Volatile Sulfides in Headspace Gases of Fresh and Processed Citrus Juices

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Hydrogen sulfide was present in parts per million in headspace gases above fresh oranges, grapefruit, tangerine, lemon, lime, tangelo, and tangor juices. Variations were studied in hydrogen sulfide content as related to time after juice extraction and the harvesting season. The most significant changes were found after fruit had been freeze-damaged. Traces of other volatile sulfur compounds present in a few fresh juices were tentatively identified as dimethyl sulfide, methanethiol, and dimethyl disulfide. No hydrogen sulfide was detected in processed juices, but dimethyl sulfide was found in canned orange and grapefruit juices and glass-packed orange juice. On the basis of the amounts present, hydrogen sulfide is probably important to aroma of fresh juices, and dimethyl sulfide is probably important to both aroma and off-flavor of some processed juices.

When citrus juices are first expressed, they manifest a unique "fresh-squeezed" flavor that soon changes either on standing or after heat treatment. Addition of volatile flavor fractions recovered during processing, such as aqueous essence or essence oil, restores some of this unique character, but the complete unique flavor of fresh juice has not been duplicated in processed citrus products. More information on the volatile flavor components present in fresh citrus juices can enhance an understanding of the basis for these unique flavor attributes. Then, if necessary, processes can be changed so that more of these volatile components can be either retained during processing or recovered and added back to the products. Also, the identification of volatile off-flavor components formed during processing can help to improve product quality by suggesting methods for their removal or for preventing their formation.

In fresh citrus, volatile sulfides are a class of compounds that has received relatively little attention. Hydrogen sulfide was first identified in citrus juices by Kirchner et al. (1950), who estimated its level at 1.6 ppm in fresh orange juice and 0.9 ppm in fresh grapefruit juice. Later, Kirchner and co-workers identified traces of hydrogen sulfide in fresh and canned orange and grapefruit juices (Kirchner and Miller, 1957; Kirchner et al., 1953). Despite many studies using gas chromatography (GC) in the ensuing years for volatile flavor constituents of orange and grapefruit, volatile sulfur compounds were not reported again in juices or flavor fractions from citrus fruit until 1967, when Wolford and Attaway (1967) showed several to be present in aqueous orange essence but did not identify individual components. More recently, Imagawa et al. (1974) identified hydrogen sulfide and dimethyl sulfide as constituents of fresh and concentrated Satsuma mandarin juices. Sawamura et al. (1976, 1977) reported hydrogen sulfide to be present in eight varieties of citrus juices and dimethyl sulfide to be presnt in two of these (Satsuma mandarin and Valencia orange). These workers showed that the level of dimethyl sulfide increased with heating of Satsuma mandarin juice and that degradation of methionine sulfonium salt was the source for the increased dimethyl sulfide in heated juice (Sawamura et al., 1978). In an earlier study at our laboratory, we reported traces of hydrogen sulfide, dimethyl sulfide, and several other volatile sulfur compounds in headspace gases above fresh orange and grapefruit juices (Shaw et al., 1980).

We now report the presence of hydrogen sulfide in headspace gases above fresh juice from all major citrus cultivars and several hybrids. The variation in quantity of hydrogen sulfide present during the harveting season is reported for several orange and grapefruit cultivars. The presence of dimethyl sulfide in certain samples of processed single-strength orange and grapefruit juice samples is also reported.

EXPERIMENTAL SECTION

To avoid confusing changes which might occur due to differences in tree location, geography, climatic factors, etc., we selected and marked individual trees of each variety for sampling during the entire study. These included the following: Hamlin, Temple, Pineapple, Valencia, and Navel oranges; Marsh, Star Ruby, and Duncan grapefruit; Dancy tangerines; Eureka lemon; Persian and Key limes. For each of these varieties, the same tree was sampled throughout the study. All other fresh fruit and all processed citrus products were purchased from a local market. Samples were analyzed within 1 day of picking.

Preparation of Juice Samples for Headspace Analyses. Fresh juice samples were prepared by hand reaming one to six washed fruit. Within 1 min after juicing, 100 mL of freshly extracted juice was poured into a 125-mL Erlenmeyer flask capped with a cork stopper through which an 8-mm glass tube had been inserted (headspace volume 40 mL). A rubber septum was attached to the upper end of the glass tube for removal of headspace gas samples by a gas syringe. Processed single-strength juices were sampled by pouring 100 mL of juice from a freshly opened container into the 125-mL Erlenmeyer flask. Frozen concentrated juices were reconstituted to single-strength juice with deionized water and sampled in the same way. A 100-mL sample of the deionized water, tested as a blank, showed no traces of volatile sulfur compounds when sampled in the same manner as the juice samples.

Headspace Sampling from a Commercial Juice Processing Plant. Duplicate 10-mL samples were taken 30 min apart from the headspace gases above 2500 gal of fresh orange juice in an enclosed 3000-gal tank. These were analyzed within 5 min after sampling. Other analyses showed 10-mL standard samples of hydrogen sulfide could be held up to 10 min in the syringe without deterioration or change in the sample.

Preparation of Standards. Standard mixtures of either 1 or 2 ppm of H_2S or Me_2S were prepared by adding either 2 or 4 μL of the appropriate sulfide from a gas-tight syringe to a 2-L Erlenmeyer flask capped with a cork stopper through which an 8-mm glass tube with a rubber septum attached had been inserted. Each standard mixture was equilibrated for 5 min before the first sample was withdrawn, and each mixture was sampled at brief intervals for up to 60 min after preparation. Calibration curves

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were prepared at three different electrometer ranges for hydrogen sulfide and at the highest sensitivity for dimethyl sulfide. In each case recorder responses for 0.25–10.0-mL (0.25–20 nL of sulfide) samples of standard were plotted vs. concentration of the standard on log–log paper. Calibration curves were obtained which showed linear log–log relationships. Reproducibility was determined from the analysis of three 10-mL samples, each containing 10 nL of H_2S. The coefficient of variation was about 10%.

Analytical Procedures. The volatile sulfur compounds were quantitatively analyzed with a gas chromatograph equipped with a flame photometric detector as described earlier (Braman et al., 1978; Ammons, 1980; Andreae, 1980) and a Hewlett-Packard Model 3380A recording integrator. The column was a 4 mm i.d. × 40 cm glass U tube packed with 15% OV-101 on 60–80-mesh Anachrom C-22 with a 200 cm long Chromel wire (1.09 Ω/m) heating coil wrapped around the U tube and connected to a powerstat at 20 V (Ammons, 1980). Helium flow through the column was 60 mL/min, and detector gas flows were 120 mL/min for hydrogen and 80 mL/min for air. The samples for gas analyses were 10-mL portions of headspace gases withdrawn through the septum with a 10-mL gas-tight syringe. At least 20 s was allowed for the syringe to fill with headspace gases before withdrawal from the septum. The headspace gases were injected onto the gas chromatographic column over a 5-s interval, and 15 s was allowed for the gases to concentrate on the column, which was cooled in liquid nitrogen. The coolant was removed, and the heater was turned on. Retention times were H_2S, 1.0 min, MeSH, 1.9 min, Me_2S, 2.1 min, and $(MeS)_2$, 2.4 min. Concentations of hydrogen sulfide and dimethyl sulfide as low as 25 ppb could be detected by this procedure.

RESULTS AND DISCUSSION

Hydrogen Sulfide in Fresh Citrus Juices. During the first 60 min after juicing hydrogen sulfide was present in parts per million in the headspace gases of most fresh citrus juices studied (Table I). Measurements were made at 2, 8, 13, 20, 40, and/or 60 min after juicing. Generally, the hydrogen sulfide level varied somewhat throughout the 60-min period but showed no definite trend toward decreasing or increasing after juicing. Thus, results from four to six determinations were averaged. These values are listed in Table I.

In an earlier study, we had found hydrogen sulfide present in headspace gases of orange and grapefruit juices in the parts per billion range when a much larger volume of headspace gas was present (Shaw et al., 1980). In the current study, headspace gas volume was reduced 230 times (from 9200 to 40 mL) compared to that of the earlier study. This resulted in the hydrogen sulfide being concentrated enough that it could be measured by direct injection rather than the more time consuming method used earlier. That method had required preconcentration on a gold trap followed by derivatization and elution from the trap.

The average values reported in Table I show changes in hydrogen sulfide levels during a season for the different cultivars. The most striking changes in hydrogen sulfide levels occurred in Hamlin and Temple oranges and in Marsh grapefruit after freeze damage. These trees were sampled weekly from Oct 1980 through early Feb 1981 and analyzed for volatile sulfides. Severe freeze damage occurred on Jan 13, 1981. Weekly sampling was continued until virtually no fruit remained on these trees. The hydrogen sulfide level generally decreased in freeze-damaged fruit, most noticeably in the Hamlin oranges and Marsh

grapefruit. One sample of Marsh grapefruit obtained on Feb 9, 1981, that was still firm and, thus, apparently not freeze damaged, contained a higher level of hydrogen sulfide than the softer, spongy, freeze-damaged fruit.

Overripe fruit usually becomes soft and spongy and has an increased degree Brix/acid ratio similar to that of freeze-damaged fruit. Because of the similarity, we compared overripe fruit with freeze-damaged fruit for volatile sulfide content. We measured hydrogen sulfide levels in overripe fruit from Pineapple (April and May), Navel (March through May), and Valencia (June and July) oranges and Duncan grapefruit (June, soft sample, Table I). The duncan grapefruit and the July sample of Valencia orange showed a decided decrease in hydrogen sulfide (to 0.1 ppm) in overripe fruit (Table I).

A headspace sample was obtained from the fresh juice holding tank of a commercial orange juice processing plant to determine whether detectable levels of volatile sulfides were present above a large quantity of commercially extracted fresh juice. Duplicate samples contained 1.45 ppm of hydrogen sulfide, and no other sulfides could be detected.

Other cultivars monitored for volatile sulfides were Eureka lemon, Persian and Key limes, Dancy tangerine, Murcott tangor, and tangelo. All had measurable levels of hydrogen sulfide in the fresh juice. Some Murcott and tangelo samples had the lowest levels of hydrogen sulfide of all fresh juices analyzed.

Duplicate samples were measured of juices from Pineapple orange, Duncan, Pink, and Marsh grapefruit, tangor, tangelo, and Persian lime, and varying results were obtained (Table I). Some of the variation in hydrogen sulfide content among samples is possibly due to individual fruit variation.

Quantitative measurements of hydrogen sulfide were reproducible only by careful repetition of the precise sampling techniques described under Experimental Section. In addition, the needle used on the 10-mL gas-tight sampling syringe had to be free of any septum material that might partially plug the opening. Even with these precautions an occasional analysis would indicate no hydrogen sulfide present and would have to be discarded. The cause of this irregularity was not identified, but other workers have experienced the same need for careful, precise sampling techniques (Ammons, 1981).

Other Volatile Sulfur Compounds in Fresh Citrus Juices. Other volatile sulfur compounds were detected in only a few fresh juice samples, and their presence was more transient than that of hydrogen sulfide. On the basis of the GC retention times, those sulfur compounds present were most likely dimethyl sulfide, methanethiol, and dimethyl disulfide. However, verification through enrichment of headspace samples with added authentic samples was not possible because of the small quantities present or the transient nature of some components. Dimethyl sulfide, when present, was at the barely detectable level and could not be quantified. Methanethiol, if present, could readily oxidize to dimethyl disulfide and thus complicate identification by GC retention times. Partial conversion to dimethyl disulfide occurred in standard mixtures of methanethiol prepared in air. In samples of Pineapple orange juice studied on March 12, 1981, March 13, 1981, and April 6, 1981 (Table I), a GC peak was present at the retention time for methanethiol, and in the two March samples, this peak was gradually replaced by a peak at the retention time for dimethyl disulfide. However, these comopunds were not positively identified.

Table I. Hydrogen Sulfide in Headspace Gases of Fresh Citrus Juices[a]

date harvested	°Brix/acid	av[b] (4-6 determns)
Hamlin Orange		
10/21/80[c]		0.8
10/28/80	10	0.4
11/03/80		1.4
11/12/80		1.7
11/17/80		0.9
11/24/80	10	1.7
12/01/80		2.0
12/08/80		1.8
12/15/80		1.7
12/22/80	13	1.5
12/29/80		2.5
01/05/81	14	1.1
01/12/81		1.5
01/19/81	21	N
01/26/81	15	0.04
02/02/81		0.04
02/09/81	19	0.06
Pineapple Orange		
01/05/81[d]		0.7; 1.4
01/12/81	12	0.8
02/11/81	15	0.5
03/12/81[c]	17	0.2
03/13/81[c]	15	0.5; 1.1
04/06/81[c]	21	0.4
04/13/81	20	1.1
05/13/81	19	0.5
Navel Orange		
12/20/80[e]		0.4
01/05/81		0.5
01/12/81	13	1.1
02/11/81	13	0.3
03/12/81	17	2.0
04/13/81	19	1.7
05/12/81	19	1.1
Valencia Orange		
01/05/81		2.2
01/12/81	8	1.5
02/11/81	10	1.4
03/12/81	12	1.8
04/13/81	18	0.7
05/12/81	15	0.2
06/15/81	22	0.4
07/20/81	27	0.1
Temple Orange		
10/21/80[c]		1.5
10/28/80		0.7
11/03/80		1.8
11/12/80		1.2
11/17/80		2.9
11/24/80		2.6
12/01/80		2.0
12/08/80		2.2
12/15/80		1.6
12/22/80		2.6
12/29/80		1.4
01/05/81	6	1.8
01/12/81		2.5
01/19/81		1.0
01/26/81	8	0.3

date harvested	°Brix/acid	av[b] (4-6 determns)
Temple Orange		
02/02/81		0.2
02/09/81	10	1.0
03/05/81	13	1.2
Pink[e] Grapefruit		
10/20/80[c,d]		0.13; 0.17
Star Ruby Grapefruit		
02/09/81	8	0.1
Marsh Grapefruit		
11/03/80		0.9
11/17/80		1.1
11/24/80		1.6
12/01/80		0.6
12/08/80[d]		0.6; 0.9
12/15/80		2.0
12/22/80		2.2
12/29/80		2.2
01/05/81	11	1.4
01/12/81		1.4
01/19/81		0.9
01/26/81		0.3
02/02/81		0.3
02/09/81[f]	10; 9	0.3; 1.3
Duncan Grapefruit		
12/05/80		0.7
01/07/81		1.2
02/06/81[d]		1.0; 1.1
03/05/81		1.0
04/07/81	8	0.9
05/06/81	10	1.5
06/09/81 (soft)	15	0.1
06/09/81 (firm)	11	1.2
Dancy Tangerine[e]		
11/21/80		1.8
01/05/81	11	1.5
Murcott Tangor		
10/17/80[c]		0.02
10/20/80[c]		0.05
10/28/80[c,d]		N
01/15/81[e]		0.9
Tangelo[e]		
10/17/80[c,d]		0.14; 0.08
10/28/80[c]		0.01
11/03/80[c]		0.1
01/27/81		N
Eureka Lemon		
01/30/81	1.3	0.7
Persian Lime		
02/11/81[g]	1.5	0.7; 0.7
03/12/81[g]	1.7; 1.8	0.4; 0.3
Key Lime		
02/12/81	1.2	0.2
04/06/81[c]	1.5	0.4
04/13/81	1.5	0.2

[a] Sample size of 10 mL taken from 40 mL of total headspace above 100 mL of juice. [b] N = not detected. [c] Other volatile sulfides also detected but not quantified. [d] Values from two-fruit samples from same source harvested on same day. [e] Date purchased in local market—harvest date not known. [f] Second sample is from firm, juicy fruit apparently not freeze-damaged. [g] First sample from yellow-colored fruit; second sample from half green-half yellow fruit.

Dimethyl Sulfide in Processed Citrus Juices. Several processed orange, grapefruit, and tangerine juices were analyzed for volatile sulfur compounds, and hydrogen sulfide was not detected in headspace gases of any sample. However, measurable quantities of dimethyl sulfide were detected in canned orange and grapefruit juices and in a sample of glass-packed orange juice that had not been prepared from reconstituted concentrate (Table II). In all cases where dimethyl sulfide was found, the level increased as time increased when the juice was allowed to stand for 60 min in a closed container. All samples that did not contain measurable dimethyl sulfide were made from frozen concentated juice except one sample of orange juice, packed in a paper carton and specifically labeled as

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Table II. Dimethyl Sulfide in Headspace Gases of Processed Citrus Juices[a]

sample	concn of DMS present,[b] ppm, in headspace gases at		
	2 min	20 min	60 min
orange juice			
canned	1.0	2.1	2.8
glass pack[c]	3.0	3.8	4.3
glass pack[d]	N	N	N
carton	N	N	N
frozen concentrate[e]	N	N	N
grapefruit juice			
canned	0.9	1.2	1.4
frozen concentrate[e]	N	N	N
tangerine juice			
frozen concentrate[e]	N	N	N

[a] Sample size of 10 mL was taken from 40 mL of total headspace (2, 20, and 60 min after opening the container) above 100 mL of juice. [b] N = not detected. [c] Not from concentrate. [d] Reconstituted from concentrate. [e] Reconstituted to single strength with deionized water prior to sampling.

"not made from concentrate".

Formation of Sulfur Compounds. Hydrogen sulfide, dimethyl sulfide, methanethiol, and dimethyl disulfide can be formed from amino acids by enzymic degradation (Meister, 1957) which might occur in fresh juice after it has been squeezed. In many organisms, cysteine is degraded enzymically to hydrogen sulfide and methionine is degraded enzymically to methanethiol and dimethyl disulfide. Methionine can also be degraded enzymically to hydrogen sulfide through homocysteine. Both cysteine and methionine have been identified in orange juice (Rockland, 1961).

S-Methylmethionine or its sulfonium ion is a precursor of dimethyl sulfide in many foods, through either enzymic or heat degradation (Hattula and Granroth, 1974). Heat degradation would be the most likely primary source for added dimethyl sulfide in processed juices. In Satsuma mandarin juice containing dimethyl sulfide, decomposition of the S-methylmethionine sulfonium ion was shown to be the source for dimethyl sulfide, and its level was higher in processed than in fresh juice (Sawamura et al., 1978). Their results indicate dimethyl sulfide might be expected to increase in citrus juices during processing.

Flavor and Aroma Considerations. The flavor and aroma thresholds for hydrogen sulfide, dimethyl sulfide, methanethiol, and dimethyl disulfide are all in the parts per billion range or lower (Fazzalari, 1978). For hydrogen sulfide, the reported aroma threshold in air ranged from 0.18 to 4.7 ppb. Most values for hydrogen sulfide found in the headspace gases of citrus juices (Table I) were about 10^3–10^4 times higher than this threshold. Thus, hydrogen sulfide probably makes a direct contribution to flavor of fresh citrus juices (Patton and Josephson, 1957).

The reported aroma threshold for dimethyl sulfide in air was 0.33–20 ppb (Fazzalari, 1978). Quantities found in processed citrus juices that contained detectable levels of this compound (Table II) were about 10^2–10^4 times higher than this threshold range. Thus, dimethyl sulfide probably constributes to the flavor of these processed juices and may be an important contributor to off-flavor of canned citrus juices in addition to off-flavor components already identified (Tatum et al., 1975).

Methanethiol and dimethyl disulfide could not be reliably quantified in headspace gases of fresh citrus juices. However, when they were detected, they were present at 10–100 times their reported threshold levels (Fazzalari, 1978). Their transient nature, their instability, and the apparent conversion of methanethiol to dimethyl disulfide complicate attempts at assessment of their contributions to flavor.

CONCLUSIONS

Hydrogen sulfide is present in the headspace gases above fresh orange, grapefruit, tangerine, lemon, lime, tangelo, and tangor juices at a level about 1000 times its threshold level in air. Thus, it probably contributes to the aroma of fresh citrus juices. Traces of other volatile sulfur compounds were also present in a few fresh juices, apparently at above aroma threshold levels. In processed citrus juices, no hydrogen sulfide was detected, but some samples contained dimethyl sulfide at levels high enough to affect their flavors, based on the reported threshold level of this sulfide in air. Since the technique used in this study is both sensitive and rapid, it should be useful in monitoring changes in volatile sulfur compounds in headspace gases of other foods as well.

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