

Comparison of Onion Varieties by Headspace Gas Chromatography-Mass Spectrometry

Heikki Kallio* and Lea Salorinne

Laboratory of Food Chemistry, Department of Chemistry and Biochemistry, University of Turku, SF-20500 Turku, Finland

Volatile aroma compounds of crushed onions (*Allium cepa* L.) were analyzed over a 6-month storage period after harvesting. Compositions of the headspace of five varieties, Jumbo, Robusta, Sturon, Hygro, and Lafort, were analyzed by a static sampling procedure using gas chromatography (GC) and GC-mass spectrometry. Most prominent among the 27 identified aroma compounds were dipropyl disulfide, methyl propyl disulfide, 1-propenyl propyl disulfides (*E* and *Z*), methyl 1-propenyl disulfides (*E* and *Z*), 1-propanethiol, dipropyl trisulfide, methyl propyl trisulfide, 2-methyl-2-pentenal, two compounds tentatively identified as 3-ethyl-1,2-dithi-5-ene and 3-ethyl-1,2-dithi-4-ene, and finally *S*-propyl thioacetate. Most prominently differing from each other were the varieties Jumbo and Lafort, whereas Hygro and Lafort were more closely related. The distribution pattern of the major compounds varied little during storage.

The *Allium* genus contains a group of herbaceous food components, the aroma and flavor of which are greatly affected by the variety, cultivation, and handling and processing of the bulbs, stems, and leaves. All this has become clear in numerous papers since the classic works of Wertheim (1844, 1845) and Semmler (1892).

The development of high-resolution capillary chromatography opened new avenues, including that into *Allium* research, in the early 1970s. Sufficient selectivity and resolution of the chromatographic systems are necessary, especially for mass spectrometry (MS) coupling, as shown by Brodnitz et al. (1969, 1971) and Boelens et al. (1971). They studied the sulfur-containing volatiles of garlic and onion.

Headspace analysis has been the tool of several research groups (Saghir et al., 1964; Bernhard, 1968; Boelens et al., 1971; Bandyopadhyay et al., 1970; Freeman and Mossadeghi, 1970; Freeman and Whenham, 1974, 1975; Tewari and Bandyopadhyay, 1977; Mazza and LeMaguer, 1979; Mazza et al., 1980; Yagami et al., 1980; Coley-Smith, 1986), though they all used low-resolution packed columns. This unusual interest in headspace methods with *Allium* studies can be explained by the possibility of sensitive detection due to the high relative volatilities and air/water partition coefficients of some typical sulfur compounds such as sulfides, thiols, and thiophenes (Mazza, 1980). This makes the analyses easier to carry out than, for example, in the case of many fruits and berries, though much care is needed to avoid identifications based solely on retention times.

The sulfur compounds identified and measured as constituents of onion by use of headspace GC-MS combinations (Boelens et al., 1971; Mazza et al., 1980) are propanethiol, dimethyl disulfide, methyl propyl disulfide, dipropyl disulfide, methyl *cis*-propenyl disulfide, methyl *trans*-propenyl disulfide, *cis*-propenyl propyl disulfide, *trans*-propenyl propyl disulfide, 2,4-dimethylthiophene, and 3,4-dimethylthiophene. The work of Boelens et al. (1971) was one of the first classic implementations in which the selected ion-monitoring technique was used in the headspace GC-MS analysis of foodstuffs using an all-glass system and capillary columns.

Most headspace measurements of onion volatiles are

made with retention time fittings (Saghir et al., 1964; Freeman and Mossadeghi, 1970; Freeman and Whenham, 1975; Mazza and LeMaguer, 1979; Yagami et al., 1980; Coley-Smith, 1986). The major volatile in onion headspace has been reported to be dipropyl disulfide, followed by the other common mono- and disulfides, thiols, and thiophenes. Headspace analysis does not give direct information about the contents or ratios of the compounds in the onion matrix. The results, however, correlate with the aroma compounds emitted into the surroundings. For example, many of the identified aroma compounds have higher relative volatilities than dipropyl disulfide (Mazza, 1980).

The overall flavor and aroma primarily set the commercial value of onions. In Finland the Stuttgarter-type onions are commonly cultivated. They are not especially suitable for industrial purposes because of their flat shape. Spherical bulbs, which keep well and have a balanced flavor, are common in industrial use. These qualities are more typical of the Rijnsburger-type varieties such as Jumbo and Robusta. The hybrids Hygro and Lafort also have good properties.

The aim of this study was to analyze the headspace aroma and compare the composition of five onion varieties by using an on-column capillary GC-MS method.

MATERIALS AND METHODS

Cultivation and Treatment of Onions. Five varieties of onion (*Allium cepa* L.), Jumbo SG, Hygro GS, Lafort LV, Sturon SG, and Robusta GS, were cultivated under identical conditions at the Agricultural Research Centre of Finland, Institute of Horticulture, Piikkiö, in 1987 (61° 23' N, 22° 33' E). Including the five varieties mentioned above, there were all together 16 trial members, 11 of which were not studied in this work. The layout was a randomized plot design (four replications) with a plot area of 4.8 m². In the fall of 1986 the soil analyses were as follows: pH 6.3; Ca 1300, K 120, Mg 110, and P 26 kg·ha⁻¹. The plots were fertilized in the spring of 1987 with MgSO₄ at a rate of 300 kg·ha⁻¹ and with NPK fertilizer (Puutarhan Y-lannos 1, Kemira Ltd., N 10%, P 5%, K 17%, Mg 2%, Fe 0.1%, B 0.15%, Cu 0.1%, Mn 0.7%, Zn 0.1%, Mo 0.01%, and Se 0.0016%) at 1200 kg·ha⁻¹. The seedlings were planted in rows (20 seedlings m⁻¹).

The precipitation in 1987 was 689 mm, which was 90 mm higher than on average, mainly due to the extremely high values in August (154 mm) and September (118 mm). The mean temperature of the year was 2.1 °C lower than the long-term average: April (long-term average 2.6 °C, 1987 1.9 °C), May (9.2, 7.5 °C), June (14.0, 11.9 °C), July (17.3, 15.3 °C), August (15.9, 12.2 °C), and September (11.0, 9.4 °C).

Normal farming practice was employed, the onions being harvested September 4, predried at 17 °C, and conditioned at 28–30 °C until November 16 under effective ventilation, during which time the moisture content of the onions decreased below 60%. After the onions were graded according to size at 20 °C, they were stored at the beginning of December at 1 °C and 90% relative humidity.

Four randomly taken grade 1 onions, diameter 50–60 mm, formed one sample. Three to six identical replica samples of each variety were prepared at time periods of 0, 1, 2, 5, and 6 months and analyzed under identical conditions. The onions were cut into pieces with a knife and macerated carefully with an onion chopper, repeating the same procedure carefully each time (crushing, timing, temperatures). A sample of 70 g was sealed in a 500-mL headspace bottle, and the volatiles were generated at 37 °C for 54 min; 37 °C was close to the optimum conditions for the enzymatic formation of onion volatiles, and after 54 min, the aroma was very intense, although not yet stale or unpleasant.

Gas Chromatographic and Mass Spectrometric Analysis. The headspace sample was introduced into the capillary GC column with a 10-mL gastight syringe equipped with a 25 cm long deactivated fused silica needle (Hamilton, Reno, NV). The solutes were focused at the front part of the column chilled with liquid nitrogen (Kallio et al., 1987). The Varian 3700 chromatograph equipped with an FI detector (250 °C) was interfaced with a Hewlett-Packard 3388A integrator (Palo Alto, CA). The fused silica capillary column (DB-1701 liquid phase, 30 m, 0.32 mm i.d., d_f 0.25 μ m, J&W Scientific, Folsom, CA) was programmed from 10 to 180 °C, 2 °C/min. The average linear flow rate of the helium carrier was 27 cm/s measured at 30 °C. The quantitative comparisons were made according to the detector responses of the compounds without correction factors or internal standards being used.

The EI mass spectra (70 eV) were recorded with a VG 7070E mass spectrometer used with the VG-11-250 data system (Wythenshawe, Manchester, U.K.) and Dani HR 2 ch gas chromatograph (Dani SPA, Milano). The same column and conditions were used as in the GC analysis mentioned earlier.

RESULTS AND DISCUSSION

Five varieties of onion (*Allium cepa*) were cultivated, harvested, conditioned, stored, and analyzed under identical conditions. Deviation of the analyses with identical reaction chambers was due to both biological variation and error in the method. When the headspace of birch syrup (Kallio et al., 1987) and roasted coffee (Kallio et al., 1990) was analyzed, the deviation in the same GC method typically varied between 1% and 10%, depending on the analytes. The principle of the method involved not using elevated temperatures, extra headspace trapping systems, or solvent extraction of the traps.

Identification of the onion volatiles was based on the MS fragmentation pattern and GC retention. The headspace method chosen excluded the possibility of using NMR owing to the small amounts of compounds collected. IR facilities, which are suitable for the analyses, were not at our disposal. The homology between certain sulfur compounds containing methyl vs propyl groups could, however, be used for indirect verification purposes. The increase in retention time was almost constant, 13.5 min, when a propyl group existed instead of a methyl group in a disulfide. The only variety analyzed with mass spectrometry was Sturon. Comparison with the other four varieties was made with retention times and co-injections of the gaseous samples.

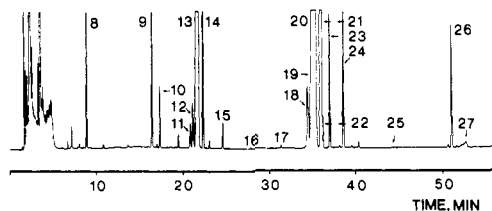


Figure 1. Gas chromatogram of the headspace of onion, variety Robusta. 10-mL on-column injection.

Table I. Compounds Identified in the Headspace of Five Onion (*A. cepa*) Varieties

peak	mol wt	formula	compound
1	76	C ₃ H ₈ S	2-propanethiol
2	76	CS ₂	carbon disulfide
3	76	C ₃ H ₈ S	1-propanethiol
4	74	C ₃ H ₆ S	methylthiirane
5	60	C ₃ H ₈ O	1-propanol
6	90	C ₄ H ₁₀ S	methyl propyl sulfide
7	90	C ₃ H ₈ OS	S-methyl thioacetate
8	94	C ₂ H ₆ S ₂	dimethyl disulfide
9	98	C ₆ H ₁₀ O	2-methyl-2-pentanol
10	118	C ₅ H ₁₀ OS	S-propyl thioacetate
11	120	C ₄ H ₈ S ₂	methyl 2-propenyl disulfide
12	120	C ₄ H ₈ S ₂	methyl 1-propenyl disulfide, Z ^a
13	122	C ₄ H ₁₀ S ₂	methyl propyl disulfide
14	120	C ₄ H ₈ S ₂	methyl 1-propenyl disulfide, E ^a
15	126	C ₂ H ₆ S ₃	dimethyl trisulfide
16	136	C ₅ H ₁₂ S ₂	ethyl propyl disulfide
17	150	C ₆ H ₁₄ S ₂	1-methylethyl propyl disulfide
18	148	C ₆ H ₁₂ S ₂	2-propenyl propyl disulfide
19	148	C ₆ H ₁₂ S ₂	1-propenyl propyl disulfide, Z ^a
20	150	C ₆ H ₁₄ S ₂	dipropyl disulfide
21	148	C ₆ H ₁₂ S ₂	1-propenyl propyl disulfide, E ^a
22	146	C ₆ H ₁₀ S ₂	3-ethyl-1,2-dithi-5-ene ^a
23	146	C ₆ H ₁₀ S ₂	3-ethyl-1,2-dithi-4-ene ^a
24	154	C ₄ H ₁₀ S ₃	methyl propyl trisulfide
25	156	C ₁₀ H ₂₀ O	decanal
26	182	C ₆ H ₁₄ S ₃	dipropyl trisulfide
27	180	C ₆ H ₁₂ S ₃	1-propenyl propyl trisulfide, (E/Z)

^a Tentative identification.

Figure 1 shows a gas chromatogram of the headspace compounds of crushed Robusta onions, collected at 37 °C. Resolution was sufficient, and quantitation of the compounds eluting after water was possible (compounds 8–27). With 10-mL injections the stationary phase was heavily overloaded by the major peaks 13 and 20. To separate the peaks 19 and 20 with at least a 50% valley, the injection volume of 1–2 mL could not be exceeded.

Table I summarizes the basic information concerning the onion volatiles. Most of the compounds listed are generally known to be components of onion aroma. The sulfur compounds, previously unknown (22 and 23), were not available in a pure form for reference purposes. This was also the case with, for example, the critical methyl propenyl disulfides and propenyl propyl disulfides.

The retention properties gave valuable additional structural information. Figure 1 and Table I show that the compounds 11–15, 18–21, and 24 consist of two analogous groups of methylalkyl (or alkenyl) and propylalkyl (or alkenyl) compounds, respectively. Comparing the mass spectra of the components with the homologous retention patterns, i.e., 11/18, 12/19, 13/20, 14/21, and 15/24, verified that the difference between the structures of the pairs was CH₂CH₂ ($M_r = 28$), the former compounds containing a methyl group instead of the propyl group in the latter. The retention time differences between the compound pairs 11/18, 12/19, etc. in the methyl and propyl groups in Figure 1 varied only in seconds, which also verified the statements.

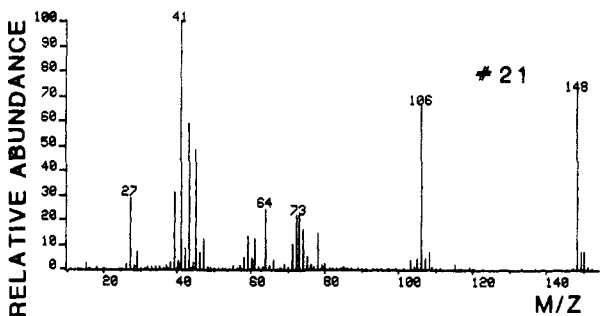
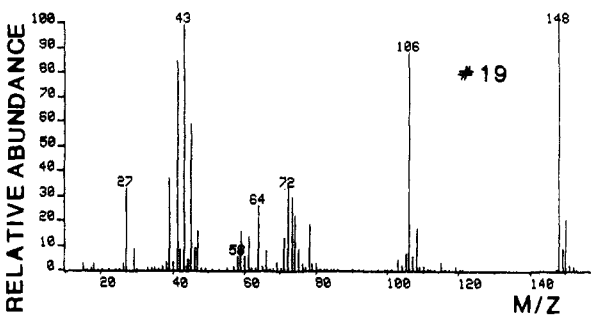
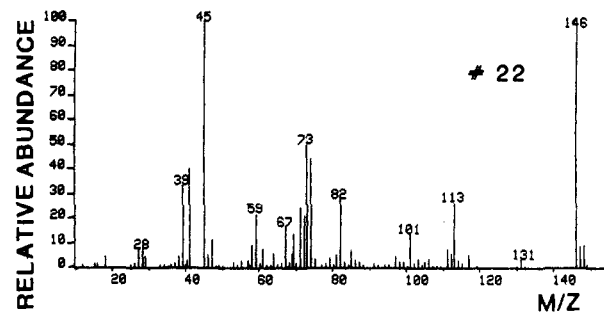
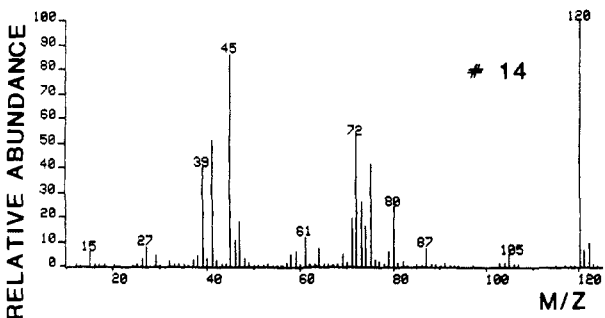
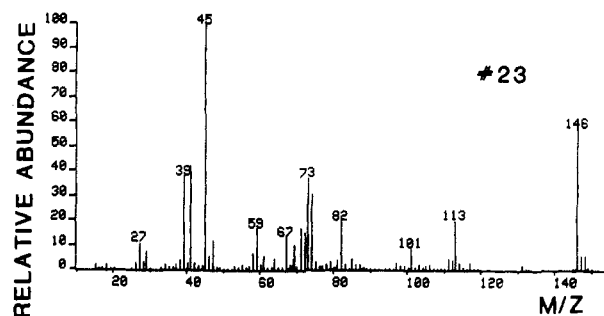
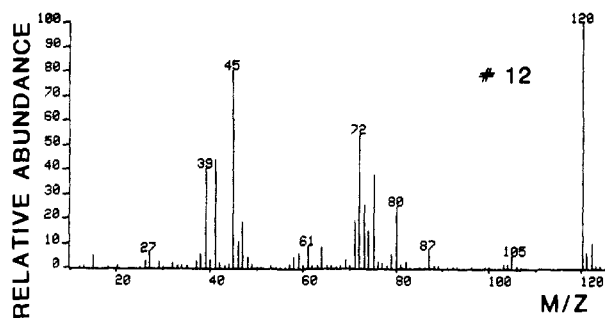


Figure 2. 70-eV EI-mass spectra of compounds 12 (methyl 1-propenyl disulfide, possibly *Z*), 14 (methyl 1-propenyl disulfide, possibly *E*), 19 (1-propenyl propyl disulfide, possibly *Z*), and 21 (1-propenyl propyl disulfide, possibly *E*), variety Sturon.

According to the mass spectral data, compounds 11, 12, and 14 are methyl propenyl disulfides and 18, 19, and 21 propenyl propyl disulfides. The close similarity of the mass spectra of the compound pairs (12/14 and 19/21) in Figure 2 verifies that they are the isomers of methyl 1-propenyl disulfide and 1-propenyl propyl disulfide, respectively. Discernment between the *E* and *Z* isomers cannot be made with the aid of the pure mass spectra. The commonly known spectra of compounds 11 and 18 clearly differed from those shown in Figure 2, which verified the structures as methyl 2-propenyl disulfide (compound 11) and 2-propenyl propyl disulfide (compound 18). This is in good agreement with the well-known work of Brodnitz et al. (1969).

Gas chromatographic analyses also tentatively indicated the presence of methyl propyl, methyl propenyl, dipropyl, and propenyl propyl tetrasulfides. For mass spectral

Figure 3. 70-eV EI-mass spectra of compounds 22 (probably 3-ethyl-1,2-dithi-5-ene) and 23 (probably 3-ethyl-1,2-dithi-4-ene), variety Sturon.

verifications larger injection volumes (30–50 mL) are, however, needed.

Compounds 22 and 23, the mass spectra of which are shown in Figure 3, are the most interesting new components found in onion. They are probably 3-ethyl-1,2-dithi-5-ene and 3-ethyl-1,2-dithi-4-ene and have not previously been identified in onions or any other vegetables. Their contents varied from 10% to 50% (peak 23, possibly 4-ene) and from 1% to 6% (peak 22, possibly 5-ene) of the total FID response of the peaks 8–27. An interesting analogue with two garlic volatiles led to these structural suggestions. Brodnitz et al. (1971) isolated two major compounds in garlic extract and verified their structures with IR, Raman, MS, and NMR analyses to be 3-vinyl-1,2-dithi-5-ene and 3-vinyl-1,2-dithi-4-ene. They assumed that the compounds were dehydration products formed nonenzymatically from diallyl thiosulfinate during GC analysis and that they were not original components of garlic.

A homologous formation mechanism for compounds 22 and 23 from 1-propenyl propyl thiosulfinate in onion might also be possible. If this dehydration occurs spontaneously in crushed onions in the reaction vial, the compounds formed are no more "artificial" than any of the major disulfides or trisulfides. So far, the new compounds may be considered only as possible structures because of the lack of synthesized reference compounds and NMR spectra.

Each onion variety was analyzed five times over the 6-month storage period. Those compounds (8–27, Table I) whose share exceeded 1% were taken into account in the quantitative analyses. The positions and integration of the fast-eluting components 1–7, i.e., 2-propanethiol, carbon disulfide, 1-propanethiol, methylthiirane, 1-propanol, methyl propyl sulfide, and *S*-methyl thioacetate, could not be measured accurately enough at the FID chromatograms (Figure 1) and were therefore excluded from the quantitative calculations. The disruption of the chromatogram at the partition ratio (*k'*) area from about 0 to 3 (including compounds 1–7) was evidently caused by the water and unnecessarily low temperature of the cold trap (liquid CO₂).

The results for all five varieties over the 6-month storage

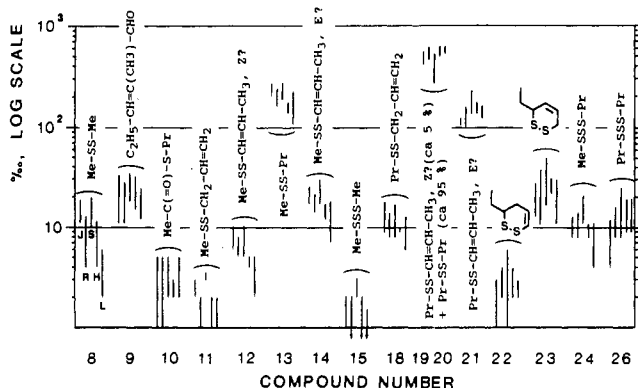


Figure 4. Relative proportions (%) of the major volatiles of onions analyzed five times over 6-month storage. Only compounds with k' values higher than that of dimethyl disulfide (DB-1701) are included. The bars join the maximum and minimum values of each compound/variety, n of each bar 15–30. Numbering of the compounds as in Table I. J = Jumbo, R = Robusta, S = Sturion, H = Hygro, L = Lafort.

period are summarized in Figure 4. The bars show the limits of the contents of each of the 16 major compounds (relative shares above 1%) shown in Table I. A general observation is the similarity of the headspace compositions of the varieties, though differences from variety to variety can be observed. Even more pronounced, and also surprising, is the stable overall distribution of the volatiles despite the storage time of up to 6 months.

Three compounds had relative proportions clearly above the others in every variety and every sampling time studied, namely, dipropyl disulfide, methyl propyl disulfide, and the later eluting 1-propenyl propyl disulfide isomers (evidently *E*). The 10% share was typically exceeded by 2-methyl-2-pentenal, methyl 1-propenyl disulfide (*E*), 2-propenyl propyl disulfide, the later of the 3-ethyl-1,2-dithiolenes, and dipropyl trisulfide.

Some of the characteristics of the varieties Jumbo and Lafort, which differed most clearly from each other, can be emphasized. In every sample of Jumbo the proportions of dimethyl disulfide (11–18%), methyl 2-propenyl disulfide (2–3%), methyl 1-propenyl disulfide (*Z*) (6–10%), and methyl 1-propenyl disulfide (*E*) (17–25%) were higher than the shares in any of the Lafort samples, which were 2–6%, 1–2%, 2–5%, and 7–18%, respectively. In Lafort, by contrast, the proportions of dipropyl disulfide (510–630%) and 1-propenyl propyl disulfide (130–170%) reached higher levels than in Jumbo (390–530% and 110–130%, respectively). For exact chemotaxonomical comparison of the onion varieties, the whole procedure including cultivation should be repeated in several years.

The total content of the volatiles emitted by the crushed onions differed from variety to variety during the half-year storage. Some preliminary observations could, however, be verified. There was a minimum in the total emission of the compounds discussed typically 2 months after harvesting. With the variety Robusta the minimum was reached as early as 1 month after harvesting. At the end of the 6-month period the total content of the compounds listed was higher in the headspace in all varieties except Jumbo as compared to the harvest-time headspace content.

No internal standards for the GC analyses were used. The standard deviations of the contents of the aroma compounds in the triplicate samples varied typically from 20% to 40%. The deviations were much less in the relative proportions of the volatiles than in the absolute integrated values in the chromatogram. This was evidently due to

difficulties in standardizing the crushing procedure of the bulbs. However, even above the nonhomogeneously crushed bulbs, the complex matrix of the sulfur-containing aroma compounds was formed under coordinative biochemical and chemical control.

ACKNOWLEDGMENT

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Registry No. 2-Propanethiol, 75-33-2; carbon disulfide, 75-15-0; 1-propanethiol, 107-03-9; methylthiirane, 1072-43-1; 1-propanol, 71-23-8; methyl propyl sulfide, 3877-15-4; *S*-methyl thioacetate, 1534-08-3; dimethyl disulfide, 624-92-0; 2-methyl-2-pentenal, 623-36-9; *S*-propyl thioacetate, 2307-10-0; methyl 2-propenyl disulfide, 2179-58-0; (*Z*)-methyl 1-propenyl disulfide, 23838-18-8; methyl propyl disulfide, 2179-60-4; (*E*)-methyl 1-propenyl disulfide, 23838-19-9; dimethyl trisulfide, 3658-80-8; ethyl pro-

pyl disulfide, 30453-31-7; 1-methylethyl propyl disulfide, 33672-51-4; 2-propenyl propyl disulfide, 2179-59-1; (*Z*)-1-propenyl propyl disulfide, 23838-20-2; dipropyl disulfide, 629-19-6; (*E*)-1-propenyl propyl disulfide, 23838-21-3; 3-ethyl-1,2-dithi-5-ene, 126790-01-0; 3-ethyl-1,2-dithi-4-ene, 126790-02-1; methyl propyl trisulfide, 17619-36-2; decanal, 112-31-2; dipropyl trisulfide, 6028-61-1; (*E/Z*)-1-propenyl propyl trisulfide, 33368-79-5.