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Potent odorants formed by lipid peroxidation as indicators of the warmed-over flavour (WOF) of cooked meat

Ute Christine Konopka, Helmut Guth, Werner Grosch

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

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Abstract. Hexanal (I), 1-octen-3-one (II), (*E*,*E*)-2,4-nonadienal (III), (*E*,*E*)-2,4-decadienal (IV) and *trans*-4,5-epoxy-(*E*)-2-decenal (V) were quantified in cooked meat (beef, pork, chicken) in which the warmed-over flavour (WOF) was observed after storage for 2 days at 4 °C. Odour activity values of the compounds on the basis of odour threshold values in water revealed that compounds I and V contributed most strongly to WOF. During the storage, free iron but not metmyoglobin catalysed the increase of compounds I, II, IV and V in models containing the lipids of beef meat.

Introduction

The flavour of cooked and uncured meat is not stable. After only a few hours of refrigerated storage and then reheating, meat can exhibit an off-flavour which is characterised by terms such as warmed-over, stale, metallic, putrid and rancid [1–3].

It is generally accepted that preoxidation of meat lipids contributes strongly to the development of the warmed-over flavour (WOF). In particular, the phospholipids having a higher concentration of polyunsaturated fatty acids are quite susceptible to autoxidation and, therefore, are considered to play a major role as precursors of WOF [2, 4].

In a previous study [5], the potent odorants resulting from the peroxidation of unsaturated fatty acids and contribute intensely to the WOF of boiled beef, were screened by aroma extract dilution analysis (AEDA). In a comparative AEDA of the volatile compounds obtained from freshly boiled as well as boiled and stored beef meat, hexanal, 1-octen-3-one, (*Z*)-2-octenal, (*Z*)-2-nonenal, (*E*,*E*)-2,4-nonadienal and *trans*-4,5-epoxy-(*E*)-2-decenal were identified as the most potent odorants contributing to WOF. Of these odorants, hexanal, 1-octen-3-one, (*E*,*E*)-2,4-nonadienal and *trans*-4,5-epoxy-(*E*)-2-decenal were selected as WOF indicators. In the present study these compounds, as well as (*E*,*E*)-2,4-decadienal, which is the most important odorant formed by lipid peroxidation in freshly boiled beef [6] and in stewed beef [7], were quantified by stable isotope dilution assays in several samples of cooked meat.

Furthermore, a model experiment was performed to clarify whether myoglobin or non-haem iron plays a role in accelerating the peroxidation of meat lipids with formation of the five above-mentioned carbonyl compounds.

Correspondence to: W. Grosch

Materials and methods

Meat. The meat samples were obtained from a local market and all excess fat was trimmed off. Beef meat (top round), cured pork (Kasseler) and chicken (brisket) were boiled [5] and stewed [8] as reported. Beef (top, round, 500 g) was ground by one pass through a ginding disc with 4-mm holes and then suspended for 30 min in an aqueous solution of $CaCl_2$ (2% w/w, 500 ml). The meat was filtered off, packaged in a polyester bag and then boiled [5]. Pork meat (shoulder, portions each of 130 g was salted (NaCl, 0.25% w/w), packaged in bags, heated for 5 min at 95 °C and then for 30 min at 80 °C. One-half of the freshly cooked meat samples was immediately analysed; the other half was packaged in polythene bags, which were flushed with nitrogen, sealed and stored as detailed in Table 2 before analysis.

Lipids. Fresh beef (bull, round, 100 g) was ground, mixed with anhydrous Na_2SO_4 (1 + 1, w/w) and then extracted with diethyl ether for 6 h in a Soxhlet apparatus. After addition of Sephadex G25 superfine (20 g, Pharmacia, Uppsala, Sweden), the solvent and the volatile compounds were distilled under high vacuum (0.1 Pa) for 3 h at 30 °C and then for 3 h at 50 °C. The lipids (3.6 g) spread on the carrier Sephadex remained in the distillation flask as powdered material.

Chemicals. Hexanal was from Merck (Darmstadt, Germany), 1-octen-3-one was from Haarmann and Reimer (Holzminden, Germany), (*E*,*E*)-2,4-nonadienal and (*E*,*E*)-2,4-decadienal were from Aldrich (Steinheim, Germany). Silica gel 60 (0.063–0.2 mm; Merck) was treated with HCl [9], dried at 180 °C and finally conditioned to a water content of 6% by mass. The following compounds were synthesised according to the cited literature: *trans*-4,5-epoxy-(*E*)-2-decenal [10], [^{2}H]hexanal [11], [^{2}H]1-octen-3-one [10], [^{2}H]-

Table 1. Thin-film capillaries, selected ions and calibration factors for mass chromatography of the odorants of cooked meat

Odorant	Capillary	Selected ion (m/z)	Internal standard	Selected ion (m/z)	Calibration factor[a]
Hexanal (I)	OV-1701	101	d-I	103–105[b]	0.81
1-Octen-3-one (II)	OV-1701	127	d-II	129	0.56
(E,E)-2,4-Nonadienal (III)	OV-1701	139	d-III	141	1.04
(E,E)-2,4-Decadienal (IV)	OV-1701	153	d-IV	155–157[b]	1.01
trans-4,5-Epoxy-(E)-2-decenal (V)	SW-10	169	d-V	171–173[b]	0.67

[a] The calibration factor refers to a 1:1 (by weight) mixture of the labelled and the unlabelled compound [10]
[b] The sum of the relative abundances of the ions was calculated
For each odorant, the internal standard was the deuteriated form (d) of the odorant

Table 2. Flavour of cooked meat samples

Sample		Meat flavour	
No.	Type	Fresh[a]	Stored[b]
1.	Beef, boiled	Meaty, boiled	Green tallowy, metallic
2	Beef, boiled	Meaty, boiled	Meaty, boiled (weaker in intensity than the fresh sample)
3.	Beef (+ Ca^{2+}), boiled[c]	Meaty, boiled, musty	Sandy, chemical
4.	Beef, stewed	Meaty, caramel-like, beef-like	Green, metallic
5.	Pork, cooked	Meaty, fatty, tallowy	Fatty, tallowy, green
6.	Chicken, cooked	Meaty, sulphurous, sweet	Green, metallic, musty

[a] The freshness of the sample was preserved by storage at − 60 °C
[b] Sample nos. 1 and 3–6 were stored for 2 days at 4 °C; sample no. 2 for 2 days at − 29 °C
[c] The beef was boiled in an aqueous solution of $CaCl_2$ (2% w/w)

(*E,E*)-2,4-nonadienal [11], [^{2}H](*E,E*)-2,4-decadienal [10], [^{2}H]-*trans*-4,5-epoxy-(*E*)-2-decenal [10]. The concentrations of the deuteriated compounds were determined as described previously [10, 11].

High-resolution gas chromatography (HRGC) – mass spectrometry (MS) analysis. HRGC was performed by means of a Carlo Erba gas chromatograph, Type 4300 (Carlo Erba, Hofheim, Germany) and by using the following fused silica thin-film capillaries: OV-1701 (30 m × 0.32 mm, 0.25 µm film thickness) from J & W Scientific (Folsom, USA) and SW-10 (30 m × 0.32 mm, 0.25 µm film thickness) from Supelco (Sulzbach, Germany). The samples (0.2–0.5 µl) were applied by the "on-column injection" technique at 35 °C. After 2 min (1 min for SW-10) the temperature of the oven was raised by 40 °C/min to 50 °C (60 °C for SW-10), held isothermally for 2 min and then raised by 4 °C/min (8 °C/min for SW-10) to 240 °C (250 °C for SW-10) which was finally held for 10 min. The flow of the carrier gas, helium, was 2.2 ml/min. For the isotope dilution assays the MS 8230 (Finnigan, Bremen, Germany) was coupled with the capillaries described above. Ion abundances were monitored in the ranges given in Table 1. By comparison of the abundance of the selected ion of the odorant to that of the internal standard (see Table 1), the data needed to carry out the quantitative calibration of the method were provided. The calibration factors were calculated from the model mixtures as described recently [10] and are listed in Table 1.

Analysis of meat samples. The ground meat sample (250 g) was mixed with anhydrous Na_2SO_4 (1 + 1, w/w), soaked overnight in diethyl ether (90 ml) and then extracted with this solvent in a Soxhlet apparatus for 6 h. Of the extract, 1% was removed accurately and, after spiking the sample with a definite amount of [^{2}H] hexanal, hexanal (I) was directly determined by HRGC-MS.

The major part of the extract was spiked with the internal standards, i.e. [^{2}H]1-octen-3-one, [^{2}H](*E,E*)-2,4-nonadienal, [^{2}H]-(*E,E*)-2,4-decadienal and [^{2}H]*trans*-4,5-epoxy-(*E*)-2-decenal and then concentrated to 150 ml by distilling the solvent on a Vigreux column (50 cm × 1 cm) at 40 °C. The solution of the volatile compounds was distilled from the nonvolatile materials under high vacuum [12–14]. After concentration to 1 ml, the condensate was fractionated at 10–12 °C on a water-cooled column (30 cm × 1.7 cm) packed with a slurry of Silica gel 60 in pentane. Elution was performed with 100 ml of the following pentane-diethyl ether mixtures: 97.5 + 2.5, v/v (fraction A), 95 + 5, v/v (fraction B) and 90 + 10, v/v (fraction C). Fractions A–C were concentrated to approximately 50 µl. The odorants (*E,E*)-2,4-nonadienal and (*E,E*)-2,4-decadienal were quantified by HRGC-MS in fraction B (Table 1). Fractions A and C were subjected to high-performance liquid chromatography (HPLC) on Hypersil [15]. Elution (flow rate 2 ml/min) of fraction A was performed with pentane-diethyl ether (97.5 + 2.5, v/v) and of fraction C with pentane-diethyl ether (90 + 10, v/v). Odorant 1-octen-3-one was localised in the effluent obtained by HPLC of fraction A and *trans*-4,5-epoxy-(*E*)-2-decenal in that of fraction C. The effluents containing these odorants were concentrated to approximately 20 µl and then subjected to HRGC/MS (Table 1).

Analysis of the model system. After addition of the internal standards to the model systems, odorants were quantified as reported in the section entitled "Analysis of meat samples".

Flavour evaluation. The freshly cooked meat sample was divided into two portions. One portion was stored at − 60 °C, and the other at the temperatures detailed in Table 2. After 2 days the samples (20 g each) in covered glass beakers (diameter, 40 mm; capacity 45 ml) were heated in a microwave oven up to 50 °C. The odour and the taste of the samples were compared by a panel consisting of at least five experienced assessors.

Results and discussion

Flavour of the meat samples

The odour and taste of the cooked meat samples were evaluated (Table 2) prior to carrying out the quantification of the selected five odorants.

In sample nos. 1 and 4–6, the meaty flavour impression had disappeared after storage for 2 days at 4 °C and an off-flavour, in which a green note predominated, had developed. In addition, a metallic note contributing to WOF was perceived in the stored cooked meat sample nos. 1, 4 and 6. Storage of boiled beef at − 29 °C delayed the formation of WOF (sample no. 2); the beef tasted meaty, but with lower intensity.

According to Graf and Panter [16], Ca^{2+} inhibits the formation of WOF. Actually, WOF was not perceivable in stored beef (sample 3) which had been boiled in an aqueous solution of calcium chloride. Nevertheless, this treatment is not advisable, because the freshly boiled beef smelled unpleasantly musty, and the meaty aroma changed into a sandy, chemical off-flavour during the storage period of 2 days.

Concentration levels of the WOF indicators

The WOF indicators and (*E*,*E*)-2,4-decadienal were quantified with stable isotope dilution assays. The results are listed in Table 2.

Storage of the boiled beef at 4 °C yielded a tenfold increase of hexanal (sample no. 1 in Table 3). Also, (*E*,*E*)-2,4-nonadienal and *trans*-4,5-epoxy-(*E*)-2-decenal increased during storage of the meat sample, but neither odorant reached the high level of hexanal. The concentration of (*E*,*E*)-2,4-decadienal, which contributes to the overall odour of cooked beef [6, 7], increased only to a small extent in samples nos. 1–4 during storage (Table 3), suggesting that it is not involved in the formation of WOF.

The slow increase of hexanal, (*E*,*E*)-2,4-nonadienal and epoxydecenal reflects the inhibition of lipid peroxidation in the boiled beef which was stored at − 29 °C (no. 2 in Table 3). Also the treatment with Ca^{2+} (sample no. 3) retarded the formation of the WOF indicators.

Beef was roasted in the presence of coconut oil for 10 min, and after addition of water, stewed for 4 h [8]. During the long heating process the Maillard reaction took place and led to 11.7 mg/kg 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone [7] which stimulates the caramel-like note in the flavour profile (Table 2). It has been suggested [17] that reductones and other reducing substances resulting from the Maillard reaction may inhibit the WOF. The storage experiment with the stewed beef (sample no. 4) did not confirm this hypothesis. The WOF was formed (Table 2) and the increases in both hexanal and *trans*-4,5-epoxy-(*E*)-2-decenal were comparable with that in boiled beef showing WOF after storage (Table 3). Furthermore, 1-octen-3-one increased strongly, while the levels of the two dienals were not influenced by the processing and by the storage of this sample.

As in the case of boiled beef, WOF in the cooked pork (sample no. 5) was indicated by increased concentrations of hexanal, (*E*,*E*)-2,4-nonadienal and *trans*-4,5-epoxy-(*E*)-2-decenal.

The WOF development (sample no. 6) during the storage of boiled chicken (Table 2) was indicated by a significant increase of the concentrations of hexanal, 1-octen-3-one, (*E*,*E*)-2,4-nonadienal and *trans*-4,5-epoxy-(*E*)-2-decenal (Table 3).

WOF is not a problem in cured meat [18]. This finding was confirmed by the determination of hexanal in cured pork (sample no. 7): the concentration of the aldehyde was low and did not change during storage.

Odour activity values (OAVs) of the WOF indicators

To gain an initial insight into the contribution of the five odorants to the odour and taste of the cooked meat samples, OAVs were calculated on the basis of their odour thresholds, which were nasally and retronasally determined by using water as solvent (Table 4). Higher OAVs

Table 3. Changes in the levels (μg/kg) of important odorants formed by lipid peroxidation during storage of cooked meat samples

Sample		Odorant									
		Hexanal		1-Octen-3-one		(E,E)-2,4-Nonadienal		(E,E)-2,4-Decadienal		*trans*-4,5-Epoxy-(E)-2-decenal	
No.	Type[a,b]	f	s	f	s	f	s	f	s	f	s
1.	Beef, boiled	390	4705	< 0.05	< 0.05	14	223	224	283	0.1	16
2	Beef, boiled	133	886	1	1	11	13	43	45	1	< 1
3	Beef (+Ca^{2+}), boiled[c]	500	1022	2	1	5	4	11	12	6	8
4	Beef, stewed	494	5871	2	12	14	13	18	23	8	13
5	Pork, cooked	165	4000	2	3	41	127	182	287	1	11
6	Chicken, boiled	175	2769	3	11	9	42	79	70	8	23
7	Cured pork, boiled	240	228[d]	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.

[a] The freshly cooked (f) and the cooked and stored (s) samples were analysed. The data are means of two assays; maximum standard deviation = ± 10%

[b,c] Refer to footnotes b and c in Table 2

[d] The sample was stored for 2 days at 4 °C

n.a., Not analysed

based on nasal odour thresholds indicate the odorants contributing to the odour of the meat samples. On the other hand, retronasally perceivable odorants, in addition to taste substances, are involved in the whole flavour impression that develops when the cooked meat is chewed.

In beef samples showing WOF, the nasal OAV of hexanal increased from 100 to more than 1000 (Table 5). Most likely, WOF has to be expected in boiled and stewed meat when the nasal OAV of hexanal surpasses values of 400 to 500. In addition to hexanal, a retronasal OAV of *trans*-4,5-epoxy-(*E*)-2-decenal higher than 800 was involved in the WOF of meat samples nos. 1, 4 and 6. On the basis of their odour qualities, hexanal is responsible for the green odour and *trans*-4,5-epoxy-(*E*)-2-decenal for the metallic odour notes. Both compounds were perceived in cooked and stored meat samples nos. 1, 4 and 6 (Table 2).

On the basis of high OAVs 1-octen-3-one contributed to the WOF formed in stewed beef (sample no. 4) and boiled chicken (sample no. 6). 1-Octen-3-one has mushroom-like, metallic odour quality [19], but it has been found [20] that small amounts of this vinylketone in combination with other aldehydes formed by lipid peroxidation result in an oxidized flavour. (*E*,*E*)-2,4-Nonadienal contributed with a high OAV to the WOF of boiled beef (sample no. 1) and cooked pork (sample no. 5).

Table 4. Odour detection thresholds in water

Compound	Threshold[a]	
	Nasal	Retronasal
1-Octen-3-one	0.05	0.0075
(E,E)-2,4-Nonadienal	0.09	0.06
(E,E)-2,4-Decadienal	0.2	0.03
trans-4,5-Epoxy-(E)-2-decenal	n.d.	0.015

[a] The threshold values (μg/kg) were determined by at least three trained judges
n.d., Not determined

Model experiment

As recently reviewed [21], there is a discussion whether haem pigments or non-haem iron plays a greater role in accelerating lipid peroxidation in cooked meat. We have performed a model experiment to clarify the contributions of myoglobin and iron ions to the peroxidation of beef lipids with regard to the five odorants.

Beef muscle contains 2–5 mg/g (wet weight) of myoglobin [22] and 17.4 μg/g (wet weight) of iron [23]. In the models studied (Table 6) the concentrations of the two

Table 5. Odour activity values (OAVs) of the five carbonyl compounds

Sample		OAV[a] of Odorant								
		Hexanal		1-Octen-3-one		(E,E)-2,4-Nonadienal		(E,E)-2,4-Decadienal		*trans*-4,5-Epoxy-2 (E)-decenal
No.	Type[b]	N	R	N	R	N	R	N	R	R
1.	Beef, boiled:									
	fresh	87	37	< 1	< 1	156	245	1120	4480	7
	stored	1045	448	< 1	< 1	2478	3912	1415	5660	1067
2.	Beef, boiled:									
	fresh	30	13	20	133	122	143	215	860	67
	stored	197	84	< 20	< 133	144	228	225	900	< 67
3.	Beef (+ Ca^{2+}), boiled[c]:									
	fresh	111	48	40	267	56	88	55	220	400
	stored	227	97	20	133	44	70	60	240	533
4.	Beef, stewed:									
	fresh	110	47	40	267	155	246	90	360	533
	stored	1305	559	240	1600	144	228	115	460	867
5.	Pork, cooked:									
	fresh	37	16	40	267	456	719	910	3640	67
	stored	889	381	60	400	1411	2228	1435	5740	733
6.	Chicken, boiled:									
	fresh	39	17	60	400	100	158	145	580	533
	stored	615	264	220	1467	467	737	350	1400	1533
7.	Cured pork:									
	fresh	53	23							
	stored	51	22							

[a] The odour activity values were calculated by dividing the concentration of the odorant (Table 3) by the mean values of its nasally and retronasally estimated threshold in water (Table 4)
[b,c] Refer to footnotes b and c in Table 2
N, Nasal; R, retronasal

Table 6. Results of the model experiment

Compound	Reaction system					
	A		B		C	
	0 days	2 days	0 days	2 days	0 days	2 days
Hexanal	5.5	5.4	5.8	6.6	5.7	54
1-Octen-3-one	0.3	0.5	0.2	0.2	0.3	1.4
(E,E)-2,4-Nonadienal	1.7	3.4	1.9	3.0	2.0	3.6
(E,E)-2,4-Decadienal	2.7	3.1	4.0	4.5	2.6	6.8
trans-4,5-Epoxy-(E)-2-decenal	2.9	3.3	1.3	1.4	2.6	5.8

Values are given in μg/kg of the reaction system
Reaction system A: meat lipids (3.6 g) spread on Sephadex G25 (20 g) were suspended in a mixture of water (75 ml) and K/Na-phosphate buffer (0.1 mol/l, pH 5.8, 10 ml); reaction system B: system A plus myoglobin (540 mg); reaction system C: system A plus $FeSO_4 \cdot 7H_2O$ (9.95 mg = 2 mg Fe)
Each reaction system was refluxed for 30 min in a nitrogen atmosphere. One-half of each reaction system was immediately analysed (0 days) and the remaining half after a storage period of 2 days at 4 °C

catalysts approached those present in 100 g of beef meat. The lipids used in the models contained 4% (by weight) linoleic acid (unpublished results), the precursor of the five carbonyl compounds [19]. The two catalysts of lipid peroxidation were absent in the reaction system A (Table 6). During the storage period of 2 days the concentration of (*E,E*)-2,4-nonadienal doubled, whereas the levels of the other carbonyl compounds varied only within the limits of the analytical error. We expect that during boiling, myoglobin in reaction system B is oxidized to metmyoglobin as occurs during the boiling of meat [24]. However, the results of the reaction system B indicate that this haem protein did not accelerate the formation of the odorants during the storage of the model. In contrast, iron in reaction system C was very effective as a catalyst of lipid peroxidation, as the five carbonyl compounds increased significantly during the storage period. In particular, hexanal was nearly tenfold higher than in the freshly boiled reaction system C. The difference between the reaction systems B and C indicates that free iron is much more effective than myoglobin or metmyoglobin in promoting the peroxidation of linoleic acid with formation of hexanal and of the other carbonyl compounds. Only (*E,E*)-2,4-nonadienal was an exception, as its concentration in the stored reaction system C was not significantly higher than in the reaction systems A and B after storage.

The result that free iron catalyses the formation of WOF odorants agrees with the observation that WOF is delayed by the addition of chelators, e.g. ethylenediaminetetraacetic acid (EDTA) [25] which trap the free iron. Also the stability of cured meat has been explained [26] by the formation of stable complexes which prevent the release of iron (II) ions from haem pigments. However, new studies [27] have shown that the nitrosylmyoglobin formed during curing acts also as an antioxidant, as it provides nitric oxide which may inhibit lipid peroxidation by the termination of free-radical processes.

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