CHANGES IN *S*-ALK(EN)YL CYSTEINE SULFOXIDES AND PRECURSOR INTERMEDIATES DURING STORAGE AMONG SHORT- AND LONG-DAY ONION CULTIVARS

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Abstract

Flavor precursor and γ-glutamyl peptide intermediate changes during storage were evaluated for two short-day (SD) and one long-day (LD) onion (*Allium cepa* L.) cultivars. Onion cultivars were greenhouse-grown, harvested, and stored under similar conditions. Cultivars differed in flavor changes during storage. (+) *S*-methyl-L-cysteine sulfoxide (MCSO) levels decreased, (+)-*S*-(1-propenyl)-L-cysteine sulfoxide (PRENCSO) levels increased linearly, and (+) *S*-propyl-L-cysteine sulfoxide levels decreased during storage. Of the peptide intermediates measured, *S*-2-carboxypropyl glutathione changes were variable, and γ-L-glutamyl-*S*-(1-propenyl)-L-cysteine sulfoxide levels decreased linearly among the cultivars. Enzymatically formed pyruvic acid levels (EPY) increased for 'Granex 33', but decreased for 'Dehydrator #3' and 'Pukekohe' during storage. The change in total *S*-alk(en)yl cysteine sulfoxide (ACSO) levels for the cultivars followed the changes in MCSO and PRENCSO. However, the behavior of MCSO and PRENCSO did not explain EPY changes for the cultivars. Because the behavior of the ACSO compounds cannot fully explain EPY changes, a more detailed understanding of the enzymatic reaction may be needed. Understanding flavor changes over time could be used to meet the specific flavor demands industry places on onions coming out of storage.

1. Introduction

Consumer demand outside the fresh market window has driven the postharvest onion market, and onions are routinely marketed and used out of short- and long-term storage. Due to physiological differences, environmental conditions during growth, and postharvest handling, onion cultivars differ in their storage duration. Onion cultivars also differ in production of enzymatically formed pyruvic acid (EPY) or pungency during storage (Kopsell *et al.*, 1997).

The precursor compounds that give rise to *Allium* flavor are termed *S*-alk(en)yl-L-cysteine sulfoxides (ACSOs). The three naturally occurring ACSO compounds in onions are *trans*-(+)-*S*-(1-propenyl)-L-cysteine sulfoxide (PRENCSO), responsible for the lacramatory or tear-producing effect, (+)-*S*-methyl-L-cysteine sulfoxide (MCSO) and (+)-*S*-propyl-L-cysteine sulfoxide (PCSO; Lancaster *et al.*, 1990). The ACSO compounds are the end product of a proposed flavor biosynthetic pathway which begins with the reduction of sulfate to sulfide and its incorporation into cysteine. Cysteine is converted into glutathione which leads to the production of *S*-2-carboxypropyl glutathione (2-CARB). 2-CARB is converted into γ-glutamyl peptide intermediates, which hydrolyze to form the ACSOs (Lancaster *et al.*, 1989).

Upon cellular disruption, the enzyme alliinase (EC 4.4.1.4) located in the vacuole is released and hydrolyzes the ACSO compounds, which reside in the cytoplasm (Lancaster *et al.* 1990). The products of ACSO hydrolysis by alliinase are alkyl sulfenic acids, pyruvic acid, and ammonia. The alkyl sulfenic acids are unstable and rearrange non-enzymatically to form thiosulfinates (Block, 1992). Pyruvate is a stable by-product of the reaction and the formation of EPY has been shown to correlate with taste perception (Wall *et al.*, 1992). However, there are instances where taste deviates from measured EPY (personal observations). Because EPY reflects only gross pyruvic acid production from all ACSOs, the amounts and behavior of individual ACSO compounds during storage is unknown. Our study was conducted to investigate the behavior of the ACSO compounds and biosynthetic intermediates during storage.

2. Materials and Methods

2.1.Plant Cultur

Onion cultivars ['Dehydrator #3' SD, (Sunseed Inc.; Hollister CA), 'Granex 33' SD, (Asgrow; Kalamazoo, MI), and 'Pukekohe' LD (Yates Seed; Auckland, New Zealand)] representing a range of storage characteristics, flavor intensity, usage types, and photoperiodic responses were selected. Onions were greenhouse-grown (34° N latitude) with day/night temperatures of 28/16 °C in hydroponic growing beds using a modified Hoagland and Arnon (1950) solution. Artificial lighting was applied to the ‘Pukekohe’ plants to ensure proper growth and development. Each cultivar was harvested when 50% of the bulbs had soft necks. At harvest, the roots and tops were clipped and the bulbs were hung in the greenhouse to cure for 7 days.

2.2. Bulb Storage

Prior to storage, four ten-bulb replicates of each cultivar were analyzed for ACSO content. The remaining ten-bulb samples of each cultivar were placed into a cooler set at 5 ± 3 °C, 80-85% RH. Every 30 days thereafter, bulbs were removed from storage and acclimatized to room temperature for 24 hours prior to the ACSO analysis. 'Granex 33' (SD) was stored for 4 months, while 'Dehydrator #3' (SD) only stored for 3 months because of severe storage losses in the fourth month. ‘Pukekohe’ was stored for 6 months.

2.3. ACSO quantification

At each sampling date, the ACSOs and precursor intermediates were extracted by placing wedges taken from each ten-bulb replicate into 12:5:3 methanol:chloroform:water and ethanol (80%; Lancaster *et al.* 1983). Samples were ion exchanged using 0.1 and 2.0 M acetic acid (HOAc) concentrations prior to being subjected to high performance liquid chromatography (HPLC) separation. Each sample fraction was derivitized with 7:1:1:1 ethanol:triethylamine:water:phenylisothiocyanate *in vacuo* and HPLC separation was achieved using a Spheri-5 RP-18 5 micron 250 $\times$ 4.6-mm column maintained at 30 °C. Sample run conditions were according to Randle *et al.* (1995).

3. Concluding Remarks

3.1. Pre-Storage ACSO and γGP levels.

Prior to storage, bulb ACSO and γGP concentration differed among cultivars (Table 1). MCSO was found in highest concentration for each cultivar. Onions grown at deficient S concentration had higher MCSO levels (Randle *et al.*, 1995), however, % bulb S at harvest was similar to field grown onions (Randle, 1992) indicating a possible upset of S metabolism inside the plant. Cultivars also differed for PRENCSO and PCSO concentration. 2-CARB and γ-glutamyl (1-propenyl)-L-cysteine sulfoxide (γGPECSO), the penultimate compound to PRENCSO, levels also differed among cultivars prior to storage (Table 1).

3.2. ACSO and γGP levels during storage.

MCSO decreased on a linear basis for the SD cultivars during storage but was non-significant for 'Pukekohe' (Figure 1). PRENCSO of each cultivar increased linearly. PCSO either displayed a decreasing trend or none at all. 2-CARB trend was quadratic for 'Dehydrator #3' (*P*=0.061), non-significant for 'Granex 33' and a linear increase for 'Pukekohe' (*P*=0.001). γGPECSO changes were similar among all cultivars tested, decreasing on a linear basis (*P*=0.001 for each cultivar) as storage duration increased.

Although ACSO and γGP changes during storage were similar among the three cultivars, the EPY changes during storage for the same samples were different. EPY levels increased linearly for 'Granex 33', but decreased linearly for 'Dehydrator #3' and 'Pukekohe' (Kopsell *et al*., 1997). Changes in EPY during storage cannot be explained by the levels and behavior of ACSO and γGP compounds. Differences in flavor expression during storage may be best understood through an understanding of the behavior of alliinase. The ACSO compounds are hydrolyzed at different rates by alliinase and isozymes of alliinase may be present in onions (Lancaster *et al*., 1995). If cultivars differences in alliinase form and activity can be identified, there exists the possibility to manipulate alliinase to change flavor expression, so that regardless of the level of ACSO compounds present in an onion bulb, the rates of hydrolysis and flavor intensity can be controlled.

References

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

Hoagland, D.R. and D.I. Arnon. 1950. The water culture method for growing plants without soil. California Agric. Expt. Sta. Circ. 347.

Kopsell, D.E. and W.M. Randle. 1997. Onion cultivars differ in pungency and bulb quality changes during storage. HortScience 32(7):1260-1263.

Lancaster, J.E. and K.E. Kelly. 1983. Quantitative analysis of the S-alk(en)yl-L-Cysteine sulfoxides in onion (Allium cepa L.). J. Sci. Food Agr. 34:1229-1235.

Lancaster, J.E. and M.J. Boland. 1990. Flavor biochemistry, p. 33-72. In: H.D. Rabinowitch and J.L. Brewster (eds.). Vol. 3: Onions and allied crops. CRC Press, Boca Raton, Fla.

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(2):455-460.

Lancaster, J., M.L.Shaw, and W.M. Randle. 1995. Hydrolysis of sulfur substrates *in vivo* by alliinase: Effects on flavor. Proceedings of the 1995 National Onion Research Conference. Madison, WI, pp. 53-58.

Randle, W.M. 1992. Sampling procedures to estimate flavor potential in onion. HortScience 27: 1116-1117.

Randle, W.M., J.E. Lancaster, M.L. Shaw, K.H. Sutton, R.L. Hay, and M.L. Bussard. 1995. Quantifying onion flavor compounds responding to sulfur fertility-Sulfur increases levels of alk(en)yl cysteine sulfoxides and biosyntehtic intermediates. J. Amer. Soc. Hort. Sci. 120(6):1075-1081.

Wall, M.M. and J.N. Corgan. 1992. Relationship between pyruvate analysis and flavor perception for onion pungency determination. Hortscience 27(9):1029-1030.

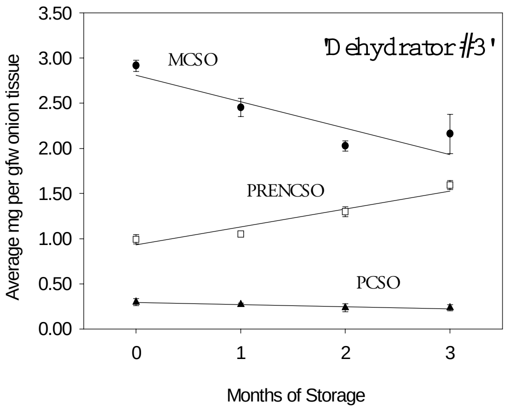
Tables

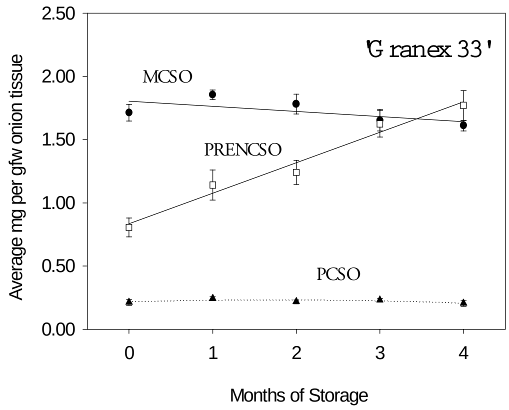
1. ACSO and γGP levels for SD and LD onion cultivars prior to storage at 5 ± 3 °C and 80% relative humidity.

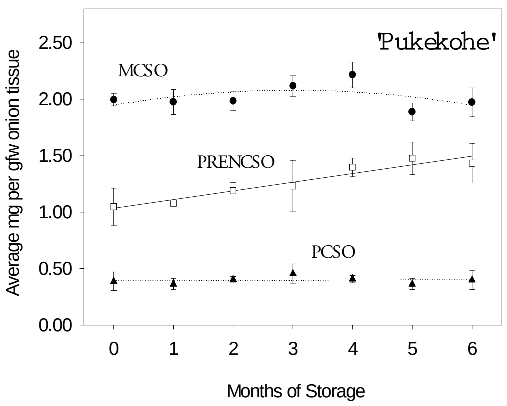
Cultivar	MCSO [z]	PRENCSO	PCSO	2-CARB	γGPECSO
‘Dehydrator #3’ (SD)	2.92 a [y]	0.99 ab	0.30 b	1.07 a	2.92 a
‘Granex 33’ (SD)	1.71 c	0.81 b	0.21 c	0.81 b	1.66 b
‘Pukekohe’ (LD)	2.00 b	1.05 a	0.39 a	0.85 b	2.63 a

[z] Average mg per gram fresh weight.
[y] Mean separation within columns by Duncan’s multiple range test, *P* = 0.05.

Figures







1. Trends of ACSO compounds for ‘Dehydrator #3’ (MCSO=3.10-0.29(Date) *P*=0.001; PRENCSO=0.73+0.20(Date) *P*=0.001; PCSO=0.32-0.02(Date) *P*=0.036) during 3 months of storage, ‘Granex 33’ (MCSO=1.84-0.40(Date) *P*=0.086; PRENCSO=0.59+0.24(Date) *P*=0.001; PCSO=N.S.) during 4 months of storage, and ‘Pukekohe’ (MCSO=N.S.; PRENCSO=0.96+0.08(Date) *P*=0.001; PCSO=N.S.) during 6 months of storage at 5 ± 3°C, 80% relative humidity