Incubation Time, Cultivar, and Storage Duration Affect Onion Lachrymatory Factor Quantification

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Abstract. The lachrymatory factor [LF, (Z,E) propanethial S-oxide] is a direct product of 1-propenyl cysteine sulfoxide (1-PRENSCO) hydrolysis and dominates onion flavor when present in high concentrations. To evaluate LF as a potential means of assessing flavor quality, two onion cultivars were greenhouse-grown and the bulbs stored for 4 months at 3 ± 1°C, 70% relative humidity. Onions were evaluated at monthly storage intervals for LF development in bulb macerates following a 120 seconds incubation time. When LF was compared to amounts of 1-PRENSCO hydrolyzed, we found that LF was severely underestimated. The relationship of LF and 1-PRENSCO also varied between cultivars during storage. As ‘Granex 33’ was stored for longer periods, the amount of LF measured at 120 seconds more closely reflected the amount of 1-PRENSCO hydrolyzed. LF from ‘Dehydrator’ #3, however, was consistently underestimated regardless of storage time. Therefore, a second experiment was conducted using individual bulbs of two onion cultivars in an attempt to determine the optimal incubation time for LF quantification. Maximum LF among bulbs was generally detected 5–10 seconds after tissue maceration for ‘Dehydrator’ and after 15–30 seconds for ‘Sweet Vidalia’. The amount of LF quantified between 5 and 120 seconds decreased linearly for nine of ten bulbs of ‘Dehydrator’, but this trend was less apparent for ‘Sweet Vidalia’. A uniform LF incubation time for individual bulbs, therefore, may not be possible for all cultivars. These data show a complex relationship among and within onion cultivars for 1-PRENSCO hydrolysis and the formation of LF in onion macerates.

Onions (Allium cepa L.) are primarily consumed for their flavors. The characteristic onion flavor develops when the tissue is cut or damaged. The enzyme allinase (EC 4.4.1.4), which is located in the vacuole, is released to hydrolyze the flavor precursors, collectively known as S-alk(en)yl-L-cysteine sulfoxides (ACSOs), located in the cytoplasm (Block, 1992; Lancaster and Collin, 1981). The three naturally occurring onion ACSOs are trans-(+)-S-(1-propenyl)-L-cysteine sulfoxide (1-PRENSCO), (+)-S-methyl-L-cysteine sulfoxide (MCSO), and (+)-S-propyl-L-cysteine sulfoxide (PCS0; Lancaster and Boland, 1990). Initial products of the hydrolytic reaction are sulfenic acids that then go on to produce the lachrymatory factor (LF), thiosulfimates, ammonia, and pyruvic acid.

The thiosulfimates are responsible for the raw flavor attribute associated with raw onions when consumed (Block, 1986; Randle et al., 1994). The thiosulfimates rearrange over time and produce disulfides and other S-compounds. (Z,E) Propanethial S-oxide, or the LF, arises from the hydrolysis of 1-propenyl cysteine sulfoxide (1-PRENSCO) and is responsible for the mouth burn and heat associated with onion consumption when in solution (Block, 1992; Randle et al., 1994). Sensory attributes from the LF can be overwhelming and dominate the experience when consuming onions with 1-PRENSCO in high concentration.

Many methods have been reported for LF quantification and quantification. The earliest attempts to separate the components of onion volatiles, including the LF, employed steam distillation and chromatography separations (Spare and Virtanen, 1963). These methods, however, were qualitative rather than quantitative. Saghir et al. (1963) developed a gas chromatography procedure using an internal standard to quantify mono- and disulfides in onion headspaces that later proved to have too high of a run time to capture the LF. Another LF quantification method used hexane extraction and spectrophotometric absorbance at 254 nm (Freeman and Whenhen, 1975). However, because other compounds were also extracted in hexane and absorbed at 254 nm, this method proved to be unreliable for LF quantification (Schmidt et al., 1996). Tewari and Bandyopadhyay (1975) developed a thin layer chromatography (TLC) procedure for LF quantification, but TLC is a slow, cumbersome process. Using TLC, LF was reported to be maximally produced within 2 min of tissue maceration, and to rapidly disappeared thereafter (Lukes, 1971). High-performance liquid chromatography (HPLC) can separate many of the onion thiosulfimates, but does not quantify the LF because it co-elutes with the C4 thiosulfimates (Block, 1992; Block et al., 1992).

Using gas chromatography–mass spectral separation, hot injection port and column temperatures caused onion chemicals to rearrange and created artifacts (Block et al., 1992). However, by using lower temperatures during GC injection and separation, LF was reliably detected but not quantified (Block et al., 1992).

Schmidt et al. (1996) investigated and developed a rapid GC method to optimize and quantify the LF by utilizing an internal standard. They reported maximum LF detection at their initial assessment time following a 2-min macerate incubation. Beyond 2 min, LF decreased and they speculated this was due to volatilization, hydrolysis, or reduction (Schmidt et al., 1996). The most recently published technique for analyzing onion thiosulfimates and LF employed supercritical fluid extraction (Calvey et al., 1997). However, LF detection was low due to its volatility and inefficient trapping on glass beads at 0°C.

Because the method of Schmidt et al. (1996) appeared to be the most reliable and rapid procedure for quantifying LF, an experiment (1) was conducted using their method to assess LF changes that may occur before and during bulb storage using two onion cultivars. However, when the LF was compared to the amount of 1-PRENSCO hydrolysis in the macerates, an inherent problem with the time of incubation became apparent. A second experiment (2) was conducted to determine the time of maximum LF detection in onion macerates and its relationship to 1-PRENSCO hydrolysis.

Materials and Methods

Experiment 1. Two short-day onion cultivars, ‘Dehydrator #3’ (Sunseeds, Hollister, Calif.) and ‘Granex 33’ (Asgrow, Kalamazoo, Mich.), were selected based on their previous reported flavor changes during storage (Kopsell and Randle, 1997). In Dec. 1997, each cultivar was seeded into Fafard 3-B substrate (Fafard, Anderson, S.C.), watered and fertilized with 20N–20P–20K nutrient solution (Grace-Sierra Co., Milpitas, Calif.) every 7 to 10 d. Seedlings were greenhouse grown (34°C, 70% relative humidity) throughout the experiment.

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Bulbs of each cultivar were harvested from 5-10 May 1998 when >50% of the plants had foliar lodging. Bulb size and maturity were similar to field-grown onions. The plants were uprooted and laid in the boxes for several days. As the leaves senesced and browned, they and the roots were removed and the bulbs placed into mesh bags and hung in the greenhouse to dry for 7 d. About 18 uniform bulbs from each cultivar were selected and placed into 16 mesh bags each.

Prior to storage, four, 10-bulb replicates of each cultivar were set aside for initial analyses. The remaining 18-bulb bags were placed into refrigerated storage (3 ± 1 °C, 70% relative humidity) using a split-block design with four blocks. Blocks were the different quadrants of the cooler, cultivars were the main plots, and months of storage were the subplots. All cultivars were stored for 4 months. At monthly intervals, four bags of onions from each cultivar were removed from storage and juiced in a pneumatic press and a 0.5-mL aliquot was taken from the onion juice for separation. The GC used a split-liner injection maintained at 200 °C. The injector temperature was maintained at 300 °C greater than the oven temperature. A flame ionization detector maintained at 250 °C was used for the GC analysis. The head pressure of 1.0 PSI (1.2 mL·min –1) with 99.999% He on a 5 mm × 0.54-mm OV-1 column. The oven temperature was 60 °C for 1 min and then increased 5 °C/min to 200 °C. The LF response with this method of Block et al. (1996). LF concentration was determined by comparing GC peak areas of the compound and the p-cymene internal standard for the same sample.

**Experiment 2.** Freshly harvested onion bulbs were obtained from industry sources (‘Sweet Vidalia’ from Terry Gerald, Statesboro, Ga., and ‘Dehydrator’ from Rogers Turlock, Calif.). ‘Sweet Vidalia’ is a mild, yellow Granex-type onion, while ‘Dehydrator’ is a high solids, pungent onion. Ten, single-bulb replicates were selected for each cultivar. 1-Propenyl cysteine sulfoxide was determined as in Expt. 1 with the following exception. 1-Propenyl cysteine sulfinate was hydrolyzed and LF produced in the onion macerates was determined after 5, 10, 15, 30, 60, 90, and 120 s of incubation to determine the time of maximum LF production.

LF was quantified using a modified procedure of Schmidt et al. (1996) to lessen the time required for LF analysis. A 1.0-mL aliquot of juice was taken from the onion juice for separation. The GC used a split-liner injection maintained at 200 °C. The injector temperature was maintained at 300 °C greater than the oven temperature. A flame ionization detector maintained at 250 °C was used for the GC analysis. The head pressure of 1.0 PSI (1.2 mL·min –1) with 99.999% He on a 5 mm × 0.54-mm OV-1 column. The oven temperature was 60 °C for 1 min and then increased 5 °C/min to 200 °C. The LF response with this method of Block et al. (1996). LF concentration was determined by comparing GC peak areas of the compound and the p-cymene internal standard for the same sample.

**Results and Discussion**

**Experiment 1: Changes in LF during bulb storage.** Data analyzed by GLM showed that the LF produced from macerated bulbs was not different to differ between cultivars (P = 0.1) among months of bulb storage (P = 0.001), and for the interaction between cultivars and months of storage (P = 0.007). 1-Propenyl cysteine sulfoxide hydrolyzed in the onion macerates differed between cultivars (P = 0.001) and among months of bulb storage (P = 0.004). Onion flavor changes as measured by pyruvic acid content (Kopsell and Randle, 1997) and flavor precursor content (Kopsell et al., 1999) have been reported during bulb storage.

Prior to storage, the amounts of 1-PRENCSO hydrolyzed were substantially more than the amounts of LF produced 120 s after tissue maceration (Table 1). For ‘Granex 33’, 11.48 µmol·mL–1 of 1-PRENCSO juice were hydrolyzed. However, only 6.96 µmol·mL–1 of LF juice were captured; yielding a 1-PRENCSO : LF ratio of 1.7:1. The difference between LF produced and 1-PRENCSO hydrolyzed was even greater for ‘Dehydrator #3’ with a 1-PRENCSO : LF ratio of 4:1. ‘Dehydrator #3’ also hydrolyzed almost three times the 1-PRENCSO as did ‘Granex 33’, yet less than twice the LF was measured. These results suggested a problem in sampling for LF using a 120-s macerate incubation time.

During storage, the amounts of 1-PRENCSO hydrolyzed and LF detected changed as did their ratios. ‘Granex 33’ LF increased linearly [LF = 6.10 + 0.91 Month; P = 0.001] while ‘Dehydrator #3’ LF decreased, then increased following a quadratic trend [LF = 11.84 – 2.17 Month + 0.34 Month²; P = 0.058] over 4 months (Table 1). Changes in LF behavior during storage have not been previously reported. Following each month of storage, the amounts of 1-PRENCSO hydrolyzed exceeded the amount of LF captured for each cultivar. By the second month of storage, however, the amount of LF captured 120 s after tissue maceration almost reflected the amount of 1-PRENCSO hydrolyzed (1:1:1) with ‘Granex 33’. The 1:1:1 ratio then remained the same through 4 months of storage (Table 1). The amount of LF captured from ‘Dehydrator #3’, however, was always less than half the amount of 1-PRENCSO hydrolyzed, regardless of the storage period.

To investigate this further, a preliminary experiment with a few remaining bulbs was performed where 1-PRENCSO and LF were sampled at earlier incubation times. 1-PRENCSO hydrolysis was slower and less complete in ‘Granex 33’ when compared to ‘Dehydrator #3’. Differences in the amount of LF captured may be explained by the differences in the way 1-PRENCSO is hydrolyzed by each cultivar. After a 10-s macerate incubation, only 64% to 70% of the 1-PRENCSO was hydrolyzed for ‘Granex 33’ while 93% to 97% was hydrolyzed for ‘Dehydrator #3’. Waiting 80 s, 70% to 90% of 1-PRENCSO was hydrolyzed in the ‘Granex 33’ macerates while continued hydrolysis in the ‘Dehydrator...
hydrolyzed differed between the cultivars (preliminary data suggest that LF should be slower and may account for a closer relationship within 5 s, sufficient time existed for the LF to be lost to volatilization or degradation. LF is, therefore, underestimated at 120 s (Table 1). With ‘Granex 33’, 1-PRENCSO hydrolysis was slower and may account for a closer relationship with the LF detected at 120 s. Our preliminary data suggest that LF should be sampled before 120 s in order to prevent loss and better reflect the quantity of 1-PRENCSO hydrolyzed.

Experiment 2: Optimum incubation time for LF. Data analyzed by GLM showed that the level of LF detected and 1-PRENCSO hydrolyzed differed between the cultivars (P = 0.001), among incubation times (P = 0.001), and for the interaction between cultivars and incubation times (P = 0.002). For most ‘Dehydrator’ bulbs, 1-PRENCSO was hydrolyzed mainly within 5 s of tissue maceration, although bulbs 2 through 4 had significant hydrolysis at longer incubation times (Table 3). The maximum of LF detection did not equate to and were often substantially lower than the amount of 1-PRENCSO hydrolyzed. LF measured also decreased linearly as incubation time (s) after tissue maceration. On average, only 41% of the maximum LF produced was detected at 120 s and this suggests that a 2-min incubation according to Schmidt et al. (1994) severely underestimated the LF maximum for this cultivar.

The time of maximum LF detection was more variable among the bulbs tested for ‘Sweet Vidalia’, and homogeneity was not found among any of the incubation times using Fisher’s exact test identified homogeneity (P = 0.01) in the LF maximum only between 10–15 s after tissue maceration. On average, only 41% of the maximum LF produced was detected at 120 s and this suggests that a 2-min incubation according to Schmidt et al. (1994) severely underestimated the LF maximum for this cultivar.

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1-PRENCSO hydrolysis occurred later and at different times in the onion macerates (Table 3). The longer it takes to reach maximum 1-PRENCSO hydrolysis, the more likely LF production and degradation will occur simultaneously. This would cause an underestimation of LF. Although time of maximum hydrolysis was variable for ‘Sweet Vidalia’, 70% of the LF levels from 15–30 s on average, only 50% of the maximum LF produced was detected at 120 s. Because LF is a direct reaction product and dominates onion flavor, its rapid quantification can be extremely valuable to researchers and marketers assessing onion flavor. Using an incubation time of 120 s after tissue maceration, LF could not be reliably quantified and was underestimated for two onion cultivars. Differences between and within the cultivars for 1-PRENCSO hydrolyzed and LF detected indicated that a shorter incubation period of the macerate was needed for maximum LF quantification. When macerates were analyzed as early as 5 s after juicing, LF reported low LF quantification following supercritical fluid extraction.

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Table 3. Noncumulative amounts of 1-propenyl cysteine sulfoxide (1-PRENCSO; μmol·mL⁻¹ onion macerate juice) hydrolysis measured at each incubation time of the individual onion bulbs of field-grown ‘Sweet Vidalia’ and ‘Dehydrator’.

<table>
<thead>
<tr>
<th>Incubation time (s)</th>
<th>1</th>
<th>2</th>
<th>3</th>
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<th>7</th>
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<th>10</th>
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<tr>
<td>Sweet Vidalia</td>
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<tr>
<td>5</td>
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<td>3.80</td>
<td>4.32</td>
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<td>3.97</td>
<td>2.24</td>
<td>4.49</td>
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Regression significance
Linear (L) P = 0.081 P = 0.004 P = 0.045 P = 0.005 P = 0.002 P = 0.007 P = 0.008 P = 0.054 P = 0.004
Quad. (Q) P = 0.002

1 Not available.
2 Non-significant.

Detection was variable between and within cultivars. Maximum LF was detected after 5–10 s for ‘Dehydrator #3’ and appeared to be a suitable incubation period to sample this cultivar. ‘Sweet Vidalia’ was more variable for the incubation time required for maximum LF detection, although 70% of the bulbs tested had an LF maximum after 15–30 s. A uniform incubation time for LF quantification of individual bulbs may not be possible for all cultivars. Because the LF is an important attribute of onion flavour, individuals conducting breeding programs that emphasize flavor improvement or modification may want to utilize LF as a selection criterion. Its value as a selection tool should take into account an acceptable variance in optimum LF incubation times for each population of bulbs. However, using multiple bulbs per treatment sample may minimize bulb-to-bulb variation, making LF sufficiently accurate to assess the flavor quality of onions destined for consumption. This approach has been used to assess gross flavor intensity by measuring enzymatically formed pyruvic acid from multiple-bulb samples in test plots and for field sampling (Randle, 1992; Randle et al., 1998).

Literature Cited