5 Aroma Compounds

5.1 Foreword

5.1.1 Concept Delineation

When food is consumed, the interaction of taste, odor and textural feeling provides an overall sensation which is best defined by the English word "flavor". German and some other languages do not have an adequate expression for such a broad and comprehensive term. Flavor results from compounds that are divided into two broad classes: Those *responsible for taste* and those *responsible for odors*, the latter often designated as aroma substances. However, there are compounds which provide both sensations.

Compounds *responsible for taste* are generally nonvolatile at room temperature. Therefore, they interact only with taste receptors located in the taste buds of the tongue. The four important basic taste perceptions are provided by: sour, sweet, bitter and salty compounds. They are covered in separate sections (cf., for example, 8.10, 22.3, 1.2.6, 1.3.3, 4.2.3 and 8.8). Glutamate stimulates the fifth basic taste (cf. 8.6.1).

Aroma substances are volatile compounds which are perceived by the odor receptor sites of the smell organ, i. e. the olfactory tissue of the nasal cavity. They reach the receptors when drawn in through the nose (orthonasal detection) and via the throat after being released by chewing (retronasal detection). The concept of aroma substances, like the concept of taste substances, should be used loosely, since a compound might contribute to the typical odor or taste of one food, while in another food it might cause a faulty odor or taste, or both, resulting in an off-flavor.

5.1.2 Impact Compounds of Natural Aromas

The amount of volatile substances present in food is extremely low (ca. 10–15 mg/kg). In general, however, they comprise a large number of components. Especially foods made by thermal processes, alone (e. g., coffee) or in combination with a fermentation process (e. g., bread, beer, cocoa, or tea), contain more than 800 volatile compounds. A great variety of compounds is often present in fruits and vegetables as well.

All the known volatile compounds are classified according to the food and the class of compounds and published in a tabular compilation (*Nijssen, L. M.* et al., 1999). A total of 7100 compounds in more than 450 foods are listed in the 1999 edition, which is also available as a database on the internet.

Of all the volatile compounds, only a limited number are important for aroma. Compounds that are considered as aroma substances are prima-

Table 5.1. Examples of key odorants

Compound	Aroma	Occurrence
(R)-Limonene	Citrus-like	Orange juice
(R)-1-p-Menthene-8-thiol	Grapefruit-like	Grapefruit juice
Benzaldehyde	Bitter almond-like	Almonds, cherries, plums
Neral/geranial	Lemon-like	Lemons
1-(p-Hydroxyphenyl)-3-butanone (raspberry ketone)	Raspberry-like	Raspberries
(R)-(−)-1-Octen-3-ol	Mushroom-like	Champignons, Camembert cheese
(E,Z)-2,6-Nonadienal	Cucumber-like	Cucumbers
Geosmin	Earthy	Beetroot
trans-5-Methyl-2-hepten-4-one (filbertone)	Nut-like	Hazelnuts
2-Furfurylthiol	Roasted	Coffee
4-Hydroxy-2,5-dimethyl-3(2H)-furanone	Caramel-like	Biscuits, dark beer, coffee
2-Acetyl-1-pyrroline	Roasted	White-bread crust

rily those which are present in food in concentrations higher than the odor and/or taste thresholds (cf. "Aroma Value", 5.1.4). Compounds with concentrations lower than the odor and/or taste thresholds also contribute to aroma when mixtures of them exceed these thresholds (for examples of additive effects, see 3.2.1.1, 20.1.7.8, 21.1.3.4).

Among the aroma substances, special attention is paid to those compounds that provide the characteristic aroma of the food and are, consequently, called key odorants (character impact aroma compounds). Examples are given in Table 5.1.

In the case of important foods, the differentiation between odorants and the remaining volatile compounds has greatly progressed. Important findings are presented in the section on "Aroma" in the corresponding chapters.

5.1.3 Threshold Value

The lowest concentration of a compound that is just enough for the recognition of its odor is called the odor threshold (recognition threshold). The detection threshold is lower, i. e., the concentration at which the compound is detectable but the aroma quality still cannot be unambiguously established. The threshold values are frequently determined by smelling (orthonasal value) and by tasting the sample (retronasal value). With a few exceptions, only the orthonasal values are given in this chapter. Indeed, the example of the carbonyl compounds shows how large the difference between the ortho- and retronasal thresholds can be (cf. 3.7.2.1.9).

Threshold concentration data allow comparison of the intensity or potency of odorous substances. The examples in Table 5.2 illustrate that great differences exist between individual aroma compounds, with an odor potency range of several orders of magnitude.

In an example provided by nootkatone, an essential aroma compound of grapefruit peel oil (cf. 18.1.2.6.3), it is obvious that the two enantiomers (optical isomers) differ significantly in their aroma intensity (cf. 5.2.5 and 5.3.2.4) and, occasionally, in aroma quality or character.

The threshold concentrations (values) for aroma compounds are dependent on their vapor pressure, which is affected by both temperature and medium. Interactions with other odor-producing substances can result in a strong increase in the odor thresholds. The magnitude of this effect is demonstrated in a model experiment in which the odor thresholds of compounds in water were determined in the presence and absence of 4-hydroxy-2,5-dimethyl-3(2H)-furanone (HD3F). The results in Table 5.3 show that HD3F does not influence the threshold value of 4-vinylguaiacol. However, the threshold values of the other odor-

Table 5.2. Odor threshold values in water of some aroma compounds (20 °C)

Compound	Threshold value (mg/l)
Ethanol	100
Maltol	9
Furfural	3.0
Hexanol	2.5
Benzaldehyde	0.35
Vanillin	0.02
Raspberry ketone	0.01
Limonene	0.01
Linalool	0.006
Hexanal	0.0045
2-Phenylethanal	0.004
Methylpropanal	0.001
Ethylbutyrate	0.001
(+)-Nootkatone	0.001
(-)-Nootkatone	1.0
Filbertone	0.00005
Methylthiol	0.00002
2-Isobutyl-3-methoxypyrazine	0.000002
1-p-Menthene-8-thiol	0.00000002

Table 5.3. Influence of 4-hydroxy-2,5-dimethyl-3(2H)-furanone (HD3F) on the odor threshold of aroma substances in water

Compound	Threshold value (μg/1)		Ratio
	I[a]	II[b]	II to I
4-Vinylguaiacol	100	90	≈1
2,3-Butanedione	15	105	7
2,3-Pentanedione	30	150	5
2-Furfurylthiol	0.012	0.25	20
β-Damascenone	2×10^{-3}	0.18	90

[a] I, odor threshold of the compound in water.

[b] II, odor threshold of the compound in an aqueous HD3F solution having a concentration (6.75 mg/1, aroma value A = 115) as high as in a coffee drink.

Table 5.4. Comparison of threshold values[a] in water and beer

Compound	Threshold (mg/kg) in	
	Water	Beer
n-Butanol	0.5	200
3-Methylbutanol	0.25	70
Dimethylsulfide	0.00033	0.05
(E)-2-Nonenal	0.00008	0.00011

[a] Odor and taste.

ants increase in the presence of HD3F. This effect is the greatest in the case of β-damascenone, the threshold value being increased by a factor of 90. Other examples in this book which show that the odor threshold of a compound increases when it is influenced by other odor-producing substances are a comparison of the threshold values in water and beer (cf. Table 5.4) as well as in water and in aqueous ethanol (cf. 20.2.6.9).

5.1.4 Aroma Value

As already indicated, compounds with high "aroma values" may contribute to the aroma of foods. The "aroma value" A_x of a compound is calculated according to the definition:

$$A_x = \frac{c_x}{a_x} \tag{5.1}$$

(c_x: concentration of compound X in the food, a_x: odor threshold (cf. 5.1.3) of compound X in the food). Methods for the identification of the corresponding compounds are described under Section 5.2.2.

The evaluation of volatile compounds on the basis of the aroma value provides only a rough pattern at first. The dependence of the odor intensity on the concentration must also be taken into account. In accordance with the universally valid law of *Stevens* for physiological stimuli, it is formulated as follows:

$$E = k \cdot (S - S_o)^n \tag{5.2}$$

E: perception intensity, k: constant, S: concentration of stimulant, S_o: threshold concentration of stimulant.

The examples presented in Fig. 5.1 show that the exponent n and, therefore, the dependency of the odor intensity on the concentration can vary substantially. Within a class of compounds, the range of variations is not very large, e. g., $n = 0.50-0.63$ for the alkanals C_4–C_9.

In addition, additive effects that are difficult to assess must also be considered. Examinations of mixtures have provided preliminary information. They show that although the intensities of compounds with a similar aroma note add up, the intensity of the mixture is usually lower than the sum of the individual intensities (cf. 3.2.1.1). For substances which clearly differ in their aroma note, however, the odor profile of a mixture is composed of the odor profiles of the components added together, only when the odor intensities are approximately equal. If the concentration ratio is such that the odor intensity of one component predominates, this component then largely or completely determines the odor profile.

Examples are (E)-2-hexenal and (E)-2-decenal which have clearly different odor profiles (cf. Fig. 5.2 a and 5.2 f). If the ratio of the odor intensities is approximately one, the odor notes of both aldehydes can be recognized in the odor profile of the mixture (Fig. 5.2 d). But if the dominating odor intensity is that of the decenal (Fig. 5.2 b), or of the hexenal (Fig. 5.2 e), that particular note determines the odor profile of the mixture.

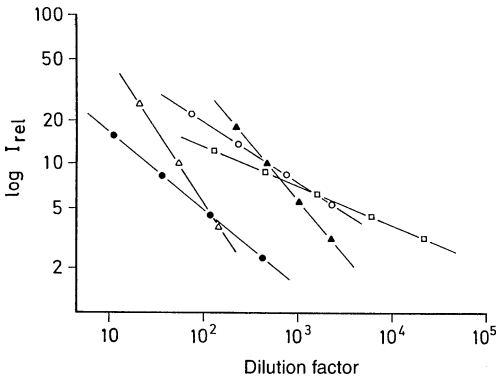


Fig. 5.1. Relative odor intensity I_{rel} (reference: n-butanol) as a function of the stimulant concentration (according to *Dravnieks*, 1977).
Air saturated with aroma substance was diluted. ● – ● – ● α-pinene, ○ – ○ – ○ 3-methylbutyric acid methyl ester, △ – △ – △ hexanoic acid, ♦ – ♦ – ♦ 2,4-hexadienal, □ – □ – □ hexylamine

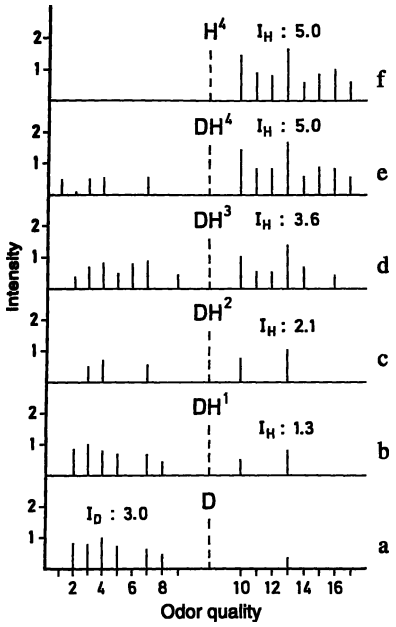
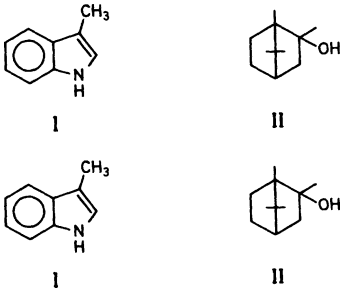


Fig. 5.2. Odor profiles of (E)-2-decenal (D), (E)-2-hexenal (H) and mixtures of both aldehydes (according to *Laing* and *Willcox*, 1983). The following concentrations (mg/kg) dissolved in di-2-ethylhexyl-phthalate were investigated: 50 (D); 2 (H^1); 3.7 (H^2); 11 (H^3) and 33 (H^4).
I_D and I_H: Odor intensity of each concentration of (E)-2-decenal and (E)-2-hexenal. Odor quality: **1**, warm; **2**, like clean washing; **3**, cardboard; **4**, oily, fatty; **5**, stale; **6**, paint; **7**, candle; **8**, rancid; **9**, stinkbug; **10**, fruity; **11**, apple; **12**, almond; **13**, herbal, green; **14**, sharp, pungent; **15**, sweet; **16**, banana; **17**, floral. The broken line separates the aroma qualities of (E)-2-decenal (*left side*) and (E)-2-hexenal

The mixture in Fig. 5.2, c gives a new odor profile because definite features of the decenal (stale, paint-like, rancid) and the hexenal (like apples, almonds, sweet) can no longer be recognized in it.
The examples show clearly that the aroma profiles of foods containing the same aroma substances can be completely dissimilar owing to quantitative differences. For example, changes in the recipe or in the production process which cause alterations in the concentrations of the aroma substances can interfere with the balance in such a way that an aroma profile with unusual characteristics is obtained.

5.1.5 Off-Flavors, Food Taints

An off-flavor can arise through foreign aroma substances, that are normally not present in a food, loss of key odorants, or changes in the concentration ratio of individual aroma substances. Figure 5.3 describes the causes for aroma defects in food. In the case of an odorous contaminant, which enters the food via the air or water and then gets enriched, it can be quite difficult to determine its origin if the limiting concentration for odor perception is exceeded only on enrichment. Examples of some off-flavors that can arise during food processing and storage are listed in Table 5.5. Examples of microbial metabolites wich may be involved in pigsty-like and earthy-muddy off-flavors are skatole (I; faecal-like, 10 µg/kg*), 2-methylisoborneol (II; earthy-muddy, 0.03 µg/kg*) and geosmin (III; earthy, (−): 0.01 µg/kg*; (+): 0.08 µg/kg*):

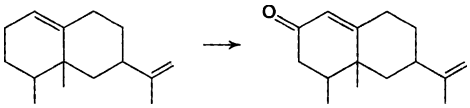
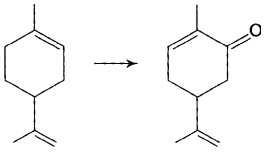
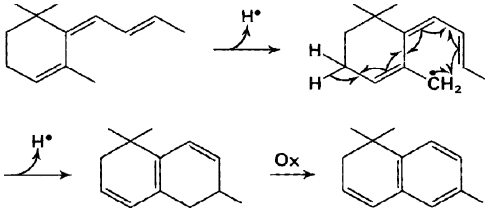


(5.3)

2,4,6-Trichloroanisole (IV) with an extremely low odor threshold (mouldy-like: $3.10^{-}5$ µg/kg, water) is an example of an off-flavor substance (cf. 20.2.7) which is produced by fungal degradation and methylation of pentachlorophenol fungicides.
To a certain extent, unwanted aroma substances are concealed by typical ones. Therefore, the threshold above which an off-flavor becomes noticeable can increase considerably in food compared to water as carrier, e. g., up to 0.2 µg/kg 2,4,6-trichloroanisole in dried fruits.

*Odor threshold in water.

Table 5.5. "Off-flavors" in food products

Food product	Off-flavor	Cause
Milk	Sunlight flavor	Photooxidation of methionine to methional (with riboflavin as a sensitizer)
Milk powder	Bean-like	The level of O_3 in air too high: ozonolyis of 8,15- and 9,15-isolinoleic acid to 6-trans-nonenal
Milk powder	Gluey	Degradation of tryptophan to o-amino-acetophenone
Milk fat	Metallic	Autoxidation of pentaene- and hexaene fatty acids to octa-1,cis-5-dien-3-one
Milk products	Malty	Faulty fermentation by *Streptococcus lactis, var. maltigenes*; formation of phenylacetaldehyde and 2-phenylethanol from phenylalanine
Peas, deep froze	Hay-like	Saturated and unsaturated aldehydes, octa-3,5-dien-2-one, 3-alkyl-2-methoxypyrazines, hexanal
Orange juice	Grapefruit note	Metal-catalyzed oxidation or photooxidation of valencene to nootkatone 
Orange juice	Terpene note	d-Limonene oxidation to carvone 
Passion fruit juice	Aroma flattening during pasteurization	Oxidation of (6-trans-2′-trans)-6-(but-2′-enyliden)-1,5,5-trimethylcyclohex-1-ene to 1,1,6-trimethyl-1,2-dihydronaphthalene: 
Beer	Sunlight flavor	Photolysis of humulone: reaction of one degradation product with hydrogen sulfide yielding 3-methyl-2-buten-1-thiol
Beer	Phenolic note	Faulty fermentation: hydrocinnamic acid decarboxylation by *Hafnia protea*

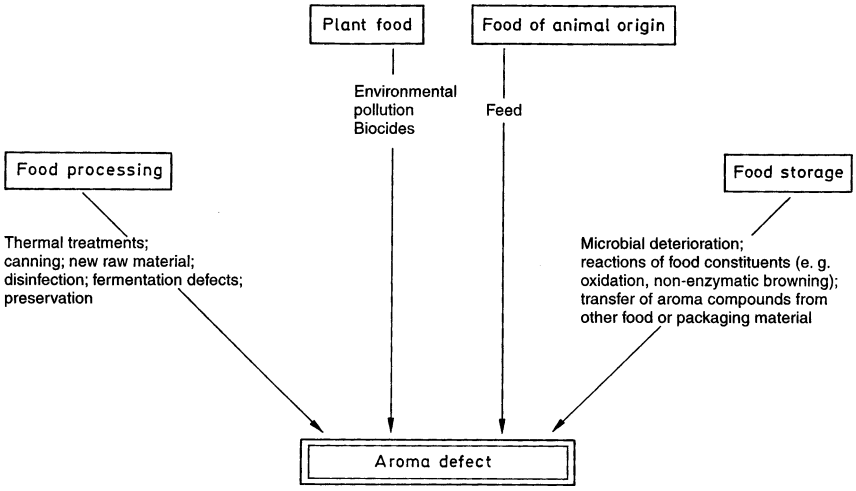


Fig. 5.3. The cause of aroma defects in food

5.2 Aroma Analysis

The aroma substances consist of highly diversified classes of compounds, some of them being highly reactive and are present in food in extremely low concentrations. The difficulties usually encountered in qualitative and quantitative analysis of aroma compounds are based on these features. Other difficulties are associated with identification of aroma compounds, elucidation of their chemical structure and characterization of sensory properties.

The results of an aroma analysis can serve as an objective guide in food processing for assessing the suitability of individual processing steps, and for assessing the quality of raw material, intermediate- and endproducts. In addition, investigation of food aroma broadens the possibility of food flavoring with substances that are prepared synthetically, but are chemically identical to those found in nature, i. e. the so-called "nature identical flavors" (cf. 5.5).

The elucidation of the aroma of any food is carried out stepwise; the following instrumental and sensory analyses are conducted:

- Isolation of the volatile compounds
- Differentiation of the aroma substances from the remaining components of the volatile fraction by dilution analyses
- Concentration and identification
- Quantification and calculation of aroma values
- Simulation of the aroma on the basis of the analytical results
- Omission experiments

5.2.1 Aroma Isolation

The amount of starting material must be selected to detect even those aroma substances which are present in very low concentrations (ppb range), but contribute considerably to the aroma because of still lower odor thresholds. The volatile compounds should be isolated from food using gentle methods because otherwise artifacts can easily be produced by the reactions listed in Table 5.6.

Additional difficulties are encountered in foods which retain fully-active enzymes, which can alter the aroma. For example, during the homogenization of fruits and vegetables, hydrolases split the aroma ester constituents, while lipoxygenase, together with hydroperoxide lyase, enrich the aroma with newly-formed volatile compounds. To avoid such interferences, tissue disintegration is done in the presence of enzyme inhibitors, e. g., $CaCl_2$ or, when possible, by rapid sample preparation. It is useful in some cases to inhibit enzyme-catalyzed reactions by the addition of methanol or ethanol. However, this can result in a change in aroma due to the formation of esters and acetals from acids and aldehydes respectively.

Table 5.6. Possible changes in aromas during the isolation of volatile compounds

Reaction
Enzymatic
1. Hydrolysis of esters (cf. 3.7.1)
2. Oxidative cleavage of unsaturated fatty acids (cf. 3.7.2.3)
3. Hydrogenation of aldehydes (cf. 5.3.2.1)
Non-enzymatic
4. Hydrolysis of glycosides (cf. 5.3.2.4 and 3.8.4.4)
5. Lactones from hydroxy acids
6. Cyclization of di-, tri-, and polyols (cf. 5.3.2.4)
7. Dehydration and rearrangement of tert-allyl alcohols
8. Reactions of thiols, amines, and aldehydes (cf. 5.3.1.4) in the aroma concentrate
9. Reduction of disulfides by reductones from the *Maillard* reaction
10. Fragmentation of hydroperoxides

At the low pH values prevalent in fruit, non-enzymatic reactions, especially reactions 4–7 shown in Table 5.6, can interfere with the isolation of aroma substances by the formation of artifacts. In the concentration of isolates from heated foods, particularly meat, it cannot be excluded that reactive substances, e. g., thiols, amines and aldehydes, get concentrated to such an extent that they condense to form heterocyclic aroma substances, among other compounds (Reaction 8, Table 5.6).

In the isolation of aroma substances, foods which owe their aroma to the *Maillard* reaction should not be exposed to temperatures of more than 50 °C. At higher temperatures, odorants are additionally formed, i. e., thiols in the reduction of disulfides by reductones. Fats and oils contain volatile and non-volatile hydroperoxides which fragment even at temperatures around 40 °C.

An additional aspect of aroma isolation not to be neglected is the ability of the aroma substances to bind to the solid food matrix. Such binding ability differs for many aroma constituents (cf. 5.4).

The aroma substances present in the vapor space above the food can be very gently detected by *headspace analysis* (cf. 5.2.1.3). However, the amounts of substance isolated in this process are so small that important aroma substances, which are present in food in very low concentrations, give no detector signal after gas chromatographic separation of the sample. These substances can be determined only by sniffing the carrier gas stream. The difference in the detector sensitivity is clearly shown in Fig. 5.4, taking the aroma substances of the crust of white bread as an example. The gas chromatogram does not show, e. g., 2-acetyl-1-pyrroline and 2-ethyl-3,5-dimethylpyrazine, which are of great importance for aroma due to high FD factors in the FD chromatogram (definition in 5.2.2.1). These aroma substances can be identified only after concentration from a relatively large amount of the food.

5.2.1.1 Distillation, Extraction

The volatile aroma compounds, together with some water, are removed by vacuum distillation from an aqueous food suspension. The highly volatile compounds are condensed in an efficiently cooled trap. The organic compounds contained in the distillate are separated from the water by extraction or by adsorption to a hydrophobic matrix and reversed phase chromatography and then prefractionated.

The apparatus shown in Fig. 5.5 is recommended for the gentle isolation of aroma substances from aqueous foods by means of distillation. In fact, a condensate can be very quickly obtained because of the short distances. As in all distillation processes, the yield of aroma substances decreases if the food or an extract is fatty (Table 5.7).

After application of high vacuum (≈5 mPa) the distillation procedure is started by dropping the

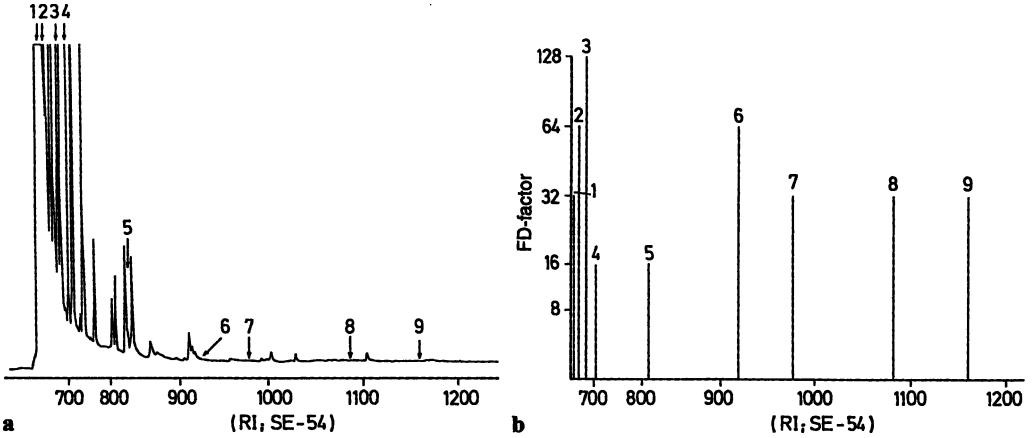


Fig. 5.4. Headspace analysis of aroma substances of white-bread crust. **a** Capillary gas chromatogram (the *arrows* mark the positions of the odorants), **b** FD chromatogram. Odorants: *1* methylpropanal, *2* diacetyl, *3* 3-methylbutanal, *4* 2,3-pentanedione, *5* butyric acid, *6* 2-acetyl-1-pyrroline, *7* 1-octen-3-one, *8* 2-ethyl-3,5-dimethylpyrazine, *9* (E)-2-nonenal (according to *Schieberle* and *Grosch*, 1992)

Table 5.7. Yields of aroma substances on distillation under vacuum[a]

Aroma substance (amount)[a]	Yield[b] (%)	
	Model I	Model II
3-Methylbutyric acid (1.9 µg)	91	31
Phenylacetaldehyde (4.2 µg)	84	21
3-Hydroxy-4,5-dimethyl-2(5H)-furanone (2.2 µg)	100	3.3
2-Phenylethanol (3.7 µg)	100	10.7
(E,E)-2,4-Decadienal (1.4 µg)	100	3.4
(E)-β-Damascenone (0.9 µg)	100	2.8
Vanillin (3.7 µg)	100	0.4

[a] Amount in the model solution: I in diethylether (50 ml), II in a mixture of diethylether (50 ml) and triglycerides (50 ml)
[b] Distillation in the apparatus shown in Fig. 5.5 at 35 °C

liquid food or the extract from the funnel (*1* in Fig. 5.5) into the distillation flask which is heated to 35–40 °C in a water bath (*2*). The volatiles including the solvent vapor are transferred into the distillation head (*3*). The distillate is condensed by liquid nitrogen in the receiver (*4*). The *Dewar* flask (*5*) protects the vacuum pump (reduced pressure 10^{-3} Pa).

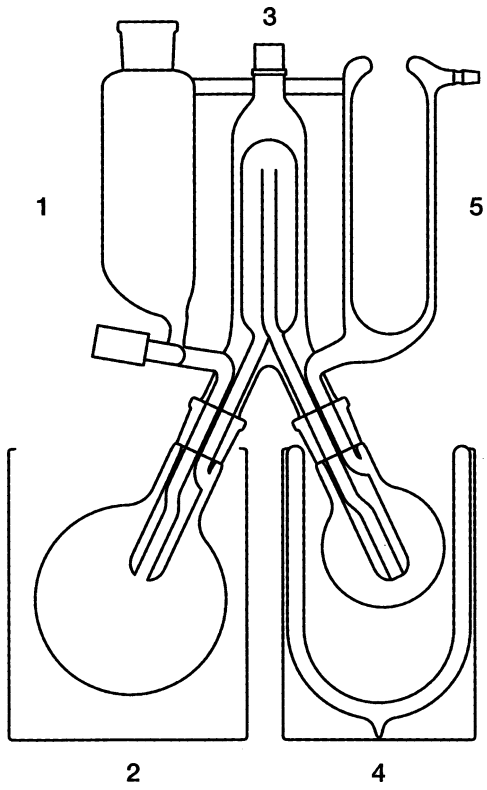


Fig. 5.5. Apparatus for the distillation of aroma substances from foods (for explanation, see text. According to *Engel* et al., 1999)

Solid foods are first extracted, the addition of water may be required to increase the yield of aroma substances.

An extraction combined with distillation can be achieved using an apparatus designed by *Likens–Nickerson* (Fig. 5.6).

In this process, low-boiling solvents are usually used to make subsequent concentration of the aroma substances easier. Therefore, this process is carried out at normal pressure or slightly reduced pressure. The resulting thermal treatment of the food can lead to reactions (examples in Table 5.6) that change the aroma composition. The example in Table 5.8 shows the extent to which some aroma substances are released from glycosides during simultaneous distillation/extraction.

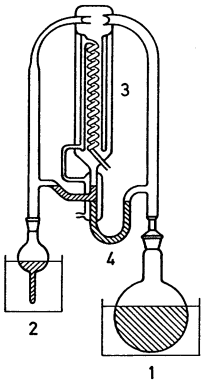


Fig. 5.6. Apparatus according to *Likens* and *Nickerson* used for simultaneous extraction and distillation of volatile compounds.
1 Flask with heating bath containing the aqueous sample, *2* flask with heating bath containing the solvent (e. g. pentane), *3* cooler, *4* condensate separator: extract is the upper and water the lower phase

Table 5.8. Isolation of odorants from cherry juice – Comparison of distillation in vacuo (I) with simultaneous distillation and extraction (II)

Odorant	I	(μg/1)	II
Benzaldehyde	202		5260
Linalool	1.1		188

Table 5.9. Relative retention time (t_{rel}) of some compounds separated by gas chromatography using Porapak Q as stationary phase (Porapak: styrene divinylbenzene polymer; T: 55 °C)

Compound	t_{rel}	Compound	t_{rel}
Water	1.0	Methylthiol	2.6
Methanol	2.3	Ethylthiol	20.2
Ethanol	8.1	Dimethylsulfide	19.8
Acetaldehyde	2.5	Formic acid	
Propanal	15.8	ethyl ester	6.0

5.2.1.2 Gas Extraction

Volatile compounds can be isolated from a solid or liquid food sample by purging the sample with an inert gas (e. g., N_2, CO_2, He) and adsorbing the volatiles on a porous, granulated polymer (Tenax GC, Porapak Q, Chromosorb 105), followed by recovery of the compounds. Water is retarded to only a negligible extent by these polymers (Table 5.9). The desorption of volatiles is usually achieved stepwise in a temperature gradient. At low temperatures, the traces of water are removed by elution, while at elevated temperatures, the volatiles are released and flushed out by a carrier gas into a cold trap, usually connected to a gas chromatograph.

5.2.1.3 Headspace Analysis

The headspace analysis procedure is simple: the food is sealed in a container, then brought to the desired temperature and left for a while to establish an equilibrium between volatiles bound to the food matrix and those present in the vapor phase. A given volume of the headspace is withdrawn with a gas syringe and then injected into a gas chromatograph equipped with a suitable separation column (static headspace analysis). Since the water content and an excessively large volume of the sample substantially reduce the separation efficiency of gas chromatography, only the major volatile compounds are indicated by the detector. The static headspace analysis makes an important contribution when the positions of the aroma sub-

stances in the chromatogram are determined by olfactometry (cf. 5.2.2.2).

More material is obtained by dynamic headspace analysis or by solid phase microextraction (SPME). In the dynamic procedure the headspace volatiles are flushed out, adsorbed and thus concentrated in a polymer, as outlined in 5.2.1.2. However, it is difficult to obtain a representative sample by this flushing procedure, a sample that would match the original headspace composition. A model system assay (Fig. 5.7) might clarify the problems. Samples (e) and (f) were obtained by adsorption on different polymers. They are different from each other and differ from sample (b), which was obtained directly for headspace analysis. The results might agree to a greater extent by varying the gas flushing parameters (gas flow, time), but substantial differences would still remain. A comparison of samples (a) and (g) in Fig. 5.7 shows that the results obtained by the distillation-extraction procedure give a relatively good representation of the composition of the starting solution, with the exception of ethanol. However, the formation of artifacts is critical (cf. 5.2.1.1).

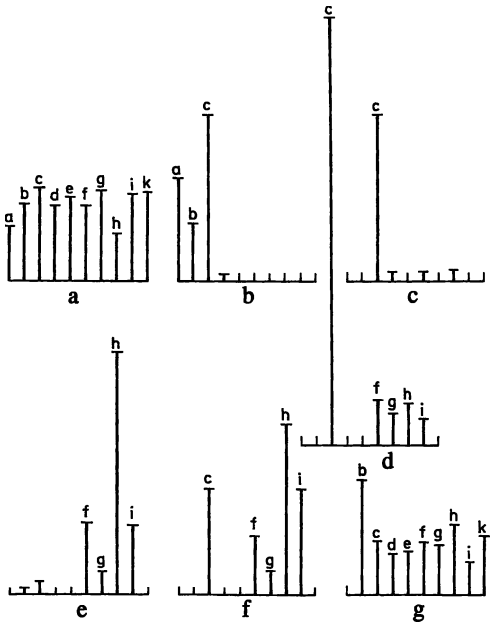


Fig. 5.7. A comparison of some methods used for aroma compound isolation (according to *Jennings* and *Filsoof*, 1977).
a *a* Ethanol, *b* 2-pentanone, *c* heptane, *d* pentanol, *e* hexanol, *f* hexyl formate, *g* 2-octanone, *h* d-limonene, *i* heptyl acetate and *k* γ-heptalactone. **b** Headspace analysis of aroma mixture **a**. *c* From aroma mixture 10 µl is dissolved in 100 ml water and the headspace is analyzed. **d** As in **c** but the water is saturated with 80% NaCl. **e** As in **c** but purged with nitrogen and trapped by Porapak Q. **f** As in **c** but purged with nitrogen and trapped by Tenax GC. **g** As in **e** but distilled and extracted (cf. Fig. 5.6)

SPME is based on the partitioning of compounds between a sample and a coated fiber immersed in it. The odorants are first adsorbed onto the fiber (e. g. nonpolar polydimethylsilo-xane or polar polyacrylate) immersed in a liquid food, a food extract or in the headspace above a food sample for a certain period of time. After adsorption is completed, the compounds are thermally desorbed into a GC injector block for further analysis.

Particularly in food applications headspace SPME is preferred to avoid possible contamination of the headspace system by non-volatile food components. Also SPME analysis is quite sensitive to experimental conditions. In addition to the stationary phase, sample, volume concentration of odorants, sample matrix and uniformity as well as temperature and time of the adsorption and desorption processes influence the yield. In quantitative SPME analysis these influences are eliminated by the use of labelled internal standards (cf. 5.2.6.1).

5.2.2 Sensory Relevance

In many earlier studies on the composition of aromas, each volatile compound was regarded as an aroma substance. Although lists with hundreds of compounds were obtained for many foods, it was still unclear which of the volatiles were really significant as odorants and to what extent important odorants occurring in very low concentrations were detected.

The studies meanwhile concentrate on those compounds which significantly contribute to aroma. The positions of these compounds in the gas chromatogram are detected with the help of dilution analyses. Here, both of the following methods based on the aroma value concept (cf. 5.1.4) find application.

5.2.2.1 Aroma Extract Dilution Analysis (AEDA)

In AEDA, the concentrate of the odorants obtained by distillation is separated by gas chromatography on a capillary column. To determine the retention times of the aroma substances, the carrier gas stream is subjected to sniffing detection after leaving the capillary column (GC/olfactometry). The sensory assessment of a single GC run, which is often reported in the literature, is not very meaningful because the perception of aroma substances in the carrier gas stream depends on limiting quantities which have nothing to do with the aroma value, e. g., the amount of food analysed, the degree of concentration of the volatile fraction, and the amount of sample separated by gas chromatography.

These limitations are eliminated by the stepwise dilution of the volatile fraction with solvent, followed by the gas chromatographic/olfactometric analysis of each dilution. The process is continued until no more aroma substance can be detected by GC olfactometry. In this way, a dilution factor 2^n (n = number of 1 + 1 dilutions) is determined for each aroma substance that appears in the gas chromatogram. It is designated as the flavor dilution factor (FD factor) and indicates the number of parts of solvent required to dilute the aroma extract until the aroma value is reduced to one.

Another more elaborate variant of the dilution analysis requires, in addition, that the duration of each odor impression is recorded by a computer and CHARM values are calculated (CHARM: acronym for combined hedonic response measurement), which are proportional to aroma values. The result of an AEDA can be represented as a diagram. The FD factor is plotted against the retention time in the form of the retention index (RI) and the diagram is called a FD chromatogram.

The FD chromatograms of the volatile compounds of white bread and French fries are presented in Fig. 5.4 and 5.8, respectively.

The identification experiments concentrate on those aroma substances which appear in the FD chromatogram with higher FD factors. To detect all the important aroma substances, the range of FD factors taken into account must not be too narrowly set at the lower end because differences in yield shift the concentration ratios. Labile compounds can undergo substantial losses and when distillation processes are used, the yield decreases with increasing molecular weight of the aroma substances.

In the case of French fries (Fig. 5.8), 19 aroma substances appearing in the FD-factor range 2^1–2^7 were identified (cf. legend of Fig. 5.8). Based on the high FD factors, the first approximation indicates that methional, 2-ethyl-3,5-dimethylpyrazine, 2,3-diethyl-5-methylpyrazine and (E,E)-2,4-decadienal substantially contribute to the aroma of French fries.

5.2.2.2 Headspace GC Olfactometry

In the recovery of samples for AEDA, highly volatile odorants are lost or are covered by the solvent peak in gas chromatography, e. g., methanethiol and acetaldehyde. For this reason, in addition to AEDA, a sample is drawn from the gas space above the food, injected into the gas chromatograph, transported by the carrier gas stream into a cold trap and concentrated there, as shown in Fig. 5.9. After quick evaporation, the sample is flushed into a capillary column by the carrier gas and chromatographed. At the end of the capillary, the experimentor sniffs the carrier gas stream and determines the positions of the chromatogram at which the odorants appear. The gas chromatogram is simultaneously monitored by a detector.

To carry out a dilution analysis, the volume of the headspace sample is reduced stepwise until no odorant is detectable by gas chromatography/olfactometry. In our example with French fries (Fig. 5.10), e. g., the odors of methanethiol, methylpropanal and dimethyltrisulfide were detectable in the sixth dilution, but only methanethiol was detectable in the seventh. The eighth dilution was odorless. Further experiments showed that methanethiol does in fact belong to the key odorants of French fries.

In GC/olfactometry, odor thresholds are considerably lower than in solution because the aroma substances are subjectied to sensory assessment in a completely vaporized state. The examples given in Table 5.10 show how great the differences can be when compared to solutions of the aroma substances in water.

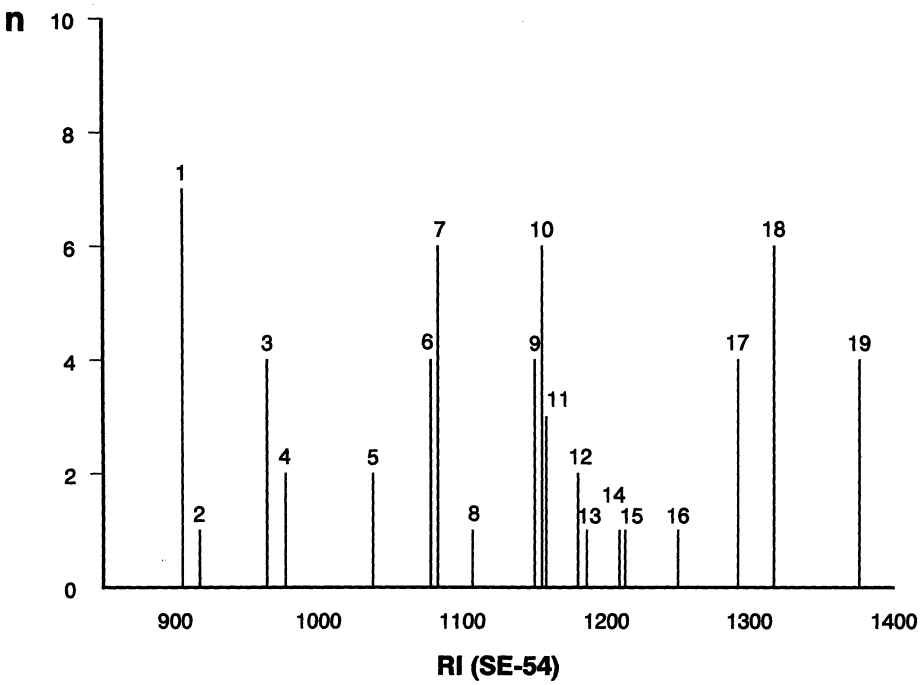


Fig. 5.8. FD chromatogram of the volatile fraction of French fries. Ordinate: n, number of $1 + 1$ dilutions. Abscissa: retention index (RI) on the capillary SE-54. The following odorants were identified: *1* methional, *2* 2-acetyl-1-pyrroline, *3* dimethyltrisulfide, *4* 1-octen-3-one, *5* phenylacetaldehyde, *6* 2-ethyl-3,6-dimethylpyrazine, *7* 2-ethyl-3,5-dimethylpyrazine, *8* nonanal, *9* (Z)-2-nonenal, *10* 2,3-diethyl-5-methylpyrazine, *11* (E)-2-nonenal, *12* 2-ethenyl-3-ethyl-5-methylpyrazine, *13* 2-isobutyl-3-methoxypyrazine, *14* dimethyltetrasulfide, *15* (E,E)-2,4-nonadienal, *16* (Z)-2-decenal, *17* (E,Z)-2,4-decadienal, *18* (E,E)-2,4-decadienal, *19* trans-4,5-epoxy-(E)-2-decenal (according to *Wagner* and *Grosch*, 1997)

Table 5.10. Odor thresholds of aroma substances in air and water

Compound	Odor thresholds in		
	Air (a) (ng/l)	Water (b) (μg/l)	b/a
β-Damascenone	0.003	0.002	6.7×10^2
Methional	0.12	0.2	1.6×10^3
2-Methylisoborneol	0.009	0.03	3.3×10^3
2-Acetyl-1-pyrroline	0.02	0.1	5×10^3
4-Vinylguaiacol	0.6	5	8.3×10^3
Linalool	0.6	6	1.0×10^4
Vanillin	0.9	20	2.2×10^4
4-Hydroxy-2,5-dimethyl-3(2H)-furanone (furaneol)	1.0	30	3×10^4

5.2.3 Enrichment

When an aroma concentrate contains phenols, organic acids or bases, preliminary separation of these compounds from neutral volatiles by extraction with alkali or acids is advantageous.

The acidic, basic and neutral fractions are individually analyzed. The neutral fraction by itself consists of so many compounds that in most cases not even a gas chromatographic column with the highest resolving power is able to separate them into individual peaks. Thus, separation of the neutral fraction is advisable and is usually achieved by liquid chromatography, or preparative gas or high performance liquid chromatography. A preliminary separation of aroma extracts is achieved

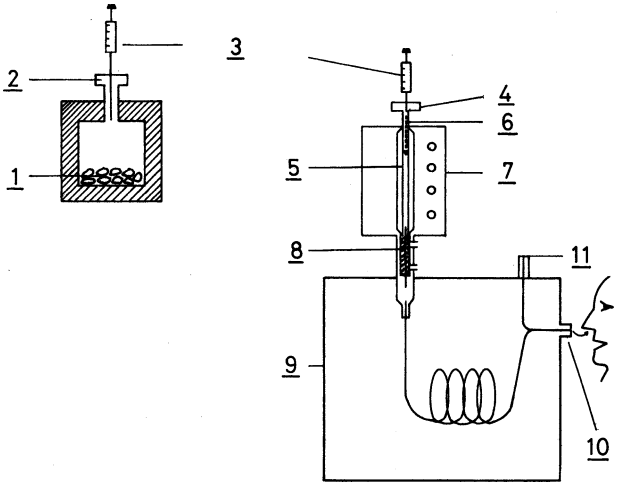


Fig. 5.9. Apparatus for the gas chromatography–olfactometry of static headspace samples. *1* Sample in thermostated glass vessel, *2* septum, *3* gastight syringe, *4* injector, *5* hydrophobed glass tube, *6* carrier gas, e. g., helium, *7* purge and trap system, *8* cold trap, *9* gas chromatograph with capillary column, *10* sniffing port, *11* flame ionization detector (according to *Guth* and *Grosch*, 1993)

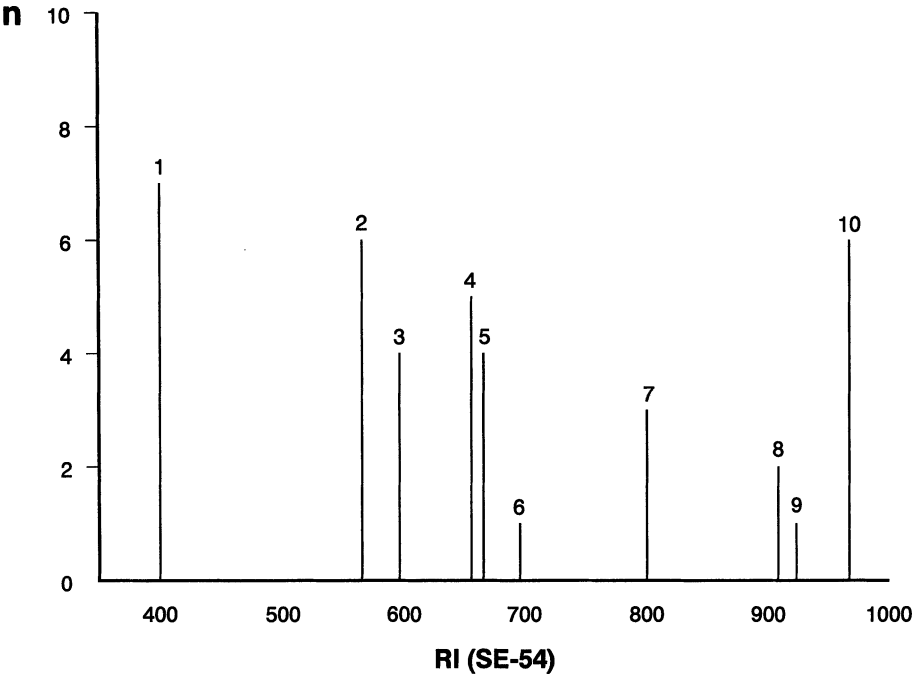


Fig. 5.10. FD chromatogram of static headspace samples of French fries. Ordinate: n, number of $1 + 1$ dilutions. Abscissa: retention index (RI) on the capillary SE-54. The following odorants were identified: *1* methanethiol, *2* methylpropanal, *3* 2,3-butanedione, *4* 3-methylbutanal, *5* 2-methylbutanal, *6* 2,3-pentanedione, *7* hexanal, *8* methional, *9* 2-acetyl-1-pyrroline, *10* dimethyltrisulfide (according to *Wagner* and *Grosch*, 1997)

by chromatography on silica gel, as shown in Table 5.11 for coffee aroma. To localize the aroma substances each of the four fractions is analyzed by gas chromatography and olfactometry. Some volatile compounds are aroma active in such low concentrations that even enrichment by column chromatography does not allow identification, e. g., 3-methyl-2-butenethiol (Fraction A in Table 5.11) and the two methoxypyrazines (Fraction B) in coffee. In most cases, further concentration is achieved with the help of multidimensional gas chromatography (MGC). The fraction which contains the unknown aroma substance is first subjected to preliminary separation on a polar capillary. The eluate containing the substance is cut out, rechromatographed on a non-polar capillary and finally analyzed by mass spectrometry. The MGC is also used in quantitative analysis for the preliminary purification of analyte and internal standard (cf. 5.2.6.1).

Table 5.11. Column chromatographic preliminary separation of an aroma extract of roasted coffee

Fraction[a]	Aroma substance
A	2-Methyl-3-furanthiol, 2-furfurylthiol, bis(2-methyl-3-furyl)disulfide, 3-methyl-2-butenethiol
B	2,3-Butanedione, 3-methylbutanal, 2,3-pentanedione, trimethylthiazole, 3-mercapto-3-methylbutylformiate, 3-isopropyl-2-methoxypyrazine, phenylacetaldehyde, 3-isobuty1-2-methoxypyrazine, 5-methyl-5(H)-cyclopentapyrazine, p-anisaldehyde, (E)-β-damascenone
C	Methional, 2-ethenyl-3,5-dimethylpyrazine, linalool, 2,3-diethyl-5-methylpyrazine, guaiacol, 2-ethenyl-3-ethyl-5-methylpyrazine, 4-ethylguaiacol, 4-vinylguaiacol
D	2-/3-Methylbutyric acid, trimethylpyrazine, 3-mercapto-3-methyl-1-butanol, 5-ethyl-2,4-dimethylthiazole, 2-ethyl-3,5-dimethylpyrazine, 3,4-dimethy1-2-cyclopentenol-1-one, 4-hydroxy-2,5-dimethyl-3(2H)-furanone, 5-ethyl-4-hydroxy-2-methyl-3(2H)-furanone, 3-hydroxy-4,5-dimethy1-2(5H)-furanone, 5-ethyl-3-hydroxy-4-methyl-2(5H)-furanone, vanillin

[a] Chromatography at 10–12 °C on a silica gel column (24 × 1 cm, deactivated with 7% water); elution with mixtures of pentane-diethylether (50 ml, 95 + 5, v/v, fraction A; 30 ml, 75 × 25, v/v, Fraction B; 30 ml, 1 + 1, v/v, Fraction C) and with diethylether (100 ml, Fraction D).

5.2.4 Chemical Structure

In the structure elucidation of aroma substances, mass spectrometry has become an indispensable tool because the substance amounts eluted by gas chromatography are generally sufficient for an evaluable spectrum. If the corresponding reference substance is available, identification of the aroma substance is based on agreement of the mass spectrum, retention indices on at least two capillary columns of different polarity, and of odor thresholds, which are compared by gas chromatography/olfactometry. If the reference substance is not available, the following procedure is suitable for the identification of the odorant:
The analyte is concentrated until a ^{1}H-NMR spectrum and, if necessary, a ^{13}C-spectrum can be measured. An example is the identification of the characteristic odorant of roasted hazelnuts. The mass spectrum of this substance (Fig. 5.11a) indicates an unsaturated carbonyl compound with a molar mass of 126. In conjunction with the structural elements shown by the ^{1}H-NMR spectrum (Fig. 5.11b), it was proposed that the odorant is 5-methyl-(E)-2-hepten-4-one (Filbertone). It goes without saying that the synthesis of the proposed aroma substance was part of the identification. It was also guaranteed that its chromatographic and sensory properties correspond with those of the unknown odorant.

5.2.5 Enantioselective Analysis

In the case of chiral aroma substances, elucidation of the absolute configuration and determination of the enantiomeric ratio, which is usually given as the enantiomeric excess (ee), are of especial interest because the enantiomers of a compound can differ considerably in their odor quality and threshold. The compound 3a,4,5,7a-

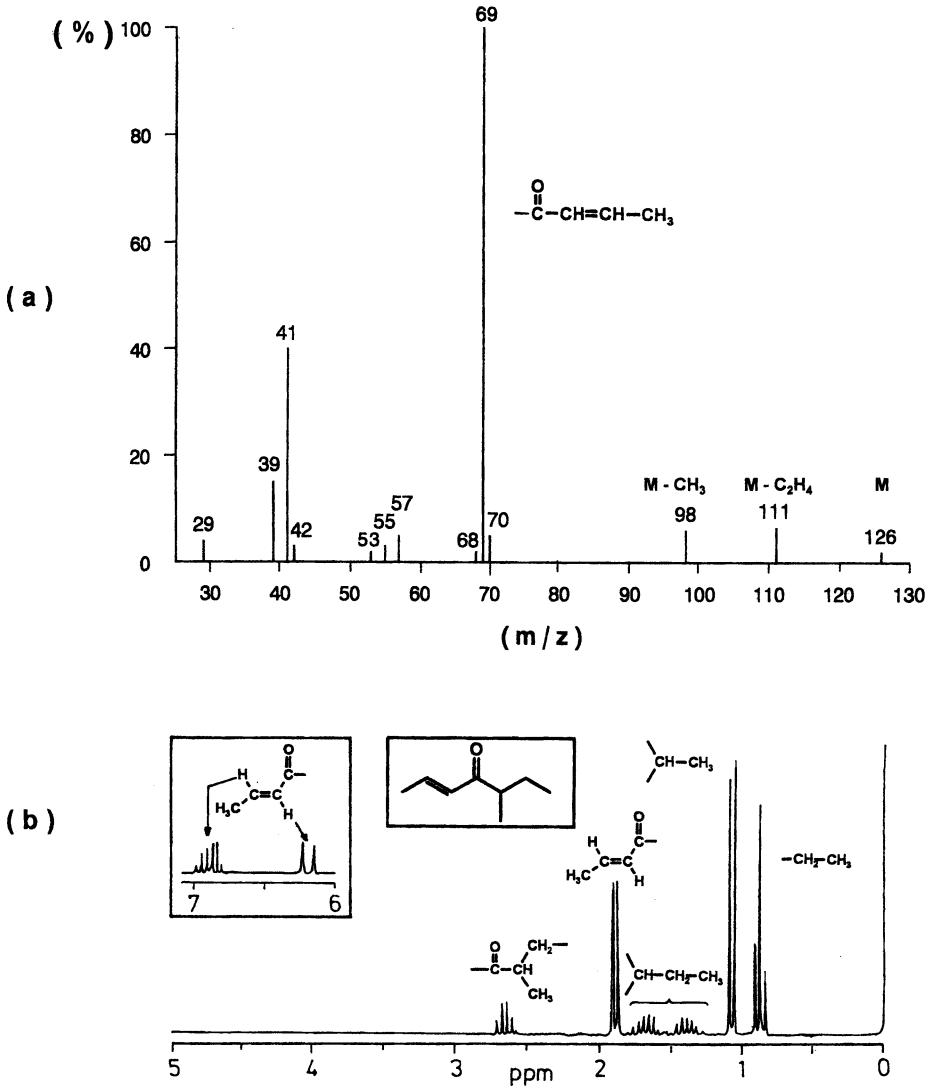


Fig. 5.11. Instrumental analysis of 5-methyl-(E)-2-hepten-4-one (according to *Emberger*, 1985) **(a)** mass spectrum, **(b)** ^{1}H-NMR spectrum (for discussion, see text)

tetrahydro-3,6-dimethyl-2(3H)-benzofuranone (wine lactone) represents an impressive example which shows how much the odor activity of enantiomers can vary. The four enantiomeric pairs of this compound have been separated by gas chromatography on a chiral phase (Fig. 5.12). The 3S,3aS,7aR-enantiomer (No. 6 in Table 5.12) has the lowest odor threshold of the eight diastereomers. The identification of this substance in wine (cf. 20.2.6.9) led to the name wine lactone. Two diastereomers (No. 3 and 8) are odorless.

The determination of the ee value can be used to detect aromatization with a synthetic chiral aroma substance because in many cases one enantiomer is preferentially formed in the biosynthesis of chiral aroma substances (examples in Table 5.13). In contrast to biosynthesis, chemical synthesis gives the racemate which is usually not separated for economic reasons. The addition of an aroma sub-

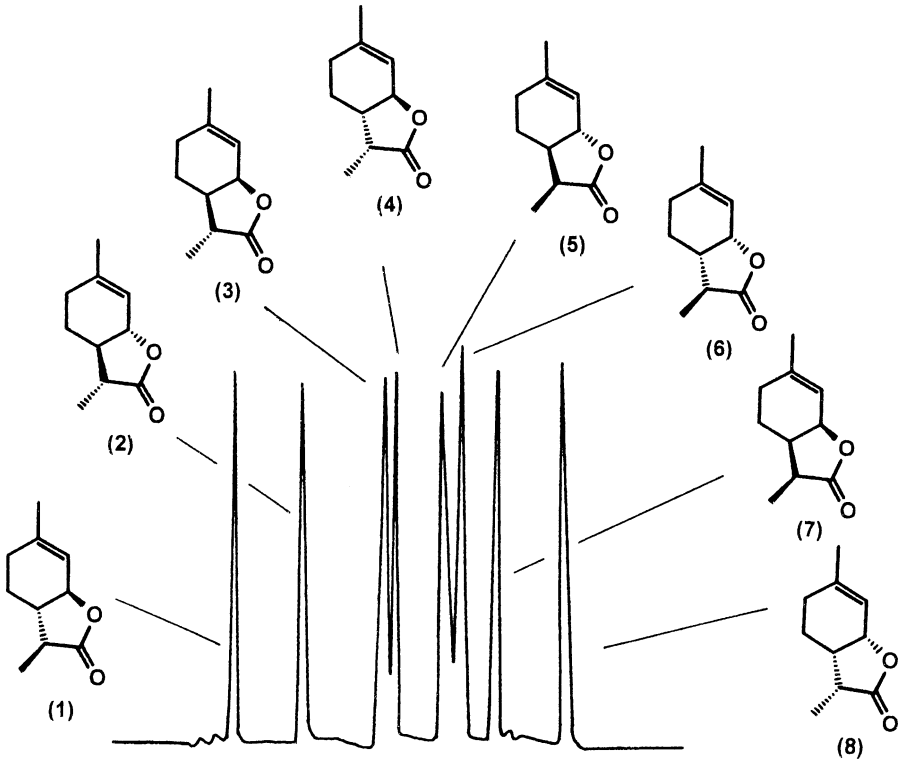


Fig. 5.12. Gas chromatogram of the diastereomers of 3a,4,5,7a-tetrahydro-3,6-dimethyl-2(3H)-benzofuranone (wine lactone) on a chiral phase (according to *Guth*, 1997)

Table 5.12. Odor threshold values of diastereomeric 3a,4,5,7a-tetrahydro-3,6-dimethyl-2(3H)-benzofuranone

No.[a]	Stereoisomer-conformation	Odor threshold (ng/l air)
1	(3S,3aS,7aS)	0.007–0.014
2	(3R,3aR,7aR)	14–28
3	(3R,3aR,7aS)	>1000
4	(3R,3aS,7aS)	8–16
5	(3S,3aR,7aR)	0.05–0.2
6	(3S,3aS,7aR)	0.00001–0.00004
7	(3S,3aR,7aS)	80–160
8	(3R,3aS,7aR)	>1000

[a] Numbering as in Fig. 5.12.

stance of this type can be determined by enantioselective analysis if safe data on the enantiomeric excess of the compound in the particular food are available. It should also be taken into account that the ee value can change during food processing, e. g., that of filbertone decreases during the roasting of hazelnuts (cf. Table 5.13).

Table 5.13. Enantiomeric excess (ee) of chiral aroma substances in some foods

Aroma substance	Food	ee (%)
R(+)-γ-Decalactone	Peach, apricot, mango, strawberry pineapple, maracuya	>80
R(+)-δ-Decalactone	Milk fat	60
R(+)-trans-α-Ionone	Raspberry	92.4
	Carrot	90.0
	Vanilla bean	94.2
R(-)-1-Octen-3-ol	Mushroom, chanterelle	>90
S(+)-E-5-Methy1-2-hepten-4-one (filbertone)	Hazelnut, raw	60–68
	Hazelnut, roasted	40–45
R-3-Hydroxy-4,5-dimethyl-2(5H)-furanone (sotolon)	Sherry	ca. 30

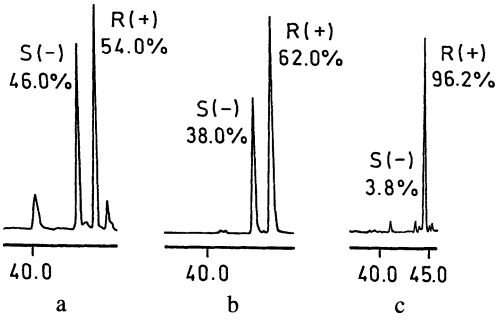


Fig. 5.13. Enantioselective gas chromatographic analysis of trans-α-ionone in aroma extracts of different raspberry fruit juice concentrates (according to *Werkhoff* et al., 1990): **a** and **b** samples with nature identical aroma, **c** natural aroma

The method frequently applied to determine ee values is the enantioselective gas chromatographic analysis of the aroma substance on a chiral phase, e. g., peralkylated cyclodextrins. This method was used, e. g., to test raspberry fruit juice concentrates for unauthorized aromatization with trans-α-ionone. The gas chromatograms of trans-α-ionone from two different samples are shown in Fig. 5.13. The low excesses of the R-enantiomer of ee = 8% (concentrate A) and ee = 24% (B) can probably be put down to the addition of synthetic trans-α-ionone racemate to the fruit juice concentrate because in the natural aroma (C), the ee value is 92.4%.

5.2.6 Quantitative Analysis, Aroma Values

5.2.6.1 Isotopic Dilution Analysis (IDA)*

The quantitative analysis of aroma substances using conventional methods often gives incorrect values. The high vapor pressure, the poor extractability especially of polar aroma substances from hydrous foods and the instability of important aroma substances, e. g., thiols, can cause unforeseeable losses in the purification of the samples and in gas chromatography.

The results of quantitative analyses are exact (standard deviation $<10\%$) and reproducible if the chemical structure of the internal standard is very similar to the structure of the analyte. An isotopomer of the analyte is the most similar. In this case, the physical and chemical properties of both substances correspond, except for a small isotope effect which can lead to partial separation in capillary gas chromatography.

The examples given in Fig. 5.14 show that for economic reasons, mostly internal standards labelled with deuterium are synthesized for IDA. The considerably more expensive carbon isotope 13 is introduced into the odorant (examples are the internal standards No. 11 and 12 in Fig. 5.14) only if a deuterium/protium exchange can occur in the course of analysis. This exchange would falsify the result. Another advantage of this isotope is the completely negligible isotope effect compared to deuterium.

It is easy to conduct an IDA because losses of analyte in the distillative recovery (cf. 5.2.1.1) and in purification do not influence the result since the standard suffers the same losses. These advantages of IDA are used in food chemistry for other analytes as well, e. g., pantothenic acid (cf. 6.3.5.2) or for the mycotoxin patulin (cf. 9.2.3).

The quantification of the odorants 2-furfurylthiol (FFT), 2-methyl-3-furanthiol (MFT) and 3-mercapto-2-pentanone (3M2P) in boiled meat will be considered as an example. Especially MFT and 3M2P are very instable, so after the addition of the deuterated standards d-FFT, d-MFT and d-3M2P (No. 13 in Fig. 5.14) to the extract, it is advisable to concentrate via a trapping reaction for thiols which is performed with p-hydroxymercuribenzoic acid. The analytes and their standards are displaced from the derivatives by cysteine in excess, separated by gas chromatography, and analyzed by mass spectrometry. In this process, mass chromatograms for the ions are monitored in which the analyte and its isotopomer differ (Fig. 5.15). After calibration, the mass chromatograms are evaluated via a comparison of the areas of analyte and standard. 2-Mercapto-3-pentanone (2M3P) is also identified in this analysis. However, this compound is of no importance for the aroma of boiled meat because of its lower concentration and higher odor threshold compared to those of 3M2P.

5.2.6.2 Aroma Values (AV)

To approach the situation in food aroma values (definition cf. 5.1.4) are calculated. It is assumed

* Most of the quantitative data on aroma substances in this book come from IDAs.

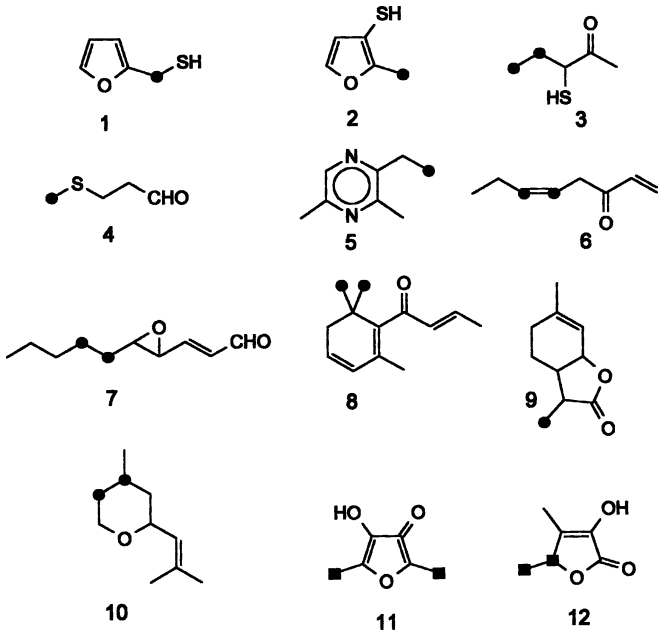


Fig. 5.14. Odorants labelled with deuterium (●) or carbon-13 (■) as internal standard substances for isotopic dilution analyses of the corresponding unlabelled odorants.
1 2-[α-$^{2}H_2$]furfurylthiol, *2* 2-[$^{2}H_3$]methyl-3-furanthiol, *3* 3-mercapto-2-[4,5-$^{2}H_2$]pentanone, *4* [4-$^{2}H_3$]methional, *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 (β-damascenone), *9* 3a,4,5,7atetrahydro-3,6-[3-$^{2}H_3$]dimethyl-2(3H)-benzofuranone (wine lactone), *10* tetrahydro-4-methyl-2-(2-methylpropenyl)-2H-[3,4-$^{2}H_3$]pyran (sotolon), *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 (rose oxide)

that the odorants showing higher AVs contribute strongly to the aroma of the food. For this purpose, the odor thresholds of the compounds dissolved in water, in oil or applied to starch are used, depending on which of these materials dominates in the food.
An example are the AVs of the odorants of French fries based on their odor thresholds in an oil (Table 5.14). Methanethiol, methional, methylpropanal and 2-methylbutanal exhibit the highest aroma values. Consequently, they should belong to the most important odorants of French fries.

5.2.7 Aroma Model, Omission Experiments

Finally, the identified odorants must actually produce the aroma in question. To test this, the determined concentrations of the odorants are dissolved in a suitable medium, which is not difficult in the case of liquid foods. The solvent for the recombination mixture called the aroma model can be adapted to the food. An ethanol/water mixture, for example, is suitable for wine. In the case of solid foods, however, compromises have to be accepted.
The aroma profile of the model is then compared to that of the food. In the example of French fries discussed in detail here, a very good approximation of the original aroma was achieved.
The selection of odorants by dilution analyses (cf. 5.2.2) does not take into account additive (cf. 20.1.7.8) or antagonistic effects (example in Fig. 5.2) because the aroma substances, after separation by gas chromatography, are sniffed individually. Therefore, in view of the last mentioned effect, the question arises whether all the compounds occurring in the aroma model really contribute to the aroma in question. To answer

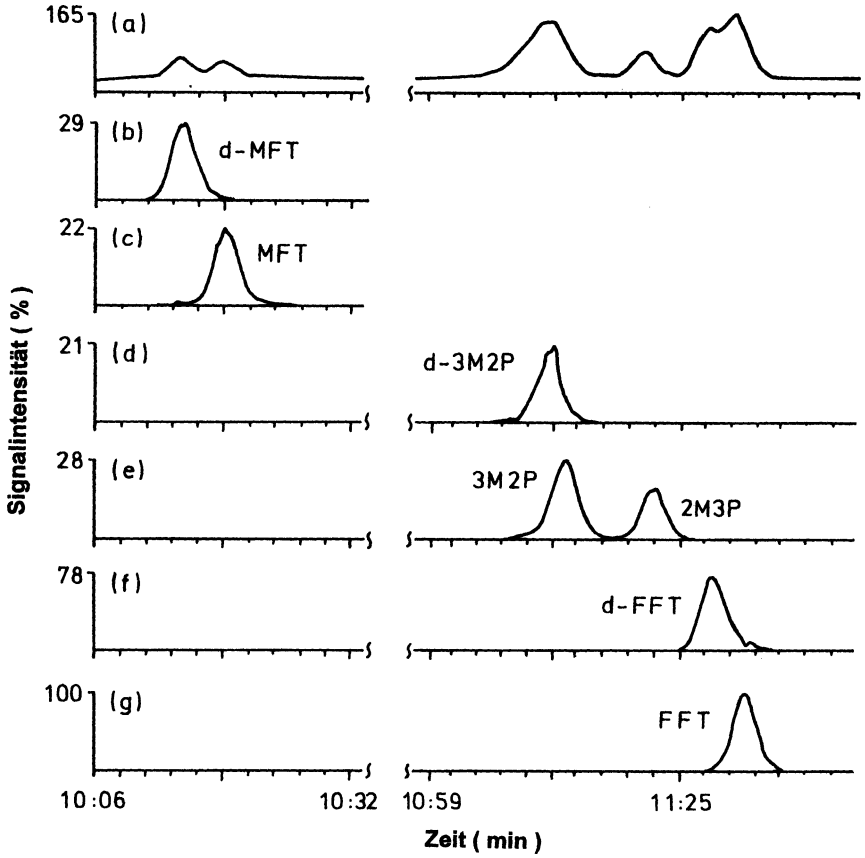


Fig. 5.15. Isotopic dilution analysis of 2-furfurylthiol (FFT), 2-methy1-3-furanthiol (MFT) and 3-mercapto-2-pentanone (3M2P).
(a) Gas chromatogram, (**b–g**) mass chromatograms of the analytes and the deuterated (**d**) internal standards; traces of the ions shown in brackets were monitored: d-MFT (m/z 118), MFT (m/z 115), d-3M2P (m/z 121), 3M2P and 2M3P (m/z 119), d-FFT (m/z 83), FFT (m/Z 81) (according to *Kerscher* and *Grosch*, 1998)

this question, one or several aroma substances are omitted in the model and a triangle test is used to examine which of three samples (two complete and one reduced aroma model) offered to the testers in random order differs in aroma from the others. If a significant number of testers determine a difference in the reduced model, it can be assumed that the odorants lacking in the reduced model contribute to the aroma and, consequently, belong to the key odorants of the food.

Some omission experiments, e. g., conducted with the aroma model for French fries, are shown in Table 5.15.

If methanethiol and the two decadienal isomers are missing in Experiments 1 and 2, the aroma has no similarity to that of French fries. All five testers were in agreement. The *Strecker* aldehydes with the malt odor (Exp. 3), 4,5-epoxydecenal (Exp. 4) and both pyrazines (Exp. 5) are also important for the aroma because their absence was noticed by four of the five testers. 1-Octen-3-one, (Z)-2- and (E)-2-nonenal are of no importance for the aroma (Exp. 6). Surprisingly, this also applies to methional (Exp. 7) although it has the second highest aroma value (cf. Table 5.14) and smells of boiled potatoes. It is obvious that methional is masked by other odorants occurring in the aroma model. In French fries, the odor note “like boiled potatoes” is probably produced by methanethiol in combination with pyrazines.

Table 5.14. Volatile compounds with high aroma values in French fries[a]

Compound	Concentration[b] (μg/kg)	Odor threshold[c] (μg/kg)	Aroma value[d]
Methanethiol	1240	0.06	2×10^4
Methional	783	0.2	3.9×10^3
Methylpropanal	5912	3.4	1.7×10^3
2-Methylbutanal	10599	10	1.1×10^3
trans-4,5-Epoxy-(E)-2-decenal	771	1.3	592
3-Methylbutanal	2716	5.4	503
(E,Z)-2,4-Decadienal	1533	4	383
4-Hydroxy-2,5-dimethyl-3 (2H)-furanone	2778	25	111
2,3-Diethyl-5-methylpyrazine	41	0.5	83
(E,E)-2,4-Decadienal	6340	180	35
2,3-Butanedione	306	10	31
2-Ethyl-3,5-dimethylpyrazine	42	2.2	19
2-Ethenyl-3-ethyl-5-methylpyrazine	5.4	0.5	11
3-Isobutyl-2-methoxypyrazine	8.6	0.8	11
2-Ethyl-3,6-dimethylpyrazine	592	57	10

[a] Potato sticks deep-fried in palm oil.
[b] Results of IDA.
[c] Odor threshold of the compound dissolved in sunflower oil.
[d] Quotient of concentration and odor threshold.

Table 5.15. Aroma model for French fries as affected by the absence of one or more odorants[a]

Exp. No.	Odorant omitted in the model	Number[b]
1	Methanethiol	5
2	(E,Z)-2,4-Decadienal and (E,E)-2,4-decadienal	5
3	Methylpropanal, 2- and 3-methylbutanal	4
4	trans-4,5-Epoxy-(E)-2-decenal	4
5	2-Ethyl-3,5-dimethylpyrazine and 3-ethyl-2,5-dimethylpyrazine	4
6	1-Octen-3-one, (Z)-2- and (E)-2-nonenal	1
7	Methional	0

[a] Models lacking in one or more components were each compared to the model containing the complete set of 19 odorants.
[b] Number of the assessors detecting an odor difference in triangle tests, maximum 5.

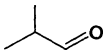
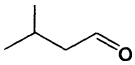
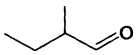
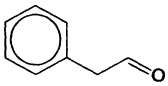
The instrumental and sensory methods presented in the French fries example have also been successfully applied in the elucidation of other aromas. The results are presented in the book for some individual foods.

5.3 Individual Aroma Compounds

The results of dilution analyses and of aroma simulation experiments show that only 5% of the more than 7000 volatile compounds identified in foods contribute to aromas. The main reason for the low number of odorants in the volatile fraction is the marked specificity of the sense of smell (for examples, cf. 5.6).

Important odorants grouped according to their formation by nonenzymatic or enzymatic reactions and listed according to classes of compounds are presented in the following sections. Some aroma compounds formed by both enzymatic and nonenzymatic reactions are covered in sections 5.3.1 and 5.3.2. It should be noted that the reaction pathways for each aroma compound are differentially established. Frequently, they are dealt with by using hypothetical reaction pathways which lead from the precursor to the odorant. The reaction steps and the intermediates of the pathway are postulated

Table 5.16. Some *Strecker* degradation aldehydes[a]

Amino acid precursor	*Strecker*-aldehyde			Odor threshold value
	Name	Structure	Aroma description	(μg/l; water)
Gly	Formaldehyde	CH_2O	Mouse-urine, ester-like	50×10^3
Ala	Ethanal		Sharp, penetrating, fruity	10
Val	Methylpropanal		Malty	1
Leu	3-Methylbutanal		Malty	0.2
Ile	2-Methylbutanal		Malty	4
Phe	2-Phenylethanal		Flowery, honey-like	4

[a] Methional will be described in 5.3.1.4.

by using the general knowledge of organic chemistry or biochemistry. For an increasing number of odorants, the proposed formation pathway can be based on the results of model experiments. Postulated intermediates have also been confirmed by identification in a numbers of cases. However, studies on the formation of odorants are especially difficult since they involve, in most cases, elucidation of the side pathways occurring in chemical or biochemical reactions, which quantitatively are often not much more than negligible.

5.3.1 Nonenzymatic Reactions

The question of which odorants are formed in which amounts when food is heated depends on the usual parameters of a chemical reaction. These are the chemical structure and concentration of the precursors, temperature, time and environment, e. g., pH value, entry of oxygen and the water content. Whether the amounts formed are really sufficient for the volatiles to assert themselves in the aroma depend on their odor thresholds and on interactions with other odorants.

Aroma changes at room temperature caused by nonenzymatic reactions are observed only after prolonged storage of food. Lipid peroxidation (cf. 3.7.2.1), the *Maillard* reaction and the related *Strecker* degradation of amino acids (cf. 4.2.4.4.7) all play a part. These processes are greatly accelerated during heat treatment of food. The diversity of aroma is enriched at the higher temperatures used during roasting or frying. The food surface dries out and pyrolysis of carbohydrates, proteins, lipids and other constituents, e. g., phenolic acids, takes place generating odorants, among other compounds.

The large number of volatile compounds formed by the degradation of only one or two constituents is characteristic of nonenzymatic reactions. For example, 41 sulfur-containing compounds, including 20 thiazoles, 11 thiophenes, 2 dithiolanes and 1 dimethyltrithiolane, are obtained by heating cysteine and xylose in tributyrin at 200 °C. Nevertheless, it should not be overlooked that even under these drastic conditions, most of the volatile compounds are only formed in concentrations which are far less than the often relatively high odor thresholds (cf. 5.6). For this reason, only a small fraction of the many volatile compounds formed in heated foods is aroma active.

5.3.1.1 Carbonyl Compounds

The most important reactions which provide volatile carbonyl compounds were presented in sections 3.7.2.1.9 (lipid peroxidation), 4.2.4.3.3 (caramelization) and 4.2.4.4.7 (amino acid decomposition by the *Strecker* degradation mechanism).
Some *Strecker* aldehydes found in many foods are listed in Table 5.16 together with the corresponding aroma quality data. Data for carbonyls derived from fatty acid degradation are found in Table 3.32. Carbonyls are also obtained by degradation of carotenoids (cf. 3.8.4.4).

5.3.1.2 Pyranones

Maltol (3-hydroxy-2-methyl-4H-pyran-4-one) is obtained from carbohydrates as outlined in 4.2.4.4.4 and has a caramel-like odor. It has been found in a series of foods (Table 5.17), but in concentrations that were mostly in the range of the relatively high odor threshold of 9 mg/kg (water).
Maltol enhances the sweet taste of food, especially sweetness produced by sugars (cf. 8.6.3), and is able to mask the bitter flavor of hops and cola.
Ethyl maltol [3-hydroxy-2-ethyl-4H-pyran-4-one] enhances the same aroma but is 4- to 6-times more powerful than maltol. It has not been detected as a natural constituent in food. Nevertheless, it is used for food aromatization.

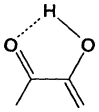
5.3.1.3 Furanones

Among the great number of products obtained from carbohydrate degradation, 3(2H)- and 2(5H)-furanones belong to the most striking aroma compounds (Table 5.18).

Table 5.17. Occurrence of maltol in food

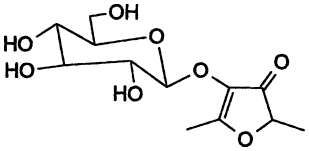
Food product	mg/kg	Food product	mg/kg
Coffee, roasted	20–45	Chocolate	3.3
Butter, heated	5–15	Beer	0–3.4
Biscuit	19.7		

Compounds I–III, V and VI in Table 5.18, as well as maltol and the cyclopentenolones (cf. 4.2.4.3.2), have a planar enol-oxo-configuration



(5.4)

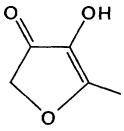
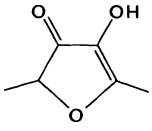
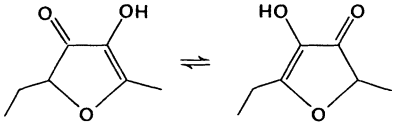
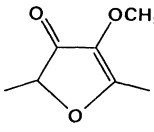
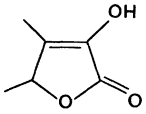
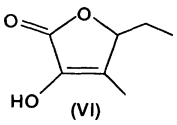
and a caramel-like odor, the odor threshold of aqueous solutions being influenced by the pH.
In Table 5.19, the examples furanone I and II show that the threshold value decreases with decreasing pH. As with the fatty acids (cf. 3.2.1.1), the vapor pressure and, consequently, the concentration in the gas phase increase with decreasing dissociation. The fact that furanone I does not appreciably contribute to food aromas is due to its high odor threshold. However, this compound is of interest as a precursor of 2-furfurylthiol (cf. 5.3.1.4). If the hydroxy group in furanone II is methylated to form IV, the caramel-like aroma note disappears.
A list of foods in which furanone II has been identified as an important aroma substance is given in Table 5.20.
As the furanones are secondary products of the *Maillard* reaction, their formation is covered in 4.2.4.3.2, 4.2.4.4.4 and 4.2.4.4.6. Whether the furanone II detected in fruit, which is partly present as the β-glycoside (e. g., in tomatoes, cf. Formula 5.5), is formed exclusively



(5.5)

by nonenzymatic reactions favored by the low pH is still not clear. Furanone V (sotolon) is a significant contributor to the aroma of, e. g., sherry, French white wine, coffee (drink) and above all of seasonings made on the basis of a protein hydrolysate (cf. 12.7.3.5). It is a chiral compound having enantiomers that differ in their odor threshold (Table 5.18) but not in their odor quality. It is formed in the *Maillard* reaction (cf. 4.2.4.4), but can also be produced from 4-hydroxyisoleucine (e. g., in fenugreek seeds, cf. 22.1.1.2.4). Furanone VI (abhexon) has

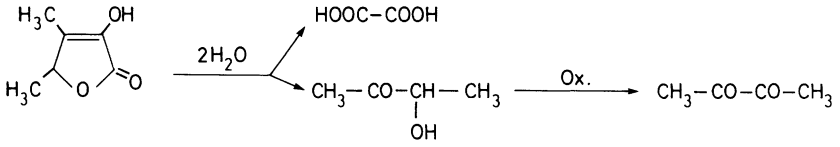
Table 5.18. Furanones in food

Structure	Substituent/trivial name or trade name (odor threshold in µg/kg, water)	Aroma quality	Occurrence
	A. *3(2H)-Furanones*		
 (I)	4-Hydroxy-5-methyl *Norfuraneol* (nasal: 23,000)	Roasted chicory-like, caramel	Meat broth
 (II)	4-Hydroxy-2,5-dimethyl *Furaneol* (nasal: 60; retronasal: 25)	Heat-treated strawberry, pineapple-like, caramel	cf. Table 5.20
 (III)	2-(5)-Ethyl-4-hydroxy-5-(2)-methyl[a] *Ethylfuraneol* (nasal: 7.5)	Sweet, pastry, caramel	Soya sauce Emmental cheese
 (IV)	4-Methoxy-2,5-dimethyl *Mesifuran* (nasal: 3400)	Sherry-like	Strawberry, raspberry[b]
	B. *2(5H)-Furanones*		
 (V)	3-Hydroxyl-4,5-dimethyl *Sotolon* (nasal, R-form 90, recemate, retronasal: 3)	Caramel, protein hydrolysate S-form 7	Coffee, sherry, seasonings, fenugreek seeds
 (VI)	5-Ethyl-3-hydroxy-*Abhexon* hydrolysate (nasal: 30, retronsal: 3)	Caramel, 4-methyl protein	Coffee, seasonings

[a] Of the two tautomeric forms, only the 5-ethyl-4-hydroxy-2-methyl isomer is aroma active.
[b] Arctic bramble (*Rubus arcticus*).

an aroma quality similar to that of sotolon and is formed by aldol condensation of 2,3-pentanedione and glycol aldehyde, which can be obtained from the *Maillard* reaction, or by aldol condensation of 2 molecules of α-oxobutyric acid, a degradation product of threonine (Fig. 5.16).

Quantitative analysis of furanones is not very easy because due to their good solubility in water, they are extracted from aqueous foods with poor yields and easily decompose, e. g., sotolon (cf. Formula 5.6). Correct values are obtained by IDA.



(5.6)

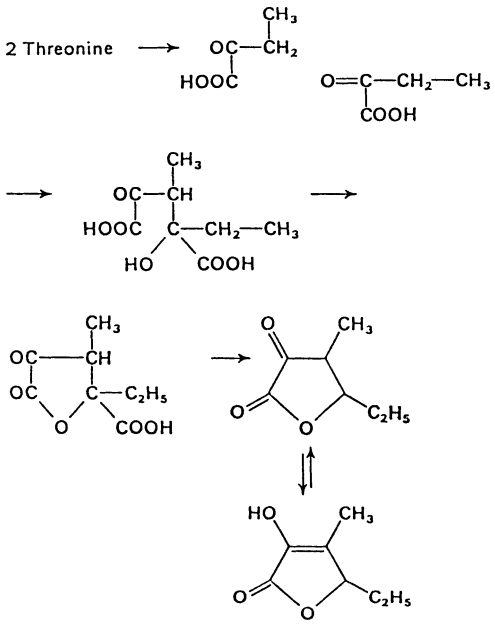


Fig. 5.16. Formation of 5-ethyl-3-hydroxy-4-methyl-2(5H)-furanone from threonine by heating

Table 5.19. Odor thresholds of 4-hydroxy-5-methyl- (I) and 4-hydroxy-2,5-dimethyl-3(2H)-furanone (II) as a function of the pH value of the aqueous solution

pH	Threshold (μg/l)	
	I	II
7.0	23,000	60
4.5	2100	31
3.0	2500	21

Table 5.20. Occurrence of 4-hydroxy-2,5-dimethyl-3(2H)-furanone

Food	mg/kg
Beer, light	0.35
Beer, dark	1.3
White bread, crust	1.96
Coffee drink[a]	1.5–7
Emmental cheese	1.2
Beef, boiled	9
Strawberry	1–30
Pineapple	1.6–35

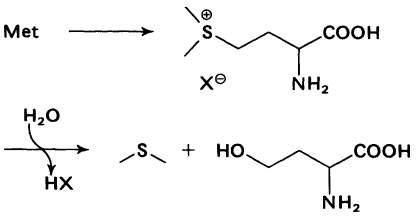
[a] Coffee, medium roasted, 54 g/l water.

5.3.1.4 Thiols, Thioethers, Di- and Trisulfides

An abundance of sulfurous compounds is obtained from cysteine, cystine, monosaccharides, thiamine and methionine by heating food. Some are very powerful aroma compounds (Table 5.21) and are involved in the generation of some delightful but also some irritating, unpleasant odor notes.

Thiols are important constituents of food aroma because of their intensive odor and their occurrence as intermediary products which can react with other volatiles by addition to carbonyl groups or to double bonds.

Hydrogen sulfide and 2-mercaptoacetaldehyde are obtained during the course of the *Strecker* degradation of cysteine (Fig. 5.17). In a similar way, methionine gives rise to methional, which releases methanethiol by β-elimination (Fig. 5.18). Dimethylsulfide is obtained by methylation during heating of methionine in the presence of pectin:



(5.7)

Methanethiol oxidizes easily to dimethyldisulfide, which can disproportionate to dimethylsulfide and dimethyltrisulfide (Formula 5.8).

Table 5.21. Sensory properties of volatile sulfur compounds

Compound	Odor	
	Quality	Threshold (μg/l)[a]
Hydrogen sulfide	Sulfurous, putrid	10
Methanethiol	Sulfurous, putrid	0.02
Dimethylsulfide	Asparagus, cooked	1.0
Dimethyldisulfide	Cabbage-like	7.6
Dimethyltrisulfide	Cabbage-like	0.01
Methional	Potatoes, boiled	0.2
Methionol	Sulfurous	5.0
3-Methyl-2-butenethiol	Animal	0.0003
3-Mercapto-2-butanone	Sulfurous	3.0
3-Mercapto-2-pentanone	Sulfurous	0.7
2-Mercapto-3-pentanone	Sulfurous	2.8
2-Furfurylthiol	Roasted, like coffee	0.012
2-Methyl-3-furanthiol	Meat, boiled	0.007
Bis(2-methyl-3-furyl)disulfide	Meat-like	0.00002
3-Mercapto-2-methylpentan-1-ol	Meat-like, like onions	0.0016

[a] In water.

Due to its very low odor threshold (Table 5.21), the trisulfide is very aroma active and is frequently found in dilution analyses as a companion substance of methanethiol. For the moment, it is unknown whether it is derived from food or whether it is an artifact obtained in the isolation and concentration of volatile compounds.

Except for the exceptionally reactive 2-mercaptoethanal, the sulfur compounds mentioned above have been identified in practically all protein-containing foods when they are heated or stored for a prolonged period of time.

The addition of H_2S to α-diketones, which are produced in the *Maillard* reaction (cf. 4.2.4.3.2 and 4.2.4.4), the elimination of water and a reaction called reductive sulfhydrylation result in mercaptoalkanes (Formula 5.9). Here, two position isomers 2-mercapto-3-pentanone (2M3P) and 3-mercapto-2-pentanone (3M2P) are produced from 2,3-pentanedione, 3M2P being an important contributor to the aroma of meat (cf. 12.9.2). Model experiments with various monosaccharides (cf. 12.9.3) show that ribose yields more 2M3P and 3M2P than glucose, the optimal pH being 5.0. The optimum probably results from the fact that while the liberation of H_2S from cysteine is favored at low pH, the fragmentation of the monosaccharides to α-diketones is favored at higher pH values.

2-Furfurylthiol (FFT) is the key odorant of roasted coffee (cf. 21.1.3.3.7). It also plays a role in meat aromas and in the aroma of rye bread crust (cf. 12.9.2 and 15.4.3.3.3). It appears on toasting when white bread is baked with a higher amount of yeast. The precursor of FFT is furfural which, according to the hypothesis, adds hydrogen sulfide to give a thiohemiacetal (Formula 5.10). Water elimination and reductive sulfhydrylation then yield FFT. On the other hand, FFT can also be formed from furfuryl alcohol after the elimination of water and addition of hydrogen sulfide. Furfuryl alcohol is one of the volatile main products of the *Maillard* reaction. Roasted coffee contains FFT and other volatile thiols not only in the free state, but also bound via disulfide bridges to cysteine, SH-peptides and proteins. The thiols can be released by reduction, e. g., with dithioerythritol.

An isomer of FFT, 2-methyl-3-furanthiol (MFT), has a similarly low odor threshold (Table 5.21), but differs in the odor quality. MFT smells like boiled meat, being one of its key odorants (cf. 12.9.2). The SH-group of MFT is considerably more instable than that of FFT because in an H-abstraction, a thiyl radical can be generated which is stabilized by resonance with the aromatic ring (Formula 5.11). The thiyl radicals dimerize to bis(2-methyl-3-furyl)

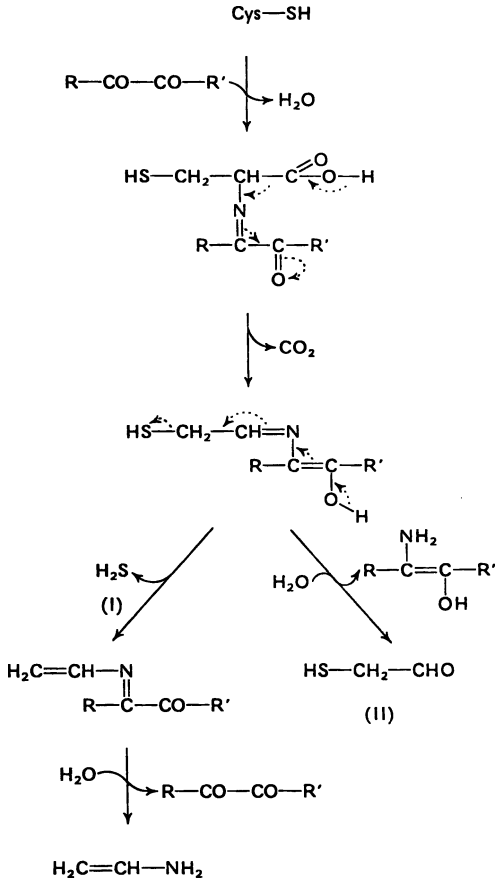


Fig. 5.17. Cysteine decomposition by a *Strecker* degradation mechanism: formation of H_2S (I) or 2-mercaptoethanal (II)

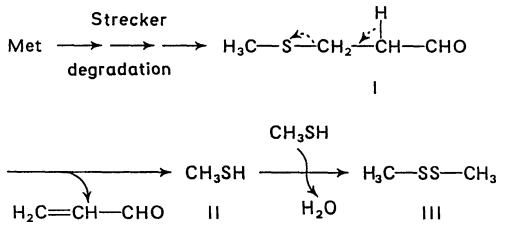
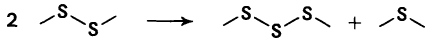


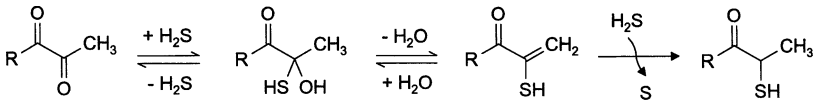
Fig. 5.18. Methionine degradation to methional, methanethiol and dimethylsulfide

disulfide, which is cleaved again at a higher temperature (Formula 5.11), e. g., during cooking. If constituents which have H-atoms abstractable by thiyl radicals, e. g., reductones, are present in food, MFT is regenerated. This is desirable because although the disulfide of MFT has a very low odor threshold (Table 5.21), its meat-like odor has a medical by-note and, unlike MFT, its *Stevens* curve is much flatter (cf. 5.1.4), i. e., the odor is not very intensive even in a higher concentration range.

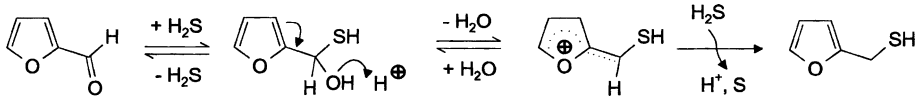
Norfuraneol (I in Table 5.18) is under discussion as the precursor of MFT. As proposed in Formula 5.12, the addition of hydrogen sulfide leads to 4-mercapto-5-methyl-3(2H)-furanone, which yields MFT after reduction, e. g., by reductones from the *Maillard* reaction, and water elimination. MFT can also be formed in meat by the hydrolysis of thiamine (Fig. 5.19). The postulated intermediate is the very reactive 5-hydroxy-3-mercaptopentan-2-one.



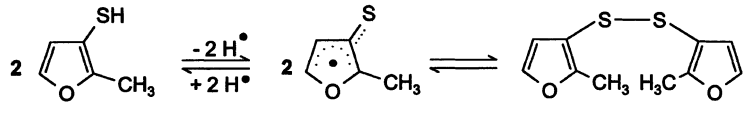
(5.8)



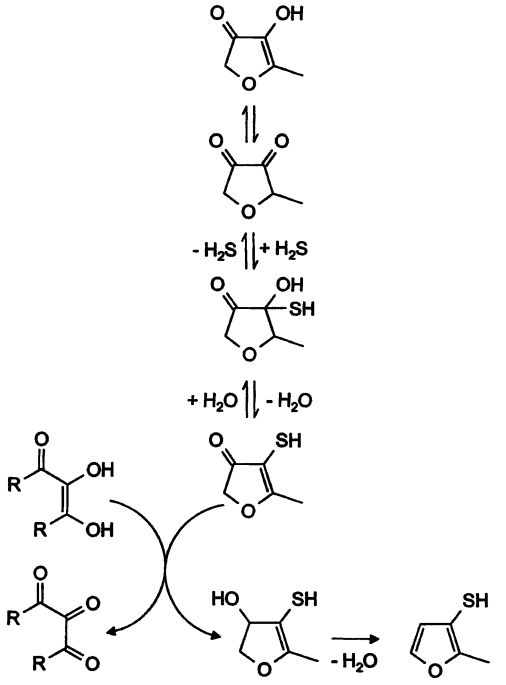
(5.9)



(5.10)



(5.11)



(5.12)

Some reaction systems, which have been described in the patent literature for the production of meat aromas, regard thiamine as precursor.

3-Methyl-2-butene-1-thiol is one of the roast odorants of coffee (cf. 21.1.3.3.7) and can cause on off-flavor in beer (cf. Table 5.5). In general, only very small amounts are formed which are still aroma active on account of the very low odor threshold (Table 5.21). The formation of the thiol is explained by the fact that the 3-methyl-2-butene radical is formed from terpenes by photolysis (beer) or under the drastic conditions of the roasting process (coffee). This radical then meets a $SH^{\bullet}$-radical formed from cysteine under these conditions. In the case of beer, humulons (cf. 20.1.2.3.2) are under discussion as the source of the alkyl radical. In coffee 3-methyl-2-butene-1-ol (prenyl alcohol) is also a possible precursor, which yields the thiol after water elimination and hydrogen sulfide addition.

It is unclear whether sulfides I–III in Fig. 5.20 and trithioacetone, analogous to trithioacetaldehyde (I), are really formed during the cooking of meat or whether these compounds are artifacts that are produced on concentration of the volatile fraction in the course of analysis (cf. 5.2.1).

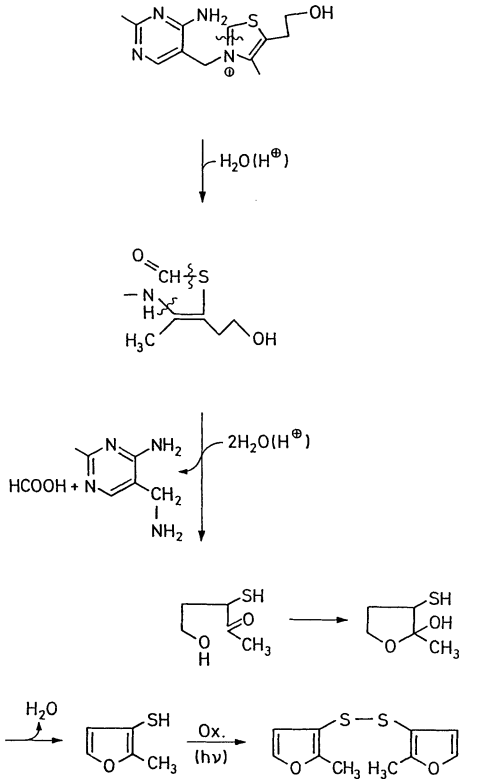


Fig. 5.19. Formation of 2-methyl-3-furanthiol and bis(2-methyl-3-furyl)disulfide from thiamine

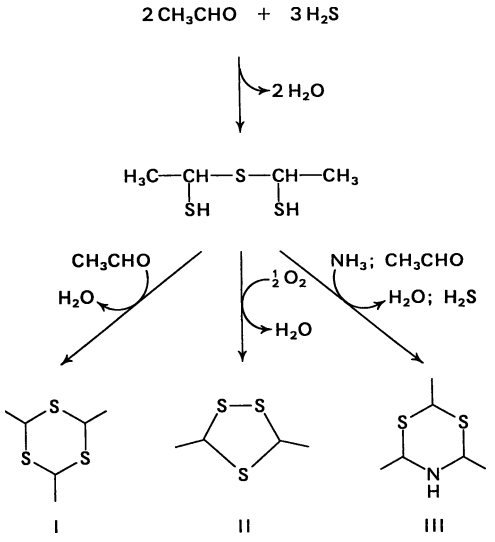


Fig. 5.20. Formation of 2,4,6-trimethyl-s-trithiane (I), 3,5-dimethyl-1,2,4-trithiolane (II) and 2,4,6-trimethyl-5,6-dihydro-1,3,5-dithiazine (III)

5.3.1.5 Thiazoles

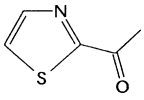
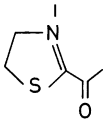
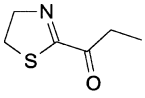
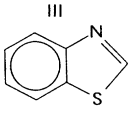
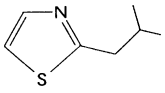
Thiazole and its derivatives are detected in foods such as coffee, boiled meat, boiled potatoes, heated milk and beer. Aroma extract dilution analyses show that among the compounds I–III in Table 5.22, 2-acetyl-2-thiazoline (II) contributes most intensively to the aroma of quick fried beef. Model experiments showed that cysteamine, formed by the decarboxylation of cysteine, and 2-oxopropanal are the precursors. It was also found that higher yields of II are obtained at pH 7.0 compared to pH 5.0. The intermediates in the reaction path to thiazoline II (Fig. 5.21) were identified as the odorless 2-(1-hydroxyethyl)-4,5-dihydrothiazole (*a*) and 2-acetylthiazolidine (*b*), which are in tautomeric equilibrium, presumably with 2-(1-hydroxyethylene)thiazolidine (*c*) as the intermediate compound (Fig. 5.21). The intermediates *a* and *b* are oxidized to thiazoline II by atmospheric oxygen in the presence of catalytic amounts of heavy metals. It is assumed that the metal ion, e. g., Cu^{2+}, oxidizes the eneaminol *c* to a resonance-stabilized radical *d* in a one-electron reaction (Fig. 5.22). This radical then traps an oxygen molecule with the formation of a peroxy radical (*e*). H-Abstraction from the eneaminol *c* results in the conversion of *e* to 2-acetyl-2-thiazolinehydroperoxide (*f*), which decomposes to thiazoline I and H_2O_2. H_2O_2 can oxidize the metal ion and regenerate it for a new cycle.

In the conversion of the precursor *b*, only the limitation of the reaction time to 10 minutes in the temperature range 50–100 °C results in the highest yield of thiazolidine II (Fig. 5.23).

This is in accord with the aroma formation during the frying of beef. The concentration of II in meat, decreases again if heating continues.

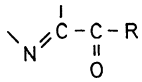
Thiazole IV (Table 5.22) can occur in milk when it is heated, and is responsible for a "stale" off-flavor. Thiazole V (Table 5.22) is a constituent of tomato aroma. The aroma of tomato products is usually enhanced by the addition of 20–50 ppb of thiazole V (for the biosynthesis of the compound, see Section 5.3.2.5).

Table 5.22. Thiazoles and thiazolines in food

Name	Structure	Aroma quality	Odor threshold (μg/kg, H_2O)
2-Acetyl-thiazole		Cereal, popcorn	10
2-Acetyl-2-thiazoline		Popcorn	1
2-Propionyl-2-thiazoline		Popcorn	1
Benzo-thiazole		Quino-line, rubber	
2-Isobutyl-thiazole		Green, tomato, wine	3

5.3.1.6 Pyrroles, Pyridines

The volatile compounds formed by heating food include numerous pyrrole and pyridine derivatives. Of special interest are the N-heterocyclic compounds with the following structural feature:



(5.13)

This characteristic feature appears to be required for a roasted odor. In fact, all the pyrrolines and pyridines listed in Table 5.23 as well as 2-acetylthiazole, 2-acetylthiazoline (cf. Table 5.22) and acetylpyrazine (cf. Table 5.23) contain this structural element and have a roasted or cracker-like odor. However, the thresholds of these compounds vary greatly. The lowest values were found for 2-acetyl-and 2-propionyl-1-pyrroline.

The length of the alkanoyl group also influences the aroma activity because in the transition from 2-propionyl- to 2-butanoyl-1-pyrroline, the roasted note suddenly disappears and the odor threshold increases by several powers of ten.

2-Acetyl-l-pyrroline (Apy) is responsible for the typical aroma of the crust of white bread and it

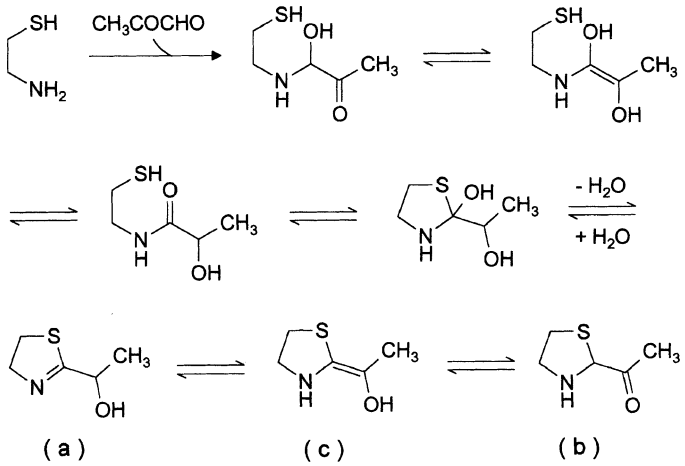


Fig. 5.21. Formation of precursors of 2-acetyl-2-thiazoline (according to *Hofmann* and *Schieberle*, 1995)

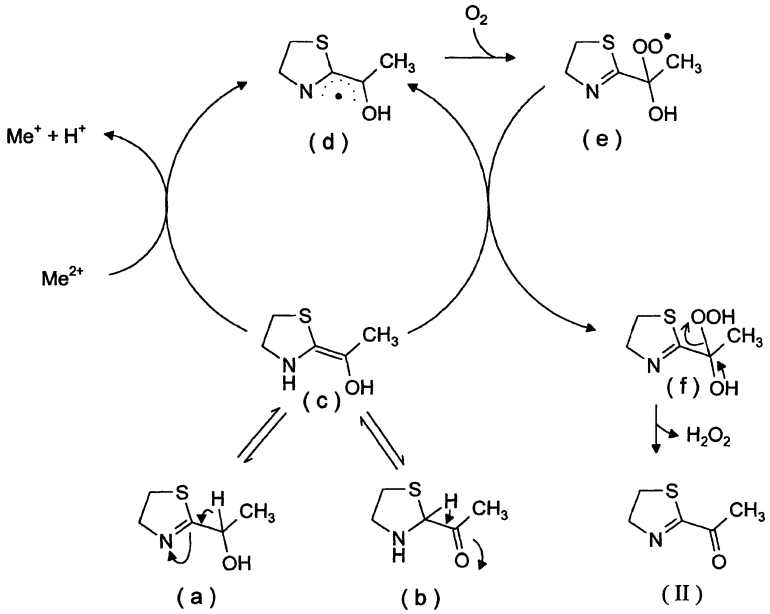


Fig. 5.22. Metal catalyzed oxidation of 2-(1-hydroxyethyl)-4,5-dihydrothiazole and 2-acetylthiazolidine (according to *Hofmann* and *Schieberle*, 1995)

produces the pleasant popcorn aroma of certain types of rice consumed mainly in Asia. In gas chromatography, Apy appears predominantly in the imine form shown in Table 5.23, whereas 2-acetyltetrahydropyridine (ATPy) appears as the eneamine and imine tautomers. Model experiments show that 1-pyrroline is the precursor of Apy and ATPy. 1-Pyrroline is formed by the *Strecker* degradation of both proline (cf. Formula 5.14) and ornithine (cf. Formula 5.15).

In the baking of white bread, ornithine comes from yeast where it is found in a concentration about four times that of free proline.

In addition, triose phosphates occurring in yeast have been identified as precursors. They yield on heating, e. g., 2-oxopropanal from di-

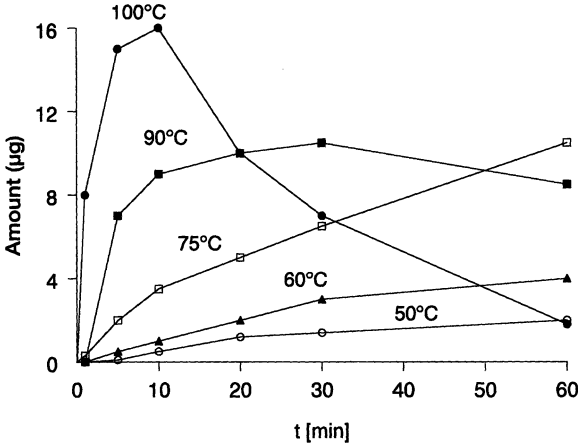
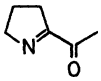
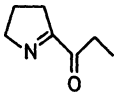
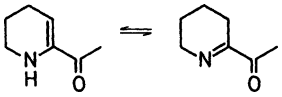
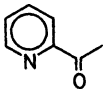


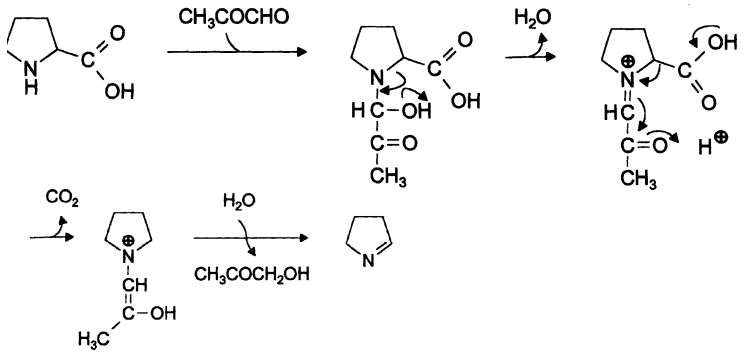
Fig. 5.23. Dependence on time and temperature of the formation of 2-acetyl-2-thiazoline from 2-(l-hydroxyethyl)-3,5-dihydrothiazole (according to *Hofmann* and *Schieberle*, 1996)

Table 5.23. Pyrrole and pyridine derivatives with a roasted aroma

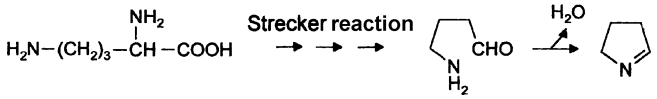
Name	Structure	Odor threshold (µg/kg, water)	Occurrence
2-Acetyl-1-pyrroline (APy)		0.1	White-bread crust, rice, cooked meat, popcorn
2-Propionyl-1-pyrroline		0.1	Popcorn, heated meat
2-Acetyltetra-hydropyridine (ATPy)		1.6	White-bread crust, popcorn
2-Acetylpyridine		19	White-bread crust

hydroxyacetone phosphate (cf. Formula 5.16), which is involved in the *Strecker* degradation (cf. Formula 5.14). Another source of 2-oxopropanal is the retroaldol condensation of 3-deoxy-1,2-dicarbonyl compounds in the course of the *Maillard* reaction (cf. 4.2.4.4.2). The reaction route which can explain the formation of Apy is based on an investigation of the model 1-pyrroline/2-oxopropanal and on labelling experiments. They show that in the reaction of proline with $[^{13}C]_6$-glucose under roasting conditions, two ^{13}C atoms are inserted into the Apy molecule. As a start in the reaction sequence to Apy, it is assumed that 2-oxopropanal (cf. 4.2.4.3.2), which is formed in the degradation of glucose, is present as a hydrate and participates in a nucleophilic attack on 1-pyrroline (Fig. 5.24). The resulting 2-(1,2-dioxopropyl)pyrrolidine is sensitive to oxygen and, consequently, rapidly oxidizes to 2-(1,2-dioxopropyl)pyrroline. After hydration, decarboxylation takes place in accord with the labelling experiment. This is followed by rearrangement and oxidation to Apy.

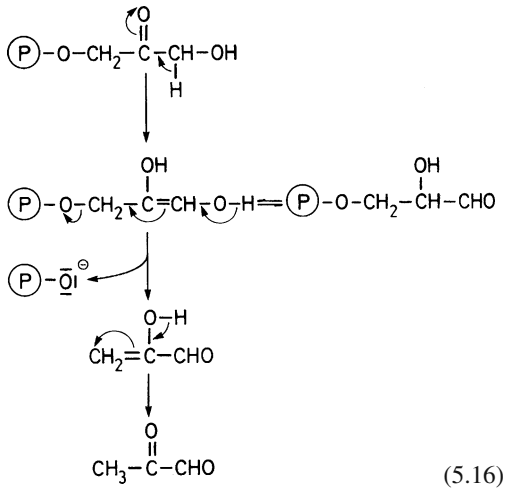
Hydroxy-2-propanone, which is formed by the *Strecker* degradation of amino acids, e. g., proline (cf. Formula 5.14), is in the enolized form the reaction partner of 1-pyrroline in the formation of ATPy (Fig. 5.25). The aldol addition of the two educts gives 2-(1-hydroxy-2-oxopropyl)-pyrrolidine (HOP) which undergoes ring opening to yield 5,6-dioxoheptylamine. The subsequent *Schiff* reaction to a 6-ring results in ATPy.



(5.14)



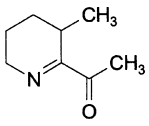
(5.15)



(5.16)

The reaction pathway shown in Fig. 5.25 can be based on the identification of HOP as an intermediate in the formation of ATPy and on a model experiment in which 2-methyl-1-pyrroline was used instead of 1-pyrroline. 2-Acetyl-3-methyl-3,4,5,6-tetrahydropyridine (cf. Formula 5.17) was produced, i. e., a displacement of the methyl group from position 2 in the 5-ring of the starting compound to position 3 in the 6-ring of the product. This shift can only be explained by the ring enlargement mechanism (Fig. 5.25).

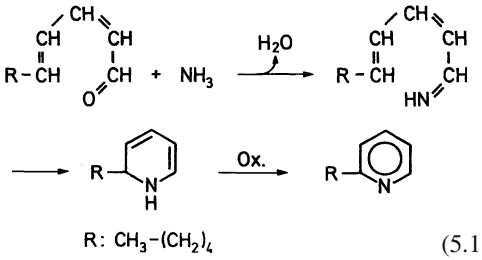
A comparison of the reaction paths in Fig. 5.24 and Fig. 5.25 allows the conclusion that the concentration ratio of 2-oxopropanal to hydroxy-2-propanone in food decides whether Apy or ATPy is preferentially formed from proline. If free amino acids are present in the food and the *Strecker* degradation dominates, then the formation of ATPy predominates. This could explain the preference for ATPy (430 µg/kg) compared to Apy (24 µg/kg) in the production of popcorn.



(5.17)

Although the odor threshold increases by about a factor of 10, the popcorn-like aroma note remains on oxidation of ATPy to 2-acetylpyridine. Substantially greater effects on the aroma are obtained by the oxidation of APy to 2-acetylpyrrole, which has an odor threshold that is more than 5 powers of ten higher and no longer smells roasted.

2-Pentylpyridine contributes to the smell of roasting lamb fat (greasy, suety odor; threshold: 0.12 µg/kg water); it produces an aroma defect in soybean products (cf. 16.3.1.1). The precursors identified were ammonia from the pyrolysis of asparagine and glutamine and 2,4-decadienal:



(5.18)

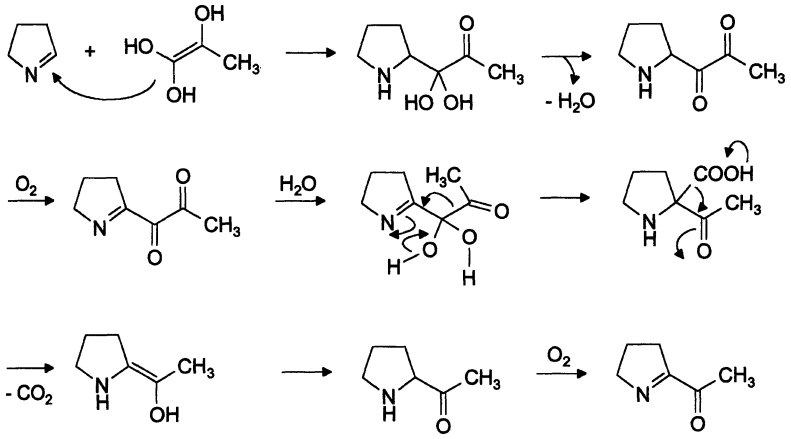


Fig. 5.24. Formation of 2-acetyl-1-pyrroline (according to *Hofmann* and *Schieberle*, 1998)

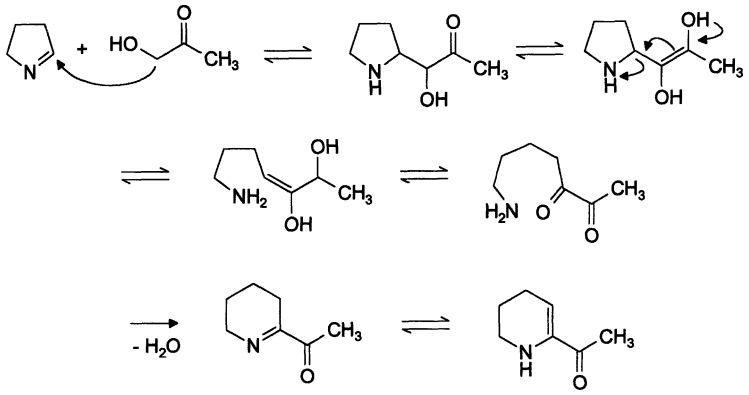


Fig. 5.25. Formation of 2-acetyltetrahydropyridine (according to *Hofmann* and *Schieberle*, 1998)

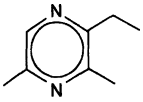
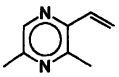
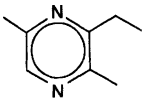
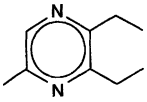
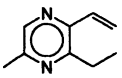
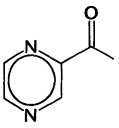
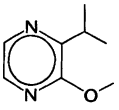
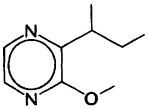
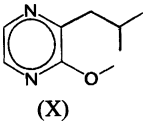
5.3.1.7 Pyrazines

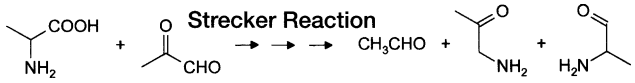
A large number of volatile pyrazines are formed on heating food. Seventy compounds are known alone in the group of alkyl pyrazines consisting only of the elements C, H and N. In dilution analyses, e. g., of coffee, bread crust, fried meat and cocoa liquor, only the first six compounds in Table 5.24 were detected; pyrazine II and V reached the highest FD factors.

According to gas chromatographic–olfactometric studies, pyrazines II, III, V and VI (Table 5.24) have the lowest odor thresholds (0.07 pmol/l air) that have ever been measured for alkyl pyrazines (cf. 5.6.3). Of these four pyrazines, II and V are produced in food in higher concentrations than III and VI (cf. example coffee, 21.1.3.3.7). As a result of this favorable ratio of concentration to odor threshold, the aroma activities of II and V exceed those of the other alkyl pyrazines.

Although the odor thresholds of pyrazines I and IV are much higher than those of pyrazines II, III, V and VI (Table 5.24), they are still detected in dilution analyses because they are formed in much higher concentrations on heating food and, consequently, can partially compensate for their "aroma weakness". 2-Oxopropanal and alanine are the precursors of pyrazines II, IV and V as well as 2-ethyl-5,6-dimethylpyrazine, which is odorless in the concentrations present in food. In accord with the formation of pyrazine in food, pyrazine IV is the main compound in model experiments (Table 5.25), followed by II and V. To explain the formation of II and IV,

Table 5.24. Pyrazines in food

Structure	Substituent	Aroma quality	Odor threshold value (μg/l; water)
 (I)	Trimethyl-	Earthy	90
 (II)	2-Ethyl-3,5-dimethyl-	Earthy, roasted	0.04
 (III)	2-Ethenyl-3,5-dimethyl-	Earthy, roasted	0.1
 (IV)	2-Ethyl-3,6-dimethyl-	Earthy, roasted	9
 (V)	2,3-Diethyl-5-methyl-	Earthy, roasted	0.09
 (VI)	2-Ethenyl-3-ethyl-5-methyl-	Earthy, roasted	0.1
 (VII)	Acetyl-	Roasted corn	62
 (VIII)	2-Isopropyl-3-methoxy-	Potatoes	0.002
 (IX)	2-sec-Butyl-3-methoxy-	Earthy	0.001
 (X)	2-Isobutyl-3-methoxy-	Hot paprika (red pepper)	0.002



(5.19)

Table 5.25. Formation of aroma active alkyl pyrazines on heating alanine and 2-oxopropanal[a]

Pyrazine[b]	Amount (µg)
2-Ethyl-3,5-dimethyl-(II)	27
2-Ethyl-3,6-dimethyl-(IV)	256
2-Ethyl-5,6-dimethyl-	2.6
2,3-Diethyl-5-methyl-(V)	18

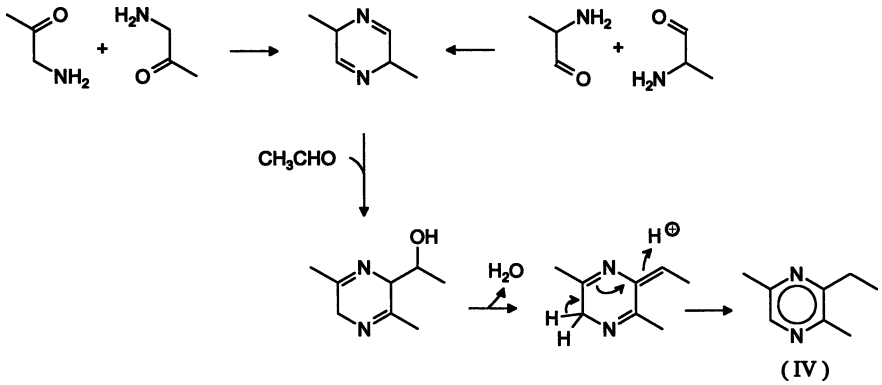
[a] The mixture of educts (2 mmol each; pH 5.6) was heated for 7 min to 180 °C.
[b] Roman numerals refer to Table 5.24.

it is postulated that the *Strecker* reaction of alanine and 2-oxopropanal represents the start, resulting in acetaldehyde, aminoacetone and 2-aminopropanal (cf. Formula 5.19).

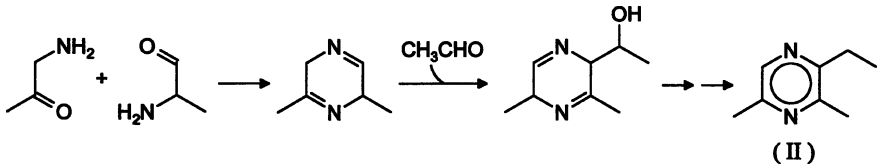
The precursor of pyrazine IV, 3,6-dimethyldihydropyrazine, is formed by the condensation both of two molecules of aminoacetone as well as two molecules of 2-aminopropanal (cf. Formula 5.20). The nucleophilic attack by dihydropyrazine on the carbonyl group of acetaldehyde and water elimination yield pyrazine IV. This mechanism also explains the formation of pyrazine II if 3,5-dimethyldihydropyrazine, which is produced by the condensation of aminoacetone and 2-aminopropanal (cf. Formula 5.21), is assumed to be the intermediate. The preferential formation of pyrazine IV in comparison with II can be explained by the fact that the *Strecker* reaction produces less 2-aminopropanal than aminoacetone because the aldehyde group in 2-oxopropanal is more reactive than the keto group. However, both aminocarbonyl compounds are required to the same extent for the synthesis of pyrazine II (cf. Formula 5.21). The powerfully odorous pyrazines VIII–X (Table 5.24) appear as metabolic by-products in some plant foods and microorganisms (cf. 5.3.2.6). Since they are very stable, they withstand, e. g., the roasting process in coffee (cf. 21.1.3.3.7).

5.3.1.8 Amines

Not only aldehydes (cf. 5.3.1.1), but also amines are formed in the *Strecker* reaction (cf. 4.2.4.4.7). The odor thresholds of these amines (examples in Table 5.26) are pH dependent. The enzymatic decarboxylation of amino acids produces the same amines as the *Strecker* reaction; the precursors are shown in Table 5.26. Both reactions take place e. g. in the production of cocoa, but the *Strecker*



(5.20)



(5.21)

Table 5.26. Precursors and sensory properties of amines

Amine	Amino acid precursor	Odor		
		Quality	Threshold (mg/l)	
			Water[a]	Oil
2-Methylpropyl	Val	Fishy, amine-like, malty	8.0	48.3
2-Methylbutyl	Ile	Fishy, amine-like, malty	4.9	69.7
3-Methylbutyl	Leu	Fishy, amine-like, malty	3.2	13.7
2-Phenylethyl	Phe	Fishy, amine-like, honey-like	55.6	89.7
3-(Methylthio)propyl	Met	Fishy, amine-like, boiled potato	0.4	0.3

[a] pH 7.5.

reaction predominates. An especially odor intensive amine, trimethylamine, is formed in the degradation of choline (cf. 11.2.4.4.4).

5.3.1.9 Phenols

Phenolic acids and lignin are degraded thermally or decomposed by microorganisms into phenols, which are then detected in food. Some of these compounds are listed in Table 5.27.
Smoke generated by burning wood (lignin pyrolysis) is used for cold or hot smoking of meat and fish products. This is a phenol enrichment process since phenol vapors penetrate the meat or fish muscle tissue. Also, some alcoholic beverages, such as Scotch whiskey, and also butter have low amounts of some phenols, the presence of which is needed to roundoff their typical aromas. Ferulic acid was identified as an important precursor in model experiments. 4-Vinylguaiacol is formed as the main product in pyrolysis, the secondary products being 4-ethylguaiacol, vanillin and guaiacol. To explain such a reaction which, for example, accompanies the process of roasting coffee or the kiln drying of malt, it has to be assumed that thermally formed free radicals regulate the decomposition pattern of phenolic acids (cf., for example, heat decomposition of ferulic acid, Fig. 5.26). In the pasteurization of orange juice, p-vinyl-guaiacol can also be formed from ferulic acid, producing a stale taste at concentrations of 1 mg/kg.

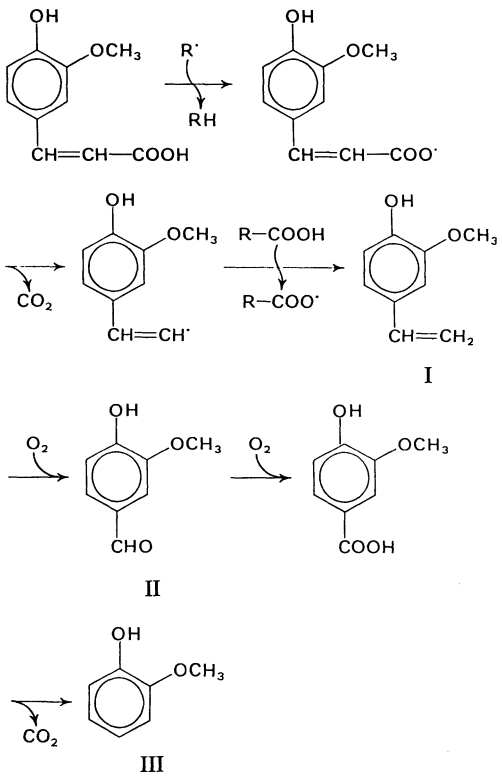


Fig. 5.26. Thermal degradation of ferulic acid. 4-Vinylguaiacol (I), vanillin (II), and guaiacol (III) (according to *Tressl* et al., 1976)

5.3.2 Enzymatic Reactions

Aroma compounds are formed by numerous reactions which occur as part of the normal metabolism of animals, plants and microorganisms. The enzymatic reactions triggered by tissue disruption, as experienced during disintegration or slicing of fruits and vegetables, are of particular importance. Enzymes can also be involved indirectly in aroma formation by providing the preliminary stage of the process, e. g. by releasing

Table 5.27. Phenols in food

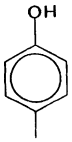
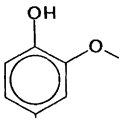
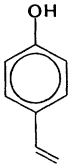
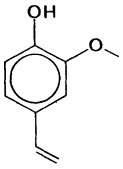
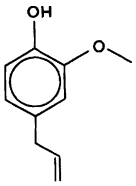
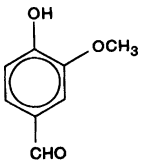
Name	Structure	Aroma quality	Odor threshold (µg/kg, water)	Occcurrence
p-Cresol	 (I)	Smoky	55	Coffee, sherry, milk, roasted peanuts, asparagus
4-Ethylphenol	 (II)	Woody		Milk, soya souce, roasted peanuts, tomatoes, coffee
Guaiacol	 (III)	Smoky, burning, sweet	1	Coffee, milk, crisp-bread, meat (fried)
4-Vinylphenol	 (IV)	Harsh, smoky	10	Beer, milk, roasted peanuts
2-Methoxy-4-vinylphenol	 (V)	Clove-like	5	Coffee, beer, apple (cooked), asparagus
Eugenol	 (VI)	Spicy	1	Tomato paste, brandy, plums, cherries
Vanillin	 (VII)	Vanilla	20	Vanilla, rum, coffee, asparagus (cooked), butter

Table 5.28. Pyrolysis products of some phenolic acids (T: 200 °C; air)

Phenolic acid	Product	Distribution (%)
Ferulic acid	4-Vinylguaiacol	79.9
	Vanillin	6.4
	4-Ethylguaiacol	5.5
	Guaiacol	3.1
	3-Methoxy-4-hydroxy-acetophenone (Acetovanillone)	2.6
	Isoeugenol	2.5
Sinapic acid	2,6-Dimethoxy-4-vinylphenol	78.5
	Syringaldehyde	13.4
	2,6-Dimethoxyphenol	4.5
	2,6-Dimethoxy-4-ethylphenol	1.8
	3,5-Dimethoxy-4-hydroxy-acetophenone (Acetosyringone)	1.1

amino acids from available proteins, sugars from polysaccharides, or ortho-quinones from phenolic compounds. These are then converted into aroma compounds by further nonenzymatic reactions. In this way, the enzymes enhance the aroma of bread, meat, beer, tea and cacao.

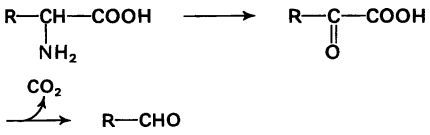
5.3.2.1 Carbonyl Compounds, Alcohols

Fatty acids and amino acids are precursors of a great number of volatile aldehydes, while carbohydrate degradation is the source of ethanal only. Due to its aroma activity at higher concentrations ethanal is of great importance for the fresh note, e. g., in orange and grapefruit juice.

Linoleic and linolenic acids in fruits and vegetables are subjected to oxidative degradation by lipoxygenase alone or in combination with a hydroperoxide lyase, as outlined in sections 3.7.2.2 and 3.7.2.3. The oxidative cleavage yields oxo acids, aldehydes and allyl alcohols. Among the aldehydes formed, hexanal, (E)-2-hexenal, (Z)-3-hexenal and/or (E)-2-nonenal, (Z)-3-nonenal, (E,Z)-2,6-nonadienal and (Z,Z)-3,6-nonadienal are important for aroma.

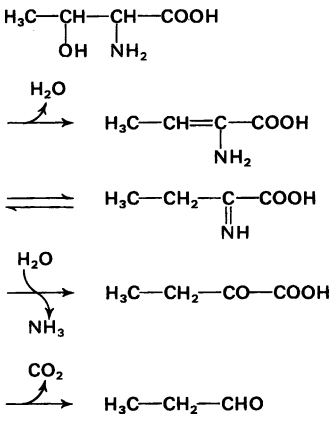
Frequently, these aldehydes appear soon after the disintegration of the tissue in the presence of oxygen. A part of the aldehydes is enzymatically reduced to the corresponding alcohols (see below). In comparison, lipoxygenases and hydroperoxide lyases from mushrooms exhibit a different reaction specificity. Linoleic acid, which predominates in the lipids of champignon mushrooms, is oxidatively cleaved to R(–)-1-octen-3-ol and 10-oxo-(E)-8-decenoic acid (cf. 3.7.2.3). The allyl alcohol is oxidized to a small extent by atmospheric oxygen to the corresponding ketone. Owing to an odor threshold that is about hundred times lower (cf. Table 3.32), this ketone together with the alcohol accounts for the mushroom odor of fresh champignons and of Camembert.

Aldehydes formed by the *Strecker* degradation (cf. 5.3.1.1; Table 5.16) can also be obtained as metabolic by-products of the enzymatic transamination or oxidative deamination of amino acids. First, the amino acids are converted enzymatically to α-keto acids and then to aldehydes by decarboxylation in a side reaction:



(5.22)

Unlike other amino acids, threonine can eliminate a water molecule and, by subsequent decarboxylation, yield propanal:



(5.23)

Many aldehydes derived from amino acids occur in plants and fermented food.

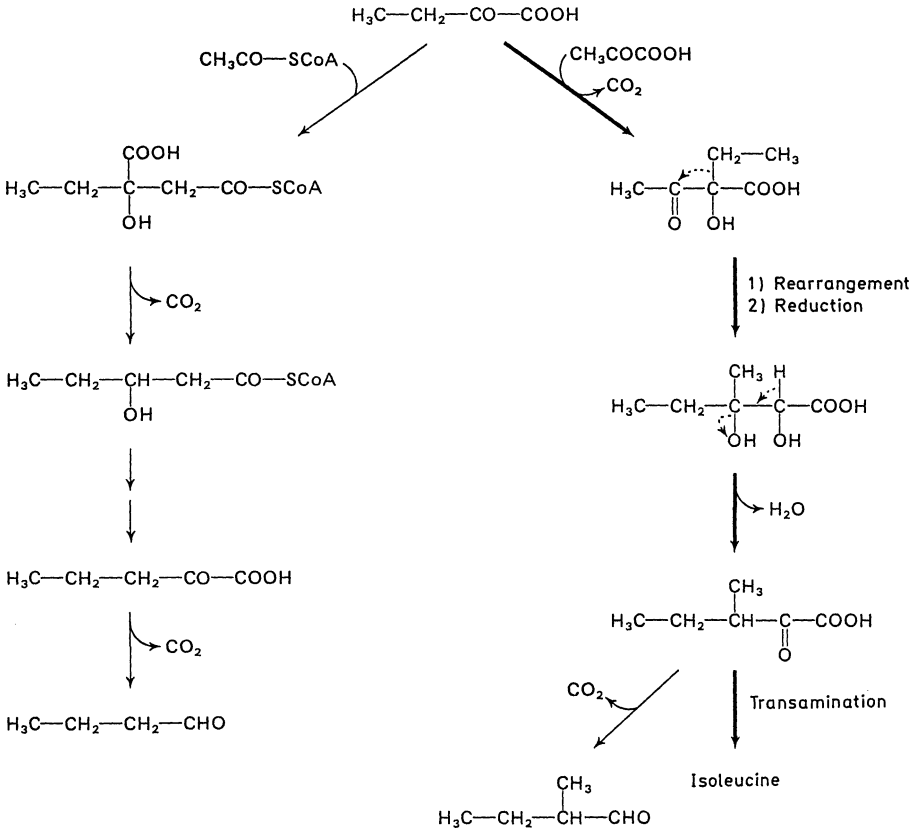


Fig. 5.27. Formation of aldehydes during isoleucine biosynthesis (according to *Piendl*, 1969). → main pathway → side pathway of the metabolism

A study involving the yeast *Saccharomyces cerevisiae* clarified the origin of methylpro-panal and 2- and 3-methylbutanal. They are formed to a negligible extent by decomposition but mostly as by-products during the biosynthesis of valine, leucine and isoleucine.

Figure 5.27 shows that α-ketobutyric acid, derived from threonine, can be converted into isoleucine. Butanal and 2-methylbutanal are formed by side-reaction pathways.

2-Acetolactic acid, obtained from the condensation of two pyruvate molecules, is the intermediary product in the biosynthetic pathways of valine and leucine (Fig. 5.28). However, 2-acetolactic acid can be decarboxylated in a side reaction into acetoin, the precursor of diacetyl. At α-keto-3-methylbutyric acid, the metabolic pathway branches to form methylpropanal and branches again at α-keto-4-methyl valeric acid to form 3-methylbutanal (Fig. 5.28).

The enzyme that decarboxylates the α-keto-carboxylic acids to aldehydes has been detected in oranges. Substrate specificity for this decarboxylase is shown in Table 5.29.

Table 5.29. Substrate specificity of a 2-oxocarboxylic acid decarboxylase from orange juice

Substrate	V_{rel} (%)
Pyruvate	100
2-Oxobutyric acid	34
2-Oxovaleric acid	18
2-Oxo-3-methylbutyric acid	18
2-Oxo-3-methylvaleric acid	18
2-Oxo-4-methylvaleric acid	15

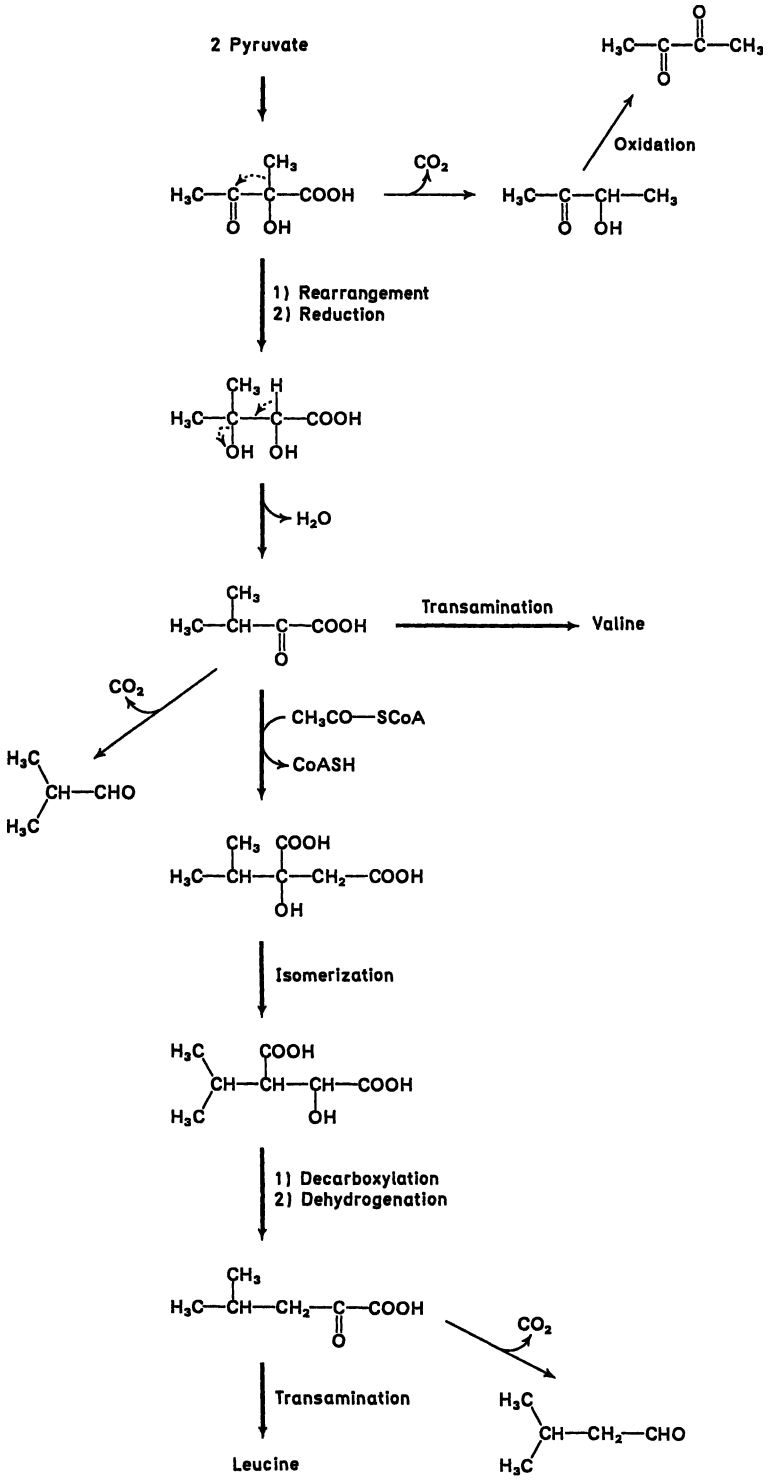


Fig. 5.28. Formation of carbonyl compounds during valine and leucine biosynthesis (according to *Piendl*, 1969). → main pathway → side pathway of the metabolism

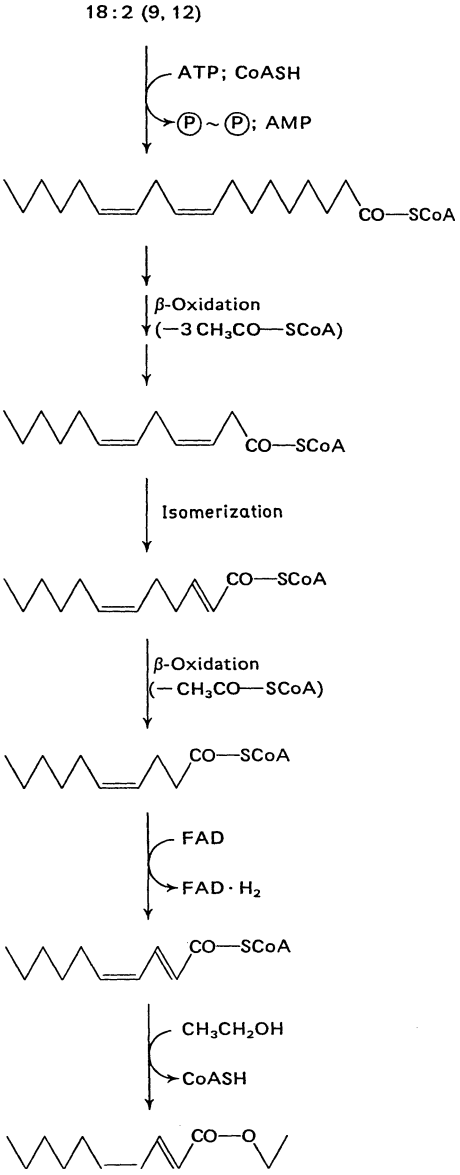


Fig. 5.29. Biosynthesis of (E,Z)-2,4-decadienoic acid ethyl ester in pears (according to *Jennings* and *Tressl*, 1974)

Alcohol dehydrogenases (cf. 2.3.1.1) can reduce the aldehydes derived from fatty acid and amino acid metabolism into the corresponding alcohols:

$$\mathrm{R{-}CH_2{-}OH + NAD^{\oplus} \rightleftharpoons R{-}CHO + NADH + H^{\oplus}} \qquad (5.24)$$

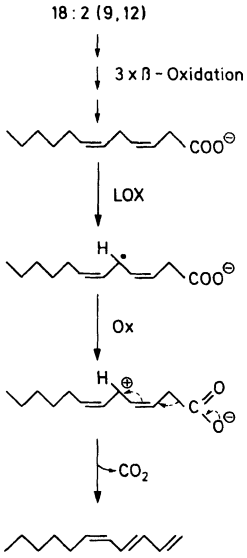
Alcohol formation in plants and microorganisms is strongly favoured by the reaction equilibrium and, primarily, by the predominance of NADH over NAD^+. Nevertheless, the enzyme specificity is highly variable. In most cases aldehydes $>C_5$ are only slowly reduced; thus, with aldehydes rapidly formed by, for example, oxidative cleavage of unsaturated fatty acids, a mixture of alcohols and aldehydes results, in which the aldehydes predominate.

5.3.2.2 Hydrocarbons, Esters

Fruits and vegetables (e. g., pineapple, apple, pear, peach, passion fruit, kiwi, celery, parsley) contain unsaturated C_{11} hydrocarbons which play a role as aroma substances. Of special interest are (E,Z)-1,3,5-undecatriene and (E,Z,Z)-1,3,5,8-undecatetraene, which with very low threshold concentrations have a balsamic, spicy, pine-like odor. It is assumed that the hydrocarbons are formed from unsaturated fatty acids by β-oxidation, lipoxygenase catalysis, oxidation of the radical to the carbonium ion and decarboxylation. The hypothetical reaction pathway from linoleic acid to (E,Z)-1,3,5-undecatrieneis shown in Formula 5.25.

$$\mathrm{R{-}CO{-}SCoA + R'{-}OH \rightarrow R{-}CO{-}O{-}R' + CoASH} \qquad (5.25)$$

Esters are significant aroma constituents of many fruits. They are synthetized only by intact cells:



(5.26)

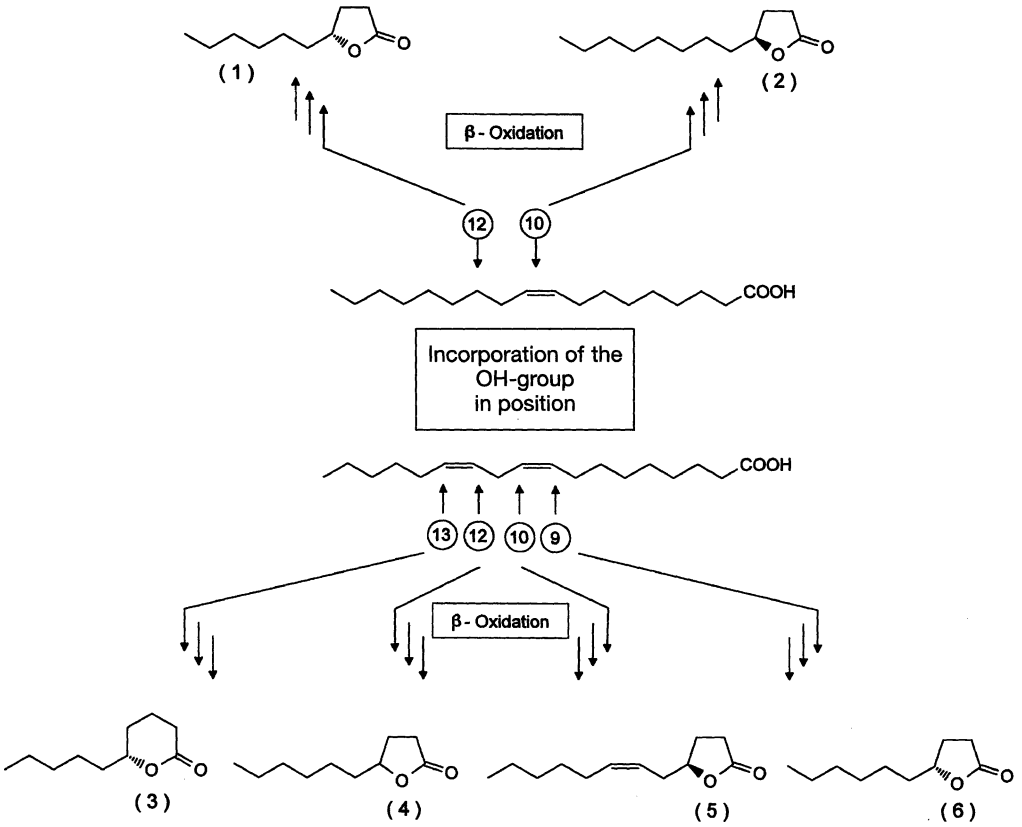


Fig. 5.30. Biosynthesis of γ- and δ-lactones from oleic and linoleic acid (according to *Tressl et al.*, 1996) (1) R-γ-decalactone, (2) S-δ-dodecalactone, (3) R-δ-decalactone, (4) γ-decalactone, (5) R-(Z)-6-γ-dodecenelactone, (6) R-γ-nonalactone

Acyl-CoA originates from the β-oxidation of fatty acids and also occasionally from amino acid metabolism. Figure 5.30 shows an example of how ethyl (E,Z)-2,4-decadienoate, an important aroma constituent of pears, is synthesized from linoleic acid.

Table 5.30 gives information on the odor thresholds of some esters. Methyl branched esters, from the metabolism of leucine and isoleucine, were found to have very low values. The odor thresholds of the acetates are higher than those of the corresponding ethylesters.

When fruits are homogenized, such as in the processing of juice, the esters are rapidly hydrolyzed by the hydrolase enzymes present, and the fruit aroma flattens.

5.3.2.3 Lactones

Numerous lactones are found in food. Some of the representatives which belong to the typical aroma substances of butter, coconut oil, and various fruits are presented in Table 5.31.

Since the aroma of lactones is partly very pleasant, these substances are also of interest for commercial aromatization of food. In the homologous series of γ- and δ-lactones, the odor threshold decreases with increasing molecular weight (Table 5.32).

The biosynthesis of lactones was studied using the yeast *Sporobolomyces odorus* and it was shown that the results are valid for animal and plant foods. Labelling with deuterium indicates

Table 5.30. Odor thresholds of esters

Compound	Odor threshold (μg/kg, water)
Methylpropionic acid methyl ester	7
2-Methylbutyric acid methyl ester	0.25
Methylpropionic acid ethyl ester	0.1
(S)-2-Methylbutyric acid ethyl ester	0.06
Butyric acid ethyl ester	0.1
Isobutyric acid ethyl ester	0.02
3-Methylbutyric acid ethyl ester	0.03
Caproic acid ethyl ester	5
Cyclohexanoic acid ethyl ester	0.001
(R)-3-Hydroxyhexanoic ethyl ester	270
Caprylic acid ethyl ester	0.1
(E,Z)-2,4-Decadienoic acid ethyl ester	100
trans-Cinnamic acid ethyl ester	0.06
Benzoic acid ethyl ester	60
Salicylic acid methyl ester	40
Butyl acetate	58
2-Methylbutyl acetate	5
3-Methylbutyl acetate	3
Pentyl acetate	38
Hexyl acetate	101
(Z)-3-Hexenyl acetate	7.8
Octyl acetate	12
2-Phenylethyl acetate	20

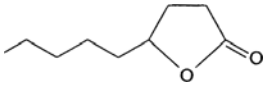
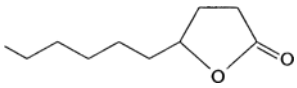
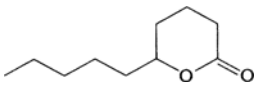
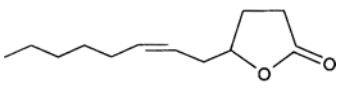
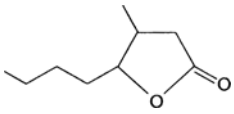
Table 5.32. Odor thresholds of lactones

Compound	Odor threshold (μg/kg, water)
γ-*Lactones*	
γ-Hexalactone	1600
γ-Heptalactone	400
γ-Octalactone	7
γ-Nonalactone	30–65
γ-Decalactone	11
γ-Dodecalactone	7
δ-*Lactones*	
δ-Octalactone	400
δ-Decalactone	100
6-Pentyl-α-pyrone	150

that the precursors oleic and linoleic acid are regio- and stereospecifically oxidized to hydroxy acids (Fig. 5.30), which are shortened by β-oxidation and cyclized to lactones. The individual steps in the biosynthesis are represented in Fig. 5.31 using (R)-δ-decalactone, a key odorant of butter (cf. 10.3.4).

Linoleic acid is metabolized by cows with the formation of (Z)-6-dodecen-γ-lactone as a secondary product (Fig. 5.30). Its sweetish odor enhances the aroma of butter. On the other hand, it is undesirable in meat.

Table 5.31. Lactones in food

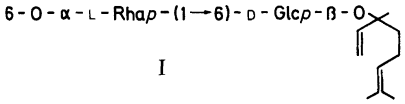
Name	Structure	Aroma quality	Occurrence
4-Nonanolide (γ-nonalactone)		Reminiscent of coconut oil, fatty	Fat-containing food, crispbread, peaches
4-Decanolide (γ-decalactone)		Fruity, peaches	Fat-containing food, cf. Table 5.13
5-Decanolide (δ-decalactone)		Oily, peaches	Fat-containing food, cf. Table 5.13
(Z)-6-Dodecen-γ-lactone		Sweet	Milk fat, peaches
3-Methyl-4-octanolide (whisky- or quercus lactone)		Coconut-like	Alcoholic beverages

The whisky or oak lactone is formed when alcoholic beverages are stored in oak barrels. 3-Methyl-4-(3,4-dihydroxy-5-methoxybenzo)octanoic acid is extracted from the wood. After elimination of the benzoic acid residue, this compound cyclizes to give the lactone. The odor thresholds of the two cis-oak lactones (3R, 4R and 3S, 4S) are about ten times lower than those of the trans diastereomers (3S, 4R and 3R, 4S).

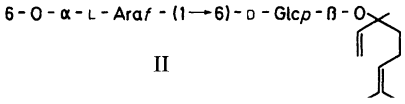
5.3.2.4 Terpenes

The mono- and sesquiterpenes in fruits (cf. 18.1.2.6) and vegetables (cf. 17.1.2.6), herbs and spices (cf. 22.1.1.1) and wine (cf. 20.2.6.9) are presented in Table 5.33. These compounds stimulate a wide spectrum of aromas, mostly perceived as very pleasant (examples in Table 5.34). The odor thresholds of terpenes vary greatly (Table 5.34). Certain terpenes occur in flavoring plants in such large amounts that in spite of relatively high odor thresholds, they can act as character impact compounds, e. g., S(+)-α-phellandrene in dill.

Monoterpenes with hydroxy groups, such as linalool, geraniol and nerol, are present in fruit juice at least in part as glycosides. Linalool-β-rutinoside (I) and linalool-6-0-α-L-arabinofuranosyl-β-D-glucopyranoside (II) have been found in wine grapes and in wine (cf. 20.2.6.9):



I



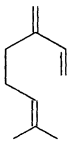
II (5.27)

Terpene glycosides hydrolyze, e. g., in the production of jams (cf. 18.1.2.6.11), either enzymatically (β-glucosidase) or due to the low pH of juices. The latter process is strongly accelerated by a heat treatment. Under these conditions, terpenes with two or three hydroxyl groups which are released undergo further reactions, forming hotrienol (IV) and neroloxide (V) from 3,7-dimethylocta-1,3-dien-3,7-diol (cf. Formula 5.28) in grape juice, or cis- and trans-furanlinalool oxides (VIa and VIb) from 3,7-dimethylocta-1-en-3,6,7-triol in grape juice and peach sap (cf. Formula 5.29).

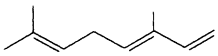
Table 5.33. Terpenes in food

Monoterpenes

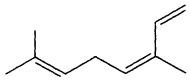
Acyclic (including cyclic derivatives)



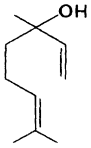
Myrcene (I)



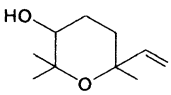
trans-Ocimene (II)



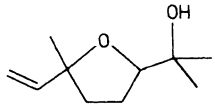
cis-Ocimene (III)



Linalool (IV)

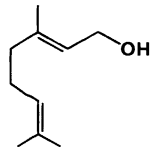


2,6,6-Trimethyl-2-vinyl-5-hydroxytetrahydropyran[a] (IVa)

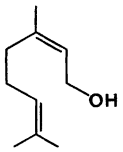


2-Methyl-2-vinyl-5-hydroxyisopropyltetrahydrofuran[a] (IVb)

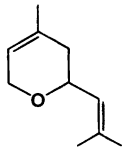
Table 5.33. (*Continued*)



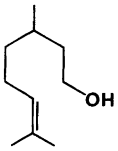
Geraniol[b] (V)



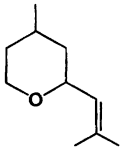
Nerol[b] (VI)



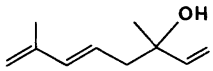
Neroloxide (VIa)



Citronellol[b] (VII)

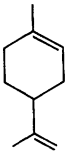


Rose oxide (VIIa)

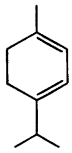


Hotrienol[c] (VIII)

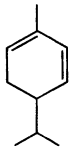
Monocyclic



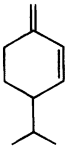
Limonene (IX)



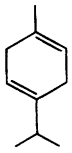
α-Terpinene (X)



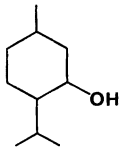
α-Phellandrene (XI)



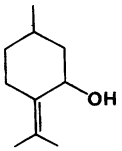
β-Phellandrene (XII)



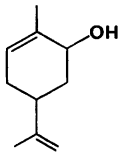
γ-Terpinene (XIII)



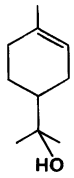
Menthol (XIV)



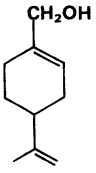
Pulegol (XV)



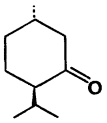
Carveol (XVI)



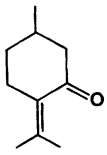
α-Terpineol (XVII)



Perilla alcohol (XVIII)

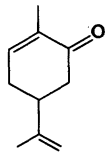


Menthone (XIX)

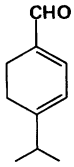


Pulegone (XX)

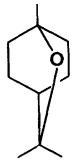
Table 5.33. (*Continued*)



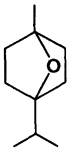
Carvone (XXI)



1,3-p-Menthadien-7-al (XXII)



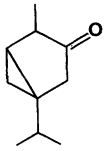
1,8-Cineole (XXIII)



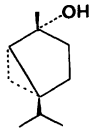
1,4-Cineole (XXIV)

Bicyclic

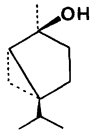
Sabinene (XXV)



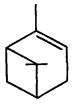
Thujone (XXVI)



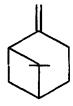
(+)-cis-Sabinene hydrate (XXVII)



(+)-trans-Sabinene hydrate (XXVIII)

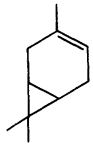


α-Pinene (XXIX)

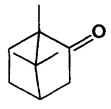


β-Pinene (XXX)

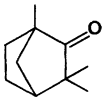
Camphene (XXXI)



Δ^3-Carene (XXXII)



Camphor (XXXIII)

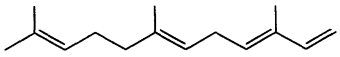


Fenchone (XXXIV)

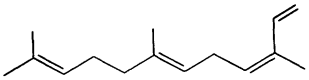
Table 5.33. (Continued)

Sesquiterpenes

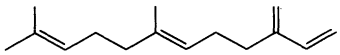
Acyclic



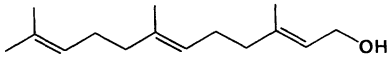
trans-α-Farnesene (XXXV)



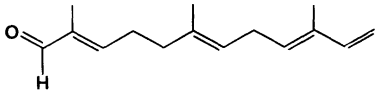
cis-α-Farnesene (XXXVI)



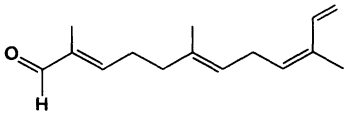
β-Farnesene (XXXVII)



Farnesol (XXXVIII)

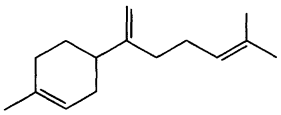


(all-trans)-α-Sinensal (XXXIX)

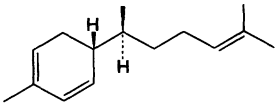


(trans,trans,cis)-α-Sinensal (XL)

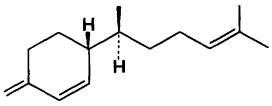
Monocyclic



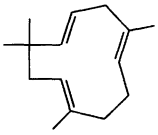
β-Bisabolene (XLI)



(–)-Zingiberene (XLII)

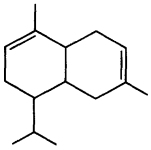


(–)-Sesquiphellandrene (XLIII)

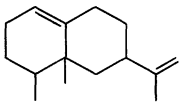


Humulene (XLIV)

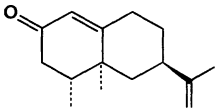
Bicyclic



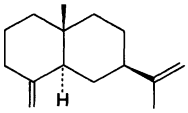
β-Cadinene (XLV)



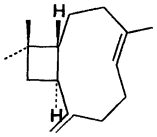
Valencene (XLVI)



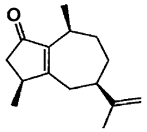
(+)-Nootkatone (XLVII)



β-Selinene (XLVIII)



β-Caryophyllene (XLIX)



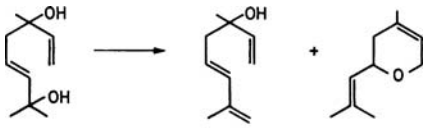
L (-) - Rotundone (L)

[a] Compounds IVa and IVb are also denoted as pyranlinalool and furanlinalool oxide, respectively.
[b] Corresponding aldehydes geranial (Va), neral (VIb) and citronellal (VIIa) also occur in food. Citral is a mixture of neral and geranial.
[c] (−)-3,7-Dimethyl-1,5,7-octatrien-3-ol (hotrienol) is found in grape, wine and tea aromas.

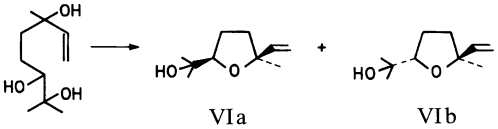
Table 5.34. Sensory properties of some terpenes

Compound[a]	Aroma quality	Odor threshold (μg/kg, water)
Myrcene (I)	Herbaceous, metallic	14
Linalool (IV)	Flowery	6
cis-Furanlinalool oxide (IVb)	Sweet-woody	6000
Geraniol (V)	Rose-like	7.5
Geranial (Va)	Citrus-like	32
Nerol (VI)		300
Citronellol (VII)	Rose-like	10
cis-Rose oxide (VIIa)	Geranium-like	0.1
R(+)-Limonene (IX)	Citrus-like	200
R(–)-α-Phellandrene (XI)	Terpene-like, medicinal	500
S(–)-α-Phellandrene (XI)	Dill-like, herbaceous	200
α-Terpineol (XVII)	Lilac-like, peach-like	330
(R)-Carvone (XXI)		50
1,8-Cineol (XXIII)	Spicy, camphor-like	12
(all-E)-α-Sinensal (XXXIX)	Orange-like	0.05
(–)-β-Caryophyllene (XLIX)	Spicy, dry	64
(–)-Rotundone (L)	Peppery	0.008

[a] The numbering of the compounds refers to Table 5.33.



IV V (5.28)



VIa VIb

(5.29)

Most terpenes contain one or more chiral centers. Of several terpenes, the optically inactive form and the l- and d-form occur in different plants. The enantiomers and diastereoisomers differ regularly in their odor characteristics. For example, menthol (XIV in Table 5.33) in the l-form

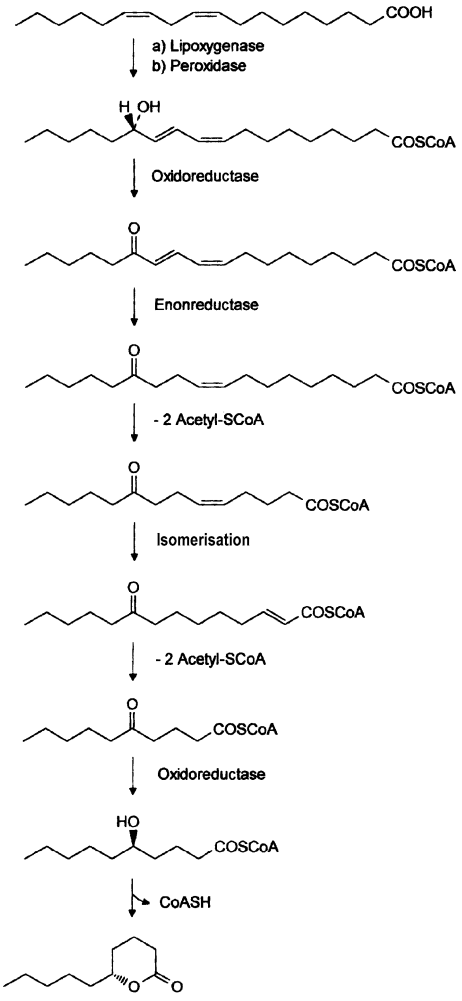


Fig. 5.31. Formation of R-δ-decalactone from linoleic acid (according to *Tressl et al.*, 1996)

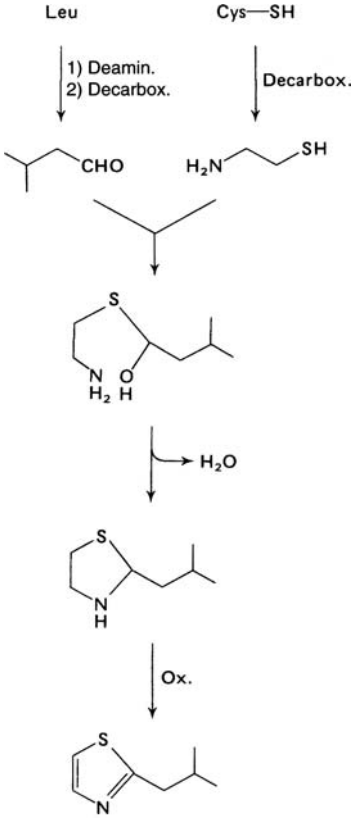
(1R, 3R, 4S) which occurs in peppermint oil, has a clean sweet, cooling and refreshing peppermint aroma, while in the d-form (1S, 3S, 4R) it has remarkable, disagreeable notes such as phenolic, medicated, camphor and musty. Carvone (XXI in Table 5.33) in the R(−)-form has a peppermint odor. In the S(+)-form it has an aroma similar to caraway. Other examples that show the influence of stereochemistry on the odor threshold of terpenes are 3a,4,5,7a-tetrahydro-3,6-dimethyl-2(3H)-benzofuranone (cf. 5.2.5) and 1-p-menthene-8-thiol (cf. 5.3.2.5).

Some terpenes are readily oxidized during food storage. Examples of aroma defects resulting from oxidation are provided in Table 5.5 and Section 22.1.1.1.

5.3.2.5 Volatile Sulfur Compounds

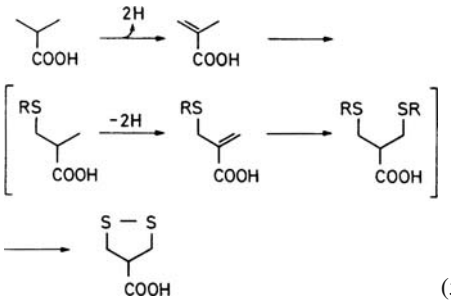
The aroma of many vegetables is due to volatile sulfur compounds obtained by a variety of enzymatic reactions. Examples are the vegetables of the plant families *Brassicacea* and *Liliaceae*; their aroma is formed by decomposition of glucosinolates or S-alkyl-cysteine-sulfoxides (cf. 17.1.2.6.7).

2-Isobutylthiazole (compound V, Table 5.22) contributes to tomato aroma (cf. 17.1.2.6.13). It is probably obtained as a product of the secondary metabolism of leucine and cysteine (cf. postulated Reaction 5.30).



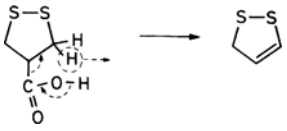
(5.30)

Isobutyric acid is the precursor of asparagus acid (1,2-dithiolane-4-carboxylic acid) found in asparagus. It is dehydrogenated to give methylacrylic acid which then adds on an unknown S-containing nucleophile (see Formula 5.31). During cooking, asparagus acid is oxidatively decarboxylated to a 1,2-dithiocyclopentene (see Formula 5.32), which contributes to the aroma of asparagus.

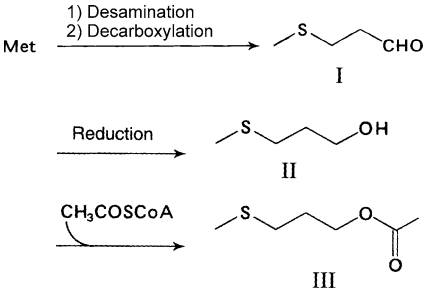


(5.31)

Volatile sulfur compounds formed in wine and beer production originate from methionine and are by-products of the microorganism's metabolism. The compounds formed are methional (I), methionol (II) and acetic acid-3-(methylthio)-propyl ester (III, cf. Reaction 5.33).



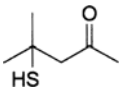
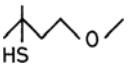
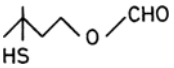
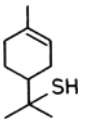
(5.32)



(5.33)

Tertiary thiols (Table 5.35) are some of the most intensive aroma substances. They have a fruity odor at the very low concentrations in which they occur in foods. With increasing concentration, they smell of cat urine and are called *catty odorants*. Tertiary thiols have been detected in some fruits, olive oil, wine (*Scheurebe*) and roasted coffee (Table 5.35). They make important contributions to the aroma and are possibly formed by the addition of hydrogen sulfide to metabolites of isoprene metabolism. In beer,

Table 5.35. Tertiary thiols in food

Name	Structure	Odor threshold (µg/kg, water)	Occurrence
4-Mercapto-4-methyl-2-pentanone		0.0001	Basil, wine (*Scheurebe*), Grapefruit
4-Methoxy-2-methyl-2-butanethiol		0.00002	Olive oil (cf. 14.3.2.1.1), black currants
3-Mercapto-3-methylbutylformate		0.003	Roasted coffee
1-p-Menthen-8-thiol		0.00002	Grapefruit

3-mercapto-3-methylbutylformate is undesirable because it causes off-flavor at concentrations as low as 5 ng/l. 1-p-Menthene-8-thiol, which contributes to grapefruit aroma, is a chiral compound. The (R)-enantiomer exhibits an extremely low odor threshold shown in Table 5.35. The (S)-enantiomer has a weak and unspecific odor.

5.3.2.6 Pyrazines

Paprika pepper (*Capsicum annum*) and chillies (*Capsicum frutescens*) contain high concentrations of 2-isobutyl-3-methoxypyrazine (X in Table 5.24 for structure). Its biosynthesis from leucine is assumed to be through the pathway shown in Formula 5.34.

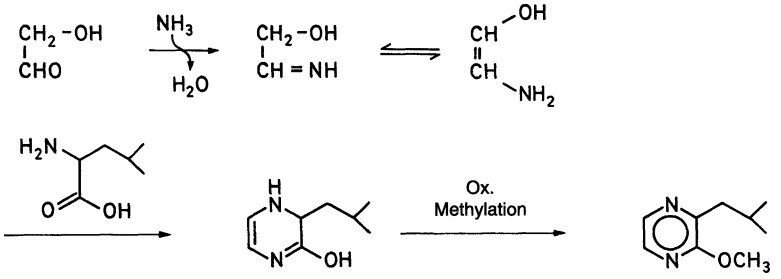
The compound 2-sec-butyl-3-methoxy-pyrazine is one of the typical aroma substances of carrots.

Pyrazines are also produced by microorganisms. For example, 2-isopropyl-3-methoxypyrazine has been identified as a metabolic byproduct of *Pseudomonas perolans* and *Pseudomonas taetrolens.* This pyrazine is responsible for a musty/earthy off-flavor in eggs, dairy products and fish.

5.3.2.7 Skatole, p-Cresol

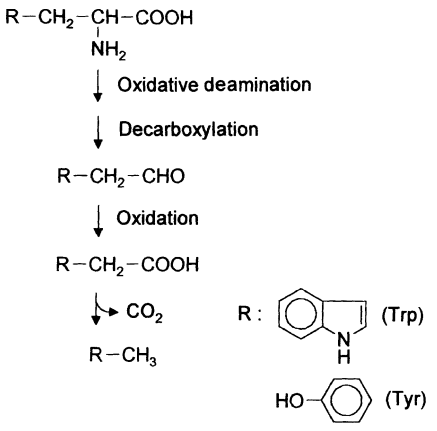
The amino acids tryptophan and tyrosine are degraded by microorganisms to skatole and p-cresol respectively (cf. Formula 5.40).

The odor thresholds of skatole have been determined in sunflower oil (15.6 µg/kg) and on starch (0.23 µg/kg). This compound plays a role in the aroma of Emmental cheese (cf. 10.3.5) and causes an aroma defect in white pepper (cf. 22.1.1.2.1). It can probably also be formed nonenzymatically from tryptophan by the



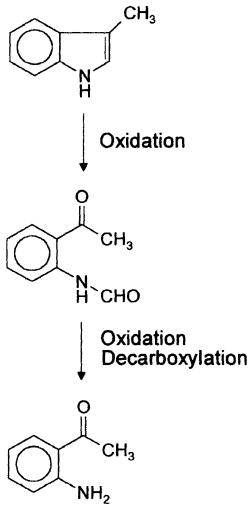
(5.34)

Strecker degradation, oxidation to indolylacetic acid and decarboxylation. The oxidative cleavage of skatole yields o-aminoacetophenone (cf. Formula 5.36), which has an animal odor and is the key aroma substance of tortillas and taco shells made of corn treated with lime (*Masa corn*). In the case of milk dry products, o-aminoacetophenone causes an aroma defect (cf. 10.3.2). Its odor threshold of 0.2 µg/kg (water) is very low. On the other hand, p-aminoacetophenone has an extremely high odor threshold of 100 mg/kg (water).



(5.35)

p-Cresol (odor threshold on starch 130 µg/kg) has been detected as an accompanying substance of skatole in samples of white pepper having an aroma defect. It is also formed in citrus oil and juice by the degradation of citral (cf. 5.5.4).



(5.36)

5.4 Interactions with Other Food Constituents

Aroma interactions with lipids, proteins and carbohydrates affect the retention of volatiles within the food and, thereby, the levels in the gaseous phase. Consequently, the interactions affect the intensity and quality of food aroma. Since such interactions cannot be clearly followed in a real food system, their study has been transferred to model systems which can, in essence, reliably imitate the real systems. Consider the example of emulsions with fat contents of 1%, 5% and 20%, which have been aromatized with an aroma cocktail for mayonnaise consisting of diacetyl, (Z)-3-hexenol, (E,Z)-2,6-nonadienol, allyl isothiocyanate and allyl thiocyanate. The sample with 20% of fat has the typical and balanced odor of mayonnaise (Fig. 5.32 a). If the fat content decreases, the aroma changes drastically. The emulsion with 5% of fat has an untypical creamy and pungent odor since there is a decrease in the intensities of the buttery and fatty notes in the aroma profile (Fig. 5.32 b). In the case of 1% of fat, pungent, mustard-like aroma notes dominate (Fig. 5.32 c).

Headspace analyses show that the drastic change in the aroma of the emulsions is based on the fact that the concentrations of the fatsoluble aroma substances (Z)-3-hexenol, allyl isothiocyanate and allyl thiocyanate in the gas phase increase with decreasing fat content (Fig. 5.33). Only the water-soluble diacetyl remains unaffected (Fig. 5.33).

The concentration of the very aroma active (E,Z)-2,6-nonadienol (cf. 10.3.6) in the head space is below the detection limit. However, this odorant can be detected by headspace GC-olfactometry (cf. 5.2.2.2). The results in Table 5.36 show that this alcohol as well as (Z)-3-hexenol no longer contribute to the aroma in the 20% fat emulsion. In the emulsion with 1% of fat, (E,Z)-2,6-nonadienol, allyl isothiocyanate and allyl thiocyanate predominate and produce the green, mustard-like aroma (Table 5.36).

A knowledge of the binding of aroma to solid food matrices, from the standpoint of food aromatization, aroma behavior and food processing and storage, is of great importance.

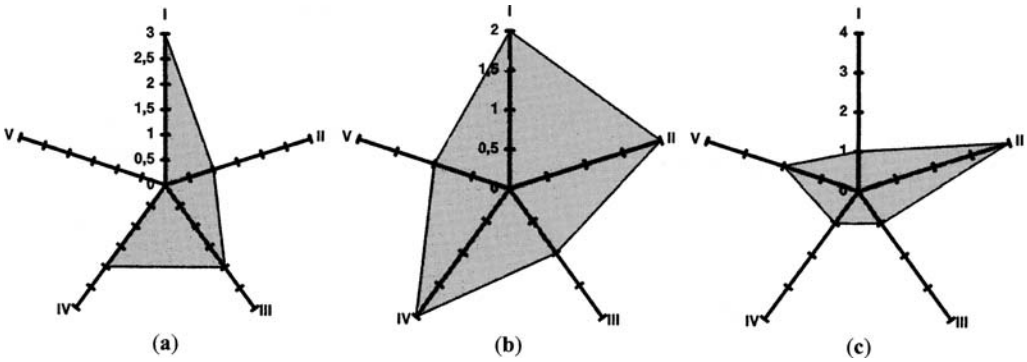


Fig. 5.32. Influence of the fat content on the aroma profile of emulsions; a) 20% fat, b) 5% fat, c) 1% fat. The intensities of the aroma qualities buttery (I), pungent, sharp (II), fatty (III), sweet (IV) and green (V) were evaluated as 1 (weak) to 4 (strong) (according to *Widder* and *Fischer*, 1996)

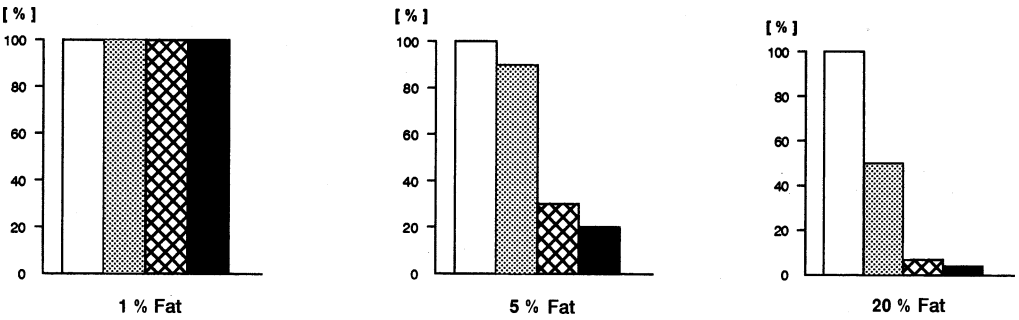


Fig. 5.33. Influence of the fat content of an emulsion on the concentration of aroma substances in the gas phase (according to *Widder* and *Fischer*, 1996).
□ diacetyl, ■ (Z)-3-hexenol, ☒ allyl isothiocyanate, ■ allyl thiocyanate

Table 5.36. Mayonnaise model: gas chromatography/olfactometry of headspace samples

Aroma substance[a]	Odor quality	Odor intensity[b]		
		1% fat	5% fat	20% fat
Diacetyl	Buttery	3	4	>4
(Z)-3-Hexenol	Green	2	1	0
(E,Z)-2-6-Nonadienol	Green, fatty	4	<1	0
Allyl isothiocyanate	Pungent, mustard-like	4	3	<1
Allyl thiocyanate	Pungent, mustard-like	4	3	<1

[a] Components of an aroma cocktail to which an oil emulsion was added.
[b] Intensity on sniffing the carrier gas stream 1 (weak)–4 (strong).

5.4.1 Lipids

In an o/w emulsion (cf. 8.15.1), the distribution coefficient, K, for aroma compounds is related to aroma activity:

$$K = \frac{C_o}{C_w} \qquad (5.37)$$

where C_o is the concentration of the aroma compound in the oil phase, and C_w the concentration of the aroma compound in the aqueous phase.
In a homologous series, e. g., n-alkane alcohols (cf. Fig. 5.34), the value of K increases with increasing chain length. The solubility in the fat or oil phase rises proportionally as the hydrophobic-

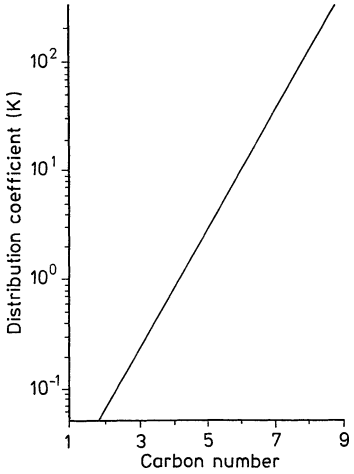


Fig. 5.34. Distribution of n-alkanols in the system oil/water (according to *McNulty* and *Karel*, 1973)

ity imposed by chain length increases. The vapor pressure behavior is exactly the reverse; it drops as the hydrophobicity of the aroma compounds increases. The vapor pressure also drops as the volume of the oil phase increases, and the odor threshold value increases at the same time. This is well clarified in Fig. 5.35. The solubility of 2-heptanone is higher in whole milk than in skim milk which, in this case, behaves as an aqueous phase. When this phase is replaced by oil (Fig. 5.35), 2-heptanone concentration in the gas phase is the lowest.

Experiments with n-alcohols demonstrate that, with increasing chain length of volatile compounds, the migration rate of the molecules from oil to water phase increases. An increase in oil viscosity retards such migration.

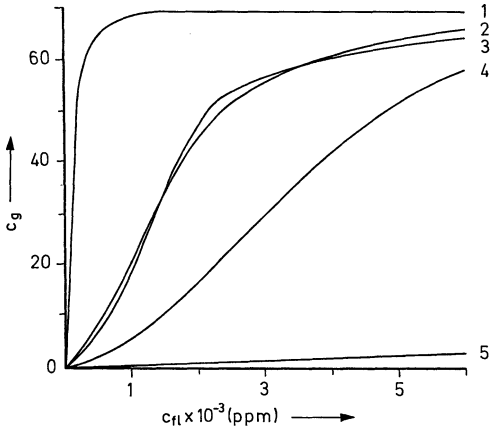


Fig. 5.35. Influence of the medium on 2-heptanone concentration in the gas phase (according to *Nawar*, 1966). 2-Heptanone alone (*1*), in water (*2*), in skim milk (*3*), in whole milk (*4*), in oil (*5*). c_{fl}: concentration in liquid; c_g: concentration in gas phase (detection signal height from headspace analysis)

5.4.2 Proteins, Polysaccharides

The sorption characteristics of various proteins for several volatile compounds are presented in Fig. 5.36. Ethanol is bound to the greatest extent, probably with the aid of hydrogen bonds. The binding of the nonpolar aroma compounds probably occurs on the hydrophobic protein surface regions. A proposal for the evaluation of data on the sorption of aroma volatiles on a biopolymer (protein, polysaccharide) is based on the law of mass action. When a biopolymer, B, has a group which attracts and binds the aroma molecule, A, then the following equation is valid:

$$K = \frac{(\mathrm{BA})}{c_\mathrm{f}(\mathrm{B})} \tag{5.38}$$

where K = a single binding constant; and c_f = concentration of free aroma compound molecules.

$$[\mathrm{BA}] = K \cdot c_\mathrm{f} \cdot (\mathrm{B}) \tag{5.39}$$

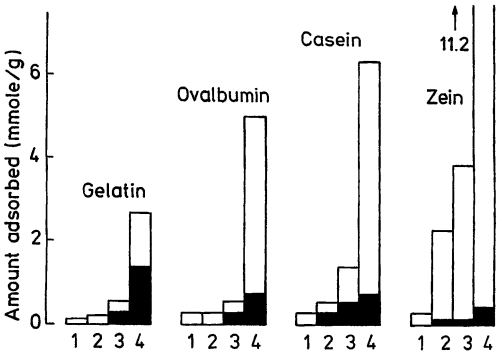


Fig. 5.36. Sorption of volatile compounds on proteins at 23 °C (according to *Maier*, 1974).
Hexane (*1*), ethyl acetate (*2*), acetone (*3*), ethanol (*4*). □ plus ■: maximal sorption, ■: after desorption

To calculate the average number of aroma molecules bound to a biopolymer, the specific binding capacity, r, has to be introduced:

$$r = \frac{(\mathrm{BA})}{(\mathrm{B}) + (\mathrm{BA})} \tag{5.40}$$

The concentration of the complex BA from Equation 5.39 is substituted in Equation 5.40:

$$r = \frac{K \cdot c_\mathrm{f}(\mathrm{B})}{(\mathrm{B}) + K(\mathrm{B})c_\mathrm{f}} = \frac{Kc_\mathrm{f}}{1 + Kc_\mathrm{f}} \tag{5.41}$$

When a biopolymer binds not only one molecular species (as A in the above case) but has a number (n) of binding groups (or sites) equal in binding ability and independent of each other, then r has to be multiplied by n, and Equation 5.41 acquires the form:

$$r = \frac{n \cdot Kc_\mathrm{f}}{1 + Kc_\mathrm{f}} \tag{5.42}$$

$$\frac{r}{c_\mathrm{f}} = K \cdot n - K \cdot r = K' - K \cdot r \tag{5.43}$$

where K' = overall binding constant.

The evaluation of data then follows Equation 5.43 presented in graphic form, i. e. a diagram of $r/C_\mathrm{f} = f(r)$. Three extreme or limiting cases should be observed:

a) A straight line (Fig. 5.37, a) indicates that only one binding region on a polymer, with n binding sites (all equivalent and independent from each other) is involved. The values n and K′ are obtained from the intersection of the straight line with the abscissa and the ordinate, respectively.

b) A straight line parallel to the abscissa (Fig. 5.37, b) is obtained when the single binding constant, K, is low and the value of n is very high. In this special case, Equation 5.48 has the form:

$$r = K' \cdot c_\mathrm{f} \tag{5.44}$$

c) A curve (Fig. 5.37, c) which in approximation is the merging of two straight lines, as shown separately (Fig. 5.37, d). This indicates two binding constants, K_1', and K_2', and their respective binding groups, n_1 and n_2, which are equivalent and independent of each other.

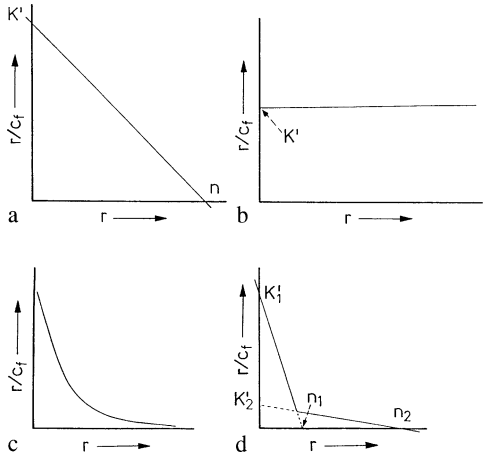


Fig. 5.37. Binding of aroma compounds by biopolymers. Graphical determination of binding parameters according to *Solms*, 1975

By plotting r versus c_f, values of K' are obtained from the slope of the curve. An example for a model system with two binding regions (case c) is given by aroma binding to starch. It should be remembered that starch binds the volatiles only after gelatinization by trapping the volatiles in its helical structure, and that starch is made up of two constituents, amylose and amylopectin. The binding parameters are listed for some aroma compounds in Table 5.37. Numerous observations indicate that K_1' and binding region n_1 are related to the inner space of the helix, while K_2' and the n_2 region are related to the outer surface of the helix. K_1' is larger than K_2', which shows that, within the helix, the aroma compounds are bound more efficiently to glucose residues of the helix. The fraction $1/n$ is a measure of the size of the binding region. It decreases, as expected, with

Table 5.37. Binding of aroma compounds by potato starch

Compounds	Binding constant			
	K_1'	n_1	K_2'	n_2
1-Hexanol	$5.45 \cdot 10^1$	0.10	–	–
1-Octanol	$2.19 \cdot 10^2$	0.05	$2.15 \cdot 10^1$	0.11
1-Decanol	$1.25 \cdot 10^2$	0.04	$1.29 \cdot 10^1$	0.11
Capric acid	$3.30 \cdot 10^2$	0.07	$4.35 \cdot 10^1$	0.19
Menthone	$1.84 \cdot 10^2$	0.012	8.97	0.045
Menthol	$1.43 \cdot 10^2$	0.007	–	–
β-Pinene	$1.30 \cdot 10^1$	0.027	1.81	0.089

Table 5.38. Binding of aroma compounds by proteins (0.4% solutions at pH 4.5)

Aroma compound	Total binding constant $K' \cdot 10^3$ ($lmol^{-1}$)			
	Bovine serum albumin		Soya protein	
	20 °C	60 °C	20 °C	60 °C
Butanal	9.765	11.362	10.916	9.432
Benzaldehyde	6.458	6.134	5.807	6.840
2-Butanone	4.619	5.529	4.975	5.800
1-Butanol	2.435	2.786	2.100	2.950
Phenol	3.279	3.364	3.159	3.074
Vanillin	2.070	2.490	2.040	2.335
2,5-Dimethyl pyrazine	0.494			
Butyric acid	0.			

Table 5.39. Use of aromas in the production of foods

Product group	Percentage (%)[a]
Non-alcoholic beverages	38
Confectionery	14
Savoury products[b], snacks	14
Bread and cakes	7
Milk products	6
Desserts	5
Ice cream	4
Alcoholic beverages	4
Others	8

[a] Approximate values.
[b] Salty product line like vegetables, spices, meat.

increasing molecular weight of alkyl alcohols, but it is still larger within the helix than on the outer surface. Altogether, it should be concluded that, within a helix, the trapped compound cannot fulfill an active role as an aroma constituent.

An unlimited number of binding sites exist in proteins dissolved or dispersed in water (case b). K' values for several aroma compounds are given in Table 5.38. The value of the constant decreases in the order of aldehydes, ketones, alcohols, while compounds such as dimethylpyrazine and butyric acid are practically unable to bind. In the case of aldehydes, it must be assumed that they can react with free amino- and SH-groups. The high values of K' can reflect other than secondary forces.

Bovine serum albumin and soya proteins are practically identical with regard to the binding of aroma compounds (Table 5.38). Since both proteins have a similar hydrophobicity, it is apparent that hydrophobic rather than hydrophilic interactions are responsible for aroma binding in proteins.

5.5 Natural and Synthetic Flavorings

Aromatized food has been produced and consumed for centuries, as exemplified by confectionery and baked products, and tea or alcoholic beverages. In recent decades the number of aromatized foods has increased greatly. In Germany, these foods account for about 15–20% of the total food consumption. A significant reason for this development is the increase in industrially produced food, which partly requires aromatization because certain raw materials are available only to a limited extent and, therefore, expensive or because aroma losses occur during production and storage. In addition, introduction of new raw materials, e. g., protein isolates, to diversify or expand traditional food sources, or the production of food substitutes is promising only if appropriate aromatization processes are available. This also applies to the production of nutraceuticals (cf. 19.1.3).

Aroma concentrates, essences, extracts and individual compounds are used for aromatization. They are usually blended in a given proportion by a flavorist; thus, an aroma mixture is "composed". The empirically developed "aroma formulation" is based primarily on the flavorist's experience and personal sensory assessment and is supported by the results of a physico-chemical aroma analysis. Legislative measures that regulate food aromatization differ in various countries.

At present, non-alcoholic beverages occupy the first place among aromatized foods (Table 5.39). Of the different types of aroma, citrus, mint and red fruit aromas predominate (Table 5.40).

5.5.1 Raw Materials for Essences

In Germany, up to about 60% of the aromas used for food aromatization are of plant origin and,

Table 5.40. Types of aroma used

Aroma type	Percentage (%)[a]
Citrus	20
Mint	15
Red fruits	11
Vanilla	10.5
Meat	10.5
Spices	8.5
Chocolate	8.5
Cheese	5.5
Nut	2.5
Others	8

[a] Approximate values.

thus, designated as "natural aroma substances". The rest of the aroma compounds are synthetic, but 99% of this portion is chemically identical to their natural counterparts. Only 1% are synthetic aroma compounds not found in nature.

5.5.1.1 Essential Oils

Essential (volatile) oils are obtained preferentially by steam distillation of plants (whole or parts) such as clove buds, nutmeg (mace), lemon, caraway, fennel, and cardamon fruits (cf. 22.1.1.1). After steam distillation, the essential oil is separated from the water layer, clarified and stored. The pressure and temperature used in the process are selected to incur the least possible loss of aroma substances by thermal decomposition, oxidation or hydrolysis.

Many essential oils, such as those of citrus fruits, contain terpene hydrocarbons which contribute little to aroma but are readily autooxidized and polymerized ("resin formation"). These undesirable oil constituents (for instance, limonene from orange oil) can be removed by fractional distillation. Fractional distillation is also used to enrich or isolate a single aroma compound. Usually, this compound is the dominant constituent of the essential oil. Examples of single aroma compounds isolated as the main constituent of an essential oil are: 1,8-cineole from eucalyptus, 1(−)-menthol from peppermint, anethole from anise seed, eugenol from clove, or citral (mixture of geranial and neral, the pleasant odorous compounds of lemon or lime oils) from litseacuba.

5.5.1.2 Extracts, Absolues

When the content of essential oil is low in the raw material or the aroma constituents are destroyed by steam distillation or the aroma is lost by its solubility in water, then the oil in the raw material is recovered by an extraction process. Examples are certain herbs or spices (cf. 22.1.1.1) and some fruit powders. Hexane, triacetin, acetone, ethanol, water and/or edible oil or fat are used as solvents. Good yields are also obtained by using liquid CO_2. The volatile solvent is then fully removed by distillation. The oil extract (resin, absolue) often contains volatile aroma compounds in excess of 10% in addition to lipids, waxes, plant pigments and other substances extractable by the chosen solvent. Extraction may be followed by chromatographic or counter-current separation to isolate some desired aroma fractions. If the solvent used is not removed by distillation, the product is called an extract. The odor intensity of the extract, compared to the pure essential oil, is weaker for aromatization purposes by a factor of 10^2 to 10^3.

5.5.1.3 Distillates

The aroma constituents in fruit juice are more volatile during the distillation concentration process than is the bulk of the water. Hence, the aroma volatiles are condensed and collected (cf. 18.2.10). Such distillates yield highly concentrated aroma fractions through further purification steps.

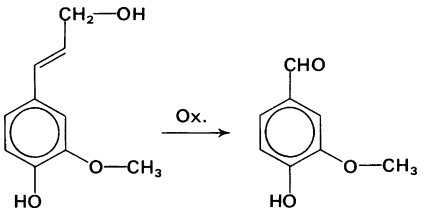
5.5.1.4 Microbial Aromas

Cheese aroma concentrates offered on the market have an aroma intensity at least 20-fold higher than that of normal cheese. They are produced by the combined action of lipases and *Penicillium roqueforti* using whey and fats/oils of plant origin as substrates. In addition to C_4–C_{10} fatty acids, the aroma is determined by the presence of 2-heptanone and 2-nonanone.

5.5.1.5 Synthetic Natural Aroma Compounds

In spite of the fact that a great number of food aroma compounds have been identified, economic factors have resulted in only a limited number of them being synthesized on a commercial scale. Synthesis starts with a natural compound available in large amounts at the right cost, or with a basic chemical. Several examples will be considered below.

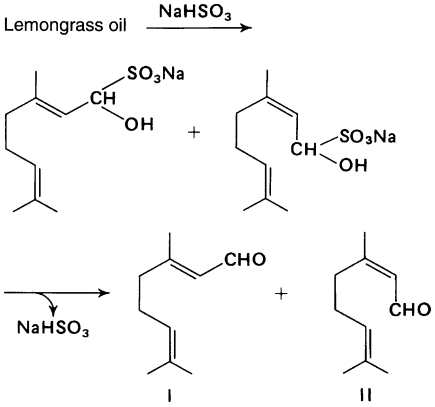
A most important aroma compound worldwide, vanillin, is obtained primarily by alkaline hydrolysis of lignin (sulfite waste of the wood pulp industry), which yields coniferyl alcohol. It is converted to vanillin by oxidative cleavage:



(5.45)

A distinction can be made between natural and synthetic vanillin by using quantitative ^{13}C analysis (cf. 18.4.3). The values in Table 5.41 show that the ^{13}C distribution in the molecule is more meaningful than the ^{13}C content of the entire molecule.

The most important source of citral, used in large amounts in food processing, is the steamdistilled oil of lemongrass (*Cymbopogon flexuosus*). Citral actually consists of two geometrical isomers: geranial (I) and neral (II). They are isolated from the oil in the form of bisulfite adducts:



(5.46)

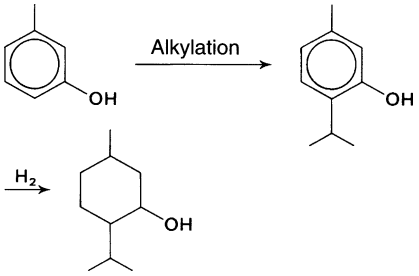
The aroma compound menthol is primarily synthesized from petrochemically obtained m-cresol.

Table 5.41. Site-specific ^{13}C isotopic analysis of vanillin from different sources

Source	R (%)[a] in			R(%)[a] total
	CHO	Benzene ring	OCH_3	
Vanilla	1.074	1.113	1.061	1.101
Lignin	1.062	1.102	1.066	1.093
Guaiacol	1.067	1.102	1.026	1.089

[a] R ($^{13}C/^{12}C$) was determined by site-specific natural isotope fractionation NMR (SNF-NMR). Standard deviation: 0.003–0.007.

Thymol is obtained by alkylation and is then further hydrogenated into racemic menthol:



(5.47)

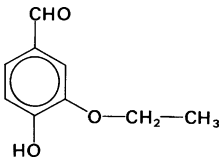
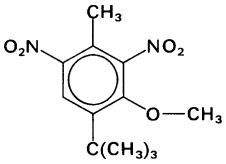
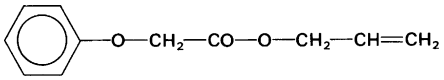
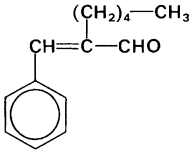
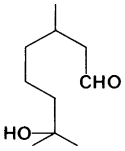
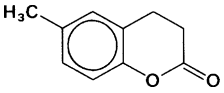
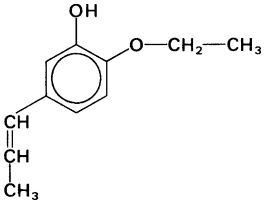
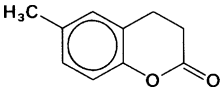
A more expensive processing step then follows, in which the racemic form is separated and 1(–)-menthol is recovered. The d-optical isomer substantially decreases the quality of the aroma (cf. 5.3.2.4).

The purity requirement imposed on synthetic aroma substances is very high. The purification steps usually used are not only needed to meet the stringent legal requirements (i. e. beyond any doubt safe and harmless to health), but also to remove undesirable contaminating aroma compounds. For example, menthol has a phenolic off-flavor note even in the presence of only 0.01% thymol as an impurity. This is not surprising since the odor threshold value of thymol is lower than that of 1(−)-menthol by a factor of 450.

5.5.1.6 Synthetic Aroma Compounds

Some synthetic flavorings which do not occur in food materials are compiled in Table 5.42. Except for ethyl vanillin, they are of little importance in the aromatization of foods.

Table 5.42. Synthetic Flavoring Materials (not naturally occurring in food)

Name	Structure	Aroma description
Ethyl vanillin		Sweet like vanilla (2 to 4-times stronger than vanillin)
Ethyl maltol	cf. 5.3.1.2	Caramel-like
Musk ambrette		Musk-like
Allyl phenoxyacetate		Fruity, pineapple-like
α-Amyl cinnamic-aldehyde		Floral, jasmin and lilies
Hydroxycitronellal		Sweet, flowery, liliaceous
6-Methyl coumarin		Dry, herbaceous
Propenylguaethol (vanatrope)		Phenolic, anise-like
Piperonyl isobutyrate		Sweet, fruity, like berry fruits

5.5.2 Essences

The flavorist composes essences from raw materials. In addition to striving for an optimal aroma, the composition of the essence has to meet food processing demands, e. g., compensation for possible losses during heating. The "aroma formulation" is an empirical one, developed as a result of long experience dealing with many problems, disappointments and failures, and

is rigorously guarded after the "know-how" is acquired.

5.5.3 Aromas from Precursors

The aroma of food that has to be heated, in which the impact aroma compounds are generated by the *Maillard* reaction, can be improved by increasing the levels of precursors involved in the reaction. This is a trend in food aromatization. Some of the precursors are added directly, while some precursors are generated within the food by the preliminary release of the reaction components required for the *Maillard* reaction (cf. 4.2.4.4). This is achieved by adding protein and polysaccharide hydrolases to food.

5.5.4 Stability of Aromas

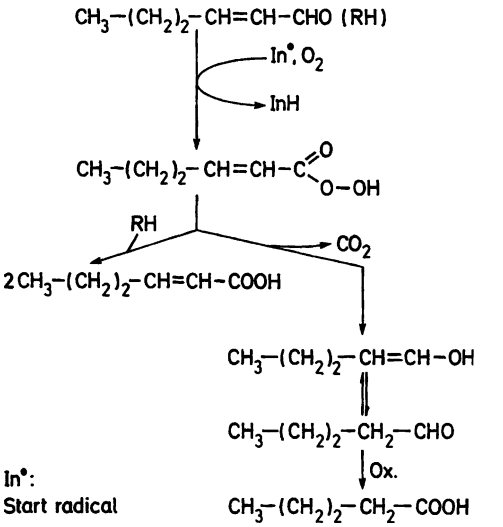
Aroma substances can undergo changes during the storage of food. Aldehydes and thiols are especially sensitive because they are easily oxidized to acids and disulfides respectively. Moreover, unsaturated aldehydes are degraded by reactions which will be discussed using (E)-2-hexenal and citral as examples. These two aldehydes are important aromatization agents for leaf green and citrus notes. (Z)-3-Hexenal, an important contributor to the aroma of freshly pressed juices, e. g., orange and grapefruit (cf. 18.1.2.6.3), is considerably more instable than (E)-2-hexenal (Table 5.43) and, consequently, hardly finds application in aromatization.

Table 5.43. Half-life periods in the degradation of C_6 and C_7 aldehydes in different solvents at 38 °C[a]

Aldehyde	Water/ Ethanol (8 + 2, v/v)	Buffer[b]/ Ethanol (8 + 2, v/v)	Triacetin
n-Hexanal	100	91	86
(E)-2-Hexenal	256	183	71
(Z)-3-Hexenal	42	36	26
n-Heptanal	79	76	73
(E)-2-Heptenal	175	137	57
(Z)-4-Heptenal	200	174	64

[a] The half-life period is given in hours.
[b] Na-citrate buffer of pH 3.5 (0.2 mol/l).

In an apolar solvent, e. g., a triacylglycerol, (E)-2-hexenal decreases much more rapidly than in a polar medium in which its stability exceeds that of hexanal (Table 5.43). It oxidizes mainly to (E)-2-hexenoic acid, with butyric acid, valeric acid and 2-penten-1-ol being formed as well. The reaction pathway to the C_{6-} and C_{5-} acids is shown in Formula 5.48.



(5.48)

At the acidic pH values found in fruit, autoxidation decreases, (E)-2-hexenal preferentially adds water with the formation of 3-hydroxy-hexanal. In addition, the double bond is iso-merized with the formation of low concentrations of (Z)-3-hexenal. As a result of its low threshold value, (Z)-3-hexenal first influences the aroma to a much greater extent than 3-hydroxyhexanal which has a very high threshold (cf. 18.1.2.6.3).
Citral is also instable in an acidic medium, e. g., lemon juice. At citral equilibrium, which consists of the stereoisomers geranial and neral in the ratio of 65:35, neral reacts as shown in Formula: 5.49. It cyclizes to give the labile p-menth-l-en-3,8-diol which easily eliminates water, forming various p-menthadien-8-ols. This is followed by aromatization with the formation of p-cymene, p-cymen-8-ol, and α,p-dimethylstyrene. p-Methylacetophenone is formed from the last mentioned compound by oxidative cleavage of the Δ^8-double bond. Together with p-cresol, p-methylacetophenone contributes apprecia-

bly to the off-flavor formed on storage of lemon juice. Citral is also the precursor of p-cresol.

In citrus oils, limonene and γ-terpinene are also attacked in the presence of light and oxygen. Carvone and a series of limonene hydroperoxides are formed as the main aroma substances.

5.5.5 Encapsulation of Aromas

Aromas can be protected against the chemical changes described in 5.5.4 by encapsulation. Materials suitable for inclusion are polysaccharides, e. g., gum arabic, maltodextrins, modified starches, and cyclodextrins. The encapsulation proceeds via spray drying, extrusion or formation of inclusion complexes. For spray drying, the aroma substances are emulsified in a solution or suspension of the polysaccharide, which contains solutizer in addition to the emulsifying agent.

In preparation for extrusion, a melt of wall material, aroma substances, and emulsifiers is produced. The extrusion is conducted in a cooled bath, e. g., isopropanol.

β-Cyclodextrins, among other compounds, can be used for the formation of inclusion complexes (cf. 4.3.2). Together with the aroma substances, they are dissolved in a water/ethanol mixture by heating. The complexes precipitate out of the cooled solution and are removed by filtration and dried. Criteria for the evaluation of encapsulated aromas are: stability of the aroma, concentration of aroma substance, average diameter of the capsules and, amount of aroma substance adhering to the surface of the capsule.

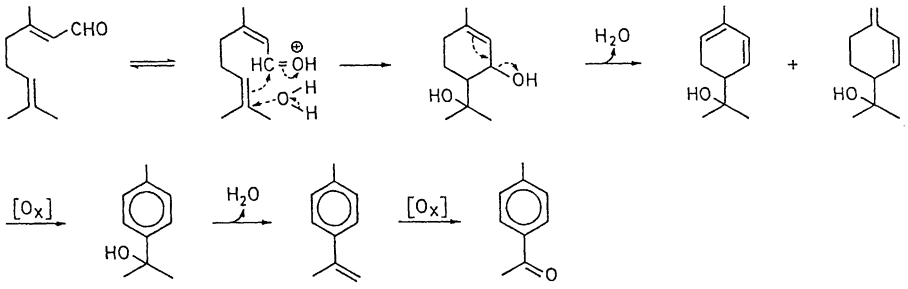
5.6 Relationships Between Structure and Odor

5.6.1 General Aspects

The effect of stimulants on the peripheral receptors of an organism results in responses that are characterized by their *quality* and their *intensity*. The intensity is quantifiable, e. g., by determining odor threshold values (cf. 5.1.3). The quality can be described only by comparison. Odor stimulants can be grouped into those of the same or similar qualities, e. g., compounds with a caramel-like odor (cf. 5.3.1.2 and 5.3.1.3) or roasted smelling N-heterocyclic compounds (cf. 5.3.1.6). The dependence of the odor threshold on the structure is of great interest since the specificity of the odor detection is the reason why aroma substances are only a fraction of the volatile compounds occurring in foods (cf. 5.3). The specificity of the sense of smell will be elucidated by using two classes of compounds as an example. Studies have shown how the odor thresholds in these classes change when the structures are systematically varied. Only odor thresholds in air are used because the influence of a solvent or a solid carrier does not have to be considered.

5.6.2 Carbonyl Compounds

In the series of saturated aldehydes C_5–C_{10}, the odor threshold reaches a minimum with octanal (Table 5.44). An E-configurated double bond in the 2-position raises the odor threshold in the case of the alkenals 5:1 to 8:1 compared with the corresponding alkanals. (E)-2-Nonenal is the ex-



(5.49)

Table 5.44. Odor thresholds (T) in air of alkanals and (E)-2-alkenals

Alkanal	T (pmol/ stimulant)	(E)-2-Alkenal	T (pmol/ stimulant)
5:0	125	5:1	1600
6:0	80	6:1	900
7:0	66	7:1	1250
8:0	4	8:1	100
9:0	7	9:1	0.4
10:0	30	10:1	25

ception with an odor threshold 17.5 times lower than that of nonanal. Decanal and (E)-2-decenal have similar intensive odors. In chiral compounds (cf. 5.2.5 and 5.3.2.4) as well as cis/trans isomers, e. g., C_6 and C_9 aldehydes with a double bond, the molecule geometry influences the odor intensity and quality (Table 5.45).

Except for the pair (E/Z)-6-nonenal, the odor threshold of the E-isomer exceeds that of the corresponding Z-isomer. In particular, the values for (E)- and (Z)-3-hexenal differ greatly. Some of the aldehydes listed in Table 5.44 and 5.45 are formed by the peroxidation of unsaturated fatty acids (cf. 3.7.2.1.9). However, they play a role in aromas only when they are produced in foods in a concentration higher than their odor threshold concentration. The aroma active aldehydes usually include hexanal, which appears as the main product in the volatile fraction of peroxidized linoleic acid and, therefore, can surmount the relatively high odor threshold (Table 5.44). (E)-2-Nonenal also belongs to this group. Although it is formed in considerably lower concentrations than hexanal, it can prevail in aromas due to its very low odor threshold. This also applies to (Z)-3-hexenal which is enzymatically formed from linolenic acid (cf. 3.7.2.3) and has a very low odor threshold. Consequently, it plays a much larger role in aromas, e. g., of fruit and vegetables, olive oil and fish, than its quantitatively more dominant companion substance (E)-2-hexenal.

Table 5.45. Dependence of the odor thresholds (T) of C_6–C_9 aldehydes on the position and geometry of the double bond

C_6-Aldehyde	T (pmol/ stimulant)	C_9-Aldehyde	T (pmol/ stimulant)
(E)-2–6:1	900	(E)-2–9:1	0.4
(Z)-2–6:1	600	(Z)-2–9:1	0.014
(E)-3–6:1	>400	(E)-3–9:1	0.5
(Z)-3–6:1	1.4	(Z)-3–9:1	0.2
(E)-4–6:1	77	(E)-4–9:1	9
		(Z)-4–9:1	1.6
		(E)-5–9:1	70
		(E)-6–9:1	0.05
		(Z)-6–9:1	1.3

5.6.3 Alkyl Pyrazines

The following example illustrates how pronounced the specificity of the sense of smell can be in cyclic compounds. The relationship between structure and odor activity was tested with 80 alkyl pyrazines. A part of the results is shown in Fig. 5.38.

In the series of mono-, di-, tri- and tetramethylpyrazines P1–P6, trimethylpyrazine (P5) shows the highest aroma activity. In the transition from dimethylpyrazines to trimethylpyrazine, the odor quality changes from nutty to earthy/roasted. If the methyl group in the ring position 2 of P5 is substituted by an ethyl group, P7 is formed, which has an odor threshold approximately 6000 times lower and an unchanged odor quality. If the ethyl group moves to the 3- (P8) or 5-position (P9), the odor threshold increases substantially. It increases even more if the ethyl group is substituted by a propyl group (P10–P12). An ethenyl group in position 2 instead of the ethyl group gives P13, but the odor threshold remains as low as with P7. If the ethenyl group moves round the ring (P14, P15), the threshold value again increases substantially. The insertion of a second ethyl group in position 3 of P7 and P13 changes neither the threshold value nor the odor quality in P16 and P17 respectively. However, if the methyl group in position 2 of P14 or in position 3 of P15 is replaced by an ethyl group, the resulting pyrazines P18 and P19 have very high threshold values. A comparison between P17 and P18 shows that whether the ethenyl group is in position 2 or 3 of ethenyl-ethyl-5-methylpyrazines is very important for the contact of the alkyl pyrazines with the odor receptor. If the methyl and ethyl group in P19 exchange positions, P20 is formed and the

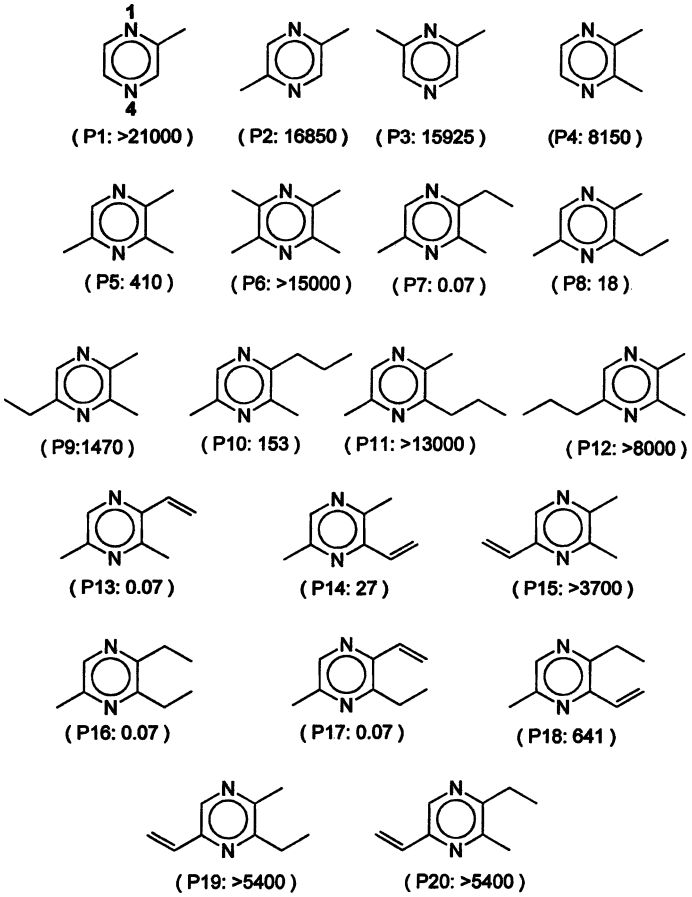


Fig. 5.38. Odor thresholds of alkyl pyrazines (according to *Wagner* et al., 1999). The odor threshold in pmol/l air is given in brackets

odor threshold remains very high. Seventy alkyl pyrazines have been identified in foods. However, in dilution analyses, the compounds which appear with a high odor intensity are only P7 and P16 in addition to P5, P13 and P17 (cf. 5.3.1.7). This is explainable by the specificity of odor detection of alkyl pyrazines discussed here.

5.7 References

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