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Quantifying Onion Flavor Compounds Responding to Sulfur Fertility-Sulfur Increases Levels of Alk(en)yl Cysteine Sulfoxides and Biosynthetic Intermediates

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Abstract. **Three onion *(Allium cepa* L.) cultivars were grown to maturity at five S fertility levels and analyzed for S-alk(en)yl-L-cysteine sulfoxide (ACSO) flavor precursors, γ– glutamyl peptide (γ– GP) intermediates, bulb S, pyruvic acid, and soluble solids content. ACSO concentration and composition changed with S fertility, and the response was cultivar dependent. At S treatments that induced S deficiency symptoms during active bulbing, (+)S-methyl-L-cysteine sulfoxide was the dominant flavor precursor, and the flavor pathway was a strong sink for available S. As S fertility increased to luxuriant levels, trans(+)-S-(1-propenyl)-L-cysteine sulfoxide (PRENCSO) became the dominant ACSO. (+)S-propyl-L-cysteine sulfoxide was found in low concentration relative to total ACSO at all S fertility treatments. With low S fertility, S rapidly was metabolized and low γ– GP concentrations were detected. As S fertility increased, γ– GP increased, especially γ– L-glutamyl-S-(1-propenyl)-L-cysteine sulfoxide, the penultimate compound leading to ACSO synthesis. Nearly 95% of the total bulb S could be accounted for in the measured S compounds at low S fertility. However, at the highest S treatment, only 40 % of the total bulb S could be attributed to the ACSO and γ– GP, indicating that other S compounds were significant S reservoirs in onions. Concentrations of enzymatically produced pyruvic acid (EPY) were most closely related to PRENCSO concentrations. Understanding the dynamics of flavor accumulation in onion and other vegetable *Alliums* will become increasing important as the food and phytomedicinal industries move toward greater product standardization and characterization.**

The characteristic flavor and aroma of plants in the family *Alliaceae* result from the enzymatic hydrolysis of the S-alk(en)yl-L-cysteine sulfoxides (ACSO) to produce volatile S compounds and the by-products pyruvic acid and ammonia. Four ACSOs have been identified in *Allium*, and the flavor variation among species is due to differences in ACSO composition and concentration (Block, 1992). Onions, the most important edible allium worldwide, have three ACSOs. Trans-(+)-S-(1-propenyl)-L-cysteine sulfoxide (PRENCSO) is normally found in the highest concentration, while (+)-S-methyl-L-cysteine sulfoxide (MCSO) and (+)-S-propyl-L-cysteine sulfoxide (PCSO) are found in lower concentrations (Lancaster and Boland, 1990). Occasionally, however, PCSO has not been detected in onion (patikkala and Virtanen, 1967; Thomas and Parkin, 1994). Thiopropanal S-oxide, the lachrymatory factor (LF) in onions, is formed from transient 1-propenyl sulfenic acid following PRENCSO hydrolysis (Block et al., 1979). The LF is responsible for the heat and burning sensation when onions are eaten raw (Randle et al., 1994).

The biosynthesis of ACSO requires S, which is taken up by the roots as sulfate (SO_4^{2-}), reduced in the plant to sulfide, and assimilated into cysteine. Some cysteine is incorporated into the glutathione cycle and then into S-2-carboxypropylglutathione (2-carb) and various γ– glutamyl peptides (r-GPs) that are intermediates in the pathway to ACSO (Lancaster and Shaw, 1989). Onion flavor can be modified by cultivar and growth environment (Bedford, 1984; Freeman and Mossadeghi, 1973; Lancaster et al., 1988; Platenius and Knott, 1935). A primary environmental factor influencing flavor is S fertility, with higher available S generally resulting in greater flavor intensity (Freeman and Mossadeghi, 1970; Randle, 1992a; Randle et al., 1994). However, poor correlations between total bulb S content and pungency among onion cultivars of broad genetic background suggested that S was differentially partitioned into flavor and nonflavor compounds (Randle, 1992a; Randle and Bussard, 1993b). The present study was initiated to determine the effect of S fertility and cultivar on the composition and concentrations of ACSOs, their biosynthetic intermediates, and other bulb quality indicators.

Materials and Methods

Plant culture. Three onion cultivars ['Rio Grande' (Rio Colorado Seed; Yuma, Ariz.), 'Savannah Sweet' (Petoseed; Saticoy, Calif.), and 'Southport White Globe' (U.S. Dept. of Agriculture plant introduction, Geneva, N.Y.)] were selected because they represented a wide response range for pungency and soluble solids content (SSC) when grown at different S fertility levels (Randle, 1992a; Randle and Bussard, 1993b). Seed was sown in artificial medium (no. 3; Fafard Co., Anderson, S.C.) in October 1991. Plants were grown at natural photoperiods (34°N) in a greenhouse, with the day/night temperatures set at 28/16C, respectively. Once

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Table 1. Elution order and retention time of authentic compounds used to identify high-performance liquid chromatography (HPLC) peaks. HPLC conditions are described in the materials and methods. R = clockwise and S = counterclockwise rotation of prioritized groups for assigning the absolute configuration of asymmetric centers according to the IUPAC labelling system.

Component name	Elution order	Retention time (min)
1-L-glutamyl-L-glutamic acid	1	2.73
Aspartic acid	2	3.20
2-Carbotypropyl glutatbione	3	3.80
(–)-S-methyl cysteine sulfoxide (S)		6.78
γ– Glutamyl-1-propenyl-L-cysteine sulfoxide	4	6.87
(+)-S-methyl-L-cysteine sulfoxide (R)	5	9.12
S-methyl glutathione	6	10.12
γ– L-glutamyl-L-methionine	7	11.37
Allyl cysteine sulfoxide (S)		12.96
(–)-S-propyl-L-cysteine sulfoxide (S)		14.03
Allyl cysteine sulfoxide (R)		14.90
Trans(+)-S-(1-propenyl)-L-cysteine sulfoxide (S)	8	15.53
γ– Glutamyl-L-phenylalanine	9	15.60
(+)-S-propyl-L-cysteine sulfoxide (R)	10	16.01
Butyl-L-cysteine sulfoxide (S)	11	17.61
Butyl-L-cysteine sulfoxide (R)	12	19.34

the true leaf emerged, seedlings were fertilized weekly with about 400 ml of a commercially available 20N-20P-20K soluble fertilizer at 200 mg·liter^{-1}. In December, seedlings were transplanted to 0.75-liter pots containing washed river sand in a split-plot design with three replications and 12 plants/replication. Cultivars were the main plots and S fertilities were the subplots. Five S-fertility treatments were used based on the results of Freeman and Mossadeghi (1970). Sulfur was varied in a modified nutrient solution of Hoagland and Amon (1950) by adjusting the $MgSO_4$ to $MgCl_2$ ratio to obtain solutions with 0.1, 0.48, 0.85, 1.6, and 3.1 meq S/liter of solution. Sulfur increased at increments or multiples of 0.375 meq S/liter with most S treatments at lower S concentrations. A S concentration of 0.1 meq/liter reduced onion growth by up to 50% compared to onions grown at 3 meq/liter (Freeman and Mossadeghi, 1970). Plants were watered twice weekly with about 100 ml of the nutrient solutions and supplemented with deionized water as needed. Media were flushed weekly with sufficient quantities of deionized water to prevent nutrient residue accumulation.

Plants were grown to maturity, as indicated by pseudostem softening and foliar lodging. Plant development and maturity were similar to field-grown plants. Plants and bulb size, however, were smaller than would be expected from field-grown plants due to restrictive pot size. Plants in a replication were considered harvestable when foliage lodged on 9 of the 12 plants. Irrigation was withheld, and the media and plants were allowed to air dry for 7 days. Bulbs were then harvested, the foliage and roots were severed, and the bulbs were cured in paper bags at ambient greenhouse temperatures for an additional 7 days. All treatments were harvested from 6 to 13 May 1992. Bulbs were stored at room temperature for 30 days before flavor analysis.

Flavor analysis. Analyses were made from the combined tissue of the 10 most-uniform bulbs from each treatment combination. A separate longitudinal wedge of tissue was taken from the 10 bulbs for each analyses. Total bulb S content was measured according to Jones and Isaac (1972). Bulb pungency was determined from enzymatically developed pyruvic acid (EPY) concentration following tissue disruption (Randle and Bussard, 1993a). Soluble solids content, an estimation of the total water-soluble carbohydrate content, was measured using a hand-held refractometer (Kemco, Corp., Tokyo, Japan).

γ– GPs and ACSOs were extracted in 12 methanol : 5 chloroform : 3 water (MCW; by volume) and ethanol (80%) according to Lancaster and Kelly (1983). S-methyl glutathione (MeGTH; 0.5 mg·g^{-1} fresh weight), γ– L-glutamyl-L glutamic acid (γgG; 0.2 mg·g^{-1} fresh weight), and (±)-S-1-butyl-L-cysteine sulfoxide (BCSO; 1 mg·g^{-1} fresh weight), not normally found in alliums, were added as internal standards to determine the percentage recovery through the extraction, derivatization, and analysis procedures. The onion extract (1 g fresh weight equivalent) was lyophilized at –36C *in vacuo* and then redissolved in 1 ml deionized water. Sample fractionation was performed by ion-exchange chromatography with Dowex 1x8 (200- to 400 mesh, 10 × 40-mm column) resin in the acetate form. A 0.5-ml sample of extract was loaded onto the column and eluted using acetic acid (HOAc) at five concentrations: 0.1, 0.2, 1, 2, and 5 M. The 0.1, 0.2, and 2 M HOAc fractions containing the ACSO and γG were eluted and lyophilized.

High-performance liquid chromatography (HPLC) analysis. Sample preparation and analysis by HPLC was according to Bidlingmeyer et al. (1984). Each sample fraction was redissolved with 1 ml deionized water, and a 20-µl sample taken and reduced *in vacuo* in alimited-volume insert (Chromacol; Waters 48 sample carriage, Millipore Corp., Milford, Mass.). Samples were then derivatized by redissolving them in a freshly prepared solution of 7 ethanol : 1 water : 1 triethylamine (TEA) : 1 phenylisothiocyanate (PITC) (by volume, 20 µl). The vials were flushed with nitrogen, sealed with Parafilm, and stored at room temperature for 20 min. The derivatizing solution was removed under vacuum, and the sample redissolved in aqueous acetonitrile (2 acetonitrile : 7 water, v/v, 100 pl) before HPLC.

A Waters (model 600) solvent delivery/control system with a Waters (WISP 712) automatic sample injector and a Waters (model 490) variable multiple-wavelength ultraviolet detector were used. The 220 × 4.6-mm column was (Aquapore RP-18; Applied Biosystems, San Jose, Calif.) fitted with a 18 × 3.5-mm guard column (Aquapore RP-18). Eluted components were detected at 254 nm, and the chromatographic traces were recorded on a personal computer (Maxima 820 software; Waters. Solvents used were A) 0.14 M sodium acetate and 0.05% TEA buffered to pH 6.35 with acetic acid and B) aqueous acetonitrile (60%). Deaeration was achieved by vacuum filtration through a 0.22-µm filter, rapid sparging with helium (100 ml·min^{-1} for 10 min), and constant slow bubbling of helium into capped, vented solvent reservoirs (5 ml·min^{-1}). Samples (10 µl) were injected onto the column, which was maintained at 30C using a Waters column heater. A flow rate of 1 ml·min^{-1} was used. The solvent gradient used was 15% to 55% B over 20 min and 55% to 100% B over 1 min, with a hold at 100% B for 14 min. The column was returned to the initial solvent over 1 min and re-equilibrated for 10 min before the next analysis.

Authentic standards were used to identify peak components (Table 1). γgGC, carb, and γ– L-glutamyl-L-phenylalanine (rgP> elute from ion-exchange chromatography in 2.0 M HOAc; γ–glutamyl-1-propenyl-L-cysteine sulfoxide, MeGTH, and γ– L-glutamyl-L-methionine elute in 1.0 M HOAc; and aspartic acid, MCSO, PRENCSO, PCSO, (±)-S-allyl-L-cysteine sulfoxide, and (±)-S-butyl-L-cysteine sulfoxide elute from ion-exchange chromatography in 0.1 M HOAc. γgG, aspartic acid, MeGTH, γ– L-glutamyl -L- methionine, a γgP were purchased from Sigma

Chemical Co. (±)-S-Methyl-L-cysteine sulfoxide, (±)-S-allyl-L-cysteine sulfoxide, (±)-S-butyl-L-cysteine sulfoxide, and (±)-S-propyl-L-cysteine sulfoxide were synthesized by the method of Lancaster and Kelly (1983). (–)-S-Methyl-L-cysteine sulfoxide (retention time 6.78 min; Table 1) and (–)-S-propyl-L-cysteine sulfoxide (14.03 min; Table 1) are isomers formed during the synthesis of these compounds, but do not occur naturally in onions and, therefore, do not appear in Fig 1. 2-Carb and γ– glutamyl-1-propenyl-L-cysteine sulfoxide were purified from onion bulbs (Shaw et al., 1989). PRENCSO was prepared by hydrolysing γ–glutamyl-S-1-propenyl-L-cysteine sulfoxide (GPRENCSO) with γ– glutamyl transpeptidase (Kuttan et al., 1974). Immediately after the methanol-chloroform split in the extraction procedure, internal standards (BCSO, γgG, and MeGTH) were added. After HPLC analysis, percent recovery was calculated and concentration was adjusted accordingly. Because of the high concentrations of MCSO detected, the peak was collected and analyzed by gas chromatography-mass spectroscopy (GC-MS). Parent [$M(H^+) = 287$] and fragmentation [$M(H^+)$-$CH_3SO = 223$] ions were identical to authentic MCSO standard eluted from HPLC and consistent with only one compound in the peak.

Statistical analysis. Data were subjected to SAS's (Cary, N.C.) general linear models procedure. Orthogonal polynomials were used to study changes with increasing S fertility by partitioning the sums of squares into components that were associated with linear and quadratic terms (Steel and Torrie, 1981). The relationship between the ACSOs and EPY was determined by regression analysis.

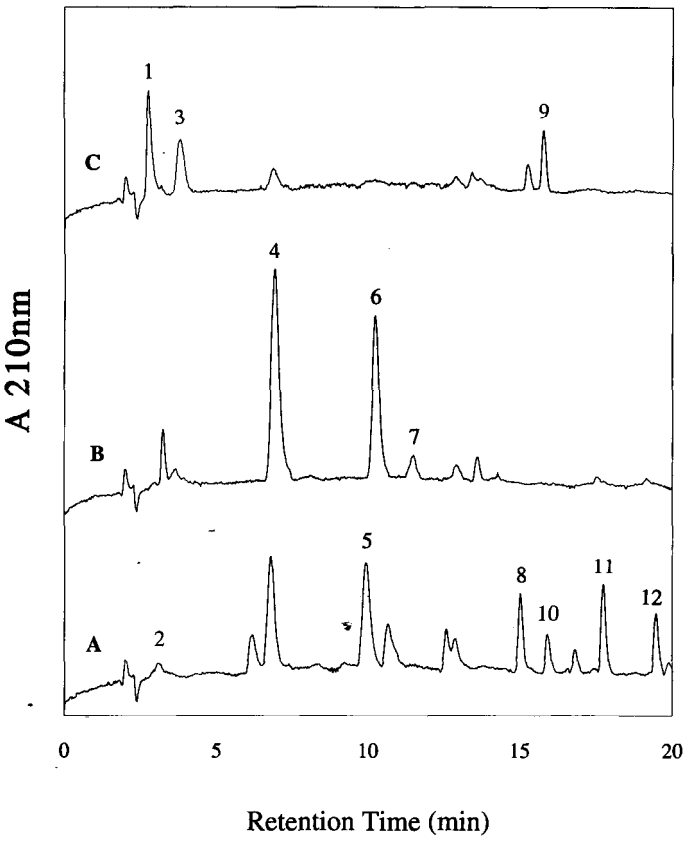

Fig. 1. Typical chromatograms of components extracted from onion bulbs ('Savannah Sweet'). **(A)** 0.1 M HOAc, **(B)** 1.0 M HOAc, **(C)** 2.0 M HOAc from ion exchange chromatography during sample fractionation. Peak assignments are as follows: 1) γ– L-glutamyl-L-glutamic acid, 2) aspartic acid, 3) 2-carboxypropyl glutathione, 4) γ– glutamyl-1-propenyl-L-cysteine sulfoxide, 5) (+)-S-methyl-L-cysteine sulfoxide (R), 6) S-methyl glutathione, 7) γ– L-glutamyl-L-methionine, 8) trans(+)-S-(l-propenyl)-L-cysteine sulfoxide (S), 9) γ– glutamyl-L-phenylalanine, 10) (+)-S-propyl-L-cysteine sulfoxide (R), 11) butyl-L-cysteine sulfoxide (S), 12) butyl-L-cysteine sulfoxide (R).

Results and Discussion

Sulfur fertility and onion growth. Sulfur fertility affected onion plant growth and development. All plants grown with 0.1 meq S/liter of nutrient solution, and a few plants among the cultivars grown with 0.48 meq S, displayed S deficiency symptoms during active bulbing. Before bulbing, plants fertilized with 0.1 and 0.48 meq S grew with no observable S deficiency symptoms. Sulfur deficiency in onions is expressed in the young, emerging leaves as a yellowing chlorosis with green strips running parallel to the leaf. In severe cases, the leaves curl, the meristem senesces and the center of the bulb becomes hollow, or both. Final bulb fresh weight differed significantly ($P = 0.01$) among cultivars, and the response to S fertility was quadratic ($P = 0.01$; Table 2). As S increased from 0.1 to 1.6 meq in the nutrient solution, Bulb fresh weight increased, but then decreased at 3.1 meq S. Sulfur fertility should therefore be considered when trying to achieve maximum bulb yields in onion. Over-fertilization with S can depress bulb yields.

Sulfur fertility and soluble solids content (SSC). A significant S × cultivar interaction for SSC ($P = 0.05$) was found. While SSC from 'Southport White Globe', a high-dry-matter cultivar, decreased with increasing S fertility, SSC from the low-dry-matter cultivar 'Savannah Sweet' increased with increasing S fertility (Table 2). Similar results for SSC were reported from onions of broad genetic background when evaluated at different S fertilities (Randle, 1992b). Some cultivars require high S fertility for maximum SSC accumulation, while others accumulate maximum SSC at low S fertility. The type of sugar accumulated, i.e., glucose, fructose, sucrose, or fructans, varies depending on the dry-matter-accumulation potential among cultivars (Darbyshire and Henry, 1979). Cultivars relatively low in dry matter accumulate mostly glucose, fructose, and sucrose, while high-dry-matter cultivars accumulate mostly fructans.

Sulfur fertility and bulb pungency. Bulb pungency, as measured by EPY, differed among cultivars and with S fertility ($P = 0.01$). At low S fertility (0.1 meq S), all three cultivars had a similar EPY. Increasing sulfur fertility to 3.1 meq S increased EPY to 5.3 and 5.7 $\mu mol \cdot g^{-1}$ fresh weight in 'Rio Grande' and 'Savannah Sweet', respectively, and 9.7 $\mu mol \cdot g^{-1}$ fresh weight in 'Southport White Globe' (Table 2). Similarresponses of EPY to increasing S fertility have been reported (Freeman and Mossadeghi, 1970; Randle and Bussard, 1993b).

Bulb S. Bulb S content increased linearly in response to increasing S fertility and did not significantly differ among cultivars (Table 2). Thus, uptake of S was similar for all three cultivars. However, differences in EPY among cultivars suggested that S metabolism to ACSO differed among cultivars.

Sulfur fertility and ACSO biosynthetic intermediates. Of the γ–GPs known to be intermediates in the flavor pathway of onion, only 2-carb and γ– GPRENCSO were identified in sufficient quantities to assess the effects of S fertility on ACSO biosynthetic intermediates. 2-Carb is synthesized toward the beginning of the flavor pathway, while γ– GPRENCSO is the penultimate peptide leading to PRENCSO synthesis (Lancaster and Boland, 1990). Among cultivars, 'Southport White Globe' had the greatest total γ– GP concentration. 2-Carb was significantly different ($P = 0.01$) among cultivars and S fertility levels, with a quadratic trend in response to increasing S fertility. Cultivars significantly interacted with S fertility for γ– GPRENCSO ($P = 0.05$). Except at very low S fertility

Table 2. Bulb S percentage (percent dry weight), enzymatically developed pyruvic acid (EPY; μmol·g^{-1} fresh weight), soluble solids content (SSC; percent), and bulb fresh weight (g) of three onion cultivars grown at five S fertility levels.

Cultivar[z]	S (meq/liter)[y]	Bulb S (% dry wt)	EPY (μmol·g^{-1} fresh wt)	SSC (%)	Bulb fresh wt (g)
RG	0.10	0.07	1.0	8.7	596
	0.48	0.08	1.1	8.3	773
	0.85	0.14	2.4	8.7	952
	1.60	0.26	3.5	8.9	1165
	3.10	0.43	5.3	9.0	1029
Contrasts					
Linear		**	NS	*	NS
Quadratic		NS	**	**	**
SS	0.1	0.07	0.8	7.5	745
	0.48	0.09	1.2	7.5	808
	0.85	0.13	2.1	7.9	954
	1.60	0.23	3.7	8.3	1177
	3.10	0.40	5.7	8.7	1080
Contrasts					
Linear		**	**	**	NS
Quadratic		NS	NS	NS	**
SWG	0.1	0.07	1.4	21.4	333
	0.48	0.12	2.0	19.5	510
	0.85	0.16	4.3	16.7	721
	1.60	0.24	5.0	16.4	858
	3.10	0.43	9.7	16.9	747
Contrasts					
Linear		**	**	NS	NS
Quadratic		NS	**	**	**

[z]RG = 'Rio Grande', SS= 'Savannah Sweet', SWG = 'Southport White Globe'.
[y]Sulfur = meq/liter of nutrient solution.
[NS],*,**Nonsignificant or significant (linear or quadratic contrast analysis) at $P = 0.05$ or 0.01, respectively.

levels, γ– GPRENCSO concentrations were higher than 2-carb when compared among the same cultivars at the same S fertility levels (Table 3). γ– GPRENCSO concentration was also higher than 2-carb in field-grown onion cultivars (Shaw et al., 1989). As S fertility increased, the relative concentrations of 2-carb to γ–GPRENCSO changed. At 0.48 meq S, γ– GPRENCSO was 120%, 110%, and 60% the concentration of 2-carb in 'Rio Grande', 'Savannah Sweet', and 'Southport White Globe', respectively. At 3.1 meq S, γ– GPRENCSO was 360%, 240%, and 240% the concentration of 2-carb in the respective cultivars. Moreover, under low S fertility, 2-carb and γ– GPRENCSO were in relatively low concentrations relative to ACSO concentration, suggesting efficient movement of S leading to ACSO synthesis. However, as S fertility increased, the greater concentrations of γ– GPRENCSO relative to 2-carb indicated less-efficient metabolism of γ–GPRBNCSO relative to 2-carb in the flavor pathway.

Sulfur fertility and ACSO. ACSO composition is important because it is responsible for the nature and intensity of the flavor, thereby influencing the organoleptic experience (Block, 1992; Randle et al., 1994). Total ACSO concentration among cultivars significantly interacted with S fertility (P = 0.05; Table 3). ACSO concentrations in 'Rio Grande' and 'Southport White Globe' increased with increasing S fertility, while ACSO concentration in 'Savannah Sweet' decreased as S fertility increased from 0.1 to 0.85 meq and then increased at 1.6 and 3.1 meq S. The concentrations of total ACSO (3.01 to 5.73 mg·g^{-1} fresh weight) among cultivars and S fertility levels were within the range of that reported by Matikkala and Virtanen (1967) at 4.2 mg·g^{-1} fresh weight, but were from two to five times the concentrations reported by Thomas and Parkin (1994) of 1.12 to 1.54 mg·g^{-1} fresh weight.

The ratio and concentration of the individual ACSOs varied with onion cultivar and S fertility. PRENCSO concentrations were significantly different among cultivars and had a quadratic response to increasing S fertility ($P = 0.01$; Table 3). Differences in PRENCSO concentrations among the lowest and highest S fertility increased about 5-fold for 'Rio Grande', 4-fold for 'Savannah Sweet', and 17-fold for 'Southport White Globe'. At fertility levels inducing S deficiency symptoms (0.1 meq S), PRENCSO was a minor ACSO, being only 5% of the total ACSO for 'Southport White Globe' and 16% of the total ACSO for 'Rio Grande' and 'Savannah Sweet'. At 3.1 meq S fertility, PRENCSO became the dominant ACSO at 46%, 64%, and 58% of the total ACSO for 'Rio Grande', 'Savannah Sweet', and 'Soutbport White Globe', respectively. Previous studies reported that PRENCSO was the dominant onion precursor, comprising 85% to 92% of the total ACSO concentration (Matikkala and Virtanen, 1967; Thomas and Parkin, 1994). A significant S × cultivar interaction (P = 0.05) was found for MCSO concentration. Interestingly, MCSO was found in unusually high concentrations (2.24 to 2.66 mg·g^{-1} fresh weight) and was the dominant ACSO at 0.1 meq S fertility, comprising 79%, 59%, and 84% of the total ACSO for 'Rio Grande', 'Savannah Sweet', and 'Southport White Globe', respectively. Most of the thiosulfinates withmethyl groups impart a cabbage-like flavor (Randle et al., 1994). No detectable trend was found for 'Rio Grande's MCSO in response to increasing S fertility, while 'Savannah Sweet' and 'Southport White Globe' had significant quadratic trends (P = 0.01; Table 3). As S fertility increased, MCSO decreased relative to total ACSO (Table 3). At 3.1 meq S, MCSO

was 37%, 26%, and 34% of the total ACSO concentration for 'Rio Grande', 'Savannah Sweet', and 'Southport White Globe', respectively. Previous reports found MCSO to be 8% to 15% of the total ACSO concentration in onions (Matikkala and Virtanen, 1967; Thomas and Parkin, 1994). Because of the unusually high concentration of MCSO detected in our study compared to others (Matikkala and Virtanen, 1967; Thomas and Parkin, 1994), composition of the putatative MCSO peak was verified by GC-MS and determined to be solely MCSO.

PCSOwas detected and was generally found in low concentration relative to total ACSO (Table 3). Cultivars significantly interacted with S fertility for PCSO (P = 0.01). At the lowest S fertility, PCSO was detected in higher concentrations than PRENCSO for all cultivars and was about 25% of the total ACSO for 'Rio Grande' and 'Savannah Sweet', and 10% of the total ACSO for 'Southport White Globe'. As S fertility increased, PCSO decreased relative to the total ACSO. At 3.1 meq S, PCSO concentration was 17%, 10%, and 8% of the total ACSO for 'Rio Grande', Savannah Sweet', and 'Southport White Globe', respectively.

Whereas EPY has been used as an indicator of onion pungency (Schwimmer and Weston, 1961; Schwimmer and Guadagni, 1962; Wall and Corgan, 1992) and is a product of ACSO hydrolysis, it was difficult to draw a clear relationship between µmol EPY and µmol total ACSO in our study (Y = 20.4 + 1.26xNS, R^2 = 0.30). A significant relationship, however, could be found between µmol EPY and µmol PRENCSO (Y = 1.7 + 1.8x, P = 0.05; R^2 = 0.81), although EPY was measured in substantially lower concentrations than ACSOs. The reasons for these discrepancies may be outlined as follows. First, not all ACSO are completely hydrolyzed by alliinase. Up to 50% of the MCSO and PCSO are left intact up to 2 h after tissue disruption (data not shown). PRENCSO, on the other hand, is 85% hydrolyzed within 6 sec of tissue disruption and completely hydrolyzed within 30 sec (data not shown). Second, alliinase content varies with cultivar, with plant development, from plant to plant within a cultivar, and with S fertility (Lancaster et al., 1993), which could affect the concentration of EPY produced. And third, the low EPY concentration compared to ACSO concentration may result from the EPY being partially metabolized during the complex chemistry that takes place after ACSO hydrolysis. Enzymatically produced pyruvic acid took about 8 to 10 min to stabilize after tissue disruption (Schwimmer and Weston, 1961).

Sulfur partitioning. Almost 95% of total bulb S (0.07% to 0.09% dry weight; Table 2) could be accounted for by the measured ACSO and γ– GP at the 0.1 meq S treatment (data not shown), suggesting that the flavor pathway was a strong sink for available S under S stress. As S fertility increased to 3.1 meq, a luxuriant S fertility level, <40% of the total bulb S could be attributed to the S compounds measured. At these higher S fertility levels, S was partitioned into compounds that were either not measured or were outside the pathway. It has been suggested that, in the bulb, the γ– GP and ACSOs function as S reserves that will support the reproductive cycle in the second year of growth (Lancaster and Boland, 1990). Yet, with about 60% of bulb S in unmeasured compounds, significant S is stored in compounds outside the flavor pathway.

Table 3. Flavor precursors and precursor intermediates[z] (mg·g^{-1} fresh weight) for three onion cultivars grown at five S fertility levels.

Cultivar[y]	S (meq/liter)[x]	PRENCSO[z]	MCSO	PCSO	Total ACSO	γGPE CSO	2-carb	Total S
RG	0.10	0.52	2.24	0.85	3.14	nd[w]	nd	3.61
	0.48	0.60	1.71	0.70	3.01	0.06	0.05	3.12
	0.85	1.42	1.99	0.94	4.35	0.51	0.20	5.06
	1.60	1.75	2.64	1.24	5.63	0.96	0.28	6.87
	3.10	2.65	2.10	0.98	5.73	1.34	0.37	7.44
Contrasts								
Linear		NS	NS	NS	NS	NS	NS	NS
Quadratic		**	NS	**	*	**	**	NS
SS	0.10	0.63	2.32	0.98	3.93	0.04	0.08	4.05
	0.48	0.85	1.77	0.79	3.41	0.08	0.07	3.56
	0.85	1.00	0.86	0.44	2.30	0.14	0.12	2.56
	1.60	2.00	1.05	0.37	3.42	0.44	0.23	4.09
	3.10	2.60	1.05	0.40	4.05	0.99	0.42	5.47
Contrasts								
Linear		NS	NS	NS	NS	**	NS	NS
Quadratic		**	**	**	*	**	**	*
SWG	0.10	0.17	2.66	0.33	3.16	0.11	0.23	3.50
	0.48	0.49	3.09	0.48	4.06	0.19	0.33	4.58
	0.85	1.31	2.49	0.47	4.27	0.49	0.40	5.16
	1.60	1.69	2.18	0.40	4.27	0.89	0.46	5.62
	3.10	3.00	1.75	0.41	5.16	2.33	0.98	8.47
Contrasts								
Linear		**	NS	NS	NS	**	*	NS
Quadratic		**	**	NS	*	**	**	*

[z]PRENCSO = 1-propenyl cysteine sulfoxide, MCSO = methyl cysteine sulfoxide, PCSO = propyl cysteine sulfoxide, ACSO = total cysteine sulfoxide precursors, γ– GPECSO = γ– glutamyl 1-propenyl cysteine sulfoxide, 2-carb = 2-carboxypropyl glutathione, Total S = total S compounds measured.
[y]RG = 'Rio Grande', SS = 'Savannah Sweet', and SWG = 'Southport White Globe'.
[x]Sulfur = meq/liter of nutrient solution.
[w]nd = Not detected.
[NS],*,**Nonsignificant or significant (linear or quadratic contrast analysis) at P = 0.05 or 0.01, respectively.

Plants grown with 0.1 meq S exhibited S deficiency symptoms only with the onset of active bulbing in our study. Of the 37 *Allium* species analyzed for quantitative and qualitative differences in ACSO concentration, all contained MCSO (Lancaster and Boland, 1990), suggesting that MCSO may be linked to a common ancestral origin. During active bulbing, MCSO synthesis became a strong sink for available S and MCSO accumulated in relatively high concentrations among the cultivars tested. MCSO accumulated even at the expense of onion growth, and its synthesis may have contributed to the expression of S deficiency symptoms.

Concentration of ACSO in onions. Conflicting accounts exist as to the relative levels of onion ACSOs and even the existence of endogenous PCSO (Lancaster and Kelly, 1983; Matikkala and Virtanen, 1967; Thomas and Parkin, 1994; Virtanen and Matikkala, 1959). While some of the differences in ACSO concentration and composition can be attributed to cultivar differences, as we report here, the method of analysis can also account for variability. The accurate quantification of endogenous ACSOs is important as the food industry moves toward greater standardization of onion flavor for fresh consumption, food processing, and using the endogenous ACSOs and their hydrolysis products in the phytomedicinal field (Augusti, 1990). Direct and indirect methods have been used to quantify *Allium* ACSO concentration. Accurate indirect measurement of ACSOs from the formation of thiosulfinates is difficult due to the complex chemistry that occurs after onion ACSO hydrolysis, especially with PRENCSO (Block, 1992). While similarities exist between thiosulfinate formation (Randle et al., 1994) and the concentrations of endogenous ACSO in this study, thiosulfinate fragments account for only a fraction of the alk(en)yl fragments nascent to onion (Thomas and Parkin, 1994).

ACSOs were first measured by direct means using an amino acid analyzer, which detected PRENCSO in the highest concentration, MCSO in a lower concentration, and PCSO in the lowest concentration (Matikkala and Virtanen, 1959). In a later study, however, Matikkala and Virtanen (1967) were unable to detect PCSO. Using two-dimensional electrophoresis and thin-layer chromatography, Granroth (1968) found only PRENCSO and MCSO, while Lancaster and Kelly (1983) unexpectedly detected PCSO in the highest concentration among the three onion ACSOs.

More recently, HPLC methods were applied to ACSO quantification in alliums. Using fluorescent detection of 9-fluorenylmethyl chloroformate-derivatized (FMOC) adducts and isocratic elution, only PRENCSO and MCSO were detected (86: 14) from aggregate onion extracts (Thomas and Parkin, 1994). Since PCSO was detected using our HPLC method but not with the FMOC method (1994) the differences between the HPLC methods were evaluated and can be explained. First, we were able to detect PCSO by fluorescence if the FMOC derivatized samples were eluted using a solvent gradient instead of isocratic elution (data not shown). In the original FMOC method (Einarsson et al., 1983), a solvent gradient was used to resolve amino acids. Second, better peak resolution was achieved when samples were first fractionated by ion-exchange chromatography into the ACSO and γ– GP components (Lancaster and Kelly, 1983). Fractionation of the aggregate methanol-ethanol-water extract is important when attempting to detect in low concentrations components, such as PCSO, which elute in close proximity or as shoulders of larger peaks. Third, the formation of n-propylated species arising in the thiosulfinates has been attributed to the partial conversion of PRENCSO to PCSO (Thomas and Parkin, 1994) due to the reducing power of aqueous onion extracts (Yagami et al., 1980). If this were true, the trend for PCSO to respond to increasing S fertility should follow the trend for PRENCSO, but it does not. As an example, where the concentration of PRENCSO increased with increasing S fertility in 'Savannah Sweet', PCSO decreased with increasing S fertility. An additional explanation for the inability to detect PCSO by the method of Thomas and Parkin (1994) could be the relatively low concentrations of total ACSOs detected. Whereas 1.1 $mg \cdot g^{-1}$ fresh weight of total ACSO was detected using the FMOC method (Thomas and Parkin, 1994) with the dehydrator cultivar, at the higher S fertility levels, we found 4.3 to 5.2 $mg \cdot g^{-1}$ fresh weight in 'Southport White Globe' (a dehydrator cultivar), which was similar to the 4.2 $mg \cdot g^{-1}$ fresh weight of total ACSO reported by Matikkala and Virtanen (1967). If percent recovery of the ACSOs is low due to sample preparation or handling, PCSO could be reduced below the detection level.

Sulfur fertility influenced total onion ACSO accumulation, the ratio of individual ACSOs, and the concentration of flavor intermediates in the biosynthetic pathway. Low S fertility resulted in efficient metabolism of S through the flavor pathway and low concentrations of γ– GP intermediates. Moreover, the flavor pathways leading to MCSO synthesis, and to a lesser extent PCSO, were strong sinks for available S during active bulbing at low S fertility. As S fertility increased, γ– GP concentrations increased, the ratio of individual ACSOs changed, and PRENCSO became the dominant ACSO at the highest fertility treatment. Commercial onions are normally grown with sufficient S to provide the needs for plant growth and ACSO accumulation (Lancaster and Boland, 1990). However, as demand increases for mild onions, plants are being subjected to decreasing S fertility and other environmental variants. Understanding the complex interaction between a plant's genetic potential for flavor accumulation and the dynamic growing environment will become increasingly important as the food and phytomedicinal industries move toward greater characterization and standardization of flavor and therapeutic compounds.

Literature Cited

Augusti, K.T. 1990. Therapeutic and medicinal values of onions and garlic, p. 93-108. In: H.D. Rabinowitch and J.L. Brewster(eds.). Onions and allied crops. vol. 3. CRC Press, Boca Raton, Fla.

Bedford, L.V. 1984. Dry matter and pungency tests on British grown onions. J. Natl. Inst. Agr. Bot. 16:58-61.

Bidlingmeyer, B.A., S.A. Cohen, and T.L. Tarvin. 1984. Rapid analysis of amino acids using pre-column derivatization. J. Chromatog. 336:93-104.

Block, E. 1992. The organosulfur chemistry of the genus *Allium*-Implications for the organic chemistry of sulfur. Angew. Chem. Intl. Ed. Engl. 31:1135-1178.

Block, E., R.E. Penn, and L.K. Revelle. 1979. Structure and origin of the onion lachrymatory factor. A microwave study. J. Amer. Chem. Soc. 101:2200-2201.

Darbyshire, B and R.J. Henry. 1979. The association of fructans with high percentage dry weight in onion cultivars suitable for dehydrating. J. Sci. Food Agr. 30:1035-1038.

Einarsson, S., B. Josefsson, and S. Lagerkvist. 1983. Determination of amino acids with 9-fluorenylmethyl chloroformate and reversed-phase high-performance liquid chromatography. J. Chromatog. 282:609-616.

Freeman, G.G. and N. Mossadeghi. 1970. Effect of sulphate nutrition on flavour components of onion *(Allium cepa)*. J. Sci. Food Agr. 21:610-615.

Freeman, G.G. and N. Mossadeghi. 1973. Studies on the relationship between water regime and flavour strength in water cress *(Rorippa nasturium-aquaticum* [L.] Hayek), cabbage *(Brassica olerucea* var. capitata) and onion *(Allium cepa)*. J. Hort. Sci. 48:365-378.

Granoth, B. 1968. Separation of *Allium* sulphur amino acids and peptides by thin layer electrophoresis and thin layer chromatography. Acta Chem. Scan. 22:3333-3335.

Hoagland, D.R. and D.I. Amon. 1950. The water culture method for

growing plants without soil. Calif. Agr. Expt. Sta. Circ. 347.
Jones, J.B. and R.A. Isaac. 1972. Determination of sulfur in plant material using a Leco sulfur analyzer. J. Agr. Food Chem. 20: 1292-l 294.
Kuttan, R., N.G. Nair, A.N. Radhakrishnan, T.F. Spande, H.J. Yeh, and B. Witkop. 1974. Isolation and characterization of γ– L-glutamyl-S-(trans-1-propenyl)-L-cysteine sulfoxide from sandal *(Santalum album* L.). An interesting occurrence of sulfoxide diastereoisomers in nature. Biochememistry 13:4394-4400.
Lancaster, J. E. and M.L. Shaw. 1989. γ– Glutamyl peptides in the biosynthesis of S-alk(en)yl-L-cysteine sulphoxides (flavour precursors) in *Allium.* Phytochemistry 28:455–460.
Lancaster, J.E, and M.J. Boland. 1990. Flavor biochemistry, p. 33-72. In: H.D. Rabinowitch and J.L. Brewster (eds.). Onions and alliedcrops. vol. 3. CRC Press, Boca Raton, Fla.
Lancaster, J.E., P.F. Reay, J.D. Mann, W.D. Bennett, and J.R. Sedcole. 1988. Quality in New Zealand grown onion bulbs-A survey of chemical and physical characteristics: Flavour precursors, dry matter, sugars, colour, and hardness. N.Z. J. Expt. Agr. 16:279-285.
Lancaster, J.E. and K.E. Kelly. 1983. Quantitative analysis of the S-alk(en)yl-L-cysteine sulphoxides in onion *(Allium cepa* L.). J. Sci. Food Agr. 34:1229-1235.
Lancaster, J.E., M.L. Shaw, S. Clark, and E. Dommisse. 1993. Biochemistry of onion flavor, p. 91-102. Proc. Natl. Onion Res. Conf. Cornell Univ., Ithaca, N.Y.
Matikkala, E.J. and A.I. Virtanen. 1967. On the quantitative determination of the amino acids and gamma-glutamyl peptides of onion. Acta Chem. Stand. 21:2891-2893.
Platenius, H. and J.E. Knott. 1935. The pungency of the onion bulb as influenced by the stage of development of the plant. Proc. Amer. Soc. Hort. Sci. 33:481–486.
Randle, W.M. 1992a. Onion germplasm interacts with sulfur fertility for plant sulfur utilization and bulb pungency. Euphytica 59:151-156.
Randle, W.M. 1992b. Sulfur nutrition affects nonstructural water-soluble carbohydrates in onion germplasm. HortScience 27:52-55.
Randle, W.M. and M.L. Bussard. 1993a. Streamlining onion pungency analyses. HortScience 28:60.
Randle, W.M. and M.L. Bussard. 1993b. Pungency and sugars of short-day onions as affected by sulfur nutrition. J. Amer. Soc. Hort. Sci. 118:766-770.
Randle, W.M., E. Block, M.H. Littlejohn, D. Putman, and M.L. Bussard. 1994. Onion *(Allium cepa* L.) thiosulfinates respond to increasing sulfur fertility. J. Agr. Food Chem. 42:2085-2088.
Schwimmer, S. and D.G. Guadagni. 1962. Relationship betweenolfactory threshold concentration and pyruvic acid content of onion juice. J. Food Sci. 27:94-97.
Schwimmer, S. and W.J. Weston. 1961. Enzymatic development of pyruvic acid in onion as a measure of pungency. J. Agr. Food Chem. 9:301-304.
Shaw, M.L., J.E. Lancaster, and G.A. Lane. 1989. Quantitative analysis of the major γ– glutamyl peptides in onion bulbs *(Allium cepa).* J. Sci. Food Agr. 48:459-467.
Steel, R.G.D. and J.H. Torrie. 1981. Principles and procedures of statistics. A biometrical approach. McGraw-Hill, New York.
Thomas, D.J. and K.L. Parkin. 1994. Quantification of alk(en)yl-L-cysteine sulfoxides and related amino acids in alliums by high-performance liquid chromatography. J. Agr. Food Chem. 42:1632-1638.
Virtanen, A. I. and E.J. Matikkala. 1959. Isolation of S-methyl cysteine sulphoxide and S-n-propyl cysteine sulphoxide from onion *(Allium cepa)* and the antibiotic activity of crushed onion. Acta Chem. Stand. 13:1898-1900.
Wall, M.M. and J.N. Corgan. 1992. Relationship between pyruvate analysis and flavor perception for onion pungency determination. HortScience 27:1029-1030.
Yagami, M., L. Kawakishi, and M. Namiki. 1980. Identification of intermediates in the formation of onion flavor. Agr. Biol. Chem. 44:2533-*2538.*