

# Odor and nutrition

## Part 3: Food odorants and their analysis

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The first two parts in this series dealt with the physiological basis of smell and the chemical properties of odorants. In this third part, foods and their respective odorants are targeted. As part of this, the challenges are highlighted that arise when analysing the aroma of foodstuff. Thereafter, modern analytical approaches and methods are discussed. And in a concluding short outlook about the key food odorant research, possible fast detection methods and their applicability are outlined.

### Abstract

Identifying those odorants in a food that actually contribute to the perceivable aroma requires a very elaborate and multi-step analytical procedure. As part of this analytical task, human sensory as well as instrumental analytical techniques are combined. Due to the large inhomogeneity of the possible odorants in the foods of interest, a specific strategy needs to be developed for each analysis. Usually, key food odorants are identified by first carefully processing the food samples and extracting the volatile fraction. These extracts are used in aroma extract dilution analyses to identify the most potent candidate substances. After quantification of the odorants, their odor thresholds are correlated to their actual concentration. In a matrix similar to the tested food, the identified odorants are recombined to model the aroma and to check whether they are sufficient. The odorants can only be proven to be required and thus be considered a key food odorant by omitting single odorants from this optimized combined mix. This elaborate process clearly shows which hurdles automatised high speed analytical tools on a microelectronic or biological basis will have to overcome.

**Keywords:** key food odorants, analytical chemistry, flavor analysis, sensory analysis

sis can rarely be interpreted without the accompanying sensory analysis. All required steps to unravel the full aroma profile of foods and to identify all relevant key food odorants (KFO) gear into each other as presented in ♦ Figure 1 [1].

### Sample preparation

The initial step of any food analysis is the selection of analytical conditions and the preparation of the sample material. Typically, for the sensory evaluation, no specific sample preparation is required except for the preparation and transformation of the test item into the ready to eat condition. A first impression of the composition of the volatile fraction can be achieved by sampling the headspace above the food. This technique only captures the volatiles that are also available for the sensory test person or a consumer when smelling the food. For the deepening instrumental analysis however, the preparation of the sample material is the crucial step. There is a broad range of established sample preparation techniques, which are only briefly outlined here. A full overview about the instrumental analysis and sensory evaluation can be found e. g. in the publication of SELZER [3].

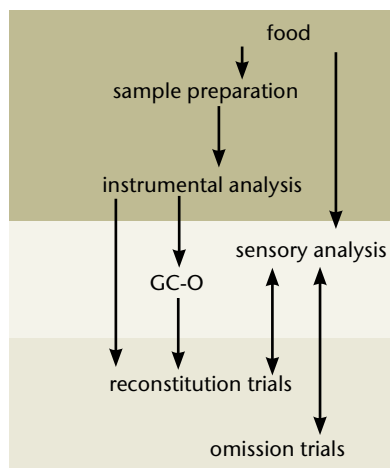
### How are key food odorants identified?

For the research and evaluation of food aromas one can principally distinguish the sensory and instrumental analysis. Actually, both methods are tightly interconnected and are frequently combined, since the purely numeric and descriptive data from the instrumental analy-

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**Fig. 1: Flow chart describing the required steps to elucidate the aroma profile of foods** (modified from [1, 2])  
GC-O = gas chromatography olfactometry

A very simple and often sensitive analytical technique is the already mentioned headspace analysis. For this, an aerial sample of the headspace above a sample is taken at a defined temperature containing the volatile odorants. This assures that only those odorants are captured that are also available for a test person or a consumer. Odorants that are only relieved from the matrix by chewing and that are thus mainly available by retronasal perception are being suppressed.

### Instrumental analysis

Once the samples are prepared in a suitable form, they can be subjected to the instrumental analysis. In the recent years, the instrumentation has been subject to a tremendous increase of performance and efficiency. Typically, odorants are separated on a capillary gas chromatographic (GC) column and are detected with a suited detector (♦ Figure 2).

The first step of this procedure is the injection of the sample into the instrument, which already shows numerous variations with respective advantages and disadvantages: e. g. a direct injection of a sample into the column (on-column injection), which prevents a degradation of sensitive molecules, an evaporation of a liquid sample in the injector, the injection of a gaseous sample or the heat supported desorption (thermo-desorption) of odorants from a solid sampling material. Even the selection of these initial conditions may be deciding for the success of the whole analysis.

As foods may contain much more than 100 volatile substances (in coffee even more than 800), that often only have minor structural differences, it is an analytical prerequisite to fully separate them [5, 6]. The separating power of a single chromatographic column may, however, not be sufficient to fulfill this task. Fractions of high interest are cut out of the first chromatographic

column and are directed to a second chromatographic column via a switch (or modulator). This second column is usually much shorter and has different separation characteristics such as polarity to allow a separation of structurally similar molecules that co-eluted from the first column. This coupling (GCxGC) generates a multidimensional information profile with enhanced separation and often also detection power [7].

For routine applications, the molecules eluting from the column are burned in a flame ionisation detector (FID) to give an unspecific signal. An assignment of the signals to a molecule is only possible if the sample to analyse is well known and only expected volatiles are targeted, or if reference substances are available to compare the retention behavior and thus verify the identity. There are also unidimensional mass spectrometers (MS) available which record the masses of the eluting volatiles (strictly speaking the ratio of mass and charge of the molecule) and a respective more or less specific fragmentation pattern. These detectors can be coupled to further mass spectrometric units (MS/MS) yielding more specific molecule fragments and may increase the sensitivity, depending on the matrix. A secure identification of odorants can be accomplished by comparing mass spectra with reference spectra from spectra databases, by a comparison of retention times with those of reference compounds, by comparison with published retention indices, specific for a substance in combination with column material, or by comparing the sensory characteristics of eluting peaks (gas chromatography olfactometry, GC-O) with reference compounds. Ideally several of these strategies are combined to increase the security of the identification.

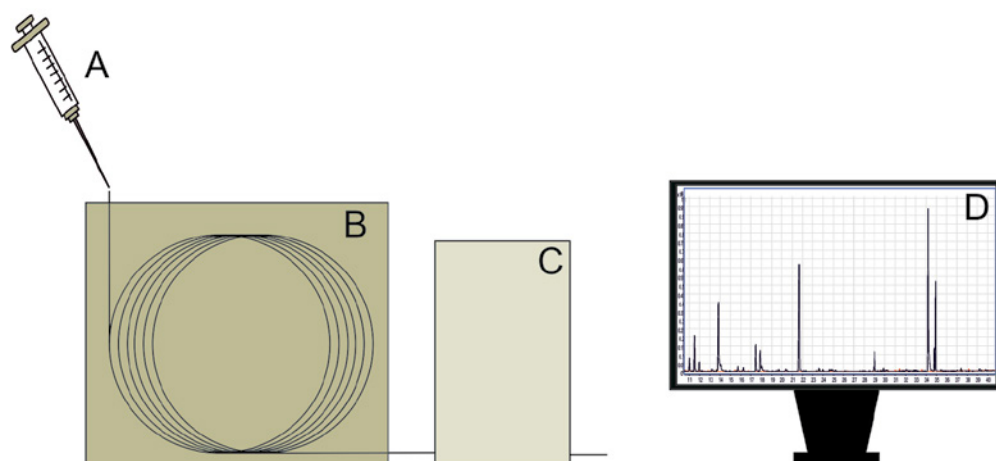
Latest advancements are detectors with highly increased sensitivity and sensors that can allocate the molecules with their accurate high resolution mass, which e. g. enhances

Two aspects are of high importance:

1. During sample preparation and subsequent analysis care must be taken to avoid artefacts in the context of sensible odorants. The application of heat (e. g. induced by friction), the activation of enzymes (thermally or due to relieve upon mechanical cell disruption), or the treatment with organic solvents can readily induce reaction of and with odorants and thus tamper with the analytical result.
2. Every sample preparation induces a bias by favored or suppressed collection of odorant groups (more or less volatile, more or less lipophilic substances).

In the most cases a combination of different procedures will lead to an approximation of an unbiased and holistic analysis.

In contrast to the headspace method, the solvent assisted flavor evaporation (SAFE) is very elaborate [4]. In the SAFE procedure aroma extracts are evaporated in a high vacuum and recondensed in a trap cooled with liquid nitrogen, which leads to less artefacts and helps to avoid a biased extraction of odorant groups due to different volatilities.



**Fig. 2: Principle of gas chromatography.** A) A sample (gaseous or liquid) is being injected to the system via the injector. (B) At high gas pressure the sample is transported over the chromatographic column (typically 30–60 m length; 0.25 mm inner diameter). (C) The inner surface of the column is coated with a specific material which can be relatively different and provides for the separation characteristics of the method. The odor molecules interact individually with this coating and are thus selectively retarded during the flow over the column. The selection of column characteristics is crucial for a successful separation of odorants and thus for the method. (D) The detector signals are recognized as so called “peaks”. The area under the peaks generated by the detector is proportional to the amount of a substance in the sample and is used for quantitative determinations.

the identification of new odorants from new sources. This technique was also used to identify relevant odorants in beer that originate from precursor substances in hops [8]. Furthermore liquid chromatography (LC), especially coupled with mass spectrometers has made a lot of progress. And so, it is imaginable that in future it is possible to analyse gustatory substances – typical goals for LC-based analytical strategies – together with a major part of the volatile fraction of a food in a common liquid chromatographic run.

In order to securely identify and determine the target odorants one usually employs stable isotope labelled reference standards that compensate for actual losses during the multistep analytical procedure (stable isotope dilution assay; SIDA) [9]. From the quantitative data of the chemical analysis one can subsequently calculate odor activity values<sup>1</sup> (OAV) for all volatile substances. All this information, however, does not necessarily allow drawing a conclusion of the importance of all the single substances for the full aroma profile of the researched food. This can only be accomplished

by evaluation of accompanying data from human sensory analysis.

### Human sensory analysis

Human sensory analysis is a very special analytical tool and reflects a scientific field of its own. Next to the work by SELZER [3], BONGARTZ [11] published a comprehensive review on sensory analysis in this journal, too. Thus, a detailed description of sensory techniques is omitted here. However, it can be fixed, that sensory analysis is required essentially to evaluate the importance of odorants and that the application of a singular method does not suffice to achieve a full understanding of food aromas.

### Coupling of GC with olfactometry

A symbiosis of particular importance is the coupling of GC separation with olfactometric detection (GC-O). For this technique, the eluent flow is split just at the end of the chromatographic column and while one part of the flow ends in one of the presented detectors (e. g. FID or MSD), the other part ends up in a special opening to relieve the flow to

the ambient air (the olfactory detector). A sensory test person can monitor the chromatographic run at this olfactory detector to correlate the detected sensory qualities and intensities with the data of the instrumental detector.

Due to the very versatile characteristics of different odor molecules there are some that may cause an intense signal in an electronic detector, but cannot or only rarely be detected by a test person using the GC-O port (e. g. tetramethyl pyrazine). There are also odorants that, due to their very low odor thresholds, can only be detected by GC-O without pre-concentration steps and are yet fundamental for the aroma of that food (e. g. p-menthene-1-thiol).<sup>2</sup>

In order to allow for an estimation of individual importance of all odorants in the complete aroma profile, the measured extracts are diluted after each analytical run 1+1 with the extraction solvent and measured

<sup>1</sup> ■■■ “Odor and nutrition. Part 2: traits of odors” in ERNÄHRUNGS UMSCHAU 63(1): 22–30, [10]

<sup>2</sup> ■■■ “Odor and nutrition. Part 1: Fundamentals of smelling” in ERNÄHRUNGS UMSCHAU 62(5): 82–91, [12]

again. This so called aroma extract dilution analysis (AEDA) is repeated until no odorant can be detected by the GC-O anymore. With the AEDA each odorant can specifically be assigned to a dilution factor (flavor dilution factor, FD-factor) at which the odorant is barely perceivable. The dilution scheme results in possible dilution factors of 1, 2, 4, 8, 16, 32, 64, and so on. The higher a dilution factor, the higher is the probability of an odorant to actually contribute to the food aroma as KFO.

### Reconstitution trials and aroma models

After identification of all potent odorants and potential KFO (e. g. OAV > 1) of the food it must be tested if they are necessary and sufficient to reconstitute the full aroma of the food and thus to reflect an accurate aroma model.

The property of being sufficient is proven if a reconstitution of the identified odorants in a matrix that resembles that of the original food (but without having an intrinsic smell) has virtually the same quality as the food. The overlap of the sensory quality is checked with human sensory analyses.

### Omission trials

Subsequent to the reconstitution the perfect reconstitute is subjected to omission trials. In these trials single odorants (or more) are omitted from the reconstitute and compared again with the aroma of the original food. Any individual odorant is only necessary for the aroma model if its omission leads to a significant deviation from the sensory properties of the original food aroma. Reconstitution and omission trials are especially required since next to a direct and active contribution of any odorant to the food aroma there may also be indirect pharmacological effects of the receptor level that may influence the perceived aroma [12].

## The importance of key food odorants

It was shown, that single odorants never appear isolated in natural foods and therefore may only contribute in part to the total perceivable odor. But why do those KFO contribute to the respective food odor and not others? Does it happen at random or may this be part of a biological program? In part 2 of this series [10] the relation of the evolutionarily shaped receptor repertoire in shed of our food preferences was outlined. There may yet be a theoretical possibility for a connection of the occurrence of some odorants in food and the abilities of our odorant receptor repertoire.

In order to check for the significance of KFO versus other non-KFO-odorants KOTTHOFF and KRAUTWURST [13] introduced a relative measure (cognate odorant receptor frequency, CORF) which is the probability to functionally assign an odorant to a specific odorant receptor (OR) (cp. part 2 of this series [10]).

To achieve this, all published cognate OR odorant pairs were compared with the odorant list that was functionally tested in the respective studies. It resulted in that despite KFO were relatively underrepresented their fraction among the functionally assigned OR odorant pairs was twice as high, compared to non-KFO. When this analysis is extended to body odors, the fraction is even higher. A meta-analysis was also applied for a work in which 60 odorants were tested of which only 26 were considered KFO. Result of this work is, that across all odorants and the entire OR repertoire the likeliness of functionally identifying cognate OR odorant pairs is about three times higher when using KFO, despite in all published bioassay based work KFO were 1.4-fold underrepresented compared to synthetic molecules [14].

On this level of data it may be expected that there is a direct correlation between odorants that supply us with valuable information and

the evolution of our OR repertoire. Most prominently the high rate of occurring single nucleotide polymorphisms (SNPs) in the genes encoding for OR suggest a high dynamic and thus allow to quickly adopt to new nutritive environments. However, if our (preferred) diet is a consequence of our given OR-mediated odor perception or if in turn our OR repertoire is a consequence of our available food sources (hunting, available fruits, or the control of fire), cannot yet be answered.

An interesting observation is the rejection of sulphur and nitrogen containing low-weight odor molecules. These molecules often reflect end points of biological degradation (e. g. proteolysis) and can thus function as a hint on spoilage and a potential danger arising from ingesting the respective food. An opposing popularity of some special foods in which these molecules contribute significantly to the aroma might be interpreted as the result of some kind of cultural development of early hunters to nowadays customers. Examples for such foods are e. g. ripened cheeses or alcoholic beverages. A complementary example from taste is the popularity of bitter tasting foods, such as coffee or chocolate which are mostly disliked from children, but may turn into acceptance or even esteem upon familiarisation or cultural development.

### Wrap up of key food odorants

Concluding it can be said that there is no tool to predict the significance of a given odorant in its food. But it is possible to divine the spectra of odorants shaping the aroma of a certain food. This is less a prediction; it rather is the absence of a surprise upon the actually identified odorants. This especially is the case for technically highly processed foods. E. g. there is a series of typical roast odorants that originate from widely spread odorant precursors, such as fatty acids, proteins, and especially sugars. For example popcorn, coffee,

cacao, and bread crust share a part of their KFO but nevertheless they all smell unequivocal due to specific odor signatures [14].

The situation is more difficult in the case of unprocessed, fresh and raw biological systems. Enzyme cascades enable the biosynthesis of highly specific and characteristic odorants and to enrich them in certain cell compartments or tissues, such as blossom, seed head, perspiratory glands, or cell membranes in general. That is why especially raw materials, fruits and herbs are often characterized by the occurrence of “character impact compounds”<sup>3</sup> or show an aroma profile consisting of a rather slim set of odorants. This is the reason why in such food specialized and rare odorants with a low distribution may often be found. Due to the enantiomeric selectivity of enzymatic processes in these foods the stereochemistry of odorants may also play an important role (compare part 2 of this series [10]). A group of prominent examples for enantiomeric odorants are terpenes. Caraway and mint for example smell strikingly different, the reason of this harsh difference, however, is mainly ruled by the respective stereoisomers of carvone, both the character impact compounds of their food.

### Fragrances, cosmetic odors and synthetic odorants

Apart from KFO and other naturally occurring food odorants not contributing to the aroma of any foods, there is a wide range of potent and prominent odorants that occur in non-food resources and such of synthetic origin. The CORF-analysis (see above) showed that KFO are especially appropriate to activate human OR, however, human OR cannot exclusively be activated with KFO. And so, there is a plethora of odorants with unique and outstanding qualitative and quantitative characteristics, which are uniquely appropriate for cosmetic applications, such are

musk-like heterocyclic musk-compounds HHCB (1,3,4,6,7,8-hexahydro-4,6,6,7,8,8-hexamethyl-cyclopenta[g]-2-benzopyran) or the discontinued musk xylol. There is a plethora of molecules being solely or much easier available via chemical synthesis, especially in the desired amounts, but also some more easily available by extraction from natural resources. Due to the structural versatility of the compounds and the unpredictability of sensory characteristics, nature will for long be an important field of research for the fragrance arena. While the whole known food aroma can be explained with little more than 220 odorants, the cosmetic odorant portfolio comprises more than 3 000 compounds [15]. The possibility to formulate all odorants in any wanted concentration for cosmetic applications is important, even those with relatively low odor thresholds, to make them sensory available, at least for a certain period of time.

### The future of aroma research and potential fields of application

The science of food odors has a major impact on many scientific and applicative areas of both physiological and economic shape. Detailed knowledge on the significance of any individual food odorant and about full odorant profiles will allow targeted and economic applications. This implies a major competitive advantage for those having access to this knowledge and those having smart ideas to serve the market. Moreover each technical advantage in this field implies an impact on the all-day food selection and thus on our nutrition and health. The most important actor in this regard will be biotechnology with its possibilities for enzymatic processing of biomass [14]. Even today a large fraction of odorants for the food industry are produced in this way. An advantage over the classical synthetic chemistry is the stereo- and

enantioselectivity of such processes and the possibility to recombine many different enzymes from different sources to tailor synthetic strategies individually. Such strategies also provide for resource efficient processes, not only because biomass can be used as a source, but also because enzymatically controlled reactions save energy as they have optimal efficiencies at comparably low temperatures (e. g. 30 °C vs. 80 °C – 100 °C) [16]. A very new prospect arises from the increased application of biotechnological strategies for legal considerations because the borders between chemical synthesis and natural resources become indistinct. Examples are the fermentation of remains from the primary food production, such as pulp or pomace, or cell cultures as well as microbiologic fermentations where enzyme cascades or single enzymes coming from bacteria, fungi, or yeast [17–20] may be applied.

An important task for the future will be the speed-up of odor analysis, which will not be possible in short term using the classical analytics presented above. The knowledge generated using the methods described above, however, may be the basis to decide what a new generation of analyses has to capture. A promising approach can be, as mentioned, the consolidation of taste and odor analysis.

A business challenge is also to speed up commodity flows in a way that the qualitative evaluation can already be finished before respective ingredients and goods, such as cocoa, oils, or spices are further processed. With the implementation of so called electronic noses (♦ box) it could be possible to evaluate the microbiologic status of fish before portioning and dosing, or to release beverage batches in ample time.

In principle it can be thought to use ambitious biosensors or bioelectron-

<sup>3</sup> Odorants that shape the aroma of a food with particular significance, e. g. vanillin in vanilla or (–)-carvone in mint.



ic noses, for which recombinant odorant receptors safeguard the required detector specificity. However, for these it is necessary to learn the activating ligands for all receptors and to develop a fundamental understanding for expression and functionality of receptors, especially with regard to the preservation of the ligand specificity [31].

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#### Conflict of Interest

The authors declare no conflict of interest.

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### Electronic nose: chances and limitations for food analysis

There have been trials for many years to detect odorants by means of artificial sensors. These so called “electronic noses”, however, are still far away from their biological paradigm in terms of sensitivity, robustness, and especially in specificity [21]. The general principle is, similar to the physiology of smell, to correlate a pattern recognition principle to odorants in order to generate a specific sensory fingerprint. In opposition to human test persons that can only test random control samples electronic noses provide for the possibility for an objective and continuous monitoring.

In the electronic nose business, one may differentiate between bio-electronic noses which apply physiologic components, especially olfactory receptors that maintain the specificity of the system and classical electronic noses on a purely microelectronic basis.

Due to the complexity of such tasks electronic nose applications can only be aspired for very specific applications with clearly defined target profiles as for today, such as the detection of a malodor in the monitoring of continuous production lines. The implementation requires special efforts in the qualification of the applied sensing elements and their calibration [22]. Recent developments of a classical electronic nose are for example the SOMSA (selective odorant measurement by multi-sensor array [23, 24]), or the SAW sensor (surface acoustic wave) [25]. With such or similar devices tests on the quality of food, e. g. coffee [26], white bread [27], or on the oxidative status of edible oils [28] have already been performed. As the practically relevant aroma profiles usually consist of a multiplicity of different volatiles, a separation of all substances by high-resolution chromatography is desirable, but very expensive and elaborate. A basic goal thus is, to increase the specificity and sensitivity of the sensing elements allowing for an omission of advanced separation techniques. Further work in this field must focus on the development of mobile devices that combine GC and detectors to simplify the analysis and to reduce the analysis time drastically [29]. Hence, the basic goal, of course, is to further increase the performance of the sensor to render the chromatographic part of the device needlessly.

To achieve this, new detector materials are being developed and fused with each other. Materials tested are for example tin dioxide (SnO<sub>2</sub>), tungsten oxide (WO<sub>3</sub>), SnO<sub>2</sub> fused with a catalytically active platinum addition, or chromium titanium oxide (Cr<sub>2-x</sub>Ti<sub>x</sub>O<sub>3+z</sub>, CTO) [30]. These individual sensor materials are further combined in sensor arrays of single sensors of different materials. The required composition of the sensor array, however, strongly depends on the matrix and the selected target molecules. Realizable fields of application are processes in which very specific odorants (e. g. with very individual functional moieties, that may serve as reactive trigger on the sensor surface) need to be captured or in which the aroma profile consists of only few volatile molecules. Potential fields of application for more complex future sensors cover the storage, the processing, and the distribution of foods, especially the monitoring of the ripening status of fruits and fresh produce is a potential field.

The major advantage of the bioelectronic noses is their high specificity, as in the physiological environment odorant binding molecules (e. g. odorant receptors) are only activated upon highly specific interaction solely with their cognate volatile ligands. The challenges of bioelectronics noses are, however, to maintain this specificity when employed in non-physiologic environments as well as the durability of the bio-tech fusion elements and also the reversibility of the detection event for repeated usage.

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