Chapter 2

Sensory Relevance of Volatile Organic Sulfur Compounds in Food

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Volatile organic sulfur compounds account for only about 10 percent of all volatile components identified in edible products. However, these compounds are important constituents of the flavor of many foods, beverages and natural isolates. Typically these compounds occur in low concentrations and contribute significantly to characteristic aroma notes due to low odor thresholds. Selected character-impact sulfur compounds found in natural and processed foods are discussed in detail and the sensory relevance is estimated on the basis of odor quality, threshold values, and sensory studies.

Volatile organic sulfur compounds are important constituents of foo flavors (*1*). The TNO compilation of volatile compounds consists of about 70 sulfur-containing molecules, which correspond to approximately 10% of th total number of volatiles listed (*2*). Sulfides represent the major chemical cla with more than 440 compounds of which the disulfide group alone accounts f about 100 different molecules. Further well-known chemical classes a thiazoles (~100), thiophenes (~100), and thiols (~60).

The most frequently listed sulfur-containing volatile molecule is dimeth disulfide reported to occur in more than 110 food products (*2*). Furth

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compounds are dimethyl sulfide (~90), benzothiazole (~80), dimethyl trisulfide (~70), methional (~60), methylmercaptan (~60), hydrogen sulfide (~50), thiazole (~30), 2-acetylthiazole (~30), 2-methylthiophene (~30), and others. However, frequent occurrence does not necessarily mean high sensory relevance. This can be estimated by various other means, such as aroma quality, intensity, and threshold values. These data, in combination with the concentration, allow a fairly good estimation of sensory contribution.

This article focuses on the sensory relevance of sulfur-containing compounds, in particular thiols, using examples from various types of natural and processed food products. The few examples chosen represent only a small part of the vast literature on odor-active sulfur compounds. A comprehensive overview on analysis, formation, and functional properties of S-compounds is given in the ACS Symposium Series 564 (*3*). The particularity of the chemistry involved in the formation of S-containing compounds is discussed by Block in an excellent review (*4*).

Experimental Procedures

It is not the purpose of this paper to discuss in great detail the various analytical and sensory approaches suitable to assess the role of individual compounds to the overall aroma of a food. Recent review articles are recommended for more information (*5-9*). However, key elements of some of the analytical and sensory approaches will be discussed to better understand sensory relevance.

Isolation of Thiols

A first and very important step in the characterization of flavor is to obtain an aroma extract with the authentic note of the food. The majority of sulfur compounds such as sulfides, thiazoles, and thiophenes are chemically stable and can easily be extracted. Thiols, however, are reactive species and susceptible to oxidation, dimerization, and reaction with carbonyls. Therefore, they deserve special attention to assure that losses during the entire sample preparation procedure are reduced to a minimum.

Trapping thiols with *p*-hydroxymercuric acid (pHMB) has been shown to be an efficient method to isolate traces of sensory-relevant thiols (Figure 1). Darriet et al. (*10*) identified 4-mercapto-4-methyl-2-pentanone (**1**) as a character impact compound of Sauvignon wines after having smelled it by the GC-sniffing technique in an aroma extract. The selective enrichment of thiols is based on the reaction of thiols with pHMB at pH 8.5 and room temperature. The derivative is water-soluble and can be separated from lipophilic materials. As the

combination of thiols with pHMB is reversible, it is then released by an excess of cysteine or glutathione for identification by GC-MS. As shown in Figure 2 odorant **1** was identified in Sauvignon blanc wines where it occurs in trace amounts.

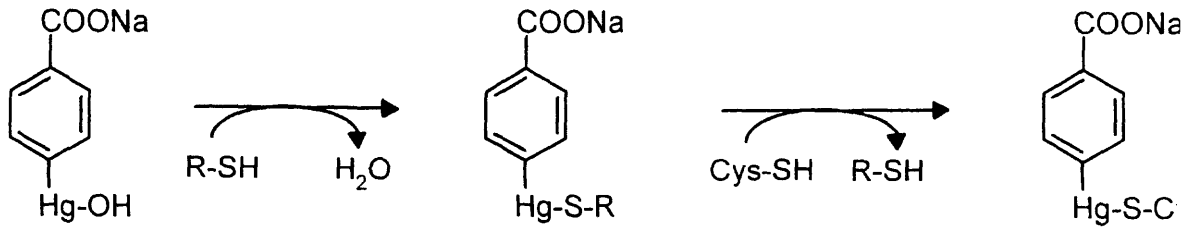


Figure 1. Enrichment of thiols using p-*hydroxymercuric acid as a selective trapping reagent.*

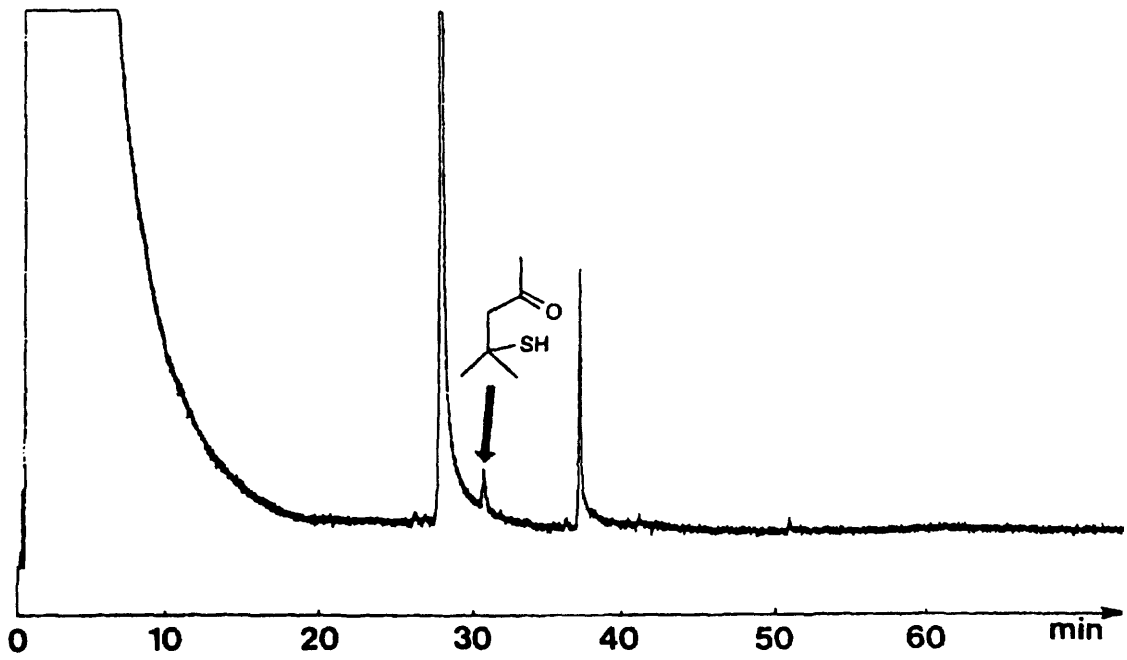


Figure 2. Chromatogram of a Sauvignon blanc wine extract after treatment wit p-hydroxymercuric acid and an excess of cysteine to isolate 4-mercapto-4-methyl-2-pentanone (1). (Reproduced with permission from reference 10. Copyright 1995 John Wiley & Sons.)

Screening of Potent Odorants

A frequently used technique to screen odorants from odorless volatile compounds is based on gas chromatography in combination with a sniffing port at which an odorant can be detected by smelling the effluent of the column (*11*). A more sophisticated approach of GC-Olfactometry (GC-O) includes sniffing of serial dilutions until no odor-active region is perceived. The two most applied methods are CHARM analysis (*12*) and Aroma Extract Dilution Analysis (AEDA) (*13*), which have been recently reviewed in detail (*5-8*).

The usefulness of GC-O is illustrated on two examples. Although the meat-like and roasty smelling compound 2-methyl-3-furanthiol (MFT, **2**) has been known since 1976 (*14*), it was only when GC-O became a generally accepted technique in flavor research that this odorant was frequently reported in food products such as tuna fish (*15*), cooked beef (*16*), boiled chicken (*17*), and roasted coffee (*18*). In some of these products, GC-O, performed on capillary columns of different polarity, was the only means of identification (*16,18*) as mass spectra could not be obtained due to inadequate concentrations for analytical detection.

1-*p*-Menthen-8-thiol (**3**), a flavor impact constituent of grapefruit juice, was first reported by Demole and coworkers in 1982 (*19*). This is one of the most powerful flavor compounds found in nature with a detection threshold of 0.0001 µg/kg water for the racemic mixture. Recently, the black currant-like smelling odorant 4-mercapto-4-methyl-2-pentanone (**1**) was additionally reported as a key constituent of grapefruit juice (*20*). Again, identification was based on GC-O data, as the concentration for GC-MS was too low. Furthermore, methional (**4**) was found as another S-containing odorant in grapefruit juice. The chemical structures of these compounds are presented in Figure 3.

These data suggest GC-O to be a particularly powerful tool for screening and identification of odor-active thiols, which have threshold values in the low and sub-ppb range, provided the compounds are known and the reference compounds available. The sensory relevance of such odorants is due to the low odor thresholds.

Quantification of Potent Odorants

As already mentioned, detection and identification of odorants with very low threshold values is rather difficult, particularly of thiols that are unstable and susceptible to chemical reactions. Therefore, the ability to obtain reliable quantitative data is a major challenge in flavor research. Isotope Dilution Assay (IDA) is a method used to overcome these limitations (*7,21,22*). It is based on the use of stable isotopomers of the analytes as internal standards. The potent odorants MFT (**2**), 2-furfurylthiol (FFT, **5**), 3-mercpato-2-pentanone (3M2P, **6**),

and 2-mercapto-3-pentanone (2M3P, 7) were simultaneously quantified in boiled beef using the deuterated analogs d-MFT, d-FFT, and d-3M2P as internal standards (*23*) (Figure 4).

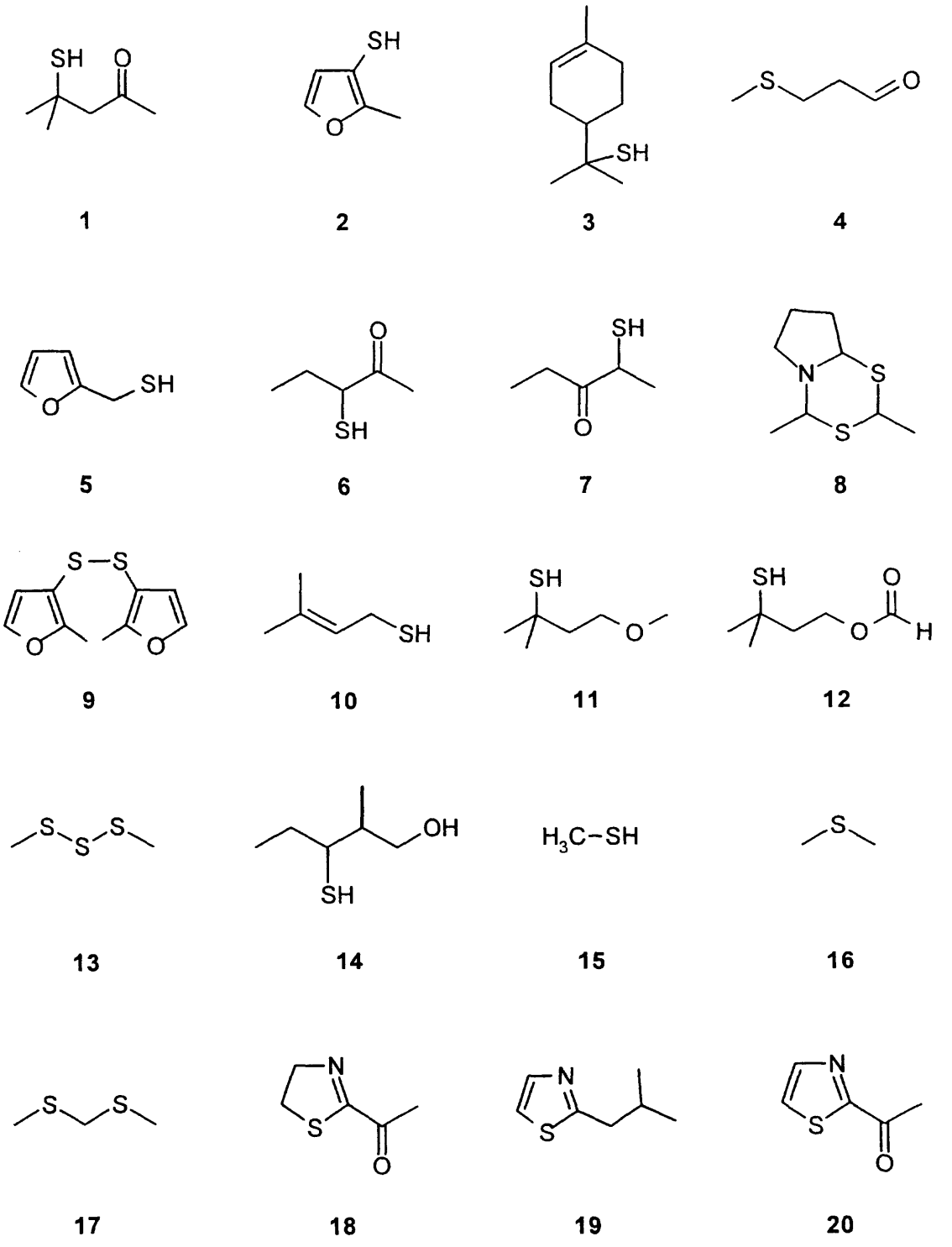


Figure 3. Examples of sulfur-containing compounds that have low sensory thresholds and often play a key role in many food flavors (1,24) (See Table I for corresponding names and threshold values).

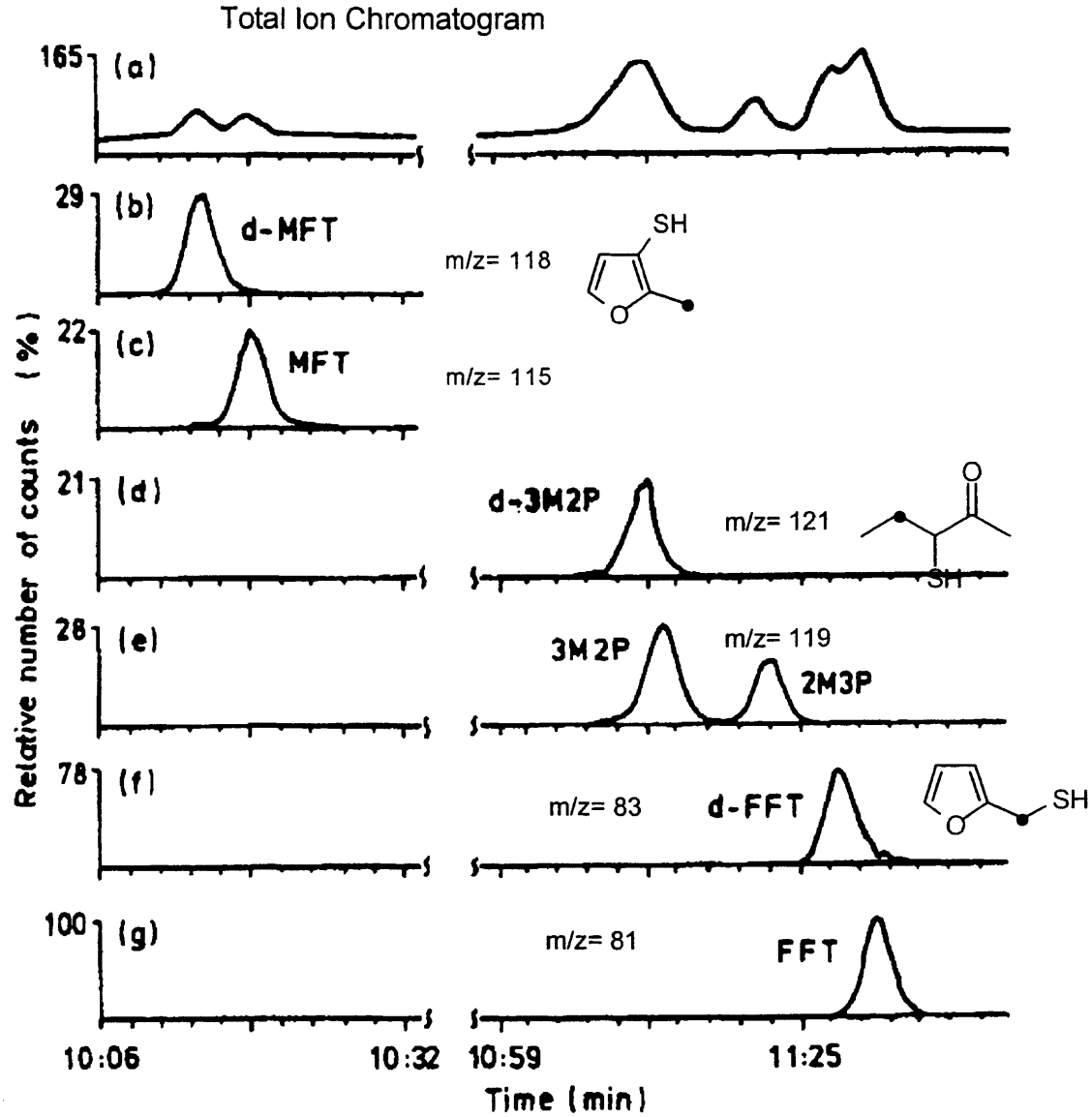


Figure 4. Quantification of 2-methyl-3-furanthiol (MFT), 2-furfurylthiol (FFT), 3-mercapto-2-pentanone (3M2P) and 2-mercapto-3-pentanone (2M3P) by isotope dilution assays using the corresponding deuterated isotopomers d-MFT, d-FFT, and d-3M2P as internal standards. (a)=total ion chromatogram; b-g=selected ion chromatograms for (b) m/z 118 for d-MFT, (c)= m/z 115 for MFT, (d)= m/z 121 for d-3M2P, (e) for m/z 119 for3M2P and 2M3P, (f)= m/z 83 for d-FFT and (g)= m/z 81 for FFT. (Adapted from reference 23. Copyright 1998 American Chemical Society).

Odor Thresholds

Apart from odor quality, the threshold value of a compound is another important characteristic for estimating sensory relevance. Threshold values can be determined orthonasally (odor) or retronasally (aroma) using various solvents, such as air, water, alcoholic solutions, oil, emulsions, starch, and

deodorized food. Perception thresholds are obtained if the odorant is different from the solvent. In the case of recognition thresholds, the characteristic aroma is still recognizable. In general, sulfur-containing compounds, such as odorants **1-21** (Figure 3), have low sensory thresholds and, therefore, often play a key role in many food flavors. Depending on the matrix used for evaluation, the threshold values may significantly differ. Some examples are listed in Table I. Odor thresholds must be carefully selected from literature, and ideally threshold values are confirmed by a trained sensory panel employed in sensory studies.

Table I. Odor Thresholds of Sulfur-Containing Compounds

Odorant	*Odor Quality*	*Air [ng/L]*	*Water [μg/kg]*
Pyrrolidino[1,2-*e*]-4*H*-2,4-dimethyl-1,3,5-dithiazine (**8**)	Roasty, shellfish		$1x10^{-11}$
bis-(2-Methyl-3-furyl)-disulfide (**9**)	Sulfury, meaty	0.001	$2x10^{-5}$
(*R*)-1-*p*-Menthen-8-thiol (**3**)	Sulfury, grape fruit		$2x10^{-5}$
4-Mercapto-4-methyl-2-pentanone (**1**)	Sulfury, black currant		$1x10^{-4}$ 0.001 [a]
3-Methyl-2-buten-1-thiol (**10**)	Sulfury, foxy	0.02	$3x10^{-4}$
4-Methoxy-2-methyl-2-butanethiol (**11**)	Sulfury, catty	0.0002	0.001 0.02 [b]
3-Mercapto-3-methylbutyl formate (**12**)	Sulfury, catty	0.0003	0.003
2-Methyl-3-furanthiol (**2**)	Sulfury, meaty	0.002	0.007
2-Furfurylthiol (**5**)	Sulfury, coffee	0.02	0.01, 0.4 [b]
Dimethyl trisulfide (**13**)	Cooked cabbage	0.1	0.01 0.2 [a], 2.5 [b]
(2*R*,3*S*)-3-Mercapto-2-methyl-1-pentanol (**14**)	Sulfury, onion	0.0001	0.03
Methional (**4**)	Cooked potato	0.2	0.2, 0.2 [b]
Methanethiol (**15**)	Sulfury, cabbage		0.2, 0.1 [b]
Dimethyl sulfide (**16**)	Sulfury, tomato		0.3 10 [a], 1.2 [b]
bis-(Methylthio)-methane (**17**)	Sulfury, truffle		0.3, 3 [b]
3-Mercapto-2-pentanone (**6**)	Sulfury, catty	0.1	0.7
2-Acetyl-2-thiazoline (**18**)	Roasty, popcorn	0.02	2
2-Isobutylthiazole (**19**)	Green ,tomato		3
2-Acetylthiazole (**20**)	Sulfury, roasty	3	10
Hydrogen sulfide	Sulfury, egg		10

a Threshold determined in an aqueous ethanol solution (10%, v/v).

b Threshold determined in deodorized oil.

SOURCE: Adapted from references 1 and 24.

The Odor Activity Value (OAV) Concept

By definition, OAV is the ratio of the concentration of an odorant to the threshold value (*12*) and is a synonym to aroma value (*25*) and odor unit (*26*). It is evident that reliable OAVs can only be calculated if correct data are obtained for the threshold and concentration of the odorant. The threshold should preferably be determined in the food itself or in a matrix closely representing the food product, e.g. in oil for margarine aroma, in water or better in an aqueous 10% ethanolic solution for wine aroma, and in starch for bread aroma.

In their work on tomato aroma, Buttery and coworkers calculated the OAVs in order to estimate the sensory relevance of volatile constituents of fresh tomato (*27*). They concluded that only a few compounds occur in concentrations well above the odor thresholds, *i.e.* only few volatile compounds have OAV>1 as shown in Table II.

2-Isobutylthiazole (**19**) with an OAV=12 belongs to that group of compounds. Interestingly, despite the relatively low OAV, this odorant is known to be a key constituent of tomato flavorings. In 1970 Kazeniac and Hall (*28*) had already reported that 2-isobutylthiazole, in amounts of 25-50 μg/kg, improves the fresh tomato note and mouthfeel of canned tomatoes.

Table II. Some Key Volatile Constituents of Fresh Tomato Aroma

Odorant	*Concentration [μg/kg]*	*Odor Threshold [μg/kg water]*	*OAV*
(*Z*)-3-Hexenal	12000	0.25	50000
Hexanal	3100	4.5	630
β-Ionone	4	0.007	630
1-Penten-3-one	520	1	520
(*E*)-β-Damascenone	1	0.002	500
3-Methylbutanal	27	0.2	130
(*E*)-2-Hexenal	270	17	16
2-Isobutylthiazole (**19**)	36	3	12

SOURCE: Adapted from reference 27. Copyright 1989 American Chemical Society.

Sensory Studies

Although the relative importance of individual odorants can be estimated on the basis of OAVs, the interactions of odorants with each other or the food matrix remain unconsidered. Flavor recombination studies, albeit time-consuming, offer an attractive way to better estimate the role of odorants in a complex food aroma. As shown for fresh basil aroma (*29*), six odorants with

OAV>1000 were found to contribute to the overall aroma (Table III). Additiona odorants with OAVs of 5 to 500 were wine lactone, methyl cinnamate, estragol α-pinene, and decanal. A model mixture of the 11 odorants revealed the typica fresh basil aroma. The similarity of the model to fresh basil was described a very good, i.e. 3.0 on a scale from 0 to 3.

Table III. Concentrations and Odor Activity Values (OAVs) of Important Odorants of Fresh Basil Leaves

Odorant	*Concentration [mg/kg]*	*OAV*
(*Z*)-3-Hexenal	12.4	413000
1,8-Cineol	64.0	246000
4-Mercapto-4-methyl-2-pentanone (**1**)	0.01	83300
Linalool	60.2	40000
4-Allyl-1,2-dimethoxybenzene	495	9900
Eugenol	89.0	8900

SOURCE: Adapted with permission from reference 29. Copyright 1997 Eigenverlag Universität Potsdam.

Omission tests were performed to better understand the relative importanc of individual odorants. The absence of eugenol or (*Z*)-3-hexenal diminishe strongly the similarity to the complete mixture, indicating the essential roles o these compounds to fresh basil aroma (Table IV). Also the absence of α-pinene 4-mercapto-4-methyl-2-pentanone, linalool or 1,8-cineol led to definint decreases in similarity.

Table IV. Omission Tests with the Basil Model Mixture

Compound removed from the model aroma	*Similarity*[a]
Eugenol	0.7
(*Z*)-3-Hexenal	1.0
α-Pinene	1.3
4-Mercapto-4-methyl-2-pentanone (**1**)	1.6
Linalool	1.8
1,8-Cineol	1.9

a The similarity was scored on a scale from 0 to 3: 1= weak, 2= middle, 3= strong.
SOURCE: Adapted with permission from reference 29. Copyright 1997 Eigenverlag Universität Potsdam.

Selected Food Aromas

Many S-compounds have been reported in the literature to have the same basic organoleptic properties as a food and are called character-impact compounds (Table V, Figures 3 and 5). These compounds are considered as essential constituents in flavor compositions, particularly if the compounds have low odor thresholds.

Table V. Character-Impact Sulfur Compounds in Foods

Sulfur Compound	*Occurrence*	*Reference*
4-Mercapto-4-methyl-2-pentanone (**1**)	Wine (Sauvignon), grape fruit	10, 30
2-Methyl-3-furanthiol (**2**)	Beef meat, cooked	14, 16
(*R*)-1-*p*-Menthen-8-thiol (**3**)	Grape fruit	19, 30
Methional (**4**)	Potato chips	31
2-Furfurylthiol (**5**)	Coffee, roasted	32, 33
Pyrrolidino[1,2-*e*]-4*H*-2,4-dimethyl-1,3,5-dithiazine (**8**)	Shellfish	34
4-Methoxy-2-methyl-2-butanethiol (**11**)	Black currant, olive oil (Spain)	35, 36
(2*R*,3*S*)-3-Mercapto-2-methyl-1-pentanol (**14**)	Onion (fresh)	37
Dimethyl sulfide (**16**)	Tomato, paste	38
bis-(Methylthio)-methane (**17**)	Truffle (white)	39
2-Acetyl-2-thiazoline (**18**)	Beef meat, roasted	40
2-Isobutylthiazole (**19**)	Tomato, fresh	41
2-Phenyl isothiocyanate (**22**)	Horseradish	42
2-Methyl-4-propyl-1,3-oxathiane (**23**)	Passion fruit	43
Ethyl 3-mercaptopropanoate (**24**)	Grape (Concorde)	44
Ethyl 3(methylthio)-propanoate (**25**)	Pineapple	45
8-Mercapto-*p*-menthan-3-one (**26**)	Buchu leaf oil	46
Propanthial *S*-oxide (**27**)	Onion, fresh	4
Propyl propanethiosulfonate (**28**)	Onion, fresh	1, 4, 47
Dipropyl disulfide (**29**)	Onion, boiled	1, 4, 47
trans-1-Propenyl propyl disulfide (**30**)	Onion, boiled	1, 4
2-(Propyldithio)-3,4-dimethylthiophene (**31**)	Onion, fried	1, 4
3,4-Dimethyl-2,3-dihydrothiophenethiol (**32**)	Onion, fried	1, 4, 48
bis-(2-Propenyl)-disulfide (**33**)	Garlic, fresh	1, 4, 49

SOURCE: Adapted from references 1 and 3.

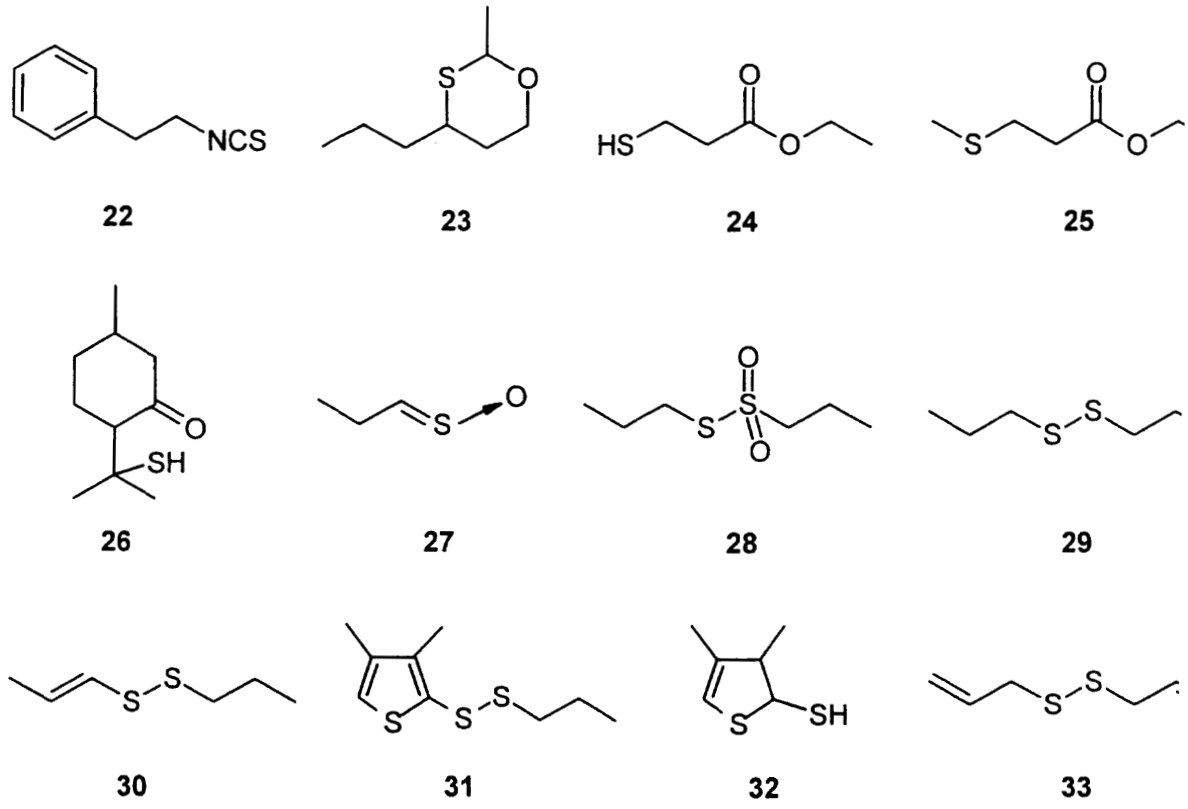


Figure 5. Examples of important S-containing character-impact compounds in foods. (See also Table V for corresponding names and odor descriptors).

The *Allium* species are known to be rich in S-containing volatile constituents. Propanthial *S*-oxide (**27**), the onion lachrymatory factor, and propyl propanethiosulfonate (**28**) are characteristic compounds of raw onions (*1,4*). Volatile compounds contributing to the aroma of cooked onions are for example dipropyl disulfide (**29**) and *trans*-1-propenyl propyl disulfide (**30**) with odor thresholds of 3.2 and 2 μg/L water, respectively (*1*). 2-(Propyldithio)-3,4-dimethylthiophene (**31**) and 3,4-dimethyl-2,3-dihydrothiophenethiol (**32**) have odors reminiscent of fried onions (*1,48*).

In an extensive study, Pickenhagen and coworkers recently identified a new chemical class of raw onion constituents, i.e. 3-mercapto-2-methyl-1-pentanol (**14**) (*37*). The *anti* enantiomers show particularly low threshold values of 0.03-0.04 μg/L water. The *syn* isomers are less odor-active and have higher threshold values of 12-30 μg/L water (*50*).

Surprisingly, little data has been published on the volatile S-compounds listed in Table V that demonstrate sensory relevance (*1*). Reliable threshold values, concentrations, and sensory studies are rarely available. This may be due to the instability of S-containing compounds, thus making identification and sensory characterization difficult.

The sensory relevance of sulfur-containing compounds will be illustrated with examples from natural and processed foods, such as olive oil, wine, cooked meat, and roasted coffee. These are only a few examples showing the importance of S-odorants to food flavors.

Olive Oil

The aroma composition of olive oils from different provinces has been studied (*36*,*51*). Surprisingly, one of the key odorants in Spanish olive oil was identified as 4-methoxy-2-methyl-2-butanethiol (**11**) (Table VI), which was absent in olive oils from Italy and Morocco (*36*). The odor was described as catty, black currant-like and with a high potency. This odorant having a high OAV amongst 21 compounds was thought to be a character-impact constituent of Spanish olive oil (*51*).

Table VI. Some Key Odorants Found in Spanish Olive Oil

Odorant	*Concentration [μg/kg]*	*Odor Threshold [μg/kg oil]*	*OAV*
Acetaldehyde	410	0.22	1865
4-Methoxy-2-methyl-2-butanethiol (**11**)	4.3	0.017	255
(*S*)-Ethyl 2-methylbutyrate	14	0.26	55
3-Methylbutanal	70	2.2	32
2-Methylbutanal	102	5.4	19
trans-4,5-Epoxy-(*E*)-2-decenal	22	1.3	17
Acetic acid	1840	124	15
Ethyl 3-methylbutyrate	5.3	0.62	8.6
Propanal	75	9.4	8.0

SOURCE: Adapted from reference 51.

A model mixture composed of 21 odorants revealed an overall aroma, which was very close to that of the authentic Spanish olive oil sample, i.e. a similarity was determined to be 2.7 ± 0.3 on a scale from 0 to 3. As shown in Figure 6, the model aroma and olive oil have very similar sensory profiles. The fruity note in the model was slightly less pronounced, and the pungent note was a bit more intense.

In further sensory experiments, individual odorants were omitted from the model aroma to study relative importance to the overall aroma. The absence of acetaldehyde and propanal (S1), acetic acid (S2), 3-/2-methylbutanal (S3), or ethyl 2-/3-methylbutyrate (S4) only slightly changed the similarity, which dropped from 2.7 to 2.3. However, when odorant **11** was removed from the

model aroma (S5), the similarity was reduced to 0.9, thus indicating that this odorant is an essential volatile constituent of Spanish olive oil (Figure 7).

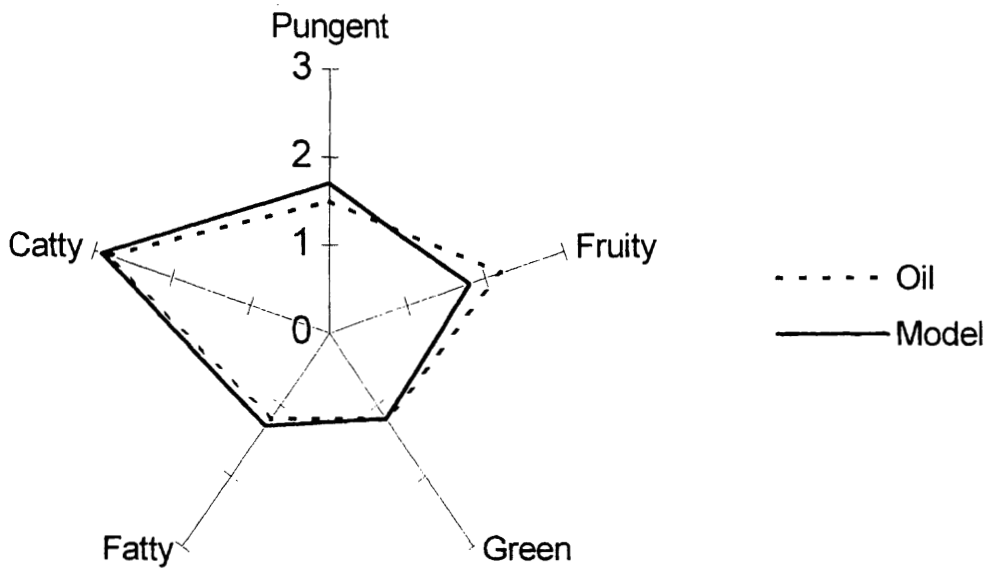


Figure 6. Sensory profiling of the model aroma consisting of 21 odorants as compared to Spanish olive oil. (Adapted from reference 51.)

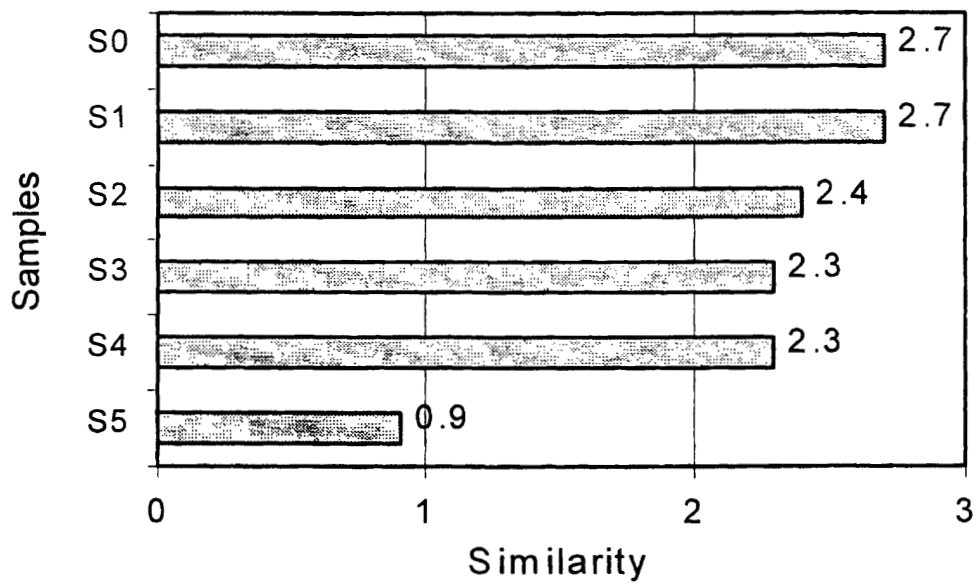


Figure 7. Effect of odorants on the aroma profile of the model mixtures SO (complete model aroma, 21 odorants), S1 (no acetaldehyde and propanal), S2 (no acetic acid), S3 (no 3-/2-methylbutanal), S4 (no ethyl 2-/3-methylbutyrate), and S5 (no 4-methoxy-2-methyl-2-butanethiol). (Adapted from reference 51.)

Wine

The important role of S-compounds, particularly thiols, has been shown in different wine varieties. 4-Mercapto-4-methyl-2-pentanone (black currant-like, **1**) was identified in Sauvignon Blanc using GC-O (*10*). As shown in Figures 8 and 9, further thiols were reported in Sauvignon Blanc (*52,53*), i.e. 4-mercapto-4-methyl-2-pentanol (citrus peel-like, **34**), 3-mercapto-3-methyl-1-butanol (broth-like, **35**), 3-mercaptohexyl acetate (passion fruit-like, **36**), and 3-mercapto-1-hexanol (grapefruit-like, **37**).

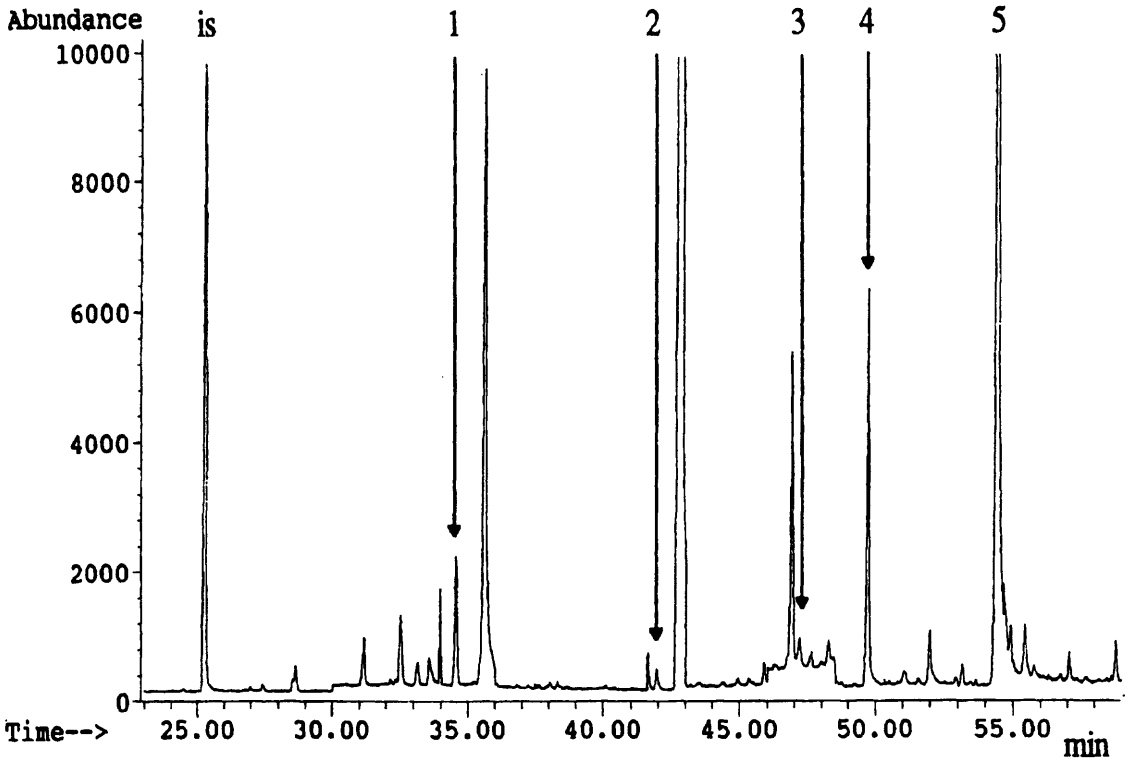
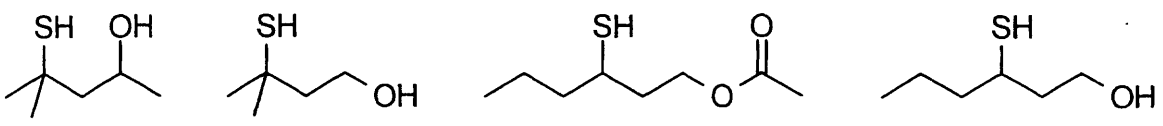


*Figure 8. Volatile S-containing constituents of Sauvignon blanc wine: 1, 4-mercapto-4-methyl-2-pentanone (**1**); 2, 4-mercapto-4-methyl-2-pentanol (**34**); 3, 3-mercapto-3-methyl-1-butanol (**35**); 4, 3-mercaptohexyl acetate (**36**); 5, 3-mercapto-1-hexanol (**37**) (structures in Figure 9). (Adapted from reference 52.)*



34 **35** **36** **37**

*Figure 9. Structures of volatile S-containing constituents of Sauvignon blanc wine. (See also Figure 3. for structure of 4-mercapto-4-methyl-2-pentanone, **1**).*

A first indication for the sensory relevance of these compounds was obtained by determining the perception odor thresholds in water and in an aqueous ethanol solution (12 %, v/v). As shown in Table VII, the thresholds differ by several orders of magnitudes.

Table VII. Perception Odor Thresholds of Selected Wine Constituents

Odorant	*Water [μg/kg]*	*Aq. EtOH (12%) [μg/kg]*
4-Mercapto-2-methyl-2-pentanone	0.0001	0.0011
4-Mercapto-4-methyl-2-pentanol	0.02	0.055
3-Mercapto-3-methyl-1-butanol	1.3	1.5
3-Mercaptohexyl acetate	0.0023	0.0043
3-Mercapto-1-hexanol	0.017	0.06

SOURCE: Adapted from references 10, 52, and 53.

The sensory relevance was estimated by calculating the odor activity values OAV, i.e. the ratio of concentration to odor threshold (Figure 10). 4-Mercapto-2-methyl-2-pentanone (**1**) showed the highest OAV followed by 3-mercapto-1-hexanol (**37**). Aroma contribution is due to relatively low odor thresholds and high concentrations, respectively, resulting in OAV of about 15 to 20. In agreement with these results, Sauvignon blanc wines with no or low amounts of odorant **1** were described as not or weakly typical (<9 ng/L), those containing higher amounts as typical (18 ng/L) or very characteristic (34 ng/L) (*54*).

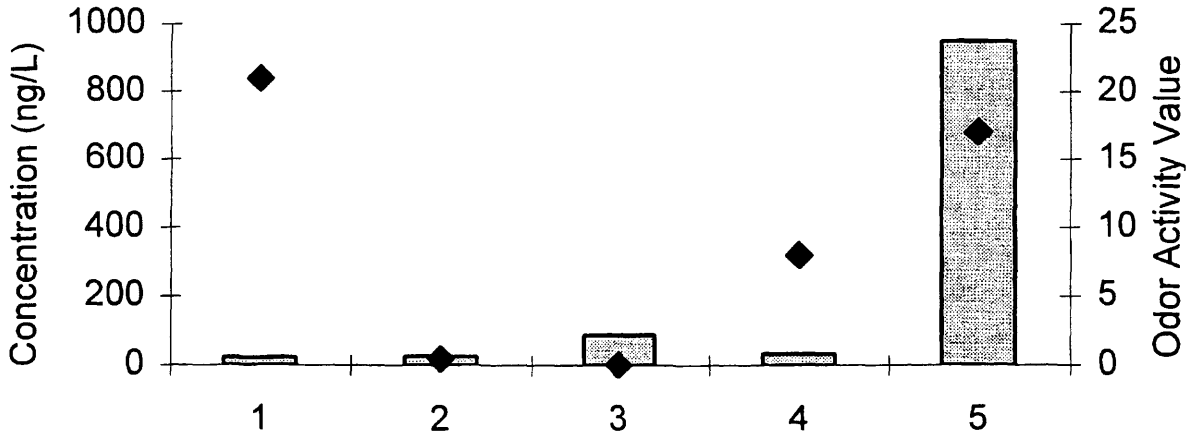


Figure 10. Sensory relevance (♦) of volatile S-constituents of Sauvignon blanc wine as related to concentration (bars): 1, *4-mercapto-4-methyl-2-pentanone (**1**);* 2, *4-mercapto-4-methyl-2-pentanol (**34**);* 3, *3-mercapto-3-methyl-1-butanol (**35**);* 4, *3-mercaptohexyl acetate (**36**);* 5, *3-mercapto-1-hexanol (**37**).* (Data are from reference 52.)

In the Scheurebe wine, four S-containing odorants were identified, i.e. 4-mercapto-4-methyl-2-pentanone (**1**), dimethyl sulfide (**16**), dimethyl trisulfide (**13**), and 3-(methylthio)-1-propanol (*55*). However, only odorant **1** was found to significantly contribute to the overall aroma; moreover, as shown in Figure 11, the OAV was the highest amongst all volatile compounds studied. The threshold values were determined in an aqueous ethanol solution (10%, v/v).

These results suggest odorant **1** is a character impact compound of Scheurebe wines (*56*). This was confirmed in omission experiments. A mixture of 42 odorants quantified in Scheurebe revealed the authentic aroma profile of this wine: similarity was determined to be 3 on a scale from 0 to 3. When odorant **1** was omitted from the model mixture, the similarity dropped to 0.5, thus indicating the significant contribution of this single odorant to the overall aroma.

In Bordeaux red wines, along with 3-mercapto-1-hexanol (**37**) and the respective acetate (**36**), the broth-like smelling odorant 3-mercapto-2-methyl-1-propanol was identified (*57*). Only the (*R*)-isomer is present in these wines. The threshold values of (*R*) and (*S*) enantiomers in water are 5 and 40 µg/L and in an aqueous ethanol solution (10%, v/v) 25 and 120 µg/L, respectively (*58*). The concentrations in young red wines were found to be much higher (25-70 µg/L) as compared to aged red wines (1-4 µg/L) and white wines (1-2 µg/L).

Cooked Meat

The role of S-compounds in meat flavors is well known. The diversity of S-containing volatile compounds in meat flavors is due to thermally induced degradation of sulfur sources, such as cysteine and thiamin, which react with sugar degradation products in the course of the Maillard reaction to generate various chemical classes such as thiols, sulfides, and S-heterocycles. Surprisingly, only a few S-compounds play a major role in the aroma of meat flavors.

In a comprehensive study on stewed beef and pork, Guth and Grosch identified the odorants contributing to the aroma of the juices (*59,60*). They have established a list of odorants obtained by GC-O analysis of the headspace and a solvent extract. Quantification experiments were performed using the IDA technique based on internal standards labeled with stable isotopes. As shown in Table VIII, three S-compounds play a certain role in the aroma profile of stewed meat juices, *i.e.* methanethiol (**15**), methional (**4**) and 2-furfurylthiol (**5**).

To check if all of the important odorants were identified, model aromas consisting of 15 odorants were sensorially evaluated and the sensory profiles compared with those of the authentic meat juices. As shown in Figure 12 for stewed beef, the model aroma was very close to the beef juice. The similarity was found to be 3 on a scale from 0 to 3. In the case of stewed pork with a similarity of 2.5, sensory profiling indicated that the model aroma was too tallowy and roasty, but not fatty enough.

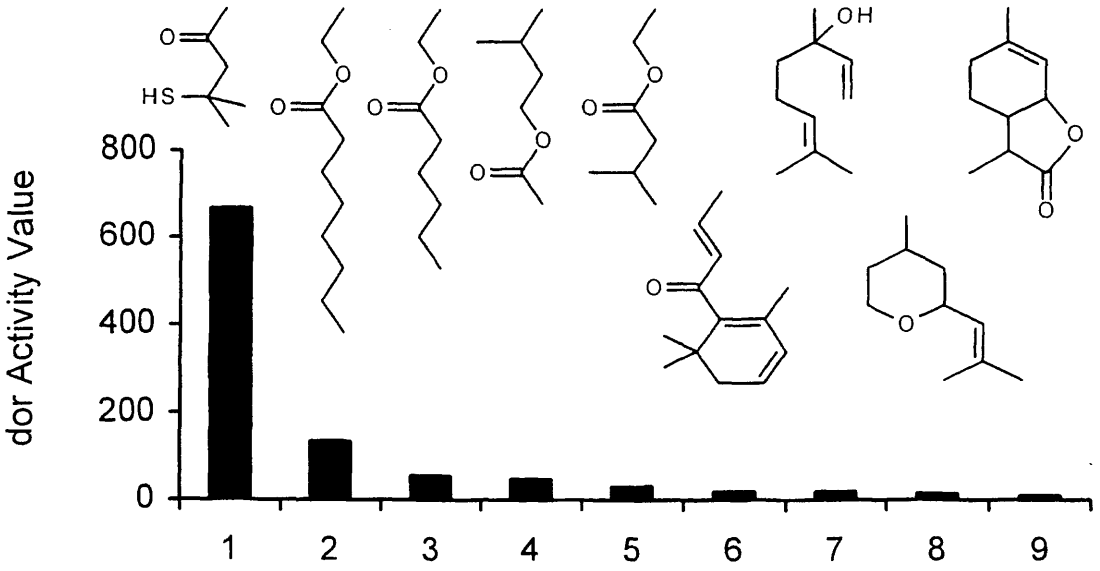


Figure 11. Estimation of the sensory relevance of odorants identified in Scheurebe wine: 1, 4-mercapto-4-methyl-2-pentanone (1); 2, ethyl octanoate; 3, ethyl hexanoate; 4, 3-methylbutyl acetate; 5, ethyl isobutyrate; 6, (E)-β-damascenone; 7, linalool; 8, cis-rose oxide; 9, wine lactone. (Data are from 56.)

Table VIII. Some Key Odorants Found in Stewed Meat Juices

Compound	*Concentration [μg/kg]*		*OAV*	
	Beef	*Pork*	*Beef*	*Pork*
Methanethiol (**15**)	300	500	1560	2500
12-Methyltridecanal	52	< 0.5	520	< 1
4-Hydroxy-2,5-dimethyl-3(2*H*)-furanone (Furaneol)	8000	2700	320	108
Acetaldehyde	6400	1500	256	60
Methional (**4**)	13	23	65	115
(*E*,*E*)-2,4-Decadienal	12	10	60	50
2-Furfurylthiol (**5**)	0.5	0.6	50	60
3-Methylbutanal	10	21	25	53
Sotolone	5	3	17	10
Hexanal	72	15	7	2
Acetic acid	200000	270000	6	8

SOURCE: Adapted with permission from reference 60. Copyright 1995 INRA.

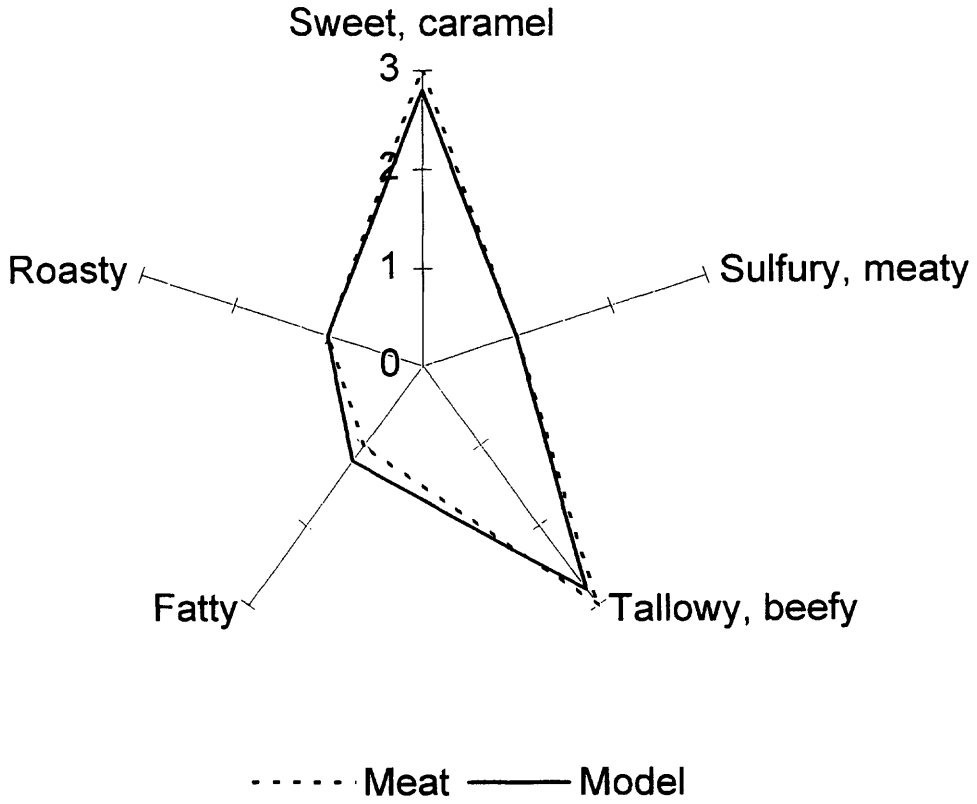


Figure 12. Odor profile of stewed beef juice (solid line) and the corresponding model aroma (dashed line). The juice matrix for sensory evaluation of the model aroma was an emulsion composed of water, coconut oil, gelatin, K_2HPO_4, lactic and glutamic acid. The pH was adjusted to 5.7 with NaOH (1 mol/L). (Data are from reference 60.)

The role of individual odorants in the stewed beef model aroma was studied by omission experiments. As shown in Figure 13, the aroma profile did not change much if 2-furfurylthiol was removed from the model aroma (S1). However, omission of 12-methyltridecanal (S5), 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone (S6), or methanethiol (S7) led to a significant decrease in similarity, thus indicating the essential role of these individual odorants to the overall aroma of stewed beef juice.

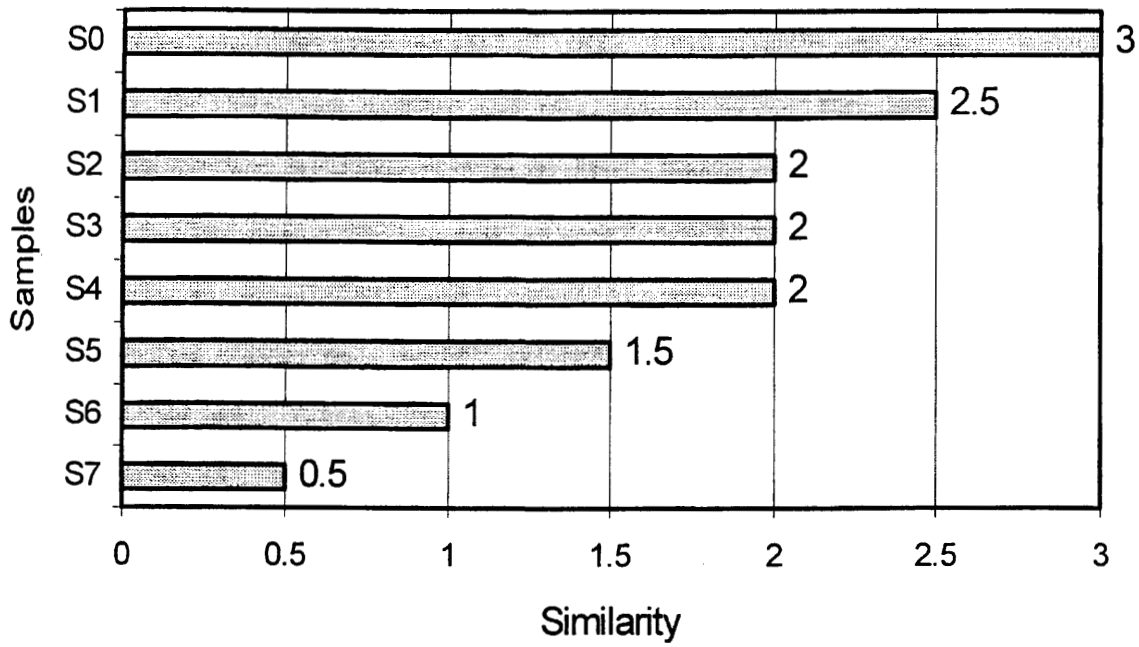


Figure 13. Effect of odorants on the aroma profile of the model mixtures S0 (complete model aroma, 15 odorants), S1 (no 2-furfurylthiol), S2 (no acetaldehyde), S3 (no sotolone), S4 (no acetic acid), S5 (no 12-methyltridecanal), S6 (no Furaneol), S7 (no methanethiol). (Data are from reference 59.)

The interaction of odorants was studied in additional sensory experiments (Table IX) by varying the composition of the model aroma (*59*). Model A was composed of the six odorants that were found to be essential in the omission experiments (Figure 13). Interestingly, the aroma of this model was very different from that of the complete model D (S0 in Figure 13). The sulfury, cabbage-like smell of methanethiol (**15**) was too intense. The predominance of **15** was reduced in model B by the addition of the S-containing odorants **4** and **5**, as well as (*E*,*E*)-2,4-decadienal and 3-methylbutanal. Also, by inclusion of these compounds the model aroma better represented the aroma of stewed beef.

The aroma profile was further improved by adding diacetyl, resulting in a similarity score of 2.5 in model C. Finally, the round and characteristic aroma of stewed beef juice was obtained by addition of butyric acid (model D). These results also show that odorants with relatively low OAVs can significantly contribute to the overall aroma of a food product.

Table IX. Aroma Recombination Studies on Stewed Beef Meat Juice

Odorant in the model	*OAV*	*Model Aroma*			
		A	*B*	*C*	*D*
Methanethiol (**15**)	1560	+	+	+	+
12-Methyltridecanal	520	+	+	+	+
4-Hydroxy-2,5-dimethyl-3(2*H*)-furanone	320	+	+	+	+
Acetaldehyde	256	+	+	+	+
Sotolone	17	+	+	+	+
Acetic acid	6	+	+	+	+
Methional (**4**)	65	-	+	+	+
(*E*,*E*)-2,4-Decadienal	60	-	+	+	+
2-Furfurylthiol (**5**)	50	-	+	+	+
3-Methylbutanal	25	-	+	+	+
Diacetyl	3	-	-	+	+
Butyric acid	3	-	-	-	+
	Similarity	0	2	2.5	3

SOURCE: Adapted from reference 59.

A similar study was performed to determine the key odorants of boiled beef meat (*9*,*23*). The model aroma of a boiled beef sample with a relatively high fat content consisted of 16 volatile compounds (Table X). Omission experiments revealed that nine odorants contributed significantly to the aroma of boiled beef, including the S-containing compounds **4**, **5**, **6**, and **15**. They can be considered as the key odorants of boiled beef.

In this sample, 12-methyltridecanal did not significantly contribute to the aroma profile in the presence of octanal, nonanal, and (*E*,*E*)-2,4-decadienal, which were responsible for the fatty note. However, in cooked lean beef meat 12-methyltridecanal is a key odorant (*59*) because this compound originates from membranes of muscle tissue and not from the fat depot.

Table X. Concentrations and Odor Activity Values (OAV) of Potent Odorants of Boiled Beef and Results of Omission Experiments

Odorant (omitted from the model) [a]	*Concentration [μg/kg]*	*OAV* [b]	*Number* [c]
2-Furfurylthiol (**5**)	29	2900	10***
4-Hydroxy-2,5-dimethyl-3(2*H*)-furanone	9075	908	10***
3-Mercapto-2-pentanone (**6**)	69	99	9***
Methanethiol (**15**)	311	1555	9***
Octanal	382	546	8**
2-Methyl-3-furanthiol (**2**)	24	3429	8**
Nonanal	1262	1262	8**
(*E*,*E*)-2,4-Decadienal	27	135	8**
Methional (**4**)	36	180	7*
12-Methyltridecanal	962	9620	6
Dimethyl sulfide (**16**)	105	350	5
(*Z*)-2-Nonenal	6.2	310	4
Acetaldehyde	1817	182	4
1-Octen-3-one	9.4	188	3
(*E*)-2-Nonenal	32	128	3
Methylpropanal	117	167	1

a Odorant omitted from the model aroma 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.
b OAVs were calculated on the basis of odor threshold values in water.
c Number of 11 assessors detecting the reduced model in triangle tests with significance * ($P < 0.05$), ** ($P < 0,01$), and *** ($P < 0.001$).
SOURCE: Adapted with permission from references 9. Copyright 2001 Oxford University Press.

Coffee

Coffee aroma is another example that demonstrates the role of S-containing odorants. The following compounds have been reported as characteristic volatile constituents of roast and ground coffee aroma (*18*,*61*): 2-methyl-3-furanthiol (**2**), methional (**4**), 2-furfurylthiol (**5**), 3-methyl-2-buten-1-thiol (**10**), 3-mercapto-3-methylbutyl formate (**12**), dimethyl trisulfide (**13**), and methanethiol (**15**). The amounts of these odorants in coffee aroma were found to be well above threshold concentrations (*62*).

Omission tests revealed 2-furfurylthiol (**5**) as an outstanding S-containing odorant of a typical roast and ground coffee aroma (*33*). The absence (Exp.1, Table XI) lowered the sulfurous/roasty note so that 15 of 20 assessors detected a difference between the complete and reduced model aroma. The important role

of odorant **5** established in the omission experiments confirmed the assumption of Reichstein and Staudinger that 2-furfurylthiol is a key compound of coffee aroma (*32*). However, its aroma impact was much lower in coffee beverages due to lower yields of this compound achieved by the brewing process (*63*).

The omission of other S-containing odorants, individually (Exp. 2-4) or in combination (Exp. 5), was detected by only 10 assessors. This detection level was below the confidence limit of 95 %, thus suggesting that these compounds are not likely essential odorants of coffee aroma. On the contrary, certain pyrazines, phenols, cyclic enolones, diones, and Strecker aldehydes are important constituents of a well-balanced coffee aroma (*33*).

Table XI. Aroma of the Roasted Arabica Coffee Model as Affected by the Absence of S-containing Compound

Exp.	*Compound(s) omitted*[a]	*Number*[b]
1	2-Furfurylthiol (**5**)	15[c]
2	2-Methyl-3-furanthiol (**2**)	10
3	3-Mercapto-3-methylbutyl formate (**12**)	10
4	3-Methyl-2-buten-1-thiol (**10**)	10
5	Odorants **2**, **10**, **12**, methional (**4**), dimethyl trisulfide (**13**), methanethiol (**15**)	10

a Odorant(s) omitted from the model aroma containing 27 compounds. The base of the model consisted of sunflower oil and water (1:20, v/v).
b Number of correct answers in triangle tests (maximum answers: 20).
c Significant aroma difference ($P < 0.05$) between the complete and reduced model.
SOURCE: Adapted from reference 33.

Structure-Odor Activity Relationships

Certain thiols (Figure 14) have particular structural features that elicit a catty type odor, which often is also described as black currant-like, foxy, grape fruit-like, buchu leaves-like, etc. The most typical catty note is shown by the odorants **1**, **26**, **39**, and **41** (*64*). The character impact odorant of tomcat's urine, however is thought to be 3-mercapto-3-methyl-1-butanol (**40**) (*65*).

Most of the odorants shown below have similar, but not identical odor characteristics. Compounds **3**, **26**, and **37** have tropical/fruity undertones, odorants **14**, **35**, **42**, and **43** elicit savory/meaty notes, whereas **10** and **24** show a more foxy/skunky character, particularly at higher concentrations. Some of these odorants can be considered as character impact compounds, e.g. **3** for grapefruit (*19*), **11** for black currant (*35*), **14** for onions (*37*), and **26** for buchu leaves (*46*).

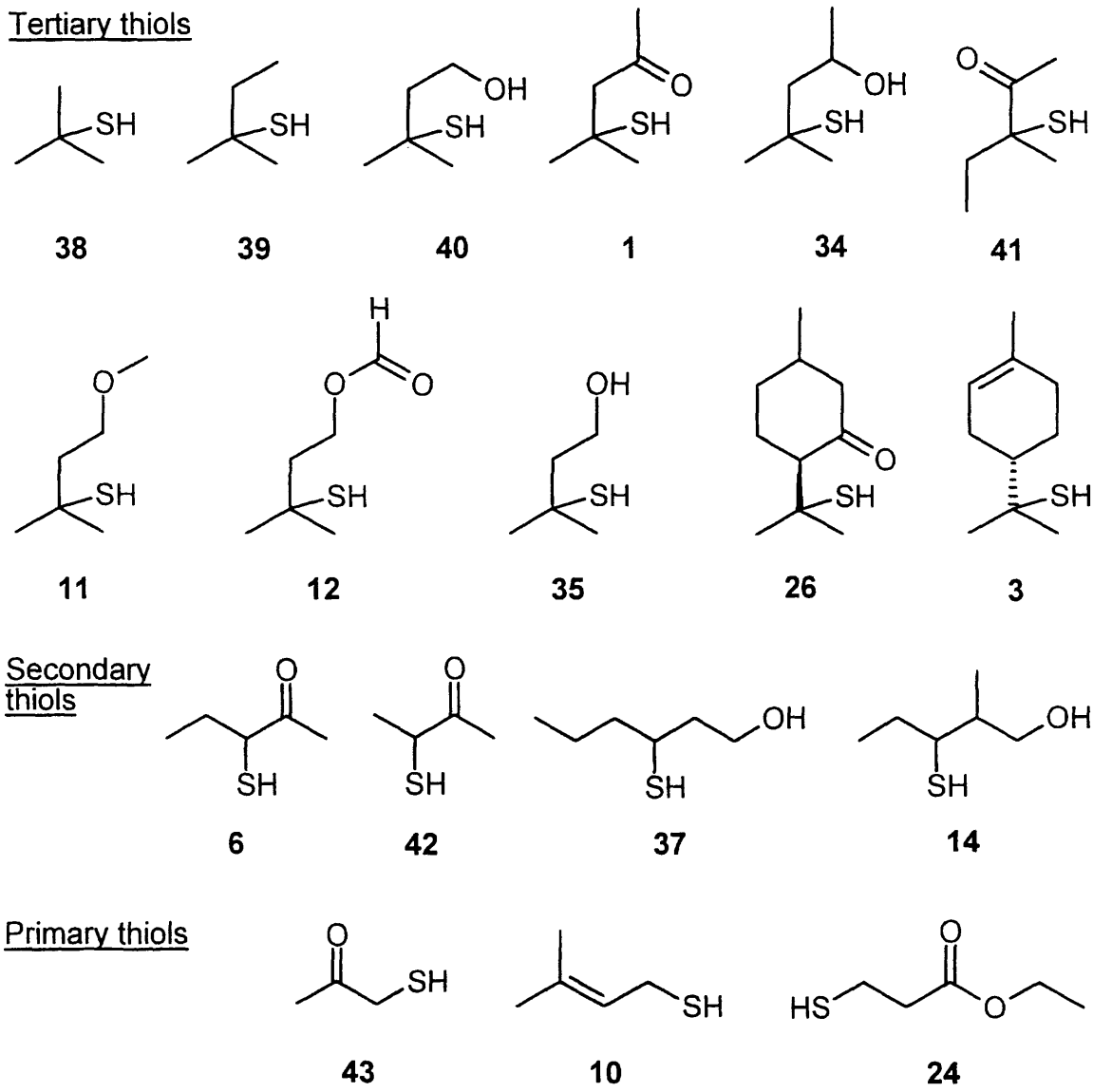


Figure 14. Examples of thiols that have particular structural features which elicit common odor-activity relationships.

According to Polak et al. (*64*), the tertiary mercapto amyl structure is the most characteristic feature shared by the majority of the compounds with the catty odor. The superimposibility of the carbon skeleton and the presence of a tertiary SH group appear to be sufficient for odor similarity. Exemptions are compounds **6** and **10**, which show typical catty notes, but are secondary and primary thiols, respectively.

The presence of a carbonyl group is not essential for the catty note. It makes little difference in odor quality whether the SH group is in the α (**41**) or β (**1**) position to the keto function. The estimated distances between SH and C=O of

about 2-4 Å do not differ markedly either in the eclipsed or staggered conformers. The α- and β- mercapto ketones might therefore both be present in conformations in which the functional groups overlap and accommodate the same receptor sites.

The most potent representatives of this group are compounds **1**, **3**, **10**, **11**, **12**, and **39**. Odor thresholds also depend strongly on structure. As shown in Table XII, tertiary thiols have lower threshold values as compared to primary and secondary thiols (*66*). Derivatization of the thiol group usually leads to an increase in threshold values.

Table XII. Odor Thresholds of S-Containing Compounds in Relation to Structure

Compound	*Structural Feature*	*Odor Threshold [μg/L beer]*
1-Butanethiol	primary SH	0.7
2-Butanethiol	secondary SH	0.6
2-Methyl-1-propanethiol	primary SH	2.5
2-Methyl-2-propanethiol (**38**)	tertiary SH	0.08
3-Methyl-2-butanethiol	secondary SH	0.2
2-Methyl-2-butanethiol (**39**)	tertiary SH	0.00007

SOURCE: Adapted from reference 66.

Off-Flavors

Sulfur containing odorants not only contribute to the characteristic aroma of a food, but can also lead to off-flavors. Food-born off-flavors occur by different causes. For example, the loss of key odorants or formation of intrinsic or new potent odorants can result during processing and/or storage, which will alter the characteristic aroma balance of the food.

Many S-compounds contribute importantly to the characteristic aroma of a food. However, in case of component overdose the overall aroma turns to off-notes. This has been reported for the foxy smelling ethyl-3-mercaptopropanoate (**24**) in Concord grape (*44*). In coffee, too high concentrations of 3-mercapto-2-buten-1-thiol (**10**) or 3-mercapto-3-methylbutyl formate (**12**) will also alter the typical coffee aroma.

Another source of off-flavors is the generation of S-compounds during processing or storage. Well-known examples are 3-mercapto-2-buten-1-thiol (**10**) in beer (*67*) or 4-mercapto-4-methyl-2-pentanone (**1**) in packaged meat-based foods (*68*). Compound **10** is formed by light-induced radical reactions of

beer constituents (Figure 15). Isohumulones decompose to 3-methyl-2-butenyl radicals whereas SH radicals stem from decomposition of S-containing amino acids and proteins in riboflavin-photosensitized reactions (*69*).

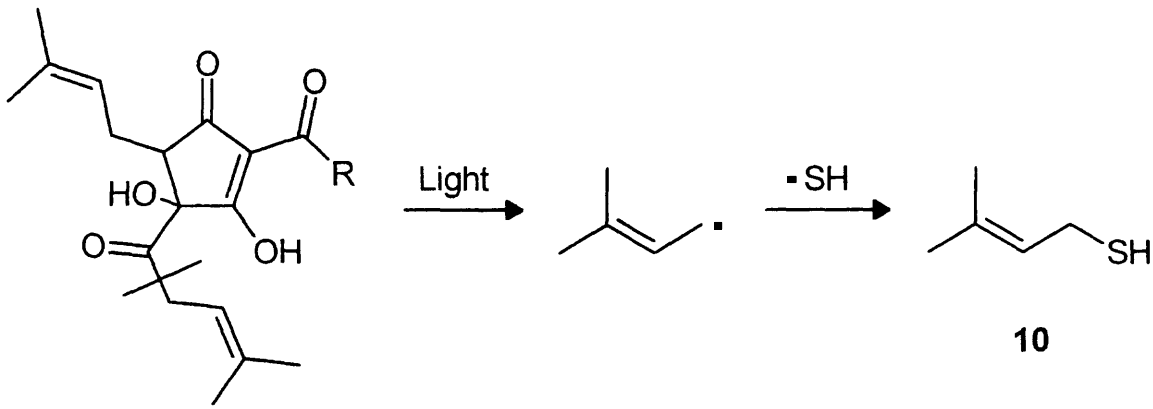


*Figure 15. Formation of 3-mercapto-2-buten-1-thiol (**10**) responsible for the sunstruck off-flavor of beer. (Adapted from reference 69.)*

As shown in Figure 16, odorant **1** can be formed from mesityl oxide and hydrogen sulfide originating from a packaging material and cooked meat, respectively (*70*). A well-known example for the formation of this off-flavor in chilled cooked meat stored in plastic bags is cooked ham (*71*).

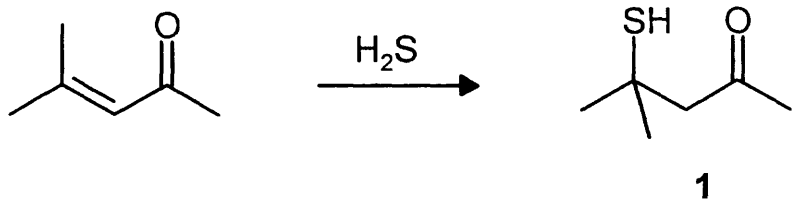


*Figure 16. Formation of 4-mercapto-4-methyl-2-pentanone (**1**) from mesityl oxide and hydrogen sulfide, responsible for the catty off-note in certain cooked meat products (Adapted from reference 70.)*

The fishy off-flavor formed in dry spinach upon storage was shown to come from two compounds present within a certain concentration range, *i.e.* (*Z*)-1,5-octadien-3-one and methional (*72*), having odor thresholds of 0.001 μg/L and 0.2 μg/L water, respectively. An aqueous solution containing 0.16 μg/L of the octadienone and 10 μg/L of methional showed a pronounced fishy odor. The

geranium-like and potato-like notes of the individual odorants were less intense. These odorants may cause fishy off-notes in many different food products as they are readily formed by lipid oxidation of unsaturated fatty acids and *Strecker* degradation of methionine, respectively. However, they can also contribute to the natural fishy odor of cooked seafood as recently demonstrated for boiled cod (*73*).

Conclusions

While the occurrence of sulfur compounds is of general interest, the sensory properties of these compounds can be critical to the flavor of a food. Recent analytical methods based on sensory directed chemical analysis in combination with aroma recombination studies and omission tests offer an attractive approach to evaluate the sensory relevance of S-compounds to food flavor. Sensory experiments are very important and should be seen as an integrated part of flavor research. As shown in this paper, S-containing organic volatile compounds play an important role in many food flavors and can be essential for a characteristic note. However, a lack of these odorants or an overdose can lead to the formation of off-flavors.

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