Changes in the Odorants of Boiled Salmon and Cod As Affected by the Storage of the Raw Material

Christian Milo[†] and Werner Grosch*

Deutsche Forschungsanstalt für Lebensmittelchemie, Lichtenbergstrasse 4, D-85748 Garching, Germany

Homogenates of salmon (A, B) and of cod (C, D) were stored at −60 °C (A, C) and at −13 °C (B, D). After boiling, A and C exhibited the mild flavor of the fresh fish, whereas B smelled fatty and train-oily and D showed a malty odor defect. The potent odorants of the four samples were screened by dilution experiments and then quantified by stable isotope dilution assays. Calculation of odor activity values (ratio of concentration to odor threshold) revealed (Z)-1,5-octadien-3-one (**I**), (E,Z)-2,6-nonadienal (**II**), propionaldehyde (**III**), acetaldehyde (**IV**), and methional (**V**) as the character impact odorants of A as well as **I**, **II**, **IV**, **V**, and (E,E)-2,4-decadienal as those of C. The off-flavors, which were formed when the raw material was stored at the higher temperature, were mainly caused by an increase of **II**, (Z)-3-hexenal, and (Z,Z)-3,6-nonadienal in B and of 3-methylbutanal in D.

Keywords: *Fish; cod; salmon; odor; off-flavor; lipid peroxidation; determination; AEDA*

INTRODUCTION

The flavor profiles of cooked salmon and cooked cod differ in the aroma notes and in the intensity of these notes. Fresh fish and a slight fish oil character were the first flavor notes observed for salmon (Chambers and Robel, 1993). Further impressions were described as bitter, metallic, nutty/buttery, and sour. In contrast, the flavor of cooked cod, which was characterized with the terms fresh fish, sour, and slight cooked potato, was of low intensity (Prell and Sawyer, 1988).

The volatiles of raw and cooked salmon have been analyzed by static and dynamic headspace methods (Girard and Nakai, 1991; Josephson et al., 1991a,b). However, the sensory relevance of the many compounds identified was not determined. In an earlier study Josephson and Lindsay (1986) have suggested that 1-octen-3-one, (Z)-1,5-octadien-3-one, (E)-2-nonenal, and (E,Z)-2,6-nonadienal play an important role in fresh fish-like odors due to their low odor threshold values.

As recently reported for boiled trout (Milo and Grosch, 1993, 1995), the character impact compounds of the aroma were identified by a procedure that started with dilution experiments. The medium and higher boiling odorants were screened by aroma extract dilution analysis (AEDA) and the lower boiling ones by gas chromatography−olfactometry of headspace samples (GCO-H). Then the simplifications implicit in the dilution experiments were corrected by the quantification of the levels of these compounds in boiled trout. Calculation of the odor activity values (OAVs, ratio of concentration to odor threshold) indicated (Z)-1,5-octadien-3-one, (E,Z)-2,6-nonadienal, and methional as character impact odorants of freshly harvested and boiled trout.

Storage of the raw material for 14 weeks at −13 °C led to an unpleasant fatty, fishy off-flavor when the trout were boiled. This off-flavor was mainly caused by a strong increase in the concentration of (Z)-3-hexenal and (Z,Z)-3,6-nonadienal (Milo and Grosch, 1993).

The aim of the present study was to compare the odorants causing the typical aromas of boiled salmon and boiled cod. In addition, the differences in the levels of important odorants of the two boiled fish species were determined as affected by the storage of the raw material at −60 and −13 °C.

EXPERIMENTAL PROCEDURES

Fish. Fillets of Atlantic cod (*Gadus morhua*) without skin and of Atlantic salmon (*Salmo salar*) with the skin were from retail trade. The fillets (4 kg each fish) were ground in a meat grinder, and the homogenate obtained was sealed in polyethylene foil in portions of 250 g and immediately frozen. Half of the samples were stored at −60 °C and the other at −13 °C. After storage, the sample was wrapped in aluminum foil and then boiled in a water bath for 15 min. The boiled samples are given the following notations: A for salmon of which the raw material was stored at −60 °C; B for salmon stored at −13 °C; C for cod stored at −60 °C; D for cod stored at −13 °C. Different storage durations are indicated in the tables by the suffixes 1−3.

Chemicals. The following pure samples of the compounds listed in the tables were obtained commercially: **1−4**, **6**, **9**, **13**, **18**, **20**, **23**, **24**, **27**, **30−33** (Aldrich, Steinheim, Germany); **8**, **15** (Alfa Products, Karlsruhe, Germany); **11** (Lancaster, Mühlheim, Germany); **26**, **29** (Fluka, Neu-Ulm, Germany). (^{13}C)Acetaldehyde (**c-1**) was from Cambridge Isotope Laboratories, MA; ($^2H_6$)dimethyl sulfide (**d-30**), ($^2H_7$)isopropyl bromide, and triethyl orthoformate were from Aldrich; ($^2H_3$)-methyl iodide was from MSD Isotopes, Montreal, PQ. The following reference substances were synthesized according to the literature cited: **7**, **12** (Ullrich and Grosch, 1988a); **10**, **28** (Milligan et al., 1963); **14** (Milo and Grosch, 1993); **17** (Buttery et al., 1983); **19** (Ullrich and Grosch, 1988b); **25** (Schieberle and Grosch, 1991).

The internal standards used for the stable isotope dilution assays (IDA) were labeled either with deuterium (**d**) or carbon-13 (**c**).

($^2H_3$)Dimethyl trisulfide (**d-10**) was obtained by a reaction of ($^2H_3$)methyl thiosulfate with sodium sulfide (Milligan et al., 1963). ($^2H_3$)CH$_3$I (15 mmol) was added to an aqueous solution of sodium thiosulfate (15 mmol, 6 mL). The mixture was stirred for 2 h at room temperature and then extracted with diethyl ether (3 × 5 mL). The organic layer was discarded, and the stirred aqueous layer was saturated with NaCl. Then aqueous solutions of formaldehyde (35 wt %, 3 mL) and sodium sulfate (1 mol/L, 7.5 mL) were added dropwise one after another, while the pH of the reaction mixture was kept at 7−8

---

* Author to whom correspondence should be addressed.

† Present address: Department of Food Science and Nutrition, University of Minnesota, St. Paul, MN.

by addition of an aqueous solution of HCl (6 mol/L). Stirring was continued for 2 h, and then **d-10** formed was extracted with diethyl ether (3 × 5 mL). The extract was dried over anhydrous Na_2SO_4.

MS-EI: 132 (M$^+$, 100%), 82 (45%), 46 (35%), 50 (20%), 64 (18%), 114 (16%).

($^2H_7$)Methylpropanal (**d-31**). The Grignard reaction of ($^2H_7$)-isopropyl bromide with triethyl orthoformate yielded ($^2H_7$)-1,1-diethoxy-2-methylpropane, which was then hydrolyzed with formation of **d-31**.

($^2H_7$)Isopropyl bromide (10 mmol) dissolved in diethyl ether (6 mL) was dropped into a stirred suspension of magnesium turnings (11 mmol) in diethyl ether (5 mL). The mixture was refluxed for 40 min and then cooled to 0 °C. After dropwise addition of triethyl orthoformate (10 mmol), the mixture was stirred for 30 min at 0 °C, then for 1 h at room temperature, and finally for 4 h at 40 °C. After the mixture was allowed to stand overnight at room temperature, diethyl ether (20 mL) and sulfuric acid (5 wt %, 20 mL) were added and then **d-31** together with the diethyl ether was distilled off. The distillate was extracted with aqueous sodium bisulfite (15 wt %, 20 mL), and the extract containing the bisulfite adduct of **d-31** was washed with diethyl ether (3 × 10 mL), acidified with aqueous HCl (6 mol/L), and saturated with NaCl. The liberated **d-31** was extracted with diethyl ether (3 × 20 mL), and the organic layer was neutralized by washing with aqueous $NaCO_3$ (0.5 mol/L) and then dried over anhydrous Na_2SO_4.

MS-EI: 50 (100%), 46 (80%), 79 (M$^+$, 70%).

The following labeled internal standards were synthesized according to the literature cited: **d-2**, **d-4**, **d-14** (Milo and Grosch, 1993); **c-3**, **d-26** (Schieberle et al., 1993); **d-6**, **d-23** (Guth and Grosch, 1993a); **d-7**, **d-11**, **d-12**, **d-15**, **d-20**, **d-24** (Guth and Grosch, 1990); **d-8** (Widder and Grosch, 1994); **d-9** (Sen and Grosch, 1991); **d-27**, **d-29** (Guth and Grosch, 1994); **d-33** (Schieberle and Grosch, 1992).

Identification of Odorants. After the fish were boiled, the volatiles were isolated from the samples, 250 g for AEDA (Milo and Grosch, 1993) and 50 g for GCO-H (Guth and Grosch, 1993b; Milo and Grosch, 1995).

Quantification of Odorants. *Analysis of Extracts.* The boiled fish sample was extracted with diethyl ether (Milo and Grosch, 1993), and the extract obtained was divided in a ratio of 1 to 5. The smaller portion, which was denoted FI, was spiked with **c-3**, **d-4**, **d-6**, **d-26**, and **d-27** and the greater portion, FII, with **d-7** to **d-9**, **d-11**, **d-12**, **d-14**, **d-15**, **d-20**, **d-23**, and **d-24**. The amounts varied between the 0.2- and the 5-fold concentration of the odorant to be estimated. FI and FII were concentrated to 150 mL, and the volatiles were distilled off from the nonvolatile materials (Milo and Grosch, 1993). Odorants **3**, **4**, **6**, **26**, and **27** were determined in FI by high-resolution gas chromatography–mass spectrometry (HRGC–MS) using the conditions previously described (Guth and Grosch, 1990; Milo and Grosch, 1993).

The distillate obtained from FII was extracted with aqueous Na_2CO_3 (0.5 mol/L, 3 × 50 mL), washed neutral with a saturated sodium chloride solution, dried over anhydrous Na_2SO_4, and finally concentrated to 200 μL. Odorants **7**–**9**, **11**, **12**, **14**, **15**, **20**, **23**, and **24** were determined by HRGC–MS (Guth and Grosch, 1990; Milo and Grosch, 1993).

Static Headspace Analysis (SHA). Odorants **1**, **2**, and **29**–**33** were determined by SHA. The boiled fish homogenate (25 g) was put into a vessel (volume 240 mL) and blended with water (25 mL) containing the internal standards **c-1**, **d-2**, **d-30**, **d-31**, and **d-33** (standard for the isomers **32** and **33**). The amount of each labeled standard varied between the 0.2- and 5-fold concentration of the odorant to be estimated. The vessel was sealed with a septum. In a separate experiment, a known amount of **d-29** (Guth and Grosch, 1994) was injected by a gastight syringe into the stirred fish sample. Stirring at 40 °C was continued for 30 min. A headspace volume of 20 mL was withdrawn by a gastight syringe and then injected into a CP-9001 gas chromatograph (Guth and Grosch, 1994; Semmelroch and Grosch, unpublished results) which was connected to the purge and trap system TCT/PTI 4001 (Chrompack, Frankfurt, Germany). The gas chromatograph was equipped with the fused silica capillaries RTX 5 (SE-54; 30 m × 0.52

Table 1. Thin Film Capillaries, Selected Ions, and Calibration Factors for Mass Chromatography of the Odorants 1, 2, and 29–33

odorant	capillary	ion (m/z)	internal standard	ion (m/z)	calibration factor
acetaldehyde (**1**)	DB-Wax	45	**c-1**	47	1.00
propionaldehyde (**2**)	DB-Wax	59	**d-2**	62	1.14
methanethiol (**29**)	SE-54	49	**d-29**	52	1.00
dimethyl sulfide (**30**)	DB-Wax	63	**d-30**	69	1.10
methylpropanal (**31**)	SE-54	73	**d-31**	80	1.00
2-methylbutanal (**32**)	SE-54	87	**d-33**	71	0.74
3-methylbutanal (**33**)	SE-54	69	**d-33**	71	0.88

mm, 1.5 μm film thickness) supplied from Amchro, Sulzbach, Germany, and DB-Wax (50 m × 0.32 mm, 1 μm film thickness) supplied from J&W Scientific, Folsom, CA. After application of the sample, the temperature of the RTX 5 capillary ws heated at 6 °C/min from 5 to 230 °C; the temperature of the DB-Wax capillary was held for 2 min at 20 °C and then heated to 230 °C at 6 °C/min. The resting conditions were identical with those reported recently (Guth and Grosch, 1993, 1994).

The gas chromatograph was coupled with the MS system INCOS XL (Finnigan, Bremen, Germany). Mass chromatograms were recorded in the chemical ionization mode with methane as reagent gas and by using the conditions listed in Table 1.

Analysis of Lipids. Lipids were extracted from the fish homogenates according to the method of Bligh and Dyer (1959), but dichloromethane was used instead of chloroform. After transesterification, the fatty acid composition was determined by gas chromatography (Christie, 1982).

RESULTS AND DISCUSSION

Raw salmon homogenates were stored for 26 weeks at −60 °C (sample A1) and −13 °C (sample B1). After boiling, sample A1 exhibited the pleasant aroma profile reported by Chambers and Robel (1993) for cooked salmon. In contrast, sample B1 smelled fatty and train-oily.

GCO-H revealed propionaldehyde (**2**) and (*Z*)-1,5-octadien-3-one (**12**) as the most potent high volatile odorants of sample A1, as they were detectable in the lowest headspace volumes of 0.5 and 1 mL, respectively, in which an odorant was perceived (Table 2). Five to tenfold higher volumes were necessary to detect the next important odorants acetaldehyde (**1**), hexanal (**6**)/(*Z*)-3-hexenal (**7**), methional (**9**), dimethyl trisulfide (**10**), and 1-octen-3-one (**11**) in the headspace of sample A1.

Strong differences between samples A1 and B1 were found for the odorants propionaldehyde (**2**), hexanal (**6**)/(*Z*)-3-hexenal (**7**), and (*Z,Z*)-2,6-nonadienal (**14**) (Table 2), as their concentrations were at least 3-fold higher in sample B1. Consequently, lower headspace volumes were sufficient for the detection of these compounds by GCO-H (Table 2).

The medium and higher boiling odorants were screened by AEDA of which the raw material had been stored for 10 weeks before boiling (Table 3). This experiment revealed (*Z*)-1,5-octadien-3-one (**12**) again as the most important odorant in both samples followed by methional (**9**) and (*E,Z*)-2,6-nonadienal (**15**).

The salmon samples screened by AEDA were stored for a shorter time at −13 °C than the samples analyzed by GCO-H. This difference might be the reason that the flavor dilution (FD) factors of the odorants did not differ greatly between samples A2 and B2 (Table 3). Only the odorants (*Z,Z*)-3,6-nonadienal (**14**), 2-acetyl-1-pyrroline (**17**), unknown (**21**), and (*E,E*)-2,4-nonadienal (**23**) were exceptions, as their FD factors were 8 and 4 times, respectively, higher in sample B2.

2368 *J. Agric. Food Chem.,* Vol. 44, No. 8, 1996

Milo and Grosch

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

compound	RI[a] on RTX 5	odor description[c]	volume[d] (mL) A1	volume[d] (mL) B1
acetaldehyde (**1**)[e]	<500	sweet	2.5	1
propionaldehyde (**2**)[e]	>500	sweet	0.5	0.1
butane-2,3-dione (**3**)[e]	595	buttery	10	10
pentane-2,3-dione (**4**)[e]	700	buttery	10	5
unknown (**5**)	748	vegetable-like	10	5
hexanal/(Z)-3-hexenal (**6**[e]/**7**)[f]	800	green	5	1
(Z)-4-heptenal (**8**)[f]	900	biscuit-like	10	5
methional (**9**)[f]	908	boiled potato-like	5	5
dimethyl trisulfide (**10**)[f]	977	cabbage-like	5	5
1-octen-3-one (**11**)[f]	980	mushroom-like	5	5
(Z)-1,5-octadien-3-one (**12**)[f]	983	geranium-like, metallic	1	1
octanal (**13**)[f]	1006	citrus-like	30	>10
(Z,Z)-3,6-nonadienal (**14**)[f]	1100	fatty, green	30	10
(E,Z)-2,6-nonadienal (**15**)[f]	1149	cucumber-like	10	10

[a] The materials were stored for 26 weeks at −60 °C (A1) and −13 °C (B1) 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. [g] RI, retention index.

Table 3. Potent Odorants (FD Factor ≥8) in Boiled Salmon: Influence of the Storage of the Raw Material on the FD Factors

compound[a]	RI on SE-54	odor description[b]	FD factor[c] A2	FD factor[c] B2
unknown (**16**)	836	vegetable-like	8	16
(Z)-4-heptenal (**8**)[d]	900	biscuit-like	32	32
methional (**9**)[d]	908	boiled potato-like	256	256
2-acetyl-1-pyrroline (**17**)[e]	925	roasty	8	32
1-octen-3-one (**11**)[d]	981	mushroom-like	64	128
(Z)-1,5-octadien-3-one (**12**)[d]	985	geranium-like	1024	1024
(E,E)-2,4-heptadienal (**18**)[e]	1011	green, tallowy	<8	8
(Z,Z)-3,6-nonadienal (**14**)[d]	1100	fatty, green	64	512
(Z)-2-nonenal (**19**)[e]	1148	fatty, green	8	16
(E,Z)-2,6-nonadienal (**15**)[d]	1154	cucumber-like	128	128
(E)-2-nonenal (**20**)[e]	1162	cardboard-like	8	<8
unknown (**21**)	1179	fruity	8	32
2,4-nonadienal (**22**)[d]	1195	green, tallowy	16	8
(E,E)-2,4-nonadienal (**23**)[d]	1216	green, tallowy	8	32
(E,E)-2,4-decadienal (**24**)[e]	1318	fatty	32	32
trans-4,5-epoxy-(E)-2-decenal (**25**)[e]	1384	metallic	16	8
butyric acid (**26**)[d,f]	1639	sweaty	16	nd[g]
2-/3-methylbutyric acid (**27**)[d,f]	1678	sweaty	8	nd

[a] Numbers **6**-**9**, **11**, **12**, **14**, **15** refer to Table 2. [b] Odor description assigned during AEDA. [c] The materials were stored for 10 weeks at −60 °C (A2) and at −13 °C (B2) before boiling. [d] The compound was identified by comparing it with the reference substance on the basis of the following criteria: RI on the capillaries SE-54 and OV-1701, mass spectra obtained by MS-EI and MS-CI, and odor quality perceived at the sniffing port. [e] 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 d. [f] RI and FD factor on capillary DB-Wax. [g] nd, not determined.

AEDA of boiled cod was performed after a longer storage period of 20 weeks (Table 4), which led to a change of the mild fishy odor (C1) into a putrid fishy and malty odor defect (D1). Greater differences in the FD factors between samples C1 and D1 were only found

Table 4. Potent Odorants (FD Factor ≥8) in Boiled Cod: Influence of the Storage of the Raw Material on the FD Factors

compound[a]	RI on SE-54	odor description[b]	FD factor[c] C1	FD factor[c] D1
pentane-2,3-dione (**4**)[d]	700	buttery	32	<8
hexanal/(Z)-3-hexenal (**6**[e]/**7**)[e]	800	green	16	8
(Z)-4-heptenal (**8**)[e]	900	biscuit-like	8	8
methional (**9**)[d]	908	boiled potato-like	128	256
2-acetyl-1-pyrroline (**17**)[e]	925	roasty	16	32
dimethyl trisulfide (**10**)[d]	957	cabbage-like	8	32
1-octen-3-one (**11**)[d]	981	mushroom-like	256	64
(Z)-1,5-octadien-3-one (**12**)[d]	985	geranium-like	64	256
(E,Z)-2,6-nonadienal (**15**)[d]	1154	cucumber-like	64	16
2,4-nonadienal (**22**)[d]	1193	fatty	32	<8
dimethyl tetrasulfide (**28**)[e]	1212	cabbage-like, putrid	16	21
(E,E)-2,4-nonadienal (**23**)[e]	1216	fatty	32	32
(E,E)-2,4-decadienal (**24**)[d]	1328	fatty	32	16
trans-4,5-epoxy-(E)-2-decenal (**25**)[e]	1384	metallic	16	8

[a] Numbers **4**, **6**-**12**, **15** refer to Table 2 and **17** and **22**-**25** to Table 3. [b,d,e] Refer to footnotes b, d and e in Table 3. [c] The materials were stored for 20 weeks at −60 °C (C1) and at −13 °C (D1).

for pentane-2,3-dione (**4**), dimethyl trisulfide (**10**), (Z)-1,5-octadien-3-one (**12**), and (E,Z)-2,6-nonadienal (**15**) (Table 4). This indicates that the concentrations of pentane-2,3-dione (**4**) and 1-octen-3-one (**11**) were higher in the sample stored at the low temperature of −60 °C (C1), whereas those of dimethyl trisulfide (**10**), (Z)-1,5-octadien-3-one (**12**), and (E,Z)-2,6-nonadienal (**15**) had increased so strongly during storage at −13 °C that their FD factors were higher in D1 than in C1.

It has been reported by Josephson et al. (1991b) that model systems consisting of carotenoid-rich salmon oil, which was adsorbed onto a Celite support and then allowed to oxidize at room temperature, rapidly developed a cooked salmon loaf-like aroma. We have repeated this model experiment and smelled indeed an intense cooked salmon loaf-like aroma when salmon oil (10 g) onto Celite (10 g) was stored for 24 h at room temperature in the dark. However, gas chromatography—olfactometry of the volatiles formed revealed only odorants listed in the Tables 2 and 3. There was no indication that an odorant originating from a breakdown of carotenoids was involved in the aroma of cooked salmons.

We have assumed that odorants found in headspace volumes of 10 mL and lower (Table 2) and those showing FD factors of 32 and higher (Table 3) contribute to the smell of boiled salmon. With the exception of dimethyl trisulfide (**10**), these odorants and in addition (E)-2-nonenal (**20**), butyric acid (**26**), and 2-/3-methylbutyric acid (**27**) were quantified by IDA in the raw salmon homogenate after storage for 14 weeks at −60 °C (raw A3 in Table 5) as well as in the boiled homogenate of which the raw material was stored for 14 weeks at −60 °C (A3 boiled) and −13 °C (B3).

A comparison of raw and boiled A3 indicates a loss of the high volatile odorants acetaldehyde (**1**) and propionaldehyde (**2**) during cooking (Table 5). Furthermore, the concentrations of butane-2,3,-dione (**3**), 1-octen-3-one (**11**), and (Z)-1,5-octadien-3-one (**12**) were somewhat smaller in boiled A3 than in the corresponding raw material, but in particular the concentrations of pentane-2,3-dione (**4**), hexanal (**6**), (Z)-3-hexenal (**7**), (Z)-4-heptenal (**8**), and methional (**9**) were increased at least by 50% (Table 5). Storage of the raw salmon homogenate at the higher temperature of −13 °C (B3 in Table

Salmon and Cod Odorants after Storage

J. Agric. Food Chem., Vol. 44, No. 8, 1996 **2369**

Table 5. Concentration and Odor Activity Values of Potent Odorants of Raw and Boiled Salmon Homogenates: Influence of the Storage of the Raw Material at Different Temperatures on the Odorants of the Boiling Aroma[a]

	concn[c,d]			odor act. value[e]		
odorant[b]	A3 (raw)	A3 (boiled)	B3	A3 (raw)	A3 (boiled)	B3
acetaldehyde (**1**)	3700	2300	2500	370	230	250
propionaldehyde (**2**)	3500	1700	4700	500	240	670
butane-2,3-dione (**3**)	57	52	nd[f]	11	10	nd
pentane-2,3-dione (**4**)	141	234	318	28	47	64
hexanal (**6**)	35	58	148	3	6	14
(*Z*)-3-hexenal (**7**)	2.6	3.9	50	87	130	1667
(*Z*)-4-heptenal (**8**)	3.0	6.0	47	50	100	783
methional (**9**)	3.0	8.0	4.4	75	200	110
1-octen-3-one (**11**)	0.5	0.4	0.4	50	40	40
(*Z*)-1,5-octadien-3-one (**12**)	0.4	0.3	0.4	1000	750	1000
(*Z,Z*)-3,6-nonadienal (**14**)	nd	5.7	49	nd	114	980
(*E,Z*)-2,6-nonadienal (**15**)	9.3	9.7	26	465	485	1300
(*E*)-2-nonenal (**20**)	2.0	2.7	6.4	25	34	80
(*E,E*)-2,4-nonadienal (**23**)	2.2	2.6	3.7	37	43	62
(*E,E*)-2,4-decadienal (**24**)	4.8	6.0	18	160	200	600
butyric acid (**26**)	1027	1137	1634	<1	<1	<1
2-/3-methylbutyric acid (**27**)	81	122	147	<1	<1	<1

[a] The materials were stored for 14 weeks at −60 °C (A3) and at −13 °C (B3). [b] Numbers **1**−**4**, **6**−**9**, **11**, **12**, **14**, and **15** refer to Table 2 and **20**, **23**, **24**, **26**, and **27** to Table 3. [c] The odorants were determined in sample A3 before and after boiling and in boiled sample B3. [d] Values are in micrograms per kilogram of raw and boiled fish, respectively. The data are mean values of duplicates. [e] The odor activity values were calculated by dividing the concentration by the retronasal threshold in water which were obtained for **3**−**9**, **11**−**15**, **20**, and **23** from Milo and Grosch (1993), for **24** from Konopka et al. (1995), and for **26** from Patton (1964); the following retronasal odor threshold values (μg/L) were determined by Guth (unpublished): **27** (750 at pH 7); the values for **1** (10) and **2** (7) were determined in this study. [f] nd, not determined.

5) resulted in significantly higher concentrations for most of the odorants. (*Z*)-3-Hexenal (**7**), (*Z*)-4-heptenal (**8**), (*Z,Z*)-3,6-nonadienal (**14**), and (*E,E*)-2,4-decadienal (**24**) were predominant examples, since their concentrations were at least 3-fold higher in B3 than in boiled A3.

Calculation of OAVs indicated (*Z*)-1,5-octadien-3-one (**12**) followed by acetaldehyde (**1**), propionaldehyde (**2**), (*E,Z*)-2,6-nonadienal (**15**), and (*E,E*)-2,4-decadienal (**24**) as the key aroma compounds of raw and boiled salmon (A3 in Table 5). The difference in the overall odor between raw and boiled A3 might be caused by the decrease in the OAVs of **1**, **2**, and **12** and by the increase in the OAVs of **7**−**9**.

The concentrations of **4**, **6**−**9**, **11**, **12**, **14**, **15**, **20**, and **23** were also determined in homogenates of boiled trout which were freshly harvested (Milo and Grosch, 1993). Although the calculation of OAVs indicated that methional (**9**), (*Z*)-1,5-octadien-3-one (**12**), and (*E,Z*)-2,6-nonadienal (**15**) were the same key odorants in boiled trout as in boiled salmon A3 (Table 5), the smells of the trout and salmon were different; in particular, the fish oil character was stronger in A3. We suggest that this difference might be caused by the higher concentrations of (*Z*)-1,5-octadien-3-one (**12**) and (*E,Z*)-2,6-nonadienal (**15**) in A3 (Table 5). Furthermore, A3 contained so much (*Z*)-3-hexenal (**7**), (*Z*)-4-heptenal (**8**), and (*Z,Z*)-3,6-nonadienal (**14**) that in contrast to the boiled trout these aldehydes contribute more to the overall odor of A3 than to that of trout. One reason for the differences in the levels of the odorants **7**, **8**, **12**, and **15**, which are all formed by a peroxidation of *n*−3 unsaturated fatty acids (PUFA) (Josephson and Lindsay, 1986; Grosch, 1987), might be the higher amounts of these PUFA in salmon than in trout (Milo, 1994).

The aroma of salmon was more affected by the storage conditions than by the boiling process. As summarized in Table 5, storage of the raw material at the higher temperature (B3) led to a strong increase of the OAV of (*Z*)-3-hexenal (**7**), (*E,Z*)-2,6-nonadienal (**15**), (*Z,Z*)-3,6-nonadienal (**14**), (*Z*)-4-heptenal (**8**), and (*E,E*)-2,4-deca-

Table 6. Fatty Acid Composition of Salmon and Cod Lipids[a]

	wt %			wt %	
fatty acid	salmon	cod	fatty acid	salmon	cod
14:0	5.0	1.1	20:1 *n*−9	14.6	1.0
16:0	13.2	19.9	20:4 *n*−6	1.4	6.0
16:1	7.0	2.4	20:4	0.3	0.8
18:0	2.2	3.2	20:5 *n*−3	4.8	16.5
18:1	15.7	13.2	22:1	14.2	<0.2
18:2 *n*−6	2.2	1.0	22:5 *n*−3	2.0	3.0
18:3 *n*−3	0.9	0.2	22:6 *n*−3	11.6	28.7
18:4 *n*−3	1.7	1.4	others	1.6	2.4

[a] Lipid content (percentage wet weight) of the salmon (16.4) and the cod (0.6).

dienal (**24**). With the exception of the latter, these odorants, as discussed above, were formed by a peroxidation of *n*−3 PUFA. Indeed, the salmon homogenate contained 16.4% lipids, in which 22:6 was the major *n*−3 fatty acid (Table 6).

Altogether, 19 odorants, which were selected on the basis of the results of both AEDA (Table 4) and GCO-H (Milo and Grosch, 1995), were quantified in boiled cod of which the raw material was stored for 14 weeks at −60 °C (C2) and −13 °C (D2).

Cod, in contrast to salmon, was a lean fish containing only 0.6% lipids (Table 6). Consequently, it was not surprising that the carbonyl compounds (*Z*)-3-hexenal (**7**), (*Z*)-4-heptenal (**8**), (*Z*)-1,5-octadien-3-one (**12**), and (*E,Z*)-2,6-nonadienal (**15**), which originate from *n*−3 PUFA (Josephson and Lindsay, 1986; Grosch, 1987), were much lower in fresh boiled cod (Table 7, C2) than in fresh boiled salmon (Table 5, A3). However, the reverse was found for hexanal (**6**), 1-octen-3-one (**11**), and (*E,E*)-2,4-nonadienal (**23**) (Table 7), which were formed by a peroxidation of *n*−6 PUFA. The reason for the preferential formation of these carbonyl compounds in cod is an open question.

On the basis of their high OAVs, acetaldehyde (**1**), methional (**9**), (*Z*)-1,5-octadien-3-one (**12**), (*E,Z*)-2,6-nonadienal (**15**), (*E,E*)-2,4-decadienal (**24**), and 3-meth-

Table 7. Concentration and Odor Activity Values of Potent Odorants of Boiled Cod Homogenates after Storage of the Raw Material at Different Temperatures[a]

odorant[b]	concn[c] C2	concn[c] D2	odor act. values[d] C2	odor act. values[d] D2
acetaldehyde (**1**)	1300	2400	130	240
butane-2,3-dione (**3**)	200	596	40	119
pentane-2,3-dione (**4**)	86	26	17	5
hexanal (**6**)	115	28	11	3
(Z)-3-hexenal (**7**)	1.3	4.3	43	143
(Z)-4-heptenal (**8**)	1.6	2.8	27	47
methional (**9**)	11	10	275	250
dimethyl trisulfide (**10**)	0.15	0.4	19	50
1-octen-3-one (**11**)	0.7	0.2	70	21
(Z)-1,5-octadien-3-one (**12**)	0.1	0.16	250	400
(Z,Z)-3,6-nonadienal (**14**)	1.3	4.2	26	84
(E,Z)-2,6-nonadienal (**15**)	3.5	2.8	175	140
(E,E)-2,4-nonadienal (**23**)	3.2	2.0	53	33
(E,E)-2,4-decadienal (**24**)	3.5	2.2	117	73
methanethiol (**29**)	100	130	50	65
dimethyl sulfide (**30**)	77	25	39	13
methylpropanal (**31**)	27	nd[e]	39	nd
2-methylbutanal (**32**)	20	270	22	386
3-methylbutanal (**33**)	51	620	204	2480

[a] The homogenate was stored for 14 weeks at −60 °C (C2) and at −13 °C (D2) before boiling. [b] Numbers **1**, **3**, **4**, **6**−**12**, **14**, and **15** refer to Table 2 and **23** and **24** to Table 3. [c] Values in micrograms per kilogram of boiled fish. The data are mean values of duplicates. [d] The odor activity values were calculated by dividing the concentration by the retronasal threshold in water which were obtained for **1**, **3**, **4**, **6**−**9**, **11**−**15**, **23**, and **24** as reported in footnote *e* of Table 5; the following retronasal odor threshold values (μg/L) were determined in this study: **10** (0.008), **30** (2), **31** (0.7), **32** (0.9), **33** (0.25); the value of **29** (2) was obtained from Fors (1988). [e] nd, not determined.

ylbutanal (**33**) are regarded as the character impact odorants of boiled cod (sample C2 in Table 7), which exhibited a pleasant mild fish flavor. The importance of compounds **9** and **12** for the flavor of boiled fish is underscored by the observation that an aqueous solution of **9** and **12** at the concentration levels found in boiled cod (C2, Table 7) smelled fishy (H. Guth, unpublished results).

Storage of the raw material at the higher temperature of −13 °C (D2 in Table 7) led to a malty odor defect in the boiled cod. We suggest that the malty-smelling 3-methylbutanal (**33**), which was 12 times higher in D2 than in C2 and reached by far the highest OAV, was responsible for the off-odor.

McGill et al. (1977) maintained a "cold storage flavor" in boiled cod of which the raw material was stored at −10 °C. The flavor defect was attributed to an increase of (Z)-4-heptenal (**8**) of which a concentration of 3.6 μg/kg was found after 200 days of storage. The data listed in Table 7 for sample D2 do not support this conclusion, as **8**, on the basis of its relatively low OAV, did not belong to the significant contributors to the overall odor of this cod sample.

Conclusion. (Z)-3-Hexenal and (Z,Z)-3,6-nonadienal, which have been detected as off-flavor substances in boiled trout, are also responsible for the flavor defect in boiled salmon when the frozen raw material is stored for a longer period. However, the levels of these odorants are higher in salmon than in trout because the former contains more *n*−3 unsaturated fatty acids, the precursors of the two aldehydes. Peroxidation of unsaturated fatty acids is not so important for the formation of an off-flavor in cod, as it is a lean fish. The malty flavor defect in boiled cod is caused by a strong increase in 3-methylbutanal.

LITERATURE CITED

Bligh, E. G.; Dyer, W. J. A rapid method for total lipid extraction and purification. *Can. J. Biochem. Physiol.* **1959**, *37*, 911−917.

Buttery, R. G.; Ling, L. C.; Juliano, B. O.; Turnbaugh, J. G. Cooked rice aroma and 2-acetyl-1-pyrroline. *J. Agric. Food Chem.* **1983**, *31*, 823−826.

Chambers, E.; Robel, A. Sensory characteristics of selected species of freshwater fish in retail distribution. *J. Food Sci.* **1993**, *58*, 508−512, 561.

Christie, W. W. *Lipid Analysis*; Pergamon Press: Oxford, U.K., 1982, p 53.

Girard, B.; Nakai, S. Static headspace gas chromatographic method for volatiles in canned salmon. *J. Food Sci.* **1991**, *56*, 1271−1274.

Grosch, W. In *Autoxidation of Unsaturated Lipids*; Chan, H. W.-S., Ed.; Academic Press: London, 1987; pp 95−139.

Guth, H.; Grosch, W. Deterioration of soya-bean oil. Quantification of primary flavour compounds using a stable isotope dilution assay. *Lebensm. Wiss. Technol.* **1990**, *23*, 513−522.

Guth, H.; Grosch, W. Odorants of extrusion products of oat meal. Changes during storage. *Z. Lebensm. Unters. Forsch.* **1993a**, *196*, 22−28 (in German).

Guth, H.; Grosch, W. Identification of potent odorants in static headspace samples of green and black tea powders on the basis of aroma extract dilution analysis (AEDA). *Flavour Fragrance J.* **1993b**, *8*, 173−178.

Guth, H.; Grosch, W. Identification of the character impact odorants of stewed beef juice by instrumental analyses and sensory studies. *J. Agric. Food Chem.* **1994**, *42*, 2862−2866.

Josephson, D. B.; Lindsay, R. C. In *Biogeneration of Aromas*; Parliment, T. H., Croteau, R., Eds.; ACS Symposium Series 317; American Chemical Society: Washington, DC 1986; pp 201−219.

Josephson, D. B.; Lindsay, R. C.; Stuiber, D. A. Influence of maturity on the volatile aroma compounds from fresh Pacific and Great Lakes salmon. *J. Food Sci.* **1991a**, *56*, 1576−1579, 1585.

Josephson, D. B.; Lindsay, R. C.; Stuiber, D. A. Volatile carotenoid-related oxidation compounds contributing to cooked salmon flavor. *Lebensm. Wiss. Technol.* **1991b**, *24*, 424−432.

Konopka, U. C.; Guth, H.; Grosch, W. Potent odorants formed by lipid peroxidation as indicators of the warmed-over flavour (WOF) of cooked meat. *Z. Lebensm. Unters. Forsch.* **1995**, *201*, 339−343.

McGill, A. S.; Hardy, R.; Gunstone, F. D. Further analysis of the volatile components of frozen cold stored cod and the influence of these on flavour. *J. Sci. Food Agric.* **1977**, *28*, 200−205.

Milligan, B.; Saville, B.; Swan, J. M. Trisulphides and tetra-sulphides from Bunte salts. *J. Chem. Soc.* **1963**, 3608−3614.

Milo, C. Thesis, Technical University of Munich, 1994 (in German).

Milo, C.; Grosch, W. Changes in the odorants of boiled trout (*Salmo fario*) as affected by the storage of the raw material. *J. Agric. Food Chem.* **1993**, *41*, 2076−2081.

Milo, C.; Grosch, W. Detection of odor defects in boiled cod and trout by gas chromatography-olfactometry of headspace samples. *J. Agric. Food Chem.* **1995**, *43*, 459−462.

Patton, S. Flavor thresholds of volatile fatty acids. *J. Food Sci.* **1964**, *29*, 679−680.

Prell, A.; Sawyer, F. M. Flavor profiles of 17 species of North Atlantic fish. *J. Food Sci.* **1988**, *53*, 1036−1042.

Schieberle, P.; Grosch, W. Potent odorants of the wheat bread crumb. *Z. Lebensm. Unters. Forsch.* **1991**, *192*, 130−135.

Schieberle, P.; Grosch, W. Changes in the concentrations of potent crust odourants during storage of white bread. *Flavour Fragrance J.* **1992**, *7*, 213−218.

Schieberle, P.; Gassenmeier, K.; Guth, H.; Sen, A.; Grosch, W. Character impact odour compounds of different kinds of butter. *Lebensm. Wiss. Technol.* **1993**, *26*, 347−356.

Salmon and Cod Odorants after Storage

J. Agric. Food Chem., Vol. 44, No. 8, 1996 **2371**

Semmelroch, P.; Grosch, W. Studies on character impact odorants of coffee brews. *J. Agric. Food Chem.* **1996**, *44*, 537−543.

Sen, A.; Grosch, W. Synthesis of six deuterated sulfur containing odorants to be used as internal standards in quantification assays. *Z. Lebensm. Unters. Forsch.* **1991**, *192*, 541−547.

Ullrich, F.; Grosch, W. Identification of the most intense odor compounds formed during autoxidation of methyl linolenate at room temperature. *J. Am. Oil Chem. Soc.* **1988a**, *65*, 1313−1317.

Ullrich, F.; Grosch, W. Flavour deterioration of soya-bean oil: identification of intense odour compounds during flavour reversion. *Fat Sci. Technol.* **1988b**, *90*, 332−336.

Widder, S.; Grosch, W. Study on the cardboard off-flavour formed in butter oil. *Z. Lebensm. Unters. Forsch.* **1994**, *198*, 297−301.