Evaluation of Key Aroma Compounds in Hand-Squeezed Grapefruit Juice (*Citrus paradisi Macfayden*) by Quantitation and Flavor Reconstitution Experiments

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Twenty-five odor-active compounds were quantified in the fresh, hand-squeezed juice of White Marsh seedless grapefruits using stable isotope dilution assays. By calculation of the odor activity values of the odorants (ratio of their concentrations in the juice to their odor thresholds in water) it was shown that the fruity esters ethyl 2-methylpropanoate, ethyl butanoate, and (*S*)-ethyl 2-methylbutanoate, and the fruity, sweet winelactone, as well as the grassy smelling (*Z*)-hex-3-enal, and *trans*-4,5-epoxy-(*E*)-dec-2-enal with metallic odor, were among the most potent odorants of the fresh grapefruit juice. The typical sulfurous, grapefruit-like odor quality was mainly due to the catty, blackcurrant-like 4-mercapto-4-methylpentan-2-one and the grapefruit-like smelling 1-*p*-menthene-8-thiol. These findings were confirmed by reconstitution experiments to simulate the aroma of the fresh grapefruit juice.

Keywords: *4-Mercapto-4-methylpentan-2-one; 1-p-menthene-8-thiol; hept-1-en-3-one; winelactone; (Z)-hex-3-enal; stable isotope dilution analysis*

INTRODUCTION

Although each citrus fruit can be distinguished from other fruits by the characteristic "citrus-like" odor with pungent, fresh notes, they clearly differ from each other by characteristic aroma attributes. These typical notes are described with expressions being closely related to the type of fruit, such as "grapefruit"- or "mandarin"-like.

Previously, the typical sulfurous, grapefruit-like odor of fresh grapefruit juice was especially referred to 1-*p*-menthene-8-thiol (*1*). This compound is often reported in the literature as one of the most powerful flavor compounds found in nature with a taste detection threshold lower than 1×10^{-4} ppb in water. Apart from this terpene thiol, we recently identified 4-mercapto-4-methylpentan-2-one as another important, sulfur-containing odorant in the aroma of hand-squeezed grapefruit juice (*2*). The very odor-active mercapto pentanone, also known as a key odorant in Sauvignon wine (*3*), was described for the first time in grapefruit juice. Based on GC/Olfactometry of serial dilutions, an approach called Aroma Extract Dilution Analysis, we recently identified hept-1-en-3-one, (*Z*)-hex-3-enal, *tr*-4,5-epoxy-(*E*)-dec-2-enal, and the winelactone as further previously unknown odorants in grapefruit juice (*2*).

The following investigations were aimed at clarifying the contribution of the 22 potent grapefruit juice odorants previously identified by quantitative measurements, a calculation of their odor activity values (ratio of concentration to odor threshold), and flavor reconstitution experiments.

MATERIALS AND METHODS

Material. Fresh grapefruits (*Citrus paradisi* MacFayden, cultivar White Marsh seedless) grown in Honduras, Central America, were purchased in a local market and were immediately used for quantitation.

Chemicals. The following compounds were obtained from the suppliers shown: [$^{13}C_2$]-Acetaldehyde (Promochem, Wesel, Germany); ethyl alcohol-*d*, 99 at. % deuterium; ethylenediamine 99%; isoprene 99%; Lawesson's reagent 97%; [$^2H_3$]-methylmagnesium iodide, 99+ at. % deuterium, 1.0 M solution in diethyl ether; methyl propenoate 99+%; sodium borodeuteride 98 at. % deuterium (Aldrich, Steinheim, Germany); Dess−Martin periodinane; hept-1-in-3-ol 98% (Lancaster, Mühlheim, Germany); nickel(II)-acetate-tetrahydrate (Merck, Darmstadt, Germany).

The following reference compounds were freshly distilled and then used for the reconstitution experiments: acetaldehyde, hexanal, octanal, decanal, (*E*,*E*)-2,4-decadienal, ethyl 2-methylpropanoate, (*R*)-α-pinene, myrcene, (*R*)-limonene (Aldrich, Steinheim, Germany), nonanal (Roth, Karlsruhe, Germany), ethyl butanoate (Fluka, Neu-Ulm, Germany), vanillin (Merck, Darmstadt, Germany).

Syntheses. *[$^2H_6$]-1-p-Menthene-8-thiol ((1′RS)-2-(4′-methylcyclohex-3′-enyl)-[1,3-$^2H_6$]-propan-2-thiol).* The thiol was prepared by a Diels−Alder-type reaction of isoprene with methyl propenoate yielding methyl (1′*RS*)-1-(4′-methylcyclohex-3′-enyl)-carboxylate according to the procedure described previously (*4, 5*), followed by a Grignard-type reaction with [$^2H_3$]-methylmagnesium iodide, yielding (1′*RS*)-2-(4′-methylcyclohex-3′-enyl)-[1,3-$^2H_6$]-propan-2-ol. The alcohol was treated with Lawesson's reagent to yield the target compound (*6*).

MS (EI) of -(1′*RS*)-1-(4′-methylcyclohex-3′-enyl)-carboxylate, *m/z* (%): 94 (100), 95 (46), 79 (45), 67 (17), 154 (16, M^+), 122 (12), 77 (11), 68 (9), 55 (8), 41 (7), 123 (7), 39 (6), 53 (6), 91 (6), 80 (5). MS (CI): 155 (100, M^++1).

MS (EI) of (1′*RS*)-2-(4′-methylcyclohex-3′-enyl)-[1,3-$^2H_6$]-propan-2-ol, *m/z* (%): 65 (100), 93 (55), 142 (55, M^+-H_2O), 124 (50), 81 (33), 46 (23). MS (CI): 143 (100, M^++1-H_2O), 161, (1, M^++1).

The mass spectrum (MS/EI) of (1′RS)-2-(4′-methylcyclohex-3′-enyl)-[1,3-$^2H_6$]-propan-2-thiol is displayed in Figure 1. MS (CI): 143 (100, M^++1-H_2S), 177 (36, M^++1).

[1,2-$^2H_2$]-Hept-1-en-3-one. After deuteration of 1-heptin-3-ol in the presence of Pd-$CaCO_3$-PbO (Lindlar catalyst), quino-

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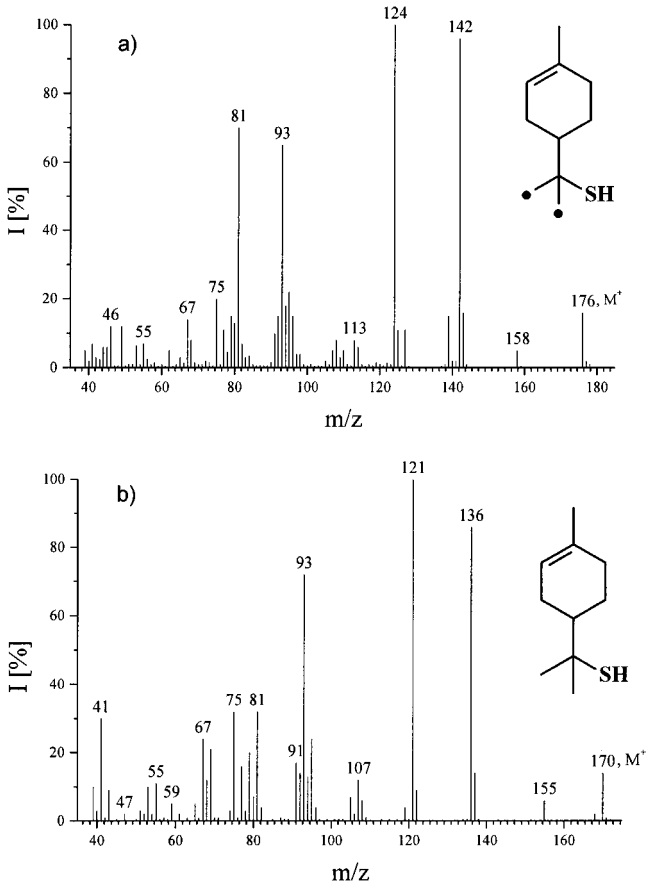

Figure 1. Mass spectra (MS/EI) of [$^2H_6$]-1-*p*-menthene-8-thiol (a) and 1-*p*-menthene-8-thiol (b).

line, and pentane (*7*), the [1,2-$^2H_2$]-hept-1-en-3-ol obtained was treated with Dess Martin periodinane (*8*) to obtain the target compound.

MS (EI) of [1,2-$^2H_2$]-hept-1-en-3-ol, *m/z* (%): 59 (100), 60, (40), 58 (32), 41 (22), 74 (22), 69 (9), 85 (7), 98 (1, M^+-H_2O). MS (CI): 99 (100, M^++1-H_2O), 100 (32), 98 (22).

The mass spectrum (MS/EI) of [1,2-$^2H_2$]-hept-1-en-3-one is displayed in Figure 2. MS (CI): 115 (100, M^++1), 116 (28), 114 (12).

The following compounds were synthesized according to the literature cited: (*Z*)-hex-3-enal (*8*), *trans*-4,5-epoxy-(*E*)-dec-2-enal (*9*), hept-1-en-3-one (*2*), 4-mercaptomethylpentan-2-one (*10*), and 1-*p*-menthene-8-thiol (*6*).

The following labeled internal standards were synthesized according to the literature cited: [2,2,2-$^2H_3$]-ethyl 2-methylpropanoate and [2,2,2-$^2H_3$]-ethyl 2-methylbutanoate (*11*), [2,2,2-$^2H_3$]-ethyl butanoate (*12*), [3,3,4,4-$^2H_4$]-hexanal (*13*), [3,4-$^2H_2$]-(*Z*)-hex-3-enal, [4,5-$^2H_2$]-oct-1-en-3-one, 4-mercapto-4-[^{13}C]-methyl-[1,3,5-$^{13}C_3$]-pentan-2-one (*14*), [$^2H_5$]-ethyl 3-hydroxyhexanoate (*15*), [2,3-$^2H_2$]-(*E*)-non-2-enal, [7,7,8,8-$^2H_4$]-(*E*,*E*)-deca-2,4-dienal, *tr*-4,5-epoxy-[7,7,8,8-$^2H_4$]-(*E*)-dec-2-enal (*16*), [$^2H_3$]-3-methylbutanol (*17*), [2,2,2-$^2H_3$]-ethyl hexanoate (*10*), [3,3,4,4-$^2H_4$]-octanal (*18*), [5,5,6,6-$^2H_4$]-nonanal (*19*), 3-([$^2H_3$]-methylthio)-1-propanal (*20*), [5,6-$^2H_2$]-decanal (*15*), [3,4-$^2H_2$]-butanoic acid (*21*), 3a,4,5,7a-tetrahydro-3-[$^2H_3$],6-dimethyl-2(3H)-benzofuranone, [$^2H_3$]-vanillin (*10*).

Concentrations of Labeled Compounds. After the syntheses, the concentrations of the labeled internal standards [2,2,2-$^2H_3$]-ethyl 2-methylpropanoate, [2,2,2-$^2H_3$]-ethyl 2-methylbutanoate, [2,2,2-$^2H_3$]-ethyl butanoate, [3,3,4,4-$^2H_4$]-hexanal, [3,4-$^2H_2$]-(*Z*)-hex-3-enal, [1,2-$^2H_2$]-hept-1-en-3-one, [4,5-$^2H_2$]-oct-1-en-3-one, [2,3-$^2H_2$]-(*E*)-non-2-enal, [7,7,8,8-$^2H_4$]-(*E*,*E*)-deca-2,4-dienal, [$^2H_3$]-3-methylbutanol, [2,2,2-$^2H_3$]-ethyl hexanoate, [3,3,4,4-$^2H_4$]-octanal, [5,5,6,6-$^2H_4$]-nonanal, 3-([$^2H_3$]-methylthio)-1-propanal, [5,6-$^2H_2$]-decanal, [3,4-$^2H_2$]-butanoic acid, [$^2H_5$]-ethyl 3-hydroxyhexanoate, and [$^2H_3$]-vanillin were determined by gas chromatography using methyl octanoate as the internal standard. Response factors (FID) were determined using defined mixtures of the respective unlabeled compounds and methyl octanoate. The concentrations of the following compounds were determined by the same approach using the internal standards given in parentheses: *tr*-4,5-epoxy-[7,7,8,8-$^2H_4$]-(*E*)-dec-2-enal ((*E*,*E*)-deca-2,4-dienal), 3a,4,5,-7a-tetrahydro-3-[$^2H_3$],6-dimethyl-2(3*H*)-benzofuranone (δ-decalactone). The concentration of [1,2-$^{13}C_2$]-acetaldehyde was determined by static headspace HRGC−MS using unlabeled acetaldehyde as the internal standard.

Isolation and Identification of the Juice Volatiles. The isolation of the juice volatiles by solvent extraction and subsequent high-vacuum distillation was performed as described previously (*6*).

Chiral Analysis. The enantiomeric ratios of limonene, α-pinene, ethyl 2-methylbutanoate, linalool, and ethyl 3-hydroxyhexanoate were determined gas chromatographically without derivatization according to the method described recently (*6*).

Quantitation of Odorants by Stable Isotope Dilution Assays. Workups with different amounts (100 mL, 1 L, or 5 L) of grapefruit juice were performed depending on the amounts of odorants present in the juice; e.g., 5 L of juice had to be used for 1-*p*-menthene-8-thiol, whereas 100 mL was sufficient for ethyl butanoate. The juice was obtained by careful hand-squeezing of the fruits using a kitchen juicer, then poured into an equal amount of aqueous saturated $CaCl_2$-solution in order to inhibit enzymic reactions, finally spiked with known amounts of the labeled internal standards listed in Table 1, and stirred for 30 min for equilibration. Solvent extraction of the samples and enrichment of the odorants for quantitation were performed as described previously (*6*). Standard curves were measured using mixtures of the labeled and unlabeled reference odorants (*22*).

High-Resolution Gas Chromatography/Mass Spectrometry (HRGC/MS). Quantitation of the volatiles was performed by two-dimensional gas chromatography (TD-HRGC) with a Mega 2 gas chromatograph (Fisons Instruments, Mainz-Kastel, Germany) as the precolumn system in tandem with a Fisons GC 5160 as the main column system. MS analyses were performed with an ITD-800 (Fisons) running in the CI-mode with methanol as the reagent gas. The following fused silica capillaries were used: DB-FFAP (30 m × 0.32 mm i.d., 0.25 μm FD, J & W Scientific, Folsom, CA) in combination with DB-5 (SE-54; 30 m × 0.32 mm i.d., 0.25 μm FD, J & W Scientific). The samples were applied by the "cool"-on-column injection technique at 40 °C. After 2 min, the temperature of the oven was raised at 40 °C/min to 50 °C (DB-5) or 60 °C (DB-FFAP), held for 2 min isothermally, raised at 6 °C/min to 180 °C, then raised at 15 °C/min to 230 °C and held for 10 min. The flow rate of the carrier gas helium was 2.5 mL/min. The cut time intervals on the main column were determined by injection of the respective reference compounds.

Other analytical details on the quantitation by mass chromatography are summarized in Table 1.

Quantitation of Terpene Hydrocarbons. Quantitation was performed by HRGC−FID using undecane as the internal standard (*15*).

Quantitation of Acetaldehyde. For the determination of acetaldehyde, 10 g of freshly squeezed grapefruit juice were poured into a vessel (100 mL), sealed with a septum, and spiked with known amounts of [1,2-$^{13}C_2$]-acetaldehyde. The analysis was done by static headspace/mass spectrometry as described in (*15*).

Sensory Evaluation. Ten assessors were recruited from the German Research Center of Food Chemistry. In preceding training sessions the panelists were asked to evaluate five suprathreshold aroma solutions of either acetaldehyde (pungent), (*Z*)-hex-3-enal (grassy), ethyl 2-methylbutanoate (fruity), (*R*)-limonene (terpene-like, peel-like), octanal (citrus-like), 4-mercapto-4-methylpentan-2-one (catty), or 1-*p*-menthene-8-thiol (grapefruit-like), by scoring the odor intensities of the solutions from 0.0 to 3.0. Sensory analyses were performed in a sensory panel room at 21 ± 1 °C at three different sessions.

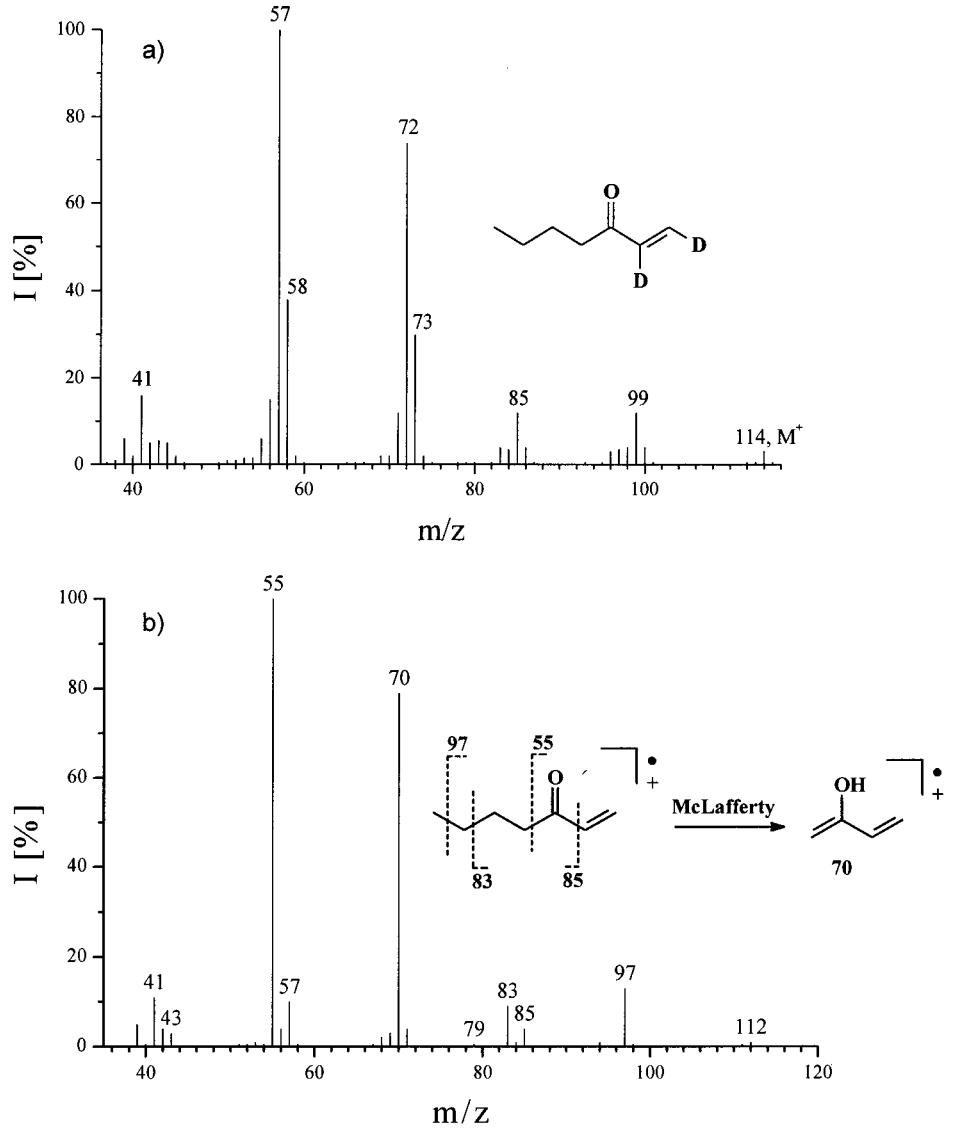

Figure 2. Mass spectra (MS/EI) of [1,2-$^2H_2$]-hept-1-en-3-one (a) and hept-1-en-3-one (b).

Fresh Grapefruit Juice. Grapefruit juice was obtained by hand-squeezing of the fruits with a kitchen juicer immediately before the sensory evaluation. The juice samples were tested orthonasally according to the method described previously (*11*). Ten assessors (five males and five females) were asked to rate given odor qualities (fruity, sweet, grassy, terpene-like, pungent, citrus-like, sulfurous, and grapefruit-like) using a seven point intensity scale from 0.0 to 3.0. These odor qualities had been selected in evaluations of fresh grapefruit juice aroma as the most intense and characteristic odor attributes for the descriptive analysis. It was not possible to find another term for "grapefruit-like" in the description of the grapefruit aroma. Therefore, this term was used for the juice evaluation by flavor profile analysis.

Grapefruit Juice Flavor Models. The following 20 compounds, dissolved in 500 μL of ethanol, were added to 1 L of an aqueous solution in concentration levels equal to those determined in the fresh grapefruit juices: acetaldehyde, (*E*,*E*)-deca-2,4-dienal, decanal, *tr*-4,5-epoxy-(*E*)-dec-2-enal, ethyl butanoate, ethyl hexanoate, (*S*)-ethyl 2-methylbutanoate, ethyl 2-methylpropanoate, 1-hepten-3-one, (*Z*)-hex-3-enal, (*R*)-limonene, linalool, 1-*p*-menthene-8-thiol, 4-mercapto-4-methylpentan-2-one, methional, myrcene, (*E*)-non-2-enal, octanal, oct-1-en-3-one, and (*R*)-α-pinene.

After the fresh juice of White Marsh grapefruits and the model mixture (15 mL each) were stirred for 30 min, they were presented to the sensory panel for comparative orthonasal evaluation in covered glass vessels (capacity, 45 mL; i.d., 40 mm) similar to the procedure described recently (*11*). The results obtained in three different sessions were averaged and plotted in spider web diagrams. The values obtained in different sessions and for the different assessors differed by not more than 10%.

Omission Experiments. Two model solutions were prepared by omitting either 4-mercapto-4-methylpentan-2-one or 1-*p*-menthene-8-thiol from the entire flavor model. Each mixture was presented in a triangle test according to §35 LMBG of the German Health Organization (*23*; Method 00.90-0 and 00.90-7) for sensory evaluation in comparison to the complete model. Panelists were asked whether the model solutions were different or not. The significance α of the differences was calculated as described in (*24*).

RESULTS AND DISCUSSION

Quantitative Analysis and Calculation of Odor Activity Values. In preliminary investigations, a total of 37 odor-active compounds had been detected by application of an aroma extract dilution analysis (AEDA), and they were subsequently identified (*2*). During AEDA, single odorants are ranked according to their odor potencies in air by sniffing serial dilutions of the flavor extract. However, odor activity values (OAVs), defined as ratio of concentration to odor threshold (*25*), give an idea of the odor potency of a single odorant in a

Table 1. Selected Ions, Calibration Factors, and Thin Film Capillaries Used for Quantitation by Stable Isotope Dilution Assays

odorant[a]	ion (*m/z*)	internal standard	ion (*m/z*)	calibration factor[b]	capillary
acetaldehyde	45	[$^{13}C_2$]-acetaldehyde	47	1.00	Rtx-5
ethyl 2-methylpropanoate	117	[2,2,2-$^2H_3$]-ethyl 2-methylpropanoate	120	0.92	DB-5
ethyl butanoate	117	[2,2,2-$^2H_3$]-ethyl butanoate	120	1.00	DB-5
(*S*)-ethyl 2-methylbutanoate	131	[2,2,2-$^2H_3$]-ethyl 2-methylbutanoate	134	0.95	DB-5
hexanal	83	[3,3,4,4-$^2H_4$]-hexanal	86−87	0.73	DB-5
(*Z*)-hex-3-enal	81	[3,4-$^2H_2$]-(*Z*)-hex-3-enal	83	0.74	DB-5
hept-1-en-3-one	113	[1,2-$^2H_2$]-hept-1-en-3-one	115	0.88	DB-5
ethylhexanoate	145	[2,2,2-$^2H_3$]-ethyl hexanoate	148	1.00	DB-5
octanal	111	[3,3,4,4-$^2H_4$]-octanal	113−115	0.87	DB-5
oct-1-en-3-one	127	[4,5-$^2H_2$]-oct-1-en-3-one	129	0.52	DB-5
4-mercapto-4-methylpentan-2-one	133	4-mercapto-4-[^{13}C]-methyl-[1,3,5-$^{13}C_3$]pentan-2-one	137	1.00	DB-5
nonanal	143	[5,5,6,6-$^2H_4$]-nonanal	147	0.87	DB-5
methional	105	3-([$^2H_3$]-methylthio)-propanal	108	0.71	DB-5
decanal	157	[5,6-$^2H_2$]-decanal	158−160	0.64	DB-5
(*E*)-non-2-enal	141	[2,3-$^2H_2$]-(*E*)-non-2-enal	143	0.83	DB-5
linalool	137	tetrahydrolinalool	141	1.61	DB-FFAP
1-*p*-menthene-8-thiol	137	2-(4′-methylcyclohex-3′-enyl)-[1,3-$^2H_6$]-propan-2-thiol	143	0.74	DB-5
ethyl 3-hydroxyhexanoate	161	[1,1,2,2,2-$^2H_5$]-ethyl 3-hydroxyhexanoate	166	0.88	DB-5
(*E*,*E*)-deca-2,4-dienal	153	[$^2H_4$]-(*E*,*E*)-deca-2,4-dienal	156−157	0.67	DB-5
tr−4,5-epoxy-(*E*)-dec-2-enal	169	*tr*-4,5-epoxy-[7,7,8,8-$^2H_4$]-(*E*)-dec-2-enal	171−173	0.67	DB-FFAP
winelactone	167	3a,4,5,7a-tetrahydro-3[$^2H_3$],6-dimethyl-2(3*H*)-benzofuranone	170	1.00	DB-5
vanillin	153	[$^2H_3$]-vanillin	156	1.01	DB-5

[a] Compounds were determined using the respective labeled internal standards by means of the ion trap detector ITD-800 (Finnigan, Bremen, Germany) running in the CI-mode with methanol as reagent gas. [b] The calibration factor was determined as reported previously (*22*). [c] Capillaries used in the stable isotope dilution assays: DB−FFAP, 30m × 0.32 mm fused silica capillary; free fatty acid phase, 0.25 μm (J&W Scientific, Folsom, CA); DB-5, 30m × 0.32 mm fused silica capillary (J&W Scientific).

Table 2. Concentrations of Potent Odorants in Hand-Squeezed Grapefruit Juice

odorant	concentration[a] (μg/kg)
acetaldehyde	6150
ethyl 2-methylpropanoate	5.8
(*R*)-α-pinene	42
ethyl butanoate	70
(*S*)-ethyl 2-methylbutanoate	3.9
hexanal	33
(*Z*)-hex-3-enal	108
myrcene	94
(*R*)-limonene	2308
hept-1-en-3-one	0.5
ethyl hexanoate	4.3
octanal	32
oct-1-en-3-one	0.8
4-mercapto-4-methylpentan-2-one	0.8
nonanal	9.3
methional	0.2
decanal	89
(*E*)-non-2-enal	0.5
linalool	76
1-*p*-menthene-8-thiol	0.01
ethyl 3-hydroxyhexanoate	117
(*E*,*E*)-deca-2,4-dienal	1.0
tr-4,5-epoxy-(*E*)-dec-2-enal	3.1
3a,4,5,7a-tetrahydro-3,6-dimethyl-2(3*H*)-benzofuranone	1.1
vanillin	69

[a] Data are mean values of at least duplicates.

food itself, based on its odor threshold in the respective food matrix. To evaluate their contribution to the overall grapefruit juice aroma, twenty-five odorants, which have been detected with high flavor dilution (FD) factors in the previous study, were selected for quantitation.

The quantitative data obtained by stable isotope dilution assays are given in Table 2. Acetaldehyde and (*R*)-limonene were by far the most abundant odorants in the fresh grapefruit juice, because of their high amounts in the mg/kg (ppm) range, followed by ethyl 3-hydroxyhexanoate, (*Z*)-hex-3-enal, myrcene, and decanal, which were present in the upper ppb levels. On the other hand, extremely low concentrations were measured for 1-*p*-menthene-8-thiol in particular, but also for hept-1-en-3-one, oct-1-en-3-one, 4-mercapto-4-methylpentan-2-one, methional, and (*E*)-non-2-enal.

In further experiments, the odor thresholds of (*R*)-α-pinene, hept-1-en-3-one, and (*R*)- and (*S*)- ethyl 3-hydroxy hexanoate were determined in air and water, respectively. The results revealed hept-1-en-3-one as another very potent, geranium-like-smelling, odorant (Table 3). Interestingly, the odor threshold of the (*S*)-isomer of ethyl 3-hydroxyhexanoate was lower by a factor of more than 100 compared to the (*R*)-isomer. The odor thresholds of the other odorants are summarized in Table 3.

A calculation of the OAVs based on the data in Tables 2 and 3 revealed that the trace compound 4-mercapto-4-methylpentan-2-one, in particular, revealed the highest OAV among all compounds under investigation (Table 4). This result indicated a high contribution of this catty-like smelling thiol to the overall aroma of grapefruit juice. In addition, high OAVs were also found for (*Z*)-hex-3-enal and decanal, the fruity-sweet smelling esters (*S*)-ethyl 2-methylbutanoate, ethyl butanoate, ethyl 2-methylpropanoate, and the winelactone, as well as for acetaldehyde and the grapefruit-like 1-*p*-menthene-8-thiol (Table 4). The OAVs of the remaining compounds were much lower. However, each of the compounds investigated revealed at least an OAV (ortho- or retro-nasal) of higher than 1. Based on these data, the compounds displayed in Table 4 are suggested as key contributors to the grapefruit juice aroma.

Reconstitution Experiments. To confirm the quantitative data obtained, sensory evaluations of grapefruit aroma models were performed. The 20 odorants marked with an asterisk in Table 4 were dissolved in water in the exact amounts determined to be present in the fresh juice. The overall aroma of the flavor model was then evaluated by the sensory panel in comparison to a hand-squeezed juice.

Table 3. Odor Thresholds of Potent Aroma Compounds Identified in Fresh, Hand-Squeezed Grapefruit Juice

odorant	odor quality	odor threshold (ng/L in air)[a]	odor threshold (μg/L in water)[b]	
			orthonasal	retronasal
acetaldehyde	fruity, pungent	41[c]	25[c]	10[c]
ethyl 2-methylpropanoate	fruity	0.1−0.2[c]	0.02[c]	0.03[c]
(*R*)-α-pinene	pine-tree	5.3	5	33
ethyl butanoate	fruity	2.7	1[c]	0.1[c]
(*S*)-ethyl 2-methylbutanoate	fruity	n.d.	0.006[c]	0.004
hexanal	green, grassy	30[c]	10.5[c]	10.5[c]
(*Z*)-hex-3-enal	green, grassy	0.09−0.36[c]	0.25[c]	0.03[c]
myrcene	mossy	44.5	14[c]	16.6[c]
(*R*)-limonene	citrus-like	424	200[c]	34
hept-1-en-3-one	geranium-like	0.08	0.04	0.04
ethyl hexanoate	fruity	3.0	5[c]	0.5
octanal	green, citrus-like	5.8−13.6[c]	8[c]	45[c]
oct-1-en-3-one	mushroom-like	0.3−0.6[c]	1[c]	0.01[c]
4-mercapto-4-methylpentan-2-one	catty, blackcurrant-like	n.d.	n.d.	0.0001
nonanal	soapy, citrus-like	5.2−12.1[c]	5[c]	3.5
methional	cooked potato	0.1−0.2[c]	1.8[c]	0.04[c]
decanal	green, soapy	1[c]	0.1[c]	7[c]
(*E*)-non-2-enal	fatty, tallowy	0.1[c]	0.8[c]	0.08[c]
linalool[e]	flowery	0.4−0.8[c]	6[c]	1.5[c]
1-*p*-menthene-8-thiol	grapefruit-like, sulfurous	n.d.	0.0001[d]	n.d.
(*R*)-ethyl 3-hydroxyhexanoate	sweet, fruity	2.1	270	63
(*S*)-ethyl 3-hydroxyhexanoate	weak, fruity	264	n.d.	n.d.
(*E*,*E*)-deca-2,4-dienal	fatty, waxy	0.13[c]	0.2[c]	0.05[c]
tr-4,5-epoxy-(*E*)-dec-2-enal	metallic	0.0006−0.0025[c]	0.12[c]	0.015[c]
winelactone	sweet, spicy	0.00001−0.00004[c]	n.d.	0.008[c]
vanillin	vanilla-like	0.6−1.2[c]	25[c]	30[c]

[a] The odor thresholds in air were determined as described elsewhere (*26*). [b] Odor thresholds in water were determined by five panelists using the triangle test as described previously (*11*). The values given are the average values of triplicates. [c] Odor thresholds according to (*27*). [d] Odor threshold as reported previously (*1*). [e] The odor thresholds of the racemic compounds are given. n.d. = not determined.

Table 4. Odor Activity Values (OAV) of Potent Odorants in Hand-Squeezed Grapefruit Juice (White Marsh)

odorant	OAV (*n*)[a]	OAV (*rn*)[a]
acetaldehyde*	246	615
ethyl 2-methylpropanoate*	290	193
(*R*)-α-pinene[b] *	8	1
ethyl butanoate*	70	695
(*S*)-ethyl 2-methylbutanoate[b] *	650	975
hexanal	3	3
(*Z*)-hex-3-enal*	430	3587
myrcene*	7	6
(*R*)-limonene[b] *	12	68
hept-1-en-3-one*	13	13
ethyl hexanoate*	<1	9
octanal*	4	<1
oct-1-en-3-one*	<1	80
4-mercapto-4-methylpentan-2-one*	- - -[d]	8000
nonanal	2	3
methional	<1	5
decanal*	890	13
(*E*)-non-2-enal*	<1	6
linalool*	13	51
1-*p*-menthene-8-thiol[c] *	100	- - -[d]
ethyl 3-hydroxyhexanoate[b]	<1	2
(*E*,*E*)-deca-2,4-dienal*	5	20
tr-4,5-epoxy-(*E*)-dec-2-enal*	26	207
3a,4,5,7a-tetrahydro-3,6-dimethyl-2(3*H*)-benzofuranone[b]	- - -[d]	138
vanillin	3	2

[a] The odor activity values (OAV; *n* = nasally, *rn* = retronasally) were calculated by dividing the concentrations of the odorants by their nasal or retronasal thresholds in water (cf. Table 3). [b] The OAVs were calculated based on the detection thresholds of the enantiomerically pure compounds. [c] The OAVs were calculated based on the detection thresholds of the racemic compounds. [d] Not calculated. [e] Odorants used in the flavor reconstitution experiments are marked with an asterisk (*).

The results, plotted as spider web diagram (Figure 3) revealed a very high similarity of the model with the natural aroma of the fresh juice, eliciting the same

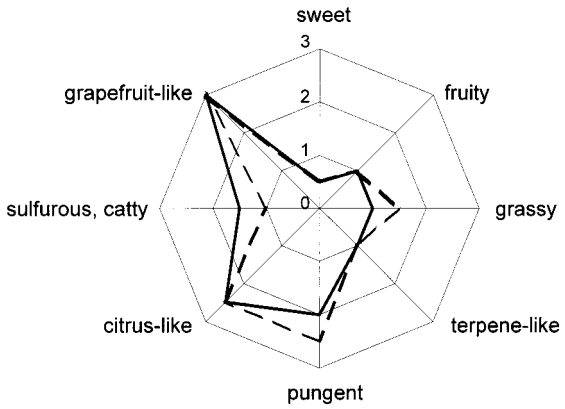

Figure 3. Comparative flavor profile analysis of hand-squeezed grapefruit juice (dotted line) and the reconstituted grapefruit aroma model solution (straight line).

intensities of the grapefruit-like, fruity, terpene-like, and citrus-like odor qualities. The grassy and pungent odor notes were rated slightly more intense in the juice, but the sulfurous, catty note was a bit more pronounced in the model. The overall aroma of the model solution was, however, described as typical fresh grapefruit-like.

A second series of experiments was aimed at clarifying the actual aroma contribution of the two highly odor-active thiols, 1-*p*-menthene-8-thiol and 4-mercapto-4-methylpentan-2-one. Each of the two compounds was singly omitted from the complete model flavor mixture and this "omission" model was orthonasally evaluated in comparison to the complete model by means of the triangle test. It was found that the omission of both thiols was detectable by the panelists. However, when 1-*p*-menthene-8-thiol was omitted, only the majority was able to detect an aroma difference. In comparison to the complete model, the overall flavor of the incomplete model was described by these panelists as less grapefruit-

like, fresh, but still a grapefruit-like odor was detectable (significance α: 5%).

On the other hand, when 4-mercapto-4-methylpentan-2-one was omitted from the complete model, all panelists reported to perceive a clear difference between the models, resulting in the highest detection significance (α = 0.1%). This model was described as orange-like and the grapefruit-like odor was lacking. Interestingly, the complete model containing 4-mercapto-4-methylpentan-2-one was not mainly described as catty but, first and foremost, as grapefruit-like. Even when panelists were asked to evaluate the catty odor quality, they reported to have mainly the impression of the grapefruit-like odor note. This effect might be due to the presence of further odorous compounds yielding the overall grapefruit-like odor as a combination, because 4-mercapto-4-methylpentan-2-one alone elicits a clear catty odor quality.

The results have shown that the typical aroma of hand-squeezed grapefruit-juice is not only due to 1-*p*-menthene-8-thiol, but is evoked much more by 4-mercapto-4-methylpentan-2-one. In mixture with fruity, citrus-like, and fresh odor notes, as they are elicited by several potent odorants, such as the esters, by (*R*)-limonene, (*Z*)-hex-3-enal, decanal, and acetaldehyde, the mercapto pentanone bears the characteristic fresh, grapefruit-like odor impression of fresh, hand-squeezed grapefruit juice.

ABBREVIATIONS USED

AEDA, aroma extract dilution analysis; ADA, aroma dilution analysis; FD, flavor dilution factor; OAV, odor activity value; SIDA, stable isotope dilution assay.

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