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Characterization of the Key Aroma Compounds in Rape Honey by Means of the Molecular Sensory Science Concept

Brigitte Ruisinger and Peter Schieberle*

Lehrstuhl für Lebensmittelchemie, Technische Universität München, Lise-Meitner-Strasse 34, D-85354 Freising, Germany

ABSTRACT: By application of aroma extract dilution analysis (AEDA) on the volatile fraction isolated by solvent extraction and solvent-assisted flavor evaporation (SAFE) from unifloral rape honey harvested in July 2009, 28 odor-active areas could be detected within a flavor dilution factor (FD) range of 4−2048. The highest FD factors were found for (*E*)-*β*-damascenone (cooked apple-like), phenylacetic acid (honey-like), 4-methoxybenzaldehyde (aniseed-like), 3-phenylpropanoic acid (flowery, waxy), and 2-methoxy-4-vinylphenol (clove-like). Twenty-three odorants were then quantitated by application of stable isotope dilution assays, and their odor activity values (OAV, ratio of concentration to odor threshold) were calculated on the basis of newly determined odor thresholds in an aqueous fructose−glucose solution. The highest OAVs were calculated for (*E*)-*β*-damascenone, 3-phenylpropanoic acid, phenylacetic acid, dimethyl trisulfide, and phenylacetaldehyde. Quantitative measurements on a rape honey produced in 2011 confirmed the results. A model mixture containing the 12 odorants showing an OAV ≥ 1 at the same concentrations as they occurred in the rape honey was able to mimick the aroma impression of the original honey. The characterization of the key odorants in rape flowers from the same field suggested 3-phenylpropanoic acid, phenylacetic acid, and three further odorants to be transferred via the bees into the honey.

KEYWORDS: *stable isotope dilution assay, odor activity value, (E)-β-damascenone, aroma recombinate, [$^2H_2$]-3-phenylpropanoic acid, solvent-assisted flavor evaporation*

INTRODUCTION

Honey is a natural product collected by honeybees from either plant nectar or honeydew and has been appreciated for thousands of years as a sweet-tasting food with a unique overall aroma. Honey mostly consists of glucose and fructose, whereas the aroma compounds are present in only small amounts.[1] Nonetheless, the aroma is one of the most prominent attributes of honey contributing to honey quality. The overall aroma is strongly influenced by the floral source, storage, and bee physiology[2] and, thus, the aroma of honeys of different floral origin varies considerably. Furthermore, although more than 600 volatile organic compounds have been identified in different honeys in the past decades,[3] studies aimed at selecting odor-active compounds from the bulk of odorless volatiles, in particular for rape honey, are rather scarce. For example, although the first investigation on honey volatiles was already published in 1962,[4] the first aroma extract dilution analysis (AEDA) was applied 25 years later, when Blank et al.[5] identified (*E*)-*β*-damascenone, phenylacetaldehyde, and *p*-anisaldehyde with the highest flavor dilution (FD) factors in linden honey. Through application of GC-O, odor-active compounds were also investigated in honeys of other floral origins.[6,7] About 35 or 46 volatiles were considered odor-active among the ∼400 volatiles reported, but most compounds were only tentatively identified. Application of the AEDA on some Brazilian honeys[8−10] suggested 2-methoxyphenol, phenylethyl alcohol, benzonitrile, 3-methoxybutanoic acid, benzaldehyde, and benzoic acid as odorants with high flavor dilution (FD) factors. Zhou et al.[11] calculated odor activity values (OAVs) for aroma compounds in buckwheat honey on the basis of odor thresholds taken from the literature. The highest OAVs were reported for (*E*)-*β*-damascenone as well as for 2- and 3-methylbutanal and sotolone.

Honey is assigned as rape nectar honey if bees have foraged the nectar mainly from flowers of rape (*Brassica napus*). Although in Germany rape honey is obtained in great quantities,[12] data on rape honey aroma compounds are scarcely available. Kaskoniene et al.[13] analyzed the volatiles of Lithuanian rape honeys and their changes during storage. The authors identified ∼100 volatile compounds, that is, alcohols, aldehydes, acids, linear and branched hydrocarbons, terpenes, or ketones. After 3 months of storage at room temperature, some compounds were no longer detectable, but, for example, dimethyl sulfide, 2-methylbutane nitrile, dimethyl disulfide, hexanal, nonane, dimethyl trisulfide, octanal, heptanoic acid, *p*-cymene, hotrienol, nonanal, lilac aldehydes C and D, *p*-cymen-8-ol, decanal, nonanoic acid, carvacrol, and *β*-damascenone were found to be formed after storage. The first study attempting to correlate volatile compounds in rape honey with sensory properties was carried out by ten Hoopen.[14] He identified six volatile carbonyl compounds, amoung which only diacetyl was detected by its odor in a GC eluate. Steeg and Montag[15,16] quantitated aromatic carboxylic acids, esters, and glycosidically bound compounds in different types of honeys. On the basis of their data, eugenol, 2-methoxyphenol, *p*-cresol, phenylacetic acid, benzaldehyde, and benzyl alcohol were suggested as possible contributors to the honey aroma. Radovic et al.[17] studied the volatile fraction of rape honeys from

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different European countries to characterize their botanical and geographical origin and detected the highest amounts for acetone, ethanol, nonanal, benzaldehyde, and benzyl alcohol. They proposed that rape honey should be characterized by both the absence of 2-methyl-1-propanal and the presence of dimethyl disulfide. Wardencki et al.[18] analyzed rape honey volatiles by application of GC-O. They found 15 odor-active areas, but were not able to unequivocally characterize the chemical structures. Plutowska et al.[19] analyzed the volatiles of some Polish honeys including rape honey. Probably because of the different extraction method, their results clearly differed from those obtained by Radovic et al.,[17] and it was assumed that the volatile fraction of rape honey lacks characteristic components, apart from benzoic acid and benzyl alcohol showing the most abundant peaks in the gas chromatogram.

The literature survey indicates that, up to now, the key aroma compounds of rape honey are still unclear, and no systematic molecular sensory science approach[20] has yet been applied on rape honey. Therefore, the aim of this study was (i) to identify the odor-active compounds in rape honey by application of an AEDA, (ii) to quantitate the most important odorants by means of stable isotope dilution assays, and, finally, (iii) to evaluate the contribution of the key aroma compounds to the overall honey aroma by aroma recombination experiments. In addition, odor-active compounds in rape flowers should be characterized for comparison.

EXPERIMENTAL PROCEDURES

Honey. Two different batches of rape honey were obtained from a local beekeeper in 2009 and 2011. To obtain a unifloral rape honey, the bee hives were placed next to a field of rape during the flowering period, and the honey was extracted directly after ripening in the hive. The fructose and glucose contents (harvest 2009) was determined to be 37.1 and 40.4 g/100 g, respectively. Moisture content was 19%, pH value was 3.9, and the invertase activity was 115.9 U/kg. Honey was harvested in July 2009 and 2011.

To confirm the botanical source, a melissopalynological analysis was performed by the Landesanstalt für Bienenkunde of the University of Hohenheim. The analysis showed 86% rape pollen. The honey was stored in a cabinet (Liebherr, Biberach, Germany) at cool conditions (4 °C) prior to analysis. Rape flowers were collected from the same field.

Chemicals. The reference compounds used for the identification of odorants were purchased from commercial sources: phenylacetic acid, phenylacetaldehyde, 3-methylbutanoic acid, 2-methylbutanoic acid, 3-phenylpropanoic acid, dimethyl trisulfide, 4-allyl-2-methoxyphenol, 3-methylbutanal, 2-methoxyphenol, γ-decalactone, (*E*,*E*)-2,4-decadienal, 3-(methylthio)propionaldehyde, and 4-methylphenol (Sigma-Aldrich Chemie, Taufkirchen, Germany); 2,3-butanedione, benzaldehyde, methyl octanoate, 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone, 2-methylpropanoic acid, and 4-methoxybenzaldehyde (Fluka, Sigma-Aldrich Chemie, Taufkirchen, Germany); 2-phenylethanol (Acros Organics, Geel, Belgium); 2-methoxy-4-vinylphenol, 2-methoxy-5-methylphenol, and 2-methylbutanal (Alfa Aesar, Karlsruhe, Germany); 4-hydroxy-3-methoxybenzaldehyde (Merck, Darmstadt, Germany). (*E*)-β-Damascenone was a gift from Symrise (Holzminden, Germany). [$^{13}C_2$]-2-Phenylacetic acid and [$^2H_5$]-benzyl alcohol were purchased from Sigma-Aldrich.

The following reference compounds were synthesized as previously reported: 2-acetyl-1-pyrroline[21] and *trans*-4,5-epoxy-(*E*)-2-decenal.[22]

Argon and liquid nitrogen were obtained from Linde (Munich, Germany). Dichloromethane and diethyl ether were freshly distilled prior to use. D-Fructose and D-glucose were from Alfa Aesar (Karlsruhe, Germany).

Synthesis of Labeled Standards. [$^2H_5$]-Benzaldehyde was prepared by oxidation of [$^2H_5$]-benzyl alcohol with Dess−Martin−periodinan, which was synthesized according to previous methods.[23,24]

[$^2H_5$]-Benzyl alcohol (110 mg, 1 mmol) was dissolved in dichloromethane (10 mL) and added dropwise to a solution of Dess−Martin−periodinane (0.5 g, 1.2 mmol, in 10 mL of dichloromethane). After stirring for 5 h, dichloromethane (20 mL) was added, and the solution was treated with sodium thiosulfate (20 mL, 0.5 M; saturated with sodium hydrogen carbonate) until the organic layer became clear. The organic layer was washed with water (20 mL) followed by a saturated aqueous solution of sodium chloride (20 mL), then dried over sodium sulfate, and purified by column chromatography on silica gel to obtain [$^2H_5$]-benzaldehyde in a yield of ∼70%.

MS-EI, *m*/*z* (%): 110 (100), 111 (93), 82 (70), 54 (25), 83 (15), 52 (14), 112 (7), 56 (7). MS-CI, *m*/*z* (%): 112 (100), 113 (8).

[$^2H_2$]-3-Phenylpropanoic acid was prepared by deuteration of cinnamic acid following a procedure reported previously for butanoic acid from butenoic acid.[25] Cinnamic acid (5 mmol) was deuterated with deuterium gas (Westfalen, Münster, Germany) for 120 min at room temperature at 5 bar in a laboratory autoclave (Roth, Karlsruhe, Germany) using platinum(IV) oxide (30 mg) as the catalyst. After filtration to remove the catalyst, [$^2H_2$]-3-phenylpropanoic acid was purified by extraction with aqueous sodium bicarbonate as described above (yield = 68%).

MS-EI, *m*/*z* (%): 91 (100), 106 (42), 152 (38), 107 (16), 78 (11), 92 (9), 105 (7), 65 (7). MS-CI, *m*/*z* (%): 153 (100), 135 (42).

The following isotopically labeled standards were synthesized according to previous papers: [$^{13}C_4$]-2,3-butanedione,[26] [$^2H_6$]-(*E*)-β-damascenone,[27] [$^2H_4$]-(*E*,*E*)-2,4-decadienal,[28] [$^2H_2$]-γ-decalactone,[29] [$^2H_6$]-dimethyl trisulfide,[30] [$^2H_3$]-3-methylbutanal[31] [$^2H_3$]-2-methoxyphenol,[32] [$^2H_2$]-3-methylbutanoic acid,[33] [$^{13}C_2$]-phenylacetaldehyde and [$^{13}C_2$]-2-phenylethanol,[34] [$^2H_{2-4}$]-4-propyl-2-methoxyphenol,[35] and [$^2H_3$]-4-hydroxy-3-methoxybenzaldehyde and [$^2H_3$]-2-methoxy-4-vinylphenol.[36]

Determination of the Concentrations of Isotopically Labeled Compounds. Because the syntheses of the labeled compounds were performed in a microscale range, only column chromatography, but neither crystallization nor distillation, could be used for purification. Thus, the concentrations of the isotopically labeled compounds were determined by means of a Trace 2000 gas chromatograph with FID detection (Thermoquest, Egelsbach, Germany) using methyl octanoate as internal standard. First, an FID response factor was determined for methyl octanoate and the respective unlabeled compound. The concentration of the labeled compound was then calculated from the peak areas of methyl octanoate using the FID response factor determined for the respective unlabeled compound.

Isolation of Honey Volatiles. An aliquot of honey (100 g) was diluted with tap water (200 mL), and the volatiles were extracted twice with freshly distilled dichloromethane (total volume = 300 mL). For separation of the aqueous and the organic layer, the mixture was centrifuged at 4500 U/min by means of at Jouan Centrifuge (Thermo Fisher, Fisher Scientific, Schwerte, Germany). The volatiles were isolated from the organic phase by means of the solvent-assisted flavor evaporation (SAFE) technique.[37] To separate the acidic from the neutral−basic volatiles, the SAFE distillate was treated with aqueous sodium carbonate (0.5 mol/L, total volume = 200 mL) to isolate the neutral−basic fraction (NBF). The combined aqueous layers were then adjusted to pH 2 with hydrochloric acid, and the acidic volatiles (AF) were extracted with dichloromethane (total volume = 200 mL). Both fractions were dried over anhydrous sodium sulfate, filtered, concentrated to ∼3 mL using a Vigreux column (50 cm × 1 cm) and, finally, to ∼200 μL by microdistillation. For identification experiments, the honey volatiles (from 500 g of rape honey) were separated by means of column chromatography on silica gel: After concentration of the NBF to 500 μL of *n*-hexane (1 mL) was added and the dichloromethane was carefully distilled off. The hexane extract was applied to a water-cooled glass column (30 cm × 1 cm) filled with a slurry of purified silica gel 60 (7% water) in pentane and separated into seven fractions using pentane/diethyl ether mixtures of increasing polarity (100:0, 95:5, 90:10, 80:20, 70:30, 50:50, 0:100, v/v, 100 mL each).[38] Each fraction was then concentrated to a final volume of ∼200 μL as described above.

Table 1. Isotopically Labeled Standards, Selected Ions, and Response Factors Used in the Stable Isotope Dilution Assays (MS-CI)

odorant	labeled standard	ion (m/z)		
		analyte	internal standard	RF[a]
4-allyl-2-methoxyphenol	[$^2H_{2-4}$]-4-propyl-2-methoxyphenol	165	169−171[b]	0.71
benzaldehyde	[$^2H_5$]-benzaldehyde	107	112	0.94
benzyl alcohol	[$^2H_5$]-benzylalcohol	91	96	0.90
2,3-butanedione	[$^{13}C_4$]-2,3-butanedione	87	91	0.97
(*E*)-β-damascenone	[$^2H_{5-7}$]-(*E*)-β-damascenone	191	196−198[b]	0.89
(*E*,*E*)-2,4-decadienal	[$^2H_{2-4}$]-(*E*,*E*)-2,4-decadienal	153	155−157[b]	1.04
γ-decalactone	[$^2H_2$]-γ-decalactone	171	173	0.93
dimethyl trisulfide	[$^2H_6$]-dimethyl trisulfide	127	133	1.00
4-hydroxy-3-methoxybenzaldehyde	[$^2H_3$]-4-hydroxy-3-methoxybenzaldehyde	153	156	1.02
4-methoxybenzaldehyde	[$^2H_3$]-4-methoxybenzaldehyde	137	140	0.89
2- and 3-methylbutanal	[$^2H_2$]-3-methylbutanal	87	89	0.99
2- and 3-methylbutanoic acid	[$^2H_2$]-3-methylbutanoic acid	103	105	0.92
2-methoxyphenol	[$^2H_3$]-2-methoxyphenol	125	128	0.86
2-methoxy-4-vinylphenol	[$^2H_3$]-2-methoxy-4-vinylphenol	151	154	0.99
phenylacetaldehyde	[$^{13}C_2$]-phenylacetaldehyde	121	123	1.00
phenylacetic acid	[$^{13}C_2$]-phenylacetic acid	137	139	0.86
2-phenylethanol	[$^{13}C_2$]-2-phenylethanol	105	107	1.02
3-phenylpropanoic acid	[$^2H_2$]-3-phenylpropanoic acid	151	153	0.76

[a]MS response factor determined by analyzing defined mixtures of the analyte and the internal standard. [b]Internal standard was used as a mixture of isotopologues.

Isolation of Rape Flower Volatiles. Rape flowers (60 g) were frozen with liquid nitrogen, removed from the stipe, and extracted for 30 min with diethyl ether. After filtration, the volatiles were isolated by SAFE distillation as described above. The distillate was dried over anhydrous sodium sulfate and concentrated to ∼200 μL.

High-Resolution Gas Chromatography−Olfactometry (HRGC-O). HRGC-O was performed by means of a gas chromatograph 8000 (Fisons Instruments, Mainz, Germany) using the following fused silica capillaries: DB-FFAP and DB-1701, each 30 m × 0.25 mm i.d., 0.25 μm film thickness (Agilent, Frankfurt/Main, Germany), and ZB-5, 30 m × 0.32 mm i.d., 0.25 μm film thickness (Phenomenex, Aschaffenburg, Germany). The samples were applied by the cold-on-column technique at 40 °C. After 2 min, the temperature was raised at 6 °C/min to 240 °C (FFAP, 235 °C) and then held for 10 min. The flow of the carrier gas helium (2.2 mL/min) was split 1:1 at the end of the capillary column into an FID (250 °C) and a heated sniffing port (200 °C) using deactivated fused silica capillaries of the same length and a Y-shaped effluent splitter.

Aroma Extract Dilution Analysis (AEDA). For determination of the flavor dilution (FD) factors as the results of an AEDA, first the original distillate was subjected to GC-O on the FFAP column to detect and evaluate the odors of all aroma-active areas. Then, the distillate was diluted stepwise with solvent in a 1:1 ratio (by vol). Each dilution was then analyzed in 1.0 μL aliquots by HRGC-O. To avoid overlooking odor-active compounds, the concentrated distillate was analyzed by at least three experienced panelists.

High-Resolution Gas Chromatography−Mass Spectrometry (HRGC-MS). Mass spectra were acquired using a gas chromatograph 5890 series II (Hewlett-Packard, Waldbronn, Germany) coupled to a MAT 95 S sector field mass spectrometer (Finnigan, Bremen, Germany). Mass spectra in the electron impact (MS-EI) mode were generated at 70 eV, and chemical ionization (MS-CI) was performed at 115 eV using isobutane as the reactant gas.

Quantitation of Odorants by Stable Isotope Dilution Assays (SIDA). For the quantitation of odor-active compounds, different amounts of honey were used (5−500 g) to obtain concentrations between 1 and 5 μg of the respective target compound. The honey samples were then spiked with the labeled standards and stirred in closed glass vessels for 120 min for equilibration. The volatile fraction was then isolated as described above.

Quantitation of 3-phenylpropanoic acid, 2- and 3-methylbutanoic acid, benzaldehyde, benzyl alcohol, and 2-phenylethanol was performed using a Varian 431 gas chromatograph coupled to a Varian 220 ion trap mass spectrometer (both Varian, Darmstadt, Germany) equipped with a 30 m × 0.25 mm, 0.25 μm, FFAP column (J&W Scientific, Folsom, CA, USA). The remaining compounds were quantitated using a two-dimensional HRGC-MS system consisting of a Trace 2000 series gas chromatograph (Thermo Quest, Egelsbach, Germany) coupled to a Varian CP 3800 gas chromatograph and a Varian Saturn 2000 ion trap mass spectrometer (both Varian, Darmstadt, Germany). Mass spectra were recorded in the chemical ionization (CI) mode using methanol as reagent gas.

Mixtures of the respective labeled and unlabeled compound were prepared in five different mass ratios (1:5, 1:3, 1:1, 3:1, and 5:1) and then analyzed by HRGC-MS to calculate the response factor (RF) for each component from the peak areas of the mass fragments (Table 1).

Determination of Orthonasal Odor Thresholds in an Aqueous Fructose−Glucose Solution. The odor thresholds were determined in an aqueous model matrix consisting of fructose (38%) and glucose (31%) adjusted to pH 4.5 with phosphate buffer. To check the purity of the reference compounds, the dilutions were first analyzed by HRGC-O.[39] The reference compounds were used only for determination of odor thresholds, if the FD factor of the target compound was at least 100-fold higher than that of the most intense contaminant in the commercial chemical.[39] A solution of the odorant was prepared in ethanol, of which 0.1 mL was dissolved in 1 kg of the aqueous fructose−glucose solution (pH 4.5) to obtain a 50-fold higher concentration than the estimated orthonasal recognition threshold. The fructose−glucose solution was then stepwise diluted (1:3), and the odor threshold was determined by a forced-choice method using increasing concentrations according to ASTM E 679-04.[40]

Aroma Profile Analysis. Aroma profiles were determined by a trained panel consisting of 18−23 panelists, who participated in weekly sensory sessions to train their ability to recognize and describe different aroma qualities. The following reference compounds were used as aroma descriptors: cooked apple-like ((*E*)-β-damascenone), sweaty (3-methylbutanoic acid), flowery (phenylacetaldehyde), honey-like (phenylacetic acid), clove-like (4-allyl-2-methoxyphenol), flowery−waxy (3-phenylpropanoic acid), and malty (3-methylbutanal). For aroma profile analysis, the intensities of the respective aroma qualities were ranked on a seven-point scale (steps of 0.5) from 0

(not perceivable) to 3 (strongly perceivable). The judgments of the panelists were averaged. Samples (20 g) were presented in Teflon vessels at room temperature.

Aroma Reconstitution Experiments. An aroma model (model I) was prepared in a fructose−glucose solution as described above using all quantitated aroma compounds in the concentrations determined in the honey sample. The recombinate and the rape honey were each placed in closed glass vessels (20 g each), presented to the panel at room temperature, and were evaluated according to the same scale used for aroma profile analysis. All evaluations were performed in triplicates.

Omission Experiments. A second model (model II) was prepared in the same way, but omitting all aroma compounds with an OAV < 1. Both aroma models were then presented to the panel using a triangle test in which all panelists were asked to identify the differing sample. A third aroma model (model III) was prepared by omitting just (*E*)-*β*-damascenone and was presented to the panel as described above. The statistical significance of the evaluation was calculated.

RESULTS AND DISCUSSION

Identification of Odor-Active Compounds. Volatiles were isolated from rape honey (harvest 2009) by solvent extraction followed by SAFE. A drop of the SAFE extract evoked a characteristic rape honey-like odor when sniffed on a strip of filter paper. Thus, it was concluded that the key aroma compounds were successfully extracted.

Application of HRGC-O to an aliquot of the distillate revealed 28 odor-active areas among the honey volatiles. These were then ranked by AEDA on the basis of FD factors.[41] Among the odorants showing FD factors ≥4, a compound with a cooked apple-like odor (**16**, Figure 1) reached the highest FD factor, followed by four odor-active compounds with a honey-like note (**31**), a flowery−waxy-smelling compound (**33**), a clove-like note (**27**), and an aniseed-like odor (**22**). Another clove-like-smelling component (**25**) and a compound with a flowery odor (**12**) were also intensely perceived.

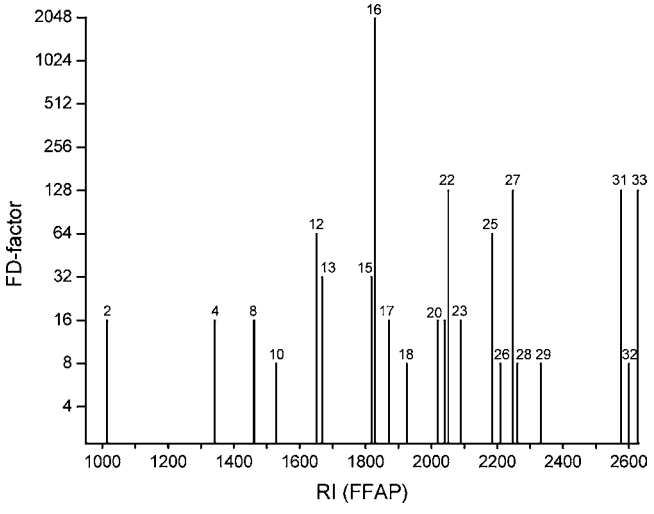

Figure 1. Flavor dilution chromatogram indicating the key odorants in the gas chromatogram of the volatile fraction isolated from rape honey.

For identification of the compounds responsible for the perceived odors, the retention indices (RI) of the odor-active areas were determined on three different stationary GC phases. A comparison of the RIs with data collected in an in-house database for about 1000 food odorants suggested a chemical structure for 25 of the 28 areas sensorially detected. The identification of the structure was then confirmed by comparing the analytical and sensory attributes of the analyte, that is, the retention index, the odor quality as well as odor intensity, and mass spectra in the EI and CI modes, with data for the respective reference compound. To obtain enough material for mass spectrometry (MS), the volatiles were isolated from 500 g of rape honey, and the NBF was further separated by column chromatography.[22] The odorants were then located by GC-O in each fraction and analyzed by MS.

The most intense aroma-active compounds in the NBF were identified as (*E*)-*β*-damascenone (**12**; FD 2048; Figure 2),

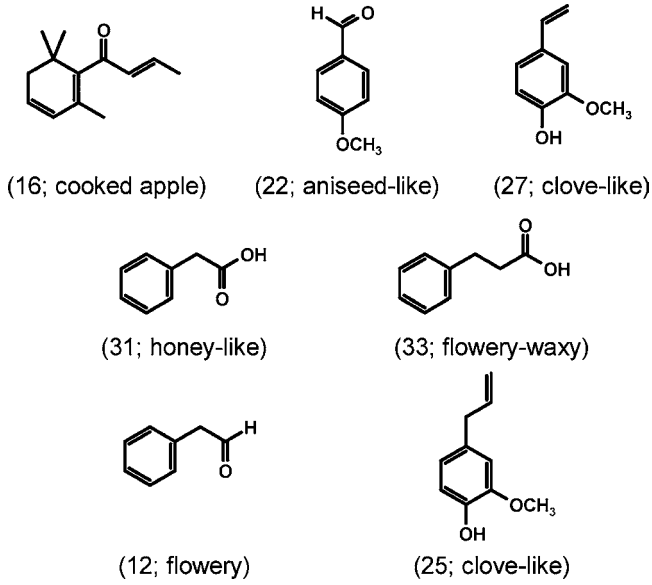

Figure 2. Structures of the most important odorants in rape honey.

smelling like cooked apple; phenylacetic acid (**31**) with a honey-like odor; 3-phenylpropanoic acid (**28**) with a flowery−waxy odor; the clove-like-smelling 2-methoxy-4-vinylphenol (**33**); and 4-methoxybenzaldehyde (**22**) with an aniseed-like odor. Somewhat lower FD factors were found for the flowery-smelling phenylacetaldehyde (**12**) and the clove-like-smelling 4-allyl-2-methoxyphenol (**28**). Altogether 28 odorants with FD factors between 4 and 2048 were identified (Table 2). The results of the AEDA suggested that several odorants with a honey-like and flowery odor (phenylacetaldehyde, phenylacetic acid) as well as clove-like smelling components (4-allyl-2-methoxyphenol, 2-methoxy-4-vinylphenol) and the cooked apple-like smelling (*E*)-*β*-damascenone should contribute significantly to the overall aroma of rape honey. Whereas most of the these compounds have been found in different honeys before,[5,9,11,15] as far as we know, 2-methoxy-4-vinylphenol is reported for the first time as a constituent of rape honey.

Because highly volatile odorants might get lost during distillation and concentration steps, rape honey was also analyzed by static headspace gas chromatography−olfactometry.[34] However, no further odor-active compounds were detected by this method. Dimethyl disulfide, previously described as a characteristic volatile in rape honey,[17] could not be detected in this study.

Quantitation of Important Odorants and Calculation of Odor Activity Values (OAVs). To evaluate of the contribution of aroma-active compounds to the overall rape

Table 2. Important Aroma Compounds (FD ≥ 4) Identified in Rape Honey (Harvest 2009)

no.	aroma compound[a]	odor quality[b]	retention index on FFAP	retention index on DB-1701	retention index on ZB-5	FD[c]	previously identified as volatile in honey
1	2- and 3-methylbutanal	malty	941	742	672	4	13, 17, 19, 43
2	2,3-butanedione	buttery	1015	704	617	16	14, 18, 19, 43
4	2-acetyl-1-pyrroline	popcorn-like	1342	1026	922	16	
6	dimethyl trisulfide	cabbage-like	1383	1045	970	4	13, 18, 19
8	3-(methylthio)propionaldehyde[d]	cooked potato-like	1461	1049	908	16	
10	benzaldehyde	bitter almond-like	1528	nd[e]	963	8	13, 16, 17, 19, 43
11	2-methylpropanoic acid	sweaty	1565	nd	nd	4	13, 19
12	phenylacetaldehyde	flowery	1651	1184	1044	64	16, 18, 19
13	2- and 3-methylbutanoic acid	sweaty, rancid	1668	1031	nd	32	13, 17, 19
14	3-methylpentanoic acid	sweaty	1792	nd	nd	4	13, 19
15	(*E*,*E*)-2,4-decadienal	fatty	1818	1465	1321	32	
16	(*E*)-*β*-damascenone	cooked apple-like	1829	1512	1387	2048	13, 18, 19
17	2-methoxyphenol	smoky	1871	1231	1091	16	16
18	2-phenylethanol	flowery	1925	1237	1095	8	16−19
19	2-methoxy-5-methylphenol[d]	smoky	1958	1332	1187	4	
20	*trans*-4,5-epoxy-(*E*)-2-decenal[d]	metallic	2020	1569	1381	16	
21	4-hydroxy-2,5-dimethyl-3(2*H*)-furanone	caramel-like	2040	1221	1055	16	
22	4-methoxybenzaldehyde	aniseed-like	2051	1442	1257	128	16, 18, 19
23	4-methylphenol	horse-like	2089	1317	1080	16	13, 16, 19
24	*γ*-decalactone	peach-like	2167	1710	1488	4	
25	4-allyl-2-methoxyphenol	clove-like	2185	1521	1360	64	16, 19
26	unknown	spicy	2210	nd	nd	8	
27	2-methoxy-4-vinylphenol	clove-like	2248	1495	1330	128	
28	unknown	spicy	2261	nd	nd	8	
29	unknown	flowery	2332	nd	nd	8	
31	phenylacetic acid	honey-like	2576	1588	1261	128	15, 16, 19, 42
32	4-hydroxy-3-methoxybenzaldehyde	vanilla-like	2600	1640	1408	8	45
33	3-phenylpropanoic acid	flowery, waxy	2628	1653	1330	128	15, 16, 19

[a]The compound was identified by comparison of its mass spectra and retention indices on three different capillaries (i.e., FFAP, DB-1701, ZB-5) as well as the odor quality and intensity perceived during sniffing with the respective reference compound. [b]Odor quality perceived at the GC sniffing port. [c]Flavor dilution factor determined by AEDA on capillary FFAP. [d]No unequivocal mass spectrum was obtained. Identification is based on the remaining criteria given in footnote *a*. [e]nd, not determined.

honey aroma, precise quantitative measurements are required. Therefore, a total of 23 odorants were quantitated in rape honey by means of SIDAs using the respective isotopically labeled reference compounds as internal standard (Table 1). In addition to odorants with high FD factors, some compounds were also quantitated, which have previously been described as major honey volatiles.[17,19]

The highest concentrations were determined for phenylacetic acid, followed by 3-phenylpropanoic acid and 2- and 3-methylbutanoic acid (Table 3). Concentrations >100 μg/kg were also found for benzyl alcohol, 2-phenylethanol, and benzaldehyde. By contrast, very low concentrations (<1 μg/kg) were determined for dimethyl trisulfide, 2-methoxyphenol, *γ*-decalactone, and (*E*,*E*)-2,4-decadienal. The quantitative results for phenylacetic acid were in good agreement with data reported earlier for German rape honeys.[42]

To estimate the contribution of the quantitated odorants to the overall rape honey aroma, OAVs (ratio of concentration to odor threshold) were calculated. Because honey mainly consists of carbohydrates, a model mixture consisting of glucose (31%) and fructose (38%) at pH 4.5 was used for the determination of odor thresholds for 13 honey aroma compounds (Table 4). Odor thresholds clearly depend on the matrix used in the sensory experiments, and even carbohydrates dissolved in water may influence the odor threshold as compared to pure water. For example, the rather high odor threshold previously

Table 3. Concentrations of Important Aroma Compounds in Rape Honey (Harvest 2009)

aroma compound	concn (μg/kg)	range (μg/kg)	*n*[a]
phenylacetic acid	5270	4850−5860	5
3-phenylpropanoic acid	1460	1320−1600	2
2-methylbutanoic acid	1120	1100−1150	2
3-methylbutanoic acid	948	921−975	2
benzyl alcohol	376	359−394	3
2-phenylethanol	276	254−310	3
benzaldehyde	123	117−130	3
4-hydroxy-3-methoxybenzaldehyde	51	49.3−52.5	3
phenylacetaldehyde	35	33.9−35.8	5
2,3-butanedione	16.1	14.2−17.7	3
2-methoxy-4-vinylphenol	14.8	14.3−15.6	6
3-methylbutanal	12.3	11.5−13.5	4
2-methylbutanal	6.6	9.3−12.0	4
4-methoxybenzaldehyde	6.4	6.2−6.8	2
(*E*)-*β*-damascenone	6.0	5.9−6.7	6
4-allyl-2-methoxyphenol	2.9	2.8−2.9	6
dimethyl trisulfide	0.8	0.8−0.8	6
2-methoxyphenol	0.8	0.7−0.8	2
γ-decalactone	0.4	0.4−0.5	2
(*E*,*E*)-2,4-decadienal	0.2	0.1−0.2	2

[a]Number of replicates.

Table 4. Orthonasal Odor Thresholds and Odor Activity Values (OAVs) of Key Odorants in Rape Honey (Harvest 2009)

aroma compound	odor threshold[a] (μg/kg)	OAV[b]
(*E*)-β-damascenone	0.01	600
3-phenylpropanoic acid	27	54
phenylacetic acid	135	39
dimethyl trisulfide	0.03	27
phenylacetaldehyde	2.5	14
2,3-butanedione	2.3	7
3-methylbutanal	2.1	6
2-methoxy-4-vinylphenol	2.8	5
2-phenylethanol	89	3
4-allyl-2-methoxyphenol	1.1	3
2-methoxyphenol	0.34	2
2-methylbutanal	3.2	2
3-methylbutanoic acid	490[c]	2
(*E*,*E*)-2,4-decadienal	0.11	1
4-hydroxy-3-methoxybenzaldehyde	66	<1
benzyl alcohol	620[d]	<1
2-methylbutanoic acid	2200[c]	<1
benzaldehyde	150[c]	<1
γ-decalactone	1.1[c]	<1
4-methoxybenzaldehyde	27[c]	<1

[a]Odor threshold was determined in an aqueous fructose-glucose solution. [b]Odor activity value; ratio of concentration to odor threshold. [c]Odor threshold in μg/kg water.[39] [d]Odor threshold in μg/kg water.[44]

determined in water for the honey-like-smelling phenylacetic acid with 6100 μg/kg water[39] suggests that this odorant should not contribute much to the overall rape honey aroma. However, the odor threshold determined in this study for phenylacetic acid amounted to 135 μg/kg in the fructose–glucose solution and, hence, indicated a greater importance for the honey aroma (Table 4).

The highest OAV among rape honey volatiles was determined for (*E*)-β-damascenone, despite its rather low concentration of 6.0 μg/kg. High OAVs were also calculated for the flowery–waxy-smelling 3-phenylpropanoic acid (**54**), the honey-like smelling phenylacetic acid (**39**), and phenylacetaldehyde (**14**) with a flowery odor quality. These results suggest that compounds with honey-like and flowery notes contribute most to the rape honey aroma. OAVs >1 were also determined for dimethyl trisulfide, 4-allyl-2-methoxyphenol, 2,3-butanedione, 2- and 3-methylbutanal, 2-methoxy-4-vinylphenol, 2-phenylethanol, 2-methoxyphenol, 3-methylbutanoic acid, and (*E*,*E*)-2,4-decadienal. However, benzaldehyde and benzyl alcohol, which were present in quite high concentrations in the rape honey, showed OAVs <1 and, thus, should not contribute to the rape honey aroma as previously suggested in the literature.

Although, for example, 4-methoxybenzaldehyde (aniseed-like) showed a high FD factor of 128, its concentration in rape honey did not reach its odor threshold. This can be explained by the fact the during AEDA, volatiles are completely vaporized and their concentrations are correlated to the odor threshold in air. Thus, the contribution of single volatiles to the aroma might be overestimated. On the other hand, the highly volatile substances 2- and 3-methylbutanal (malty) showed OAVs >1 and were, thus, likely to influence the honey aroma.

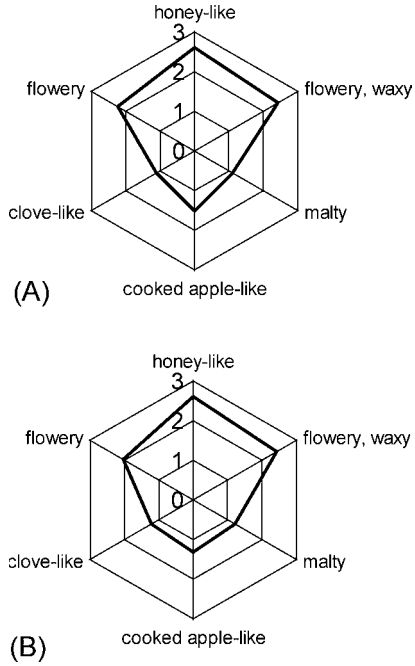

Figure 3. Aroma profile of rape honey (A) and the aroma recombinate (B).

Aroma Recombination and Omission Experiments. As food aroma is a mixture of certain key aroma compounds, it is important to validate the quantitative data on the basis of reconstitution experiments. The aroma recombinate of the respective rape honey was prepared in the same fructose–glucose matrix as used for determination of odor thresholds. The model mixture contained all reference compounds in the concentrations given in Table 3. The cabbage-like-smelling dimethyl trisulfide was omitted in the aroma reconstitute, because its odor quality impaired the overall honey-like aroma of the rape honey recombinate. Either this might be caused by binding of the odorant by honey ingredients, which could not be mimicked in the model mixture, or the odorant might be formed as an artifact during workup.

A trained sensory panel then compared the aroma recombinate and the authentic rape honey. The aroma qualities honey-like, flowery, clove-like, baked apple-like, flowery/wax-like, and malty were nearly similar in both the rape honey sample and the aroma reconstitute (Figure 3). The overall similarity between the rape honey and the recombinate was ranked with 2.6 on a scale from 0 to 3. A second recombinate was prepared containing only odorants with OAV >1 (model II) and was presented to the sensory panel in a triangle test in comparison to model I. The omission of the six compounds with an OAV <1 (Table 4) could be recognized by only 7 of 23 panelists, thus proving that these compounds did not show a substantial effect on the overall honey aroma. Model III, in which only (*E*)-β-damascenone was omitted, was compared to model I in the same way, and only 6 of 16 panelists could distinguish between both models. Hence, it can be concluded that despite its high OAV this aroma compound does not much contribute to the overall honey aroma.

To verify the analytical data and conclusions drawn, a rape honey was collected in 2011. By GC-O, the same odor-active

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substances were detected as in the rape honey from 2009 (data not shown). Then, the 14 aroma compounds, which had also shown the highest OAVs in the 2009 sample, were quantitated and their OAVs calculated (Table 5). The results confirmed the highest OAVs for β-damascenone, phenylacetic acid, and phenylacetaldehyde as found for the honey from 2009. On the other hand, 2,3-butanedione was higher, whereas dimethyl trisulfide was lower. As rape honey is a food product, its composition is influenced by parameters such as climatic conditions or bee behavior. Hence, certain deviation in quantitative results can be assigned to annual variations.

Table 5. Concentrations, Orthonasal Odor Thresholds, and Odor Activity Values (OAVs) of Key Odorants in Rape Honey (Harvest 2011)

aroma compound	concn (μg/kg)	odor threshold[a] (μg/kg)	OAV[b]
(*E*)-β-damascenone	7.6	0.01	760
phenylacetic acid	6860	135	51
3-phenylpropanoic acid	1040	27	39
phenylacetaldehyde	79	2.5	32
2,3-butanedione	55	2.3	24
2-methoxy-4-vinylphenol	18.6	2.8	7
dimethyl trisulfide	0.16	0.03	5
3-methylbutanal	11.1	2.1	5
4-allyl-2-methoxyphenol	3.1	1.1	3
2-phenylethanol	258	89	3
2-methylbutanal	8.5	3.2	3
3-methylbutanoic acid	1530	490[c]	3
2-methoxyphenol	0.6	0.34	1
(*E*,*E*)-2,4-decadienal	0.1	0.11	1

[a]Odor threshold was determined in an aqueous fructose−glucose solution. [b]Odor activity value; ratio of concentration to odor threshold. [c]Odor threshold in μg/kg water.[39]

Identification of Odor-Active Compounds in Rape Flowers. To get a first insight into the sources of the honey odorants, rape flowers were collected from the same rape plants next to where the bees were located. The volatiles were isolated, and the odor-active compounds were located by GC-O and then ranked by AEDA. The green, grassy-smelling (*Z*)-3-hexenal was identified with the highest FD factor in the flower distillate followed by phenylacetic acid and phenylacetaldehyde (Table 6). High FD factors were also found for 2-acetyl-1-pyrroline, dimethyl trisulfide, 2- and 3-methylbutanoic acid, 4-methoxybenzaldehyde, and indole. A comparison between the most intense odorants in the rape honey and the rape flowers (Tables 2 and 6) suggested, in particular, phenylacetic acid, 3-phenylpropanoic acid, phenylacetaldehyde, 4-methoxybenzaldehyde, and 2- and 3-methylbutanoic acid to be transferred by the bees from the flowers into the honey. However, odor-active constituents of the flowers, such as (*Z*)-3-hexenal, 2-isobutyl-3-methoxypyrazine, or indole, did not appear in the final honey. On the other hand, (*E*)-β-damascenone was the most important odorant in the final honey, which did not occur in the flower distillate. Because the flowers had to be macerated for extraction of the volatiles, for example, enzymatic metabolization reactions leading to odorant degradation, could not be completely inhibited.

Table 6. Odorants with High FD Factors (FD ≥ 16) in Rape Flowers

		retention index on		
aroma compound[a]	odor quality[b]	FFAP	ZB-5	FD[c]
(*Z*)-3-hexenal	green, grassy	1141	800	2048
2-acetyl-1-pyrroline[d]	popcorn-like	1335	924	256
(*Z*)-1,5-octadien-3-one[d]	metallic	1371	983	64
dimethyl trisulfide	cabbage-like	1376	968	256
acetic acid	sour	1429	nd[e]	64
3-(methylthio) propionaldehyde[d]	cooked potato-like	1449	904	128
2-isobutyl-3-methoxypyrazine[d]	bell pepper-like	1526	1182	128
phenylacetaldehyde	flowery	1648	1044	512
2- and 3-methylbutanoic acid	sweaty, rancid	1660	nd	256
trans-4,5-epoxy-(*E*)-2-decenal[d]	metallic	2009	1380	128
4-methoxybenzaldehyde	aniseed-like	2041	1263	256
4-allyl-2-methoxyphenol	clove-like	2170	1359	64
indole	mothball-like	2475	1389	256
phenylacetic acid	honey-like	2587	1254	512
3-phenylpropanoic acid	flowery, waxy	2612	1330	16

[a]The compound was identified by comparison of its mass spectra and retention indices on two different capillaries (i.e., FFAP, ZB-5) as well as the odor quality and intensity perceived during sniffing with the respective reference compound. [b]Odor quality perceived at the GC sniffing port. [c]Flavor dilution factor determined by AEDA on capillary FFAP. [d]No unequivocal mass spectrum was obtained. Identification is based on the remaining criteria given in footnote *a*. [e]nd: not determined.

In summary, the results suggest that the rape honey aroma can be closely mimicked using only 12 aroma compounds in their natural concentrations. In particular, amino acid degradation products, such as phenylacetaldehyde and 3-methylbutanal, fermentation products such as 2,3-butanedione, and some phenols such as 4-allyl-2-methoxyphenol and 2-methoxy-4-vinylphenol as well as (*E*)-β-damascenone contributed to rape honey aroma. Honey odorants either can be derived from the floral source, can be changed by the bee, or may be generated during storage and temperature. Because several odorants were also found in rape flowers, some odor-active compounds in rape honey clearly originate from the flower. Because most odorants found in rape honey have previously also been reported in honeys of other floral origin, further investigations on odor-active compounds in different types of honey on the basis of the molecular sensory science concept should be performed to find typical marker odorants for rape honey.

AUTHOR INFORMATION

Corresponding Author

*Phone: +49 8161 71 2932. Fax +49 8161 71 2970. E-mail: Peter.Schieberle@ch.tum.de.

Notes

The authors declare no competing financial interest.

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