

Characterization of the Major Odor-Active Compounds in Thai Durian (*Durio zibethinus* L. 'Monthong') by Aroma Extract Dilution Analysis and Headspace Gas Chromatography–Olfactometry

Jia-Xiao Li, Peter Schieberle, and Martin Steinhaus*

Deutsche Forschungsanstalt für Lebensmittelchemie (German Research Center for Food Chemistry), Lise-Meitner-Strasse 34, 85354 Freising, Germany

S Supporting Information

ABSTRACT: An aroma extract dilution analysis applied on the volatile fraction isolated from Thai durian by solvent extraction and solvent-assisted flavor evaporation resulted in 44 odor-active compounds in the flavor dilution (FD) factor range of 1–16384, 41 of which could be identified and 24 that had not been reported in durian before. High FD factors were found for ethyl (2*S*)-2-methylbutanoate (fruity; FD 16384), ethyl cinnamate (honey; FD 4096), and 1-(ethylsulfanyl)ethanethiol (roasted onion; FD 1024), followed by 1-(ethyldisulfanyl)-1-(ethylsulfanyl)ethane (sulfury, onion), 2(*S*)-ethyl-4-hydroxy-5(2)-methylfuran-3(2*H*)-one (caramel), 3-hydroxy-4,5-dimethylfuran-2(*SH*)-one (soup seasoning), ethyl 2-methylpropanoate (fruity), ethyl butanoate (fruity), 3-methylbut-2-ene-1-thiol (skunky), ethane-1,1-dithiol (sulfury, durian), 1-(methylsulfanyl)-ethanethiol (roasted onion), 1-(ethylsulfanyl)propane-1-thiol (roasted onion), and 4-hydroxy-2,5-dimethylfuran-3(2*H*)-one (caramel). Among the highly volatile compounds screened by static headspace gas chromatography–olfactometry, hydrogen sulfide (rotten egg), acetaldehyde (fresh, fruity), methanethiol (rotten, cabbage), ethanethiol (rotten, onion), and propane-1-thiol (rotten, durian) were found as additional potent odor-active compounds. Fourteen of the 41 characterized durian odorants showed an alkane-1,1-dithiol, 1-(alkylsulfanyl)alkane-1-thiol, or 1,1-bis(alkylsulfanyl)alkane structure derived from acetaldehyde, propanal, hydrogen sulfide, and alkane-1-thiols. Among these, 1-(propylsulfanyl)ethanethiol, 1-[[1-(methylsulfanyl)ethyl]-sulfanyl]ethanethiol, and 1-[[1-(ethylsulfanyl)ethyl]sulfanyl]ethanethiol were reported for the first time in a natural product.

KEYWORDS: dithiohemiacetal, dithioacetal

■ INTRODUCTION

Durian is the fruit of the durian tree (*Durio zibethinus* L.) belonging to the mallow family (Malvaceae).¹ The genus *Durio* is native to Southeast Asia with the center of diversity in Borneo. Several wild *Durio* species produce edible fruits, but only *D. zibethinus* is cultivated on a large scale.² Main producers of durian are Thailand, Malaysia, and Indonesia.³ In the United States and Europe, durian is available in Asian food shops.

Durian fruits are of a spherical to ovoid shape and typically 2–3 kg in weight. The thorny husk has a green to brownish olive color. The fruit is a capsule, normally divided into five locules. Each locule contains one to five seeds covered by a fleshy yellow aril.^{2,3} These arils constitute the edible part, the pulp, and represent 20–30% of the fruit weight.^{2,3} They are highly nutritious with ~30% carbohydrates, ~3% fat, and ~3% protein. Durian is mainly consumed as fresh fruit, but is locally also used as an ingredient in curry, relish, cake, yogurt, sweets, and ice cream. Traditional Malaysian products are tempoyak (fermented durian) and lempuk (a durian preserve).³ In Thailand, fried durian chips are a popular snack.

The sensory properties of fresh durian combine a pleasant creamy consistency, a pronounced sweet taste, and a strong, penetrating odor, not comparable to that of any other kind of fruit. The aroma profile can be best described as a combination of an intense sulfury, roasted onion-like odor with fruity, sweet, caramel-like, and soup seasoning-like notes. In Southeast Asia, durian is deeply appreciated and often referred to as the “king

of fruits”, whereas some people in the Western hemisphere regard the durian odor as offensive and nauseous.

The unique odor properties of durian have repeatedly attracted the attention of chemists. A pioneering work on durian odorants was published by Baldry et al. in 1972.⁴ After isolation of durian volatiles by vacuum distillation, they identified propane-1-thiol as an onion-like-smelling odorant by thin layer chromatography and mass spectrometry of the phenylmercury thiolate. Methane- and ethanethiol as well as dimethyl and diethyl sulfide were tentatively identified. By dynamic headspace gas chromatography using a packed column they identified hydrogen sulfide and diethyl disulfide. Gas chromatography–olfactometry (GC-O) of a solvent extract obtained from durian led to the identification of acetaldehyde, propanal, and a series of esters, of which ethyl 2-methylbutanoate was estimated to be the most odor-active. Using a dynamic headspace GC approach, Moser et al.⁵ assigned the sulfury odor of durian to hydrogen sulfide and dialkyl sulfides, particularly diethyl disulfide and diethyl trisulfide. Wong and Tie⁶ later identified 63 volatiles in 3 clones of Malaysian durian, among them 30 esters and 16 sulfur compounds. The major volatile was found to be 3-

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Table 1. Aroma-Active Compounds (FD ≥ 1) in the SAFE Distillate Obtained from Durian Pulp

no.	odorant ^a	odor ^b	RI ^c		FD factor ^d	fraction(s) ^e	previously reported
			FFAP	DB-5			
1	ethyl propanoate	fruity	954	713	16	NBF, SGF2	4, 6, 9, 10, 12–14, 17–19
2	ethyl 2-methylpropanoate	fruity	961	753	256	NBF, SGF2	6, 8–10, 12–14, 18, 19
3	methyl (2 <i>S</i>)-2-methylbutanoate	fruity	1006	772	64	NBF, SGF2	4–6, 8–10, 12–14, 18, 19
4	(2 <i>Z</i>)-but-2-ene-1-thiol	skunky	1009	720	4	NBF, SGF1–2, TF	
5	ethyl butanoate	fruity	1031	800	256	NBF, SGF2	6, 9, 10, 12–14, 18, 19
6	ethyl (2 <i>S</i>)-2-methylbutanoate	fruity	1048	847	16384	NBF, SGF2	4, 6, 8–10, 12–14, 16–19
7	3-methylbut-2-ene-1-thiol	skunky	1104	819	256	NBF, SGF1–2, TF	
8	propyl (2 <i>S</i>)-2-methylbutanoate	fruity	1145	947	16	NBF, SGF2	4, 6, 12–14, 17–19
9	ethane-1,1-dithiol	sulfury, durian	1174	730	256	TF	
10	diethyl disulfide	roasted onion	1210	919	2	NBF, SGF1	4–15, 17–19
11	1-(methylsulfanyl)ethanethiol	roasted onion	1235	843	256	NBF, SGF2, TF	
12	3-hydroxybutan-2-one	buttery	1283	700	2	AF, NBF, SGF5	6, 8, 9, 12, 13
13	1-(ethylsulfanyl)ethanethiol	roasted onion	1285	916	1024	NBF, SGF2, TF	6
14	1,1-bis(methylsulfanyl)ethane	metallic	1285	935	2	NBF, SGF2	7, 10
15	ethyl (2 <i>R</i>)-2-hydroxy-2-methylbutanoate	fruity	1300	917	8	NBF, SGF3	
16	1-(methylsulfanyl)propane-1-thiol	roasted onion	1322	936	16	NBF, SGF2, TF	
17	unknown	roasted peanut	1330		128	NBF, SGF2	
18	1-(ethylsulfanyl)-1-(methylsulfanyl)ethane	metallic	1339	1011	2	NBF, SGF2	7, 14
19	1-(ethylsulfanyl)propane-1-thiol	roasted onion	1365	1011	256	NBF, SGF2, TF	
20	1-(propylsulfanyl)ethanethiol	roasted sesame	1371	1009	32	NBF, SGF2, TF	
21	1,1-bis(ethylsulfanyl)ethane	rubbery, burnt	1389	1083	4	NBF, SGF2	10, 12, 13, 15
22	ethyl (methylsulfanyl)acetate	sulfury, fruity	1445	983	4	NBF, SGF2	6, 8
23	unknown	boiled cabbage	1451	983	16	NBF, SGF3	
24	diethyl trisulfide	fried shallot	1498	1136	16	NBF, SGF1	5–7, 10, 12–15, 17, 19
25	ethyl (2 <i>E</i> ,4 <i>E</i>)-hexa-2,4-dienoate	honey	1505	1092	1	NBF, SGF2	
26	3-isobutyl-2-methoxypyrazine ^f	bell pepper	1523	1181	2	NBF, SGF3	
27	linalool	floral, citrusy	1544	1098	2	NBF, SGF4	
28	butanoic acid	cheese	1626		1	AF	
29	3-sulfanylbutan-1-ol	onion, leek	1654	924	2	NBF, TF	
30	(2 <i>S</i>)-2-methylbutanoic acid	cheese	1668		2	AF	16, 19
31	1-[[1-(methylsulfanyl)ethyl]sulfanyl]ethanethiol (I)	roasted onion	1708	1215	4	NBF, SGF2, TF	
32	pentanoic acid	cheese	1733		2	AF	15
33	1-(ethyldisulfanyl)-1-(ethylsulfanyl)ethane	sulfury, onion	1735	1314	512	NBF, SGF2	7, 8
34	1-[[1-(ethylsulfanyl)ethyl]sulfanyl]ethanethiol (I)	roasted onion	1756	1285	32	NBF, SGF2, TF	
35	1-[[1-(methylsulfanyl)ethyl]sulfanyl]ethanethiol (II)	roasted onion	1770	1237	2	NBF, SGF2, TF	
36	1-[[1-(ethylsulfanyl)ethyl]sulfanyl]ethanethiol (II)	roasted onion	1811	1304	16	NBF, SGF2, TF	
37	hexanoic acid	cheese	1843		2	AF	
38	ethyl 3-phenylpropanoate	honey	1884	1351	32	NBF, SGF2	
39	<i>trans</i> -4,5-epoxy-(2 <i>E</i>)-dec-2-enal ^f	metallic	2009	1379	4	NBF, SGF3	
40	4-hydroxy-2,5-dimethylfuran-3(2 <i>H</i>)-one	caramel	2036	1056	128	AF	
41	unknown	caramel	2044		64	AF	
42	2(5)-ethyl-4-hydroxy-5(2)-methylfuran-3(2 <i>H</i>)-one	caramel		1135 ^g			
			2085	1142 ^g	512	AF	
43	ethyl cinnamate	honey	2133	1466	4096	NBF, SGF2	
44	3-hydroxy-4,5-dimethylfuran-2(5 <i>H</i>)-one	soup seasoning	2210	1100	512	AF	

^aOdorants were identified by comparing their retention indices on FFAP and DB-5 columns, their mass spectra obtained by GC-MS, and their odor characteristics as perceived during GC-O with data of reference compounds. ^bOdor quality perceived at the sniffing port during GC-O. ^cRI = retention index; calculated from the retention time of the compound and the retention times of adjacent *n*-alkanes by linear interpolation. ^dFD factor = flavor dilution factor; dilution factor of the highest dilution of the concentrated SAFE distillate in which the odorant was detected during GC-O; average of three trained panelists (one female, two males). ^eFraction(s) in which the odorant was detected by GC-O after fractionation of the initial extract: AF = fraction of acidic volatiles, NBF = fraction of neutral and basic volatiles, SGF1–5 = silica gel fractions 1–5; TF = thiol fraction. ^fAn unequivocal mass spectrum could not be obtained in the durian fruit extract fractions; identification was based on the resting criteria detailed in footnote a. ^gIsomers were separated on capillary DB-5, but not on capillary FFAP.

hydroxybutan-2-one. The ester fraction was dominated by ethyl 2-methylbutanoate and ethyl propanoate. Dithiohemiacetals

and cyclic dithioacetals were discovered, but hydrogen sulfide, methanethiol, dimethyl sulfide, and diethyl sulfide were not

reported. More recent studies led to the identification of ~200 compounds.^{7–19} However, in most cases, no attempt was made to assess the odor activity of the individual volatiles identified. As the only exception, Weenen et al.⁸ applied GC-O and a rough aroma extract dilution analysis (AEDA) (only dilution 5 and 50 were analyzed) on an extract obtained from Indonesian durian fruits. In the 50× diluted sample, 17 odor-active compounds were detected by GC-O. Among them, 12 compounds had a sulfury odor, but only a few odorants were structurally characterized. The most potent odorants were identified as ethyl 2-methylbutanoate and 3,5-dimethyl-1,2,4-trithiolane.

Despite the quite high number of studies on durian volatiles, it is still unclear which odorants predominately contribute to its aroma. Therefore, our aim was to systematically assess the odor contribution of individual durian volatiles. The volatile fraction of durian obtained from Thailand was isolated under mild conditions and screened for odor-active compounds by AEDA.²⁰ Additionally, screening for highly volatile odorants was accomplished by static headspace GC-O (SH-GC-O).

MATERIALS AND METHODS

Durian. Fruits, organically grown in Thailand, were purchased from a local Internet shop. They were obtained from trees of *D. zibethinus* 'Monthong', the most common cultivar on the Thai market. Fruits were handpicked almost fully ripe and sent to Germany by air freight within 2 days. After arrival, the fruits were stored for another 1–2 days to reach full ripeness, indicated by cracks in the husk and release of the typical odor.

Reference Odorants. Compounds **1**, **2**, **5**, **8**, **10**, **12**, **22**, **25–28**, **30**, **32**, **37**, **38**, **40**, **42–44**, **46**, **48**, **49** (Table 1), ethyl (2*R*/2*S*)-2-methylbutanoate, (2*R*/2*S*)-2-methylbutanoic acid, methyl (2*R*/2*S*)-2-methylbutanoate, and propyl (2*R*/2*S*)-2-methylbutanoate were obtained from Aldrich (Sigma-Aldrich-Chemie, Taufkirchen, Germany), and compound **24** was from Chemos (Regenstauf, Germany). 3-Sulfanylbutan-1-ol (**29**) was a gift from Johannes Polster, Technische Universität München, Freising, Germany. Further reference odorants were synthesized as detailed below.

Chemicals and Reagents. (2*R*)-2-Hydroxy-2-methylbutanoic acid was from Chemgenx (New Brunswick, NJ, USA). Other chemicals were from Sigma-Aldrich (Taufkirchen, Germany) or VWR (Darmstadt, Germany). Acetaldehyde and propanal were freshly distilled before use. Dichloromethane, diethyl ether, and pentane were distilled through a column (120 cm × 5 cm) packed with Raschig rings. Ethanol was dried above a molecular sieve (3 Å). Acetate buffer of pH 5 was prepared from sodium acetate (20.5 g) and acetic acid (23.5 g) made up to 100 mL with water. Purified silica was prepared from silica 60, 0.040–0.063 mm (VWR): After extraction with hydrochloric acid (32%; 3 h), the gel was washed with water until the eluate was acid free, dried at 120 °C until constant weight, and then adjusted to 7% water content. Mercurated agarose gel was prepared from Affi-Gel 10 (Bio-Rad, Munich, Germany).²¹

Syntheses. Hydrogen sulfide (**45**) and methanethiol (**47**) were generated in situ by adding hydrochloric acid (2 mol/L) to aqueous sodium sulfide and sodium methanethiolate, respectively.

The following reference odorants were synthesized according to previously published procedures: (2*Z*)-but-2-ene-1-thiol (**4**),²² *trans*-4,5-epoxy-(2*E*)-dec-2-enal (**39**),²³ 3-methylbut-2-ene-1-thiol (**7**).²⁴ Methyl- (**3**), ethyl- (**6**), and propyl (2*S*)-2-methylbutanoate (**8**) were synthesized from the respective alcohol and (2*S*)-2-methylbutanoic acid by acid-catalyzed esterification using the procedure recently detailed for [2,2,2-²H₃]-ethyl butanoate.²⁵

Odorants **9**, **11**, **13–16**, **18–21**, **31**, and **34–36** were synthesized as described below. NMR and MS data are provided in the Supporting Information.

Ethane-1,1-dithiol (9). Acetaldehyde (0.66 g, 15 mmol) and acetate buffer (pH 5; 20 mL) were added to a mixture of sodium sulfide

nonahydrate (9.0 g, 37.5 mmol) and dichloromethane (15 mL) with constant stirring at –60 °C in an argon atmosphere. After further stirring for 3 h at –60 °C, followed by 24 h at room temperature, the organic layer was separated and the aqueous phase was further extracted with dichloromethane (20 mL). The combined solvent extracts were washed with water (40 mL) and brine (40 mL) and then dried over anhydrous sodium sulfate, and finally the solvent was evaporated in vacuo. The residue was purified by bulb-to-bulb distillation (61 °C, 7–8 kPa) to afford 0.12 g of **9** in 93% purity (GC-FID) equivalent to 8% yield.

1-(Methylsulfanyl)ethanethiol (11). The synthesis was performed following an approach reported by Schutte²⁶ with some modifications. Acetaldehyde (1.10 g, 25 mmol), sodium methanethiolate (1.75 g, 25 mmol), and acetate buffer (pH 5; 20 mL) were successively added to a mixture of sodium sulfide nonahydrate (6.0 g, 25 mmol) and dichloromethane (10 mL) with constant stirring at –60 °C in an argon atmosphere. After further stirring for 3 h at –60 °C, followed by 3 days at room temperature, the organic layer was separated and the aqueous phase was extracted with dichloromethane (20 mL). The combined solvent extracts were washed with water (40 mL) and aqueous sodium carbonate (5%; 40 mL), and the target compound was extracted with aqueous potassium hydroxide (1 mol/L; 50 mL). After the pH had been adjusted to 2 (aqueous HCl; 16%), the target compound was re-extracted with dichloromethane (200 mL). The organic extract was dried over anhydrous sodium sulfate, and the solvent was evaporated in vacuo. The residue was purified by flash chromatography. Elution with pentane afforded 0.62 g of **11** in 94% purity (GC-FID) equivalent to 23% yield.

1-(Ethylsulfanyl)ethanethiol (13). By applying the same approach as detailed for **11**, the compound was synthesized from sodium sulfide, acetaldehyde, and ethanethiol (1.55 g, 25 mmol). Finally, 1.06 g of **13** was obtained in 95% purity (GC-FID) equivalent to 33% yield.

1,1-Bis(methylsulfanyl)ethane (14). Dimethyl disulfide (1.32 g, 21.3 mmol) and acetaldehyde (0.94 g, 21.3 mmol) were successively added to tributylphosphine (purity ≥ 97%; 5 mL, 19.4 mmol) at room temperature under an argon atmosphere.²⁷ The mixture was shaken for 10 min and then subjected to flash chromatography. Elution with pentane afforded 0.57 g of **14** in 91% purity (GC-FID) equivalent to 22% yield.

Ethyl (2*R*/2*S*)-2-Hydroxy-2-methylbutanoate. Boric acid (62 mg, 1 mmol) was added in one portion to a solution of 2-hydroxy-2-methylbutanoic acid (1.18 g, 10 mmol) in anhydrous ethanol (15 mL) with constant stirring.²⁸ The mixture was further stirred at room temperature for 48 h and then concentrated in vacuo to afford a crude pale yellow oil. Dichloromethane (50 mL) was added, and the mixture was washed with aqueous sodium carbonate (0.5 mol/L; 50 mL) and water (100 mL). The organic fraction was dried over anhydrous sodium sulfate, and the solvent was removed in vacuo to afford 0.39 g of ethyl (2*R*/2*S*)-2-hydroxy-2-methylbutanoate in 99% purity (GC-FID) equivalent to 27% yield and an *R/S* ratio of 1:1.

Ethyl (2*R*)-2-Hydroxy-2-methylbutanoate (15). Using the procedure described above for the racemate, **15** was prepared from enantiopure (2*R*)-2-hydroxy-2-methylbutanoic acid (0.3 g, 2.5 mmol). Finally, 0.14 g of **15** was obtained in 99% purity (GC-FID) equivalent to 38% yield and an *R* percentage of 99.5% (chiral GC).

1-(Methylsulfanyl)propane-1-thiol (16). By applying the same approach as detailed for **11**, the target compound was synthesized from sodium sulfide, propanal (1.45 g, 25 mmol), and sodium methanethiolate. Finally, 0.90 g of **16** was obtained as a colorless oil in 88% purity (GC-FID) equivalent to 26% yield.

1-(Ethylsulfanyl)-1-(methylsulfanyl)ethane (18). 1-(Ethylsulfanyl)ethanethiol (**13**; 0.51 g, 4.2 mmol) was added to a mixture of aqueous sodium hydroxide (10 mol/L; 0.4 mL) and methanol (5 mL) at 0 °C.²⁹ After 5 min of stirring, methyl iodide (0.26 mL, 4.2 mmol) was added dropwise. Stirring was continued at room temperature for 2 h, and then methanol was removed using a Vigreux column (60 cm). The residue was poured into water (20 mL) and extracted with diethyl ether (3 × 20 mL). The organic layer was washed with brine (60 mL) and dried over anhydrous sodium sulfate. The solvent was evaporated in vacuo, and the residue (0.37 g) was purified by flash

chromatography. Elution with pentane afforded 0.24 g of **18** in 93% purity (GC-FID) equivalent to 39% yield.

1-(Ethylsulfanyl)propane-1-thiol (19). By applying the same approach as detailed for **11**, the target compound was synthesized from sodium sulfide, propanal (1.45 g, 25 mmol), and ethanethiol (1.55 g, 25 mmol). Finally, 0.92 g of **19** was obtained as a colorless oil in 91% purity (GC-FID) equivalent to 27% yield.

1-(Propylsulfanyl)ethanethiol (20). By applying the same approach as detailed for **11**, the target compound was synthesized from sodium sulfide, acetaldehyde, and propane-1-thiol (1.90 g, 25 mmol). Finally, 1.1 g of **20** was obtained as a colorless oil in 93% purity (GC-FID) equivalent to 30% yield.

1,1-Bis(ethylsulfanyl)ethane (21). Copper(I) bromide (72 mg, 0.5 mmol) was added to acetaldehyde (0.44 g, 10 mmol) and ethanethiol (1.36 g, 22 mmol) in acetonitrile (20 mL) at 0 °C.³⁰ After 16 h of stirring at room temperature, aqueous sodium hydroxide (4 mol/L, 20 mL) was added, and the mixture was extracted twice with dichloromethane (2 × 60 mL). The combined organic phases were washed with water (120 mL) and dried over anhydrous sodium sulfate. The solvent was evaporated in vacuo, and the crude product was purified by flash chromatography. Elution with pentane afforded 1.16 g of **21** as a colorless oil in 99% purity (GC-FID) equivalent to 77% yield.

1-[[1-(Methylsulfanyl)ethyl]sulfanyl]ethanethiol (31/35). The synthesis of the two diastereomers of 1-[[1-(methylsulfanyl)ethyl]sulfanyl]ethanethiol started as detailed for **11**. The crude dichloromethane extract of the reaction mixture, containing 77% **11** and 15% **9** (GC-FID), was vigorously shaken with aqueous sodium carbonate (5%; 40 mL). The organic phase was dried over anhydrous sodium sulfate, and the solvent was evaporated in vacuo. The residue was purified by flash chromatography. Elution with pentane afforded 43 mg of **31** in 83% purity and 31 mg of **35** in 89% purity (GC-FID).

1-(Ethylsulfanyl)-1-(ethylsulfanyl)ethane (33). Following a procedure described for the synthesis of mixed disulfides from the corresponding thiols,³¹ an aqueous solution of copper(II) sulfate (2.25 mol/L; 20 mL) was added to a solution of 1-(ethylthio)ethanethiol (73 mg, 0.6 mmol) and ethanethiol (64 mg, 1.0 mmol) in diethyl ether (15 mL) at room temperature. After 30 min of continuous stirring, ammonium acetate (2 g; 26 mmol) was added. The ethereal layer was separated, washed with brine (3 × 20 mL), aqueous sodium carbonate (5%; 60 mL), and finally water (60 mL), before drying over anhydrous sodium sulfate. The solvent was evaporated in vacuo, and the crude product was purified by flash chromatography. Elution with pentane/diethyl ether (95:5, v/v) afforded **33** as a colorless oil in 95% purity (GC-FID).

1-[[1-(Ethylsulfanyl)ethyl]sulfanyl]ethanethiol (34/36). A mixture of **9** (94 mg, 1 mmol) and **13** (114 mg, 0.9 mmol) in dichloromethane (50 mL) was vigorously shaken with aqueous sodium carbonate (5%; 50 mL) to yield the two diastereomers of 1-[[1-(ethylsulfanyl)ethyl]sulfanyl]ethanethiol in a ratio of 65:35 (GC-FID). The organic phase was dried over anhydrous sodium sulfate, and the solvent was evaporated in vacuo. The residue was purified by flash chromatography. Elution with pentane afforded 30 mg of **34** in 83% purity and 28 mg of **36** in 86% purity (GC-FID) equivalent to 15 and 14% yields, respectively.

Flash Chromatography. Synthetic raw products were applied onto silica gel 60 (0.040–0.063 mm; 50 g) (VWR) in a glass column (2 cm i.d.). Elution was performed at 100 kPa of nitrogen pressure. The eluate was monitored by GC-FID; fractions containing the target compound were pooled, and the solvent was removed in vacuo.

Isolation of Durian Volatiles. Durian pulp (10 g), obtained from a freshly opened fruit, was added to dichloromethane (50 mL), and the mixture was homogenized under ice cooling and the addition of anhydrous sodium sulfate (10 g). The homogenate was filtered through defatted cotton wool, and the residue was further extracted with dichloromethane (50 mL). The extracts were combined, and nonvolatile material was removed by solvent-assisted flavor evaporation³² (SAFE) at 40 °C.

GC-O and GC-FID Analyses. A Trace GC Ultra gas chromatograph (Thermo Scientific, Dreieich, Germany) was equipped with a

cold-on-column injector, a FID, and a tailor-made sniffing port. The following fused silica columns were used: (1) DB-FFAP, 30 m × 0.32 mm i.d., 0.25 μm film, (2) DB-5, 30 m × 0.32 mm i.d., 0.25 μm film (Agilent J&W, Waldbronn, Germany), and (3) BGB-176, 30 m × 0.25 mm i.d., 0.25 μm film (BGB Analytik, Schlossböckelheim, Germany). The carrier gas was helium at 70 kPa (DB-FFAP, DB-5) and 120 kPa (BGB-176), respectively. The initial oven temperature was 40 °C (2 min). Gradients were 6 °C/min for DB-FFAP and DB-5 and 2 °C/min for BGB-176. For GC-O analyses, the column effluents were divided 1:1 using a deactivated Y-shaped glass splitter and two deactivated fused silica capillaries (50 cm × 0.25 mm i.d.) (Chromatographie Handel Müller, Fridolfing, Germany) connecting the splitter to the FID and the sniffing port, respectively. The sniffing port consisted of a cylindrically shaped aluminum device (105 mm × 24 mm diameter) with a beveled top and a central drill hole (2 mm) housing the capillary. It was mounted on a heated (200 °C) detector base of the GC. During a GC-O run, the panelist placed her/his nose closely above the top of the sniffing port and evaluated the odor of the effluent. If an odor was perceived, the retention time was marked in the FID chromatogram printed by a recorder and the odor quality was noted.

For the GC-O analysis, the SAFE distillate obtained from 10 g of durian pulp was concentrated to a volume of 1 mL using a Vigreux column (60 cm). GC-O was performed by six trained panelists (three females, three males), and the results were summarized. Linear retention indices (RI) of the odor-active compounds were calculated from their retention times and the retention times of adjacent *n*-alkanes by linear interpolation.

Aroma Extract Dilution Analysis. Dilutions of the concentrated SAFE distillate of 1:2, 1:4, 1:8, 1:16, etc., were prepared with dichloromethane and analyzed by GC-O using column 1 (FFAP). Each odorant was assigned a flavor dilution (FD) factor, representing the highest dilution in which the odorant was detected.²⁰ The FD factors obtained by three trained panelists (one female, two males) were averaged. The mean FD factors were calculated as 2ⁿ, with *n* being the arithmetic mean of the log₂ values of the FD factors of the individual panelists rounded to the nearest integer.

Fractionation of Durian Volatiles. The SAFE distillate obtained from 50 g of durian pulp was extracted with aqueous sodium hydrogen carbonate (0.5 mol/L; three times, total volume = 200 mL) to remove the acidic volatiles. The organic phase was dried over anhydrous sodium sulfate and concentrated (Vigreux column) to 1 mL (fraction of neutral and basic volatiles, NBF). The combined aqueous phases were washed with dichloromethane (50 mL), acidified to pH 2 with hydrochloric acid (16%), and extracted with dichloromethane (three times, total volume = 200 mL). The combined solvent extracts were dried over anhydrous sodium sulfate and concentrated to 1 mL (fraction of acidic volatiles, AF). The NBF was further fractionated by column chromatography using purified silica gel (10 g) in a cooled (12 °C) glass column (1 cm i.d.) using the following pentane/diethyl ether mixtures: 100:0, 90:10, 70:30, 50:50, and 0:100 (50 mL each). The eluate was collected in 50 mL portions, and each portion was concentrated to 1 mL (silica gel fractions, SGF1–5).

The volatile thiol fraction (TF) of durian was isolated from the SAFE distillate obtained from 200 g of durian pulp by affinity chromatography. The SAFE distillate was concentrated (5 mL) and applied onto mercerated agarose gel (1 g) in a glass column (0.5 cm i.d.). After the column had been rinsed with pentane/dichloromethane (2:1; 50 mL), the thiols were eluted with dithiothreitol (10 mmol/L) in pentane/dichloromethane (2:1; 50 mL).³³ The excess of dithiothreitol was removed by SAFE distillation, and the distillate was concentrated to 200 μL using a Vigreux column (60 cm) and a microdistillation device.³⁴

Gas Chromatography–Mass Spectrometry (GC-MS). Mass spectra in the electron ionization (MS-EI) mode were generated at 70 eV using a HP 5890 gas chromatograph (Hewlett-Packard, Heilbronn, Germany) connected to an MAT 95 mass spectrometer (Finnigan, Bremen, Germany). Mass spectra in the chemical ionization (MS-CI) mode were acquired with a 3800 gas chromatograph connected to a

Saturn 2200 mass spectrometer (Varian, Darmstadt, Germany) using methanol as reactant gas.

Two-Dimensional Heart-Cut Gas Chromatography–Mass Spectrometry (GC–GC–MS). A Combi PAL autosampler (CTC Analytics, Zwingen, Switzerland) was mounted on a Trace GC Ultra equipped with a cold on-column injector (Thermo Scientific, Dreieich, Germany) and an FFAP capillary, 30 m × 0.32 mm i.d., 0.25 μm film (Agilent J&W, Waldbronn, Germany). The column end was connected to a moving column stream switching system (MCSS) (Thermo Scientific), conveying the eluate retention time programmed through deactivated fused silica capillaries (0.32 mm i.d.) either simultaneously to an FID and a sniffing port or via a heated (250 °C) transfer line to a cold trap localized in the oven of a CP 3800 GC (Varian). The tailor-made trap consisted of a piece of steel tubing housing the capillary and could be cooled by means of liquid nitrogen. Downstream of the trap, the capillary was connected to a BGB-176 column, 30 m × 0.25 mm i.d., 0.25 μm film (BGB Analytik, Schlossböckelheim, Germany). The end of this column was connected to a Saturn 2000 mass spectrometer (Varian) operated in the CI mode with methanol as the reactant gas. For the determination of the enantiomeric distribution of 3, 6, 8, 15, and 30, start temperatures were 40 °C and temperature gradients were 6 °C/min in the first and 2 °C/min in the second dimension. A heart-cut of the eluate of the first column containing the respective target compound was transferred via the MCSS and the transfer line to the precooled trap. Then the trap cooling was turned off, and the second oven was started. The retention times of the target compounds in the first and second dimensions were previously determined using reference compounds.

Static Headspace Gas Chromatography–Olfactometry (SH–GC–O) and Static Headspace Gas Chromatography–Mass Spectrometry (SH–GC–MS). Durian pulp (10 g) was placed into a 120 mL septum-sealed vial and equilibrated for 10 min at 37 °C. Volumes of 5, 2.5, 1.25, 0.63, 0.31, and 0.16 mL of the headspace were subsequently injected using a Combi PAL autosampler (CTC Analytics) via a cold on-column injector (helium, 110 kPa) onto a deactivated fused silica precolumn (0.2 m × 0.53 mm i.d.) installed in a Trace GC Ultra gas chromatograph (Thermo Scientific). Volatiles were trapped on the precolumn using a 915 cold trap at –150 °C. The precolumn was connected to the main column and an outlet solenoid valve via a three-way connector. During trapping, the solenoid valve was open to maintain a flow of 20 mL/min through the precolumn. After injection was finished, the solenoid valve was closed and the trap was heated to 250 °C to quantitatively transfer the trapped volatiles onto the main column, a DB-5, 30 m × 0.25 mm i.d., 1 μm film (Agilent J&W). The oven start temperature was 0 °C maintained by a liquid nitrogen oven cooling. After 2 min, the temperature was raised at 6 °C/min. The end of the main column was connected to a pressure-controlled stream switching system (S+H Analytik, Mönchengladbach, Germany), which transferred the eluate to an FID and a sniffing port and/or a Saturn 2100 mass spectrometer (Varian). Each odor-active compound in the eluate was assigned an FD factor, calculated as the ratio of the initial volume (5 mL) and the lowest injection volume in which the compound was detected at the sniffing port. The FD factors obtained by three trained panelists (one female, two males) were averaged as detailed above.

RESULTS AND DISCUSSION

Odorant Screening. Arils of fully ripened durian fruits were extracted with dichloromethane, and nonvolatile constituents were removed by SAFE distillation.³² The distillate, when tested on a strip of filter paper, still elicited the characteristic durian odor. The solution was concentrated and then submitted to AEDA using a GC capillary with FFAP phase. In the FD factor range of 1–16384, 44 odor-active areas were detected (Table 1).

Structural Assignment of Odorants. To identify the compounds responsible for the respective odorant areas

detected during AEDA, first, RIs and odor qualities were compared to published data compiled in an in-house database. This approach allowed tentative assignment of the structures of 25 of the 44 odorants (1–8, 10, 12, 22, 24, 26–30, 32, 37–40, and 42–44). Assigned structures were then confirmed by comparing the RIs, the odor qualities, and the mass spectra of the odorants with the respective data of reference compounds analyzed in parallel. To avoid coelution problems, the durian volatiles were fractionated before GC–MS analysis. First, the acidic volatiles were separated from neutral and basic volatiles. The latter were further fractionated by silica gel chromatography. Additionally, volatile thiols were isolated by covalent chromatography on mercurated agarose gel. The odorants were localized in the fractions by GC–O, and then the fractions were submitted to GC–MS analysis. Only for two compounds (26 and 39) with low FD factors (2) did the mass spectral confirmation fail. However, their RIs on two capillaries of different polarities (DB-5, FFAP) and their odor properties were identical with those of 3-isopropyl-2-methoxypyrazine (26) and *trans*-4,5-epoxy-(2*E*)-dec-2-enal (39).

The remaining 19 yet to be identified odorants were also localized in the fractions by GC–O, and the respective fractions were analyzed by GC–MS. A mass spectrum was assigned to an unknown odorant when identical spectra were obtained on two separation systems of different polarities (DB-5, FFAP) at the respective retention time at which the odor was perceived. The mass spectra of three odorants (14, 21, and 25) produced hits when compared to the NIST database.³⁵ Structures were confirmed with the reference compounds, either obtained from a commercial supplier (25) or synthesized following published procedures (14 and 21). Thus, these odorants were identified as 1,1-bis(methylsulfanyl)ethane (14), 1,1-bis(ethylsulfanyl)ethane (21), and ethyl (2*E*,4*E*)-hexa-2,4-dienoate (25).

Three compounds (17, 23, and 41) remained unidentified, because no unequivocal mass spectra could be obtained. The structures of the remaining 13 compounds were assigned as follows.

Identification of Odorant 9. The sulfury, durian-like-smelling compound was detected by GC–O in TF, thus indicating a sulfhydryl group. Its mass spectrum (Figure 1)

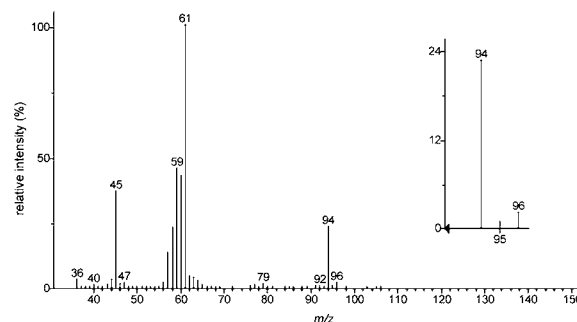


Figure 1. Mass spectrum (MS–EI) of odorant 9. Enlarged detail shows molecular ions.

suggested an M_r of 94 and an isotopologue molecular ion at m/z 96. The intensity of the isotopologue ion m/z 96 was 9% relative to m/z 94, thus suggesting the presence of two sulfur atoms in the molecule. Because the spectrum did not match the spectrum of ethane-1,2-dithiol³⁵ and no fragment m/z 47 corresponding to $[\text{CH}_3\text{S}]^+$ was observed, 9 was proposed to be

ethane-1,1-dithiol, which was finally confirmed by synthesis of the reference compound.

Identification of Odorants 11, 13, and 20. The compounds showed roasted onion-like (11 and 13) and roasted sesame-like (20) odors and, using GC-O, were also detected in TF. Their mass spectra (Figure 2) suggested

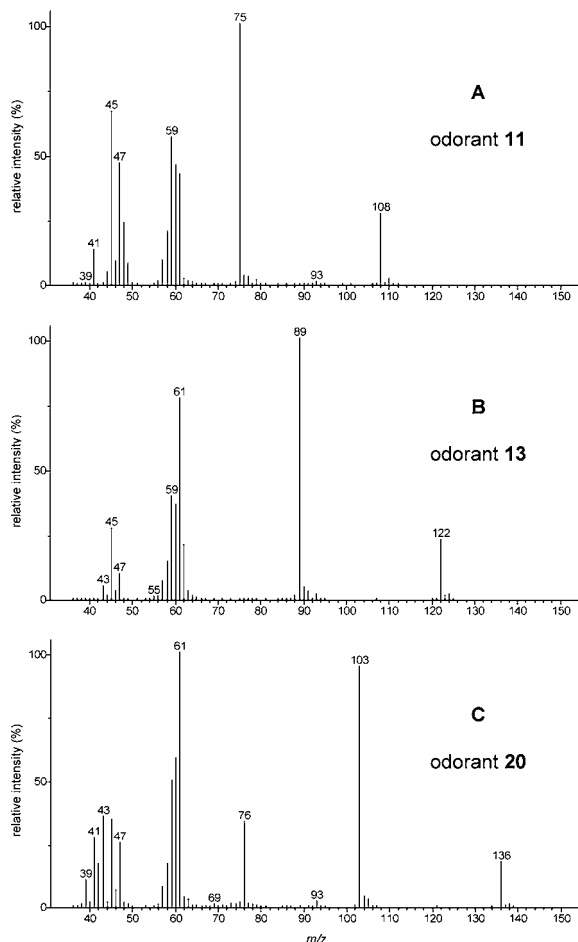


Figure 2. Mass spectra (MS-EI) of odorants 11 (A), 13 (B), and 20 (C).

homologues with M_r of 108, 122, and 136. The presence of a sulfhydryl group was confirmed by fragments $[M - HS]^+$ at m/z 75, 89, and 103, respectively. The intensities of isotopologue peaks at m/z 110, 124, and 138 indicated two sulfur atoms in each molecule. The signal at m/z 61, also observed in the spectrum of 9 and indicating a fragment $[CH_3CHSH]^+$, suggested 11, 13, and 20 to be 1-(methylsulfanyl)ethanethiol, 1-(ethylsulfanyl)ethanethiol, and 1-(propylsulfanyl)ethanethiol, respectively. Synthesis of the three dithiohemiacetals confirmed the structural assignment.

Identification of Odorant 15. The fruity-smelling compound was recovered in SGF3. Whereas the odor quality suggested 15 to be an ester, its occurrence in SGF3 indicated a higher polarity. Its EI mass spectrum (Figure 3) showed a high similarity to the published³⁵ spectrum of methyl 2-hydroxy-2-methylbutanoate. However, its CI spectrum clearly indicated an M_r of 146, thus suggesting the ethyl analogue. This assumption was confirmed by synthesis of ethyl 2-hydroxy-2-methylbutanoate via a selective esterification with boric acid catalysis.²⁸

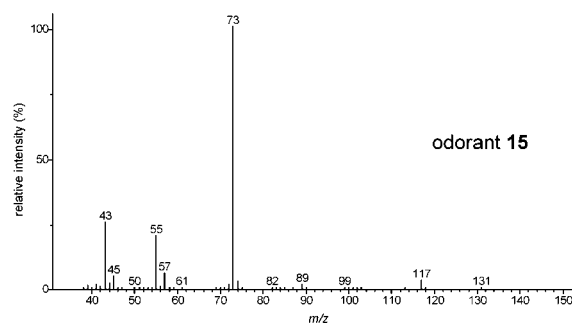


Figure 3. Mass spectrum (MS-EI) of odorant 15.

Identification of Odorants 16 and 19. Both odorants appeared in the thiol fraction and showed roasted onion-like odor notes. Their mass spectra (Figure 4) suggested an M_r of

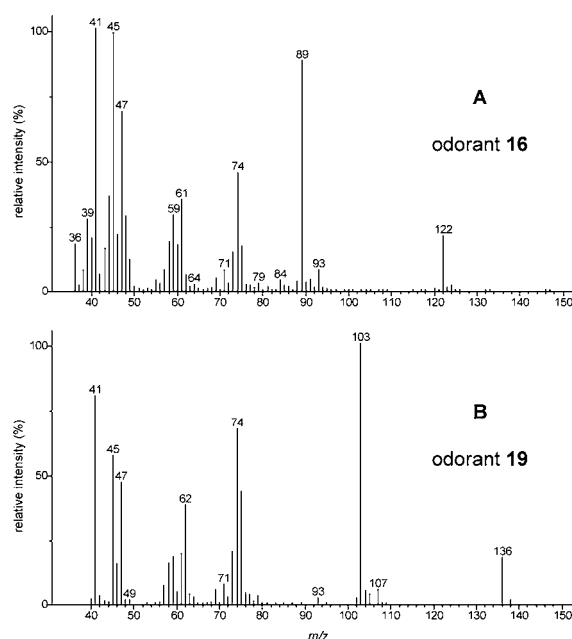


Figure 4. Mass spectra (MS-EI) of odorants 16 (A) and 19 (B).

122 and 136, respectively, and confirmed the presence of a sulfhydryl group through a main fragment $[M - HS]^+$. The intensities of isotopologue peaks at m/z 124 and 138, respectively, indicated two sulfur atoms in each molecule. Mass spectral fragmentation suggested 16 and 19 to be 1-(methylsulfanyl)propane-1-thiol and 1-(ethylsulfanyl)propane-1-thiol, which was finally confirmed by synthesis of the reference compounds.

Identification of Odorant 18. This metallic-smelling compound was detected in NBF and SGF2, but not in TF. Its mass spectrum (Figure 5) suggested an M_r of 136 and, with the intensity of m/z 138 being ~9% of the intensity of m/z 136, the presence of two sulfur atoms. Fragments at m/z 89 and 75 corresponded to the loss of $[CH_3S]^+$ and $[CH_3CH_2S]^+$, respectively, thus suggesting 18 to be 1-(ethylsulfanyl)-1-(methylsulfanyl)ethane. Synthesis of the reference compound finally confirmed this structure.

Identification of Odorants 31 and 34–36. All four compounds were detected in TF and exhibited the same roasted onion-like odor as odorants 11, 13, 16, and 19, thus suggesting similar dithiohemiacetal structures. Compounds 31

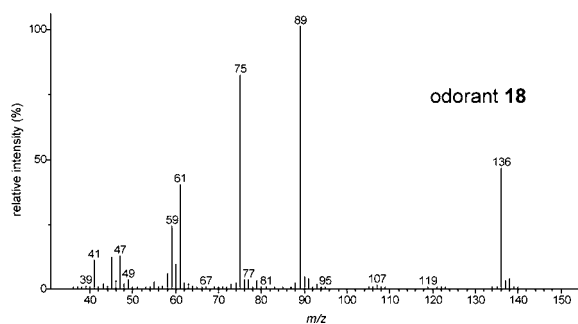


Figure 5. Mass spectrum (MS-EI) of odorant 18.

and 35 showed identical mass spectra (Figure 6A). The fragmentation pattern in the range of $m/z \leq 75$ was virtually

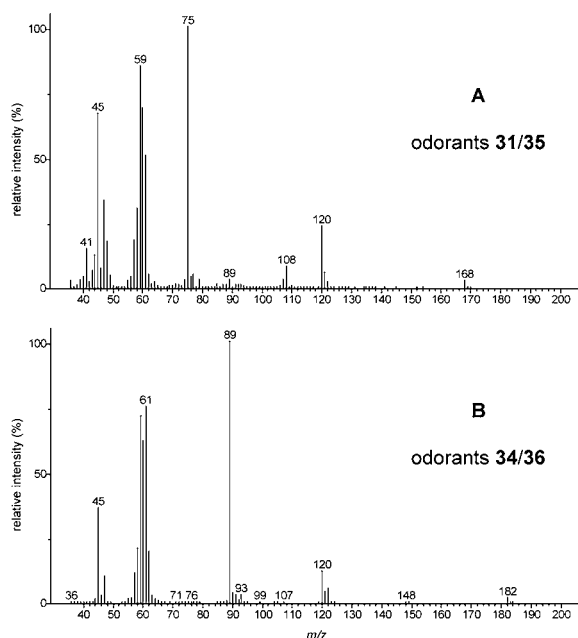


Figure 6. Mass spectra (MS-EI) of odorants 31/35 (A) and 34/36 (B).

the same as found for 1-(methylsulfanyl)ethanethiol (11) (Figure 2), thus suggesting that the base peak ion m/z 75 was $[\text{CH}_3\text{CHSCH}_3]^+$. Provided that the small peak at m/z 168 was the molecular ion, the fragment ion m/z 120 would correspond to a neutral loss of methanethiol. In summary, all observations were in agreement with a structure proposal of 1- $\{[1\text{-(methylsulfanyl)ethyl]sulfanyl}\}$ ethanethiol. Because this structure includes two chiral centers, odorants 31 and 35 would correspond to the two diastereomers. The 1- $\{[1\text{-(methylsulfanyl)ethyl]sulfanyl}\}$ ethanethiol diastereomers were finally synthesized by condensation of ethane-1,1-dithiol (9) and 1-(methylsulfanyl)ethanethiol (11), followed by preparative separation on silica gel, where 31 was eluted before 35. In an analogous manner, odorants 34 and 36 were identified as diastereomers of 1- $\{[1\text{-(ethylsulfanyl)ethyl]sulfanyl}\}$ ethanethiol. Their mass spectra were identical (Figure 6B) and showed the same fragmentation pattern in the m/z range ≤ 89 as found in the spectrum of 1-(ethylsulfanyl)ethanethiol (13) (Figure 2), thus suggesting $[\text{CH}_3\text{CHSCH}_2\text{CH}_3]^+$ as the base peak ion, as well as m/z 182 as molecular ion and m/z 120 as the neutral loss of ethanethiol. The reference compounds

were obtained by condensation of ethane-1,1-dithiol (9) and 1-(ethylsulfanyl)ethanethiol (13) followed by preparative separation on silica gel, where 34 was eluted before 36.

Identification of Odorant 33. The compound with a sulfury, onion-like smell was recovered in SGF2, but not in TF, indicating the absence of a sulfhydryl group. However, its MS (Figure 7) showed analogies to the spectra of 1-(ethylsulfanyl)-

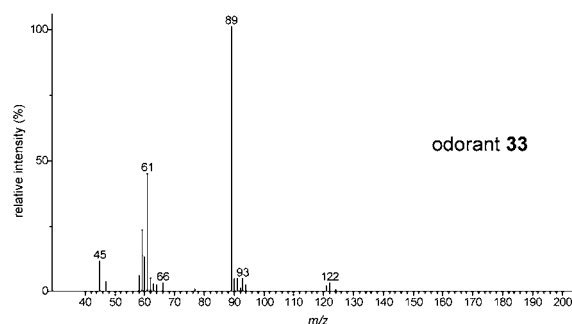


Figure 7. Mass spectrum (MS-EI) of odorant 33.

ethanethiol (13) and $\{[1\text{-(ethylsulfanyl)ethyl]sulfanyl}\}$ ethanethiol (34/36), suggesting a related structure. A compound with a very similar mass spectrum was reported in durian earlier⁷ and tentatively assigned to 1-(ethyldisulfanyl)-1-(ethylsulfanyl)ethane, although the structure could not be confirmed due to the lack of the reference compound in that study. Therefore, we synthesized 1-(ethyldisulfanyl)-1-(ethylsulfanyl)ethane. Its sensory properties, retention data, and mass spectrum were identical with the data of the sought substance. Thus, odorant 33 was unequivocally identified as 1-(ethyldisulfanyl)-1-(ethylsulfanyl)ethane.

Enantiomeric Distribution of Chiral Odorants. The enantiomeric composition of 2-methylbutanoic acid and its methyl, ethyl, and propyl esters, as well as of ethyl 2-hydroxy-2-methylbutanoate, were determined by heart-cut GC-GC-MS using a chiral β -cyclodextrin phase in the second dimension and enantiopure reference compounds. Results indicated that 2-methylbutanoic acid and its esters all showed the *S* configuration, whereas ethyl 2-hydroxy-2-methylbutanoate exhibited the *R* configuration. Due to their low FD factors, no effort was made to clarify the enantiomeric distribution of linalool (27), 3-sulfanylbutan-1-ol (29), and *trans*-4,5-epoxy-(2*E*)-dec-2-enal (39). The enantiomeric ratio of the dithiohemiacetals and the dithioacetals (11, 13, 16, 18–20, 31, and 33–36) will be subject to further investigations.

Screening for Highly Volatile Odorants by SH-GC-O.

During the workup of the sample for AEDA, particularly during distillate concentration, odor-active compounds with boiling points lower than that of the extraction solvent will be lost. Therefore, SH-GC-O was suggested as a complementary technique to AEDA to cover highly volatile odorants.³⁶ Its application on durian pulp resulted in the identification of six additional odorants, five of which could be unequivocally identified. These compounds were hydrogen sulfide, acetaldehyde, methanethiol, ethanethiol, and propane-1-thiol (Table 2).

In summary, 50 odor-active compounds were detected by application of an AEDA and by SH-GC-O in this study, 46 of which could be identified. The highest FD factors (≥ 128) were determined for the 13 odorants displayed in Figure 8. Among them, a total of 8 compounds (7, 9, 11, 19, 40, and 42–44) were previously unknown in durian.

Table 2. Aroma-Active Compounds in the Headspace above Durian Pulp (RI < 800)

no.	odorant ^a	odor ^b	RI ^c DB-5	FD factor ^d	previously reported
45	hydrogen sulfide ^e	rotten egg	<500	16	4, 5, 9
46	acetaldehyde	fresh, fruity	<500	≥32	4, 12, 13, 17–19
47	methanethiol	rotten, cabbage	<500	≥32	4, 9, 17, 19
48	ethanethiol	rotten, onion	510	≥32	4, 6, 9, 10, 12, 14, 17–19
49	propane-1-thiol	rotten, durian	618	≥32	4, 6, 9, 10, 12–14, 17–19
50	unknown	onion, sulfury	677	4	
1	ethyl propanoate	fruity	717	4	4, 6, 9, 10, 12–14, 17–19
9	ethane-1,1-dithiol ^e	sulfury, durian	730	16	
2	ethyl 2-methylpropanoate	fruity	757	≥32	6, 8–10, 12–14, 18, 19

^aOdorants were identified by comparing their retention indices on the DB-5 column, their mass spectra obtained by SH-GC-MS, and their odor characteristics as perceived during SH-GC-O with respective data of reference compounds. ^bOdor quality as perceived at the sniffing port during SH-GC-O. ^cRI = retention index; calculated from the retention time of the compound and the retention times of adjacent *n*-alkanes by linear interpolation. ^dFD factor = flavor dilution factor; calculated as ratio of the initial volume (5 mL) and the lowest injection volume in which the compound was detected during SH-GC-O; average of three trained panelists (one female, two males). ^eAn unequivocal mass spectrum could not be obtained by SH-GC-MS, identification was based on the resting criteria detailed in footnote a.

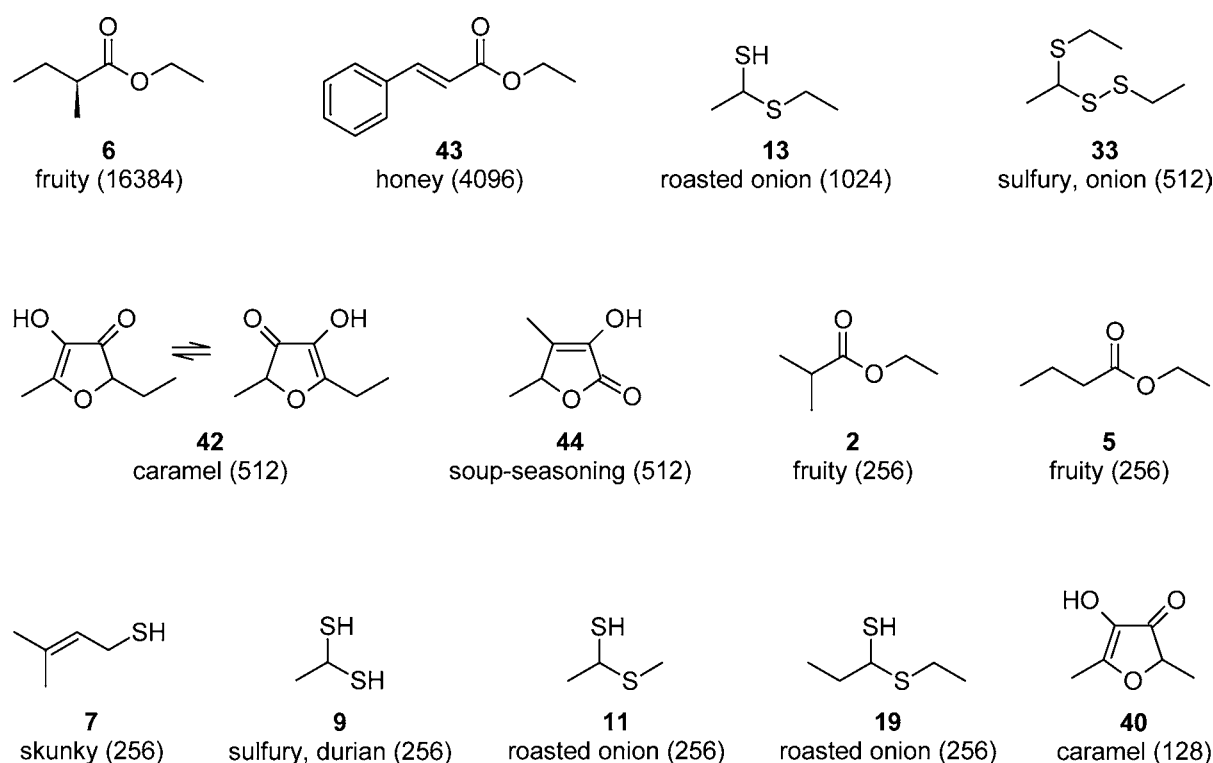


Figure 8. Most aroma-active compounds in the SAFE distillate obtained from durian pulp (FD factors in parentheses).

Esters. According to their FD factors, ethyl (2*S*)-2-methylbutanoate (**6**) (FD 16384), ethyl 2-methylpropanoate (**2**) (FD 256), ethyl butanoate (**5**) (FD 256), and methyl (2*S*)-2-methylbutanoate (**3**) (FD 64) were the most potent fruity-smelling compounds. The extraordinary high FD factor of ethyl (2*S*)-2-methylbutanoate was well in line with the early work of Baldry,⁴ who had already suggested that ethyl 2-methylbutanoate is a key aroma compound of durian, as well as with the results of Weenen et al.,⁸ who stated that this ester contributes “most to the nonsulfur part of durian flavor”. In our study, the compound was furthermore elucidated to be enantiopure ethyl (2*S*)-2-methylbutanoate. Another potent odor-active ester was honey-like-smelling ethyl cinnamate (**43**) (FD 4096), not previously reported in durian.

Sulfur Compounds. SH-GC-O analysis revealed hydrogen sulfide, methanethiol, ethanethiol, and propane-1-thiol as highly

volatile potent odorants. These compounds had been reported previously (Table 2). Their odors, however, when evaluated during GC-O, were rather unpleasant, with a pronounced rotten note.

Another group of sulfur compounds with primarily sulfury and roasted onion-like odors was likely derived from acetaldehyde, propanal, hydrogen sulfide, methanethiol, ethanethiol, and propane-1-thiol by a formation of geminal dithiols (**9**), dithiohemiacetals (**11**, **13**, **16**, **19**, **20**, **31**, and **34–36**), and dithioacetals (**14**, **18**, **21**, **31**, **33**, and **34–36**). Within this group of compounds, the roasted onion-like-smelling 1-(ethylsulfanyl)ethanethiol (**13**) (Figure 8) showed the highest FD factor of 1024, followed by 1-(ethylsulfanyl)ethane (**33**) (Figure 8), ethane-1,1-dithiol (**9**) (Figure 8), 1-(methylsulfanyl)ethanethiol (**11**) (Figure 8), and 1-(ethylsulfanyl)propane-1-thiol (**19**) (Figure 8). These

compounds have rarely been found in natural products so far and even more rarely unequivocally identified using structurally characterized reference compounds. Ethane-1,1-dithiol (**9**) was unequivocally identified only in yeast extract,³⁷ but tentatively also identified in cooked mutton³⁸ and beef.³⁹ 1-(Methylsulfanyl)ethanethiol (**11**) was first identified in beef broth⁴⁰ and later reported in cooked beef,³⁹ yeast extract,³⁷ dried squid,⁴¹ and leek.⁴² 1-(Ethylsulfanyl)ethanethiol (**13**) was first reported in durian⁶ and also tentatively identified in leek.⁴² 1,1-Bis(methylsulfanyl)ethane (**14**) was tentatively identified in peanuts,⁴³ yeast extract,³⁷ durian,^{7,10} and more recently also reported in anchovy sauce.⁴⁴ 1-(Methylsulfanyl)propane-1-thiol (**16**) was reported in yeast extract³⁷ and onions⁴⁵ and 1-(ethylsulfanyl)propane-1-thiol (**19**) in leek.⁴² 1-(Ethylsulfanyl)-1-(methylsulfanyl)ethane (**18**), 1,1-bis(ethylsulfanyl)ethane (**21**), and 1-(ethylsulfanyl)-1-(ethylsulfanyl)ethane (**33**) were tentatively identified in durian before (Table 1), but not reported from any other natural source. 1-(Propylsulfanyl)ethanethiol (**20**), 1-[[1-(methylsulfanyl)ethyl]sulfanyl]ethanethiols (**31/35**), and 1-[[1-(ethylsulfanyl)ethyl]sulfanyl]ethanethiols (**34/36**), to our knowledge, have not been reported from a natural source before.

Cyclic dithioacetals, such as trithianes and trithiolanes, previously reported as volatiles in durian,^{6–14,19} were localized in the durian extract by GC-MS during our study (data not shown), but were not found to be odor-active during AEDA. Dialkyl sulfides such as diethyl disulfide (**10**) and diethyl trisulfide (**24**) were also previously suggested to contribute to the sulfury note of durian;^{4,5} however, their low FD factors determined in our study do not support this assumption.

Furanones. The results of the AEDA suggested that 2(5)-ethyl-4-hydroxy-5(2)-methylfuran-3(2H)-one (**42**) (Figure 8) and 4-hydroxy-2,5-dimethylfuran-3(2H)-one (**40**) (Figure 8) might account for the sweet, caramel-like note in the flavor profile of durian, whereas the soup seasoning-like note is most probably due to 3-hydroxy-4,5-dimethylfuran-2(SH)-one (**44**) (Figure 8). None of these furanones had been reported in durian before.

Miscellaneous Compounds. Acetaldehyde, found as a potent odorant during SH-GC-O, might additionally contribute to the fruity aroma. 3-Hydroxybutan-2-one, earlier described as one of the major volatiles in durian^{6,8} and suggested as responsible for a creamy note,⁹ despite its abundance, was detected with an FD factor of only 2, reflecting its rather high odor threshold. A rancid, cheesy note, frequently assigned to durian, pointed to the presence of short-chain carboxylic acids. Butanoic acid (**28**), pentanoic acid (**32**), and hexanoic acid (**37**) were detected as odor-active compounds in our study, but their FD factors were rather low (≤ 2).

In conclusion, several new aroma compounds with interesting odors were identified in durian. The systematic application of AEDA and SH-GC-O provided some suggestions as to which compounds might be vitally contributing to the overall aroma of durian. However, to unequivocally assess the contribution of individual odorants to durian aroma, omission tests with aroma models based on exact quantitative data of major odor-active durian volatiles will be necessary. This will require further investigations.

■ ASSOCIATED CONTENT

📄 Supporting Information

Spectral data (NMR, MS) of synthesized compounds. This material is available free of charge via the Internet at <http://pubs.acs.org>.

■ AUTHOR INFORMATION

Corresponding Author

*Phone: +49 8161 71 2991. Fax: +49 8161 71 2970. E-mail: ms@lrz.tum.de.

Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS USED

AEDA, aroma extract dilution analysis; AF, fraction of acidic volatiles; FD factor, flavor dilution factor; GC-O, gas chromatography–olfactometry; MCSS, moving column stream switching system; NBF, fraction of neutral and basic volatiles; RI, retention index; SAFE, solvent-assisted flavor evaporation; SGF, silica gel fraction; SH, static headspace; TF, thiol fraction.

Nomenclature

Dithioacetal, IUPAC: 1,1-bis(alkylsulfanyl)alkane; dithiohemiacetal, IUPAC: 1-(alkylsulfanyl)alkane-1-thiol; diethyl disulfide, IUPAC: (ethylsulfanyl)ethane; diethyl trisulfide, IUPAC: diethyltrisulfan; ethyl cinnamate, IUPAC: ethyl (2E)-3-phenylprop-2-enoate; 2(5)-ethyl-4-hydroxy-5(2)-methylfuran-3(2H)-one, also called homofuraneol, tautomeric mixture of 2-ethyl-4-hydroxy-5-methylfuran-3(2H)-one and 5-ethyl-4-hydroxy-2-methylfuran-3(2H)-one; linalool, IUPAC: 3,7-dimethylocta-1,6-dien-3-ol; 4-hydroxy-2,5-dimethylfuran-3(2H)-one, also called Furaneol; 3-hydroxy-4,5-dimethylfuran-2(SH)-one, also called sotolon; *trans*-4,5-epoxy-(2E)-dec-2-enal, IUPAC: (2E)-3-[(2R,3R)/(2S,3S)-3-pentylloxiran-2-yl]prop-2-enal.

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