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**Insights into the Key Compounds of Durian (*Durio zibethinus* L. 'Monthong') Pulp Odor  
by Odorant Quantitation and Aroma Simulation Experiments**

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**ABSTRACT:** Sixteen compounds, previously identified as potent odorants by application of an aroma extract dilution analysis and the gas chromatography-olfactometry analysis of static headspace samples were quantitated in the pulp of durians, variety Monthong, and odor activity values (OAVs) were calculated by dividing the concentrations obtained by the odor thresholds of the compounds in water. In combination with data recently reported for hydrogen sulfide and short-chain alkanethiols, OAVs > 1 were obtained for 19 compounds, among which ethyl (2*S*)-2-methylbutanoate (fruity; OAV 1700000), ethanethiol (rotten onion; OAV 480000), and 1-(ethylsulfanyl)ethanethiol (roasted onion; OAV 250000) were the most potent, followed by methanethiol (rotten, cabbage; OAV 45000), ethane-1,1-dithiol (sulfury, durian; OAV 23000), and ethyl 2-methylpropanoate (fruity; OAV 22000). Aroma simulation and omission experiments revealed that the overall odor of durian pulp could be mimicked by only two compounds, namely ethyl (2*S*)-2-methylbutanoate and 1-(ethylsulfanyl)ethanethiol, when combined in their natural concentrations.

**KEYWORDS:** *durian, Durio zibethinus, stable isotope dilution assay, SIDA, omission test, ethyl (2S)-2-methylbutanoate, 1-(ethylsulfanyl)ethanethiol, dithiohemiacetal.*

## Introduction

Durian fruit is exceedingly popular in many East Asian countries. Its edible part, a yellowish pulp, consists of the arils encasing the seeds. The pulp exhibits a sweet taste and a pleasant custard-like consistency. Its odor, which combines some fruitiness with a dominating sulfury note, however, is extremely strong and, from the point of view of an American or European not used to durian consumption, very extraordinary and rather repellent. To approach the compounds being responsible for the characteristic odor properties, we recently screened the volatile fraction of durian pulp, variety Monthong, by aroma extract dilution analysis (AEDA) and gas chromatography–olfactometry (GC-O) of headspace samples.<sup>1</sup> Results revealed high flavor dilution (FD) factors for fruity smelling ethyl (2*S*)-2-methylbutanoate, honey-like smelling ethyl cinnamate, and roasted onion-like smelling 1-(ethylsulfanyl)ethane-1-thiol, followed by 1-(ethyldisulfanyl)-1-(ethylsulfanyl)ethane (sulfury, onion), 2(5)-ethyl-4-hydroxy-5(2)-methylfuran-3(2*H*)-one (caramel), 3-hydroxy-4,5-dimethylfuran-2(5*H*)-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)ethane-1-thiol (roasted onion), 1-(ethylsulfanyl)propane-1-thiol (roasted onion), and 4-hydroxy-2,5-dimethylfuran-3(2*H*)-one (caramel). Hydrogen sulfide (rotten egg), acetaldehyde (fresh, fruity), methanethiol (rotten, cabbage), ethanethiol (rotten, onion), and propane-1-thiol (rotten, durian) were additionally identified as potent odorants by headspace GC-O analysis. The concentrations of hydrogen sulfide, methanethiol, ethanethiol, and propane-1-thiol in Monthong durian pulp were recently determined by newly developed stable isotope dilution assays employing sodium (<sup>34</sup>S)sulfide, sodium (<sup>2</sup>H<sub>3</sub>)methanethiolate, (<sup>2</sup>H<sub>5</sub>)ethanethiol, and (<sup>2</sup>H<sub>7</sub>)propane-1-thiol as internal standards and 3-buten-2-one as derivatization reagent.<sup>2</sup> Results showed an exceptional high odor activity

value (OAV; ratio of concentration to odor threshold) for ethanethiol (480000) and also a comparably high OAV for methanethiol (45000), whereas OAVs for propane-1-thiol (6300) and hydrogen sulfide (330) were lower. Data therefore suggested that in particular ethanethiol contributes to the overall aroma of Monthong durian pulp. To verify this assumption and clarify the odor impact of other compounds, the aim of the present study was to complete the quantitation of all odorants detected with high FD factors during the screening by AEDA, validate the results of identification and quantitation through sensory evaluation of an aroma reconstitution model prepared accordingly, and finally perform omission tests.

## Materials and Methods

**Durian Pulp.** Durians were purchased from a local internet shop during years 2012 and 2013. They were handpicked from trees of *Durio zibethinus* 'Monthong' in Thailand and sent to Germany by air. The fruits were allowed to reach full ripeness indicated by cracks in the husk and release of the typical odor, which was typically the case one or two days after arrival. The husk was opened by hand and the pulp was carefully removed from the seeds with a spoon.

**Reference Odorants.** Isotopically unmodified odorants were obtained as detailed recently.<sup>1</sup>

**Stable Isotopically Substituted Durian Odorants.** (<sup>2</sup>H<sub>6</sub>)-**13** was purchased from aromaLAB, Planegg, Germany. (<sup>13</sup>C<sub>2</sub>)-**11**<sup>3</sup> and (<sup>13</sup>C<sub>2</sub>)-**12**<sup>4</sup> were synthesized as detailed in the literature. The esters (<sup>2</sup>H<sub>3</sub>)-**3**, (<sup>2</sup>H<sub>3</sub>)-**4**, (<sup>2</sup>H<sub>5</sub>)-**5**, (<sup>2</sup>H<sub>3</sub>)-**8**, and (<sup>2</sup>H<sub>3</sub>)-**14** were synthesized from the corresponding acids and alcohols through Fischer esterification.<sup>5</sup> In brief, sulfuric acid (50 µL) was added to a mixture of carboxylic acid (20 mmol) and alcohol (2 mmol), the mixture was heated to 80 °C for 30 min, diluted with water (50 mL), and extracted with diethyl ether (100 mL). The ethereal extract was washed with aqueous sodium carbonate (0.5 mol/L; 3 × 100 mL)

and water (100 mL), dried over anhydrous sodium sulfate, and the solvent was finally removed in vacuo. ( $^2\text{H}_{10}$ )-**1**, ( $^{13}\text{C}_2$ )-**2**, ( $^{13}\text{C}_2$ )-**6**, ( $^{13}\text{C}_2$ )-**7**, ( $^{13}\text{C}_2$ )-**9**, ( $^2\text{H}_5$ )-**10**, and ( $^{13}\text{C}_2$ )-**15** were synthesized as detailed below.

**Miscellaneous Chemicals.** ( $^{13}\text{C}_2$ )Acetaldehyde, bromo( $^2\text{H}_5$ )ethane, di-*tert*-butyl dicarbonate, dithiothreitol, ethynylmagnesium bromide (0.5 M in THF), lithium diisopropylamide mono(tetrahydrofuran) (1.5 M in cyclohexane), and sodium sulfide nonahydrate were purchased from Sigma-Aldrich (Taufkirchen, Germany). Dichloromethane, and diethyl ether were freshly distilled before use. Mercurated agarose gel was prepared from Affi-Gel 10 (Bio-Rad, Munich, Germany).<sup>6</sup>

**Syntheses.** ( $^2\text{H}_{10}$ )Diethyl trisulfide (( $^2\text{H}_{10}$ )-**1**). The compound was synthesized following an approach detailed for the synthesis of the isotopically unmodified compound.<sup>7</sup> To ethanol (10 mL), powdered sulfur (0.077 g, 2.4 mmol) and sodium hydroxide (0.097 g, 2.4 mmol) were added. The mixture was refluxed under continuous stirring for 1 h. After cooling, bromo( $^2\text{H}_5$ )ethane (0.182 g, 1.6 mmol) in ethanol (5 mL) was added. The mixture was stirred for 3 h, poured into cold water (70 mL), and extracted with diethyl ether (2 × 100 mL). The combined organic phases were washed with water (50 mL), dried over anhydrous sodium sulfate, and concentrated using a Vigreux column (60 cm) at 40 °C. The concentrate was taken up in dichloromethane (50 mL). GC-MS analysis indicated a mixture of ( $^2\text{H}_{10}$ )diethyl disulfide and ( $^2\text{H}_{10}$ )diethyl trisulfide. The concentration of ( $^2\text{H}_{10}$ )diethyl trisulfide in the solution was determined by GC-FID using methyl octanoate as internal standard and a response factor previously determined from the analysis of a mixture of isotopically unmodified diethyl trisulfide and methyl octanoate. MS-EI,  $m/z$  (intensity in %) 46 (12), 64 (21), 66 (70), 82 (7), 94 (10), 98 (38), 130 (5), 164 (100;  $\text{M}^+$ ); MS-CI (methanol),  $m/z$  (intensity in %) 165 (100;  $[\text{M}+\text{H}]^+$ ). The

detailed MS-CI spectrum is provided in the Supporting Information.

*(<sup>13</sup>C<sub>2</sub>)Ethane-1,1-dithiol ((<sup>13</sup>C<sub>2</sub>)-2).* The compound was synthesized from (<sup>13</sup>C<sub>2</sub>)acetaldehyde (0.35 g; 7.5 mmol) and sodium sulfide nonahydrate (3.6 g, 15 mmol) using the approach recently detailed for the synthesis of the isotopically unmodified compound.<sup>1</sup> MS-EI, *m/z* (intensity in %) 46 (27), 59 (10), 60 (18), 61 (36), 62 (32), 63 (100), 96 (26; M<sup>+</sup>); MS-CI (methanol), *m/z* (intensity in %) 63 (100). The detailed MS-CI spectrum is provided in the Supporting Information.

*1-(Ethylsulfanyl)-1-(ethylsulfanyl)(<sup>13</sup>C<sub>2</sub>)ethane ((<sup>13</sup>C<sub>2</sub>)-6).* The compound was synthesized from (<sup>13</sup>C<sub>2</sub>)-**9** (0.061 g; 0.5 mmol) and ethanethiol (0.062 g; 1 mmol) using the approach recently detailed for the synthesis of the isotopically unmodified compound.<sup>1</sup> MS-EI, *m/z* (intensity in %) 46 (8), 61 (28), 62 (11), 63 (51), 66 (4), 91 (100), 121 (3); MS-CI (methanol), *m/z* (intensity in %) 123 (100). The detailed MS-CI spectrum is provided in the Supporting Information.

*(1,2-<sup>13</sup>C<sub>2</sub>)But-3-yn-2-ol.* A solution of (<sup>13</sup>C<sub>2</sub>)acetaldehyde (1.0 g, 21.7 mmol) in anhydrous THF (15 mL) was added dropwise to a solution of ethynylmagnesium bromide (0.5 M in THF; 100 mL) at -78 °C in an argon atmosphere. Then, the mixture was stirred at room temperature for 3 h. Saturated aqueous ammonium chloride solution (300 mL) was added and the mixture was extracted with dichloromethane (150 mL). The organic phase was washed with brine (200 mL) and dried over anhydrous sodium sulfate. The solvent was removed in vacuo to afford 0.75 g of the target compound as a colorless oil in 80% purity (GC-FID) corresponding to 38% yield.

*tert-Butyl (1,2-<sup>13</sup>C<sub>2</sub>)but-3-yn-2-yl carbonate.* The compound was synthesized by adapting the approach published for the synthesis of the isotopically unmodified compound.<sup>4, 8</sup> Pyridine (2.1 mL, 25.8 mmol) and 4-(dimethylamino)pyridine (0.105 g, 0.86 mmol) were added to a

108 solution of (1,2-<sup>13</sup>C<sub>2</sub>)but-3-yn-2-ol (0.60 g, 8.6 mmol) in dichloromethane (20 mL). To the  
109 mixture, di-*tert*-butyl dicarbonate (4.12 g, 18.9 mmol) in dichloromethane (10 mL) was added  
110 dropwise. After stirring for 4 h, the mixture was poured into an aqueous citric acid solution (0.5  
111 M; 50 mL) and the phases were separated. The aqueous phase was further extracted with  
112 dichloromethane (50 mL). The combined organic phases were dried under anhydrous sodium  
113 sulfate. The solvent was removed in vacuo to afford 1.63 g of the target compound as a colorless  
114 oil.

115 *Di-tert-butyl (1,2-<sup>13</sup>C<sub>2</sub>)hept-3-yne-2,5-diyl biscarbonate.* The compound was synthesized  
116 by adapting the approach published for the synthesis of the (<sup>2</sup>H<sub>3</sub>)-substituted isotopologue.<sup>4</sup> The  
117 *tert*-butyl (1,2-<sup>13</sup>C<sub>2</sub>)but-3-yn-2-yl carbonate obtained above was dissolved in anhydrous THF (15  
118 mL). Lithium diisopropylamide mono(tetrahydrofuran) (1.5 M in cyclohexane; 5.7 mL, 8.6  
119 mmol) was added dropwise at -78 °C under an argon atmosphere. After stirring (30 min),  
120 propanal (0.6 g, 10.3 mmol) in anhydrous THF (5 mL) was added and stirring was continued for  
121 1 h. Di-*tert*-butyl dicarbonate (2.25 g, 10.3 mmol) dissolved in anhydrous THF (10 mL) was  
122 added dropwise and stirring continued for further 1 h. The mixture was allowed to warm up to  
123 room temperature, diluted with diethyl ether (30 mL), and washed with aqueous sodium  
124 hydroxide (2 M; 100 mL), followed by water (100 mL) and brine (100 mL). The organic phase  
125 was dried under anhydrous sodium sulfate and the solvent was removed in vacuo to afford 3.8 g  
126 of the target compound as a colorless oil.

127 *Di-tert-butyl (1,2-<sup>13</sup>C<sub>2</sub>)hept-3,4-dione-2,5-diyl biscarbonate.* The compound was  
128 synthesized by adapting the approach published for the synthesis of the (<sup>2</sup>H<sub>3</sub>)-substituted  
129 isotopologue.<sup>4</sup> Potassium permanganate (1.63 g, 10.3 mmol) was dissolved in a mixture of  
130 acetone (50 mL), water (10 mL), and acetic acid (1.5 mL) at 0 °C. The di-*tert*-butyl (1,2-



$^{13}\text{C}_2$ )hept-3-yne-2,5-diyl biscarbonate obtained above was added and the mixture was stirred for 3.5 h at 0 °C. Aqueous sodium hydrogen sulfite (5 %; 15 mL) was added and the mixture was filtered through sea sand. The acetone was removed in vacuo and the residual aqueous phase was extracted with dichloromethane (3 × 30 mL). The combined organic extracts were washed with saturated aqueous sodium hydrogen carbonate (100 mL), dried over anhydrous sodium sulfate and the solvent was removed in vacuo to afford 2.25 g of the target compound as a colorless oil.

*2(5)-Ethyl-4-hydroxy-5(2)-( $^{13}\text{C}$ )methyl(2(5)- $^{13}\text{C}$ )furan-3(2H)-one* ( $^{13}\text{C}_2$ )-7). The compound was synthesized by adapting the approach published for the synthesis of the ( $^2\text{H}_3$ )-substituted isotopologue.<sup>4</sup> The Di-*tert*-butyl (1,2- $^{13}\text{C}_2$ )hept-3,4-dione-2,5-diyl biscarbonate obtained above was refluxed in a mixture of water (20 mL) and oxalic acid (1.8 g) for 6 h under an argon atmosphere. After cooling to room temperature, brine (20 mL) was added and the mixture was extracted with dichloromethane (5 × 30 mL). The combined organic extracts were washed with saturated aqueous sodium hydrogen carbonate (150 mL), and dried over anhydrous sodium sulfate to afford a stock solution of the target compound. GC-MS (DB-5 column) revealed a tautomeric mixture in the ratio of 29% (first eluted isomer) to 71% (second eluted isomer). MS-EI (minor tautomer),  $m/z$  (intensity in %) 57 (100), 59 (42), 69 (6), 87 (14); MS-EI (major tautomer),  $m/z$  (intensity in %) 41 (24), 43 (37), 45 (100), 46 (18), 53 (13), 55 (11), 57 (23), 71 (63), 99 (30), 116 (10), 129 (33), 144 (81;  $\text{M}^+$ ); MS-CI (methanol; both tautomers),  $m/z$  (intensity in %) 145 (100;  $[\text{M}+\text{H}]^+$ ). The detailed MS-CI spectrum is provided in the Supporting Information.

*1-(Ethylsulfanyl)( $^{13}\text{C}_2$ )ethane-1-thiol* ( $^{13}\text{C}_2$ )-9). The compound was synthesized from ( $^{13}\text{C}_2$ )acetaldehyde (0.22 g; 5 mmol) and ethanethiol (0.31 g, 5 mmol) using the approach recently detailed for the synthesis of the isotopically unmodified compound.<sup>1</sup> The synthesis

afforded 0.28 g of the target compound as colorless oil in 80% purity (GC-FID) corresponding to 36% yield. MS-EI,  $m/z$  (intensity in %) 46 (12), 47 (6), 60 (11), 61 (35), 62 (40), 63 (59), 91 (100), 124 (29;  $M^+$ ); MS-CI (methanol),  $m/z$  (intensity in %) 91 (100), 125 (13;  $[M+H]^+$ ). The detailed MS-CI spectrum is provided in the Supporting Information.

*1-(Ethylsulfanyl)(2,2,3,3,3- $^2H_5$ )propane-1-thiol* ( $(^2H_5)$ -**10**). The compound was synthesized from (2,2,3,3,3- $^2H_5$ )propanal (0.32 g; 5 mmol), sodium sulfide nonahydrate (1.2 g, 5 mmol), and ethanethiol (0.31 g; 5 mmol) using the approach recently detailed for the synthesis of the isotopically unmodified compound.<sup>1</sup> The synthesis afforded 0.21 g of the target compound as colorless oil in 52% purity (GC-FID) corresponding to 15% yield. MS-EI,  $m/z$  (intensity in %) 46 (17), 45 (49), 47 (11), 49 (11), 62 (24), 77 (11), 79 (38), 80 (27), 108 (100), 141 (29;  $M^+$ ); MS-CI (methanol),  $m/z$  (intensity in %) 108 (100), 142 (11;  $[M+H]^+$ ). The detailed MS-CI spectrum is provided in the Supporting Information.

*1-(Methylsulfanyl)( $^{13}C_2$ )ethane-1-thiol* ( $(^{13}C_2)$ -**15**). The compound was synthesized from ( $^{13}C_2$ )acetaldehyde (0.22 g; 5 mmol), sodium sulfide nonahydrate (1.2 g, 5 mmol), and sodium methanethiolate (0.35 g, 5 mmol) using the approach recently detailed for the synthesis of the isotopically unmodified compound.<sup>1</sup> The synthesis afforded 0.21 g of the target compound as a colorless oil in 95% purity (GC-FID) corresponding to 36% yield. MS-EI,  $m/z$  (intensity in %) 43 (24), 45 (25), 46 (43), 47 (40), 48 (17), 49 (12), 60 (18), 61 (46), 62 (34), 63 (53), 77 (100), 110 (26;  $M^+$ ); MS-CI (methanol),  $m/z$  (intensity in %) 77 (100), 111 (15;  $[M+H]^+$ ). The detailed MS-CI spectrum is provided in the Supporting Information.

**Enzymatic Quantitation.** The concentration of acetaldehyde in Monthong durian pulp was determined by employing a commercial enzymatic assay (Roche Yellow line, R-Biopharm, Darmstadt, Germany). In the presence of aldehyde dehydrogenase, acetaldehyde was

quantitatively oxidized by  $\text{NAD}^+$  to acetic acid and the NADH formed was photometrically determined at 340 nm.

**Stable Isotope Dilution Assays (SIDAs).** Portions of durian pulp (10-170 g) were spiked with stable isotopically substituted durian odorants (0.5-50  $\mu\text{g}$ ) in dichloromethane (100 mL) and each mixture was homogenized using a stainless steel blender. The organic phase was separated, dried over anhydrous sodium sulfate, and nonvolatile material was removed by solvent-assisted flavor evaporation (SAFE)<sup>9</sup> at 40 °C. For the quantitation of non-thiol compounds, the distillate was concentrated to final volumes of 0.2-1 mL, first using a Vigreux column (60 cm) and subsequently a Bemelmans microdistillation device,<sup>10</sup> and aliquots (1-2  $\mu\text{L}$ ) of this concentrate were directly subjected to two-dimensional heart-cut gas chromatography–mass spectrometry (GC-GC-MS). For the quantitation of thiols, including the dithiohemiacetals, the distillate obtained by SAFE was concentrated to 5 mL and applied onto mercurated agarose gel (1 g). Using the approach detailed recently,<sup>11</sup> non-thiols were washed off, the thiol fraction was eluted with an excess of dithiothreitol, and the bulk of dithiothreitol was removed by SAFE. The distillate was concentrated (0.2 mL) and aliquots (1-2  $\mu\text{L}$ ) were subjected to GC-GC-MS.

Peak areas corresponding to analyte and internal standard were obtained from the extracted ions chromatograms using the quantifier ions detailed in Table 1. The concentration of each target compound in the durian pulp was then calculated from the area counts of the analyte peak, the area counts of the standard peak, the amount of durian pulp used and the amount of standard added, by employing a calibration line equation previously obtained from the analysis of analyte/standard mixtures in known concentrations.<sup>2</sup>

**Gas Chromatography–Flame Ionization Detector (GC-FID).** For the exact quantitation of stable isotopically substituted durian odorants in stock solutions used in the SIDA experiments

and for the determination of the purity of synthetic products, a system consisting of a Trace GC Ultra gas chromatograph equipped with a cold-on-column injector and an FID (Thermo Scientific, Dreieich, Germany) was employed. The GC was equipped either with a fused silica column DB-FFAP, 30 m  $\times$  0.32 mm i.d., 0.25  $\mu$ m film, or with a fused silica column DB-5, 30 m  $\times$  0.32 mm i.d., 0.25  $\mu$ m film (both Agilent, Waldbronn, Germany). The carrier gas was helium at 70 kPa. Injection volume was 1  $\mu$ L. The initial oven temperature was 40  $^{\circ}$ C (2 min), followed by a gradient of 6  $^{\circ}$ C/min, which was run until an end temperature of 240  $^{\circ}$ C. The FID was connected to a computer and chromatogram recording and peak area count calculation was performed by ChromQuest 5.0 (Thermo).

**Gas Chromatography–Mass Spectrometry (GC-MS).** Mass spectra of synthetic products in the electron ionization mode (MS-EI) were recorded 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 mode (MS-CI) were acquired with a 3800 gas chromatograph connected to a Saturn 2200 mass spectrometer (Varian, Darmstadt, Germany) and methanol as reagent gas. The GC column employed was a DB-FFAP, 30 m  $\times$  0.25 mm i.d., 0.25  $\mu$ m film, or a DB-5, 30 m  $\times$  0.25 mm i.d., 0.25  $\mu$ m film (both Agilent). Oven programs corresponded to those detailed for GC-FID analyses.

**Two-Dimensional Heart-Cut Gas Chromatography–Mass Spectrometry (GC-GC-MS).** A Trace GC Ultra (Thermo) was equipped with a Combi PAL autosampler (CTC Analytics, Zwingen, Switzerland), a cold-on-column injector (Thermo) and a DB-FFAP capillary, 30 m  $\times$  0.32 mm i.d., 0.25  $\mu$ m film (Agilent). The column end was connected to a moving column stream switching system (MCSS) (Thermo). The computer-operated MCSS conveyed the column eluate through uncoated fused silica capillaries (0.32 mm i.d.) either to an FID (Thermo)

and a tailor-made sniffing port<sup>12</sup> serving as monitor detectors or via a transfer line to a second column inside the oven of a CP 3800 GC (Varian). This GC was connected to a Saturn 2000 mass spectrometer (Varian) operated in CI mode with methanol as the reactant gas.

For the analysis of the SIDA samples, the column in the second oven was a DB-5, 30 m × 0.25 mm i.d., 0.25 µm film (Agilent). Start temperatures were 40 °C and temperature gradients were 4-6 °C/min in the first and second dimension. For the determination of the enantiomeric distribution of 1-(ethylsulfanyl)ethanethiol, the column in the second oven was a BGB-175, 30 m × 0.25 mm i.d., 0.25 µm film (BGB Analytik, Rheinfelden, Germany). Start temperatures were 40 °C and temperature gradients were 6 °C/min in the first and 2 °C/min in the second dimension.

During analysis, a heart-cut of the eluate of the first column containing the respective target compound(s) was transferred during 1-1.5 min via the MCSS and the transfer line to the second oven. Transferred substances were refocused by a stream of cold (−196 °C) nitrogen gas directed at the end of the transfer line inside the second oven. Then, cooling was turned off and second oven and mass spectrometer were started. The retention times of the target compounds in the first and second dimensions were previously determined using reference compounds.

**Odor Thresholds.** On the basis of the procedure for the determination of odor and taste thresholds by a forced-choice ascending concentration series method of limits of American Society for Testing and Materials (ASTM),<sup>13</sup> orthonasal odor thresholds in water were determined using the experimental setup detailed previously.<sup>14</sup>

**Aroma Model Solutions.** Aliquots (50-200 µL) of aqueous or ethanolic stock solutions of reference odorants were combined and diluted with aqueous buffer (5 mmol citric acid; pH 6.2). Sunflower oil was added to obtain a final oil concentration of 3 %, which is the approximate

natural lipid content in durian pulp. The concentrations of the stock solutions, the size of the aliquots, and the dilution with the water/oil matrix were adjusted to yield final concentrations of each odorant in the model solution identical to the concentrations previously determined in the durian fruit pulp and to maintain final ethanol concentration below 1 g/L.

**Quantitative Descriptive Analysis (QDA).** Samples of freshly homogenized durian pulp (10 g) and samples of an aroma simulation model (20 compounds, OAVs  $\geq 1$ ; 10 g) prepared as described above were placed into cylindrical (40 mm  $\times$  65 mm high) ground neck borosilicate glasses with lids (VWR, Darmstadt, Germany) and orthonasally evaluated by trained panelists. Descriptors were defined on the basis of the odor of a reference compound dissolved in water at a concentration of 100 $\times$  its respective orthonasal odor threshold value. Reference odorants used in the sensory experiments were ethyl (2*S*)-2-methylbutanoate (fruity), 4-hydroxy-2,5-dimethylfuran-3(2*H*)-one (caramel), 3-hydroxy-4,5-dimethylfuran-2(5*H*)-one (soup seasoning), 1-(ethylsulfanyl)ethanethiol (roasted onion), ethanethiol (rotten onion), hydrogen sulfide (rotten egg), and acetaldehyde (fresh). Panelists rated the intensity of each descriptor in both samples on a seven-point scale ranging from 0 to 3 with 0.5 increments and with 0 = not detectable, 1 = weak, 2 = moderate, and 3 = strong. Ratings of 14 panelists (males and females, ages 20 to 46) were averaged by calculating the arithmetic mean.

**Omission Tests.** Aroma model solutions were prepared as described above and presented to the trained panelists in the borosilicate glasses detailed before. The models from which one or more odorants had been omitted were orthonasally tested against two samples of a reference model in a triangle test with forced choice. For each omission experiment, the *p*-value was calculated from the total number of panelists and the number of correct answers.<sup>15</sup>

## Results and Discussion

**Development of Stable Isotope Dilution Assays.**

Sixteen substances recently detected among the most odor-active compounds during the application of an AEDA and the GC-O analysis of static headspace samples were quantitated in the pulp of durians, variety Monthong. These 16 compounds included the entire set of 12 odorants for which FD factors  $\geq 256$  had been determined.<sup>1</sup> Except for acetaldehyde, which was quantitated employing a commercial enzymatic assay, quantitation was aspired by SIDA using  $^2\text{H}$ - or  $^{13}\text{C}$ -substituted analogues of the target compounds as internal standards to compensate for losses during the work-up procedures.

The majority of isotopically substituted odorants needed was obtained by synthesis, either following literature procedures or by adapting procedures published for the synthesis of the respective isotopically unmodified compound. In detail, synthesis of ( $^2\text{H}_{10}$ )diethyl trisulfide (( $^2\text{H}_{10}$ )-**1**) was accomplished by reacting bromo( $^2\text{H}_5$ )ethane with trisulfide, which was generated together with the disulfide in situ from elemental sulfur in alkaline solution (Figure 1).<sup>7</sup> Isotopically substituted analogues of the 1,1-dithio compounds ethane-1,1-dithiol (**2**), 1-(ethyldisulfanyl)-1-(ethylsulfanyl)ethane (**6**), 1-(ethylsulfanyl)ethane-1-thiol (**9**), 1-(ethylsulfanyl)propane-1-thiol (**10**), and 1-(methylsulfanyl)ethanethiol (**15**) were synthesized as described for the isotopically unmodified compounds,<sup>1</sup> but using ( $^{13}\text{C}_2$ )acetaldehyde instead of acetaldehyde and (2,2,3,3,3- $^5\text{H}_5$ )propanal instead of propanal. Isotopically substituted esters were synthesized from the corresponding acids with the deuterated alcohols (2,2,2- $^2\text{H}_3$ )ethanol, ( $^2\text{H}_5$ )ethanol, or ( $^2\text{H}_3$ )methanol.<sup>5</sup> For the synthesis of 2(5)-ethyl-4-hydroxy-5(2)-( $^{13}\text{C}$ )methyl(2(5)- $^{13}\text{C}$ )furan-3(2*H*)-one (( $^{13}\text{C}_2$ )-**7**), a method published by Blank et al.<sup>4</sup> for the synthesis of the deuterated compound was adapted. Blank et al. reported the synthesis of 2(5)-(2,2,2- $^2\text{H}_3$ )ethyl-4-hydroxy-5(2)-methylfuran-3(2*H*)-one starting from but-3-yn-2-ol. Protection by a *tert*-butyloxycarbonyl (Boc) group yielded *tert*-butyl but-3-yn-2-yl carbonate. This

compound was reacted with lithium diisopropylamide, followed by (3,3,3- $^2\text{H}_3$ )propanal and the resulting alkoxide was in situ derivatized with di-*tert*-butyl dicarbonate to yield di-*tert*-butyl (7,7,7- $^2\text{H}_3$ )hept-3-yne-2,5-diyl biscarbonate. We used the same approach, but started from (1,2- $^{13}\text{C}_2$ )but-3-yn-2-ol, which was prepared from ( $^{13}\text{C}_2$ )acetaldehyde and ethynylmagnesium bromide, and employed isotopically unmodified propanal instead of the deuterated compound to obtain di-*tert*-butyl (1,2- $^{13}\text{C}_2$ )hept-3-yne-2,5-diyl biscarbonate (Figure 2). As reported for the deuterated analogue,<sup>4</sup> oxidation of the triple bond to the diketone by aqueous potassium permanganate followed by removal of the Boc groups and cyclisation under acidic conditions finally led to the target compound.

In summary, for each of the 15 target compounds a stable isotopologue was finally available, which then was used as internal standard in the quantitation assays (Figure 3).

**Concentrations and OAVs of Monthong Durian Odorants.** Results of the SIDA experiments and the enzymatic quantitation of acetaldehyde revealed concentrations roughly ranging between 1  $\mu\text{g/kg}$  and 100  $\text{mg/kg}$  (Table 2), thus covering five orders of magnitude. In combination with the data recently reported for hydrogen sulfide (**19**) and short-chain alkane thiols in Monthong durians,<sup>2</sup> high concentrations were particular determined for acetaldehyde (**16**; 98400  $\mu\text{g/kg}$ ), ethanethiol (**17**; 45400  $\mu\text{g/kg}$ ), methanethiol (**18**; 26700  $\mu\text{g/kg}$ ), and ethyl (2*S*)-2-methylbutanoate (**5**; 13800  $\mu\text{g/kg}$ ), whereas concentrations of 3-methylbut-2-ene-1-thiol (**13**), ethyl cinnamate (**4**), and 1-(ethylsulfanyl)propane-1-thiol (**10**) were in the low  $\mu\text{g/kg}$  range.

To achieve an approximation of the odor potency of the individual compounds, an OAV was calculated for each odorant as ratio of its natural concentration in the Monthong durians to its orthonasal odor detection threshold in water (Table 3). Nineteen of the 20 compounds exceeded their respective odor threshold value, many of them by far. Exceptional high OAVs were



obtained for fruity smelling ethyl (2*S*)-2-methylbutanoate (**5**; OAV 1700000), rotten onion-like smelling ethanethiol (**17**; OAV 480000), and roasted onion-like smelling 1-(ethylsulfanyl)ethanethiol (**9**; OAV 250000). OAVs of this size are beyond the range typically found for aroma-active compounds in food,<sup>16</sup> however they very well corresponded to the extraordinary intensity of durian pulp odor.

The high OAV calculated for 1-(ethylsulfanyl)ethanethiol prompted us to investigate its enantiomeric distribution in durian, as enantiomers may substantially differ in their olfactory properties. Analysis of a fruit pulp extract by GC-GC-MS using a chiral column in the second chromatographic dimension revealed the presence of a racemic mixture in durian. This suggested a nonenzymatic formation from acetaldehyde, hydrogen sulfide and ethanthiol in the fruit pulp and retrospectively justified OAV calculation based on the odor threshold of the racemate.

A key role of ethyl (2*S*)-2-methylbutanoate (**5**) for the fruity odor note of Monthong durian pulp has already been suggested from its high FD factor obtained by AEDA.<sup>1</sup> Ethyl 2-methylbutanoate has widely been reported as a major volatile in the pulp of different durian varieties,<sup>17-28</sup> although in some varieties e.g. ethyl propanoate was more abundant.<sup>18, 24, 25</sup> A crucial impact of ethyl 2-methylbutanoate for durian odor has been claimed before such as by Baldry et al.<sup>17</sup>, Weenen et al.,<sup>19</sup> and Voon et al.<sup>23</sup> Ethanethiol (**17**) has also for a long time been known as durian volatile,<sup>17, 18, 21, 22, 25</sup> however, its high odor potency was yet not appropriately recognized. 1-(Ethylsulfanyl)ethanethiol (**9**) has been reported from durian only by Wong and Tie,<sup>20</sup> who assumed that the sum of sulfur compounds including 1-(ethylsulfanyl)ethanethiol account for the oniony note in durian odor, but did not assess the impact of individual compounds. Other authors suggested rather propane-1-thiol (**20**),<sup>17</sup> 3,5-dimethyl-1,2,4-trithiolane,<sup>19</sup> or methyl propyl disulfide<sup>22</sup> as crucial component for the oniony odor note in

durians. Näf and Velluz<sup>20</sup> reported a series of dithioacetals from durian, but did not find dithiohemiacetals, which, according to our results,<sup>1</sup> are the more potent odorants. The workup procedures used might account for that. Näf and Velluz applied simultaneous distillation-extraction, whereas Wong and Tie<sup>20</sup> applied a rather mild volatile isolation technique, which was similar to our approach and was based on a vacuum distillation at moderate temperatures of 28-30 °C.

Apart from ethyl (2*S*)-2-methylbutanoate, ethanethiol, and 1-(ethylsulfanyl)ethanethiol, high OAVs in the range of 330 to 45000 were also calculated for 13 further compounds (**18**, **2**, **8**, **14**, **3**, **20**, **16**, **15**, **10**, **13**, **1**, **6**, and **19**) (Table 3). 2(5)-ethyl-4-hydroxy-5(2)-methylfuran-3(2*H*)-one (**7**), 3-hydroxy-4,5-dimethylfuran-2(5*H*)-one (**11**), ethyl cinnamate (**4**), and 4-hydroxy-2,5-dimethylfuran-3(2*H*)-one (**12**), on the other hand, exhibited comparatively low OAVs of 1 to 22, although their FD factors obtained by AEDA were quite high (128 to 4096). The odor potency of these substances might have been overestimated during AEDA, where compounds are assessed completely vaporized. Their rather high boiling points and the good water solubility of the furanones may substantially contribute to a low release from aqueous solution, which would have resulted in rather low OAVs.

**Monthong Durian Odor Simulation.** To demonstrate that the 20 odor-active compounds quantitated in this study included the key players in the overall olfactory profile of durian pulp, an odor simulation model was prepared. The model was based on a buffered (pH 6.2) mixture of 97 % water and 3 % sunflower oil serving as an approximation of fresh durian pulp matrix and included all 20 compounds in their natural concentrations as compiled in Table 2. The simulation model exhibited a strong and characteristic durian odor and, when orthonasally compared to fresh Monthong durian pulp in a QDA, showed a good agreement in the overall rating of major

odor descriptors. Both, the fruit material and the model, exhibited a dominating roasted onion-like odor, followed by fruity, rotten onion-like, and rotten egg-like notes, whereas fresh, caramel-like, and soup seasoning-like notes were rated less intense (Figure 4).

**Omission Tests.** The successful simulation of Monthong durian odor allowed for the application of omission tests to study the contribution of individual odorants to the overall odor profile. In an omission test, one or more compounds are omitted from the odor simulation model and this incomplete model is then compared to the complete model in a triangle test. In case the test results in a significant difference, a singly omitted compound has proven its odor impact.<sup>29</sup>

In a first experiment (Table 4, test O1), the complete Monthong durian odor simulation model (20 odorants, 1–20) was tested against a model from which all ten odorants with OAVs < 5000 (1, 4, 6, 7, 10–13, 15, 19) had been omitted. The resulting mixture of ten odorants (2, 3, 5, 8, 9, 14, 16–18, 20) could not significantly be distinguished from the complete model ( $p \gg 5\%$ ), indicating that the omitted odorants are of minor importance. Therefore, the model containing only the 10 odorants with OAVs > 5000 was used as reference for the following tests. From this model, the omission of single odorants was performed in ten separate experiments (Table 4, tests O2–O11). None of the ten tests resulted in a significant difference between test and reference sample and therefore were not helpful to rank the odor contribution of the individual odorants. Obviously, omission of any single odorant from the model could be compensated by the other nine compounds. A similar observation was recently reported from a mango aroma model.<sup>30</sup> In the case of durian, the compensating effect is very plausible. First, because the ten odorants basically exhibited only two types of odor qualities, fruity and sulfury-oniony, and second, because the concentrations of some odorants were so far above their odor threshold values that the corresponding olfactory receptors might already act beyond their saturation level. In this

case, e.g. the omission of ethyl (2*S*)-2-methylbutanoate (**5**), although exhibiting an OAV as high as 1700000, might be fully compensated by ethyl 2-methylpropanoate (**8**; OAV 22000), methyl (2*S*)-2-methylbutanoate (**14**; OAV 13000), and ethyl butanoate (**3**; OAV 11000), which in combination would be able to saturate the odor receptor for fruitiness. In the same way, the omission of oniony smelling ethanethiol (**17**; OAV 480000) could be compensated by 1-(ethylsulfanyl)ethanethiol (**9**; OAV 250000) and vice versa. Following this idea further, it seemed to be possible that the typical durian odor could possibly be evoked by the combination of only two potent compounds, a fruity smelling one and an oniony smelling one. This hypothesis was tested in omission experiment O12 (Table 4). A model only containing the odorants ethyl (2*S*)-2-methylbutanoate (**5**) and 1-(ethylsulfanyl)ethanethiol (**9**) was tested against the reference model of ten compounds. The result indicated no significant difference. Thus, the typical durian pulp odor could be evoked by a mixture of only two odor-active compounds present in their natural concentrations. In contrast, when ethyl (2*S*)-2-methylbutanoate (**5**) was additionally omitted, thus 1-(ethylsulfanyl)ethanethiol (**9**) alone was tested against the reference model of ten compounds, the result was a clear sensory difference (Table 4, test O13). Although the oniony note is clearly dominating durian pulp odor, the fruity note is obviously of vital importance, too. Similar binary mixtures such as a mixture of ethyl (2*S*)-2-methylbutanoate (**5**) and ethanethiol (**17**) in the respective natural concentrations were also able to evoke a smell clearly reminiscent of durian pulp (data not shown).

In summary, the molecular sensory science approach including screening of odor-active compounds by AEDA and static headspace GC-O, structural assignment of the most odor-active compounds, their exact quantitation, calculation of OAVs, and finally the execution of omission tests revealed a surprisingly small number of only two key compounds, namely ethyl (2*S*)-2-

methylbutanoate and 1-(ethylsulfanyl)ethanethiol, being sufficient to evoke the characteristic smell of durian pulp.

## Associated Content

## Supporting Information

MS-CI spectra of synthesized isotopically substituted odorants (PDF)

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## Notes

The authors declare no competing financial interest.

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## Abbreviations Used

AEDA, aroma extract dilution analysis; Boc, *tert*-butyloxycarbonyl; CI, chemical ionization; EI, electron ionization; FD factor, flavor dilution factor; FFAP, free fatty acid phase; FID, flame ionization detector; GC, gas chromatography; MS, mass spectrometry; GC-O, gas chromatography–olfactometry; GC-GC-MS, two-dimensional heart-cut gas chromatography–mass spectrometry; MCSS, moving column stream switching system; NAD, nicotinamide adenine dinucleotide; OAV, odor activity value; QDA, quantitative descriptive analysis; SAFE, solvent-assisted flavor evaporation; SIDA, stable isotope dilution assay.

## Nomenclature

Dithiohemiacetal, 1-(alkylsulfanyl)alkane-1-thiol; diethyl trisulfide, diethyltrisulfane; ethyl

430 cinnamate, ethyl (2*E*)-3-phenylprop-2-enoate; 2(5)-ethyl-4-hydroxy-5(2)-methylfuran-3(2*H*)-  
431 one, also called homofuraneol, tautomeric mixture of 2-ethyl-4-hydroxy-5-methylfuran-3(2*H*)-  
432 one and 5-ethyl-4-hydroxy-2-methylfuran-3(2*H*)-one; 2(5)-ethyl-4-hydroxy-5(2)-  
433 (<sup>13</sup>C)methyl(2(5)-<sup>13</sup>C)furan-3(2*H*)-one, tautomeric mixture of 2-ethyl-4-hydroxy-5-  
434 (<sup>13</sup>C)methyl(2-<sup>13</sup>C)furan-3(2*H*)-one and 5-ethyl-4-hydroxy-2-(<sup>13</sup>C)methyl(5-<sup>13</sup>C)furan-3(2*H*)-  
435 one; 3-hydroxy-4,5-dimethylfuran-2(5*H*)-one, also known as sotolon; 4-hydroxy-2,5-  
436 dimethylfuran-3(2*H*)-one, also known as Furaneol<sup>®</sup>; 4-hydroxy-2(5)-methyl-5(2)-  
437 (<sup>13</sup>C)methyl(5(2)-<sup>13</sup>C)furan-3(2*H*)-one, tautomeric mixture of 4-hydroxy-2-methyl-5-  
438 (<sup>13</sup>C)methyl(5-<sup>13</sup>C)furan-3(2*H*)-one and 4-hydroxy-5-methyl-2-(<sup>13</sup>C)methyl(2-<sup>13</sup>C)furan-3(2*H*)-  
439 one.

## References

1. Li, J.-X.; Schieberle, P.; Steinhaus, M. Characterization of the major odor-active compounds in Thai durian (*Durio zibethinus* L. 'Monthong') by aroma extract dilution analysis and headspace gas chromatography-olfactometry. *J. Agric. Food Chem.* **2012**, *60*, 11253–11262.
2. Li, J.-X.; Schieberle, P.; Steinhaus, M. Development of stable isotope dilution assays for the quantitation of the food odorants hydrogen sulphide, methanethiol, ethanethiol, and propane-1-thiol and application to durian (*Durio zibethinus* L.) pulp. *Eur. Food Res. Technol.* DOI: 10.1007\_s00217-016-2723-z.
3. Blank, I.; Schieberle, P.; Grosch, W. Quantification of the flavour compounds 3-hydroxy-4,5-dimethyl-2(5H)-furanone and 5-ethyl-3-hydroxy-4-methyl-2(5H)-furanone by a stable isotope dilution assay. In *Progress in Flavour Precursor Studies*; Schreier, P., Winterhalter, P., Eds.; Allured Publishing: Carol Stream, IL, 1993; pp 103–109.
4. Blank, I.; Fay, L. B.; Lakner, F. J.; Schlosser, M. Determination of 4-hydroxy-2,5-dimethyl-3(2H)-furanone and 2(or 5)-ethyl-4-hydroxy-5(or 2)-methyl-3(2H)-furanone in pentose sugar-based Maillard model systems by isotope dilution assays. *J. Agric. Food Chem.* **1997**, *45*, 2642–2648.
5. Fischer, E.; Speier, A. Darstellung der Ester. *Ber. Dtsch. Chem. Ges.* **1895**, *28*, 3252–3258.
6. Bio-Rad Laboratories, Hercules, CA. [www.bio-rad.com/webroot/web/html/lsr/tech\\_support\\_faqs/FAQ268439460.html](http://www.bio-rad.com/webroot/web/html/lsr/tech_support_faqs/FAQ268439460.html) (November 4, 2014).
7. Chorbadjiev, S.; Roumian, C.; Markov, P. New preparative synthesis of dialkyl disulfides. *J. Prakt. Chem.* **1977**, *319*, 1036–1038.

- 462 8. Eren, D.; Keinan, E. Total synthesis of linear polyprenoids. 3. Syntheses of ubiquinones via  
463 palladium-catalyzed oligomerization of monoterpene monomers. *J. Am. Chem. Soc.* **1988**,  
464 *110*, 4356–4362.
- 465 9. Engel, W.; Bahr, W.; Schieberle, P. Solvent assisted flavour evaporation – a new and  
466 versatile technique for the careful and direct isolation of aroma compounds from complex  
467 food matrices. *Eur. Food Res. Technol.* **1999**, *209*, 237–241.
- 468 10. Bemelmans, J. M. H. Review of isolation and concentration techniques. In *Progress in*  
469 *Flavour Research*; Land, G. G., Nursten, H. E., Eds.; Applied Science: London, U.K., 1979;  
470 pp 79–88.
- 471 11. Steinhaus, M. Characterization of the major odor-active compounds in the leaves of the  
472 curry tree *Bergera koenigii* L. by aroma extract dilution analysis. *J. Agric. Food Chem.*  
473 **2015**, *63*, 4060–4067.
- 474 12. Steinhaus, M.; Sinuco, D.; Polster, J.; Osorio, C.; Schieberle, P. Characterization of the  
475 aroma-active compounds in pink guava (*Psidium guajava*, L.) by application of the aroma  
476 extract dilution analysis. *J. Agric. Food Chem.* **2008**, *56*, 4120–4127.
- 477 13. American Society of Testing and Materials. Standard E679-04. Standard practice for  
478 determination of odor and taste thresholds by a forced-choice ascending concentration series  
479 method of limits In *ASTM Book of Standards*; American Society of Testing and Materials:  
480 West Conshohocken, PA, 2005; Vol. 15.08, pp 38–44.
- 481 14. Czerny, M.; Christlbauer, M.; Christlbauer, M.; Fischer, A.; Granvogl, M.; Hammer, M.;  
482 Hartl, C.; Hernandez, N.; Schieberle, P. Re-investigation on odour thresholds of key food  
483 aroma compounds and development of an aroma language based on odour qualities of  
484 defined aqueous odorant solutions. *Eur. Food Res. Technol.* **2008**, *228*, 265–273.



- 485 15. Quadt, A.; Schönberger, S.; Schwarz, M. *Statistische Auswertungen in der Sensorik:*  
486 *Leitfaden für die Praxis*, 1st ed.; Behr's Verlag: Hamburg, Germany, 2009.
- 487 16. Dunkel, A.; Steinhaus, M.; Kotthoff, M.; Nowak, B.; Krautwurst, D.; Schieberle, P.;  
488 Hofmann, T. Nature's chemical signatures in human olfaction: A foodborne perspective for  
489 future biotechnology. *Angew. Chem. Int. Ed.* **2014**, *53*, 7124–7143.
- 490 17. Baldry, J.; Dougan, J.; Howard, G. E. Volatile flavouring constituents of durian.  
491 *Phytochemistry* **1972**, *11*, 2081–2087.
- 492 18. Wong, K. C.; Tie, D. Y. Volatile constituents of durian (*Durio zibethinus* Murr.). *Flavour*  
493 *Fragrance J.* **1995**, *10*, 79–83.
- 494 19. Weenen, H.; Koolhas, W. E.; Apriyantono, A. Sulfur-containing volatiles of durian fruits  
495 (*Durio zibethinus* Murr.) *J. Agric. Food Chem.* **1996**, *44*, 3291–3293.
- 496 20. Näf, R.; Velluz, A. Sulfur compounds and some uncommon esters in durian (*Durio*  
497 *zibethinus* Murr.). *Flavour Fragrance J.* **1996**, *11*, 295–303.
- 498 21. Jiang, J.; Choo, S. Y.; Omar, N.; Ahamad, N. GC-MS analysis of volatile compounds in  
499 durian (*Durio zibethinus* Murr.). In *Developments in Food Science 40. Food Flavors:*  
500 *Formation, Analysis and Packaging Influences*; Contis, E. T., Ho, C.-T., Mussinan, C. J.,  
501 Parliament, T. H., Shahidi, F., Spanier, A. F., Eds.; Elsevier: Amsterdam, The Netherlands,  
502 1998; pp 345–352.
- 503 22. Voon, Y. Y.; Hamid, N. S. A.; Rusul, G.; Osman, A.; Quek, S. Y., Characterisation of  
504 Malaysian durian (*Durio zibethinus* Murr.) cultivars: Relationship of physicochemical and  
505 flavour properties with sensory properties. *Food Chemistry* **2007**, *103*, 1217–1227.

- 506 23. Voon, Y. Y.; Sheikh Abdul Hamid, N.; Rusul, G.; Osman, A.; Quek, S. Y. Volatile flavour  
507 compounds and sensory properties of minimally processed durian (*Durio zibethinus* cv.  
508 D24) fruit during storage at 4 °C. *Postharvest Biol. Technol.* **2007**, *46*, 76–85.
- 509 24. Chin, S. T.; Nazimah, S. A. H.; Quek, S. Y.; Che Man, Y. B.; Abdul Rahman, R.; Mat  
510 Hashim, D. Analysis of volatile compounds from Malaysian durians (*Durio zibethinus*)  
511 using headspace SPME coupled to fast GC-MS. *J. Food Compos. Anal.* **2007**, *20*, 31–44.
- 512 25. Chin, S. T.; Hamid Nazimah, S. A.; Quek, S. Y.; Che Man, Y. B.; Rahman, R. A.; Hashim,  
513 D. M. Changes of volatiles' attribute in durian pulp during freeze- and spray-drying process.  
514 *Food Sci. Technol.* **2008**, *41*, 1899–1905.
- 515 26. Chawengkijwanich, C.; Sa-nguanpuag, K.; Tanprasert, K. Monitoring volatile compounds  
516 emitted by durian pulp (*Durio zibethinus* Murr.) at mature and ripe stage using solid phase  
517 microextraction (SPME). *Acta Hortic.* **2008**, *804*, 321–326.
- 518 27. Jaswir, I.; Che Man, Y. B.; Selamat, J.; Ahmad, F.; Sugisawa, H. Retention of volatile  
519 components of durian fruit leather during processing and storage. *J. Food Process. Preserv.*  
520 **2008**, *32*, 740–750.
- 521 28. Chin, S. T.; Hamid Nazimah, S. A.; Quek, S.-Y.; Che Man, Y. B.; Rahman, R. A.; Hashim,  
522 D. M. Effect of thermal processing and storage condition on the flavour stability of spray-  
523 dried durian powder. *Food Sci. Technol.* **2010**, *43*, 856–861.
- 524 29. Grosch, W. Evaluation of the key odorants of foods by dilution experiments, aroma models  
525 and omission. *Chem Senses* **2001**, *26*, 533–545.
- 526 30. Munafo, J. P.; Didzbalis, J.; Schnell, R. J.; Steinhaus, M. Insights into the key aroma  
527 compounds in mango (*Mangifera indica* L. 'Haden') fruits by stable isotope dilution  
528 quantitation and aroma simulation experiments. *J. Agric. Food Chem.* **2016**, *64*, 4312–4318.

**Figure Captions.**

**Figure 1.** Synthesis of ( $^2\text{H}_{10}$ )diethyl trisulfide based on the approach reported earlier for the isotopically unmodified compound.<sup>7</sup>

**Figure 2.** Synthesis of 2(5)-ethyl-4-hydroxy-5(2)-( $^{13}\text{C}$ )methyl(2(5)- $^{13}\text{C}$ )furan-3(2*H*)-one based on the approach reported earlier for the deuterated compound.<sup>4</sup> Dots indicate the positions of  $^{13}\text{C}$  atoms.

**Figure 3.** Structures of the stable isotopically substituted durian odorants used as internal standards in the stable isotope dilution assays. Dots indicate the positions of  $^{13}\text{C}$  atoms.

**Figure 4.** Aroma profile of the Monthong durian aroma model (20 compounds,  $\text{OAV} \geq 1$ ) in comparison with the aroma profile of fresh pulp obtained from ripe Monthong durians. Panelists rated the intensity of each descriptor orthonasally on a scale from 0 to 3 with 0.5 increments and with 0 = not detectable, 1 = weak, 2 = moderate, and 3 = strong.

**Table 1.** Isotopically Substituted Compounds and Quantitation Parameters Used in the Stable Isotope Dilution Assays

target analyte	isotopically substituted internal standard	quantifier ion ( <i>m/z</i> )		calibration line <sup>a</sup>
		analyte	standard	
diethyl trisulfide ( <b>1</b> )	( <sup>2</sup> H <sub>10</sub> )diethyl trisulfide	155	165	y = 0.98 x – 0.02
ethane-1,1-dithiol ( <b>2</b> )	( <sup>13</sup> C <sub>2</sub> )ethane-1,1-dithiol	61	63	y = 0.88 x + 0.07
ethyl butanoate ( <b>3</b> )	(2,2,2- <sup>2</sup> H <sub>3</sub> )ethyl butanoate	117	120	y = 1.00 x – 0.02
ethyl cinnamate ( <b>4</b> )	(2,2,2- <sup>2</sup> H <sub>3</sub> )ethyl cinnamate	177	180	y = 0.90 x + 0.01
ethyl (2 <i>S</i> )-2-methylbutanoate ( <b>5</b> )	( <sup>2</sup> H <sub>5</sub> )ethyl 2-methylbutanoate	131	136	y = 0.94 x + 0.02
1-(ethylsulfanyl)-1-(ethylsulfanyl)ethane ( <b>6</b> )	1-(ethylsulfanyl)-1-(ethylsulfanyl)( <sup>13</sup> C <sub>2</sub> )ethane	121	123	y = 0.94 x + 0.11
2(5)-ethyl-4-hydroxy-5(2)-methylfuran-3(2 <i>H</i> )-one ( <b>7</b> )	2(5)-ethyl-4-hydroxy-5(2)-( <sup>13</sup> C)methyl(5(2)- <sup>13</sup> C)furan-3(2 <i>H</i> )-one	143	145	y = 0.94 x + 0.02
ethyl 2-methylpropanoate ( <b>8</b> )	(2,2,2- <sup>2</sup> H <sub>3</sub> )ethyl 2-methylpropanoate	117	120	y = 0.93 x + 0.03
1-(ethylsulfanyl)ethane-1-thiol ( <b>9</b> )	1-(ethylsulfanyl)( <sup>13</sup> C <sub>2</sub> )ethane-1-thiol	89	91	y = 1.10 x + 0.00
1-(ethylsulfanyl)propane-1-thiol ( <b>10</b> )	1-(ethylsulfanyl)(2,2,3,3,3- <sup>5</sup> H <sub>5</sub> )propane-1-thiol	75	80	y = 0.99 x – 0.20
3-hydroxy-4,5-dimethylfuran-2(5 <i>H</i> )-one ( <b>11</b> )	3-hydroxy-4-methyl-5-( <sup>13</sup> C)methyl(5- <sup>13</sup> C)furan-2(5 <i>H</i> )-one	129	131	y = 0.98 x – 0.02
4-hydroxy-2,5-dimethylfuran-3(2 <i>H</i> )-one ( <b>12</b> )	4-hydroxy-2(5)-methyl-5(2)-( <sup>13</sup> C)methyl(5(2)- <sup>13</sup> C)furan-3(2 <i>H</i> )-one	129	131	y = 1.03 x – 0.01
3-methylbut-2-ene-1-thiol ( <b>13</b> )	3-( <sup>2</sup> H <sub>3</sub> )methyl(4,4,4- <sup>2</sup> H <sub>3</sub> )but-2-ene-1-thiol	69	75	y = 0.98 x – 0.20
methyl (2 <i>S</i> )-2-methylbutanoate ( <b>14</b> )	( <sup>2</sup> H <sub>3</sub> )methyl 2-methylbutanoate	117	120	y = 1.02 x – 0.02
1-(methylsulfanyl)ethanethiol ( <b>15</b> )	1-(methylsulfanyl)( <sup>13</sup> C <sub>2</sub> )ethane-1-thiol	75	77	y = 0.97 x + 0.05

<sup>a</sup>y = peak area standard / peak area analyte; x = concentration standard (μg/mL) / concentration analyte (μg/mL).

**Table 2.** Concentrations of Major Odor-Active Compounds in Monthong Durian Pulp

odorant	conc <sup>a</sup> (μg/kg)	CV <sup>b</sup> (%)
acetaldehyde ( <b>16</b> )	98400	18
ethanethiol ( <b>17</b> )	45400 <sup>c</sup>	20 <sup>c</sup>
methanethiol ( <b>18</b> )	26700 <sup>c</sup>	13 <sup>c</sup>
ethyl (2 <i>S</i> )-2-methylbutanoate ( <b>5</b> )	13800	12
ethyl butanoate ( <b>3</b> )	8380	8
hydrogen sulfide ( <b>19</b> )	3340 <sup>c</sup>	6 <sup>c</sup>
propane-1-thiol ( <b>20</b> )	3070 <sup>c</sup>	22 <sup>c</sup>
ethane-1,1-dithiol ( <b>2</b> )	2940	16
ethyl 2-methylpropanoate ( <b>8</b> )	2000	8
methyl (2 <i>S</i> )-2-methylbutanoate ( <b>14</b> )	602	6
1-(ethylsulfanyl)ethanethiol ( <b>9</b> )	403	34
2(5)-ethyl-4-hydroxy-5(2)-methylfuran-3(2 <i>H</i> )-one ( <b>7</b> )	376	32
diethyl trisulfide ( <b>1</b> )	196	46
1-(methylsulfanyl)ethanethiol ( <b>15</b> )	176	9
4-hydroxy-2,5-dimethylfuran-3(2 <i>H</i> )-one ( <b>12</b> )	51	30
1-(ethyldisulfanyl)-1-(ethylsulfanyl)ethane ( <b>6</b> )	16	34
3-hydroxy-4,5-dimethylfuran-2(5 <i>H</i> )-one ( <b>11</b> )	13	25
1-(ethylsulfanyl)propane-1-thiol ( <b>10</b> )	7.7	20
ethyl cinnamate ( <b>4</b> )	4.5	20
3-methylbut-2-ene-1-thiol ( <b>13</b> )	0.9	37

<sup>a</sup>Mean of triplicates. <sup>b</sup>Coefficient of variation; calculated by dividing the standard deviation by the mean value. <sup>c</sup>Data taken from a previous publication.<sup>2</sup>

**Table 3.** Odor Thresholds and OAVs of Major Odor-Active Compounds in Monthong Durian Pulp

odorant	odor quality	odor threshold <sup>a</sup> (μg/kg)	OAV <sup>b</sup>
ethyl (2 <i>S</i> )-2-methylbutanoate ( <b>5</b> )	fruity	0.0080	1700000
ethanethiol ( <b>17</b> )	rotten onion	0.095 <sup>c</sup>	480000 <sup>c</sup>
1-(ethylsulfanyl)ethanethiol ( <b>9</b> )	roasted onion	0.0016	250000
methanethiol ( <b>18</b> )	rotten, cabbage	0.59 <sup>c</sup>	45000 <sup>c</sup>
ethane-1,1-dithiol ( <b>2</b> )	sulfury, durian	0.13	23000
ethyl 2-methylpropanoate ( <b>8</b> )	fruity	0.089	22000
methyl (2 <i>S</i> )-2-methylbutanoate ( <b>14</b> )	fruity	0.048	13000
ethyl butanoate ( <b>3</b> )	fruity	0.76	11000
propane-1-thiol ( <b>20</b> )	rotten, durian	0.49 <sup>c</sup>	6200 <sup>c</sup>
acetaldehyde ( <b>16</b> )	fresh, fruity	16	6100
1-(methylsulfanyl)ethanethiol ( <b>15</b> )	roasted onion	0.038	4600
1-(ethylsulfanyl)propane-1-thiol ( <b>10</b> )	roasted onion	0.0063	1200
3-methylbut-2-ene-1-thiol ( <b>13</b> )	skunky	0.00076	1200
diethyl trisulfide ( <b>1</b> )	fried shallot	0.20	980
1-(ethyldisulfanyl)-1-(ethylsulfanyl)ethane ( <b>6</b> )	sulfury, onion	0.018	890
hydrogen sulfide ( <b>19</b> )	rotten egg	10 <sup>c</sup>	330 <sup>c</sup>
2(5)-ethyl-4-hydroxy-5(2)-methylfuran-3(2 <i>H</i> )-one ( <b>7</b> )	caramel	17	22
3-hydroxy-4,5-dimethylfuran-2(5 <i>H</i> )-one ( <b>11</b> )	soup seasoning	1.7	7
ethyl cinnamate ( <b>4</b> )	honey	1.7	2
4-hydroxy-2,5-dimethylfuran-3(2 <i>H</i> )-one ( <b>12</b> )	caramel	54	1

<sup>a</sup>Orthonasal odor threshold in water. <sup>b</sup>Odor activity value, ratio of concentration (cf. Table 2) to odor threshold. <sup>c</sup>Data taken from a previous publication.<sup>2</sup>

**Table 4.** Omission Tests Applied to the Monthong Durian Aroma Model

test	odorant(s) omitted <sup>a</sup>	reference model <sup>a</sup>	correct answers/ panelists <sup>a</sup>	<i>p</i> value (%)	level of significance <sup>b</sup>
O1	10 odorants, OAV < 5000	20 odorants, OAV ≥ 1	6/18	59	—
O2	ethyl (2 <i>S</i> )-2-methylbutanoate ( <b>5</b> )	10 odorants, OAV > 5000	8/18	22	—
O3	ethanethiol ( <b>17</b> )	10 odorants, OAV > 5000	9/18	11	—
O4	1-(ethylsulfanyl)ethanethiol ( <b>9</b> )	10 odorants, OAV > 5000	9/18	11	—
O5	methanethiol ( <b>18</b> )	10 odorants, OAV > 5000	7/18	39	—
O6	ethane-1,1-dithiol ( <b>2</b> )	10 odorants, OAV > 5000	8/18	22	—
O7	ethyl 2-methylpropanoate ( <b>8</b> )	10 odorants, OAV > 5000	6/18	59	—
O8	methyl (2 <i>S</i> )-2-methylbutanoate ( <b>14</b> )	10 odorants, OAV > 5000	7/18	39	—
O9	ethyl butanoate ( <b>3</b> )	10 odorants, OAV > 5000	5/18	77	—
O10	propane-1-thiol ( <b>20</b> )	10 odorants, OAV > 5000	8/18	22	—
O11	acetaldehyde ( <b>16</b> )	10 odorants, OAV > 5000	7/18	39	—
O12	all except <b>5</b> and <b>9</b>	10 odorants, OAV > 5000	9/19	15	—
O13	all except <b>9</b>	10 odorants, OAV > 5000	17/19	< 0.1	***

<sup>a</sup>Number of correct answers resulting from the triangle test and total number of panelist participating. <sup>b</sup>—, not significant ( $p > 5\%$ ); \*, significant ( $5\% \geq p > 1\%$ ); \*\*, highly significant ( $1\% \geq p > 0.1\%$ ); \*\*\*, very highly significant ( $p \leq 0.1\%$ ).

Figure 1.

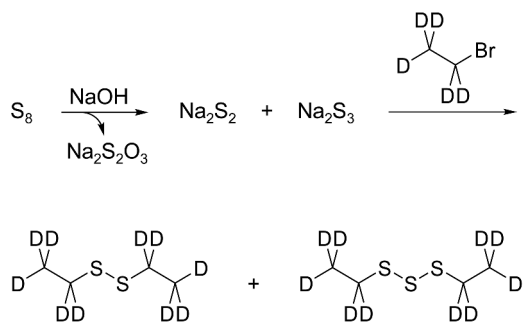
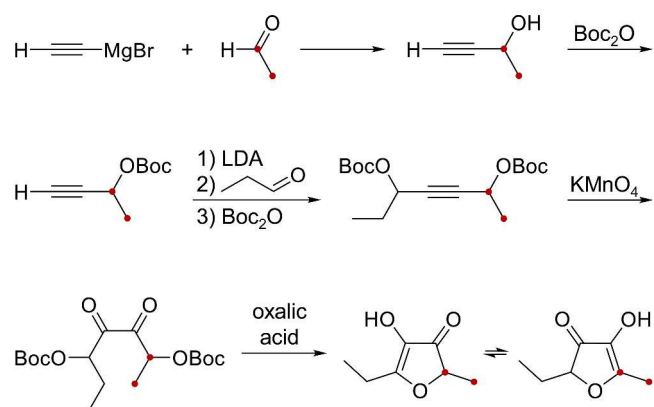
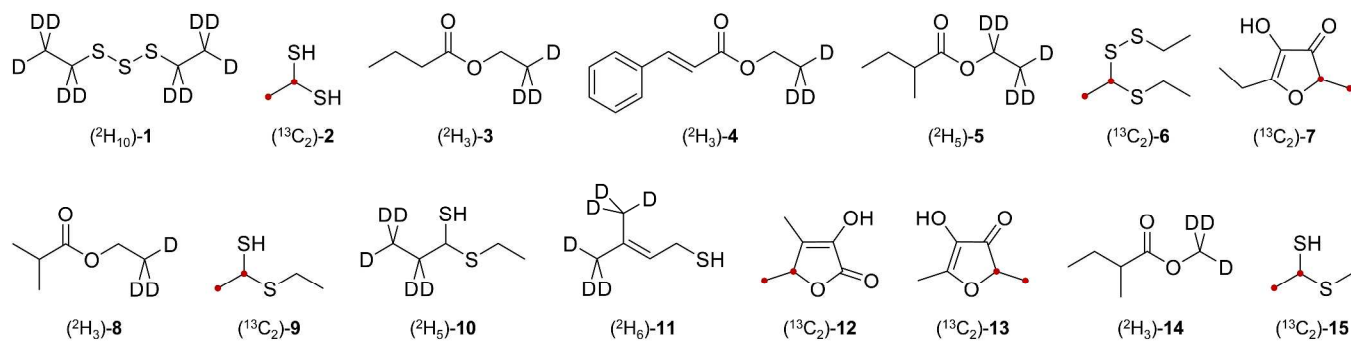
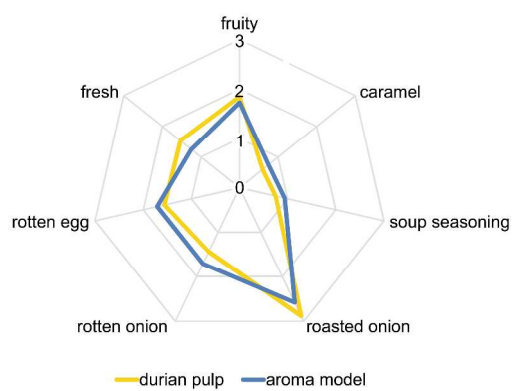




Figure 2.



**Figure 3.**

**Figure 4.**

TOC graphic.

