Changes in the Odorants of Boiled Trout (*Salmo fario*) As Affected by the Storage of the Raw Material

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Aroma extract dilution analysis of freshly harvested and boiled trouts (A) resulted in 24 odorants, of which 19 were identified. Twelve compounds were quantified by isotope dilution assays in sample A, in boiled trouts of which the raw material had been stored for 17 weeks at −13 °C (B), and in homogenates which were freshly prepared and boiled (C) or stored for 14 weeks at −13 °C and then boiled (D). Calculation of odor activity values (OAVs, ratio of concentration to odor threshold) revealed (Z)-1,5-octadien-3-one, (E,Z)-2,6-nonadienal, and methional are the most potent odorants of samples A and C. After storage of the raw material, the OAVs of (Z)-3-hexenal and (Z,Z)-3,6-nonadienal were strongly enhanced. Consequently, both aldehydes contributed substantially to the fatty, fishy off-flavor of samples B and D. (Z)-3-Hexenal is proposed as an indicator substance to objectify the fatty, fishy off-flavor of boiled trouts.

Fish has a characteristic flavor which is influenced by the species and the conditions used for its storage and processing (Prell and Sawyer, 1988; Lindsay, 1990). More than 280 volatile compounds have been identified in freshly harvested and processed fish (Maarse and Visscher, 1991), but little is known about the compounds that actually contribute to the flavor. Only Josephson et al. (1983, 1984), Josephson and Lindsay (1986), and Hirano et al. (1992) have shown that carbonyl compounds and alcohols with six, eight, and nine carbon atoms are involved in the flavor of freshly harvested fish. These odorants are formed by a lipoxygenase-initiated peroxidation of the n–3 and n–6 polyunsaturated fatty acids occurring in fish tissues (German and Kinsella, 1985; Hsieh and Kinsella, 1986, 1989; Zhang et al., 1992).

As recently reviewed (Grosch, 1993), the potent odorants of a food are localized in the capillary gas chromatogram by gas chromatography–olfactometry (GCO) of serial dilutions of the extract. This procedure is denoted aroma extract dilution analysis (AEDA), and the results are expressed as flavor dilution (FD) factors. The FD factor for a compound is the ratio of its concentration in the initial extract to its concentration in the most dilute extract in which odor was detected by GCO. After AEDA screening, the odorants with the highest FD factors are identified. Then the simplifications implicit in AEDA (Grosch, 1993) are corrected by the quantification of the levels of these compounds in the food and by the calculation of the odor activity values (OAVs, ratio of concentration to odor threshold). Odorants with high OAVs are important contributors to the characteristic flavors or off-flavors and are suitable as indicator substances for the objective determination of flavor differences in foods.

In the present study the AEDA procedure was applied to ascertain the odorants causing the typical odor of boiled trouts. To reveal the influence on the odor of boiled trouts dependent on a longer storage period of the raw material at −13 °C, the levels of the odorants showing high FD factors were determined by stable isotope dilution assays (IDA). To accelerate off-flavor formation, the storage temperature for the frozen trouts was higher in this study than that used in fish industry.

EXPERIMENTAL PROCEDURES

Trouts. Freshly harvested trouts (*Salmo fario*, 250–350 g each fish) were obtained from a local breeding station. After removal of the guts, 10 fish were individually wrapped in polyethylene foil and stored at −13 °C. Approximately 1.5 h after catch (sample A) and after the storage period of 17 weeks (sample B), each fish was wrapped in aluminum foil and then boiled in a water bath for 15 min. Before this treatment, the polyethylene foil was removed from the stored samples. In a further experiment the fillets with skins were separated from the trouts and then ground in a meat grinder, and the homogenate obtained in portions of 300 g was sealed in polyethylene foil. The freshly prepared homogenate (sample C) and the homogenate stored for 14 weeks at −13 °C were boiled as described for samples A and B.

Chemicals. Pure samples of the compounds in Table I were obtained commercially: 1–3, 6, 7, 12, and 13 (Aldrich, Steinheim, Germany), 15 (Alfa Products, Karlsruhe, Germany), 26 (ICN, Meckenheim, Germany). 10 was a gift from Dr. R. Emberger (Haarmann and Reimer, Holzminden, Germany). Acetaldehyde, bismuth(III) oxide, 3-benzyl-5-(2-hydroxyethyl)-4-methylthiazolium chloride, and linolenic acid were from Aldrich. Deuterium gas, 99.9% isotopic purity, was from Linde, Unterschleissheim, Germany, and [3,3,3-²H]propanol was from MSD Isotopes, Montreal, Canada. (Z)-4-Heptenal was from Alfa Products, and triphenyl phosphine was from Merck, Darmstadt, Germany. Silica gel 60 (Merck) was treated with HCl and deactivated with 1.5 or 4.5% (w/w) water according to the method of Esterbauer (1968).

Synthesis of Unlabeled Compounds. (Z,Z)-3,6-Nonadienal was prepared by reductive ozonolysis of linolenic acid in analogy to the procedure which had been used for the ozonolysis of methyl α-eleostearate (Fischer et al., 1989).

Oxygen gas containing ozone was passed for 20 min through methylene chloride (60 mL) which was cooled to −78 °C. In an aliquot the ozone concentration was determined by titration (Kienitz, 1953): 8.6 μmol/mL. Linolenic acid (100 mg, 0.43 mmol) dissolved in methylene chloride (2 mL) was mixed with 50 mL of the solution of ozone, while still at −78 °C. After 10 min, the ozonides were reduced by addition of triphenyl phosphine (300 mg, 1.15 mmol). In the apparatus recently described (Sen et al., 1991a), (Z,Z)-3,6-nonadienal together with the solvent was distilled off *in vacuo* (4 mPa, 38 °C) from the nonvolatile material. The distillate obtained was washed with aqueous Na_2CO_3 (0.5 mol/L, 2 × 50 mL) and with water (2 × 25 mL). It was dried over anhydrous Na_2SO_4 and, finally, concentrated to 0.5 mL by distilling off the solvent on a Vigreux column (60 × 1 cm). (Z,Z)-3,6-Nonadienal was purified by preparative gas chromatography (GC) on a SE-30 column [2.5 m × 2 mm stainless steel column packed with SE-30 (5%, w/w) on Chromosorb W AW DMCS,

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

		RI on				FD factor[b]	
no.	compound	SE-54	OV-1701	DB-Wax	odor description[a]	A	B
1	butane-2,3-dione[c]	600	700	980	buttery-like	8	8
2	3-methylbutanal[c]	660	731	918	malty	16	8
3	pentane-2,3-dione[c]	700	785	1026	buttery-like	16	32
4	(Z)-3-hexenal[c,e]	800	880	1145	green	<8	32
5	hexanal	800	875	1083	green	8	16
6	(Z)-4-heptenal[c]	900	986	1245	biscuit-like	16	64
7	methional[c]	908	1033	1458	boiled potato	64	256
8	2-acetyl-1-pyrroline[d]	925	1010	1343	roasty, popcorn-like	32	16
9	unknown	974			flowery	16	8
10	1-octen-3-one[c]	981	1063	1305	mushroom-like	128	128
11	(Z)-1,5-octadien-3-one[c]	985	1079	1377	geranium-like	128	512
12	octanal[c]	1005	1082	1293	citrus-like	32	<8
13	(E,E)-2,4-heptadienal[c]	1011	1130	1498	lard-like	8	16
14	unknown	1027			roasty	8	<8
15	(E)-2-octenal[c]	1058	1164	1434	nut-like	8	<8
16	unknown	1081			mushroom-like, earthy	8	<8
17	(Z,Z)-3,6-nonadienal[c,e]	1100	1187		fatty, green	<8	128
18	2-acetyl-2-thiazoline[d]	1105	1232	1767	roasty, popcorn-like	32	32
19	(Z)-2-nonenal[d]	1148	1251	1510	fatty, green	8	8
20	(E,Z)-2,6-nonadienal[c]	1154	1269	1591	cucumber-like	128	128
21	(E)-2-nonenal[c]	1162	1272	1540	green, tallowy	128	32
22	unknown	1179			fruity	8	16
23	(E,E)-2,4-nonadienal[c,e]	1216	1342	1708	green, tallowy	<8	32
24	3-methylnonane-2,4-dione[d]	1253	1393	1726	strawy	8	<8
25	unknown	1280			flowery	8	<8
26	(E,E)-2,4-decadienal[c]	1318	1446	1816	fried fat	16	8
27	4,5-epoxy-(E)-2-decenal[d]	1384	1553	2020	metallic	16	<8

[a] Odor description assigned during AEDA. [b] The trouts were freshly harvested (A) and stored for 17 weeks at −13 °C (B) before boiling. [c] The compound was identified by comparing it with the reference substance on the basis of the following criteria: RI on the capillaries given in the table, mass spectra obtained by MS-EI and MS-CI, and odor quality perceived at the sniffing port. [d] 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 c. [e] The compounds were enriched from sample B for identification.

100–120 mesh]: ¹H NMR (CDCl₃) δ 0.96 (t, *J* = 7.6 Hz, H-9), 2.05 (q, *J* = 7.9 Hz, H-8), 2.77 (t, *J* = 7.9 Hz, H-5), 3.20 (d, *J* = 7.2 Hz, H-2), 5.27 (m, *J* = 10.6/7.4 Hz, H-6), 5.40 (m, *J* = 10.6/7.4 Hz, H-7), 5.55 (m, *J* = 10.6/7.2 Hz, H-3), 5.67 (m, *J* = 10.6/7.5 Hz, H-4), 9.66 (t, *J* = 2.0 Hz, H-1). The δ values of the olefinic protons were assigned after decoupling from the 5-methylene group (δ 2.77). The mass spectra in the electron impact mode (MS-EI) and in the chemical ionization mode (MS-CI) are shown in parts a and b, respectively, of Figure 1.

The following compounds were synthesized according to the literature cited: 2-acetyl-1-pyrroline (Buttery and Ling, 1982); (Z)-2-nonenal (Ullrich and Grosch, 1988a); (Z)-3-hexenal, and (Z)-1,5-octadien-3-one (Ullrich and Grosch, 1988b); 3-methylnonane-2,4-dione (Guth and Grosch, 1989); 4,5-epoxy-(E)-2-decenal (Schieberle and Grosch, 1991); 2-acetyl-2-thiazoline (Cerny and Grosch, 1992).

Synthesis of Labeled Compounds. [²H]-(Z,Z)-3,6-Nonadienal was synthesized on the route for the unlabeled compound (Kajiwara et al., 1977): the Grignard reaction of 1-bromo-2-pentyne with 2-(3′-butynyloxy)tetrahydropyran resulted in 3,6-nonadiyn-1-ol after hydrolysis of the pyranyl ether. The title compound was obtained by stereoselective deuteration of the diyne system and oxidation of the alcohol with chromium trioxide in pyridine.

1-Bromo-2-pentyne. Pyridine (60 μL) and phosphorus tribromide (6 g, 20 mmol) dissolved in diethyl ether (5 mL) were successively added to a stirred solution of 2-pentyn-1-ol (504 mg, 60 mmol) in diethyl ether (10 mL). After refluxing for 2 h in an atmosphere of nitrogen, the reaction was quenched with ice-water (5 mL). The organic layer was washed with aqueous Na₂CO₃ (0.5 mol/L, 2 × 25 mL) and aqueous saturated NaCl (2 × 5 mL) and dried over anhydrous Na₂SO₄. 1-Bromo-2-pentyne was purified by distillation *in vacuo*: bp 52–55 °C (25 mmHg).

3,6-Nonadiyn-1-ol. In an atmosphere of dry nitrogen a solution of ethyl magnesium bromide (15 mmol) and 2-(3′-butynyl-1-oxy)tetrahydropyran (2.3 g, 15 mmol) in dry tetrahydrofuran (THF, 10 mL) was stirred for 2 h at 60 °C. The mixture was cooled to room temperature, and then cuprous bromide (60 mg) was added under stirring. After 15 min, 1-bromo-2-pentyne

(1.46 g, 10 mmol) in THF (3 mL) was dropped into the reaction mixture which was stirred for 1 h at room temperature and then for 18 h at 40 °C. The mixture was poured into aqueous saturated ammonium chloride (10 mL), and the product was extracted with diethyl ether (3 × 15 mL). The residue obtained after evaporation of the solvent was taken up in methanol (5 mL) and then mixed with a solution of *p*-toluenesulfonic acid (100 mg) in methanol (10 mL). The pyranyl ether was hydrolyzed for 2 h at 55–60 °C. The cooled reaction mixture was dissolved in diethyl ether (30 mL) and washed with aqueous Na₂CO₃ (0.5 mol/L, 2 × 5 mL) and with aqueous saturated NaCl (3 × 5 mL). After drying over anhydrous Na₂SO₄, the solvents were removed *in vacuo*, and the 3,6-nonadiyn-1-ol was purified by flash chromatography (Still et al., 1978). The sample was applied onto a column (1.9 × 20 cm) which was packed with a slurry of silica gel (40 μm) in pentane–diethyl ether (90:10 v/v). Stepwise elution was performed with 100 mL of 90:10 (v/v) pentane–diethyl ether followed by 185 mL of pentane–diethyl ether (70:30 v/v); 3,6-nonadiyn-1-ol appeared in the elution range 125–285 mL.

[²H]-(Z,Z)-3,6-Nonadien-1-ol. Nickel acetate tetrahydrate (550 mg, 2.2 mmol) in [²H₁]methanol was mixed with a freshly prepared solution of sodium borodeuteride (110 mg) in [²H₁]methanol (2.6 mL). Then ethyleneamine (0.3 mL) and 3,6-nonadiyn-1-ol (400 mg, 2.8 mmol) in [²H₁]methanol (5 mL) were successively added. The autoclave containing the complete mixture was flushed two times with deuterium gas, and then 3,6-nonadiyn-1-ol was deuteriated for 90 min at a pressure of 4 × 10⁵ Pa. After dilution with water (50 mL), the reaction mixture was extracted with diethyl ether (3 × 50 mL). The organic layer was washed with water (5 × 30 mL) and then dried over anhydrous Na₂SO₄.

[²H]-(Z,Z)-3,6-Nonadienal. The oxidation of [²H]-(Z,Z)-3,6-nonadien-1-ol with chromium trioxide in pyridine and the purification of the aldehyde by filtration through a Florisil column were performed as reported for the synthesis of [²H]-3-(Z)-hexenal (Guth and Grosch, 1990). The effluent of the Florisil column was washed with aqueous HCl (1 mol/L, 2 × 25 mL) and then with aqueous saturated NaCl until the acid was removed. The residue from the ether extract was chromatographed with 75 mL
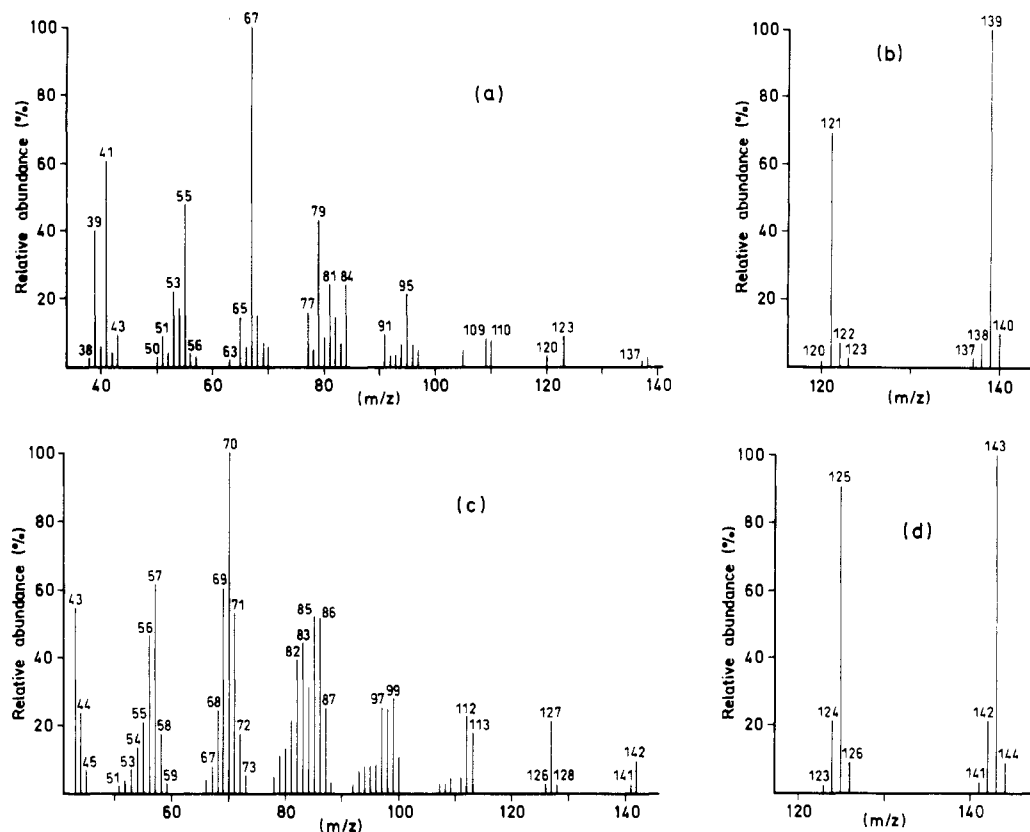
Figure 1. Mass spectra of (Z,Z)-3,6-nonadienal [(a) MS-EI; (b) MS-CI] and of $[^2H]$-(Z,Z)-3,6-nonadienal [(c) MS-EI; (d) MS-CI].

of pentane and 110 mL of pentane–diethyl ether (97.5:2.5 v/v) on a silica gel column (20 × 1 cm, silica gel deactivated with 1.5%, w/w, water), maintained at 10 °C by a cooling jacket, to afford $[^2H]$-(Z,Z)-3,6-nonadienal, of which the MS-EI and MS-CI are shown in parts c and d, respectively, of Figure 1.

[²H]Pentane-2,3-dione. The key step in the preparation of [²H]pentane-2,3-dione was the condensation of [3,3,3-²H]propanal with acetaldehyde by using a thiazolium salt as catalyst (Stetter et al., 1976). Oxidation of the [²H]-3-hydroxy-2-pentanone obtained with bismuth(III) oxide (Rigby, 1951) yielded the corresponding dione.

[3,3,3-²H]Propanal was obtained by oxidation of [3,3,3-²H]-propanol (300 mg, 5 mmol) with pyridinium chlorochromate (2.15 g, 10 mmol) as reported for [²H]hexanol (Guth and Grosch, 1993a).

Acetaldehyde (176 mg, 4 mmol), 3-benzyl-5-(2-hydroxyethyl)-4-methylthiazolium chloride (270 mg) in methanol (3 mL), and triethylamine (1 mL) were successively added to a solution of [3,3,3-²H]propanal (232 mg, 4 mmol) in diethyl ether (35 mL). After stirring overnight at 35–40 °C, the reaction mixture was acidified with acetic acid (15 mL) and then concentrated to 15 mL by distilling off the low-boiling solvents. Bismuth(III) oxide (530 mg, 1.15 mmol) was added to the mixture which was held at 100 °C for 10 min. Together with acetic acid the [²H]pentane-2,3-dione formed was distilled off. After neutralization with aqueous Na₂CO₃, the distillate was extracted with diethyl ether (2 × 50 mL). The combined ether extract was dried over anhydrous Na₂SO₄ and then concentrated to 2.5 mL by distilling off the solvent on a Vigreux column. [²H]Pentane-2,3-dione was purified by preparative GC on the SE-30 column reported above: MS-EI 43 (100%), 60 (60%), 103 (M⁺, 30%).

[²H]Hexanal, [²H]-(Z)-3-hexenal, [²H]-1-octen-3-one, [²H]-(Z)-1,5-octadien-3-one, [²H]-(E)-2-nonenal, [²H]-(E,Z)-2,6-non-adienal, [²H]-(E,E)-2,4-nonadienal, [²H]methional, and [¹³C]-butane-2,3-dione were prepared and purified according to the methods of Guth and Grosch (1990, 1993a), Sen and Grosch (1991), and Schieberle et al. (1993).

High-Resolution Gas Chromatography (HRGC)–Mass Spectrometry (MS) Analysis. HRGC was performed by means of a Carlo Erba gas chromatograph, Type 4200 (Carlo Erba, Hofheim, Germany) and by using the following thin-film capillaries: SE-

54 (30 m × 0.32 mm) glass capillary, coated with SE-54 according to the procedure of Grob and Grob (1979); fused silica capillaries OV-1701 and DB-Wax (30 m × 0.32 mm, 0.25- and 0.5-μm film thicknesses, respectively) which were supplied from J&W Scientific, Folsom, CA. The samples (0.4 μL) were applied by the on-column injection technique at 35 °C, and the temperature of the capillaries was raised at 40 °C/min to 50 °C, held isothermal for 2 min, and then raised at 6 °C/min to 200 °C. The flow rate of the helium carrier gas was 2.0 mL/min. Retention data of the compounds are presented as retention indices (RI) calculated from the retention times of alkanes by using a program for cubic spline interpolation (Halang et al., 1978). Mass spectrometry was performed on an MS 8230 (Finnigan, Bremen, Germany) or on a ion trap detector (Sen et al., 1991). The conditions used for the measurement of the mass spectra in the electron impact mode (MS-EI) and in the chemical ionization mode (MS-CI) as well as those used for the registration of the mass chromatograms have been earlier reported (Schieberle and Grosch, 1987; Sen et al., 1991). For the isotope dilution assays the MS 8230 and the ion trap detector were coupled with the capillaries given in Table II; the conditions used for HRGC were the same as reported above. Ion abundances were monitored in the ranges given in Table II. By comparison of the selected ion of the odorant to that of the abundance of the selected ion of the internal standard (see Table II), the data needed to carry out the quantitative calibration of the method were provided. The calibration factors (Table II) were calculated from the model mixtures as recently described (Sen et al., 1991).

HRGC–Olfactometry (HRGC–O). HRGC on the capillaries SE-54 and OV-1701 was performed as described above. At the end of the capillary, the effluent was split into an FID and a sniffing port (Blank et al., 1989). The potent odorants occurring in the neutral volatile fraction were located in the capillary gas chromatograms by AEDA (Ullrich and Grosch, 1987; Schieberle and Grosch, 1987, 1988; Blank et al., 1989).

Proton Magnetic Resonance Spectra (¹H NMR). ¹H NMR was recorded with an AMX 500 spectrometer (Bruker, Karlsruhe, Germany) operating at 500 MHz. The substance was dissolved in CDCl₃.

Concentrations of Labeled Compounds. The concentrations of the labeled compounds were determined by HRGC with

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Table II. Thin-Film Capillaries, Selected Ions, and Calibration Factors for Mass Chromatography of the Odorants

odorant[a,b]	capillary	selected ion, m/z	int std[c]	selected ion, m/z	calibrn factor
butane-2,3-dione (1)[b,d]	SE-54	159	c-1[d]	161	1.00
pentane-2,3-dione (2)[b]	DB-Wax	101	d-2	104	1.00
(Z)-3-hexenal (4)	OV-1701	99	d-4	101	0.93
hexanal (5)[b]	DB-Wax	83	d-5	86 + 87[e]	1.23
methional (7)	OV-1701	105	d-7	108	1.00
(Z)-4-heptenal (6)	SE-54	113	d-6	115	1.00
1-octen-3-one (10)	SE-54	127	d-10	129	0.53
(Z)-1,5-octadien-3-one (11)	SE-54	125	d-11	127	0.77
(Z,Z)-3,6-nonadienal (17)	OV-1701	121 + 139[e]	d-17	125 + 143[e]	0.76
(E)-2-nonenal (19)	SE-54	141	d-19	143	0.83
(E,Z)-2,6-nonadienal (20)	SE-54	139	d-20	141	0.81
(E,E)-2,4-nonadienal (23)	SE-54	139	d-23	141	1.04

[a] The numbering of the compounds refers to Table I. [b] Compounds 1, 2, and 5 with their internal standards were determined by the ion trap detector ITD-800 and the remaining compounds with their standards by the MS 8230. [c] Abbreviation of the labeling: c, carbon-13; d, deuterium. [d] Butane-2,3-dione was determined after conversion into dimethylquinoxaline (Schieberle et al., 1993). [e] The sum of the relative abundances of the two ions was calculated.

methyl octanoate as an internal standard. With exception of [^{2}H]-(Z,Z)-3,6-nonadienal, the response factors were estimated by HRGC of mixtures that consisted of known amounts of methyl octanoate and of the corresponding unlabeled compounds. The response factor obtained for [^{2}H]-(E,Z)-2,6-nonadienal was also used for the calculation of the [^{2}H]-(Z,Z)-3,6-nonadienal concentration.

Isolation of the Volatiles. After boiling, each sample was frozen in liquid nitrogen and then ground in a Waring Blendor. The powdered material (1.8 kg in portions of 200 g for the identification of the odorants; 250 g for IDA) was mixed with anhydrous Na$_2$SO$_4$ (1:1 w/w), soaked overnight in diethyl ether (900 mL/250 g of fish), and then extracted with this solvent for 7 h in a Soxhlet apparatus. In an IDA the extract was spiked with known amounts of the labeled internal standard substances. After concentration to 150 mL by distilling off the solvent on a Vigreux column (50 × 1 cm) at 40 °C, the solution of the volatiles was distilled off from the nonvolatile materials under high vacuum (4 mPa) in the apparatus earlier described by Guth and Grosch (1989), but with four cooling traps (Sen et al., 1991). When a pressure of 4 mPa was reached, the extract was slowly dropped into the distillation flask, which was heated to 37 °C. After addition of the extract, distillation at high vacuum (4 mPa) was continued for 90 min at 37 °C.

The distillate obtained was washed with aqueous Na$_2$CO$_3$ (0.5 mol/L, 2 × 50 mL) and with saturated aqueous NaCl solution (3 × 15 mL). After drying over anhydrous Na$_2$SO$_4$, the distillate was concentrated to 200 μL by distilling off the solvent on a Vigreux column (50 × 1 cm) and by microdistillation (Bemelmans, 1979).

Isotope Dilution Assay (IDA). An aliquot (0.5 μL) of the volatile fraction (total volume 200 μL) was analyzed by HRGC–MS for the concentrations of methional, hexanal, and pentane-2,3-dione (Table II). In a further aliquot (20 μL) butane-2,3-dione was quantified after reaction with o-phenylenediamine as 2,3-dimethylquinoxaline (Schieberle et al., 1993). The major part of the volatiles was separated on a silica gel column (Schieberle and Grosch, 1987). The fraction, which was eluted with 50 mL of 85:15 (v/v) pentane–diethyl ether, was analyzed by HRGC–MS for the concentrations of the eight carbonyl compounds listed in Table II.

RESULTS AND DISCUSSION

The volatiles from 1.8 kg of trouts which were freshly harvested and then boiled (sample A) were isolated by solvent extraction and distillation. AEDA of this fraction revealed 24 odorants in the FD factor range 8–128 (Table I), of which compounds 7 (smelling boiled potato-like), 10 (mushroom-like), 11 (geranium-like), 20 (cucumber-like), and 21 (green, tallowy) showed the highest FD factors. Of the 24 odorants, 14 were identified on the basis of HRGC and MS data and on the agreement of the odor quality with that of the corresponding reference substance. The results summarized in Table I show that methional (7), 1-octen-3-one (10), (Z)-1,5-octadien-3-one (11), (E,Z)-2,6-

Table III. Concentration and Odor Activity Values of Potent Odorants of Boiled Trouts before and after Storage of the Raw Material[a]

	concn[b]		odor act. value[c]	
odorant	A	B	A	B
butane-2,3-dione (1)	268	363	54	73
pentane-2,3-dione (2)	140	167	28	33
(Z)-3-hexenal (4)	1.6	13	53	430
hexanal (5)	37	98	4	9
(Z)-4-heptenal (6)	2.8	6.0	46	100
methional (7)	5.8	7.5	145	188
1-octen-3-one (10)	0.6	0.9	60	90
(Z)-1,5-octadien-3-one (11)	0.36	0.64	900	1600
(Z,Z)-3,6-nonadienal (17)	<1.2	14.0	<24	280
(E)-2-nonenal (19)	2.7	5.0	34	62
(E,Z)-2,6-nonadienal (20)	8.0	10.5	400	525
(E,E)-2,4-nonadienal (23)	4.7	6.4	78	107

[a] The trouts were freshly harvested (A) and stored for 17 weeks at −13 °C (B) before boiling. [b] Values in micrograms per kilogram of boiled fish. The data are mean values of duplicates. [c] The odor activity values were calculated by dividing the concentration by the odor threshold listed in Table IV.

nonadienal (20), and (E)-2-nonenal (21) belong to the most important odorants of freshly boiled trouts. In addition, five odorants (8, 18, 19, 24, and 27 in Table I) were tentatively identified on the basis of HRGC data and the odor quality.

After storage of the raw material for 17 weeks at −13 °C (sample B), the boiled trouts exhibited a fatty, fishy odor. AEDA of the volatiles isolated from this sample indicated an increase of the FD factors for (Z)-4-heptenal (6), methional (7), and (Z)-1,5-octadien-3-one (11). Furthermore, (Z)-3-hexenal (4), (E,E)-2,4-nonadienal (23), and compound 17 were found, from which 17 showed the high FD factor of 128.

The MS-EI and MS-CI of compound 17 agreed with data corresponding to (Z,Z)-3,6-nonadienal (Figure 1a,b), which was prepared by reductive ozonolysis of linolenic acid.

The odorants showing an increase of the FD factor after storage of the trouts (Table I) were quantified by IDA. In these experiments, butane-2,3-dione and pentane-2,3-dione were included, because their low FD factors found in AEDA might have been caused by losses of these very volatile compounds during the concentration of the aroma extracts.

The results summarized in Table III indicate that sample A was high in butane-2,3-dione and pentane-2,3-dione. The origin of these odorants in the boiled trout is unknown. Hexanal and (E,Z)-2,6-nonadienal, which are formed by peroxidation of n–6 and n–3 polyunsaturated fatty acids,

Table IV. Detection Odor Thresholds in Water of Some Carbonyl Compounds

compound	threshold[a]
butane-2,3-dione (1)	5.0
pentane-2,3-dione (2)	5.0
(Z)-3-hexenal (4)	0.03
hexanal (5)	10.5
(Z)-4-heptenal (6)	0.06
methional (7)	0.04
1-octen-3-one (10)	0.01
(Z)-1,5-octadien-3-one (11)	0.0004
(Z,Z)-3,6-nonadienal (17)	0.05
(E)-2-nonenal (19)	0.08
(E,Z)-2,6-nonadienal (20)	0.02
(E,E)-2,4-nonadienal (23)	0.06

[a] The threshold values (micrograms per kilogram) were retronasally (Guth and Grosch, 1993b) determined by at least three trained judges. Mean values were calculated from duplicates.

Table V. Concentration and Odor Activity Values of Potent Odorants of Boiled Trout Homogenates before and after Storage of the Raw Material[a]

	concn[b]		odor act. value[c]	
odorant	C	D	C	D
butane-2,3-dione (1)	255	297	51	59
pentane-2,3-dione (2)	17	59	3	12
(Z)-3-hexenal (4)	1.4	24	47	800
hexanal (5)	14	33	1	3
(Z)-4-heptenal (6)	1.1	6.0	18	100
methional (7)	4.0	5.2	100	130
1-octen-3-one (10)	0.16	0.16	16	16
(Z)-1,5-octadien-3-one (11)	0.17	0.16	400	400
(Z,Z)-3,6-nonadienal (17)	1.2	22	24	440
(E)-2-nonenal (19)	1.1	2.1	14	26
(E,Z)-2,6-nonadienal (20)	2.1	4.8	105	240
(E,E)-2,4-nonadienal (23)	2.7	2.6	45	43

[a] The homogenate was boiled (C) immediately after preparation and (D) after storage for 14 weeks at -13 °C. [b,c] Refer to footnotes b and c in Table III.

respectively, as well as the Strecker aldehyde methional predominated in the fraction of aldehydes. Storage of the trouts led to a strong increase of (Z)-3-hexenal and (Z,Z)-3,6-nonadienal, but the concentrations of hexanal and (Z)-4-heptenal were at least twice as high in sample B.

Calculation of the OAVs (Table III) on the basis of the corresponding odor thresholds in water (Table IV) revealed (Z)-1,5-octadien-3-one, (E,Z)-2,6-nonadienal, and methional as the most potent odorants of sample A.

The OAVs of the compounds listed in Table III increased when the trouts were stored for 17 weeks at -13 °C. In particular, the OAV of (Z)-3-hexenal and (Z,Z)-3,6-nonadienal increased so strongly that these aldehydes, in addition (Z)-1,5-octadien-3-one and (E,Z)-2,6-nonadienal, showed the highest OAVs in sample B.

In a separate experiment the homogenate prepared from freshly harvested trouts was boiled (sample C). The odorants formed were quantified and their OAVs calculated. In agreement with the results obtained for the whole fishes (sample A in Table III), (Z)-1,5-octadien-3-one, (E,Z)-2,6-nonadienal, and methional were the most potent odorants of sample C (Table V), but the OAVs of the first two carbonyl compounds were considerably lower in sample C than in sample A.

When the homogenate was stored for 14 weeks at -13 °C and then boiled (sample D in Table V), the concentrations of pentane-2,3-dione, (Z)-3-hexenal, hexanal, (Z)-4-heptenal, (Z,Z)-3,6-nonadienal, and (E,Z)-2,6-nonadienal were significantly higher than in sample C, whereas the concentrations of the two C₈ vinyl ketones did not change. After a storage period of 21 weeks, the levels of (Z)-3-

hexenal and (Z,Z)-3,6-nonadienal were increased to 51 and 29 µg/kg, respectively (data not shown in Table V).

Sample D smelled more intensely fatty/fishy than sample B. As the OAVs of (Z)-3-hexenal and (Z,Z)-3,6-nonadienal were substantially higher in sample D than in sample B, we suggest that this increase is mainly responsible for the rise in the intensity of the off-flavor.

Conclusion. The concentrations of (Z)-3-hexenal and (Z,Z)-3,6-nonadienal are very low in freshly harvested and boiled trouts. They increase when the frozen raw material is stored for a longer period to levels which contribute strongly to the fatty, fishy off-flavor of boiled trouts. As (Z)-3-hexenal is more stable than (Z,Z)-3,6-nonadienal, it is more a suitable indicator for objective determination of off-flavors. An analytical procedure for the accurate determination of (Z)-3-hexenal in boiled trouts using (Z)-3-hexenal labeled with deuterium as internal standard and quantification by HRGC–MS has been developed.

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