

TECHNIQUES FOR ANALYZING FOOD AROMA

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10

Gas Chromatography–Olfactometry in Food Aroma Analysis

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I. INTRODUCTION

Progress in instrumental analysis has led to long lists of volatiles (1). Unfortunately, the sensory relevance of these volatile compounds has not been as extensively evaluated, although the use of the human nose as a sensitive detector in gas chromatography (GC) was proposed by Fuller and coworkers as early as 1964 (2). In the meantime, much has been published on food aroma, often without identifying the impact compounds. Therefore, one of the major problems in aroma research is to select those compounds that significantly contribute to the aroma of a food.

Flavor is usually divided into the subsets of taste and smell, which are perceived in the mouth and the nose, respectively. However, “flavor” is frequently used in publications exclusively dealing with volatiles. The terms “aroma” and “odor” are not well defined and are often used as synonyms.

Odor is best reserved for the smell of food before it is put into the mouth (nasal perception) and aroma for the retronasal smell of food in the mouth.

In general, the aroma of a food consists of many volatile compounds, only a few of which are sensorially relevant. A first essential step in aroma analysis is the distinction of the more potent odorants from volatiles having low or no aroma activity. In 1963, Rothe and Thomas calculated the ratio of the concentration of an odorant to its odor threshold and denoted it "aroma value" (3). This approach was the first attempt to estimate the sensory contribution of single odorants to the overall aroma of a food. Since that time, similar methods have been developed: odor unit (4) based on nasal odor thresholds, flavor unit (5) using retronasal odor thresholds, and odor activity value (OAV) (6). However, this concept requires identification and quantification of a great number of volatile compounds and determination of their threshold values, which is time-consuming. Furthermore, there is no guarantee that all of the important odorants were considered, unless a screening step for the most important aroma compounds was used.

GC in combination with olfactometric techniques (GC-O) is a valuable method for the selection of aroma-active components from a complex mixture (7). Experiments based on human subjects sniffing GC effluents are described as GC-O. This technique helps to detect potent odorants, without knowing their chemical structures, which might be overlooked by the OAV concept (ratio of concentration to threshold) if the sensory aspect is not considered from the very beginning of the analysis. Experience shows that many key aroma compounds occur at very low concentrations; their sensory relevance is due to low odor thresholds. Thus, the peak profile obtained by GC does not necessarily reflect the aroma profile of the food.

The purpose of this contribution is to discuss recent developments in food aroma analysis from the chemist's point of view. It will particularly focus on qualitative aroma composition obtained by GC-O. Potential and limitations of the GC-O approach will be discussed and comments made to allow a more realistic interpretation of data. This overview is addressed to flavor scientists from both industry and academia.

II. GAS CHROMATOGRAPHY-OLFACTOMETRY TECHNIQUES

In general, it is very difficult to judge the sensory relevance of volatiles from a single GC-O run. Several techniques have been developed to objectify GC-O data and to estimate the sensory contribution of single aroma components. This issue seems to be of great concern, as a considerable part of the 7th

Weurman Symposium was dedicated to this topic (8). Dilution techniques and time-intensity measurements are the two main GC-O methods.

A. Time-Intensity Measurements

McDaniel et al. (9) have developed the technique Osme, measuring the perceived odor intensity of a compound in the GC effluent. The subject rates the aroma intensity by using a computerized 16-point scale time-intensity device and indicates the corresponding aroma characteristics. This technique provides an FID-style aromagram called an osmogram (Fig. 1). Ideally, it requires only one injection when working with well-trained assessors. Human subjects were found to be reliable "instruments" for reporting odor intensity changes in response to changes in odorant concentration (10). Similar methods based on olfactive intensity measurements have recently been reported (11).

B. Dilution Techniques

Two techniques based on dilution have been developed: CharmAnalysis by Acree and coworkers (6,12,13) and aroma extract dilution analysis (AEDA) by Grosch and his group (7,14,15). Both evaluate the odor activity of individual compounds by sniffing the GC effluent of a series of dilutions of the origi-

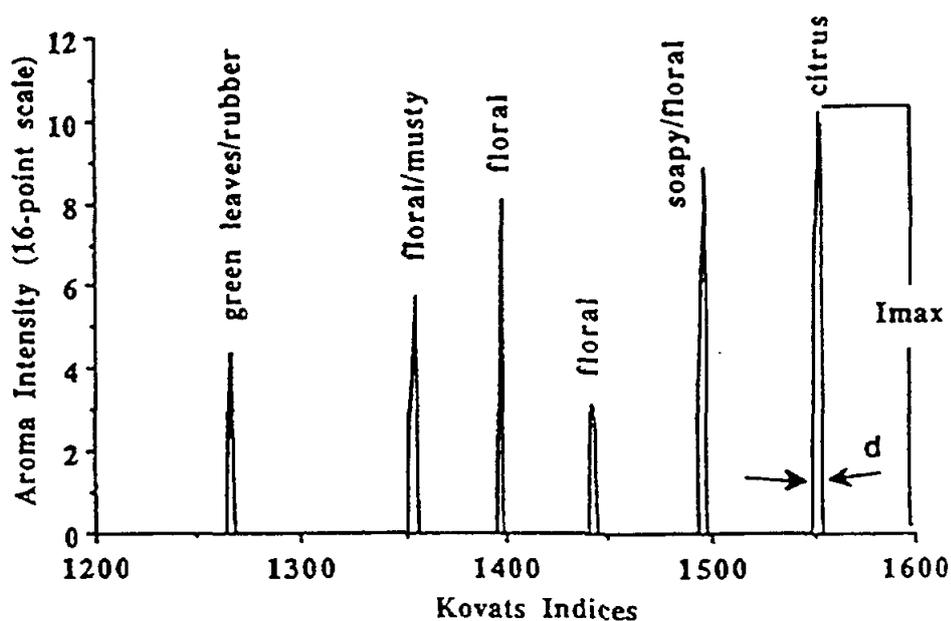


FIGURE 1 Osmogram with odor duration time (d) and maximum odor intensity (I_{\max}). (From Ref. 10.)

nal aroma extract. Both methods are based on the odor-detection threshold. The dilution value obtained for each compound is proportional to its OAV in air, i.e., its concentration. Several injections are required to reach a dilution of the aroma extract in which odorous regions are no longer detected.

In CharmAnalysis, the dilutions are presented in randomized order to avoid bias introduced by knowledge of the samples. The assessor detects the beginning and the end of each aroma perception (duration of the smell) and notes the sensory attributes (Fig. 2). The dilution value is measured over the entire time of the eluting peak. From these data, the computerized system constructs chromatographic peaks where the peak areas are proportional to the amount of the odorant in the extract. The Charm value is calculated according to the formula $c = d^{n-1}$, where n is the number of coincident responses and d the dilution value. The result of the CharmAnalysis is displayed in a Charm chromatogram. As shown in Figure 3, the sensory relevant volatile glucose-proline reaction products were detected by Charm analysis and then, based on these results, identified as detailed in Figure 4. The major peak found in the sample, 5-acetyl-2,3-1H-pyrrolizine, was almost odorless (16).

In AEDA, the assessor indicates whether or not an aroma can be perceived and notes the sensory descriptor. The result is expressed as the flavor dilution (FD) factor that corresponds to the maximum dilution value detected, i.e., the peak height obtained in CharmAnalysis. The FD factor is a relative measure and represents the odor threshold of the compound at a given concentration. The data are presented in an FD chromatogram (Fig. 5) indicating the retention indices (x-axis) and FD factors in a logarithmic scale (y-axis).

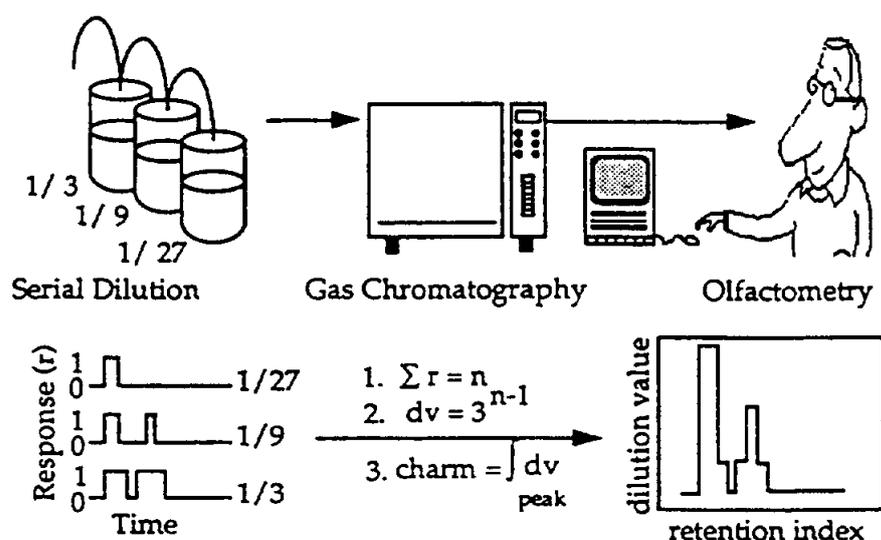


FIGURE 2 Schematic procedure for gas chromatography-olfactometry using CharmAnalysis. (From Ref. 12.)

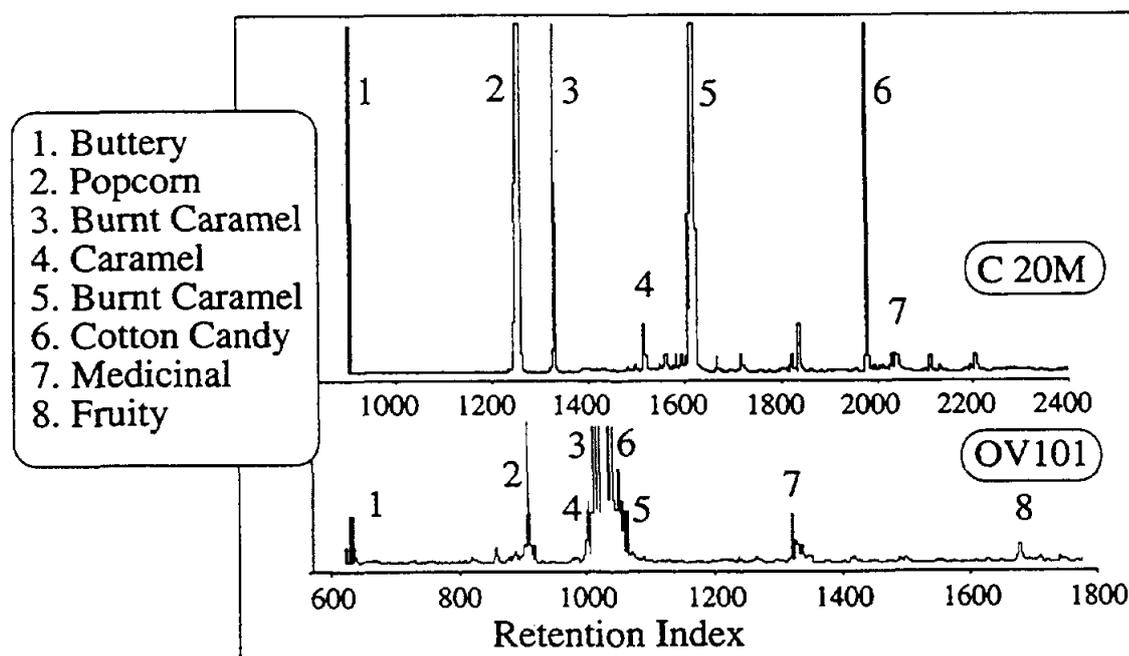


FIGURE 3 Charm chromatograms of the volatile 200°C glucose-proline reaction products on OV 101 and Carbowax 20M columns. (From Ref. 16.)

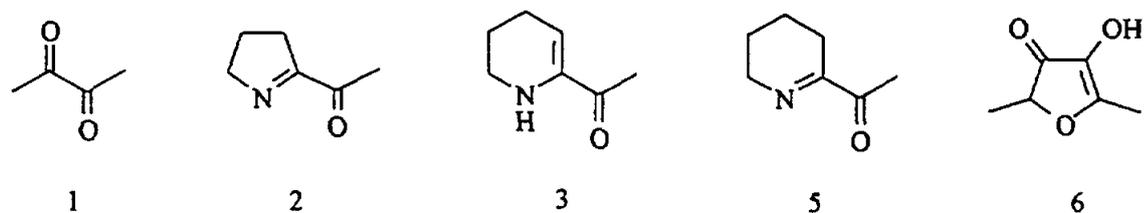


FIGURE 4 Major odor-potent compounds in the glucose-proline reaction detected by CharmAnalysis: diacetyl (no. 1), 2-acetyl-1-pyrroline (no. 2), 2-acetyl-1,4,5,6-tetrahydropyridine (no. 3), 2-acetyl-3,4,5,6-tetrahydropyridine (no. 5), and furaneol (no. 6). Compound numbers refer to Figure 3.

AEDA has been proposed as a screening method for potent odorants as the results are not corrected for losses during isolation (7).

The results obtained for freshly roasted *Arabica* coffee are illustrated in Figure 5 (17). From more than 1000 volatiles detected in the original aroma extract by FID, only about 60 odor-active regions were selected by GC-O. AEDA revealed 38 odorants with FD factors of 16 or higher. Odorants 5, 14, 19, 26, 30, and 32 have been newly identified in coffee aroma. Their identification stemmed from the high FD factors. They would most likely have been overlooked without using GC-O as a screening method for odor-active compounds. Odorants with FD factors of 128 or higher are shown in Figure 6.

C. Static Headspace GC-O

The GC-O techniques described above mainly deal with aroma extracts (liquids) isolated from the food. Recently, Guth and Grosch reported a new concept in aroma research using static headspace in combination with GC-O (18). The equipment is composed of a purge-and-trap system for introducing various volumes of gaseous samples without artefact formation, a suitable capillary column, and an effluent splitter to simultaneously perform GC-O and detection by

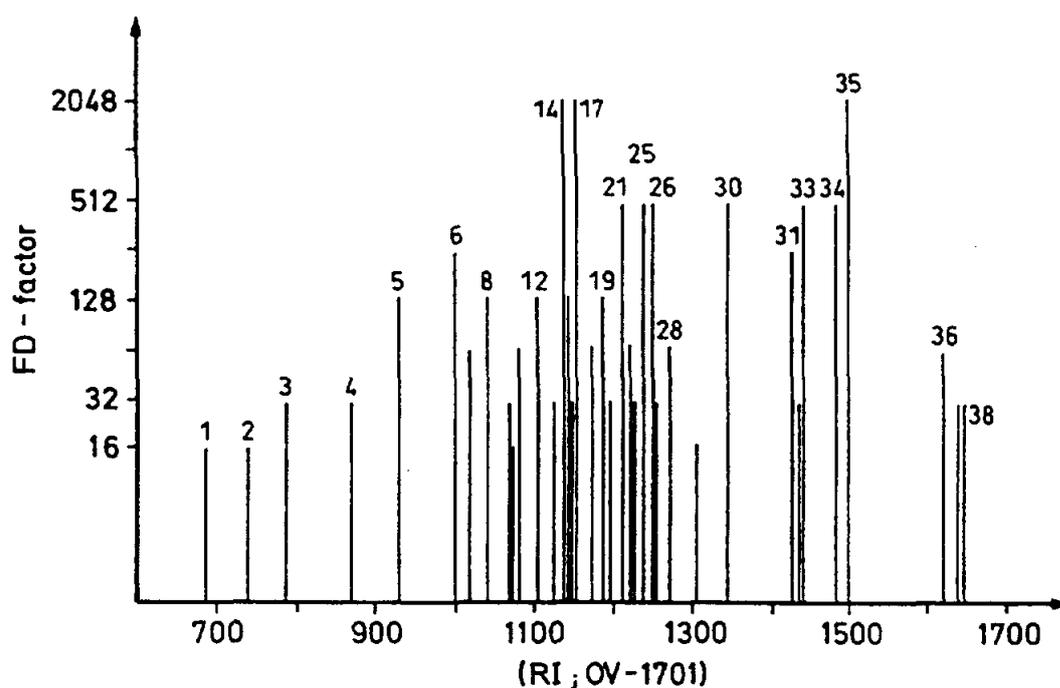


FIGURE 5 FD chromatogram of an aroma extract obtained from roast and ground *Arabica* coffee. (From Ref. 17.)

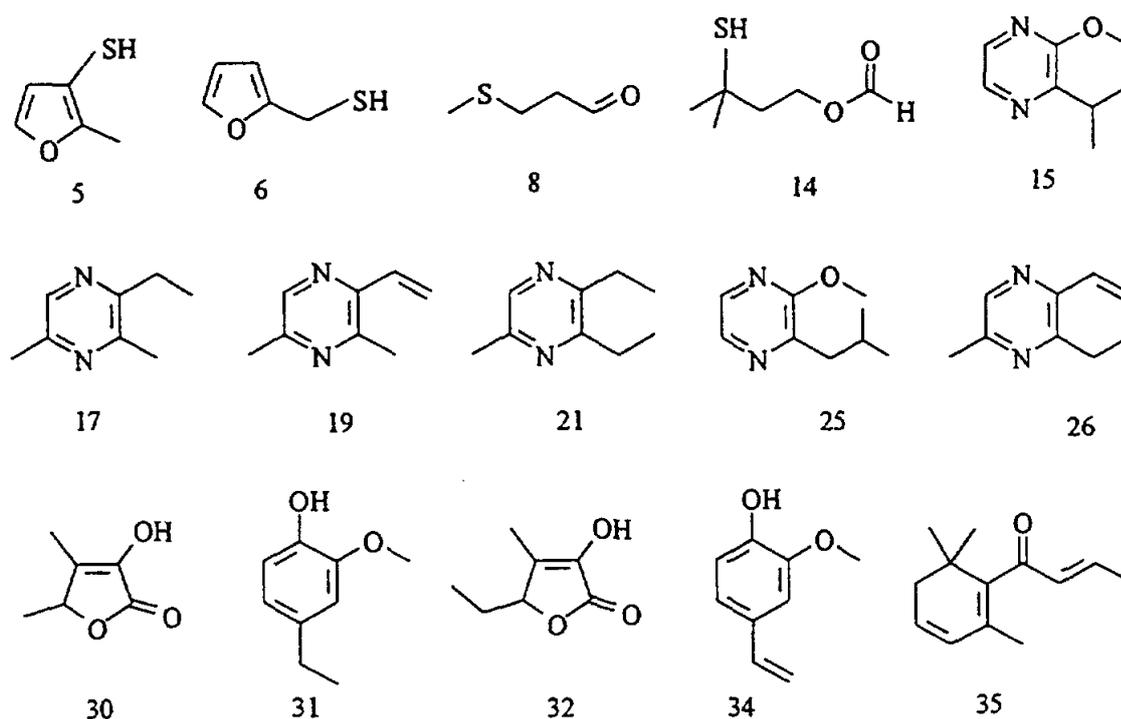


FIGURE 6 Chemical structures of some aroma impact compounds ($FD \geq 128$) found in an aroma extract of roast and ground *Arabica* coffee: 2-methyl-3-furanthiol (no. 5), 2-furfurylthiol (no. 6), methional (no. 8), 3-mercapto-3-methylbutyl formate (no. 14), 3-isopropyl-2-methoxypyrazine (no. 15), 2-ethyl-3,5-dimethylpyrazine (no. 17), 2-ethenyl-3,5-dimethylpyrazine (no. 19, W. Grosch, personal communication), 2,3-diethyl-5-methylpyrazine (no. 21), 3-isobutyl-2-methoxypyrazine (no. 25), 2-ethenyl-3-ethyl-5-methylpyrazine (no. 26, W. Grosch, personal communication), sotolon (no. 30), 4-ethylguaiacol (no. 31), abhexon (no. 32), 4-vinylguaiacol (no. 34), and (*E*)- β -damascenone (no. 35). The numbering corresponds to that in Figure 5.

FID or MS (Fig. 7). A defined volume of the headspace is injected into a pre-cooled trap to focus the volatiles. After flushing the air present in the gas volume, GC separation is started by raising the oven temperature. Dilution steps are made by injecting decreasing headspace volumes to evaluate the relative odor potencies. The problem of identification is solved by using the same analytical conditions (capillary, temperature program) as for AEDA, so that identification can be performed on the basis of odor qualities and RI values (18).

The sensory relevance of individual odorants can be estimated by injecting various headspace volumes. This is equivalent to AEDA of liquid samples. In contrast to AEDA where aroma compounds are separated from the food matrix,

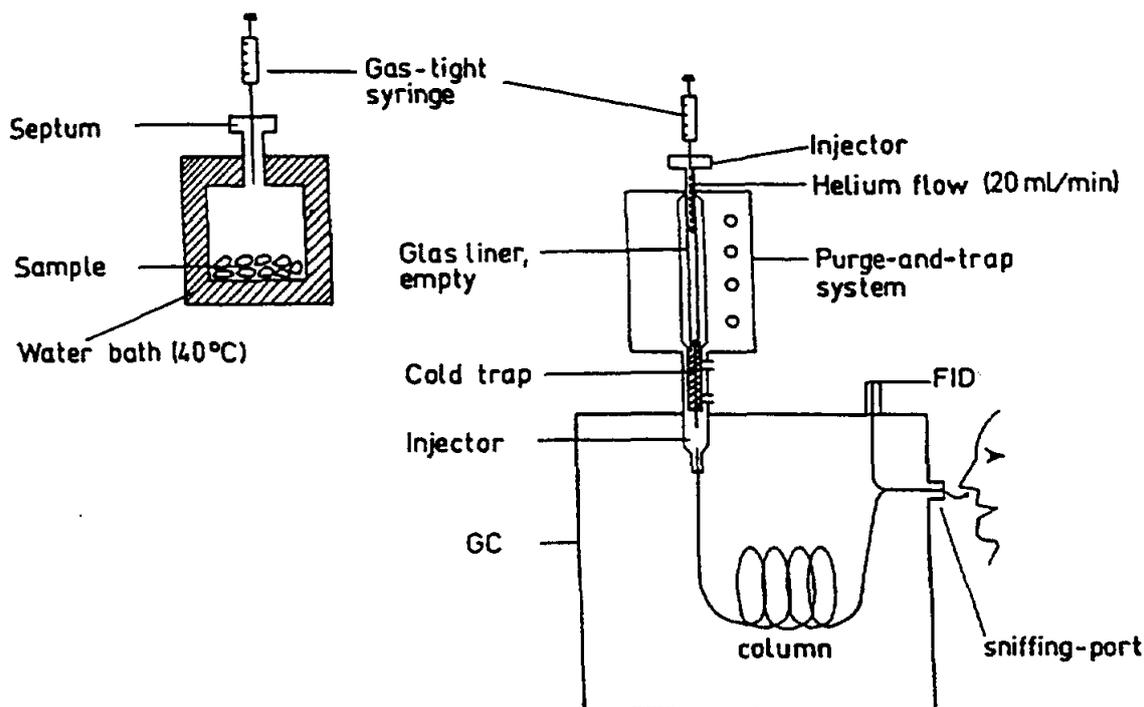


FIGURE 7 Schematic presentation of the static headspace GC-O technique. (Adapted from Guth and Grosch, Annual Report of the Deutsche Forschungsanstalt für Lebensmittelchemie, 1993, p. 27.)

static headspace GC-O provides data about the aroma above the food. This technique is suitable for studying the effect of the food matrix on the aroma profile. Therefore, AEDA and static headspace GC-O result in complementary data.

The analysis of coffee aroma is an excellent example of the potential of static headspace GC-O (19). Compared to AEDA (17), compounds 1–4 and 7 were additionally detected (Table 1). The sensory contribution of odorants was different from that obtained by AEDA. In general, very volatile compounds were underestimated by AEDA, most likely due to losses during sample preparation. 2,3-Pentanedione (no. 8), diacetyl (no. 5), 3-methyl-2-butene-1-thiol (no. 9), acetaldehyde (no. 1), and 3-methylbutanal (no. 6) are key odorants of *Arabica* coffee (Fig. 8). Methanethiol (no. 2) and 2-methylbutanal (no. 7) contribute more significantly to the aroma of *Robusta* coffee.

III. POTENTIAL OF THE GC-O APPROACH

A. Screening and Identification of Potent Odorants

Detection of odorous regions in a gas chromatogram is the first useful information that can be obtained from a single GC-O run. In the first GC-O run, all

TABLE 1 Aroma Impact Odorants of Roast and Ground *Arabica* and *Robusta* Coffee Detected by Static Headspace GC-O^a and Expressed as FD Factors^b

| No. | Compound | Aroma quality (on GC-O) | FD factor | |
|-----|---------------------------|---|----------------|----------------|
| | | | <i>Arabica</i> | <i>Robusta</i> |
| 1. | Acetaldehyde | Fruity, pungent | 25 | 25 |
| 2. | Methanethiol | Cabbagelike, sulfury | 5 | 12.5 |
| 3. | Propanal | Fruity | 5 | 5 |
| 4. | Methylpropanal | Fruity, malty | 5 | 5 |
| 5. | Diacetyl | Buttery | 62.5 | 125 |
| 6. | 3-Methylbutanal | Malty | 12.5 | 25 |
| 7. | 2-Methylbutanal | Malty | 5 | 12.5 |
| 8. | 2,3-Pentanedione | Buttery | 125 | 125 |
| 9. | 3-Methyl-2-butene-1-thiol | Sulfury, <i>Allium</i> -like, foxy ^c | 62.5 | 62.5 |

^aModified from Ref. 19.

^bThe headspace volumes of 25, 5, 2, 1, 0.4, and 0.2 ml correspond to the FD factors of 1, 5, 12.5, 25, 62.5, and 125, respectively.

^cAroma qualities depend on concentration.

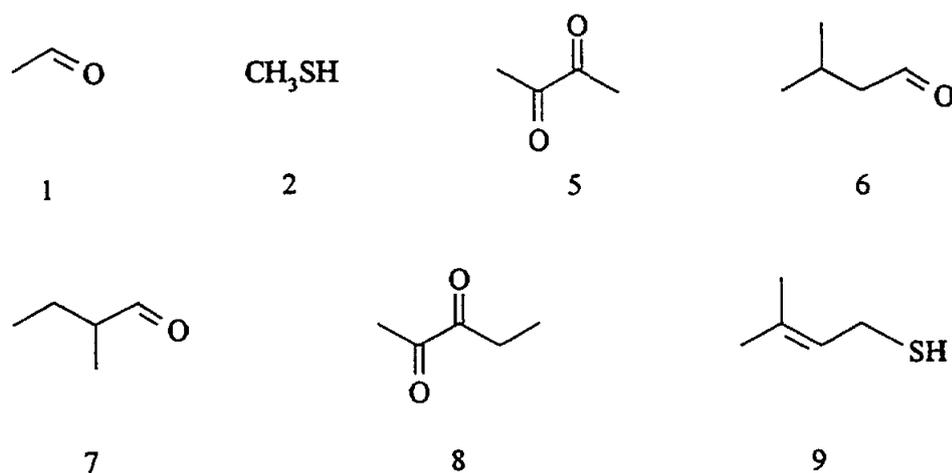


FIGURE 8 Chemical structures of some aroma impact compounds (FD \geq 12.5) found in the headspace of roast and ground *Arabica* and *Robusta* coffee: acetaldehyde (no. 1), methanethiol (no. 2), diacetyl (no. 5), 3-methylbutanal (no. 6), 2-methylbutanal (no. 7), 2,3-pentanedione (no. 8), and 3-methyl-2-butene-1-thiol (no. 9). The numbers correspond to those in Table 1.

volatiles are detected whose concentrations in the GC effluent are higher than their odor thresholds. The corresponding volatiles are then characterized by their aroma quality and intensity as well as by their chromatographic properties, i.e., retention index (RI). The RI increments, obtained on stationary phases with different polarities, provide additional information about the nature of the aroma-active component, such as functional groups. Aroma qualities and intensities are very useful data for flavorists, who can then use these to create characteristic and complex aroma notes.

As mentioned in Section II, chromatograms obtained by FID detection and olfactory response are different. Aroma-active compounds usually do not correspond to the major volatile components in the food. As shown in Figure 9, many important odorants of white bread crust were not visible in the gas chromatogram, for example, 2-acetyl-1-pyrroline (no. 11) (20). This can be explained by the low odor threshold of these compounds. Identification of such minor components (Fig. 10) is a challenging task.

Once the aroma-active regions have been selected by a dilution analysis, the often time-consuming identification experiments can be focused on the most potent odorants. If further fractionation and clean-up steps are required, GC-O may again serve as a screening method and guide sample-purification work. This approach, called sensory directed chemical analysis, is particularly useful when identifying unknown compounds with very low threshold values. An impressive example is the identification of 1-*p*-menthene-8-thiol as the aroma principle of grapefruit juice (21). Its threshold is the lowest ever reported for a naturally occurring compound: 2×10^{-8} mg/liter water. More examples are listed in Table 2. Most of these compounds occur at very low concentrations and are difficult to identify using conventional analytical techniques.

In general, the aroma quality of a volatile component combined with more than one RI value is considered as equivalent to identification by GC-MS. However, the reference compound should be available because of shifts of RI values, especially on polar capillaries. The presence of an odorant can be verified by coelution with the reference compound on capillaries of differing polarity. This approach is helpful for the identification of odorants with very low threshold values and unique aroma qualities (Table 3). RI values may also provide crucial data for the identification of unknown odorants, even if the reference compound is not available. In some cases, verification by GC-MS is possible by tuning the detection technique, e.g., looking at typical fragments of the target molecule in a well-defined region of the gas chromatogram, recording in the SIM mode, and applying GC-MS/MS. It should be mentioned that, with certain experience, time-consuming identification work can be limited to a few, still unknown, compounds.

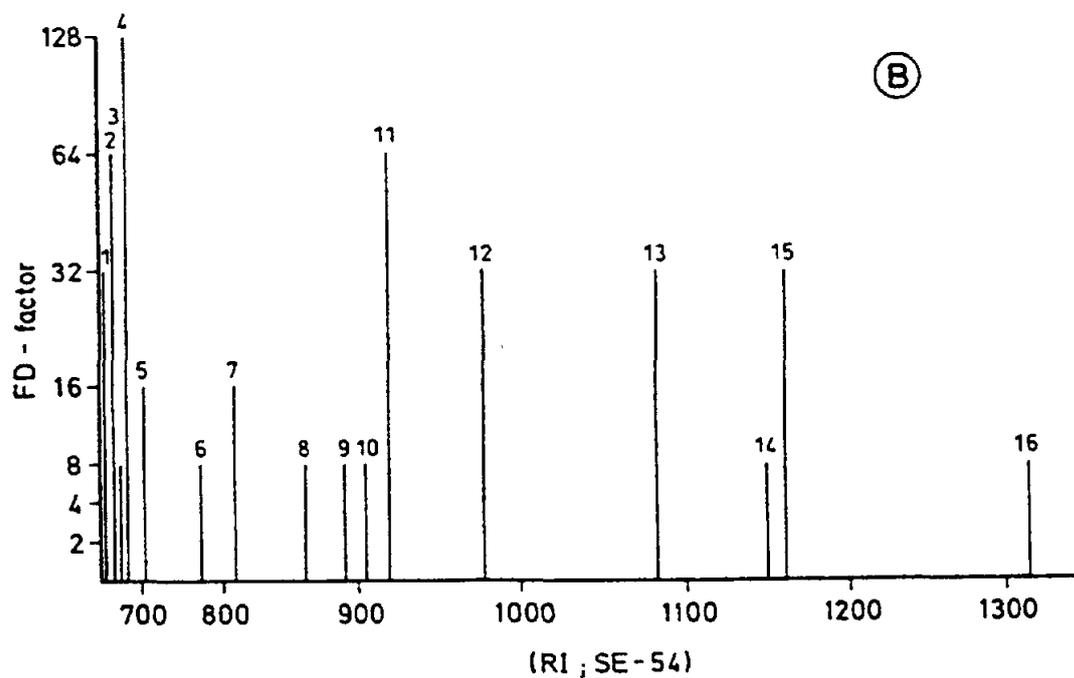
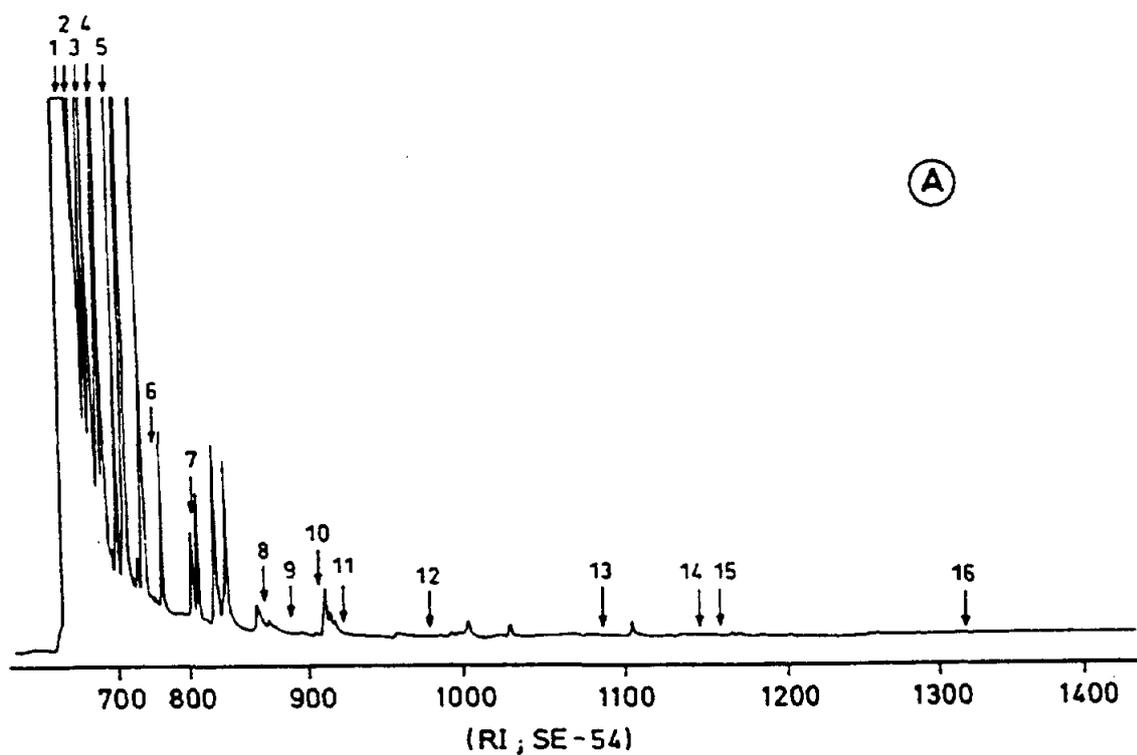


FIGURE 9 Gas chromatogram (A) and FD chromatogram (B) of the head-space volatiles of fresh white bread crust. (From Ref. 20.)

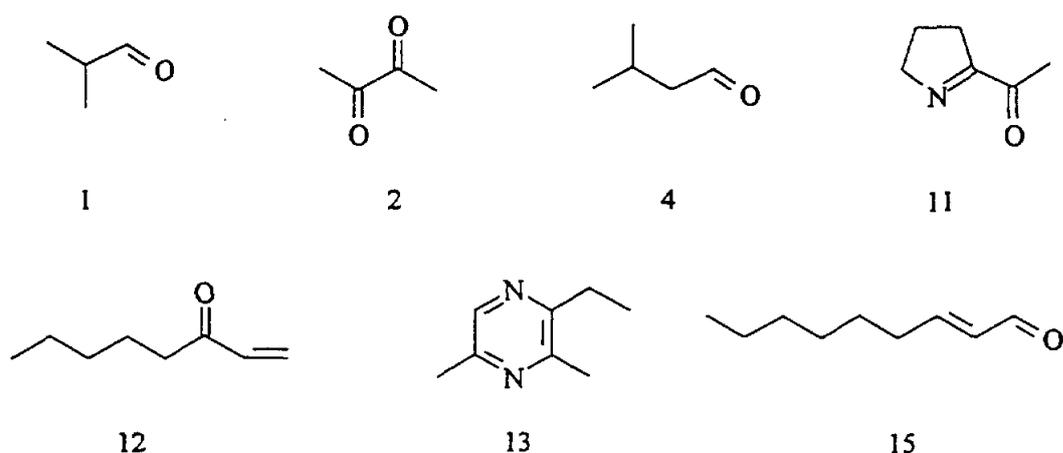


FIGURE 10 Chemical structures of the odor-active components identified in the headspace of fresh white bread crust: 2-methylpropanal (no. 1), diacetyl (no. 2), 3-methylbutanal (no. 4), 2-acetyl-1-pyrroline (no. 11), 1-octene-3-one (no. 12), 2-ethyl-3,5-dimethylpyrazine (no. 13), and (*E*)-2-nonenal (no. 15). The numbering corresponds to that in Figure 9.

TABLE 2 Aroma Impact Components Newly Identified in Foods on the Basis of GC-O

| Food | Compound | Ref. |
|---------------------|---|------|
| Bread crust, wheat | 2-Acetyl-1-pyrroline | 22 |
| Beef meat, boiled | 2-Methyl-3-furanthiol ^a | 23 |
| Beef meat, roasted | 2-Acetyl-2-thiazoline | 24 |
| Beef meat, stewed | 12-Methyltridecanal ^a | 25 |
| Coffee, roasted | 3-Mercapto-3-methylbutyl formate ^a | 26 |
| Cheese (Emmentaler) | Furaneol, homofuraneol | 27 |
| Grapefruit | 1- <i>p</i> -Menthene-8-thiol ^a | 21 |
| Wine (Sauvignon) | 4-Mercapto-4-methyl-2-pentanone | 28 |
| Tea, green | 3-Methyl-2,4-nonanedione | 18 |
| Lovage | Sotolon | 29 |

^aOdorant was reported for the first time as food constituent.

TABLE 3 Selected Examples for Compounds that Can Be Identified by GC-O on the Basis of Aroma Quality and Retention Indices

| Compound | Aroma quality (at sniffing port) | Linear retention indices | | |
|---|-------------------------------------|--------------------------|---------|------|
| | | SE-54 | OV-1701 | FFAP |
| 1-Octene-3-one ^a | Mushroomlike | 982 | 1065 | 1315 |
| (Z)-1,5-Octadiene-3-one ^a | Metallic, geranium-like | 985 | 1085 | 1395 |
| <i>trans</i> -4,5-Epoxy-(<i>E</i>)-2-decenal ^a | Fatty, metallic | 1385 | 1550 | 1990 |
| 3-Methyl-2,4-nonanedione ^a | Strawy, haylike | 1316 | 1400 | 1700 |
| δ -Decalactone | Coconutlike | 1685 | 1733 | 2185 |
| (<i>E</i>)- β -Damascenone ^a | Honeylike, cooked apples | 1395 | 1500 | 1825 |
| Sotolon | Seasoninglike | 1107 | 1350 | 2215 |
| Furaneol | Caramellike | 1065 | 1240 | 2045 |
| 2-Isopropyl-3-methoxypyrazine | Earthy, potato-like | 1097 | 1146 | 1430 |
| 2-Isobutyl-3-methoxypyrazine | Earthy, paprikalike | 1186 | 1237 | 1520 |
| 2-Ethyl-3,5-dimethylpyrazine | Earthy, roasty | 1083 | 1150 | 1455 |
| 2,3-Diethyl-5-methylpyrazine | Earthy, roasty | 1155 | 1219 | 1485 |
| 2-Acetyl-1-pyrroline ^a | Roasty | 923 | 1013 | 1345 |
| 2-Acetyl-2-thiazoline | Roasty, popcornlike | 1110 | 1245 | 1720 |
| Methional | Cooked potato-like | 909 | 1042 | 1465 |
| 3-Mercapto-2-pentanone ^a | Catty, sulfury | 907 | 1018 | 1365 |
| 2-Furfurylthiol | Roasty, sulfury | 913 | 1000 | 1445 |
| 2-Methyl-3-furanthiol | Meaty, roasty, sweet | 870 | 932 | 1325 |
| 3-Methyl-2-butene-1-thiol ^a | Sulfury, <i>Allium</i> -like, foxy | 821 | 874 | 1105 |
| 3-Mercapto-3-methylbutyl formate ^a | Catty, sulfury | 1023 | 1138 | 1515 |

^aCompound is not commercially available.

B. Formation of Potent Odorants

The identity of a key aroma compound is a flavor chemist's first information. However, it will not automatically lead to product quality improvement. Additional work on precursors and formation mechanisms is required (Table 4). This may result in conditions favoring the generation of positive aromas by processing. It may also support selection of raw materials and give some indications for a more efficient enzymatic and/or thermal treatment to liberate precursors of key aroma components.

TABLE 4 Precursors of Some Aroma Impact Compounds Found in Food

| Compounds | Food | Precursor systems | Ref. ^a |
|------------------------------|-----------------------|---------------------------------------|-------------------|
| 12-Methyltridecanal | Stewed beef | Plasmaologens | 25 |
| 2-Methyl-3-furanthiol | Boiled beef | Thiamine/Cysteine (H ₂ S) | 31 |
| 2-Ethyl-3,5-dimethylpyrazine | Roasted beef, coffee | Alanine/Methylglyoxal | 32 |
| 2-Acetyl-2-thiazoline | Meatlike model system | Cysteamine/Methylglyoxal ^b | 33 |
| 3-Methyl-2-butene-1-thiol | Roasted coffee | Prenyl alcohol/H ₂ S | 34 |
| 2-Acetyl-1-pyrroline | Wheat bread crust | Ornithine/Methylglyoxal ^c | 35 |
| Furaneol | Wheat bread crust | Fructose-1,6-bisphosphate | 36 |
| Homofuraneol | Soy sauce | Sedoheptulose-7-phosphate | 37 |
| Sotolon | Fenugreek | 4-Hydroxy-L-isoleucine | 38 |
| 3-Methyl-2,4-nonanedione | Soy beans, green tea | Furanoid fatty acids | 39 |

^aRecently published references are preferably cited.

^b2-(1-Hydroxyethyl)-4,5-dihydrothiazole is the key intermediate.

^c1-Pyrroline is an important intermediate.

Recently, furaneol and homofuraneol were detected by GC-O in Maillard model reactions based on pentoses and different amino acids. This initiated a systematic study to explain these surprising findings (30). As shown in Figure 11, the Strecker aldehydes of glycine and alanine were actively involved in the formation of furaneol and homofuraneol, respectively. The results were obtained using the ¹³C-labeled precursors and GC-MS/MS as a selective and sensitive detection technique.

C. Off-Flavor Analysis

GC-O is the method of choice for selecting those components that are responsible for aroma deviation in food, i.e., an off-flavor. In general, it can be applied to both foodborne off-flavor formation and off-flavor problems related to contamination. The latter is caused by odorants that normally do not belong to the overall aroma of the product, i.e., external contaminants (e.g., packaging) or compounds formed upon processing and storage (e.g., microbial spoilage). In both cases, the comparison of the off-flavor of the contaminated food with the reference product usually results in a limited number of sensory relevant compounds, which reflect the difference in aroma profiles. Identification work can then be focused on these odorants.

Recent work by Spadone et al. (40) on the Rio defect in green coffee from Brazil and by Marsili et al. (41) on the off-flavor of sugar beet impres-

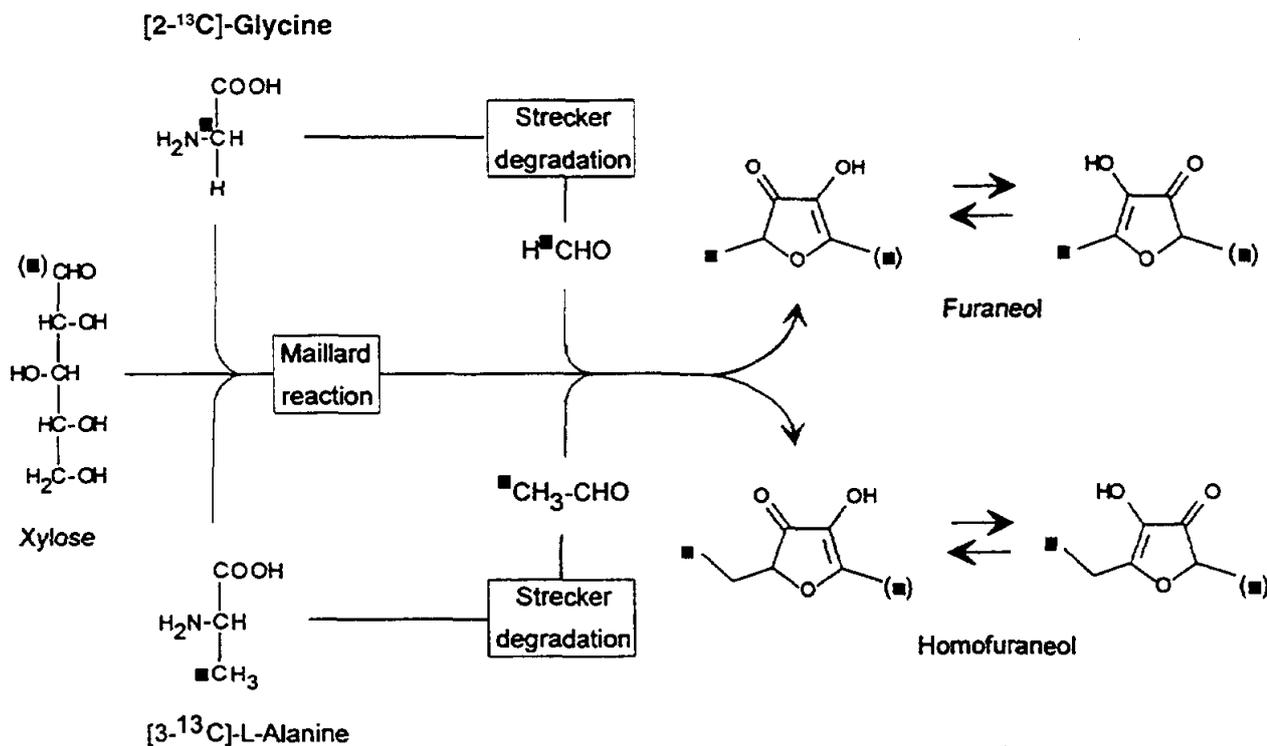


FIGURE 11 Schematic formation of furaneol and homofuraneol from pentoses (e.g., xylose) in the presence of glycine and alanine elucidated by labeling experiments. The marked positions (■) represent ¹³C-atoms. (Adapted from Ref. 30.)

sively illustrates the potential of this approach: 2,4,6-trichloroanisole and geosmin were identified as off-flavor compounds, respectively. Both odorants have very low odor thresholds in water: 5×10^{-8} and 5×10^{-7} mg/liter, respectively. Identification work was completed by quantitative data, and the off-flavor activity was confirmed by sensory evaluation. The approach, based on sensory techniques and a strong analytical support, provides a good basis for solving off-flavor problems in a reasonable time (Table 5).

Foodborne off-flavor is mainly caused by concentration shifts in aroma-active food constituents. This is much more difficult to handle due to the subtle changes that finally result in an unbalanced aroma. The warmed-over flavor (WOF) of cooked meat is a well-known example in the food industry. Quantitative results of odorants contributing to the off-flavor are indispensable for obtaining reliable data about changes in the aroma profile. Using this approach, hexanal and *trans*-4,5-epoxy-(*E*)-2-decenal have recently been found to be the main contributors of WOF (48).

TABLE 5 Examples of GC-O Recently Applied to Off-Flavors Caused by Direct or Storage-Related Contamination

| Product | Off-flavor compound | Ref. ^a |
|------------------------------|---|-------------------|
| Coffee, green | 2,4,6-Trichloroanisole | 40 |
| Beet sugar | Geosmin, butyric, and isovaleric acids | 41 |
| Water, river | 2-Methylisoborneol, geosmin | 42 |
| Water, mineral | C ₃ -Alkyl benzenes | 43 |
| Cognac, cork taint | 2,4,6-Trichloroanisole, 2,3,4,6-tetrachlorophenol | 44 |
| Wine | 2-Acetyltetrahydropyridines, furaneol | 45,46 |
| Pearl millet, ground, wetted | 2-Acetyl-1-pyrroline | 47 |

^aRecently published references are given preference.

IV. ANALYTICAL CONSIDERATIONS RELATED TO GC-O

A. Representativeness of the Aroma Extract

An excellent review of sample preparation has recently been published by Teranishi and Kint (49). In general, heat treatment should be limited to avoid formation of artefacts and decomposition of aroma impact components. Enzymatic activity in natural products is another critical parameter that should be controlled during sample preparation. In general, there is no ideal extraction method in food aroma analysis. The choice of an extraction procedure depends on the food and is always a compromise.

The issue of representativeness of the aroma extract has recently been discussed in detail by Etiévant and coworkers (50,51). Apparently, little attention is paid in the literature to the quality of the aroma extract, although it is well known that aroma composition depends on the extraction method used. Indeed, the first objective in aroma analysis is to ensure that the extract is representative of the original product. The conditions of extraction and concentration should be designed in such a way as to obtain a sample with an authentic aroma. To make sure that an aroma extract merits further analytical and sensory characterization, it is highly recommended to check its representativeness, e.g., using triangle, similarity scaling, descriptive, or matching tests. This is the basis for obtaining reliable results.

As illustrated in Table 6, the isolation method used to prepare sample B changed the unique meaty/savory note of the original product (sample A) to "boiled vegetables, grilled, burnt." In contrast, the isolation method used to obtain sample C resulted in an extract that revealed the authentic aroma of the

TABLE 6 Sensory Evaluation to Check the Representativeness of the Aroma Extract Obtained from a Commercially Available Food Flavoring with Savory Character

| Sample | Isolation method | Sensory attributes |
|---|-----------------------------|----------------------------------|
| A (original product) | | Meaty, roasty, savory, onionlike |
| B (extract of A) | SDE (boiling conditions) | Cooked vegetables, burnt, meaty |
| C (extract of A) | SDE (static vacuum at 35°C) | Meaty, roasty, savory, onionlike |
| D (extract of SDE residue) ^a | Direct solvent extraction | Caramel-like, savory, acidic |

^aSample D was obtained from the SDE residue of sample C by extraction with diethyl ether. SDE = simultaneous distillation-extraction.

Source: Modified from Ref. 52.

original product. Consequently, this extract was further characterized by GC-O and other instrumental and analytical techniques. The results and the isolation techniques used will be discussed in the following section.

B. Comparison of Different Isolation Techniques

1. SDE Under Reduced Pressure

The simultaneous distillation-extraction (SDE) under atmospheric pressure (53) is not always the most appropriate technique, and its use should be carefully considered. This technique is an elegant and rapid extraction method resulting in an aroma extract that is ready to be injected into a GC system after concentration. However, heat-induced artefact formation, decomposition of labile compounds, and loss of very volatile compounds are serious drawbacks. Furthermore, only steam-distillable volatiles are extracted. Polar compounds, such as hydroxyfuranones and phenols, are particularly poorly recovered.

Considerable effort has been made to overcome at least one of the limitations of the SDE technique, i.e., heat-induced changes of the aroma extract. A modified SDE apparatus was designed to work under static vacuum (SDE-SV), thus allowing extraction at 30–35°C (54). Several solvents were tested, of which butylethyl ether showed good results for various classes of substances (52). The extract (SDE fraction) can be directly analyzed by GC without any concentration step. SDE-SV is more time-consuming than conventional SDE and also more delicate in handling: exact control of three temperatures (aqueous sample, organic solvent, cooling by cryostat) is necessary, but results in a ready-to-inject aroma extract free of artefacts.

GC-O of the SDE-SV extract of a commercial meaty/savory flavoring

(sample C, Table 6) revealed 15 odor-active components out of about 100 volatiles. However, only 6 odorants showed FD factors of 2⁸ and higher (Table 7). The meaty/savory note was mainly imparted by odorants containing sulfur. The *cis*-isomer of 2-methyl-3-tetrahydrofuranthiol was also found, but did not contribute to the overall aroma. Identification was mainly based on GC-MS and NMR and was verified by commercially available or synthesized reference compounds. These are essential for unequivocal identification. The chemical structures of the aroma impact compounds identified in the flavoring are shown in Figure 12.

2. SDE Combined with Direct Solvent Extraction

As shown in Table 8, the acidic fraction (sample D in Table 6) obtained from the residue after extraction by SDE-SV was a good source of additional information, particularly about polar aroma compounds. They were isolated from the SDE residue by direct solvent extraction with diethyl ether, purified by extraction with sodium carbonate (0.5 mol/liter) and after acidification reextracted with the solvent. Furaneol (no. 8) was the dominating odorant in this extract; accordingly sample D was mainly described as caramel-like.

3. SDE Versus Static Headspace

As mentioned earlier (Sec. II.C.), headspace GC analysis yields additional data about very volatile compounds, which are usually lost during conventional sample preparation. The sensory relevance of odorants present in the

TABLE 7 Odorants Identified on the Basis of AEDA in the SDE-SV Extract (sample C) of a Commercially Available Food Flavoring with Savory Character

| No. | Compound ^a | Retention index | | Odor quality (GC-O) | FD factor (2 ⁿ) |
|-----|---|-----------------|------|------------------------|--------------------------------|
| | | OV-1701 | FFAP | | |
| 1 | 2-Methyl-3-furanthiol (MFT) | 931 | 1325 | Meaty, roasty, sweet | 10-11 |
| 2 | <i>trans</i> -2-Methyl-3-tetrahydrofuranthiol | 992 | 1315 | Meaty, savory, onion | 10-11 |
| 3 | 2-Furfurylthiol | 1000 | 1450 | Sulfury, roasty | 14-15 |
| 4 | Methional | 1044 | 1465 | Cooked potato, boiled | 10-11 |
| 5 | S-(2-methyl-3-furyl)-ethanethioate | 1238 | 1700 | Meaty, roasty | 10-11 |
| 6 | 4-Acetyloxy-2,5-dimethyl-3(2H)-furanone | 1430 | 2005 | Caramel-like, savory | 8-9 |

^aCompounds nos. 1-6 were detected in the SDE fraction obtained under static vacuum.
Source: Modified from Ref. 52.

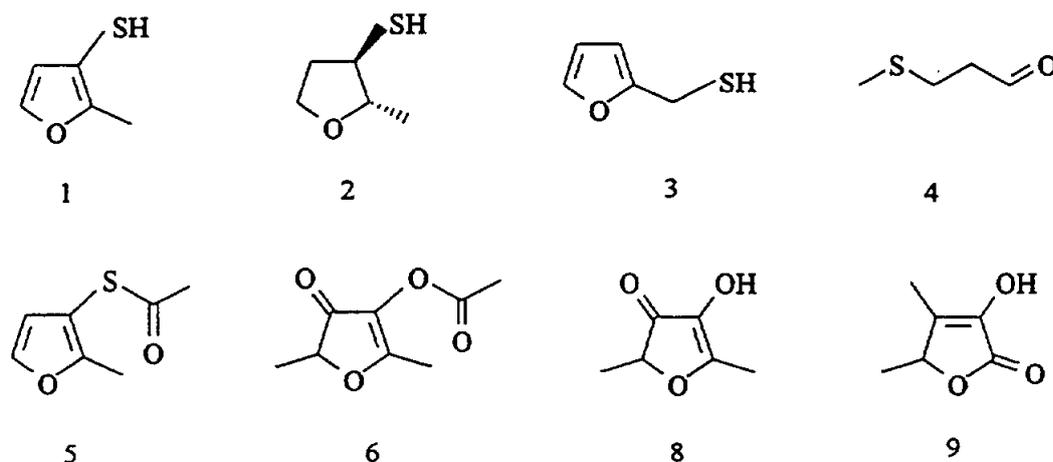


FIGURE 12 Aroma impact compounds identified in a meaty/savory food flavoring: 3-methyl-2-furanthiol (no. 1), *trans*-2-methyl-3-tetrahydrofuranthiol (no. 2), 2-furfurylthiol (no. 3), methional (no. 4), S-(2-methyl-3-furyl)ethanethioate (no. 5), 4-acetyloxy-2,5-dimethyl-3(2H)-furanone (no. 6), furaneol (no. 8), and sotolon (no. 9). Acetic acid (no. 7) is not shown. The numbering corresponds to that in Tables 7–9.

TABLE 8 Odorants Identified on the Basis of AEDA in the Acidic Fraction (sample D) of a Commercially Available Food Flavoring with Savory Character

| No. | Compound ^a | Retention index | | Odor quality (GC-O) | FD factor (2 ⁿ) |
|-----|-----------------------|-----------------|------|------------------------|--------------------------------|
| | | OV-1701 | FFAP | | |
| 7 | Acetic acid | 785 | 1460 | Acetic, pungent | 10–11 |
| 8 | Furaneol | 1240 | 2045 | Caramel-like, sweet | 16–17 |
| 9 | Sotolon | 1350 | 2220 | Seasoninglike | 11–12 |

^aCompounds nos. 7–9 were detected in the acidic fraction (SDE residue of sample C).

Source: Modified from Ref. 52.

headspace above a food can be evaluated by combining AEDA with the static headspace technique (18). This new approach, called static headspace GC-O, was applied to the food flavoring discussed above.

The odorants listed in Table 9 were identified on the basis of their chromatographic and sensory properties using the same analytical conditions as applied to AEDA. A stepwise reduction of the headspace volume revealed the most potent odorants. The medium, dry or aqueous, significantly influenced the results. For example, thiols nos. 1 and 2 were completely lacking in the headspace of the solid product, while in the aqueous medium they showed high odor potencies. These compounds could have been either efficiently encapsulated or liberated from nonvolatile precursors by hydrolysis.

Compounds 1–4 and 10–13 were more abundant in the headspace of the aqueous sample. Others dominated in the headspace of the dry product, such

TABLE 9 Static Headspace GC-O of a Commercially Available Food Flavoring with Savory Aroma Character

| No. | Compound ^a | Aroma quality (on GC-O) | FD factor (2 ⁿ) ^b | |
|-----|---|----------------------------|--|-------------------|
| | | | Solid sample | Aqueous sample |
| 1+2 | MFT + <i>trans</i> -2-methyl-3-tetrahydrofuranthiol | Meaty, savory | — | 8 |
| 3 | 2-Furfurylthiol | Sulfury, roasty | 4 | 8 |
| 4 | Methional | Cooked potato | 4 | 8 |
| 5 | S-(2-Methyl-3-furyl)-ethanethioate | Meaty, roasty | 2 | 2 |
| 6 | 4-Acetyloxy-2,5-dimethyl-3(2H)-furanone | Carmel-like | 5 | 2 |
| 7 | Acetic acid | Acidic, pungent | 2 | — |
| 8 | Furaneol | Carmel-like | 2 | — |
| 9 | Sotolon | Seasoninglike | — | — |
| 10 | Diacetyl | Buttery, sweet | <2 | 4 |
| 11 | Hexanal | Green, fatty | <2 | 4 |
| 12 | 1-Octene-3-one | Mushroomlike | 3 | 7 |
| 13 | (E)-2-Nonenal | Fatty | 2 | 7 |

^aNumbering corresponds to that in Tables 7 and 8.

^bThe initial headspace volume of 20 ml was set as FD = 1. The headspace volume was stepwise reduced and the GC-O procedure repeated to 0.1 ml, which corresponds to FD = 2⁸.

Source: Modified from Ref. 52.

as the polar and well water-soluble compounds 6–8. Diacetyl and the lipid degradation products 11–13 contributed more significantly to the aroma of the headspace than the liquid aroma extract obtained by SDE-SV.

4. Vacuum Distillation Versus Direct Solvent Extraction

The analysis of furaneol is an excellent example to illustrate that, unless appropriate isolation techniques are used, an important odorant may be missed. Contradictory results have been published concerning the occurrence and concentration of furaneol and its methylether (MDMF) in strawberries. Therefore, no clear conclusion can be drawn about their sensory relevance. As shown in Table 10, it was rather difficult to detect furaneol in vacuum distillats (Refs. A and B). It is highly oxygenated and, therefore, does not steam-distil due to its low vapor pressure in aqueous samples (58). Consequently, furaneol must be extracted with solvent (Refs. C and D). Cold on-column injection should ideally be used to avoid thermally induced decomposition of furaneol (59) (see Sec. IV.C).

C. Optimized Chromatographic Conditions

1. Aroma Alteration Prior to Chromatography

As many odorants are rather labile and occur at low concentrations, a long storage period between sample preparation and GC-O should be avoided. The choice of solvent is another critical parameter. Certain thiols rapidly dimerize

TABLE 10 Results Reported in the Literature on the Presence of Furaneol and Its Methylether in Strawberries—Effect of Analytical Conditions

| Analytical parameter | Ref. A (56) | Ref. B (57) | Ref. C (58) | Ref. D (59) |
|---------------------------|-------------|-------------|-------------|----------------|
| Extraction | | | | |
| Vacuum distillation | x | x | | |
| Direct solvent extraction | | | x | x |
| Injection mode | | | | |
| Split/Splitless | x | x | x | |
| Cold on-column | | | | x |
| Concentration (mg/kg) | | | | |
| Furaneol | <0.01 | — | 2.2–6.3 | 2.7–16.2 |
| MDMF | 0.2 | 0.1–2.6 | 0.5–10.9 | Not determined |

in diethyl ether upon refrigerated storage (60), for example, 2-methyl-3-furanthiol, which is an aroma impact compound of boiled beef (23). In such cases, the aroma concentrate should preferably be stored in pentane at -30°C , if possible under an inert gas, to avoid alteration of the aroma profile.

Furthermore, the aroma extract should be injected using the cold on-column technique. Unstable volatiles readily decompose in a heated injector block and form artefacts, e.g., hydroxyfuranones and thiols. Sulfur-containing compounds are particularly susceptible to heat-induced decomposition that can take place during split/splitless injection, GC separation, or in the GC-MS interface (61). Indeed, many newly reported constituents in *Allium* chemistry are artefacts (Fig. 13). HPLC and low-temperature GC and GC-MS conditions have been proposed for their analysis (62,63).

2. Effect of Chromatography on GC-O Data

The problems involved in analyzing very low amounts of often labile components should not be neglected in GC-O (64). Testing column quality on a regular basis is indispensable. Several mixtures are commercially available for testing polar and apolar capillaries. The so-called Grob test is highly recom-

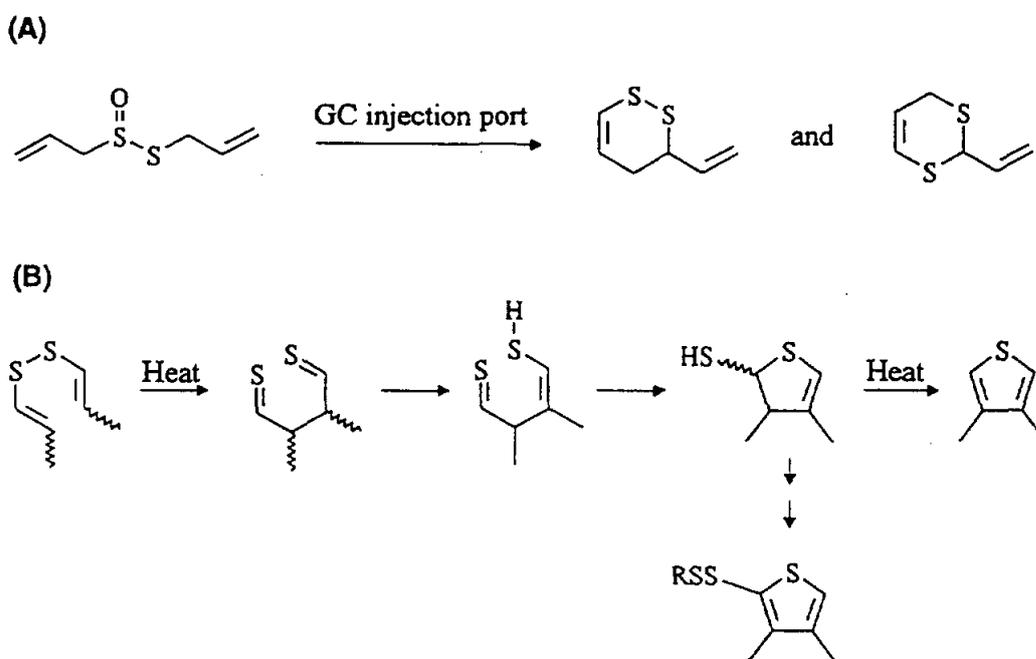


FIGURE 13 Formation of artefacts in *Allium* chemistry. (A) Allicin readily decomposes in a heated injector block forming two thioacrolein isomers before GC separation (62). (B) Bis-(1-propenyl)-disulfide rearranges at 85°C to thienyl compounds commonly found in *Allium* distillates (63).

mended, as it rapidly indicates the quality of the analytical capillary (65). For example, adsorption effects due to active surfaces are indicated by tailing of the 1-octanol peak. The quality of an FFAP column can be tested by injecting mixtures of alkanes and free fatty acids.

Many products, especially heat-processed and fermented foods, contain a large variety of substance classes. Therefore, an aroma extract should be analyzed on at least two capillaries of different polarity: an apolar phase (e.g., OV-1, SE-54) and a polar phase (e.g., Carbowax, FFAP). This may help to achieve a better separation of odor-active compounds, as shown in Figure 3. The medium polar capillary OV-1701 is a good compromise for analyzing both apolar and rather polar compounds. In general, chromatography may affect the FD factor and Charm value, particularly at high dilution levels when picogram amounts are analyzed.

As shown in Table 11, odor thresholds determined by GC-O may vary by several orders of magnitude depending on the stationary phase used. Consequently, such effects will also influence the FD factor and Charm value since they represent the odor threshold of the compound at a given concentration. Indeed, different FD factors were determined for MFT on SE-54 and FFAP: 2^{14} and 2^6 , respectively. On the contrary, abhexon showed higher FD factors on FFAP than SE-54: 2^{16} and 2^5 , respectively. Consequently, FD factors should be determined on suitable capillaries (64). Compounds with low threshold values are much more affected by this phenomenon, i.e., sotolon compared to furaneol. This can be explained by a chromatographic discrimination at low concentration as discussed below.

TABLE 11 Odor Thresholds^a (ng/liter air) of Some Selected Odorants as Affected by the Stationary Phase^b

| Compound | SE-54 | OV-1701 | FFAP |
|------------------------------|-------------|---------|-------------|
| 2-Methyl-3-furanthiol (MFT) | 0.001–0.002 | n.d. | 5–10 |
| Abhexon | 2–4 | n.d. | 0.002–0.004 |
| Sotolon | n.d. | 0.6–1.2 | 0.01–0.02 |
| Furaneol | n.d. | 1–2 | 0.5–1.5 |
| 3,4-Dimethylcyclopentenolone | n.d. | 1–2 | 0.05–0.1 |
| Cyclotene | n.d. | 10–20 | 10–20 |

^aOdor thresholds were determined by GC-O (14) using (*E*)-2-decenal as internal standard.

^bCapillaries were selected using the Grob test.

3. Discrimination of Odorants on Stationary Phases

The phenomenon of adsorption/instability of enoloxo compounds during GC analysis is illustrated in Figure 14. Furaneol and its methylether were analyzed at different concentrations by diluting the samples 1:1, i.e. FD-1, FD-2, FD-4, and FD-8. The chromatography of 4-methoxy-2,5-dimethyl-3(2H)-furanone (no. 1) was not affected by the stationary phase. On the contrary, furaneol (no. 2) was partially 'lost' on SE-54. Chromatographic behavior on FFAP was acceptable.

Discrimination of several compounds at low concentrations on different stationary phases was studied using furaneol, cyclotene, and their methylethers (Fig. 15). Decreasing amounts were injected via cold on-column, and the yields were determined by projecting the peak areas onto that of the undiluted sample, which was set at 100%. These experiments were continued until the detection limit of the FID was reached. FFAP was found to be the most suitable stationary phase for the analysis of polar compounds followed by Carbowax. On the contrary, yields on SE-54 and OV-1701 decreased strongly with increasing dilution. The corresponding methylethers showed much better chromatographic properties, most likely due to the blocked hydroxyl group, which reduces interaction with the stationary phase.

About 1 ng of furaneol injected on-column onto a FFAP still revealed a symmetrical peak by FID detection which can be integrated for quantification. It has been reported that, using a heated injector block, 10 ng of furaneol still gives a well-defined, sharp peak on a Carbowax fused silica capillary (66). However, we found that about 20–30 ng of furaneol on a OV-1701 resulted in an almost undetectable broad peak. Therefore, the FD factors of furaneol determined on FFAP are usually higher than those on OV-1701. The same phenomenon was observed for homofuraneol, sotolon, and abhexon, which are potent odorants and contribute to the flavor of several heat-processed and fermented foods.

V. SENSORY ASPECT OF GC-O

A. Role of Odor Thresholds in GC-O

It is very useful to have an approximate idea about the threshold value of odorants. The odor threshold of a compound (O_x), measured as ng/liter of air, can be determined by GC-O using (*E*)-2-decenal as "sensory" internal standard according to the following equation (14):

$$O_x = \frac{O_1 \cdot D_1}{C_1} \cdot \frac{C_x}{D_x} \quad (1)$$

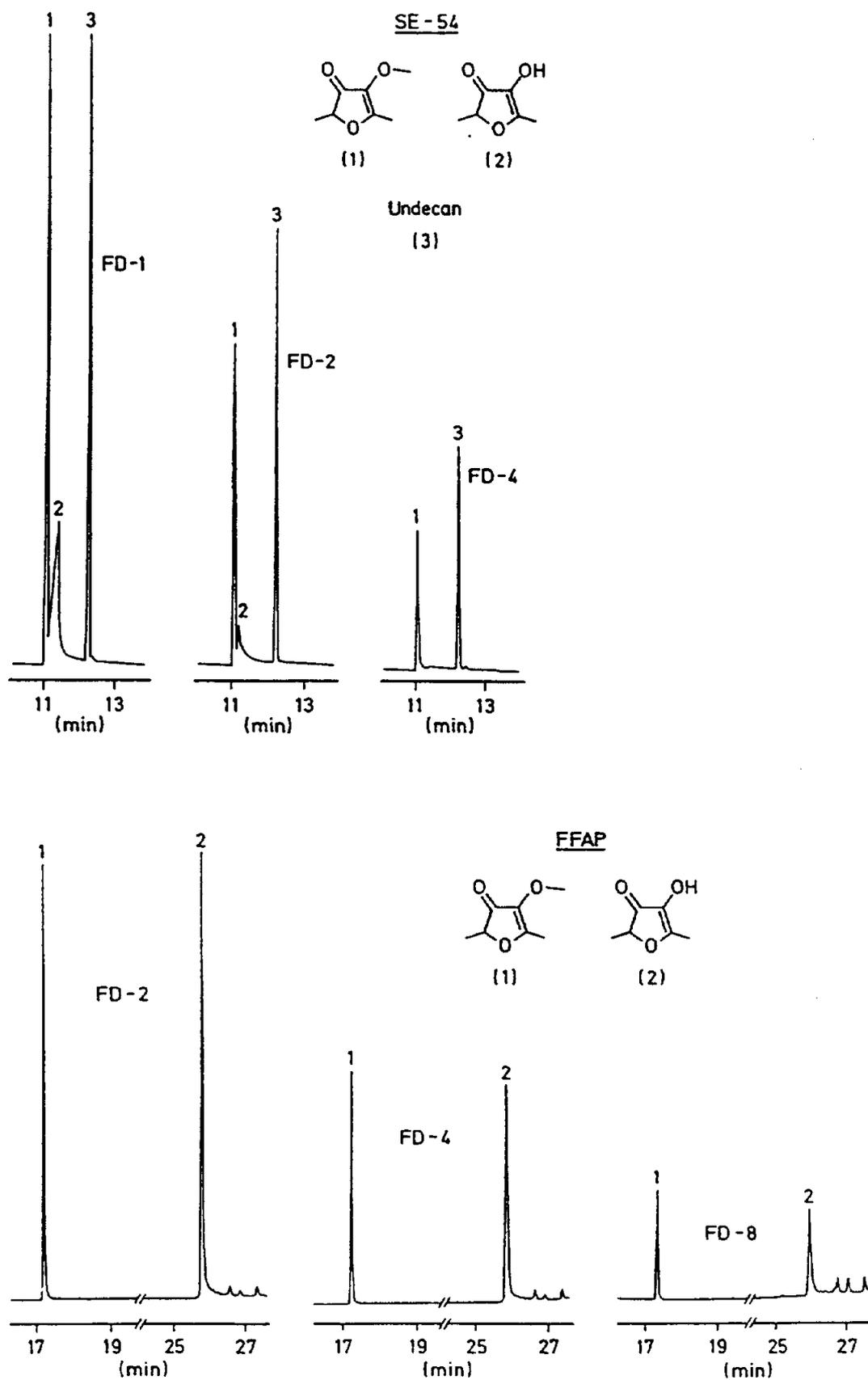


FIGURE 14 Gas chromatography of 4-methoxy-2,5-dimethyl-3(2H)-furanone (no. 1) and furaneol (2) on SE-54 and FFAP fused silica capillaries (I. Blank and W. Grosch, unpublished results). The original sample (FD-1) was stepwise diluted (FD-2, FD-4, FD-8) and analyzed using the same conditions (injection: cold on-column).

where C_i and C_x represent the concentrations and D_i and D_x the dilution values of the internal standard and the odorant, respectively. The term O_i is the odor threshold of the internal standard, (*E*)-2-decenal, which has previously been determined: 2.7 ng/liter air (67). This compound must be present in the solution containing the odorant(s). Therefore, all thresholds listed in Table 12 are related to the "sensory" internal standard, which allows an objective comparison of the values.

The information about odor thresholds determined by GC-O can be of great help in identifying sensory relevant compounds of both positive and off-flavors, particularly in case of separation problems (peak overlapping) or similar mass spectra. 2-Ethyl-3,5-dimethylpyrazine and 2-ethyl-3,6-dimethylpyrazine have similar mass spectra but can easily be distinguished based on

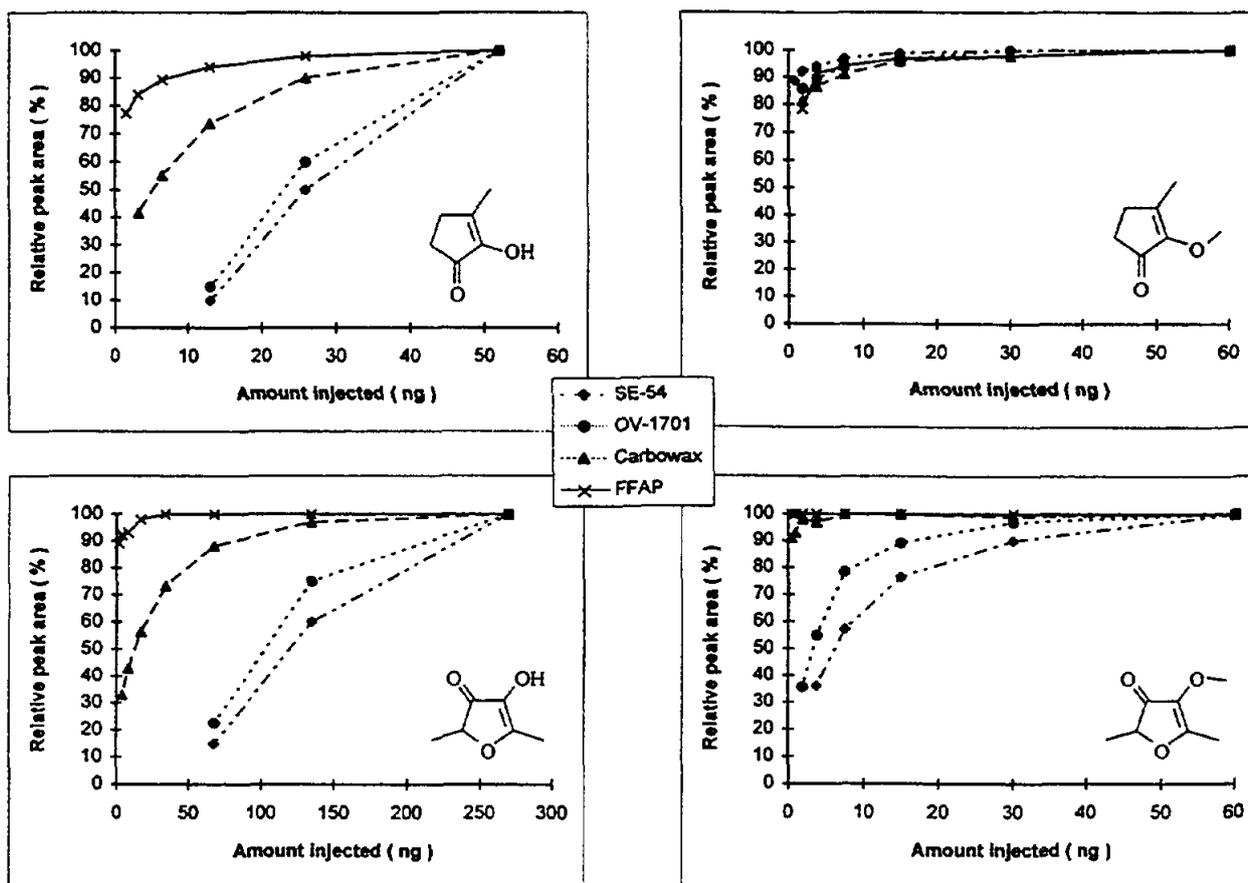


FIGURE 15 Yields of cyclotene, 2-methoxy-3-methyl-2-cyclopentene-1-one, furaneol, and 4-methoxy-2,5-dimethyl-3(2H)-furanone as a function of different polar stationary phases on GC with fused silica capillaries (injection: cold on-column). (Adapted from Ref. 64.)

TABLE 12 Odor Thresholds Determined by GC-O^a of Some Aroma Impact Components^b Found in Thermally Processed Foods^c

| Compound | Odor threshold (ng/liter air) |
|--|-------------------------------|
| <i>trans</i> -4,5-Epoxy-(<i>E</i>)-2-decenal | 0.0005–0.005 |
| (<i>Z</i>)-2-Nonenal | 0.002–0.008 |
| (<i>E</i>)-2-Nonenal | 0.04–0.16 |
| (<i>E,E</i>)-2,4-Decadienal | 0.05–0.2 |
| (<i>E,Z</i>)-2,6-Nonadienal | 0.1–0.4 |
| Hexanal | 15–45 |
| (<i>E</i>)- β -Damascenone | 0.002–0.004 |
| (<i>Z</i>)-1,5-Octadiene-3-one | 0.003–0.006 |
| 3-Methyl-2,4-nonanedione ^d | 0.007–0.014 |
| 1-Octene-3-one | 0.05–0.1 |
| 4-Methylacetophenone | 2–4 |
| Diacetyl | 10–20 |
| 4-Ethylguaiacol | 0.01–0.03 |
| 4-Vinylguaiacol | 0.4–0.8 |
| Eugenol | 0.2–0.4 |
| Vanillin | 0.6–1.2 |
| 4-Methylphenol | 0.3–1 |
| Myristicin | 1–2 |
| 2-Isopropyl-3-methoxypyrazine | 0.0005–0.001 |
| 2,3-Diethyl-5-methylpyrazine | 0.009–0.018 |
| 2-Ethyl-3,5-dimethylpyrazine | 0.007–0.014 |
| 2-Isobutyl-3-methoxypyrazine | 0.002–0.004 |
| 2-Acetyl-1-pyrroline | 0.02–0.04 |
| 2-Acetyltetrahydropyridine | 0.1–0.2 |
| 3-Mercapto-3-methylbutyl formate | 0.0002–0.0004 |
| 2-Methyl-3-furanthiol ^e | 0.001–0.002 |
| 2-Furfurylthiol | 0.01–0.02 |
| Dimethyltrisulfide | 0.06–0.12 |
| Methional | 0.1–0.2 |
| 2-Acetylthiazol | 2–5 |

^aOdor thresholds were determined by GC-O (14) on an OV-1701 using (*E*)-2-decenal as 'sensory' internal standard.

^bFrom Refs. 32, 64, 68–70.

^cThe threshold values of enoloxo compounds determined on FFAP are listed in Table 11, i.e., abhexon, sotolon, furaneol, cyclotene, and 3,4-dimethylcyclopentenolone.

^dOdor threshold was determined on a Carbowax.

^eOdor threshold was determined on a SE-54.

their odor thresholds: 0.007–0.014 and 2.5–5 ng/liter air, respectively (32). Although 1-octene-3-ol and 1-octene-3-one coelute on apolar capillaries, the presence of the latter can be verified by dilution of the sample due to its 100-fold lower threshold. Thus, dilution techniques provide additional data for positive identification. Compounds with odor thresholds lower than 1 ng/liter air are usually below the detection limit of an FID.

B. Limitations of the GC-O Approach

The importance of the representativeness of the aroma extract and the possible effects of the GC analysis on GC-O data have already been discussed in detail (see Sec. IV). Several recently published articles are recommended regarding practical aspects of the GC-O procedure (12,13,71–73). Therefore, only a few additional remarks will be made here.

All GC-O runs of a dilution analysis should be performed within one week to reduce variability in GC analysis and sensorial perception. If analysis takes longer to complete, assessors can have “gaps” during sniffing, i.e., they do not detect a substance at a certain dilution, but detect it again at a higher dilution (73). The first step is to establish a profile of the original aroma extract by detecting the odor-active regions and describing their aroma characteristics. This should be done on two capillaries of different polarity. A medium polar capillary is proposed for the dilution analysis. All odor-active regions detected in the original aroma extract should be sniffed throughout the entire dilution series, consisting of not more than 10 samples. The last 5 dilutions should be repeated on the second capillary, preferably an FFAP. Some of the dilutions should be evaluated by additional assessors. The use of humidified air is recommended to reduce olfactory fatigue by nasal dehydration. Consider that the number of odorants detectable by GC-O depends on the extraction method and the threshold of the volatiles, but also on parameters that are arbitrarily selected, i.e., amount of food sample, concentration factor, sample volume injected.

Problems such as representativeness, GC analysis, and “gaps” during sniffing can be solved by taking the necessary precautions. More serious limitations of the GC-O approach originate from the sensory area. Methods based on odor threshold detection, i.e., GC-O using dilution techniques (Charm, AEDA) and the OAV concept (ratio of concentration to threshold), are not consistent with psychophysical views (74). The major problems are that thresholds vary depending on the experimental conditions, differing intensity functions for volatiles above the threshold are not accommodated, and no prediction about the activity of volatiles in a mixture is possible, especially if they occur at concentrations below threshold.

Differing intensity functions for volatiles account for a well-known phenomenon in GC-O: some intensely smelling compounds disappear after a few dilution steps (e.g., vanillin), while others with a lower aroma intensity in the original extract have the highest FD factors. The sensory relevance of the latter is overestimated. (*E*)- β -Damascenone is a typical representative for such compounds, which are characterized by a relatively flat dose/intensity function. This is most likely why (*E*)- β -damascenone does not play a major role in the aroma of coffee, despite low threshold values (Table 12), i.e., high FD factors and OAVs. In other words, threshold concentration does not necessarily correlate with aroma potency.

A more satisfactory but more difficult approach is to provide intensity measures, which can only be carried out with a well-trained panel (75). A fundamental weakness of all of these techniques is that they do not account for interactions arising in the olfactory system or between taste and smell. The chemical bases of these senses are still not sufficiently understood.

C. Interpretation of GC-O Data

Properly performed GC-O and adequate knowledge about the possible limitations of the technique are the basis for a realistic interpretation of the results. The flavor scientist's major questions are: "What can be concluded from GC-O data?" and "Where is the limit of a realistic interpretation?"

Certainly, GC-O is a first essential step for distinguishing odor-active compounds from volatiles without odor impact. This screening procedure is the basis for identification experiments. GC-O also provides a first indication about the odor potency of volatile compounds, i.e., to what extent they individually contribute to the overall aroma. However, in most cases final conclusions about their sensory relevance cannot be drawn and further work is necessary (see Sec. VI.).

Analytical and sensorial data cannot be presented with the same precision. While RI values and mass spectra can be precisely determined, GC-O data lack comparable accuracy and reproducibility. FD factors and Charm values are approximations of the sensory relevance of an odorant. In fact, a 256-fold dilution is a rough estimation, depending on extraction yields and the assessor, and could also be 128 or 512. Such exact values are rather misleading: GC-O techniques are not this accurate. The use of 2^n , where n is the number of dilution steps, may help to avoid overinterpretation of GC-O data (76). Though 256 and 2^8 represent the same value, the latter gives a more realistic idea of the odor potency of a compound. It should be mentioned, however, that FD factors do not normally differ by more than two dilution steps.

Furthermore, Charm or FD chromatograms can be divided into three regions, represented by compounds with high, medium, or low dilution values. This classification of odorants could be used as selection criteria for identification, which would be focused on the first two categories, i.e., odorants of high and medium potencies. Less effort would be attributed to those odorants contributing to the "background" aroma. However, the role of the aroma quality should not be neglected in this context: several background odorants with a typical note may also contribute to the overall aroma.

The approach presented above could be standardized by setting the highest dilution value at 2^{10} (= 1024) and relating the remaining values accordingly. Odorants of high and medium potencies would be grouped depending on their dilution values, i.e., 2^{8-10} and 2^{5-7} , respectively. In this way, the role of an odorant in different foods could easily be estimated. Moreover, it would allow a better comparison of GC-O data from different laboratories.

In summary, GC-O techniques should be seen as screening methods to gain an insight into important contributors to a characteristic aroma (7,71). GC-O performed as Charm analysis and Osme have also been claimed as quantitative bioassays (10,77). However, more time is needed for training of assessors and verification using statistical means.

VI. OUTLOOK

The aim of GC-O techniques in food aroma research is to determine the relative odor potency of compounds present in the aroma extract. This method gives the order of priority for identification and thus indicates the chemical origin of olfactory differences (7). The value of the results obtained by GC-O depends directly on the effort invested in sample preparation and analytical conditions. Analysis of an aroma extract by dilution techniques (AEDA, Charm) combined with static headspace GC-O provides a complete characterization of the qualitative aroma composition of a food. However, this is only the first step in understanding the complex aroma of a food.

State of the art in food aroma research today is based on a combined sensory and analytical approach. It is basically composed of the following three steps, which can be applied to the characterization of both positive aroma and off-flavors:

1. Qualitative aroma composition (based on GC-Olfactometry)
2. Quantitative aroma composition (odor activity value concept)
3. Aroma recombination studies (aroma simulation based on quantitative data)

Work starts with the analysis of the aroma composition and is completed when the aroma of the food can be simulated in an appropriate matrix on the basis of the quantitative data obtained. The last step is essential and validates the analytical results. Recently published data on stewed beef (78) and coffee brew (79) impressively demonstrate the potential of this approach. However, a crucial step is accurate quantification of the aroma impact compounds. Special techniques are necessary to quantify labile odorants at low concentrations, i.e., isotope dilution assay using the labeled odorant as internal standard. A major concern is the availability of these labeled compounds. The recently published review articles of Grosch (80) and Schieberle (72) are recommended for more details.

In conclusion, there is a clear need to improve the quality and stability of food aromas and flavors. The techniques presented above represent an attractive approach for analyzing aromas more purposefully. Depending on the case, it is possible to simplify the approach and find compromises in all three phases so that essential work can be done in a reasonable time. The food and flavor industry is well advised to profit from this development and to adapt the different techniques to their specific needs.

ACKNOWLEDGMENTS

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APPENDIX: ABBREVIATIONS AND TRIVIAL AND TRADE NAMES

Chemicals

| | |
|------------------------------------|---|
| Abhexon | 5-Ethyl-3-hydroxy-4-methyl-2(5H)-furanone |
| Cyclotene | 2-Hydroxy-3-methyl-2-cyclopentene-1-one |
| (<i>E</i>)- β -Damascenone | 1-(2,6,6-Trimethyl-1,3-cyclohexadienyl)-(<i>E</i>)-2-butene-1-one |
| 3,4-Dimethylcyclopentenolone | 2-Hydroxy-3,4-dimethyl-2-cyclopentene-1-one |
| 4-Ethylguaiaicol | 4-Ethyl-2-methoxyphenol |
| Eugenol | 4-(1-Propenyl)-2-methoxyphenol |
| Furaneol® | 4-Hydroxy-2,5-dimethyl-3(2H)-furanone |
| | Furaneol is a trade name of Firmenich, Geneva, Switzerland |

| | |
|-----------------|---|
| Homofuraneol | 2(5)-Ethyl-4-hydroxy-5(2)-methyl-3(2H)-furanone |
| Isovaleric acid | 3-Methylbutanoic acid |
| MDMF | 4-Methoxy-2,5-dimethyl-3(2H)-furanone |
| Methional | 3-Methylthio-1-propanal |
| Methylglyoxal | 2-Oxopropanal |
| MFT | 2-Methyl-3-furanthiol |
| Sotolon | 3-Hydroxy-4,5-dimethyl-2(5H)-furanone |
| 4-Vinylguaiacol | 4-Vinyl-2-methoxyphenol |

Analytical Techniques

| | |
|----------|--|
| AEDA | Aroma Extract Dilution Analysis |
| Charm | Combined Hedonic and Response Measurement |
| FID | Flame Ionization Detector |
| FFAP | Free Fatty Acid Phase (polar stationary phase for GC) |
| GC | Gas Chromatography (using capillary columns) |
| GC-O | GC-Olfactometry |
| GC-MS | GC-Mass Spectrometry |
| GC-MS/MS | GC-Tandem-MS |
| HPLC | High-Performance Liquid Chromatography |
| MS | Mass Spectrometry |
| OAV | Odor Activity Value (ratio of concentration to odor threshold) |
| Osme | from the Greek word $\delta\mu\eta$, meaning 'smell' |
| OV-101 | Ohio Valley apolar stationary phase for GC |
| OV-1701 | Ohio Valley medium polar stationary phase for GC |
| RI | Retention Index |
| SDE | Simultaneous Distillation Extraction |
| SDE-SV | SDE under Static Vacuum |
| SE-54 | Apolar stationary phase for GC |
| SIM | Selective Ion Monitoring (GC-MS technique) |

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