Changes in the Odorants of Boiled Carp Fillet (*Cyprinus carpio* L.) As Affected by Increasing Methionine Levels in Feed

S. Schlüter and H. Steinhart*

Institute of Biochemistry and Food Chemistry, University of Hamburg, Grindelallee 117, 20146 Hamburg, Germany

F. J. Schwarz and M. Kirchgessner

Institute of Physiology of Nutrition, Technical University of Munich, 85356 Freising-Weihenstephan, Germany

Sixty common carp in groups of five in four tanks per treatment were given three diets containing different increasing amounts of methionine. The aroma extract dilution analysis (AEDA) of the boiled carp fillets resulted in 32 odorants, of which 26 were identified. Ten compounds were quantified using an internal standard (IS), and the very low concentrations of 2-acetyl-1-pyrroline, (Z)-1,5-octadien-3-one, and methional were calculated by the nasally determined detection limit. The concentration of methional seemed to increase with increasing methionine in the diet. Because the unstable methional could be converted by β-elimination into methanethiol, the impact resulting in an off-flavor was investigated by headspace analysis. The investigation revealed no difference in the methanethiol contents between the treatments with the lowest and highest methionine supplies.

Keywords: *Carp flavor; AEDA; 2-acetyl-1-pyrroline; (Z)-1,5-octadien-3-one; methionine*

INTRODUCTION

The sensory aspects of fish are essential for consumer satisfaction. More than 280 volatile compounds have been identified in freshly harvested and processed fish (Nijssen et al., 1996). Many researchers have reported on enzymatic reactions of lipoxygenases in fish tissue with polyunsaturated fatty acids for the characterization of many carbonyl and alcohol compounds that contribute to the distinctive plant-like odors of some freshwater fishes (Josephson and Lindsay, 1986; Karahadian and Lindsay, 1989), but little is known about the odor of carp. An earthy, mushroom-like, and fishy odor seems to have prejudiced some consumers against eating carp. As recently reviewed by Schlüter et al. (1996) the odor of boiled carp fillet correlates with the diet of the carp. If the foodstuff has a high content of fat, the resulting odor of boiled carp fillet is pleasant. The earthy, mushroom-like, and fishy odor seems to be affected by an increasing content of (Z)-4-heptenal and 1-octen-3-one caused by other foodstuffs.

Schwarz et al. (1998) recently showed the need of additional methionine in carp feed, but little is known about the contribution of methionine derivatives to the odor of boiled carp fillets. In this experiment, the carp were fed with increasing amounts of methionine in the diet and the influence of this feed additive on the changes of impact aroma compounds in boiled carp fillets was investigated.

EXPERIMENTAL PROCEDURES

Carp. The carp were obtained from the experiment of Schwarz et al. (1998), selecting three treatments (TI−TIII) with increasing amounts of methionine in the diets. The methionine level ranged from 0.49% (TI) to 0.79% (TII) and 1.34% (TIII) in the dry matter of the diet. A total of 60 carp were divided into groups of 5 fish per tank with four tanks assigned to each dietary treatment. The average initial weight per fish was 569 ± 65 g. The experiment lasted for 85 days (Schwarz et al., 1998). At the end of the experiment, the carp had mean final weights of 779 g (TI), 1033 g (TII), and 1069 g (TIII). The carp were fasted for 18 h, stunned by a blow to the head, killed, gutted, and filleted. The fillets were stored without skin at −70 °C. For the analysis four samples of 220 g of each group were investigated.

Chemicals. Potent odorants with their odor description and flavor dilution (FD) factor are listed in Table 2. They were purchased commercially: pentane-2,3-dione was from Merck, Darmstadt, Germany; compounds **5**, **11**, **19**, **20**, **24**, and **28** were from Aldrich, Steinheim, Germany; compounds **12**, **17**, and **25** were from Janssen, Germany; compounds **15**, **16**, and **31** were a gift of Prof. Schieberle and Dr. Guth, Garching, Germany; compounds **13**, **29**, and **30** were synthesized according to the literature cited [**13** and **30** (Organikum, 1988); **29** (Guth and Grosch, 1989)].

Isolation of Volatiles. Four samples of 220 g of carp fillet of each group were wrapped in a polyethylene bag. The samples were mixtures of two or three carp fillets of each group. The fillets were steam-boiled for 30 min. After boiling, each sample was frozen in liquid nitrogen and then ground in a Moulinex (a household blender). The powdered material was mixed with anhydrous Na_2SO_4 (1:1, w/w), soaked overnight in diethyl ether [freshly distilled, 900 mL/250 g fish, spiked with internal standard compounds (Figure 1; Table 2)]; and then extracted with this solvent for 7 h in a Soxhlet apparatus. The diethyl ether was concentrated to 150 mL after Soxhlet extraction by distilling off the solvent on a Vigreux column (45 × 1 cm) at 40 °C. The solution of the volatiles was distilled off from the nonvolatile materials under vacuum (6.7 Pa). The samples were cooled with liquid nitrogen and afterward slowly heated to 37 °C during the distillation.

The distillate was washed with aqueous Na_2CO_3 (0.5 mol/

* Author to whom correspondence should be addressed (fax 0049-40-42838-4342; e-mail Steinhart@lc.chemie.uni-hamburg.de).

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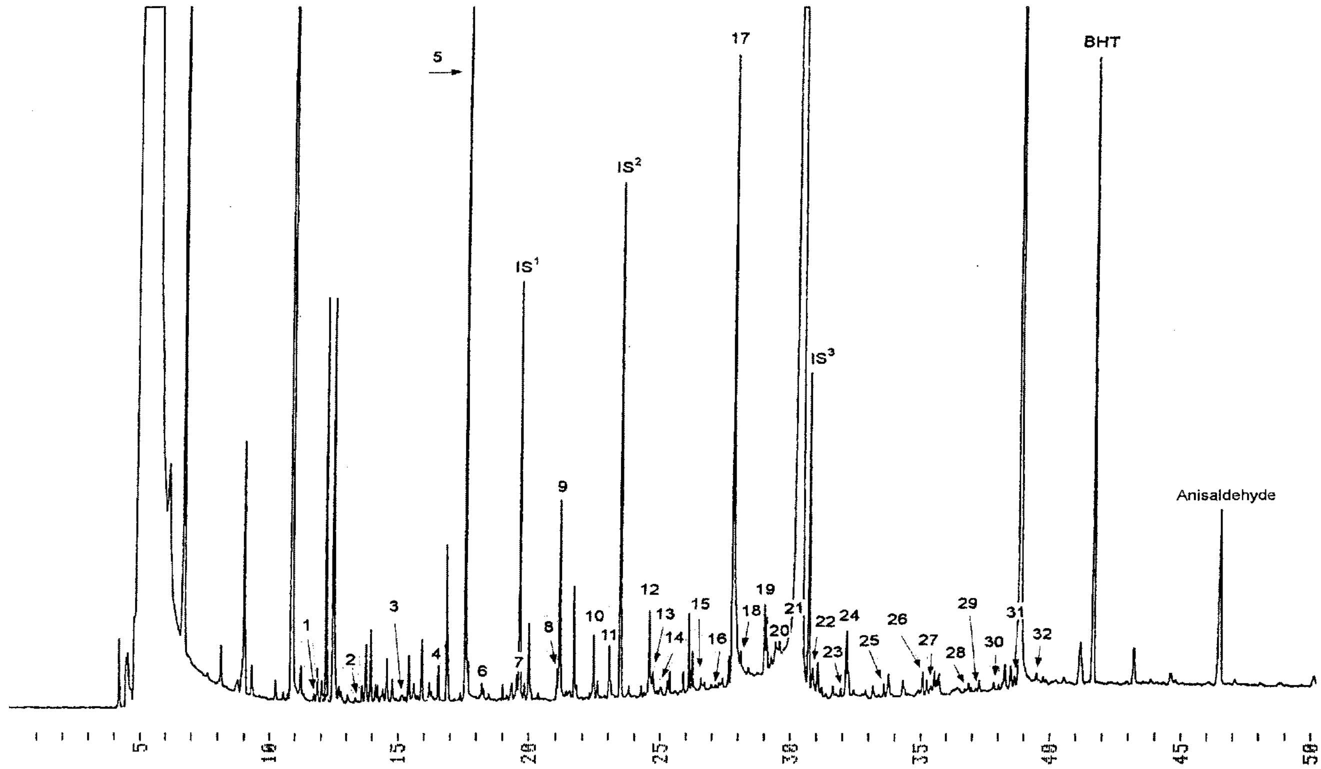

(IS[1] internal standard 5-methylhexanone (48.6 μg/mL), IS[2] = 3-octanone (48.8 μg/mL), IS[3] = 2-decanone (49.2μg/mL))

Figure 1. Capillary gas chromatogram by HRGC/O of boiled carp fillet of diet TI.

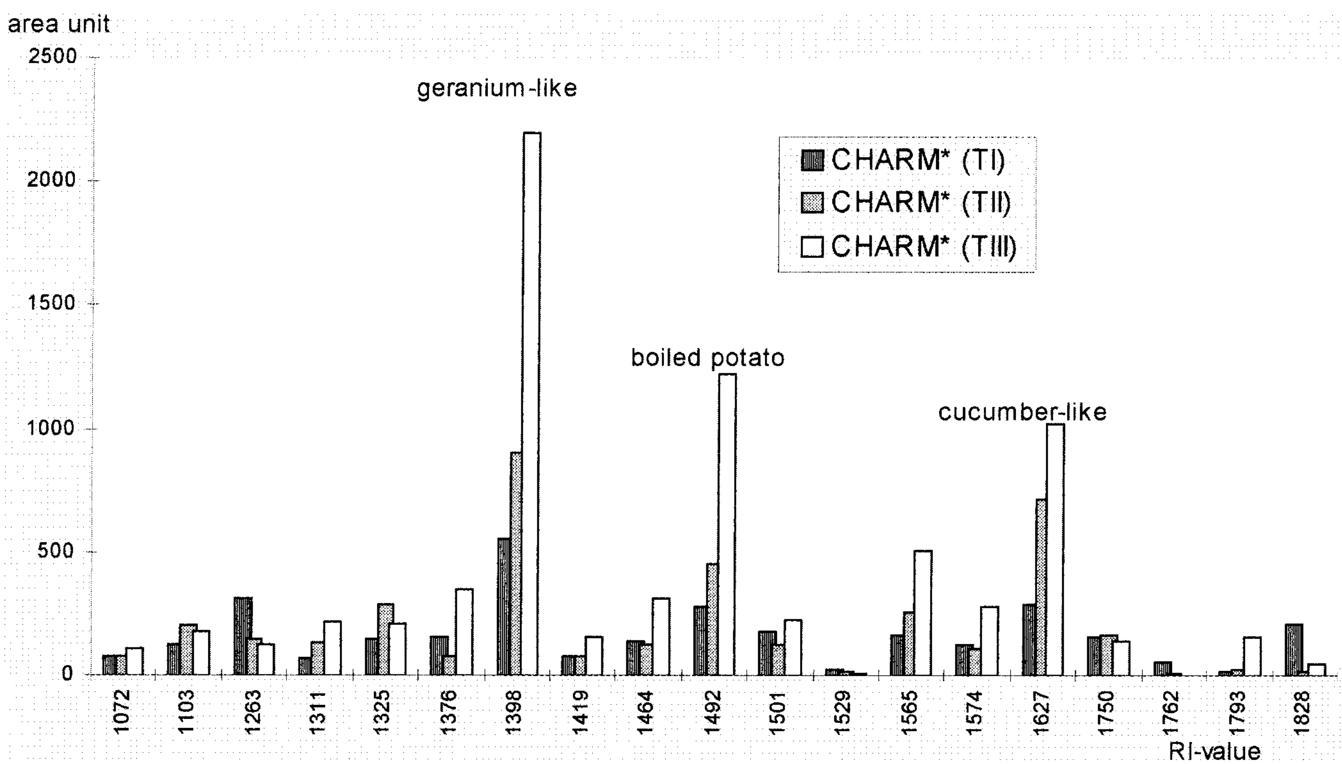

Figure 2. Combined hedonic and response measurement (CHARM) values of boiled carp fillet (TI, TII, and TIII).

L, 2 × 50 mL) and with saturated aqueous NaCl solution (3 × 15 mL). After drying over anhydrous Na_2SO_4, the distillate was concentrated to 0.5 mL by distilling off the solvent on a Vigreux column (45 × 1 cm) and by microdistillation (neutral volatile fraction). Compared to the aqueous extract, this fraction contained the neutral volatile components.

High-Resolution Gas Chromatography (HRGC). HRGC was performed with a Carlo Erba gas chromatograph, Type 4200 (Carlo Erba, Hofheim, Germany) by using a fused silica capillary DB-Wax (60 m × 0.32 mm, 0.5 μm film thickness) supplied by J&W Scientific, Folsom, CA. The samples (2 μL) were applied by the splitless injection technique at 34 °C. The initial temperature of 34 °C was held for 3 min and then raised at 5 °C/min to 200 °C and hold 10 min at 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)

Table 1. Identified and Unknown Odorants in Boiled Carp Fillet

no.	odorant	capillary DB-Wax 0.5 μm	capillary DB-Wax 0.25 μm	capillary DB-5 0.25 μm
1	3-methylbutanal	$957_{\text{malt-like}}$	$906_{\text{malt-like}}$	$664_{\text{malt-like}}$
2	butane-2,3-dione	$987_{\text{buttery-like}}$	$954_{\text{buttery-like}}$	$<600_{\text{buttery-like}}$
3	thiophene	$1041_{\text{sulfur-containing}}$	$995_{\text{sulfur-containing}}$	
4	pentane-2,3-dione	$1072_{\text{buttery-like}}$	$1044_{\text{buttery-like}}$	$700_{\text{buttery-like}}$
5	hexanal	1103_{green}	1075_{green}	798_{green}
6	unknown	1121_{stinky}	1111_{stinky}	
7	(*Z*)-3-hexenal	1161_{green}	1131_{green}	798_{green}
8	unknown	$1172_{\text{sulfur-containing}}$	1146_{tainted}	
9	heptanal	1204_{fruity}	1171_{fruity}	895_{fruity}
10	diethyl disulfide	$1248_{\text{sulfur-containing}}$	$1187_{\text{sulfur-containing}}$	$916_{\text{sulfur-containing}}$
11	(*Z*)-4-heptenal	1263_{fishy}	1222_{fishy}	814_{tainted}
12	octanal	1311_{orange}	1273_{orange}	999_{orange}
13	1-octen-3-one	$1325_{\text{mushroom-like}}$	$1279_{\text{mushroom-like}}$	$974_{\text{mushroom-like}}$
14	bis(methylthio)methan	$1330_{\text{earth-like}}$	$1260_{\text{earth-like}}$	$881_{\text{earth-like}}$
15	2-acetyl-1-pyrroline	$1376_{\text{popcorn-like}}$	$1317_{\text{popcorn-like}}$	$918_{\text{popcorn-like}}$
16	(*Z*)-1,5-octadien-3-one	$1398_{\text{geranium-like}}$	$1352_{\text{geranium-like}}$	$979_{\text{geranium-like}}$
17	nonanal	1419_{fruity}	1380_{fruity}	1102_{fruity}
18	unknown	$1435_{\text{urine-like}}$	$1411_{\text{urine-like}}$	$1130_{\text{urine-like}}$
19	(*E*)-2-octenal	$1464_{\text{fat-like, nut-like}}$		$1156_{\text{fat-like, nut-like}}$
20	methional	$1492_{\text{boiled potato-like}}$	$1426_{\text{boiled potato-like}}$	$898_{\text{boiled potato-like}}$
21	unknown	1501_{tainted}	1471_{tainted}	960_{tainted}
22	(*Z*)-2-nonenal	$1529_{\text{fat-like, green}}$	$1492_{\text{fat-like, green}}$	$1142_{\text{fat-like, green}}$
23	unknown	1565_{stinky}		
24	(*E*)-2-nonenal	$1574_{\text{lin-like}}$	$1524_{\text{lin-like}}$	$1155_{\text{lin-like}}$
25	(*E*,*Z*)-2,6-nonadienal	$1627_{\text{cucumber-like}}$	$1567_{\text{cucumber-like}}$	$1148_{\text{cucumber-like}}$
26	unknown	$1687_{\text{fat-like, sweaty}}$	$1605_{\text{fat-like, sweaty}}$	$1174_{\text{fat-like, sweaty}}$
27	phenylacetaldehyde	1697_{honey}	1621_{flowery}	1032_{flowery}
28	(*E*,*E*)-2,4-nonadienal	1750_{fettig}	1687_{fettig}	1204_{fettig}
29	3-methyl-2,4-nonandione	$1762_{\text{anise-like}}$	$1706_{\text{anise-like}}$	
30	(*E*,*Z*)-2,6-nonadienol	$1793_{\text{cucumber-like}}$		
31	2-acetyl-2-thiazoline	$1828_{\text{popcorn-like}}$	$1721_{\text{popcorn-like}}$	$1098_{\text{popcorn-like}}$
32	(*E*,*E*)-2,4-decadienal	$1862_{\text{fatty, tallowy}}$	$1818_{\text{fatty, tallowy}}$	$1311_{\text{fatty, tallowy}}$

calculated from the retention indices of alkanes according to the Kovats index system.

HRGC/Olfactometry (HRGC/O). HRGC was performed as described above. At the end of the capillary, the effluent was split between an FID and a sniffing port (1:1; Schlüter et al., 1996). The location of the potent odorants of the neutral volatile fraction in the capillary gas chromatograms was determined by aroma extract dilution analysis (AEDA; Schieberle and Grosch, 1987) and modified combined hedonic and response measurement (Schlüter et al., 1996) by a trained panel. In the modified combined hedonic and response measurement (CHARM) method the assessor recognizes the odorants at the sniffing port during half an hour (between 10 and 40 min of the run, Figure 1). With a button, he describes the beginning and the end of the odorant impression. The areas of the compounds were calculated by different factors depending on the recognized time (3 s = factor 1, 6 s = 2, 9 s = 3; results in Table 3 and Figure 2). The listed values are averaged over all assessors. For comparison, the odor activity values (ratio of concentration to odor threshold; Table 4) were calculated. Ten compounds were quantified using an internal standard (IS), and the concentrations of 2-acetyl-1-pyrroline, (Z)-1,5-octadien-3-one, and methional were calculated by the nasally determined detection limit.

HRGC/O–Headspace. Analysis of the headspace samples was performed with the gas chromatographic system described above. The samples were obtained by cooking 5 g of the homogenized carp samples of TI and TIII, in headspace vials (10 mL volume), for 30 min in an oil bath of 100 °C. A volume of 300 μL was injected.

RESULTS

The volatiles of fillets of boiled carp that had been fed with diets with different levels of methionine were isolated by solvent extraction and distillation (Figure 1 displays the chromatograms of all diets). AEDA of these different fractions showed the same important odorants

Table 2. Potent Odorants (FD Factors ≥8) in Boiled Carp Fillet

no.	compound	FD factor
4	pentane-2,3-dione	8
5	hexanal	32
11	(*Z*)-4-heptenal	32
12	octanal	32
13	1-octen-3-one	128
15	2-acetyl-1-pyrroline	32
16	(*Z*)-1,5-octadien-3-one	256
17	nonanal	8
19	(*E*)-2-octenal	8
20	methional	128
21	unknown	16
22	(*Z*)-2-nonenal	16
23	unknown	8
24	(*E*)-2-nonenal	64
25	(*E*,*Z*)-2,6-nonadienal	256
28	(*E*,*E*)-2,4-nonadienal	16
29	3-methylnonane-2,4-dione	8
30	(*E*,*Z*)-2,6-nonadienol	8
31	2-acetyl-2-thiazoline	8

in the FD factor range of 8 to 256 (Table 2), of which compounds **13** (smelling mushroom-like), **16** (geranium-like), **20** (boiled potato), and **25** (cucumber-like) showed the highest FD factors. Twenty-six of 32 odorants were identified (Table 1) on the basis of HRGC data and their agreement of odor quality with that of the corresponding reference substances. The results summarized in Table 2 show that 1-octen-3-one (**13**), (*Z*)-1,5-octadien-3-one (**16**), methional (**20**), and (*E*,*Z*)-2,6-nonadienal (**25**) are among the most important odorants in boiled carp fillet. The results were confirmed by the modified CHARM method; 10 trained persons smelled the different fractions of TI–TIII at the sniffing port (Table 3; Figure 2).

The concentrations of potent odorants except for methional (Table 4) showed no correlation with the increasing dietary methionine level. The concentration

Table 3. Combined Hedonic and Response Measurement (CHARM) Values of Boiled Carp Fillet (TI, TII, and TIII)[a]

no.	compound	CHARM (TI)	CHARM (TII)	CHARM (TIII)
4	pentane-2,3-dione	74	75	111
5	hexanal	128	204	181
11	(*Z*)-4-heptenal	315	151	122
12	octanal	67	136	221
13	1-octen-3-one	144	289	212
15	2-acetyl-1-pyrroline	158	79	353
16	(*Z*)-1,5-octadien-3-one	551	906	2199
17	nonanal	79	75	153
19	(*E*)-2-octenal	140	128	314
20	methional	282	453	1225
21	unknown	177	121	228
22	(*Z*)-2-nonenal	23	15	10
23	unknown	164	260	503
24	(*E*)-2-nonenal	128	106	283
25	(*E*,*Z*)-2,6-nonadienal	289	720	1021
28	(*E*,*E*)-2,4-nonadienal	158	166	141
29	3-methylnonane-2,4-dione	53	8	0
30	(*E*,*Z*)-2,6-nonadienol	13	20	157
31	2-acetyl-2-thiazoline	210	15	47

[a] The CHARM values are averaged over 10 judges. T, treatment.

of methional in the carp fillet seemed to be increased. This cannot be proved because the concentrations are averages of two analyses (TI, 7.0 μg/kg; TII, 15.9 ± 3.3 μg/kg; and TIII, 26.35 ± 5.25 μg/kg).

To find out if the methional has an influence in the off-flavor of boiled carp fillet, the methionine derivatives were analyzed by static headspace of TI and TIII (Figure 3). The unstable methional could convert into methanethiol by β-elimination, which finally oxidizes to dimethyl sulfide. The retention time, RI value, and odor description of all unknown headspace compounds of the diet TI are shown (Table 5). The compounds were identified by adding the suspected reference substance to the headspace vial. Methanethiol was identified at 5.86 min; however, dimethyl sulfide (17.2 min) was absent. Figure 4 shows the percentages of total HRGC area of each headspace compound. The mean area of each compound was calculated by five replicates. Although the content of methional increased in the plasma with increasing dietary methionine, there is no difference in the content of methanthiol, which had standard deviations of 40% for TI and 8% for TIII (data not shown).

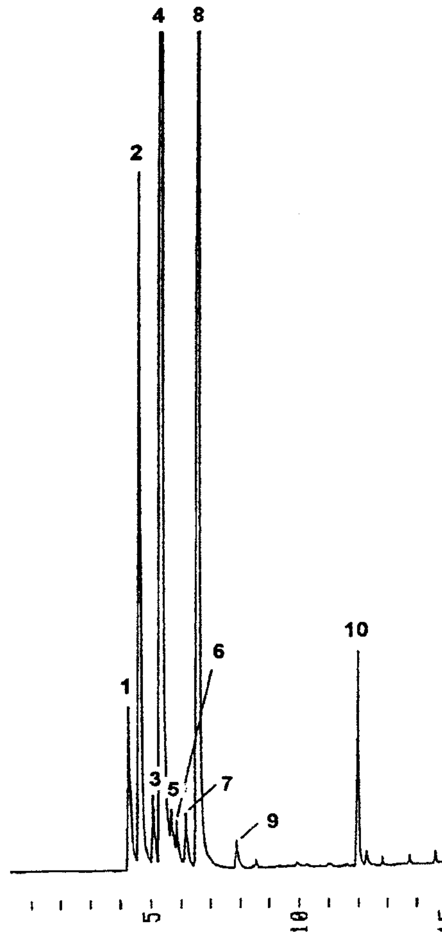

Figure 3. Capillary gas chromatogram obtained by HRGC/O–headspace of boiled carp fillet.

DISCUSSION

The identified volatiles were already reported in different fish species but not in carp fillet (Josephson et al., 1984; Josephson and Lindsay, 1986; Milo and Grosch, 1993; Cadwallader et al., 1995; Kawai, 1996).

Volatiles that are formed by peroxidation of $n-6$ and $n-3$ polyunsaturated fatty acids, for example, hexanal, (*Z*)-4-heptenal, octanal, nonanal, and (*E*,*Z*)-2,6-nonadienal, predominated in the fraction of aldehydes. The content of (*Z*)-4-heptenal seems to be responsible for an

Table 4. Concentrations and Odor Activity Values of Potent Odorants of Boiled Carp Fillet (TI, TII, and TIII)

no.	compound	odor threshold[a,b]	TI (concn)[d]	odor act. value TI[e]	TII (concn)[d]	odor act. value TII[e]	TIII (concn)[d]	odor act. value TIII[e]
4	pentane-2,3-dione	5.00	60.1	12.0	116.4	23.3	138	27.6
5	hexanal	45.20	430.9	9.5	481.6	10.7	470	10.4
11	(*Z*)-4-heptenal	0.06	39.9	665.0	43.1	718.3	35.7	595.0
12	octanal	0.130	51.7	73.9	117.9	168.4	71.0	101.4
13	1-octen-3-one	0.001	19.8	396.0	19.8	396.0	16.7	334.0
15	2-acetyl-1-pyrroline[c]	0.13	5.3	40.8	6.7	51.5	1.45	11.2
16	(*Z*)-1,5-octadien-3-one[c]	0.001	4.35	4350.0	3.3	3300.0	9.3	9300.0
17	nonanal	1.00	456.2	456.2	719.2	719.2	539.2	539.2
19	(*E*)-2-octenal	0.50	60.5	121.0	55.2	110.4	61.6	123.2
20	methional[c]	0.11	7.0	63.6	15.9	144.5	26.35	239.5
24	(*E*)-2-nonenal	0.08	50.4	629.8	39.7	496.0	45.6	570.4
25	(*E*,*Z*)-2,6-nonadienal	0.02	31.8	1590.0	19.2	960.0	38.0	1900.0
28	(*E*,*E*)-2,4-nonadienal	0.06	8.95	149.2	7.4	123.5	11.1	185.0

[a] The threshold values were retronasally determined by Milo and Grosch (1993), except hexanal and methional. [b] The threshold values of hexanal and methional were determined nasally by five and seven trained assesors, respectively. [c] The concentrations of 2-acetyl-1-pyrroline, (*Z*)-1,5-octadien-3-one, and methional were calculated on the nasally determined detection limit. [d] The concentration values are in micrograms per kilogram of boiled carp fillet. The data are mean values of four replicates. [e] The odor activity values were calculated by dividing the concentration by the odor threshold.

Table 5. Retention Time, Retention Index (RI) Values, and Odor Description of Headspace Compounds[a]

no.	retention time	RI (DB-Wax)	odor description	identification
1	4.32	<500		
2	4.66	513		
3	5.06	598		
4	5.27	620		
5	5.68	661		
6	5.86	679	stinky, sulfurous	methanethiole
7	6.15	704		ethanal
8	6.54	725		
9	7.90	799		propanal
10	11.99	937		

[a] Compounds are detected by static headspace.

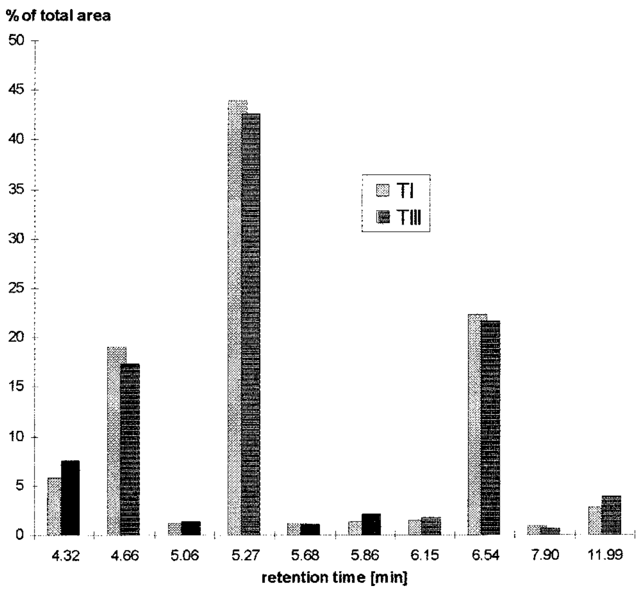

Figure 4. Percentages of an HRGC area of each headspace compound (TI and TIII).

off-flavor in boiled carp fillet. Badings (1973) determined that >15 μg/kg of this substance caused a fishy and tallowy off-flavor in vegetable oil. Milo and Grosch (1993) described the odor of (*Z*)-4-heptenal as biscuit-like. They found 2.8−6.0 μg/kg in boiled trout. (*Z*)-4-Heptenal could also be responsible for the odor difference between boiled trout and carp fillet.

Another compound responsible for the earthy odor of boiled carp fillet could be 1-octen-3-one (formed by peroxidation of arachidonic acid) with a content ∼20 μg/kg. Milo and Grosch (1993) found 0.6−0.8 μg/kg in boiled trout, whereas Josephson et al. (1984, 1986) found ∼30 μg/kg in whitefish.

Sulfur-containing compounds do not contribute significantly to the carp fillet odor, although, besides the Strecker aldehyde methional, other S compounds were identified, for example, thiophene, diethyl disulfide, and bis(methylthio)methane according to Runge et al. (1990) (data not shown; FD factor <8). The concentration of methional was slightly increased with increasing dietary methionine without a simultaneous increase of methanethiol, which could lead to an off-flavor. This result correlates with the results of Schwarz et al. (1998).

They showed that the methionine content in the plasma of fasted carp increased significantly from TI to TII and reached a plateau at this dietary level. The protein content of the fillet also increased significantly from 17.2% (TI) to 18.5% (TIII) with increasing dietary methionine, whereas the fat content of the fillet was reduced from 6.99% (TI) to 5.43% (TII) and 4.97% (TIII).

Experimental evidence shows that methionine as an essential amino acid is used effectively for growth in carp (Schwarz et al., 1998). Protein deposition increased significantly with higher methionine content up to a methionine requirement of 0.86% in dry matter. The methionine is almost completely converted into protein, although in TIII there was a higher dietary supply in comparison to the requirement. Methional has a pleasant impact of boiled potato on the odor of boiled carp fillet, although methanethiol causes an off-flavor. We suppose that the pleasant flavor of boiled carp fillet will not be influenced if the methionine supply in the diet is calculated by taking into consideration the methionine requirement.

LITERATURE CITED

Badings, H. T. Fishy off-flavors in autoxidized oils. *J. Am. Oil Chem. Soc.* **1973**, *50*, 334.

Cadwallader, K. R.; Tan, Q.; Chen, F.; Meyers, S. P. Evaluation of the aroma of cooked spiny lobster tail meat by aroma extract dilution analysis. *J. Agric. Food Chem.* **1995**, *43*, 2432−2437.

Guth, H.; Grosch, W. 3-Methylnonene-2,4-dione—an intense odour compound formed during flavour reversion of soyabean oil. *Fat Sci. Technol.* **1989**, *91*, 225−230.

Josephson, D. B.; Lindsay, R. C. Enzymic generation of volatile aroma compounds from fresh fish. 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. Identification of volatile aroma compounds from oxidized frozen whitefish (*Coregonus clupeaformis*). *Can. Inst. Food Sci. Technol. J.* **1984**, *17*, 178−182.

Karahadian, C.; Lindsay, R. C. Role of oxidative processes in the formation and stability of fish flavors. In *Flavor Chemistry, Trends and Developments*; Teranishi, R., Buttery, G., Shahidi, F., Eds.; ACS Symposium Series 388; American Chemical Society: Washington, DC, 1989; pp 60−75.

Kawai, T. Fish flavor. *Crit. Rev. Food Sci. Nutr.* **1996**, *35*, 257−298.

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.

Nijssen, L. M., Visscher, C. A., Maarse, H., Willemsens, L. C., Boelens, M. H., Eds. *Volatile Compounds in Food, Qualitative and Quantative Data*, 7th ed.; TNO Nutrition and Food Research Institute: Zeist, The Netherlands, 1996.

Organikum. *Organisch-chemisches Grundpraktikum; 17. Auflage*; VEB Deutscher Verlag der Wissenschaften: Berlin, Germany, 1988 (in German).

Runge, G.; Steinhart, H.; Schwarz, F. J.; Kirchgessner, M. Amounts of volatile sulfur compounds in the edible part of carp (*Cyprinus carpio* L.) fed different fat and α-tocopheryl acetate dietary supplements. *Agribiol. Res.* **1990**, *43*, 183−190.

Schieberle, P.; Grosch, W. Quantitative analysis of aroma compounds in wheat and rye bread crusts using a stable isotope dilution assay. *J. Agric. Food Chem.* **1987**, *35*, 252−257.

Schlüter, S.; Steinhart, H.; Schwarz, F. J.; Oberle, M.; Kirchgessner, M. Influence of feed on the flavor of boiled carp fillet. *Lebensmittelchemie* **1996**, *50*, 109−112 (in German).

Schwarz, F. J.; Kirchgessner, M.; Deuringer, U. Studies on the methionine requirement of carp (*Cyprinus carpio* L.). *Aquaculture* **1998**, *161*, 121−129.

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