Detection of Odor Defects in Boiled Cod and Trout by Gas Chromatography–Olfactometry of Headspace Samples

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The most potent high volatile odorants occurring in the air above boiled cod and trout were evaluated by GCO of decreasing headspace volumes. Acetaldehyde, dimethyl sulfide, dimethyl trisulfide, and (*Z*)-1,5-octadien-3-one were found in small headspace samples of boiled cod (*Gadus morhua*), and acetaldehyde, propionaldehyde, methional, 1-octen-3-one, and (*Z*)-1,5-octadien-3-one in that of boiled trout (*Salmo fario*). Storage of the raw material at −13 °C led to odor defects in the boiled fish. The increase of trimethylamine, butane-2,3-dione, methylpropanal, and 2- and 3-methylbutanal in cod and that of acetaldehyde, propionaldehyde, butane-2,3-dione, pentane-2,3-dione, and C_6, C_8, and C_9 carbonyl compounds in trout contributed to the formation of the odor defects.

Keywords: *Aroma; boiled fish; headspace analysis*

As recently reviewed (Acree, 1993; Grosch, 1993) two techniques, Charm Analysis and Aroma Extract Dilution Analysis (AEDA), are suitable to screen the potent odorants of foods. However, the two dilution techniques are limited to odorants boiling higher than the solvent used for the extraction and dilution steps. Furthermore, odorants boiling in the same range as the extraction solvent are partially lost during the concentration of the extract by distilling off the solvent. To overcome these limitations, AEDA was completed by gas chromatography–olfactometry of headspace samples (GCO-H).

The new technique has been applied to identify the potent high volatile odorants of roasted coffee (Holscher and Steinhart, 1992) as well as to objectify the odor differences of green and black tea (Guth and Grosch, 1993), of two virgin olive oil samples (Blekas et al., 1994), and of oatmeal extrusion products stored with and without the addition of α-tocopherol (Guth and Grosch, 1994). In these studies a series of decreasing headspace volumes was drawn from each sample and then analyzed by GCO. The odorants in the lowest headspace volume in which odor was detected by GCO show the highest odor unit value (Guadagni et al., 1966), which is defined as the ratio of the concentration of the compound in the headspace to its odor threshold. It is suggested that these potent odorants in addition to those revealed by AEDA contribute strongly to the odor of a food (Guth and Grosch, 1993).

In the present study the GCO-H procedure was applied to objectify the changes in the odors of boiled trout (*Salmo fario*) and boiled cod (*Gadus morhua*) as affected in both cases by the storage of the raw material at −13 °C. In the case of boiled trouts the AEDA reported earlier (Milo and Grosch, 1993) was completed by the GCO-H.

EXPERIMENTAL PROCEDURES

Fish. Fillets of Atlantic cod (*G. morhua*, 3 kg), free of skins, were obtained from a local market. The fillets were ground in a meat grinder, and the mince was divided in portions of 150 g each. Ten portions were sealed under vacuum in polyethylene bags (sample A), and the remaining 10 were sealed in the presence of air (sample B). Samples A and B were stored for 26 weeks at −60 and −13 °C, respectively. After storage, the samples were thawed overnight at 6 °C, freed from the polyethylene bags, then wrapped in aluminum foil in portions of 50 g, and finally boiled in a water bath for 10 min. Freshly harvested trouts (*S. fario*) were obtained from a local breeding station. After removal of the guts, five fish were individually put in polyethylene bags and stored at −13 °C. Approximately 1.5 h after catch (sample C) and after a storage period of 1 year (sample D), each fish was wrapped in aluminum foil and then boiled in a water bath for 15 min. After boiling, the trouts were cut into pieces, frozen in liquid nitrogen, and then homogenized in a Waring Blendor. The homogenates obtained (samples C and D) were immediately frozen and stored at −60 °C until the day of the experiment.

Chemicals. Pure samples of the compounds in Tables 1 and 2 were obtained commercially: **1**, hydrochloride of **3**, **4**–**8**, **10**, **16**, and **19** (Aldrich, Steinheim, Germany), **2** (Fluka, Neu-Ulm, Germany), **21** (Merck, Darmstadt, Germany), **23** and **26** (Alfa-Products, Karlsruhe, Germany). **12** was a gift from Dr. R. Emberger (Haarmann and Reimer, Holzminden, Germany).

The following compounds were synthesized according to the literature cited: (*Z*)-1,5-octadien-3-one and (*Z*)-3-hexenal (Ullrich and Grosch, 1988); dimethyl trisulfide and dimethyl tetrasulfide (Milligan et al., 1963); (*Z,Z*)-3,6-nonadienal (Milo and Grosch, 1993).

GCO-H. Analysis of the headspace samples was performed with a CP-9001 gas chromatograph connected to the purge and trap system TCT/PTI 4001 (Chrompack, Frankfurt, Germany). The TCT/PTI 4001 system was programmed and controlled via the keyboard of the gas chromatograph. The glass tube in the desorption heating block of the purge and trap facility was empty. The gas chromatograph was equipped with a cooling system for the oven (Chrompack) and with an RTX 5 (SE-54) fused silica capillary (30 m × 0.52 mm, film thickness 1.5 μm; Amchro, Sulzbach/Taunus, Germany). At the exit end of the capillary, the effluent was split (1 + 1, v/v) into an FID and a sniffing port by using deactivated fused silica capillaries (30 cm × 0.10 mm). The FID and the sniffing port were held at a temperature of 250 °C. Nitrogen (20 mL/min) was used as makeup gas for the FID. After each GCO-H run, the purge system was automatically cleaned (cleanup flow, 50 mL of helium; cleanup temperature, 275 °C). After boiling, the fish sample (50 g) was left for 5 min at room temperature, freed from the aluminum foil, put into a vessel (volume, 240 mL) which was sealed with a septum, and then held in a water bath at 40 °C. After 1 h and during a period of 1.5 h at the most, the headspace volumes detailed in Tables 1 and 2 were drawn by a gastight syringe and then injected with a velocity of 10 mL/min into the purge system which operated in the

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0021-8561/95/1443-0459$09.00/0

Table 1. Lowest Headspace Volumes Required To Perceive the Odorant at the Sniffing Port in GCO-H of Boiled Cod Minces after Storage of the Raw Material at Different Temperatures[a,b]

compound	RI[f] on RTX 5	odor description[c]	volume[d] (mL) A	B
acetaldehyde (**1**)[e]	<400	sweet	5	2.5
methanethiol (**2**)[e]	<400	sulfurous	10	20
trimethylamine (**3**)[e]	<500	amine-like	>20	5
dimethyl sulfide (**4**)[e]	505	cabbage-like	5	10
2-methylpropanal (**5**)[e]	≈550	malty	>20	5
butane-2,3-dione (**6**)[e]	595	buttery-like	10	2.5
3-methylbutanal (**7**)[e]	653	malty	20	0.5
2-methylbutanal (**8**)[e]	663	malty	>20	5
unknown (**9**)	785	vegetable-like, pungent	>20	10
methional (**10**)[f]	908	boiled potato-like	10	5
dimethyl trisulfide (**11**)[e]	977	cabbage-like, putrid	1	1
1-octen-3-one (**12**)[f]	980	mushroom-like	10	5
(*Z*)-1,5-octadien-3-one (**13**)[f]	983	geranium-like	5	5
unknown (**14**)	1077	mushroom-like, earthy	>20	10
dimethyl tetrasulfide (**15**)[f]	1232	cabbage-like, putrid	10	10

[a] The cod minces were stored for 26 weeks at −60 (A) and −13 °C (B) before boiling. [b] Samples A and B were thermostated at 40 °C during GCO-H. [c] Odor description assigned during GCO-H. [d] The lowest headspace volume required to perceive the odorant at the sniffing port. [e] The compound was identified by comparing it with the reference substance on the basis of the following criteria: retention time on the capillary RTX 5, mass spectra obtained by MS-EI and MS-CI, and odor quality perceived at the sniffing port. [f] The MS signals were too weak for an interpretation; the compound was identified by comparing it with the reference substance on the basis of the remaining criteria reported in footnote *e*. RI, retention index.

Table 2. Lowest Headspace Volumes Required To Perceive the Odorant at the Sniffing Port in GCO-H of Boiled Trouts before and after Storage of the Raw Material[a,b]

compound[c]	RI on RTX 5	odor description[d]	volume[e] (mL) C	D
acetaldehyde (**1**)[f]	<400	sweet	5	1
propionaldehyde (**16**)[f]	≈450	sweet	2.5	0.5
2-methylpropanal (**5**)[f]	≈550	malty	>30	5
butane-2,3-dione (**6**)[f]	595	buttery-like	10	2.5
unknown (**17**)	645	vegetable-like, pungent	30	2.5
3-methylbutanal (**7**)[f]	653	malty	>30	5
unknown (**18**)	685	pungent, ethereal	>30	10
pentane-2,3-dione (**19**)[f]	700	buttery-like	10	2.5
unknown (**20**)	748	vegetable-like	30	2.5
hexanal (**21**)[f]				
(*Z*)-3-hexenal (**22**)[f]	800	green	10	1
(*Z*)-4-heptenal (**23**)[f]	900	biscuit-like	30	5
methional (**10**)[g]	908	boiled potato-like	5	5
1-octen-3-one (**12**)[g]	980	mushroom-like	5	2.5
(*Z*)-1,5-octadien-3-one (**13**)[g]	983	geranium-like	2.5	0.5
octanal (**24**)[f]	1006	citrus-like	30	10
unknown (**14**)	1077	mushroom-like, earthy	30	10
(*Z*,*Z*)-3,6-nonadienal (**25**)[g]	1100	fatty, green	30	10
(*E*,*Z*)-2,6-nonadienal (**26**)[g]	1149	cucumber-like	30	5

[a] The trouts were freshly harvested (C) and stored for 1 year at −13 °C (D) before boiling. [b] Samples C and D were thermostated at 40 °C. [c] Numbers **1**, **5**−**7**, **10**, and **12**−**14** refer to Table 1. [d,e,f,g] Refer to footnotes *c*, *d*, *e*, and *f*, respectively, in Table 1.

desorption mode for 10 min at a temperature of 250 °C. The carrier gas helium (flow, 20 mL/min) swept the headspace sample into the trap (40 cm × 0.53 mm fused silica capillary coated with CP-sil 8CP, film thickness 5 μm), which was precooled with liquid nitrogen at −110 °C for 10 min. To start the GC run, the trap was heated very rapidly to 200 °C. This temperature was held for 1 min, and the sample was flushed by the helium (flow rate, 8 mL/min) onto the RTX 5 capillary which was held at a temperature of 5 °C. Immediately, the GC run was started, and the temperature of the oven was raised at 6 °C/min to 230 °C.

Mass Spectrometry. The apparatus used for GCO-H was modified. The RTX 5 capillary was replaced by a SE-54 fused silica capillary (30 m × 0.32 mm, film thickness 0.25 μm; J&W Scientific, Folsom, CA). The exit of the capillary was coupled with the mass spectrometer Incos XL (Finnigan, Bremen, Germany). The flow of the carrier gas helium was 2 mL/min, and after the start of a GC run, the temperature of the oven was held at 0 °C for 2 min and then raised at a rate of 6 °C/min to 230 °C. Headspace samples of 20 mL were analyzed as reported under GCO-H. Mass spectra in the electron impact mode (MS-EI) were generated at 70 eV and in the chemical ionization mode (MS-CI) at 110 eV with methane as reagent gas.

RESULTS AND DISCUSSION

Raw cod minces were stored for 26 weeks at −60 °C (sample A) and at −13 °C (sample B). After boiling, sample A exhibited a pleasant mild fishy odor, whereas sample B smelled putrid, fishy, and malty.

Before the beginning of GCO-H, the air above these samples, which had been positioned in the sealed vessel (cf. Experimental Procedures), was drawn by a gastight syringe and then directly sniffed by three assessors. They agreed that a volume of 10 mL was entirely sufficient to perceive the characteristic odors of samples A and B.

GCO-H was started with a headspace volume of 20 mL. As summarized in Table 1, 10 and 15 odorants were found in cod samples A and B. Then the headspace volume was stepwisely reduced to show the most potent odorants of the boiled cod (Table 1). In the first step, the volume was reduced from 20 to 10 mL. As displayed in the capillary gas chromatograms (Figure 1), GCO-H of the smaller volume indicated only 9 and 14 odorants in samples A and B, respectively. 3-Methylbutanal was not more detectable by GC-sniffing in sample A, and methanethiol was now lacking in sample B (Figure 1 and Table 1). Reduction of the headspace volume to 5 mL even enlarged the difference between samples A and B. Only 4 odorants (no. **1**, **4**, **11**, **13**) were perceivable in sample A and 10 (no. **1**, **3**, **5**−**8**, **10**−**13**) in sample B. A further reduction of the headspace volume indicated dimethyl trisulfide (**11**) as the most potent odorant of sample A, as a volume of only 1 mL was sufficient for its detection (Table 1). The trisulfide **11** was also perceived in 1 mL of headspace of sample B, but the malty odorant 3-methylbutanal was the sole compound that was sniffed in the volume of 0.5 mL (Table 1).

It is assumed that the odorants of boiled cod differing in the headspace volume by a factor greater than 2, which is within the limit of error of the GCO-H (Guth and Grosch, 1993), are responsible for the change of the pleasant mild fishy odor (A) into a putrid fishy and malty odor defect (B). According to Table 1 the headspace volume of the amine **3** as well as those of **5**−**8** and **14** was lower in sample B than in sample A, indicating their strong increase in the boiled cod which was prepared from the raw material stored at −13 °C.

The formation of the amine **3** is the characteristic feature for the deterioration of fish belonging to the gadoid species (Amano et al., 1963; Miller et al., 1972; Krzymien and Elias, 1990).

On the basis of their low headspace volume of 2.5 mL, propionaldehyde (**16**) and (*Z*)-1,5-octadien-3-one (**13**) were the most potent odorants of the freshly boiled trouts (C) followed by acetaldehyde (**1**), methional (**10**),

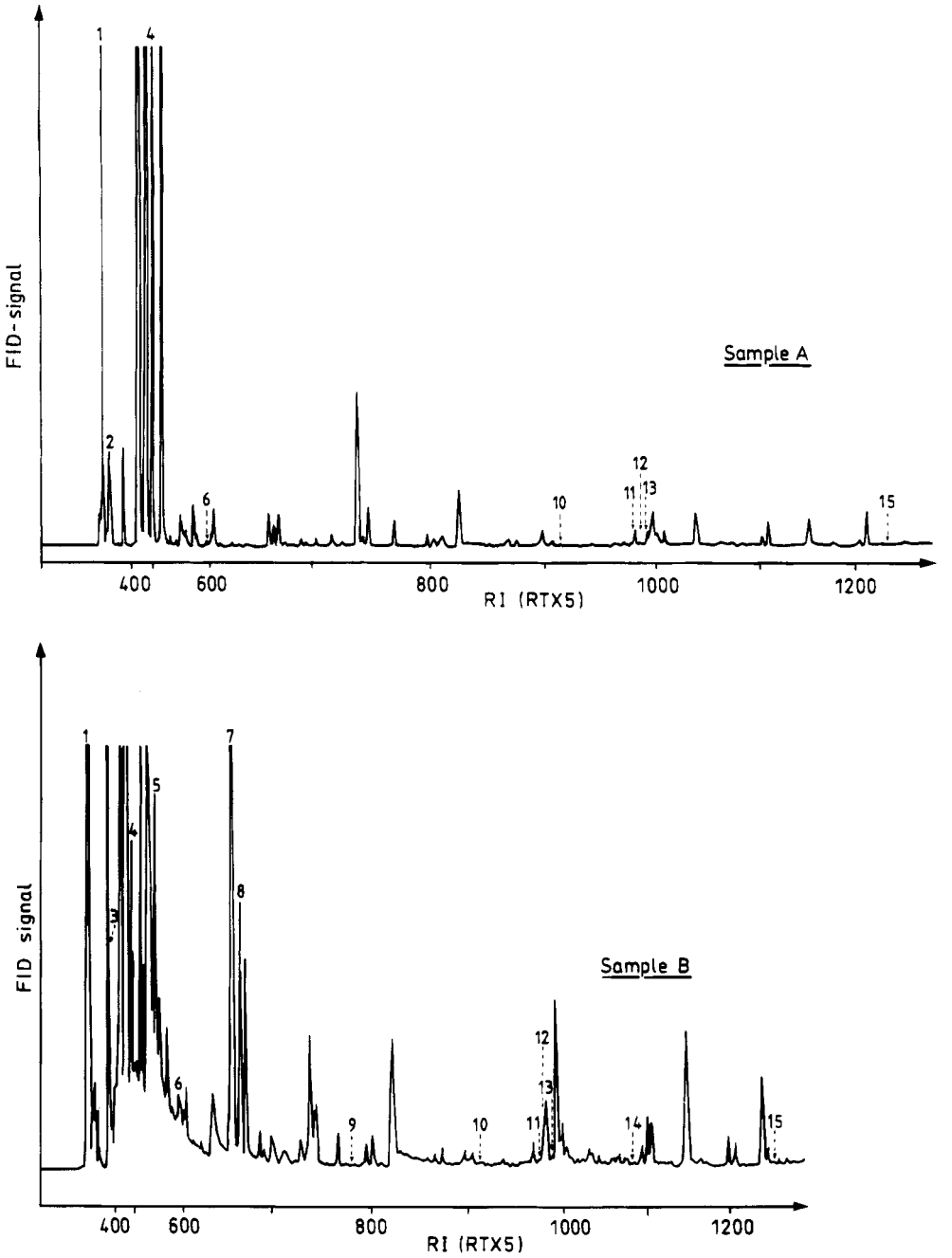

Figure 1. Capillary gas chromatograms obtained by GCO-H of cod samples A and B. A headspace volume of 10 mL was drawn from boiled cod mince of which the raw material had been stored for 26 weeks at −60 °C (sample A) and at −13 °C (sample B). The numbering of the odorants refers to Table 1.

and 1-octen-3-one (**12**) for which a volume of 5 mL was required (Table 2). Of these compounds, **10**, **12**, and **13** have been quantified in boiled trouts, and their odor activity values (OAVs, ratio of concentration to odor threshold) have been calculated in an earlier study on the basis of their corresponding odor thresholds in water (Milo and Grosch, 1993). The OAVs indicated that of the three compounds (*Z*)-1,5-octadien-3-one followed by methional was the most potent odorant of the freshly boiled trouts.

After storage of the raw material for 1 year at −13 °C (sample D), the boiled trouts exhibited a strong, train-like off-odor which was perceived in a headspace volume of 5 mL. The results of GCO-H summarized in Table 2 revealed that with the exception of methional and 1-octen-3-one, the concentrations of all of the odorants had increased in sample D. Hence, in the headspace volume of 5 mL and lower the odorants **5**−**7**, **17**, **19**−**23**, and **26**, in addition to **1**, **10**, **12**, **13** and **16**, already detected in the freshly boiled trouts (C), were additionally perceived in the boiled trouts prepared from the stored material (D). Of these odorants, **12**, **13**, **21**−**23**, **25**, and **26** were formed by the peroxidation of $n-3$ and $n-6$ polyunsaturated fatty acids (Josephson and Lindsay, 1986; Grosch, 1987) and were most likely important contributors of the trainy odor defect of sample D. The increase of (*Z*)-1,5-octadien-3-one and of the C_9 aldehydes **25** and **26**, when the trouts were stored at −13 °C, agreed with the quantitative results reported recently (Milo and Grosch, 1993). The absence of C_6 and C_9 aldehydes in cod sample B (Table 1) indicates that the peroxidation of unsaturated lipids with formation of these aldehydes did not play such an important role in the deterioration of cod as in that of trouts. Hexanal (**21**) and (*Z*)-3-hexenal (**22**) were not separated on the RTX 5 capillary used for the GCO-H

of the trout samples (Table 2). According to quantitative data (Milo and Grosch, 1993) the latter aldehyde might be more strongly involved than hexanal in the off-odor of sample D.

CONCLUSION

The odor difference of boiled cod minces and of boiled trouts can be objectified by gas chromatography–olfactometry of decreasing headspace samples. The new analytical method is suitable to show the high volatile odorants contributing to the off-odors that are formed when the raw material is inappropriately stored.

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Received for review June 16, 1994. Accepted November 10, 1994.[⊗]

JF9403200

[⊗] Abstract published in *Advance ACS Abstracts,* December 15, 1994.