-one (97%) was from Lancaster Synthesis Ltd. (Morecambe, U.K.). 2,4-Decadienal (85−90%) was from Fluka (Buchs, Switzerland). Refined and deodorized fish oil was a gift from the pilot plant at Department of Biotechnology, Technical University of Denmark.

The traps consisted of 225 mg of Tenax-GR (Chrompack, Bergen op Zoom, The Netherlands), placed in $^1/_4$-inch steel tubes (Perkin-Elmer, Buckinghamshire, U.K.). An antifoaming preparation [antifoam B, Sigma (St. Louis, MO)] was added to a level of 1 μg/mL of water.

Chewing Experiments. Two persons chewed in duplicates 10 g of boiled salmon meat for 10 s. For cooking, salmon samples of 60 g were heated for 16 min at 100 °C in a hot-air oven. After the samples were chewed, these were spit into a 100 mL pear-shaped flask, 25 mL of distilled water was added, and volatiles were collected as described in Table 1.

Thermal Desorption. A Perkin-Elmer (Norwalk, CN) ATD-400 automatic thermal desorber with a Tenax TA-packed cold trap was used for thermally desorbing the collected volatiles. Helium was used as carrier gas. The gas flow from the trap to the transfer line to the capillary column in the gas chromatograph (GC) was split in the ratio of 5.0 mL min^{-1}/1.3 mL min^{-1}.

GC−MS. The transfer line of the ATD was connected to a Hewlett-Packard (Palo Alto, CA) 5890 IIA gas chromatograph equipped with a HP 5972 A mass-selective detector. A DB 1701 column (30 m × 0.25 mm × 1.0 μm, J&W Scientific, Folsom,

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Table 1. Parameters for Dynamic Headspace Sampling of Volatiles in Fish

parameter	
sample amount	20 g of fish powder + 25 mL of water
adsorbent	Tenax-GR
purge flow	340 mL/min
purge time	30 min
sampling temperature	45 °C

CA) with a flow of 1.3 mL of helium/min and the following temperature program was used: 25 °C for 1 min, 25 to 175 °C at 4 °C/min, 175 to 240 °C at 20 °C/min, and finally hold at 240 °C for 7 min. The GC−MS transfer line temperature was kept at 280 °C. The ionization energy of the mass spectrometer was set at 70 eV in EI mode and the detector operated with a mass range of 30−350 amu with a repetition rate at 2.2 scans/s and a threshold of 50.

Quantification. *Headspace Sampling of Standards.* For quantification purposes C_5−C_{10} aldehydes and some ketones dissolved in fish oil in five sets of concentrations were added to samples of 20 g of fresh salmon powderized as described in Results. The standards were collected as described in Table 1. Results from the collections of standards were used to prepare a calibration curve for each standard compound, using the HP ChemStation software.

Limit of Detection. Detection limits were determined as 3× noise (Knoll, 1985). The noise was determined from six GC-MS analyses on blank tubes and was recorded over a period of 1 min. The response calculated as 3× noise was converted to concentrations by use of the calibration curve prepared for each standard compound.

RESULTS AND DISCUSSION

The present study of headspace sampling methodology aims at establishing a set of sampling conditions allowing an acceptable reproducibility of the collection and quantification of volatiles from fish samples. The basic study used salmon meat as the food matrix to be analyzed.

Sample Preparation. A sample preparation methodology was developed where fish meat was cut in pieces of 1−3 cm^3, which were immersed in liquid nitrogen for approximately 30 s. The deep-frozen pieces were transferred to a small kitchen chopper and chopped for about 1 min or until all fish cubes had been comminuted into a fine powder.

To provide an efficient and reproducible transfer of volatiles from the fish powder to the trap, the powder had to be slurried in water. The ratio of fish to water was studied and it was found that a suspension of approximately 20 g of the frozen fish powder in a 100 mL pear-shaped flask with 25 mL of distilled water was usable. At increased water content the release of volatiles from cheese, fruit, and ham decrease (Rosa et al., 1994), but an aqueous slurry of the fish powder was necessary for securing stirring during sampling and for providing an even distribution of the slurried powder. Without water addition the level of collected volatiles was very low. To minimize evaporation of volatiles the stoppered flask was kept on ice for a maximum of 30 min until headspace sampling commenced. Immediately prior to headspace sampling, the 25 mL of distilled and purified water was added to the flask.

To assess the oxidation hazard during sample preparation, parallel samplings were made by the method described but carrying out the comminution of the frozen fish in a nitrogen atmosphere and flushing the purge flasks with nitrogen before placing the powder in the flask. The results of the sample preparation study are given in Table 2. It is indicated that the technique of nitrogen blanketing does not change the levels of aldehydic oxidation products recovered. We conclude that within the relatively short time of sample preparation there is no risk of artifactual formation of oxidation products.

To assess the advantage of the described liquid nitrogen freezing procedure, the results obtained by this methodology were compared to results obtained by placing a cold (not frozen) fish mince in the purge flask and using a tissue homogenizer in the flask for the fine grinding of the fish tissue. This method of sample preparation was performed both directly and with nitrogen blanketing in all homogenization steps.

Between the two methodologies there was no marked difference in the level of carbonyl compounds determined in the fish tissue. There is a tendency to a larger variability in the results obtained when purging the sample prepared by aqueous tissue homogenization is purged. This may be related to the observation that the solids from the latter preparation tend to aggregate in the purge flask, whereas the powder from the frozen preparation remains in constant suspension during the purging. Therefore, we find the liquid nitrogen freezing and subsequent grinding to frozen powder to be the better sample preparation method.

Headspace Sampling of Volatiles: *Purge Flow.* Volatiles were stripped from the fish powder in the aqueous suspension by a stream of nitrogen carrying the volatiles to an adsorbent trap (Figure 1). Different purge flows were tested and a rather high purge gas flow of 340 mL/min was chosen, thereby securing an efficient stirring of the sample slurry. The nitrogen flow was led to the sample suspension through a washing bottle head. The outlet from the bottle head was connected by a stainless steel fitting and Teflon ferrule to the adsorbent Tenax-GR trap.

Sampling Time. The influence of sampling time (6, 12, 18, 24, 30, and 42 min) on the level of volatiles was investigated. An increase in sampling time caused an increase in the collected volatiles with a maximum at 30 min of sampling for the more volatile compounds (Table 3). After sampling for 42 min, lower levels of volatiles were found compared to 30 min of sampling. This can be explained by loss of volatiles due to breakthrough of the traps. Loss of volatiles due to breakthrough was tested by collection of volatiles for 30 min on three traps connected in series. On the second trap the most volatile compounds such as pentanal and (*E*)-2-pentenal were collected. On the third trap trimethylamine was found and also other very volatile compounds in trace amounts.

Sampling Temperature. The sampling temperature is important for the volatility of the volatile compounds. However a high sampling temperature can cause formation or conversion of the volatiles (Spanier et al., 1994; Spanier and Boylston, 1994). Different sampling temperatures (45, 55, and 60 °C) were tested and 45 °C was chosen. At 60 °C so much water was collected on the traps that it disturbed the subsequent chromatography. Judged from TIC (total ion current) areas, the highest levels of the most volatile compounds were determined with 45 °C as sampling temperature (data not shown). At 55 °C compounds less volatile than heptanal (RI = 988) resulted in higher TIC areas than found by sampling at 45 °C. An advantage of the chosen sampling conditions is the low sampling temperature,

Table 2. Concentrations of Volatiles in Salmon Flesh,[a] Determined after Different Sample Preparation Procedures[b]

		frozen powder[d]		aqueous homogenization[d]	
volatile	RI[c]	no	nitrogen blanketing	no	nitrogen blanketing
(*E*)-2-pentenal	863	80 ± 16	83 ± 17	129 ± 29	91 ± 17
hexanal	885	176 ± 16	203 ± 11	202 ± 25	175 ± 7
(*E*)-2-hexenal	964	54 ± 15	66 ± 8	53 ± 37	54 ± 10
3-heptanone	973	5.3 ± 14	3.2 ± 37	2.4 ± 17	2.7 ± 9
heptanal	988	38 ± 22	39 ± 14	47 ± 22	49 ± 7
octanal	1093	41 ± 10	44 ± 6	56 ± 23	59 ± 10
(*E*,*E*)-2,4-heptadienal	1148	359 ± 14	427 ± 11	342 ± 44	355 ± 18
2-nonanone	1190	6.3 ± 14	6.8 ± 11	7.1 ± 28	7.0 ± 9
nonanal	1198	318 ± 28	286 ± 23	288 ± 36	312 ± 18
(*E*,*E*)-2,4-decadienal	1453	156 ± 42	154 ± 29	95 ± 82	177 ± 22

[a] Salmon stored for ≈24 months at −20 °C. [b] Sampling parameters: Purge flow 340 mL/min at 45 °C, Tenax-GR traps. Mean values (micrograms per kilogram) and relative standard deviations (%) from six determinations are given. The following compounds were included in the search but were not detected in this fish: methional (RI = 1047), 1-octen-3-ol (1070), (*E*)-2-heptenal (1073), 1-octen-3-one (1080), (*E*)-2-octenal (1179), (*E*,*Z*)-2,6-nonadienal (1293), decanal (1301), and (*E*,*E*)-2,4-nonadienal (1362). [c] RI, retention index on DB 1701 column. [d] See details in Results and Discussion.

Table 3. Concentrations of Volatiles in Salmon Flesh,[a] Determined after Different Headspace Sampling Times[b]

volatile	6 min	12 min	18 min	24 min	30 min	42 min
(*E*)-2-pentenal	4.5 ± 0.6	11.6 ± 0.1	21.7 ± 4.6	14.3 ± 9.4	29.9 ± 3.0	28.2 ± 11.8
hexanal	4.9 ± 2.3	19.8 ± 2.5	51.8 ± 9.2	47.3 ± 14.2	64.0 ± 5.7	57.7 ± 24.2
(*E*)-2-hexenal	nd[c]	nd	nd	tr[d]	tr	nd
heptanal	nd	nd	nd	10.1 ± 5.0	15.5 ± 0.6	14.7 ± 8.2
octanal	7.3 ± 0.5	10.0 ± 0.8	13.4 ± 0.5	14.1 ± 1.1	15.4 ± 0.7	16.6 ± 4.6
(*E*,*E*)-2,4-heptadienal	nd	7.0 ± 2.5	27.3 ± 3.6	35.9 ± 4.7	44.8 ± 25	70.4 ± 36.8
(*E*)-2-octenal	nd	nd	nd	nd	nd	tr
nonanal	nd	6.9 ± 1.0	21.3 ± 3.5	27.6 ± 3.5	35.4 ± 2.2	61.0 ± 46.9
(*E*,*E*)-2,4-decadienal	nd	nd	nd	nd	45.8 ± 48.7	61.7 ± 46.4

[a] Salmon stored for ≈18 months at −20 °C. [b] Sampling parameters: Purge flow 340 mL/min at 45 °C, Tenax-GR traps. Mean values (micrograms per kilogram) and standard deviations from triplicate determinations are given. The following compounds were included in the search but were not detected in this fish: pentanal, 3-heptanone, methional, 1-octen-3-ol, (*E*)-2-heptenal, 1-octen-3-one, and (*E*,*Z*)-2,6-nonadienal, decanal, and (*E*,*E*)-2,4-nonadienal. [c] nd, not detected. [d] *tr*, trace; peak too small for quantification.

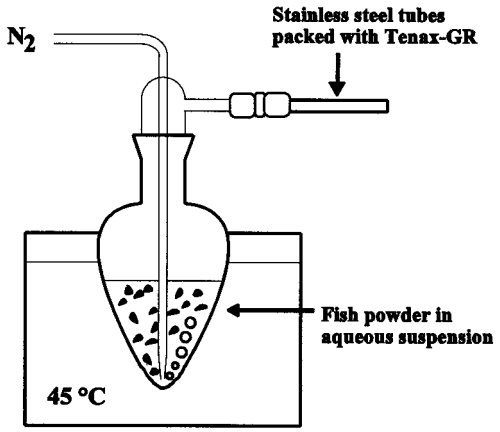

Figure 1. Dynamic headspace sampling of volatiles in fish.

which minimizes the risk of thermal or chemical modification of the volatile compounds.

The Tenax trap was maintained at room temperature during the sampling. Thereafter, water was removed from the Tenax trap by purging the trap in the opposite direction for 15 min (50 mL of N_2/min).

Reproducibility of the Headspace Sampling Method. The reproducibility of the method was determined by carrying out five analyses over a 2 month period on a fresh salmon stored in pieces at −80 °C. For the volatiles, variation coefficients of 10−40% were determined (Table 4). Six successive analyses for determination of a compound, e.g., hexanal, in one salmon had a variation coefficient in the same magnitude, i.e., about 30%.

Data for coefficients of variation relating to headspace analysis of food volatiles were not easy to find in the literature 10 years ago (Poll and Hansen, 1990). This situation has not changed much during the 1990s. Standard deviations reported for analysis of volatiles from heterogeneous matrixes ranged from 7% to 20% (Poll and Hansen, 1990). Volatiles in fresh grapefruit juice were determined with relative standard variations from 5% to 40% (Cadwallader and Xu, 1994). The relatively high coefficients of variation found in the present study do not preclude the use of the methodology in, e.g., storage studies, where changes in levels of volatiles easily become sufficiently large to reveal statistically significant changes (Refsgaard et al., 1998a). This is also related to the fact that the relative standard deviations decrease with an increasing level of volatiles in stored fish. Some of the data reported in the present study are based on analyses of fresh fish, with low levels of most of the volatiles, and consequently with high relative standard deviations.

Table 4. Reproducibility of the Headspace Sampling Method

	reproducibility[a]			reproducibility[b]		
volatile	mean (μg/kg)	SD[c]	CV[d] (%)	mean (μg/kg)	SD	CV (%)
(*E*)-2-pentenal	nd[e]			47.9	9.2	19
hexanal	10.2	4.2	41	80.0	32	40
(*E*)-2-hexenal	9.2	1.1	12	26.6	5.9	22
heptanal	nd			19.2	5.0	26
octanal	nd			19.6	4.9	25
(*E*,*E*)-2,4-heptadienal	30.3	9.9	33	107	23.0	21
nonanal	55.4	18	33	61.5	20.5	33

[a] Measured five times on the same fresh salmon with six determinations each time. [b] Measured by four persons on the same salmon (stored at −20 °C for 2 years) with six determinations for each person. [c] SD, standard deviation. [d] CV, coefficient of variation. [e] nd, not detected.

Table 5. Limits of Detection and Levels of Volatiles in Five Species of Fresh Fish[a]

volatile	limits of detection[b] (μg/kg)	levels of volatiles (μg/kg of meat)				
		salmon[c]	cod	saithe	redfish	mackerel
pentanal	1.57	nd[d]	nd	nd	nd	nd
(*E*)-2-pentenal	0.42	nd	12 ± 9.3	17 ± 10	nd	138 ± 14
hexanal	0.37	28 ± 18	28 ± 14	16 ± 15	50 ± 18	144 ± 55
(*E*)-2-hexenal	0.19	1.4 ± 2.0[e]	nd	10 ± 2.3	nd	43 ± 3.6
3-heptanone	0.06	nd	nd	nd	nd	13 ± 18
heptanal	0.46	7.8 ± 3.3	7.6 ± 2.2	1.6 ± 2.3	1.5 ± 0.53	5.4 ± 2.2
methional	12	nd	nd	nd	nd	nd
1-octen-3-ol	0.16	2.7 ± 3.8[e]	nd	nd	nd	nd
(*E*)-2-heptenal	0.27	nd	nd	nd	nd	nd
1-octen-3-one	0.56	nd	28 ± 9.1	21 ± 8.6	9.9 ± 1.2	73 ± 13
octanal	0.21	8.8 ± 7.1	15 ±2.4	7.3 ± 1.0	9.3 ± 0.89	19 ± 0.98
(*E*,*E*)-2,4-heptadienal	0.17	11 ± 5.6	19 ± 6.2	36 ± 15	8.1 ± 3.3	218 ± 40
(*E*)-2-octenal	0.56	11 ± 15[e]	nd	8.9 ± 8.7	13 ± 2.6	23 ± 1.8
2-nonanone	0.04	0.13 ± 0.28[e]	12 ± 0.58	16 ± 3.1	13 ± 0.79	13 ± 0.25
nonanal	1.83	29 ± 20	92 ± 20	56 ± 50	37 ± 14	19 ± 11
(*E*,*Z*)-2,6-nonadienal	0.82	3.3 ± 4.6[e]	nd	nd	nd	18 ± 1.8
decanal	2.71	nd	nd	nd	nd	nd
(*E*,*E*)-2,4-nonadienal	0.69	nd	nd	nd	nd	nd
(*E*,*E*)-2,4-decadienal	2.29	nd	nd	nd	nd	nd

[a] One fish of each species was analyzed in triplicate. For quantification, standard curves were prepared by collection of standards added to samples of powderized fresh salmon. Mean values and standard deviations are given. [b] Determined in fresh salmon (see description in Materials and Methods). [c] Mean concentration for five fresh salmon each analyzed in triplicate. [d] nd, not detected. [e] The compound was not detected in all five salmon.

Further, the person to person reproducibility of the method was studied by comparing the results obtained when four different persons performed collection of volatiles on samples from a salmon stored 2 years at −20 °C. The variation coefficients were 20−40% (Table 4), i.e., the same magnitude as the variation coefficient found on the concentration measurements of a single compound.

Quantification of Volatiles. The compounds collected by the method were volatiles having five carbon atoms or more. Compounds of higher volatilities could not be reproducibly determined. This limitation was due to the removal of water and thereby the most volatile compounds from the tubes before the chromatography but also due to breakthrough of volatiles from the traps as described previously. The compound of the lowest volatility detected by the headspace sampling method was 2,4-decadienal.

Character impact odorants in fresh cod and fresh salmon are (*Z*)-1,5-octadien-3-one, (*E*,*Z*)-2,6-nonadienal, acetaldehyde, and methional (Milo and Grosch, 1996). For salmon propanal is also an important odorant, as is (*E*,*E*)-2,4-decadienal for cod. The very volatile compounds acetaldehyde, propanal, and pentanal could not be reproducibly detected by the dynamic headspace method presented. The volatiles we quantified were pentenal and C_6−C_{10} aldehydes, including the commercially available compounds of the odorants reported in fresh cod: (*E*,*Z*)-2,6-nonadienal and (*E*,*E*)-2,4-decadienal (Milo and Grosch, 1996). Some ketones were also quantified, whereas methional was not detected.

For quantification purposes we used calibration curves that were based on addition of standards to a fish (salmon) matrix. For standards we used relevant fish volatiles that were commercially available. This basis for quantification eliminates some of the errors related to the frequently used internal standard calibration (Drozd et al., 1990). The calibration curves were based on abundance (signal size) determined by mass spectrometry. The target ion abundance was selected on the basis of two qualifier ions and retention time.

In Table 5 are given the limits of detection of volatiles collected by the presented method. The majority of the aldehydes could be detected in 0.2−0.6 μg/kg level. For the most volatile compound quantified, pentanal, and for the less volatile compounds, nonanal, (*E*,*Z*)-2,6-nonadienal, decanal, (*E*,*E*)-2,4-nonadienal, and (*E*,*E*)-2,4-decadienal, the detection limits were higher and in the 0.7−2.7 μg/kg level. For two of the quantified ketones, 3-heptanone and 2-nonanone, very low detection limits were determined (0.04−0.06 μg/kg).

The limits of detection presented here are derived from the instrumental noise level, multiplied by a factor of 3 (Knoll, 1985) and converted to a concentration, using the calibration curve for the compound in question. This is a frequently used definition of a detection limit, but it should be realized that it takes into account only the instrumental variation (Kucharczyk, 1993). A detection limit based on actual observations, and thus including sampling uncertainties, presumably would be considerably higher. However, for direct chromatographic measurements, the 3 times SD_{blank} definition has been claimed to be 2−10 times higher than the limit determined from the actual signals from a calibration curve (Casado et al., 1996).

Applicability of the Method. For the set of parameters shown in Table 1 the methodology developed for salmon meat was tested on four other species of fish: cod, saithe, mackerel, and redfish. For headspace sampling from cod, it was found necessary to add an antifoaming preparation.

Table 5 summarizes the levels of the quantified volatiles in fresh fish of the five species. Hexanal, heptanal, octanal, (*E*,*E*)-2,4-heptadienal, 2-nonanone, and nonanal were detected in all five species. The level of these volatiles was 1−100 μg/kg except for (*E*,*E*)-2,4-heptadienal in mackerel (≈200 μg/kg). The levels of hexanal and (*E*,*Z*)-2,6-nonadienal in fresh salmon are the same as reported elsewhere, 35 and 9.3 μg/kg, respectively (Milo and Grosch, 1996). Most of the volatiles quantified in salmon gave rather high standard deviations (Table 5). This could be due to biological variation of lipid and lipid constituents in the five analyzed salmon. The biological variations, in e.g., lipid content and levels of different fatty acids are high even in farmed salmon (Refsgaard et al., 1998b). Milo and Grosch (1996)

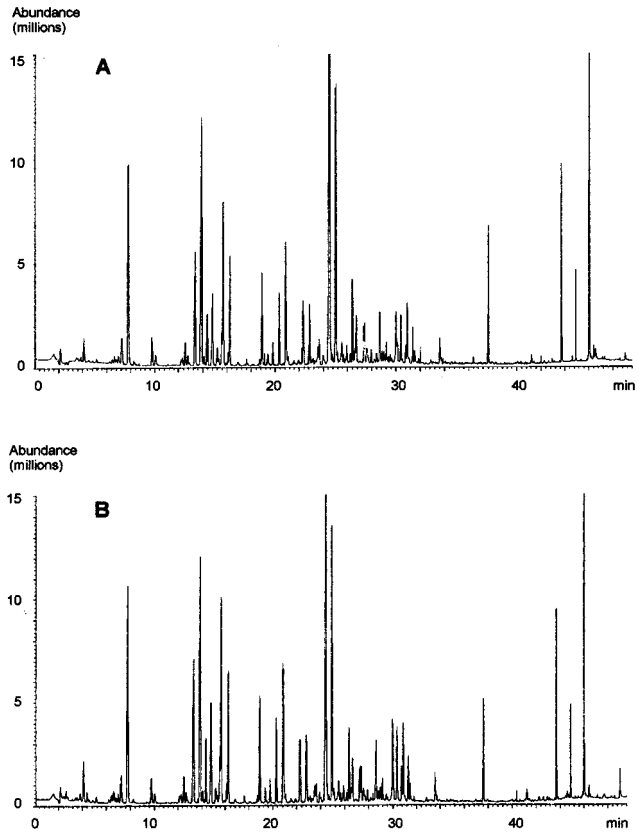

Figure 2. Total ion chromatograms of volatiles collected from a boiled sample of a salmon stored at −20 °C for 2 years. (A) Frozen in liquid nitrogen and powderized; (B) chewed for 10 s.

quantified (*E, E*)-2,4-nonadienal and (*E,E*)-2,4-decadienal in fresh cod and salmon (2−5 μg/kg) using vacuum distillation for collection of volatiles. These compounds could not be detected in fresh fish by the method presented here (Table 5). Of the volatiles quantified in this study, *(E)*-2-pentenal, hexanal, *(E)*-2-hexenal, and heptanal have previously been identified in mackerel (Alasalvar et al., 1997). To our knowledge the data given in Table 5 represent the first report on quantification of volatiles in fresh saithe, redfish, and mackerel.

After determination of the set of parameters to be used for the headspace sampling (Table 1), we investigated whether the sampled volatiles were representative of the volatiles in a chewed sample. This was done by real mouth experiments where the fish samples were chewed and the masticated fish samples were analyzed for volatiles by the dynamic headspace sampling method. The same volatile patterns were determined for the real mouth experiments and for the fish powder sampling method (Figure 2). This indicates that the developed sample preparation method gave a representation of the volatiles released from a sample that has been in the oral cavity and that no extra volatiles were released due to the mastication.

The presented headspace sampling method is accurate and fast and can be used for both identification and quantification of volatiles in fish.

ABBREVIATIONS USED

ATD, automatic thermal desorber/desorption; CV, coefficient of variation; GC−MS, gas chromatography−mass spectrometry; RI, retention index; SD, standard deviation; TIC, total ion current/chromatogram.

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