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Identification of new key aroma compounds in roasted sesame seeds with emphasis on sulfur components

Hitoshi Tamura

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Vorabveröffentlichungen

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List of abbreviations and trivial names

AEDA	aroma extract dilution analysis
BHT	2,6-di-*tert*-butyl-4-methylphenol
CI	chemical ionization
DMAP	*N*,*N*-dimethyl-4-aminopyridine
DMF	*N*,*N*-dimethylformamide
DMSO	dimethyl sulfoxide
EI	electron impact ionization
FD-factor	flavor dilution-factor
FFAP	free fatty acid phase
FID	flame ionization detector
FVP	flash vacuum pyrolysis
GC	gas chromatograph, gas chromatography
GC-MS	gas chromatography-mass spectrometry
GC-O	gas chromatography-olfactometry
HPLC	high performance liquid chromatography
HRGC	high resolution gas chromatography
HRGC-O	high resolution gas chromatography-olfactometry
HRGC-MS	high resolution gas chromatography-mass spectrometry
ITD	ion trap detector
MCSS-system	moving column stream switching system
MS	mass spectroscopy, mass spectrum
NMR	nuclear magnetic resonance
NOESY	nuclear Overhauser effect spectroscopy
OAV	odor activity value
Pyrogallol	benzene-1,2,3-triol
RI	retention index
SAFE	solvent assisted flavor evaporation
SIDA	stable isotope dilution assay
THF	tetrahydrofuran

1 Introduction

1.1 Sesame seeds

1.1.1 Botany of sesame

Sesame belongs to the *Sesamum* genus under the family Pedaliaceae. The *Sesamum* genus has approximately 30–40 species, most of which are wild species and naturally grow in the African savanna. *Sesamum indicum* L. is almost only one cultivated species, and therefore, the commercially available sesame for edible use is generally *Sesamum indicum* L. The Swedish naturalist Carl von Linne (Carolus Linnaeus, 1707–1778) named this species *Sesamum indicum* L., because he thought that the origin of sesame was India. However, in the literature, the origin of sesame has been a matter of discussion for more than one century among hundreds of writers. Along with the Indian subcontinent, the other candidate is central African savanna, because many wild sesame species grow there and show much variability. (*Kobayashi, 1986 and 1989a; Namiki, 1995; Bedigian, 2011a*).

Sesame (**Figure 1**) is an erect annual herbaceous plant which is 50–200 cm tall, and has simple or branched stems with opposite or alternate leaves at each node. Its flowers, which are bell-shaped or tubular, labiate, pink or white colored, and about 3–5 cm long come out at axils. They blossom at least 30–40 days after the sowing, and the pollination is completed soon after blooming. The fruit which is 1.5–5 cm long is an oblong capsule with small seeds inside. After maturity, the fruits burst open along the line between carpel, and the seeds are forcibly scattered. The Arabic incantation “open sesame” in the Arabian Nights Entertainment might have originated from this movement of the sesame seeds capsule opening. Each of the capsules contains 50–100 or more oval-, pear-, or teardrop-shaped seeds. The seed is 1.5–4.0 mm long, 1.0–2.0 mm wide, and 0.5–1.0 mm thick, and the weight is 1.8–5.0 g/1000 seeds. The seed color varies from white, yellow, gray, brown, violet to black. Black sesame commonly has a thick and rough seed coat, while white sesame seeds have a thin and smooth one.

Figure 1: *Sesamum indicum* L. (taken from Köhler‘s Medizinal-Pflanzen, *Köhler, 1887*).

Sesame is cultivated in the tropical and subtropical zone between latitudes of approximately 45º north and 45º south, for example, India, Myanmar, China, and Sudan. The cultivation requires a frost-free growing period, and it is favorable that the average daily temperature is around 25 ºC. The seed can germinate at a soil temperature of about 20 ºC, and the plant matures mostly in 110 days or less. It is necessary to harvest the fruits containing the seeds when the fruits take on a yellow tinge, or they burst open (*Kobayashi, 1989a; Namiki, 1995; Bedigian, 2011b*).

1.1.2 World production

Data from the *Food and Agriculture Organization of the United Nations* (*2008*) report a world *sesame seed* production of around 3.5 million metric tons in 2008 (**Table 1**), with an increasing tendency in the past fifty years (**Figure 2**).

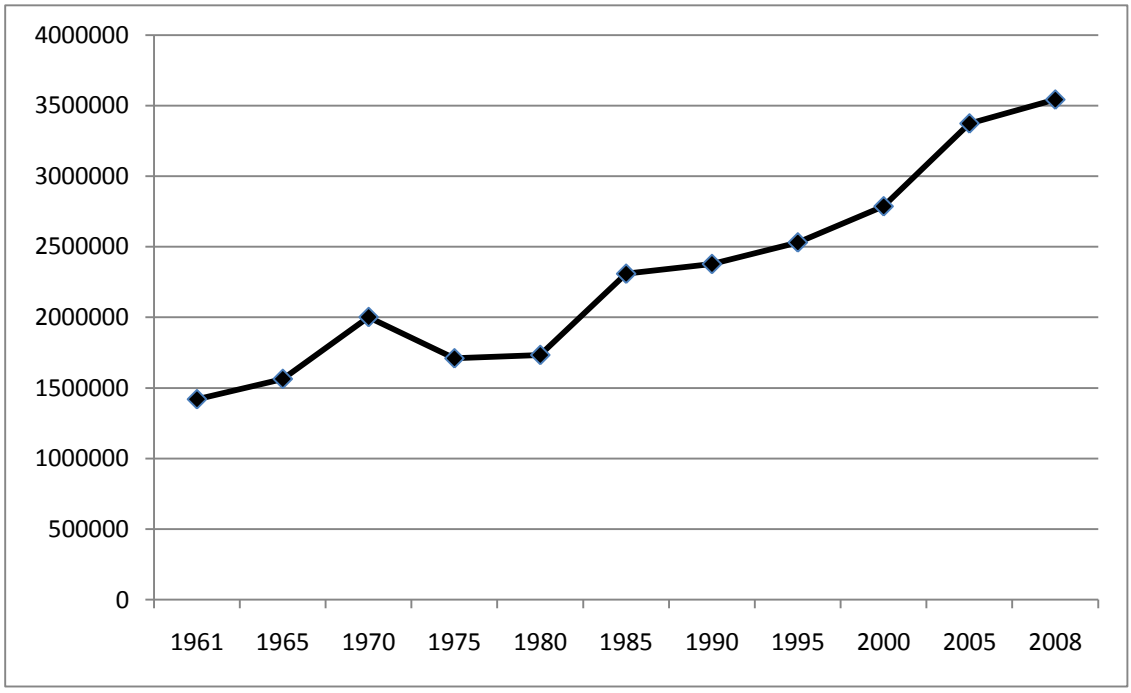


Figure 2: Annual worldwide production of sesame seeds since 1961 (*Food and Agriculture Organization, 2008*).

The major sesame seed producing countries (2008) are India, Myanmar, China, and Sudan (**Table 1**). These four countries cover 62% of the world total production of 3.5 million metric tons.

Table 1: Worldwide production of sesame seeds in 2008 (*Food and Agriculture Organization, 2008*).

country	production [t]	country	production [t]
India	640,000	Uganda	173,000
Myanmar	620,000	Nigeria	110,000
China	586,408	Paraguay	50,049
Sudan	350,000	Niger	50,646
Ethiopia	186,772	Central African Republic	49,027
		total	3,542,129

Like soybean and rapeseed, sesame is in the category of an oilseed, because the main use of sesame seeds is to produce edible oil. World total productions of main oilseeds are listed in **Table 2**. Among them, soybean shows the most quantity of production, followed by cotton seed, rapeseed, and peanut. The production of sesame seeds is approximately only one-tenth of sunflower seed and one-seventieth of soybean.

However, most of the oilseeds have other faces for use. Although soybean is the most produced oil seed among them (**Table 2**), it is also needed as a source of protein or a feed crop. Cotton seeds are a by-product of cotton yarn, which is a more important commodity. Peanut is popular because of its characteristic flavor as well as sesame. In the case of rapeseeds and sunflower seeds, their productivities have been improved using the means of cultivar improvement, while the productivity of sesame seeds still has difficulties because most of the sesame-producing countries are less developed countries (**Table 1**), and therefore, their investment in agriculture is limited. Even if the sesame seed production is not higher than the other oilseeds due to its lower productivity, sesame is, however, consumed through the ages in whichever form of oil or seed itself because of its pleasant unique flavor, which is favored especially in Africa and all over Asia (*Nishino, 1989*).

Table 2: Worldwide productions of main oilseeds in 2008 (*Food and Agriculture Organization, 2008*).

oilseed	production [t]	country	production [t]
soybean	230,581,106	sunflower seed	35,657,834
cotton seed	65,423,810	sesame seed	3,542,129
rapeseed	58,061,092	linseed	2,170,639
peanut	38,216,299	castor seed	1,603,772

1.1.3 Use of sesame seeds as food

Raw sesame seeds elicit only a weak grassy odor, but the oil obtained by pressing is used as valuable cooking and frying oil, especially in the Asian cuisine. However, for other sesame products the seeds are roasted resulting in a unique and highly attractive odor characterized by sulfurous, roasty, nutty and meaty notes.

In Europe and in the United States the roasted seeds are a popular topping for baked wheat flour products, such as biscuits, breads, and crackers, while in Asia the oil isolated from the roasted seeds is used as a seasoning or cooking oil in many dishes and also the roasted seeds are consumed in various forms, e.g., ground, paste, or just as it is. In the Middle East countries, sesame is consumed predominantly as a paste, for example 'Tahini' which is used as the base ingredient for a variety of the local cuisines. In India, sesame paste seasoned with condiments or spices is served together with baked bread named chapatti or naan. In East Asia the black sesame variety is also popular as well as the white one. In China, roasted sesame oil is mainly used to fry vegetable, meat, or fish. Another popular Chinese dish is a steamed flour dumpling, which contains a mixture of sesame paste, fats, and sugar and coated by roasted sesame seeds.

In Japan, intact roasted sesame seeds are frequently added onto a rice ball, which is made by rolling and pressing rice by hand to a palm-size so that it is portable and easy to eat. Although it is a typical way in Japan to grind roasted sesame seeds in a conical ceramic mortar by a wooden pestle, occasionally they are just pressed with fingers or cut with a kitchen knife on a cutting board as a simple method to increase its flavor. Sesame paste, which is made by fine grinding of the roasted seeds, is also utilized for products like a dressing for salad or sauce to dip boiled meat. The preferred way in Japan is to grind them by a small pestle in a small mortar just before consumption in order to ensure a fresh aroma. *Awazuhara* (*1980*) reported that the pleasant aroma of ground roasted sesame seeds was not preserved for a long period of time after they were ground or roasted (*Kobayashi, 1989a and 1989b; Namiki, 1995; Takeda and Fukuda, 1996*).

1.2 Composition of sesame seeds

1.2.1 Nutritional value

The major constituents of sesame are approximately 50% lipids, 18% proteins, 23% carbohydrates, and 12% total dietary fiber (*USDA National Nutrient Database, 2011*).

A half the weight of sesame seeds is fat, which consists of triacylglycerols (95%), diacylglycerols (2.6%), free fatty acids (0.1%), and other lipids including phospholipids and unsaponifiables (2.3%). Fatty acids composition of the sesame oil includes mainly oleic (35–50%) and linoleic acids (35–50%), with lower amounts of palmitic (2–7%) and stearic acids (7–17%) but only trace amounts of linolenic acid (*Namiki, 1995; Kamal-Eldin, 2011*).

The content of protein in sesame is approximately 18–20%. The amino acid composition of sesame seeds is shown in **Table 3**. Sesame is a rich amino acid source, for example it has abundant essential amino acids such as glutamic acid, arginine, leucine, and glycine (*Namiki, 1995; Shahidi and Tan, 2011*). However, the sesame intake is usually less than the proposed amount of daily amino acid intake (*FAO/WHO/UNU, 2007*). For example, to eat the recommended value of leucine for a person of 70 kg weight, a consumption of 200 g sesame seeds per a day would be needed, and it is unrealistic in a normal food life.

Table 3: Amino acid composition of sesame seed (taken from *FAO, 1981*).

amino acid	concentration (mg/100 g sesame seeds)	amino acid	concentration (mg/100 g sesame seeds)
isoleucine	773	arginine	2586
leucine	1433	histidine	523
lysine	585	alanine	964
methionine	602	aspartic acid	1754
cystine	386	glutamic acid	4148
phenylalanine	947	glycine	1043
tyrosine	667	proline	790
threonine	763	serine	995
tryptophan	–	total essential amino acid	7428
valine	985		
		total amino acid	20233

Sesame contains about 23% of carbohydrates, in which 3.6% D-glucose and 3.4% D-fructose were reported. As minor constituents of sesame carbohydrates D-galactose (0.4%), sucrose (0.2%), and raffinose (0.6%) were also measured (*Wankhede and Tharanathan, 1976*).

In roasted sesame seeds, a considerable level of the B vitamins is present (thiamine: 0.49 mg/100 g, riboflavin: 0.23 mg/100 g, and niacin: 5.3 mg/100 g) (*Subdivision on Resource Study, 2010*). However, the seeds are actually not used as the major supply source of B vitamins in the regular diet due to the low consumption of sesame seeds.

The tocopherol content (vitamin E) in roasted sesame seeds was reported to be around 24 mg/100 g (α-: 0.1, β-: 0.2, γ-: 23.4, and δ-: 0.4 mg/100 g) (*Subdivision on Resource Study, 2010*). The tocopherol found in sesame is mostly γ-tocopherol, and the α-tocopherol content is smaller. Although there is a report that the vitamin E activity of γ-tocopherol is less than about 1/10 of α-tocopherol *in vivo* (*Bieri and Evarts, 1974*), γ-tocopherol has a higher antioxidative activity than α-tocopherol *in vitro*.

Sesame oil has been appreciated for a long time as frying oil, because it is not easily deteriorated even if used at a higher temperature for a long period of time. The γ-tocopherol

might play a part to make sesame oil more resistant to the oxidative degradation (*Yamashita, 1989*).

1.2.2 Antioxidants

In addition to the tocopherols, lignans were investigated as significant antioxidative compounds in sesame seeds. Lignans are defined as low molecular weight compounds formed by the oxidative coupling of *p*-hydroxyphenylpropane units. Sesamin and sesamolin are the major lignans in sesame which have an antioxidative activity and are not found in other edible oils (**Figure 3**).

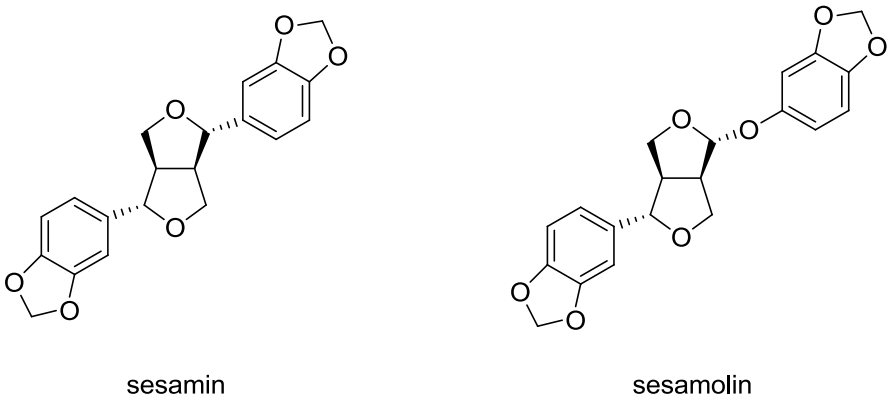


Figure 3: Chemical structure of characteristic major sesame lignans.

Besides the lignans, a phenol, sesamol is also important as an antioxidant in sesame (**Figure 4**). Sesamol has a strong antioxidant activity similar to butyrated hydroxytoluene (BHT) and butyrated hydroxyanisole (BHA) (*Namiki, 1989 and 1995; Shahidi and Tan, 2011*).

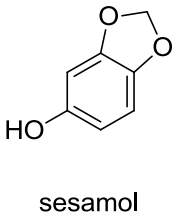


Figure 4: Structure of sesamol.

In conjunction with the nutritional values of sesame, these antioxidants also should contribute to the good reputation of sesame as a healthy food.

1.2.3 Volatile compounds

Many investigations have already been performed on the volatile components of roasted sesame seeds and oil. The first attempts to clarify the compounds involved in the aroma of roasted sesame seeds and oil were performed by Yamanishi's group from the 1960s (*Yamanishi et al., 1960 and 1967; Takei et al., 1969; Kinoshita and Yamanishi, 1973; Soliman et al., 1975*). These studies have led to the identification of 19 volatile components in roasted sesame oil. Most of them were carbonyl compounds. In roasted seeds 28 volatile compounds, mainly pyrazines, were identified. The authors estimated that sulfur-containing compounds, carbonyl compounds and pyrazines might be responsible for the aroma of roasted sesame. They also described that an aroma concentrate of lipid-free sesame seeds did not show the characteristic aroma of roasted sesame seeds.

In 1974, *Manley et al.* reported the positive and tentative identification of 25 pyrazines and the identification of 6 phenols and 7 aldehydes in roasted sesame seeds using the distillation-extraction apparatus developed by *Nickerson and Likens* (*1966*).

In 1985, *Soliman et al.* investigated an effect of antioxidants (sesamin and sesamolin) on the volatile compounds in roasted white sesame seeds. They mentioned that the predominant change by removing the antioxidants was a decrease of 2,4-undecadienal and an increase of 2,4,6-dodecatrienal, and they reported that the addition of sesamin and sesamolin to defatted sesame caused a much stronger roasted sesame-like aroma in its neutral-acidic fraction. They also showed (*Soliman et al., 1986*) that the non-carbonyl fraction of the neutral-acidic fraction from roasted white sesame seeds had the typical roasted sesame seed flavor.

In 1988, *Takei* compared the aroma of roasted sesame and roasted, huskless sesame and newly identified 43 volatile compounds. This literature said that the roasted huskless sesame exhibited only a much weaker sesame-like odor and the roasted husk had just a hay-like odor, and that the mixture of them did not represent the same odor of roasted sesame but hay and slightly sesame-like odor. One year later, *Takei* (*1989*) compared the roasted odor of fractions afforded by the solvent extraction from raw sesame seeds. Sesame seeds were extracted with hexane, the residue was extracted with chloroform, and then with methanol. Every fraction and residue was roasted, however no single fraction but a mixture of the hexane fraction and its residue showed typical roasted sesame-like odor. Consequently, the author concluded that all the fractions were necessary to generate the roasted sesame-like aroma.

In 1989, *Nakamura et al.* identified a total of 221 constituents in roasted sesame oil. Among them, they identified 45 sulfur-containing compounds using flame photometric detector (FPD). The most abundant compound detected with the FPD was newly identified in roasted sesame aroma as 1-methyldithio-2-propanone, which was described as a boiled-cabbage-like and meaty.

In the same year, *El-Sawy et al*. (*1989*) investigated the volatile compounds of black sesame seeds. It was reported that the most abundant peak detected by GC-MS was 2,4-undecadienal in the neutral-acidic fraction and 2-methylpyrazine in the basic fraction.

Thus, to put this section together, during about 30 years from the 1960s the identification of more than three-hundred volatile compounds was reported. However, the sensory contribution of the individual compounds to the overall aroma of roasted sesame was not necessarily taken into account in the studies of this period, probably because a systematic approach to clarify the contributions of aroma compounds was not established at that time.

1.3 Definition of aroma

Humans recognize volatile compounds as an odor, when those molecules are perceived by the olfactory receptors in the nose. The receptors are present in the olfactory epithelium in the nasal cavity. The odorants reach the receptors by two ways: either by the intake air through the nostrils (orthonasal perception), or by the exhaled air through the nasal cavity while chewing and swallowing food (retronasal perception). A nerve impulse from the receptors is generated and transmitted to the brain, only if the concentration of the volatile compound exceeds its odor threshold, which is a specific parameter of each volatile compound and has a broad extent of concentrations depending on the chemical structure of the volatile. Therefore, only the compound which has the concentration above its odor threshold (odor-active compounds) should be able to influence the overall aroma of a given food. Hence, a methodology to differentiate odor-active compounds from 'odor-inactive' ones is of great significance for aroma research.

1.4 Methodology to determine key odorants of food aroma

In former investigations on the volatile components of roasted sesame (cf. **1.2.3**), the application of high resolution gas chromatography-mass spectrometry (HRGC-MS) showed its efficacy by the identification of over 300 volatile compounds existing in sesame. The sensory contribution of individual odorants to the total roasted sesame flavor was, however, not taken into consideration.

Among numerous odor compounds present in food, sometimes only one compound has a responsible odor note for the characteristic aroma of the food, for example the cucumber-like smelling (*E*,*Z*)-2,6-nonadienal, the coffee-like smelling 2-furfurylthiol, the vanilla-like smelling 4-hydroxy-3-methoxybenzaldehyde (vanillin), and so on. These examples indicate that a food aroma does not consist of all volatile components contained but is caused by "key odorants".

Hence, one of the most important questions in aroma researches is to find out these key odorants contributing significantly to the overall aroma of the investigated materials.

Grosch and *Schieberle* (*Grosch, 1993 and 2001; Schieberle, 1995a*) developed a methodology to discover volatile components which predominantly and substantially contribute to food aroma based on combining olfactometry and sensory evaluation techniques with instrumental analyses.

1.4.1 Isolation of volatile components

Analysis of aroma compounds in food starts with isolation of volatile components from the food matrix. Non-volatile components such as fat, protein, or carbohydrates present in food samples must be eliminated before instrumental analyses using gas-chromatography (GC) or GC-mass spectrometry (GC-MS), because GC and GC-MS are designed only for the analyses of volatile compounds, and the injection of non-volatile compounds therefore, may damage the stationary phase, and the analytical data measured by those contaminated instruments is obviously unreliable. Furthermore, the total amount of volatile components present in food is generally quite low (ca. 10–15 mg/kg).

Thus, selection of an appropriate technique to isolate the volatile compounds from the matrix is the first key point in aroma analyses. Careful extraction of the volatile compounds from food without heating using a low-boiling organic solvent (e.g. dichloromethane, ether, or pentane) should be selected. All the organic compounds in the food including the aroma substances and also non-volatile organic components as fat, are supposed to be extracted by this step. And then, the extract is submitted to a distillation in high vacuum to separate a volatile fraction from the non-volatiles. It is critical to ensure that the distillate elicits a representative aroma profile of the original product as applying it on a strip of a filter paper, and the distillate has to show identical or at least very similar aroma profile to that of the investigated food. By this approach, losses of the odor-active compounds and generation of artifacts, which are aroma compounds not contained in the original food aroma but produced during aroma concentrate preparation, are evaluated to the utmost extent.

Certainly, the higher the temperature to extract or distill volatile compounds is used, the higher quantity of volatile components should be available for the instrumental analysis. However, the preparation of the aroma concentrate at the high temperature causes some problems in aroma analyses. First, the volatile compounds might be simply decomposed by the heating process. Second, the heat increases the possibility to form new odorants from precursors still unreacted and contained in the food during the extraction. This kind of the precursors should not be reacted and generate their products.
Schieberle has reported a good example for the second problem (*1995b*). He showed that the concentrations of the roasty smelling 2-acetyltetrahydropyridine and the popcorn-like smelling 2-acetyl-1-pyrroline quantitated in an extract obtained from fresh hot-air popped corn using

simultaneous steam distillation-extraction (SDE) (*Likens and Nickerson, 1964*) were higher by factors of 2.7 and 2.3, respectively, than those from the extract isolated by extraction/vacuum sublimation. This result indicated that heating of unreacted precursors caused formations of these odorants during boiling of the popcorn suspension in the SDE apparatus. Therefore, it indicated that isolation techniques which require higher temperatures, like steam distillation or SDE, were unsuitable for aroma compounds isolation.

To overcome this problem, solvent assisted flavor evaporation (SAFE) (*Engel et al. 1999*, cf. **3.4.1.2**) is one of the solutions. This distillation apparatus is designed to enable high vacuum (approx. 10^{-3}~10^{-4} Pa), and therefore the distillation only needs a lower temperature. Structure of the apparatus combining with a high performance pump like a diffusion pump or a turbo pump permits an immediate evaporation of the volatile compounds after feeding the sample extract, and hence the time of aroma compounds exposed to heat becomes as short as possible. Furthermore, if the distillation flasks are frequently changed during the distillation, the heating to the residue can be limited, and so, the generation of artifacts can be controlled at a lower level. This kind of gentle method with minimized heating to both of the volatiles and the residue are recommended to separate aroma compounds from food matrix.

Using this technique, the volatile part is isolated from the non-volatile material. Subsequently, the solvent in the distillate is gently removed at mild condition to obtain aroma concentrate.

1.4.2 Aroma extract dilution analysis

The aroma concentrate obtained as described above is then analyzed by high resolution gas chromatography-olfactometry (HRGC-O) (*Day et al., 1957; Fuller et al., 1964; Dravnieks and O'Donnell, 1971*). For this instrumental analysis combining a sensory evaluation, the effluent is divided into two equal portions at the end of the capillary column in the GC. One of the divided effluents was led to a flame ionization detector (FID) generally, and another part of the effluent was evaluated by sniffing using a heated sniffing-port at the same time (**Figure 5**).

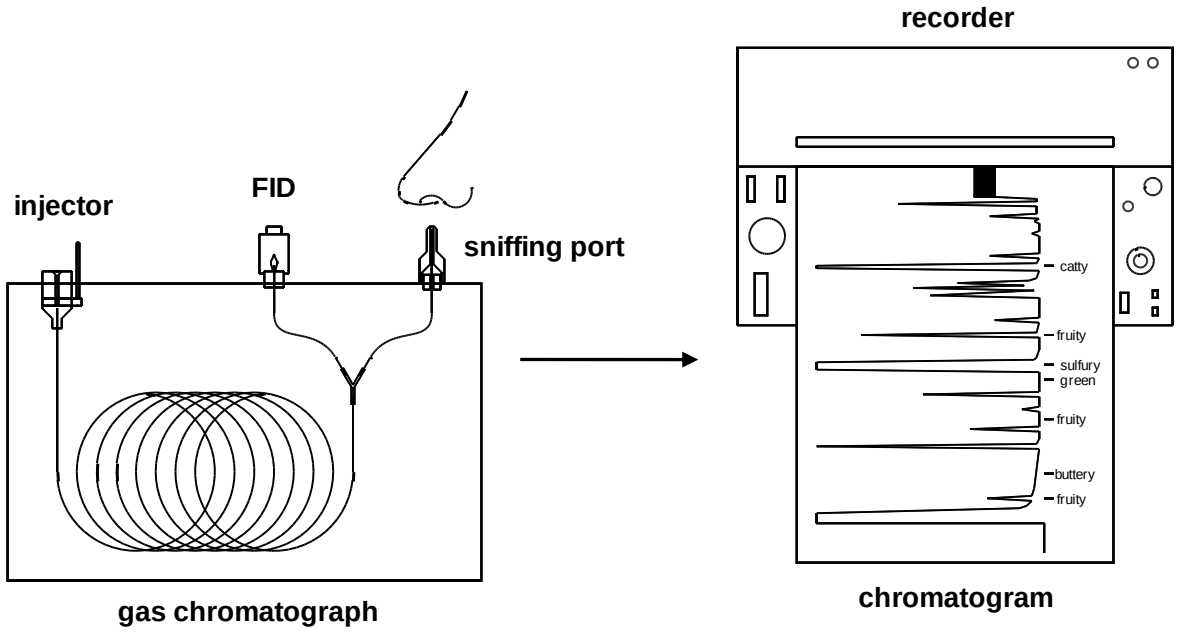


Figure 5: Principle of GC-Olfactometry (taken from *Steinhaus, 2001*).

The perceived odor qualities at the sniffing port are noted at the corresponding elution times directly on the FID-chromatogram paper chart while performing the GC-O analysis (**Figure 5**). By using this technique, it is possible to separate aroma-active compounds from odorless volatile components and to characterize them by their aroma quality as well as by their retention indices. Another profit is that a human nose is a very sensitive detector for aroma-active volatile compounds comparing with detectors commonly used for the GC like the FID, so that highly potent odorants present in very low concentrations can be located, even if the sensitivity of the FID is not sufficient to detect their peaks. The peak-area profile obtained by HRGC-FID or other detectors does not necessarily reflect the aroma profile of the investigated food because it only shows the proportion of the abundances of volatile constituents. Thus, the GC-O is an appropriate approach for aroma research to screen odorless and uninteresting volatiles and, thus, to sort out potent odorants from hundreds of volatile components present in food aromas.

But, the sensory assessment by a single run of the undiluted aroma distillate using HRGC-O has one drawback. The number of odorants detectable by HRGC-O depends not only on the thresholds and the concentrations of the volatiles but also on parameters that can easily be changed consciously or subconsciously, such as the amount of food extracted by solvent, the concentration degree of the aroma extract, and the amount of injected sample into GC, and also the stability of each aroma compound. Eventually, this drawback means that only one run

of HRGC-O is not reliable enough to distinguish between the aroma-active potent odorants that contribute strongly to the target food aroma and the unimportant volatile compounds.

The Osme-technique (*Miranda-Lopez et al., 1992*), in which panelists rate the perceived odor intensity of each compound separated in HRGC-O, also should be inappropriate to rank and compare the relative sensorial contributions of the detected individual odorants to the overall aroma, because of the lower objectivity and the lower accuracy to rate the perceived odor intensity which is subjectively judged, and because the perceived odor intensities of different odorants do not vary in a same manner according to their concentrations (*Dravnieks, 1977*).

To conquer the above mentioned limitations of a single HRGC-O analysis, an extract dilution technique was developed by *Schmid and Grosch* (*1986*) and *Ullrich and Grosch* (*1987*), and established and denominated as an Aroma Extract Dilution Analysis (AEDA) by *Schieberle and Grosch* (*1987a*). The procedure of this technique is as follows: The aroma concentrate isolated from the food material is stepwise diluted with solvent in a volume ratio of 1 to 1, that goes like 1, 2, 4, 8, 16… 1024, and so on. Then, each dilution is analyzed by HRGC-O starting from the original extract in descending concentrations. These GC-O runs of a series of the dilutions are performed until no longer odorants can be perceived at the sniffing port. The highest dilution at which the odorant was smelled is defined as its Flavor Dilution-factor (FD-factor). Consequently, the FD-factor has to be determined for each of the detected aroma-active compounds in the GC-O runs. The FD-factor is not an absolute measure but a relative one, and the ranking of the FD-factors is supposed to be proportional to that of the odor activity values (OAV, cf. **1.4.5**) of aroma compounds in air, if all aroma compounds were exhaustively extracted from the food sample.

As an illustration of the AEDA, its application to elucidate key compounds responsible for moderately roasted white sesame seeds aroma by *Schieberle* (*1996*) is presented hereto. As the result following the procedure written above, 39 aroma-active regions were detected in the FD-factor range of 8 through 1024. The result of the AEDA is usually displayed as a diagram of the FD-factors (y-axis) in a logarithmic scale versus the retention indices (x-axis) which is called a Flavor Dilution chromatogram (FD chromatogram) (**Figure 6**). Based on their FD-factors, the relative importance of odorants to the aroma of roasted white sesame seeds was estimated.

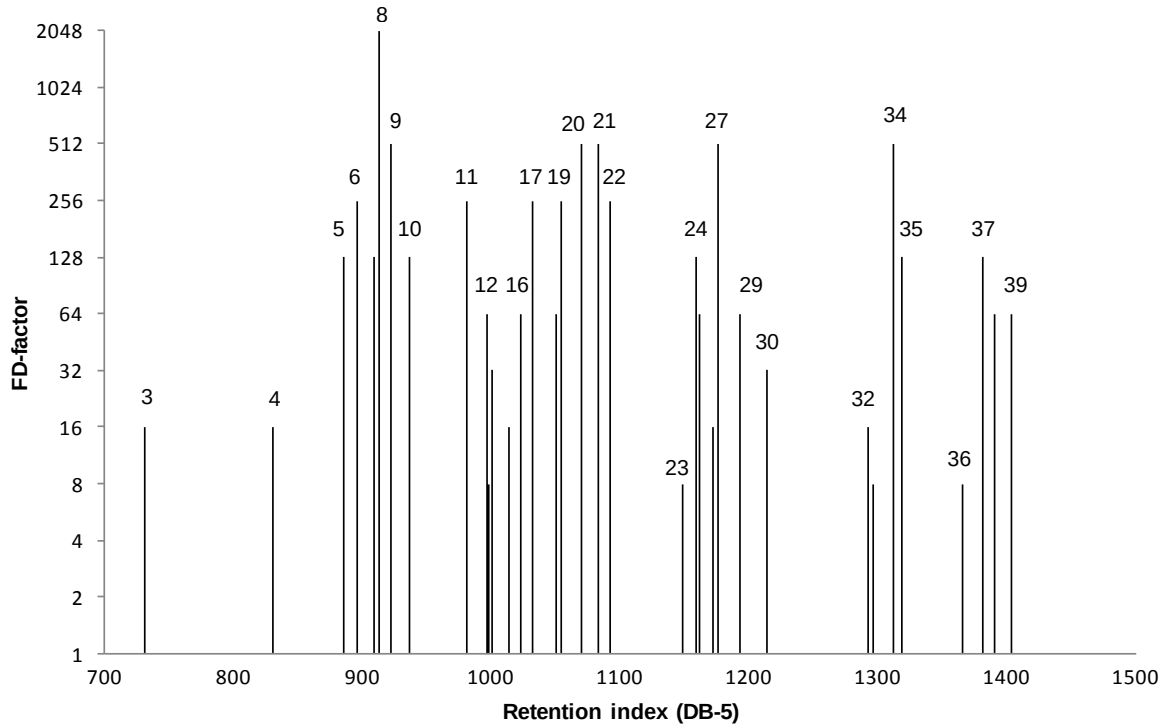


Figure 6: Flavor dilution (FD)-chromatogram of the odor-active compounds in a distillate from moderately roasted sesame seeds (*Schieberle, 1996*).

During the HRGC-O run, the odorants are supposed to be completely volatilized at the sniffing port which is normally kept at 200–250 ºC, so they are evaluated irrespective of their boiling points. This means that high-boiling compounds may be overestimated compared to low-boiling substances. Moreover, since the aroma compounds are isolated from food matrix and separated to individual odorants by HRGC, their solubility in the food matrix as well as their interactions with each other or non-volatile constituents is not taken into account to evaluate their contributions to the overall aroma. For instance, polar compounds isolated from a food with a polar matrix like water are overrated rather than apolar substances. On the other hand, the reverse phenomena ought to take place if the matrix is an apolar one, like oil. Additionally, the yields of the odorants during solvent extraction … high-vacuum distillation also affect the FD-factors, and at the same time, unstable compounds might decompose during those isolation steps. Consequently, highly important compounds can be underestimated because of the lower FD-factors as the result of the loss by the isolation procedure.

For these reasons, the FD-factors obtained by the AEDA do not necessarily indicate which compounds make the greatest contribution to the aroma of food. Hence, the aroma extract dilution analysis (AEDA) must be utilized as a screening method to estimate the likely key

compounds and to concentrate the effort of the identification experiments on the compounds with the higher FD-factors.

As another screening methodology using the "dilution to threshold" concept, the Charm analysis has been developed by *Acree et al.* (*1984*). The term "Charm" is an acronym standing for Combined Hedonic Aroma Response Measurements. In the Charm analysis, the assessor detects not only the odor and its quality but also the duration how long the odorant can be sniffed in the GC-O run, and "charm" values are produced as areas of chromatographic peaks calculated from dilution values (same as the FD-factor) (y-axis) vs. the duration smelled as retention indices (x-axis). So the difference of the Charm analysis from the AEDA is that the duration of the odor in HRGC-O is taken into account. However, basically their results should indicate the same ranking of aroma-active compounds, because both of them calculate the odor activity value in air of each aroma compound in the aroma extract.

The AEDA is supposed to be the most reasonable screening method at the moment to rank the contributions of aroma compounds in the investigated food aroma extract, because to check if the odor can be sniffed is quite an objective way, and the FD-factor of the odorant in the GC-O depends on its concentration in the extract and its odor threshold in air. By this method, only a few dozens of compounds can be suggested as candidates of the key compounds from the vast numbers of volatile components in food. Then, identification experiments can be focused on the aroma-active compounds with the higher FD-factors. Thus, the use of the AEDA allows to focus the identification experiments on volatile components potentially contributing to a food aroma.

1.4.3 Identification experiments

As described in the previous chapter, identification experiments are carried out on volatile constituents with the higher FD-factors as the next step. To confirm that all highly potent odorants were included, the identification experiments should be focused on a somewhat wide FD-factor range of aroma-active compounds, particularly in the 50–100 fold dilution range (*Grosch, 1993*), because the FD-factor has some uncertainties to definitely know the contributions of aroma compounds, as described in **1.4.2**.

For example, *Schieberle* (*1996*) attempted to identify 39 aroma regions in roasted white sesame seeds aroma using a little bit wider FD-factor range 8 through 2048, and then

identified 31 aroma-active compounds among them (**Table 4**). Considering the fact that more than 300 volatile constituents in roasted sesame had been reported (cf. **1.2.3**), the work for the identification of the flavor compounds was reduced to only about a tenth.

Table 4: Odor-active compounds (FD ≥8) in moderately roasted sesame seeds (*Schieberle, 1996*).

No.	odorant [a]	odor quality [b]	RI on		FD-factor
			DB-5	FFAP	
1	2,3-butanedione	buttery	<600	980	8
2	3-methylbutanal	malty	<600	905	128
3	unknown	sulfury, meaty	730	–	16
4	unknown	sulfury, wort-like	830	–	16
5	unknown	meat-like, sulfury	885	–	128
6	unknown	catty	895	–	256
7a	3-(methylthio)propanal (methional)	cooked potato-like	908	1449[c]	128
7b	unknown	roasty, catty	–	–	
8	2-furfurylthiol	roasty, coffee-like	912	1430[c]	2048
9	2-acetyl-1-pyrroline	roasty, popcorn-like	921	1314[c]	512
10	4-methyl-3-thiazoline	garlic, carbide-like	936	1438	128
11	1-octen-3-one	mushroom-like	980	1278	256
12	unknown	catty	–	–	64
13	2-ethyl-(5)6-methylpyrazine	pyrazine-like, fruity	998	1390	8
14	trimethylpyrazine	burnt, potato-like	1000	1287	32
15a	2-acetylthiazole	roasty, sulfury	1013	1137[d]	16
15b	5-methyl-2-furfurylthiol	roasty, sulfury	1016	1527[c]	
16	acetylpyrazine	roasty	1023	1603	64
17	unknown	sulfury	–	–	256

Table 4: Continued.

No.	odorant [a]	odor quality [b]	RI on		FD-factor
			DB-5	FFAP	
18	phenylacetaldehyde	honey-like	1050	1610	64
19	unknown	sulfury	–	–	256
20	4-hydroxy-2,5-dimethyl-3(2*H*)-furanone [e]	caramel-like	1070	2066	512
21	2-ethyl-3,5-dimethylpyrazine	potato-like, roasty	1083	1458	512
22	2-methoxyphenol	burnt, sweet	1092	1863	256
23	(*Z*)-2-nonenal	green, tallowy	1148	1479	8
24	2,3-diethyl-5-methylpyrazine	potato-like, roasty	1158	1483	128
25	(*E*)-2-nonenal	green	1161	1520	64
26	2-methyl-(3-methyldithio)-furan	meat-like	1172	1676[c]	16
27	2-phenylethylthiol	burnt-rubbery	1176	1622	512
28	unknown	green, leaf-like	–	–	–
29	2-pentylpyridine	fatty, tallowy	1192	1507	64
30	(*E*,*E*)-2,4-nonadienal	fatty, waxy	1213	1704	32
31	unknown	green, fatty	–	–	32
32	indole	mothball-like	1292	1550[c]	16
33	(*E*,*Z*)-2,4-decadienal	green, tallowy	1296	1413[c]	8
34	2-methoxy-4-vinylphenol	spicy	1312	1474[c]	512
35	(*E*,*E*)-2,4-decadienal	fatty, waxy	1318	1800	128
36	(*E*)-2-undecenal	fatty, green	1366	1465	8

Table 4: Continued.

No.	odorant [a]	odor quality [b]	RI on		FD-factor
			DB-5	FFAP	
37	*trans*-4,5-epoxy-(*E*)-2-decenal	metallic	1381	1962	128
38	3-methyl-1*H*-indole (skatole)	mothball-like	1390	1629[d]	64
39	4-hydroxy-3-methoxybenzaldehyde (vanillin) [e]	vanilla-like	1403	2577	64

[a] The odorant was identified by comparing it with the reference compound on the basis of the following criteria: Retention indices on the stationary phases given in the table, mass spectrum obtained by MS-EI or MS-CI, odor quality perceived at the sniffing port, and odor threshold in air. [b] Odor quality assigned during AEDA. [c] The RI was determined on capillary DB-WAX. [d] The RI was determined on capillary OV-1701. [e] The acidic volatiles were isolated by treatment of the ethereal extract with an aqueous sodium bicarbonate solution (0.1 M).

The identification of aroma-active compounds is performed by comparing them with reference compounds on the basis of the following criteria:

1. retention indices (RI) on at least two stationary phases of different polarity,
2. mass spectra generated by electron impact ionization (EI) mode or chemical ionization (CI) mode using HRGC-MS,
3. odor quality and intensity perceived at the sniffing-port.

Before working on these criteria, the preceding structure estimation of the aroma-active compounds is required. After performing AEDA on two stationary phases, both results of the AEDA are combined, and retention indices on the two columns as well as odor quality of the aroma-active compounds are compared with those in the literature. Using the reference substances of the estimated structures, instrumental analyses by HRGC-O and HRGC-MS on the two columns are performed. If all the data given in the criteria are identical between the aroma-active compound in the extract of a food sample and the corresponding reference compound, the aroma-active compound is labeled as “identified”.

It is not unusual that the aroma-active compound in food is present in a very low concentration, so that a large amount of the investigated food sample must be extracted to obtain an unequivocal mass spectrum of the odorant.
Further fractionation and purification are often effective to avoid the overlap of aroma-active compounds with the other volatile components in the gas chromatogram. For the identification of the aroma-active volatiles in roasted sesame *Schieberle* (*1996*) employed fractionation by silica gel column chromatography with *n*-pentane/diethyl ether mixtures of increasing polarity.

For the identification experiments in the extract either with or without the above-mentioned fractionation, the two dimensional GC-MS is a powerful method (cf. **3.7.3**). This technique permits separation of volatile constituents using two kinds of capillary columns of different polarity in a single run. Volatiles are separated in the first GC, but after the elution only the peaks of interest are collected in a cooling trap. Then, the collected compounds are transferred into the second column, and analyzed in the mass spectrometer connected to the end of the second column. This method enables one not only to obtain the more unambiguous mass spectrum, but also to check the retention indices on the two different stationary phases at the same time.

The purification step is by far more important when the target compound is “unknown”, which means that there is no information described in the literature ever before about the volatile compound, because it is critical to get the clear mass spectrum or the other information to assume its chemical structure.

Steinhaus and Schieberle (*2000*) reported the fractionation of the volatiles in a hop extract by silica gel column chromatography, and they also applied silica coated with silver nitrate on chromatography of commercial-available undecatetraenes mixture to newly identify (3*E*,5*Z*)-undeca-1,3,5,9-tetraene present among aroma-active compounds in the hop extract. One of the separated undecatetraenes had the same properties as those of the aroma-active compound, and its structure was clarified as the (3*E*,5*Z*)-undeca-1,3,5,9-tetraene using GC-MS, UV measurements, and ^{1}H-NMR analysis. The same group separated 4-mercapto-4-methylpentan-2-one as a potent odorant in one of the hop varieties using affinity chromatography which harnessed the affinity of mercury to thiols for their isolation (*Steinhaus et al., 2007*). As the other methods, it is also possible to use high performance liquid chromatography (HPLC) or preparative gas chromatography for the isolation of volatiles.

In the case that there is no reference compound commercially available, it is necessary to synthesize a reference compound to identify an unknown compound. This step includes the confirmation of the synthesized structure using mass spectrometry (MS) and nuclear magnetic resonance measurements (NMR) of ^{1}H or ^{13}C.

Miyazawa et al. (*2009a*) synthesized 6 isomers of (8*E*)-undeca-6,8,10-trienones, and then identified (6*Z*,8*E*)-undeca-6,8,10-trien-3-one and (6*Z*,8*E*)-undeca-6,8,10-trien-4-one as novel aroma compounds in galbanum oil. Furthermore, they also newly identified (6*Z*,8*E*)-undeca-6,8,10-trien-4-ol as the aroma-active flavor constituent in yuzu (*Citrus junos* Sieb. ex Tanaka) peel oil by synthesizing 4 isomers of (8*E*)-undeca-6,8,10-trienols (*Miyazawa et al., 2009b*).

Hence, for the identification of aroma-active components, fractionation techniques, instrumental analyses, especially using GC-MS, and organic syntheses play an important role. During those identification experiments, HRGC-O can again be a great help to locate the unknown aroma-active odorant even if its peak on the GC cannot be observed, because the human nose is still the much more sensitive detector to odorants.

1.4.4 Quantitation by stable isotope dilution assay

The prerequisite for the calculation of odor activity values (OAV: ratio of concentration to odor threshold) of aroma compounds to clarify the unequivocal contribution of volatile compounds is a precise quantitation methodology to know their real concentrations in food. As described above, however, the analysis needs some enrichment steps of aroma compounds, during which usually the original amount of the analytes should undergo a change. Thus, the internal standard used for this purpose is urged to be a compound with a similar chemical stability and similar physical properties to the target compound.

In *1940, Rittenberg and Foster* for the first time quantitated amino acids and fatty acids with their isotope labeled internal standards, and *Sweeley et al.* (*1966*) demonstrated to use this technique for the quantitation of glucose by GC-MS analysis with deuterated glucose. Later on, this method was applied for the first time in aroma analysis on the quantitation of aroma-active compounds in bread crusts by *Schieberle and Grosch* (*1987b*), and was called stable isotope dilution assay (SIDA). In this SIDA method, odorants are quantitated by the use of the stable isotope labeled analogues of the analytes as internal standards. Since they have nearly identical chemical and physical properties as the analyte, losses during the enrichment of aroma compounds are satisfactorily compensated.

The most time-consuming effort of the SIDA is the development of the synthetic route to prepare isotopically labeled odorants, because most of them are commercially not available. Deuterium is mostly used to label the internal standard by replacing with hydrogen in the molecule of the analyte because of its affordable price. At least two atoms of deuterium are needed in the molecule, so that isotopes naturally contained in the analyte should not affect the quantitation results. The stability of the labeled element in the molecule is also of importance to obtain accurate results. For example, the buttery-smelling odorant 2,3-butanedione does not have such a suitable hydrogen to be replaced by deuterium, as all the hydrogens exist at the α-positions of carbonyl groups. The deuterium at the α-position might exchange with protium during the work-up procedure. Hence, the SIDA of 2,3-butanedione is normally performed with $^{13}C_4$-2,3-butanedione as the labeled internal standard. The ^{13}C-atom is not inexpensive but extremely chemically-stable.

By way of example for the SIDA procedure, the roasted white sesame seeds were frozen with liquid nitrogen, ground, and suspended in diethyl ether containing known amounts of labeled isotopologs of the aroma-active compounds screened by AEDA beforehand. After stirring, the extract was filtered, and the volatile fraction was isolated under high vacuum. Subsequently,

the roasted sesame aroma isolate containing the labeled internal standards and the analytes was analyzed by GC-MS in the chemical ionization (CI) mode. Each peak area of the labeled internal standard and the odorant were separately measured by using either their protonated molecular ions or their main fragments. The concentration of the analyte was calculated from the amount of labeled standard added and sesame seeds extracted, and the peak areas (**Table 5**) (*Schieberle, 1996*).

The chemical ionization (CI) mass spectroscopy is a milder ionization mode than the electron impact ionization (EI) so that the molecular weight plus proton is normally the most abundant in its spectrum. The use of the CI mode in SIDA minimizes the loss of labeled atoms in the molecule of internal standards during mass spectroscopy and also to avoid disturbances by fragment ions from other co-eluting compounds.

Table 5: Concentrations and odor activity values (OAVs) of 10 selected aroma compounds in sesame (*Schieberle, 1996*).

compound	odor quality	concentration [µg/kg]	odor threshold [µg/kg]	OAV
2-acetyl-1-pyrroline	roasty, popcorn-like	30	0.1	300
2-furfurylthiol	roasty, coffee-like	54	0.4	135
2-phenylethylthiol	burnt-rubbery	6	0.05	120
4-hydroxy-2,5-dimethyl-3(2*H*)-furanone	caramel-like	2511	50	50
2-ethyl-3,5-dimethylpyrazine	potato-like, roasty	53	3	18
2-methoxyphenol	burnt, sweet	269	19	14
2-pentylpyridine	fatty, tallowy	19	5	4
2-acetylpyrazine	roasty	26	10	3
4-vinyl-2-methoxyphenol	spicy	72	50	1
(*E*,*E*)-2,4-decadienal	fatty, waxy	89	180	<1

1.4.5 Determination of odor activity values

The concentrations of aroma-active compounds alone are not sufficient to assess their aroma impact in the investigated material, because interactions between odorants and food matrix can considerably differ by the kind of the matrices. For example, these interactions may result in different odor thresholds dependent on the matrix, as shown in **Table 6**. The great difference between the odor threshold in water and oil of (*E*,*E*)-2,4-decadienal clearly shows that the concentration is not enough to measure the contribution of volatile compounds to food aroma. If 100 µg/kg of (*E*,*E*)-2,4-decadienal is contained in a water-based food, this compound is very significant in the food aroma because the concentration by far exceeds its odor threshold in water (0.2 µg/kg). However, the same compound, (*E*,*E*)-2,4-decadienal at the same concentration in an oily food does not smell, because it is below the threshold in oil (180 µg/kg).

Table 6: Comparison of odor thresholds determined in water and oil (data taken from *Rychlik et al., 1998*).

compound	odor threshold [µg/kg] in	
	water	oil
(*E*,*E*)-2,4-decadienal	0.2	180
2-furfurylthiol	0.005	0.4
acetaldehyde	25	0.22
butanoic acid	1000	135

As a concept to clarify a significance of individual aroma compounds, *Patton and Josephson* (*1957*) first described that the aroma compound which has a concentration higher than its odor threshold should be significant for a food aroma. *Rothe and Thomas* (*1963*) designated this concept as “Aromawert” that means “aroma value” in German.

The odor activity value (OAV), which is equivalent to the aroma value, is defined as the ratio of the concentration of an aroma compound X to its odor threshold in the corresponding matrix to the investigated food, thereby indicating how many fold of dilution by the matrix is necessary to delete the odor of the compound X from the food aroma.

$$\text{OAV (X)} = \frac{\text{concentration (X)}}{\text{odor threshold (X)}}$$

Thus, an OAV is a theoretical value if the investigated food can be diluted by the matrix in which the odor threshold used for the OAV calculation is measured. In the case of roasted sesame, the odor threshold in sunflower oil is normally used to calculate the OAV, but the matrix of roasted sesame does not only consist of oil but also contains solid materials.
However, odor activity values (OAVs) are useful to evaluate the aroma contribution of a single odorant in overall food aroma. Odorants with an OAV smaller than 1 should not contribute to the aroma of a food, and the higher the OAV each of odorants has, the more contribution to the food aroma can be assumed.

For the determination of the OAVs of the 10 quantitated odorants in the roasted sesame, *Schieberle* (*1996*) used the odor thresholds determined in sunflower oil, because the roasted sesame contains 50% of oil. As shown above in **Table 5**, the calculated OAVs elucidated that 2-acetyl-1-pyrroline had the highest contribution, followed by 2-furfurylthiol, 2-phenylethylthiol, and 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone.

1.5 Odor-active compounds in roasted sesame seeds

As mentioned earlier (cf. **1.2.3**), there were a lot of investigations on the identification of the volatile components in roasted sesame seeds or oil. However, the importance of the individual volatiles as odor-active compounds for the total sesame aroma was not sufficiently taken into account.

The first systematic study focusing on the odor-active compounds in roasted sesame aroma was introduced by Schieberle and his group in the 1990s (*Schieberle, 1993, 1994, 1996; Schieberle et al., 1996*).

In *1993, Schieberle* applied the aroma extract dilution analysis (AEDA) on short-roasted white sesame and long-roasted one. The following stable isotope dilution assay (SIDA) and the calculation of odor activity values (OAV) showed the odor difference caused by the roasting time (**Table 7**). As the roasting time was lengthened, basically all the OAVs of the odor-active compounds increased, but it drastically enhanced the coffee-like smelling 2-furfurylthiol which was the most important compound in the long-roasted sesame. On the other hand, the OAV of the 2-acetyl-1-pyrroline which had the highest OAV in the short-roasted one was lowered.

Table 7: Odor activity values (OAV) of important aroma compounds in long- and short-roasted sesame seeds (taken from *Schieberle, 1993*).

odorants	OAV	
	short-roasted	long-roasted
4-hydroxy-2,5-dimethyl-3(2*H*)-furanone	50	183
4-vinyl-2-methoxyphenol	1	12
2-methoxyphenol	14	262
2-acetylpyrazine	3	191
2-ethyl-3,5-dimethylpyrazine	18	79
2-pentylpyridine	4	51
2-furfurylthiol	135	6152
2-acetyl-1-pyrroline	300	120

Furthermore, the difference of aroma between commercial roasted sesame oil and the long-roasted sesame oil was also assessed using the same concept (**Table 8**) to find out the reason why the commercial oil had a fatty and tallowy odor note. The comparison of their OAV presented that in the commercial oil the OAV of 2-furfurylthiol was lower while that of 2-

pentylpyridine was higher. This fatty, tallowy smelling 2-pentylpyridine should, thus, cause the significant tallowy smell in the oil in conjunction with the effect of the lowered 2-furfurylthiol.

Table 8: Odor activity values (OAV) of five primary odorants in a commercial "pure sesame oil" manufactured from roasted seeds – comparison with fresh long-roasted sesame seeds (taken from *Schieberle, 1993*).

odorants	OAV	
	commercial oil	long-roasted
2-furfurylthiol	790	6152
2-methoxyphenol	95	262
4-hydroxy-2,5-dimethyl-3(2*H*)-furanone	78	183
2-acetylpyrazine	163	191
2-pentylpyridine	383	51

Schieberle (*1994*) also investigated the aroma of a black sesame variety using the odor activity value concept to elucidate its odor-active compounds and to compare them with those of white sesame one. To get an insight of the aroma difference between roasted black and white sesame seeds, the OAVs were calculated for both varieties (**Table 9**). The results showed that 2-furfurylthiol and 2-phenylethylthiol were the most important in both varieties. He mentioned that the higher OAV ratio of the fatty, tallowy smelling 2-pentylpyridine compared to the most odor-active 2-furfurylthiol in the black seeds than that in white seeds might cause the fatty, oily note in the black variety. It was also estimated that a degradation of linoleic acid might be favored in the black variety, because both, the amounts of (*E*,*E*)-2,4-decadienal and 2-pentylpyridine were higher in the black compared to white seeds.

Table 9: Odor activity values of seven important odorants in black and white sesame seeds (taken from *Schieberle, 1994*).

odorants	odor activity value	
	black seeds	white seeds
(*E*,*E*)-2,4-decadienal	6	1
2-methoxyphenol	139	262
2-pentylpyridine	181	51
2-furfurylthiol	1682	6152
2-ethyl-3,5-dimethylpyrazine	131	79
4-hydroxy-2,5-dimethyl-3(2*H*)-furanone	234	183
2-phenylethylthiol	240	880

In addition to the difference caused by the sesame variety, the influence of roasting temperature was also studied (**Table 10**). At the lower temperature (180 and 200 ºC) the typical roasted, sweet-sulfury note of sesame predominated, whereas the burnt, rubbery, and fatty odor was enhanced at higher temperatures (220 and 240 ºC). All the OAVs of odor-active compounds increased as the temperature was raised, but to a different extent (**Table 10**). With an increase of the temperature from 180 ºC to 240 ºC, the OAV of 2-furfurylthiol increased by a factor of 4, while the OAVs of the other three aroma-active compounds increased by factors of about 30. Thus, the comparatively higher OAVs of 2-methoxyphenol, 2-pentylpyridine, and 2-phenylethylthiol might influence the aroma difference of the seeds roasted at the higher temperature compared to those at the lower temperature.

Table 10: Influence of roasting temperature on the odor activity values (OAV) of four key odorants in white sesame seeds (*Schieberle, 1994*).

odorants	OAV in sample			
	180 ºC	200 ºC	220 ºC	240 ºC
2-furfurylthiol	1225	3165	5080	5235
2-methoxyphenol	44	340	1334	1458
2-pentylpyridine	18	74	277	525
2-phenylethylthiol	360	2160	8960	10800

As already described above in **1.4**, the detailed paper about the aroma of moderately roasted white sesame seeds, which was equal to the short-roasted sesame described in **Table 7**

(*Schieberle, 1993*), was then published (*Schieberle, 1996*). The application of AEDA revealed 41 aroma-active volatiles (**Table 4**). Ten major aroma-active compounds were subsequently quantitated by SIDA (**Table 5**). According to their high OAVs, 2-acetyl-1-pyrroline (popcorn-like), 2-furfurylthiol (coffee-like), 2-phenylethylthiol (rubbery) and 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone (caramel-like) were identified as key aroma compounds of the roasted white sesame seeds. However, in these studies the structures of eight aroma-active compounds with sulfurous or catty aroma notes remained open.

Since the studies by Schieberle, some further investigations on odor-active compounds in roasted sesame were published.

In 1996, *Shimoda et al.* attempted to isolate sesame-oil-seed-like aroma compounds from roasted sesame seed oil using thin layer chromatography (TLC) followed by preparative GC. The authors summarized that 1-(5-methyl-2-furanyl)-1-propanone, 3-formylthiophene, 2-propyl-4-methylthiazole, and 2-ethyl-4-methyl-1*H*-pyrrole might be the principal contributors. However, their results might be affected by unidentified trace odor-active compounds present in each of fractions, because they did not check the aroma of the compounds responsible for the fractions using the GC-O or another appropriate technique.

In 2001, *Cadwallader and Heo* investigated the odor-active compounds in roasted sesame oil using direct thermal desorption GC-O (DTD-GC-O) and sample dilution analysis (SDA). They quantitated the aroma-active compounds screened by the DTD-GC-O-SDA, and also calculated odor activity values (OAV). As a result, acetaldehyde, 2-methylphenol, 2-ethyl-3,5-dimethylpyrazine, 3-methylbutanal, 2-pentylpyridine, 1-octen-3-one, and 2-acetylpyrazine showed the highest OAVs.

In 2006, *Ikeda et al.* analyzed sesame-flavored dressings using the Charm analysis (*Acree et al., 1984*), and concluded that sulfurous compounds were important for the aroma. As the sulfurous compounds, butanethiol, 3-methyl-2-butene-1-thiol, 2-methyl-3-furanthiol, and dimethyl trisulfide were identified.

In 2008, *Takeda et al.* analyzed roasted sesame seeds aroma using solvent assisted flavor evaporation (SAFE) and their headspace using solid phase micro extraction (SPME). In the SAFE distillate 2-acetyl-1-pyrroline, (*E*,*E*)-2,4-decadienal, 2-methoxyphenol, and 2,5-dimethyl-4-hydroxy-3(2*H*)-furanone showed the highest flavor dilution (FD) factors, while by

the headspace analysis 2-furfurylthiol showed the highest FD factor. In addition, 2-mercapto-3-pentanone was identified for the first time as an aroma-active compound in roasted sesame.

1.6 Objectives

As described above, roasted sesame seeds are a popular food because of their high nutritive values and their unique and highly attractive odor generated by roasting. In Asia, the oil isolated from the roasted seeds is used as a seasoning in many dishes, while in Europe and the United States the roasted seeds are quite common as a topping on bakery goods. In Japan, sesame in a ground roasted state is most often consumed. Moreover, a lot of Japanese prefer to grind roasted sesame seeds in a small mortar with a small pestle just before consumption to ensure an unaltered fresh aroma, because by experience the attractive sulfurous odor cannot be preserved.

After the numerous researches on volatile compounds in sesame, Schieberle and his group introduced their systematic approach of aroma analyses into the investigation of roasted sesame odor in the 1990s. It was elucidated that 2-acetyl-1-pyrroline (roasty), 2-furfurylthiol (coffee-like), 2-phenylethylthiol (rubbery), and 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone (caramel-like) were the key compounds of roasted sesame aroma based on their high odor activity values (OAV). However, eight aroma-active sulfurous, meaty, or catty unknown odorants were not discovered in this research.
The odor qualities of these unknown compounds implied, however, that they might be sulfur-containing compounds, and furthermore, could contribute to the quickly vanishing attractive sulfurous odor of freshly ground roasted sesame.

The aim of the present research was, therefore, to reinvestigate the aroma-active compounds present in roasted white sesame seeds with special emphasis on the presently unknown sulfurous and catty smelling odorants. The odorants were evaluated by aroma extract dilution analysis (AEDA), and the aroma-active unknown sulfurous and catty odorants were subsequently identified. Their concentrations were quantitated by stable isotope dilution assay (SIDA), and the application of odor activity value concept should lead to evaluate the contributions of the sulfurous and catty unknown odorants to the overall roasted ground sesame seeds aroma.

2 Results

2.1 Identification of novel aroma-active thiols in pan-roasted white sesame seeds

To re-investigate the aroma-active compounds responsible for the aroma of freshly roasted sesame seeds, and to identify the sulfurous unknown components, white sesame seeds from Egypt were analyzed. The seeds were roasted for 15 min at 200 °C in a pan, and then ground with a pestle in a mortar. The ground roasted sesame seeds elicited a typical roasted sesame-like odor, which was characterized as nutty, sulfurous, roasty, and meaty. Then, ground roasted seeds (2.5 g) were extracted with dichloromethane, distilled by means of solvent assisted flavor evaporation (SAFE) to isolate volatiles from oil, and concentrated to obtain 200 μL of the aroma concentrate which exhibited the typical aroma of roasted sesame seed, when checked on a strip of filter paper.

2.1.1 Aroma extract dilution analysis (AEDA)

To characterize the aroma-active compounds in the distillate, an aroma extract dilution analysis (AEDA) was applied on the roasted sesame aroma concentrate. The application of AEDA revealed thirty-two aroma-active regions in the flavor dilution (FD) factor range of 2 to 2048 (**Figure 7**).

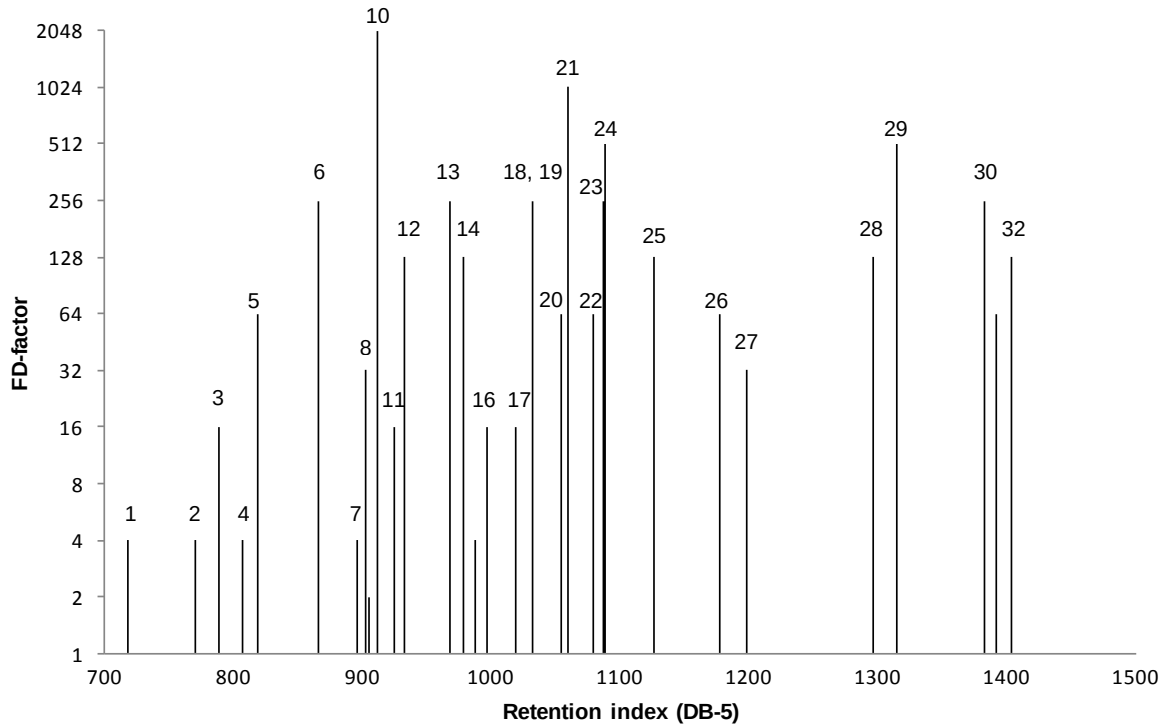


Figure 7: Flavor-dilution (FD)-chromatogram (FD≥2) of a distillate prepared from roasted white sesame seeds.

The coffee-like smelling compound **10** and the caramel-like smelling compound **21** showed the highest FD-factors (FD-2048 and 1024), followed by the coffee-like smelling compound **24** and the clove-like smelling compound **29** with FD-factors of 512. With somewhat lower aroma activities (FD-256), compound **6** (meaty), compound **13** (sulfurous, onion-like), compound **23** (earthy, potato-like), and compound **30** (metallic) were detected. Another region showing FD-factor of 256 was compound **18** (roasty) and **19** (sulfurous, catty), which were detected at the same retention index on the DB-5 capillary column. As aroma compounds with one step lower aroma intensities (FD-128), the earthy and burnt smelling compound **12**, the mushroom-like smelling compound **14**, the roasty smelling compound **25**, the fatty smelling compound **28**, and the vanilla-like smelling compound **32** were located.

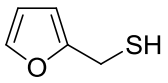
2.1.2 Identification experiments

For the identification of the aroma-active compounds detected by AEDA, their retention indices on two stationary phases of different polarity (DB-5 and DB-FFAP), their odor

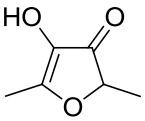
attributes perceived at the sniffing port by high resolution GC-olfactometry (HRGC-O), and their mass spectra in the EI-mode were determined. Chemical structures of the compounds were estimated using the data obtained from the previous literature, and the data of the aroma-active compounds were compared with those of reference compounds of the estimated structure. The odorants were described as 'identified', if all the data of the investigated aroma-active compound matched those of the reference substances.

Comparing the retention indices and the odor qualities of the aroma-active compounds (**Table 11**) with those listed in the previous study (**Table 4**, *Schieberle, 1996*), the structures of 16 odorants could readily be assigned, and their mass spectra (MS) were checked with those of their reference compounds (cf. **3.7.1** and **3.7.3**). Among the 16 odorants, five compounds were identified from the aroma concentrate used for the AEDA, which was obtained by the extraction from 2.5 g of ground roasted sesame seeds.

The coffee-like smelling compound **10** was identified as 2-furfurylthiol with the highest FD-factor (2048) in agreement with earlier results (**Table 4**, *Schieberle, 1996*). The caramel-like smelling compound **21** was identified as 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone showing the second most aroma activity (FD-1024), also in agreement with the former investigation by *Schieberle* in *1996*.



2-furfurylthiol (**10**)



4-hydroxy-2,5-dimethyl-3(2*H*)-furanone (**21**)

The sulfurous and onion-like compound **13** and the earthy and potato-like compound **23** were identified as dimethyl trisulfide and 2-ethyl-3,5-dimethylpyrazine with FD-factors 256, respectively. Compound **11** (nutty) with an FD-factor 16 was identified 2-acetyl-1-pyrroline, which had shown the highest odor activity value (OAV) in the previous research (**Table 5**, *Schieberle, 1996*).

The HRGC-MS and two dimensional HRGC-MS analyses of the aroma concentrate from 2.5 g of ground roasted sesame did not allow to obtain mass spectra of most of the other aroma-active odorants due to their low concentrations and co-eluting substances. Therefore, the amount of ground roasted sesame seeds extracted was scaled up, and fractionation of the

distillate was done in order to avoid co-elutions and to obtain unequivocal mass spectra of the aroma-active compounds.

First, one thousand times more weight of ground roasted sesame seeds (2.5 kg) than those used for the AEDA (2.5 g) were extracted. Then, column chromatography on silica gel using *n*-pentane/diethyl ether mixtures of different polarity was performed, and the eluate was separated into five fractions. (cf. **3.4.1.3**). Fraction A showed a sulfurous and meaty odor. Fraction B exhibited a predominant fatty aroma with sulfurous and coffee-like odor qualities. Metallic and spicy odor notes predominated in fraction C, while sweet and caramel-like odor was dominant in fraction D. Fraction E had almost no odor. In all fractions the odorants were located by GC-O, and thereafter the GC-MS analysis was applied to all fractions. The corresponding fractions to each of the odorants are listed in **Table 11**.

These scale up and fractionation permitted the identification of 2-thenylthiol (**24**; thiophen-2-yl-methylthiol; coffee-like) and 2-methoxy-4-vinylphenol (**29**; clove-like) with FD-factors of 512, followed by *trans*-4,5-epoxy-(*E*)-2-decenal (**30**) which showed an FD-factor of 256.

As shown in **Table 11**, most of the remaining unidentified aroma-active odorants could be identified on the basis of the previous data (**Table 4**, *Schieberle, 1996*).

However, the structures of fourteen odorants (**1**–**7**, **9**, **15**–**16**, **18**–**20**, **25**) could not be assigned based on the former data, although eight of them (**1, 5**–**7**, **9**, **15, 19**–**20**) had already been detected as aroma-active compounds in roasted sesame before, but remained unidentified (**Table 4**, *Schieberle, 1996*). Due to their sulfurous, meaty, catty and/or black-currant-like odor attributes, it was, however, assumed that these odorants might play a crucial role for the characteristic sulfurous aroma of roasted sesame seeds, in particular in the freshly ground state. As the next step, therefore, the identification experiments were focused on these compounds.

Table 11: Odor-active compounds (FD ≥2) identified in the aroma distillate obtained from roasted white sesame seeds.

no.	odorant [a]	odor quality [b]	RI on		fraction [c]	FD-factor	ref. [g]
			DB-5	FFAP			
1	2-methyl-1-propene-1-thiol	sulfurous, meaty	718	1013	A	4	–
2	(*Z*)-3-methyl-1-butene-1-thiol	sulfurous, meaty	770	n.d. [d]	A	4	–
3	(*E*)-3-methyl-1-butene-1-thiol	sulfurous, meaty	788	n.d. [d]	A	16	–
4	(*Z*)-2-methyl-1-butene-1-thiol	sulfurous, meaty	807	1098	A	4	–
5	(*E*)-2-methyl-1-butene-1-thiol	sulfurous, meaty	818	1105	A	64	–
6	2-methyl-3-furanthiol	meaty	865	1300	A	256	(1)
7	3-mercapto-2-pentanone	catty, black currant-like	895	1344	B	4	–
8	3-(methylthio)propanal (methional)	potato-like	902	1452	C	32	(2)
9	2-mercapto-3-pentanone	catty, black currant-like	905	1361	B	2	(3)
10	2-furfurylthiol	coffee-like	911	1426	B	2048	(4)
11	2-acetyl-1-pyrroline	nutty	924	1326	B	16	(2)
12	4-methyl-3-thiazoline [e]	earthy, burnt	932	1422	D	128	(2)
13	dimethyl trisulfide	sulfurous, onion-like	967	1370	A	256	(5)
14	1-octen-3-one	mushroom-like	978	1295	B	128	(2)
15	4-mercapto-3-hexanone	catty, black currant-like	987	1405	B	4	–
16	unknown	roasty	996	n.d.	C	16	–
17	2-acetylthiazole [e]	popcorn-like	1019	1611	C	16	(4)
18	unknown	roasty	1032	n.d.	C	256	–
19	3-mercapto-3-methylbutyl formate	sulfurous, catty	1032	1517	B		–

Table 11: Continued.

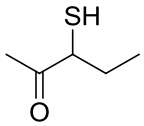
no.	odorant [a]	odor quality [b]	RI on		fraction [c]	FD-factor	ref. [g]
			DB-5	FFAP			
20	2-methyl-3-thiophenethiol	meaty, sulfurous	1054	1556	A	64	–
21	4-hydroxy-2,5-dimethyl-3(2*H*)-furanone	caramel-like	1059	2029	D	1024	(4)
22	2-ethyl-3,6-dimethylpyrazine	earthy, potato-like	1079	1449	D	64	(6)
23	2-ethyl-3,5-dimethylpyrazine	earthy, potato-like	1086	1449	D	256	(6)
24	2-thenylthiol	coffee-like	1088	1676	B	512	–
25	unknown	roasty	1126	n.d.	B	128	–
26	2-phenylethylthiol	rubber-like	1177	1611	B	64	(2)
27	2-pentylpyridine [e]	fatty	1198	1507	B	32	(5)
28	(*E*,*Z*)-2,4-decadienal [f]	fatty	1296	1763	B	128	(2)
29	2-methoxy-4-vinylphenol	clove-like	1315	2193	C	512	(4)
30	*trans*-4,5-epoxy-(*E*)-2-decenal	metallic	1383	1997	C	256	(2)
31	3-methyl-1*H*-indole (skatole)	fecal	1391	2497	D	64	(5)
32	4-hydroxy-3-methoxybenzaldehyde (vanillin)	vanilla-like	1404	2569	D	128	(5)

[a] The odorant was identified by comparing it with the reference compound on the basis of the following criteria: Retention indices on the stationary phases detailed in the table, odor quality perceived at the sniffing port and mass spectrum obtained by MS-EI. [b] Odor quality perceived at the sniffing port. [c] Silica gel fractions (*n*-pentane/diethyl ether, v/v); A: 100:0, B: 90:10, C: 70:30, D: 50:50. [d] Due to bad chromatographic behavior a retention index on FFAP could not be determined. Identification was based on the remaining criteria given in footnote [a]. [e] Tentative identification based on published data (*Schieberle, 1996*). [f] No unequivocal mass spectrum was obtained. Identification was based on the remaining criteria given in footnote [a]. [f] Reference where the odorant was first reported as the volatile compound in sesame; (1): *Ikeda et al., 2006*, (2): *Schieberle, 1996*, (3): *Takeda et al., 2008*, (4): *Takei, 1988*, (5): *Nakamura et al., 1989*, (6): *Kinoshita and Yamanishi, 1973*. n.d.: not determined.

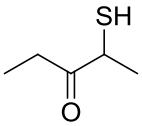
2.1.3 Identification of unknown sulfur-containing compounds

2.1.3.1 Identification of odorants 7 and 9

Compounds **7** and **9**, both of which showed a catty, black-currant-like odor at the low FD-factors of 4 and 2, respectively, could be detected in fraction B (**Table 11**). Their mass spectra were successfully obtained from the enriched aroma concentrate using 2.5 kg of ground roasted sesame, and finally, by means of the respective reference odorants, these compounds could be identified as 3-mercapto-2-pentanone (**7**) and 2-mercapto-3-pentanone (**9**). The latter, 2-mercapto-3-pentanone was previously also reported by *Takeda et al.* (*2008*) as an aroma-active constituent detected by headspace dilution analysis using solid phase micro extraction (SPME) in ground roasted sesame seeds, while 3-mercapto-2-pentanone was newly identified in roasted sesame.



3-mercapto-2-pentanone (**7**)



2-mercapto-3-pentanone (**9**)

2.1.3.2 Identification of odorants 6 and 20

The meaty smelling compound **6**, showing the highest FD-factor among the previously unknown eight odorants, and, also, the meaty and sulfurous smelling unknown compound **20** were concentrated in fraction A. Since the odor qualities suggested that these compounds were also sulfur-containing compounds and especially that the meaty-smelling unknown **6** was estimated to be 2-methyl-3-furanthiol from its odor attribute and retention indices on DB-5 and DB-FFAP (**Table 11**), selective enrichment of thiols by affinity chromatography on mercurated agarose gel (prepared shown in **3.3.1**) was applied (cf. **3.4.1.4**).

Fraction A was applied on a column packed with agarose gel to which phenylmercuric acetate was bound. When the fraction passed through the column, only thiols were reversibly bound to the gel because of the affinity between thiol and mercury. Then, compounds except thiols were removed by washing with solvent. Desorption of the target thiols from the gel was achieved by using a dithiothreitol solution, and separation of the volatile target thiols from dithiothreitol was carried out by SAFE distillation.

The scheme of the enrichment of the aroma-active thiols by means of affinity chromatography is shown in **Figure 8**.

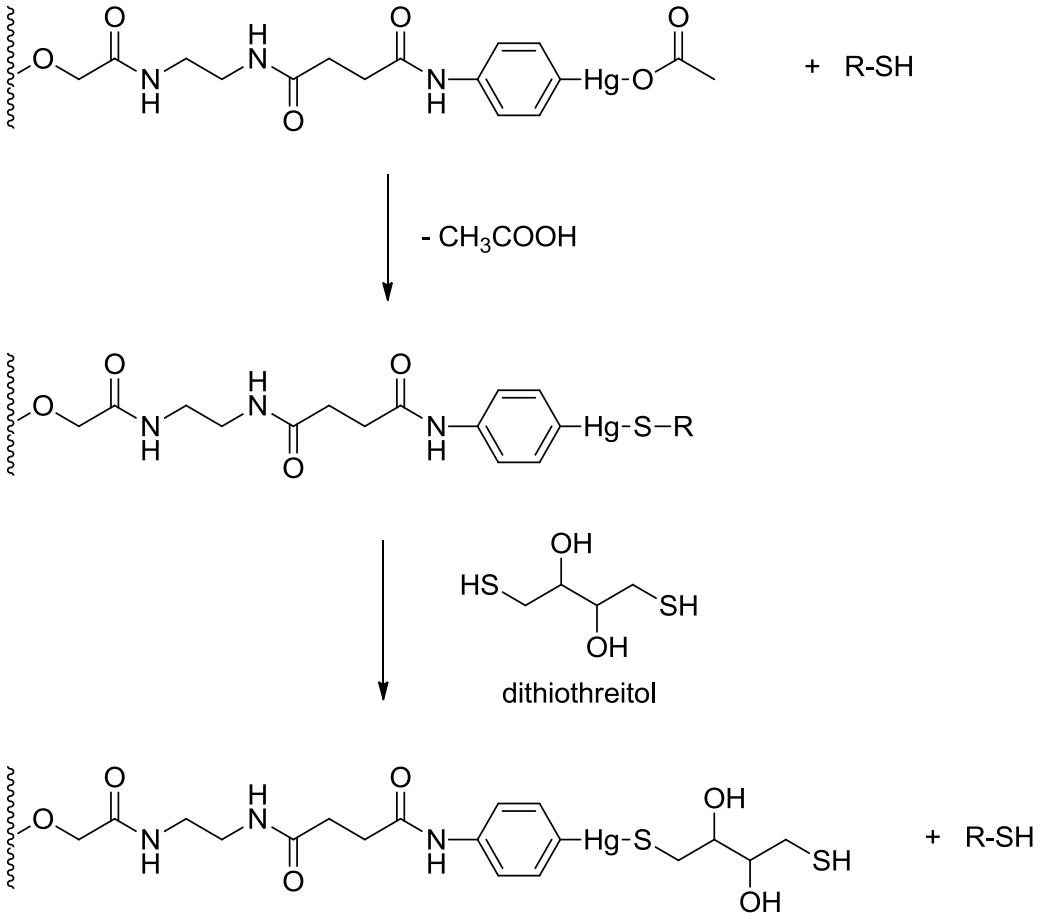
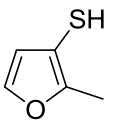
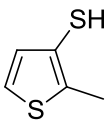


Figure 8: Scheme for the isolation of the aroma-active thiols from roasted sesame seeds.

After concentration of the thiol isolate, the sample was analyzed using a GC-O/GC-O/MS-system (cf. **3.7.3**) with a heart-cut interface to monitor their mass spectra, by which a further increase in sensitivity was achieved. This approach allowed to identify **6** as 2-methyl-3-furanthiol and **20** as the corresponding 2-methyl-3-thiophenethiol (**Table 11**). *Ikeda et al.* had already reported the identification of 2-methyl-3-furanthiol as a key constituent in commercially available sesame-flavored dressings in *2006*, whilst 2-methyl-3-thiophenethiol was identified here from roasted sesame for the first time.



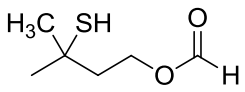
2-methyl-3-furanthiol (**6**)



2-methyl-3-thiophenethiol (**20**)

2.1.3.3 Identification of odorant 19

The sulfurous and catty smelling compound **19** was found to elute at the same retention index with the roasty smelling odorant (**18**) on DB-5. They comprised one region which showed an FD-factor of 256, but could be separated by silica gel chromatography into different fractions (**Table 11**). The isolation of the sulfurous and catty unknown **19** from compound **18** allowed to identify 3-mercapto-3-methylbutyl formate because of its odor quality and retention indices. However, due to very small amount of **19** a further purification using the mercurated agarose was also necessary to obtain a mass spectrum. Finally, the structure of **19** was confirmed as 3-mercapto-3-methylbutyl formate using the two-dimensional HRGC-MS system (cf. **3.7.3**). First time ever, 3-mercapto-3-methylbutyl formate was identified in sesame seeds.



3-mercapto-3-methylbutyl formate (**19**)

2.1.4 Identification of further new aroma compounds

2.1.4.1 Identification of odorant 1

Using the two-dimensional HRGC-MS-system, a mass spectrum of the sulfurous and meaty smelling unknown odorant **1** was obtained by isolating all the thiols in fraction A using the mercurated agarose gel (**Figure 9**). However, although it was possible to get a mass spectrum, no reference spectrum could be found in the literature.

The mass spectrum suggested a formula weight of 88 and the additional fragment *m*/*z* 90 amounting exactly to 4.3% of the intensity of *m*/*z* 88, proposed the presence of one sulfur atom in the molecule. In addition, the fragment ion *m*/*z* 55 indicated the cleavage of a thiol group (M^+–33) and, also, the presence of a butenyl moiety. Thus, the structure of odorant **1** was proposed to be a butenethiol.

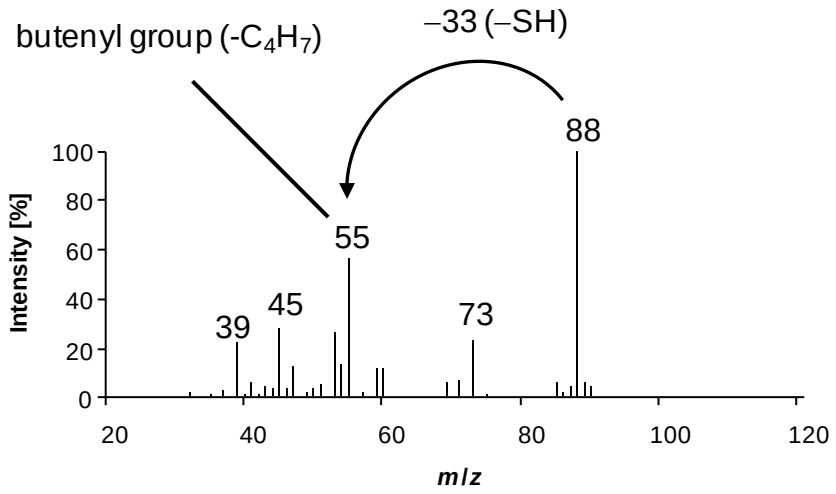


Figure 9: Mass spectrum (MS-EI) of the sulfurous and meaty smelling compound **1**.

Estimation of odorant 1 structure using newly synthesized butenethiols

To check this assumption, all five possible structures of butenethiol isomers were synthesized (cf. **3.3.2.1**–**3.3.2.4**), namely (*E*)- and (*Z*)-2-butene-1-thiol, 3-butene-1-thiol, 3-butene-2-thiol and 2-methyl-2-propene-1-thiol (**Figure 10**).

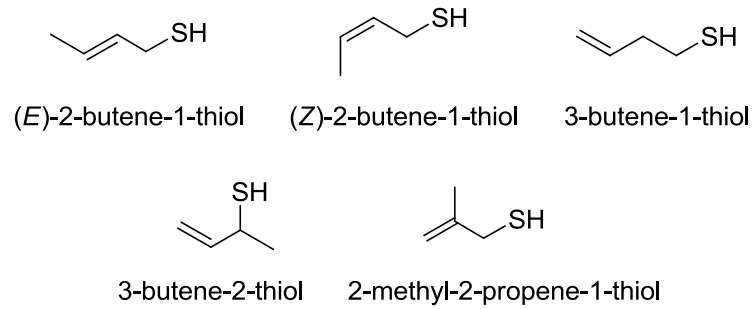


Figure 10: Structures of the synthesized butenethiols as possible candidates for compound **1**.

First, their retention indices and odor qualities were compared to those of compound **1**. Due to their clearly different retention indices, 3-butene-1-thiol, 3-butene-2-thiol and 2-methyl-2-propene-1-thiol could be excluded (**Table 12**).

Table 12: Comparison of odor qualities and retention indices of the synthesized butenethiols and compound **1**.

compound	odor quality [a]	RI[b] on	
		DB-5	FFAP
3-butene-2-thiol	sulfurous	677	< 900
2-methyl-2-propene-1-thiol	sulfurous	689	996
3-butene-1-thiol	rotten	700	980
(*E*)-2-butene-1-thiol	sulfurous	717	1006
odorant **1**	sulfurous, meaty	718	1013
(*Z*)-2-butene-1-thiol	sulfurous	721	1014

[a] Odor quality perceived at the sniffing port.
[b] RI = retention index correlated to a homologous series of *n*-alkanes.

On the other hand, (*E*)- and (*Z*)-2-butene-1-thiol had similar retention indices on both capillary columns (DB-5 and DB-FFAP) to those of compound **1** (**Table 12**). Thus, their mass spectra were compared using two dimensional HRGC-MS, but these differed somehow in the mass spectrometric fragmentation patterns, especially in the intensities of *m*/*z* 55 (**Figure 11**), as well as in their odor qualities (**Table 12**).

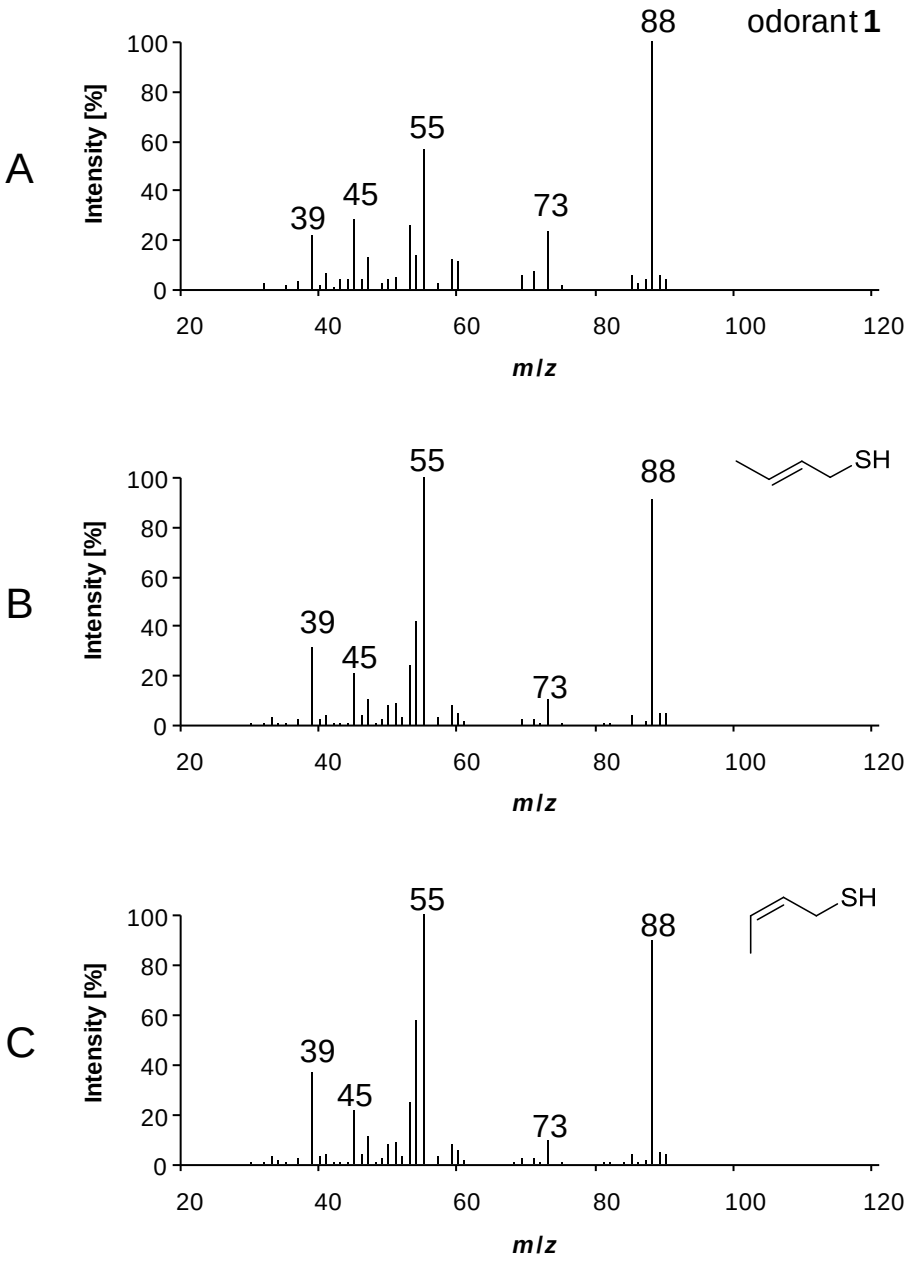
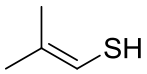


Figure 11: Mass spectra (MS-EI) of odorant **1** (A), (*E*)-2-butene-1-thiol (B), and (*Z*)-2-butene-1-thiol (C).

At this point it was considered that the thiol group in the chemical structure of the unknown compound **1** might be directly bound to the double bond. Since the fragment ion *m*/*z* 73 (M^+–15) indicating the cleavage of a methyl group was more abundant in the mass spectrum of odorant **1** (**Figure 11A**) than in the mass spectra of both isomers of the 2-butene-1-thiols (**Figure 11B** and **11C**), also a branched carbon skeleton was assumed. Consequently, the structure of unknown odorant **1** was proposed to be 2-methyl-1-propene-1-thiol.



2-methyl-1-propene-1-thiol

Synthesis of the 1-alkene-1-thiol structure and identification of odorant 1

To confirm the proposed structure, its reference compound was necessary, however 2-methyl-1-propene-1-thiol was unavailable from commercial sources. Approaches to synthesize 2-methyl-1-propene-1-thiol had already been reported (*Sidhu et al., 1966; Brandsma, 1970; Zhang et al., 1998*), but these procedures were far from practical to synthesize the reference substance. Thus, a new synthetic route leading from the corresponding aldehydes to 1-alkene-1-thiols via the alkane-1,1-dithiols was developed (**Figure 12**). First, 2-methylpropanal was converted to 2-methylpropane-1,1-dithiol by treatment with H_2S, which was then submitted to flash vacuum pyrolysis (FVP) (*Schiess et al., 1995; Kreilein et al., 2005*) in order to induce H_2S elimination leading to the target compound 2-methyl-1-propene-1-thiol.

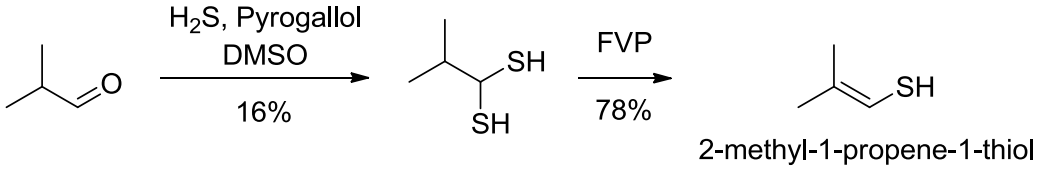


Figure 12: Synthetic approach used in the preparation of 2-methyl-1-propene-1-thiol.

The analytical data of the synthesized 2-methyl-1-propene-1-thiol, such as retention indices (**Table 12**), the mass spectrum (**Figure 13B**), and the odor quality were identical with those of odorant **1**, and thus, the compound was identified as 2-methyl-1-propene-1-thiol (**Table 11**). To the best of our knowledge, 2-methyl-1-propene-1-thiol (**1**) was identified for the first time from natural products (*Tamura et al., 2010 and 2011*).

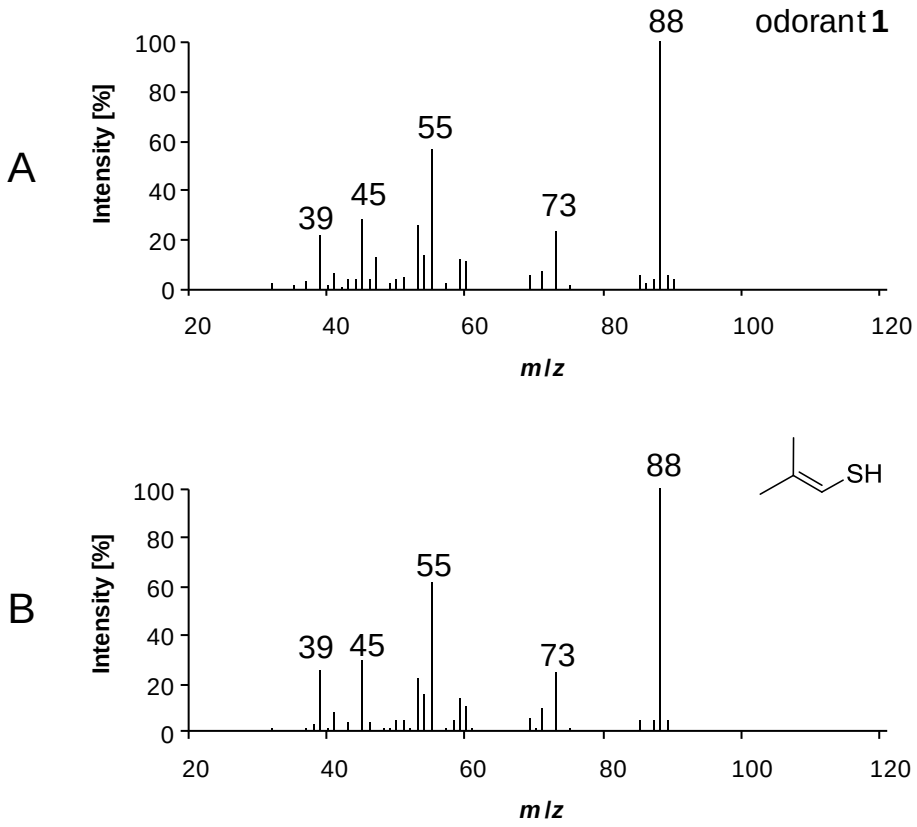


Figure 13: Mass spectra (MS-EI) of odorant **1** (A) and 2-methyl-1-propene-1-thiol (B).

2.1.4.2 Identification of odorants 2 – 5

Structural estimation of odorant 5

In the first stage of the structure estimation of odorant **5** (**Table 11**), it was suggested to be 3-methyl-2-butene-1-thiol, because its retention indices (DB-5: 818, DB-FFAP: 1105) were quite similar to those of 3-methyl-2-butene-1-thiol (DB-5: 820, DB-FFAP: 1101). In addition, both exhibited a sulfurous, meaty odor quality. However, two-dimensional HRGC-MS analysis yielded a mass spectrum (**Figure 14A**) which clearly differed in the intensities from that of 3-methyl-2-butene-1-thiol (**Figure 14B**).

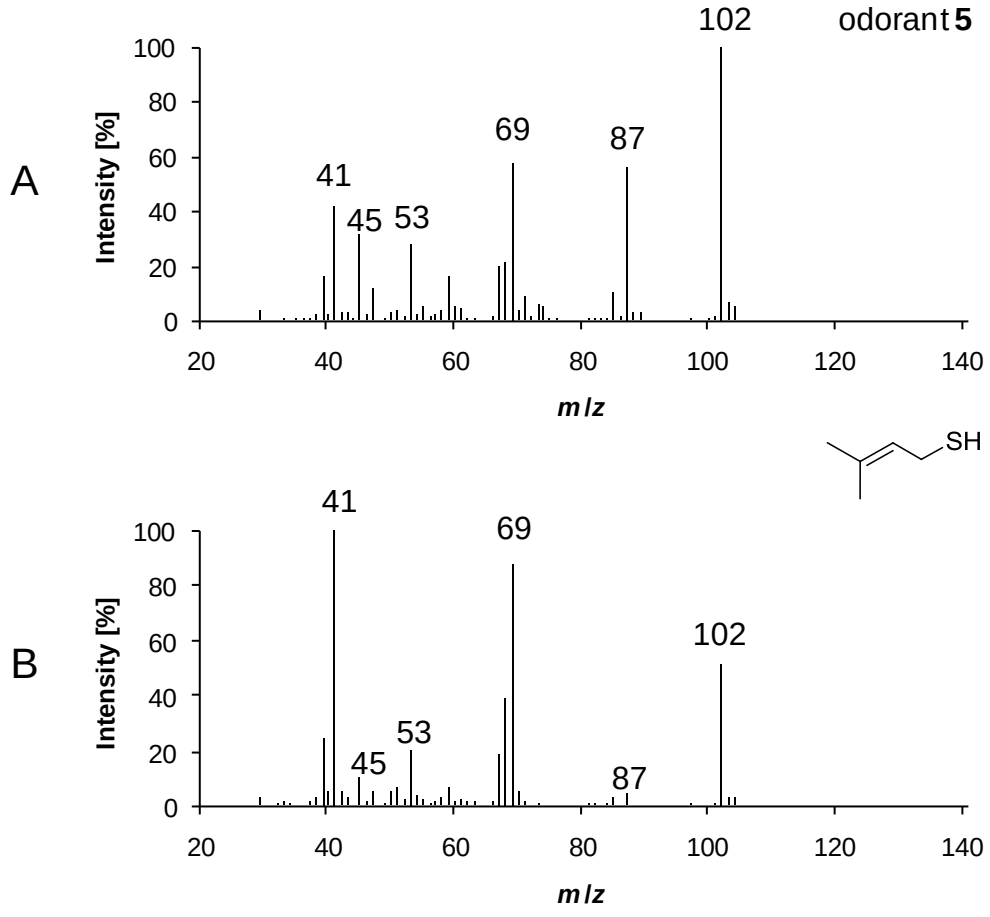


Figure 14: Mass spectra (MS-EI) of odorant **5** (A) and 3-methyl-2-butene-1-thiol (B).

Furthermore, the mass spectra of unknown odorants **2**, **3**, and **4** in the thiol fraction isolated by the mercurated agarose gel from fraction A, exhibited similar fragmentation patterns to that of unknown odorant **5**, and also showed the same molecular ion (*m*/*z* 102) (**Figure 15**). Their

odor qualities were also quite comparable (**Table 11**, sulfurous and meaty). Therefore, it was considered that all four unknown compounds (**2**–**5**) might be isomers of a similar structure.

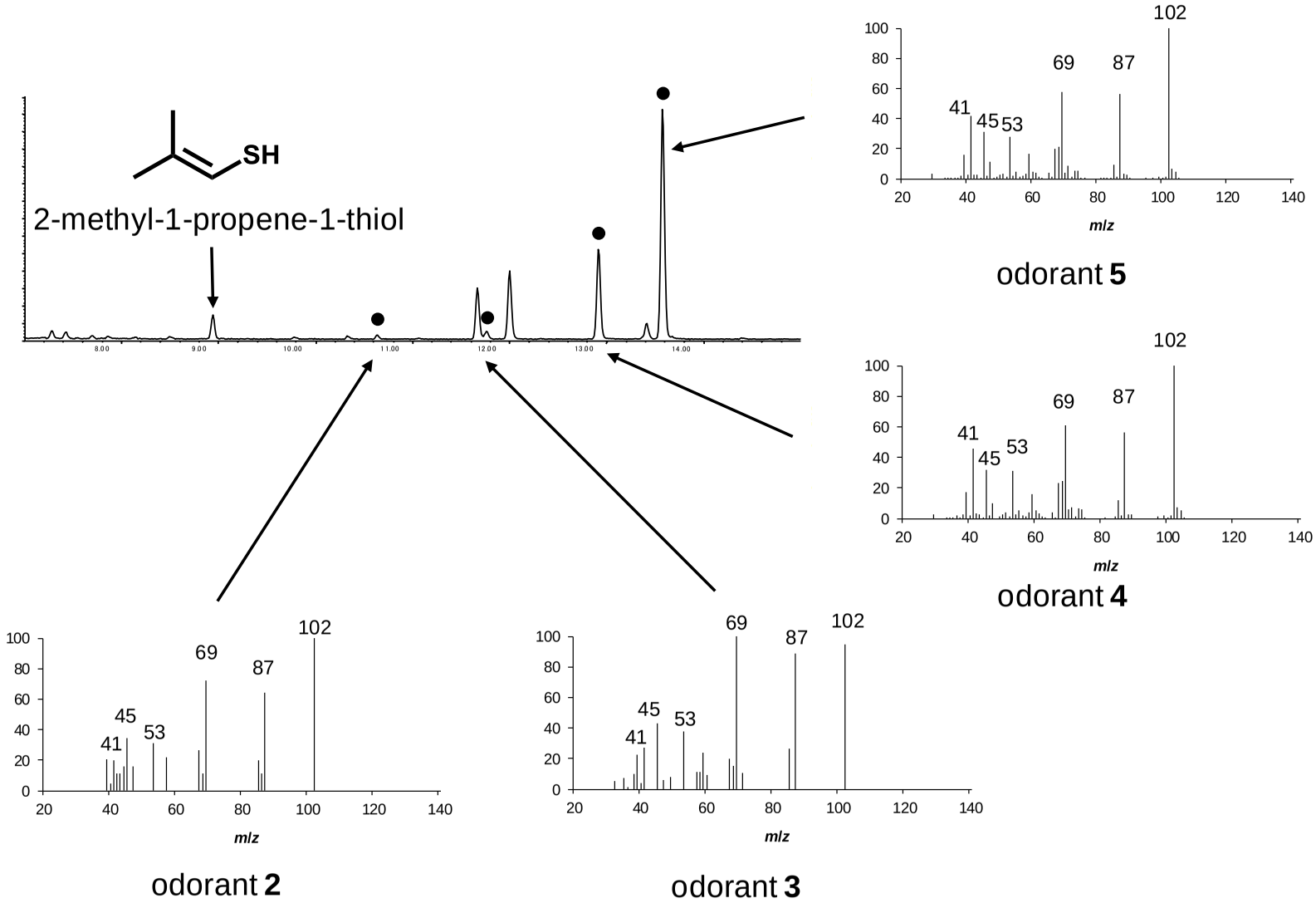


Figure 15: Peaks of odorants **2**–**5** in the gas chromatogram and their mass spectra (MS-EI).

A comparison of the mass spectrum of **5** (**Figure 16A**) with that of 2-methyl-1-propene-1-thiol (**1**; **Figure 16B**) indicated clear similarities. Both mass spectra showed the molecular ions as the highest peaks (**Figure 16**, *m*/*z*: 102 for odorant **5**, and *m*/*z*: 88 for 2-methyl-1-propene-1-thiol (**1**)). Moreover, they showed similar cleavage patterns: both had (M^+–15) indicating a cleavage of a methyl group (*m*/*z*: 87 for odorant **5**, and *m*/*z*: 73 for **1**), and (M^+–33) indicating a cleavage of a thiol group (*m*/*z*: 69 for odorant **5**, and *m*/*z*: 55 for **1**). These similarities mean that all fragments in **5** were shifted 14 mass units higher as compared to 2-methyl-1-propene-1-thiol (**1**).

Thus, it was estimated that odorant **5** might have a skeleton consisting of five instead of four carbon atoms, and was proposed to be either (*E*)-2-methyl-1-butene-1-thiol, (*Z*)-2-methyl-1-butene-1-thiol, (*E*)-3-methyl-1-butene-1-thiol, or (*Z*)-3-methyl-1-butene-1-thiol (**Figure 17**).

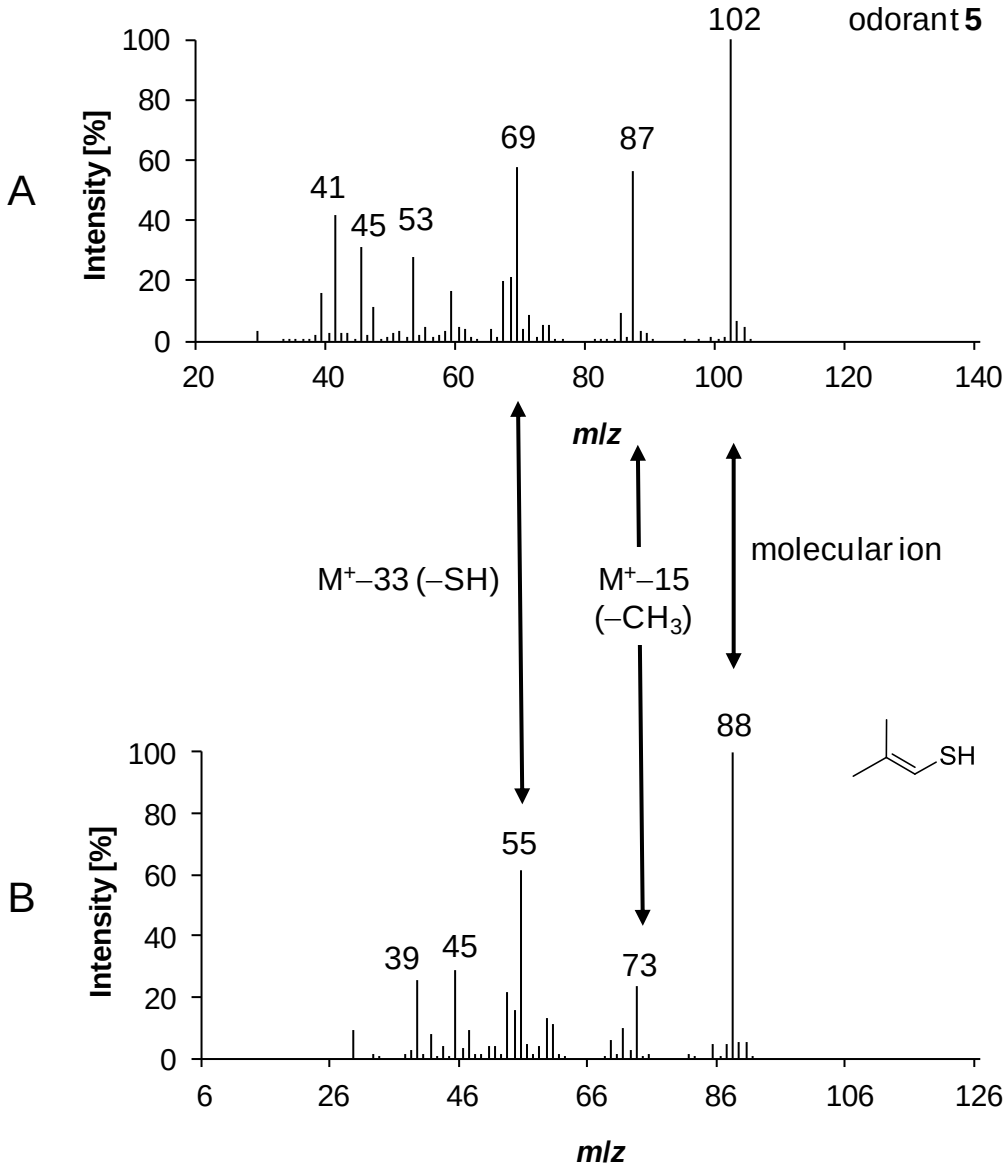
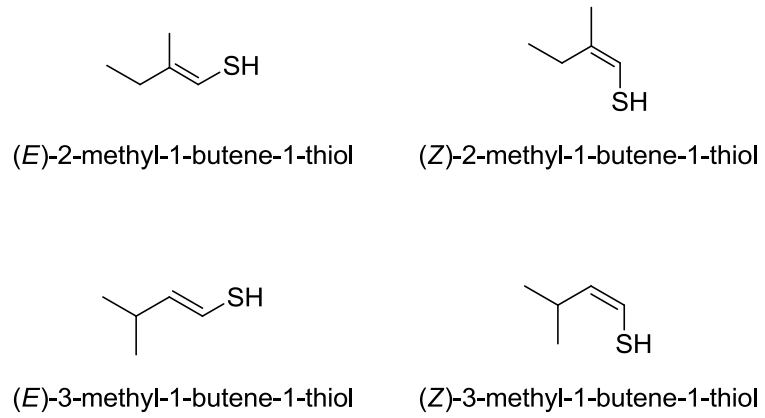


Figure 16: Comparison of the mass spectra (MS-EI) of odorant **5** (A) and 2-methyl-1-propene-1-thiol (B; **1**).



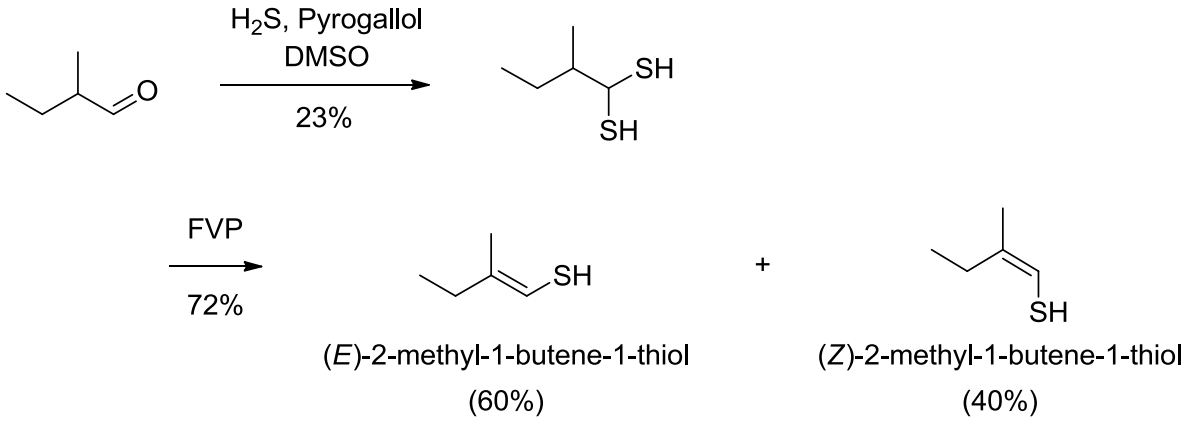
(*E*)-2-methyl-1-butene-1-thiol (*Z*)-2-methyl-1-butene-1-thiol

(*E*)-3-methyl-1-butene-1-thiol (*Z*)-3-methyl-1-butene-1-thiol

Figure 17: Structures of (*E*)- and (*Z*)-2-methyl-1-butene-1-thiol and (*E*)- and (*Z*)-3-methyl-1-butene-1-thiol.

Synthesis of 2-methyl-1-butene-1-thiols and identification of odorant 4 and 5

To corroborate this assumption, first (*E*)-2-methyl-1-butene-1-thiol and (*Z*)-2-methyl-1-butene-1-thiol were synthesized (**Figure 18**) following the synthetic approach used for 2-methyl-1-propene-1-thiol (**Figure 12**), but using 2-methylbutanal as the starting material instead of 2-methylpropanal. The aldehyde was converted via 2-methylbutane-1,1-dithiol into 2-methyl-1-butene-1-thiol, which was obtained as mixture of (*E*)- and (*Z*)-isomers. The GC peaks of these two isomers had slightly different retention indices on both columns (DB-5 and DB-FFAP) (cf. **Table 11**), attempts to separate the (*E*)- and (*Z*)-isomers using preparative gas chromatography, however failed because the odorants were too unstable to survive the heated transfer line of the preparative GC.



(*E*)-2-methyl-1-butene-1-thiol (60%) (*Z*)-2-methyl-1-butene-1-thiol (40%)

Figure 18: Synthetic approach used in the preparation of (*E*)- and (*Z*)-2-methyl-1-butene-1-thiol.

Finally, the two GC peaks of 2-methyl-1-butene-1-thiol were assigned the correct stereochemistry according to data obtained by nuclear Overhauser effect spectroscopy-nuclear magnetic resonance (NOESY-NMR) without isolation of the (*E*)- and (*Z*)-isomers (**Figure 19**). The NOESY-NMR analysis of the major isomer (60% of the total GC peak area) indicated that the hydrogen at the 4-position was not so close to the thiol group, but to the hydrogen at 1-position in the molecule. On the other hand, the data for the minor isomer (40% of the total GC peak area) showed no nuclear Overhauser effect between the hydrogen at the 4-position and the hydrogen at 1-position, but it was indicated that the hydrogen at the 4-position was close to the thiol group. Hence, it was determined that the major GC peak was (*E*)-2-methyl-1-butene-1-thiol, and that the minor one was (*Z*)-2-methyl-1-butene-1-thiol.

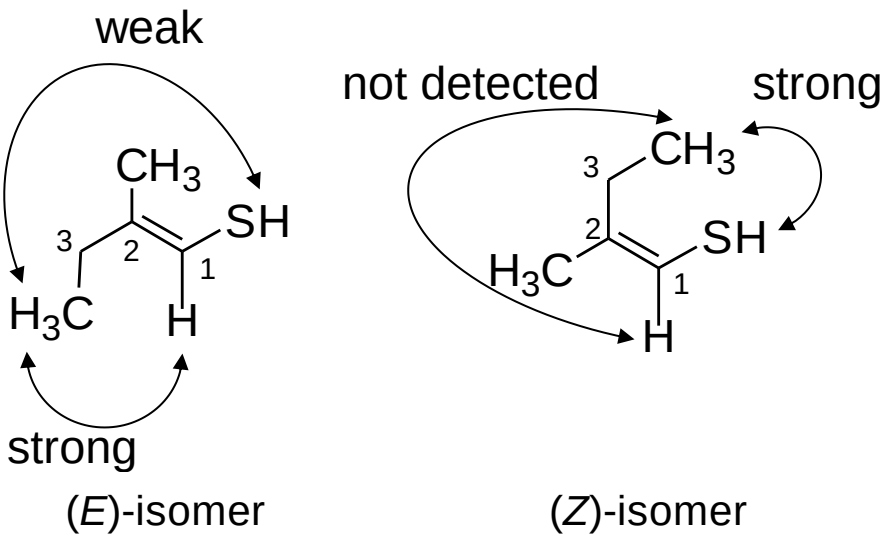


Figure 19: NOE observed for synthesized (*E*)-2-methyl-1-butene-1-thiol and (*Z*)-2-methyl-1-butene-1-thiol.

Comparison of the retention indices, the olfactory properties, and the mass spectra (**Figure 20**) of synthesized (*E*)-2-methyl-1-butene-1-thiol and (*Z*)-2-methyl-1-butene-1-thiol with those of compounds **4** and **5**, identified **4** as (*Z*)-2-methyl-1-butene-1-thiol, and **5** as (*E*)-2-methyl-1-butene-1-thiol.

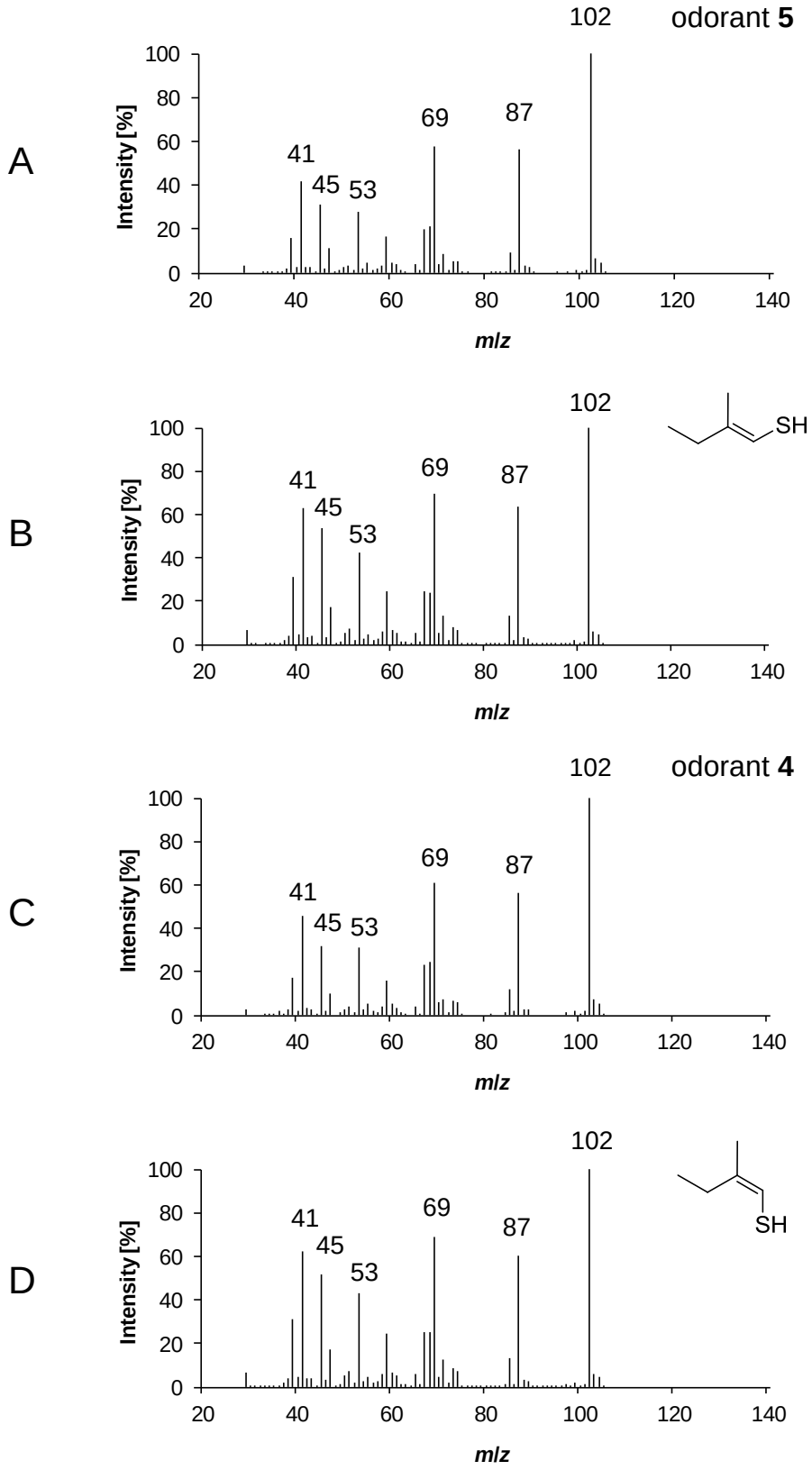


Figure 20: Mass spectra (MS-EI) of odorant **5** (A), (*E*)-2-methyl-1-butene-1-thiol (B), odorant **4** (C), and (*Z*)-2-methyl-1-butene-1-thiol (D)

Synthesis of 3-methyl-1-butene-1-thiols and identification of odorant 2 and 3

As already indicated in **Figure 15**, also the mass spectra of compounds **2** and **3** were quite identical and also showed similarities to those of compounds **4** and **5**. It was, thus, suggested that these odorants might be (*Z*)- and (*E*)-3-methyl-1-butene-1-thiol. Following the approach shown in **Figure 21**, both isomers were synthesized starting from 3-methylbutanal. The two peaks obtained during synthesis were assigned the correct stereochemistry according to the (*E*)/(*Z*) ratio, which was determined by ^{1}H-NMR on the basis of the different coupling constants between each of protons at the double bond (cf. **3.3.2.9**).

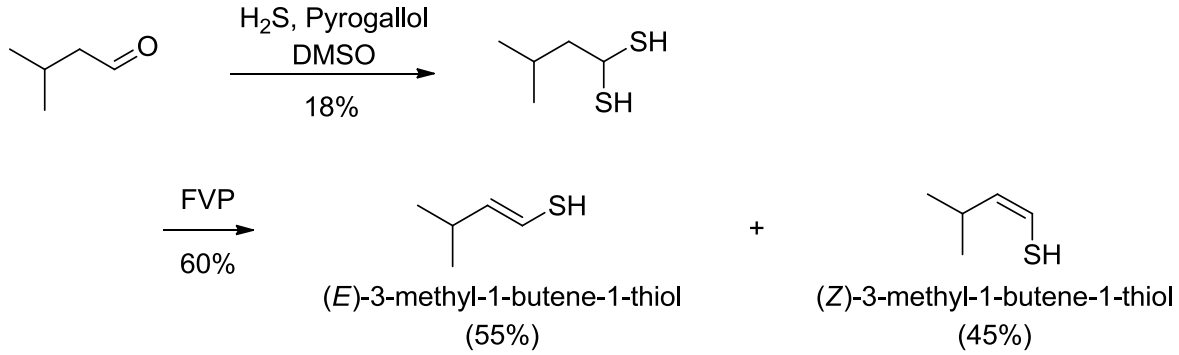


Figure 21: Synthetic approach used in the preparation of (*E*)-3-methyl-1-butene-1-thiol and (*Z*)-3-methyl-1-butene-1-thiol.

By comparing the mass spectra, the retention indices and the odor properties (**Table 11**) of synthesized (*Z*)-3-methyl-1-butene-1-thiol (**Figure 21**) and (*E*)-3-methyl-1-butene-1-thiol (**Figure 21**) with those of the unknown compounds, **2** was identified as (*Z*)-3-methyl-1-butene-1-thiol and **3** as (*E*)-3-methyl-1-butene-1-thiol.

To the best of my knowledge, (*E*)-2-methyl-1-butene-1-thiol (**5**), (*Z*)-2-methyl-1-butene-1-thiol (**4**), (*E*)-3-methyl-1-butene-1-thiol (**3**), and (*Z*)-3-methyl-1-butene-1-thiol (**2**) were novel compounds, neither identified in food before nor addressed in any other scientific study (*Tamura et al., 2010 and 2011*).

2.1.4.3 Identification of odorant 15

The two-dimensional HRGC-MS analysis of the thiol isolate of fraction B yielded a mass spectrum for the catty, black currant-like smelling odorant **15** (**Figure 22**). The identical odor quality suggested that **15** might be a homologue of 3-mercapto-2-pentanone or 2-mercapto-3-pentanone (molecular weight 118). The retention indices (**Table 13**) and the molecular mass of 132 pointed to a C-6 compound, e.g., a mercapto hexanone structure. Moreover, the fragment *m*/*z* 57 suggested a propionyl moiety and *m*/*z* 75 indicated a mercapto propyl group in the molecule. Thus, the structure was proposed to be 4-mercapto-3-hexanone.

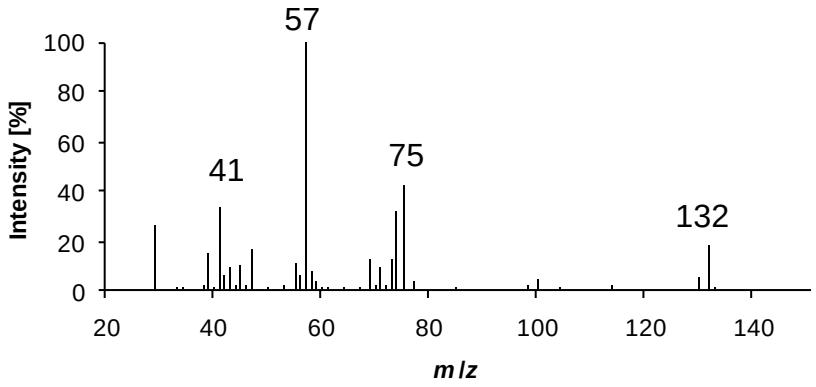


Figure 22: Mass spectrum (MS-EI) of odorant **15**.

Table 13: Odor qualities and retention indices of catty, black-currant-like smelling odorants in roasted sesame seeds.

No.	compound	odor quality [a]	RI on	
			DB-5	FFAP
7	3-mercapto-2-pentanone	catty, black currant-like	895	1344
9	2-mercapto-3-pentanone	catty, black currant-like	905	1361
15	unknown	catty, black currant-like	987	1405

[a] Odor quality perceived at the sniffing port.

To check this, 4-mercapto-3-hexanone was synthesized following a three-step synthesis (**Figure 23**). Because the reference compound exhibited the same odor quality as odorant **15** and yielded the same mass spectrum as well as the same retention indices, **15** was finally identified as 4-mercapto-3-hexanone.

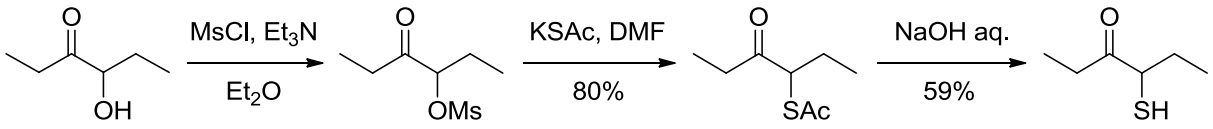


Figure 23: Synthetic approach used in the preparation of 4-mercapto-3-hexanone.

As result of the identification experiments, eleven aroma-active thiols including all eight sulfurous or catty unknown compounds in the former study (*Schieberle, 1996*) were identified. Among them, nine aroma-active thiols were identified for the first time in roasted sesame through this study, namely 2-methyl-1-propene-1-thiol (**1**), (*Z*)-3-methyl-1-butene-1-thiol (**2**), (*E*)-3-methyl-1-butene-1-thiol (**3**), (*Z*)-2-methyl-1-butene-1-thiol (**4**), (*E*)-2-methyl-1-butene-1-thiol (**5**), 3-mercapto-2-pentanone (**7**), 4-mercapto-3-hexanone (**15**), 3-mercapto-3-methylbutyl formate (**19**), and 2-methyl-3-thiophenethiol (**20**). Particularly, (*E*) and (*Z*)-2-methyl-1-butene-1-thiol and (*E*) and (*Z*)-3-methyl-1-butene-1-thiol are novel compounds.
However, to only identify novel compounds does not make sense to discover the total flavor of roasted sesame, especially in the freshly ground state. Thus, their contribution using the odor activity value concept must be evaluated as the next step.

2.2 Assessing the aroma impact of aroma-active thiols by odor activity values

In order to obtain exact concentrations and to compensate for losses occurring during workup procedures, stable isotope dilution assays (SIDA) were developed for the quantitation of the following eleven aroma-active thiols which have been identified in ground pan-roasted white sesame seeds as described above (cf. **2.1.3** and **2.1.4**): 2-methyl-1-propene-1-thiol (**1**), (*Z*)-3-methyl-1-butene-1-thiol (**2**), (*E*)-3-methyl-1-butene-1-thiol (**3**), (*Z*)-2-methyl-1-butene-1-thiol (**4**), (*E*)-2-methyl-1-butene-1-thiol (**5**), 2-methyl-3-furanthiol (**6**), 3-mercapto-2-pentanone (**7**), 2-mercapto-3-pentanone (**9**), 4-mercapto-3-hexanone (**15**), 3-mercapto-3-methylbutyl formate (**19**), and 2-methyl-3-thiophenethiol (**20**).

2.2.1 Development of stable isotope dilution assays for aroma-active thiols

As a first step of the SIDA method, stable isotope labeled analogs of the above eleven aroma-active thiols were synthetically prepared. Among them, three deuterium labeled odorants namely [$^2H_3$]-4-mercapto-3-hexanone, [$^2H_6$]-2-methyl-1-propene-1-thiol, and [$^2H_3$]-2-methyl-1-butene-1-thiol, were synthesized for the first time within the scope of this study. In addition, the new synthetic route for [$^2H_6$]-3-mercapto-3-methylbutyl formate was exploited to achieve higher yield and purity.

2.2.1.1 [$^2H_3$]-4-Mercapto-3-hexanone (d-15)

Deuterium labeled 4-mercapto-3-hexanone (**d-15**) was synthesized following the route described above for the unlabeled compound (cf. **2.1.4.3** and **Figure 23**), but using [$^2H_3$]-4-hydroxy-3-hexanone instead of 4-hydroxy-3-hexanone as the starting material. The [$^2H_3$]-4-hydroxy-3-hexanone was prepared in a three step procedure from [$^2H_3$]-bromoethane as presented in **Figure 24**. A nucleophilic substitution using 2-lithio-1,3-dithiane as nucleophile yielded [$^2H_3$]-2-ethyl-1,3-dithiane, which was deprotonated by butyl lithium and then reacted with propanal. This nucleophilic addition resulted in [$^2H_3$]-1-(2-ethyl-1,3-dithian-2-yl)propan-1-ol, which underwent hydrolysis to finally yield [$^2H_3$]-4-hydroxy-3-hexanone. Then, [$^2H_3$]-4-mercapto-3-hexanone (**d-15**) was successfully synthesized following the same three steps as described for the unlabeled compound.

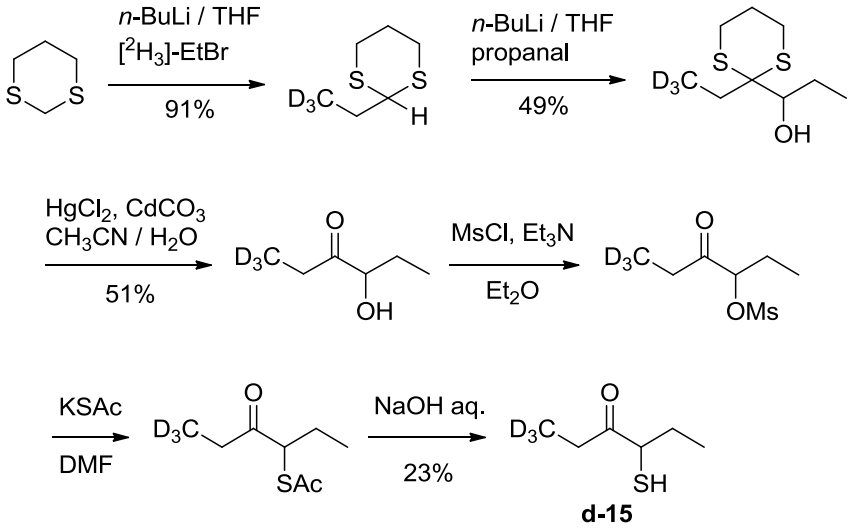


Figure 24: Synthetic approach used in the preparation of [$^2H_3$]-4-mercapto-3-hexanone (**d-15**).

The MS-CI of the synthesized [$^2H_3$]-4-mercapto-3-hexanone showed *m/z* 136 $(M+1)^+$ as the molecular mass peak (**Figure 25**, left). Hence, the incorporation of three deuterium atoms was confirmed by comparing the spectrum with the MS-CI of 4-mercapto-3-hexanone (**Figure 25**, right).

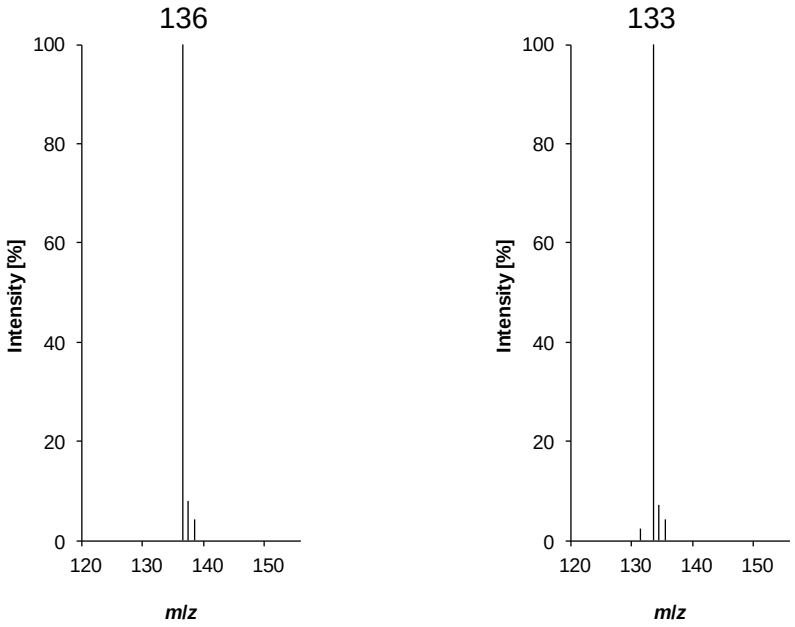


Figure 25: Mass spectra (MS-CI; methanol) of [$^2H_3$]-4-mercapto-3-hexanone (left) and 4-mercapto-3-hexanone (right).

The main fragments of the labeled compound (*m*/*z* 136) and of the unlabeled substance (*m*/*z* 133) were used to determine the response factor. For this purpose, seven mixtures involving known amounts of 4-mercapto-3-hexanone and the labeled one in the ratio 1:10, 1:3, 1:2, 1:1, 3:1, 5:1 and 10:1 were analyzed by two dimensional HRGC-MS (cf. **3.7.4**). The calibration curve was obtained by plotting the weight ratios of analyte to standard versus the respective area ratios of *m*/*z* 133 to 136 (**Figure 26**). A response factor (0.94) was calculated based on the gradient of the calibration curve.

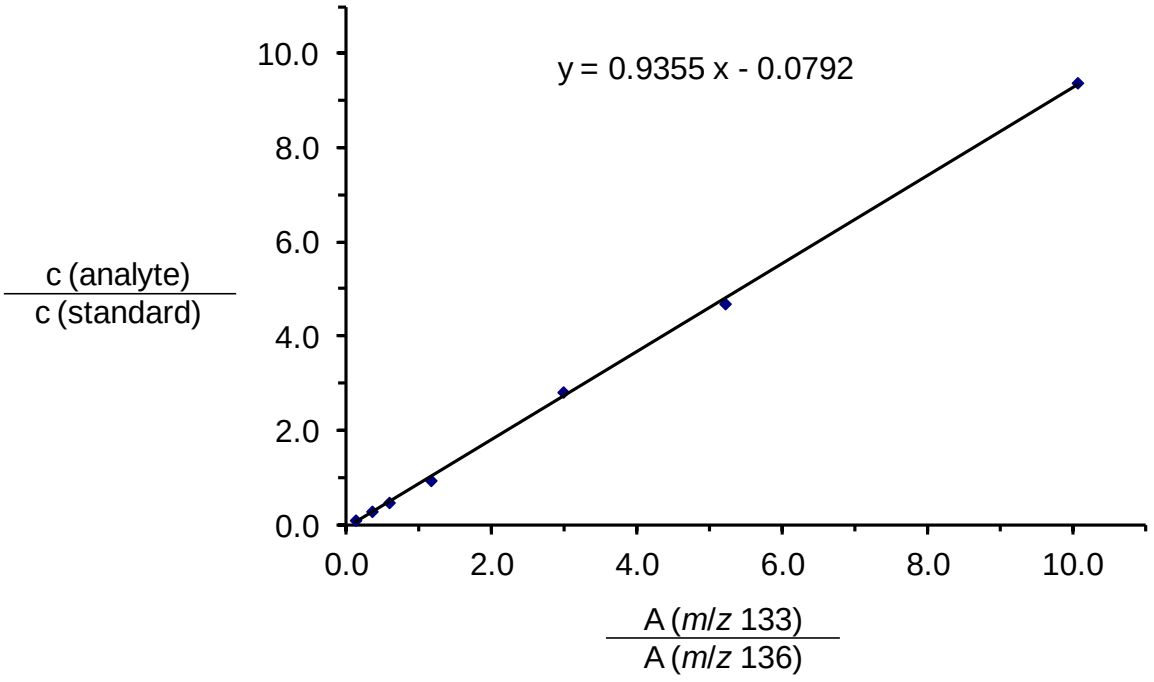


Figure 26: Calibration curve used to determine the response factor of [$^2H_3$]-4-mercapto-3-hexanone (**d-15**).

2.2.1.2 [$^2H_6$]-3-Mercapto-3-methylbutyl formate (d-19)

Although the preparation of [$^2H_6$]-3-mercapto-3-methylbutyl formate (**d-19**) was accomplished before (*Masanetz et al., 1995*), a new synthetic route was developed (**Figure 27**) aiming at higher yields and purity. First, [$^2H_6$]-acetone was converted into [$^2H_6$]-ethyl 3-methyl-2-butenoate by the Horner-Wadsworth-Emmons reaction. Then, the tertiary thiol functional group was regioselectively inserted by addition of H_2S to the olefinic double bond. Reduction of the ester with lithium aluminum hydride yielded the corresponding alcohol,

which was finally esterified with formic acid to obtain [$^2H_6$]-3-mercapto-3-methylbutyl formate (**d-19**). Using this new method, due to the direct insertion of the tertiary thiol group, a higher purity and a higher yield of the target compound was achieved (cf. **3.3.3.2**) as compared to the formerly published procedure (*Masanetz et al., 1995*).

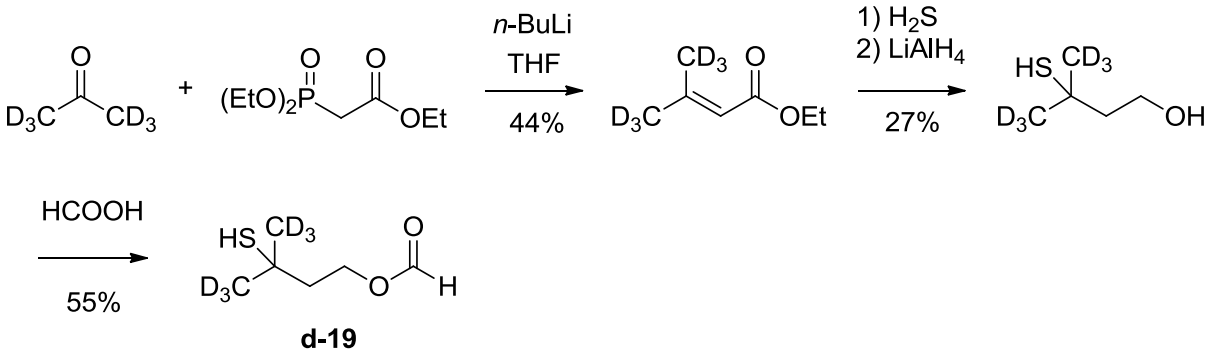


Figure 27: Synthetic approach developed for the preparation of [$^2H_6$]-3-mercapto-3-methylbutyl formate (**d-19**).

2.2.1.3 Deuterium labeled 1-alkene-1-thiols

Syntheses of labeled 1-alkene-1-thiols followed the procedures applied for the preparation of the unlabeled compounds, as drawn for 2-methyl-1-propene-1-thiol (**1**) in **Figure 12**. In general, the corresponding stable isotope labeled aldehydes were reacted with H_2S and the resulting dithiols subsequently underwent flash vacuum pyrolysis (FVP) to yield the target compounds.

2.2.1.3.1 [$^2H_6$]-2-Methyl-1-propene-1-thiol (d-1)

The deuterium labeled aldehyde [$^2H_6$]-2-methylpropanal, needed for the preparation of [$^2H_6$]-2-methyl-1-propene-1-thiol (**d-1**), was synthesized from [$^2H_6$]-acetone (**Figure 28**). For that purpose, [$^2H_6$]-acetone was first converted to [$^2H_6$]-1,1-dimethyloxirane by the Johnson-Corey-Chaykovsky reaction. Acid catalyzed opening of the oxirane ring then resulted in the [$^2H_6$]-2-methylpropanal formation. Hydrogen sulfide addition followed by FVP on the labeled aldehyde afforded the target compound [$^2H_6$]-2-methyl-1-propene-1-thiol (**d-1**) (**Figure 28**).

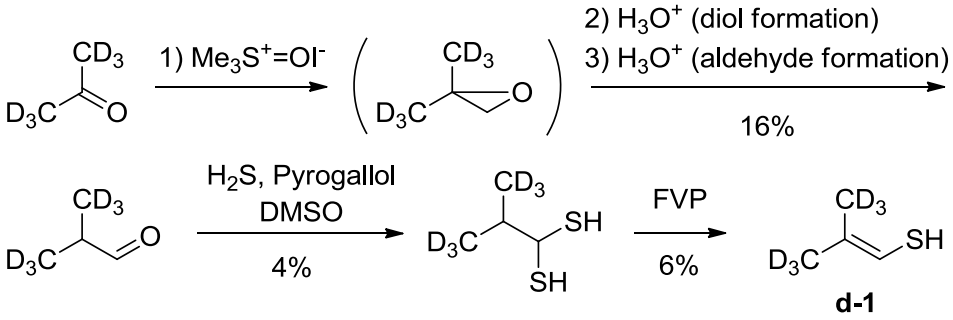


Figure 28: Synthetic approach used in the preparation of [$^2H_6$]-2-methyl-1-propene-1-thiol (**d-1**).

The mass spectrum of [$^2H_6$]-2-methyl-1-propene-1-thiol in the CI-mode with methanol as reagent gas exhibited *m/z* 95 $(M+1)^+$ as the molecular peak (**Figure 29**, left). A comparison with the MS-CI of 2-methyl-1-propene-1-thiol (*m/z* 95, **Figure 29**, right) proved the incorporation of six deuterium atoms in the molecule.

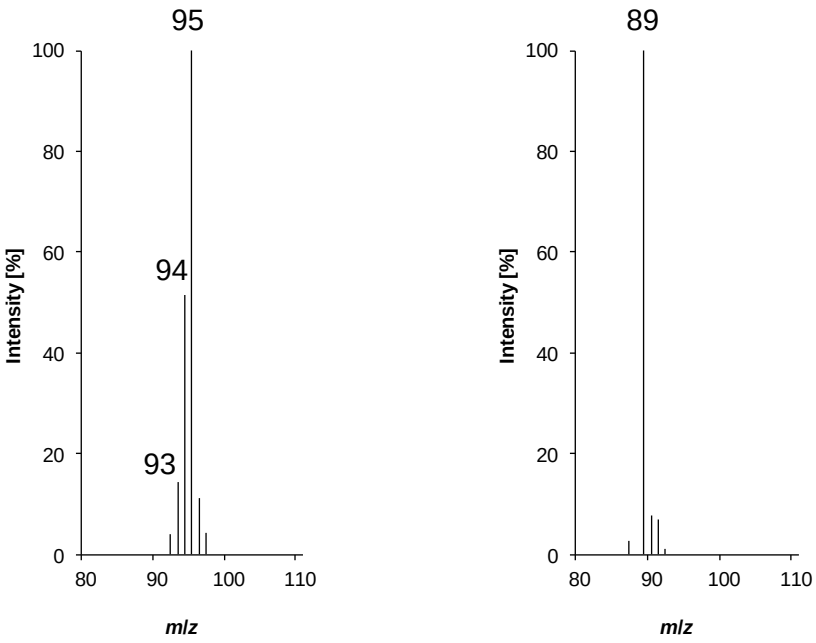


Figure 29: Mass spectra (MS-CI; methanol) of [$^2H_6$]-2-methyl-1-propene-1-thiol (left) and 2-methyl-1-propene-1-thiol (right).

The main fragments of the labeled compound (*m*/*z* 95) and of the unlabeled one (*m*/*z* 89) were used to determine the response factor. For this purpose, five mixtures containing known amounts of 2-methyl-1-propene-1-thiol and [$^2H_6$]-2-methyl-1-propene-1-thiol in the ratio 1:10, 1:3, 1:1, 3:1, and 5:1 were analyzed by two dimensional HRGC-MS (cf. **3.7.4**). The calibration curve was obtained by plotting the weight ratios of analyte to standard versus the respective area ratios of *m*/*z* 89 to 95 (**Figure 30**). A response factor (0.67) was calculated based on the gradient of the calibration curve.

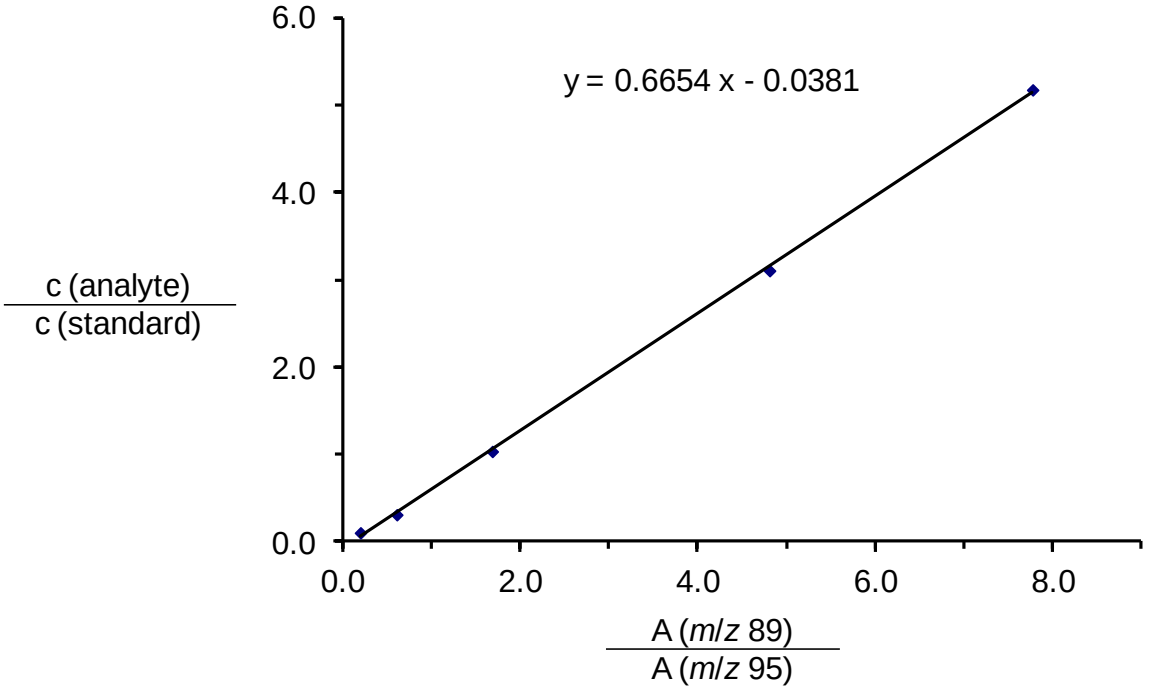


Figure 30: Calibration curve used to determine the response factor of [$^2H_6$]-2-methyl-1-propene-1-thiol (**d-1**).

2.2.1.3.2 [$^2H_3$]-(*E*)- and [$^2H_3$]-(*Z*)-2-Methyl-1-butene-1-thiol (d-5 and d-4)

The preparation of [$^2H_3$]-2-methylbutanal was necessary for the syntheses of [$^2H_3$]-(*E*)-2-methyl-1-butene-1-thiol (**d-5**) and [$^2H_3$]-(*Z*)-2-methyl-1-butene-1-thiol (**d-4**). The labeled aldehyde was synthesized from butyronitrile according to *Goering and Tseng* (*1981*) (**Figure 31**). Using diisobutylaluminium hydride, butyronitrile was converted to the corresponding aluminum imide. Deprotonation by lithium diisopropyl amide yielded the dianion, which was alkylated using methyl iodide. Acid hydrolysis finally afforded [$^2H_3$]-2-methylbutanal. The [$^2H_3$]-(*E*)-2-methyl-1-butene-1-thiol (**d-5**) and [$^2H_3$]-(*Z*)-2-methyl-1-butene-1-thiol (**d-4**) (**Figure 31**) were finally prepared by the general pathway described in **Figure 12**.

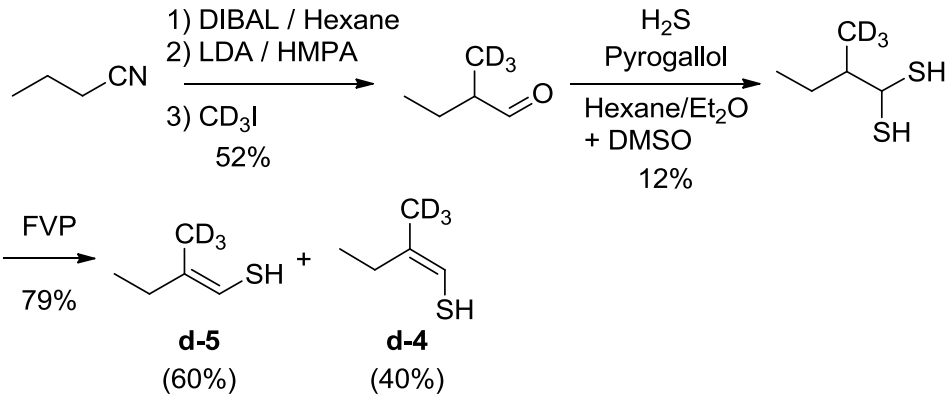


Figure 31: Synthetic approach used in the preparation of [$^2H_3$]-(*E*)-2-methyl-1-butene-1-thiol (**d-5**) and [$^2H_3$]-(*Z*)-2-methyl-1-butene-1-thiol (**d-4**).

The mass spectra of [$^2H_3$]-(*E*)-2-methyl-1-butene-1-thiol (**d-5**) (**Figure 32**, left) and [$^2H_3$]-(*Z*)-2-methyl-1-butene-1-thiol (**d-4**) (**Figure 33**, left) showed a molecular mass of *m*/*z* 106 $(M+1)^+$. Thus, compared with the MS-CI of (*E*)-2-methyl-1-butene-1-thiol (**Figure 32**, right) and (*Z*)-2-methyl-1-butene-1-thiol (**Figure 33**, right) it was proven that three deuterium atoms were incorporated into the structures of both isomers.

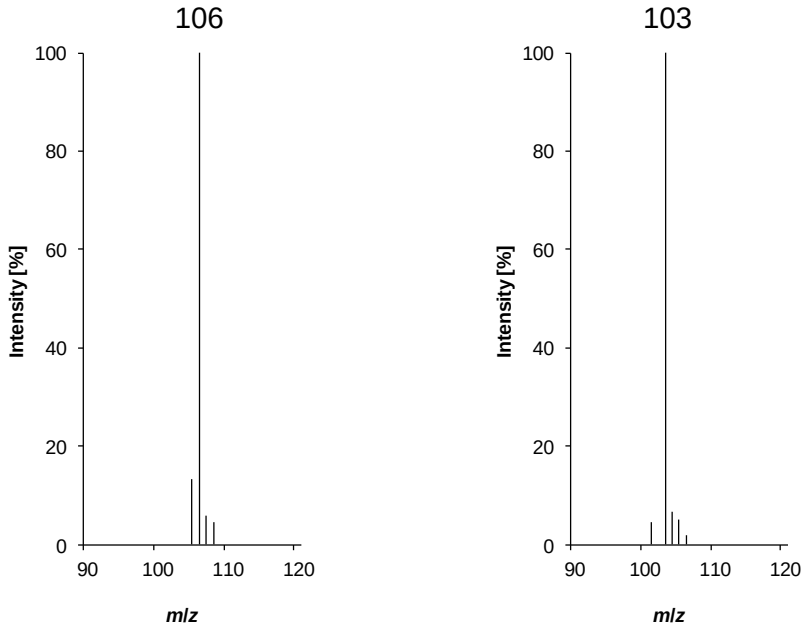


Figure 32: Mass spectra (MS-CI; methanol) of [$^2H_3$]-(*E*)-2-methyl-1-butene-1-thiol (left) and (*E*)-2-methyl-1-butene-1-thiol (right).

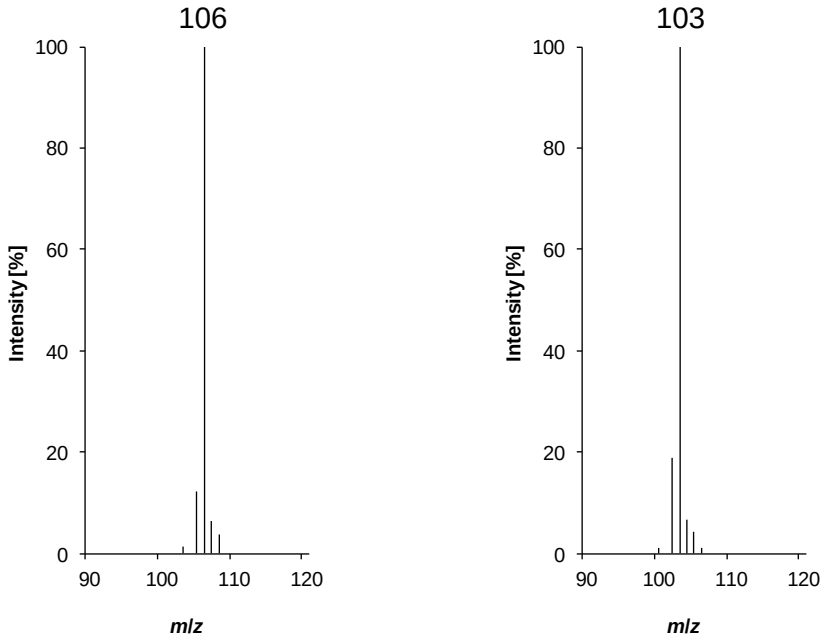


Figure 33: Mass spectra (MS-CI; methanol) of [$^2H_3$]-(*Z*)-2-methyl-1-butene-1-thiol (left) and (*Z*)-2-methyl-1-butene-1-thiol (right).

The main fragments of the labeled compound (*m*/*z* 106) and of the unlabeled one (*m*/*z* 103) were used to determine the response factor. For this purpose, three mixtures containing known amounts of (*E*)- or (*Z*)-isomers of 2-methyl-1-butene-1-thiol and the corresponding labeled standard in the ratio 1:2, 1:1, and 3:1 were analyzed by two dimensional HRGC-MS (cf. **3.7.4**), respectively. The calibration curves for both isomers were obtained by plotting the weight ratios of analytes to standards versus the respective area ratios of *m*/*z* 103 to 106 (**Figure 34** and **35**). Response factors (1.31 and 1.36, respectively) were calculated based on the gradient of the calibration curve.

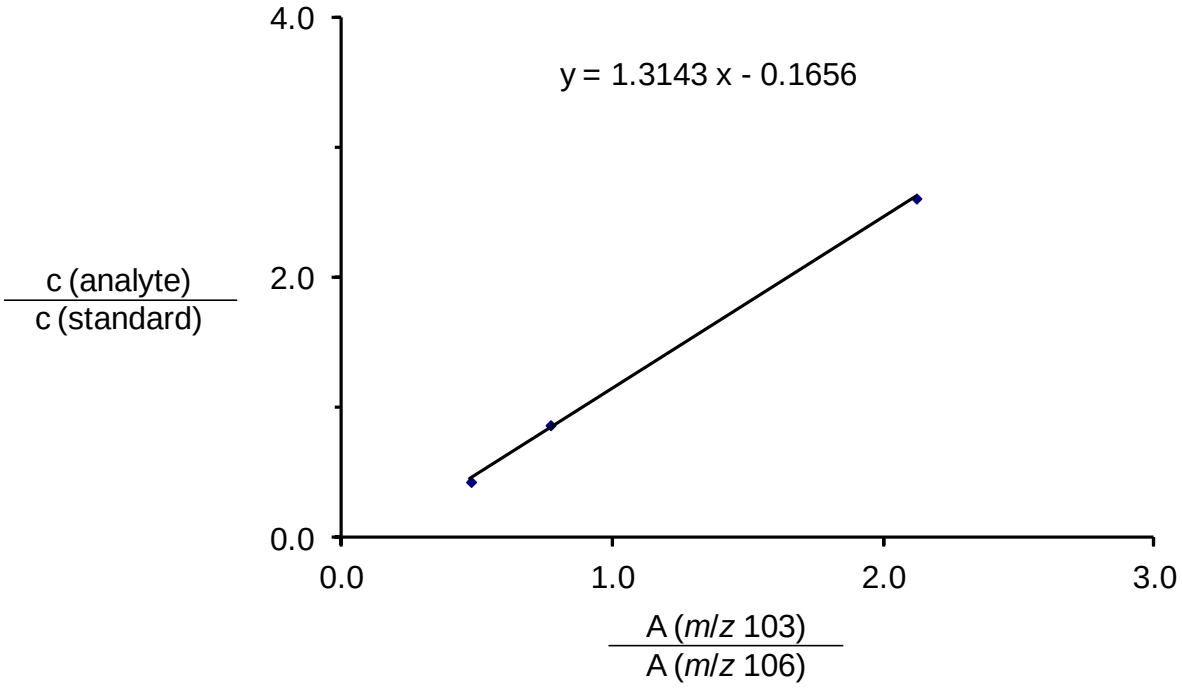


Figure 34: Calibration curve used to determine the response factor of [$^2H_3$]-(*E*)-2-methyl-1-butene-1-thiol (**d-5**).

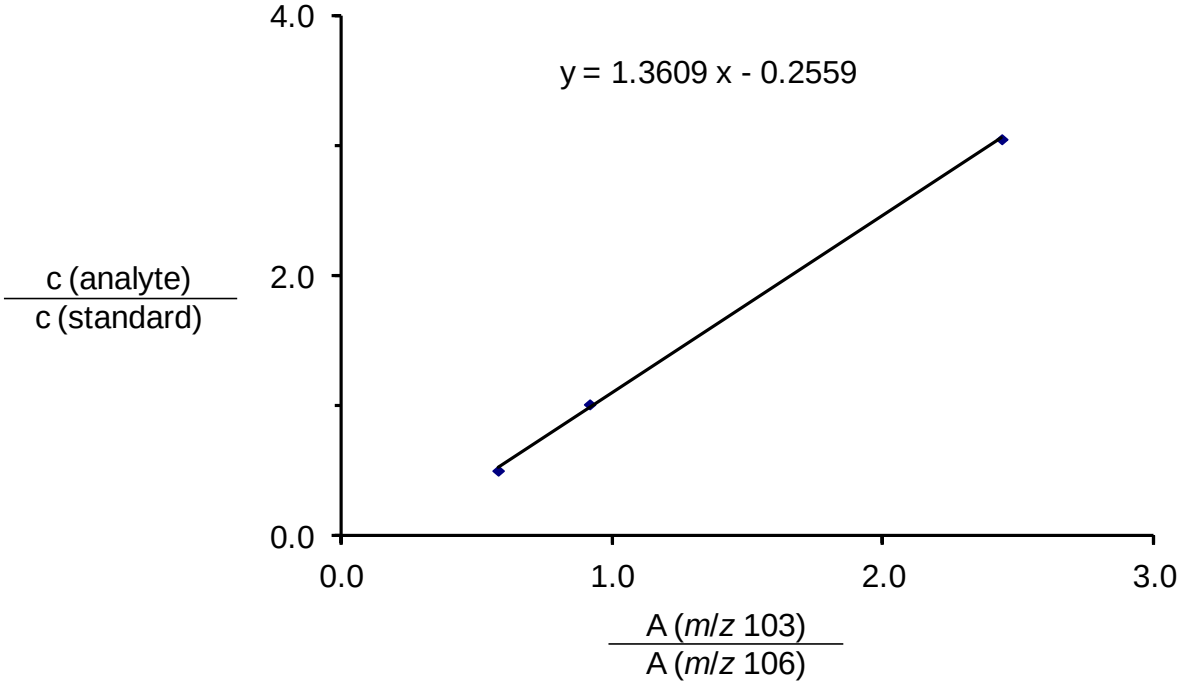


Figure 35: Calibration curve used to determine the response factor of [$^2H_3$]-(*Z*)-2-methyl-1-butene-1-thiol (**d-4**).

2.2.1.4 Preparation of further deuterium labeled thiols

The syntheses of deuterium labeled internal standards for 2-methyl-3-furanthiol and 3-mercapto-2-pentanone, namely [$^2H_3$]-2-methyl-3-furanthiol (**d-6**) and [$^2H_2$]-3-mercapto-2-pentanone (**d-7**), followed already published procedures (*Sen and Grosch, 1991*).

Preparation of [$^2H_3$]-2-methyl-3-thiophenethiol (**d-20**) was also completed using the same approach as described for the synthesis of [$^2H_3$]-2-methyl-3-furanthiol (**d-6**) by *Sen and Grosch* (*1991*). For that purpose, the starting compound was simply changed from 3-bromofuran to 3-bromothiophene, and the target compound [$^2H_3$]-2-methyl-3-thiophenethiol (**d-20**) was suitably synthesized (cf. **3.3.3.5**).

Attempts to synthesize [$^2H_3$]-(*E*)-3-methyl-1-butene-1-thiol and [$^2H_3$]-(*Z*)-3-methyl-1-butene-1-thiol failed so far because of the enormous instability of these compounds. For that reason concentrations of (*E*)- and (*Z*)-3-methyl-1-butene-1-thiol were approximated using [$^2H_3$]-(*E*)-2-methyl-1-butene-1-thiol as the internal standard.

For this purpose, three mixtures containing known amounts of (*E*)- or (*Z*)-isomers of 3-methyl-1-butene-1-thiol and [$^2H_3$]-(*E*)-2-methyl-1-butene-1-thiol in the ratio 1:2, 1:1, and 3:1 were analyzed by two dimensional HRGC-MS (cf. **3.7.4**). The calibration curves for both isomers were obtained by plotting the weight ratios of analytes to standards versus the respective area ratios of *m*/*z* 103 to 106 (**Figure 36** and **37**). Response factors (1.25 and 1.26, respectively) were calculated based on the gradient of the calibration curve.

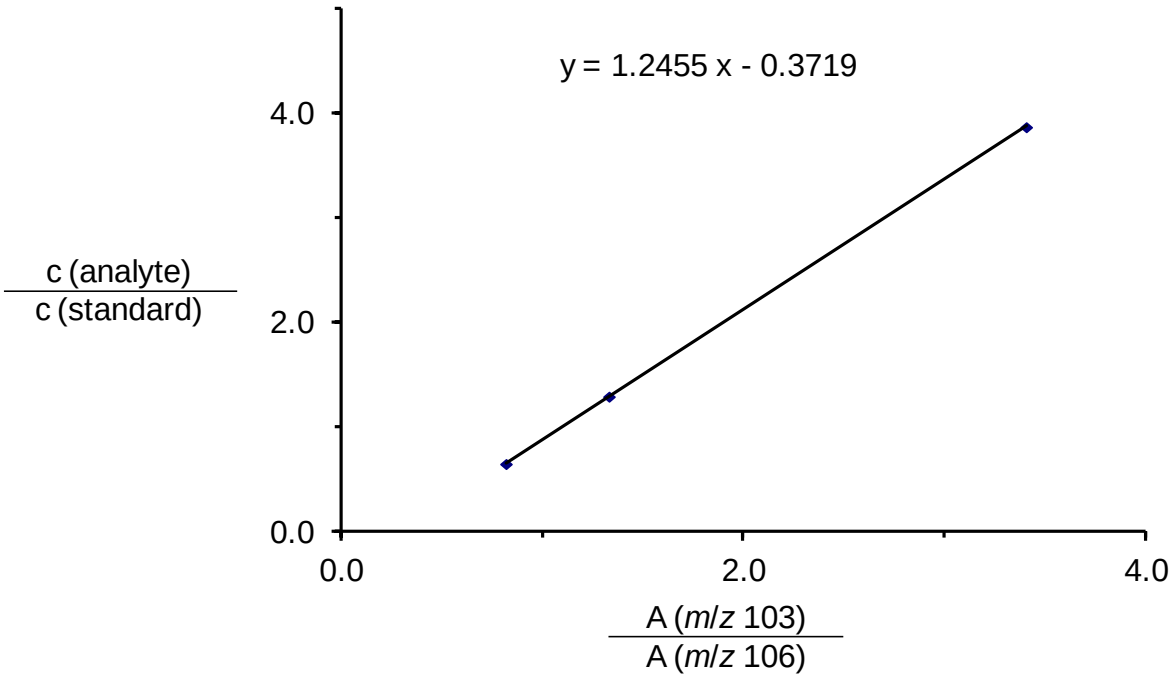


Figure 36: Calibration curve used to determine the response factor of [$^2H_3$]-(*E*)-2-methyl-1-butene-1-thiol (**d-5**) to (*E*)-3-methyl-1-butene-1-thiol.

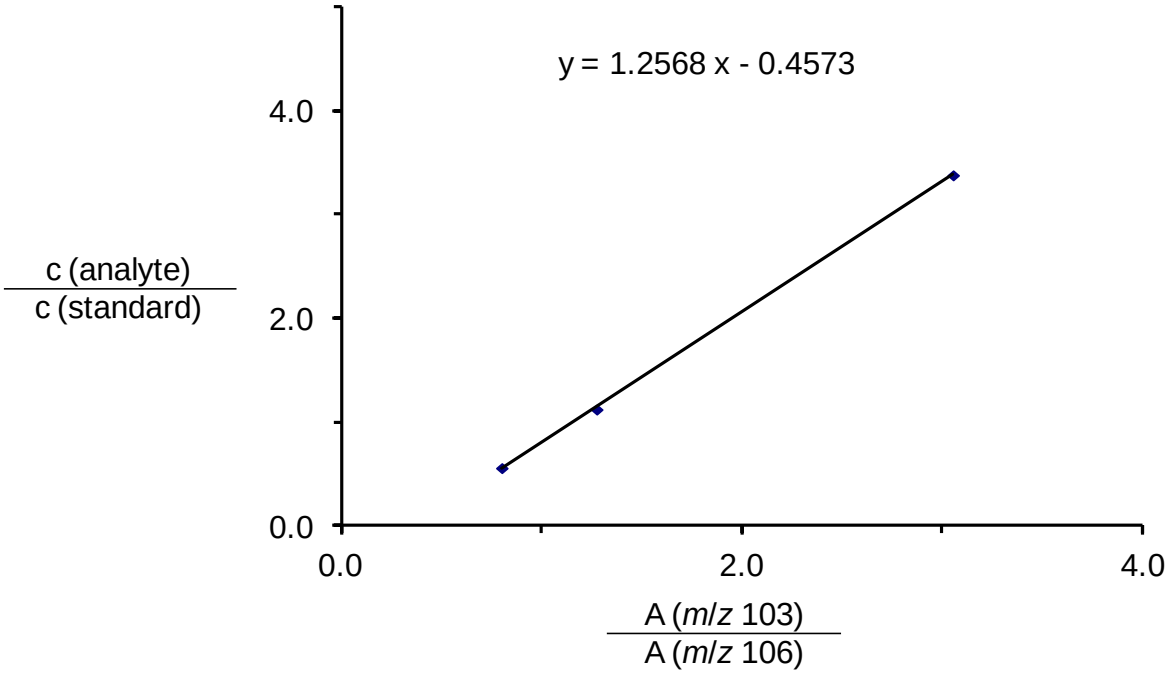


Figure 37: Calibration curve used to determine the response factor of [$^2H_3$]-(*E*)-2-methyl-1-butene-1-thiol (**d-5**) to (*Z*)-3-methyl-1-butene-1-thiol.

In the same way, quantitation of 2-mercapto-3-pentanone was achieved using [$^{2}H_{2}$]-3-mercapto-2-pentanone (**d-7**) as internal standard as previously described by *Kerscher and Grosch* (*1998*) .

2.2.2 Concentrations and odor activity values of aroma-active thiols

By now, all isotopically labeled internal standards for the assessment of their aroma impacts on ground roasted sesame seeds aroma were available. The structures of the deuterium labeled internal standards are displayed in **Figure 38**.

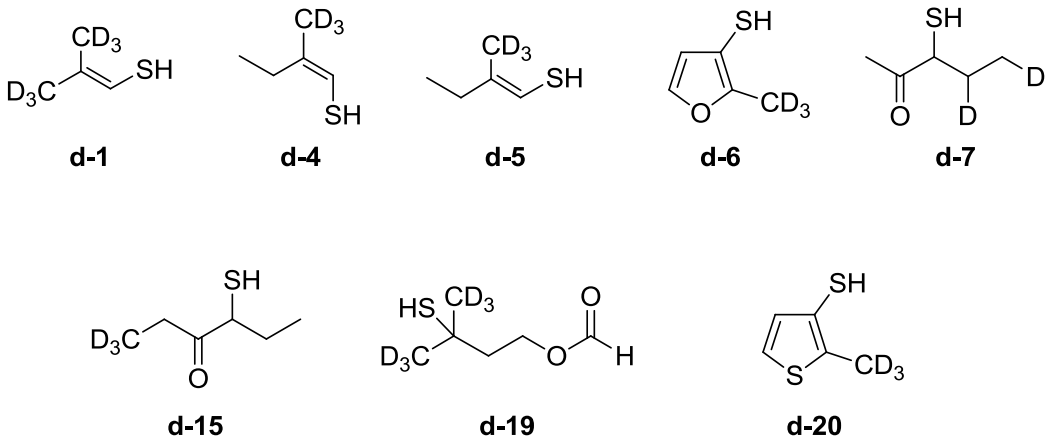


Figure 38: Structures of the deuterium labeled internal standards: [$^2H_6$]-2-methyl-1-propene-1-thiol (**d-1**), [$^2H_3$]-(*Z*)-2-methyl-1-butene-1-thiol (**d-4**), [$^2H_3$]-(*E*)-2-methyl-1-butene-1-thiol (**d-5**), [$^2H_3$]-2-methyl-3-furanthiol (**d-6**), [$^2H_2$]-3-mercapto-2-pentanone (**d-7**), [$^2H_3$]-4-mercapto-3-hexanone (**d-15**), [$^2H_6$]-3-mercapto-3-methylbutyl formate (**d-19**), and [$^2H_3$]-2-methyl-3-thiophenethiol (**d-20**).

2.2.2.1 Quantitation of the aroma-active thiols using stable isotope dilution assay (SIDA)

For the execution of the SIDAs for the aroma-active thiols, defined amounts of the stable isotope labeled standards were added to known amounts of freshly ground pan-roasted white sesame seeds together with the extraction solvent. Extracts were then filtered, and distilled using the SAFE apparatus.

To obtain clear mass spectra of the thiols, the SAFE distillate was fractionated by affinity chromatography using mercurated agarose gel (cf. **2.1.3.2** and **3.4.1.4**). By this technique, the target thiols as well as the deuterium labeled thiols were selectively enriched. The thiol isolate was subsequently concentrated and analyzed by mass chromatography using a GC/GC-MS system with heart-cutting (cf. **3.7.4**).

The results of the quantitation by stable isotope dilution assay revealed concentrations of the aroma-active thiols in the range of 11 ng/kg up to 0.8 mg/kg (**Table 14**). The highest value (800 µg/kg) was found for 2-methyl-1-propene-1-thiol and (*E*)-2-methyl-1-butene-1-thiol, followed by (*Z*)-3-methyl-1-butene-1-thiol (690 µg/kg). However, as a sum of (*E*)- and (*Z*)-isomers, the concentrations of 2-methyl-1-butene-1-thiol (1200 µg/kg) was highest, and 3-methyl-1-butene-1-thiol (970 µg/kg, sum of isomers) followed in the mg/kg scale, therefore all 1-alkene-1-thiols had the concentrations around one mg/kg in roasted white sesame seeds. Moreover, 2-mercapto-3-pentanone (170 µg/kg) and 2-methyl-3-furanthiol (100 µg/kg) followed the 1-alkene-1-thiols in high concentration. The lowest amount was measured for 3-mercapto-3-methylbutyl formate (11 ng/kg). The other three thiols were present in concentrations between 4.7 and 29 µg/kg.

Table 14: Concentrations of major aroma-active thiols in pan-roasted white sesame seeds using stable isotope dilution assay (SIDA).

compound	odor quality	concentration [a] [µg/kg]	RSD [b] [%]
2-methyl-1-propene-1-thiol	sulfurous, meaty	800	13
(*Z*)-3-methyl-1-butene-1-thiol	sulfurous, meaty	690	23
(*E*)-3-methyl-1-butene-1-thiol	sulfurous, meaty	280	11
(*Z*)-2-methyl-1-butene-1-thiol	sulfurous, meaty	400	32
(*E*)-2-methyl-1-butene-1-thiol	sulfurous, meaty	800	27
2-methyl-3-furanthiol	meaty	100	21
3-mercapto-2-pentanone	catty, black currant-like	29	11
2-mercapto-3-pentanone	catty, black currant-like	170	2
4-mercapto-3-hexanone	catty, black currant-like	4.7	3
3-mercapto-3-methylbutyl formate	sulfurous, catty	0.011	43
2-methyl-3-thiophenethiol	meaty, sulfurous	11	39

[a] Mean values of triplicates.

[b] Relative standard deviation of the concentration values (cf. **3.5.4**).

2.2.2.2 Odor activity values of the aroma-active thiols

In order to assess the aroma impact of the individual thiols, their concentrations needed to be related to their respective odor thresholds which were determined in the same matrix as the target food.

With respect to the high fat content of sesame seeds, sunflower oil was selected as matrix for threshold determination. Although some of the quantitated thiols were well-known aroma compounds, their odor thresholds in oleaginous matrix had not been published before except those of 2-methyl-3-furanthiol and 3-mercapto-2-pentanone by *Kerscher* (*2000*). Therefore, odor thresholds in sunflower oil of the rest of the aroma-active thiols in roasted sesame were newly determined by a set of forced-choice triangular tests with ascending concentrations of the test compounds (*Czerny et al., 2008*).

Since 2- and 3-methyl-1-butene-1-thiols were not available as pure (*E*)- and (*Z*)-compounds and because of their instability, their thresholds were determined as 60:40 mixture for 2-methyl-1-butene-1-thiol and 55:45 for 3-methyl-1-butene-1-thiol of the (*E*)- and (*Z*)-isomers, respectively.

The thresholds ranged between 0.7 μg/kg (4-mercapto-3-hexanone) and 2.2 mg/kg (2-methyl-3-thiophenethiol) as shown in **Table 15**. The newly identified 4-mercapto-3-hexanone had the lowest odor threshold in oil (0.07 μg/kg) among the thiols found in roasted sesame in this study. It was about one third of 3-mercapto-2-pentanone (0.19 μg/kg) and about one ninth of 2-mercapto-3-pentanone (0.60 μg/kg). Among the newly identified 1-alkene-1-thiols, 3-methyl-1-butene-1-thiol exhibited the lowest odor threshold (0.4 μg/kg) compared to 2-methyl-1-propene-1-thiol (30 μg/kg) and 2-methyl-1-butene-1-thiol (1.3 μg/kg). The highest odor threshold was found for 2-methyl-3-thiophenethiol possibly because it had more oil solubility than the other thiols.

Using these data, odor activity values (OAV) were calculated as values of concentrations divided by odor thresholds (**Table 15**). OAVs of 3-mercapto-3-methylbutyl formate and 2-methyl-3-thiophenethiol were far below 1, thus indicating that these compounds are not odor-active in the overall aroma of ground roasted sesame seeds. Concentrations of the other seven compounds clearly exceeded their respective odor threshold values, with 3-methyl-1-butene-1-thiol (2400) and 2-methyl-1-butene-1-thiol (920) showing the highest OAVs. The OAV of 2-mercapto-3-pentanone was also higher (280), followed by 2-methyl-3-furanthiol (180) and 3-mercapto-2-pentanone (150). 4-Mercapto-3-hexanone and 2-methyl-1-propene-1-thiol showed relatively high OAVs of 65 and 27, respectively. Thus, the seven thiols were found to

significantly contribute to the aroma of ground roasted sesame seeds, namely 2-methyl-1-propene-1-thiol, 3-methyl-1-butene-1-thiol, 2-methyl-1-butene-1-thiol, 2-methyl-3-furanthiol, 3-mercapto-2-pentanone, 2-mercapto-3-penatanone, and 4-mercapto-3-hexanone.

Table 15: Odor activity values (OAV) of aroma-active thiols in pan-roasted white sesame seeds.

compound	odor quality	concentration [a] [µg/kg]	odor threshold [µg/kg]	OAV
2-methyl-1-propene-1-thiol	sulfurous, meaty	800	30	27
3-methyl-1-butene-1-thiol	sulfurous, meaty	970 [b]	0.40 [c]	2400
2-methyl-1-butene-1-thiol	sulfurous, meaty	1200 [d]	1.3 [e]	920
2-methyl-3-furanthiol	meaty	100	0.56 [f]	180
3-mercapto-2-pentanone	catty, black currant-like	29	0.19 [f]	150
2-mercapto-3-pentanone	catty, black currant-like	170	0.60	280
4-mercapto-3-hexanone	catty, black currant-like	4.7	0.07	65
3-mercapto-3-methylbutyl formate	sulfurous, catty	0.011	0.17	<<1
2-methyl-3-thiophenethiol	meaty, sulfurous	11	2200	<<1

[a] Mean values of triplicates.

[b] Sum of isomers: 29% (*E*)- and 71% (*Z*)-isomer.

[c] Odor threshold of a mixture of the (*E*)- and (*Z*)-isomer: 55% (*E*)- and 45% (*Z*)-isomer.

[d] Sum of isomers: 66% (*E*)- and 34% (*Z*)-isomer.

[e] Odor threshold of a mixture of the (*E*)- and (*Z*)-isomer: 60% (*E*)- and 40% (*Z*)-isomer.

[f] Odor threshold published in *Kerscher* (*2000*).

2.3 Discussion

The aroma of roasted white sesame seeds shows sulfurous, roasty, nutty and meaty odor attributes. The reinvestigation of this roasted sesame aroma using aroma extract dilution analysis (AEDA) confirmed the previous research conducted by *Schieberle* (*1996*). As shown in **Table 11**, the application of AEDA revealed 32 aroma-active odorants having FD-factors between 2 and 2048, among which the highest FD-factor of 2048 was found for 2-furfurylthiol, followed by 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone showing the second highest one (FD-1024). Moreover, eleven unknown aroma-active compounds which had sulfurous, meaty, or catty, blackcurrant-like odor qualities were detected, eight of which also remained unidentified in the previous investigation (*Schieberle, 1996*). Identification experiments on these aroma-active unknown odorants employing isolation techniques such as silica gel column chromatography and affinity chromatography using mercurated agarose gel, followed by instrumental analyses using the two-dimensional gas chromatograph-mass spectrometer were carried out.

As a result, nine aroma-active thiols with sulfurous, meaty or catty, black currant-like odor notes were identified for the first time in roasted sesame, namely 2-methyl-1-propene-1-thiol (**1**), (*Z*)-3-methyl-1-butene-1-thiol (**2**), (*E*)-3-methyl-1-butene-1-thiol (**3**), (*Z*)-2-methyl-1-butene-1-thiol (**4**), (*E*)-2-methyl-1-butene-1-thiol (**5**), 3-mercapto-2-pentanone (**7**), 4-mercapto-3-hexanone (**15**), 3-mercapto-3-methylbutyl formate (**19**), and 2-methyl-3-thiophenethiol (**20**) (**Figure 39**).

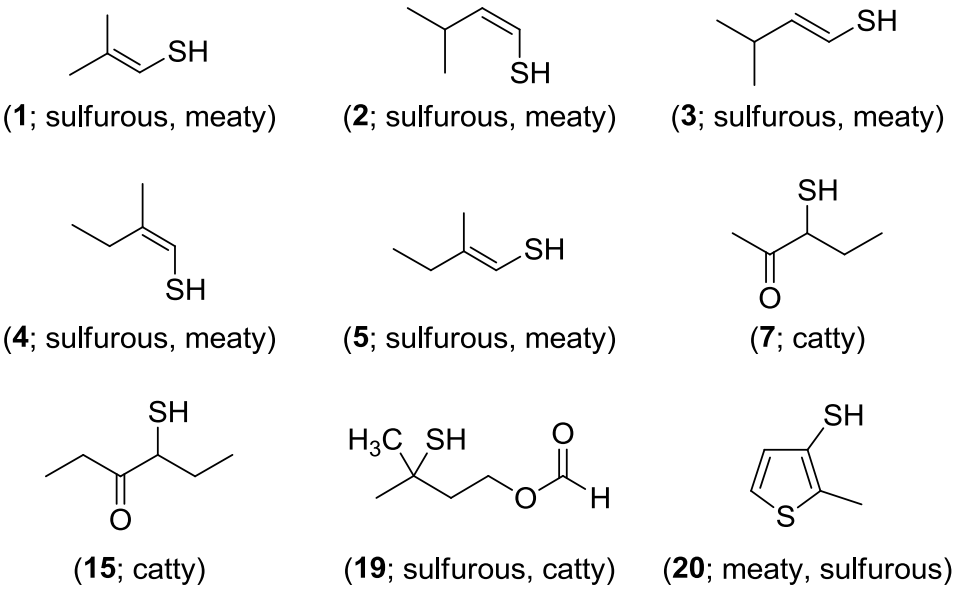


Figure 39: Chemical structures of the compounds identified for the first time in roasted sesame, or in foods (**1**-**5**, **15**).

Among them, to the best of my knowledge, 2-methyl-1-propene-1-thiol (**1**), (*Z*)-3-methyl-1-butene-1-thiol (**2**), (*E*)-3-methyl-1-butene-1-thiol (**3**), (*Z*)-2-methyl-1-butene-1-thiol (**4**), (*E*)-2-methyl-1-butene-1-thiol (**5**), and 4-mercapto-3-hexanone (**15**) were identified for the first time in natural food products. Four of them, (*Z*)-3-methyl-1-butene-1-thiol (**2**), (*E*)-3-methyl-1-butene-1-thiol (**3**), (*Z*)-2-methyl-1-butene-1-thiol (**4**), (*E*)-2-methyl-1-butene-1-thiol (**5**) were novel compounds. In particular, the 1-alkene-1-thiols might play an important role in the characteristic and intense aroma of freshly ground roasted sesame seeds. Their instability might explain that roasted sesame loses its fresh aroma after grinding and may also account for their relatively low FD factors detected in AEDA (**Table 11**).

Some studies reported that 3-methyl-2-butene-1-thiol was identified as a key compound of roasted sesame aroma (*Ikeda et al., 2006*; *Takeda et al., 2008*). As written above (cf. **2.1.4.2**), however, the thiol has almost the same retention indices, the same molecular weight, and the similar odor quality of (*E*)-2-methyl-1-butene-1-thiol (**5**). Hence, the aroma-active region identified before as 3-methyl-2-butene-1-thiol could be (*E*)-2-methyl-1-butene-1-thiol (**5**) because 3-methyl-2-butene-1-thiol was not detected in the GC-MS run of the thiol fraction obtained by affinity chromatography in this study.

To unequivocally evaluate the aroma impact of these newly identified thiols in roasted sesame, the precise quantitation of their concentration in roasted sesame seeds by stable isotope dilution assays (SIDA), followed by calculation of odor activity values (OAV: ratio of concentration to odor threshold) using the odor threshold measured in sunflower oil were performed. For that purpose, the SIDA of the new compounds were developed by synthesizing their deuterium labeled analogues.

In consequence, the concentration of seven thiols clearly exceeded their respective odor thresholds, namely 2-methyl-1-propene-1-thiol, 3-methyl-1-butene-1-thiol, 2-methyl-1-butene-1-thiol, 2-methyl-3-furanthiol, 3-mercapto-2-pentanone, 2-mercapto-3-pentanone, and 4-mercapto-3-hexanone (**Figure 40**). The novel compounds, 3-methyl-1-butene-1-thiol (OAV: 2400) and 2-methyl-1-butene-1-thiol (OAV: 960) showed the highest OAVs. Therefore, these newly identified thiols, 1-alkene-1-thiols should contribute to the sulfurous note of freshly ground roasted sesame aroma.

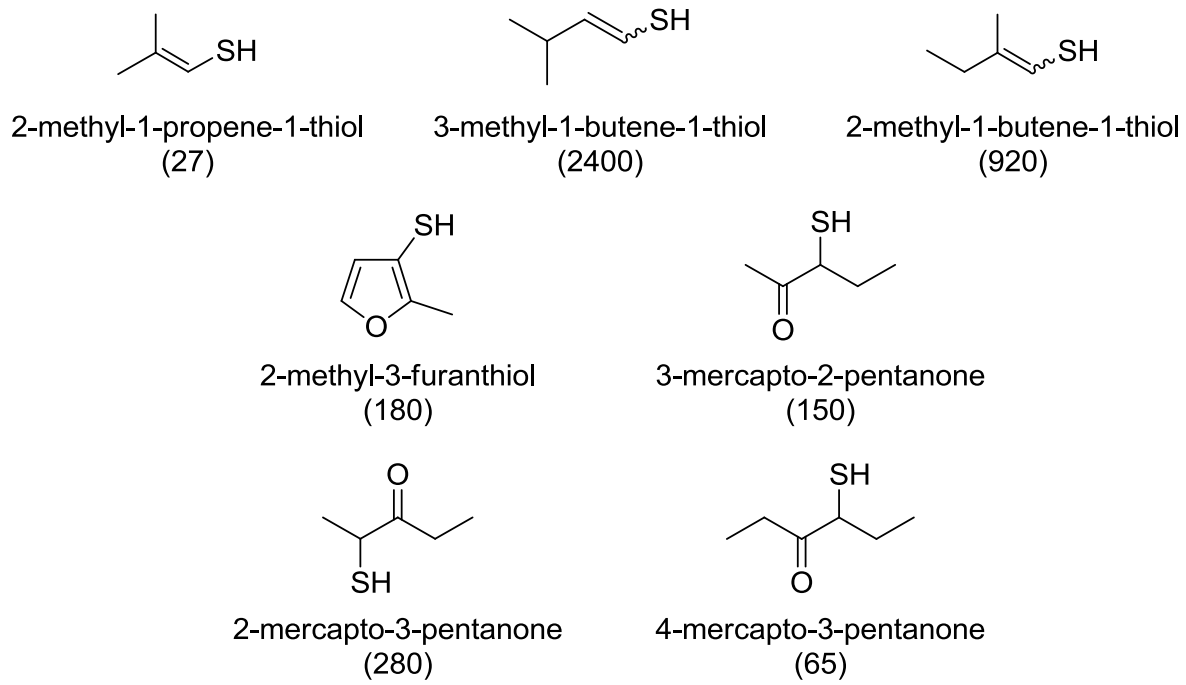


Figure 40: Odor-active thiols in roasted sesame (odor activity value in parenthesis).

However, it must be taken into account that the OAV of 3-methyl-1-butene-1-thiol was afflicted with some uncertainty, because the isomeric distribution in the roasted sesame seeds (**Table 14**) is somewhat different from that of the synthetic mixture (**Figure 21**), whereas for 2-methyl-1-butene-1-thiol it was virtually the same (cf. **Table 14** and **Figure 18**).

Nevertheless, the 2- and 3-methyl-1-butene-1-thiols seemed to be the compounds mainly responsible for the characteristic sulfury aroma of freshly pan-roasted white sesame seeds. Their OAVs even exceeded the OAVs of the most odor-active aroma compounds reported in the previous study on roasted sesame flavor (*Schieberle, 1996*, **Table 5**). Whether the (*E*)- or the (*Z*)-isomers of both of 2- and 3-methyl-1-butene-1-thiols contribute more to the sulfury note cannot be judged before the individual compounds are available in an appropriate isomeric purity. This will be subject of further research.

Although the odor activity value is a convenient tool to reveal whether an individual odorant should be able to contribute to the overall food aroma, any interaction during the perception of a mixture of aroma compounds is not taken into consideration (*Schieberle, 1995*). Therefore, the next step should be to prepare an aroma model based on the data in **Table 15** and the former data described by *Schieberle* (*1996*) (**Table 5**) and to compare the orthonasal odor characteristics of this model to a freshly prepared sample of ground roasted white sesame seeds (*Schieberle et al., 1993*). When this experiment yields a good agreement, then finally omission tests (*Grosch, 2001*) would reveal which odorants definitely contribute to the roasted sesame overall aroma.

The reason why the 1-alkene-1-thiols have been identified so far only in roasted sesame is a very interesting question. The most simple way to think of this pathway is the synthetic route used in this research for the 1-alkene-1-thiol reference compounds (**Figure 12**, **Figure 18**, and **Figure 21**). The syntheses only needed hydrogen sulfide and the corresponding Strecker aldehydes, namely 2-methylpropanal, 2-methylbutanal, and 3-methylbutanal as starting materials for the generation of 2-methyl-1-propene-1-thiol, 2-methyl-1-butene-1-thiol, and 3-methyl-1-butene-1-thiol, respectively. Hydrogen sulfide and all the three Strecker aldehydes have been already reported to be present in roasted sesame by the former researchers (*Yamanishi et al., 1960*; *Soliman et al. 1985; Cadwallader and Heo, 2001; Takei et al., 2002*), and all of them are supposed to be formed from their precursor amino acids, namely cysteine/cystine, valine, isoleucine, and leucine, which are constituents of roasted sesame (cf. **1.2.1**). Hence, the hypothesis outlined in **Figure 41** may be possible for the generation of 1-alkene-1-thiols.

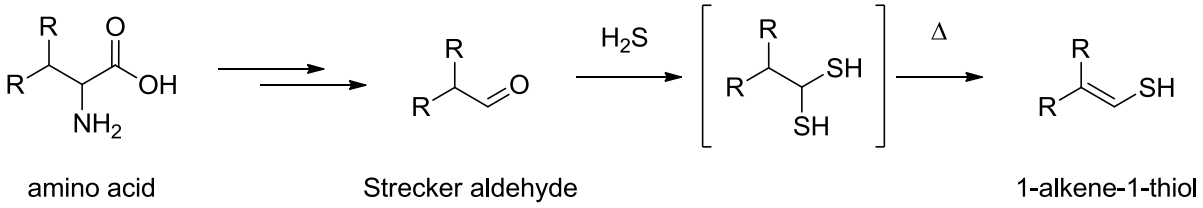


Figure 41: The hypothetical pathway of 1-alkene-1-thiol generation.

However, these amino acids are, of course, present in a variety of natural foods. The roasting step is necessary to generate the 1-alkene-1-thiols because the raw seeds did not smell sulfury. Other than sesame, however, there are a lot of roasted foods containing the amino acids.
1-Alkene-1-thiols are unstable probably due to its easily oxidizable structure. The π-electron of the double bond in a 1-alkene-1-thiol structure should stabilize the existence of a radical or a cation on the sulfur atom. In that case, the hydrogen on the sulfur atom should be oxidized more easily as well as that in the case of 2-methyl-3-furanthiol, and then various kinds of decompositions such as an oxidative dimerization or a polymerization, may occur. Actually, leaving the 1-alkene-1-thiol reference substance for a couple of days in high concentrations without solvent dilution after the synthesis resulted in a tarry precipitate which was speculated to be a polymer.

As described in the introduction, sesame contains specific antioxidative compounds like sesamin (cf. **1.2.2**). This might be the reason why the 1-alkene-1-thiols have been identified only in roasted sesame. The antioxidative activity of sesame might allow the 1-alkene-1-thiol to survive in the roasted seeds or oil.

Furthermore, one more additional peak was observed in the GC analysis of 1-alkene-1-thiol on the polar capillary column. The peak had the same molecular weight and a very close RI to those of the 1-alkene-1-thiol. Thus, it was assumed that this peak might be a thial formed through the estimated route as exemplified in **Figure 42**. This phenomenon could mean that 1-alkene-1-thiols are also unstable in a polar environment such as water.

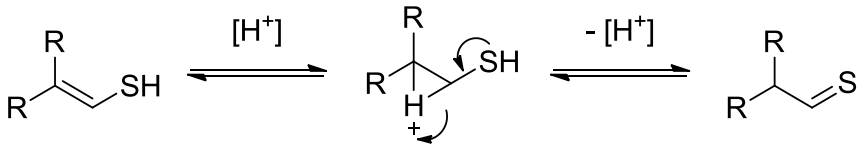


Figure 42: Hypothetical thial formation from a 1-alkene-1-thiol in a polar environment.

Hence, the fact that 50% of sesame consists of fat (cf. **1.2.1**) could also help to prevent the decomposition of 1-alkene-1-thiols. Aroma compounds in roasted sesame are likely to be dissolved in the oil, and so the 1-alkene-1-thiols should be surrounded by oil and protected from polar ingredients.

Thus, to summarize the points described above, the rare situation in sesame, where the aroma compounds are enclosed in oil and also potent antioxidants exist, might be the reason why 1-alkene-1-thiol could be found only in roasted sesame seeds at present.

As described in **1.2.3**, hitherto *Soliman et al.* (*1985*) researched the difference between sesame aroma with and without the antioxidants, sesamin and sesamolin, in which it was mentioned that addition of sesamin and sesamolin to defatted roasted sesame created much stronger roasted sesame-like aroma, although not surprisingly they did not focus on the 1-alkene-1-thiol, and could not firmly find out the responsible odorants.
Hence, to confirm the hypothesis of the 1-alkene-1-thiol pathway, it is needed to identify and quantitate 1-alkene-1-thiols in a heated mixture of amino acids with and without the antioxidants, for example in sunflower oil. It will also be a challenging topic in the future.

On the other hand, *Schieberle* (*1993*) had investigated the precursor of 2-furfurylthiol in roasted sesame, and he found that 2-furfurylthiol was not so much generated from the water-soluble fraction of ground unroasted sesame but mainly from its residue when both of the fractions were heated in sunflower oil. This result shows that 2-furfurylthiol in roasted sesame is not principally formed from sugars and amino acids but from some sort of water-insoluble constituents. Thus, this kind of another possibility also should deserve considering for the generation of 1-alkene-1-thiols in roasted sesame seeds.

3 Experimental part

3.1 Chemicals

All commercially available compounds were purchased from the suppliers listed below. Commercially unavailable substances were synthesized according to the literatures cited or were kindly supplied as gift. Especially, I thank T. Hasegawa Co., Ltd. for synthesizing many of the commercially unavailable compounds.

3.1.1 Reference odorants

2-acetyl-1-pyrroline (*Buttery et al., 1982; Schieberle, 1991*)
(*E*/*Z*)-2-butene-1-thiol (*synthesis by T. Hasegawa Co., Ltd., cf.* ***3.3.2.1***)
3-butene-1-thiol (*synthesis by T. Hasegawa Co., Ltd., cf.* ***3.3.2.2***)
3-butene-2-thiol (*synthesis by T. Hasegawa Co., Ltd., cf.* ***3.3.2.4***)
dimethyl disulfide, Acros Organics, Geel, Belgium
trans-4,5-epoxy-(*E*)-2-decenal (*Schieberle and Grosch, 1991*)
2-ethyl-3,5(6)-dimethylpyrazine, Acros Organics, Geel, Belgium
2-furfurylthiol, Aldrich, Steinheim
4-hydroxy-2,5-dimethyl-3(2*H*)-furanone, Aldrich, Steinheim
4-hydroxy-3-methoxybenzaldehyde (vanillin), Merck, Darmstadt
4-mercapto-3-hexanone (*synthesis by T. Hasegawa Co., Ltd., cf.* ***3.3.2.6***)
3-mercapto-3-methylbutyl formate (*Czerny et al., 1999*)
2-mercapto-3-pentanone (*synthesis by T. Hasegawa Co., Ltd., cf.* ***3.3.2.5***)
3-mercapto-2-pentanone, Alfa Aesar, Karlsruhe
2-methoxy-4-vinylphenol, Alfa Aesar, Karlsruhe
(*E*/*Z*)-2-methyl-1-butene-1-thiol (*synthesis by T. Hasegawa Co., Ltd., cf.* ***3.3.2.8***)
(*E*/*Z*)-3-methyl-1-butene-1-thiol (*synthesis by T. Hasegawa Co., Ltd., cf.* ***3.3.2.9***)
3-methyl-2-butene-1-thiol (*Horscher et al., 1992*)
2-methyl-3-furanthiol, Acros Organics, Geel, Belgium
3-methyl-1*H*-indole (skatole), Tokyo Chemical Industry Co., Tokyo, Japan
2-methyl-1-propene-1-thiol (*synthesis by T. Hasegawa Co., Ltd., cf.* ***3.3.2.7***)
2-methyl-2-propene-1-thiol (*synthesis by T. Hasegawa Co., Ltd., cf.* ***3.3.2.3***)
2-methyl-3-thiophenethiol (*Hofmann and Schieberle, 1995*)
3-methylthiopropanal (methional), Aldrich, Steinheim

1-octen-3-one, T. Hasegawa Co., Ltd., Tokyo, Japan

2-phenylethylthiol, Aldrich, Milwaukee, USA

2-thenylthiol, Acros Organics, Geel, Belgium

3.1.2 Isotopically labeled compounds

[$^2H_3$]-4-mercapto-3-hexanone (*synthesis by T. Hasegawa Co., Ltd., cf.* ***3.3.3.1***)

[$^2H_6$]-3-mercapto-3-methylbutyl formate (*synthesis cf.* ***3.3.3.2***)

[$^2H_2$]-3-mercapto-2-pentanone (*Sen and Grosch, 1991*)

[$^2H_3$]-(*E*/*Z*)-2-methyl-1-butene-1-thiol (*synthesis by T. Hasegawa Co., Ltd., cf.* ***3.3.3.4***)

[$^2H_3$]-2-methyl-3-furanthiol (*Sen and Grosch, 1991*)

[$^2H_6$]-2-methyl-1-propene-1-thiol (*synthesis by T. Hasegawa Co., Ltd., cf.* ***3.3.3.3***)

[$^2H_3$]-2-methyl-3-thiophenethiol (*synthesis by T. Hasegawa Co., Ltd., cf.* ***3.3.3.5***)

3.1.3 Chemicals

acetic acid, BP Japan, Tokyo, Japan

[$^2H_6$]-acetone, Taiyo Nippon Sanso, Tokyo, Japan

acetonitrile, Junsei Chemical, Tokyo, Japan

Affi-gel 10, Bio-Rad Laboratories, Helcules, California

2-aminoethanol, Sigma-Aldrich Chemie, Steinheim

p-aminophenylmercuric acetate, Sigma, Sigma-Aldrich Chemie, Taufkirchen

argon (99.996 Vol.%), Westfalen, Münster

benzene-1,2,3-triol (pyrogallol), Junsei Chemical, Tokyo, Japan

[$^2H_3$]-bromoethane, CDN isotopes, Tokyo, Japan

4-bromo-1-butene, Tokyo Chemical Industry, Tokyo, Japan

3-bromothiophene, Sigma-Aldrich Chemie, Taufkirchen

2-buten-1-ol, Sigma-Aldrich Japan, Tokyo, Japan

n-butyl lithium (1.59 M in hexane), Kanto Chemical, Tokyo, Japan

butyronitrile, Tokyo Chemical Industry, Tokyo, Japan

cadmium carbonate, Kanto Chemical, Tokyo, Japan

carbon disulfide, Junsei Chemical, Tokyo, Japan

3-chloro-2-methyl-1-propene, Tokyo Chemical Industry, Tokyo, Japan

citric acid monohydrate, Junsei Chemical, Tokyo, Japan

1,8-diazabicyclo[5.4.0]-7-undecene, Tokyo Chemical Industry, Tokyo, Japan
dichloromethane, Merck, Darmstadt
diethyl ether, Merck, Darmstadt
diethylphosphonoacetic acid ethyl ester, Tokyo Chemical Industry, Tokyo, Japan
diisobutylaluminum hydride (1M in hexane), Kanto Chemical, Tokyo, Japan
diisopropylamine, Junsei Chemical, Tokyo, Japan
N,*N*-dimethyl-4-aminopyridine (DMAP), Aldrich, Sigma-Aldrich Chemie, Taufkirchen
N,*N*-dimethylformamide, Aldrich, Sigma-Aldrich Chemie, Steinheim
dimethyl sulfoxide, Junsei Chemical, Tokyo, Japan
1,3-dithiane, Tokyo Chemical Industry, Tokyo, Japan
dithiothreitol, Sigma, Sigma-Aldrich Chemie, Taufkirchen
ethanol (95%), Japan Alcohol Corporation, Tokyo, Japan
ethyl acetate, Junsei Chemical, Tokyo, Japan
formic acid, Junsei Chemical, Tokyo, Japan
helium (99.996 Vol.%), Westfalen, Münster
hexamethylphosphoramide, Tokyo Chemical Industry, Tokyo, Japan
n-hexane, Merck, Darmstadt
hydrochloric acid (32%), Merck, Darmstadt
hydrogen (99.999 Vol.%), Westfalen, Münster
hydrogen sulfide, Tomoe Shokai, Tokyo, Japan
hydroquinone, Junsei Chemical, Tokyo, Japan
4-hydroxy-3-hexanone, Tokyo Chemical Industry, Tokyo, Japan
iodomethane, Merck, Darmstadt
[$^{2}H_3$]-iodomethane, CDN isotopes, Tokyo, Japan
isopropyl alcohol, anhydrous, Merck, Darmstadt
lithiuim aluminium hydride, Aldrich, Sigma-Aldrich Chemie, Steinheim
magnesium sulfate, anhydrous, Junsei Chemical, Tokyo, Japan
mercury(II) chloride, Junsei Chemical, Tokyo, Japan
methanesulfonyl chloride, Wako Pure Chemical Industries, Osaka, Japan
2-methylbutanal, Tokyo Chemical Industry, Tokyo, Japan
3-methylbutanal, Toyo Gosei, Tokyo, Japan
methyl octanoate, Fluka, Sigma-Aldrich Chemie, Taufkirchen
2-methylpropanal, Toyo Gosei, Tokyo, Japan
nitrogen (99.999 Vol.%), Westfalen, Münster
nitrogen, liquid, Westfalen, Münster

n-pentane, Merck, Darmstadt
3-pentanone, Tokyo Chemical Industry, Tokyo, Japan
potassium thioacetate, Tokyo Chemical Industry, Tokyo, Japan
propanal, Junsei Chemical, Tokyo, Japan
silica gel 60, 0.063–0.200 mm, Merck, Darmstadt
sodium bicarbonate, Junsei Chemical, Tokyo, Japan
sodium bisulfite, Junsei Chemical, Tokyo, Japan
sodium carbonate, Tokuyama, Tokyo, Japan
sodium chloride, Merck, Darmstadt
sodium hydride (60% in oil), Junsei Chemical, Tokyo, Japan
sodium hydroxide, Junsei Chemical, Tokyo, Japan
sodium sulfate, anhydrous, Merck, Darmstadt
sulfuric acid, Junsei Chemical, Tokyo, Japan
tetrahydrofuran, Aldrich, Sigma-Aldrich Chemie, Taufkirchen
triethylamine, Junsei Chemical, Tokyo, Japan
thiourea, Merck, Darmstadt

3.1.4 Purification of chemicals

Diethyl ether, dichloromethane and *n*-pentane were freshly distilled on a Vigreux column or a column (150 × 5 cm) packed with Raschig rings prior to use.

Purification of silica gel according to Esterbauer (1968):

Silica gel 60 (0.063–0.200 mm) was mixed with hydrochloric acid (32%) and the mixture was allowed to stand overnight. The next day the intensely yellow colored hydrochloric acid was decanted off and the silica gel was washed with water until a neutral pH was reached. Additionally, the acid-free silica gel was washed with 4 L of distilled water and was subsequently filtered by suction. Then the moist silica gel was oven-dried for 3 h at 150 °C. In order to adjust the water content of the silica gel to 7%, the silica gel was activated at a rotary evaporator at 35 °C and 16 mbar for more than 100 minutes (*Esterbauer, 1968*).

3.2 Investigated materials

White sesame seeds (*Sesamum Indicum* L.) from Egypt were purchased in a local supermarket in Garching, Germany. The seeds (250 g) were washed with tap water (500 mL), filtered with a strainer, and then roasted in a frying pan with continuous stirring for 15 min at 200 °C. After freezing with liquid nitrogen, the seeds were ground in a commercial blender.

3.3 Syntheses

3.3.1 Mercurated agarose gel

Mercurated agarose gel (equal to Bio-Rad Affi-gel 501) was used in affinity chromatography for the isolation of thiols. The agarose gel was an organomercurial derivative of *N*-hydroxysuccinimide activated agarose gel (Bio-Rad Affi-gel 10). The mercurated agarose gel was prepared by reacting Affi-gel 10 with *p*-aminophenylmercuric acetate in isopropyl alcohol/DMF, then blocking unreacted succinimide groups with ethanolamine and a final solvent wash followed by slurry in isopropyl alcohol to bottle the gel. Bio-Rad Laboratories, Inc. specified this procedure.

The synthesis route is shown in **Figure 43**.

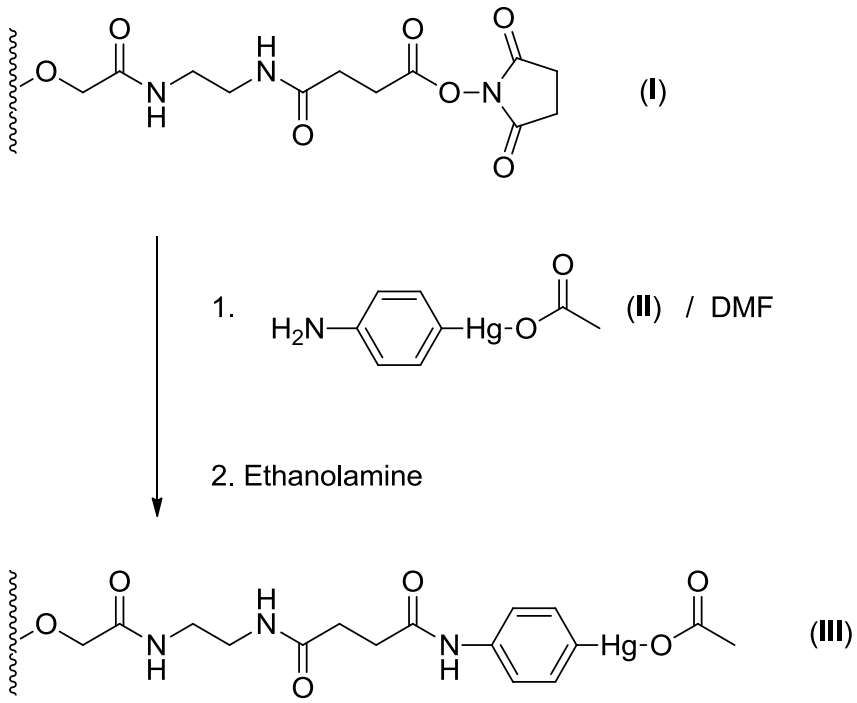


Figure 43: Synthesis route of mercurated agarose gel.

Mercurated agarose gel (III)

The settled Affi-Gel 10 (**I**, 25 mL) was transferred into a Büchner funnel and washed with 100 mL of anhydrous isopropyl alcohol by means of a water-pump.

The washed Affi-Gel 10 (25 mL) was transferred into a 100 mL flask. *p*-Aminophenyl-mercuric acetate (**II**, 0.375 g) was dissolved in *N*,*N*-dimethylformamide (DMF, 7.5 mL) and

this solution was added to the Affi-Gel 10 (**I**). After stirring of the slurry with an overhead stirrer for 4 h at room temperature, ethanolamine (0.25 mL) was added, and this suspension was stirred for another 1 h. Then the material was transferred onto a Büchner funnel and washed successively with *N*,*N*-dimethylformamide (100 mL) and anhydrous isopropyl alcohol (200 mL). After the final solvent wash, the mercurated agarose gel (**III**) was resuspended in 75 mL of anhydrous isopropyl alcohol and kept at −20 ºC in a dark bottle.

3.3.2 Reference odorants

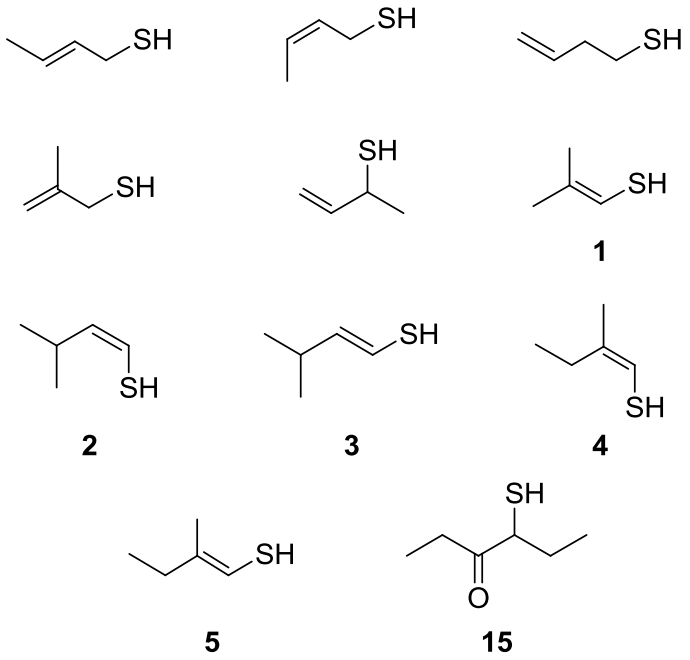


Figure 44: Structures of the eleven thiols prepared by syntheses. Only compounds also identified in sesame seeds are numbered.

3.3.2.1 (*E*)-2-Butene-1-thiol and (*Z*)-2-butene-1-thiol

A mixture of (*E*)- and (*Z*)-2-buten-1-ol (96:4, 21.5 g, 300 mmol) was dropwise added to a solution of thiourea (29.5 g, 390 mmol) in hydrochloric acid (6 mol/L, 65 mL) kept at room temperature during 15 min. After stirring for further 4 h at 45 ºC, sodium hydroxide (10% in water, 158 g) was added. The reaction mixture was heated to 100 ºC, and the crude product was distilled off together with the water. The organic layer was separated, dried over

anhydrous Na_2SO_4, and distilled under atmospheric pressure (b.p.: 101 ºC) to yield an oil consisting of (*E*)- and (*Z*)-2-butene-1-thiol (5.1 g, yield: 19%). The (*E*)/(*Z*) ratio was determined by GC to be 93:7.

Odor quality (HRGC-O): (*E*-isomer): sulfurous.
Odor quality (HRGC-O): (*Z*-isomer): sulfurous.
RI (FFAP): (*E*-isomer): 1006. **RI (DB-5)**: (*E*-isomer): 717.
RI (FFAP): (*Z*-isomer): 1014. **RI (DB-5)**: (*Z*-isomer): 721.
MS (EI): (*E*-isomer): cf. **Figure 11B**.
MS (EI): (*Z*-isomer): cf. **Figure 11C**.

3.3.2.2 3-Butene-1-thiol

3-Butene-1-thiol

Thiourea (6.8 g, 88.9 mmol) and 4-bromo-1-butene (10.0 g, 74.1 mmol) were dissolved in ethanol (95%, 50 mL) under an atmosphere of nitrogen. After stirring for 8 h at 80 ºC, the reaction mixture was cooled to room temperature, and sodium hydroxide (50% in water, 12 g) was added. After stirring for further 8 h at 80 ºC, the reaction mixture was cooled to 0 ºC, acidified to ~pH 3 with citric acid (27% in water, 35.5 g), and extracted with *n*-pentane. The organic phase was dried over anhydrous $MgSO_4$ and the solvent was removed under atmospheric pressure. The crude product was then distilled under atmospheric pressure (b.p.: ~70–80 ºC) to yield 3-butene-1-thiol (560 mg, yield: 9%).

Odor quality (HRGC-O): rotten.
RI (FFAP): 980. **RI (DB-5)**: 700.
^{1}H NMR: δ 1.43 (t, J = 7.6, 1H); 2.38 (dt, J = 7.2, 7.2, 2H); 2.59 (dt, J = 7.2, 7.6, 2H); 5.07–5.13 (m, 2H); 5.77 (ddt, J = 7.2, 10.0, 17.2, 1H).
^{13}C NMR: δ 23.9, 37.9, 116.8, 136.0.
MS (EI): *m*/*z* (%): 88 (65), 60 (39), 59 (10), 55 (26), 54 (38), 53 (15), 47 (100), 46 (28), 45 (26), 41 (16), 39 (42).

3.3.2.3 2-Methyl-2-propene-1-thiol

The compound was prepared from 3-chloro-2-methyl-1-propene following the method described above for 3-butene-1-thiol (**3.3.2.1**, yield: 12%).

Odor quality (HRGC-O): sulfurous

RI (FFAP): 996. **RI (DB-5)**: 689.

^{1}H NMR: δ 1.47 (t, J = 8.0, 1H); 1.85 (s, 3H); 3.14 (d, J = 8.0, 2H); 4.78 (s, 1H); 4.91 (s, 1H).

^{13}C NMR: δ 20.6, 31.8, 112.1, 144.5.

MS (EI): *m*/*z* (%): 88 (98), 73 (18), 60 (15), 59 (11), 55 (100), 54 (46), 53 (34), 51 (10), 50 (10), 47 (20), 45 (31), 41 (13), 39 (66), 29 (22).

3.3.2.4 3-Butene-2-thiol

The target compound was synthesized in a two-step procedure as follows:

3-Buten-2-yl methyl dithiocarbonate

NaH (60%, 48.5 g, 1.21 mol, washed with *n*-hexane prior to use) was suspended in a solution of *N*,*N*-dimethyl-4-aminopyridine (DMAP, 1.22 g, 10 mmol) in tetrahydrofuran (THF, 500 mL). Then, 2-buten-1-ol (30.0 g, 416 mmol) in THF (1 L) was slowly added, and after stirring for 1.5 h at room temperature, CS_2 (158 g, 2.08 mol) was added to the reaction mixture within 45 min. After further stirring for 30 min at room temperature, methyl iodide (285 g, 2.01 mol) was slowly added within 1 h. The mixture was stirred overnight and then acetic acid (131 g, 2.18 mol), followed by water (1 L) was added. The organic layer was separated, and the aqueous layer was extracted with ethyl acetate. The combined organic layers were successively washed with water and brine, then dried over anhydrous $MgSO_4$, filtered, and concentrated under reduced pressure to yield a colored oil (105.8 g). The crude product was purified by distillation (b.p.: 69–77 ºC/0.8 kPa) yielding 3-buten-2-yl methyl dithiocarbonate (65.2 g, yield: 97%, purity: 79%) as an oil.

MS (EI): *m*/*z* (%): 102 (31), 87 (5), 75 (10), 55 (100), 54 (19), 53 (12), 47 (10), 45 (15), 39 (11), 29 (11).

3-Butene-2-thiol

The synthesis of 3-butene-2-thiol was finished following a procedure as given by *Taguchi et al.* (*1969*). A mixture of 3-buten-2-yl methyl dithiocarbonate (20.0 g, 123 mmol), 2-aminoethanol (7.5 g, 123 mmol), and 2,6-di-*tert*-butyl-4-methylphenol (BHT, 0.1 g) was heated to 80 ºC with continuous stirring. After a few minutes, the reaction mixture was distilled under atmospheric pressure (b.p.: ~25–47 ºC) to yield a colorless oil (2.0 g). The crude product obtained was further purified by distillation under atmospheric pressure (b.p.: ~79–80 ºC) to yield 3-butene-2-thiol (400 mg, yield: 4%, purity: 89%).

Odor quality (HRGC-O): sulfurous.
RI (FFAP): <900. **RI (DB-5)**: 677.
^{1}H NMR: δ 1.41 (d, J = 7.2, 3H); 1.66 (d, J = 5.6, 1H); 3.59 (ddq, J = 5.6, 7.2, 7.2 1H); 4.93 (d, J = 10.0, 1H); 5.09 (d, J = 17.2, 1H); 5.90 (ddd, J = 7.2, 10.0, 17.2, 1H).
^{13}C NMR: δ 24.0, 37.4, 112.6, 142.9.
MS (EI): *m*/*z* (%): 88 (50), 73 (10), 59 (17), 55 (100), 54 (26), 53 (22), 51 (10), 45 (20), 39 (29), 29 (19).

3.3.2.5 2-Mercapto-3-pentanone

The compound was synthesized in a three-step procedure starting from 3-pentanone.

2-Bromo-3-pentanone

3-Pentanone (13.0 g, 151 mmol) was added to a solution of potassium chlorate (2.2 g, 18.0 mmol) in water (150 mL) at room temperature. The reaction mixture was warmed up to 50 ºC, and then bromine (37.3 g, 233 mmol) was added dropwise at 45–50 ºC within 10 min. After stirring at 45 to 50 ºC within 3 h, the reaction mixture was cooled to room temperature, and poured into 10% aq. sodium thiosulfate (500 mL). The aqueous solution was extracted with diethyl ether. The organic layers were combined, washed consecutively with 10% aq. sodium thiosulfate, saturated aq. $NaHCO_3$ and finally brine, and dried over $MgSO_4$. The organic solution was filtered, and concentrated under reduced pressure to yield crude 2-bromo-3-pentanone (32 g), which was directly used in the next step.

2-Acetylthio-3-pentanone

A solution of the crude 2-bromo-3-pentanone (32 g) in *N*,*N*-dimethylformamide (DMF, 80 mL) was added dropwise to a solution of potassium thioacetate (24.4 g, 213 mmol) in DMF

(500 mL) at 35 to 40 ºC within 20 min. After stirring for 3 h at 35 to 40 ºC, the reaction mixture was extracted with diethyl ether. The organic layer was washed with saturated aq. $NaHCO_3$ and brine, and then dried over $MgSO_4$. The organic solution was filtered, and concentrated under reduced pressure to yield crude 2-acetylthio-3-pentanone (20 g), which was directly used in the next step.

2-Mercapto-3-pentanone

The crude 2-acetylthio-3-pentanone (20 g) was added to 5% aq. NaOH (360 g) at room temperature. After stirring for 2 h at room temperature, the reaction mixture was washed with diethyl ether. The aqueous layer was acidified with 20% aq. citric acid (pH = 5), and extracted with diethyl ether. The organic layer was washed with brine, and dried over $MgSO_4$. The solution was filtered and concentrated under reduced pressure. The residue (10 g) was distilled (b.p.: ~30 ºC/3 kPa) to yield crude 2-mercapto-3-pentanone (0.5 g, purity: 75%). The distillate (0.5 g) was dissolved in 5% aq. NaOH, and then washed with diethyl ether. The aqueous layer was acidified with 20% aq. citric acid (pH = 5), and extracted with diethyl ether. The organic layer was washed with brine, and dried over $MgSO_4$. The organic solution was filtered and concentrated under reduced pressure. The residue (0.3 g) was distilled (b.p.: ~30 ºC/2.6 kPa) into a colorless oil of 2-mercapto-3-pentanone (50 mg, yield: 0.3%, purity: 96%). The compound was characterized by comparing the MS-EI data with results reported by *Mottram et al.* (*1995*).

MS (EI): *m*/*z* (%): 118 (20), 90 (4), 61 (53), 60 (8), 59 (7), 58 (6), 57 (100), 56 (5), 45 (5), 35 (5), 29 (37).

3.3.2.6 4-Mercapto-3-hexanone

The target compound was synthesized in a three-step procedure starting from 4-hydroxy-3-hexanone as outlined in **Figure 23**.

4-(Methanesulfonyloxy)-3-hexanone

4-Hydroxy-3-hexanone (5.0 g, 43.0 mmol) and triethylamine (13.0 g, 129.0 mmol) were dissolved in diethyl ether (43 mL), and methanesulfonyl chloride (5.9 g, 51.6 mmol) in diethyl ether (10 mL) was added to the solution at 0 ºC. After stirring for 5.5 h, water was added, and the organic layer was separated and successively washed consecutively with citric acid (10%

in water), brine, an aqueous sodium carbonate solution (10%), then again with brine, and was finally dried over anhydrous $MgSO_4$. The organic phase was filtered and concentrated under reduced pressure to yield crude 4-(methanesulfonyloxy)-3-hexanone (7.3 g), which was directly used in the next step.

MS (EI): *m*/*z* (%): 137 (7), 136 (6), 79 (13), 69 (3), 59 (11), 58 (4), 57 (100), 55 (4), 41 (7), 29 (18).

4-Acetylthio-3-hexanone

Potassium thioacetate (9.1 g, 80.0 mmol) was dissolved in *N*,*N*-dimethylformamide (DMF, 100 mL) and the crude 4-(methanesulfonyloxy)-3-hexanone (14.0 g) in DMF (140 mL) was added to the solution between 20 to 30 ºC within 1 h. After stirring for 3 h at 35 ºC, the reaction mixture was extracted with diethyl ether. The organic layer was washed with an aqueous sodium bicarbonate solution (10 %) and brine, dried over anhydrous $MgSO_4$, filtered, and concentrated under reduced pressure to obtain 4-acetylthio-3-hexanone, which was purified by distillation (b.p.: ~55–58 ºC/0.4 kPa) to yield 4-acetylthio-3-hexanone (10.0 g, yield: 80%, purity: >99%).

MS (EI): *m*/*z* (%): 174 (5), 132 (16), 131 (24), 117 (20), 99 (5), 75 (25), 74 (5), 58 (5), 57 (100), 55 (7), 45 (6), 43 (94), 41 (12), 39 (6), 29 (18).

4-Mercapto-3-hexanone

To obtain the target compound, an aqueous solution of NaOH (5%, 140.0 g, 175.0 mmol) was added to a solution of 4-acetylthio-3-hexanone (8.7 g, 50.0 mmol) in diethyl ether (62.0 g) at ~0–10 ºC within 30 min. After stirring for 2 h, the reaction mixture was separated, the aqueous layer was acidified to pH 4.0 with citric acid (10% in water) and extracted with diethyl ether. The combined organic layers were washed with brine, dried over anhydrous $MgSO_4$, filtered, and concentrated under reduced pressure. The residue (9.0 g) was distilled (b.p.: ~45–46 ºC/0.8 kPa) to yield the target compound (3.9 g, yield: 59%, purity: >99%).

Odor quality (HRGC-O): catty, black currant-like

RI (FFAP): 1405. **RI (DB-5)**: 987.

^{1}H NMR: δ 0.98 (t, J = 7.2, 3H); 1.11 (t, J = 7.2, 3H); 1.70 (ddq, J = 7.2, 7.2, 14.4, 1H); 1.71 (d, J = 10.4, 1H); 1.95 (ddq, J = 7.2, 7.2, 14.4, 1H); 2.53 (dq, J = 7.2, 17.2, 1H); 2.74 (dq, J = 7.2, 17.2, 1H); 3.24 (ddd, J = 7.2, 7.2, 10.4, 1H).

^{13}C NMR: δ 8.2, 11.9, 27.7, 33.3, 48.6, 208.8.

MS (EI): cf. **Figure 22**.

MS (CI): cf. **Figure 25**.

3.3.2.7 2-Methyl-1-propene-1-thiol

The target compound was prepared in a two-step synthesis starting from 2-methylpropanal as shown in **Figure 12**.

2-Methylpropane-1,1-dithiol

Pyrogallol (0.5 g, 4 mmol) and dimethyl sulfoxide (DMSO, 400 g) were added to 2-methylpropanal (132.3 g, 1835 mmol) at room temperature, and the mixture was slowly cooled to 10 ºC. H_2S (102.1 g, 2996 mmol) was passed through the solution for 2 h while maintaining the temperature below 20 ºC. Then, the reaction mixture was poured onto ice water (600 g), and extracted with diethyl ether. The combined organic phases were washed with water and brine, dried over anhydrous $MgSO_4$, and concentrated under reduced pressure. The residue (77.8 g) was subjected to vacuum distillation, and the distillate was washed with aqueous sodium bisulfite (10%), dried over anhydrous $MgSO_4$ and filtered to yield a pale yellowish oil of 2-methylpropane-1,1-dithiol (36.7 g, yield: 16%, purity: 97%).

^{1}H NMR: δ 1.03 (d, J = 6.8, 6H); 2.01 (double septuplet, J = 4.4, 6.8, 1H); 2.20 (d, J = 6.8, 2H); 4.08 (dt, J = 4.4, 6.8, 1H).

^{13}C NMR: δ 18.8, 37.2, 45.7.

MS (EI): m/z (%): 122 (25), 89 (69), 88 (40), 79 (28), 73 (17), 59 (15), 55 (100), 47 (16), 45 (41), 41 (17), 39 (23).

2-Methyl-1-propene-1-thiol

To obtain the target compound, 2-methylpropane-1,1-dithiol (1.1 g, 9.0 mmol) was subjected to flash vacuum pyrolysis (FVP, 380 ºC/0.4 kPa) (*Schiess et al., 1995; Kreilein et al., 2005*) over 20 min, and a yellowish oil of 2-methyl-1-propene-1-thiol (0.6 g, yield: 78%, purity: 86%) was collected.

Odor quality (HRGC-O): sulfurous, meaty.

RI (FFAP): 1013. **RI (DB-5)**: 718.

^{1}H NMR: δ 1.73 (s, 3H); 1.77 (s, 3H); 2.52 (d, J = 6.8, 1H); 5.69 (d, J = 6.8, 1H).

^{13}C NMR: δ 18.8, 25.3, 107.3, 135.8.

MS (EI): cf. **Figure 13B**.

MS (CI): cf. **Figure 29**.

3.3.2.8 (*E*)-2-Methyl-1-butene-1-thiol and (*Z*)-2-methyl-1-butene-1-thiol

The target compounds were synthesized starting from 2-methylbutanal following the reaction scheme for 2-methyl-1-propene-1-thiol (**3.3.2.7**) as reported above in **Figure 18**.

2-Methylbutane-1,1-dithiol

2-Methylbutanal was treated with H_2S using the method described above for the synthesis of 2-methyl-1-propene-1-thiol (**3.3.2.7**) (yield: 24%, purity: 97%).

^{1}H NMR: δ 0.92 (t, J = 7.2, 3H); 1.04 (d, J = 6.4, 3H); 1.32 (ddq, J = 7.2, 7.2, 15.2, 1H); 1.51–1.61 (m, 1H); 1.71–1.80 (m, 1H); 2.14 (d, J = 6.4, 1H); 2.29 (d, J = 6.4, 1H); 4.23 (ddd, J = 3.6, 6.4, 6.4, 1H).

^{13}C NMR: δ 11.6, 14.8, 26.7, 43.7, 44.2.

MS (EI): *m*/*z* (%): 136 (22), 103 (81), 102 (70), 87 (33), 79 (30), 69 (100), 61 (46), 53 (23), 47 (23), 45(53), 41 (81), 39 (27).

(E)-2-Methyl-1-butene-1-thiol and (Z)-2-methyl-1-butene-1-thiol

The target compounds were prepared by FVP of 2-methylbutane-1,1-dithiol using the parameters described above for the synthesis of 2-methyl-1-propene-1-thiol (**3.3.2.7**) (yield: 72%, purity: 92%). The (*E*)/(*Z*)-ratio was determined by GC to be 60:40. The correct assignment of the GC peaks was achieved by NOESY-NMR of the mixture (**Figure 19**).

Odor quality (HRGC-O): (*E*-isomer): sulfurous, meaty.

Odor quality (HRGC-O): (*Z*-isomer): sulfurous, meaty.

RI (FFAP): (*E*-isomer): 1105. **RI (DB-5)**: (*E*-isomer): 818.

RI (FFAP): (*Z*-isomer): 1098. **RI (DB-5)**: (*Z*-isomer): 807.

^{1}H NMR (*E*-isomer): δ 1.00 (t, J = 7.6, 3H); 1.72 (s, 3H); 2.07 (q, J = 7.6, 2H); 2.54 (d, J = 7.2, 1H); 5.71 (d, J = 7.2, 1H).

^{1}H NMR (*Z*-isomer): δ 1.00 (t, J = 7.6, 3H); 1.75 (s, 3H); 2.16 (q, J = 7.6, 2H); 2.51 (d, J = 7.2, 1H); 5.67 (d, J = 7.2, 1H).

^{13}C NMR (*E*-isomer): δ 12.4, 22.4, 32.1, 106.4, 140.6.

^{13}C NMR (*Z*-isomer): δ 11.4, 17.0, 25.8, 106.6, 141.1.

MS (EI) (*E*-isomer): cf. **Figure 20B**.

MS (EI) (*Z*-isomer): cf. **Figure 20D**.

MS (CI) (*E*-isomer): cf. **Figure 32**.

MS (CI) (*Z*-isomer): cf. **Figure 33**.

3.3.2.9 (*E*)-3-Methyl-1-butene-1-thiol and (*Z*)-3-methyl-1-butene-1-thiol

The target compounds were synthesized by a two-step synthesis as outlined in **Figure 21**.

3-Methylbutane-1,1-dithiol

The target compound was prepared by treatment of 3-methylbutanal with H_2S as described above for 2-methyl-1-propene-1-thiol (**3.3.2.7**) (yield: 18%, purity: 98%).

^{1}H NMR: δ 0.92 (d, J = 6.4, 6H); 1.74 (dd, J = 7.2, 7.2, 2H); 1.79–1.93 (m, 1H); 2.36 (d, J = 6.4, 2H); 4.11 (tt, J = 6.4, 7.2, 1H).

^{13}C NMR: δ 21.9, 26.7, 37.2, 52.6.

MS (EI): *m*/*z* (%): 136 (21), 103 (65), 102 (58), 87 (40), 69 (100), 61 (42), 60 (36), 59 (27), 45(40), 43 (46), 41 (62), 39 (22).

(E)-3-Methyl-1-butene-1-thiol and (Z)-3-methyl-1-butene-1-thiol

The 1-alkene-1-thiol isomers were prepared by FVP of 3-methylbutane-1,1-dithiol (yield: 60%, purity: 96%). The (*E*)/(*Z*)-ratio was determined by GC to be 55:45. The correct assignment of the GC peaks was achieved by ^{1}H NMR of the mixture using the coupling constants of the neighboring olefinic protons at a chemical shift of δ 5.74 and δ 5.48, respectively.

Odor quality (HRGC-O): (*E*-isomer): sulfurous, meaty.

Odor quality (HRGC-O): (*Z*-isomer): sulfurous, meaty.

RI (FFAP): (*E*-isomer): n.d. **RI (DB-5)**: (*E*-isomer): 788.

RI (FFAP): (*Z*-isomer): n.d. **RI (DB-5)**: (*Z*-isomer): 770.

^{1}H NMR (*E*-isomer): δ 0.97 (d, J = 6.4, 6H, $(CH_3)_2$); 2.30 (double septuplet, J = 6.4, 6.4, 1H, $CH(Me)_2$); 2.68 (d, J = 6.4, 1H, SH); 5.74 (dd, J = 6.4, 15.2, 1H, *i*-Pr–CH=R); 5.76 (dd, J = 6.4, 15.2, 1H, R=CH–SH).

^{1}H NMR (*Z*-isomer): δ 0.98 (d, J = 6.8, 6H, $(CH_3)_2$); 2.61 (double septuplet, J = 6.8, 9.2, 1H, $CH(Me)_2$); 2.62 (d, J = 9.2, 1H, SH); 5.48 (dd, J = 9.2, 9.2, 1H, *i*-Pr–CH=R); 5.87 (dd, J = 9.2, 9.2, 1H, R=CH–SH).

^{13}C NMR (*E*-isomer): δ 22.1, 31.9, 111.0, 141.4.

^{13}C NMR (*Z*-isomer): δ 22.0, 27.8, 112.0, 138.5.

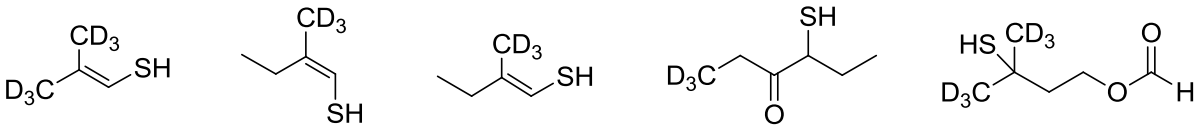
MS (EI) (*E*-isomer): cf. **Figure 48A**.

MS (EI) (*Z*-isomer): cf. **Figure 48B**.

MS (CI) (*E*-isomer): cf. **Figure 49**.

MS (CI) (*Z*-isomer): cf. **Figure 49**.

3.3.3 Isotopically labeled odorants



d-1 **d-4** **d-5** **d-15** **d-19**

Figure 39: Structures of deuterium labeled thiols prepared by synthesis.

3.3.3.1 [$^2H_3$]-4-Mercapto-3-hexanone (d-15)

The compound (**d-15**) was synthesized in a six-step procedure starting from [$^2H_3$]-bromoethane (**Figure 24**).

[$^2H_3$]-2-Ethyl-1,3-dithiane

Under an atmosphere of argon, *n*-butyl lithium in hexane (15.4 mL, 1.59 mol/L, 24.5 mmol) was added dropwise to a solution of 1,3-dithiane (2.9 g, 24.5 mmol) in THF (25 mL) at –30 °C for 10 min. After stirring for 2 h at –30 °C, a solution of [$^2H_3$]-bromoethane (2.5 g, 22.3

mmol) in THF (5 mL) was dropwise added at –20 ºC for 5 min. After stirring overnight at 0 ºC in a refrigerator, the reaction mixture was warmed to room temperature, and poured onto ice water (50 mL). The aqueous solution was extracted with diethyl ether. The organic layer was washed with brine, and dried over $MgSO_4$. The solution was filtered, and concentrated under high vacuum. The residue (4.2 g) was distilled (b.p.: 71–72 ºC/0.5 kPa) into an oil of [$^2H_3$]-ethyl-1,3-dithiane (3.1 g, yield: 91%, purity: 86%).

MS (EI): *m*/*z* (%): 151 (47), 121 (9), 120 (7), 119 (100), 77 (12), 76 (7), 75 (7), 74 (13), 73 (7), 59 (5), 58 (5), 47 (6), 46 (15), 45 (21), 44 (7), 43 (9), 41 (12).

[$^2H_3$]-1-(2-Ethyl-1,3-dithian-2-yl)propan-1-ol

Under an atmosphere of argon, *n*-butyl lithium in hexane (13.8 mL, 1.59 mol/L, 22.0 mmol) was added dropwise to a solution of [$^2H_3$]-ethyl-1,3-dithiane (3.1 g, 20.0 mmol) in THF (100 mL) at –30 ºC for 10 min. After stirring for 2 h at –30 ºC, a solution of propanal (1.7 g, 30.0 mmol) in THF (5 mL) was dropwise added at –20 ºC within 5 min. After stirring overnight at 0 ºC in a refrigerator, the reaction mixture was warmed to room temperature, and poured into ice water (50 mL). The aqueous solution was extracted with diethyl ether. The organic layers were combined, washed with brine, and dried over $MgSO_4$. The organic solution was filtered, and concentrated under high vacuum. The residue (4.6 g) was purified by silica gel (150 g) chromatography using hexane/ethyl acetate (15:1, v/v) as eluent. The fractions containing the target compound were combined and concentrated under high vacuum into an oil of [$^2H_3$]-1-(2-ethyl-1,3-dithian-2-yl)propan-1-ol (2.1 g, yield: 49%, purity: 97%).

MS (EI): *m*/*z* (%): 209 (1), 191 (14), 152 (9), 151 (9), 150 (100), 108 (5), 106 (37), 76 (16), 75 (6), 73 (9), 59 (5), 57 (5), 46 (7), 45 (12), 41 (14).

[$^2H_3$]-4-Hydroxy-3-hexanone

Under a nitrogen atmosphere, $HgCl_2$ (5.4 g, 20.0 mmol), $CdCO_3$ (3.4 g, 20.0 mmol) and [$^2H_3$]-1-(2-ethyl-1,3-dithian-2-yl)propan-1-ol (2.1 g, 9.8 mmol) were dissolved in water/acetonitrile (1:10, v/v, 110 mL). After stirring for 2 h at 50–60 ºC, the reaction mixture was cooled to room temperature, diluted with diethyl ether, and filtered. The filtrate was washed with brine and dried over $MgSO_4$. The solution was filtered, and concentrated under high vacuum. The residue (1.0 g) was purified by silica gel (45 g) chromatography using hexane/ethyl acetate (10:1–3:1, v/v) as eluent. The fractions containing the target compound

were combined and concentrated under high vacuum into an oil of [$^2H_3$]-4-hydroxy-3-hexanone (0.6 g, yield: 51%, purity: 94%).

MS (EI): *m*/*z* (%): 119 (2), 62 (23), 60 (41), 59 (100), 58 (23), 57 (13), 41 (20), 32 (24), 31 (34), 29 (14).

[$^2H_3$]-4-Methanesulfonyloxy-3-hexanone

The compound was prepared by reaction of [$^2H_3$]-4-hydroxy-3-hexanone and methanesulfonyl chloride, according to the synthesis of 4-methanesulfonyloxy-3-hexanone reported above in **3.3.2.6**. The crude product (0.51 g) was directly used in the next step.

MS (EI): *m*/*z* (%): 139 (5), 137 (5), 79 (11), 69 (4), 60 (100), 59 (12), 57 (4), 41 (7), 32 (21), 29 (6).

[$^2H_3$]-4-Acetylthio-3-hexanone

The compound was prepared by a treatment of the crude [$^2H_3$]-4-methanesulfonyloxy-3-hexanone with potassium thioacetate, according to the synthesis of 4-acetylthio-3-hexanone reported above in **3.3.2.6** (yield: 0.53 g). The crude product was directly used in the next step.

MS (EI): *m*/*z* (%): 177 (4), 135 (13), 134 (20), 117 (18), 102 (5), 75 (24), 74 (5), 60 (100), 55 (5), 45 (6), 43 (95), 41 (11), 39 (5), 32 (24).

[$^2H_3$]-4-Mercapto-3-hexanone (d-15)

The compound **d-15** was prepared by treatment of the crude [$^2H_3$]-4-acetylthio-3-hexanone with aq. NaOH, according to the synthesis of 4-mercapto-3-hexanone reported above in **3.3.2.6** (156 mg, yield: 23%, purity: 97%).

^{1}H NMR: δ 0.96 (dd, J = 7.2, 7.2 Hz, 3H); 1.67 (ddq, J = 7.2, 7.2, 14.4 Hz, 1H); 1.68 (d, J = 10.0 Hz, 1H); 1.92 (ddq, J = 7.2, 7.2, 14.4 Hz, 1H); 2.48 (d, J = 18.0 Hz, 1H); 2.70 (d, J = 18.0 Hz, 1H); 3.21 (ddd, J = 7.2, 7.2, 10.0 Hz, 1H).
^{13}C NMR: δ 7.4, 11.9, 27.7, 33.1, 48.6, 208.9.
MS (EI): cf. **Figure 50**.
MS (CI): cf. **Figure 25**.

3.3.3.2 [$^2H_6$]-3-mercapto-3-methylbutyl formate (d-19)

The compound (**d-19**) was prepared in a four-step synthesis starting from [$^2H_6$]-acetone (**Figure 27**).

[$^2H_6$]-Ethyl 3-methyl-2-butenoate

Under an atmosphere of argon, *n*-butyl lithium in hexane (76 mL, 1.59 mol/L, 120.9 mmol) was added dropwise to a solution of diethylphosphonoacetic acid ethyl ester (40.63 g, 181.4 mmol) in THF (730 mL) at 0 ºC within 20 min. After stirring for 20 min at 0 ºC, a solution of [$^2H_6$]-acetone (7.74 g, 120.9 mmol) in THF (40 mL) was dropwise added at 0 ºC for 10 min. After stirring for 1.5 h at 0 ºC, the reaction mixture was warmed up to room temperature, stirred overnight, and poured into water (800 mL). The aqueous solution was extracted with diethyl ether. The organic layer was washed with brine and dried over $MgSO_4$. The solution was filtered, and concentrated under reduced pressure. The residue (103 g) was distilled (b.p.: 48–50 ºC/2.1 kPa) into a colorless oil of [$^2H_6$]-ethyl 3-methyl-2-butenoate (7.08 g, yield: 44%, purity: 98%).

MS (EI): *m*/*z* (%): 134 (34), 106 (17), 89 (100), 88 (19), 87 (11), 86 (9), 61 (24), 42 (4), 41(4).

[$^2H_6$]-Ethyl 3-mercapto-3-methylbutyrate

Diazabicyclo[5.4.0]undec-7-ene (DBU, 0.7 g, 4.6 mmol) and hydroquinone (0.25 g, 2.3 mmol) were added to a solution of [$^2H_6$]-ethyl 3-methyl-2-butenoate (6.27 g, 46.8 mmol) in dimethyl sulfoxide (DMSO, 13 g) at 0 ºC. Hydrogen sulfide was passed through the solution at a rate of 50 mL/min for 2 h at 0 ºC. The reaction mixture was then warmed up to 40 ºC, stirred overnight, and poured into aq. HCl (1 mol/L; 100 mL) at 10 ºC. The aqueous solution was extracted with diethyl ether. The organic layer was washed with saturated aq. $NaHCO_3$ and brine, and dried over Na_2SO_4. The solution was filtered, and concentrated under reduced pressure into a crude oil of [$^2H_6$]-ethyl 3-mercapto-3-methylbutyrate (8.83 g). The crude product was directly used in the next step.

MS (EI): *m*/*z* (%): 168 (47), 135 (50), 123 (20), 95 (24), 93 (100), 88 (15), 81 (77), 80 (16), 65 (64), 63 (19), 62 (62), 61 (18), 60 (36), 46 (23), 45 (16), 44 (20), 29 (38).

[$^2H_6$]-3-Mercapto-3-methylbutanol

Under an atmosphere of argon, the crude [$^2H_6$]-ethyl 3-mercapto-3-methylbutyrate (8.83 g, 52.6 mmol) in diethyl ether (50 mL) was added dropwise to a suspension of lithium aluminium hydride (2.13 g, 55.9 mmol) in diethyl ether (150 mL) at 0 ºC within 30 min. After stirring for 2 h at 0 ºC, water (11 mL) was added dropwise to the reaction mixture at 0 ºC for 5 min. After stirring for 40 min at 10 ºC, aq. HCl (2 mol/L; 250 mL) was added. The organic layer was separated and the aqueous layer was extracted with diethyl ether. The organic layer was dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue (7.07 g) was distilled (b.p.: 52 ºC/5 kPa) into a colorless oil of [$^2H_6$]-3-mercapto-3-methylbutanol (1.57 g, 2 steps' yield: 27%, purity: 99%).

MS (EI): *m*/*z* (%): 126 (8), 93 (16), 92 (54), 81 (26), 74 (100), 73 (62), 63 (13), 62 (16), 60 (10), 49 (13), 46 (21), 45 (16), 44 (27), 43 (13).

[$^2H_6$]-3-mercapto-3-methylbutyl formate (d-19)

[$^2H_6$]-3-mercapto-3-methylbutanol (0.88 g, 7.0 mmol) and formic acid (3.21 g, 69.8 mmol) were mixed at room temperature. After stirring for 5 h at room temperature, the reaction mixture was diluted with diethyl ether (100 mL), washed with water and brine, and dried over Na_2SO_4. The solution was filtered, and concentrated under reduced pressure. The residue (7.93 g) was distilled (b.p.: 30–35 ºC/3 kPa) into a colorless oil of [$^2H_6$]-3-mercapto-3-methylbutyl formate (**d-19**) (0.59 g, yield: 55%, purity: 98%).

^{1}H NMR: δ 1.73 (s, 1H); 1.97 (t, J = 7.2 Hz, 2H); 4.38 (t, J = 7.2 Hz, 2H); 8.05 (s, 1H).
^{13}C NMR: δ 32.1, 42.4, 44.1, 61.5, 160.9.
MS (EI): *m*/*z* (%): 154 (3), 108 (36), 90 (7), 81 (17), 75 (100), 74 (20), 62 (9), 60 (4), 46 (9), 45 (11), 44 (15), 43 (6), 31 (4), 29 (5).
MS (CI): *m*/*z* (%): 121 (16), 109 (31), 76 (6), 75 (100), 74 (16).

3.3.3.3 [$^2H_6$]-2-Methyl-1-propene-1-thiol (d-1)

The compound (**d-1**) was prepared in a three-step synthesis starting from [$^2H_6$]-acetone (**Figure 28**).

[$^2H_6$]-2-Methylpropanal

Under an atmosphere of nitrogen, sodium hydride (22.0 g, 550 mmol) was suspended in DMSO (500 mL) at room temperature. Then, trimethylsulfoxonium iodide (121.0 g, 550 mmol) was added to the solution within 40 min keeping the temperature under 25 ºC, and then [$^2H_6$]-acetone (32.1 g, 500 mmol) was added to the reaction mixture within 5 min at room temperature. After stirring for 2 h, the reaction mixture was poured into ice water (750 g), and extracted with diethyl ether. The organic layer was dried over Na_2SO_4, and filtered (ca. 500 mL). A solution of citric acid monohydrate (3 g) in water (27 g) was added, and after stirring the mixture overnight at room temperature, Na_2CO_3 (1.8 g) was added to adjust the pH to 6 ~ 7. The organic layer was separated and concentrated under atmospheric pressure. The residue (13.6 g) was combined with the water layer and added to H_2SO_4 (5% in water, 300 g). This mixture was then submitted to steam distillation. The distillate was extracted with diethyl ether. The extract was dried over Na_2SO_4, filtered, and distilled under atmospheric pressure (b.p.: 60 ºC) to yield an oil of [$^2H_6$]-2-methylpropanal (6.2 g, yield: 16%, purity: 92%).

MS (EI): *m*/*z* (%): 78 (M^+, 42), 50 (12), 49 (100), 48 (14), 46 (35), 45 (49), 42 (15), 41 (9), 32 (11), 30 (20), 29 (25).

[$^2H_6$]-2-Methylpropane-1,1-dithiol

[$^2H_6$]-2-Methylpropanal was treated with H_2S using the method reported above for the synthesis of the unlabeled 2-methylpropane-1,1-dithiol (**3.3.2.7**) (yield: 4%, purity: 95%).

MS (EI): *m*/*z* (%): 128 (11), 95 (62), 94 (59), 79 (20), 76 (18), 62 (35), 61 (61), 60 (100), 59 (16), 49 (27), 48 (16), 46 (45), 45 (56), 42 (22), 41 (19), 34 (35) , 33 (23) , 32 (18).

[$^2H_6$]-2-Methyl-1-propene-1-thiol (d-1)

The compound **d-1** was prepared by flash vacuum pyrolysis (FVP) (*Schiess et al., 1995; Kreilein et al., 2005*) of [$^2H_6$]-2-methylpropane-1,1-dithiol using the parameters reported above for the synthesis of the unlabeled 2-methyl-1-propene-1-thiol (**3.3.2.7**) (yield: 6%).

MS (EI): cf. **Figure 51**.

MS (CI): cf. **Figure 29**.

3.3.3.4 [$^2H_3$]-(*E*)-2-Methyl-1-butene-1-thiol (d-5) and [$^2H_3$]-(*Z*)-2-methyl-1-butene-1-thiol (d-4)

The compounds (**d-5** and **d-4**) were prepared in a three-step synthesis starting from butyronitrile (**Figure 31**).

[$^2H_3$]-2-Methylbutanal

The compound was synthesized following a procedure given in (*Goering and Tseng, 1981*). Under N_2 atmosphere, diisobutylaluminum hydride (DIBAL) in hexane (200 mL, 1 mol/L, 200 mmol) was added to a solution of butyronitrile (13.8 g, 200 mmol) in diethyl ether (50 mL) at −10 ºC, and the reaction mixture was stirred for 1 h. In the meantime, lithium diisopropylamide was prepared as follows: Under an atmosphere of nitrogen, *n*-butyl lithium in hexane (127 mL, 1.58 mol/L, 200 mmol) was added to a solution of diisopropylamine (20.2 g, 200 mmol) in diethyl ether (270 mL) at −70 ºC, and the solution was warmed to −30 ºC, and stirred for 30 min. This solution was then added to the main reaction mixture at −10 ºC within 30 min. Hexamethylphosphoramide (45 g, 251 mmol) was added slowly to the reaction mixture at −5 ºC, which was then warmed to 20 ºC and stirred for 1 h. Then, [$^2H_3$]-iodomethane (31.2 g, 220 mmol) was added to the reaction mixture at −10 ºC. After stirring overnight, the reaction mixture was warmed up to 40 ºC, followed by further stirring for 1 h. Then, H_2SO_4 (20% in water, 400 g) was added to the reaction mixture at 5 ºC. The reaction mixture was heated to 100 ºC, and the crude product was distilled off together with the water. The organic layer was separated, dried over anhydrous Na_2SO_4, and filtered to yield a solution of crude [$^2H_3$]-2-methylbutanal (ca. 500 mL). This crude solution was directly used in the next step.

MS (EI): *m*/*z* (%): 89 (8), 61 (40), 60 (100), 59 (44), 58 (10), 44 (22), 43 (54), 42 (35), 41 (20), 39 (13), 32 (18), 31 (31), 30 (27), 29 (29).

[$^2H_3$]-2-Methylbutane-1,1-dithiol

The crude solution of [$^2H_3$]-2-methylbutanal was treated with H_2S using the method reported above for the synthesis of the unlabeled 2-methylbutane-1,1-dithiol (**3.3.2.8**) (yield: 6%, purity: 84%).

MS (EI): *m*/*z* (%): 139 (19), 106 (100), 105 (51), 79 (37), 72 (84), 71 (33), 64 (37), 61 (27), 60 (29), 47 (27), 46 (24), 45 (62), 44 (30), 43 (50), 42 (38), 41 (25), 34 (23).

[$^2H_3$]-(E)-2-Methyl-1-butene-1-thiol (d-5) and [$^2H_3$]-(Z)-2-methyl-1-butene-1-thiol (d-4)

The compounds (**d-5** and **d-4**) were prepared by flash vacuum pyrolysis (FVP) of [$^2H_3$]-2-methylbutane-1,1-dithiol using the parameters previously reported for the synthesis of the unlabeled (*E*)/(*Z*)-2-methyl-1-butene-1-thiol (**3.3.2.8**) (yield: 79%, purity: 90%). The (*E*)/(*Z*)-ratio was determined by GC to be 60:40. The correct assignment of the GC peaks was achieved by comparing their retention times with those of the unlabeled (*E*) and (*Z*)-2-methyl-1-butene-1-thiol.

^{1}H NMR (*E*-isomer, **d-5**): δ 0.98 (t, J = 7.6 Hz, 3H); 2.05 (q, J = 7.6 Hz, 2H); 2.51 (d, J = 7.2 Hz, 1H); 5.69 (d, J = 7.2 Hz, 1H).

^{1}H NMR (*Z*-isomer, **d-4**): δ 0.98 (t, J = 7.6 Hz, 3H); 2.14 (q, J = 7.6 Hz, 2H); 2.48 (d, J = 7.2 Hz, 1H); 5.65 (d, J = 7.2 Hz, 1H).

^{13}C NMR (*E*-isomer, **d-5**): δ 12.4, 32.1, 106.4, 140.6.

^{13}C NMR (*Z*-isomer, **d-4**): δ 11.5, 25.8, 106.6, 141.1.

MS (EI) (*E*-isomer, **d-5**): cf. **Figure 52A**.

MS (EI) (*Z*-isomer, **d-4**): cf. **Figure 52B**.

MS (CI) (*E*-isomer, **d-5**): cf. **Figure 32**.

MS (CI) (*Z*-isomer, **d-4**): cf. **Figure 33**.

3.3.3.5 [$^2H_3$]-2-methyl-3-thiophenethiol (d-20)

The compound (**d-20**) was synthesized following a procedure published for the synthesis of [$^2H_3$]-2-methyl-3-furanthiol (**d-6**) (*Sen and Grosch, 1991*), but using 3-bromothiophene instead of 3-bromofuran as the starting material.

^{1}H NMR: δ 3.01 (s, 1H); 6.87 (d, J = 5.2 Hz, 1H); 7.04 (d, J = 5.2 Hz, 1H).

MS (EI): *m*/*z* (%): 135 (9), 134 (9), 133 (100), 132 (22), 131 (22), 100 (36), 99 (26), 88 (8), 70 (11), 69 (10), 62 (17), 56 (7), 46 (11), 45 (13).

MS (CI): *m*/*z* (%): 136 (12), 135 (7), 134 (100).

3.4 Identification of aroma-active compounds

3.4.1 Isolation of the volatiles

3.4.1.1 Solvent extraction

Roasted, ground white sesame seeds (2.5 g) prepared as described in **3.2** were extracted with dichloromethane (50 mL) at room temperature for 1 h. After filtration, the residue was twice extracted for 30 min with dichloromethane (total volume 100 mL), and finally the organic phases were combined.

3.4.1.2 High vacuum distillation

The volatiles of the extract were isolated from non-volatile substances by means of high-vacuum distillation using solvent assisted flavor evaporation (SAFE) (*Engel et al., 1999*). For this purpose the apparatus shown in **Figure 46** was used.

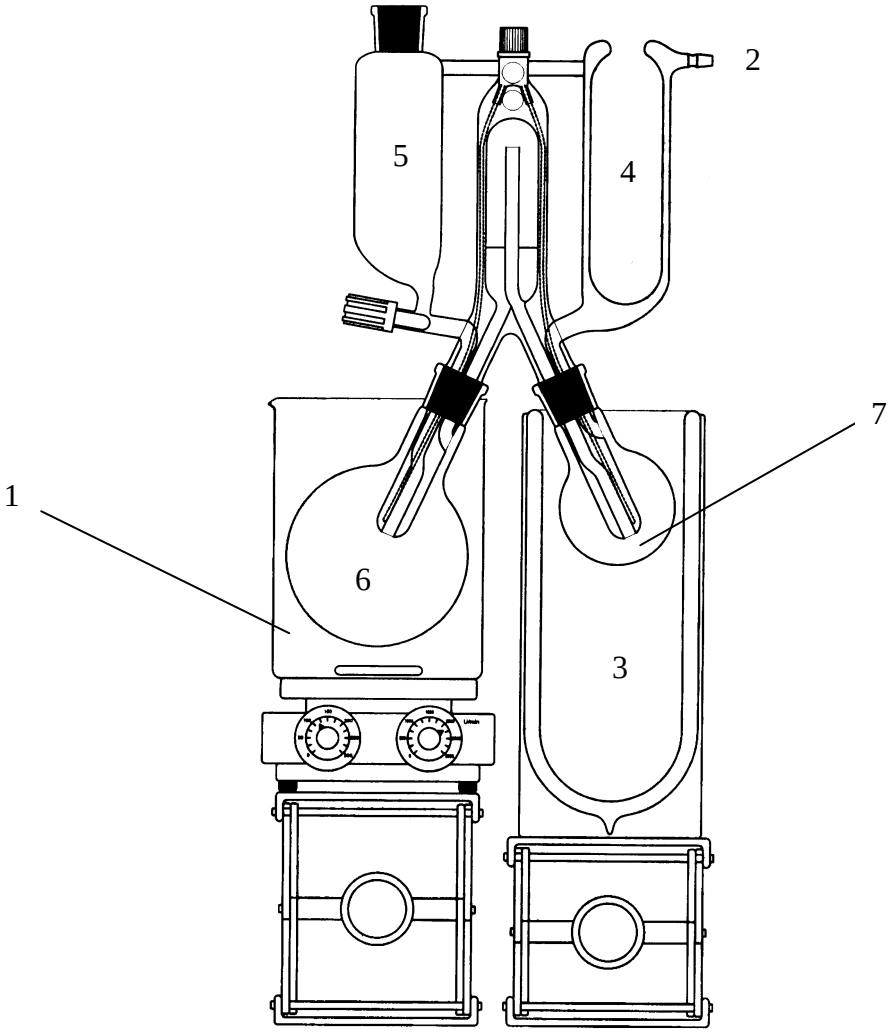


Figure 46: Apparatus used for the solvent assisted flavor evaporation (SAFE) (*Engel et al., 1999*).

Before starting the distillation, the water bath (1) and heatable parts of the apparatus were thermostated at 40 ºC. Then high vacuum (10^{-3} to 10^{-4} Pa) was applied to the solvent assisted flavor evaporation (SAFE) apparatus by a diffusion pump or a turbo pump (Leybold, Cologne) via outlet 2 while the high-vacuum stopcock of the dropping funnel (5) was carefully closed. Then liquid nitrogen was poured into the traps (3 and 4).

The distillation procedure was started by dropping aliquots of the sample from the dropping funnel (5) into the distillation flask (6). This addition was done very slowly and continuously. From the vapor spray, which was formed immediately, the volatiles and the solvent were transferred into the distillation head. Non-volatile substances remained in the distillation flask (6). The distillate entered the liquid nitrogen cooled flask and the volatiles and the solvent were condensed along the inner walls of the flask (7).

To finish the distillation, the connection to the pump was interrupted and the apparatus was ventilated via the high-vacuum stopcock.

After defrosting, the SAFE distillate was dried over anhydrous sodium sulfate, filtered, and concentrated to 200 µL at 45 ˚C using a Vigreux column (60 cm × 1 cm).

3.4.1.3 Fractionation of volatiles by column chromatography

For identification experiments, the distillate prepared from 2.5 kg of ground roasted sesame seeds was fractionated at 10–12 ºC by column chromatography on purified silica gel 60 (0.063–0.200 mm) (cf. **3.1.4**, *Esterbauer, 1968*).

The ground roasted white sesame seeds (2.5 kg) prepared (cf. **3.2**) were extracted with dichloromethane (2.5 L) at room temperature for 1 h. After filtration, the residue was further extracted for 30 min with two more portions of dichloromethane (1.25 L). The combined extracts were concentrated to 2 L, and the volatiles were isolated by SAFE distillation (cf. **3.4.1.2**). The distillate was concentrated to 5 mL at 45 ˚C using a Vigreux column. Then 5 mL of *n*-hexane was added and the distillate was concentrated again to 5 mL by distilling off the solvent on a Vigreux column to replace dichloromethane with *n*-hexane as solvent.

The bottom of a water-cooled glass column (39 cm × 2.5 cm i.d.) was stuffed with silylated fiberglass on which sea sand (1.0 g) was added, and the column was filled with a slurry of the purified silica gel (75 g, 7% water) in *n*-pentane.

The roasted sesame seeds aroma concentrate (5 mL) was applied on the column by means of a pipette. Elution was performed using five *n*-pentane/diethyl ether mixtures of increasing polarity as detailed in **Table 16**.

Table 16: Elution scheme for column chromatography on silica gel (water content 7%).

fraction	mobile phase	ratio (v/v)	volume (mL)
A	*n*-pentane	100	450
B	*n*-pentane/diethyl ether	90/10	450
C	*n*-pentane/diethyl ether	70/30	450
D	*n*-pentane/diethyl ether	50/50	450
E	diethyl ether	100	450

All fractions were dried over anhydrous sodium sulfate, filtered, and concentrated to 200 µL at 45 ˚C. The concentrated fractions were analyzed by means of HRGC-O, HRGC-MS, and two-dimensional HRGC-MS (cf. **3.7.3**).

3.4.1.4 Enrichment of thiols by affinity chromatography using mercurated agarose gel

For selective enrichment of thiols, fractions A and B were concentrated to 5 mL and thiols were isolated by affinity chromatography on a cross-linked organomercury agarose gel (equivalent to Bio-Rad Affi-Gel 501 which is now unavailable) prepared described above in **3.3.1** referring to *Full and Schreier* (*1994*) and *Steinhaus et al.* (*2007*).

The bottom of a glass column (15 cm × 0.7 cm i.d.) was stuffed with silylated fiberglass, on which 2 mL of mercurated agarose gel suspended in isopropyl alcohol was added, and then it was conditioned with 5 mL of isopropyl alcohol. The fraction was applied on the column using a pipette. Then the gel was washed with 60 mL of *n*-pentane/dichloromethane mixture (2/1, v/v). This fraction was discarded. Then thiols, which were reversibly covalently and selectively bound to the gel, were eluted with 50 mL of 10 mM dithiothreitol solution in *n*-pentane/dichloromethane mixture (2/1, v/v). Separation of the target volatile thiols from the dithiothreitol was achieved by solvent assisted flavor evaporation (SAFE, cf. **3.4.1.2**).

The distillate was thawed, dried over anhydrous sodium sulfate, filtered, and then concentrated to 200 µL at 45 ˚C using a Vigreux column (60 cm × 1 cm i.d.). The thiol concentrate was analyzed by HRGC-O, HRGC-MS, and two-dimensional HRGC-MS (cf. **3.7.3**).

3.4.2 High-resolution gas chromatography-olfactometry (HRGC-O)

The high-resolution gas chromatography-olfactometry (HRGC-O) served to identify aroma-active volatiles in the solvent extract of ground roasted white sesame seeds.

The volatiles were separated by the high-resolution gas chromatography and the effluent from the capillary gas chromatography column split 1:1 (by volume) into a flame ionization detector (FID) and a sniffing-port was examined by sniffing to assess the odor of the compounds in parallel with their FID detection.

3.4.3 Aroma extract dilution analysis (AEDA)

The SAFE (cf. **3.4.1.2)** distillate (200 µL) of the ground roasted sesame extract obtained in **3.4.1.1** was stepwise diluted with dichloromethane (1+1 by volume) to prepare dilutions of 1:2, 1:4, 1:8, 1:16 … etc. of the original extract. The HRGC-O was performed with aliquots (0.5 µL) of the diluted extracts on capillary DB-5 and DB-FFAP, and dilution was continued until no odor was detected during the whole run (*Ullrich and Grosch, 1987*).

Each single odorant was, thus, assigned a flavor dilution factor (FD factor) representing the highest dilution in which the odorant was detected at the sniffing port. The undiluted sample had an FD-factor of 1 by definition.

3.4.4 High-resolution gas chromatography-mass spectrometry (HRGC-MS)

For the identification of the odorants in roasted sesame, the extract obtained for aroma extract dilution analysis (cf. **3.4.3**) and all the fractions obtained by silica gel column chromatography (cf. **3.4.1.3**) were examined by high-resolution gas chromatography (used fused silica capillary columns: DB-5 and DB-FFAP) coupled with a sector field mass spectrometer (cf. **3.7.1**) generating mass spectra at 70 eV in the electron impact ionization mode (EI-mode).

If it was not possible to obtain their unequivocal mass spectra with interfering substances, two-dimensional gas chromatography coupled with an ion trap (cf. **3.7.3**) was employed. Here, mass spectra were also generated by MS-EI and compared with those of reference compounds obtained under the same conditions of the GC run.

3.5 Quantitation of odorants

The aroma-active thiols were quantitated by the application of stable isotope dilution assays (SIDA) (cf. **3.5.4**).

3.5.1 Determination of the concentration of isotopically labeled odorants

The concentration of the stable isotopically labeled odorants, namely [$^2H_6$]-2-methyl-1-propene-1-thiol, [$^2H_3$]-(*E*)-2-methyl-1-butene-1-thiol, [$^2H_3$]-(*Z*)-2-methyl-1-butene-1-thiol, [$^2H_3$]-4-mercapto-3-hexanone, and [$^2H_6$]-3-mercapto-3-methylbutyl formate, was determined by means of HRGC-FID with methyl octanoate as internal standard. In the case of [$^2H_2$]-3-mercapto-2-pentanone, 2,3-pentanedione was used as an internal standard, and also 2-

thenylthiol was selected for [$^2H_3$]-2-methyl-3-thiophenethiol. First, a solution of the corresponding unlabeled odorant (ca. 100 µg/mL) and a solution of the corresponding internal standard (ca. 100 µg/mL) were prepared. Then, for the determination of response factors, a solution with the reference odorants solution and the internal standards solution in the ratio 1:1 was made. The mixed solutions were analyzed by means of HRGC-FID. Each of the response factors were calculated by using following formula:

$$R_f = \frac{c_u \cdot A_{is}}{c_{is} \cdot A_u}$$

R_f response factor
c_u concentration of the unlabeled reference odorant
c_{is} concentration of the internal standard
A_u peak area of the unlabeled reference odorant
A_{is} peak area of the internal standard

After the calculations of the response factors, the solution of the labeled odorant (0.5 mL) were mixed with the corresponding internal standard solution (0.5 mL), and analyzed by HRGC-FID as well. The concentration of the stable isotopically labeled odorants was determined by the following formula:

$$c_I = R_f \cdot \frac{c_{is} \cdot A_I}{A_{is}}$$

c_I concentration of the isotopically labeled odorant
R_f response factor
c_{is} concentration of the internal standard
A_I peak area of the isotopically labeled odorant
A_{is} peak area of the internal standard

The concentration of [$^2H_3$]-2-methyl-3-furanthiol was determined by means of 2-furfurylthiol. The response factor was defined as 1.

3.5.2 Sample preparation for stable isotope dilution assay (SIDA)

Ground roasted white sesame seeds (amounts from 10 to 350 g), prepared as described above (cf. **3.2**), were suspended in dichloromethane (volumes from 15 to 500 mL), and the labeled internal standards (amounts from 0.1 to 10 µg) were added in dichloromethane solution. Amounts of sample and of internal standards were adjusted according to preliminary experiments in order to yield appropriate responses during mass chromatography and response ratios of analytes and respective standards in the range of 1:3 to 3:1. After stirring at room temperature for 1 h, the mixture was filtered and the residue was extracted twice for 30 min with dichloromethane (volumes from 10 to 350 mL). The organic phases were combined and the nonvolatile material was removed by solvent assisted flavor evaporation (SAFE) (cf. **3.4.1.2**), only in the case that needed higher sample amounts and thus the extracts contained too much oil to have an appropriate flow rate of the following affinity chromatography described below. After the distillates were thawed, the distillates were dried over anhydrous sodium sulfate and filtered. The extract was concentrated by distilling off the solvent on a Vigreux column (60 cm × 1 cm i.d.) at 45 °C to 10 mL.

The aroma-active thiols were then isolated by affinity chromatography on mercurated agarose gel (equivalent to Affi-Gel 501 which is now unavailable and was synthesized according to **3.3.1**) using the method described above in **3.4.1.4**. Finally 200 µL of the thiol concentrate was obtained. The thiol isolate was analyzed using the two dimensional HRGC-ITD-system described under **3.7.4**.

3.5.3 Mass chromatography

The quantitation of the aroma-active thiols was carried out by means of mass chromatography in the application of stable isotope dilution assay. The mass chromatogram were recorded by the two dimensional HRGC-ITD-system described under **3.7.4** in the chemical ionization (CI) mode with methanol as reactant gas.

The peak area counts of specific ions of the labeled standard and the analyte (**Table 17**) were separately determined in the mass chromatogram. From the amounts of the added standard and the extracted roasted sesame seeds, and by using response factors obtained with definite mixtures of standard and analyte, the concentration of the thiol odorants in ground roasted white sesame seeds could be accurately determined:

$$c_u = R_f \cdot \frac{m_d}{g} \cdot \frac{A_u}{A_d}$$

c_u concentration of the analyte
R_f response factor
m_d amount of the isotopically labeled internal standard added
g amount of the sample analyzed
A_u peak area of the ion *m*/*z* of the unlabeled analyte
A_d peak area of the ion *m*/*z* of the isotopically labeled standard

The response factor (R_f) was a factor to compensate an uncompleted isotope labeling of the standard. For the determination of the response factors, calibration solutions with known amounts of isotopically labeled and unlabeled odorants in the ratio 1:3, 1:1 and 3:1 were analyzed by means of the two dimensional HRGC-ITD.
The response factor (R_f) was calculated by using the following formula:

$$R_f = \frac{m_u \cdot A_d}{m_d \cdot A_u}$$

R_f response factor
m_u amount of the analyte
m_d amount of the isotopically labeled standard
A_u peak area of the ion *m*/*z* of the unlabeled analyte
A_d peak area of the ion *m*/*z* of the isotopically labeled standard

The quantitated odorants, the standards used for the quantitation experiments and the selected ions are summarized in **Table 17**.

Table 17: Odorants, labeled standards and mass traces [*m*/*z*] used for the quantitation of aroma compounds in ground roasted white sesame seeds.

odorant	labeled odorant	selected ion (*m*/*z*)	
		unlabeled odorant	labeled odorant
2-methyl-1-propene-1-thiol	[$^2H_6$]-2-methyl-1-propene-1-thiol	89	95
(*Z*)-3-methyl-1-butene-1-thiol	[$^2H_3$]-(*E*)-2-methyl-1-butene-1-thiol	103	106
(*E*)-3-methyl-1-butene-1-thiol	[$^2H_3$]-(*E*)-2-methyl-1-butene-1-thiol	103	106
(*Z*)-2-methyl-1-butene-1-thiol	[$^2H_3$]-(*Z*)-2-methyl-1-butene-1-thiol	103	106
(*E*)-2-methyl-1-butene-1-thiol	[$^2H_3$]-(*E*)-2-methyl-1-butene-1-thiol	103	106
2-methyl-3-furanthiol	[$^2H_3$]-2-methyl-3-furanthiol	115	118
3-mercapto-2-pentanone	[$^2H_2$]-3-mercapto-2-pentanone	119	121
2-mercapto-3-pentanone	[$^2H_2$]-3-mercapto-2-pentanone	119	121
4-mercapto-3-hexanone	[$^2H_3$]-4-mercapto-3-hexanone	133	136
3-mercapto-3-methylbutyl formate	[$^2H_6$]-3-mercapto-3-methylbutyl formate	103	109
2-methyl-3-thiophenethiol	[$^2H_3$]-2-methyl-3-thiophenethiol	131	134

3.5.4 Relative standard deviation

The relative standard deviation (RSD) to measure variability of the quantitated value was calculated using the following formula:

$$RSD[\%] = \sqrt{\frac{1}{n}\sum_{i=1}^{n}(x_i - \bar{x})^2} \cdot \frac{100}{\bar{x}}$$

n: number of determinations

$\bar{x}$: arithmetic average of single determined values

x_i: single determined value

3.6 Chromatographic methods

3.6.1 High-resolution gas chromatography: HRGC-FID and HRGC-O

High-resolution gas chromatography (HRGC) of aroma extracts with detection by a flame ionization detector (FID) and olfactometry was performed using a gas chromatograph Trace GC (Thermo Scientific, Dreieich). At the end of the capillary the effluent was split 1:1 (by volume) into an FID and a sniffing-port using two deactivated fused silica capillaries (50 cm × 0.20 mm) and a Y-shaped glass splitter.

HRGC conditions

gas chromatograph	Thermo Scientific HRGC Trace GC, Dreieich
carrier gas	helium (preliminary pressure 70 kPa)
sample injection	on-column; 40 ˚C
injection volume	0.5–2 µL
splitter	glass splitter (Chrompack, Frankfurt); split in a 1:1 ratio to FID and to the sniffing-port, between FID resp. sniffing-port and splitter deactivated fused silica capillaries (50 cm × 0.20 mm; Chromatograhie Handel Müller, Fridolfing) were used
detector	FID
FID gases	hydrogen (70 kPa)
	synthetic air (100 kPa)
make-up gas	nitrogen (100 kPa)
FID temperature	250 ˚C
sniffing-port temperature	250 ˚C
recorder	SERVOGOR 124 (Kipp & Zonen, Schwerte)
sensitivity	100 mV
paper transport	60 cm/h

Used fused silica capillary columns

DB-5	DB-5, WCOT Fused Silica, 30 m × 0.32 mm i.d., 0.25 µm film thickness (J&W Scientific, Agilent Technologies, Waldbronn)
DB-FFAP	DB-FFAP, WCOT Fused Silica, 30 m × 0.32 mm i.d., 0.25 µm film thickness (J&W Scientific, Agilent Technologies, Waldbronn)

DB-1701 DB-1701, WCOT Fused Silica, 30 m × 0.32 mm i.d., 0.25 µm film thickness (J&W Scientific, Agilent Technologies, Waldbronn)

Temperature programs

for DB-5:

40 °C, 2 min $\xrightarrow{6\ ^\circ C\ /\ min}$ 250 °C, 5 min

for DB-FFAP:

40 °C, 2 min $\xrightarrow{6\ ^\circ C\ /\ min}$ 230 °C, 5 min

for DB-1701:

40 °C, 2 min $\xrightarrow{6\ ^\circ C\ /\ min}$ 240 °C, 5 min

The high-resolution gas chromatography-olfactometry (HRGC-O) was used for the aroma extract dilution analysis, for the quantitation of isotopically labeled standard solutions, and for the determination of the odor quality of aroma-active compounds and their retention indices.

3.6.2 Determination of retention indices (RI)

The retention index (*Kováts, 1958*) of a compound was determined by co-chromatography with a solution of *n*-alkanes mixture (C_6–C_{18} for DB-5, C_6–C_{26} for DB-FFAP). According to *Van den Dool and Kratz* (*1963*) the retention index (RI) was calculated by linear interpolation:

$$RI = 100 \cdot \left[N + \frac{RT_v - RT_n}{RT_{n+1} - RT_n} \right]$$

RI retention index

RT_v retention time of the compound

RT_n retention time of the alkane with n carbon atoms

RT_{n+1} retention time of the alkane with n+1 carbon atoms

N number of carbon atoms of the alkane n

3.7 High-resolution gas chromatography-mass spectrometry (HRGC-MS)

High-resolution gas chromatographic-mass spectrometric examinations were performed with the following systems.

3.7.1 HRGC-MAT 95S

For the identification of aroma-active compounds, mass spectra were generated in the electron impact mode (EI-mode) using a gas chromatograph type 5890 series II (Hewlett Packard, Waldbronn) coupled with a sector field mass spectrometer type MAT 95 S (Finnigan, Bremen). The sample was applied by the cold-on-column injection technique. For HRGC the following fused silica capillaries were used: DB-5 (J&W Scientific, Agilent Technologies, Waldbronn) and DB-FFAP (J&W Scientific, Agilent Technologies, Waldbronn). The used temperature programs and capillary column parameters corresponded to those listed under **3.6.1**. Mass spectra in the electron impact mode (EI-mode) were generated at 70 eV.

3.7.2 HRGC-Agilent 5973

Mass spectra of the synthesized reference odorants and the intermediates were obtained using an Agilent 6890 gas chromatograph connected to a 5973 mass selective detector equipped with a TC-1 capillary column (60 m × 0.25 mm i.d., 0.25 µm film thickness, GL Sciences Co., Tokyo). Samples were applied in the split mode (1:50) at 250 ˚C, and helium at a flow rate of 1.8 mL/min served as the carrier gas. The temperature program began from 40 ˚C, held for 2 min, raised at a rate of 3 ˚C/min to 280 ˚C, and held for 5 min. Mass spectra were generated in the EI-mode at 70 eV.

3.7.3 Two dimensional HRGC-ITD 800

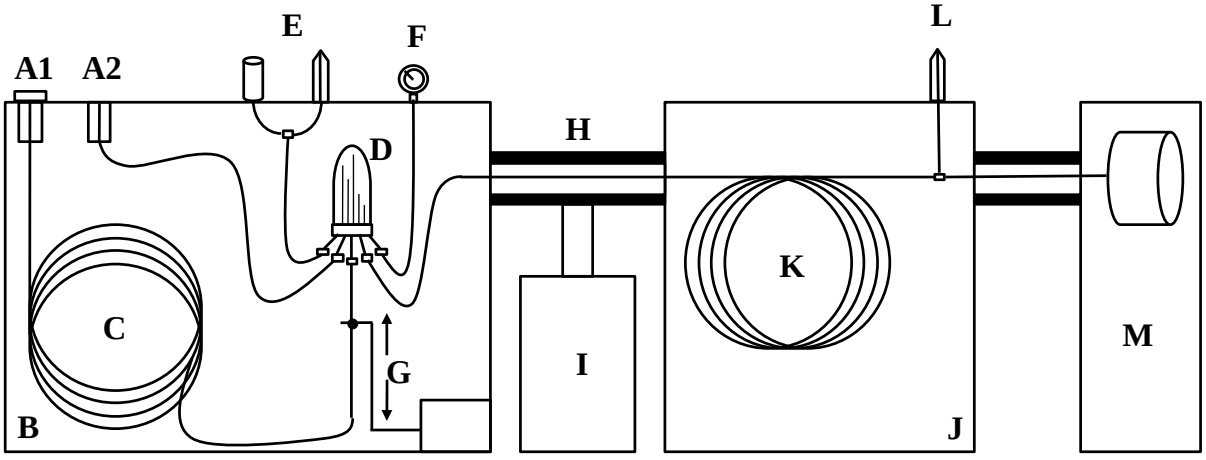
Where co-eluting compounds did not allow to obtain unequivocal mass spectra of the target compound, a two-dimensional gas chromatographic heart-cut technique coupled with mass spectrometry was exploited. A schematic diagram is shown in **Figure 47**.

A gas chromatograph type Mega 2 series (GC1; Fisons Instruments, Egelsbach) was coupled with a gas chromatograph type 5160 Mega series (GC2; Carlo Erba Instruments, Hofheim)

over a moving column stream switching system (MCSS-system; Thermo Finnigan, Egelsbach) (*Weber et al., 1995*).

The samples were injected by means of cold-on-column onto the column in the first dimension (DB-FFAP with the same parameters for HRGC-O described in **3.6.1**). The elution time of the investigated odorant was determined in the off-line modus of the MCSS-system by HRGC-O and FID of reference substances; because in this modus the effluent was directed to an FID and a sniffing-port via a deactivated fused silica transfer line. At the elution time of the examined odorants, the effluent was transferred via a deactivated fused silica transfer line into a cold trap section of the transfer line (–100 °C) using the MCSS-system in the transfer-modus. After the cooling was turned off, the trap was heated to 250 °C to flush the trapped compound onto the second column and the trapped compounds were further separated using a DB-5 column (with the same parameters for HRGC-O described in **3.6.1**) installed in the second gas chromatograph oven.

The end of the second column was simultaneously connected to an ion trap mass spectrometer ITD 800 (Finnigan, Bremen, Germany) and a second sniffing port via a Y-shaped glass splitter (Chrompack, Frankfurt). The effluent was monitored using the second sniffing-port, and its mass spectrum was simultaneously recorded by the ion trap mass spectrometer. Mass spectra in the electron impact mode (EI) were generated at 70 eV.



A1: cold-on-column injector

A2: injector for the maintenance of the carrier gas pressure of the glass cap tube of the MCSS-system

B: first gas chromatograph

C: first capillary column (DB-FFAP)

D: glass cap tube of the MCSS-system
E: detector: flame ionization detector (FID) and sniffing-port
F: manometer of the glass cap tube
G: control unit of the MCSS-system for the vertical moving of the capillary column (C)
H: thermostated transfer line
I: control unit for the cooling and heating of the cold trap
J: second gas chromatograph
K: second capillary column (DB-5)
L: sniffing port for the second GC
M: mass spectrometer (ITD 800)

Figure 47: Schematic diagram of a two-dimensional HRGC-MS-system.

3.7.4 Two dimensional HRGC-ITD Saturn 2200

For quantitation by stable isotope dilution assay, a two dimensional HRGC system connected to an ion trap mass spectrometer (Saturn 2200, Varian, Darmstadt) was used. The operational sequence basically agreed with the description under **3.7.3**.

The system consisted of a gas chromatograph (GC 1) type Trace GC Ultra (Thermo Scientific, Dreieich) equipped with a DB-FFAP column, a CP-3800 gas chromatograph (GC 2; Varian, Darmstadt, Germany) equipped with a DB-1701 column (both columns 30 m × 0.32 mm, 0.25 µm film thickness; J&W Scientific, Agilent, Waldbronn, Germany), and a COMBI PAL autosampler (CTC Analytics, Zwingen, Switzerland). The first GC housed a moving column stream switching system (MCSS-system; Thermo Finnigan, Egelsbach) leading the effluent of the first column either to an FID and a sniffing port or via a deactivated fused silica transfer line to the column in the second oven. The end of the second column was connected to the MS Saturn 2200 (Varian, Darmstadt, Germany). To obtain mass spectra of the odorant and the internal standard, at its elution time from the first column, which was determined by GC-O in a preliminary run, the MCSS was switched to the transfer line. While collecting the effluent of the first column, a trap section of the transfer line located in the second oven was cooled. This was achieved by a stream of cold nitrogen gas, which before was passed through liquid nitrogen. After collecting was finished, cooling was stopped and the second oven was started.

The recording of the spectra in the chemical ionization (CI) mode was carried out with chemical ionization energy of 115 eV with methanol as reagent gas.

3.8 Nuclear magnetic resonance spectroscopy (NMR)

^{1}H and ^{13}C nuclear magnetic resonance spectra were recorded by a JEOL JNM-LA400 spectrometer (JEOL Ltd., Tokyo) at 400 or 100 MHz, respectively. The samples were dissolved in deuterochloroform ($CDCl_3$). The chemical shift was recorded in parts per million (ppm) using tetramethylsilane (TMS) as the internal standard (δ = 0.00 ppm). Coupling constants *J* are denoted in Hz.

3.9 Determination of odor thresholds in oil

Reference compounds were checked for odor-active impurities by GC-O prior to use, and, if necessary, purified by distillation. The odorant was dissolved in sunflower oil in a 100-fold higher concentration than the estimated orthonasal recognition threshold. The stock solution was vigorously shaken, and was then diluted stepwise 1:5 (w/w) with sunflower oil. Each test sample was orthonasally evaluated in a triangular test with two blank samples (sunflower oil only). The samples were presented with increasing concentrations of the odorant. The sensory experiments were performed at 23 °C in a sensory room with single booths. The sensory panel consisted of 12–19 assessors in the age of 26–60 years, all of them being employees of the Technical Research Institute, R&D Center of T. Hasegawa Co., Ltd., Kawasaki, Japan. They were trained to recognize over 100 aroma chemicals. Odor thresholds were calculated according to the method reported in (*Czerny et al., 2008*).

4 Summary

From a long time ago sesame has been widely consumed as an oil source, especially in Asian countries, due to its good reputation for health and also for the pleasant aroma of ground roasted seeds. In the Western world, the roasted seeds are commonly used as a topping for bakery goods, while in Asia not only the roasted seeds but also the oil are popularly used in various kinds of cuisines. In Japan the pleasant aroma during grinding of the roasted sesame seeds is favored, and, therefore, the seeds are directly ground in a small mortar with a small pestle just before consumption to ensure the fresh aroma.

Many researchers have already reported studies on the volatile constituents of sesame. Among those, the most sophisticated analysis to discover the sensory contribution of individual aroma compounds in roasted sesame was performed by *Schieberle* in *1996*. The application of an aroma extract dilution analysis (AEDA) revealed 41 aroma-active volatiles. Ten major aroma-active compounds were subsequently quantitated by stable isotope dilution assays (SIDA). According to their high odor activity values (OAV: ratio of concentration to odor threshold), 2-acetyl-1-pyrroline, 2-furfurylthiol, 2-phenylethylthiol, and 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone were identified as key aroma compounds of roasted sesame seeds. However, structures of eight compounds with sulfurous or catty notes remained unknown.
Therefore, the aim of this study was to reinvestigate the odor-active compounds in ground roasted sesame seeds with special emphasis on the currently unknown sulfurous or catty-smelling odorants, expecting to obtain further insights on the sulfury aroma in freshly ground roasted sesame seeds.

Screening for aroma-active compounds in an aroma distillate of freshly ground roasted white sesame seeds by AEDA revealed 32 odorants in the FD-factor range of 2 to 2048, twenty-nine of which could be identified. The highest FD-factors were found for 2-furfurylthiol and 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone. In addition, nine odor-active thiols with sulfurous, meaty, or catty, black currant-like odors were identified for the first time in roasted sesame seeds. Among them, to the best of my knowledge, 2-methyl-1-propene-1-thiol, (*E*)- and (*Z*)-2-methyl-1-butene-1-thiol, (*E*)- and (*Z*)-3-methyl-1-butene-1-thiol, and 4-mercapto-3-hexanone were previously unknown ever as food constituents. Four of them, (*E*)- and (*Z*)-2-methyl-1-butene-1-thiol and (*E*)- and (*Z*)-3-methyl-1-butene-1-thiol were completely novel compounds. Their structures were confirmed by comparing their mass spectra, retention

indices, and sensory properties with those of synthesized reference compounds, some of which, especially the synthetic route of 1-alkene-1-thiols, were newly developed. The relatively unstable 1-alkene-1-thiols represent a new class of food odorants, and their instability might explain that the aroma of roasted sesame loses its freshness soon after grinding. Therefore, the 1-alkene-1-thiols are suggested as the key contributors to the characteristic sulfurous aroma of freshly ground roasted sesame seeds.

In order to unequivocally evaluate the aroma impact of these novel odorants, their quantitation using SIDA, followed by calculation of OAV were performed. For that purpose, their deuterium-labeled analogues were synthesized by using the synthetic pathway for the non-labeled reference odorants. Especially, the development of the SIDA for the newly identified 1-alkene-1-thiols and 4-mercapto-3-hexanone with their internal standards, which were synthesized for the first time, were accomplished. The precise quantitation by SIDA using the synthesized labeled odorants was performed, and revealed concentrations of the aroma-active thiols in ground roasted sesame in the range of 11 ng/kg (3-mercapto-3-methylbutyl formate) up to 1.2 mg/kg (2-methyl-1-butene-1-thiol, sum of isomers).

For assessments of the aroma contribution of the quantitated aroma-active thiols, the odor activity value concept was applied. To complete this task, odor thresholds in oil of the thiols were determined because roasted sesame seeds contain about 50% of oil. Considering the difficulty to separate (*E*)- and (*Z*)-isomers of the unstable 2- and 3-methyl-1-butene-1-thiol, the odor thresholds of their (*E*)/(*Z*) mixtures with ratios of their synthesized reference compounds (approximately 1 to 1) were determined. Concentrations of seven aroma-active thiols other than 3-mercapto-3-methylbutyl formate and 2-methyl-3-thiophenethiol clearly exceeded their odor threshold values, with 3-methyl-1-butene-1-thiol (2400) and 2-methyl-1-butene-1-thiol (960) showing the highest OAVs.

Thus, the results indicate that 2-methyl-1-butene-1-thiol and 3-methyl-1-butene-1-thiol should be the compounds mainly responsible for the characteristic odor note of freshly ground roasted white sesame seeds. Their OAVs even exceeded the OAVs of the most odor-active aroma compound 2-acetyl-1-pyrroline (300) in the previously reported study on roasted white sesame flavor. Whether the (*E*)- or the (*Z*)-isomers contribute more to the sulfurous note and how closely the reconstitute with these novel thiols and the previous data resembles the original roasted sesame sample, will be interesting subjects for further researches.

5 Zusammenfassung

Aufgrund seines Gesundheitswerts und des angenehmen Aromas der gerösteten und gemahlenen Samen wurde Sesam seit alters her, vor allem in asiatischen Ländern, als Fettlieferant genutzt. In der westlichen Welt werden die gerösteten Samen verbreitet zur Garnierung von Backwaren eingesetzt, während in Asien nicht nur die gerösteten Samen als solche, sondern auch das daraus gewonnene Öl gerne zum Aromatisieren verschiedener Gerichte verwendet wird. In Japan schätzt man besonders das angenehme Aroma, das beim Mahlen der gerösteten Sesamsamen freigesetzt wird. Deshalb werden die Samen mit Hilfe eines kleinen Mörsers erst direkt vor dem Verzehr gemahlen, um ein besonders frisches Aroma sicherzustellen.

Viele Arbeiten sind bereits über die flüchtige Fraktion von Sesam publiziert worden. Die gründlichste Arbeit über den sensorischen Beitrag einzelner Aromastoffe zum Sesamaroma wurde 1996 von Schieberle verfasst. Die Anwendung einer Aromaextraktverdünnungsanalyse ergab 41 aromaaktive Verbindungen. Die zehn potentesten Aromastoffe wurden anschließend über Stabilisotopenverdünnungsanalysen quantifiziert. Auf der Basis hoher Aromawerte (Quotient aus Konzentration und Geruchsschwellenwert) wurden 2-Acetyl-1-pyrrolin, 2-Furfurylthiol, 2-Phenylethanthiol und 4-Hydroxy-2,5-dimethyl-3(2*H*)-furanon als Schlüsselaromastoffe gerösteter Sesamsamen identifiziert. Die Identität von acht Substanzen mit schwefliger bzw. katzenurinartiger Geruchsqualität blieb jedoch ungeklärt.

Das Ziel dieser Arbeit war es entsprechend, ein Screening des Aromadestillats aus frisch gemahlenen, gerösteten weißen Sesamsamen auf geruchsaktive Verbindungen, durchzuführen, um einen genaueren Einblick in ihr Aroma zu erhalten. Besondere Berücksichtigung sollte dabei den bisher unbekannten, schweflig bzw. katzenurinartig riechenden Aromastoffen zukommen.

Das Screening nach aromaaktiven Verbindungen in einem Destillat aus frisch gemahlenen, gerösteten weißen Sesamsamen durch eine Aromaextraktverdünnungsanalyse ergab 32 Substanzen im FD-Faktorbereich zwischen 2 und 2048. Davon konnten 29 identifiziert werden. Die höchsten FD-Faktoren wurden für 2-Furfurylthiol und 4-Hydroxy-2,5-dimethyl-3(2*H*)-furanon gefunden. Darüber hinaus wurden neun aromaaktive Thiole mit schwefligem, fleischigem, oder katzenurinartigem Geruch, bzw. einem Geruch nach schwarzen

Johannisbeeren, erstmals in gerösteten Sesamsamen identifiziert. Sechs davon, nämlich 2-Methyl-1-propen-1-thiol, (*E*)- und (*Z*)-2-Methyl-1-buten-1-thiol, (*E*)- und (*Z*)-3-Methyl-1-buten-1-thiol, sowie 4-Mercapto-3-hexanon waren, nach bestem Wissen, bisher nicht als Lebensmittelinhaltsstoffe bekannt. Bei vier Verbindungen, nämlich (*E*)- und (*Z*)-2-Methyl-1-buten-1-thiol, sowie (*E*)- und (*Z*)-3-Methyl-1-buten-1-thiol handelte es sich um komplett neue Verbindungen. Ihre Strukturen wurden über einen Vergleich ihrer Massenspektren, ihrer Retentionsindices und ihrer sensorischen Eigenschaften mit den entsprechenden Daten von synthetisch dargestellten Referenzsubstanzen bestimmt. Einige Synthesestrategien, insbesondere die Synthesewege zu den 1-Alken-1-thiolen, wurden dazu neu entwickelt. Diese vergleichsweise instabilen 1-Alken-1-thiole stellen eine neue Klasse an Lebensmittelaromastoffen dar. Ihre Instabilität könnte erklären, warum das Aroma von geröstetem Sesam seine Frische nach dem Mahlen relativ schnell verliert.

Um den Einfluss dieser neuartigen Aromastoffe auf das Gesamtaroma eindeutig bewerten zu können, wurden sie mit Hilfe von Stabilisotopenverdünnungsanalysen quantifiziert und anschließend wurden ihre Aromawerte bestimmt. Dazu wurden zunächst deuteriummarkierte Analoga synthetisiert, wobei derselbe Syntheseansatz verwendet wurde, der auch bei den unmarkierten Verbindungen zum Einsatz kam. Insbesondere für die neu entdeckten 1-Alken-1-thiole und das 4-Mercapto-3-hexanon wurden erstmals markierte, interne Standards synthetisiert und Stabilisotopenverdünnungsanalysen entwickelt. Die exakte Quantifizierung über Stabilisotopenverdünnungsanalysen unter Verwendung der markierten Standards ergab Konzentrationen im Bereich von 11 ng/kg (3-Mercapto-3-methylbutylformiat) bis 1,2 mg/kg (2-Methyl-1-buten-1-thiol; Summe der Isomere).

Die Bewertung des Aromabeitrags der quantifizierten aromaaktiven Thiole erfolgte durch Anwendung des Aromawertkonzepts. Nachdem die gerösteten Sesamsamen rund 50 % Lipide enthalten, wurden dazu die Geruchsschwellenwerte in geruchsarmem Speiseöl bestimmt. Da die (*E*)- und (*Z*)-Isomere der instabilen 2- und 3-Methyl-1-buten-1-thiole präparativ nicht getrennt werden konnten, wurden die Geruchsschwellen mit den synthetisierten, etwa 1:1-Mischungen aus (*E*)- und (*Z*)-Isomer bestimmt. Mit Ausnahme von 3-Mercapto-3-methylbutylformiat und 2-Methyl-3-thiophenthiol lagen die Konzentrationen der sieben aromaaktiven Thiole deutlich über ihren Geruchsschwellenwerten, wobei 3-Methyl-1-buten-1-thiol mit 2400 und 2-Methyl-1-buten-1-thiol mit 960 die höchsten Aromawerte aufwiesen.

Entsprechend deuten die Ergebnisse darauf hin, dass 2-Methyl-1-buten-1-thiol und 3-Methyl-1-buten-1-thiol wesentlich für die charakteristische Aromanote von frisch gemahlenen, gerösteten Sesamsamen verantwortlich sind. Ihre Aromawerte lagen sogar höher als die von 2-Acetyl-1-pyrrolin, dem Aromastoff mit dem höchsten Aromawert (300) in der ursprünglichen Arbeit zum Aroma von geröstetem weißen Sesam. Ob jeweils die (*E*)- oder (*Z*)-Isomere mehr zur schwefligen Aromanote beitragen und wie ähnlich ein Aromarekonstitutionsmodell auf der Basis der hier für diese neuen Thiole erhaltenen Daten und der bereits bekannten Daten einer Originalprobe gerösteten Sesams sind, wird Gegenstand weiterer, interessanter Forschungsarbeiten sein.

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7 Appendix

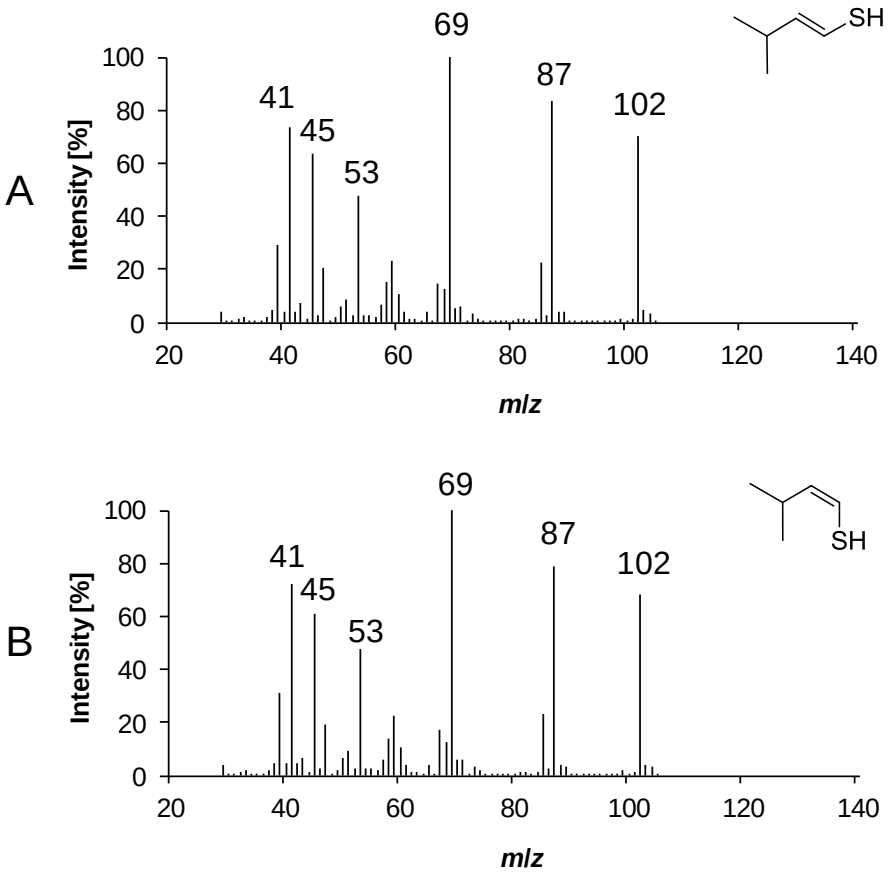

Figure 48: Mass spectra (MS-EI) of (*E*)-3-methyl-1-butene-1-thiol (A) and (*Z*)-3-methyl-1-butene-1-thiol (B).

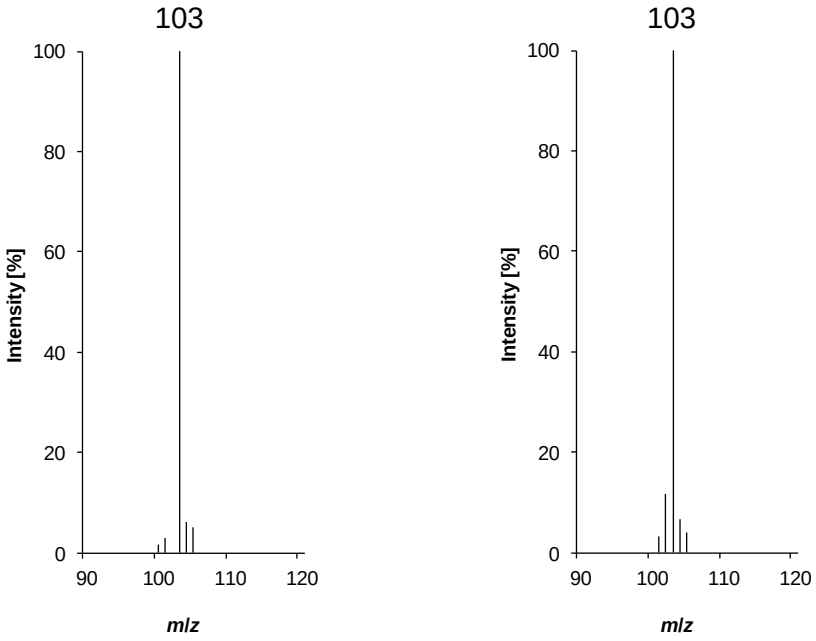

Figure 49: Mass spectra (MS-CI; methanol) of (*E*)-3-methyl-1-butene-1-thiol (left) and (*Z*)-3-methyl-1-butene-1-thiol (right).

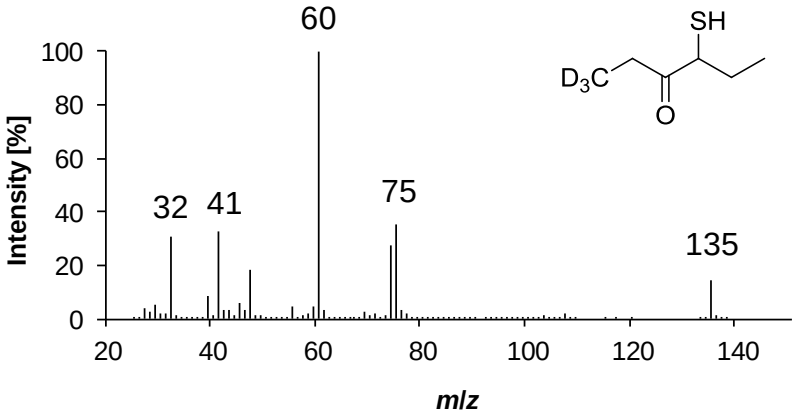

Figure 50: Mass spectra (MS-EI) of [$^2H_3$]-4-mercapto-3-hexanone (**d-15**).

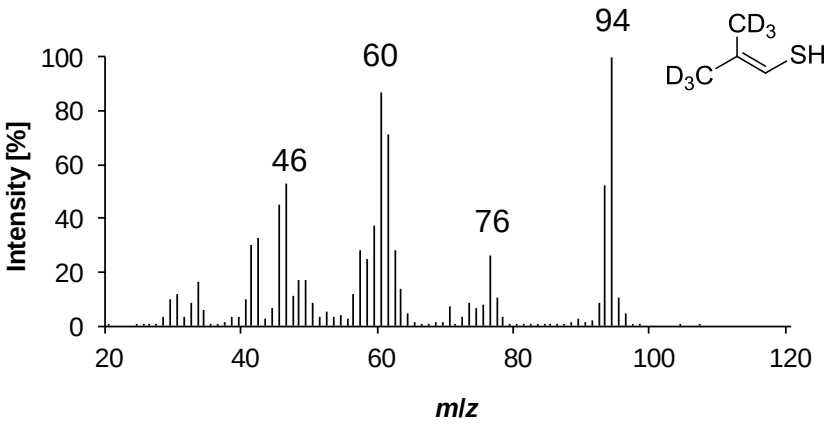

Figure 51: Mass spectra (MS-EI) of [$^2H_6$]-2-methyl-1-propen-1-thiol (**d-1**).

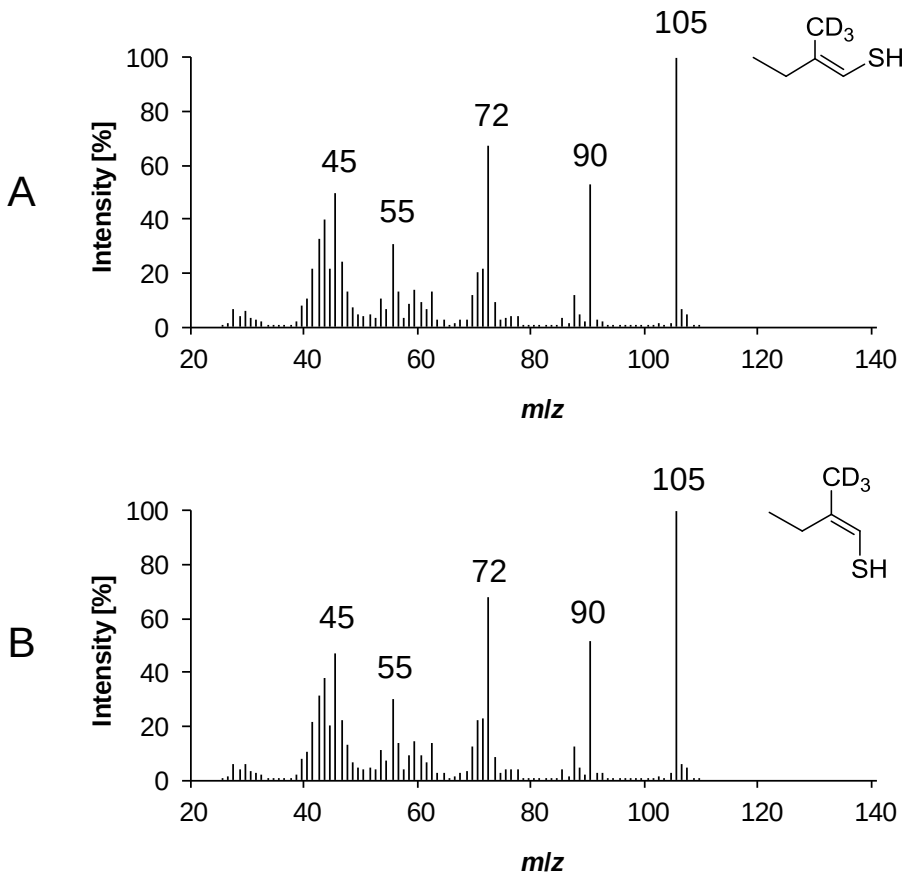

Figure 52: Mass spectra (MS-EI) of [$^2H_3$]-(*E*)-2-methyl-1-butene-1-thiol (A; **d-5**) and [$^2H_3$]-(*Z*)-2-methyl-1-butene-1-thiol (B; **d-4**).