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REVIEW

Evaluation of the Key Odorants of Foods by Dilution Experiments, Aroma Models and Omission

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Abstract

The state of the art in aroma analysis is reviewed with emphasis on aroma-recombination studies using synthetic blends of odorants (aroma models) which have been prepared on the basis of analytical data. The model that matches the original aroma is the starting material for omission experiments which are performed to establish the odorants that actually contribute to the aroma. These experiments are discussed in detail for the aromas of two wine varieties, three olive oils of different provenance, French fries, boiled beef and coffee. The results indicate that odorants with higher odour activity values (OAV, the ratio of the concentration to the odour threshold) are frequently essential for the aroma. However, there are exceptions where odorants with high OAVs are suppressed in the aroma and compounds with lower OAVs are important contributors. These findings are discussed in the light of model experiments which have been reported in the literature to obtain an insight into the perceptual interactions of odorants in mixtures.

Introduction

During the first period of aroma research, analytical investigations were performed under the assumption that all the volatiles occurring in food contribute to its aroma. Consequently, the analytical procedure, mostly a combination of gas chromatography and mass spectrometry, was confined to the identification of the volatiles appearing as peaks in the gas chromatogram. Rijkens and Boelens (Rijkens and Boelens, 1975) calculated that 2600 substances had been identified up to the year 1974 and predicted the occurrence of a total number of 10 000 odorants in foods. Indeed, ~8000 volatiles were reported up to 1997 (Nijssen *et al.*, 1997). However, Rijkens and Boelens (Rijkens and Boelens, 1975) were also beginning to doubt whether all of these volatiles actually contributed to the aromas. These doubts increased further as researchers began to calculate odour activity values (OAVs, synonymous with 'odour unit' and 'odour value') of volatile compounds in terms of the ratio of the concentration of the odorant in the material to the odour threshold (Rothe and Thomas, 1963). For example, Buttery (Buttery, 1993) found that of the 400 volatiles identified in tomatoes, only 16 reached an OAV of one or more.

The knowledge that not all of the volatiles occurring in a food contribute to its aroma was the reason for changing the methodology of analysis. Since 1984, when the procedure for charm analysis was published (Acree *et al.*, 1984), techniques were developed that focused on the identification of compounds with higher OAVs contributing to the aroma. The new methods were successful as the results allowed the preparation of synthetic blends whose aromas were very close to those of the original foods. Furthermore, the results led to the conclusion that <5 % of the volatiles identified in foods contributed to aromas (Grosch, 2000).

The aim of the following review is to present examples in which the aroma was simulated on the basis of instrumental and sensory analyses. In particular, omission experiments showing character impact odorants of foods will be discussed in some detail. However, the state of the art in the methodology of aroma analysis (Table 1) first needs to be considered.

Aroma analysis

Screening for potent odorants

The analytical procedure starts with dilution experiments (Table 1). As reviewed by Acree (Acree, 1993, 1997) and Grosch (Grosch, 1993, 1994), two techniques—charm analysis and aroma extract dilution analysis (AEDA)—are

Table 1 Outline of aroma analysis

Step	Procedure
I	Separation of the extract containing the volatile fraction of a food by high-resolution gas chromatography and localization of potent odorants by charm analysis or aroma extract dilution analysis (AEDA)
II	Detection of highly volatile potent odorants by gas chromatography–olfactometry of static headspace samples (GCOH)
III	Enrichment and identification of potent odorants
IV	Quantification of potent odorants and calculation of their odour activity values (OAVs)
V	Preparation of a synthetic blend of the potent odorants on the basis of the quantitative data obtained in step IV. Critical comparison of the aroma profile of the synthetic blend, denoted aroma model, with that of the original
VI	Comparison of the overall odour of the aroma model with that of models in which one or more components are omitted (omission experiments)

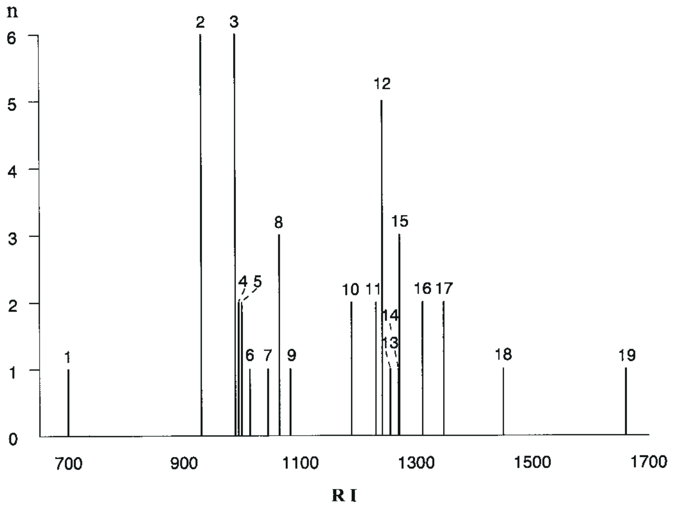

Figure 1 FD chromatogram of the volatile fraction isolated from boiled beef (Kerscher and Grosch, 1997). Ordinate: *n*, exponent of FD factor 2^n. Abscissa: retention index (RI) on the capillary OV-1701. The odorants were identified as: **(1)** 2,3-butanedione; **(2)** 2-methyl-3-furanthiol; **(3)** 2-furfurylthiol; **(4)** butyric acid; **(5)** 3-mercapto-2-pentanone; **(6)** 2-acetyl-1-pyrroline; **(7)** methional; **(8)** 1-octen-3-one; **(9)** octanal; **(10)** nonanal; **(11)** guaiacol; **(12)** 4-hydroxy-2,5-dimethyl-3(2H)-furanone; **(13)** (*Z*)-2-nonenal; **(14)** (*E*,*Z*)-2,6-nonadienal; **(15)** (*E*)-2-nonenal; **(16)** *p*-cresol; **(17)** 3-hydroxy-4,5-dimethyl-2(5H)-furanone; **(18)** (*E*,*E*)-2,4-decadienal; **(19)** 12-methyltridecanal.

used in most cases to screen the potent, medium and lower volatile odorants on which the identification experiments are then focused. In both procedures, an extract obtained from the food is diluted, usually as a series of 1:1 or 1:2 dilutions, and each dilution is analysed by gas chromatography–olfactometry (GCO).

In the case of AEDA, the result is expressed as flavour dilution (FD) factor (Grosch, 1993), which is the ratio of the concentration of the odorant in the initial extract to its concentration in the most dilute extract in which the odour is still detectable by GCO. Consequently, the FD factor is a relative measure and is proportional to the OAV of the compound in air. As an example, Figure 1 shows a plot of the FD factors of the odorants of boiled beef versus their retention indices (RI) on a gas chromatography (GC) capillary; this plot is termed an FD chromatogram. The highest FD factors were found for 2-methyl-3-furanthiol (No. 2), 2-furfurylthiol (No. 3), 1-octen-3-one (No. 8), 4-hydroxy-2,5-dimethyl-3(2H)-furanone (HD3F, No. 12) and (*E*)-2-nonenal (No. 15).

Charm analysis constructs chromatographic peaks, the areas of which are proportional to the amount of the chemical in the extract (Acree, 1993). The primary difference between the two methods is that charm analysis measures the dilution value over the entire time the compounds elute, whereas AEDA simply determines the maximum dilution value detected (Acree, 1993).

Charm values, FD factors and OAVs are functions of the odorant's concentration in the extract, but are not psychophysical measures for perceived odour intensity (Frijters, 1978). To take this criticism into account, Acree's group defined the odour spectrum value (OSV) which is the normalized charm value modified with an approximate Steven's law exponent (Ong and Acree, 1998; Ong *et al.*, 1998).

GCO techniques which are rarely applied are the Osme method (McDaniel *et al.*, 1990; Miranda-Lopez *et al.*, 1992) and olfactometry global analysis or OGA (van Ruth and Roozen, 1994; van Ruth *et al.*, 1995). In the Osme method (Osme is derived from the Greek word meaning smell) the non-diluted aroma extract is examined by GCO (Miranda-Lopez *et al.* 1992). The odour intensities perceived in replicates by several assessors are averaged, yielding a consensus aromagram. Osme takes into consideration Stevens' law of psychophysics, as it measures the response to odorants on a scale of time-intensity. However, in a study on the aroma of apples (Plotto *et al.*, 1998) the ranking of aroma-active esters according to Osme resulted in an order comparable with ranking based on OAV. This indicates that the results were not significantly affected when Stevens' law was not taken into account, as in the case of OAV calculations. OGA is similar to Osme, but each peak in the aromagram is not related to the odour intensity of the volatile, but to its detection frequency. Recently, a first critical comparison of AEDA, Osme and OGA in the case of a food product has indicated that the three methods agree in the evaluation of the most potent odorants of cooked mussels (LeGuen *et al.*, 2000).

A further method—aroma extract concentration analysis (AECA)—is the reverse of AEDA. It starts with the

Table 2 GCOH of boiled beef[a]

No.	Odorant	Volume[b] (ml)	FD factor[c]
1	Methanethiol	0.5	40
2	Dimethyl sulphide	1.0	20
3	Dimethyl trisulphide	2.5	8
4	Octanal	2.5	8
5	Nonanal	2.5	8
6	Acetaldehyde	5.0	4
7	Methylpropanal	5.0	4
8	Hexanal	5.0	4
9	2-Methyl-3-furanthiol	5.0	4
10	Methional	5.0	4
11	2-Furfurylthiol	5.0	4
12	3-Methylbutanal	10.0	2
13	1-Octen-3-one	10.0	2
14	Dimethyl tetrasulphide	10.0	2
15	2-Methylbutanal	20.0	1
16	(E)-2-nonenal	20.0	1

[a] Source: Kerscher and Grosch (Kerscher and Grosch, 2000).

[b] Lowest headspace volume required to perceive the odorant at the sniffing port.

[c] The highest headspace volume (20 ml) was equated to the flavour dilution (FD) factor of one. The FD factor values of the other odorants were calculated on this basis.

undiluted extract which then is concentrated stepwise and after each step an aliquot is analysed by GCO (Kerscher and Grosch, 1997). This procedure avoids an underestimation of the FD factors of unstable odorants which are degraded in AEDA.

In most cases, the screening methods discussed above are applied to aroma extracts. The highly volatile odorants are not evaluated, as they are lost during the concentration of the aroma extract or are masked in the gas chromatogram by the solvent peak. To overcome this limitation, the screening has to be completed by GCO of decreasing headspace volumes—step II in Table 1 (Holscher and Steinhart, 1992; Guth and Grosch, 1993). In the example of boiled beef (Table 2), the analysis was started with a headspace volume of 20 ml, in which GCO of the headspace (GCOH) revealed 16 odorants. Then the headspace volume was reduced in a series of steps to find the most potent, highly volatile odorants. GCOH of volumes of 10 and 5 ml indicated only 14 and 11 odorants, respectively (Table 2). GCOH of a volume of 2.5 ml still showed five odorants (Nos 1–5 in Table 2); after reduction to 0.5 ml, only methanethiol (No. 1) was found. According to this method, compound No. 1 was the most potent, highly volatile odorant of boiled beef.

Identification

The results obtained in the screening procedures are not corrected for losses of odorants during the extraction and concentration procedures. For this reason, the identification experiments should be focused not only on compounds with the highest FD factors, but also on those perceived at lower dilutions (Grosch, 1993). Furthermore, it is necessary to enrich odorants which do not appear as peaks in the gas chromatogram, or are concealed by the large peaks of odourless volatiles. Enrichment (step III in Table 1) is performed by column chromatography (Schieberle and Grosch, 1987; Blank *et al.*, 1992a) or with the new technique of multidimensional gas chromatography or MDGC (Weber *et al.*, 1995). In MDGC the aroma extract is separated on a polar pre-column, then a section of the effluent containing the analyte is cryofocused with liquid nitrogen and subsequently transferred to an unpolar main column which is combined with a mass spectrometer (MS) and a sniffing port.

In the identification experiment it is necessary to compare by GCO the odour quality of the analyte with that of an authentic sample at approximately equal levels. Only when, in addition to GC and MS data, there is an agreement in the sensorial properties, is the analyte, which has been perceived by GCO in the volatile fraction, correctly identified. All of the odorants reported here were identified in this way.

Quantification and calculation of OAVs

Due to the complexity of the volatile fraction and the large differences in concentration, volatility and reactivity of the odorants, it is not possible in most cases to quantify the odorants precisely (error < 10 %) by using conventional methods (Schieberle and Grosch, 1987; Grosch, 1993; Schieberle, 1995; Blank *et al.*, 1999). Not only losses during the extraction and the cleaning-up of the analyte, but even those caused by adsorption during GC (Blank *et al.*, 1992a), which are often overlooked, may lead to incorrect results. However, precise quantitative measurements of the odorants can be performed by the use of stable isotopomers of the analytes as internal standards (examples in Figure 2) in the so-called ‘stable isotope dilution assays’ (SIDA). Each assay consists of the followings steps (Schieberle, 1995; Maarse and Grosch, 1996): after the food sample or its extract has been spiked by the addition of known amounts of the corresponding labelled odorants, the volatile fraction is distilled off; the volatiles are then enriched by column chromatography or MDGC; finally, the subfractions containing the mixture of the unlabelled analytes and their isotopomers are analysed by capillary GC in combination with MS. To differentiate between the labelled and the unlabelled odorants, the traces of distinct ions are recorded. As an example, the mass chromatograms obtained for quantification of the levels of 2-methyl-3-furanthiol, 3-mercapto-2-pentanone, 2-mercapto-3-pentanone and 2-furfurylthiol in cooked beef are shown in Figure 3.

The precision of SIDA has been confirmed in model experiments (Schieberle and Grosch, 1987; Guth and Grosch, 1990). Although after cleaning-up, the yields of

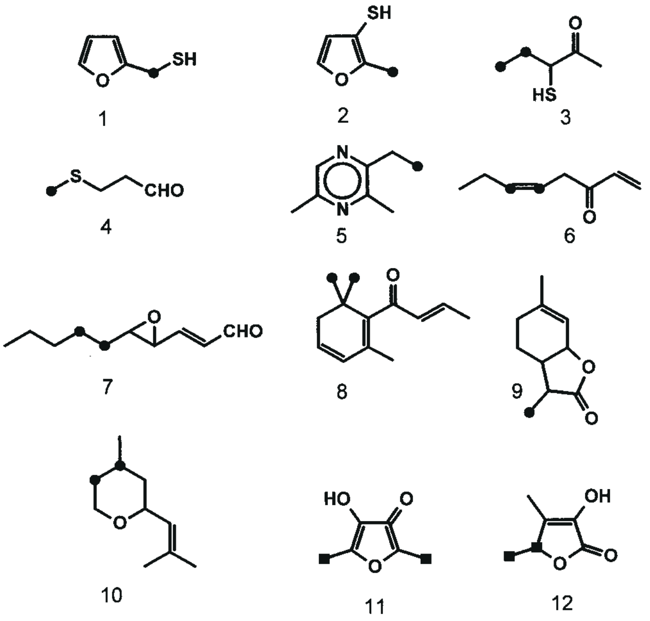

Figure 2 Odorants labelled with deuterium (●) or carbon-13 (■) used as internal standards in stable isotope dilution assays of the corresponding unlabelled analytes. **(1)** 2-[α-$^{2}H_2$]furfurylthiol; **(2)** 2-[$^{2}H_3$]methyl-3-furanthiol; **(3)** 3-mercapto-2-[4,5-$^{2}H_2$]pentanone; **(4)** [4-$^{2}H_3$]methional; **(5)** 2-[$^{2}H_3$]ethyl-3,5-dimethylpyrazine; **(6)** (*Z*)-1,5-[5,6-$^{2}H_2$]octadien-3-one; **(7)** *trans*-4,5-epoxy-(*E*)-2-[6,7-$^{2}H_4$]decenal; **(8)** 1-(2,6,6-[6,6-$^{2}H_6$]trimethyl-1,3-cyclohexadienyl)-2-buten-1-one (d-β-damascenone); **(9)** 3a,4,5,7a-tetrahydro-3,6-[3-$^{2}H_3$]dimethyl-2(3H)-benzofuranone, (d-wine lactone); **(10)** tetrahydro-4-methyl-2-(2-methylpropenyl)-2H-[3,4-$^{2}H_3$]pyran (d-rose oxide); **(11)** 4-hydroxy-2,5-[$^{13}C_2$]dimethyl-3(2H)-furanone; **(12)** 3-hydroxy-4,5-[4-^{13}C]dimethyl-2(5H)-[5-^{13}C]furanone.

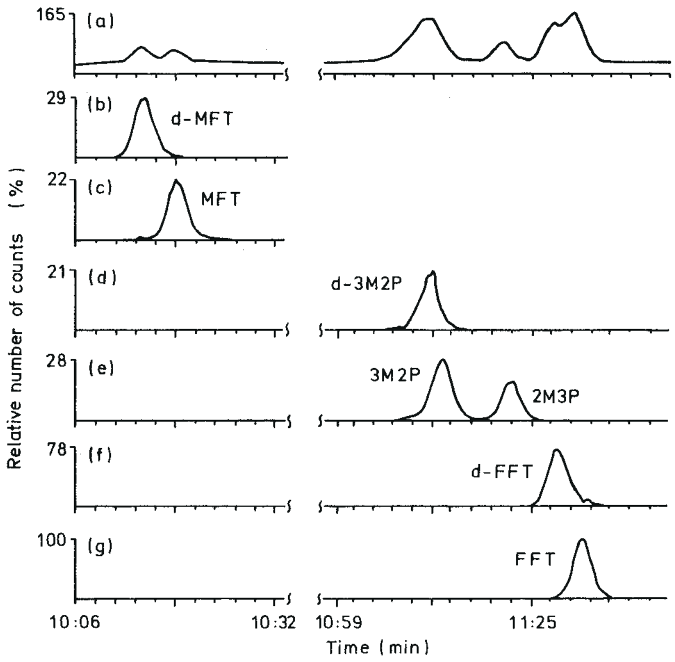

Figure 3 Stable isotope dilution assay mass chromatograms of the sulphur odorants from boiled beef (Kerscher and Grosch, 1998). **(a)** Total ion chromatogram. **(b–g)** Chromatograms of the ions given in parentheses: deuteriated 2-methyl-3-furanthiol (d-MFT, 118 *m/z*); unlabelled MFT (115 *m/z*); deuteriated 3-mercapto-2-pentanone (d-3M2P, 121 *m/z*); unlabelled 3M2P and 2-mercapto-3-pentanone (119 *m/z*); deuteriated 2-furfurylthiol (FFT, 83 *m/z*); and unlabelled FFT (81 *m/z*). The number of counts was set to 100% for FFT; *m/z*, ratio of mass to the number of charges on the ion.

some analytes were <10 %, the results of quantification were correct as the standards showed equal losses. Unless otherwise stated, the quantitative data reported here were obtained by SIDA.

To approach the situation in food, OAVs of the odorants are calculated (step IV in Table 1). For this purpose, odour threshold values of these volatiles are estimated in a medium that predominates in the food, e.g. water, oil, starch. As an example, the OAVs of the potent odorants of tomato paste, calculated as ratio of concentration to odour threshold value in water are listed in Table 3. The highest OAVs were found for β-damascenone, followed by dimethyl sulphide and methional. In agreement with the suggestion of Frijters (Frijters, 1978) that the odour activity concept can give some guidance in flavour research, it is assumed that the odorants showing higher OAVs contribute strongly to the aroma of tomato paste (Guth and Grosch, 1999).

Aroma models

Flavour chemists have always wanted to prove that the results of aroma analyses are correct. Therefore, synthetic blends of odorants (aroma models) were prepared on the basis of the obtained analytical data and their aromas were compared with those of the originals (step V in Table 1). Early experiments, e.g. to reconstitute the aroma of olive oil (Flath *et al.*, 1973) and that of orange juice (Dürr and Schobinger, 1981), were not very successful. Lack of odorants which are only detectable in the food by GCO and not by the GC detector as well as incorrect quantitative data are the main reasons that the original and the corresponding aroma model disagree.

Progress was made by Buttery (Buttery, 1993) and Buttery *et al.* (Buttery *et al.*, 1990, 1994, 1995) in a study on the aromas of fresh ripe tomatoes and tomato paste. The authors identified >400 volatiles and calculated OAVs for 40 odorants which had been quantified with conventional analytical methods. On the basis of these results and supported by GCO, they prepared aroma models for fresh tomatoes and tomato paste. Sensory panel studies showed these mixtures to have aromas very similar to the originals.

Recently, the study of the aroma of tomato paste was repeated using AEDA, GCOH and SIDA (Guth and Grosch, 1999). The composition of the resulting aroma model II is compared in Table 3 with model I obtained by Buttery (Buttery, 1993).

The concentrations of the first six odorants were in the same range in the two models. 1-Nitro-2-phenylethane was lacking in model II because it was not perceived in AEDA. Due to relatively low OAVs, linalool and 2-phenylethanol

Table 3 Comparison of two aqueous aroma models for tomato paste[a]

Odorant	Concentration (μg/l)		
	Model I	Model II	
Dimethyl sulphide	2000	1390	(1390)[b]
3-Methylbutyric acid	2000	1840	(3)
Eugenol	100	95	(95)
Methional	30	26	(650)
3-Methylbutanal	24	38	(152)
(*E*)-β-damascenone	14	5.7	(5700)
1-Nitro-2-phenylethane	70	—[c]	
Acetic acid	n.a.	218 000	(10)
Linalool	—[c]	5.3[d]	(4)
2-Phenylethanol	—[c]	1280	(28)
4-Hydroxy-2,5-dimethyl-3(2H)-furanone (HD3F)	—[c]	690	(138)
5-Ethyl-4-hydroxy-2-methyl-3(2H)-furanone	n.a.	31	(6)
4-Vinylguaiacol	n.a.[e]	80	(16)
4-Hydroxy-4,5-dimethyl-2(5H)-furanone (HD2F)	n.a.	17	(213)
Vanillin	n.a.	240	(8)
Methylpropanal	n.a.	28	(40)
Butyric acid	n.a.	720	(1)

[a] Source: model I (Buttery, 1993), model II (Guth and Grosch, 1999).

[b] The OAVs given in brackets were calculated by dividing the concentration by the odour threshold value of the compound in water.

[c] The authors suggest that the compound does not contribute to the aroma of tomato paste.

[d] Quantified with tetrahydrolinalool as internal standard.

[e] Not analysed.

were not added to model I. HD3F was not taken into consideration, since addition of 1 mg/l of this compound to model I did not confer any improved tomato paste aroma (Buttery *et al*., 1994, 1995). Also, the aroma of model II was very close to that of the original (Guth and Grosch, 1999). The authors had the impression that of the compounds lacking in model I, 3-hydroxy-4,5-dimethyl-2(5H)-furanone (HD2F) belonged to the key odorants of tomato paste.

The preparation of aroma models is simple for liquid foods, as it is easy to obtain a homogenous blend of odorants. Difficulties arise, however, in the case of solid foods, because in general it is not possible to reproduce the composition and distribution of the non-volatile components. As a compromise simple materials, e.g. oil/water mixtures, cellulose and starch, are used as the base (Czerny *et al*., 1999). However, the aroma model for a solid food can be improved when it is not affected by interactions of the odorants with the material used as the base. This would be the case when the model is identical with the composition of the odorants in the air above the food. An approach to gain data for the preparation of such models was developed and successfully applied to reconstitute the aroma of bread crust (Zehentbauer and Grosch, 1997) and roasted coffee (Grosch and Mayer, 2000).

Table 4 Aroma models prepared on the basis of dilution experiments

Aroma model for	Reference
Dill herb[a]	(Blank *et al*., 1992b)
Sour cream butter[a]	(Schieberle *et al*., 1993)
Stewed beef juice[a]	(Guth and Grosch, 1994)
Swiss cheese[a,,b]	(Preininger *et al*., 1996)
Coffee brew[a]	(Semmelroch and Grosch, 1996; Mayer *et al*., 2000)
Wine (Gewürztraminer, Scheurebe)[a,b]	(Guth, 1997a, 1998)
Basil[a]	(Guth and Murgoci, 1997)
Strawberry juice[a]	(Schieberle and Hofmann, 1997)
Camembert cheese	(Kubickova and Grosch, 1998)
Parsley (leaves)	(Masanetz and Grosch, 1998)
Olive oil (three provenances)[a]	(Reiners and Grosch, 1998)
French fries[a]	(Wagner and Grosch, 1998)
Baguette crust	(Zehentbauer and Grosch, 1998)
Roasted coffee[a]	(Czerny *et al*., 1999)
Tomato paste	(Guth and Grosch, 1999)
Pepper[a]	(Jagella and Grosch, 1999)
Boiled beef[a]	(Grosch *et al*., 2000)

[a] Omission experiments were performed.

[b] The model includes also the taste compounds.

Table 5 Odorants showing OAVs ≥10 in Gewürztraminer and/or Scheurebe wines[a]

Odorant	Gewürztraminer		Scheurebe	
	Conc. (µg/l)	OAV[b]	Conc. (µg/l)	OAV[b]
Ethyl octanoate	630	315	270	135
Ethyl hexanoate	490	98	280	56
3-Methylbutyl acetate	2900	97	1450	48
Ethyl isobutyrate	150	10	480	32
(E)-β-damascenone	0.84	17	0.98	20
Linalool	175	12	307	20
Wine lactone	0.10	10	0.10	10
Ethyl butyrate	210	11	184	9
cis-Rose oxide	21	105	3.0	15
4-Mercapto-4-methylpentan-2-one	<0.01	n.d.[c]	0.40	667

[a] Source: Guth (Guth, 1997a, 1998).

[b] The odour activity values (OAVs) were calculated as the ratio of concentration to odour threshold values of the compound in water/ethanol (9 + 1, w/w).

[c] Not determined.

Omission experiments

Aroma models based on the results of dilution experiments have been prepared for the foods listed in Table 4. In most cases the aroma of the model matched very well that of the original. However, in the dilution experiments, the odour impact of the volatiles was evaluated after separation by GC (example in Figure 1). Perceptual interactions of odorants, which were characterized by inhibition and suppression (Laing, 1988a, b; Acree, 1993), are abolished. Therefore, the question of which compound among the potent odorants actually contributes to the aroma has to be answered by omission experiments (step VI in Table 1).

To answer this question, the impact of components of the aroma model was evaluated by omission experiments for those foods which are listed in Table 4. Some details of the results obtained in studies of the aromas of wine, olive oil, French fries and boiled beef will be discussed in the following.

Wine

In a study on the aroma of the two wine varieties, Gewürztraminer and Scheurebe (Guth 1997a, 1998), OAVs were calculated on the basis of odour threshold values in water/ethanol (9 + 1, w/w). According to Table 5, esters, which are responsible for the fruity note in the aroma, showed high OAVs in the two wines. Characteristic differences in the OAVs were found for *cis*-rose oxide and 4-mercapto-4-methylpentan-2-one. The former was highly aroma-active in Gewürztraminer and the latter in Scheurebe, although its concentration amounted only to 0.4 µg/l (Table 5).

An aroma model for Gewürztraminer containing nine odorants with OAV ≥_10 (Table 5) was not satisfactory. However, when the model was completed by the odorants showing OAVs of 1–9 (Table 6), the aroma matched very well that of the original wine. In contrast, addition of a further 13 odorants with OAVs <1 did not affect the aroma of the model. Also, the aroma of the Scheurebe wine was imitated by a synthetic blend of the odorants with OAVs ≥1. When *cis*-rose oxide and 4-mercapto-4-methylpentan-2-one were absent in the models for Gewürztraminer and Scheurebe, respectively, the aromas changed drastically and did not resemble those found in the original samples. In contrast, omission of e.g. acetaldehyde, β-damascenone and geraniol, had only a small effect. It might be of interest that neither of the odorants listed in Tables 5 and 6 occur only in wine. *cis*-Rose oxide, for example, contributes to the aroma of linden honey (Blank *et al.*, 1989) and 4-mercapto-4-methyl-2-pentanone to that of basil (Guth and Murgoci, 1997) and grapefruit (Schieberle and Büttner, 2000).

To investigate the effect of ethanol on the aroma and the taste of Gewürztraminer, an aqueous ethanolic solution was investigated. The solution contained, in addition to the odorants listed in Tables 5 and 6, the taste compounds screened and quantified in previous experiments (Guth, 1998). Then the ethanol concentration was lowered in the flavour model (Table 7). Reduction from nearly 10 % to 9 % ethanol did not change the flavour. However, further reduction to a concentration of 7 % affected the flavour as the intensities of the fruity, flowery odour and the acid taste increased. This change increased with the decrease of the ethanol concentration. In the model containing only 3 % ethanol, the characteristic flavour of the wine was completely destroyed.

The drastic change in the flavour of the model might be caused by an increase in the partial pressure of the odorants due to the reduction of the ethanol concentration. A model

Table 6 Odorants of Gewürztraminer wine with OAV 1–9[a]

Odorant	Concentration (mg/l)	OAV
Acetaldehyde	1.86	4
Acetic acid	280	1
Geraniol	0.22	7
3-Hydroxy-4,5-dimethyl-2(5H)-furanone	0.0054	1
Ethyl acetate	63.5	8
1,1-Diethoxyethane	0.375	8
Butane-2,3-dione	0.15	2
Ethyl 2-methylbutyrate	0.0044	4
Methylpropanol	52	1
3-Methylbutanol	127.8	4
Dimethyl trisulphide	0.00025	1
(3-Methylthio)-1-propanol	1.41	3
Hexanoic acid	3.2	1
2-Phenylethanol	18	2
Ethyl *trans*-cinnamate	2.0	2
4-Allyl-2-methoxyphenol	0.0054	1
(Z)-6-dodecenoic acid-γ-lactone	0.00027	3

[a] Source: Guth (Guth, 1997a, 1998).

experiment (Guth, 1997b) indicates that doubling of the odorant concentrations in the gas phase was observed at the most when ethanol was lacking. Then, the experiments summarized in Table 8 were performed to clarify whether ethanol affects the odour-activity of aroma compounds. The odour threshold values of some components of the wine flavour were determined, both in the absence and in the presence of ethanol in the gas phase. The values listed in Table 8 indicate a strong increase in the odour threshold when ethanol was present. The increase varied between factors of 10 and 312 which were found for ethyl hexanoate and methylpropanol, respectively. The results suggest that the inhibition of the odour activity of the wine volatiles by ethanol is much larger than the reduction of their partial pressure.

Olive oil

The different aromas of olive oils originating from Italy, Morocco and Spain were reconstituted by solutions of 25, 14 and 21 odorants, respectively, in a refined plant oil (Reiners and Grosch, 1998). Omission experiments indicated that the green smelling (*Z*)-3-hexenal and (*Z*)-3-hexenol had the greatest odour impact in the oil from Italy. This corresponds to their relatively higher OAVs. On the other hand, acetaldehyde, which showed the highest OAV of all potent odorants occurring in the oil from Italy, was only of minor importance.

The aroma of the oil from Morocco differed from that of the other two oil samples in a special fruity note which was caused by ethyl cyclohexyl carboxylate. This ester, showing the highest OAV (550) of all odorants, is formed by fermentation of the olive fruits which takes place in the traditional process used for oil production in Morocco. The omission experiments revealed in addition that guaiacol, despite of its lower OAV of 3, was essential for the aroma of this oil variety.

Table 7 Influence of ethanol reduction on the aroma and taste of Gewürztraminer wine models[a]

Ethanol concentration (g/l)	Similarity[b]	Intensity[c] of the	
		Odour	Taste
100	3	1.5	0.5
90	3	1.5	0.5
80–70	2	2.0	1.0
60–50	1.5	2.5	2.0
30	1	3.0	2.5
10–0	0.5	3.0	3.0

[a] Source: Guth (Guth, 1998).

[b] The similarity of the model with the original Gewürztraminer wine was scored from 0 (none) to 3 (identical).

[c] The intensities of odour and taste were scored from 0 (none) to 3 (strong).

Table 8 Effect of ethanol on the odour threshold of some wine odorants in air[a]

Compound	Odour threshold[b] (ng/l)		Factor *b*/*a*
	Without ethanol (*a*)	With ethanol[c] (*b*)	
Ethyl isobutanoate	0.3	38	127
Ethyl butanoate	2.5	200	80
Ethyl hexanoate	9	90	10
Methylpropanol	640	200 000	312
3-Methylbutanol	125	6300	50

[a] Source: Guth (Guth, 1997b).

[b] The odour threshold was determined with an olfactometer.

[c] Concentration of ethanol in the gas phase: 55.6 mg/l.

Apart from acetaldehyde, 4-methoxy-2-methyl-2-butanethiol appeared with the highest OAV in the oil from Spain. This blackcurrant-like smelling thiol, which has also been found in the essential oil of blackcurrant buds (Rigaud *et al.*, 1986), was indeed the key odorant of this oil variety.

French fries

A model with aroma very similar to that of French fries processed in palm oil (Wagner and Grosch, 1998) contained 19 odorants dissolved in sunflower oil. The composition of this solution was varied to clarify whether all of the

Table 9 Odour of the aroma model for French fries as affected by the absence of one or more odorants[a]

Exp.	Odorant omitted in the model[b]	Number[c]	Intensity of the odour difference[d]
1	Methanethiol (20667)[e]	5	2.5
2	(*E*,*Z*)-2,4-decadienal (383) and (*E*,*E*)-2,4-decadienal (35)	5	2.5
3	2-Ethyl-3,5-dimethylpyrazine (19) and 3-ethyl-2,5-dimethylpyrazine (10)	4	1.5
4	2,3-Diethyl-5-methylpyrazine (82) and 2-ethenyl-3-ethyl-5-methylpyrazine (11)	0	
5	Methylpropanal (1739), 2-methylbutanal (1059) and 3-methylbutanal (503)	4	0.5
6	*trans*-4,5-Epoxy-(*E*)-2-decenal (592)	4	0.5
7	1-Octen-3-one (<1), (Z)-2-nonenal (3) and (*E*)-2-nonenal (<1)	1	0.5
8	Methional (3915)	0	

[a] Source: Wagner and Grosch (Wagner and Grosch, 1998).

[b] Models lacking in one or more components were each compared to the model containing the complete set of 19 odorants.

[c] Number of five assessors detecting an odour difference in triangle tests.

[d] Odour difference between complete and reduced models; rating scale: 0 (no deviation) to 3 (strong deviation).

[e] OAVs calculated on the basis of odour threshold values in oil are given in parentheses.

components took part in the generation of the aroma. An extract of the results is shown in Table 9.

Methanethiol, the odorant with the highest OAV, was missed in exp. 1 by each member of the panel because a boiled-potato-like note was lacking in the aroma profile of French fries. In exp. 2 the judges agreed that the decadienal stereoisomers had a great impact on the aroma and were responsible for the deep fried, fatty odour note. Of the four pyrazines, the absence of 2-ethyl-3,5-dimethylpyrazine and 3-ethyl-2,5-dimethylpyrazine was clearly recognized in exp. 3, but the absence of the two pyrazines, 2,3-diethyl-5-methyl- and 2-ethenyl-3-ethyl-5-methylpyrazine, was not noted in exp. 4. This means that 2,3-diethyl-5-methylpyrazine did not play a role in the aroma of French fries, although its OAV was much higher than the OAV of the other three pyrazines. Absence of the three Strecker aldehydes and of *trans*-4,5-epoxy-(*E*)-2-decenal changed the aroma of the model in exps 5 and 6, respectively, but the deviation in the odour intensity and quality was only small. In accordance with their low OAV, the carbonyl compounds which were lacking in exp. 7 did not significantly contribute to the aroma. The result of exp. 8 was very surprising. Although methional was the odorant showing the second highest OAV of all of the volatiles occurring in French fries (Table 9), the aroma of the model in which this odorant was lacking did not differ from that of the complete model. This result was also remarkable for the reason that the odour quality of methional is reminiscent of boiled potatoes. Obviously, other odorants, e.g. methanethiol and the pyrazines lacking in exp. 3, might cause the boiled-potato-like note in the odour profile of French fries.

Boiled beef

The aroma model of a boiled beef sample with a relatively higher fat content consisted of 16 volatiles (Table 10). Their selection based on the results of AEDA and GCOH is shown in Figure 1 and Table 2. Omission experiments (Table 10) revealed that nine odorants contributed significantly to the aroma of boiled beef. 2-Furfurylthiol, HD3F, methylfuranthiol, but also mercaptopentanone and methional belonged to this group. In a further triangle test, the seven odorants whose contribution to the overall odour was not significantly established in exps 10–16 (Table 10) were omitted. When compared with the model containing the complete set of 16 odorants, only 6 of the 11 assessors identified the reduced model (Grosch *et al.*, 2000). This means that the aroma of the reduced model does not differ significantly from that of the complete model. Therefore, it was concluded that compounds Nos 1–9 in Table 10 are the key odorants of boiled beef. 12-Methyltridecanal did not significantly contribute to the aroma of the model, although its OAV was very high (Table 10). Obviously, it did not successfully compete with octanal, nonanal and decadienal for the stimulation of the fatty note in the aroma profile of boiled beef. However, it belonged to the key odorants when lean beef was cooked because it originates from the membranes of the muscle tissue and not from the fat depot (Guth and Grosch, 1994; Kerscher *et al.*, 2000).

Roasted coffee

In a further experiment, a model which imitates the odour profile of a medium-roasted coffee sample from Colombia was prepared using 27 volatiles (Czerny *et al.*, 1999). Then 20 omission experiments were carried out. An extract of the results is shown in Table 11.

A comparison of exps 1 and 2 indicated that 2-furfurylthiol was the outstanding odorant in the group of sulphur compounds. Its absence in exp. 1 lowered so strongly the

Table 10 Concentrations, odour activity values (OAV) of potent odorants of boiled beef and the results of omission experiments[a]

No.	Odorant (omitted[b])	Concentration (μg/kg)	OAV[c]	Number[d]
1	2-Furfurylthiol	29	2900	10***
2	4-Hydroxy-2,5-dimethyl-3(2H)-furanone (HD3F)	9075	908	10***
3	3-Mercapto-2-pentanone	69	99	9***
4	Methanethiol	311	1555	9***
5	Octanal	382	546	8**
6	2-Methyl-3-furanthiol	24	3429	8**
7	Nonanal	1262	1262	8**
8	(*E*,*E*)-2,4-decadienal	27	135	8**
9	Methional	36	180	7*
10	12-Methyltridecanal	962	9620	6
11	Dimethyl sulphide	105	350	5
12	(*Z*)-2-nonenal	6.2	310	4
13	Acetaldehyde	1817	182	4
14	1-Octen-3-one	9.4	188	3
15	(*E*)-2-nonenal	32	128	3
16	Methylpropanal	117	167	1

[a] Source: Kerscher and Grosch (Kerscher and Grosch, 2000), Grosch *et al*. (Grosch *et al*., 2000).

[b] Odorant which was omitted in the aroma model containing the compounds listed in the table. The base of the model consisted of 10% sunflower oil in a phosphate buffer of pH 5.7.

[c] OAVs were calculated on the basis of odour threshold values in water.

[d] Number of 11 assessors detecting the reduced model in triangle tests.

*Significant ($P < 0.05$); **highly significant ($P < 0.01$); ***very highly significant ($P < 0.001$).

sulphurous/roasty note odour of the model that 15 of 20 answers were correct. The important role established in these omission experiments for 2-furfurylthiol confirmed the assumption of Reichstein and Staudinger (Reichstein and Staudinger, 1955) that it was a key component of coffee flavour. However, its impact was much lower in the coffee beverage due to its lower yield in the brewing process (Mayer *et al.*, 2000). Absence of the four furanones in exp. 3 (Table 3) was clearly recognized. However, when HD3F and 2(or 5)-ethyl-4-hydroxy-5(or 2)-methyl-3(2H)-furanone (EHM3F) were lacking in exp. 4, or HD2F and EHM2F in exp. 5, the aromas of the complete and the reduced model were not significantly different. Obviously, two of the four furanones were sufficient for the coffee aroma.

Experiments 6 and 7 indicated that the aroma of the model was significantly changed by the absence of the alkyl pyrazines PI, PII and PIII. Methoxypyrazine PIV which, in contrast to exp. 6, was present in exp. 7 did not affect the aroma of the model (Grosch *et al.*, 2001). This was confirmed in omission experiments for the coffee brew aroma (Mayer *et al.*, 2000). This result is of interest as PIV is the most aroma-active constituent of raw coffee and is stable during roasting (Czerny and Grosch, 2000). The authors assumed that roasting of the raw material not only produces the pleasant aroma which is characteristic for coffee but, in addition, generates odorants which suppress the peasy odour note caused by PIV.

Table 11 Aroma of the model for a medium-roasted Arabica coffee sample as affected by the absence of compounds[a]

Exp.	Compound(s) omitted[b]	Number[c]
1	2-Furfurylthiol	15[d]
2	2-Methyl-3-furanthiol, dimethyl trisulphide, methional, 3-mercapto-3-methylbutylformate, 3-methyl-2-buten-1-thiol, methanethiol	10
3	4-Hydroxy-2,5-dimethyl-3(2H)-furanone (HD3F), 2(or 5)-ethyl-4-hydroxy-5(or 2)-methyl-3(2H)-furanone (EHM3F), 3-hydroxy-4,5-dimethyl-2(5H)-furanone (HD2F), 5-ethyl-3-hydroxy-4-methyl-2(5H)-furanone (EHM2F)	11[d]
4	HD3F and EHM3F	8
5	HD2F and EHM2F	9
6	2-Ethyl-3,5-dimethylpyrazine (PI), 2-ethenyl-3,5-dimethylpyrazine (PII), 2,3-diethyl-5-methylpyrazine (PIII), 3-isobutyl-2-methoxypyrazine (PIV)	13[d]
7	Pyrazines PI–PIII as in exp. 6	12[d]

[a] Source: Czerny *et al*. (Czerny *et al*., 1999).

[b] Models lacking in one or more components were singly compared to the model containing the complete set of 27 odorants.

[c] Number of correct answers. Maximum possible number of correct answers in the triangle test: 20.

Final discussion

The sensory studies reviewed here indicate that complex odours, such as those of different types of wine and olive oil, French fries, boiled beef and coffee, can be reconstituted by aroma models that contain only a small fraction of the great number of volatiles occurring in these foods. Omission experiments are then performed to identify the character impact odorants.

The ability of human subjects to detect changes in odour mixtures after omission of one compound is poor when the odour activities of the components are similar (Laska and Hudson, 1992). However, in the experiments reported here, the odour activities (expressed as OAV) of the food volatiles are different, and this fact facilitates the identification of a mixture with a single component omitted. The results obtained for boiled beef, for example, indicate that omission of 2-furfurylthiol or HD3F or 3-mercapto-2-pentanone or methanethiol in the aroma model was recognized by a highly significant number of assessors.

The omission experiments reviewed here have revealed that some of the odorants with higher OAVs cause the characteristic note of the aroma, e.g. *cis*-rose oxide and 4-mercapto-4-methylpentan-2-one in Gewürztraminer and Scheurebe wines, respectively, or 4-methoxy-2-methyl-2-butanethiol in an olive oil variety and 2-furfurylthiol in roasted coffee. But these experiments have also shown negative perceptual interactions of odorants. For example, methional and 12-methyltridecanal, although appearing with high OAVs, are suppressed in the aroma model for French fries and boiled beef, respectively. A further impressive example, as already mentioned above, is the masking of the peasy smell of methoxypyrazines in coffee by odorants formed during the roasting process.

In simple experiments with food-relevant odorants, antagonistic effects have been reported for mixtures of (*Z*)-3-hexenal and (*E*,*Z*)-2,4-heptadienal (Meijboom, 1964), as well as for (*E*)-2-decenal and (*E*)-2-hexenal (Laing and Willcox, 1983).

The perceptual interaction of odorants was also investigated using complex mixtures. A large study was performed by Meilgaard (Meilgaard, 1975, 1982) to evaluate the odorants contributing to the aroma of beer. He found that the odour threshold values of most of the volatiles are higher in beer than in water, e.g. that of butanol, 3-methylbutanol and dimethyl sulphide, by factors of 400, 280 and 152, respectively. (*E*)-2-nonenal is an exception, since its threshold is approximately as low in beer as in water. In 20 separate experiments the odour threshold values of mixtures containing up to five alcohols, fatty acids, esters or carbonyl compounds, as well as alcohols and esters, were estimated using beer as the solvent. A comparison of the OAV of the mixture with the sum of the OAVs of the components led to the general conclusion that most of the beer aroma compounds exhibit partial addition: 0.5 OAV + 0.5 OAV produces 0.8 ± 0.2 OAV. No obvious examples of synergism or antagonism were observed in these studies.

A strict additive effect was shown by Guadagni *et al.* (Guadagni *et al.*, 1963, 1966) for mixtures containing ten compounds, each at 1/10 of their threshold in aqueous solution. This was found with 20 different combinations of sub-threshold concentrations and was independent of the chemical nature of the compound. Indications for a synergistic effect of odorants at sub-threshold concentrations were reported by Laska and Hudson (Laska and Hudson, 1991). Their suggestion was derived from the observation that the threshold of an odorant in the mixed state is lower than that in the unmixed state. The greatest difference was reported for decyl acetate. Its threshold decreases by five orders of magnitude when it is a component of a mixture consisting of cineole, linalool, (–)-carvone, *tert*-butylcyclohexyl acetate and 2-pentanone. However, as discussed here, aroma models containing all compounds with OAVs >1 imitate very well the corresponding food aromas. There are no indications that in these cases additional volatiles with OAV <1 might play an important role. In contrast, as mentioned above, antagonistic effects causing a suppression of odorants showing high OAVs have been ascertained.

Another aspect of the perceptual interactions of odorants was investigated by Laing (Laing, 1994). He found that humans can only discriminate and identify up to three or four odorants in a mixture. When more than four odorants are mixed, as shown here for the aroma models, then they produce on overall aroma that differs from the individual odours. Laing (Laing, 1994) suggested that the new aroma is the result of a large loss of the odour properties of the individual constituents. This agrees with the observation that omission of one or two odorants in the aroma model often causes only small changes in odour quality, which are difficult to describe and hardly affect the odour intensity of the model. Laing (Laing, 1994) proposed two mechanisms—spatial and temporal filtering—to explain the perceptual interactions of odorants in mixtures. The spatial filtering hypothesis starts with the observation that in the olfactory epithelium an odorant interacts with a substantial number of receptor sites and cells which are often widely distributed. Consequently, stimulation by an odorant produces a pattern of responsive cells. However, when a mixture of two odorants enters the nose, information about one or both odorants can be lost through competition for receptor sites or cells. Consequently, the greater the number of constituents in a mixture, the greater is the chance of an overlapping of response pattern (Laing, 1994).

The concept of temporal filtering is based on the observation that different odorants may differ greatly in the reaction time for the recognition of odour quality (Laing and MacLeod, 1992; Laing 1994). This time was termed ‘latency’, and it was shown that it is inversely proportional to the odorant concentration. When, for example, two odorants compete for receptor sites and cells, it is proposed that the 'faster' odorant will be the first to occupy receptor sites and thus to reduce the chance of stimulation by the 'slower' odorant, or might act as an antagonist (Laing, 1994).

Application of these mechanisms, for example to the results of the omission experiments which have been discussed before for the boiled beef aroma model, might lead to the following proposal: 12-methyltridecanal does not successfully compete with octanal, nonanal and decadienal for the receptor sites and cells, and/or its latency is longer than those of the aldehydes having a shorter carbon chain.

Conclusion

It has been proven by aroma models that the odorants occurring in complex aromas can be identified by a multi-step analytical procedure based on the odour activity concept. As this procedure has been successfully applied to the majority of important foods, most of the volatiles which might play a role in aromas have been now identified. Omission experiments have revealed that odorants with higher OAVs are frequently essential for the aroma. However, there are also exceptions where odorants with high OAVs are suppressed in the aroma and compounds with lower OAVs are important contributors. These results are in agreement with the observation of Laing (Laing, 1994) that there is a large loss of sensory properties about the individual constituents when odorants are mixed. To understand the perceptual interactions of odorants which result in well-known aromas, corresponding experiments should be performed with those volatiles that have been identified as odour-active components of foods.

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