

# Identification of New Strawberry Sulfur Volatiles and Changes during Maturation

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**ABSTRACT:** Two Florida strawberry cultivars, ‘Strawberry Festival’ and ‘Florida Radiance’, were harvested at five fruit developmental stages (white, half red, three-quarter red, full ripe, and overripe) at four harvest dates. A static headspace solid-phase microextraction (SPME) sampling technique coupled with gas chromatography (GC) using pulsed flame photometric detection (PFPD) was employed to measure 16 sulfur volatiles in these strawberries. A total of 7 sulfur volatiles have been previously reported, and 9 are reported for the first time in strawberries. Newly identified sulfur volatiles include methyl thiopropionate, ethyl thiobutanoate, methyl thiohexanoate, methyl (methylthio)acetate, ethyl (methylthio)acetate, methyl 2-(methylthio)butyrate, methyl 3-(methylthio)propionate, ethyl 3-(methylthio)propionate, and methyl thiooctanoate. Identifications were based on matching sulfur peak linear retention indexes (LRIs) of unknowns with authentic standards and gas chromatography–mass spectrometry (GC–MS) data. Concentrations were determined using both internal and external standards. Most sulfur volatiles increased with increasing maturity, with only concentrations of hydrogen sulfide and methanethiol remaining relatively consistent at all five stages. At the white and half red stages, most sulfur volatiles consisted of various alkyl sulfides. At three-quarter red (commercial ripe), full ripe, and overripe stages, the majority of sulfur volatiles consisted of sulfur esters. Most sulfur volatiles increased dramatically between the commercial ripe, full ripe, and overripe stages, increasing as much as 100% between full ripe and overripe. Principal component analysis indicated that sulfur volatiles could be used to distinguish overripe from full ripe and commercial ripe berries.

**KEYWORDS:** Strawberry thioesters, SPME, PCA

## INTRODUCTION

Volatile sulfur compounds are an important class of odor-active compounds because of their low thresholds, strong odor impact, and wide distribution in food products. Generally, sulfur volatiles are degradation products of cystine, cysteine, methionine, glutathione, and some vitamins. Sulfur volatiles can be present in many different chemical forms, including thiols, sulfides, polysulfides, thioesters, and heterocyclic compounds, which make the detection and identification of these compounds more difficult. These sulfur volatiles have an enormous range of olfactory qualities: rotten egg, tropical fruit, cabbage, garlic, onion, potato, coffee, and grapefruit.<sup>1</sup> Some of these volatiles can be responsible for off-flavors, while some of them have been identified as favorable character-impact substances in foods, such as coffee,<sup>2</sup> wine,<sup>1</sup> beer,<sup>3</sup> truffles,<sup>4</sup> allium species,<sup>5</sup> and various fruits.<sup>6–12</sup> Fruits with sulfur-aroma-impact compounds include melon,<sup>10</sup> pineapple,<sup>8</sup> yellow passion fruit,<sup>11</sup> grapefruit,<sup>12</sup> and durian.<sup>9</sup> Sulfur volatiles have also been reported in strawberries.<sup>6,13,14</sup> Seven sulfur volatiles, including hydrogen sulfide, methanethiol, dimethyl sulfide, dimethyl disulfide, dimethyl trisulfide, methyl thioacetate, and methyl thiobutyrate, have been reported in various strawberry cultivars. Even though they are present at very low concentrations, some of these can be important to the aroma of certain strawberry cultivars. Specifically, methanethiol, dimethyl disulfide, dimethyl sulfide, methyl thioacetate, and methyl thiobutanoate are considered to be important constituents in some “older” strawberry cultivars.<sup>14</sup> However, the information on strawberry sulfur volatiles is still quite limited.

Strawberry is a nonclimacteric fruit in which ripening is characterized by texture softening, pigment, flavor, and aroma synthesis, acidity decreases, and sugar increases. Information on the changes in aroma composition during strawberry maturation is still very limited.<sup>15</sup> To date, no study has focused on the changes of strawberry sulfur volatiles at different maturity stages. Furthermore, the overall sulfur volatile composition of strawberries is still not well-characterized.

Strawberry is the second most important fruit product in Florida and a major source of winter and early spring strawberries in the U.S. market. Sulfur volatiles are difficult to identify and quantify because they typically exist at sub-microgram per kilogram levels. However, several have even lower aroma thresholds. Sulfur volatiles are only found in some strawberry cultivars and not others.<sup>13</sup> ‘Strawberry Festival’ is the industry standard cultivar in Florida, while ‘Florida Radiance’ is a new complementary strawberry cultivar. The sulfur volatile content of these two strawberry cultivars has not been previously reported. The objective of the current study is to identify and quantify the sulfur volatiles in Florida strawberries and determine their relative concentrations at five maturity stages using static headspace solid-phase microextraction (SPME) coupled with gas chromatography (GC) using pulsed flame photometric detection (PFPD).

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## MATERIALS AND METHODS

**Chemical.** The follow standards were obtained from Sigma-Aldrich (St. Louis, MO): methyl thioacetate, methyl thiobutanoate, methyl (methylthio)acetate, ethyl (methylthio)acetate, methyl 3-(methylthio)propionate, and methyl thiooctanoate. One internal standard, allyl isothiocyanate, was obtained from Fluka (Milwaukee, WI), and the other internal standard, isopropyl disulfide, was obtained from SAFC (St. Louis, MO). Dimethyl sulfide (DMS), dimethyl disulfide (DMDS), and dimethyl trisulfide (DMTS) were obtained from Acros Organics (Fair Lawn, NJ). Methyl thiopropionate was obtained from Oxford Chemical Limited (Seaton Carew, Hartlepool, U.K.), and methyl thiohexanoate was obtained from Charkit Chemical Corporation (Norwalk, CO). Methyl 2-(methylthio)butyrate and ethyl 3-(methylthio)propionate were obtained from R. C. Treatt and Co., Ltd. (Bury St. Edmunds, Suffolk, U.K.). The two internal standards were employed as isopropyl disulfide in methanol at 17.86  $\mu\text{g}/\text{kg}$  and allyl isothiocyanate prepared similarly at 111.34  $\mu\text{g}/\text{kg}$ . Sodium fluoride (ACS grade) was obtained from Acros Organics. Sodium chloride and methanol was purchased from Fisher Scientific (Fair Lawn, NJ).

**Strawberry Samples.** ‘Strawberry Festival’ and ‘Florida Radiance’ strawberries were grown at the Gulf Coast Research and Education Center, University of Florida, Wimauma, FL. They were hand-harvested at five fruit developmental stages, including “white”, “half red”, “three-quarter red”, “full ripe”, and “overripe”, between February and March during the 2010 growing season. Harvest dates were February 9, February 25, March 8, and March 22.

After the fruit was harvested, strawberry samples were stored at 5 °C overnight, allowed to warm to room temperature, and washed before processing the next morning. A total of 200 g of fresh strawberry fruit were pureed in a Waring blender (Waring Products Division, Dynamics Corporation, New Hartford, CO), with equal weights of freshly distilled water, 80 g of sodium chloride, and 4 g of sodium fluoride. Blending was performed using a high-speed pulse for 20 s. The puree was stored in 300 mL brown glass bottles and frozen until analysis.

**SPME of Sulfur Volatiles in Strawberries.** Two sampling protocols were employed to analyze strawberry sulfur volatiles. For the high-concentration sulfur volatiles, including methyl thioacetate and methyl thiobutanoate, 1 g of strawberry puree was diluted with 9 g of water in a 40 mL vial, which was flushed with nitrogen. A 4 mm stir bar and 50  $\mu\text{L}$  of internal standard of isopropyl disulfide were added to the sample. Then, the sample was equilibrated with stirring at 30 °C for 20 min in a water bath. After equilibration, a divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS, 2 cm, Supelco, Bellefonte, PA) fiber was exposed to the headspace of the vial for 15 min.

For the remaining sulfur volatiles, 10 g of the undiluted strawberry puree was mixed with 15  $\mu\text{L}$  of internal standard (allyl isothiocyanate). The sample was equilibrated with stirring at 30 °C for 20 min in the water bath. After equilibration, the same fiber was exposed to the headspace of the vial for 40 min. After extraction, the fiber was then introduced to a GC injection for a 3 min desorption.

**GC–PFPD Identification of Sulfur Compounds.** Volatile sulfur analyses was performed using an Agilent 7890A gas chromatograph system equipped with sulfur-specific PFPD [model 5380 pulsed flame photometric detector (PFPD), OI Analytical Co., College Station, TX]. Compound separation was achieved using a Stable Wax column (30 m  $\times$  0.32 mm inner diameter cross-linked polyethylene glycol, 0.50  $\mu\text{m}$  film thickness, Restek, Bellefonte, PA). The column flow was 1.5 mL/min (helium). The oven temperature was programmed at 35 °C for a 1 min initial hold, then increased at 3 °C/min to 65 °C, then increased at 6 °C/min to 170 °C, and finally increased at 10 °C/min to a final temperature of 240 °C, with a 5 min hold. The GC injection temperature was 200 °C, and the detector temperature was 250 °C. The sulfur gate time was 6–24.9 ms, and the pulse frequency was approximately 3 pulses/s. Compound identification was based on the authentic sulfur

standards. Retention indices (RIs) were estimated using standard alkanes  $\text{C}_5$ – $\text{C}_{25}$ .

**Gas Chromatography–Mass Spectrometry (GC–MS) Identification of Sulfur Compounds.** GC–MS analyses were performed using a Perkin-Elmer Clarus 500 gas chromatograph and quadrupole mass spectrometer (PerkinElmer, Waltham, MA). Compound separation was achieved using a DB Wax column (60 m  $\times$  0.25 mm inner diameter cross-linked polyethylene glycol, 0.50  $\mu\text{m}$  film thickness, J&W Scientific, Agilent Technique, Foster City, CA). The column flow rate was 2.0 mL/min (helium). The initial oven temperature was 35 °C with a 1 min hold and then increased to 190 °C at 4 °C/min, and at 190 °C, the rate was increased to 8 °C/min until 240 °C, with a 5 min hold. Injection, MS transfer line, and ion-source temperatures were 230, 250, and 180 °C, respectively. Electron ionization mass spectrometric data for  $m/z$  30–300 were collected using a scan model, with an ionization voltage of 70 eV. Compound identifications were made by comparing mass spectral data from the Wiley 275.L (G1035) or National Institute of Standards and Technology (NIST) database and confirmed by matching retention values and fragmentation patterns with authentic sulfur standards.

**Calibration Plots and Quantification.** Plots of the concentration versus the PFPD peak area were developed by adding authentic sulfur standards of known concentration to a strawberry puree, whose volatiles had been removed using vacuum. Individual sulfur stock solutions were prepared in freshly distilled, deionized water to make the first level mixed standard solution, which was then diluted 1:200 (v/v) with freshly distilled water to obtain the more dilute set of standards. Each sulfur standard mixture was separately added to a “blank” devolatilized strawberry puree. A series of concentration levels of the standards in the devolatilized strawberry puree was prepared. Coupled with the internal standard, the added sulfur compounds were extracted with SPME, as performed for the sample. Standard calibration curves were developed for each individual sulfur volatile and were used to calculate the concentrations of sulfur volatiles in samples. Duplicate analysis was performed for each sample.

**Statistical Analysis.** SAS statistical software (SAS System Software, version 9.1, SAS Institute, Cary, NC) was used to test the statistical variances of sulfur volatiles from four harvest dates and five fruit stages at each harvest date. Analysis of variance (ANOVA) was applied for variance testing of each sulfur volatile among different maturity stages and multiple harvest dates. The Unscrambler software, version 7.6 (CAMO ASA, Oslo, Norway) was used for principal component analysis (PCA). Variables were mean-centered and scaled.

## RESULTS AND DISCUSSION

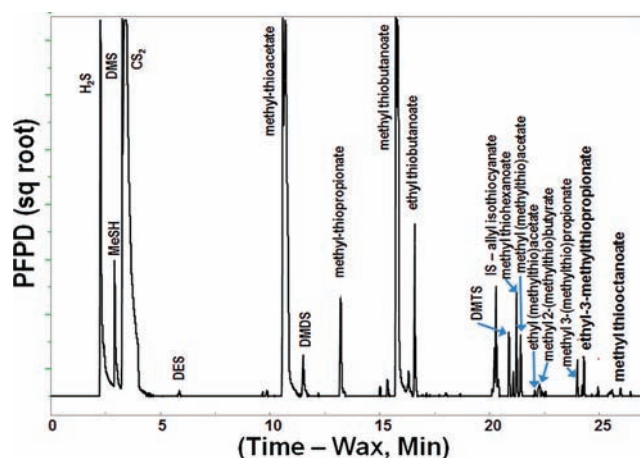
**Identification of Strawberry Sulfur Volatiles.** Sulfur volatiles are known to play an important role in many vegetable flavors. However, few sulfur volatiles have been identified in fruits, such as strawberry. As indicated in Table 1, Winter<sup>14</sup> identified  $\text{H}_2\text{S}$ , methanethiol, and a trace of dimethyl disulfide in freshly crushed strawberries using MS. Dirinck and co-workers<sup>13</sup> quantified the volatiles in freshly macerated strawberries using a flame ionization detector (FID) with two internal standards. They also employed a flame photometric detector (FPD) and identified two thioesters, methyl thioacetate and methyl thiobutanoate. These sulfur volatiles were not found in most strawberry cultivars that they examined and are thought to be a distinct feature in strawberry flavor. More recently, Schulbach and co-workers<sup>6</sup> employed PFPD to identify nine sulfur volatiles from intact, unbruised strawberries.

In this study, nine new sulfur esters were identified, specifically, methyl thiopropionate, ethyl thiobutanoate, methyl thiohexanoate, methyl (methylthio)acetate, ethyl (methylthio)acetate,

Table 1. Sulfur Standards and Calibration Plots Used for Quantification

| name                            | source, purity               | regression equation          | R <sup>2</sup> | range <sup>a</sup> |
|---------------------------------|------------------------------|------------------------------|----------------|--------------------|
| isopropyl disulfide (IS)        | SAFC                         |                              |                |                    |
| methyl thioacetate              | Aldrich, 97%                 | $y = 0.3987 \ln(x) + 0.117$  | 0.9412         | 0.06–10            |
| methyl thiobutanoate            | Aldrich, 99%                 | $y = 0.4225 \ln(x) + 0.4847$ | 0.9734         | 0.04–6             |
| allyl isothiocyanate (IS)       | Fluka, ≥98%                  |                              |                |                    |
| dimethyl sulfide (DMS)          | ACROS                        | $y = 0.2148 \ln(x) + 0.1723$ | 0.9565         | 0.07–11            |
| dimethyl disulfide (DMDS)       | ACROS, 99%                   | $y = 0.4739 \ln(x) + 1.312$  | 0.9721         | 0.10–17            |
| methyl thiopropionate           | Oxford Chemical              | $y = 0.5673 \ln(x) + 1.1236$ | 0.9819         | 0.07–11            |
| dimethyl trisulfide (DMTS)      | ACROS, ≥98%                  | $y = 0.4739 \ln(x) + 1.312$  | 0.9721         | 0.08–12            |
| methyl thiohexanoate            | Charkit Chemical Corporation | $y = 0.4282 \ln(x) + 1.0705$ | 0.9479         | 0.08–13            |
| methyl (methylthio)acetate      | Aldrich, 99%                 | $y = 0.4209 \ln(x) + 0.4222$ | 0.9606         | 0.06–10            |
| ethyl (methylthio)acetate       | Aldrich, 98%                 | $y = 0.0093x + 0.0547$       | 0.9204         | 0.07–11            |
| methyl 2-(methylthio)butyrate   | R. C. Treatt and Co.         | $y = 0.4551 \ln(x) + 1.108$  | 0.9765         | 0.07–11            |
| methyl 3-(methylthio)propionate | Aldrich, 98%                 | $y = 0.4018 \ln(x) + 0.2037$ | 0.956          | 0.13–21            |
| ethyl 3-(methylthio)propionate  | R. C. Treatt and Co.         | $y = 0.4001 \ln(x) + 0.2974$ | 0.9423         | 0.09–14            |
| methyl thiooctanoate            | Aldrich                      | $y = 0.3141 \ln(x) + 0.8289$ | 0.9177         | 0.01–1             |

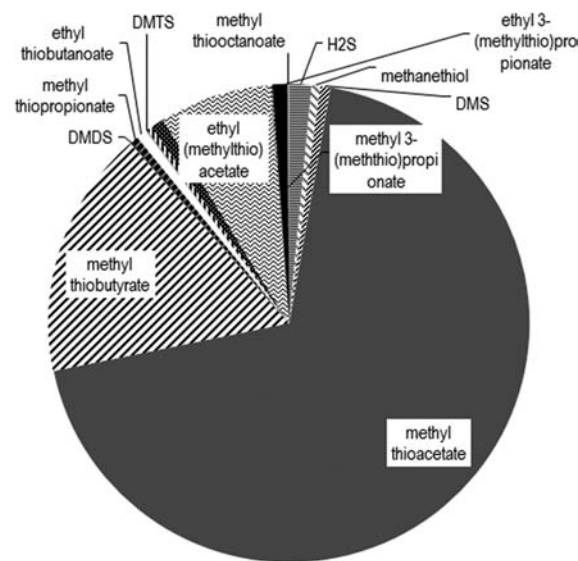
<sup>a</sup> In units of  $\mu\text{g}/\text{kg}$ .



**Figure 1.** Chromatogram of sulfur volatiles from 'Strawberry Festival' (harvested on March 8) at the full ripe stage. MeSH, methane thiol; DMS, dimethyl sulfide; CS<sub>2</sub>, carbon disulfide; DES, diethyl sulfide; DMDS, dimethyl disulfide; DMTS, dimethyl trisulfide; IS, internal standard.

methyl 2-(methylthio)butyrate, methyl 3-(methylthio)propionate, ethyl 3-(methylthio)propionate, and methyl thiooctanoate. It is worth noting from the PFPD chromatogram shown in Figure 1 that these nine additional compounds are for the most part smaller peaks, which might explain why these volatiles have not been identified until now. Even though the PFPD is highly selective and sensitive for sulfur, it provides little identification information other than chromatographic retention time. Therefore, additional identification procedures were employed.

Two of the thioesters previously identified<sup>13</sup> were present in concentrations high enough to be seen in the MS total ion current (TIC) chromatogram. As shown in Figure 2, these two esters comprise almost 70% of all sulfur volatiles in terms of concentration. The sulfur ester present at the third highest concentration (6% in terms of total concentration) was ethyl (methylthio)acetate. Its concentration was not sufficient to produce a TIC peak, as illustrated in the bottom chromatogram in Figure 3, but, as noted in the upper chromatogram, could be

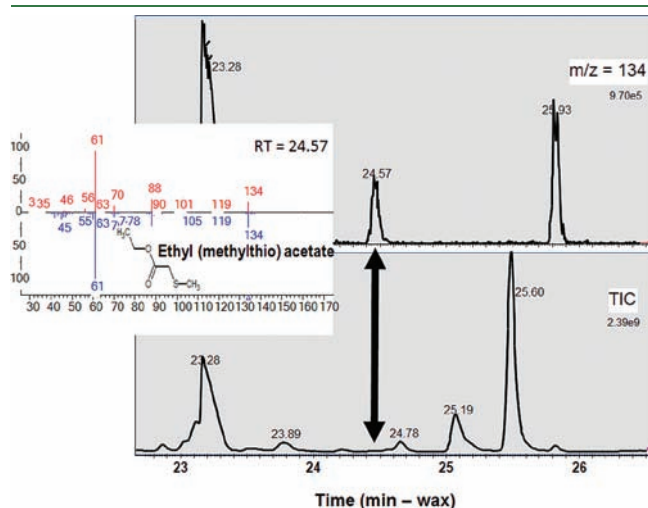


**Figure 2.** Distribution of sulfur volatiles from 'Strawberry Festival' at full ripeness harvested on March 8, 2010 (excluding CS<sub>2</sub>).

detected using selected ion monitoring (SIM) at  $m/z$  143 [molecular ion of ethyl (methylthio)acetate, the baseline-corrected fragmentation spectrum shown in the upper inset]. When the spectra of standard ethyl (methylthio)acetate is compared to library spectra, a match >800 was obtained.

As noted in Figure 2, the concentrations of the remaining sulfur volatiles were quite low and could only be detected with PFPD. Their PFPD peaks were small and did not produce a MS TIC peak. To identify them, SIM MS was employed. Each of the remaining trace volatiles were identified first from matching sample linear retention indexes (LRI) values from sulfur-specific PFPD to those of standards. Multiple standards for each candidate sulfur volatile were run to find a retention time match. A final MS confirmation was determined by matching the LRI value from SIM MS to at least two characteristic ions, so that an ion ratio could also be obtained with those of standards. These ions are listed in Table 1 for each sulfur volatile. When retention index

values are compared between PFPD sulfur and SIM MS, it should be kept in mind that two different chromatographic systems were employed. Although both were wax columns, the column lengths and diameters were different and there were different outlet pressures for both systems. These differences were most severe for early eluting volatiles. However, these volatiles have been previously reported, and there was excellent agreement between the two systems for most of the newly identified compounds. For example, for the newly identified methyl thiopropanoate, the PFPD LRI value was 1129 and the SIM MS value at  $m/z$  104 and 57 was 1132. One additional criteria was used, which was the ratio of the two ions. All but two of the nine newly identified compounds had the same ratio of the monitoring ions as authentic standards. Methyl thiopropanoate was one of the



**Figure 3.** Full spectrum identification of ethyl (methylthio)acetate using MS in both TIC and SIM modes. The top chromatogram is a SIM at  $m/z$  134. The bottom chromatogram is the TIC. (Inset) Top, baseline-corrected spectra of the peak at 24.57 min; bottom, standard ethyl (methylthio)acetate.

compounds that did not have the same ratio as the standard. This could be due to impurities because  $m/z$  59 is not a very unique mass and coeluting volatiles would alter the ratio; nevertheless, the identification of this compound should be considered tentative.

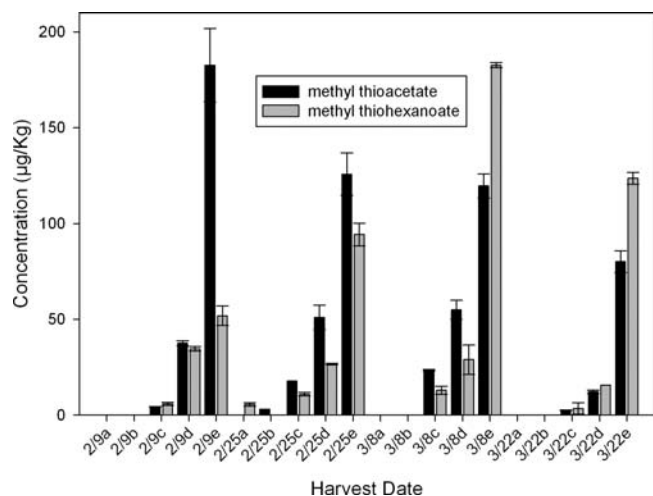
**Sulfur Esters.** These esters comprised the major portion of all sulfur volatiles in all but the most immature strawberry maturity stages. As shown in Table 2, total esters ranged from 94 to 99% of all sulfur volatiles in maturity stages 3–5 and methyl thioacetate was the dominant sulfur ester. Sulfur esters have been found in other tropical/subtropical fruits, such as melon,<sup>10</sup> pineapple,<sup>8</sup> durian,<sup>9</sup> kiwi,<sup>7</sup> and passion fruits.<sup>11</sup> Methyl 3-(methylthio)propionate, ethyl 3-(methylthio)propionate, methyl (methylthio)acetate, and ethyl (methylthio)acetate have been identified in some melon cultivars.<sup>10</sup> These sulfur esters are considered to be important to the aroma profiles of specific melons. Methyl 3-(methylthio)propionate and ethyl 3-(methylthio)propionate have also been identified in pineapple<sup>8</sup> and impart a tropical fruit flavor. Methyl (methylthio)acetate and ethyl (methylthio)acetate have been identified in Indonesian durians,<sup>9</sup> in which sulfur volatiles provide a major component of the overall aroma perception. Methyl thioacetate and methyl thiobutanoate are both aroma-active strawberry volatiles with very low thresholds and appear in relatively high concentrations. Other thioesters, such as methyl thiopropanoate, methyl thiohexanoate, and methyl thiooctanoate, have thresholds only slightly higher than methyl thiobutanoate.<sup>16</sup> However, the role of these same sulfur esters in strawberry aroma is unclear at this time and was not an objective of this study.

Sulfur volatiles in general are thought to be degradation or reaction products of the sulfur-containing amino acids, such as cystine, cysteine, methionine, and glutathione. However, sulfur esters are specifically thought to derive from cysteine and methionine<sup>1</sup> alone. The reason that these sulfur esters are only observed in the later ripening stages is probably due to the lack of enzyme activity in unripened fruits or low levels of a necessary precursor that increases with maturity.

**Table 2.** Identification of Strawberry Sulfur Volatiles from PFPD and MS Data<sup>a</sup>

| PFPD LRI <sup>b</sup> | compounds                           | previous identification <sup>c</sup> | odor descriptors <sup>d</sup> | standard <sup>e</sup> | MS LRI            | ions ( $m/z$ ) monitored |
|-----------------------|-------------------------------------|--------------------------------------|-------------------------------|-----------------------|-------------------|--------------------------|
| 457                   | hydrogen sulfide (H <sub>2</sub> S) | a, c                                 | rotten egg, sulfurous         | LRI                   | 501               | 34, 33                   |
| 510                   | methanethiol                        | a, b, c                              | cabbage, sulfurous            | LRI                   | 585               | 48, 47                   |
| 678                   | dimethyl sulfide (DMS)              | b, c                                 | cabbage, sulfurous            | LRI, std              | 752               | 62, 47                   |
| 1056                  | <b>methyl thioacetate</b>           | b, c                                 | cheesy, garlic                | LRI, std              | 1061              | 90, 43                   |
| 1081                  | dimethyl disulfide (DMDS)           | a, b, c                              | garlic, sulfurous             | LRI, std              | 1082              | 94, 79                   |
| 1129                  | methyl thiopropanoate               |                                      | cheesy, rotten egg            | LRI, std              | 1132 <sup>f</sup> | 104, 57                  |
| 1208                  | <b>methyl thiobutanoate</b>         | b, c                                 | cheesy, garlic, cabbage       | LRI, std              | 1209              | 118, 71                  |
| 1237                  | ethyl thiobutanoate <sup>g</sup>    |                                      | no standard                   | LRI                   | 1236 <sup>f</sup> | 132, 76                  |
| 1401                  | dimethyl trisulfide (DMTS)          | c                                    | onion, sulfurous              | LRI, std              | 1399              | 126, 79                  |
| 1416                  | methyl thiohexanoate                |                                      | green, fruity, onion          | LRI, std              | 1417              | 131, 99                  |
| 1424                  | methyl (methylthio)acetate          |                                      | garlic, sulfuric              | LRI, std              | 1425              | 120, 74                  |
| 1453                  | <b>ethyl (methylthio)acetate</b>    |                                      | green, onion                  | LRI, std              | 1463              | 134, 88                  |
| 1461                  | methyl 2-(methylthio)butyrate       |                                      | fruity, floral, garlic        | LRI, std              | 1476              | 148, 102                 |
| 1542                  | methyl 3-(methylthio)propionate     |                                      | sweet, spicy, garlic          | LRI, std              | 1554              | 134, 74                  |
| 1574                  | ethyl 3-(methylthio)propionate      |                                      | grassy, cabbage               | LRI, std              | 1584              | 148, 74                  |
| 1647                  | methyl thiooctanoate <sup>g</sup>   |                                      | fruity, pineapple             | LRI, std              | 1641 <sup>f</sup> | 159, 127                 |

<sup>a</sup> Compounds in bold were identified on the basis of full spectrum matching with standards and library spectra. <sup>b</sup> LRI = linear retention index in a Stable Wax column. <sup>c</sup> a, from ref 14; b, from ref 13; and c, from ref 6. <sup>d</sup> Odor description of standards. <sup>e</sup> std = authentic standard. <sup>f</sup> SIM peak found at appropriate RT but ratio different from that of standards. <sup>g</sup> Identification tentative.



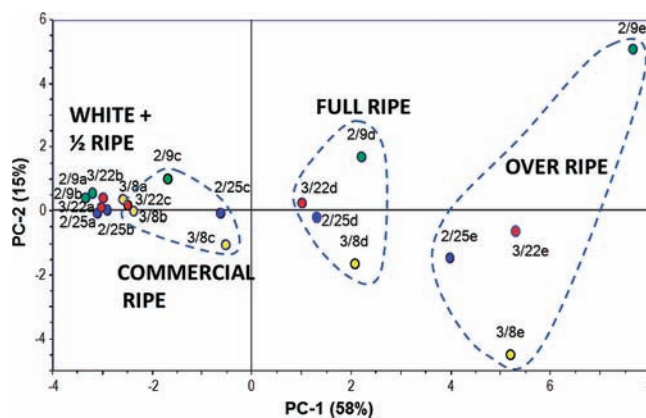
**Figure 4.** Concentrations of methyl thioacetate and methyl thiohexanoate (graphed at  $100\times$  actual concentration) in 'Strawberry Festival' at five different maturity stages from four different harvest dates. Harvest dates designated by month/day. Maturity stages designated by letter: a, white; b, half red; c, three-quarter red/commercial ripe; d, full ripe; and e, overripe.

**Harvest Date Sulfur Volatile Differences.** There was a general trend of increasing sulfur volatile concentrations for the first three harvest dates. This is clearly shown in Figure 4. Additional examples can be seen from the data in Table 2. Sulfur volatile concentrations were consistently lower at the fourth harvest date for almost all volatiles and most maturity stages, because of heavy rains and cool weather after the third harvest (between March 8 and March 22, 2010).

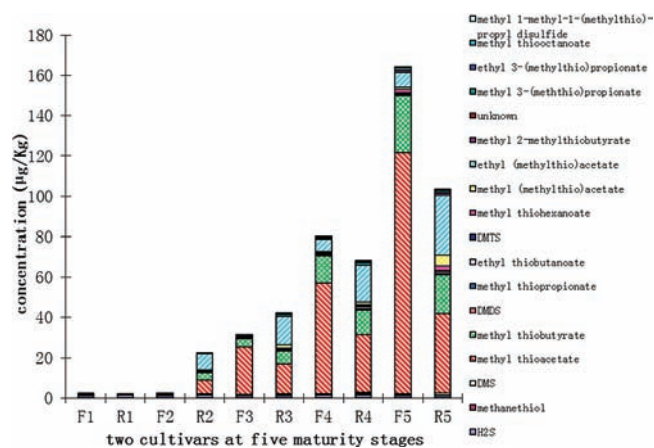
Variance in sulfur volatile concentrations from different harvest dates was obvious, as compared at the same stage during four harvest dates. It is well-documented that fruit volatile profiles are affected by environmental factors, such as growing seasons, weather, and temperature.<sup>17</sup> In strawberry, although volatile sulfur changes at fruit maturity stages have not been reported, it has been reported that ester concentrations of eight Florida strawberries are highly affected by harvest dates.<sup>18</sup>

**Sulfur Volatile Composition at Five Maturity Stages.** Most fruit flavor volatiles are secondary metabolites and absent during the early stages of fruit formation. This is also true for strawberry sulfur volatiles and clearly shown in Table 2. Only hydrogen sulfide, methanethiol, and dimethyl sulfide were detected in the most immature (white) maturity stage. Concentration changes shown in Figure 4 were typical of most sulfur volatiles in this study, where concentrations were very low or zero for the two most immature stages. Even at three-quarter red (commercial or shipping ripe), sulfur volatile concentrations were low. However, at full ripe and overripe maturity stages, concentrations increased exponentially. This is one of the first studies of sulfur volatile changes during fruit maturity.

On the basis of the data in Table 2, volatile strawberry sulfur changes during fruit maturity could be separated into two groups. The first group consists of those volatiles whose concentration was relatively constant at all five maturity stages. Included in this group were hydrogen sulfide and methanethiol. Both hydrogen sulfide and methanethiol have been previously reported in mature strawberry fruits.<sup>14</sup> In durian, hydrogen sulfide was synthesized in the mature fruit. There was little or no hydrogen sulfide in unripe durian.<sup>18</sup> In this study, both compounds were



**Figure 5.** PCA score plot of 'Strawberry Festival' sulfur volatiles at five maturity stages and four harvest dates. Maturity stages designated by letter: a, white; b, half red; c, three-quarter red/commercial ripe; d, full ripe; and e, overripe. Harvest dates designated by month/day: green, 2/9; blue, 2/25; yellow, 3/8; and red, 3/22.



**Figure 6.** Relative distribution of sulfur volatiles of 'Strawberry Festival' (F) and 'Florida Radiance' (R) at five maturity stages harvested on March 8, 2010: 1, white; 2, half red; 3, three-quarter red or commercial ripe; 4, full ripe; 5, overripe.

found in the most immature stage (white) and all other stages. They might also be responsible for the "offensive" aroma of immature fruits because there are few other volatiles to mask their presence.

The second group consists of those compounds whose concentrations are strongly influenced by the maturity state. This group consists of dimethyl sulfide, dimethyl disulfide, dimethyl trisulfide, methyl thioacetate, methyl thiobutanoate, methyl thiopropionate, ethyl thiobutanoate, methyl thiohexanoate, methyl (methylthio)acetate, ethyl (methylthio)acetate, methyl 2-(methylthio)butyrate, methyl 3-(methylthio)propionate, ethyl 3-(methylthio)propionate, and methyl thiooctanoate. These sulfur volatiles were essentially absent at the early maturity stages, such as the white and half red stages. They were found at low levels at the three-quarter red stage (commercial ripe) and increased dramatically between commercial ripe, full ripe, and overripe stages.

**PCA.** Shown in Figure 5 is the PCA score plot for the 'Strawberry Festival' sulfur volatile data. Principal component 1 (PC1) accounted for 58% of the total variance, and principal

**Table 3. Concentrations of Sulfur Volatiles in ‘Strawberry Festival’ Strawberry at Five Maturity Stages from Four Harvest Dates ( $\mu\text{g}/\text{kg}$ )<sup>4a</sup>**

| RI <sup>b</sup> | compounds                        | date        | white     | half red | three-quarter red | full ripe | overripe  |
|-----------------|----------------------------------|-------------|-----------|----------|-------------------|-----------|-----------|
| 457             | hydrogen sulfide <sup>c</sup>    | February 9  | 0.48 fgh  | 0.33 i   | 0.56 efg          | 0.66 de   | 1.04 c    |
|                 |                                  | February 25 | 0.44 ghi  | 0.39 hi  | 0.55 efg          | 0.57 efg  | 0.57 defg |
|                 |                                  | March 8     | 1.13 bc   | 1.12 bc  | 1.12 c            | 1.21 ab   | 1.06 c    |
|                 |                                  | March 22    | 0.56 efg  | 0.7 d    | 0.56 efg          | 0.59 def  | 1.29 a    |
| 510             | methanethiol <sup>c</sup>        | February 9  | 0.28 fgh  | 0.22 gh  | 0.48 bcd          | 0.55 b    | 0.72 a    |
|                 |                                  | February 25 | 0.28 fgh  | 0.3 efg  | 0.47 bcd          | 0.44 bcde | 0.39 cdef |
|                 |                                  | March 8     | 0.48 bcd  | 0.46 bcd | 0.46 h            | 0.47 bc   | 0.22 gh   |
|                 |                                  | March 22    | 0.37 defg | 0.48 bcd | 0.44 bcde         | 0.51 bcd  | 0.72 a    |
| 678             | dimethyl sulfide                 | February 9  | 0.14 ef   | 0.11 f   | 0.39 def          | 1.01 b    | 1.41 a    |
|                 |                                  | February 25 | 0.16 ef   | 0.14 ef  | 0.61 cd           | 0.73 bcd  | 0.82 bc   |
|                 |                                  | March 8     | 0.22 ef   | 0.23 ef  | 0.23 def          | 0.51 cde  | 0.72 bcd  |
|                 |                                  | March 22    | 0.17 ef   | 0.21 ef  | 0.26 ef           | 0.79 bc   | 1.36 a    |
| 1056            | methyl thioacetate               | February 9  | 0.00 j    | 0.00 j   | 4.36 i            | 37.54 e   | 182.55 a  |
|                 |                                  | February 25 | 0.00 j    | 3.15 ij  | 17.77 g           | 50.97 d   | 125.72 b  |
|                 |                                  | March 8     | 0.00 j    | 0.00 j   | 23.42 f           | 54.95 d   | 119.57 b  |
|                 |                                  | March 22    | 0.00 j    | 0.00 j   | 2.58 ij           | 12.20 h   | 80.05 c   |
| 1208            | methyl thiobutyrate              | February 9  | 0.00 i    | 0.00 i   | 1.03 i            | 5.24 f    | 16.89 c   |
|                 |                                  | February 25 | 0.00 i    | 0.00 i   | 2.05 h            | 7.91 e    | 27.07 a   |
|                 |                                  | March 8     | 0.00 i    | 0.00 i   | 4.08 g            | 13.31 d   | 28.39 a   |
|                 |                                  | March 22    | 0.00 i    | 0.00 i   | 0.00 i            | 2.20 h    | 19.90 b   |
| 1081            | dimethyl disulfide               | February 9  | 0.00 b    | 0.00 b   | 0.04 b            | 0.08 b    | 9.19 a    |
|                 |                                  | February 25 | 0.03 b    | 0.04 b   | 0.07 b            | 0.08 b    | 0.09 b    |
|                 |                                  | March 8     | 0.03 b    | 0.04 b   | 0.08 b            | 0.13 b    | 0.14 b    |
|                 |                                  | March 22    | 0.03 b    | 0.03 b   | 0.03 b            | 0.08 b    | 0.16 b    |
| 1129            | methyl thiopropionate            | February 9  | 0.00 i    | 0.00 i   | 0.08 i            | 0.15 h    | 0.21 ef   |
|                 |                                  | February 25 | 0.00 i    | 0.07 i   | 0.17 gh           | 0.25 de   | 0.32 ab   |
|                 |                                  | March 8     | 0.00 i    | 0.05 i   | 0.21 fg           | 0.36 a    | 0.30 bc   |
|                 |                                  | March 22    | 0.00 i    | 0.00 i   | 0.06 i            | 0.23 ef   | 0.27 cd   |
| 1237            | ethyl thiobutanoate <sup>d</sup> | February 9  | 0.00 e    | 0.06 e   | 0.07 e            | 0.24 d    | 0.47 bc   |
|                 |                                  | February 25 | 0.00 e    | 0.05 e   | 0.22 d            | 0.41 bc   | 0.53 abc  |
|                 |                                  | March 8     | 0.00 e    | 0.00 e   | 0.19 d            | 0.55 ab   | 0.69 a    |
|                 |                                  | March 22    | 0.00 e    | 0.00 e   | 0.05 e            | 0.15 de   | 0.40 c    |
| 1401            | dimethyl trisulfide              | February 9  | 0.00 g    | 0.00 g   | 0.04 fg           | 0.08 d    | 0.15 b    |
|                 |                                  | February 25 | 0.03 g    | 0.03 g   | 0.06 ef           | 0.07 d    | 0.11 c    |
|                 |                                  | March 8     | 0.03 g    | 0.04 g   | 0.08 d            | 0.11 c    | 0.18 a    |
|                 |                                  | March 22    | 0.03 g    | 0.00 g   | 0.04 g            | 0.07 de   | 0.12 bc   |
| 1416            | methyl thiohexanoate             | February 9  | 0.00 h    | 0.00 h   | 0.06 gh           | 0.35 e    | 0.52 d    |
|                 |                                  | February 25 | 0.00 h    | 0.00 h   | 0.11 fg           | 0.27 e    | 0.94 c    |
|                 |                                  | March 8     | 0.00 h    | 0.00 h   | 0.13 f            | 0.29 e    | 1.83 a    |
|                 |                                  | March 22    | 0.00 h    | 0.00 h   | 0.03 h            | 0.16 f    | 1.24 b    |
| 1424            | methyl (methylthio)acetate       | February 9  | 0.00 d    | 0.00 d   | 0.26 cd           | 0.62 bcd  | 1.95 a    |
|                 |                                  | February 25 | 0.00 d    | 0.00 d   | 0.47 cd           | 0.61 bcd  | 0.92 bc   |
|                 |                                  | March 8     | 0.00 d    | 0.00 d   | 0.40 cd           | 0.70 bcd  | 0.94 bc   |
|                 |                                  | March 22    | 0.00 d    | 0.00 d   | 0.16 d            | 1.34 b    | 1.34 b    |
| 1453            | ethyl (methylthio)acetate        | February 9  | 0.00 d    | 0.00 d   | 0.00 d            | 6.89 c    | 11.96 a   |
|                 |                                  | February 25 | 0.00 d    | 0.00 d   | 0.00 d            | 5.54 c    | 9.47 b    |
|                 |                                  | March 8     | 0.00 d    | 0.00 d   | 0.00 d            | 6.03 c    | 7.41 c    |
|                 |                                  | March 22    | 0.00 d    | 0.00 d   | 0.00 d            | 5.90 c    | 10.20 b   |
| 1461            | methyl 2-methylthiobutyrate      | February 9  | 0.00 i    | 0.06 fg  | 0.05 gh           | 0.06 f    | 0.06 fg   |
|                 |                                  | February 25 | 0.00 i    | 0.06 f   | 0.06 f            | 0.07 f    | 0.28 b    |
|                 |                                  | March 8     | 0.00 i    | 0.05 gh  | 0.05 fg           | 0.08 e    | 0.96 a    |
|                 |                                  | March 22    | 0.00 i    | 0.04 hi  | 0.05 gh           | 0.09 d    | 0.15 c    |

Table 3. Continued

| RI <sup>b</sup> | compounds                         | date        | white  | half red | three-quarter red | full ripe | overripe |
|-----------------|-----------------------------------|-------------|--------|----------|-------------------|-----------|----------|
| 1506            | unknown <sup>c</sup>              | February 9  | 0.00 c | 0.00 c   | 0.00 c            | 0.05 b    | 0.05 b   |
|                 |                                   | February 25 | 0.00 c | 0.00 c   | 0.05 b            | 0.05 b    | 0.07 a   |
|                 |                                   | March 8     | 0.00 c | 0.00 c   | 0.00 c            | 0.00 c    | 0.05 b   |
|                 |                                   | March 22    | 0.00 c | 0.00 c   | 0.00 c            | 0.06 b    | 0.05 b   |
| 1542            | methyl 3-(methylthio)propionate   | February 9  | 0.00 f | 0.31 f   | 0.67 de           | 2.51 b    | 3.29 a   |
|                 |                                   | February 25 | 0.00 f | 0.34 ef  | 0.48 ef           | 0.65 de   | 0.53 ef  |
|                 |                                   | March 8     | 0.00 f | 0.00 f   | 0.46 ef           | 0.80 d    | 1.39 c   |
|                 |                                   | March 22    | 0.00 f | 0.00 f   | 0.33 ef           | 1.40 c    | 1.71 c   |
| 1574            | ethyl 3-(methylthio)propionate    | February 9  | 0.00 d | 0.00 d   | 0.00 d            | 0.34 c    | 2.90 a   |
|                 |                                   | February 25 | 0.00 d | 0.00 d   | 0.00 d            | 0.23 d    | 0.35 b   |
|                 |                                   | March 8     | 0.00 d | 0.00 d   | 0.00 d            | 0.00 d    | 0.00 d   |
|                 |                                   | March 22    | 0.00 d | 0.00 d   | 0.00 d            | 0.27 c    | 0.30 c   |
| 1647            | methyl thiooctanoate <sup>f</sup> | February 9  | 0.00 e | 0.00 e   | 0.05 d            | 0.11 b    | 0.16 a   |
|                 |                                   | February 25 | 0.00 e | 0.00 e   | 0.00 e            | 0.06 d    | 0.08 c   |
|                 |                                   | March 8     | 0.00 e | 0.00 e   | 0.04 de           | 0.05 d    | 0.07 c   |
|                 |                                   | March 22    | 0.00 e | 0.00 e   | 0.00 e            | 0.00 e    | 0.05 d   |

<sup>a</sup>The same letter means no significant difference between means of each compound at five maturity stages from four harvested dates using Tukey's honest significant difference (HSD) test at  $p < 0.05$ . <sup>b</sup>RI = retention index in a Stable Wax column. <sup>c</sup>Concentration estimated by the internal standard of allyl isothiocyanate. <sup>d</sup>Concentration estimated using the response factor of methyl thiopropionate. <sup>e</sup>Concentration estimated using methyl 2-methylthiobutyrate. <sup>f</sup>Concentration estimated using methyl thiooctanoate.

component 2 (PC2) accounted for an additional 15% variance. Loading values indicated that PC1 was primarily influenced by methyl thioacetate (+) and CS2 (−) values. In a similar fashion, PC2 was influenced most strongly by ethyl 3-(methylthio)propanoate (+) and methyl 2-methylthiobutyrate (−) values. Individual PC1 score values increased with an increasing maturity stage at all four harvest dates, as evidenced by the progression of data points from left to right in Figure 5. It is clear that the overripe (OR) berry volatile group on the right-hand side (positive PC1 values) of the plot are completely separate from all of the other maturity stage values. Full ripe is also completely separated from other maturity stages. However, commercial ripe (dates ending in “c”) is slightly overlapping in maturity with a single value of the half red group. The most immature groups (white and half red) were not separated and could not be differentiated using sulfur volatiles. These results indicated that sulfur volatile concentrations were appreciably different among the later three fruit developmental stages, with the largest differences at the later maturity stages.

**Differences between ‘Strawberry Festival’ and ‘Florida Radiance’.** The average content of individual volatile sulfides in ‘Strawberry Festival’ and ‘Florida Radiance’ from four harvest dates at five fruit maturity stages are presented as stacked bars in Figure 6. Although total volatiles were similar in the first four maturity stages, ‘Strawberry Festival’ had approximately 60% greater total sulfur volatiles than ‘Florida Radiance’ at the overripe stage. Individual sulfur volatiles were profoundly different at almost every maturity stage. As seen in Figure 6 and Table 3, only small concentrations of volatile sulfur compounds are present at the most immature stage (white, 1), consisting primarily of hydrogen sulfide and methanethiol. At the half red stage (maturity stage 2), there were major differences between the two cultivars. ‘Strawberry Festival’ had much the same sulfur profile as it had in the white stage, but ‘Florida Radiance’ had appreciable amounts of sulfur esters and about 10× higher total sulfur concentrations. At the commercial ripe stage (maturity stage 3), the total amount of volatile sulfur compounds were

similar but the distribution of sulfur volatiles was profoundly different. Methyl thioacetate was the single greatest sulfur volatile in the last four maturity stages, but its relative contribution to total sulfur volatiles varied considerably between the two cultivars. In ‘Strawberry Festival’, methyl thioacetate was the dominant sulfur volatile in maturity stages 3–5, comprising 75% of all sulfur volatiles at the commercial ripe stage (stage 3) and continued to increase with increasing maturity stages, reaching the highest level at the overripe stage (stage 5), with a concentration range of 80–180 μg/kg. Even at the overripe stage, methyl thioacetate comprised 73% of the total sulfur volatiles. As seen in Figure 6, ‘Florida Radiance’ strawberry had a relatively smaller portion of methyl thioacetate, ranging from 30 to 38% of total volatiles in stages 3–5. This cultivar was characterized as having lower methyl thioacetate concentrations but higher methyl thiobutyrate and ethyl (methylthio)acetate concentrations compared to ‘Strawberry Festival’. ‘Florida Radiance’ had almost 4 times as much ethyl (methylthio)acetate compared to ‘Strawberry Festival’ at the overripe stage.

Methyl thioacetate and methyl thiobutanoate have thresholds of approximately 5 μg/kg.<sup>19</sup> Therefore, according to the values in Table 3, these two compounds might contribute aroma notes at the fully ripe and overripe stages in both cultivars because their concentrations exceed their thresholds. They will probably make a stronger contribution in ‘Florida Radiance’ because of the higher concentrations of these volatiles in this cultivar.

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