Changes in the *S*-alk(en)yl Cysteine Sulfoxides and their Biosynthetic Intermediates during Onion Storage

David E. Kopsell¹ and William M. Randle²

Department of Horticulture, University of Georgia, Athens, GA 30602-7273

Mark A. Eiteman²

Department of Biological and Agricultural Engineering, University of Georgia, Athens, GA 30602-4435

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ABSTRACT. Onion (*Allium cepa* L.) pungency changes during storage. To better understand these flavor changes, seven onion cultivars representing different storage duration, photoperiodic requirement, and flavor intensity were greenhouse grown and the bulbs stored for 3 or 6 months at 5 ± 3 °C, 0.8 to 1.1 kPa vapor pressure deficit. Bulbs were evaluated using high-pressure liquid chromatography quantification for changes in *S*-alk(en)yl cysteine sulfoxide (ACSO) flavor precursors and γ -glutamyl peptide (γ -GP) biosynthetic intermediates before storage and monthly thereafter. Before and during storage, cultivars differed in total ACSO, (+) *S*-methyl-L-cysteine sulfoxide (MCSO), *trans*-(+)-*S*-(1-propenyl)-L-cysteine sulfoxide (PRENCOSO), (+) propyl-L-cysteine sulfoxide (PCSO), *S*-2 carboxypropyl glutathione (2-CARB), and γ -L-glutamyl-*S*-(1-propenyl)-L-cysteine sulfoxide (γ GPECSO) concentration. During storage MCSO generally decreased while PRENCOSO increased in concentration for most cultivars. The linear increase in PRENCOSO concentration during storage was accompanied by a linear decrease in γ GPECSO concentration. While not measured in this study, these trends indicate γ -glutamyl transpeptidase activity throughout bulb storage. γ -Glutamyl transpeptidase was previously reported to be active only in the later stages of bulb storage or during bulb sprouting. Changes in ACSO and γ -GP compounds during storage did not follow previously reported changes during storage for enzymatically formed pyruvic acid (EPY) for these cultivars. To better understand what causes flavor changes in onions during storage, future investigations should include analysis of the enzymes involved in flavor development and ACSO hydrolysis products.

Despite its low nutritional value, the onion (*Allium cepa*) has long been valued in the human diet. Its value arises from the unique onion flavors, and their ability to enhance other foods during processing. Onion flavor development is well understood. Upon cellular disruption, the enzyme alliinase (EC 4.4.1.4) in the vacuole is released to hydrolyze the flavor precursors collectively termed *S*-alk(en)yl-L-cysteine sulfoxides (ACSOs) in the cytoplasm (Block, 1992). The three naturally occurring onion ACSO compounds are *trans*-(+)-*S*-(1-propenyl)-L-cysteine sulfoxide (PRENCOSO), normally found in the highest concentration, (+)-*S*-methyl-L-cysteine sulfoxide (MCSO), and (+)-*S*-propyl-L-cysteine sulfoxide (PCSO) (Lancaster and Boland, 1990). Hydrolysis of the ACSO compounds by alliinase yields unstable alk(en)yl sulfenic acids, which rearrange nonenzymatically to form thiosulfonates that contribute to flavor perceived (Block, 1992). Pyruvic acid and ammonia are nonflavor products of this enzymatic reaction. Thiopropanal *S*-oxide, the lachrymatory factor that causes the tearing and burning sensation associated with onions, is formed only from 1-propenyl sulfenic acid following hydrolysis of PRENCOSO (Block, 1992).

The flavor biosynthetic pathway begins with the absorption of sulfate (SO_4^{2-}) by onion roots. Sulfate is reduced to sulfide and assimilated into cysteine. The tripeptide glutathione is then formed and converted to *S*-2-carboxypropyl glutathione (2-CARB), metabolized through various other γ -glutamyl peptides, terminating in ACSO synthesis (Lancaster and Shaw, 1989).

Onions are routinely marketed and used out of short- and long-term storage. Conditions in U.S. onion storage facilities usually are in the range of 0 to 3 °C and 60% to 75% relative humidity. Storage potential differs among onion cultivars and is influenced by the duration of dormancy, rate of water loss (Corgan and Kedar, 1990), and susceptibility to disease (Maude, 1990). Short-day (SD) onion cultivars, which characteristically have a short dormancy period, large bulb necks, and high rates of water loss after harvest, generally do not store well (Woodman and Barnell, 1937). Long-day (LD) onion cultivars, in contrast, generally have a longer dormancy period, smaller diameter bulb necks, low rates of water loss, and as a consequence a significantly greater storage potential. Short-day cultivars, however, have been developed with improved storage characteristics (Madisa, 1994; Peters et al., 1989), while some LD onion cultivars store poorly.

Onion pungency changes during storage. After 4 months of storage at 15 to 20 °C, bulb pungency, as measured by EPY (enzymatically formed pyruvic acid) significantly decreased for three long-day cultivars (Peterson et al., 1986). On the other hand, for the LD cultivar 'Spartan Banner', EPY increased during 20 °C storage for the first 16 weeks and then leveled off through the 32nd week (Hanum et al., 1995). Recently, using a broad cross-section of cultivars grown under similar conditions and stored at 5 °C, EPY decreased over time except for the SD cultivar 'Granex 33', which increased during storage (Kopsell and Randle, 1997). Measurement of onion flavor is routinely carried out through the quantification of EPY because of stability of pyruvic acid (Schwimmer and Weston, 1961; Randle and Bussard, 1993) and good correlation with overall taste perception (Wall and Corgan, 1992). Because EPY reflects only gross pyruvic acid production from all ACSOs, the amounts and behavior of individual ACSO compounds during

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¹Graduate research assistant.

²Associate professor.

Fig. 1. Typical chromatograms of the compounds extracted from onion bulb tissue ('Granex 33') with ion exchange chromatography using (A) 0.1 M HOAc and (B) 2.0 M HOAc for fractionation. Identified peaks are as follows: 1) (+)-S-methyl-L-cysteine sulfoxide, 2) trans(+)-S-(1-propenyl)-L-cysteine sulfoxide, 3) (+)-S-propyl-L-cysteine sulfoxide, 4) butyl-L-cysteine sulfoxide (S), 5) butyl-L-cysteine sulfoxide (R), 6) γ -L-glutamyl-L-glutamic acid, 7) 2-carboxypropyl glutathione, 8) γ -glutamyl-1-propenyl-L-cysteine sulfoxide, 9) S-methyl glutathione.

storage is unknown. The composition and concentration of the different ACSOs are important. The ACSOs give rise to various thiosulfinates which elicit the different flavors such as heat, pungency, and the fresh onion taste (Randle et al., 1994). Our study was conducted to investigate the behavior of the different ACSO compounds and several of their biosynthetic intermediates during cold-temperature storage.

Materials and Methods

PLANT CULTURE. Eight onion cultivars ['Dehydrator #3' SD, 'Walla Walla Sweet' Intermediate-day (ID), 'Zenith' LD (Sunseed Inc.; Hollister, Calif.), 'Granex 33' SD, 'Sweet Sandwich' LD (Asgrow; Kalamazoo, Mich.), 'Candy' ID (PetoSeed; Saticoy, Calif.), and 'Pukekohe' LD (Yates Seed; Auckland, New Zealand)] were selected to represent a wide range in storage potential, flavor intensities, usage type, and photoperiodic response. In January 1994, each cultivar was seeded in 400 growing cubes (Grodan A/S, DK-2640 Hedehusene, Denmark), covered with fine vermiculite, watered as needed, thinned to one plant per cube 2 weeks after planting, and fertilized with 20N–20P–20K nutrient solution (Grace-Sierra Co., Milpitas, Calif.) every 7 to 10 d. Seedlings were greenhouse grown (34° N latitude) with day/night temperatures of 28/16 °C for 9.5 weeks before transplanting into greenhouse hydroponic growing tanks according to the method of Kopsell and Randle (1997). A modified Hoagland and Arnon (1950) solution was used and the total dissolved salts in each tank were maintained at ≈ 1000 mg·L⁻¹ throughout the experiment. Because onion flavor is derived from sulfur (S) compounds, the SO₄⁻² content in the nutrient solution was

monitored weekly (Gaines and Mitchell, 1979) in each tank and maintained at 60 mg·L⁻¹. Onions were arranged on five tanks and blocked according to photoperiodic response. To prevent premature bulbing, SD cultivars were covered with black plastic on 21 Apr. to maintain an 11 h photoperiod. On 18 May, the SD cultivars were left uncovered and bulbs were allowed to mature under natural photoperiods. To stimulate bulbing of the ID and LD cultivars, the natural photoperiod was extended 1 h at the end of the day with 150-W lights (18 μ mol·m⁻²·s⁻¹) suspended over the beds on 1 May. On 15 May, the natural photoperiod was extended 2 h, and on 27 May the photoperiod was extended 3 h which allowed the plants to mature. Bulb size and maturity were similar to field-grown onions.

Each cultivar was harvested when 50% of the bulbs had soft pseudostems. At harvest, the roots were clipped, and the onions were laid in the greenhouse for several days with leaf blades intact. After the onion leaves had browned, they were removed, and the bulbs placed in mesh bags and hung in the greenhouse to cure for 7 d. Uniform bulbs of each cultivar were selected and placed in mesh bags for storage.

BULB STORAGE. Before storage, four 10-bulb replicates of each cultivar were analyzed for ACSO and γ GP content. The remaining 10-bulb samples of each cultivar were placed in refrigerated storage (5 $\pm$ 3 °C, 0.8 to 1.1 kPa vapor pressure deficit) using a split block design and four replications. Blocks were the four quadrants of the cooler, cultivars were the main plots, and months of storage were the subplots. 'Granex 33' was stored for 4 months, while 'Dehydrator #3' was stored for only 3 months because of severe storage losses in the fourth month. The ID and LD cultivars were stored for 6 months. Every 30 d after the beginning of storage, bulbs were removed from storage and acclimated to room temperature for 24 h before the ACSO analyses.

HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC) ANALYSIS. At each sampling date, a 0.30 cm thick wedge was taken from each of the ten bulbs per replicate of each cultivar. The wedges from each cultivar/replicate were combined and placed in jars containing 12 methanol : 5 chloroform : 3 water, and then in jars containing 80% ethanol to extract the precursor intermediates (γ GP) and ACSOs (Lancaster and Kelly, 1983). S-Methyl glutathione (MeGTH; 0.5 mg·g⁻¹ fresh tissue mass), γ -L-glutamyl-L-glutamic acid (γ GG; 0.2 mg·g⁻¹ fresh tissue mass), and ($\pm$)-S-1-butyl-L-cysteine sulfoxide (BCSO; 1.0 mg·g⁻¹ fresh tissue mass), were used as internal standards. Eighteen milliliters of solution (1 g fresh mass equivalent) was placed into a 40 mL test tube along with each internal standard, dried with a gentle stream of ambient air, and redissolved in 1.0 mL of deionized/distilled water. A 0.5 mL aliquot was then subjected to ion exchange chromatography using a 10 $\times$ 40-mm column (Bio-Rad, Hercules, Calif.) with 3 mL Dowex 1 $\times$ 8 resin (200 to 400 mesh; Bio-Rad, Hercules, Calif.). Fractionation was carried out using acetic acid (HOAc) at four concentrations: 0.1, 0.2, 2, and 5 M. The fractions containing the ACSO and γ GP compounds (0.1 and 2 M) were each collected and dried using ambient air.

This method was modified from Randle et al. (1995) which used three concentrations of acetic acid (HOAc; 0.1, 1, and 2 M respectively) during ion exchange in order to elute the flavor compounds and intermediates. According to the method of Randle et al. (1995), 0.1 M HOAc fraction eluted the ACSO compounds and the BCSO standard, 1 M HOAc eluted γ -L-glutamyl-S-(1-propenyl)-L-cysteine sulfoxide (γ GEPCSO) and the MeGTH standard, and 2 M HOAc eluted S-2 carboxypropyl glutathione (2-CARB) and the γ GG standard. Visual inspection of the chromatograms using the 1995 procedure reveal that the 1 M fraction contained a small unassigned peak which corresponded to the

retention time of 2-CARB in the 2 M fraction. A small unassigned peak in the 2 M fraction also corresponded to the retention time of γ GEPCSO eluting in the 1 M fraction. Line spectra obtained from photodiode array detection (PDA; Waters Model 996 photodiode array detector, Milford, Mass.) substantiated the identity of the fractions (data not shown). Thus both 2-CARB and γ GEPCSO were eluting in both the 1 and 2 M HOAc fractions, thereby slightly underestimating their concentration if not quantified in both fractions. The internal standards eluted only in their respective HOAc fraction, and there is no other peak contamination at their retention times. In light of these observations, it was decided to omit the use of 1 M acetic acid and elute 2-CARB, γ GEPCSO, MeGTH, and γ gG with 2 M HOAc (Fig. 1). The addition of this change decreased the total analysis time substantially since the number of HPLC runs (50 min per run) was decreased by a third.

HPLC sample preparation and analysis were carried out according to Randle et al. (1995). Each dried sample fraction was resuspended in 1.0 mL deionized-distilled water, a 100- μ L sample was transferred to a 1.5 mL microcentrifuge vial, and dried *in vacuo* using a Labconco Centrivap Concentrator (Kansas City, Mo.). Upon dryness, 250 μ L of 1 ethanol : 1 triethylamine (TEA) : 1 water was added to each sample and dried again. Derivatization was performed by the addition of 100 μ L of freshly prepared 7 ethanol : 1 TEA : 1 phenylisothiocyanate (PITC) : 1 water to each sample. The vials were immediately flushed with nitrogen, capped, and stored at room temperature for 20 min. The derivatizing solution was then removed *in vacuo*. Samples were redissolved using 500 μ L of 2 acetonitrile (HPLC grade): 7 water and transferred to 1.5-mL glass vials before HPLC analysis.

A Gilson (Madison, Wis.) HPLC unit with an auto injector (model 231) and an Applied Biosystems absorbance detector (model 759A; San Jose, Calif.) were used. A Spheri-5 RP-18 5 micron 250 $\times$ 4.6-mm column (Applied Biosystems, San Jose, Calif.) fitted with a 15 $\times$ 3.2-mm 7.0 micron guard column (RP-18 Newgard; Applied Biosystems) was used for separation. The column temperature was maintained at 30 °C using a Bio-Rad column heater (Hercules, Calif.). Eluents were A) 0.14 M sodium acetate with 0.05% TEA buffered to pH 6.5 with glacial acetic acid, and B) 60% acetonitrile in water (v/v). Each eluent was filtered through a 0.45- μ m Magna nylon filter (MSI, Westboro, Mass.). The eluent flow rate was 1.0 mL·min⁻¹ and the gradient was 15% B for 1.10 min, 15% to 45% B over 20 min, 45% to 0% B over 1 min, and a hold at 0% B for 14 min. The eluent composition was returned to the initial condition over one minute, and the column reequilibrated for ten minutes before the next injection. Eluted compounds from a 10 μ L injection of each HOAc fraction were

detected at 254 nm, and data were collected, recorded, and integrated using a personal computer (Gilson 718 sample manager software; Fig. 1). Peak assignment was carried out by comparing retention times with authentic standards (supplied by J.E. Lancaster, Crop and Food Research, Christchurch, New Zealand) as described by Randle et al., (1995).

DATA ANALYSIS. Prestorage and storage data were analyzed using the GLM procedures of SAS (Cary, N.C.) and Duncan's means separation. The SD cultivars, and the ID and LD cultivars were grouped according to storage duration before analyzing the data from the stored onions. The relationship between storage duration and ACSO or γ GP content was determined by regression analysis.

Results and Discussion

All precursor and peptide variables measured before and during storage differed significantly among the cultivars tested in the ANOVA ($P = 0.01$). Total ACSO, PRENCISO, 2-Carb and γ GEPCSO had significant ($P = 0.01$) cultivar by storage date interactions.

ACSO AND γ GP LEVELS BEFORE STORAGE. Before being placed into storage, bulb ACSO and γ GP concentration differed among cultivars (Table 1). Total ACSO concentration was highest for 'Dehydrator #3' (4.2 mg·g⁻¹ fresh mass) and lowest for 'Granex 33' and 'Walla Walla Sweet' (2.73 and 2.47 mg·g⁻¹ fresh mass, respectively). Total ACSO levels were similar to the range previously reported for onions (Edwards et al., 1994; Thomas and Parkin, 1994; Randle et al., 1995). Of the individual ACSOs, MCSO was measured in the highest concentration among the cultivars. MCSO concentration was highest for 'Dehydrator #3' (2.92 mg·g⁻¹ fresh mass), and lowest for 'Walla Walla Sweet' and 'Candy' (1.31 and 1.47 mg·g⁻¹ fresh mass, respectively). Previously, white or dehydrator type cultivars were reported to accumulate similar levels of MCSO and PRENCISO (Thomas and Parkin, 1994) or higher levels of MCSO than PRENCISO (Randle et al., 1995). The LD and ID cultivars tested were not significantly different for MCSO concentration within photoperiodic groups. In previous studies using HPLC analysis, PRENCISO was the predominant onion ACSO (Edwards et al., 1994; Thomas and Parkin, 1994). However, when plants were grown at deficient to luxuriant S concentration, MCSO became the dominant ACSO at the lower S concentrations (Randle et al., 1995). It is possible that while the concentrations of S in the hydroponic nutrient solutions were thought to be adequate and available at the half-strength Hoagland and Arnon rate (1950), SO₄²⁻ metabolism was affected by the

Table 1. Flavor precursor and precursor intermediates² (mg·g⁻¹ fresh mass) for short- (SD), intermediate- (ID), and long-day (LD) onion cultivars before storage.

Cultivar ^a	Type	MCSO	PRENCISO	PCSO	ACSO	2-CARB	γ GEPCSO
Dehydrator #3	SD	2.92 a ^x	0.99 abc	0.30 cd	4.20 a	1.07 a	2.92 a
Granex 33	SD	1.71 c	0.81 cd	0.21 e	2.73 ef	0.81 b	1.66 c
Walla Walla Sweet	ID	1.31 d	0.90 bcd	0.26 de	2.47 f	0.83 b	1.58 c
Candy	ID	1.47 cd	1.21 a	0.26 cde	2.94 de	0.78 b	1.49 c
Pukekohe	LD	2.00 b	1.05 ab	0.39 ab	3.43 bc	0.85 b	2.63 a
Zenith	LD	2.09 b	1.18 a	0.41 a	3.68 b	0.89 b	2.17 b
Sweet Sandwich	LD	2.09 b	0.73 d	0.33 bc	3.16 cd	0.83 b	1.81 c

²MCSO = methyl cysteine sulfoxide, PRENCISO = 1-propenyl cysteine sulfoxide, PCSO = propyl cysteine sulfoxide, ACSO = total amount of cysteine sulfoxide precursors, 2-CARB = 2-carboxypropyl glutathione, γ GEPCSO = γ -glutamyl 1-propenyl cysteine sulfoxide.

^aSeed Sources: 'Dehydrator #3', 'Walla Walla Sweet', and 'Zenith', SunSeed Inc., Hollister, Calif.; 'Granex 33' and 'Sweet Sandwich', Asgrow, Kalamazoo, Mich.; 'Candy', PetoSeed, Saticoy, Calif.; 'Pukekohe', Yates Seed, Auckland, New Zealand.

^xMean separation within columns by Duncan's multiple range test, $P = 0.05$.

nutrient balance of the solution. The percent bulb S for the cultivars (data not shown) at harvest in this study was similar to field grown onions (Randle, 1992). Sulfate uptake, therefore, did not appear to be affected by the hydroponic solutions used. In a recent study, however, lowering the N concentration in the hydroponic solution decreased MCSO levels and increased PRENCISO levels similar to those found in field-grown onions (unpublished data). How available N or other ions affect S metabolism through the flavor

pathway warrants further investigation.

Cultivars also differed for PRENCISO concentration (Table 1). The highest concentrations were measured with 'Candy', 'Zenith', 'Pukekohe', and 'Dehydrator #3' (1.21, 1.18, 1.05, and 0.99 mg·g⁻¹ fresh mass, respectively), while the lowest concentrations were measured from 'Sweet Sandwich', 'Granex 33', and 'Walla Walla Sweet' (0.73, 0.81, and 0.90 mg·g⁻¹ fresh mass, respectively). Differences also existed for PCSO levels among the cultivars and

Table 2. Effects of storage time at 5 ± 3 °C and 0.8 to 1.1 kPa vapor pressure deficit on flavor precursor^z means and standard errors (mg·g⁻¹ fresh mass) of the short-day 'Dehydrator #3' (D3) and 'Granex 33' (G33), intermediate-day 'Candy' (Ca) and 'Walla Walla Sweet' (WWS), and long-day 'Pukekohe' (PLK), 'Sweet Sandwich' (SS), and 'Zenith' (Ze) onion cultivars.

Months	Cultivar						
	D3	G33	Ca	WWS	PLK	SS	Ze
MCSO							
0	2.92 ± 0.11 ^y	1.71 ± 0.14	1.47 ± 0.11	1.31 ± 0.29	2.00 ± 0.11	2.09 ± 0.11	2.09 ± 0.09
1	2.45 ± 0.18	1.86 ± 0.08	1.24 ± 0.03	1.28 ± 0.20	1.98 ± 0.22	1.87 ± 0.33	2.06 ± 0.07
2	2.03 ± 0.10	1.78 ± 0.16	1.51 ± 0.17	1.29 ± 0.06	1.99 ± 0.17	2.26 ± 0.20	2.05 ± 0.09
3	2.16 ± 0.31	1.65 ± 0.17	1.54 ± 0.17	1.10 ± 0.13	2.12 ± 0.18	2.08 ± 0.12	2.06 ± 0.22
4	NA ^x	1.61 ± 0.09	1.32 ± 0.13	1.08 ± 0.20	2.22 ± 0.23	2.00 ± 0.18	2.36 ± 0.87
5	NA	NA	1.11 ± 0.15	0.97 ± 0.18	1.89 ± 0.16	1.96 ± 0.26	1.75 ± 0.15
6	NA	NA	1.14 ± 0.12	1.01 ± 0.14	1.97 ± 0.25	1.93 ± 0.33	1.69 ± 0.18
Regression significance							
Linear (L)	P = 0.001	P = 0.086	P = 0.004	P = 0.001	P = 0.230	P = 0.427	P = 0.135
Quadratic (Q)	P = 0.026	P = 0.095	P = 0.055	P = 0.960	P = 0.191	P = 0.434	P = 0.131
PRENCISO							
0	0.99 ± 0.05	0.81 ± 0.08	1.21 ± 0.03	0.90 ± 0.06	1.05 ± 0.16	0.73 ± 0.11	1.18 ± 0.25
1	1.05 ± 0.02	1.14 ± 0.12	1.18 ± 0.05	1.04 ± 0.05	1.08 ± 0.02	0.80 ± 0.08	1.28 ± 0.09
2	1.30 ± 0.06	1.24 ± 0.10	1.28 ± 0.12	1.20 ± 0.03	1.19 ± 0.07	0.84 ± 0.07	1.22 ± 0.05
3	1.60 ± 0.05	1.63 ± 0.11	1.44 ± 0.11	1.45 ± 0.12	1.23 ± 0.23	1.03 ± 0.09	1.38 ± 0.08
4	NA	1.77 ± 0.12	1.58 ± 0.09	1.58 ± 0.10	1.40 ± 0.08	1.12 ± 0.13	1.45 ± 0.12
5	NA	NA	1.67 ± 0.06	1.71 ± 0.08	1.48 ± 0.14	1.35 ± 0.13	1.53 ± 0.13
6	NA	NA	1.80 ± 0.14	1.78 ± 0.17	1.43 ± 0.18	1.29 ± 0.17	1.66 ± 0.14
Regression significance							
L	P = 0.001	P = 0.001	P = 0.001	P = 0.001	P = 0.001	P = 0.001	P = 0.001
Q	P = 0.003	P = 0.664	P = 0.117	P = 0.131	P = 0.647	P = 0.917	P = 0.265
PCSO							
0	0.30 ± 0.04	0.21 ± 0.04	0.26 ± 0.02	0.26 ± 0.04	0.39 ± 0.08	0.33 ± 0.02	0.41 ± 0.04
1	0.27 ± 0.02	0.24 ± 0.02	0.21 ± 0.02	0.24 ± 0.03	0.36 ± 0.05	0.36 ± 0.06	0.42 ± 0.04
2	0.24 ± 0.05	0.47 ± 0.50	0.22 ± 0.02	0.27 ± 0.01	0.40 ± 0.03	0.38 ± 0.05	0.38 ± 0.04
3	0.24 ± 0.04	0.23 ± 0.01	0.21 ± 0.02	0.22 ± 0.03	0.46 ± 0.09	0.34 ± 0.11	0.38 ± 0.06
4	NA	0.21 ± 0.03	0.21 ± 0.05	0.22 ± 0.04	0.41 ± 0.03	0.36 ± 0.02	0.45 ± 0.12
5	NA	NA	0.16 ± 0.02	0.23 ± 0.02	0.36 ± 0.05	0.36 ± 0.07	0.33 ± 0.04
6	NA	NA	0.16 ± 0.03	0.26 ± 0.04	0.40 ± 0.08	0.34 ± 0.06	0.34 ± 0.03
Regression significance							
L	P = 0.036	P = 0.939	P = 0.001	P = 0.098	P = 0.271	P = 0.397	P = 0.062
Q	P = 0.582	P = 0.195	P = 0.857	P = 0.094	P = 0.278	P = 0.387	P = 0.316
ACSO							
0	4.20 ± 0.08	2.73 ± 0.22	2.94 ± 0.15	2.47 ± 0.26	3.43 ± 0.16	3.16 ± 0.13	3.68 ± 0.35
1	3.78 ± 0.17	3.24 ± 0.19	2.63 ± 0.05	2.63 ± 0.05	3.49 ± 0.11	3.03 ± 0.46	3.76 ± 0.07
2	3.57 ± 0.11	3.24 ± 0.23	3.01 ± 0.24	2.75 ± 0.06	3.60 ± 0.11	3.48 ± 0.22	3.64 ± 0.11
3	3.99 ± 0.40	3.51 ± 0.09	3.18 ± 0.30	2.77 ± 0.11	3.80 ± 0.13	3.45 ± 0.04	3.82 ± 0.23
4	NA	3.58 ± 0.18	3.10 ± 0.18	2.87 ± 0.20	4.02 ± 0.28	3.47 ± 0.23	4.24 ± 1.04
5	NA	NA	2.93 ± 0.13	2.91 ± 0.22	3.73 ± 0.28	3.67 ± 0.46	3.62 ± 0.23
6	NA	NA	3.09 ± 0.17	3.05 ± 0.31	3.80 ± 0.22	3.55 ± 0.46	3.68 ± 0.26
Regression significance							
L	P = 0.058	P = 0.001	P = 0.071	P = 0.001	P = 0.002	P = 0.017	P = 0.287
Q	P = 0.006	P = 0.111	P = 0.398	P = 0.709	P = 0.058	P = 0.512	P = 0.308

^zMCSO = methyl cysteine sulfoxide, PRENCISO = 1-propenyl cysteine sulfoxide, PCSO = propyl cysteine sulfoxide, ACSO = total amount of cysteine sulfoxide precursors (ACSO = MCSO + PRENCISO + PCSO).

^yAverage milligram per gram fresh weight ± standard error.

^xNA = not available.

Table 3. Effects of storage time at 5 ± 3 °C and 0.8 to 1.1 kPa vapor pressure deficit on flavor precursor intermediate² means and standard error (mg·g⁻¹ fresh mass) of the short-day 'Dehydrator #3' (D3) and 'Granex 33' (G33), the intermediate-day 'Candy' (Ca) and 'Walla Walla Sweet' (WWS), and long-day 'Pukekohe' (PLK), 'Sweet Sandwich' (SS), and 'Zenith' (Ze) onion cultivars.

Months	Cultivar						
	D3	G33	Ca	WWS	PLK	SS	Ze
2-CARB							
0	1.07 ± 0.01 ^y	0.81 ± 0.14	0.78 ± 0.06	0.83 ± 0.10	0.85 ± 0.03	0.83 ± 0.06	0.89 ± 0.04
1	1.15 ± 0.11	0.88 ± 0.14	0.62 ± 0.02	0.92 ± 0.06	0.93 ± 0.12	0.77 ± 0.01	1.03 ± 0.04
2	1.31 ± 0.17	0.84 ± 0.09	0.97 ± 0.05	0.82 ± 0.04	1.22 ± 0.09	1.00 ± 0.05	0.90 ± 0.02
3	1.06 ± 0.08	0.90 ± 0.05	0.97 ± 0.06	0.89 ± 0.13	1.35 ± 0.09	0.88 ± 0.05	0.85 ± 0.05
4	NA ^x	0.85 ± 0.04	0.93 ± 0.08	0.86 ± 0.10	1.21 ± 0.04	0.76 ± 0.05	0.96 ± 0.07
5	NA	NA	0.76 ± 0.07	0.73 ± 0.12	1.09 ± 0.09	0.81 ± 0.01	1.07 ± 0.04
6	NA	NA	0.77 ± 0.04	0.75 ± 0.08	1.31 ± 0.12	0.99 ± 0.18	1.07 ± 0.15
Regression significance							
Linear (L)	<i>P</i> = 0.465	<i>P</i> = 0.522	<i>P</i> = 0.496	<i>P</i> = 0.039	<i>P</i> = 0.001	<i>P</i> = 0.329	<i>P</i> = 0.013
Quadratic (Q)	<i>P</i> = 0.061	<i>P</i> = 0.380	<i>P</i> = 0.002	<i>P</i> = 0.134	<i>P</i> = 0.004	<i>P</i> = 0.029	<i>P</i> = 0.045
γGPECSEO							
0	2.92 ± 0.25	1.66 ± 0.15	1.49 ± 0.26	1.58 ± 0.15	2.63 ± 0.27	1.81 ± 0.08	2.17 ± 0.12
1	2.65 ± 0.21	1.53 ± 0.34	1.16 ± 0.08	1.41 ± 0.16	2.43 ± 0.10	1.60 ± 0.26	1.92 ± 0.14
2	2.49 ± 0.17	1.26 ± 0.12	1.08 ± 0.04	1.11 ± 0.05	2.26 ± 0.15	1.72 ± 0.18	1.86 ± 0.18
3	2.15 ± 0.13	1.03 ± 0.19	1.14 ± 0.05	0.63 ± 0.08	2.27 ± 0.11	1.41 ± 0.01	1.49 ± 0.02
4	NA	0.74 ± 0.04	0.81 ± 0.10	0.31 ± 0.08	2.08 ± 0.12	1.20 ± 0.21	1.51 ± 0.10
5	NA	NA	0.67 ± 0.15	0.19 ± 0.05	1.74 ± 0.10	0.89 ± 0.22	1.26 ± 0.26
6	NA	NA	0.44 ± 0.09	0.12 ± 0.05	1.76 ± 0.24	0.52 ± 0.08	1.26 ± 0.16
Regression significance							
L	<i>P</i> = 0.001	<i>P</i> = 0.001	<i>P</i> = 0.001	<i>P</i> = 0.001	<i>P</i> = 0.001	<i>P</i> = 0.001	<i>P</i> = 0.001
Q	<i>P</i> = 0.797	<i>P</i> = 0.440	<i>P</i> = 0.452	<i>P</i> = 0.011	<i>P</i> = 0.833	<i>P</i> = 0.014	<i>P</i> = 0.156

²2-CARB = 2-carboxypropyl glutathione, γGPECSEO = γ-glutamyl 1-propenyl cysteine sulfoxide.

³Average milligram per gram fresh weight ± standard error.

^xNot available.

PCSO was found in the lowest concentration of the ACSOs (Table 1). Propyl cysteine sulfoxide levels reported in this study were generally lower than previously reported for different onion cultivars grown at a similar S fertility (Randle et al., 1995).

Flavor precursor intermediate levels also differed among cultivars before storage. 'Dehydrator #3' differed in 2-CARB concentration, while 'Dehydrator #3' and 'Pukekohe' had the highest concentrations of γGPECSEO (Table 1). S-2-Carboxypropyl glutathione occurs early in the flavor pathway, and γGPECSEO is the penultimate peptide in the synthesis of PRENCSEO (Lancaster and Boland, 1990). These peptide intermediates values were generally measured in higher concentrations, but within the range of those previously reported (Randle et al., 1995).

ACSO AND γGP LEVELS DURING STORAGE. The levels of the total ACSOs, individual ACSO, and precursor intermediates changed during storage for all the cultivars tested (Tables 2 and 3). The trends for total ACSO changes during storage were variable among the cultivars tested. Total ACSO concentration for 'Dehydrator #3' decreased then increased quadratically (*P* = 0.002) during 3 months of storage while the total ACSO concentration of 'Granex33' increased linearly during 4 months of storage (*P* = 0.001). Significant linear increases for ACSO concentration were identified for 'Candy' (*P* = 0.072), 'Walla Walla Sweet' (*P* = 0.039), 'Pukekohe' (*P* = 0.001), and 'Sweet Sandwich' (0.024) during 6 months of storage, but no significant trend was displayed for 'Zenith'. Levels of total free flavor precursors and peptide intermediates of 'Southport White Globe' onions increased during 5 months of storage at 10 °C and ambient humidity (Lancaster and Shaw, 1991). The changes in the levels of MCSO, PRENCSEO, and PCSO during storage were cultivar dependent. Before storage, MCSO

was in the highest concentration (Table 1). Methyl cysteine sulfoxide remained in the highest concentration throughout storage for 'Dehydrator #3', 'Pukekohe', 'Sweet Sandwich', and 'Zenith' (Table 2). For 'Granex 33', 'Candy', and 'Walla Walla Sweet', the concentrations of PRENCSEO became greater than MCSO during storage. For 'Walla Walla Sweet', PRENCSEO levels exceeded the MCSO levels during the third storage month while it was not until the fourth storage month for 'Granex 33' and 'Candy' (Table 2). Of the individual ACSO compounds, PCSO was always found in the lowest concentration for each cultivar throughout storage. These trends demonstrate that the biosynthetic flavor pathway in onions is active during bulb storage.

Of the individual ACSOs, MCSO decreased linearly for each SD and ID cultivar during storage (Table 4). However, no significant trends were displayed for the LD cultivars. The monthly rate of MCSO decrease, as indicated by the slope of the regressions, ranged from -0.40 mg·g⁻¹ fresh weight for 'Granex 33' to -0.05 mg·g⁻¹ fresh weight for 'Candy' (Table 4). The monthly MCSO decrease was much greater for the SD onions than for the ID cultivars (Table 4). The slopes of the cultivars were separated using a generalized linear model procedure (GLM) of SAS, and differences were determined using an F statistic at the α = 0.05 level. Based on this confidence level, the rates of MCSO degradation differed among the SD and ID cultivars. The regression slope for 'Dehydrator #3', 'Granex 33', and 'Walla Walla Sweet' differed significantly, but the slopes of 'Walla Walla Sweet' and 'Candy' were not significantly different. The MCSO slope during storage could be associated with each cultivars dormancy characteristics. 'Dehydrator #3' and 'Granex 33' both had very short dormancy periods (Kopsell and Randle, 1997).

1-Propenyl-cysteine sulfoxide concentration linearly increased during storage for all cultivars (Table 4). A comparison of the

Table 4. Significance of the linear regression equations of methyl cysteine sulfoxide (MCSO), 1-propenyl cysteine sulfoxide (PRENCISO), and γ -glutamyl 1-propenyl cysteine sulfoxide (γ GPECSO) of the short-day (SD) 'Dehydrator #3' (D3) and 'Granex 33' (G33), intermediate-day (ID) 'Candy' (Ca) and 'Walla Walla Sweet' (WWS), and long-day (LD) 'Pukekohe' (PLK), 'Sweet Sandwich' (SS), and 'Zenith (Ze) onions during storage at 5 ± 3 °C and 0.8 to 1.1 kPa vapor pressure deficit.

Cultivar	Type	Linear regression equation					
		MCSO ^z		PRENCISO		γ GPECSO	
		b	m	b	m	b	m
D3	SD	3.10	-0.29***	0.73	0.20***	3.17	-0.24***
G33	SD	1.84	-0.40*	0.59	0.24***	1.94	-0.23***
Ca	ID	1.54	-0.05**	1.01	0.11***	1.60	-0.16***
WWS	ID	1.40	-0.06***	0.75	0.16***	1.85	-0.27***
PLK	LD	NS		0.96	0.08***	2.76	-0.15***
SS	LD	NS		0.59	0.11***	2.14	-0.21***
Ze	LD	NS		1.07	0.08***	2.27	-0.16***

^zb = Y intercept and m = slope of the regression equation. Units are mg·g⁻¹ fresh mass onion tissue.

NS, *, **, *** Nonsignificant or significant at $P = 0.10$, 0.01, or 0.001, respectively.

slopes of each regression equation using GLM indicated that the rate of the increase differed among cultivars. The slopes of the SD cultivars were not different at the $\alpha = 0.05$ level, but their slopes were different from the ID and LD cultivars. The rates at which PRENCISO increased for the ID cultivars were significantly different, but there was no difference between the slopes of 'Candy' and 'Sweet Sandwich'. Nor were there any differences among the slopes of the LD cultivars. The slope of PRENCISO during storage also appeared to be associated with a cultivars dormancy characteristics (Kopsell and Randle, 1997). Those cultivars with longer dormancy generally had smaller regression slope values.

The trends of 2-CARB were also very variable among the cultivars. Quadratic trends which first increased, then decreased, were charted for 'Dehydrator #3' ($P = 0.06$), 'Candy' ($P = 0.002$), and 'Sweet Sandwich' ($P = 0.024$). S-2 carboxypropyl glutathione increased linearly for 'Pukekohe' ($P = 0.001$) and 'Zenith' ($P = 0.013$) while a linear decrease was found for 'Walla Walla Sweet' ($P = 0.039$).

All cultivars decreased linearly for γ GPECSO concentration during storage (Table 4). The regression slopes for γ GPECSO were statistically different among cultivars. The slopes of 'Walla Walla Sweet' and 'Granex 33', 'Granex 33' and 'Candy', and 'Sweet Sandwich' and 'Candy' were different, but no differences were found between the slopes of 'Walla Walla Sweet' and 'Dehydrator #3', and between 'Dehydrator #3' and 'Granex 33'.

An interesting relationship was observed between γ GPECSO and PRENCISO (Table 4). With all the cultivars, PRENCISO linearly increased while γ GPECSO linearly decreased during storage (Table 4). In the proposed biosynthetic flavor pathway, γ -glutamyl transpeptidase [EC 2.3.2.2] is responsible for the hydrolysis of the γ -glutamyl moiety from γ GPECSO to produce PRENCISO (Schwimmer, 1971; Lancaster and Shaw, 1994). Actively growing onions and sprouting onion bulbs that have broken dormancy exhibited relatively high γ -glutamyl transpeptidase activity, whereas dormant onion bulbs showed little or no activity (Lancaster and Shaw, 1991). During eight months of storage, Hanum et al. (1995) reported that the yellow pungent cultivar 'Spartan Banner' displayed no measurable γ -glutamyl transpeptidase activity *in vitro* through the second month of storage, but activity increased from 0 to over 2 units of activity/mg protein between months 3 and 8. Hanum et al. (1995) however did not measure levels of γ GPECSO or PRENCISO, nor did they report when 'Spartan Banner' broke dormancy. A decrease in the levels of γ GPECSO and an increase in the levels of PRENCISO for each cultivar in our study suggested γ -glutamyl transpeptidase activity

throughout the storage period (Fig. 2). The conversion of γ GPECSO to PRENCISO was not associated with bulb dormancy. For example, 'Dehydrator #3' broke dormancy after the first month of storage while all the LD cultivars remained dormant for the entire storage period (data not shown). Because the regression slopes at which γ GPECSO decreased and PRENCISO increased were different among the cultivars, there also may be differences in the activity of γ -glutamyl transpeptidase among the cultivars as well (Table 4).

In a previous report, bulbs from these same cultivars were evaluated for gross flavor intensity as measured by enzymatically formed pyruvic acid (EPY) (Kopsell and Randle, 1997). Although EPY is not a flavor compound responsible for the characteristic onion flavors, it is a stable primary product of ACSO hydrolysis that was well correlated with flavor perception (Wall and Corgan, 1992). Direct comparison of the changes in the ACSOs during storage can be made with EPY in the previous study because the tissues in the different analyses were taken from the same bulbs. Interestingly, changes in EPY during storage could not universally explain the changes in total ACSO content or changes in the individual ACSOs. For example, EPY increased during storage only for 'Granex 33'. However, total ACSO content increased for all the cultivars except 'Dehydrator #3' and 'Zenith', which

Fig. 2. Linear regression trends for 1-propenyl cysteine sulfoxide (PRENCISO ●) and γ -glutamyl 1-propenyl cysteine sulfoxide (γ GPECSO ◆) for 'Granex 33' during 4 months of storage at 5 ± 3 °C and 0.8 to 1.1 kPa vapor pressure deficit.

decreased or remained unchanged respectively. One would expect an increase in total ACSO content during storage to accompany an increase in EPY. In a study evaluating S-supply on onion ACSO accumulation, it was also difficult to draw a clear relationship between $\mu\text{mols EPY}$ and $\mu\text{mols total ACSO}$ (Randle et al., 1995).

We are currently investigating the role of alliinase activity during storage to possibly explain the discrepancy between the changes in ACSO content and EPY. Alliinase specific activity (units/mg protein) varied among cultivars and with plant development (Lancaster et al., 1993). In addition, the *in vitro* K_m of alliinase differs with the individual ACSOs. 1-Propenyl cysteine sulfoxide had the lowest value, indicating that it appears to be the major substrate for alliinase (Schwimmer, 1969; Hanum et al., 1995). *In vivo* decomposition of PRENCISO was immediate and almost 100% within 20 s of maceration, while 40% of the PCISO and 30% of the MCISO remained intact up to 2 h after maceration (Lancaster et al., 1995). Matching individual ACSO changes with *in vivo* alliinase specific activity during storage may explain why EPY decreases do not follow total ACSO increases.

Differences in ACSO and γGP accumulation exist among onion cultivars when grown under similar conditions. Likewise, changes during storage in ACSO and γGP concentrations were cultivar dependent, and could not be associated with photoperiodic classification. These differences can be attributed to the heterogeneity found with in the *Allium cepa* germplasm. Despite these differences, similarity in the behavior of PRENCISO and $\gamma\text{GPECISO}$ among onion cultivars was discovered. All the cultivars tested decreased in $\gamma\text{GPECISO}$ and increased in PRENCISO concentration during storage, but differences in the rates of $\gamma\text{GPECISO}$ decomposition and PRENCISO accumulation were identified among cultivars. The enzyme γ -glutamyl transpeptidase is responsible for the conversion of $\gamma\text{GPECISO}$ to PRENCISO. Although γ -glutamyl transpeptidase activity has only been reported in actively growing onions, or onions that have broken dormancy, in this study conversion of $\gamma\text{GPECISO}$ to PRENCISO was found in dormant bulbs. Changes in the ACSO compounds for the onion cultivars during storage did not reflect EPY changes in a previous report. The differences in flavor expression among onion cultivars may be best understood through a more detailed understanding of the concentration and behavior of alliinase in relation to ACSO content. Understanding the mechanisms responsible for flavor changes that occur during storage will allow for better product utilization after storage.

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