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Characterization of the Aroma-Active Compounds in Pink Guava (*Psidium guajava*, L.) by Application of the Aroma Extract Dilution Analysis

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The volatiles present in fresh, pink-fleshed Colombian guavas (*Psidium guajava*, L.), variety *regional rojo*, were carefully isolated by solvent extraction followed by solvent-assisted flavor evaporation, and the aroma-active areas in the gas chromatogram were screened by application of the aroma extract dilution analysis. The results of the identification experiments in combination with the FD factors revealed 4-methoxy-2,5-dimethyl-3(2*H*)-furanone, 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone, 3-sulfanylhexyl acetate, and 3-sulfanyl-1-hexanol followed by 3-hydroxy-4,5-dimethyl-2(5*H*)-furanone, (*Z*)-3-hexenal, *trans*-4,5-epoxy-(*E*)-2-decenal, cinnamyl alcohol, ethyl butanoate, hexanal, methional, and cinnamyl acetate as important aroma contributors. Enantioselective gas chromatography revealed an enantiomeric distribution close to the racemate in 3-sulfanylhexyl acetate as well as in 3-sulfanyl-1-hexanol. In addition, two fruity smelling diastereomeric methyl 2-hydroxy-3-methylpentanoates were identified as the (*R,S*)- and the (*S,S*)-isomers, whereas the (*S,R*)- and (*R,R*)-isomers were absent. Seven odorants were identified for the first time in guavas, among them 3-sulfanylhexyl acetate, 3-sulfanyl-1-hexanol, 3-hydroxy-4,5-dimethyl-2(5*H*)-furanone, *trans*-4,5-epoxy-(*E*)-2-decenal, and methional were the most odor-active.

KEYWORDS: Extract dilution analysis; 3-sulfanyl-1-hexanol; 3-sulfanylhexyl acetate; methyl (*R,S*)- and (*S,S*)-2-hydroxy-3-methylpentanoate

INTRODUCTION

The fruits of *Psidium guajava*, L., a tree native to tropical America and a member of the myrtle family Myrtaceae, are widely cultivated in different tropical and subtropical countries with increasing popularity all over the world. Guava fruits are spherical in shape, about 4 to 10 cm in diameter, and covered by a leathery, green to yellowish skin (exocarp). Depending on the variety, either a white or a pink fruit flesh (meso- and endocarp) houses numerous small seeds.

The fruits are rich in pectin, carotenoids, vitamins, in particular vitamin C, and dietary fiber and are consumed as whole fruits, either fresh or cooked. In addition, a major part of the harvest is processed into jam, sherbet, pastes, or puree, which is a common ingredient in multifruit juices popular in many Northern countries. The estimated guava production in Colombia amounts to about 100 000 tons/year, a major part of which is processed to a candy bar named "bocadillo".

Guava fruits elicit a pleasant aroma in combination with a sour-sweet taste. The characteristic aroma profile combines a green, grassy note with fruity and sweet attributes and a distinct grapefruit-like aroma contributes to the characteristic tropical fruit character. First attempts to identify the odorous principle of guavas date back to the 1960s (*1, 2*). Since then numerous studies on guava volatiles (*3–21*) have been performed, which have led to the identification of ~400 volatile compounds until today (*22*). However, little attention has been paid so far to the contribution of individual compounds to the typical aroma. Because it is well-documented in the recent literature (*23*) that only a limited share of the volatiles present in a food actually contribute to a given aroma, closing this gap would be helpful, for example, to assess aroma changes caused by industrial fruit processing. Whether or not an individual volatile actually plays a role in generating the overall food aroma depends on its individual odor threshold and its concentration in the food. Since the odor thresholds of food volatiles often cover several orders of magnitude, minor compounds may be important odor contributors, while major volatiles might be negligible.

Only two investigations were aimed at clarifying the aroma activity of individual guava constituents. McLeod and de Troconis (*6*) applied GC-Olfactometry on an extract isolated from Venezuelan guavas and detected 37 odor-active regions. Chemical structures were tentatively assigned for 28 areas based on GC-MS measurements. However, identifications were not

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confirmed by using reference compounds and thus, unidentified minor volatiles with high odor potencies may have coeluted with major peaks in the chromatogram, thereby erroneous correlations between odor perception and the mass spectrum recorded may occur. Attempts to overcome this problem, e.g., by fractionation of the extract, were not performed, and also no efforts were made to rank the detected odorants according to their individual odor contribution. By applying GC-O on a pink guava puree, Jordán et al. (*19*) recently detected 46 odor-active compounds, 40 of which could be characterized. These authors tried to rank the importance of a single odorant on the basis of the number of assessors detecting an odor during GC-O. According to this approach, 23 volatiles were suggested to be of major importance, because these were detected by all assessors in all runs.

To obtain more knowledge on the aroma contribution of volatiles present in fresh guava, the aim of the present study was to screen the odor-active compounds by application of the aroma extract dilution analysis (AEDA) on an extract obtained by careful solvent extraction and SAFE-distillation from fresh, ripe Colombian pink-fleshed guavas, variety *regional roja*.

MATERIALS AND METHODS

Fruits. Fresh pink-fleshed guavas (*Psidium guajava*, L.), variety *regional roja*, grown in Puente Nacional (Santander, Colombia) were purchased at a local market in the full ripe state (skin greenish yellow) and transported overnight to Germany by air freight.

Reference Odorants. The following compounds were purchased from the commercial sources given in parentheses: numbers **1**, **2**, **4**, **7**, **10**−**15**, **17**, **19**, **20**, **22**, **23**, **25**−**31** (Aldrich, Sigma-Aldrich Chemie, Taufkirchen, Germany) and **5** and **18** (Alfa Aesar, Karlsruhe, Germany).

Syntheses. Compounds **21** and **24** were synthesized by reacting either (*E,E*)-2,4-decadienal (Aldrich) or (*E,E*)-2,4-dodecadienal (Alfa Aesar) with 3-chloroperbenzoic acid (Aldrich) as described in ref *24*.

(*E*)-3-Hexenal (3). To a solution of 1,1,1-tris(acetyloxy)-1,1-dihydro-1,2-benz-iodoxol-3-(1*H*)-one (Dess−Martin-periodinane; 1.3 mmol; 550 mg) in dichloromethane (5 mL) was added (*25*) (*E*)-3-hexenol (1 mmol; 100 mg) in dichloromethane (5 mL), then the mixture was stirred for 1 h at room temperature. After addition of diethyl ether (80 mL), the solution was washed with an aqueous sodium thiosulfate solution (1 mol/L; 100 mL; saturated with sodium hydrogen carbonate), followed by aqueous sodium hydrogencarbonate (0.5 mol/L; 100 mL), and finally water (50 mL). The solution was dried over anhydrous sodium sulfate, filtered, and, after addition of hexane (2 mL), concentrated to 2 mL with use of a Vigreux column (50 × 1 cm i.d.). To isolate the target compound, the concentrate was applied onto a water-cooled (12 °C) glass column (25 × 1 cm i.d.) filled with a slurry of purified silica gel (9 g; silica 60, 0.040−0.063 mm, Merck, Darmstadt, Germany) in pentane (*23*). The aldehyde was eluted with pentane/diethyl ether (90:10, by volume, 50 mL), and its concentration was determined by GC-FID, using (*E*)-2-hexenal as the internal standard.

Yield: 44 mg of (*E*)-3-hexenal = 44%. MS-EI: *m/z* (intensity in %) 41 (100), 69 (44), 39 (27), 55 (24), 42 (15), 83 (12), 98 (12), 70 (12), 80 (11), 53 (10). MS-CI (isobutane): *m/z* (intensity in %) 99 (100; M + H^+).

(*Z*)-1,5-Octadien-3-one (6). (*Z*)-3-Hexenal (3 mmol; 294 mg) was reacted with vinyl magnesium bromide (10 mmol) (*26*) and the resulting (*Z*)-1,5-octadien-3-ol was subsequently oxidized into the target compound with use of Dess−Martin-periodinane (2.2 mmol, 933 mg) as described above. The concentration of the target compound was determined by using 1-octen-3-one as the internal standard.

Yield: 87 mg = 35%. MS-EI: *m/z* (intensity in %) 55 (100), 41 (23), 39 (9), 95 (8), 109 (7), 67 (6), 69 (5). MS-CI (isobutane): *m/z* (intensity in %) 125 (100; M + H^+).

Methyl 2-Hydroxy-3-methylpentanoates. The synthesis of the four stereoisomers of methyl 2-hydroxy-3-methylpentanoate started from the four chiral amino acids L-isoleucine, D-isoleucine, L-allo-isoleucine, or D-allo-isoleucine, respectively, according to the procedure described recently for the respective ethyl 2-hydroxy-3-methylpentanoates (*27*). The following data were obtained:

Methyl (2*R*,3*S*)-2-Hydroxy-3-methylpentanoate. Yield: 52 mg = 40%. MS-EI: *m/z* (intensity in %): 90 (100), 87 (71), 45 (70), 41 (47), 57 (38), 69 (25), 43 (14), 58 (10), 39 (9). MS-CI: *m/z* (intensity in %) 147 (100; M + H^+).

Methyl (2*S*,3*S*)-2-Hydroxy-3-methylpentanoate. Yield: 29 mg = 22%. MS-EI: *m/z* (intensity in %) 90 (100), 87 (62), 45 (55), 41 (44), 57 (41), 69 (23), 39 (8). MS-CI: *m/z* (intensity in %) 147 (100; M + H^+).

Methyl (2*R*,3*R*)-2-Hydroxy-3-methylpentanoate. Yield: 57 mg = 43%. MS-EI: *m/z* (intensity in %) 90 (100), 45 (62), 87 (59), 41 (44), 57 (36), 69 (20), 43 (14), 39 (9), 58 (9). MS-CI: *m/z* (intensity in %) 147 (100; M + H^+).

Methyl (2*S*,3*R*)-2-Hydroxy-3-methylpentanoate. Yield: 41 mg = 31%. MS-EI: *m/z* (intensity in %): 90 (100), 87 (65), 45 (51), 57 (35), 41 (34), 69 (21), 85 (8), 58 (7). MS-CI: *m/z* (intensity in %) 147 (100; M + H^+).

(*R/S*)-3-Sulfanylhexyl Acetate. The compound was prepared following closely a method previously described (*28*). To a solution of racemic 3-sulfanyl-1-hexanol (20 mmol; 2.69 g) in dichloromethane (15 mL) was added acetyl chloride (50 mmol; 3.93 g) dissolved in dichlormethane (15 mL) at 0 °C under argon atmosphere, and the mixture was stirred for 2 h at room temperature. The solvent and the excess of acetyl chloride were removed in vacuo, and the (*R*/*S*)-3-sulfanylhexyl acetate formed was purified by distillation in vacuo.

Yield: 2.64 *g* = 75%. MS-EI: *m/z* (intensity in %) 43 (100), 55 (49), 88 (45), 116 (45), 83 (38), 73 (33), 87 (25), 67 (22), 41 (21), 82 (14). MS-CI: *m/z* (intensity in %) 117 (100; M + H^+ − CH_3COOH).

To separate both isomers in a preparative scale, the racemic mixture was reacted with enantiomerically pure (1*S*,4*R*)-camphanic acid chloride to yield two diastereoisomers (*28*). Separation was done by the following procedure:

The diastereomers of 3-{[(1*S*,4*R*)-camphanoyl]sulfanyl}hexyl acetates (5.6 mmol; 2 g) were applied onto a water-cooled (12 °C) glass column (60 × 3.5 cm i.d.) packed with a slurry of purified silica gel in pentane. Elution was performed with pentane/diethyl ether (80:20; by volume; 4 L). The eluate was collected in portions of 50 mL and monitored for the analytes by TLC (silica gel 60 F_{254}, 5 × 10 cm, Merck, Darmstadt, Germany), using the solvent mixture pentane/diethyl ether, 80:20, v/v. The target compounds were detected in fractions 39−72 (1900−3600 mL). These fractions were submitted to HPLC analysis to determine the isomeric distribution. Fractions 45 ((*R*)-derivative) and 69 ((*S*)-derivative), respectively, showed the highest purities and were used to prepare the enantiopure thiols for the sensory experiments.

Fractions 39−44 and 46−48 ((3*R*)-derivative) as well as 65−68 and 70−72 ((3*S*)-derivative) were combined and used for the preparation of the {[(2*S*)-2-phenylpropanoyl]sulfanyl}hexyl acetates needed in the NMR experiments.

(*R*)-3-Sulfanyl-1-hexanol and (*S*)-3-Sulfanyl-1-hexanol. The enantiopure 3-{[(1*S*,4*R*)-camphanoyl]sulfanyl}hexyl acetates were treated with lithium aluminum hydride (*28*) to obtain the free thiols. The concentrations of the target compounds were determined by GC-FID, using methyl octanoate as the internal standard. To take into account the different detector responses, a correction factor was determined from a mixture of known amounts of racemic 3-sulfanyl-1-hexanol and methyl octanoate.

Yield: 7.46 mg of (3*R*)-3-sulfanyl-1-hexanol with an enantiomeric purity of 99.8% and 6.78 mg of (3*S*)-3-sulfanyl-1-hexanol with an enantiomeric purity of 99.7%. MS-EI: *m/z* (relative intensity in %) 55 (100), 100 (64), 57 (57), 41 (49), 61 (43), 67 (35), 82 (34), 83 (28), 47 (19), 73 (16), 88 (15), 39 (15), 43 (14), 134 (14, M^+), 56 (13), 71 (13), 69 (13), 45 (12); 42 (11), 59 (11).

(*R*)-3-Sulfanylhexyl Acetate and (*S*)-3-Sulfanylhexyl Acetate. Enantiopure 3-sulfanylhexyl acetates were prepared from (*R*)-3-sulfanyl-1-hexanol (0.028 mmol; 3.73 mg) or (*S*)-3-sulfanyl-1-hexanol (0.025 mmol; 3.39 mg), respectively, as described above for the racemic compound. The crude products were purified by column chromatography, using a water-cooled (12 °C) glass column (15 × 2 cm i.d.)

filled with a slurry of purified silica gel in pentane. Elution was performed with pentane/diethylether (90:10, by volume, 300 mL). The eluate was collected in fractions of 50 mL, and the target compound was obtained in fractions 3−4 (100−200 mL). The concentration was determined by GC-FID, using methyl octanoate as the internal standard. To take into account the different detector responses, a correction factor was determined from a mixture of known amounts of racemic 3-sulfanylhexyl acetate and methyl octanoate.

Yield: 2.30 mg of (3*R*)-3-sulfanylhexyl acetate = 41% with an enantiomeric purity of 100% and 2.71 mg of (3*S*)-3-sulfanylhexyl acetate = 53% with an enantiomeric purity of 99.5%.

The absolute configuration of the thiol group was derived from ^{1}H NMR data of the hydratropic acid thiol esters prepared by a reaction with (*R*)-2-phenylpropanoyl chloride as described in ref *28*. The chloride was generated by a treatment of (*R*)-2-phenylpropanoic acid with oxalyl chloride.

Isolation of the Volatiles. Whole fruits (100 g) were blended using a commercial stainless steel blender. After 5 min, dichloromethane (300 mL) was added to the puree, and the mixture was cooled in an ice bath. With continuous stirring and cooling, sodium sulfate (250 g) was added in small portions. The mixture was filtered through defatted cotton wool and the sodium sulfate/guava powder obtained was washed with another portion of dichloromethane (200 mL). The combined organic phases, exhibiting the characteristic aroma of guava fruits, were submitted to SAFE distillation (*29*). The distillate obtained was extracted three times with an aqueous sodium carbonate solution (0.5 mol/L; total volume: 400 mL) to remove the acidic volatiles. The organic phase, containing the neutral and basic volatiles (NBV), was dried over anhydrous sodium sulfate and concentrated to 1 mL by distilling off the solvent by means of a Vigreux column (50 × 1 cm). The combined aqueous phases were washed with dichloromethane (50 mL), acidified (pH 2) with hydrochloric acid (16%), and extracted with dichloromethane (total volume: 300 mL) to yield the fraction of acidic volatiles (AV), which was also concentrated to 1 mL.

Gas Chromatography-FID and Gas Chromatography-Olfactometry (GC-O). GC-FID and GC-O analyses were performed with a gas chromatograph 8160 (Fisons Instruments, Mainz, Germany) with helium as the carrier gas. Samples were applied by the cold-on-column injection technique. Capillaries used were DB-5, DB-1701, and DB-FFAP (each 30 m × 0.32 mm i.d., 0.25 μm film thickness, 70 kPa head pressure) (J&W Scientific, Chromatographie-Handel Müller, Fridolfing, Germany). Columns BGB-175 and BGB-176 (each 30 m × 0.25 mm i.d., 0.25 μm film thickness, 140 kPa head pressure) were obtained from BGB Analytik (Schlossböckelheim, Germany).

For GC-O applications, the end of the capillaries were connected to a deactivated Y-shaped glass splitter (Chromatographie Handel Mueller, Fridolfing, Germany) dividing the effluent into two equal parts, which were transferred via two deactivated fused silica capillaries (50 cm × 0.25 mm) to a sniffing port and an FID, respectively. The sniffing port consisted of a cylindrically shaped aluminum device (80 mm × 25 mm i.d.) with a beveled top and a central drill hole (2 mm) housing the capillary. It was mounted on a detector base of the GC and heated to 180 °C. The FID was operated at 250 °C with hydrogen (20 mL/min) and air (200 mL/min). Nitrogen (30 mL/min) was used as the makeup gas. The injection volume was 0.5 μL. During a GC-O run, the nose of the panelist was placed closely above the top of the sniffing port and the odor of the effluent was evaluated. If an odor was recognized, the retention time was marked in the chromatogram, and the odor quality was assigned. The panelists were trained on a "flavor language" in several sessions, in which pure reference odorants were evaluated. The GC-O analyses were performed by at least three panelists.

Aroma Extract Dilution Analysis (AEDA). Fractions NBV and AV were stepwise diluted to obtain dilutions of 1:1, 1:2, 1:4, 1:8, 1:16, ..., 1:4096 of the original solutions (*23, 30*). Each dilution was submitted to GC-O, using capillary FFAP. The oven temperature was programmed as follows: 2 min at 40 °C, then at 6 deg/min to 190 °C and finally at 12 deg/min to 240 °C. The odor-active compounds were located in the chromatograms, and each odorant detected was assigned an FD factor representing the highest dilution in which the odorant was detectable. The FD factors obtained by three panelists were averaged.

Fractionation of Volatiles by Column Chromatography. Fraction NBV, isolated as detailed above, was concentrated to 1 mL with use of a Vigreux column (50 × 1 cm) and applied at 12 °C onto a water-cooled glass column (25 × 1 cm i.d.) filled with a slurry of purified silica gel (9 g) in pentane. Elution was performed with pentane (50 mL) followed by pentane/diethyl ether (99:1; v/v; 50 mL), pentane/diethyl ether (90:10; v/v; 50 mL), pentane/diethyl ether (70:30; v/v; 50 mL), and finally diethyl ether (50 mL). The eluate was collected in 10 mL fractions. The odorants detected during AEDA were localized in the fractions by GC-O, and mass spectra were recorded by GC-MS, using capillaries DB-5 and FFAP, respectively.

Selective Enrichment of Thiols. Fraction NBV was concentrated to 5 mL, and the thiols were isolated by affinity chromatography on mercurated agarose gel as recently reported (*31*).

Gas Chromatography−Mass Spectrometry (GC-MS). Mass spectra were recorded on a gas chromatograph 5890 series II (Hewlett-Packard, Waldbronn, Germany) connected to a sector field mass spectrometer MAT 95 S (Finnigan, Bremen, Germany). Mass spectra in the electron ionization mode (MS-EI) were recorded at 70 eV ionization energy, and mass spectra in the chemical ionization mode (MS-CI) at 115 eV with isobutane as the reactant gas.

Ratio of 2-Methylbutanoic Acid to 3-Methylbutanoic Acid. The ratio of the amounts of 2- and 3-methylbutanoic acid was determined by GC-MS calculating the intensities of the fragments *m/z* 60 (3-methylbutanoic acid) and *m/z* 74 (2-methylbutanoic acid). Seven defined mixtures of 2- and 3-methylbutanoic acid (100:0; 90:10; 70:30; 50:50; 30:70; 10:90; 0:100) were analyzed under the same conditions and a calibration line was drawn plotting the intensity ratio of *m/z* 60 over the sum of *m/z* 60 + *m/z* 74 against the percentage of 3-methylbutanoic acid in the mixture.

Two-Dimensional Gas Chromatography−Mass Spectrometry (TD-GC-MS). The system consisted of a Combi PAL autosampler (CTC Analytics, Zwingen, Switzerland), a Trace GC 2000 series, equipped with a cold on column injector and a moving column stream switching system (Thermo Finnigan, Egelsbach, Germany), a heated (250 °C) transfer line to the cold trap (SGE, Darmstadt, Germany) localized in the second GC (CP 3800, Varian, Darmstadt, Germany), and an ion trap mass spectrometer (Saturn 2000, Varian). Spectra were recorded either in the EI or in the CI mode with methanol as the reactant. For the determination of the enantiomeric distribution of **16** and **18**, the first oven was equipped with an FFAP column and the second with a BGB-175 column. The determination of the enantiomeric distribution in the methyl 2-hydroxy-3-methylpentanoates was performed on a DB-5 column in the first and a BGB-176 column in the second dimension. The start temperature was 40 °C in all cases. Temperature gradients were 6 deg/min in the first and 2 deg/min in the second dimension.

Determination of Odor Thresholds. These were determined by aroma extract dilution analysis of a mixture containing known amounts of the target odorant and (*E*)-2-decenal as internal standard. Thresholds were calculated from the FD factors determined by using the method of Ullrich and Grosch (*32*) and a threshold of 2.7 ng/L for (*E*)-2-decenal (*33*).

High-Performance Liquid Chromatography (HPLC). To determine the isomeric purity of (*S*)- and (*R*)-3-{[(1*S*,4*R*)-camphanoyl]sulfanyl}hexyl acetate, an HPLC-System consisting of a Jasco PU-1580 HPLC pump (Jasco, Grossumstadt, Germany), a Nucleosil-100-5 C18 column (Macherey-Nagel, Düren, Germany), and a Jasco UV-1575 UV/vis detector was used. The acetates were isolated with hexane/ethyl acetate (90:10; by volume; 1 mL/min) as the eluent, which was monitored at 254 nm.

RESULTS AND DISCUSSION

Aroma-Active Compounds in Guavas. Guava fruits were blended and mixed with anhydrous sodium sulfate to obtain a dry powder, and the homogenate was extracted with dichloromethane by vigorous stirring. The addition of sodium sulfate was necessary to achieve a homogeneous mixture because, due to the high pectin content (*34*), the fruit mash otherwise formed an unextractable, gum-like matter.

Table 1. Aroma-Active Compounds (FD ≥ 8) Detected in a Solvent Extract Obtained from Fresh, Pink Guavas

no.[a]	odorant[b]	odor quality[c]	RI[d] (FFAP)	RI[d] (DB-5)	FD factor[e]	earlier reported as volatile constituent, ref no.
1	ethyl butanoate	fruity	1028	802	256	*4*
2	hexanal	grassy	1075	802	256	*3*
3	(*E*)-3-hexenal	grassy	1130	800	8	*7*
4	(*Z*)-3-hexenal	grassy	1135	796	512	*7*
5	1-octen-3-one[f]	mushrooms	1289	978	8	*7*
6	(*Z*)-1,5-octadien-3-one[f]	geranium leaves	1364	987	8	
7	methional	cooked potato	1443	903	256	
8	methyl (2*R*,3*S*)-2-hydroxy-3-methylpentanoate	fruity	1473	992	32	
9	methyl (2*S*,3*S*)-2-hydroxy-3-methylpentanoate	fruity	1493	994	16	
10	linalool[f]	flowery, citrus	1540	1103	8	*10*
11	4-methoxy-2,5-dimethyl-3(2*H*)-furanone	caramel	1591	1041	2048	*17*
12	methyl benzoate	violet, floral	1610	1096	64	*3*
13	butanoic acid	rancid, cheese	1615	817	8	*7*
14	ethyl benzoate	violet, floral	1652	1170	32	*7*
15	2- and 3-methylbutanoic acid[g]	rancid, cheese	1658	885	8	*9*
16	3-sulfanylhexyl acetate	black currant	1708	1249	2048	
17	pentanoic acid	rancid, cheese	1726	918	32	*9*
18	3-sulfanyl-1-hexanol	grapefruit	1831	1418	2048	
19	γ-octalactone	coconut	1906	1257	8	*10*
20	heptanoic acid	rancid, cheese	1943	1112	8	*7*
21	*trans*-4,5-epoxy-(*E*)-2-decenal[f]	metallic	2006	1383	512	
22	γ-nonalactone[f]	coconut	2021	1370	32	*10*
23	4-hydroxy-2,5-dimethyl-3(2*H*)-furanone	caramel	2023	1081	2048	*7*
24	*trans*-4,5-epoxy-(*E*)-2-undecenal[f]	metallic	2094	1486	32	
25	γ-decalactone	peach	2138	1468	8	*7*
26	cinnamyl acetate	floral, honey	2144	1453	128	*3*
27	δ-decalactone[f]	coconut	2188	1495	64	*11*
28	3-hydroxy-4,5-dimethyl-2(5*H*)-furanone	seasoning-like	2200	1136	1024	
29	cinnamyl alcohol	floral, honey	2284	1335	512	*7*
30	γ-dodecalactone[f]	peach	2383	1678	64	*11*
31	phenylacetic acid[f]	honey	2555	1261	8	*9*

[a] Odorants were consecutively numbered according to their retention time on the FFAP column. [b] Odorants were identified by comparing their retention indices on the FFAP and DB-5 column, their mass spectra obtained by GC-MS, and their odor characteristics with respective data of reference compounds. [c] Odor quality as perceived at the sniffing port during GC-O. [d] RI = retention index. [e] FD factor = flavor dilution factor. [f] Unequivocal mass spectra could not be obtained in the guava extract, identification was based on the resting criteria detailed in footnote *b*. [g] 2- and 3-methylbutanoic acid were not separated on the GC-column used; mass spectral data showed a mixture of both isomers and their ratio was determined from the intensities of characteristic mass fragments as 78% 2- and 22% 3-methylbutanoic acid.

To avoid odorant degradation and/or artifact formation, high temperatures were avoided throughout the entire workup procedure. In particular, addition of sodium sulfate to the homogenate was done in small portions under ice-cooling to compensate for the heat liberated by hydration. Extraction with dichloromethane was performed at ambient temperature and during SAFE distillation the temperature was always kept below 40 °C. The distillate obtained represented the typical guava flavor with its green, sweet, tropical-fruit and also grapefruit-like notes.

The volatiles were separated into a fraction containing the neutral and basic volatiles (NBV) and a fraction containing the acidic volatiles (AV) by treatment with aqueous sodium carbonate. Both fractions were concentrated at 40 °C with use of a Vigreux column, and the aroma-active compounds in the concentrates were screened by application of the aroma extract dilution analysis. A total of 31 odor-active regions were detected in both fractions, among which two areas with caramel-like aromas (**11** and **23**), a grapefruit-like odor (**18**), and a black-currant aroma quality (**16**) showed the highest flavor dilution (FD)-factors (**Table 1**). Structural assignments of the odorants detected were performed by comparing retention indices, odor qualities, and odor intensities with data available in an in-house database consisting of nearly 1000 compounds previously identified as odorants in foods. On the basis of the suggested structure, mass spectra of the analyte and the corresponding reference compound were recorded. To avoid coelutions during mass spectrometric analysis, the volatiles present in the NBV were further fractionated by column chromatography on silica gel. In a parallel experiment, thiols were selectively enriched by affinity chromatography on mercurated agarose gel (*35*).

In the FD factor range of 8 to 2048 a total of 31 aroma-active compounds were identified. The highest FD factors (range 512−2048), were found for eight compounds, the structures of which are displayed in **Figure 1**. The two compounds eliciting a caramel-like odor note at the highest FD factor of 2048 were identified as 4-methoxy-2,5-dimethyl-3(2*H*)-furanone (**11**) and 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone (**23**). In addition, the 3-sulfanylhexyl acetate (**16**) with a black-currant-like aroma and the grapefruit-like smelling 3-sulfanyl-1-hexanol (**18**) were identified with high FD factors. Also the seasoning-like smelling 3-hydroxy-4,5-dimethyl-2(5*H*)-furanone (**28**), the green, grassy (*Z*)-3-hexenal (**4**), the metallic *trans*-4,5-epoxy-(*E*)-2-decenal (**21**), and the floral cinnamyl alcohol (**28**), followed by ethyl butanoate (**1**), hexanal (**2**), methional (**7**), and cinnamyl acetate (**26**) showed high odor activity. Among these compounds, 3-sulfanylhexyl acetate, 3-sulfanyl-1-hexanol, 3-hydroxy-4,5-dimethyl-2(5*H*)-furanone, *trans*-4,5-epoxy-(*E*)-2-decenal, and methional were identified for the first time in guavas.

Structural Assignment of 16 and 18. Several substituted cyclodextrine derivatives have already been applied to separate either 3-sulfanylhexyl acetate (**16**) or 3-sulfanyl-1-hexanol (**18**) into its enantiomers (*36–38*). Good separations were found in our study with 2,3-diacetyl-6-*tert*-butyldimethylsilyl-β-cyclodextrin dissolved in 1701 polysiloxane as the stationary phase.

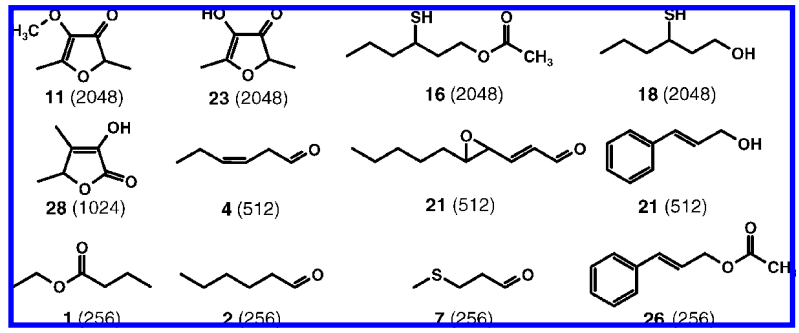

Figure 1. Aroma-active compounds showing the highest FD factors in pink guava fruits (*Psidium guajava* L.).

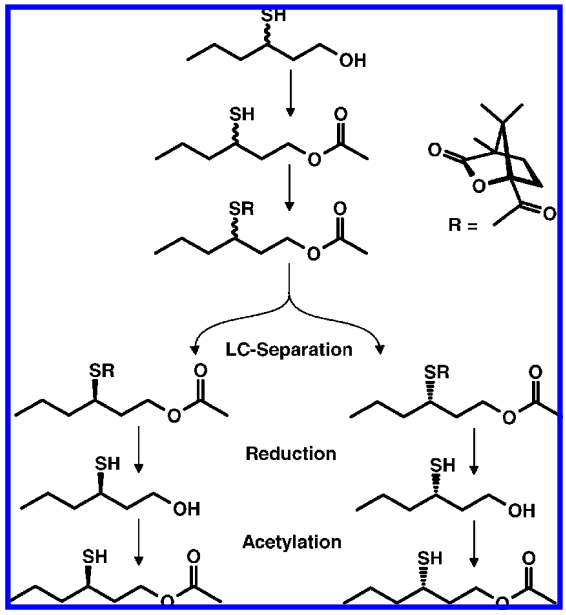

Figure 2. Synthetic pathway used in the preparation of enantiopure (*R*)- and (*S*)-3-sulfanyl-1-hexanol and the respective *O*-acetates.

To assign the correct elution order, enantiopure samples of both isomers were prepared. This was achieved by a separation of the diastereomeric compounds obtained by forming a derivative of 3-sulfanylhexyl acetate with the enantiomerically pure (1*S*,4*R*)-camphanoyl chloride (*28*). The diastereomers were separated by column chromatography on silica gel and, after a reductive cleavage of the esters, the enantiopure (*R*)- and (*S*)-3-sulfanyl-1-hexanols were obtained in an enantiomeric purity of nearly 100%. The entire procedure applied to prepare the enantiopure compounds is schematically shown in **Figure 2**. The assignment of the correct stereochemistry was performed by ^{1}H NMR of the *O*-acetyl-*S*-[(2*S*)-2-phenylpropanoyl] derivatives (*28*).

The results revealed that 3-sulfanyl-1-hexanol occurred almost in a racemic ratio (**Table 2**) with the (*R*)- and the (*S*)-isomer eliciting the same grapefruit-like odor at the extremely low odor threshold of 70−80 pg per liter in air. The corresponding acetates also occurred as a nearly racemic mixture with both enantiomers eliciting a black-currant like aroma. The odor thresholds were in the same order of magnitude as those of the corresponding thiols (**Table 2**).

Structural Assignment of Diastereomeric Methyl 2-Hydroxy-3-methylpentanoates. The four possible stereoisomers were separately synthesized from the respective chiral amino acid. All four isomers were characterized by their retention indices on different GC columns, their mass spectra, and their odor properties (**Table 3**).

Table 2. Chromatographic Parameters and Sensory Properties of the Enantiomers of 3-Sulfanyl-1-hexanol and 3-Sulfanylhexyl Acetate and Their Distribution in Guavas.

compd	RI[a] (BGB-175)	odor quality[b]	odor threshold[c] [ng/L in air]	enantiomeric distribution in guavas [%]
(*R*)-3-sulfanyl-1-hexanol	1497	grapefruit	0.08	43
(*S*)-3-sulfanyl-1-hexanol	1490	grapefruit	0.07	57
(*R*)-3-sulfanylhexyl acetate	1461	black-currant	0.10	42
(*S*)-3-sulfanylhexyl acetate	1466	black-currant	0.03	58

[a] RI = retention index. [b] Odor quality as perceived at the sniffing port during GC-O. [c] Odor threshold in air as determined according to ref *32*.

Table 3. Analytical and Sensory Data of the Four Isomeric Methyl 2-Hydroxy-3-methylpentanoates

isomer	RI[a] (DB-5)	RI[a] (1701)	RI[a] (FFAP)	RI[a] (BGB-176)	odor quality[b]	odor threshold[c] [ng/L]
(2*S*,3*S*)-	991	1101	1492	1114	fruity	10
(2*R*,3*R*)-	991	1101	1492	1100		>200
(2*S*,3*R*)-	990	1095	1471	1086	fruity	110
(2*R*,3*S*)-	990	1095	1471	1092	fruity	11

[a] RI = retention index. [b] Odor quality perceived at the sniffing port during GC-O. [c] Odor threshold in air as determined according to ref *26*.

As to be expected, mass spectra of all isomers were quite similar and were, thus, inappropriate for differentiation. Also no reliable differentiation was possible on the basis of their retention indices on nonpolar GC columns like DB-1 or DB-5 (**Table 3**). However, the diastereomers showed good separation on polar GC columns, and on a chiral β-cyclodextrine column (BGB-176) all four isomers were well separated. Interestingly, the odor thresholds of the (2*S*,3*S*)- and (2*R*,3*S*)-isomer were much lower as compared to the respective (3*R*)-isomers (**Table 3**). A comparison of the retention indices of **8** and **9** with those of the reference compounds (**Figure 3**) showed the presence of the fruity smelling methyl (*R*,*S*)-2-hydroxy-3-methylpentanoate (**8**) and methyl (*S*,*S*)-2-hydroxy-3-methylpentanoate (**9**) in a ratio of 60% to 40%, while the sensorially less active (*S*,*R*)- and (*R*,*R*)-isomers where absent.

As a result of the identification experiments, 31 key odorants of pink guavas could be identified (**Table 1**), among which nine aroma compounds, namely (*Z*)-1,5-octadien-3-one, methional, methyl (2*R*,3*S*)-2-hydroxy-3-methylpentanoate, methyl (2*S*,3*S*)-2-hydroxy-3-methylpentanoate, 3-sulfanylhexyl acetate, 3-sulfanyl-1-hexanol, *trans*-4,5-epoxy-(*E*)-2-decenal, *trans*-4,5-epoxy-(*E*)-2-undecenal, and 3-hydroxy-4,5-dimethyl-2(5*H*)-furanone were identified for the first time in guavas.

The results of the AEDA suggested that the most important aroma compounds of guavas are the following: (*Z*)-3-hexenal,

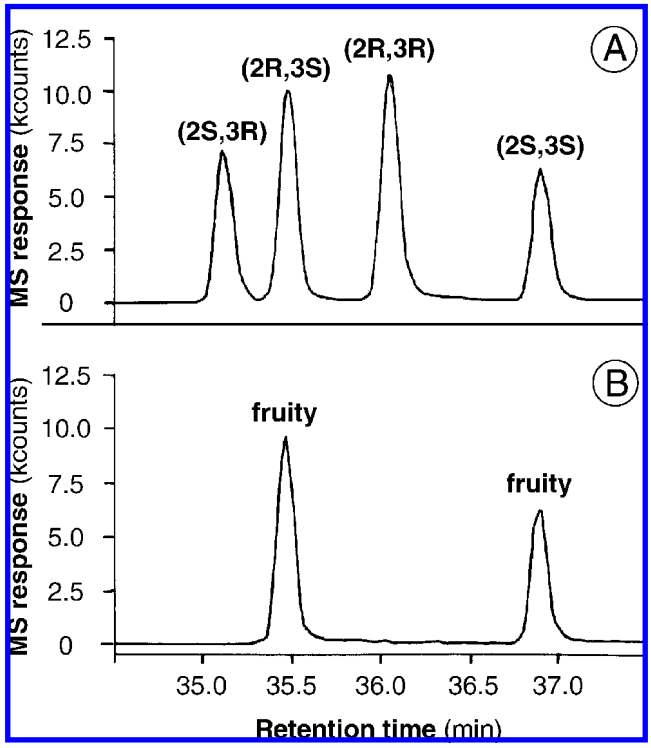

Figure 3. GC-separation of the synthesized isomers of methyl 2-hydroxy-3-methylpentanoate (**A**) and their occurrence in the distillate obtained from pink guava (**B**)

hexanal, 3-sulfanyl-1-hexanol, 3-sulfanylhexyl acetate, 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone, and 4-methoxy-2,5-dimethyl-3(2*H*)-furanone, with (*Z*)-3-hexenal and hexanal being responsible for the green aroma note, and 3-sulfanyl-1-hexanol and 3-sulfanylhexyl acetate for the tropical and grapefruit-like note. 4-Hydroxy-2,5-dimethyl-3(2*H*)-furanone, together with some other compounds, such as ethyl butanoate, methyl and ethyl benzoate, cinnamyl alcohol, and cinnamyl acetate, might account for the sweet and fruity character.

(*Z*)-3-Hexenal and hexanal have frequently been reported in *Psidium guajava* (*3, 9–15, 18, 19*). Together with (*E*)-2-hexenal and the corresponding odorless alcohols these compounds have previously been reported as major volatiles in guavas (*11*) and are suggested to be formed by an enzymatic degradation of unsaturated fatty acids upon disruption of the plant tissue (*39*).

Several thiols other than the 3-sulfanyl-1-hexanol and 3-sulfanylhexyl acetate have been reported in *Psidium guajava* before (*11, 16*). Nishimura et al. (*11*) identified 2-methylpropanethiol and 6-sulfanyl-1-hexanol, while Bassols and Demole (*16*) detected 2-pentanethiol, a compound earlier found by Idstein and Schreier (*7*), but was assigned as 3-pentanethiol by these authors. However, neither 3-sulfanyl-1-hexanol (**18**) nor 3-sulfanylhexyl acetate (**16**) have been reported as a constituent of pink guavas up to now. The crucial problem with these compounds might be their instability during workup. In earlier studies on guava volatiles often high temperatures were applied, either to isolate the volatiles, e.g., by steam-distillation (*6*), or by using split injection in GC analysis (*19*).

3-Sulfanyl-1-hexanol was first identified in yellow passion fruit (*Passiflora edulis* f. *flavicarpa*) by Engel et al. (*40*). Later it was also found in wine (*41*), grapefruit juice (*42*), and beer (*43*). It was also suggested to play a role in the odor of human axiliary sweat (*44, 45*). Also 3-sulfanylhexyl acetate (**16**) was previously reported in passion fruit (*40*), and later in wine (*41*) and grapefruit juice (*42*).

Our results demonstrate that the enantiomeric distribution in both **16** and **18** was virtually the same and was close to the racemate (**Table 2**). But in passion fruits, the (*S*)-isomers prevailed with 58−90% for the alcohol (*36–38*) and 91−96% for the acetate (*36, 38*). In wine, also the (*S*)-isomer predominated with 68−73% in the alcohol, whereas the ester showed an *R*/*S*-ratio of 41/59 to 57/43, except for wine made from botrytized grapes, in which the (*S*)-isomer predominated (*46*).

The low odor thresholds in air determined for **18** and **16** (**Table 2**) corresponded well with the low thresholds in water previously published by Bouchiloux et al. (**18**: 12−15 ng/L; **16**: 2−3 ng/L) (*47*). The two enantiomers of **18** exhibited nearly the same odor thresholds, which was in agreement with results of Tominaga et al. (*46*). However, these authors found some differences in the odor quality of the enantiomers. They described the (*R*)-isomer as grapefruit, citrus-peel-like, while the (*S*)-isomer was found to be more passion fruit-like.

For the two enantiomers of 3-sufanylhexyl acetate (**16**), the thresholds in air differed by a factor of 3−4 with the (*S*)-isomer being more odor-active than the (*R*)-isomer. This result also confirmed recent data found in a hydro-alcoholic wine model solution (*46*). However, while in our study, both enantiomers were described as black-currant-like during GC-O, Tominaga et al. (*46*) and Weber et al. (*36*) described the odor of the (*R*)-isomer as more passion fruit-like and that of the (*S*)-isomer as more herbaceous.

In passion fruits (*48*), grapes (*41, 49*), and also in axilliary sweat (*44, 45*), 3-sulfanyl-1-hexanol was shown to be derived from the cleavage of a nonvolatile precursor, (*S*)-(1-hydroxy-3-hexyl)cysteine, most probably by the action of a β-lyase (*45, 50*). In grapes, this precursor is located predominantly in the skin (*49, 51*) and liberates the thiol during fermentation (*42*).

The gasoline-like smelling 2-pentanethiol, earlier held responsible for the tropical odor note in white-fleshed guavas by König et al. (*52*), was not detected in our study.

4-Hydroxy-2,5-dimethyl-3(2*H*)-furanone (**23**) was earlier found by Idstein and Schreier (*7*) and also by Jordan et al. (*19*) in guavas, while its methoxy derivative **11** was only reported by Pino et al. (*17*) so far. Methyl and ethyl benzoate have been reported as guava constituents in numerous studies (*3, 7, 10–12, 15, 17, 19, 34*) as well as cinnamyl alcohol (*12, 13, 19*) and cinnamyl acetate (*7, 14, 15, 34*).

Although the chiral methyl 2-hydroxy-3-methylpentanoates have already been found in other fruits, both esters were found for the first time as aroma compounds of guavas in this study. The enantiomeric distribution in the methyl 2-hydroxy-3-methylpentanoates (**Figure 2**) in guavas suggests their biogeneration from L-isoleucine via the corresponding β-keto-acid followed by a nonstereospecific reduction to the alcohol.

The aroma compounds of different varieties may also be different. Therefore, quantitative studies are under way to confirm the contribution of the odor-active compounds to the overall aroma of pink and also white guavas.

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