Identification of Volatile Flavor Compounds with High Aroma Values from Shallow-Fried Beef

Kirsten Specht and Werner Baltes*

Institute for Food Chemistry, Technical University of Berlin, Gustav-Meyer-Allee 25, 13355 Berlin, Germany

Aroma extract dilution analysis of fried beef steak volatiles trapped during frying with two different cooking conditions resulted in 50 primary odor compounds with flavor dilution (FD) factors between 4 and 2048. Beef steak frying at hot plate temperatures of 280 °C for 3 min per side or of 300 °C for 1 min per side, respectively, yielded statistically significant different results determined in a sensory sequence test. Differences in flavor qualities of the meat samples obtained by the varied frying conditions became manifest by employing the sensory aroma profile analysis. The flavor extracts were analyzed by high-resolution gas chromatography effluent sniffing and GC/MS. Preparative fractionation of sensorially interesting regions of the effluent sniffing gas chromatogram resulted in identification of a large number of overlapping compounds, which would not otherwise have been possible. Pleasant flavor qualities were much stronger for the 280 °C extract than for the 300 °C extract. This was mainly due to the pleasant odorous combination of 2-ethyl-3,5-dimethylpyrazine and 2-propyl-3-methylpyrazine with FD factors of 2048 in the 280 °C extract and 256 in the 200 °C extract, respectively.

Keywords: *Fried beef flavor; dynamic headspace analysis; aroma extract dilution analysis; flavor dilution factors; aroma profile analysis*

INTRODUCTION

To date more than 800 beef flavor compounds have been identified. Flavor development appears to be due to a combination of thermal degradation products of sugars, amino acids, and nucleotides as well as to the products of the Maillard reaction and lipid oxidation (Shahidi *et al.*, 1986). Differences in the flavor of beef are mainly due to different cooking methods such as boiling, roasting, frying, pressure-cooking, and retort heating (Morton and MacLeod, 1982). According to MacLeod and Seyyedian-Ardebili (1981) the cooking method contributes significantly to the formation of volatile compounds and hence relates to differences in overall meat flavor. Concerning the question as to which of the identified volatile compounds are important for the characteristic odor of foodstuffs, Ullrich and Grosch (1987) and Grosch (1990) presented a screening procedure for important volatile odor compounds, called aroma extract dilution analysis. Volatiles of stepwise-diluted extracts were analyzed by high-resolution gas chromatography (HRGC) and effluent sniffing to determine flavor dilution (FD) values, defined as the lowest concentration at which a compound is still sensorially detectable. Application of this technique enabled the identification of potent odorants with high aroma values in a series of foodstuffs, such as cherry juice (Schmid and Grosch, 1986), autoxidized linoleic acid (Ullrich and Grosch, 1987), wheat and rye bread crusts (Schieberle and Grosch, 1987), meat broths of chicken, ox, and cow (Gasser and Grosch, 1988, 1990), reheated boiled beef (Konopka and Grosch, 1991), and roast beef (Cerny and Grosch, 1992).

The objective of the present study was the determination of potent odorants by use of the aroma extract dilution analysis of shallow-fried beef steak volatiles trapped during frying without oil in a specially constructed apparatus. Aroma compounds responsible for optimal flavor development were determined by comparing two extracts, each trapped under different frying conditions. The use of oil for heat transfer has been avoided since heated oil would give a large number of thermal degradation products probably overlapping important meat aroma compounds which could also originate from lipid degradation.

EXPERIMENTAL PROCEDURES

Materials. Three longissimus dorsi of young bulls, hung for 14 days, were trimmed of all visible tendons and fat and cut into 8 × 4 cm large and 2 cm thick slices. The slices of each of the three ribeyes were uniformly distributed, eight being placed in each polythene bag. The bags were sealed and stored at −10 °C. For investigation, the meat was allowed to thaw overnight at 7 °C and was brought to room temperature before frying.

Tenax GC 60/80 was purchased from Alltech GmbH, Unterhaching, Germany. Diethyl ether of analar standard was twice distilled before use. The Tenax GC trap was prepared by using a Pasteur pipet without tip, filled with 0.3 g of Tenax GC and sealed on both sides with a plug of silanized glass wool. The trap was ready to use after elution with 5 mL of twice distilled diethyl ether. For estimation of the relative concentrations of the volatiles and the dilution of the samples, respectively, 0.0024 g of α-pinene diluted in 20 mL of diethyl ether was used as external standard. External standard solution (100 μL) was applied on the prepared Tenax trap. Diethyl ether was removed by evaporation with purified air at a flow rate of 1800 mL/min.

Isolation of the Volatiles. A new method for direct isolation of the headspace volatiles of beef steak during shallow frying without oil has been developed including the construction of an appropriate apparatus (Figure 1). A joint clip (3) fastens a commercially available Teflon-coated pan (1) to a lid adapter with flange LF 150 and center neck NS 29/32, one angled side neck NS 14/23, and two parallel side necks NS 29/32 (2). The apparatus was sealed with a viton gasket (4) between the pan and lid flange. The meat slice was fixed with four Teflon screws to a perforated 5 mm thick Teflon plate of 10 cm diameter (5) which was attached to a stainless steel tube (6). The tube was fixed with a cone screw connecting adapter NS 29/32 with plastic cap and seal in the center neck.

0021-8561/94/1442-2246$04.50/0

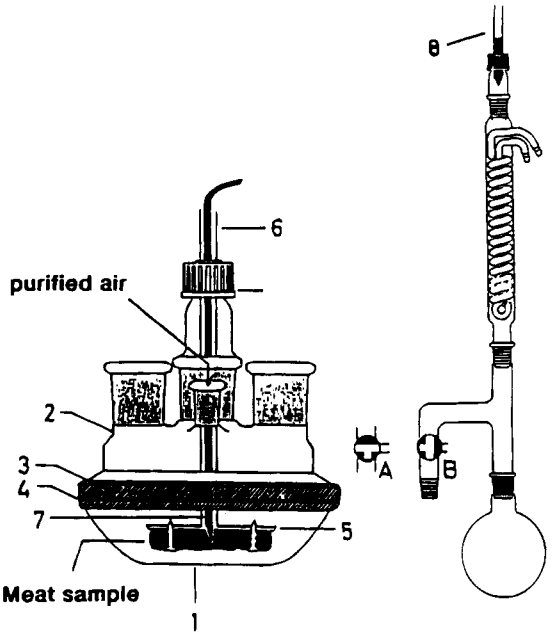

Figure 1. Apparatus for direct isolation of steak volatiles.

Internal temperature measurement during frying was accomplished by a thermocouple (7) passed down the middle of the stainless steel tube, the junction being in good contact with the meat. One of the two side necks NS 29/32 was connected to a modified distilling head with a three-way PTFE stopcock and a Dimroth condenser; the other neck was sealed with a glass stopper.

After a standardized conditioning period, during which the apparatus was flushed with purified air through the side neck NS 14/29, the Teflon plate with the meat slice was pressed to the bottom of the pan by means of the stainless steel tube to obtain optimal heat transfer. At the same time, the three-way stopcock was adjusted from position A to position B to pass the headspace volatiles through the Tenax GC trap (8) at the top of the reflux condenser with an air flow of 1800 mL/min. At the end of frying the Teflon plate was raised and the stopcock was adjusted to the initial position. Frying of the second meat side was carried out in the same way followed by trapping of the volatiles for an additional standardized period of time after heating. With the described technique reproducible composed extracts could be obtained under standardized experimental conditions.

Frying Conditions. Determination of potent odorants which probably could indicate a pleasant aroma development requires comparison of extracts of beef steak volatiles trapped at frying conditions which would give statistically significant different sensory results such as overall pleasant or unpleasant acceptability. Frying conditions were compared in a sensory sequence test according to the procedure of Neumann *et al.* (1983).

A test panel of 12 members evaluated seven meat samples fried at different temperatures and times: sample I, 250 °C, 2 min per side; sample II, 250 °C, 3 min per side; sample III, 250 °C, 4 min per side; sample IV, 280 °C, 2 min per side; sample V, 280 °C, 3 min per side; sample VI, 300 °C, 1 min per side; sample VII, 300 °C, 2 min per side. Statistically significant differences were determined between samples V and VI. Differences in flavor qualities of the meat samples obtained at the varied frying conditions became manifest by employing the sensory aroma profile analysis according to the methods of Neumann *et al.* (1983) and Rothe *et al.* (1981a,b).

On the basis of these results, each side was fried either for 3 min at a hot plate temperature of 280 °C (overall pleasant acceptability) or for 1 min at a hot plate temperature of 300 °C (overall unpleasant acceptability). A precision hot plate provided constant temperatures up to 300 ± 1 °C. To obtain comparable sampling times of 16 min per meat slice, the volatiles were purged on Tenax GC during frying and additionally for standardized periods of 10 min (280 °C extract) and 14 min (300 °C extract), respectively, after heating. Frying eight slices of beef yielded extracts of sufficient concentrations leading to total sampling times of 128 min. After each slice was fried, the pan was wiped out with a dry cloth. Investigation of the volatiles was carried out after solvent desorption with diethyl ether, collecting the first 100 μL of extracts of sufficient concentrations.

Preparative gas chromatography was performed with a combination of eight extracts (altogether 800 μL) yielding three fractions each of 100 μL which were analyzed by combined gas chromatography/mass spectrometry by use of two capillary columns of different polarities.

Preparative Capillary Gas Chromatography. Preparative HRGC was performed, in manual operation mode with a commercially available chromatography system (Gerstel MCS), on a combination of two wide bore fused silica DB-1 capillary columns (5 m × 0.53 mm i.d., 5 μm film thickness, and 30 m × 0.53 μm i.d. 1.5 mm film thickness in series). The flow rate of the helium carrier was 5 mL/min, and the GC oven was temperature programmed from 70 °C, 5 min isotherm, to 130 °C at 3 °C/min and from 130 to 260 °C at 10 °C/min. A mass flow controlled automated multidimensional switching system MCS (multiple column switching, Gerstel GmbH, Mühlheim a. d. Ruhr, Germany) was employed for the preparative isolation of three fractions of sensorially interesting regions in the gas chromatogram in combination with an automated fraction collection system and a temperature-programmed cold injection system (CIS) from 60 °C at a heating rate of 12 °C/min to 130 °C held for 180 s, followed by a heating rate of 12 °C/min to 350 °C held for 60 s. The fractions were trapped at −30 °C.

Capillary Gas Chromatography (HRGC) Effluent Sniffing. Aroma extract dilution analysis of the collected headspace volatiles was performed on a Carlo Erba HRGC 5300 gas chromatograph with FID detector and sniffing port. The flavor dilution (FD) factors of the odorants were determined by effluent sniffing by the following dilution series: 200 μL of the 280 or 300 °C extracts, respectively, were stepwise (2^1, 2^2, 2^3, ...) diluted with diethyl ether until no single odorous compound was detectable by effluent sniffing. The original or diluted extracts (1 μL) were injected on a J&W DB-1 fused silica capillary column (60 m × 0.32 mm i.d., 1.0 μm film thickness); the flow rate of the helium carrier was 30 cm/s (35 °C), and the GC oven was held at 35 °C for 10 min after injection, raised at 2 °C/min to 280 °C, and finally held at 280 °C for 10 min. The end of the column was fitted to a quick-seal splitter, splitting the effluent 1:1. Splitter went to the FID detector and on the other end to a second heated outlet. Sniffing analysis of the carrier gas effluent was carried out by a five-member trained test panel, each specifying odor characteristics and strength of the odorants leaving the capillary column. The aromagram [logarithm of the odorants *vs* their retention indices (RI) values] was plotted.

Gas Chromatography/Mass Spectrometry (GC/MS). GC/MS analysis of extracts and fractions obtained by preparative gas chromatography, respectively, was performed on a Finnigan MAT 9610 gas chromatograph coupled to a Finnigan 4500 mass spectrometer equipped with an Incos 2100 data system. Mass spectra in the electron impact mode (EI) were generated at 70 eV. Extracts (1 μL) were separated on a J&W DB-1 fused silica capillary column as mentioned above but without quick seal splitter. Each of the three fractions obtained by preparative gas chromatography was injected (1 μL), either on the DB-1 column or on a J&W Carbowax fused silica capillary column (60 m × 0.25 mm i.d., 0.25 μm film thickness).

With regard to the Carbowax column the flow rate of the helium carrier was 30 cm/s (40 °C) and the GC oven was temperature programmed for 5 min isotherm at 40 °C, then raised at 2 °C/min to 210 °C, and held for 30 min. Kovats indices (RI) were calculated with the aid of a modified method for temperature-programmed gas chromatography developed by van den Dool and Kratz (1963).

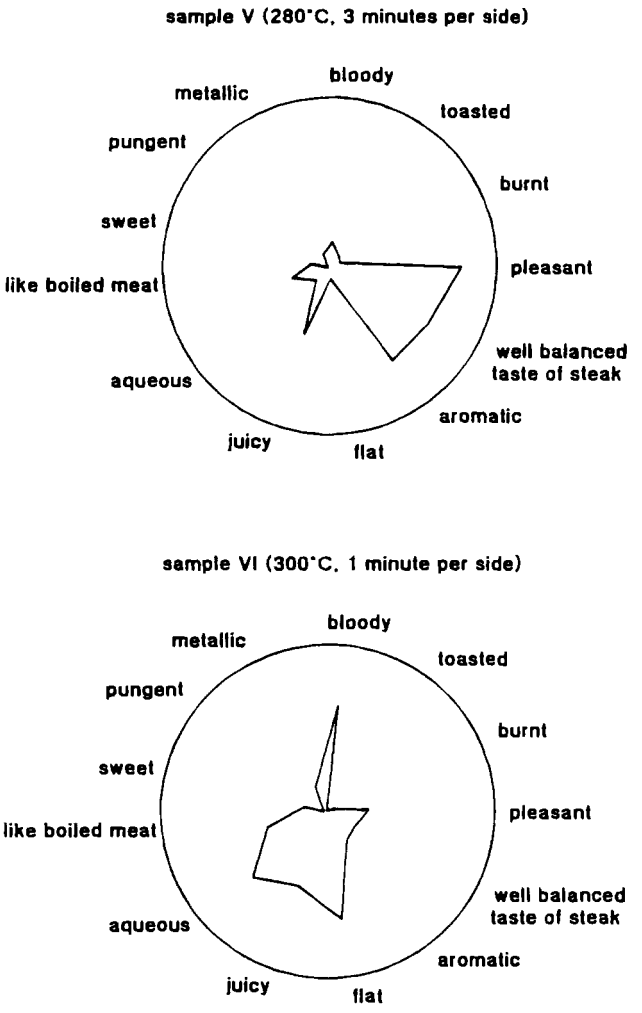

Figure 2. Aroma profile diagrams of the meat samples obtained by different frying conditions.

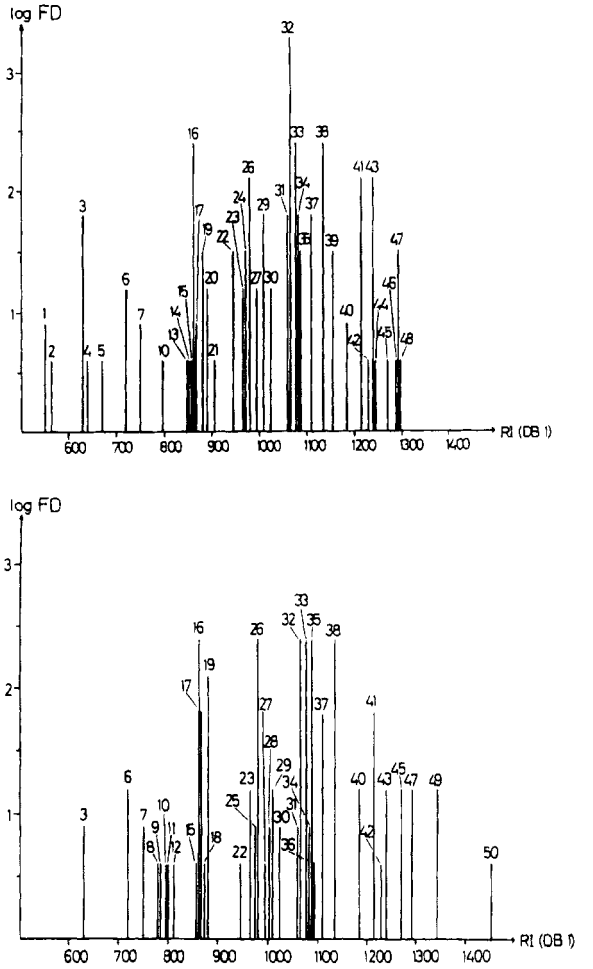

Figure 3. Aromagrams of the 280 (top) and 300 °C (bottom) extracts (logarithm of the FD *vs* their RI values).

RESULTS

Sensory Aroma Profile Analysis. The chosen conditions of frying at hot plate temperatures of either 280 °C for 3 min per side or of 300 °C for 1 min per side led to statistically significant different results determined using a sensory sequence test. The aroma profile analysis of the resulting meat samples particularly showed statistically significant differences in flavor qualities such as *pleasant*, *well-balanced*, *aromatic*, *bloody*, *aqueous*, and *flat*. Between the other flavor descriptions such as *juicy*, *boiled*, *sweet*, *pungent*, *metallic*, *toasted*, and *burnt* no statistically significant differences were obvious. The resulting aroma profile diagrams are shown in Figure 2.

Aroma Extract Dilution Analysis. With reference to the FD values, the description of the odors recognized during HRGC effluent sniffing and the responsible aroma compounds are summarized in Table 1, comparing the 280 and 300 °C extracts.

Aromagrams. The aromagrams of the two extracts obtained under different frying conditions are shown in Figure 3.

In the total volatile fraction of the 280 °C extract, 43 odorants showed FD factors of 4 and higher compared to 40 odorants of the 300 °C extract. The combination of 2-ethyl-3,5-dimethylpyrazine and 2-propyl-3-methylpyrazine with odor descriptions such as *burnt*, *fragrant*, *meaty*, *green*, and *bread-crust-like* reached FD factors of 2048 in the 280 °C extract compared to 250 in the 300 °C extract. Considering the 300 °C extract, no aroma compound showed an FD factor above 250.

Preparative Gas Chromatography. With the help of preparative fractionation of sensorially interesting regions of the effluent sniffing gas chromatogram, it was possible to identify a large number of overlapping compounds, which would not otherwise have been possible. This fact is demonstrated in the case of the following EI mass spectrum (Figure 4, peak 26), which was formed from a mixture of octanal, 2,3,5-trimethylpyrazine, amylfuran, 1,3,4-dimethylbenzene, and 2-ethyl-5(6)-methylpyrazine.

The large number of overlapping compounds hindered the identification of characteristic odorants determined by sniffing analysis. For this reason every available compound detected in odorous ranges, particularly in those with high FD values, was analyzed in test mixtures by HRGC effluent sniffing with regard to its odor and aroma intensity. Only substances whose RI and aroma impressions agreed with those of the standards were considered as identified with the exception of such compounds which were identified only by means of comparable literature EI mass spectral data. Odor characterizations of about 300 standard substances were carried out in the described way.

Concerning the five compounds identified under peak 26, the resulting *fruity*, *green*, *sweet*, *roasty*, and *pungent* aroma impression is due to the combination of octanal, 2,3,5-trimethylpyrazine, and 2-ethyl-5-methylpyrazine, respectively. Although 2-pentylfuran and 1,3,5-trimethylbenzene showed also weakly fruity and pungent

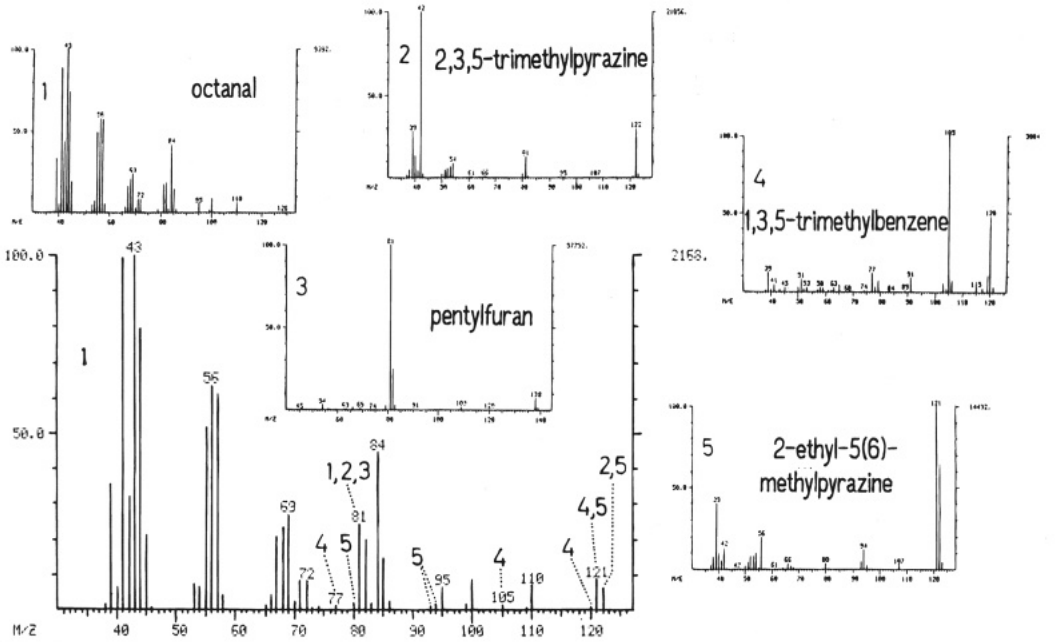

Figure 4. Original mass spectrum and mass spectra of the overlapped compounds (EI mode).

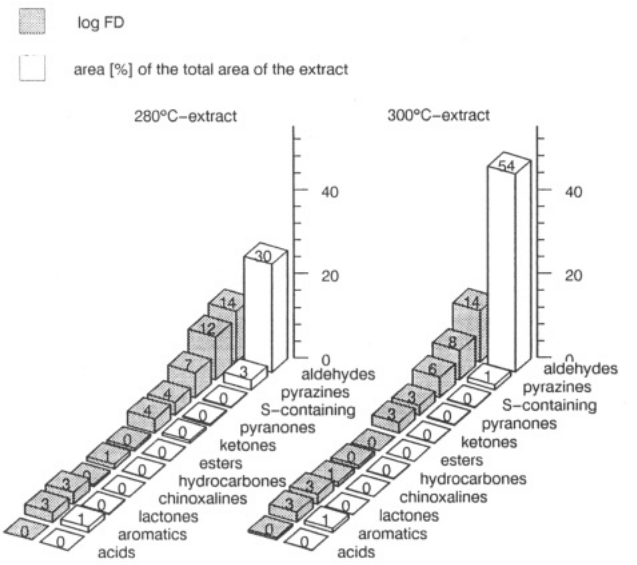

Figure 5. Total of the relative area units of odorous aroma compounds distributed in substance classes compared to the summarized logarithm of their related FD factors.

odors, they presumably would be masked by the other more intense aroma compounds.

DISCUSSION

Influence of Different Substance Classes on the Aroma of Shallow-Fried Beef. Figure 5 shows the total of the relative area units of odorous aroma compounds distributed in substance classes compared to the summarized logarithm of their related FD factors. Concerning the total of their relative area units as well as the summarized logarithms of their FD factors, the aldehydes with mainly fatty, fruity, green, and sweet flavor qualities are dominant in both of the two different extracts. Compounds belonging to the classes of pyrazines, sulfur-containing compounds, furanones, ketones, and aromatic compounds are altogether represented at significantly lower concentrations in proportion to the summarized logarithms of their FD factors. Thus, even lower concentrations of substances in these classes could strongly influence the aroma of fried beef.

Classification of Compounds with High Aroma Values in Pleasant and Unpleasant Categories. The overall acceptability is influenced not only by the odor intensity of an aroma compound but also by the kind of flavor quality such as pleasant or unpleasant. For that purpose, compounds with high aroma values being classed with flavor dilution factors ≥64 were divided into those with pleasant and those with unpleasant flavor qualities (Table 2).

Summarizing the FD factors in each case and subtraction of the sum of contributors to unpleasant flavor qualities from that of contributors to pleasant flavor qualities, estimating an average tolerance of $\pm 2^3$ per FD factor, yielded a positive value of $+1280 \pm 112$ for the 280 °C extract, compared to a negative value of -776 ± 112 for the 300 °C extract. Even with an estimated average tolerance of $\pm 2^4$ per FD factor, the lower limit in the case of the 280 °C extract comes to +1056 compared to the upper limit of −552 in the case of the 300 °C extract.

Twelve peaks of the 280 °C extract and nine peaks of the 300 °C extract with FD factors ≥64 should be, per definition, considered as compounds with high aroma values and therefore as important contributors to beef steak aroma.

Mode of Formation of Potent Odorants of Shallow-Fried Beef. Highly concentrated compounds with FD factors ≥64 such as heptanal, octanal, nonanal, 2(*E*)-nonenal, and 2(*E*)-decenal are derived from thermally induced degradation of saturated or unsaturated fatty acids (Forss, 1972). When a fatty acid is heated, the intermediate hydroperoxide spontaneously decomposes to an aldehyde and a radical, which may in turn be oxidized, dehydrated, and decarboxylated to a further aldehyde molecule (Grosch, 1982). Other potent odorous lipid degradation products are 4,5-dihydro-5-propyl-2(3*H*)-furanone (γ-heptalactone) and 4,5-dihydro-5-butyl-2(3*H*)-furanone (γ-octalactone). Watanabe and Sato (1971) identified a series of γ-lactones such as γ-hepta-, γ-octa-, and γ-nonalactone and in smaller concentrations also δ-lactones among the volatile compounds from beef fats heated at 145 °C. Presumably, during autoxidation oxygen attacks γ- and/or δ-carbon atoms and in turn

Table 1. Volatile Odor Compounds of Beef Steaks Fried at Two Different Heating Conditions (Hot Plate Temperature of 280 °C, 3 min/Side, or Hot Plate Temperature of 300 °C, 1 min/Side)

compd no.	name	RI (DB-1)	FD (280 °C extract)	FD (300 °C extract)
1	2,3-butanedione (sweet, buttery)	<600	8	
2	unknown (pungent, moldy, musty, mushroom-like)	<600	4	
3	3-methylbutanal (solvent-like, pungent, green)	629	64	8
4	2-methylbutanal (pungent, sweet, roasty)	639	4	
5	2,3-pentanedione (buttery, lemon-like, sweet, fruity)	669	4	
6	dimethyl disulfide (moldy, pungent, rubbery, onion-like)	722	16	16
7	toluol (synthetic, fruity, pungent, rubbery, solvent-like)	751	8	8
8	hexanal (sweet, fatty, green, grassy)	775		4
9	butyric acid (sweet, unpleasant)	778		4
10	unknown (moldy, fruity, sweet)	796	4	4
11	unknown (pungent, sulfury)	799		4
12	unknown (pungent, roasty, smoky)	817	2	4
13	unknown (fragrant, bread-like)	856	4	
14	unknown (pungent, burnt, fragrant)	860	4	
15	2,4-dimethylthiazole (rubbery, moldy, fruity, pungent)	861	4	4
16	methional (cooked potatoes, meat broth, fragrant)	864	256	256
17	methional + unknown compound (cooked potatoes, fruity, fatty, roasty)	866	8	32
18	ethenylbenzene (pungent, aromatic, fragrant, roasty)	876	2	4
19	heptanal (fruity, fatty, sweet)	879	32	128
20	2,5-dimethylpyrazine + 1-nonene (fried rice, popcorn, pungent, green)	889	16	
21	4,5-dimethylthiazole (smoky, roasty, fragrant, nutty)	906	4	
22	dimethyl trisulfide (fragrant, musty, roasty, rubbery)	944	32	4
23	1-octen-3-one (fresh, mushrooms, pungent, rubbery)	958	16	16
24	3-octanone + furfuryl acetate (fruity, nutty, moldy, fatty, earthy, pungent, fresh mushrooms)	967	32	2
25	2-ethyl-5-methylpyrazine (fruity, sweet, pungent)	976		8
26	octanal + 2,3,5-trimethylpyrazine + 2-ethyl-5-methylpyrazine (fruity, green, sweet, pungent, roasty)			
27	2-ethenyl-5(6)-methylpyrazine (Bondarovich *et al.*, 1967) + 1-decene (smoky, roasty, break-like, cooked rice, popcorn, coffee-like)	989	16	4
28	unknown (flowery, fruity, green)	1004		4
29	phenylacetaldehyde (sweet, fruity, flowery)	1008	64	16
30	2,5-dimethyl-4-hydroxy-3(2*H*)-furanone (roasted almonds, sweet)	1024	16	8
31	2-ethyl-3,6-dimethylpyrazine (burnt, pungent, roasty)	1062	64	8
32	2-ethyl-3,5-dimethylpyrazine + 2-propyl-3-methylpyrazine (burnt, fragrant, meaty, green, bread-crust-like)	1064	2048	256
33	2-ethenyl-3,6(5)-dimethylpyrazine (Bondarovich *et al.*, 1967) (pungent, sweet, cooked rice, fatty)	1078	256	256
34	2-methyl-3-hydroxypyran-4-one + 2-ethenyl-3,6(5)-dimethylpyrazine (pungent, fruity, fatty, flowery, sweet)			
35	nonanal (fragrant, sweet, fatty, green, pungent)	1084	32	256
36	1-undecene (fatty, burnt, nutty, rubbery)	1089		4

Table 1 (Continued)

compd no.	name	RI (DB-1)	FD (280 °C extract)	FD (300 °C extract)
37	4,5-dihydro-5-propyl-2(3*H*)-furanone (fruity, fatty, sweet, pungent, roasty)	1113	64	64
38	2(*E*)-nonenal (pungent, fragrant, fruity, roasty)	1136	256	256
39	2,3-diethyl-5-methylpyrazine (meaty, roasty, fragrant, sweet)	1147	32	
40	decanal (sweet, fruity, like aldehydes, roasty)	1186	8	16
41	4,5-dihydro-5-butyl-2(3*H*)-furanone (caramel-like, pungent, roasty, fruity)	1220	128	64
42	unknown (pungent, green, fragrant, fruity)	1233	4	4
43	2(*E*)-decenal (pungent, green, sweet, fruity, fatty)	1239	128	16
44	unknown (aromatic, pungent, roasty, nutty, sweet, fruity)	1248	4	2
45	2-methylchinoxaline (aromatic, roasted, nutty, sweet, fruity, fatty)	1272	4	16
46	undecanal (sweet, pungent, green)	1288	4	
47	2(*E*),4(*E*)-decadienal (sweet, rubbery, fatty, pungent)	1291	32	16
48	2-isopentyl-3,6-dimethylpyrazine (Liardon and Philipossian, 1978) (sweet, fragrant, fatty, fruity, pungent)	1295	4	2
49	2(*E*)-undecenal (sweet, fruity, fatty)	1342	2	16
50	2(*E*)-dodecenal (sweet, fruity, roasty, pungent)	1448		4

Table 2. Classification of Compounds with High Aroma Values in Pleasant and Unpleasant Categories

unpleasant				pleasant			
		FD factor				FD factor	
peak no.	compd	280 °C extract	300 °C extract	peak no.	compd	280 °C extract	300 °C extract
3	3-methylbutanal	64	8	16	methional	256	256
19	heptanal	32	128	29	phenylacetaldehyde	64	8
26	octanal + 2,3,5-trimethylpyrazine + 2-ethyl-5-methylpyrazine	128	256	31	2-ethyl-3,6-dimethylpyrazine	64	16
33	2-ethenyl-3,6(5)-dimethylpyrazine	256	256	32	2-ethyl-3,5-dimethylpyrazine + 2-propyl-3-methylpyrazine	2048	256
34	2-methyl-3-hydroxypyran-4-one + 2-ethenyl-3,6(5)-dimethylpyrazine	64	8				
35	nonanal	32	256	Σ FD factors (pleasant)		2432	536
37	4,5-dihydro-5-propyl-2(3*H*)-furanone	64	64				
38	2(*E*)-nonenal	256	256				
41	4,5-dihydro-5-butyl-2(3*H*)-furanone	128	64				
43	2(*E*)-*D*ecenal	128	16				
Σ FD factors (unpleasant)		1152	1312				

the resulting hydroperoxides are converted to γ- or δ-hydroxy acids and lactones (Grosch, 1982). 3-Methylbutanal, methional, and phenylacetaldehyde are Strecker degradation products of the amino acids leucine, methionine, and phenyalanine (Baltes, 1980) Liebich *et al.* (1972) established that carbonyl compounds including 3-methylbutanal, were probably responsible for roasted beef flavor.

2-Methyl-3-hydroxypyran-4-one (maltol) with fruity, fatty, and sweet odors is a thermal degradation product of glycogene. It also derives from the 1-deoxyosone pathway of the Maillard reaction (Baltes, 1980). Experimentally, maltol has been formed by the caramelization of maltose and certain maltooligosaccharides but has not been formed in detectable amounts from glucose, sucrose, or pure starch. It is said to enhance the flavor and sweetness of soft drinks, fruit juices, syrups, cakes, and other carbohydrate-rich foods and is generally recognized as safe as a food flavoring substance (Hodge, 1967).

Pyrazines are very important constituents of fried aromas. The proposed formation pathway is the reaction of sugars with amino acids with the formation of α-amino carbonyl intermediates which condense to produce pyrazine compounds. There is still much speculation as to the way in which the nitrogen atoms are incorporated into the pyrazine molecule. Free ammonia formed as a result of the decomposition of amino acids may combine with sugar and/or lipid degradation products to form pyrazines (Wong and Bernhard, 1988; Whitfield, 1992). Another hypothesis suggests that nitrogen still bound to the amino acid may react with sugars (Wong and Bernhard, 1988). The identification of long-chain-alkyl-substituted pyrazines in some cooked foods prompted an investigation of the reaction of fatty aldehydes with acetol and ammonium acetate at 100 °C (Chiu *et al.*, 1990). The compounds formed included, among others, 2,3,5-trimethylpyrazine, 2-ethyl-5(6)-methylpyrazine, and 2-ethyl-3,5(and 3,6)-dimethylpyrazine, which were also identified among the potent odorants of shallow fried beef. Presumably, 2-ethyl-3,5-dimethylpyrazine could be formed by reaction of 2-hydroxypropanal and acetol followed by condensation with glycolaldehyde (Figure 6). A similar

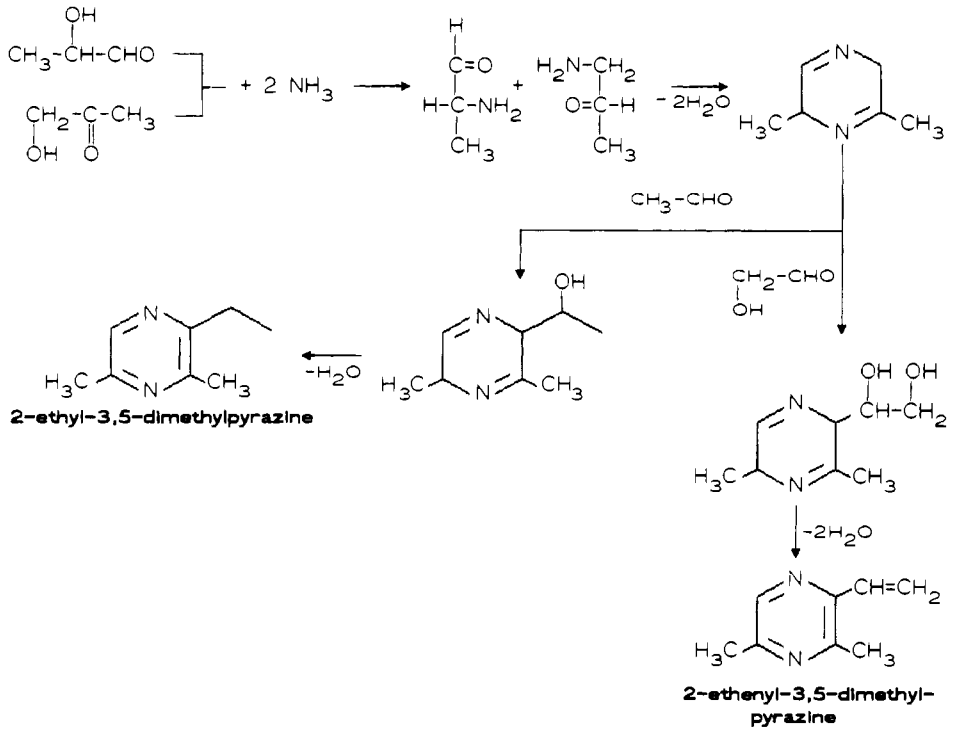

Figure 6. Proposed formation of 2-ethyl-3,5-dimethylpyrazine and 2-ethenyl-3,5-dimethylpyrazine.

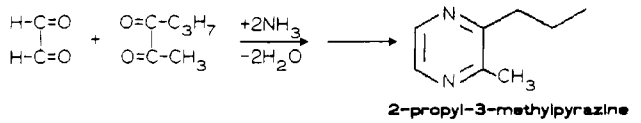

Figure 7. Proposed formation of 2-propyl-3-methylpyrazine.

reaction pathway has recently been proposed from Grosch (1993). 2-Propyl-3-methylpyrazine could be derived from condensation of glyoxal and 2,3-hexanedione (Figure 7). Cerny and Grosch (1992) identified 2-ethyl-3,5-dimethylpyrazine with an earthy–roast odor among the compounds with high aroma values of roasted beef.

Conclusion. With the aid of the described isolation and identification techniques, it was possible to work out characteristic differences between the extracts. The influence of aroma compounds with pleasant flavor qualities is stronger for the 280 °C extract (beef steak shallow pan fried for 3 min per side at 280 °C) than for the 300 °C extract (beef steak shallow pan fried for 1 min per side at 300 °C). This is mainly due to different FD factors determined for the combination of 2-ethyl-3,5-dimethylpyrazine and 2-propyl-3-methylpyrazine. In the 280 °C extract this combination showed the highest FD factor of 2048 compared to the 300 °C extract which showed an FD factor of 256.

With the exception of methional, no sulfur-containing compounds, which are considered to be responsible for interesting meaty flavor notes (Werkhoff *et al.*, 1990) were identified as potent odorants. This agrees with the results of Cerny and Grosch (1992), who identified among the volatile flavor compounds of fried beef only 2-acetylthiazoline in addition to methional but with a more roasty than meaty odor. It may be concluded that the majority of compounds with high aroma values are responsible for fatty, sweet, or roasted flavor qualities contributing to the roasted meat character of shallow-fried beef.

ACKNOWLEDGMENT

The work was supported by means of the Deutsche Forschungsgemeinschaft (DFG), Bonn, Germany. We thank Mr. D. Seidl for technical assistance, Ms. Chr. Kloth for performing the preparative gas chromatography, and Mr. R. Hatton for assistance with translation.

LITERATURE CITED

Baltes, W. The significance of the Maillard reaction on the development of flavor in foods. *Lebensmittelchem. Gerichtl. Chem.* **1980**, *34*, 34–38 (in German).

Bondarovich, H. A.; Friedel, P.; Krampl, V.; Renner, J.; Shephard, F. W.; Gianturco, M. A. Volatile constituents of coffee—Pyrazines and other compounds. *J. Agric. Food Chem.* **1967**, *15*, 1093–1099.

Cerny, C.; Grosch, W. Evaluation of potent odorants in roasted beef by aroma extract dilution analysis. *Z. Lebensm. Unters. Forsch.* **1992**, *194*, 1–4.

Chiu, E.-M.; Kuo, M.-C.; Bruechert, L. J.; Ho, C.-T. Substitution of pyrazines by aldehydes in model systems. *J. Agric. Food Chem.* **1990**, *38*, 58–61.

Forss, D. A. Odor and flavor compounds from lipids. *Prog. Chem. Fats Other Lipids* **1972**, *13*, 181–258.

Gasser, U.; Grosch, W. Identification of volatile flavour compounds with high aroma values from cooked beef. *Z. Lebensm. Unters. Forsch.* **1988**, *186*, 489–494.

Gasser, U.; Grosch, W. Primary odorants of chicken broth. A comparative study with meat broths from cow and ox. *Z. Lebensm. Unters. Forsch.* **1990**, *190*, 3–8.

Grosch, W. Lipid degradation products and flavour. In *Food Flavours*; Morton, I. D., MacLeoad, A. J., Eds.; Elsevier: Amsterdam, 1982; pp 325–386.

Grosch, W. Aroma compound analysis. *Chem. Unserer Zeit* **1990**, *24*, 82–89.

Grosch, W. New Developments in Flavor Analysis. *Lebensmittelchemie* **1993**, *47*, 129–134 (in German).

Hodge, J. E. Origin of flavor in food—Nonenzymatic browning reactions. In *Symposium on Food, The Chemistry and Physiology of Flavors*; Schultz, H. W., Day, E. A., Libbey, L. M., Eds.; AVI Publishing: Westport, CT, 1967; pp 465–491.

Konopka, U. K.; Grosch, W. Potent odorants causing the warmed-over flavour in boiled beef. *Z. Lebensm. Unters. Forsch.* **1991**, *193*, 123–125.

Liardon, R.; Philipossian, G. Volatile components of fermented soya hydrolysate I. Identification of some lactones. *Z. Lebensm. Unters. Forsch.* **1978**, *167*, 180–185.

Liebich, H. M.; Douglas, D. R.; Zlatkis, A.; Müggler-Chavan, F.; Donzel, A. Volatile compounds in roast beef. *J. Agric. Food Chem.* **1972**, *20*, 96–99.

MacLeod, G.; Seyyedain Ardebili, M. Natural and simulated meat flavors (with particular reference to beef). *CRC Crit. Rev. Food Sci. Nutr.* **1981**, *14*, 309–381.

Morton, I. D., MacLeod, A. J., Eds. *Food Flavors Part A. Introduction*; Elsevier: New York, 1982.

Neumann, R., Molnar, P., Arnold, S., Eds. In *Sensorische Lebensmitteluntersuchung*; VEB Fachbuchverlag: Leipzig, 1983.

Rothe, M.; Engst, W.; Specht, M. Examples for the solution of Aroma Problems via Sensory Profile Methods. *Chem. Mikrobiol. Technol. Lebensm.* **1981a**, *7*, 1–8 (in German).

Rothe, M.; Specht, M.; Erhardt, V. Studies on quality of characterization of meat-like flavourings. In *Flavour '81*; Schreier, P., Ed.; de Gruyter: Berlin, 1981b; pp 203–210.

Schieberle, P.; Grosch, W. Evaluation of the flavour of wheat and rye bread crusts by aroma extract dilution analysis. *Z. Lebensm. Unters. Forsch.* **1987**, *185*, 111–113.

Schmid, W.; Grosch, W. Identification of highly aromatic volatile flavour compounds from cherries (Prunus cerasus L.) *Z. Lebensm. Unters. Forsch.* **1986**, *182*, 407–412.

Shahidi, F.; Rubin, L. J.; D'Souza, L. A. Meat flavor volatiles: A review of the composition, techniques of analysis, and sensory evaluation. *CRC Crit. Rev. Food Sci. Nutr.* **1986**, *24*, 141–243.

Ullrich, F.; Grosch, W. Identification of the most intense volatile flavour compounds formed during autoxidation of linoleic acid. *Z. Lebensm. Unters. Forsch.* **1987**, *184*, 277–282.

Van den Dool, H.; Kratz, P. D. A. Generalization of the retention index system including linear temperature programmed gas liquid partition chromatography. *J. Chromatogr.* **1963**, *11*, 463–471.

Watanabe, K.; Sato. Y. Gas chromatographic and mass spectral analysis of heated flavor compounds of beef fats. *Agric. Biol. Chem.* **1971**, *35*, 756–763.

Wekhoff, P.; Brüning, J.; Emberger, R.; Güntert, M.; Köpsel, M.; Kuhn, W.; Surburg, H. Isolation and characterization of volatile sulfur-containing meat flavor components in model systems. *J. Agric. Food Chem.* **1990**, *38*, 777–791.

Whitfield, F. B. Volatiles from interactions of maillard reactions and lipids. *CRC Crit. Rev. Food Sci. Nutr.* **1992**, *31*, 1–58.

Wong, J. M.; Bernhard, R. A. Effect of nitrogen source on pyrazine formation. *J. Agric. Food Chem.* **1988**, *36*, 122–129.

Received for review April 1, 1994. Accepted July 11, 1994.[⊗]

[⊗] Abstract published in *Advance ACS Abstracts,* August 15, 1994.