Evaluation of the Character Impact Odorants in Fresh Strawberry Juice by Quantitative Measurements and Sensory Studies on Model Mixtures

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Twelve odorants, previously identified with high flavor dilution (FD) factors, were quantified in a fresh strawberry juice by using stable isotope dilution assays. Calculation of odor activity values (ratio of concentration to odor threshold), on the basis of odor thresholds in water, revealed especially the six compounds (*Z*)-3-hexenal (green), 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone (caramel-like, sweet), methyl butanoate (fruity), ethyl butanoate (fruity), methyl 2-methylpropanoate (fruity), and 2,3-butanedione (buttery) as the key flavor compounds in the typical strawberry-like odor of the juice. These results were corroborated by the following sensory experiments: A mixture of the 12 most potent odorants added to a model juice matrix, consisting of pectin, sugars, and nonvolatile acids, in concentrations equal to those in the fresh juice, resulted in an overall odor profile very similar to that of the fresh juice. Furthermore, comparison of the overall odors of 11 model mixtures lacking in 1 of the 12 odorants under investigation, with the odor evoked by the complete set of odorants, showed a clear flavor difference, especially when 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone, (*Z*)-3-hexenal, or methyl butanoate was lacking in the mixture.

Keywords: *Strawberry odor; stable isotope dilution assay; odor activity value; sensory study; [$^{13}C]_2$-2,3-butanedione; [$^2H_3$]methyl butanoate; [$^2H_3$]methyl 2-methylbutanoate; [$^2H_3$]ethyl butanoate; [$^2H_3$]-4-methoxy-2,5-dimethyl-3(2H)-furanone*

INTRODUCTION

The volatile components of strawberries have been extensively studied [cf. review by Latrasse (1991)], and more than 360 volatiles are assumed to be involved in strawberry flavor. By application of an aroma extract dilution analysis (AEDA) on a fresh strawberry juice, we (Schieberle, 1994) recently found that the 15 odor-active compounds listed in Table 1 showed the highest flavor dilution (FD) factors among the juice volatiles. These data corroborated previous results obtained for compounds **I**−**IV**, **Vb**, **VIa**, **IX**, **Xb**, and **XII** (Table 1) also using a combination of analytical and sensory analysis (Larsen and Poll, 1992; Fischer and Hammerschmidt, 1992).

Using AEDA, the contribution of odor-active compounds to the overall food odor is estimated by ranking the odor activities of single odorants on the basis of their odor thresholds in air. To link results of AEDA experiments with the original strawberry aroma, it is a necessary further step to exactly determine the amounts of the odorants in the fruit material and, finally, to perform sensory studies on model mixtures containing the flavor compounds in the same concentrations as determined in the fresh strawberry material. This concept has recently been successfully applied in studies on the flavors of stewed beef juice (Guth and Grosch, 1994) or the aroma of coffee brew (Semmelroch and Grosch, 1995).

The purpose of the this paper was, therefore, to quantitate selected key flavor compounds in fresh strawberry juice and, then, to compare the aroma of various model mixtures of key odorants with that of the original strawberry juice. Several juice odorants are known to be relatively labile [e.g. (*Z*)-3-hexenal or 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone] and others (e.g. 2,3-butanedione or methyl butanoate) are very volatile. To compensate losses during the workup procedure, stable isotope dilution assays were used in the quantification experiments.

Table 1. Flavor Dilution (FD) Factors of Odor-Active Volatiles in a Fresh Strawberry Juice [According to Schieberle (1994)]

no.	odorant	odor quality	FD
I	4-hydroxy-2,5-dimethyl-3(2*H*)-furanone	caramel-like	4096
II	(*Z*)-3-hexenal	green, leaflike	1024
III	methyl butanoate	fruity	256
IV	ethyl butanoate	fruity	512
Va	ethyl 2-methylbutanoate[a]	fruity	128
Vb	ethyl 3-methylbutanoate[a]	fruity	
VIa	methyl 2-methylbutanoate[a]	fruity	256
VIb	methyl 3-methylbutanoate[a]	fruity	
VII	acetic acid	sour	1024
VIII	2,3-butanedione	buttery	256
IX	butanoic acid	sweaty	2048
Xa	3-methylbutanoic acid[a]	sweaty	256
Xb	2-methylbutanoic acid[a]	sweaty	
XI	ethyl 2-methylpropanoate	fruity	128
XII	4-methoxy-2,5-dimethyl-3(2*H*)-furanone	caramel-like, burnt	64

[a] Compounds were not separated by the HRGC stationary phase used in the GC/Olfactometry. Identification is based on the different spectra recorded by MS/EI.

EXPERIMENTAL PROCEDURES

Materials. *Strawberries.* Strawberries (fruits from Spain) were purchased at a local market.

Chemicals. Compounds **I**−**XII** (cf. Table 1) were supplied by Aldrich (Steinheim, Germany). Prior to sensory experiments the compounds were purified according to known

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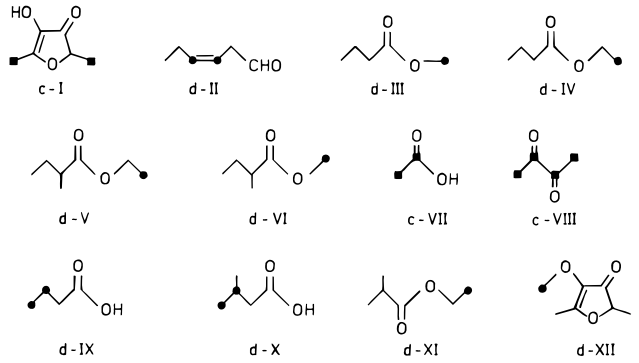

Figure 1. Labeled internal standards used in the quantitation of strawberry odorants: (●) deuterium label; (■) carbon-13 label.

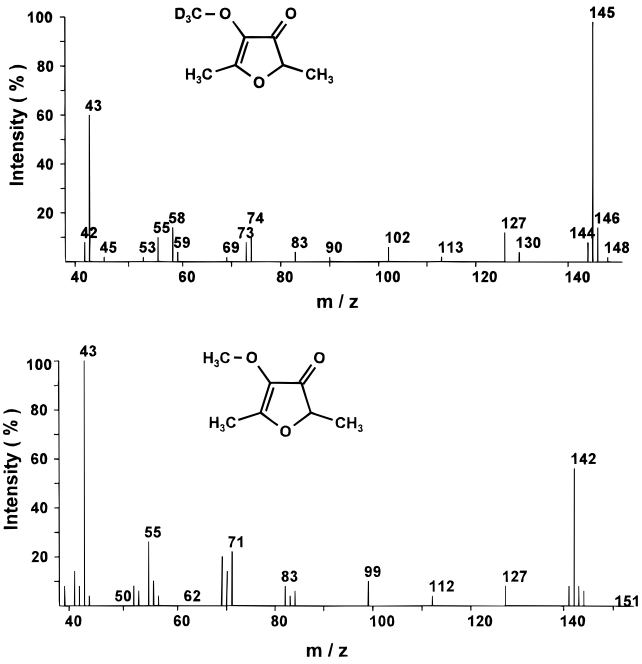

Figure 2. Mass spectra (MS/EI) of (A, top) the labeled internal standard [$^2H_2$]-4-methoxy-2,5-dimethyl-3(2*H*)-furanone and (B, bottom) the unlabeled aroma compound

procedures using either distillation in vacuum or crystallization, respectively. (*Z*)-3-Hexenal was freed from its diethyl acetal (ICN, Heidelberg, Germany) by treatment with hydrochloric acid (0.5 mol/L) for 2 h at room temperature.

*Labeled Internal Standards. [$^2H_3$]-4-Methoxy-2,5-dimethyl-3(2H)-furanone (d-**XII**; Figure 1).* 4-Hydroxy-2,5-dimethyl-3(2*H*)-furanone (2 mmol) and [$^2H_3$]methyl iodide (6 mmol) were refluxed in acetone (8 mL) for 8 h in the presence of potassium carbonate. After dilution with tap water (40 mL), the mixture was extracted four times with dichloromethane (total volume: 120 mL); the combined organic layer was then treated with aqueous sodium hydroxide (0.1 mol/L; 20 mL) and, after washing with brine, dried over Na_2SO_4. d-**XII** was obtained gas chromatographically pure in an overall yield of 75%. In line with the synthesis, the mass spectrum in Figure 2A, compared with the data for the unlabeled compound **XII** (Figure 2B), confirmed the incorporation of three deuterium atoms in d-**XII**.

*[$^{13}C_4$]-2,3-Butanedione (c-**VIII**; Figure 1).* Freshly distilled [$^{13}C_2$]acetaldehyde (11.4 mmol) was condensed to [$^{13}C_4$]-2-hydroxy-3-butanone in dry triethylamine (3.43 mmol) using 3-benzyl-5-(2-hydroxyethyl)-4-methylthiazolium chloride (0.57 mmol; Aldrich, Germany) as the catalyst. After heating in an argon atmosphere for 1.5 h at 80 °C, the mixture was cooled and then acetic acid (20 mL) was added. The [$^{13}C_4$]-3-hydroxy-2-butanone formed was then oxidized into c-**VIII** by heating in the presence of bismuth(III) oxide (2 mmol). The labeled

Table 2. Mass Spectral Data (MS/EI) of Labeled Esters[a]

ester	*m/z* (%)
[$^2H_3$]ethyl butanoate	71 (100), 43 (80), 91 (55), 41 (35), 61 (20), 74 (15), 48 (12), 104 (10)
[$^2H_3$]methyl butanoate	77 (100), 71 (65), 43 (64), 41 (38), 62 (32), 90 (23), 39 (22), 55 (13)
[$^2H_3$]methyl 2-methylbutanoate	43 (100), 71 (48), 113 (18), 89 (10), 74 (8), 104 (5)

[a] Spectra were recorded by means of a mass spectrometer type 95 S (Finnigan, Bremen, Germany) running in the electron impact mode (MS/EI) at 70 eV.

dione formed was continuously distilled off together with the acetic acid, and the latter was removed by treating the ice-cooled distillate, first, with solid Na_2CO_3 followed by aqueous sodium bicarbonate (0.5 mol/L, adjusted to pH 8.0 with 2 N HCl). The target compound was then isolated by extracting the mixture with diethyl ether (total volume: 80 mL).

*[$^2H_3$]Methyl butanoate (d-**III**; Figure 1); [$^2H_3$]methyl 2-methylbutanoate (d-**VI**; Figure 1), and [$^2H_3$]ethyl butanoate (d-**IV**; Figure 1)* were prepared by reacting the corresponding acids with either [$^2H_3$]methanol or [$^2H_3$]ethanol, respectively, using the reaction conditions previously described for the preparation of [$^2H_3$]ethyl octanoate (Schieberle et al., 1993). The incorporation of the three deuterium atoms was checked by mass spectrometry (MS 95S; Finnigan, Bremen, Germany) in the chemical ionization mode using isobutane as the reactant gas. The data obtained by mass spectrometry in the electron impact mode (MS/EI) are given in Table 2.

The following compounds were prepared as described previously: [$^{13}C_2$]-4-hydroxy-2,5-dimethyl-3(2*H*)-furanone (c-**I** in Figure 1; Sen et al., 1991a); [$^2H_3$]-(*Z*)-3-hexenal (d-**II** in Figure 1; Guth and Grosch, 1990); [$^2H_3$]-3-methylbutanoic acid (d-**X** in Figure 1; Guth and Grosch, 1994); [$^2H_3$]butanoic acid (d-**IX** in Figure 1; Schieberle et al., 1993); [$^2H_3$]ethyl 2-methylpropionate (d-**XI** in Figure 1); and [$^2H_3$]ethyl 2-methylbutanoate (d-**V** in Figure 1; Guth and Grosch, 1993). [$^{13}C_2$]Acetic acid (c-**VII**; Figure 1) was purchased from Aldrich (Steinheim, Germany). The concentrations of the labeled standards were determined gas chromatographically by using ethyl pentanoate as the internal standard and by using response factors determined from mixtures of ethyl pentanoate and of the corresponding unlabeled compounds.

Quantitation by Stable Isotope Dilution Analyses (SIDA). *Isolation of the Flavor Compounds and the Labeled Internal Standards.* The concentrations of the odorants under investigation varied from the micrograms per kilogram to the milligrams per kilogram range. However, an amount of about 20 μg of standard is sufficient to perform the SIDA experiments. Because the labeled standards are relatively expensive, three different workups using different amounts of strawberries were run in parallel, which had been adapted to the concentrations of the odorants under investigation.

*Odorants **I**, **VII**, and **IX**.* One strawberry fruit was frozen in liquid nitrogen and then powdered in a cooled mortar. An aliquot of the powder (2 g) was suspended in saturated $CaCl_2$ solution (30 mL; pH 3.5) and, after addition of c-**I** (20 μg), c-**VII** (200 μg), and d-**IX** (20 μg), stirred for 30 min at room temperature. The mixture was extracted five times with diethyl ether (total volume: 100 mL); the combined etheral extracts were washed with brine, then dried over Na_2SO_4, and concentrated to 200 μL as previously described (Schieberle, 1991).

*Odorants **II**–**IV**, **VIII**, and **IX**.* Strawberries (75 g) were homogenized with a saturated $CaCl_2$ solution (75 g; pH 3.5) containing the labeled internal standards d-**II** (30 μg), d-**III** (60 μg), d-**IV** (30 μg), c-**VIII** (40 μg), and d-**X** (100 μg) for 120 s and then stirred for 30 min at room temperature. The mixture was centrifuged at 4 °C (10 000 rpm; 30 min) and the supernatant extracted six times with diethyl ether (total volume: 240 mL). The combined organic layers were washed with brine, dried over Na_2SO_4, and finally concentrated to 200 μL as previously reported (Schieberle, 1991).

*Odorants **V**, **VI**, **XI**, and **XII**.* Strawberries (500 g) were homogenized in a saturated $CaCl_2$ solution (500 mL; pH 3.5)

Table 3. Thin Film Capillaries, Selected Ions, and Calibration Factors Used in the Stable Isotope Dilution Assays

no.	odorant[a]	capillary[b]	selected ion *m/z*	int std[c]	selected ion *m/z*	calibr factor[d]
I	4-hydroxy-2,5-dimethyl-3(2*H*)-furanone	DB-FFAP	129	c-**I**	131	1.00
II	(Z)-3-hexenal	DB-1701	81	d-**II**	83	0.74
III	methyl butanoate	DB-1701	103	d-**III**	106	0.92
IV	ethyl butanoate	DB-1701	117	d-**IV**	120	1.00
Va	ethyl 2-methylbutanoate[e]	DB-1701	131	d-**V**	134	0.95
VIb	methyl 2-methylbutanoate[e]	DB-1701	117	d-**VI**	120	0.93
VII	acetic acid	DB-FFAP	61	c-**VII**	63	1.00
VIII	2,3-butanedione	DB-FFAP	87	c-**VIII**	91	1.00
IX	butanoic acid	DB-FFAP	89	d-**IX**	91−93[f]	0.89
Xb	3-methylbutanoic acid[e]	DB-FFAP	103	d-**X**	105	0.70
XI	ethyl 2-methylpropionate	DB-1701	117	d-**XI**	120	0.92
XII	4-methoxy-2,5-dimethyl-3(2*H*)-furanone	DB-1701	143	d-**XII**	146	0.95

[a] Compounds were determined with their internal standards by the ion trap detector ITD-800 (Finnigan, Bremen, Germany) running in the chemical ionization mode with methanol as the reagent gas. [b] DB-FFAP: 30 m × 0.32 mm fused silica capillary; free fatty acid phase, 0.25 μm (Chrompack, Frankfurt, Germany) was used for acidic compounds. DB-OV-1701: 30 m × 0.32 mm fused silica capillary, silicone OV-1701, 0.25 μm (J&W Scientific, Fisons, Mainz, Germany) was used preferentially for the neutral volatiles. [c] Cf. Figure 1. Abbreviation of the labeling: c, carbon-13; d, deuterium. [d] The calibration factor was determined as reported by Sen et al. (1991b). [e] Being inseparable by HRGC, the sum of both the 3- and 2-methyl isomers was determined. [f] The sum of the cluster of ions present in the internal standard was used in the calculation of concentrations.

containing the internal standards d-**V**, c-**VI**, d-**XI**, and d-**XII** (10 μg each) for 120 s and then stirred for 30 min at room temperature. The mixture was centrifuged at 4 °C (10 000 rpm; 30 min) and the supernatant extracted eight times with diethyl ether (total volume: 800 mL). To remove the excess of acidic compounds, the combined organic layers were treated with an aqueous sodium bicarbonate solution (0.5 mol/L; total volume: 300 mL). The organic layer was washed with brine and dried over Na_2SO_4.

The concentrated extracts of the three workups were finally analyzed by mass chromatography as described below.

High-Resolution Gas Chromatography/Mass Chromatography (HRGC/MC). The experimental details of the mass chromatographic measurements are summarized in Table 3. After application of the sample (0.5 μL) on the capillary column by the cold on-column injection technique at 35 °C, the temperature of the oven was held for 2 min, then raised by 40 °C/min to 60 °C, held isothermally for 5 min, and finally raised at 6 °C/min to 240 °C. The linear velocity of the carrier gas helium was 35 cm/s. The effluent of the HRGC column, which was coupled to an ITD 800 (Finnigan, Bremen, Germany), was monitored in the mass range *m/z* 50−200. The intensities of selected ions (cf. Table 3), obtained by chemical ionization (MS/CI) with methanol as the reagent gas, were recorded by the computer system of the mass spectrometer. Then, the concentrations of the odorants were calculated from the amounts of the internal standards and subsequently corrected by using calibration factors (cf. Table 3), which had been obtained from mixtures of labeled and unlabeled odorants (Sen et al., 1991b). Analyses were run in triplicate. The values obtained from the same sample differed by not more than ±5%, whereas a difference of ±10% was found in parallel workups of the same batch.

Sensory Evaluation. Assessors were recruited from the German Research Center for Food Chemistry and were trained to describe aroma qualities of about 40 defined odorous chemicals. The assessors were also subjected to a ranking test with a series of seven suprathreshold aqueous solutions of (*Z*)-3-hexenal (green, leaflike), 2-methylbutanoic acid (sweaty), 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone (caramel-like and sweet), methyl butanoate (fruity), 2,3-butanedione (buttery), and acetic acid (sour and pungent) and were asked to score the odor intensities of the solutions. Six panelists were then selected. Sensory analyses were performed in a sensory panel room at 21 ± 1 °C at three different sessions.

Fresh Strawberry Juice. Fresh strawberries were homogenized at 4 °C in a commercial blender and centrifuged for 10 min at 20 000 rpm and 4 °C. The supernatant (strawberry juice) was used for sensory evaluations, which were carried out about 20 min after centrifugation. No significant change in the overall aroma of the juice was observed within 1 h after homogenization. The evaluation of the odor (orthonasal) and flavor (retronasal) of the fresh juice was performed similarly to the procedure described recently (Guth and Grosch, 1993). The assessors were asked to evaluate the intensity of eight given odor qualities (green, leaflike, caramel-like, fruity, sweet, sour, pungent, sweety, buttery) in a freshly prepared strawberry juice using a seven point intensity scale from 0.0 to 3.0. The odor qualities (cf. Figure 3) corresponded to the odor notes evoked by the key strawberry odorants under investigation (cf. Table 1). The results obtained in the three different sessions were averaged for each odor note and plotted in a spider web diagram. The values judged by the six assessors and, also, determined in the different sessions differed by not more than 10%.

Strawberry Flavor Models. Pectin (4 g), glucose (23 g), fructose (23 g), sucrose (10 g), citric acid (7 g), and malic acid (1 g) were dissolved in tap water (1 L), and the color of the solution was adjusted in comparison to the color of the original strawberry juice by using synthetic red (E 122) and synthetic yellow (E 104) food colorings. The concentrations of the nonvolatile ingredients were adapted from data given in the *Souci-Fachmann-Kraut* nutrition table (Scherz and Senser, 1994).

The following mixture of 12 odorants, dissolved in ethanol (300 μL), was added to 1 L of this matrix: 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone (8740 μg), acetic acid (74510 μg), butanoic acid (1790 μg), 2-methylbutanoic acid (2200 μg), 2,3-butanedione (1290 μg), methyl butanoate (4960 μg), ethyl butanoate (410 μg), (*Z*)-3-hexenal (330 μg), methyl 2-methylbutanoate (48 μg), ethyl 2-methylpropionate (44 μg), ethyl 2-methylbutanoate (7 μg), and 4-methoxy-2,5-dimethyl-3(2*H*)-furanone (25 μg). After 20 min of stirring, the overall odor of the model was evaluated by odor profiling as described above for the fresh juice. Because the odor qualities and odor thresholds of 2- and 3-methylbutanoic acid as well as the corresponding 2-methyl- and 3-methylbutanoic acid esters were not very different (cf. Table 4), only the 2-methyl isomers were used in the sensory experiments.

Detection of Flavor Differences. Eleven model juices were singly prepared by omitting only one odorant from the complete flavor model mixture (cf. above). Each of the 11 mixtures was presented to the panelists in comparison with the complete model by using the triangle test. Panelists were asked whether the solutions were identical in the overall odor or not. In each session, a maximum of six different model mixtures (20 g each) was presented in covered glass vessels (capacity: 45 mL; i.d., 40 mm). Due to the instability of some components, exclusively fresh preparations were tested.

Determination of Odor Thresholds. Odor thresholds were determined by the triangle test as recently described (Guth and Grosch, 1993) using tap water (pH was adjusted to 3.5) instead of sunflower oil as the solvent. The samples were presented in order of increasing concentrations (1:1 dilutions),

Table 4. Odor Thresholds, Concentrations, and Odor Activity Values (OAV) of Potent Odorants in the Fresh Strawberry Juice

no.	odorant	odor quality	threshold (μg/L in water)	concn[a] (μg/kg)	OAV[b]
I	4-hydroxy-2,5-dimethyl-3(2*H*)-furanone	caramel-like	10	16239	1624
II	(*Z*)-3-hexenal	green, leaflike	0.25	333	1332
III	methyl butanoate	fruity	5	4957	991
IV	ethyl butanoate	fruity	1	410	410
Va	ethyl 2-methylbutanoate	fruity	0.15	} 7[c]	} ≈42
Vb	ethyl 3-methylbutanoate	fruity	0.20		
VIa	methyl 2-methylbutanoate	fruity	0.25	} 48[c]	} ≈150
VIb	methyl 3-methylbutanoate	fruity	0.40		
VII	acetic acid	sour	60000	74513	1
VIII	2,3-butanedione	buttery	3	1292	431
IX	butanoic acid	sweaty, rancid	2730	1831	<1
Xa	3-methylbutanoic acid[a]	sweaty	540	} 2237[c]	} 3−4
Xb	2-methylbutanoic acid[a]	sweaty	740		
XI	ethyl 2-methylpropanoate	fruity	0.1	43	430
XII	4-methoxy-2,5-dimethyl-3(2*H*)-furanone	sweet, smoky	25	20	<1

[a] The data are mean values of triplicates. [b] Odor activity values were calculated by dividing the concentrations by the odor thresholds. [c] The sum of the 3- and 2-methyl isomers is given, because they were not completely separated on the stationary phase used. Using characteristic fragments in the MS/EI (*m*/*z* 60, 3-methylbutanoic acid; *m*/*z* 74, 2-methylbutanoic acid; *m*/*z* 88, methyl 2-methylbutanoate; *m*/*z* 74, methyl 3-methylbutanoate; *m*/*z* 102, ethyl 2-methylbutanoate; *m*/*z* 88, ethyl 3-methylbutanoate) a nearly 1:1 ratio in each isomeric mixture was estimated. The conformation of the 2-methylbutanoic acid and the corresponding esters was not analyzed.

and the threshold values evaluated in three separate sessions were averaged. The values between individuals and separate sessions differed by not more than one dilution step; i.e., a threshold value of 0.25 μg/L represents a range from 0.1 to 0.4 μg/L.

RESULTS AND DISCUSSION

In a preliminary experiment, six different batches of strawberries were hedonically evaluated to select a fruit material having a typical ripe strawberry odor. The volatile fraction from the fruits selected (Spanish strawberries) was isolated by extraction and sublimation in vacuo (Schieberle, 1991). Application of an AEDA resulted in the same odor-active volatiles as found previously (cf. Table 1). The 12 flavor compounds showing the highest FD factors were then quantified in a fresh juice by stable isotope dilution assays and by using the 12 labeled internal standards displayed in Figure 1. 2- and 3-methylbutanoic acid as well as the corresponding ethyl and methyl esters of both acids were quantified by using one labeled isomer (cf. Table 1 and Figure 1).

The highest concentrations were determined for acetic acid (no. **VII**; Table 4), followed by 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone (no. **I**) and methyl butanoate (no. **III**), while, e.g., ethyl 2- and 3-methylbutanoate were present in comparatively lower amounts (no. **Va/b**). The latter esters were not completely separable on the stationary phase used. Therefore, the concentrations given in Table 4 represent the sum of both isomers.

The determination of odor thresholds, needed for the calculation of odor activity values (OAVs; ratio of concentration to odor threshold), showed the lowest odor thresholds for ethyl 2-methyl propionate (no. **XI**), ethyl 2-methylbutanoate (no. **Va**), and ethyl 3-methylbutanoate (no. **Vb**), while acetic acid (no. **VII**) and butanoic acid (no. **IX**) showed relatively high odor thresholds (Table 4).

Calculation of the odor activity values (Table 4) then revealed six compounds as the most odor-active, namely (*Z*)-3-hexenal, 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone, methyl and ethyl butanoate, ethyl 2-methylpropanoate, and 2,3-butanedione.

To correlate the analytical results with the sensory data, the odor profile of the fresh strawberry juice was determined. A comparison of the odor profile of the

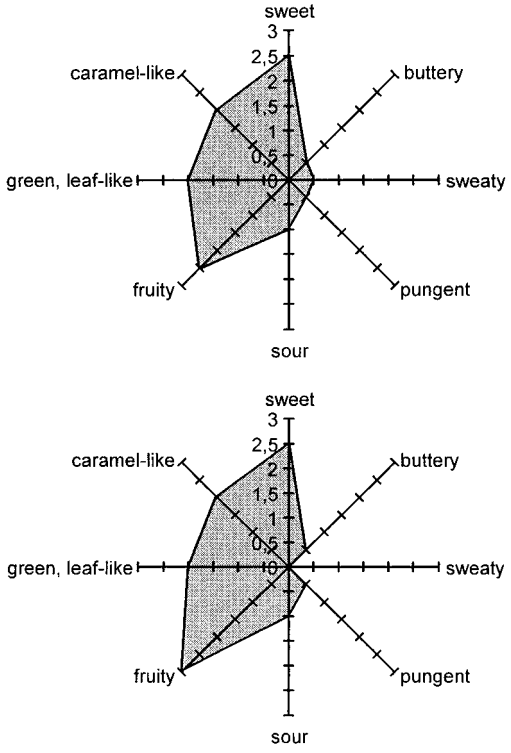

Figure 3. Flavor profiles of the strawberry model juice (A, top) and a freshly prepared strawberry juice (B, bottom).

fresh juice (Figure 3A) with the OAVs of the odorants showing the same odor notes suggests that the predominating notes in the odor profile are caused by the following odorants: green, leaflike (Figure 3A) by (*Z*)-3-hexenal (no. **II**; Table 4); caramel-like and sweet odor quality (Figure 3A) by 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone (no. **I**); and fruity note (Figure 3A) mostly by the three esters (no. **III**, **IV**, and **XI**; Table 4). However, it should be stressed that the overall odor impression was strawberry-like, which was only evoked by the complete mixture of the 12 key odorants (cf. Table 4) as discussed below.

To establish that the odorants showing high OAVs are actually the key aroma compounds in the strawberry juice, two further sets of sensory experiments were performed. First, the 12 odorants (Table 4) were dissolved in a model juice matrix of pH 3.5, in concen-

Table 5. Odor of the Model Juice[a] Affected by the Absence of One Component

expt no.	odorant omitted in the model juice	no. of panelists detecting an oder difference[b]
1	4-hydroxy-2,5-dimethyl-3(2*H*)-furanone	6 (6)
2	(*Z*)-3-hexenal	5 (5)
3	methyl butanoate	4 (3)
4	ethyl butanoate	4 (3)
5	ethyl 2-methylbutanoate	4 (4)
6	methyl 2-methylbutanoate	3 (2)
7	acetic acid	3 (3)
8	2,3-butanedione	3 (2)
9	butanoic acid	2 (3)
10	2-methylbutanoic acid	2 (1)
11	ethyl 2-methylpropanoate	2 (1)
12	4-methoxy-2,5-dimethyl-3(2*H*)-furanone	1 (0)

[a] Model juices lacking in one component (no. **I**−**XII**; Table 4) were singly compared to the model juice containing the complete set of 12 odorants (cf. Sensory Evaluation) by using the triangle test. [b] The model solutions were judged orthonasally by six trained panelists. The results of the retronasal judgment (tasting) are given in parentheses.

trations equal to those determined in the strawberry juice. Then, the odor profile of the mixture was evaluated by the sensory panel. The result of the sensory evaluation is shown in Figure 3B. A comparison with the odor profile of the fresh juice (cf. parts A and B of Figure 3) showed a clear similarity, thereby corroborating the flavor impact of the 12 odorants under investigation.

To gain some insights into the relative importance of single odorants, in a second set of experiments, 11 model mixtures were prepared, each lacking in only one odorant (Table 5). The sensory difference was judged in triangle tests using the complete mixture of the 12 odorants as the control. Omission of 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone (expt 1, Table 5) or (*Z*)-3-hexenal (expt 2), respectively, led to a significant change in the overall odor of the mixture, which was easily detectable by all six or five members of the panel, respectively. To the contrary, a lack of butanoic acid, 3-methylbutanoic acid, ethyl 2-methylpropionate, or 4-methoxy-2,5-dimethyl-3(2*H*)-furanone was only detected by one or two panelists, respectively, indicating a lower flavor impact of these compounds. The latter compound has been reported as an important odor-active strawberry volatile in studies by Fischer and Hammerschmidt (1992) and Ulrich et al. (1995). The findings of both groups were based on AEDA experiments ranking odorants on the basis of odor thresholds in air. However, it has been shown for different odorants that no linear correlation between odor threshold in air and in matrices (e.g. oils) exists [cf. review by Schieberle (1995)]. However, the different results might also be caused by the different strawberry varieties used or their maturation stage (Osamu et al., 1990).

An overall sensory evaluation of three of the model juices (Table 5) revealed a typical strawberry-like odor in the model mixture containing the complete set of odorants (mixture A; Table 6), whereas the lack of 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone as well as a lack of (*Z*)-3-hexenal caused a clear change in the overall strawberry-like odor, thereby showing that both are character impact odorants in strawberry flavor.

The overall composition of strawberry volatiles significantly depends on the variety used (Douillard et Guichard, 1990; Ulrich et al., 1995). So, further work is needed to show whether the differences in the overall odors of strawberry varieties are caused by changes in the quantitative composition of the odorants identified in the present work or are due to the presence of additional odorants.

Table 6. Sensory Evaluation of Model Mixtures of Strawberry Odorants in a Synthetic Juice Matrix[a]

mixture	overall odor description	odorant lacking	similarity[b]
A	typical strawberry-like	no	3
B	green, fruity	4-hydroxy-2,5-dimethyl-3(2*H*)-furanone	1
C	sweetish, caramel-like	(*Z*)-3-hexenal	2

[a] The overall similarity of mixtures containing (A) the complete set of the twelve odorants (cf. Table 4) or lacking in either 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone (mixture B) or (*Z*)-3-hexenal (mixture C) was evaluated in comparison with the fresh strawberry juice as the control. [b] Similarity to the odor of fresh strawberry juice: 1, weak; 2, medium; 3, strong.

CONCLUSION

The results clearly indicate that a systematic approach to locate key odorants in food flavors using the odor activity concept, followed by experiments to mimic the original food flavor in model solutions, is a powerful tool to identify character impact food odorants. Such data strongly suggest those compounds are useful as indicators, e.g., to select varieties in plant breeding or to objectify flavor changes in strawberry products caused by, e.g., raw materials, processing conditions, or storage.

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