

Assessment of the Aroma Impact of Major Odor-Active Thiols in Pan-Roasted White Sesame Seeds by Calculation of Odor Activity Values

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ABSTRACT: Eleven odor-active thiols, namely, 2-methyl-1-propene-1-thiol, (*Z*)-3-methyl-1-butene-1-thiol, (*E*)-3-methyl-1-butene-1-thiol, (*Z*)-2-methyl-1-butene-1-thiol, (*E*)-2-methyl-1-butene-1-thiol, 2-methyl-3-furanthiol, 3-mercapto-2-pentanone, 2-mercapto-3-pentanone, 4-mercapto-3-hexanone, 3-mercapto-3-methylbutyl formate, and 2-methyl-3-thiophenethiol, recently identified in an extract prepared from white sesame seeds, were quantitated in sesame using stable isotope dilution analyses. For that purpose, the following deuterium-labeled compounds were synthesized and used as internal standards in the quantitation assays: [²H₆]-2-methyl-1-propene-1-thiol, [²H₃]-(*E*)- and [²H₃]-(*Z*)-2-methyl-1-butene-1-thiol, [²H₃]-2-methyl-3-furanthiol, [²H₂]-3-mercapto-2-pentanone, [²H₃]-4-mercapto-3-hexanone, [²H₆]-3-mercapto-3-methylbutyl formate, and [²H₃]-2-methyl-3-thiophenethiol. On the basis of the results obtained, odor activity values (OAVs) were calculated as ratio of the concentration and odor threshold of the individual compounds in cooking oil. According to their high OAVs, particularly the 3-methyl-1-butene-1-thiols (OAV: 2400) and the 2-methyl-1-butene-1-thiols (OAV: 960) were identified as the most odor-active compounds in pan-roasted white sesame seeds. These compounds were therefore suggested to be mainly responsible for the characteristic but rather unstable sulfury aroma of freshly pan-roasted white sesame seeds.

KEYWORDS: Roasted white sesame seeds, stable isotope dilution assay, odor activity value, 2-methyl-1-propene-1-thiol, 2-methyl-1-butene-1-thiol, 3-methyl-1-butene-1-thiol

INTRODUCTION

In the Western world, roasted sesame seeds prevalently serve as a topping for different bakery products. However, they are more widely used in Asian cuisines, where either the roasted seeds or the oil obtained from them are used as a common flavoring for a variety of dishes. In Japan, roasted sesame seeds are freshly ground at the table to ensure an unaltered fresh aroma characterized by pleasant roasty, nutty, and sulfurous notes.

In our previous study,^{1,2} the aroma-active compounds present in fresh pan-roasted white sesame seeds were identified by application of aroma extract dilution analysis (AEDA). A total of 32 odorants were detected in the flavor dilution factor range of 2–2048 of which 29 could be structurally assigned. Confirming earlier results,^{3–5} high flavor dilution factors were found for the coffeelike smelling 2-furfurylthiol, the caramel-like smelling 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone, the coffeelike smelling 2-thenylthiol, and the clovelike smelling 2-methoxy-4-vinylphenol. Nine odor-active compounds with sulfurous, meaty, and/or catty, black currantlike odors were previously unknown in roasted sesame. Among them, 2-methyl-1-propene-1-thiol, (*Z*)-3-methyl-1-butene-1-thiol, (*E*)-3-methyl-1-butene-1-thiol, (*Z*)-2-methyl-1-butene-1-thiol, (*E*)-2-methyl-1-butene-1-thiol, and 4-mercapto-3-hexanone were reported for the first time as natural aroma compounds. Moreover, the unstable 1-alkene-1-thiols represented a completely new class of food odorants.

The aim of the present study was to unequivocally evaluate the impact of the major aroma-active thiols on the characteristic but quickly vanishing sulfury aroma of freshly pan-roasted white

sesame seeds on the basis of odor activity values. For that purpose, isotopically labeled analogues of the target compounds were synthesized and used as internal standards in stable isotope dilution analysis (SIDA). Odor activity values of individual compounds were then calculated as the ratio of the concentrations obtained and their odor thresholds in oil.

MATERIALS AND METHODS

Materials. White sesame seeds (Egypt) were purchased in a local supermarket in Germany. The seeds (250 g) were washed with tap water (500 mL), filtered with a strainer, and then roasted in a frying pan with continuous stirring for 15 min at 200 °C. After they were frozen with liquid nitrogen, the seeds were ground in a commercial blender.

Reference Odorants. The following compounds were obtained from commercial sources: 2-methyl-3-furanthiol (Acros Organics, Geel, Belgium) and 3-mercapto-2-pentanone (Alfa Aesar, Karlsruhe, Germany). 3-Mercapto-3-methylbutyl formate⁶ and 2-methyl-3-thiophenethiol⁷ were synthesized according to the literature cited. 2-Methyl-1-propene-1-thiol, (*E*)-/(*Z*)-2-methyl-1-butene-1-thiol, (*E*)-/(*Z*)-3-methyl-1-butene-1-thiol, and 4-mercapto-3-hexanone were synthesized as reported previously.¹

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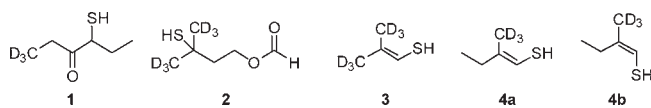


Figure 1. Deuterium-labeled thiols prepared by synthesis: $[^2\text{H}_3]$ -4-mercapto-3-hexanone (1), $[^2\text{H}_6]$ -3-mercapto-3-methylbutyl formate (2), $[^2\text{H}_6]$ -2-methyl-1-propene-1-thiol (3), $[^2\text{H}_3]$ -(*E*)-2-methyl-1-butene-1-thiol (4a), and $[^2\text{H}_3]$ -(*Z*)-2-methyl-1-butene-1-thiol (4b).

Syntheses. Reference Odorants. 2-Mercapto-3-pentanone.

The compound was synthesized in a three-step procedure starting from 3-pentanone.

(a). *2-Bromo-3-pentanone*. 3-Pentanone (13.0 g, 151 mmol) was added to a solution of potassium chlorate (2.2 g, 18.0 mmol) in water (150 mL) at room temperature. The reaction mixture was warmed to 50 °C, and then, bromine (37.3 g, 233 mmol) was added dropwise at 45–50 °C within 10 min. After it was stirred at 45–50 °C for 3 h, the reaction mixture was cooled to room temperature, and poured into 10% aqueous sodium thiosulfate (500 mL). The aqueous solution was extracted with diethyl ether (500 mL \times 2). The organic layers were combined, washed consecutively with 10% aqueous sodium thiosulfate, saturated aqueous NaHCO_3 , and finally brine, and dried over MgSO_4 . The organic solution was filtered and concentrated under reduced pressure to yield crude 2-bromo-3-pentanone (32 g), which was directly used in the next step.

(b). *2-Acetylthio-3-pentanone*. A solution of the crude 2-bromo-3-pentanone (32 g) in *N,N*-dimethylformamide (DMF, 80 mL) was added dropwise to a solution of potassium thioacetate (24.4 g, 213 mmol) in DMF (500 mL) at 35–40 °C within 20 min. After it was stirred for 3 h at 35–40 °C, the reaction mixture was extracted with diethyl ether (500 mL \times 2). The organic layer was washed with saturated aqueous NaHCO_3 and brine and then dried over MgSO_4 . The organic solution was filtered and concentrated under reduced pressure to yield crude 2-acetylthio-3-pentanone (20 g), which was directly used in the next step.

(c). *Target Compound*. To obtain the target compound, the crude 2-acetylthio-3-pentanone (20 g) was added to 5% aqueous NaOH (360 g) at room temperature. After it was stirred for 2 h at room temperature, the reaction mixture was washed with diethyl ether (300 mL \times 2). The aqueous layer was acidified with 20% aqueous citric acid (pH 5) and extracted with diethyl ether (300 mL \times 2). The organic layer was washed with brine and dried over MgSO_4 . The solution was filtered and concentrated under reduced pressure. The residue (10 g) was distilled (bp \sim 30 °C/3 kPa) to yield crude 2-mercapto-3-pentanone (0.5 g; purity, 75%). The distillate (0.5 g) was dissolved in 5% aqueous NaOH (10 mL) and then washed with diethyl ether (20 mL \times 2). The aqueous layer was acidified with 20% aqueous citric acid (pH 5) and extracted with diethyl ether (20 mL \times 2). The organic layer was washed with brine and dried over MgSO_4 . The organic solution was filtered and concentrated under reduced pressure. The residue (0.3 g) was distilled (bp \sim 30 °C/2.6 kPa) into a colorless oil of 2-mercapto-3-pentanone (50 mg; yield, 0.3%; purity, 96%). The compound was characterized by comparing the MS-EI data with results reported previously.⁸

MS-EI, m/z (%): 118 (M^+ , 20), 90 (4), 61 (53), 60 (8), 59 (7), 58 (6), 57 (100), 56 (5), 45 (5), 35 (5), 29 (37).

Deuterium-Labeled Odorants. The structures of the newly synthesized compounds are given in Figure 1.

$[^2\text{H}_3]$ -4-Mercapto-3-hexanone (1). The compound was synthesized in a six-step procedure starting from $[^2\text{H}_3]$ -bromoethane (Figure 2).

(a). $[^2\text{H}_3]$ -2-Ethyl-1,3-dithiane. Under an atmosphere of argon, *n*-butyl lithium in hexane (15.4 mL, 1.59 mol/L, 24.5 mmol) was added dropwise to a solution of 1,3-dithiane (2.9 g, 24.5 mmol) in THF (25 mL) at -30 °C for 10 min. After it was stirred for 2 h at -30 °C, a solution of $[^2\text{H}_3]$ -bromoethane (2.5 g, 22.3 mmol) in THF (5 mL) was dropwise added at -20 °C for 5 min. After it was stirred overnight at 0 °C in a refrigerator, the reaction mixture was warmed to room temperature and poured into ice water (50 mL). The aqueous solution was extracted with diethyl ether (50 mL \times 3). The organic layer was washed with brine and dried over MgSO_4 . The solution was filtered and concentrated under high vacuum. The residue (4.2 g) was distilled (bp 71–72 °C/0.5 kPa) into an oil of $[^2\text{H}_3]$ -ethyl-1,3-dithiane (3.1 g; yield, 91%; purity, 86%). MS-EI, m/z (%): 151 (M^+ , 47), 121 (9), 120 (7), 119 (100), 77 (12), 76 (7), 75 (7), 74 (13), 73 (7), 59 (5), 58 (5), 47 (6), 46 (15), 45 (21), 44 (7), 43 (9), 41 (12).

(b). $[^2\text{H}_3]$ -1-(2-Ethyl-1,3-dithian-2-yl)propan-1-ol. Under an atmosphere of argon, *n*-butyl lithium in hexane (13.8 mL, 1.59 mol/L, 22.0 mmol) was added dropwise to a solution of $[^2\text{H}_3]$ -ethyl-1,3-dithiane (3.1 g, 20.0 mmol) in THF (100 mL) at -30 °C for 10 min. After it was stirred for 2 h at -30 °C, a solution of propanal (1.7 g, 30.0 mmol) in THF (5 mL) was dropwise added at -20 °C within 5 min. After it was stirred overnight at 0 °C in a refrigerator, the reaction mixture was warmed to room temperature and poured into ice water (50 mL). The aqueous solution was extracted with diethyl ether (100 mL \times 3). The organic layers were combined, washed with brine, and dried over MgSO_4 . The organic solution was filtered and concentrated under high vacuum. The residue (4.6 g) was purified by silica gel (150 g) chromatography using hexane/ethyl acetate (15:1, v/v) as the eluent. The fractions containing the target compound were combined and concentrated under high vacuum into an oil of $[^2\text{H}_3]$ -1-(2-ethyl-1,3-dithia-2-yl)propan-1-ol (2.1 g; yield, 49%; purity, 97%). MS-EI, m/z (%): 209 (M^+ , 1), 191 (14), 152 (9), 151 (9), 150 (100), 108 (5), 106 (37), 76 (16), 75 (6), 73 (9), 59 (5), 57 (5), 46 (7), 45 (12), 41 (14).

(c). $[^2\text{H}_3]$ -4-Hydroxy-3-hexanone. Under a nitrogen atmosphere, HgCl_2 (5.4 g, 20.0 mmol), CdCO_3 (3.4 g, 20.0 mmol), and $[^2\text{H}_3]$ -1-(2-ethyl-1,3-dithian-2-yl)propan-1-ol (2.1 g, 9.8 mmol) were dissolved in water/acetonitrile (1:10, v/v, 110 mL). After it was stirred for 2 h at 50–60 °C, the reaction mixture was cooled to room temperature, diluted with diethyl ether (500 mL), and filtered through Celite. The filtrate was washed with brine and dried over MgSO_4 . The solution was filtered and concentrated under high vacuum. The residue (1.0 g) was purified by silica gel (45 g) chromatography using hexane/ethyl acetate (10:1–3:1, v/v) as the eluent. The fractions containing the target compound were combined and concentrated under high vacuum into an oil of $[^2\text{H}_3]$ -4-hydroxy-3-hexanone (0.6 g; yield, 51%; purity, 94%). MS-EI, m/z (%): 119 (M^+ , 2), 62 (23), 60 (41), 59 (100), 58 (23), 57 (13), 41 (20), 32 (24), 31 (34), 29 (14).

(d). $[^2\text{H}_3]$ -4-Methanesulfonyloxy-3-hexanone. The compound was prepared by reaction of $[^2\text{H}_3]$ -4-hydroxy-3-hexanone and methanesulfonyl chloride, according to the synthesis of 4-methanesulfonyloxy-3-hexanone reported in our previous study.¹ The crude product (0.51 g; purity, 76%) was directly used in the next step. MS-EI, m/z (%): 139 (5), 137 (5), 79 (11), 69 (4), 60 (100), 59 (12), 57 (4), 41 (7), 32 (21), 29 (6).

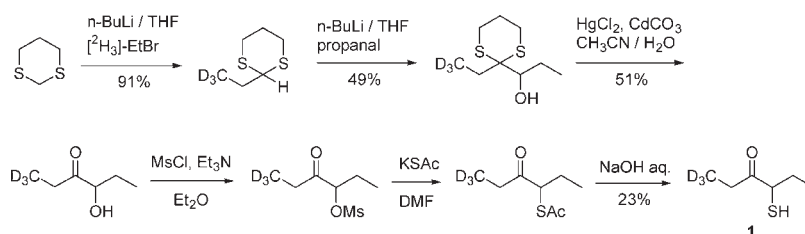


Figure 2. Synthetic approach used in the preparation of [$^2\text{H}_3$]-4-mercapto-3-hexanone (1).

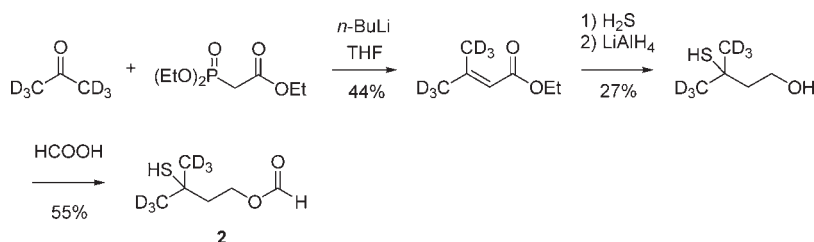


Figure 3. Synthetic approach used in the preparation of [$^2\text{H}_6$]-3-mercapto-3-methylbutyl formate (2).

(e). [$^2\text{H}_3$]-4-Acetylthio-3-hexanone. The compound was prepared by a treatment of the crude [$^2\text{H}_3$]-4-methanesulfonyloxy-3-hexanone with potassium thioacetate, according to the synthesis of 4-acetylthio-3-hexanone reported in our previous study¹ (yield, 0.53 g). The crude product was directly used in the next step. MS-EI, m/z (%): 177 (M^+ , 4), 135 (13), 134 (20), 117 (18), 102 (5), 75 (24), 74 (5), 60 (100), 55 (5), 45 (6), 43 (95), 41 (11), 39 (5), 32 (24).

(f). [$^2\text{H}_3$]-4-Mercapto-3-hexanone. The compound was prepared by treatment of the crude [$^2\text{H}_3$]-4-acetylthio-3-hexanone with aqueous NaOH, according to the synthesis of 4-mercapto-3-hexanone reported in our previous study¹ (156 mg; yield, 23%; purity, 97%). ^1H NMR: δ 0.96 (dd, $J = 7.2, 7.2$ Hz, 3H), 1.67 (ddq, $J = 7.2, 7.2, 14.4$ Hz, 1H), 1.68 (d, $J = 10.0$ Hz, 1H), 1.92 (ddq, $J = 7.2, 7.2, 14.4$ Hz, 1H), 2.48 (d, $J = 18.0$ Hz, 1H), 2.70 (d, $J = 18.0$ Hz, 1H), 3.21 (ddd, $J = 7.2, 7.2, 10.0$ Hz, 1H). ^{13}C NMR: δ 7.4, 11.9, 27.7, 33.1, 48.6, 208.9. MS-EI, m/z (%): 135 (M^+ , 13), 75 (35), 74 (27), 60 (100), 55 (5), 47 (18), 45 (6), 43 (5), 41 (33), 39 (9), 32 (34), 29 (6).

[$^2\text{H}_6$]-3-Mercapto-3-methylbutyl Formate (2). The compound was prepared in a four-step synthesis starting from [$^2\text{H}_6$]-acetone (Figure 3).

(a). [$^2\text{H}_6$]-Ethyl 3-Methyl-2-butenolate. Under an atmosphere of argon, *n*-butyl lithium in hexane (76 mL, 1.59 mol/L, 120.9 mmol) was added dropwise to a solution of diethylphosphonoacetic acid ethyl ester (40.63 g, 181.4 mmol) in THF (730 mL) at 0 °C within 20 min. After it was stirred for 20 min at 0 °C, a solution of [$^2\text{H}_6$]-acetone (7.74 g, 120.9 mmol) in THF (40 mL) was dropwise added at 0 °C for 10 min. After it was stirred for 1.5 h at 0 °C, the reaction mixture was warmed up to room temperature, stirred overnight, and poured into water (800 mL). The aqueous solution was extracted with diethyl ether (800 mL \times 2). The organic layer was washed with brine and dried over MgSO_4 . The solution was filtered and concentrated under reduced pressure. The residue (103 g) was distilled (bp 48–50 °C/2.1 kPa) into a colorless oil of [$^2\text{H}_6$]-ethyl 3-methyl-2-butenolate (7.08 g; yield, 44%; purity, 98%). MS-EI, m/z (%): 134 (M^+ , 34), 106 (17), 89 (100), 88 (19), 87 (11), 86 (9), 61 (24), 42 (4), 41(4).

(b). [$^2\text{H}_6$]-Ethyl 3-Mercapto-3-methylbutyrate. Diazabicyclo[5.4.0]undec-7-ene (DBU, 0.7 g, 4.6 mmol) and hydroquinone (0.25 g, 2.3 mmol) were added to a solution of [$^2\text{H}_6$]-ethyl 3-methyl-2-butenolate (6.27 g, 46.8 mmol) in dimethyl sulfoxide (DMSO, 13 g) at 0 °C. Hydrogen sulfide was passed through the solution at a rate of 50 mL/min for 2 h at 0 °C. The reaction mixture was then warmed up to 40 °C, stirred overnight, and poured into aqueous HCl (1 mol/L; 100 mL) at 10 °C. The aqueous solution was extracted with diethyl ether (100 mL \times 2). The organic layer was washed with saturated aqueous NaHCO_3 and brine and dried over Na_2SO_4 . The solution was filtered and concentrated under reduced pressure into a crude oil of [$^2\text{H}_6$]-ethyl 3-mercapto-3-methylbutyrate (8.83 g; purity, 69%). The crude product was directly used in the next step. MS-EI, m/z (%): 168 (M^+ , 47), 135 (50), 123 (20), 95 (24), 93 (100), 88 (15), 81 (77), 80 (16), 65 (64), 63 (19), 62 (62), 61 (18), 60 (36), 46 (23), 45 (16), 44 (20), 29 (38).

(c). [$^2\text{H}_6$]-3-Mercapto-3-methylbutanol. Under an atmosphere of argon, the crude [$^2\text{H}_6$]-ethyl 3-mercapto-3-methylbutyrate (8.83 g, 52.6 mmol) in diethyl ether (50 mL) was added dropwise to a suspension of lithium aluminum hydride (2.13 g, 55.9 mmol) in diethyl ether (150 mL) at 0 °C within 30 min. After the mixture was stirred for 2 h at 0 °C, water (11 mL) was added dropwise to the reaction mixture at 0 °C for 5 min. After it was stirred for 40 min at 10 °C, aqueous HCl (2 mol/L; 250 mL) was added. The organic layer was separated, and the aqueous layer was extracted with diethyl ether (125 mL \times 2). The organic layer was dried over Na_2SO_4 , filtered, and concentrated under reduced pressure. The residue (7.07 g) was distilled (bp 52 °C/5 kPa) into a colorless oil of [$^2\text{H}_6$]-3-mercapto-3-methylbutanol (1.57 g; 2 steps; yield, 27%; purity, 99%). MS-EI, m/z (%): 126 (M^+ , 8), 93 (16), 92 (54), 81 (26), 74 (100), 73 (62), 63 (13), 62 (16), 60 (10), 49 (13), 46 (21), 45 (16), 44 (27), 43 (13).

(d). [$^2\text{H}_6$]-3-Mercapto-3-methylbutyl Formate. [$^2\text{H}_6$]-3-mercapto-3-methylbutanol (0.88 g, 7.0 mmol) and formic acid (3.21 g, 69.8 mmol) were mixed at room temperature. After it was stirred for 5 h at room temperature, the reaction mixture was diluted with diethyl ether (100 mL), washed with water and brine, and dried over Na_2SO_4 . The solution was filtered and

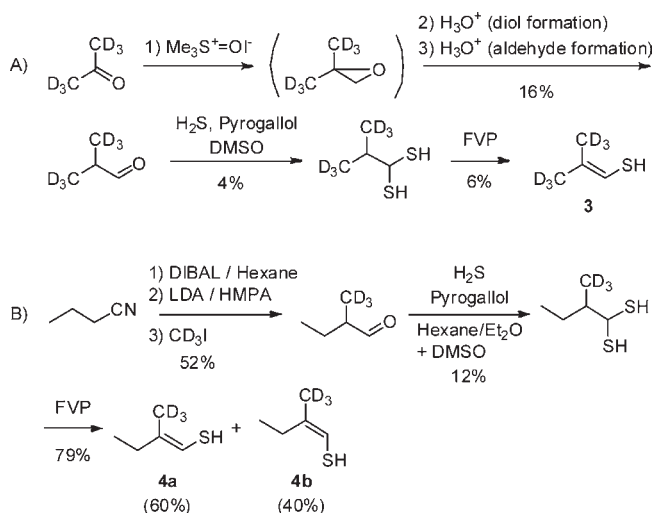


Figure 4. Synthetic approach used in the preparation of (A) $[^2\text{H}_6]$ -2-methyl-1-propene-1-thiol (**3**) and (B) $[^2\text{H}_3]$ -(E)-2-methyl-1-butene-1-thiol (**4a**) and $[^2\text{H}_3]$ -(Z)-2-methyl-1-butene-1-thiol (**4b**).

concentrated under reduced pressure. The residue (7.93 g) was distilled (bp 30–35 °C/3 kPa) into a colorless oil of $[^2\text{H}_6]$ -3-mercapto-3-methylbutyl formate (0.59 g; yield, 55%; purity, 98%). ^1H NMR: δ 1.73 (s, 1H), 1.97 (t, $J = 7.2$ Hz, 2H), 4.38 (t, $J = 7.2$ Hz, 2H), 8.05 (s, 1H). ^{13}C NMR: δ 32.1, 42.4, 44.1, 61.5, 160.9. MS-EI, m/z (%): 154 (M^+ , 3), 108 (36), 90 (7), 81 (17), 75 (100), 74 (20), 62 (9), 60 (4), 46 (9), 45 (11), 44 (15), 43 (6), 31 (4), 29 (5).

$[^2\text{H}_6]$ -2-Methyl-1-propene-1-thiol (**3**). The compound was prepared in a three-step synthesis starting from $[^2\text{H}_6]$ -acetone (Figure 4A).

(a). $[^2\text{H}_6]$ -2-Methylpropanal. Under an atmosphere of nitrogen, sodium hydride (22.0 g, 550 mmol) was suspended in DMSO (500 mL) at room temperature. Then, trimethylsulfoxonium iodide (121.0 g, 550 mmol) was added to the solution within 40 min keeping the temperature under 25 °C, and then, $[^2\text{H}_6]$ -acetone (32.1 g, 500 mmol) was added to the reaction mixture within 5 min at room temperature. After it was stirred for 2 h, the reaction mixture was poured into ice water (750 g) and extracted with diethyl ether (1 L \times 2). The organic layer was dried over Na_2SO_4 and filtered (ca. 500 mL). A solution of citric acid monohydrate (3 g) in water (27 g) was added, and after it was stirred the mixture overnight at room temperature, Na_2CO_3 (1.8 g) was added to adjust the pH to 6–7. The organic layer was separated and concentrated under atmospheric pressure. The residue (13.6 g) was combined with the water layer and added to H_2SO_4 (5% in water, 300 g). This mixture was then submitted to steam distillation. The distillate was extracted with diethyl ether (300 mL \times 2). The extract was dried over Na_2SO_4 , filtered, and distilled under atmospheric pressure (bp 60 °C) to yield an oil of $[^2\text{H}_6]$ -2-methylpropanal (6.2 g; yield, 16%; purity, 92%). MS-EI, m/z (%): 78 (M^+ , 42), 50 (12), 49 (100), 48 (14), 46 (35), 45 (49), 42 (15), 41 (9), 32 (11), 30 (20), 29 (25).

(b). $[^2\text{H}_6]$ -2-Methylpropane-1,1-dithiol. $[^2\text{H}_3]$ -2-Methylpropanal was treated with H_2S using the method previously reported for the synthesis of the unlabeled 2-methylpropane-1,1-dithiol¹ (yield, 4%; purity, 95%). MS-EI, m/z (%): 128 (M^+ , 11), 95 (62), 94 (59), 79 (20), 76 (18), 62 (35), 61 (61), 60 (100), 59 (16), 49 (27), 48 (16), 46 (45), 45 (56), 42 (22), 41 (19), 34 (35), 33 (23), 32 (18).

(c). $[^2\text{H}_6]$ -2-Methyl-1-propene-1-thiol. The compound was prepared by flash vacuum pyrolysis (FVP)^{9,10} of $[^2\text{H}_3]$ -2-methylpropane-1,1-dithiol using the parameters previously reported for the synthesis of the unlabeled 2-methyl-1-propene-1-thiol¹ (yield, 6%). MS-EI, m/z (%): 95 (21), 94 (M^+ , 100), 76 (23), 62 (32), 61 (74), 60 (57), 58 (20), 57 (20), 49 (17), 46 (50), 45 (35), 42 (35), 41 (22), 33 (17).

$[^2\text{H}_3]$ -(E)-2-Methyl-1-butene-1-thiol (**4a**) and $[^2\text{H}_3]$ -(Z)-2-Methyl-1-butene-1-thiol (**4b**). The compounds were prepared in a three-step synthesis starting from butyronitrile (Figure 4B).

(a). $[^2\text{H}_3]$ -2-Methylbutanal. The compound was synthesized following a previous procedure.¹¹ Under N_2 atmosphere, diisobutylaluminum hydride (DIBAL) in hexane (200 mL, 1 mol/L, 200 mmol) was added to a solution of butyronitrile (13.8 g, 200 mmol) in diethyl ether (50 mL) at -10 °C, and the reaction mixture was stirred for 1 h. In the meantime, lithium diisopropylamide (LDA) was prepared as follows: Under an atmosphere of nitrogen, *n*-butyl lithium in hexane (127 mL, 1.58 mol/L, 200 mmol) was added to a solution of diisopropylamine (20.2 g, 200 mmol) in diethyl ether (270 mL) at -70 °C, and the solution was warmed to -30 °C and stirred for 30 min. This solution was then added to the main reaction mixture at -10 °C within 30 min. Hexamethylphosphoramide (45 g, 251 mmol) was added slowly to the reaction mixture at -5 °C, which was then warmed to 20 °C and stirred for 1 h. Then, $[^2\text{H}_3]$ -iodomethane (31.2 g, 220 mmol) was added to the reaction mixture at -10 °C. After it was stirred overnight, the reaction mixture was warmed up to 40 °C, followed by further stirring for 1 h. Then, H_2SO_4 (20% in water, 400 g) was added to the reaction mixture at 5 °C. The reaction mixture was heated to 100 °C, and the crude product was distilled off together with the water. The organic layer was separated, dried over anhydrous Na_2SO_4 , and filtered to yield a solution of crude $[^2\text{H}_3]$ -2-methylbutanal (ca. 500 mL). This crude solution was directly used in the next step. MS-EI, m/z (%): 89 (M^+ , 8), 61 (40), 60 (100), 59 (44), 58 (10), 44 (22), 43 (54), 42 (35), 41 (20), 39 (13), 32 (18), 31 (31), 30 (27), 29 (29).

(b). $[^2\text{H}_3]$ -2-Methylbutane-1,1-dithiol. The crude solution of $[^2\text{H}_3]$ -2-methylbutanal was treated with H_2S using the method previously reported for the synthesis of the unlabeled 2-methylbutane-1,1-dithiol¹ (yield, 6%; purity, 84%). MS-EI, m/z (%): 139 (M^+ , 19), 106 (100), 105 (51), 79 (37), 72 (84), 71 (33), 64 (37), 61 (27), 60 (29), 47 (27), 46 (24), 45 (62), 44 (30), 43 (50), 42 (38), 41 (25), 34 (23).

(c). $[^2\text{H}_3]$ -(E)-2-Methyl-1-butene-1-thiol and $[^2\text{H}_3]$ -(Z)-2-Methyl-1-butene-1-thiol. The compounds were prepared by FVP of $[^2\text{H}_3]$ -2-methylbutane-1,1-dithiol using the parameters previously reported for the synthesis of the unlabeled (E)/(Z)-2-methyl-1-butene-1-thiol¹ (yield, 79%; purity, 90%). The (E)/(Z)-ratio was determined by GC to be 60:40. The correct assignment of the GC peaks was achieved by comparing their retention times with those of (E)- and (Z)-2-methyl-1-butene-1-thiol. ^1H NMR (E-isomer): δ 0.98 (t, $J = 7.6$ Hz, 3H), 2.05 (q, $J = 7.6$ Hz, 2H), 2.51 (d, $J = 7.2$ Hz, 1H), 5.69 (d, $J = 7.2$ Hz, 1H). ^1H NMR (Z-isomer): δ 0.98 (t, $J = 7.6$ Hz, 3H), 2.14 (q, $J = 7.6$ Hz, 2H), 2.48 (d, $J = 7.2$ Hz, 1H), 5.65 (d, $J = 7.2$ Hz, 1H). ^{13}C NMR (E-isomer): δ 12.4, 32.1, 106.4, 140.6. ^{13}C NMR (Z-isomer): δ 11.5, 25.8, 106.6, 141.1. MS-EI (E-isomer), m/z (%): 105 (M^+ , 100), 90 (54), 72 (70), 71 (22), 70 (21), 55 (31), 46 (25), 45 (53), 44 (23), 43 (43), 42 (36), 41 (23). MS-EI (Z-isomer), m/z (%): 105 (M^+ , 100), 90 (51), 72 (72), 71 (23), 70 (23), 55 (31), 46 (25), 45 (53), 44 (24), 43 (45), 42 (37), 41 (24).

Table 1. Selected Ions Used for the Stable Isotope Dilution Assays of Major Aroma-Active Thiols in Pan-Roasted White Sesame Seeds

odorant	labeled odorant	selected ion (<i>m/z</i>)	
		unlabeled odorant	labeled odorant
2-methyl-1-propene-1-thiol	[² H ₆]-2-methyl-1-propene-1-thiol	89	95
(<i>Z</i>)-3-methyl-1-butene-1-thiol	[² H ₃]-(<i>E</i>)-2-methyl-1-butene-1-thiol	103	106
(<i>E</i>)-3-methyl-1-butene-1-thiol	[² H ₃]-(<i>E</i>)-2-methyl-1-butene-1-thiol	103	106
(<i>Z</i>)-2-methyl-1-butene-1-thiol	[² H ₃]-(<i>Z</i>)-2-methyl-1-butene-1-thiol	103	106
(<i>E</i>)-2-methyl-1-butene-1-thiol	[² H ₃]-(<i>E</i>)-2-methyl-1-butene-1-thiol	103	106
2-methyl-3-furanthiol	[² H ₃]-2-methyl-3-furanthiol	115	118
3-mercapto-2-pentanone	[² H ₂]-3-mercapto-2-pentanone	119	121
2-mercapto-3-pentanone ^b	[² H ₂]-3-mercapto-2-pentanone	119	121
4-mercapto-3-hexanone	[² H ₃]-4-mercapto-3-hexanone	133	136
3-mercapto-3-methylbutyl formate	[² H ₆]-3-mercapto-3-methylbutyl formate	103	109
2-methyl-3-thiophenethiol	[² H ₃]-2-methyl-3-thiophenethiol	131	134

[²H₃]-2-Methyl-3-thiophenethiol. The compound was synthesized following a procedure published for the synthesis of [²H₃]-2-methyl-3-furanthiol¹² but using 3-bromothiophene instead of 3-bromofuran as the starting material. ¹H NMR: δ 3.01 (s, 1H), 6.87 (d, *J* = 5.2 Hz, 1H), 7.04 (d, *J* = 5.2 Hz, 1H). MS-EI, *m/z* (%): 135 (9), 134 (9), 133 (M⁺, 100), 132 (22), 131 (22), 100 (36), 99 (26), 88 (8), 70 (11), 69 (10), 62 (17), 56 (7), 46 (11), 45 (13).

[²H₃]-2-methyl-3-furanthiol and [²H₂]-3-mercapto-2-pentanone were synthesized as reported in ref 12.

Quantitation of Thiols by SIDAs. Ground roasted white sesame seeds (10–350 g), prepared as described above, were suspended in dichloromethane (15–500 mL), and the labeled internal standards (0.1–10 μg) were added in dichloromethane solution. Amounts of sample and of internal standards were adjusted according to preliminary experiments to yield appropriate responses during mass chromatography and response ratios of analytes and respective standards in the range of 1:3 to 3:1. After it was stirred at room temperature for 1 h, the mixture was filtered, and the residue was twice extracted for 30 min with dichloromethane (10–350 mL). The organic phases were combined, and nonvolatile material was removed by solvent-assisted flavor evaporation (SAFE).¹³ The thiol fraction was then isolated by affinity chromatography on mercurated agarose gel¹⁴ and concentrated to 200 μL at 45 °C using a Vigreux column (60 cm × 1 cm).

Mass chromatography was accomplished by injecting the concentrate into a GC/GC-MS system with a heart-cut interface. This system consisted of a Trace GC Ultra (Thermo Scientific, Dreieich, Germany) equipped with a 30 m × 0.32 mm i.d. × 0.25 μm film thickness DB-FFAP column (J&W Scientific, Agilent, Waldbronn, Germany), a CP-3800 gas chromatograph (Varian, Darmstadt, Germany) equipped with a 30 m × 0.32 mm i.d. × 0.25 μm film thickness DB-1701 column (J&W Scientific), and a COMBI PAL autosampler (CTC Analytics, Zwingen, Switzerland). The first GC housed a moving column stream switching system (MCSS) leading the effluent of the first column either in equal parts to an FID and a sniffing port or via a deactivated fused silica transfer line to the column in the second oven. The end of the second column was connected to the MS Saturn 2200 (Varian), running in the chemical ionization (CI) mode with methanol as reactant gas (ionization energy, 115 eV). To obtain mass spectra of the odorant and the internal standard, at its elution time from the first column, which was determined

by gas chromatography-olfactometry (GC-O) in a preliminary run, the MCSS was switched to the transfer line. While collecting the effluent of the first column, a trap section of the transfer line located in the second oven was cooled. This was achieved by a stream of cold nitrogen gas, after being passed through liquid nitrogen. After collection was finished, cooling was stopped, and the second oven was started. Temperature programs were 40 °C for 2 min, then 6 °C/min to 230 °C for the FFAP column, and 240 °C for the 1701 column. Mass chromatograms were recorded, and concentrations of the analytes were calculated from the area counts of specific ions of labeled standards and analytes (Table 1), amounts of sesame seeds, and amounts of standards added, and corrected according to response factors determined from defined mixtures of labeled and unlabeled compounds.

Determination of Odor Thresholds. Reference compounds were checked for odor-active impurities by GC-O prior to use and, if necessary, purified by distillation. The odorant was dissolved in sunflower oil in a 100-fold higher concentration than the estimated orthonasal recognition threshold. The stock solution was vigorously shaken and was then diluted stepwise 1:5 (w/w) with sunflower oil. Each test sample was orthonasally evaluated in a triangular test with two blank samples of pure sunflower oil. The samples were presented with increasing concentrations of the odorant. The sensory experiments were performed at 23 °C in a sensory room with single booths by the sensory panel of the Technical Research Institute, R&D Center of T. Hasegawa Co., Ltd. (Kawasaki, Japan). The panel was trained to recognize over 100 aroma chemicals. Individual sensory tests were performed by at least 12 assessors, male and female, in the age of 25–60 years. All panelists gave informed consent to participate in the sensory tests of the present investigation. Odor thresholds were calculated according to the method reported previously.¹⁵

Mass Spectrometric Characterization of Synthesized Products. Mass spectra of the synthesized reference odorants and the intermediates were obtained using an Agilent 6890 gas chromatograph connected to a 5973 mass selective detector and equipped with a 60 m × 0.25 mm i.d. × 0.25 μm film thickness TC-1 capillary column (GL Sciences Co., Tokyo, Japan). Samples were applied in the split mode (1:50) at 250 °C, and helium at a flow rate of 1.8 mL/min served as the carrier gas. The initial oven temperature was 40 °C. It was held for 2 min, and then, the temperature was raised at 3 °C per min to

Table 2. OAVs of Major Aroma-Active Thiols in Pan-Roasted White Sesame Seeds

compd	odor quality	concentration ^a ($\mu\text{g}/\text{kg}$)	RSD ^b (%)	odor threshold ($\mu\text{g}/\text{kg}$)	OAV
2-methyl-1-propene-1-thiol	sulfurous, meaty	800	13	30	27
3-methyl-1-butene-1-thiol	sulfurous, meaty	970 ^c	13	0.40 ^d	2400
2-methyl-1-butene-1-thiol	sulfurous, meaty	1200 ^c	28	1.25 ^f	960
2-methyl-3-furanthiol	meaty	100	21	0.56 ^g	180
3-mercapto-2-pentanone	catty, black currantlike	29	11	0.19 ^g	150
2-mercapto-3-pentanone	catty, black currantlike	170	2	0.60	280
4-mercapto-3-hexanone	catty, black currantlike	4.7	3	0.07	65
3-mercapto-3-methylbutyl formate	sulfurous, catty	0.011	43	0.17	$\ll 1$
2-methyl-3-thiophenethiol	meaty, sulfurous	11	39	2200	$\ll 1$

^a Mean values of triplicates. ^b Relative standard deviation of the concentration values. ^c Sum of isomers: 29% (*E*)- and 71% (*Z*)-isomer. ^d Odor threshold of a mixture of the (*E*)- and (*Z*)-isomer: 55% (*E*)- and 45% (*Z*)-isomer. ^e Sum of isomers: 66% (*E*)- and 34% (*Z*)-isomer. ^f Odor threshold of a mixture of the (*E*)- and (*Z*)-isomer: 60% (*E*)- and 40% (*Z*)-isomer. ^g Odor threshold published in ref 17.

a final temperature of 280 °C. Mass spectra were generated in the MS-EI mode at 70 eV.

NMR Spectra. ¹H and ¹³C NMR spectra were recorded in CDCl₃ by means of a JEOL JNM-LA400 spectrometer (JEOL Ltd., Tokyo, Japan) at 400 and 100 MHz, respectively. The chemical shift was recorded in parts per million (ppm) using tetramethylsilane as the internal standard ($\delta = 0.00$ ppm). Coupling constants *J* are denoted in Hertz.

RESULTS AND DISCUSSION

Development of SIDA for the Quantitation of Major Aroma-Active Thiols in Pan-Roasted White Sesame Seeds. To obtain exact concentrations and to compensate for losses occurring during workup procedures, SIDAs were developed for the quantitation of the following 11 major aroma-active thiols in pan-roasted white sesame seeds: 2-methyl-1-propene-1-thiol, (*Z*)-3-methyl-1-butene-1-thiol, (*E*)-3-methyl-1-butene-1-thiol, (*Z*)-2-methyl-1-butene-1-thiol, (*E*)-2-methyl-1-butene-1-thiol, 2-methyl-3-furanthiol, 3-mercapto-2-pentanone, 2-mercapto-3-pentanone, 4-mercapto-3-hexanone, 3-mercapto-3-methylbutyl formate, and 2-methyl-3-thiophenethiol. As a first step, stable isotope labeled analogues of these compounds were synthesized.

Deuterium-labeled 4-mercapto-3-hexanone was synthesized as reported for the unlabeled compound¹ using [²H₃]-4-hydroxy-3-hexanone instead of 4-hydroxy-3-hexanone as educt. The [²H₃]-4-hydroxy-3-hexanone was prepared in a three-step procedure from [²H₃]-bromoethane (Figure 2). A nucleophilic substitution using 2-lithio-1,3-dithiane as nucleophile yielded [²H₃]-2-ethyl-1,3-dithiane. [²H₃]-2-ethyl-1,3-dithiane was deprotonated by butyl lithium and reacted with propanal. This nucleophilic addition resulted in [²H₃]-1-(2-ethyl-1,3-dithian-2-yl)propan-1-ol, which underwent hydrolysis to finally yield [²H₃]-4-hydroxy-3-hexanone.

Although the preparation of [²H₆]-3-mercapto-3-methylbutyl formate was accomplished previously,¹⁶ a new synthetic route was developed (Figure 3). [²H₆]-Acetone was converted into [²H₆]-ethyl 3-methyl-2-butenolate by the Horner–Wadsworth–Emmons reaction. Then, the tertiary thiol function was regioselectively inserted by addition of H₂S to the olefinic double bond. Reduction with LiAlH₄ yielded the corresponding alcohol, which was esterified with formic acid to [²H₆]-3-mercapto-3-methylbutyl formate. Using this method, because of the direct insertion of the tertiary thiol group, a higher purity and a higher yield of the

target compound as compared to the formerly published procedure¹⁶ were achieved.

Syntheses of labeled 1-alkene-1-thiols followed the procedures applied for the preparation of the unlabeled compounds.¹ In general, the corresponding stable isotope-labeled aldehydes were reacted with H₂S, and the resulting dithiols subsequently underwent FVP to yield the target compounds (Figure 4). Syntheses of the labeled aldehydes were accomplished as follows: [²H₆]-2-methylpropanal used to prepare [²H₆]-2-methyl-1-propene-1-thiol was synthesized from [²H₆]-acetone (Figure 4A). For that purpose, [²H₆]-acetone was first converted to [²H₆]-1,1-dimethyloxirane by a Johnson–Corey–Chaykovsky reaction. Acid-catalyzed opening of the oxirane ring then resulted in [²H₆]-2-methylpropanal. [²H₃]-2-Methylbutanal, needed for the preparation of [²H₃]-(*E*)-2-methyl-1-butene-1-thiol (**4a**), and [²H₃]-(*Z*)-2-methyl-1-butene-1-thiol (**4b**) was synthesized from butyronitrile according to Goering and Tseng¹¹ (Figure 4B). Using diisobutylaluminum hydride, butyronitrile was converted to the corresponding aluminum imide. Deprotonation by lithium diisopropyl amide yielded the dianion, which was alkylated using methyl iodide. Acidic hydrolysis finally afforded [²H₃]-2-methylbutanal. Attempts to also synthesize [²H₃]-(*E*)-3-methyl-1-butene-1-thiol and [²H₃]-(*Z*)-3-methyl-1-butene-1-thiol failed so far because of the enormous instability of these compounds. For that reason, concentrations of (*E*)- and (*Z*)-3-methyl-1-butene-1-thiol were approximated using [²H₃]-(*E*)-2-methyl-1-butene-1-thiol as the internal standard. In the same way, quantitation of 2-mercapto-3-pentanone was achieved using [²H₂]-3-mercapto-2-pentanone as an internal standard.

Syntheses of deuterium-labeled 2-methyl-3-furanthiol and 3-mercapto-2-pentanone followed already published procedures.¹² [²H₃]-2-Methyl-3-thiophenethiol was prepared using the approach detailed in ref 12 for the synthesis of [²H₃]-2-methyl-3-furanthiol.

Concentrations and Odor Activity Values of Major Aroma-Active Thiols in Pan-Roasted White Sesame Seeds. The labeled aroma compounds synthesized were used as internal standards for the quantitation of the target compounds in freshly ground pan-roasted white sesame seeds by SIDAs. The results of the quantitation (Table 2) showed concentrations in the range of 11 ng/kg (3-mercapto-3-methylbutyl formate) up to 1.2 mg/kg (2-methyl-1-butene-1-thiol, sum of isomers). However, to assess the aroma impact of the individual thiols, their concentrations needed to be related to their respective odor thresholds. For that purpose, odor thresholds were determined by a set of forced-choice

Table 3. OAVs of Key Odorants in Roasted Sesame Seeds According to Schieberle⁵

compd	odor quality	concentration ($\mu\text{g}/\text{kg}$)	odor threshold ($\mu\text{g}/\text{kg}$)	OAV
2-acetyl-1-pyrroline	roasty, popcornlike	30	0.1	300
2-furfurylthiol	roasty, coffeelike	54	0.4	135
2-phenylethanethiol	burnt-rubbery	6	0.05	120
4-hydroxy-2,5-dimethyl-3(2H)-furanone	caramel-like	2511	50	50
2-ethyl-3,5-dimethylpyrazine	potato-like, roasty	53	3	18
2-methoxyphenol	burnt, sweet	269	19	14
2-pentylpyridine	fatty, tallowy	19	5	4
2-acetylpyrazine	roasty	26	10	3
4-vinyl-2-methoxyphenol	spicy	72	50	1
(E,E)-2,4-decadienal	fatty, waxy	89	180	<1

triangular tests with ascending concentrations of the test compounds.¹⁵ With respect to the high fat content of sesame seeds, sunflower oil was used as matrix in the threshold determinations. Because 2- and 3-methyl-1-butene-1-thiols were not available as pure (*E*)- and (*Z*)-compounds, thresholds were determined with approximately 1:1 mixtures of the (*E*)- and (*Z*)-isomers, respectively, as they were obtained using the synthetic approach detailed previously.¹ The thresholds determined ranged between 70 ng/kg (4-mercapto-3-hexanone) and 2.2 mg/kg (2-methyl-3-thiophenethiol). Using these data, odor activity values (OAVs) were calculated as the ratio of concentration and odor threshold. OAVs of 3-mercapto-3-methylbutyl formate and 2-methyl-3-thiophenethiol were by far below 1, thus indicating that these compounds are not odor-active in roasted sesame seeds. Concentrations of the other seven compounds were clearly exceeding their respective threshold values, with 3-methyl-1-butene-1-thiol (2400) and 2-methyl-1-butene-1-thiol (960) showing the highest OAVs. However, it must be taken into account that the OAV of 3-methyl-1-butene-1-thiol was afflicted with some uncertainty, because the isomeric distribution in the roasted sesame seeds somewhat differed from that in the synthetic mixture, whereas for 2-methyl-1-butene-1-thiol it was virtually the same. Nevertheless, the data clearly indicated that the 2- and 3-methyl-1-butene-1-thiols are of major importance for the characteristic sulfury aroma of freshly pan-roasted white sesame seeds, in particular, because their OAVs also exceeded the OAVs of the most odor-active aroma compounds reported in our previous study on roasted sesame flavor (Table 3). Furthermore, this was the first time that 1-alkene-1-thiols were identified as major impact compounds of a food flavor. However, whether the (*E*)- or the (*Z*)-isomers of 2- and 3-methyl-1-butene-1-thiol contribute more to the sulfury note of roasted sesame seeds may not be judged before the individual compounds are available in an appropriate isomeric purity. This will be subject to further research.

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ABBREVIATIONS USED

AEDA, aroma extract dilution analysis; CI, chemical ionization; DBU, diazabicyclo[5.4.0]undec-7-ene; DIBAL, diisobutylaluminum hydride; FVP, flash vacuum pyrolysis; GC-O, gas chromatography-olfactometry; LDA, lithium diisopropylamide; MCSS, moving column stream switching system; OAV, odor activity value; SAFE, solvent-assisted flavor evaporation; SIDA, stable isotope dilution assay

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