DOI: 10.1002/anie.201309508

Chemistry of Smell

Nature's Chemical Signatures in Human Olfaction: A Foodborne Perspective for Future Biotechnology

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Keywords:
biotechnology · electronic noses · key food odorants · olfaction · receptors

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The biocatalytic production of flavor naturals that determine chemosensory percepts of foods and beverages is an ever challenging target for academic and industrial research. Advances in chemical trace analysis and post-genomic progress at the chemistry–biology interface revealed odor qualities of nature's chemosensory entities to be defined by odorant-induced olfactory receptor activity patterns. Beyond traditional views, this review and meta-analysis now shows characteristic ratios of only about 3 to 40 genuine key odorants for each food, from a group of about 230 out of circa 10000 food volatiles. This suggests the foodborn stimulus space has co-evolved with, and roughly match our circa 400 olfactory receptors as best natural agonists. This perspective gives insight into nature's chemical signatures of smell, provides the chemical odor codes of more than 220 food samples, and beyond addresses industrial implications for producing recombinants that fully reconstruct the natural odor signatures for use in flavors and fragrances, fully immersive interactive virtual environments, or humanoid bioelectronic noses.

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1. Introduction

For more than 8000 years, natural biochemical processes have been employed in the production of foods, such as bread, beer, and wine, cheese and yogurt, vinegar, soy or fish sauce. Thus, by generating new aromas and tastes through microbial fermentation or enzymatic activity, the birth of flavor biotechnology was introduced.[1]

Although the metabolic performance of multiple microorganisms promises an enormous potential for de novo flavor biosynthesis, the yields of valuable compounds found in nature are usually too low for commercial applications.[2] With the exception of some flavor compounds derived from primary metabolism, such as L-glutamic acid and citric acid, metabolic diversity often leads to a rather broad spectrum of closely related compounds, for example, a series of fusel alcohols derived from amino acid metabolism.[2]

Within the last century, the enormous progress in organic synthesis allowed the cost-efficient preparation of highly purified, naturally occurring odorous molecules and chiral odorants.[3] For example, the industrial production of the minty smelling und cooling active (1*R*,2*S*,5*R*)-configured (−)-menthol was achieved already in the mid-1960s by Haarmann & Reimer yielding racemic menthol, from which (−)-menthol is separated by chiral resolution.[4] In the late 1980s, a team led by Ryōji Noyori, who won the 2001 Nobel Prize for Chemistry in recognition of his work on this process by using BINAP ruthenium catalysts, was the first to develop the myrcene-based synthesis of (−)-menthol, today known as the "Takasago process", involving the asymmetric isomerization of diethylgeranylamine to 3-(*R*)-citronellal enamine using the catalyst $[\{(S)\text{-BINAP}\}_2\text{Ru}]\text{ClO}_4$ as a novel key reaction step.[5] As synthetic (−)-menthol is today one of the world's most-sold flavor ingredients, reaching a global demand of 25000 to 30000 metric tons per year, BASF developed a new process in 2012 involving the assymetric hydrogenation of (*Z*)-neral using a chiral rhodium catalyst.[6] Moreover, organic chemistry was successful to produce non-natural homologues of even higher impact, for example, the nowadays widely used ethylvanillin showing a circa four-fold enhanced flavor intensity when compared to its natural analogue vanillin.[7]

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Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/anie.201309508.

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Despite the achievements in industrial flavor production, there is a growing aversion of alienated consumers towards non-natural chemicals added to foods, cosmetics, or household products, thus creating an increasing request for truly authentic flavor signatures, as well as the demand for flavor molecules of "organic" or biological origin.[2] Over the last decades, this led to a significant shortage of several plant resources, such as vanilla and peppermint, and has pushed the employment of "greener" chemistry and more "eco-friendly" biotechnological manufacturing processes towards the generation of bioflavors by means of plant cells, tissue cultures, or microbial processes involving bacteria, fungi, yeast, and their enzymes, respectively.[2,8–10] Even insect-derived enzymes were recently proposed as a yet-underestimated treasure for industrial biotechnology.[11] The biotechnological production of fine chemicals such as, for example, organic acids, amino acids, nucleotides, vitamins, and alcohols,[12] biocatalytic regio- and stereoselective transformations,[13] rational protein design, and computer-aided enzyme design coupled with directed evolution techniques targeting the development of novel biological catalysts,[14,15] multistep processes employing sequential bio- or chemocatalyzed transformations or designed recombinant whole-cell expressing multiple enzymes,[16] as well as selective recovery of target molecules by efficient down-stream processing have been rapidly expanding over the last years to become mature disciplines in today's chemical industry.[2,17] Although volatile alcohols such as the fusel alcohols, odor-active acids, and esters such as 2-phenylethyl acetate, aldehydes such as (*Z*)-3-hexenal and vanillin, ketones, and 3- and 5-alkanolides are already biocatalytically produced in industry, their chemical diversity makes odor molecules still a truly challenging target for biotechnology with wide applications in food, feed, cosmetics, as well as pharmaceutical sectors.[2,9,10]

The advancement in our understanding of the molecular basis of olfaction is imperative to more effectively navigate post-genomic flavor biotechnology towards those target molecules which are evolutionary selected by nature to create the truly authentic olfactory percept of the various foods and beverages. However, this requires new knowledge on how our sense of olfaction is able to deconvolute the puzzling world of food odors on a molecular level. Working at the interface between the chemical world of volatile molecules and the sensory perception in the brain, a repertoire of more than 400 rhodopsin-like G protein-coupled seven transmembrane helix receptors, named odorant receptors (OR), translate external chemical stimuli into internal information that can be processed by neural circuits.[18] Assuming that these receptors have evolved to cope with this task, the analysis of their coding strategy promises to yield valuable insight in how to encode nature's chemical information in an efficient way.[19] To meet this demand, the chemical odor space needs to be defined and the comprehensive population of sensory active key molecules, coined "sensometabolome", to be decoded that reflect the sensory phenotype and trigger the flavor signature of a given food.[20,21]

Although tremendous technological progress has been made in recent years in molecular sensory science to elucidate the sensometabolome of foods on a molecular level ("sensomics"),[1,20,21] a new quality of knowledge is required in particular at the chemistry–biology interface of olfaction.[22]

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In other words, our understanding of odorant coding critically depends on knowledge on the interaction of key odorants with their best cognate receptor proteins and on deciphering the combinatorial code in which the identity of odorants is encoded by the particular subset of receptors that they activate.[22]

Elucidating the human receptor codes for biologically relevant single odorants and, even more importantly, those for nature's chemosensory mixtures that coding for the olfactory images perceived by our brain during food consumption, will be an important milestone for the efficient and cost-competitive biotechnological reconstruction of truly authentic odor signatures—all constructed from the same supply of biologically produced key odorants.

2. Analytical Coverage of the Chemical Odor Space of Food Needs to be Comprehensive and Quantitative

The hunt for the odorous molecules in our daily diet started with the introduction of gas chromatography (GC) in the early sixties.[23] At that time, research was performed with the assumption that the entire set of volatiles occurring in either foods, body odors, or environmental odors contributes to the specific smell of the chemosensory entity. However, although about 8000 volatiles were identified up to the year 2013[24] and a total number of 10000 volatiles was predicted to occur in foods,[25] early experiments to reconstitute the aroma of foods such as olive oil[26] and orange juice[27] by the volatiles identified were not successful. The lack of high-impact trace odorants that were not detectable by the analytical equipment, that is, the flame ionization detector of a GC, as well as incorrect quantitative data were considered the main reasons for the disagreement in the sensory percepts of authentic foods and the corresponding biomimetic odorant cocktails.[28]

Thus dose/activity considerations increasingly fueled upcoming doubts that all of the suggested 10000 volatiles present in foods actually contributed to the specific smell of the food.[28–30] This induced a paradigm shift in the search for the key odorants of foods and introduced the birth of the "sensomics" approach. Adapting the idea of coupling gas chromatography to the antenna of insects, known as GC-electro-antennography and widely used in experimental entomology for the detection of volatiles perceived by the antennal olfactory apparatus of insects,[31] GC-olfactometry (GC-O) was developed to locate the odorants among the bulk of sensorially inactive volatiles in the chromatographic effluent by "sniffing" detection using the human nose as a most sensitive and selective biological detector.[28,30,32–34] Techniques based on repeated GC-O analysis of serially diluted aroma distillates such as, for example, charm analysis[35,36] or aroma extract dilution analysis,[28,30] enabled the comprehensive detection of the set of odor-active molecules and allowed their ranking with respect to their sensory impact based on relative thresholds in air.

This activity-guided strategy tremendously helped to focus the laborious identification experiments on the most odor-active molecules in foods. However, because the olfactory screening of the lead molecules by GC-O is based on their threshold in air and not in the respective food matrix, researchers began to investigate the contribution of individual odorants to a given food aroma on the basis of "odor units" or "odor activity values" (OAV) defined as the ratio of the concentration of an odorant in the food and its odor threshold in an appropriate matrix.[29,30,37,38] However, the enormous chemical complexity of the volatile fraction and the large differences in concentration, volatility, and chemical stability of the key odorants challenged their accurate and precise quantitation.[28,30]

The breakthrough then came by using stable isotope (^{13}C, ^{2}H)-labeled twin molecules of the key odorants as most suitable internal standards for high-resolution gas chromatography/mass spectrometric analysis.[21,28,34,39–42] Accounting for analyte discrimination during extraction, sample clean-up,

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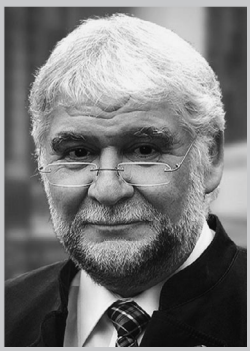

Peter Schieberle studied chemistry at the University of Aachen and food chemistry at the University of Bonn and received his PhD from the TU München (TUM) in 1980. After his habilitation at TUM, he was appointed as lecturer at the University of Erlangen-Nürnberg (1988), as Privatdozent at TUM (1989), and Full Professor for Food Chemistry at the Bergische Universität Wuppertal (1993–95). Since 1995, he holds the Chair for Food Chemistry at TUM and is the Director of the German Research Center for Food Chemistry of the Leibniz Society and of the Hans-Dieter-Belitz Institute for Cereal and Protein Research.

Thomas Hofmann studied food chemistry at the University of Erlangen-Nürnberg and received his PhD (1995) and habilitation (1998) at the Chemistry Department of the TU München (TUM). From 1999 to 2002, he was deputy director of the German Research Center for Food Chemistry of the Leibniz Society. In 2002, he took over the Chair of Food Chemistry of the University of Münster, and since 2007 he holds the Chair of Food Chemistry and Molecular Sensory Science at TUM. Since 2009, he is senior vice-president for research and innovation of TUM.

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and chromatography, this so-called stable isotope dilution analysis (SIDA) allowed the robust quantitative analysis of the key food odorants with a required precision of less than 10%.[28]

This so-called "sensomics" approach comprising the bioactivity-guided discovery of key odorants, their accurate quantitation, followed by aroma reconstitution and omission experiments showed that a comprehensive and quantitative analytical coverage of the chemical odor space of a given food is required to fully create the hedonically complex odor image of a food. Consequently, omitting just one key odorant, or having incorrect concentrations of one or more volatiles in odor blends, will induce significant deviations in the perception of the aroma as compared to the food itself.[28,30]

3. Mixing Odorants Wipes Out Individuality for the Benefit of New Odor Percepts

As GC-O evaluates the odor impact of the volatiles individually after chromatographic separation, any perceptual interactions of odorants are not taken into account.[36,43,44] This describes a non-natural, artificial setting, as in our daily life the chemosensory machinery is continuously exposed to complex cocktails of volatile chemicals, with huge variations in both their chemical structure as well as their concentration ranges, rather than to single compounds.[24,25] Although processing of complex stimuli patterns by the olfactory system is a central issue in understanding the perception of nature's chemosensory entities such as food aromas, body odors, or environmental cues, the various rules underlying the olfactory decoding of these chemical signatures remain largely obscure.[18,45]

Human psychophysical studies convincingly demonstrated that the perception of mixtures of odorants, even if each correctly identified alone, is not just a simple sum of the percepts of the individual components.[44–49] For mixtures containing more than four components, the odorants were found to lose their individuality and produce a new odor percept conveying a unique odor quality not elicited by the single components. For example, studies on human volunteers[48] and newborn rabbits[49] have strongly suggested a pineapple-like smelling mixture of three compounds to be processed as a new entity. Also the geranium-like smelling (*Z*)-1,5-octadien-3-one and the cooked potato-like smelling 3-(methylthio)propanal (methional), reported as key molecules evoking the fishy aroma of boiled cod,[37] stored sardines,[50] as well as a fishy "off-odor" in dry spinach,[51] lose their individual odor character when present in a ratio of 1:100, giving rise to a characteristic fishy note.[51] This phenomenon, coined synthetic or configural processing,[49,52] was recently confirmed by neurophysiological experiments demonstrating that selected cortical neurons respond to binary odorant mixtures but not to their individual components.[49,53] This implies that merely using the chemical structure of individual odorants is insufficient to identify and predict characteristic odor qualities of natural chemosensory entities. However, the rules governing the involvement of either elemental or configural processes in human odor perception still remain poorly understood.[18,45]

Despite being unable to identify individual odorants in a mixture,[47,52,54] humans can easily discriminate mixtures from each other, and even multiple compound mixtures were demonstrated by human sensory experiments to create the impression of distinguishable olfactory percepts, coined "odor object".[45] Recently, evidence was found that the identification and discrimination of complex olfactory stimuli rely on the formation and modulation of such "odor objects" in the piriform cortex.[45] Convergent findings from human and rodent models suggest that distributed piriform ensemble patterns of olfactory qualities and categories are crucial for maintaining the perceptual constancy of ecologically inconstant stimuli.[45]

Artificial re-engineering of such complex olfactory objects by using synthetic blends of 3, 12, 27, and 28 odor-active key molecules, each in its natural concentration as present in the given food, was convincingly demonstrated to reconstruct the authentic chemosensory percept of, for example, sour-cream butter,[55] fresh strawberry,[34] Arabica coffee,[56] and red wine,[41] respectively. On the other hand, compound mixtures showed an increasingly similar smell with increasing the number of their components.[57] Odorant mixtures comprising more than 30 volatiles were reported to reach a generic quality coined "olfactory white" under two conditions; first, when the mixture components span olfactory space and, second, when the individual odorants are of equal intensity.[57] To understand how the plethora of food odors is coded and why these are different from "olfactory white", first of all, the chemical stimulus space of nature's chemosensory entities needs to be defined on a molecular level.

4. Less than 3% of Foodborne Volatiles Constitute the Chemical Odorant Space

Aimed at defining the olfactory space of molecules in our daily diet, a systematic review (meta-analysis) was performed on the basis of a comprehensive literature survey by means of a Scifinder search using the terms "aroma analysis" and "flavor analysis" to give, after removal of replicates and patent literature and after further refinement using the search term "food", a total of 5642 publications in the period from 1980 to 2013. Furthermore, the German National Library was screened for the terms "aroma" and "flavor" to give 949 hits, including books, monographs, and PhD theses.

As the chemical characterization of the olfactory space of food and beverages requires the correct and comprehensive qualitative and quantitative capture of the entire set of key odorants[28] rather than a constrained consideration of only individual subgroups of volatiles, the following pre-specified inclusion criteria were applied for the meta-analysis:

1) bioactivity-directed discovery of most intense odorants based on GC-O analysis of serially diluted volatile extracts;[30,33,35,58]
2) unequivocal identification of key odorants by comparing chromatographic retention time, mass spectrometric, as

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well as sensory data with those of independently synthesized reference compounds;[59]

3) comprehensive quantitation of the entire set of key odorants using accurate and robust techniques, such as stable isotope dilution analyses.[21,28,34,42,60]

Using this quality-oriented strategy, we considered a total of 119 publications reporting on the key food odorants (KFO) in 227 food samples from a wide range of categories, such as alcoholic beverages, meat products, fish and sea food, cereal and bakery products, dairy products, fats and oil seeds, fruits, vegetables, mushrooms, spices and herbs, cocoa and chocolate, coffee, tea, and some others including soy sauce, balsamic vinegar, honey, caramalt, and popcorn (Figure 1). The assignment of the 227 individual food samples as well as the source literature can be found in the Supporting Information, Figure S1.

In 81 out of these 119 publications, biomimetic mixtures of the KFOs, each in its natural concentration as determined in the respective food, were confirmed to match the odor profile and intensity of the authentic food by performing aroma recombination/omission and/or spiking experiments using highly purified synthetic reference odorants, thus demonstrating that the entire set of KFOs has been completely elucidated.[34,41,55,56]

As the odorants identified could not be considered in their perceived supra-threshold intensity, we approximated their odor impact by using their odor activity values (OAV) calculated as the ratio of concentration to odor threshold of a given volatile.[29,30,38] This procedure led to the identification of a total of 226 key food odorants (KFOs), defined by an OAV ≥ 1 at least in one of the 227 food samples considered in the present study. This allows the conclusion that less than 3% of the 10000 volatiles expected in foods contribute to their specific smell, thus implying that not much more than these circa 230 KFOs define the volatile stimulus space of most of our food and beverage categories.

5. Sensomics Analysis Reveals "Generalists" and "Individualists"

To visualize the results of this meta-analysis, the 226 KFOs were arranged in order of increasing abundance in the food

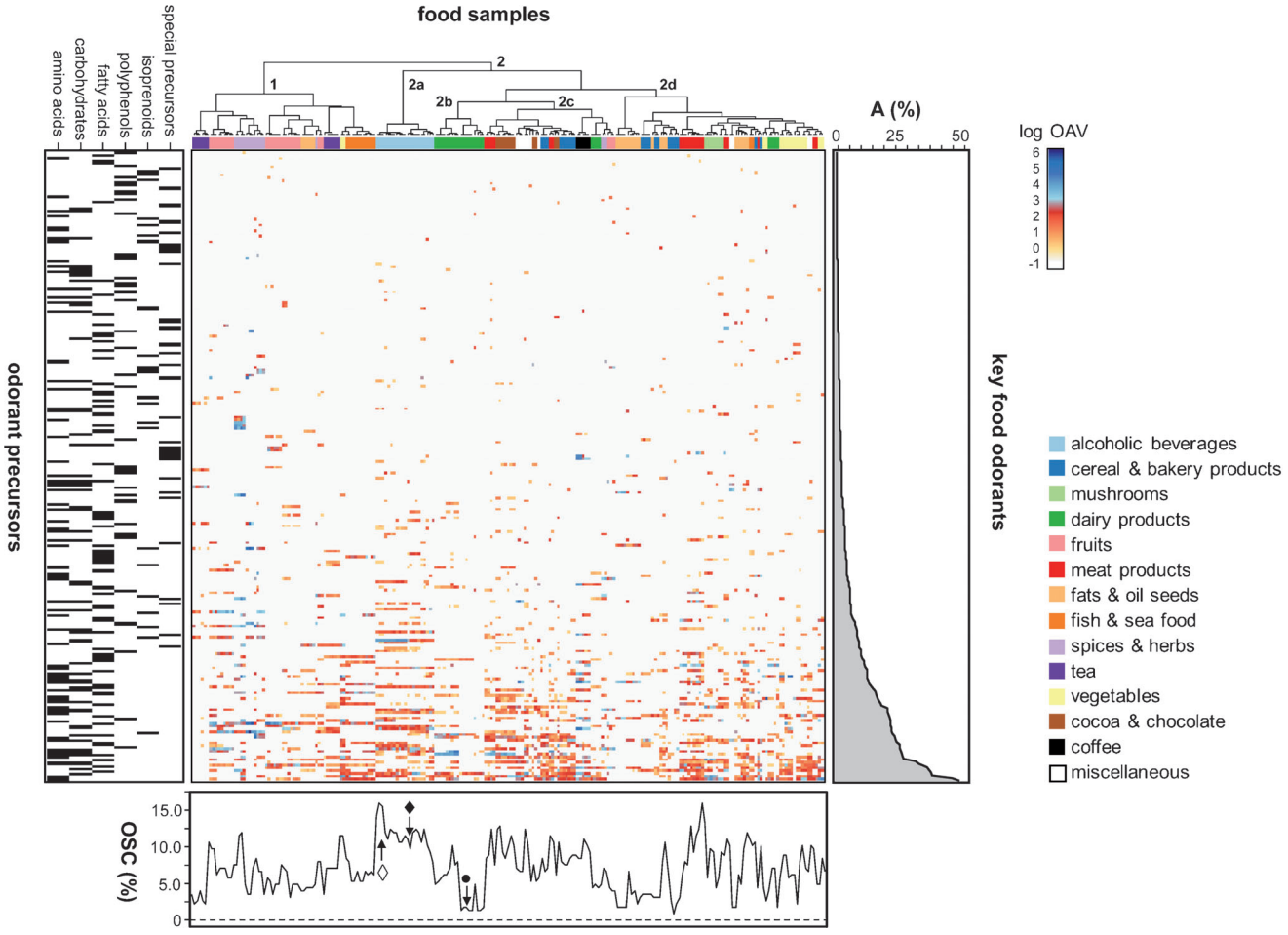

Figure 1. Heatmap displaying the odor activity values (OAVs) and the relative abundance (A, [%]) of the 226 key food odorants (KFOs) characterized in 227 food samples. The odorant space coverage (OSC, [%]) defines the percentage of the olfactory space (226 KFOs) covered by the number of KFOs in the individual food samples; as examples, cognac (◇; 36 KFOs), beer (◆; 18 KFOs), and cultured butter (●; 3 KFOs) are highligthed with an arrow. Precursor molecules leading to KFOs are grouped into classes and are given in the Supporting Information, Table S1. The assignment of the 227 individual food samples as well as the source literature can be found in the Supporting Information, Figure S1.

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samples, whereas the 227 food samples were clustered in accordance with the multivariate distances between the patterns of their KFOs (calculated on the basis of OAVs) using an hierarchical cluster analysis to result in a total of 51302 bins, either occupied with the color-coded stimulus strength (expressed by a $\log \mathrm{OAV} \geq 0$) of each odorant in each food, or remaining empty ($\log \mathrm{OAV} < 0$; Figure 1).

Based on dose-activity considerations, the KFOs can be classified into two groups of volatiles: on one hand, high-threshold volatiles reaching their odor impact by their high levels in foods, for example, the high odor thresholds of 16 and 13 $\mu g\,L^{-1}$ for acetaldehyde and (*R*)-limonene, respectively, are compensated by the high concentrations of 6150 and 2308 $\mu g\,L^{-1}$ found for these odorants in hand-squeezed grapefruit juice.[61] On the other, trace-level volatiles exceeding their low threshold concentrations at low concentrations, for example, the grapefruit-like smelling 1-*p*-menthene-8-thiol and the cooked apple-like smelling (*E*)-β-damascenone showed concentrations of 0.01 and 0.9 $\mu g\,L^{-1}$ in grapefruit juice and red wine, respectively, but exceeded their extraordinarily low odor threshold of 0.0002 and 0.01 $\mu g\,L^{-1}$ by a factor of 50 and 90 (Table 1).[41,61]

Ranking the total of 226 KFOs in the order of their abundance in the considered food samples revealed 16 "generalists" which were detected as KFOs in more than 25% of the 227 food samples investigated (Figure 1, abundance diagram on right hand side). For example, the cooked potato-like smelling 3-(methylthio) propanal (methional) and the malty smelling 2- and 3-methylbutanal contribute to the aroma of more than 50% of all the food samples, followed by butane-2,3-dione (buttery), (*E*,*E*)-2,4-decadienal (fatty, fried), 2,5-dimethyl-4-hydroxy-3(2*H*)-furanone (caramel-like), hexanal (green, grassy), 4,5-dimethyl-3-hydroxy-2(5*H*)-furanone (sotolon; seasoning-like, maple syrup-like), 1-octene-3-one (mushroom-like), acetic acid (vinegar-like), acetaldehyde (fresh, fruity), ethyl 2- and 3-methylbutanoate (fruity), (*E*)-2-nonenal (cardboard-like), vanillin (vanilla-like), 2-acetyl-1-pyrroline (roasty, popcorn-like), 2- and 3-methylbutanoic acid (sweaty), and butanoic acid (sweaty; Figure 1). The names and abundance of generalists can be found in the Supporting Information, Table S1.

Table 1: Odorant qualities and threshold concentrations of selected key food odorants (KFO).

KFO	Odor quality	Threshold conc. [$\mu g\,kg^{-1}$ water][a]
ethanol	alcoholic	990000
2-methyl-1-propanol	malty	19000
acetic acid	vingar-like	5600
1-hexanol	green, grassy	590
(*E*)-2-hexenal	green, apple-like	110
2-phenylethanol	flowery, wine-like	18
(*R*)-limonene	citrus-like	13
2-methoxy-4-vinylphenol	smoky	5
3-hydroxy-4,5-dimethyl-2(5*H*)-furanone	seasoning-like	2
butan-2,3-dione	butter-like	1
3-methylbutanal	malty	0.5
3-(methylthio)propanal	cooked potato-like	0.4
(*E*)-2-hexenal	green, grassy	0.1
2-acetyl-1-pyrroline	popcorn-like	0.05
(*E*,*E*)-2,4-decadienal	fatty, French fries-like	0.03
wine lactone	coconut-like	0.02
(*E*)-β-damascenone	cooked apple-like	0.01
(*E*,*Z*)-2,6-nonadienal	cucumber-like	0.005
(*Z*)-1,5-octadien-3-one	geranium-like	0.0003
1-*p*-menthene-8-thiol	grapefruit-like	0.0002
2-methyl-3-furanthiol	meaty, bouillon-like	0.00003

[a] Threshold concentrations were taken from the in-house database of the German Research Centre for Food Chemistry. Threshold concentrations were determined in bottled water using a three-alternative forced-choice procedure in ascending concentrations as recommended by the American Society of Testing and Materials (ASTM).

The wide-spread "generalists" seem to be primarily released from carbohydrates, amino acids, and unsaturated fatty acids as ubiquitously present biosynthetic precursors (Figure 1, left hand side, lower end of bar-code; Supporting Information, Table S1). These non-volatile bulk components are either transformed by enzymatic reactions, for example, in plant-derived food and/or fermentations, or are non-enzymatically degraded by Strecker- and Maillard-type reactions, for example, upon drying, boiling, baking, or roasting processes: methional (**1**) is generated from methionine,[8,62] 2- and 3-methylbutanal (**2**) and 2- and 3-methylbutanoic acid (**3**) from isoleucine and leucine,[8,62] 2-acetyl-1-pyrroline (**4**) from proline,[62–64] and acetaldehyde as well as acetic acid from alanine,[8,62] butane-2,3-dione (**5**),[8,64,65] 4,5-dimethyl-3-hydroxy-2(5*H*)-furanone (**6**),[65,66] 2,5-dimethyl-4-hydroxy-3(2*H*)-furanone (**7**),[62,64,67] acetaldehyde,[8,62] and acetic acid[8,62] are carbohydrate transformation products, (*E*,*E*)-2,4-decadienal, 1-octene-3-one, and (*E*)-2-nonenal are formed from unsaturated fatty acids by lipid oxidation,[64] butanoic acid is generated upon anaerobic fermentation of carbohydrates as well as lipolysis of triglycerides,[8,68] ethyl 2- and 3-methylbutanoate is originating from amino acid catabolism,[8] and vanillin from phenylpropenoic acid or lignin transformation.[8,69] As an example, the reaction routes leading to generalists **1**–**7** are highlighted in Figure 2.

Apart from the 16 "generalists", another 57 KFOs contributed to the aroma of 5–25% of the food samples ("intermediaries"), while a large body of 151 "individualists" was present in less than 5% of the foods (Supporting Information, Table S1). Among the latter group some highly distinctive odorants contribute to the typical odor signature of only a single or a very few food entities. Among the individualists are, for example, the balsamic smelling (*E*,*E*,*Z*,*E*)-1,3,5,9-undecatetraene (**8**) in hops,[70] the sulfury, garlic-like smelling diallyl disulfide (**9**) in garlic,[71] the green onion-like smelling dipropyl disulfide (**10**) in onion,[72] the fruity, grapefruit-like smelling 1-*p*-menthene-8-thiol (**11**) in grapefruit juice,[61] and the coconut-like smelling (3*S*,3a*S*,7a*R*)-3a,4,5,7a-tetrahydro-3,6-dimethyl-benzofuran-2(3*H*)-one (wine lactone; **12**) in red and white wine[41,73] as well as orange juice (Figure 3a).[74] Names and abundance of individualists can be found in the Supporting Information,

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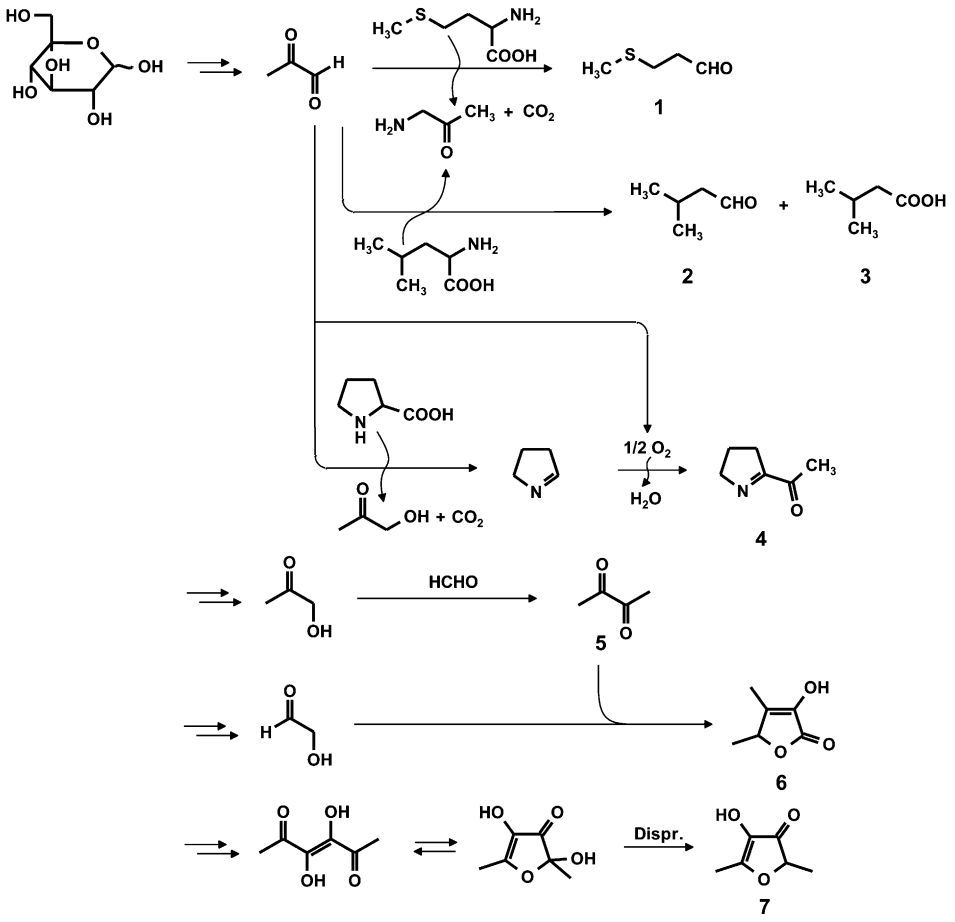

Figure 2. Reaction routes leading to the "generalist" odorants methional (**1**), 3-methylbutanal (**2**), 3-methylbutanoic acid (**3**), 2-acetyl-1-pyrroline (**4**), butan-2,3-dione (**5**), 4,5-dimethyl-3-hydroxy-2(5*H*)-furanone (**6**), and 2,5-dimethyl-4-hydroxy-3(2*H*)-furanone (**7**) by non-enzymatic reactions of reducing carbohydrates and amino acids.

Table S1. Most of these "individualists" exhibit very low odor threshold concentrations, for example, 0.02 $\mu g\,kg^{-1}$ by wine lactone, 0.0002 $\mu g\,kg^{-1}$ by 1-*p*-menthene-8-thiol, and 0.00003 $\mu g\,kg^{-1}$ by the meaty, bouillon-like smelling 2-methyl-3-furanthiol (Table 1).

Consequently, the lack of such single high-impact molecules in biomimetic aroma recombinants of foods does not enable the authentic odor character of the respective food to be reconstructed. For example, the omission of the coconut-like-smelling wine lactone and the fruity, grapefruit-like-smelling 1-*p*-menthene-8-thiol from aroma recombinants of white wine (28 KFOs) and grapefruit (24 KFOs), respectively, strongly reduced the similarity of the odorant cocktails with the original food.[61,75]

In contrast to the "generalists", the conversion of highly distinctive precursor molecules comprising polyphenols, isoprenoids, and other largely unkown biosynthetic metabolites contributes mainly to the generation of "individualists" (Figure 1, left-hand side, upper end of bar-code; Supporting Information, Table S1): For example, the hunt for the wine lactone precursor revealed (*E*)-2,6-dimethyl-6-hydroxyocta-2,7-dienoic acid glucose ester (**13**) as well as its aglycon as candidate molecules in grapes,[76] among which the free acid was demonstrated by wine-relevant model studies and stereoselective deuterium-labeling experiments to give rise to the wine lactone (**12**) via a non-enzymatic stereoselective cationic cyclization cascade including a 1,3-hydride shift (Figure 3b).[77,78] In other words, many of the "individualists" imply the presence of specialized precursor molecules characteristic for one or just a very few food products. Although knowledge on these precursors is still in its infancy, the bioengineered overproduction of such metabolites and their ability to generate desired odorants under mild and controlled conditions will be of high value for biotechnological applications.

6. Combinatorial Food Odor Codes Comprise 3–40 Key Odorants

The hierarchical cluster analysis of the 227 food samples showed two large clusters, cluster **1**, containing the samples of the categories tea, fruit, spices, and seafood, and cluster **2** with four subclusters: subcluster **2a** grouping alcoholic beverages, subcluster **2b** comprising most of the dairy products, and the rather scattered subclusters **2c** and **2d** containing the various meat products, vegetables, fats & oil seeds, cocoa, coffee, cereal & bakery products (Figure 1). The individual "odor codes" of the 227 food samples, considered in the present meta-analysis, are surprisingly small and comprise a center group of a maximum of 36 key odorants (cognac) out of the 226 KFOs in distinct concentration ratio.

Unlike the artificial "olfactory white" conditions,[57] the complex olfactory object of an authentic food does not span stimulus space as demonstrated by the low odorant space coverage (OSC) of maximally 16% across all the food categories (Figure 1, bottom part). Despite the low OSC value, there are significant differences in the complexity of the odor codes of the different foods, for example, the odor of the various alcoholic beverages comprises 18 (beer) to 36 KFOs (cognac), corresponding to 8-16% of all KFOs, while only 1% of natural KFOs is used to create the authentic olfactory percept of cultured butter with only three volatiles, namely butan-2,3-dione (buttery), δ-decalactone (coconut-like), and butanoic acid (sweaty).[55]

The olfactory perception of foods and beverages is, however, not coded exclusively by the identity of its individual KFOs, but also by huge differences in the odor

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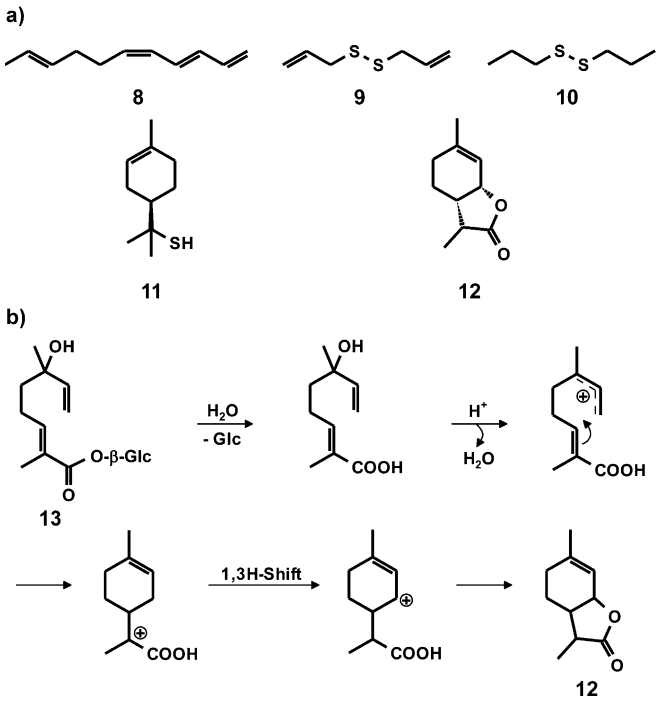

Figure 3. a) Chemical structures of "individualist" odorants (*E*,*E*,*Z*,*E*)-1,3,5,9-undecatetraene (**8**),[70] diallyl disulfide (**9**),[71] dipropyl disulfide (**10**),[72] 1-*p*-menthene-8-thiol (**11**)[61] and (3*S*,3a*S*,7a*R*)-3a,4,5,7a-tetrahydro-3,6-dimethylbenzofuran-2(3*H*)-one (wine lactone, **12**),[41,73] and b) reaction route leading to wine lactone (**12**) from its precursor (*E*)-2,6-dimethyl-6-hydroxyocta-2,7-dienoic acid glucose ester (**13**).[76]

impact of the individual stimuli which is determined by the ratio of the concentration and the odor threshold level of the components. For instance, the 28 KFOs recombined in natural concentrations to match the odor percept of a Dornfelder red wine ranged from 0.9 $\mu g\,L^{-1}$ for the cooked apple-like smelling (*E*)-1-(2,6,6-trimethylcyclohex-1-en-1-yl)but-2-en-1-one, known as (*E*)-β-damascenone, over 79040 $\mu g\,L^{-1}$ for the flowery smelling 2-phenylethanol up to 641900 $\mu g\,L^{-1}$ for the vinegar-like smelling acetic acid.[41] Considering their odor detection thresholds of 0.01, 18, and 5600 $\mu g\,L^{-1}$ (Table 1), respectively, (*E*)-β-damascenone, 2-phenylethanol, and acetic acid show largely different odor activity values (OAVs) of 90, 4391, and 115, thus demonstrating that the key food odorants in a natural food setting show huge differences in their individual odor impact and flavor contribution and are far from being present in equal intensity.

7. Combinatorial Food Odor Codes Carry Information on Food Manufacturing Practice

To visualize the influence of processing on a food's combinatorial odor code, a quantitative metrics describing network structure was constructed by using the KFO meta-analysis data to quantify the topological importance of a node (food) in a network and to identify community structures within the network by studying the relationship between the topology of the individual food odor codes (Figure 4).

Sorting and color-coding the food samples according to the manufacturing process that was applied revealed that the topology of the network is highly nested, with separate clusters of non-processed (raw), fermented, aqueous thermally processed (boiled, cooked), and dry thermally processed (roasted, deep-fried, baked) foods (Figure 4). Highly distinct modular structures are found for fermented foods like cheeses (circle 1, Figure 4) and alcoholic beverages (circle 2), for thermally processed fish (circle 3), as well as for non-processed foods, such as citrus (circle 4) and apple juices (circle 5). Interestingly, some of the food samples showed high correlation between two categories owing to the combined application of fermentation and thermal treatment in the manufacturing of, for example, heated yeast extract (circle 6).

To investigate the underlying odor code of beverages, their KFO data were extracted, treated by means of a hierarchical cluster analysis, and visualized as a heatmap comprising 123 key odorants and 51 beverage samples (Figure 5). The source literature can be found for the respective food samples in the Supporting Information, Figure S1. The samples of green tea (8 KFOs), black tea (16 KFOs), roasted coffee (23 KFOs), fennel tea (9 KFOs), and buttermilk (4 KFOs) were separated in cluster **A1**. Cluster **A2** was split into the subclusters **A2′** containing fruit juices made from orange (22 KFOs), grapefruit (24 KFOs), apple (12 KFOs), pineapple (9 KFOs), and strawberry (10 KFOs), and subcluster **A2″** comprising all alcoholic beverages including white wine (22–27 KFOs), red wine (24–27 KFOs), beer (17–20 KFOs), cognac and brandies (36 KFOs), American bourbon (24–26 KFOs), and Scotch single malt whiskies (28–30 KFOs).

All alcoholic beverages showed major similarities in KFO subclusters **B1** and **B2** comprising expected yeast fermentation products,[79] namely ethanol (alcoholic), butan-2,3-dione (buttery), 3-methyl-1-butanol (malty) and 2-phenylethanol (flowery), as well as the fruity smelling esters ethyl butanoate, ethyl 2- and 3-methylbutanoate, ethyl 2-methylpropanoate, ethyl hexanoate, and ethyl octanoate (Figure 5). Moreover, the cooked apple-like smelling norisoprenoide (*E*)-β-damascenone, known to be generated from glycosidic precursors originating from the plant raw material (grapes, barley, corn, rye),[80] contributes to the typical odor signature of all alcoholic beverages.

Along with this general set of fermentation metabolites, the specific odor of spirits (whisky, brandy, cognac) is characterized by 1,1-diethoxyethane (fruity), phenylethyl acetate (flowery), and methylbutyl acetate (fruity), the smoky smelling phenols 2-methoxyphenol and 4-ethyl-2-methoxyphenol (phenolic, clove-like), vanillin (vanilla-like), 2-methyl-1-butanol (malty), (*E*)-2-nonenal (cardboard-like), and δ-nonalactone (peach-like; **B1** in Figure 5). In contrast to the Bourbon whiskies, the characteristic smoky/peaty note of Scotch Islay single malts is elicited by the higher odor activity values of phenolics, such as for example, propyl-2-methoxyphenol (phenolic), 3- and 4-ethylphenol (phenolic), 4- and 5-methyl-2-methoxyphenol (smoky), 2- and 4-methylphenol (smoky, phenolic), as well as 2-allylmethoxyphenol (clove-like) originating from kilning the malt over peat fire.[81]

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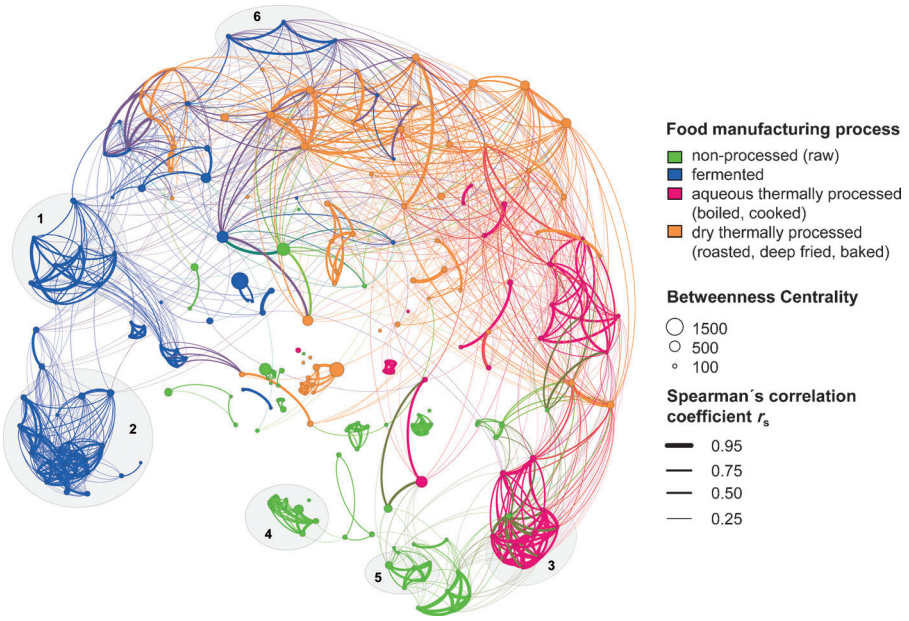

Figure 4. Quantitative matrix describing network structure of the combinatorial odor codes separate foods depending on their history of processing. The betweenness centrality decomposition method was used to quantify the topological importance of a node (food) in the network and to identify community structures within the network by studying the relationship between the topology of the individual food odor codes. Node size measures how often a node appears on shortest paths between nodes in the network; in other words, it indicates both the load and importance of a food sample in the network. Thickness of connecting lines represent the size of the correlation coefficient between the chemical odor code of the food sample. For clarity, correlation coefficients of <0.25 are not shown. Highlighted food groups are cheeses (circle 1), alcoholic beverages (circle 2), cooked fish (circle 3), citrus juice (circle 4), apple juice (circle 5), and heat-treated yeast (circle 6).

Moreover, oak cask maturation or oak chip treatment, used to spice up the flavor signature of barrel aged alcoholic beverages, is reflected by a high odor impact of (3*S*,4*S*)-*cis*-whisky lactone (woody, coconut-like)[88] and vanillin,[8,69] which are thermally released from 4-*O*-(6-*O*-galloylglucosyl)-3-methyloctanoic acid[89] and lignin,[69] respectively, upon toasting the wood prior to use for beverage storage.

Sharing the same esters present in alcoholic beverages, such as ethyl butanoate, ethyl 2-methylpropanoate, ethyl 2- and 3-methylbutanoate, and ethyl hexanoate, the odor code of orange and grapefruit juice (subcluster **A2′**) showed high similarity in cluster **B3** comprising the terpenoids limonene, linalool, myrcene, and pinene, as well as the aldehydes (*Z*)-3-hexenal, octanal, nonanal, and decanal (Figure 5).[90] Also hexanal (green, grassy) contributed equally to both types of juices, whereas the black currant-like smelling 4-methyl-4-sulfanyl-2-pentanone and the grapefruit-like smelling 1-*p*-menthene-8-thiol coined solely the typical odor signature of grapefruit.[90]

Along with the general fermentation metabolites (cluster **B2**), the aroma of beer is characterized by the high odor impact of 2,5-dimethyl-4-hydroxy-3(2*H*)-furanone (caramel-like), 4,5-dimethyl-3-hydroxy-2(5*H*)-furanone (maple-syrup like), methional (cooked potato-like), and 2- and 3-methylbutanal (malty), all thermally formed during the malting process by Maillard-type reactions,[62,63] the clove-like 4-ethenyl-2-methoxyphenol, primarily generated from ferulic acid upon fermentation and contributing to the odor of wheat beer,[8,82] and, in case of the Pilsner-type beer, the flowery linalool released upon yeast fermentation from hop-derived glycosides.[83]

In comparison, the odor code of red and white wines is extended by a high odor impact of additional fermentation products, such as acetic acid, butanoic acid, octanoic acid, decanoic acid, δ-decalactone, and methionol.[8,84] Furthermore, the characteristic signature of individual wine varieties is coined by an increased odor activity of highly distinctive key odorants, such as for example the rose-like smelling *cis*-rose oxide generated from the terpenoid precursor 3,7-dimethyl octa-2,5-dien-1,7-diol (geranyl diol) in Gewürztraminer upon reductive yeast metabolism and acid-catalyzed cyclization[85,86] and the catty, black-currant-like smelling 4-methyl-4-sulfanyl-2-pentanone released from its *S*-cysteine conjugate 4-*S*-cysteinyl-4-methyl-2-pentanone in Scheurebe upon action of the yeast's lyase.[85,87]

The green-smelling volatiles hexanal and (*Z*)-3-hexenal, and the fruity ethyl 2- and 3-methylbutanoate also contributed to the odor code of apple juice and were extended by (*E*)-β-damascenone, (*E*)-2-hexenal, 1-octen-3-one, 1,5-octadien-3-one, dimethylsulfide, methional, 1-butanol, and 1-hexanol.[91]

Along with the green smelling (*Z*)-3-hexenal and the set of fruity smelling esters (methyl butanoate, methyl 2- and 3-methylbutanoate, ethyl butanoate, ethyl 2-methylpropanoate, ethyl hexanoate), the typical odor profile of strawberry and pineapples is created by high OAVs of the caramel-like smelling 2,5-dimethyl-4-hydroxy-3(2*H*)-furanone,[34,92] whereas (*E*,*Z*)-1,3,5-undecatriene (fresh, pineapple-like) and ethyl 2-methyl-3-sulfanylpropanoate (fruity, sulfury) differentiates the pineapple odor code from that of strawberries.[92]

8. Odorant–Receptor Hits Cluster to Phylogenetic Receptor Subsets

Being immersed in a world of chemicals, the chemical senses of mammals have evolved to, for instance, efficiently seek for nutritive foods, and to avoid the ingestion of potentially toxic substances.[93] In contrast to the hard-wired translation of bitter and sweet taste receptor activation into an aversive and attractive hedonic behavior, respectively,

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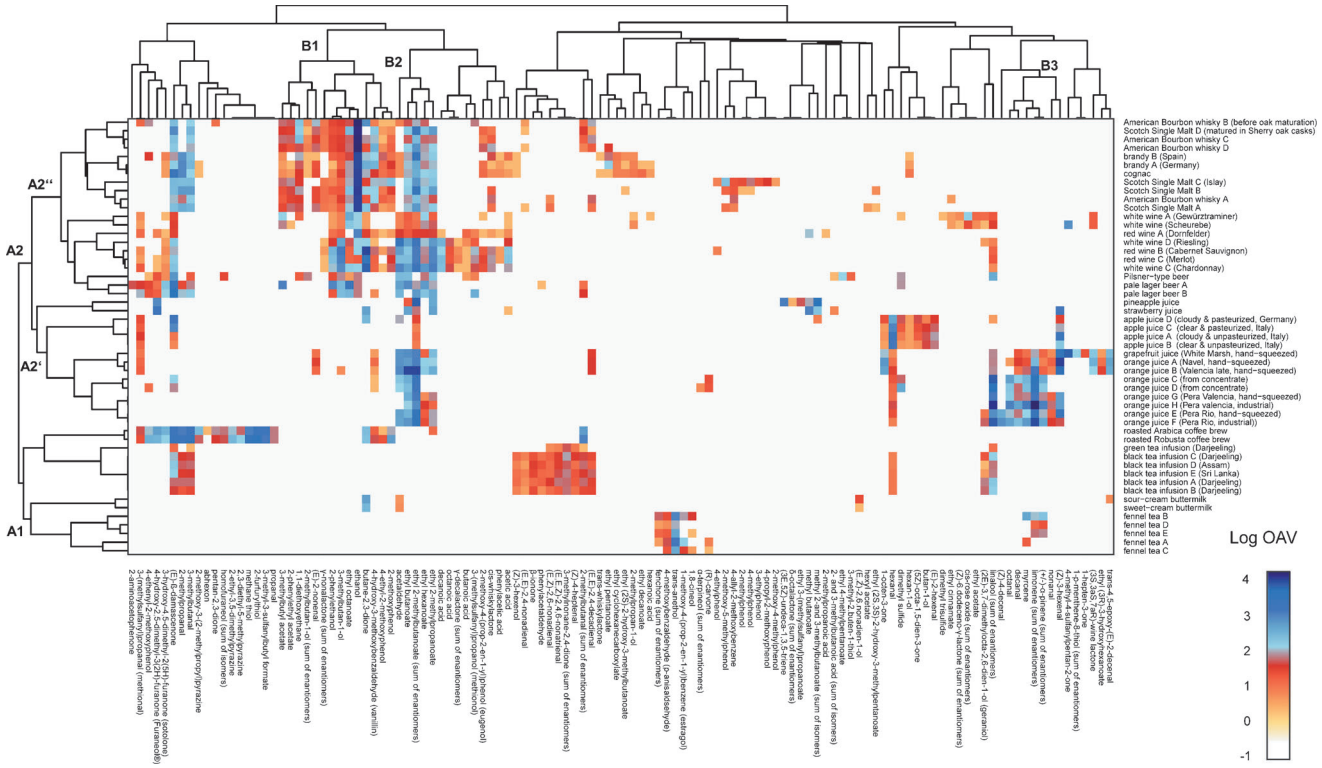

Figure 5. Hierarchical cluster analysis and heat map visualization of beverages (row dendrograms) and their corresponding odor codes (column dendrograms). This heat map is available in the Supporting Information, Figure S2 as a high-resolution, expandable version.

which may, at least in part, be culturally imprinted,[94] innate preferences for food-related odors are much less clear, but are being discussed.[95] Reflecting the prime importance of odor for telling good food from bad food and for being able to hedonically differentiate among foods, the high discriminatory power of the multidimensional stimulus-detector system of olfaction arises from a large multigene family coding for about 400 rhodopsin-like G protein-coupled seven transmembrane helix receptors (GPCR), named odorant receptors (OR).[96] An olfactory function of other receptor multigene families, for example, trace amine-associated receptors (TAAR),[97] or vomeronasal type-I receptors (VN1R),[98] which also detect food-related odors, is inferred from experiments with rodents, but has not been demonstrated in humans to date.

Although the receptors are exposed to ten thousands of volatile chemicals in the surrounding environment, the amount needed for receptor activation differs by several orders of magnitude, for example, spanning from 0.00003 $\mu g\,kg^{-1}$ (2-methyl-3-furanthiol) up to 990000 $\mu g\,kg^{-1}$ (ethanol) (Table 1). However, notably, the number of 226 qualitatively verified, quantitatively determined, and sensorically validated KFOs, which based on our current knowledge represent our foodborne stimulus space, is covered about twice by the number of circa 400 identified putative functional human OR genes.[99] This observation emphasizes the role of KFOs as potent or even best natural activators of a significant portion of our OR repertoire.[100,116] Compared to the sense of taste, comprising few receptors and even fewer taste qualities, the enormous flexibility of a complex and dynamic odorant/receptor space,[97] has the evolutionary benefit of being able to detect a wide range of odors, favoring the hedonic behavior to open up new food sources, and to develop food preferences across cultural imprinting. To date, only 42 human ORs (ca. 10%) were functionally assigned ("de-orphaned") to at least one odorant.[100,116] Correlating all published odorant hits with the phylogenetic relationships of all human OR suggests at first view that odorants with certain physicochemical characteristics, such as functional groups, activate OR from distinct phylogenetic clades (Figure 6). Mammalian OR genes are classified into class I and class II according to their amino acid sequence similarity.[101,102] Here, class I OR is the evolutionary oldest OR class, which is encoded in a single genomic cluster in human and mouse, and is also found in fish,[101] suggesting rather soluble odorant agonists.[102] Indeed, this is reflected by a marked difference in clogD, which is the logarithm of its partition coefficient between *n*-octanol and water, $\log(c_{octanol}/c_{water})$, compared over all published agonists for human and mouse class I OR and class II-OR (Supporting Information, Table S2). Several groups independently found carboxylic acids and esters as agonists for 5 of the 55 human class I OR (Figure 6; Supporting Information, Tables S2, S3).[103–106] This agrees well with an earlier report describing mostly mono- and dicarboxylic acids as agonists for 10 of the 127 mouse class I ORs (Supporting Information, Table S4).[107] Within class II OR, five of the 36 published de-orphaned human class II OR, namely OR1A1, OR1A2, OR3A1, OR1D2, and OR1G1, are encoded by genes from one of the best characterized human OR gene clusters on chromosome 17@3.1.[108] Interestingly, these OR share their best agonists,

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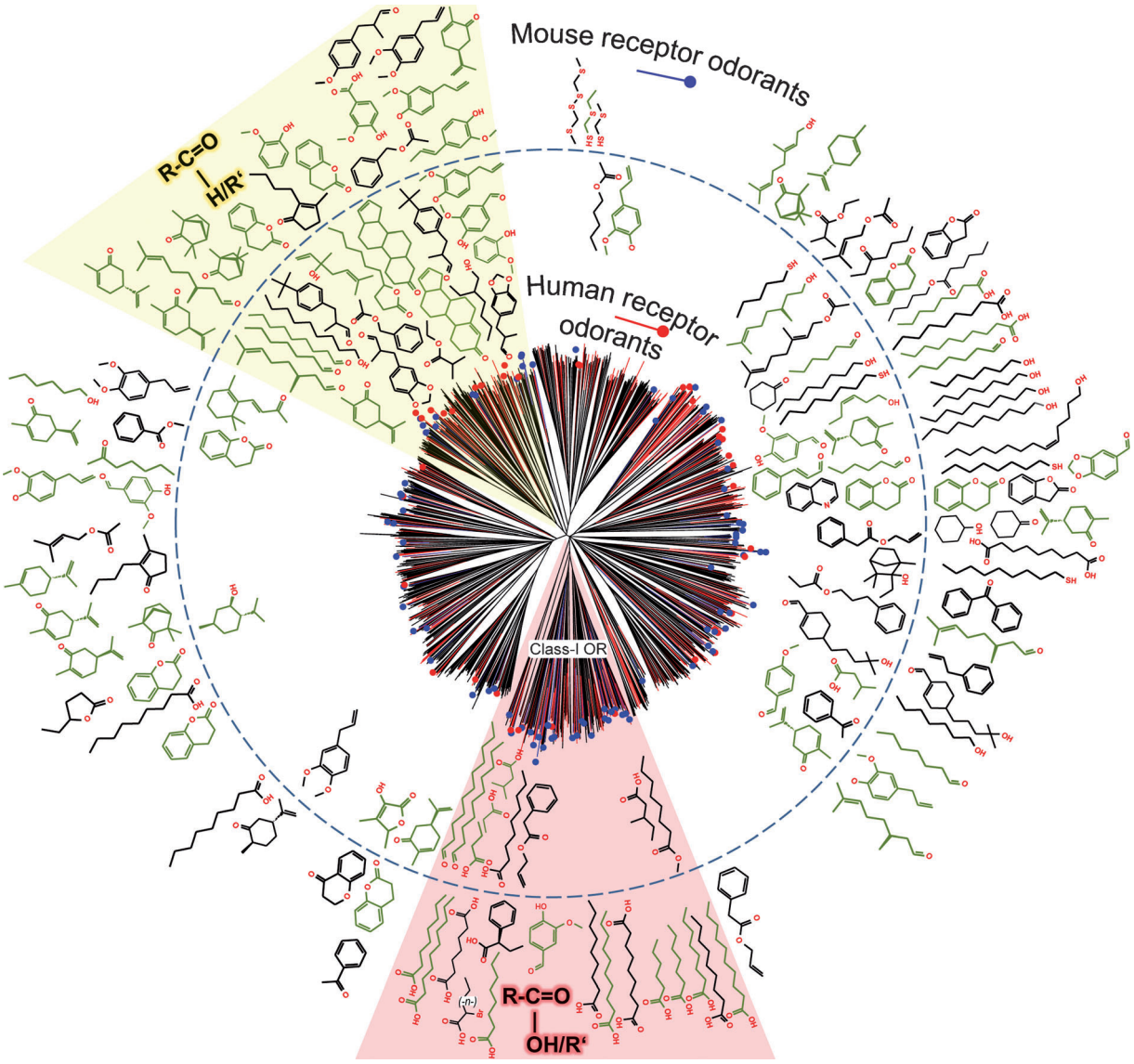

Figure 6. Chemical structures of bioassay-active odorants mapped onto the phylogenetic relationships of all human and mouse odorant receptors (OR). Radial phylogenetic dendrogram of all human (red) and mouse (black/blue) OR. Each line represents one receptor, with the branch length representing evolutionary distance. The bootstrap method was applied to determine the statistical reliability of existing nodes ($n = 500$ replicates), using MEGA5 software. The clade with the class I OR is oriented downward (shaded red). Filled circles depict human (red circles) and mouse (blue circles) receptors with bioassay-based odorant information (Supporting Information, Tables S3, S4).[100–110,114–116] Chemical structures of OR-activating odorants were taken from the Chemical Entities of Biological Interest (ChEBI) library, and were aligned to their respective cognate OR in the dendrogram. Key food odorants are highlighted in green. Note that the number of de-orphaned OR (filled circles) and bioassay-active odorant structures may not match because of combinatorial odorant coding of OR.

centered around aliphatic and aromatic, fruity and floral smelling aldehydes (Figure 6, upper highlighted clade; Supporting Information, Table S3).[105,106,109,110] Other than that, some subfamilies of Class II OR appear to show a rather broad spectrum of chemically versatile agonists (Figure 6).

9. Odorant Receptors are De-orphaned More Likely with Key Food Odorants

At first glance, functionally identified KFOs and non-key food odorants (non-KFO) may seem to be equally represented across the human OR space (Figure 6). However, the meta-analysis including information on all published, as at February 2014, bioassay-tested KFOs as compared to non-KFOs, versus the entire human OR repertoire, as well as data from mouse OR orthologues, suggested a bias toward KFO as best ligands for OR.[100] As a statistical evaluation parameter of the correlation of odorant/receptor hits, the cognate odorant receptor frequency (CORF) was introduced:[100]

$$\mathrm{CORF} = \frac{n(\text{odorant receptor hits})^2}{n(\text{Bioassay tested odorants})\, n(\text{Bioassay active odorants})} \quad (1)$$

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with *n*(odorant receptor hits) representing the number of published bioassay-active best cognate odorant–receptor pairs, *n*(bioassay tested odorants) representing the number of published bioassay-tested odorants, and *n*(bioassay active odorants) representing the number of odorants activating at least one receptor. Up to February 2014, the CORF data suggest a 1.8-times enhanced chance of identifying odorant–receptor pairs when using KFOs (Table 2). This becomes more evident when testing KFO versus specific receptor subsets, such as the ancient class I OR. Here, the CORF value for KFO is even 2.3 times higher compared to non-KFO (Table 2).

Table 2: Comparison of key food odorants (KFO) and other olfactory stimuli (non-KFOs) versus ancient class I and class II odorant receptors (OR).

	OR class I		OR class II	
	KFOs	non-KFOs	KFOs	non-KFOs
bioassay-tested odorants[a]	121	171	121	171
bioassay-active odorants[b]	10	7	26	32
hits[c]	23	15	56	64
CORF[d]	0.44	0.19	1.00	0.75

[a] Number of published bioassay-tested odorants. [b] Number of odorants that could activate at least one receptor. [c] Number of published bioassay-active best cognate odorant–receptor pairs. [d] Cognate odorant receptor frequency[100] reflecting the chance of functional cognate odorant–receptor pairs. Numbers were taken from literature (Supporting Information, Tables S3, S4).[100,114,116a,b]

Most recently, Mainland et al. reported on the comprehensive testing of an entire human OR library, including numerous non-synonymous receptor alleles, against a collection of 60 single odorants, with only 26 of those being KFOs (Supporting Information, Table S5).[116c] With 18 hits from 16 bioassay-active KFOs (CORF: 0.78) versus 8 hits from 8 bioassay-active non-KFOs (CORF: 0.24), confirmed by concentration–response relations, the CORF value again suggests a more than three times enhanced chance of identifying cognate odorant–receptor pairs when using KFOs. In conclusion, over all human OR, the meta-analysis showed that cognate KFO–receptor pairs are about three times more frequent than non-KFO–receptor pairs, despite the fact that KFO are under-represented among all odorants tested in all to date published bioassay-based screening experiments.[100,116c]

Notably, there is growing evidence that for few substances within homologous series or stereoisomers of food-related volatiles, a KFO function and the lowest odor thresholds are strictly correlated. For example, among the eight possible stereoisomers of wine lactone, the coconut-like smelling (3a*S*,4a*S*,7a*R*)-isomer was not only found as the sole naturally occurring stereoisomer in white wines and citrus juices, but even showed an extraordinarily low human odor threshold of 0.00002 $ng\,L^{-1}$ (air), while most of the other stereoisomers elicited higher odor thresholds above 1000 $ng\,L^{-1}$.[73,74] For another group of important food volatiles, the pyrazines, structure–odor activity studies performed by a trained sensory panel revealed by far the lowest human odor thresholds of 0.007–0.018 $\mu g\,L^{-1}$ (water) for the earthy and baked potato-like smelling 2-ethyl-3,5-dimethylpyrazine, 2-ethenyl-3,5-dimethylpyrazine, and 2,3-diethyl-5-methylpyrazine among 70 pyrazines tested,[111] and exactly these high-impact stimuli are those most frequently detected as KFOs in thermally treated foods such as, for example, roasted coffee,[56] French fries,[112] chocolate,[113] cocoa,[42,113] and peanut butter.[42] The low detection thresholds for the KFOs could have been adjusted to match the low concentrations at which these molecules occur in nature, thus implying a coevolution of OR genes and nature's plethora of odorants. This adds to the notion that an odorant space defined by the most abundant KFOs may largely overlap as best cognate matches with the reminiscent functional human OR space. At present it is, however, completely unclear to which extent the natural volatile stimulus space of foods spans our olfactory stimulus space.

Owing to combinatorial receptor codes for odors,[107] that is, a single odorant can activate several odorant receptors, and a single receptor may be activated by several odorants, albeit in a concentration-dependent way, the receptor activity patterns elicited by single odorants may be overlapping, but are largely specific.[104,106,110,114,116] For example, the odorant receptor activity patterns recorded for the sweet, grassy smelling 2*H*-chromen-2-one (coumarin), the fruity smelling allyl phenylacetate, and the urine-like smelling 5α-androst-16-en-3-one are displayed in Figure 7 as EC_{50}-based barcodes. These barcodes show receptor complementation, for example, by activation of OR2B11, OR2J2, OR2W1, and OR5P3 by 2*H*-chromen-2-one, and receptor co-activation, for example, for OR2J2 by 2*H*-chromen-2-one and allyl phenylacetate.[104,106]

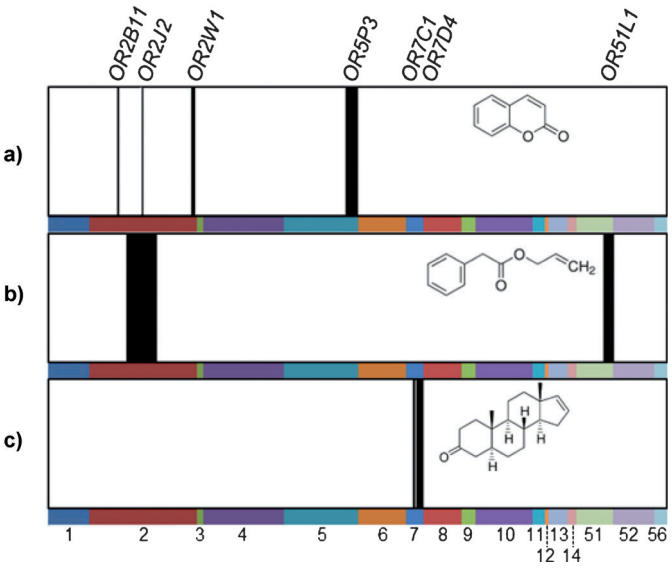

Figure 7. Odorant-specific receptor activity patterns. Examples of odorant receptor activity patterns are given as EC_{50}-based barcodes for a) 2*H*-chromen-2-one (coumarin), b) allyl phenylacetate, and c) 5α-androst-16-en-3-one, with their potencies to activate single receptors coded by the width of the bars, which are logarithmically proportional. Odorant receptor families (*x*-axis) are color-coded and depicted by numbers. For receptor information, their EC_{50} values, and references, see the Supporting Information, Table S3.

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However, foodborne aromas are always rather complex mixtures of volatile chemicals, which may interact at the odorant receptor level in a syntopic competitive or non-competitive way.[115] Thus, while aroma mixtures will not be easily analyzed into their components, mixture-specific and concentration-based receptor activity patterns of validated foodborne aroma recombinates may, nevertheless, be objectified as aroma-specific barcodes, rendering a new and powerful method for food quality control and for knowledge based improvement of food flavors. A comprehensive approach toward de-orphaning our entire OR repertoire (Figure 6) with the entire set of known KFOs (Figure 1) may finally result in a highly resolved picture of the olfactory coding of physiologically relevant, foodborne odorants and their natural mixtures.

10. Future Implications for Applications in the Chemical Industry

In case of the multidimensional sense of olfaction, the sheer unlimited variations in biologically relevant chemosensory entities are, in contradiction to traditional views, defined by a "combinatorial odor code", which comprises a center group of up to 40 out of the circa 230 high-impact odorants identified among the circa 10000 food volatiles in a distinct concentration ratio. This chemical olfactory landscape is finally translated into specific receptor activity patterns ("receptor barcode") elicited by the interaction of the key odorant mixture with their best cognate receptors out of the circa 400 member odorant receptor repertoire. Most impressively, minimal cocktails of 3–40 key odorants, each in its natural concentration, are truly sufficient to synthesize the authentic percept of the specific odor quality of most, if not all foods, although the odor quality of the food is not represented by any of the single ingredients alone.[34,41,55,56] Similarly, cortical patterns of neural activity induced by a compound mixture are unique, and are not evoked by a combination of neural activities induced by the mixtures' components.[117,118] In other words, a key mechanism by which the brain internalizes the external chemical olfactory landscape and encodes perceptual representations of behaviourally relevant odors is through the synthesis of different olfactory inputs into a unitary perceptual experience, that is an odor object, rather than through a analytical combination of components.[45,57,118,119] Given the combinatorial nature of the chemical odor code and the large number of circa 400 different ORs, the number of different perceived odors seems almost infinite,[18] in particular, when compared to our trichromatic color vision, in which we perceive millions of colors although equipped with only three different kinds of photoreceptor cones, the "red-type" L cones, "green-type" M cones, and "blue-type" S cones, plus rods.[120] Unlike the artificial "olfactory white",[57] the complex olfactory object of an authentic food neither contains components of equal intensity as demonstrated by the wide range of OAVs from 1 up to 2 730000 in a food's odor code, nor does it span stimulus space, for example, computing the spread of the KFOs of an individual food in the olfactory space of the 226 key odorants reveals that they reflect extreme clusters (Supporting Information, Figure S1).

This molecular knowledge on chemosensory odor perception at the chemistry–biology intersection is opening up new avenues for biotechnological applications. For example, understanding the odor code of crops and fruits on a molecular level holds promise for improvement of modern breeding strategies which in the past have been typically targeted toward field performance, yield, and storage characteristics while ignoring quality traits, such as aroma and taste.[121] An integrated systems approach combining "sensomics" data, proteomics, gene sequence, and expression data will help to unravel the yet mainly unknown causal links between specific molecular and quality traits, with subsequent application to efficient and targeted crop and fruit improvement strategies.[121,122] Analytical assessment of the KFOs would greatly facilitate the accurate evaluation of a large number of progeny, thus moving the selection of flavor earlier in the selection sequence and increasing the chance of finding truly superior new cultivars for a wide cross-section of food crops.[123] Evenly important and following the consumer's request for truly authentic flavor signatures using odorants of biological origin, a huge economic potential is expected from targeting biocatalytic production of flavor molecules toward the circa 230 naturally occurring KFOs which are essentially needed to create our food's olfactory signatures.

Although the world market of flavors and fragrances, including flavor blends, fragrance blends, essential oils, and aroma chemicals, has a current volume of about $22.2 billion and is projected to rise 4.4% annually through 2016, reaching $26.5 billion, which is still less than 10% of the supply derived from bioprocesses.[124] Examples, such as ethyl (*E*,*Z*)-2,4-decadienoate, a KFO of Barlett pears and products made thereof, which is nowadays cheaper to produce using enzyme catalysis than chemosynthesis, should encourage further research on KFOs as the prime chemosensory target molecules of nature.[10] Although just about 400 enzymes comprising hydrolytic enzymes (for example, lipases, glycosidases, proteases), transferases (for example, cyclodextrin glucanotransferase), oxireductases (for example, alcohol dehydrogenase lipoxygenase, peroxidases, laccase), and lyases (for example, D-fructose-1,6-bisphosphate aldolase, sesquiterpene synthase) have been commercialized primarily for enantioselective organic synthesis as well as the biotechnological production of flavor compounds, the potential for new catalytic reactions in industrial applications is far from being exploited considering the circa 25000 enzymes expected to be present in nature.[125] Moreover, the introduction of new genes into microorganisms and plants has become more or less a routine. But as long as the regulation mechanisms of biosynthetic pathways are not thoroughly understood, increased levels of desired molecules will only be achieved randomly by metabolic engineering.[126] Detailed biochemical analysis of recombinant proteins and studies with transgenic lines, where the gene has been down/up-regulated, are essential. Advancement in research on flavor-coding genes and in genetic engineering is expected to support the identification of metabolic bottlenecks and will help creating novel high-yielding plants and microbial strains,[8,10,126] and by

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complementary bioengineering may provide promising solutions such as improved substrate dosage, optimized reaction media, and in situ product recovery.[8,10,127] This will help to prepare the grounds for the next generation of bioflavor processes utilizing the potential of overproducers and metabolically engineered biosynthetic pathways toward the production of high-value key food odorants.[10,126,128]

Once nature's key odorants are made industrially available by biological means, appropriate quantitative reconstitution will enable companies in food, feed, cosmetic, or pharmaceutical sectors to develop biomimetic recombinants fully constructing the very authentic odor impression of each and every food or environmental entity, for example, convenience and snack products delivering the time-resolved hedonic impression of a "flavor movie" playing the very realistic smell of strawberry fields, children's medicine with attractive flavors, or air fresheners delivering the illusion of being in an English garden or a pine wood forest—all constructed from the same biologically produced key odorants. Although the "scent organ" depicted already 1932 in *Brave New World* remains a literary construction,[129] the reconstruction and fine-tuning of nature's odors can be realized with increasing precision by means of new software-assisted technological devices, such as Givaudan's "virtual aroma synthesizer".[130]

Besides being of economic value, such odor recombinants might be used as highly standardized molecular probes in future olfaction research. Although the olfactory system typically encounters complex chemical mixtures in the environment, yet many scientific studies in olfaction research, rightly striving for precise stimulus control, present single pure chemicals against an odorless background.[131] Findings based on such an approach may fail to uncover important mechanisms that support the detection of complex odor objects against a background of chemical "noise", an arguably crucial and routine aspect of olfactory perception in humans.[131]

The biotechnological reconstruction of biologically relevant chemosensory mixtures will also have a major impact in the creation of fully immersive interactive virtual environments,[132] for example, in serious gaming to augment the virtual experience for reinforcement of learning,[133] improvement of concentration levels,[134] retention of information,[135] and promotion of independent thinking by dispersing authentic aromatic sensations by means of scent delivery technologies in response to specific gaming actions or situations ("olfactory feedback").[136] Moreover, mobile communication devices such as smart phones and tablets vaporizing all type of scents upon demand might open up a new era of olfactory enhanced mobile messaging or deliver a new sensory experience during e-book reading. Although some computer-controlled technological devices delivering smell are already commercially available, it will not necessarily be possible to produce, on demand, nature's authentic odors until the accuracy of smell reproduction is almost perfect.[137] The biotechnological reconstruction of the chemical code of food, body, and environmental odors will be a key step in accomplishing the required smell authenticity.[137] Moreover, these odor recombinants might be helpful for medical applications,[138] for example, to reinforce hedonic experience as part of rehabilitation of patients with olfactory hyperesthesia where the scent to be dispersed can be programmed in real-time by the clinician in response to conversation with the patient, or in therapy of severe and chronically ill cancer or immune deficiency patients, which often suffer from a severely reduced food intake owing to impaired chemosensory abilities and hedonic experience.

Furthermore, such biotechnologically produced odor recombinants might be promising in nutritional research targeting sensory-induced appetite regulation.[139] Orthonasal odor perception of food, one of the complex environmental influences on appetite, is supposed to be a main factor in short-term over-eating, and the increased availability of highly palatable, energy-dense foods is often attributed to the worldwide increase in prevalence of obesity.[140] As retronasal aroma stimulation inhibits the process of prolonged eating, contributing to meal termination,[141] a sensory-induced manipulation of, for instance, a craving state by means of the delivery of highly standardized aroma recombinants ("hedonizers") imparting the very realistic chemosensory profile of high-caloric foods (creamy/dairy, deep-fried, chocolate, savory foods), for which a craving is existing, might open a new avenue to suppress an emerging craving and to counteract phases of overeating.

After a decade of post-genomic progress in cell-based G-protein-coupled receptor assay technologies, it is still one of the major unsolved riddles how the chemically diverse KFOs interact with their respective chemoreceptors to elicit cellular and ultimately systemic responses.[142] Compared to the recent progress in applying the cell-based high-throughput screening technologies for the discovery for novel taste modulators (sweet, salt, and umami enhancers, bitter blockers),[143] the task of characterizing the interactions of odorant receptors and their natural ligands by robust screening platforms is technically more challenging and is still at an earlier stage of development; however, it is a far more cumbersome undertaking.[142] Beyond the shear number of OR, complexity is increased by the excessive occurrence of natural mutations by single nucleotide polymorphisms (SNPs) or copy number variations (CNVs), which on one hand may lead to up to 600 allelic OR variants in an individual, and on the other may strongly affect the function of about 66% of our OR repertoire.[99,116] Indeed, for four OR it was reported that genetic variations by SNPs are correlated with a specific hyperosmia for isovaleric acid, or an anosmia for 2-heptanone, androstenone, and β-ionone.[144] Moreover, syntopic competitive, as well as non-competitive interactions of odorants in mixtures at the receptor level may shape an olfactory perception of odorant mixtures according to pharmacological principles, such as agonism, antagonism, or allosteric modulation.[145] Monitoring binding kinetics of volatile lipophilic odorants to their specific receptors is still in its infancy.[146] Thus, only little is known about human receptor codes for nature's single odorants (Supporting Information, Tables S2, S3),[100,116] and nothing is known about receptor codes for biologically relevant chemosensory mixtures.

Using the biomimetic recombinants of the KFOs, the entire human olfactory receptor repertoire might be chal-

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lenged using ex vivo cell-based assays to elucidate for the first time intensity- and type-specific receptor activity patterns ("receptor barcode") elicited by chemosensory entities of nature. After calibration with the KFO mixture, these might be used to screen for novel molecules that exhibit similar interaction patterns or to "fingerprint" food aromas, body odors, or environmental cues. There is growing evidence that olfactory communication plays a key role in offspring identification and mother recognition, with distinctive olfactory patterns of the para-axillary area, or the nipple-areola region, being important for newborns to recognize their own mother, or as a guide to nourishment, respectively.[147] Moreover, human milk exhibits olfactory clues that foster the newborns' ability to orient themselves versus the human milk source.[148] Infants can distinguish the odor of their mother's breast milk from that of other mothers;[149] however, they prefer the odor of other mothers' breast milk to that of artificial infant formula,[150] thus implying that the odor codes in infant formula differ from that of human breast milk. Identification and quantitation of the odor-active key volatiles in smallscale human milk samples[151] set the ground for the development of improved infant formula containing a mother' breast milk aroma composition to improving the acceptance of nutraceutical compositions for babies and newborns such as infant formula.[152] In the near future, the aforementioned receptor-based technologies may allow the high-throughput analysis of the odor signature of breast milk from individual donors, on the basis of which personalized biomimetic recombinants can be developed to improve the acceptance of hypoallergenic baby formula and breast comfort aid systems, and to help fathers or caregivers being scented with the odor recombinants to endure less rejection by the babies, thus keeping working mothers' life more managable.[152] Moreover, these odor recombinants might support the development of devices designed to pacify a baby when feeling discomfort or pain. First scientific evidence was given by demonstrating that the odor of mother's milk lowered behavioral pain responses (crying, grimacing, and motor activities) in human newborns undergoing routine heelsticks when compared to a control experiment performed with the odor of an infant milk formula.[153]

Elucidating the human receptor codes for biologically relevant single odorants and, in particular, those for biologically relevant chemosensory mixtures will be an important milestone to take, for example, to create highly distinctive, biomimetic high-performance electronic noses which operate, fundamentally different from the discrimination strategy of conventional electronic noses equipped with metal oxide semiconductor detectors or polymer-coated surface acoustic wave sensor arrays,[154] as humanoid odor detectors. After calibration using biomimetic recombinants of KFOs, these systems are empowered to detect and discriminate the truly complex patterns of nature's odors in real-time with highest sensitivity and selectivity, ideally by employing a biosensor-suited array of the circa 400 different human olfactory receptor types together with fabricated nanosensor devices, which produce and amplify the electrical signals from the biological interaction of odors with receptors.[99,155,156] The information of complex odor mixtures will then be converted into a multivariate "odor image", holding a record of the key odorants' binding affinities, as well as their antagonistic, additive, and synergistic interactions at the level of individual proteins of the full human olfactory receptor repertoire. Such non-invasive systems hold promise in many fields, including the food and beverage industry, environmental monitoring, and biomedical applications such as disease diagnostics (urine, blood, saliva, exhaled breath, body odor) or detection of bacterial infections.[155]

Although the gap between the expectations on utilizing principles of chemosensation and what scientists can deliver has often been wide, we fully anticipate that, through multidisciplinary collaboration, chemists, biologists, sensory scientists, experimental psychologists, and (bio)engineers will be able to bridge this gap and will significantly help the chemical industry translating knowledge on the biological mechanisms of olfaction and nature's chemical signatures of smell into novel applications using a new quality of biomimetic mixtures of flavor-active fine chemicals.

11. List of Abbreviations

A	(relative) abundance
CORF	cognate odorant receptor frequency
GPCR	G protein-coupled seven-transmembrane helix receptor(s)
GC-O	GC-olfactometry
KFO/KFOs	key food odorant/odorants
OAV	odor acticity value
OR	odorant receptor(s)

Received: November 1, 2013
Revised: February 2, 2014
Published online: June 18, 2014

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