

## ARTICLES

Onion (*Allium cepa* L.) Thiosulfinates Respond to Increasing Sulfur Fertility

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Onions (*Allium cepa* L.) were greenhouse grown with various sulfur fertilities and assayed for changes in thiosulfinates and related compounds. The concentrations of methyl, *n*-propyl, and 1-propenyl thiosulfinates, the zwiebelanes, and the onion lachrymatory factor (LF) increased with a linear trend as sulfur fertility increased with the exception of methanesulfinothioic acid *S*-*n*-propyl ester (MeS(O)SP<sub>n</sub>) and methanesulfinothioic acid *S*-methyl ester (MeS(O)SMe). The (thiosulfinate plus zwiebelanes):LF ratio increased with increased sulfur fertility, suggesting that the former were being formed at a greater rate at higher sulfur fertility. Variation in absolute and relative concentrations of these three classes of sulfur compounds with changing S fertility could cause subtle flavor differences in onion.

**Keywords:** Onion; *Allium cepa*; flavor; fertility; sulfur; thiosulfinate

## INTRODUCTION

The chemistry and biochemistry of onion (*Allium cepa* L.) and other *Allium* spp. have been the subject of numerous reviews (Block, 1992). Upon tissue disruption, the characteristic flavor of onion results from the catabolism of endogenous *S*-alk(en)yl-L-cysteine sulfoxide (CSO) flavor precursors by the enzyme alliinase (EC 4.4.1.4). Primary products produced by this reaction include pyruvate, ammonia, and thiosulfinates. Thiosulfinates are responsible for fresh onion flavor (Freeman and Whenham, 1976) and participate in a variety of subsequent reactions to form an array of organosulfur compounds (Block, 1992).

Thiosulfinates generated by onions are dependent on the alkyl groups present in the flavor precursors. Precursors in onion are dominated by 1-propenyl-CSO, lesser amounts of the methyl-CSO, and minor amounts of *n*-propyl-CSO (Lancaster and Kelly, 1983; Thomas and Parkin, 1994). Relative concentrations of thiosulfinates and other compounds in *Allium* spp. have been reported (Block, 1992). The 1-propenyl moieties in onion, however, are underestimated because most 1-propenesulfenic acid ends up as propanethial *S*-oxide (lachrymatory factor, LF), which is lost during thiosulfinate analysis.

Flavor potential in onions is genetically determined, but environmentally influenced. Onion cultivars differ widely in pungency when grown in a single environment (Randle and Bussard, 1993). Specific environmental factors that influence flavor intensity are temperature (Platenius and Knott, 1941), irrigation (Freeman and Mossadeghi, 1973), and N-form (Gamiely et al., 1991).

Sulfur (S) fertility also influences flavor intensity (Freeman and Mossadeghi, 1970; Randle, 1992) and is of particular interest because of the S-containing flavorants in onions. Poor correlations between total bulb S concentration and pungency in onions of broad genetic background suggest that S is differentially partitioned into flavor and nonflavor compounds among cultivars (Randle, 1992). Our purpose in the present study is to determine if changes in S fertility influence the quantity and composition of onion thiosulfinates and other flavorants.

## MATERIALS AND METHODS

Seeds of cv. Southport White Globe onions were sown in Fafard No. 3 artificial medium (Fafard Co., Anderson, SC) in October of 1991. Plants were greenhouse grown in Athens, GA, under natural photoperiod (34° N latitude) with day and night temperatures set at 28 and 16 °C, respectively. Seedlings were fertilized weekly with approximately 400 mL at 200 ppm of a commercially available 20-20-20 soluble fertilizer once the true leaf emerged. In December, seedlings were transplanted to 0.75-L pots containing washed river sand in a randomized complete block design with three replications, 12 plants per replication. Five S fertility treatments were used to test the effect of low S fertility on onion thiosulfinates. S was varied in a modified Hoagland and Arnon (1950) solution by adjusting the ratio of magnesium sulfate and magnesium chloride to obtain solutions with 0.1, 0.48, 1.60, and 3.10 mequiv of S/L. An S concentration of 0.1 mequiv/L has been shown to reduce onion growth by up to 50% compared to onions grown at 3.0 mequiv/L (Freeman and Mossadeghi, 1970). Plants were watered twice weekly with approximately 100 mL of the nutrient solutions, supplemented with deionized water as needed, and the pots were leached weekly.

Plants were grown to maturity, characterized by a softening of the leaf sheath area and lodging of the foliage. Plants and bulb size were smaller than would be expected from field-grown plants due to the pot size restrictions. Plant development and maturity, however, were similar to those of field-

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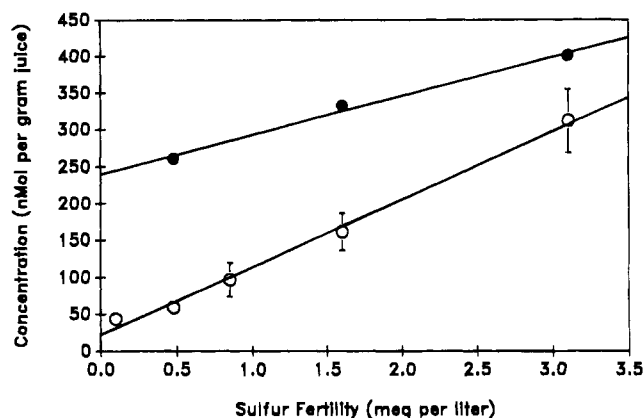
grown plants. Replications were considered harvestable when the foliage lodged on 9 of the 12 plants. All treatments were harvested between May 6 and 13, 1992. Irrigation was withheld, and the plants dried for 7 days. Bulbs were then harvested, the foliage and roots severed, and the bulbs cured in paper bags at ambient greenhouse temperatures for 7 days. Bulbs were stored at room temperature for approximately 30 days prior to compound analysis.

Juice was obtained from onion bulb quarters (from which the outer scales had been first removed) of known weight (ca. 200 g) using a Hamilton Beach Model 395W juice extractor. The previously reported flavorant isolation procedure (Block et al., 1992b) was employed, with minor changes noted here. Thus, following each juicing procedure, the juicer was rinsed with 50 mL of distilled water which was added to the juice, and after 5 min, the combined liquid was rapidly vacuum filtered through a thin pad of Celite in a Büchner funnel. To this combined liquid was added 100 mL of saturated saline, to minimize emulsion formation and salt out the LF, and the resultant liquid was extracted with fresh reagent grade ether ( $3 \times 200$  mL). The ether layers were separated using a separatory funnel, combined, dried ( $\text{MgSO}_4$ ), and concentrated *in vacuo* at a bath temperature of  $17^\circ\text{C}$ . When the concentrate had been sufficiently reduced in volume, it was transferred to a 25-mL pear-shaped flask, where the volume was further reduced to 1–1.5 mL. This liquid was placed in a 2-mL centrifuge tube and carefully concentrated at  $17^\circ\text{C}$  with a gentle stream of air to a volume of ca. 0.5 mL for analysis of LF 10 as well as compounds 1–9 or to a minimal volume if only 1–9 were to be analyzed. Benzyl alcohol (0.5–5  $\mu\text{L}$ ) was added on the basis of GC–MS analyses of representative samples (less benzyl alcohol was used for samples containing lower levels of S compounds). In view of the thermal instability of thiosulfinates and other onion flavorants, samples were analyzed immediately. If this proved impossible, samples were kept at  $-78^\circ\text{C}$  prior to analysis. Mass spectrometric ion ratio methods were used to determine concentrations of individual components. Thus, under standard operating conditions, the mass spectrometer was calibrated using authentic samples of thiosulfinates 1–7, zwiebelanes 8 and 9, and lachrymatory factor (LF) propanethial S-oxide (10), each containing known weights of benzyl alcohol, comparing the intensity of the *m/e* 107 ion of benzyl alcohol with the intensity of an ion previously determined to be characteristic of each compound 1–10 (e.g., *m/e* 73 for 1 and 4, *m/e* 75 for 2 and 6, *m/e* 88 for 3, *m/e* 96 for 5, *m/e* 95 for 7, *m/e* 99 and 113 for 8 and 9, and *m/e* 90 for 10).

Mass spectra were collected using a Hewlett-Packard 5898 mass spectrometer (MS Engine) equipped with a GC with EI analysis at 70 eV. A dual column Hewlett-Packard 5890 II GC with programmable on-column injector and cryogenic cooling ( $\text{CO}_2$ ) was used for GC separations. Data processing was achieved using an HP/Apollo 400 series computer employing standard Hewlett-Packard HP UX Chemstations software. GC separations were accomplished using a 15 m  $\times$  0.54 mm i.d. J&W Scientific DB-1 (Durabond) column or a 30 m  $\times$  0.53 mm i.d. HP-5 (cross-linked 5% phenyl methyl silicone gum) column, using 99.999% helium as a carrier gas. The temperature profiles employed were as follows:  $0\text{--}200^\circ\text{C}$  at  $5^\circ\text{C}/\text{min}$ , injector under oven tracking control, transfer line at  $100^\circ\text{C}$ , and a column head pressure of 5 psi; or  $0^\circ\text{C}$  hold for 5 min to  $200^\circ\text{C}$  at  $2^\circ\text{C}/\text{min}$ , injector under oven tracking control, transfer line at  $80^\circ\text{C}$ , and a column head pressure of 10 psi. The MS source and quadrupole magnet temperatures were maintained at 200 and  $100^\circ\text{C}$ , respectively. Samples were analyzed in duplicate, and average variation between runs was  $\pm 7\%$ . Data were analyzed using the GLM and REG procedures of SAS (Cary, NC).

## RESULTS AND DISCUSSION

All plants given 0.1 mequiv of S/L and several plants given 0.48 mequiv of S/L produced S-deficiency symptoms during active bulbing. Total moles of 1–9 (thiosulfinates 1–7, zwiebelanes 8 and 9) as well as moles of LF 10 increased linearly ( $p \geq 0.01$ ) with increasing



**Figure 1.** Total moles of assayed thiosulfinates plus zwiebelanes ( $\circ$ ;  $Y = 22.1 + 91.6X$ ) and lachrymatory factor ( $\bullet$ ;  $Y = 239.8 + 53.0X$ ) from cv. Southport White Globe onions grown at different sulfur fertility concentrations.

S fertility (Figure 1), with the slope of the LF (10) increase ( $Y = 239.8 + 53.0X$ ) smaller than that of total moles of 1–9 ( $Y = 22.1 + 91.6X$ ).

The concentrations of the thiosulfinates and zwiebelanes 1–9 in extracts of onions generally increased with a linear trend ( $p \geq 0.01$ ) with increasing S fertility (Table 1). The exceptions were 6, which responded with a quadratic trend ( $p \geq 0.01$ ), and 7, which showed no trend in response to increasing S fertility. While moles of total Me–, MeCH=CH–, and *n*-Pr–, summed from 1–9, increased with a linear trend with increasing S fertility, their ratios varied. As S fertility increased, total MeCH=CH– increased in concentration relative to total Me– and *n*-Pr–. At 0.1 mequiv S fertility, the ratio of Me–:MeCH=CH–:*n*-Pr– was 1.2:0.9:1, while at 0.48 mequiv of S it was 1.3:1.3:1. At 0.85 mequiv of S it was 1.4:1.8:1, at 1.6 mequiv of S it was 1.4:2.2:1, and at 3.1 mequiv of S it was 1.5:2.5:1. Total Me– increased slightly relative to *n*-Pr– with increasing S fertility. The observed increase in the concentrations of 1–9 is consistent with their origin from common precursor compounds (e.g.,  $\text{RS(O)CH}_2\text{CH(NH}_2\text{)COOH}$ , 11) when analyzed from onions grown at similar S fertility (Randle and Lancaster, 1993).

The LF (10) also increased with a linear trend ( $p \geq 0.05$ ) to increasing S fertility. An interesting trend is noted if the relative increases in the amounts of 1–9 with increasing S fertility are compared to the increase of 10. At higher levels of S fertility, the (thiosulfinates + zwiebelanes)/LF ratio ( $\Sigma 1\text{--}9/10$ ) increases, as also seen by comparing the slopes of the lines in Figure 1. While additional data to verify this observation would be desirable, this trend may simply reflect the local concentrations of sulfenic acids, which should increase on cutting onions grown at higher S fertility, favoring more efficient formation of the thiosulfinates from bimolecular condensation of the sulfenic acid precursor relative to the unimolecular rearrangement of 1-propenesulfenic acid to the LF.

Our work constitutes the first attempt to closely examine the variation of key onion flavorants with levels of S fertility. The only significant flavorants whose variations were not monitored were MeCH=CHS(O)-SP $\text{r-n}$  and OSCHCHMeCHMeCHSO, neither of which survives GC–MS analysis; however, on the basis of previous HPLC work, the levels of both of these compounds should be similar to that of MeCH=CHS(O)-Pr-*n* (Block, 1992a). The qualitative and quantitative data presented here for onions grown under conditions

**Table 1. Thiosulfinates and Related Compounds from Extracts of Cv. Southport White Globe Onions, Greenhouse Grown, and Receiving Sulfur Fertility of 0.1, 0.48, 0.85, 1.6, and 3.1 mequiv of S/L of a Modified Hoagland Solution (Determinations Were Made by GC-MS, and Concentrations Are in Nanomoles per Gram of Juice)**

compound <sup>a</sup>	S fertility					significance	
	0.1	0.48	0.85	1.60	3.10	linear	quadratic
(1) <i>n</i> -PrS(O)S-1-Propenyl-( <i>E,Z</i> )	5.6	8.2	20.0	32.5	67.0	**	ns
(2) <i>n</i> -PrS(O)SP <i>n</i>	8.0	8.6	10.0	13.0	17.5	**	ns
(3) MeSS(O)-1-Propenyl-( <i>E</i> )	6.8	10.3	30.5	44.5	94.5	**	ns
(4) MeS(O)S-1-Propenyl-( <i>E,Z</i> )	10.0	18.3	21.5	34.5	67.0	**	ns
(5) MeSS(O)Pr- <i>n</i>	3.4	4.1	5.7	7.1	17.1	**	ns
(6) MeS(O)SP <i>n</i>	2.9	3.0	3.5	4.3	4.2	ns	**
(7) MeS(O)SMe	5.7	3.6	3.8	4.3	2.8	ns	ns
(8) <i>cis</i> -zwiebelane	0.6	2.5	6.8	16.0	31.0	**	ns
(9) <i>trans</i> -zwiebelane	0.2	0.6	2.5	5.0	10.5	**	ns
(10) EtCH=SO (LF)	ND <sup>b</sup>	261.0	ND <sup>b</sup>	332.0	401.0	*	ns
total Me- <sup>c</sup>	34.5	42.9	68.8	99.1	188.4	**	ns
total MeCH=CH- without 10 <sup>c,d</sup>	24.0	43.0	90.6	153.5	311.5	**	ns
total MeCH=CH- with 10 <sup>c,d</sup>	ND <sup>b</sup>	304.0	ND <sup>b</sup>	485.5	712.5	**	ns
total <i>n</i> -Pr- <sup>c</sup>	27.9	32.5	49.2	70.0	123.3	**	ns
total except 10	43.2	59.2	96.6	161.3	311.6	**	ns
total with 10	ND <sup>b</sup>	320.2	ND <sup>b</sup>	493.3	712.6	**	ns
(Σ1-9)/10 <sup>e</sup>	ND <sup>b</sup>	0.23	ND <sup>b</sup>	0.49	0.78		

<sup>a</sup> Chemical Abstracts Service names of compounds (provided by the author): 1, 1-propanesulfinothioic acid *S*-(*E,Z*)-1-propenyl ester; 2, 1-propanesulfinothioic acid *S*-1-propyl ester; 3, (*E*)-1-propenesulfinothioic acid *S*-methyl ester; 4, methanesulfinothioic acid *S*-(*Z,E*)-1-propenyl ester; 5, 1-propanesulfinothioic acid *S*-methyl ester; 6, methanesulfinothioic acid *S*-1-propyl ester; 7, methanesulfinothioic acid *S*-methyl ester; 8, (1 $\alpha$ ,2 $\beta$ ,3 $\beta$ ,4 $\alpha$ ,5 $\beta$ )-2,3-dimethyl-5,6-dithiabicyclo[2.1.1]hexane 5-oxide; 9, ( $\pm$ )-(1 $\alpha$ ,2 $\alpha$ ,3 $\beta$ ,4 $\alpha$ ,5 $\alpha$ )-2,3-dimethyl-5,6-dithiabicyclo[2.1.1]hexane 5-oxide; 10, propanethial *S*-oxide. The presence and concentrations of *n*-PrSS(O)-1-propenyl-(*Z,E*), and OS=CHCH(Me)CH(Me)CH=SO were not determined. These compounds have been previously detected in onion extracts using HPLC conditions (Block et al., 1992a); they do not survive GC-MS analysis. <sup>b</sup> ND, not determined, but should be substantial. <sup>c</sup> Sum of the concentrations of each alkyl group. Contributions toward the total for each alkyl group are doubled when the same alkyl group occurs twice in the same molecule (e.g., 2 in the case of *n*-Pr, 7 in the case of Me, and 8/9 in the case of MeCH=CH; in the latter case, MeCH=CHS(O)SCH=CHMe is assumed to be the immediate precursor (Block, 1992) so 8/9 are included in calculating total MeCH=CH-. <sup>d</sup> Given without 10; with 10. <sup>e</sup> Sum of the concentrations of 1-9 divided by the concentration of 10. <sup>f</sup> ns, \*, \*\*, nonsignificant or significant at *p* = 0.05 or 0.01, respectively.

of midrange S fertility (e.g., 1.6 mequiv of S) are similar to those previously reported for store-purchased onions (Block et al., 1992a).

Different organosulfur components contribute unique odor and taste to the onion (Block, 1992). Sensory information on 1-10 at concentrations similar to those reported here includes the following: 1 has a green, raw fresh onion flavor with a 0.1 ppm threshold; 2 has a fresh onion, chive, and green onion flavor with a 0.5 ppm threshold; 3 has a cabbage, slight radish, meaty sulfur flavor with a 0.01 ppm threshold; 4 has a sulfur, cabbage, shallot-like, creamy flavor with a 0.01 ppm threshold, becoming more onion-like and livery/meaty sulfur, slightly cabbage-like at 0.5-1.0 ppm; 5/6 have creamy, cabbage/sulfur flavors with slight green notes with a 0.5 ppm threshold; 7 has a creamy, sulfury, cabbage, broccoli, cauliflower-like, green-onion-like flavor with a 0.1 ppm threshold; 8 imparts a sweet or brown sauté taste with liver and hydrogen sulfide notes with a 0.5 ppm threshold; 9 has a green or raw onion and sweet sulfur taste with a 0.1 ppm threshold; 10 has onion, sulfury, rubbery notes with some tongue burn at 0.1-0.5 ppm and sulfur, slight green, gaseous, livery, mouth burn/heat at 1-2 ppm.

In conclusion, total assayed volatile organosulfur concentration depended on S fertility. Individual compounds 1-10 generally increased with a linear trend with increasing S fertility with the exceptions of 6 and 7. The ratio of total methyl:1-propenyl:*n*-propyl concentrations summed from 1-9 changed with S fertility. Total 1-propenyl from 1-9 was the minor radical at low S fertility but became the dominant radical with higher S fertility. Changes in thiosulfinate concentration and composition due to S fertility could influence onion flavor.

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