

Original paper

Primary odorants of chicken broth

A comparative study with meat broths from cow and ox

Uwe Gasser and Werner Grosch

Deutsche Forschungsanstalt für Lebensmittelchemie, Lichtenbergstrasse 4, D-8046 Garching, Federal Republic of Germany

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Primäre Geruchsstoffe bei Hühnerbrühe. Eine vergleichende Untersuchung mit Brühen aus Kuh- und Ochsenfleisch

Zusammenfassung. Durch Aromaextraktverdünnungsanalyse (AEVA) der flüchtigen Verbindungen, isoliert durch simultane Destillation/Extraktion aus Hühnerbrühe, wurden 16 primäre Aromastoffe mit FD-Faktoren im Bereich 64 bis 2048 wahrgenommen. Von diesen Verbindungen wurden 14 identifiziert: 2-Methyl-3-furanthiol, 2-Furfurylthiol, Methional, 2,4,5-Trimethylthiazol, Nonanal, 2(E)-Nonenal, 2-Formyl-5-methylthiophen, *p*-Kresol, 2(E),4(E)-Nonadienal, 2(E),4(E)-Decadienal, 2-Undecenal, β -Ionon, γ -Decalacton, γ -Dodecalacton. Die primären Geruchsstoffe der Hühnerbrühe wurden mit denen verglichen, die aus einer AEVA von Kuh- und Ochsenfleischbrühe stammten. Hauptunterschiede waren: 2(E),4(E)-Decadienal (fettig) und γ -Dodecalacton (talig, fruchtig) überwogen in Hühnerbrühe, während die Schwefelverbindungen Bis(2-methyl-3-furyl)disulfid (fleischartig) und Methional (gekochte Kartoffeln) in den Brühen aus Rindfleisch dominierten. Die Geruchsschwellen (in Luft) wichtiger Fleischaromastoffe wurden bestimmt.

Summary. Aroma extract dilution analysis (AEDA) of the volatiles obtained by the simultaneous distillation/extraction of a chicken broth resulted in 16 primary odour compounds with FD factor values between 64 and 2048. Fourteen of these compounds were identified as: 2-methyl-3-furanthiol, 2-furfurylthiol, methional, 2,4,5-trimethylthiazole, nonanal, 2(E)-nonenal, 2-formyl-5-methylthiophene, *p*-cresol, 2(E),4(E)-nonadienal, 2(E),4(E)-decadienal, 2-undecenal, β -ionone, γ -decalactone and γ -dodecalactone. The primary odorants of chicken broth were compared with those resulting from the AEDA of broths from cow and ox meat. The major differences were that 2(E),4(E)-decadienal (fatty) and γ -dodecalactone (tallowy, fruity) prevailed in the chicken

broth, whereas the sulphur compounds, bis(2-methyl-3-furyl)disulphide (meat-like-aroma) and methional (aroma like cooked potatoes), predominated in broths prepared from cow and ox meats. The odour thresholds (in air) of important meat aroma compounds are reported.

Introduction

The composition of the volatile fraction produced during the heating of chicken meat has been analysed by many authors. Review articles [1–4] and the TNO list [5] indicate that more than 300 compounds have been identified, but no attempt has been made to determine the actual significance of these volatiles for the aroma of cooked chicken meat. As recently reported [6] aroma extract dilution analysis (AEDA) is a systematic approach to evaluate the significance of odorants of boiled beef, since AEDA results in FD factors that are directly proportional to the aroma values of the compounds occurring in the aroma extract isolated from a food [7]. The volatile fraction of a chicken broth was investigated by AEDA in order to identify the primary odorants. These compounds and their FD factors were then compared with those resulting from the AEDA of broths obtained from cow and ox meat.

Experimental

Materials

Frozen chicken meat (boiling fowl grade A, average weight 2600 g) was obtained from a local market and stored at -25°C until use. Meat of cow and ox (top round cut) was purchased in the minced form from a butcher. After 1 day of storage at $+4^{\circ}\text{C}$, the volatiles were isolated from 500 g of each meat and suspended in 500 ml tap water, by simultaneous distillation/extraction [6].

Pure compounds, corresponding to those in Table 2, were obtained commercially: nos. 1, 3, 4, 9, 11–14, 17–19, 22–25, 27, 28, 32,

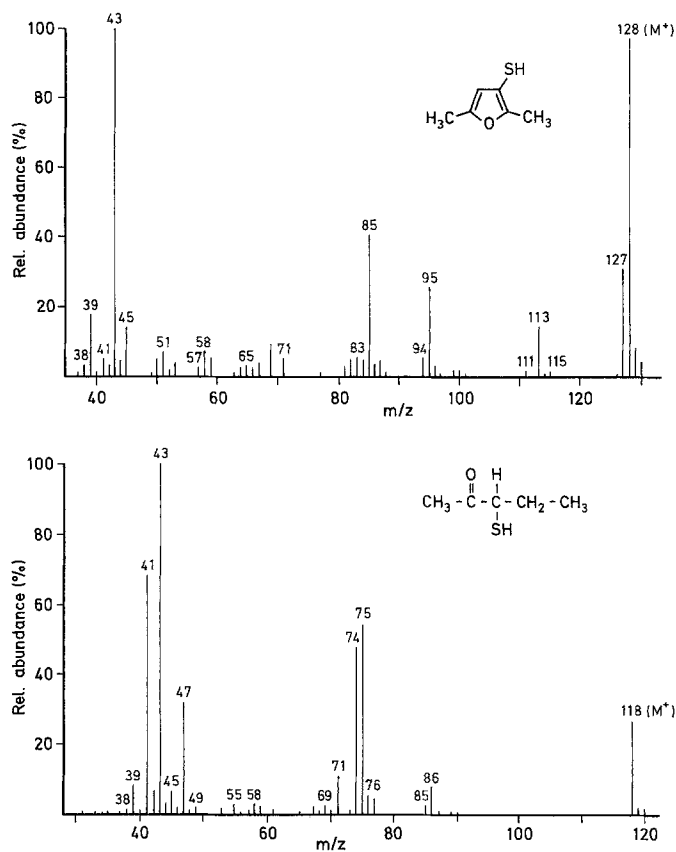


Fig. 1. Mass spectra (EI mode) of 2,5-dimethyl-3-furanthiol and 3-mercapto-2-pentanone

33, 36, 37, 40, 41 were from Aldrich (Steinheim, FRG); no. 8 was from Sigma (Taufkirchen/Munich, FRG); no. 10 was from Haarmann and Reimer (Holzminden, FRG); no. 39 was from Roth (Karlsruhe, FRG); γ -dodecalactone was a gift from Professor Mosandl (University of Frankfurt, FRG).

2,5-Dimethyl-3-furanthiol was synthesized according to Evers [8]. The resulting bis(2,5-dimethyl-3-furyl)disulphide was reduced with NaBH_4 (dissolved in methanol) to the corresponding thiol, which was then purified by preparative gas chromatography. 3-Mercapto-2-pentanone was synthesized according to Asinger et al. [9] and purified by distillation. The mass spectra (EI mode) of 2,5-dimethyl-3-furanthiol and 3-mercapto-2-pentanone are shown in Fig. 1. The solvents used were purified according to Schieberle and Grosch [10]. Silica gel 60 (Merck, Darmstadt, FRG) was treated with HCl and deactivated with 7% (w/w) water [11].

Isolation of volatiles

After thawing, the chicken was cut into pieces (diameter approx. 4 cm) and trimmed of all excess fat. Approximately 500 g of the meat pieces (with bones, without innards) were suspended in 500 ml tap water and then continuously extracted with 60 ml pentane/diethyl ether [6]. The extracts obtained from 21 kg chicken were combined and dried over anhydrous Na_2SO_4 . The solvent was removed by distillation on a Vigreux column (50 \times 1 cm) and the residual solution (40 ml) was stored under nitrogen at -60°C .

Column chromatography

The whole extract was fractionated at 10 – 12°C on a water-cooled, jacket column (30 \times 1.5 cm i.d.), packed with a slurry of silica gel in

pentane. The volume of each sample was 3 ml per run. The elution was successively performed with pentane (fraction A, 100 ml), pentane/diethyl ether (95 + 5, v/v, fraction B, 100 ml), pentane/diethyl ether (9 + 1, v/v, fraction C, 100 ml), pentane/diethyl ether (8 + 2, v/v, fraction D, 100 ml), pentane/diethyl ether (1 + 1, v/v, fraction E, 100 ml) and diethyl ether (fraction F, 100 ml). The fractions were concentrated as reported [6]. Fraction A was separated by preparative high resolution chromatography (HRGC) and fractions D and E were separated by high performance liquid chromatography (HPLC); fractions B, C and F were analysed by HRGC-MS [6].

Preparative HRGC of fraction A

Preparative HRGC was performed with a Carlo Erba gas chromatograph, Type 4200, using a wide-bore capillary column (20 m \times 0.5 mm) coated with OV-1701 according to Grob et al. [12]. The flow rate of the carrier gas was 1.2 ml/min. The effluent containing the odorants to be analysed (2–28 min; subfraction AI), was isolated from the remainder of the volatiles (> 28 min) by condensation in a trap cooled with liquid nitrogen and was then dissolved in pentane/diethyl ether (30 μl ; 2 + 1, v/v). Thus, the material of 35 runs was combined, concentrated and analysed by HRGC-MS [6].

HPLC of fractions D and E

HPLC was performed with the column described previously [6] and at a flow rate of 2.0 ml/min. The following solvents were used: pentane/diethyl ether (9 + 1, v/v) for fraction D and pentane/diethyl ether (8 + 2, v/v) for fraction E. The effluents were monitored at 220 nm. The elution ranges of the subfractions collected are reported in Table 1. In order to obtain enough material for MS, the material of 86 runs (HPLC of fraction D) and 31 runs (fraction E) were collected and concentrated. The subfractions were analysed by HRGC-MS as described [6].

HRGC-effluent sniffing

HRGC-effluent sniffing was carried out as described previously [6]. The FD factors of the odorants were determined by AEDA [6] of the concentrates containing all of the volatile fractions isolated from 500 g meat (cow, ox, chicken). Odour threshold values were approximated by an olfactometric method [7] using 2(E)-decenal as the internal standard. HRGC was performed on the capillaries OV-1701 or SE-54.

Table 1. Elution ranges of the subfractions obtained by HPLC of fractions D and E

Separation of			
Fraction D ^a		Fraction E ^a	
Subfraction	Elution range (ml)	Subfraction	Elution range (ml)
DI	3.0– 6.6	EI	0.8– 3.3
DII	6.6–17.2	EII	3.3– 5.6
DIII	17.2–23.1	EIII	5.6–10.2
DIV	23.1–30.7	EIV	10.2–20.8

^a The fractions were obtained by column chromatography on silica gel

Results

Odorants of chicken broth

The extract containing the volatile fraction of boiled chicken meat smelled intensely like a chicken broth. As summarized in Table 2, 43 odour compounds with FD factors between 16 and 2048 were detected in the extract. The chemical structures of 31 of these odorants were evaluated on the basis of HRGC and MS data and on the

agreement with the odour quality of the corresponding reference substance (Table 2). Compounds 3, 4, 33 and 42, with the highest FD factor values, were identified as 2-methyl-3-furanthiol, 2-furfurylthiol, 2(E),4(E)-decadienal and γ -dodecalactone. HRGC on a capillary column coated with a chiral phase indicated that the γ -dodecalactone was a racemate.

In addition to 2-methyl-3-furanthiol, compound no. 7 also smelled "meaty" and showed a relatively high FD factor value of 256. The RI of no. 7 on the two capillary

Table 2. Volatile odour compounds of chicken broth. Results of gas chromatography-effluent sniffing and identification experiments

No.	Compound	Fraction ^a	RI		Odour description ^b	FD factor
			OV-1701	SE-54		
1	2-Methylthiophene ^c	B	827	765	Sulphurous	16
2	Unknown	–	855	–	Putrid, musty	16
3	2-Methyl-3-furanthiol ^c	AI	924	868	Meat-like, sweet	1024
4	2-Furfurylthiol ^c	B	985	911	Roasty	512
5	3-Mercapto-2-pentanone ^d	EI	998	898	Sulphurous	128
6	2-Acetyl-1-pyrroline ^c	AI	1012	923	Roasty	16
7	2,5-Dimethyl-3-furanthiol ^d	B	1022	968	Meaty	256
8	Methional ^c	AI	1039	903	Cooked potato	128
9	2(E)-Heptenal ^c	DII	1062	958	Fatty	32
10	1-Octen-3-one ^c	AI	1067	980	Mushroom-like	32
11	2,4,5-Trimethylthiazole ^c	EIV	1072	995	Earthy	128
12	2-Formylthiophene ^c	DII	1133	995	Sulphurous	16
13	2-Acetylthiazole ^c	AI	1140	1020	Roasty	16
14	Phenylacetaldehyde ^c	DII	1176	1055	Honey-like	16
15	Unknown	–	1157	–	Sulphurous	16
16	Unknown	EIII	1172	–	Sulphurous	32
17	Nonanal ^c	B	1190	1104	Tallowy, green	128
18	2-Methoxyphenol ^c	DIII	1220	1087	Phenolic	32
19	2-Acetylthiophene ^c	DIII	1240	1090	Sulphurous	16
20	2-Acetyl-2-thiazoline ^c	EIII	1246	–	Roasty	32
21	Unknown	–	1253	–	Unpleasant	16
22	2(E)-Nonenal ^c	DII	1260	1160	Tallowy, fatty	64
23	2-Formyl-5-methylthiophene ^c	EIII	1272	1119	Sulphurous	64
24	4-Methylphenol ^c	DIV	1290	1073	Phenolic	64
25	Decanal ^c	EI	1286	1207	Tallowy	16
26	Unknown	DIV	1301	–	Musty	16
27	2(E),4(E)-Nonadienal ^c	DII	1335	1212	Fatty	64
28	2(E)-Decenal ^c	DII	1368	1262	Tallowy	32
29	Unknown	–	1372	–	Sulphurous	16
30	Unknown	–	1383	–	Fruity	16
31	Unknown	–	1401	–	Unpleasant	16
32	2,4-Decadienal ^c	DII	1417	1295	Fatty, tallowy	128
33	2(E),4(E)-Decadienal ^c	DII	1439	1316	Fatty	2048
34	Unknown	–	1444	–	Sulphurous	16
35	Unknown	–	1449	–	Meaty	16
36	2-Undecenal ^c	DII	1455	1350	Tallowy, sweet	256
37	Indole ^c	DIII	1524	1292	Sweet, burnt	32
38	Unknown	–	1540	–	Burnt	32
39	β -Ionone ^d	B	1620	1493	Violet-like	64
40	Tridecanol ^c	EIV	1695	1593	Tallowy, musty	32
41	γ -Decalactone ^c	EIV	1697	1473	Peach-like	64
42	γ -Dodecalactone ^c	EIV	1898	1685	Tallowy, fruity	512
43	Unknown	–	1977	–	Tallowy	16

^a Fraction or subfraction in which most of the compound appeared after enrichment (column chromatography, HPLC)

^b Odour description assigned during AEDA

^c The compound was identified by comparing it with the reference substance on the basis of the following criteria: RI on the two capillaries detailed in the table, mass spectra obtained by MS (EI) and MS (CI) and odour quality perceived at the sniffing port

^d The MS signals of the substances were too weak for an unequivocal interpretation. The compound was only identified by comparing it with the reference substance on the basis of the RI on the two capillaries and odour quality perceived at the sniffing port

^e The peak was identified by comparison with data from the library of mass spectra

Table 3. Odour thresholds of some volatiles identified in boiled meat

Compound	Threshold ^a (ng/l; air)
2-Methyl-3-furanthiol	0.0025–0.001
Bis (2-methyl-3-furyl)disulphide	0.0007–0.0028
2-Furfurylthiol	0.0045–0.002
2,5-Dimethyl-3-furanthiol	0.0035–0.014
3-Mercapto-2-pentanone	0.045 –0.18
2,4,5-Trimethylthiazole	1.8 –7.2
2-Formyl-5-methylthiophene	1.75 –7.4
2(E),4(E)-Decadienal	0.04 –0.16

^a The range was established by the lowest and the highest value found by three judges; the reference substance for the calculation of the odour thresholds was 2(E)-decenal, odour threshold in air: 2.7 ng/l [15]

columns and the odour quality were identical with the data on 2,5-dimethyl-3-furanthiol (Table 2). Even after enrichment of no. 7 by preparative HRGC, the amount of material was too small to give unequivocal MS signals.

3-Mercapto-2-pentanone (no. 5), which also showed no clear MS signals, methional (no. 8) and 2,4,5-trimethylthiazole (no. 11) were further sulphur containing compounds that contributed significantly to the flavour of the chicken broth (Table 2). 3-Mercapto-2-pentanone has been identified as the product of a thermal degradation of thiamine [13, 14] and is proposed to be a precursor of 3-methyl-2-furanthiol [13].

The odour thresholds of some of the aroma-active compounds of boiled chicken meat were evaluated. The data listed in Table 3 demonstrated that the threshold values of the heterocyclic compounds were very different. Compared to 2,4,5-trimethylthiazole and 2-formyl-5-methylthiophene, the four furan derivatives had extremely low threshold values.

Species-related differences

For a comparative study of the primary odorants of the chicken broth with those of broths from bovine meat (cow and ox), only compounds with an FD factor value of 64, or higher, were selected (Table 4).

The FD factors of the odorants of boiled cow and ox meat were identical, differing, at the most, by a factor of two, which is within the limit of error of the AEDA. By contrast, significant differences were found between odorants of boiled bovine meat and chicken. Particularly bis(2-methyl-3-furyl)disulphide with its “meat-like” odour and the Strecker aldehydes, methional and phenylacetaldehyde, predominated in the aroma of boiled cow and ox. The “fatty” odorants, 2(E),4(E)-decadienal, γ -dodecalactone and 2-undecenal (stereochemistry unknown), prevailed in that of boiled chicken meat. The FD factors of two important odorants, namely 2-methyl-3-furanthiol and 2-furfurylthiol, did not differ significantly in bovine and chicken meat.

Table 4. Comparison of FD factors of odorants appearing in broths from chicken, cow and ox meats^a

Compound	FD factor		
	Chicken	Cow	Ox
2-Methyl-3-furanthiol	1024	512	512
Bis(2-methyl-3-furyl)disulphide	< 16	2048	1024
2-Furfurylthiol	512	512	256
2,5-Dimethyl-3-furanthiol	256	< 16	< 16
3-Mercapto-2-pentanone	128	32	32
Methional	128	512	1024
2,4,5-Trimethylthiazole	128	< 16	< 16
2-Formyl-5-methylthiophene	64	< 16	< 16
Phenylacetaldehyde	16	64	32
2(E),4(E)-Decadienal	2048	64	32
2,4-Decadienal	128	< 16	< 16
2-Undecenal	256	< 16	< 16
γ -Dodecalactone	512	< 16	< 16
γ -Decalactone	64	< 16	< 16
Nonanal	128	< 16	< 16
2(E)-Nonenal	64	32	64
2(E),4(E)-Nonadienal	64	< 16	< 16
β -Ionone	64	64	64
<i>p</i> -Cresol	64	< 16	< 16

^a The compounds which appeared in one of the meat species with an FD factor of at least 64 are compared

Table 5. Fat content and fatty acid composition of the chicken and cow meat samples

	Chicken ^a (%)	Cow (top round) (%)
Fat ^b	14.6	8.3
Fatty acid ^c		
14:0	0.6	2.2
16:0	20.7	23.7
16:1	4.5	3.5
18:0	5.7	20.2
18:1	42.7	44.8
18:2	23.4	2.2
18:3	0.8	0.2
20:4	0.4	< 0.1
Other	1.2	3.1

^a Chicken was minced and trimmed of all excess fat (cf. section on “Isolation of volatiles”)

^b The fat content was determined according to a standard method [19]

^c The fatty acid composition was determined by gas chromatography [20]

Differences in the amount and composition of fat have previously been discussed [16–18] as causes of the formation of different aromas during the boiling of chicken and bovine meat. These data were again determined for the meat samples of chicken and cow used in the present study. As shown in Table 5, the chicken meat contained nearly twice as much fat as the cow meat and a 10-fold higher level of linoleic acid. These differences agree with the data published in the literature [21].

Discussion

It has been reported [22, 23] that volatile sulphur compounds play a major role in the flavour of chicken broth. The results reported here demonstrate that the sulphur compounds 2-methyl-3-furanthiol, 2-furfurylthiol, 2,4,5-trimethylthiazole and methional were identified as the primary odorants of the chicken broth. In addition, some evidence was found that 2,5-dimethyl-3-furanthiol and 3-mercapto-2-pentanone contributed to the flavour of the chicken. Of these sulphur compounds, 2-methyl- and 2,5-dimethyl-3-furanthiol (each with a meat-like odour quality) and 2-furfurylthiol (roasty) have extremely low odour thresholds in air. In the case of the 2-furfurylthiol, a very low odour threshold was also evaluated for a solution of the compound in water [24].

The S-containing heterocyclic compounds 2,4,5-trimethylthiazole and 2-formyl-5-methylthiophene contributed to the odours of the broths, although they had lower FD factor values than the furan derivatives. This difference may be due to the odour threshold values, which in the case of the thiazole and the thiophene derivatives are some orders of magnitude higher than those found for the furan derivatives. The role of H₂S, which has been suggested by Pippen and Mecchi [25] as a further sulphur compound contributing to the chicken flavour, was not analysed in our study.

Carbonyl compounds formed by oxidative degradation of unsaturated acyl lipids have been discussed by Minor et al. [26] as a cause of the "chicken" aroma, since the removal of the carbonyls from the volatile fraction resulted in a loss of the "chicken-odour" and an intensification of the "meaty odour". In particular, the 2,4-decadienal was found by Pippen and Nonaka [27] to contribute to the aroma of chicken. The importance of 2(E),4(E)-decadienal was confirmed in our study, since it showed the highest FD factor of all the aroma compounds extracted from the chicken broth. γ -Dodecalactone and 2-undecenal were also potent odorants arising from a breakdown of lipids.

Rothe et al. [17] reported that the addition of sunflower oil changed the odour quality of a beef broth to that of a chicken broth. In agreement with the mentioned results of Minor et al. [26], the authors [17] concluded that carbonyl compounds, formed by the autoxidation of unsaturated lipids, change the "meat-like" odour into a "chicken-like" odour.

The results reported in this paper indicate that odorants formed by peroxidation of unsaturated lipids prevailed in boiled chicken in comparison to boiled bovine (cow and ox) meat. In particular 2(E),4(E)-decadienal, which is formed by the autoxidation of linoleic acid [28], appeared to be a major odorant of the chicken broth and played a minor role in the broth from cow and ox meat. This difference most likely results from the 10-fold higher level of linoleic acid in the chicken meat in comparison to the bovine meat.

On the other hand, the levels of meat-like odorants were lower in the chicken broth than in the beef broth. The difference was especially striking for bis(2-methyl-3-furfuryl)disulphide, the major odorant of beef, as its FD

factor was 256-fold lower in the chicken. By contrast, the level of its reduction product 2-methyl-3-furanthiol and also the level of 2-furfurylthiol in the chicken and beef were in the same range. The differences found for the levels of the thiol and its disulphide suggest that the oxidation of 2-methyl-3-furanthiol to its disulphide was inhibited during boiling of the chicken meat. An explanation could be a competition of the thiols and the linoleic acid for the gaseous oxygen. It is assumed that the relatively high level of linoleic acid in the chicken meat captures most of the oxygen for peroxidation reactions and, in this way, protects the thiol against oxidation to the disulphide.

Model experiments by Whitfield et al. [29] showed that lipids reduce the formation of heterocyclic compounds by the Maillard reaction. In particular, the levels of 2-methyl-3-furanthiol and 2-furfurylthiol were lowered to 33% and 50%, respectively, after heating a mixture of cysteine and ribose in the presence of lecithin. The authors [29] assumed that this effect could be the result of volatile carbonyl compounds, derived from the autoxidation of the lipid, capturing reactants (e.g. H₂S) essential for the formation of the heterocyclic compounds.

As reported in the results section, the sum of 2-methyl-3-furanthiol and its disulphide was much lower in the chicken than in the bovine volatiles. This difference may be due to the reactions proposed by Whitfield et al. [29], as the level of unsaturated lipids was much higher in the chicken. Also, the higher FD factor of methional in the beef compared to the chicken volatiles indicates that the Strecker degradation of methionine is partially inhibited during boiling of the chicken meat.

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