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S-Alk(en)yl-L-cysteine Sulfoxides and Relative Pungency Measurements of Photosynthetic and Nonphotosynthetic Tissues of *Allium porrum*

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Three standard assays for pyruvate gave equivalent measurements of relative pungency for two leek cultivars (**'Tadorna'** and **'Ramona'**). Background pyruvate levels varied depending on the assay used, ranging from 0.4 (lactate dehydrogenase) to 1.5 (high-performance liquid chromatography, HPLC) μmol g^{-1} fresh weight (FW) on average. The relative pungencies of the two leek cultivars were also compared to total concentrations of the *S*-alk(en)yl-L-cysteine sulfoxides (RCSOs). The average ratio of EPy to total RCSOs was 10.9, indicating that standard pungency assays underestimate the levels of RCSOs in the tissue. A detailed analysis of **'Tadorna'** leaves showed that total RCSO concentrations decreased acropetally. Profiles were composed of (−/+)-methyl-, (−/+)-ethyl-, (+)-propyl-, and (+)-1-propenyl-L-cysteine sulfoxide (MCSO, ECSO, PCSO, and 1-PeCSO, respectively). (+)-PCSO was the most prominent in green (2.4 mg g^{-1} FW), yellow (5.5 mg g^{-1} FW), and white (3.8 mg g^{-1} FW) tissues. The prop(en)yl-L-cysteine sulfoxide derivatives were dominant in tissues that had photosynthetic capacity. The (+)-MCSO levels were high in the bulb (3.6 mg g^{-1} FW). Interestingly, detectable levels of (−/+)-ECSO were measured in the leaves (~0.5 mg g^{-1} FW). RCSO profiles of the different tissue regions were similar, but more (+)-PCSO and (+)-1-PeCSO were detected in the bulb. In general, mature upper leaf tissues had lower levels of total RCSOs. Overall, mild extraction methods and a low-temperature HPLC protocol (preferably with long retention times) achieved adequate compound separation and resolution of the diastereomers.

KEYWORDS: Leek; *Allium porrum* L.; photosynthesis; cysteine sulfoxide; RCSO; relative pungency; pyruvate; flavor precursors; onion; garlic

INTRODUCTION

The *Alliums* have been a spice crop and source of medicinal agents in most world cultures for centuries. Their major culinary and medicinal characteristics are often attributed to flavor agents, which constitute their tissue pungency. *Allium* pungency can be attributed to a small set of flavor precursors, the *S*-alk(en)yl-L-cysteine sulfoxides (RCSOs) (*1*, *2*). Their breakdown products react to produce a myriad of thiosulfinate and polysulfide derivatives and are the origin of the flavors that characterize fresh and prepared foods and herbal preparations (*3*, *4*). A relative tissue pungency measurement can be obtained when a detailed chemical analysis of RCSOs is not required. This is typically accomplished by quantifying the enzymatic byproducts of RCSO breakdown, pyruvate (*2*), ammonia (*5*), or volatiles (*4*, *6*, *7*). Alternatively, the concentrations of RCSOs can be determined using complex analytical procedures such as high-performance liquid chromatography (HPLC) or gas chromatography (GC) (*7*).

Relative pungency measurements that quantify enzymatically produced pyruvate (EPy) provide a quick estimate of RCSO concentration in *Allium* tissues and can reveal subtle differences in the total RCSOs present (*2*). Quantification of pyruvate can be accomplished with differing levels of specificity, typically relying on a classical chemical analysis by derivatization, chromatographic separation, or monitoring enzymatic coupling reactions.

The first pungency assay measuring EPy in onion tissues was developed by Schwimmer and Weston, (*2*) who quantified the hydrolysis of RCSOs by alliinase, *S*-alky(en)yl-L-cysteine sulfoxide lyase (C-*S* lyase) [E.C. 4.4.1.4] (*2*). When *Allium* cells are broken, vacuole-bound alliinase mixes with RCSOs in the cytoplasm and reacts to produce pyruvic acid, ammonia, and various sulfenic acids whose specific structures are dictated by the variety of RCSOs present (*3*, *4*). Derivatization of pyruvate with 2,4-dinitrophenyl hyrdrazine (DNPH) yields a colored

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product that can be quantified spectrophotometrically (*3, 4*). Since DNPH reacts with any free carbonyl group (*8*), the naturally occurring background level of pyruvate and other carbonyl-containing compounds must be measured in a parallel sample in which the alliinase enzyme has been denatured. The relative pungency is given by the difference of the EPy and the background reading (BKGD). The pyruvate assay using DNPH remains an industry and research standard for pungency assessment despite its low specificity for pyruvate (*9*).

A more specific assay for pyruvate measures the activity of the alliinase enzyme indirectly, providing a measure of the total RCSO concentration (*1, 2, 5, 10, 11*). Pyruvate formed from the hydrolysis of RCSOs is converted to lactate by lactate dehydrogenase (LDH). The amount of NADH consumed by the LDH reaction is measured spectrophotometrically and is theoretically directly proportional to the amount of RCSOs processed. A background pyruvate level must also be measured and subtracted from the EPy of the sample (*1*). This method reduces the possibility of false positives associated with the DNPH assay since LDH is highly specific for pyruvate.

HPLC analysis of EPy provides yet another way to assess *Allium* pungency (*12*). Chromatographic isolations of pyruvate and quantification provide a high degree of specificity for this enzymatic byproduct, but care must still be taken in measuring background levels of pyruvate. In addition, HPLC involves detailed laboratory procedures and sophisticated equipment, not available to all researchers, especially those working under field conditions.

RCSOs can be extracted from *Allium* tissues and characterized directly when compared to laboratory-synthesized standards. Alternatively, thiosulfinate and volatile byproducts of the alliinase reaction can be extracted and determined in a similar fashion (*3, 4*). In both cases, liquid or gas chromatography is most often used to separate and quantify the compounds in question. The earliest methods relied on ion-exchange chromatography, thin-layer chromatography, and electrophoresis, which lacks the ability to resolve the prop(en)yl derivatives adequately and often has low compound recoveries (*6*).

Researchers who are interested in downstream flavor characteristics analyze thiosulfinates and their related breakdown products (*13*). Careful examination of the sample constituents afforded estimates of the precursor levels, that is, RCSO concentrations. However, these methods are subject to misinterpretation because thiosulfinates and dialk(en)yl polysulfides undergo spontaneous rearrangement, decomposition, and cyclization reactions, making a retroanalysis of RCSO precursors difficult (*13, 14*). Misidentification of chromatographic peaks that may not be well-resolved or vary in retention time is also a danger (*14*). Due to the complexities of this chemistry, many researchers opt to analyze flavor precursors directly (*7, 15–18*).

The leek (*Allium porrum* L.) is our model *Allium* species, since it is closely related to onions and garlic. However, unlike the tubular leaves of onions, the flat and firm leaves of leeks are more suitable for photosynthetic studies coupled to flavor precursor analysis (*19*). However, a comprehensive analysis of the RCSOs found in this species is currently not available. As such, we sought to compare detailed measurements of the RCSO profile of photosynthetic leaf tissues and the nonphotosynthetic storage tissue of the pseudostem with measurements of relative pungency. Although measurements of pyruvate can provide a picture of the total RCSO profile, there is disagreement on the level of accuracy and precision needed to evaluate flavor and pungency. In addition, the wide range of methods, method variations, and published values for background pyruvate levels and relative pungency has become the source of disagreement among studies (*9*). In this paper, we compare relative pungency measurements of leek tissues with analogous measurements of RCSOs to determine the locations of specific RCSOs in these tissues and the usefulness of relative pungency assays to detect subtle differences in the total RCSO concentrations in different tissues.

MATERIALS AND METHODS

Chemical Reagents. Tris(hydroxymethyl)aminomethane base (TRIZMA), pyruvic acid (standard solution, 0.45 mM), trichloroacetic acid (TCA), pyridoxal 5′-phosphate (PLP), DNPH, *o*-phthaldialdehyde (OPA), acetonitrile, monosodium phosphate, and a lactate dehydrogenase (suspended in ammonium sulphate, 1000 units mL^{-1}) were obtain from Sigma-Aldrich Canada, Ltd. (Oakville, ON, Canada). NADH was obtained from Calbiochem (San Diego, CA). A 0.3 mM solution of NADH was prepared by dissolving 5 mg of NADH in 4 mL of 1.5 M Tris buffer, which was adjusted to pH 7.5 using HCl. The extract concentration was determined by measuring the light absorbance of a diluted solution at 340 nm and converting it to a concentration using the millimolar extinction coefficient of NADH (6.220 mM^{-1} cm^{-1}). The stock NADH solution was then diluted with Tris buffer to obtain a concentration of 0.30 mM.

Plant Material. Tissue was obtained from greenhouse-grown leeks, cvs. **'Tadorna'** (Stokes, Thorold, ON, Canada) and **'Ramona'** (Bejo Seeds, Geneva, NY). Leeks were grown in the greenhouse in ProMix in 1.7 L plastic pots placed in ebb-and-flow watering troughs, set to water twice per week with a modified Hoagland's solution (6:15:6 N/P/K) (*20*). The greenhouse conditions were controlled using an Argus System (White Rock, BC, Canada). A 12 h photoperiod was maintained using a combination of ambient outdoor light and supplemental high-pressure sodium lamps. Day and night temperatures were 20 and 15 °C, respectively. Whole plants (4–5 months from sprouting) were collected for the sampling of mature leaves. Consistently, leaf numbers four to seven were utilized for all experiments. The plants were sprayed with Lorsban (active ingredient: chlorpyrifos; Dow AgroSciences, Indianapolis, IN) to control thrips when needed.

Tissue Extracts for Pyruvate Analysis. Leek leaf extracts were prepared (*2*). Midleaf sections of two fully mature leaves (leaf numbers four or five) of 6–8-month-old leek plants were longitudinally bisected and weighed (<0.5 g). Homogenization of all leek tissue extracts proceeded as follows. Half of each leaf section was used to determine EPy, and the other half was used to determine BKGD. The tissue was homogenized in 1.5 mL of water for a period of 2 min. This was followed by an additional 2 mL of water and 1.5 mL of a 5% TCA solution and one additional minute of homogenization. To determine the BKGD, extracts were first homogenized in 1.5 mL of a 5% TCA solution for 2 min to deactivate alliinase. This was followed by an addition of 3.5 mL of water and 1 additional minute of homogenization. All samples were then filtered through qualitative #1 filter paper (Whatman Inc., Florham Park, NJ) and immediately frozen.

Instrumentation. A UV–visible spectrophotometer (Beckman DU-640, Mississauga, ON, Canada) was used for the semimicro LDH assays and DNPH colorimetric analysis. A spectrophotometric microplate reader (Bio-Rad 3550-UV, Mississauga, ON, Canada) was used for the microwell LDH assays. Semimicro 1 mL quartz cuvettes were used for the LDH assay, a Corning brand UV transparent 96-well microplate was used for the microwell LDH assays, and disposable semi-microwell cuvettes were used for the DNPH colorimetric analysis. HPLC analysis of pyruvate and RCSOs was performed using a Beckman System Gold 126 (Mississauga, ON, Canada) solvent module pump equipped with a Bio-Rad Aminex HPX-87H 300 × 7.8 column and Beckman System Gold 168 diode array detector. All extracts were filtered using a Waters (Mississauga, ON, Canada), Sep-Pak cartridge prior to injection. A polytron homogenizer (Brinkmann Instruments, Westbury, NY) was used for all tissue macerations (2 min duration on high speed), unless otherwise stated.

LDH Assays. The standard LDH and microwell assay procedures utilized 0.1 mM NADH, 0.5 M TRIZMA base, and LDH at 15 units

mL^{-1}. The microwell assay had a total volume of 305 μL, being exactly one-third the volume of the standard LDH method. All measurements were taken at pH 7.5 and 25 °C.

The difference in absorbance (ΔA) was determined from the difference of the initial absorbance (A_0) and the final absorbance (A_f) reading, ($\Delta A = A_0 - A_f$). The absorbance difference of a blank (ΔA_{bl}; 15 μL of water were added in place of LDH) was subtracted from each ΔA. The corrected absorbance difference (ΔA_c) was then calculated as $\Delta A_c = \Delta A - \Delta A_{bl}$. This correction was found to be insignificant for the microwell assay. A standard curve was created from the semimicro- ($Y = 0.0061X$, $R^2 = 0.9999$) and microwell ($Y = 0.0047X$, $R^2 = 0.9989$) assays using concentrations of pyruvate within the range 5–75 μM (at 15 μM intervals). Reactions went to completion within 10 min. The concentration of pyruvate in the extract was then converted to micromoles per gram of fresh weight (FW) of tissue by dividing by the mass of the tissue used to prepare the extract.

DNPH Assay. The pyruvate concentrations were determined using a modified colormetric assay (*1*, *21*). Between 50 and 100 μL of extract were diluted to 200 μL using distilled water, and 100 μL of a 0.6 mM DNPH solution (prepared using 2N HCl as a solvent) was added. A blank consisting of 200 μL of water and 100 μL of 0.6 mM DNPH was also prepared. Following 10 min of incubation at 37 °C, 500 μL of 0.6N NaOH were added. The absorbance at 420 nm was measured using the blank as a reference, and the concentration of pyruvate was determined ($Y = 0.0083X$, $R^2 = 0.9967$).

HPLC of Pyruvic Acid. Pyruvate concentrations were also determined by HPLC. A Beckman System Gold HPLC was used with a reverse-phased C18 (octadecyl) Nucleosil (100 A) column, 250 mm × 4.6 mm column, 5μ, with an additional guard column (Sigma-Aldrich Canada Ltd., Oakville, ON, Canada). The pyruvate concentration was determined following isocratic separation using a 0.008N H_2SO_4 mobile phase with a 0.3 mL min^{-1} flow rate. Total runtime was 60 min, and pyruvate had a retention time of 18.5 min. A standard curve was generated ($Y = 3.188 \times 10^6X$, $R^2 = 0.9996$).

Preparation of RCSO Standards. Diastereomeric (+/−)-*S*-methyl-L-cysteine sulfoxide (MCSO), (+/−)-*S*-ethyl-L-cysteine sulfoxide (ECSO), (+/−)-*S*-propyl-L-cysteine sulfoxide (PCSO), and (+/−)-*S*-butyl-L-cysteine sulfoxide (BCSO) were synthesized (*21*). L-Cysteine, *S*-methyl-L-cysteine, *S*-ethyl-L-cysteine, 1-bromopropane, 3-bromopropene (allyl bromide), and 1-bromobutane were obtained from Sigma-Aldrich Canada Ltd., (Oakville, ON, Canada). The syntheses of (+)-*S*-1-propenyl-L-cysteine sulfoxide ((+)-1-PeCSO) and (+)-*S*-2-propenyl-L-cysteine sulfoxide ((+)-ACSO) were conducted separately (*22–24*). Attempts to synthesize usable quantities of (+)-1-PeCSO were not successful. All other standards showed yields greater than 75%. (+/−)-BCSO was used as an internal standard in all HPLC analyses of RCSOs in fresh tissue. All standards were dried and stored at −20 °C until needed. Fresh standards were synthesized periodically to ensure purity.

Mass Spectrometry of Laboratory-Synthesized RCSOs. Compound identities were confirmed by electrospray ionization mass spectrometry at the McMaster Regional Center for Mass Spectrometry, Hamilton, ON, Canada. Positive ion detection was used. The identity of each RCSO standard was confirmed by comparing the expected molecular mass (g mol^{-1}) with the observed mass/charge (*m/z*) and the expected major fragments. Specifically, these were as follows: MCSO (151.112 g mol^{-1}) 152/174, $[M + H]^+/[M + Na]^+$; ECSO (165.159 g mol^{-1}), 166/188, $[M + H]^+/[M + Na]^+$; PCSO (179.142 g mol^{-1}), 180/202, $[M + H]^+/[M + Na]^+$; ACSO, (177.150 g mol^{-1}), 178/179/200/223, $[M + H]^+/[M + 2H]^+/[M + Na]^+/[M + 2Na]^+$; and BCSO, (193.193 g mol^{-1}), 194/216, $[M + H]^+/[M + Na]^+$.

Extraction of RCSOs. RCSOs were extracted for HPLC analysis by soaking the leaf tissues for a minimum of 12 h in 20 mL of 100% methanol at room temperature, repeated three times. The tissue was macerated with a mortar and pestle before the final 12 h extraction. Extracts were filtered through No. 42 filter paper (Whatman Inc., Florham Park, NJ), and the filtrate was evaporated to dryness under a gentle stream of air. Lastly, samples were resuspended in the OPA reagent (*11*) to a final volume of 5 mL, which provided an excess of the derivatizing agent (*25*). A minimum of 30 min was allowed for derivatization, after which all samples were filtered through a 0.45 μm, 3 mm, Cameo 3N nylon syringe filter (Osmonics Laboratory Products, Minnetonka, MN). Samples not analyzed immediately were stored at −80 °C. Peak areas were consistent for up to 2 months. A total of 154 μL of 0.0675 M (+/−)-BCSO solution was added to each sample before tissue processing to act as an internal standard, and recovery was consistently over 80%. This resulted in a final concentration of 0.4 mg mL^{-1} in the OPA solution.

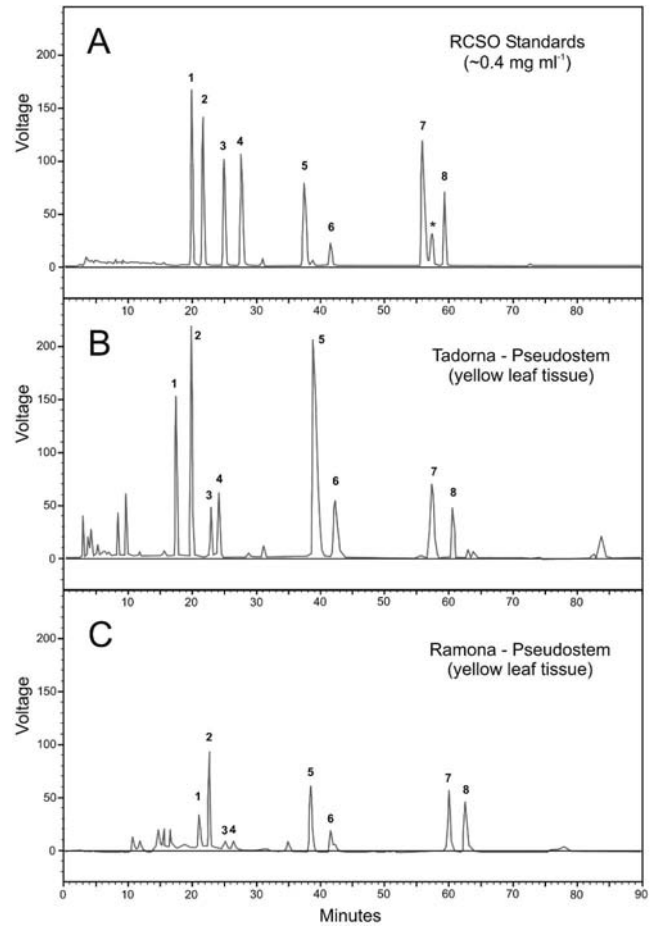

Figure 1. (**A**) HPLC chromatograph of RCSO standards used in the analysis of *Allium porrum* tissues. Sample chromatograph of yellow, developing photosynthetic leaf tissue of cv. Tadorna (**B**) and cv. Ramona (**C**). Peak identities are as follows: 1, (−)-MCSO; 2, (+)-MCSO; 3, (−)-ECSO; 4, (+)-ECSO; 5, (+)-PCSO; 6, (+)-1-PeCSO; 7, (−)-BCSO, * (+)-propyl-*L*-cysteine impurity; 8, (+)-BCSO.

HPLC Analysis of RCSOs. Most HPLC procedures for RCSO separation and determination have short run times of 30–55 min (*11*, *16*, *25*, *17*), whereas the modified HPLC protocol used in our investigation extended the run time to 90 min. This was done in order to eliminate peak misidentification and resolve diastereomers. Excellent peak separation was achieved by using a 90 min runtime. A separation of approximately 1 min was seen between all diastereomeric pairs (**Figure 1A**).

Separations of RCSO standards and tissue extracts were achieved using a method modified from Krest et al. (*11*) and Ziegler and Sticher (*25*), and HPLC standard curves were generated for individual RCSO standards as well as mixtures, using concentrations ranging from 0.05 to 0.75 mg mL^{-1} (**Figure 1**). The retention time of (+)-1-PeCSO was determined from a minimal quantity of impure synthesized standard; however, quantification utilized standard curves generated for (+)-ACSO. The assignment of (−) and (+) isomer peak order relied on previous designations (*25*).

A Beckman System Gold HPLC was used with a reverse-phased C18 (octadecyl) Nucleosil (100 A) column, 250 mm × 4.6 mm column, 5μ, with an additional guard column (Sigma-Aldrich Canada Ltd., Oakville, ON, Canada). The acetonitrile/phosphate buffer solvent mixture had a flow rate of 1.0 mL min^{-1}. Absorbance of OPA derivatives was monitored using a Beckman System Gold 168 Diode

Table 1. Background (BKGD) Pyruvate Levels and Relative Pungencies (PUNG) of Two Leek Cultivars (**'Ramona'** and **'Tadorna'**) As Determined by Four Pungency Assays[a]

	concentration (μmol g^{-1} FW)			
	BKGD		PUNG	
assay	Tadorna	Ramona	Tadorna	Ramona
DNPH	1.257 ± 0.245 A	1.364 ± 0.200 A	4.193 ± 0.887 a	1.097 ± 0.263 b
LDH	0.484 ± 0.058 C	0.363 ± 0.086 C	3.516 ± 0.883 a	1.054 ± 0.348 b
LDH micro	0.855 ± 0.068 B	0.777 ± 0.123 B	3.650 ± 0.887 a	0.994 ± 0.311 b
HPLC	1.507 ± 0.216 A	1.303 ± 0.201 A	3.984 ± 1.147 a	0.967 ± 0.309 b
mean value			3.836 ± 0.951 a	1.028 ± 0.308 b

[a] Green photosynthetic leaf tissue samples were used and ranged in mass from 0.2 to 0.4 g. Methods were compared for their measurements of BKGD and PUNG for both cultivars. Standard errors are given, and differences between assays are read down the columns and are indicated by upper (BKGD) and lower (PUNG) case letter respectively, where $p < 0.05$. Background pyruvate levels varied depending on the assay utilized; however, the resulting relative pungency values were not different.

Table 2. Background Pyruvate Level and Relative Pungency Profile of Leek Leaves (Cv. Tadorna, Sixth Leaf, $n = 26$) from Nonphotosynthetic Pseudostem Tissue to the Photosynthetic Tissues of the Upper Leaf Regions, As Measured by the 2,4-Dinitrophenyl Hydrazine Assay[a]

% distance from leaf base	tissue color	background pyruvate level (μmol g^{-1} FW)	relative pungency (μmol g^{-1} FW)
87–100 (leaf tip)	green	3.97 ± 0.71 a	5.46 ± 0.97 b
73–87 (upper mid leaf)	green	2.66 ± 0.20 a	3.69 ± 2.13 b
33–44 (pseudostem)	yellow	4.23 ± 0.35 a	5.67 ± 2.03 b
0–11 (bulb)	white	2.33 ± 0.16 a	3.60 ± 1.36 b
pooled values		3.34 ± 0.25 a	4.58 ± 0.35 b

[a] The standard errors are given; differences are indicated by lowercase letter where $p < 0.05$, and comparisons are to be read down the columns. No differences in background pyruvate level or relative pungency were detected along the leaf blades.

Array detector at 335 nm. The injection volume was 20 μL. A two-solvent mixture of acetonitrile and 0.05 M phosphate buffer at pH 6.5 ran as follows: 19% acetonitrile was adjusted to 24% over 10 min, maintained for 10 min, and then adjusted to 27% over 30 min. At the 50 min mark, the mixture was increased to 30% acetonitrile for 30 min. Finally, at the 80 min mark, it was returned to 19% acetonitrile over 10 min and was ready for another injection. A single run lasted a total of 90 min. Retention times and peak areas of extracts were compared to RCSO standard curves.

Data Processing and Statistical Analysis. All data were compiled and calculated using a combination of Microsoft Excel 2002 (Microsoft Corporation, Redmond, WA) and Sigma Plot version 9.0 (Systat Software Inc., Point Richmond, CA). Means were compared using Student's t statistics, with a type I error rate of 5% ($p < 0.05$). The mean standard error (MSE) is reported. The least significant difference was used to compare large groups of means.

RESULTS AND DISCUSSION

Background Levels of Pyruvate in Leek Leaves. One value for the leek pseudostem of 0.43 μmol mL^{-1} has been published (*12*). In comparison, many background pyruvate values have been published for other *Allium* species. Fresh onion juice was first reported to be within the range of 0.1–4.1 μmol mL^{-1} (*2*). Subsequent studies have reported values of 0.1–1.0 μmol mL^{-1} (*26*) and, more recently, 0.5 μmol mL^{-1} (*12*). It has been suggested that the typical background level could be ignored in most *Allium* species since the alliinase reaction commonly yields many times the amount of pyruvate found in the background (*12*).

In our investigation, the background pyruvate levels in the leek leaves of each cultivar (**'Tadorna'** and **'Ramona'**) were nearly identical (**Table 1**), with respect to the assay utilized. The microscale LDH assay measured a background level twice as great as that of the bench-scale LDH assay (**Table 1**). The DNPH and HPLC techniques determined similar background levels of pyruvate that were higher than the other assays (**Table 1**). The LDH assay measured the lowest pyruvate background, with an average of 0.42 μmol g^{-1} FW. The highest background reading (1.4 μmol g^{-1} FW) was determined by the HPLC procedure.

Assuming that the density of the leek juice, obtained from the leaf tissue extracts, is close to that of water (1 g mL^{-1}), a value of 0.42 μmol g^{-1} FW from **Table 1** would be equivalent to 0.42 μmol mL^{-1} of pyruvate in the juice. On the other end of this range, the HPLC background reading would be equivalent to 1.4 μmol mL^{-1}. This conversion provides a basis for comparison to other reported values.

The background values determined in our study are comparable to those reported for the leaves of onion seedlings, 0.84–0.95 μmol mL^{-1}, and of leek the pseudostem, 0.43 μmol mL^{-1}, (*12*) and are well within the range of commonly reported values, 0.1–4.1 μmol mL^{-1} (*2*, *12*, *26*, *27*).

Interestingly, the background pyruvate readings in **Table 2** (DNPH method) had a mean of 3.34 ± 0.25 μmol g^{-1} FW, which did not vary significantly along the leaf blade. Although higher than the values reported in **Table 1**, this background reading is still within the range reported for onion (*26*) and may be due to other unknown metabolic factors.

There has been little agreement about the background concentration of pyruvate typically found in *Allium* tissues, such as onion scales or garlic cloves. As a result, there is always debate surrounding the results of relative pungency assays (*9*). It appears that the LDH assay (or microwell variation) provides the most accurate measure of background pyruvate, although variability is consistently high among all the assays (**Table 1**). The LDH assays reported values in the range of 0.48–0.86 μmol mL^{-1}, which correspond well to the 0.79 μmol mL^{-1} reported for onion bulb tissue (*27*) and the 0.43 μmol mL^{-1} for the leek pseudostem (*12*). Although 0.8 μmol mL^{-1} is a relatively low level of naturally occurring pyruvate, it should not be deemed insignificant. In *Allium* species with pungencies in the range of 3–7, disregarding the background pyruvate could inflate the relative pungency readings by 10–20%.

Relative Pungency in Leek Leaves. The relative pungency values were not different for each assay despite their differing specificity for pyruvate (**Table 1**). However, differences were detected between the relative pungencies of the two cultivars. Ramona leaves were found to have an average relative pungency of 1.028 μmol g^{-1} FW, while Tadorna leaves were nearly 4

Table 3. RSCO Profiles of Leek Leaf Tissues (Cv. **'Tadorna'** and **'Ramona'**) in Leaf (Green, Photosynthetic Tissue), Pseudostem (Yellow, Developing Photosynthetic Leaf Tissue), and Bulb (White, Nonphotosynthetic Leaf Tissue)[a]

	tissue concentration (mg g^{-1} FW)						
tissue	(−)-MCSO	(+)-MCSO	(−)-ECSO	(+)-ECSO	(+)-PCSO	(+)-1-PeCSO	total RCSO
Tadorna							
leaf	0.351 ± 0.002 C	1.170 ± 0.031 B	0.440 ± 0.002 A	0.532 ± 0.003 A	2.404 ± 0.043 C	1.543 ± 0.027 A	6.439 ± 0.071 C
pseudostem	0.447 ± 0.007 B	0.702 ± 0.013 C	0.450 ± 0.004 A	0.453 ± 0.005 A	5.548 ± 0.117 A	1.179 ± 0.021 B	8.779 ± 0.160 B
bulb	0.713 ± 0.003 A	3.560 ± 0.020 A	0.337 ± 0.001 B	0.500 ± 0.002 A	3.781 ± 0.029 B	0.685 ± 0.006 C	9.576 ± 0.042 A
Ramona							
leaf	0.140 ± 0.001 D	0.219 ± 0.005 D	0.108 ± 0.002 C	0.111 ± 0.001 B	0.347 ± 0.017 D	0.170 ± 0.004 D	1.279 ± 0.029 D
pseudostem	0.151 ± 0.002 D	0.248 ± 0.006 D	0.099 ± 0.001 C	0.125 ± 0.001 B	0.377 ± 0.012 D	0.189 ± 0.004 D	1.386 ± 0.026 D
bulb	0.117 ± 0.001 D	0.249 ± 0.004 D	0.111 ± 0.001 C	0.147 ± 0.002 B	0.384 ± 0.016 D	0.196 ± 0.004 D	1.404 ± 0.029 D

[a] Mean concentrations are expressed on a milligram per gram fresh weight basis (mg g^{-1} FW) with standard errors. The MSE was 0.029 mg g^{-1} FW. Differences between individual RCSO concentrations of each tissue region are given by uppercase letter where $p < 0.05$ and are read vertically.

times as pungent, 3.836 μmol g^{-1} FW. Further experiments with the more pungent cultivar, **'Tadorna'** (**Table 2**), confirmed the relative pungency values, with a pooled mean of a 4.58 ± 0.35 μmol g^{-1} FW. Leek pseudostem pungencies were previously reported to be in the range of 3.5–4.5 μmol g^{-1} FW (*28, 29*). In our case, the difference was considered cultivar-specific as the leeks were grown under identical conditions in the same greenhouse section. This would suggest that **'Tadorna'** had approximately 4 times the level of RCSOs found in **'Ramona'**.

Pungency Profile of Leek Leaves. The flavor precursors (RCSOs) are synthesized in photomixotrophic tissues (*15*). Investigations of onion leaves suggested that RCSOs are synthesized in the developing leaves of onions and are likely exported to the pseudostem as the plant matures. Pungency gradients have been identified in *Allium* tissues (*30–32*). Specifically, levels of pyruvate decrease from the outer to the inner leaves of the leek, within the pseudostem region (*31*).

The pyruvate profile of mature leek leaves (cv. **'Tadorna'**) was assessed to determine if a pungency gradient was present (**Table 2**). When the standard Schwimmer and Weston DNPH method was used, (*1*) no significant difference in relative pungency was present along the leaf blade. In addition, the background pyruvate level was constant throughout the leaf but was noticeably higher than the values reported in **Table 1**.

The absence of a pyruvate gradient along the leaf blades suggested that either EPy levels are similar because the alliinase reaction proceeded to the same degree at any point along the leaf or that the RCSO concentration was homogenous throughout the leaf. Overall, this does not eliminate the possibility that the specific types of flavor precursors found in different regions of leek leaves vary. Elucidation of these RCSO profiles would provide more insight into the nature of their biosynthesis or catalysis in leek leaf tissue.

Photosynthetic and Nonphotosynthetic Leek Leaf Tissues: RCSO Profiles. RCSO profiles differ from *Allium* to *Allium* (*3, 4, 33*). The most commonly detected flavor precursors are (+)-MCSO, (+)-PCSO, (+)-1-PeCSO, and (+)-2-PeCSO, commonly called (+)-ACSO or alliin (*2–4*). It is typical for distinct *Allium* species to have one or two dominant flavor precursors. Garlic exhibits large amounts of (+)-ACSO, whereas onions display (+)-1-PeCSO, the only RCSO that yields the lachrymatory factor (*2–4*). PCSO has been said to be dominant in the leek, whereas MCSO tends to be found in generous amounts in all *Allium* species, especially wild types (*12, 34*). (+/−)-ECSO is typically found in very low concentration or not found at all (*11*). Recently, (+/−)-BCSO was shown to be a dominant RCSO in *A. siculum* (*35*), but it is typically not a major component of the RCSO profile of most *Allium* species (*36*). The presence of the (+/−) diastereomers is an additional characteristic of the RCSO profile (*25*), where the (+) isomer is typically dominant (*11*).

In the **'Tadorna'** leek tissue extracts analyzed, endogenous RCSO levels varied in different parts of the leaf (**Figure 1B**). Total RCSOs decreased acropetally, from 9.6 mg g^{-1} FW in white tissues to 6.4 mg g^{-1} FW in green tissues (**Table 3**). (+)-PCSO was the most prominent RCSO in all tissue regions analyzed, showing the highest levels in yellow leaf tissue (5.55 mg g^{-1} FW). In white tissues, the second most prominent RCSO was (+)-MCSO (3.56 mg g^{-1} FW), whereas in the yellow and green leaf tissue, it was (+)-1-PeCSO (1.18 and 1.54 mg g^{-1} FW, respectively). The third most prominent RCSO in white tissues was (−)-MCSO (0.71 mg g^{-1} FW), whereas in the yellow and green tissues, it was (+)-MCSO (0.70 and 1.17 mg g^{-1} FW, respectively). All other RCSOs were detected at levels at or below 0.5 mg g^{-1} FW in all tissue regions (**Table 3**, **Figure 1B**).

In the **'Ramona'** leek tissue extracts, endogenous RCSO levels showed little variation between the different leaf regions (**Figure 1C**). Total RCSOs ranged from 1.3 mg g^{-1} FW in white tissues to 1.4 mg g^{-1} FW in green tissues, but concentrations were not significantly different (**Table 3**). (+)-PCSO was the most prominent RCSO in all tissue regions analyzed, showing concentrations between 0.35 to 0.38 mg g^{-1} FW. The second most prominent RCSO was (+)-MCSO which ranged from 0.22 to 0.25 mg g^{-1} FW. The third most prominent RCSO was (+)-PeCSO which ranged from 0.17 to 0.20 mg g^{-1} FW. All of the other RCSOs were detected at levels at or below 0.15 mg g^{-1} FW across all tissue regions (**Table 3**, **Figure 1C**).

The presence of (+/−)-ECSO in leek tissue (*36*) was confirmed by our results (**Table 3**). (+/−)-ECSO was easily detected in all tissue regions at concentrations ranging from 0.3 to 0.5 mg g^{-1} FW in **'Tadorna'** and 0.10 to 0.15 mg g^{-1} FW in **'Ramona'**. **Figure 1B** and **C** show the distinct peaks of (−/+) ECSO in both cultivars.

(+)-PCSO is often prominent in leek pseudostems (*6, 37*). Yet, other studies have found zero or just trace amounts of (+)-PCSO, and dominant concentrations of (+)-1-PeCSO ranging from 0.2 to 2.2 mg g^{-1} FW (*16, 17, 36*); these values are comparable to our results for the white tissues of **'Tadorna'** and **'Ramona'**. It is possible that the disagreement between the studies may have resulted from compound breakdown during analysis or high-temperature GC as opposed to low-temperature HPLC (*6*). Other discrepancies could result from peak misidentification caused by a shifting of retention times or peak overlap if run times are too short (*14*). HPLC protocols that run from 5 to 30 min have reported that it is difficult to get good peak separation of the three prop(en)yl derivatives, PCSO,

1-PeCSO, and ACSO (*25*). In addition, the individual peaks of the (+/−) isomers straddle or overlap one another, making peak analysis very difficult and subject to error.

Trends in RCSO Localization. RCSOs are synthesized in the leaves of onion seedlings, (*15*) and it has been speculated that they are exported to the bulb or pseudostem region as the crop matures. Total RCSO concentration is generally measured in the highest concentration in young onion leaves and decreases proportionally with leaf age and tissue maturity, and this is also true for pseudostem or bulb tissue (*6*).

The leek leaves studied in this investigation were taken from mature plants, consistently sampling leaves four to seven of 4–5 month old plants. Due to the initiation of *Allium* leaves from the basal meristem, a maturity differential can be mapped onto the leaf blades themselves—green leaf tips being the oldest and pseudostem tissue the youngest. Maturity regions along the leaf blades of leeks have been identified previously (*38*). Specifically, epicuticular wax formation does not occur until after cell elongation. Wax deposition and elongation were always complete in green tissues, asynchronous in yellow tissues, and incomplete in white tissue. In this sense, the green upper-leaf regions are fully mature, while yellow and white regions are considered still-developing. It was noted that total RCSOs of green, yellow, and white tissues corresponded well with tissue maturity, where total RCSO concentration decreased upward along the leaf blade.

Interestingly, (+)-PCSO and (+)-1-PeCSO were in high concentrations in the green and yellow tissues (**Table 3**), perhaps corresponding to regions with photosynthetic capability. The prop(en)yl-L-cysteine derivatives found in onion leaves increase in response to additions of their biosynthetic precursors to the leaf blades (*39*). High levels of PCSO in photosynthetic regions of leek leaf tissues suggest that the biosynthetic precursors to PCSO (and potentially other RCSOs) might be synthesized within or in close proximity to photosynthetic tissue but that other RCSOs respond to different physiological processes. Studies show that undifferentiated onion and garlic callus tissues had little to no free flavor precursors (*40–42*), whereas callus cells differentiating into shoots exhibit the typical complement of RCSOs found in the leaves of seedlings (*15*). Interestingly, callus cells grown in darkness and differentiating into root tissue exhibit mainly (+)-MCSO and only trace amounts of other flavor precursors which are found in high concentrations in the leaves (*39*).

The majority of RCSO biosynthesis takes place in developing tissues (*6, 15, 42*). When no light is supplied, fast-growing callus tissue shows dominant concentrations of MCSO (*39*). Our work confirms these findings. We found high levels of (+/−)-MCSO in bulb tissues and a diverse RCSO profile in the photosynthetic leaf tissue (**Figure 1B**,**C**).

The concentration of (−/+)-ECSO was not significantly different in yellow and green tissues (values ranged from ∼0.1 to 0.5 mg g^{-1} FW in the cultivars studied, **Table 3**). This may suggest that the biosynthesis of (−/+)-ECSO is not necessarily dependent on being associated with green tissues and may respond to signals intermediate to those of methyl or prop(en)yl derivatives.

Previous studies of leek pseudostem tissue showed only traces of ECSO (*36*). In the leaves of other *Allium* species, higher levels of ECSO are found, such as in *A. ochotense* (0.05 mg g^{-1} FW) and *A. ampeloprasum* L. (elephant garlic, the same species as the domestic leek, 0.03 mg g^{-1} FW). The reported concentrations are a factor of 10 lower than what we found. Variation between growing conditions and plant specimens could explain our unexpectedly high concentrations. Another contributing factor could be RCSO and thiosulfinate breakdown if extraction or analysis methods are too harsh (*43*).

Occurrence of the (−)-RCSO Isomers. Earlier studies of the properties of alliinase have shown that the enzyme reacts preferentially with the (+) isomer of any RCSO (*1*), but reactions with the (−) isomers do occur, only at slower rates (*11*). The (−) RCSOs were not formed in garlic tissue cultures, and this was likely due to the selectivity of the *S*-alk(en)yl-L-cysteine oxidase for the (+) isomer (*42*). However, it is clear from our study that (−)-MCSO and (−)-ECSO are present in leek tissue, and in greater than trace amounts as previously reported (*36*) (**Figure 1**). Similarly, the (+/−) isomers of MCSO and ACSO have been resolved; however, only (+)-MCSO and (+)-ACSO could be resolutely determined in the garlic extracts used (*25*) (a room temperature 50% methanol extraction was used, and samples were extracted for 5 min, perhaps not long enough for total tissue extraction). Verification of the presence of the (−) isomers in leek tissue, by liquid chromatography–mass spectroscopy, for example, could be done in future studies.

We speculate that our gentle 100% methanol extraction procedure allowed us to extract higher quantities of the RCSOs present, including the (−) isomers. In addition, compound degradation was minimized by the low-temperature HPLC protocol. The (−) isomers, previously thought to be only trace compounds, were easily resolved. It should be noted that we also found trace amounts of (−)-PCSO and (−)-1-PeCSO in some of the samples, but the small peak areas could not be quantified reliably.

Pyruvate - RCSOs Ratio. The concentrations of RCSOs in the green leaf extracts (**Table 3**) were converted from milligrams per gram of fresh weight to micrograms per gram of fresh weight so they could be directly compared to the pyruvate measurements taken for the same tissue regions, either photosynthetic (green), developing photosynthetic (yellow), or nonphotosynthetic (white).

Photosynthetic tissue (green) from **'Ramona'** leaves had total RCSO concentrations of 8.9 μmol g^{-1} FW, on average, with an average relative pungency of 1.0 μmol of pyruvate g^{-1} FW. Comparatively, the leaf tissue extracts of **'Tadorna'** had average total RCSO concentrations of 44.4, 60.6, and 66.1 μmol g^{-1} FW in the green, yellow, and white tissue regions, respectively. The relative pungency readings obtained for these same tissues were 4.5 (green), 6.0 (yellow), and 4.0 (white). On the basis of the total RCSO measurements (**Table 3**) and the pungency readings of the pyruvate assays (**Tables 1** and **2**), the ratio of total RCSOs to relative pungency was 10.9:1 (**Table 4**).

Previous work has determined that levels of EPy in onion bulbs and the concentration of RCSO approach a 1:1 ratio, as expected theoretically (*32*). However, numerous studies and reviews have stated that this does not occur in vivo (*6, 12, 22, 45*). In onions, the DNPH and LDH assays typically underestimate the level of RCSOs due to the fact that the alliinase reaction does not typically go to completion (*45*) and is further complicated by the dependence on a suitable concentration of its enzymatic cofactor PLP (*1*).

Despite the deviation from the expected ratio, the LDH or DNPH assay can still provide an indication of total RCSO pools and qualitative differences between tissues or cultivars. In addition, the DNPH can be scaled down to the microplate level. (*46*) At this scale, it would acquire some of the benefits of the LDH microwell procedure used in our investigation and would be useful in breeding programs seeking mild or pungent seed stock. Furthermore, **Tables 1**, **2**, and **4** indicate that the crude

Table 4. Comparison of the Ratio of the Total RCSOs and Relative Pungency Measurements Obtained in this Investigation of Leek Cultivars, '**Tadorna**' and '**Ramona**'

leek cultivar	tissue type (color)	total RCSOs (μmol g^{-1} FW)	relative pungency (μmol pyruvate g^{-1} FW)	ratio
Ramona	photosynthetic (green)	8.9	1.0	8.9:1
Tadorna	photosynthetic (green)	44.4	4.0	11.1:1
			5.5	8.1:1
			4.0	11.1:1
			(mean = 4.5)	(mean = 9.9:1)
Tadorna	developing photosynthetic (yellow)	60.6	6.0	10.1:1
Tadorna	nonphotosynthetic (white)	66.1	4.0	16.5:1
				mean = 10.9:1

assays can be used to compare cultivars or target tissues for further and more detailed analyses of specific RCSOs. In this sense, the various assays for pyruvate can be used to probe leaf, pseudostem, and bulb or root tissues in order to locate differences in pungency among the different organs of the plant.

The overall accuracy and speed of the LDH enzymatic method (or LDH microwell variation) make it suitable for the analysis of large numbers of samples. Similarly, the DNPH assay provides reliable measurements of relative pungency and is suitable for most assessments of *Allium* tissues. DNPH was also able to differentiate between the pungencies of two leek cultivars and suggest that pungency was consistent along the leaf blade.

However, any analysis of EPy as a measure of tissue pungency must take into account that the crude assays for pyruvate underestimate the concentration of total RCSOs by a factor of 10.9 and do not differentiate between individual RCSOs. Therefore, pungency studies cannot reveal specific flavor traits or biochemical patterns resulting from changes in the RCSO profile; these changes may be due to environmental stress or the processes of photosynthesis, when carbon, nitrogen, and sulfur reduction are active.

In general, assays for relative tissue pungency are useful for cultivar comparisons and quick assessments of the general pungencies of bulb or leaf tissues of *Allium* species. They are a simple way to probe various tissues for pyruvate and provide an estimate of the levels of RCSOs present. These assays would be particularly useful for cultivar selection in breeding programs but do not provide any insight into the specific RCSO present. However, relative pungency assays cannot be employed to characterize differences in the RCSOs profiles of *Allium* tissues. In order to properly study the flavor profile of any *Allium*, the individual RCSOs must be isolated and quantified.

In the leek cultivars studied in this investigation, photosynthetic and nonphotosynthetic tissue regions showed differing profiles of RCSOs despite showing no differences in relative pungency. These tissues also differed with relative tissue maturity—the highest total concentrations were in the newly developing basal leaf tissue (bulb) and the lowest in the mature tissues of the upper leaf regions. The presence of high levels of (+)-prop(en)yl-L-cysteine sulfoxides in photosynthetic tissues suggests that the biochemical precursors to (+)-PCSO and (+)-1-PeCSO are present in these tissues. Compounds such as (−/+)-MCSO and (−/+)-ECSO respond to more ubiquitous signals throughout the leaf.

As a result of our observations, we recommend that, when analyzing the RCSO profiles of *Allium* tissues, mild extraction procedures (100% methanol at room temperature) should be employed. In addition, we recommend that detailed investigations of the flavor precursors should rely on a suitable HPLC protocol, preferably with long retention times so as to achieve adequate separation of the (+/−) isomers of the RCSOs.

ABBREVIATIONS USED

(+)-*S*-2-propenyl-L-cysteine sulfoxide, (+)-ACSO; (+/−)-*S*-butyl-L-cysteine sulfoxide, BCSO; background pyruvate concentration, BKGD; 2,4-dinitrophenyl hyrdrazine, DNPH; (+/−)-*S*-ethyl-L-cysteine sulfoxide, ECSO; enzymatically produced pyruvate, EPy; fresh weight, FW; gas chromatography, GC; high-performance liquid chromatography, HPLC; lactate dehydrogenase, LDH; (+/−)-*S*-methyl-L-cysteine sulfoxide, MCSO; *o*-phthaldialdehyde reagent, OPA; (+)-*S*-1-propenyl-L-cysteine sulfoxide, (+)-1-PeCSO; (+/−)-*S*-propyl-L-cysteine sulfoxide, PCSO; *S*-alk(en)yl-*L*-cysteine sulfoxides, RCSOs.

LITERATURE CITED

(1) Schwimmer, S.; Mazelis, M. Characterization of alliinase of *Allium cepa* (onion). *Arch. Biochem. Biophys.* **1963**, *100*, 66–73.

(2) Schwimmer, S.; Weston, W. J. Enzymatic development of pyruvic acid in onion as a measure of pungency. *J. Agric. Food Chem.* **1961**, *9*, 301–304.

(3) Block, E. The chemistry of garlic and onions. *Sci. Am.* **1985**, *252*, 114–119.

(4) Block, E. The organosulfur chemistry of the genus *Allium* - Implications for the organic chemistry of sulfur. *Angew. Chem., Int. Ed. Engl.* **1992**, *31* (9), 1135–1178.

(5) Miron, T.; Rabinkov, A.; Mirelman, D.; Weiner, L.; Wilchek, M. A spectrophotometric assay for allicin and alliinase (alliin lyase) activity: Reaction of 2-nitro-5-thiobenzoate with thiosulfinates. *Anal. Biochem.* **1998**, *265*, 317–325.

(6) Lancaster, J. E.; Boland, L. Flavor Chemistry. In *Onions and Allied Crops*; Rabinowitch, H. D., Brewster, J. K., Eds.; CRC Press: Boca Raton, FL, 1990, *Vol. III*, pp 33–72.

(7) Kubec, R.; Svobodová, M.; Velísek, J. Gas-chromatographic determination of *S*-alk(en)ylcysteine sulfoxides. *J. Chromatogr. A* **1999**, *862* (1), 85–94.

(8) Williamson, K. L. *Macroscale and Microscale Organic Experiments*, 2nd ed.; D.C. Health and Company: Lexington, 1994; p 710.

(9) Havey, M. J.; Cantwell, M.; Jones, M. G.; Jones, R. W.; Schmidt, N. E.; Uhlig, J.; Watson, J. F.; Yoo, K. S. Significant variation exists among laboratories measuring onion bulb quality traits. *HortScience* **2002**, *37* (7), 1086–1087.

(10) Lohmüller, E. M.; Landshuter, J.; Knobloch, K. On the isolation and characterization of a C-S-lyase preparation from leek, *Allium porrum*. *Planta Med.* **1994**, *60*, 337–342.

(11) Krest, I.; Glodek, J.; Keugsen, M. Cysteine sulfoxides and alliinase activity of some *Allium* species. *J. Agric. Food Chem.* **2000**, *48*, 3753–3760.

(12) Yoo, K. S.; Pike, L. M. Determination of background pyruvic acid concentrations in onions, *Allium* species, and other vegetables. *Sci. Hortic.* **2001**, *89*, 249–256.

(13) Block, E.; Naganathan, S.; Putnam, D.; Zhao, S. H. *Allium* chemistry: HPLC analysis of thiosulfinates from onion, garlic, wild garlic (ramsoms), leek, scallion, shallot, elephant (great-headed) garlic, chive and chinese chive. Uniquely high allyl to

methyl ratios in some garlic samples. *J. Agric. Food Chem.* **1992**, *40*, 2418–2430.
(14) Block, E. Flavor artifacts: correspondence. *J. Agric. Food Chem.* **1993**, *41*, 692.
(15) Lancaster, J. E.; Reynolds, P. H. S.; Shaw, M. L.; Dommisse, E. M.; Munro, J. Intra-cellular localization of the biosynthetic pathway to flavor precursors in onion. *Phytochemistry* **1989**, *28* (2), 461–464.
(16) Thomas, D. J.; Parkin, K. L. Quantification of alk(en)yl-L-cysteine sulfoxides and related amino acids in *Alliums* by high-performance liquid chromatography. *J. Agric. Food Chem.* **1994**, *42* (8), 1632–1638.
(17) Yoo, K. S.; Pike, L. M. Determination of flavor precursor compound *S*-alk(en)yl-L-cysteine sulfoxides by an HPLC method and their distribution in *Allium* species. *Sci. Hortic.* **1998**, *75* (1/2), 1.
(18) Hughes, J.; Tregova, A.; Tomsett, A. B.; Jones, M. G.; Cosstick, R.; Collin, H. A. Synthesis of the flavor precursor, alliin, in garlic tissue cultures. *Phytochemistry* **2005**, *66*, 187–194.
(19) Doran, J. A.; Lairson, L.; Grodzinski, B.; McDonald, M. R. Leaf photosynthesis and transpiration of two leek cultivars with differing pseudostem pungency levels. *Acta Hortic.* **2004**, *633*, 51–57.
(20) Salisbury, F.; Ross, C. *Plant Physiology*; Wadsworth Publishing Co.: Belmont, CA, 1992.
(21) Randle, W. M. Onion flavor compound analysis. http://www.uga.edu/onion/onionflavorchemistry.htm (accessed 2000 to 2005).
(22) Shen, C.; Parkin, K. L. In vitro biogeneration of pure thiosulfinates and propanethial-*S*-oxide. *J. Agric. Food Chem.* **2000**, *48*, 6254–6260.
(23) Koch, I.; Keusgen, M. Diastereoselective synthesis of alliin by asymmetric sulfur oxidation. *Pharmazie* **1998**, *53*, 668–671.
(24) Lancaster, J. E.; Kelly, K. E. Quantitative analysis of the *S*-alk(en)yl-L-cysteine sulphoxides in onion (*Allium cepa* L.). *J. Sci. Food Agric.* **1983**, *34*, 1229–1235.
(25) Ziegler, S. J.; Sticher, O. HPLC of *S*-alk(en)yl-L-cysteine derivatives in garlic including quantitative determination of (+)-S-allyl-L-cysteine sulfoxide (alliin). *Planta Med.* **1989**, *55*, 372–378.
(26) Randle, W. M.; Bussard, M. L. Streamlining onion pungency analysis. *Hortic. Sci.* **1993**, *28* (1), 60.
(27) Luo, C.; Ewart, L. A new pyruvic acid (PA) procedure for rapid evaluation of onion pungency. Abstract and Program of the 1995 National Onion Research Conference, Wisconsin Center, Madison, WI, Dec 6–9, 1995; p 26.
(28) Hovius, M. H. Y. Department of Horticulture, University of Wisconsin; personal communication, Muck Crops Vegetable Conference, Bradford, Ontario, April 2–3, **2003**.
(29) Hovius, M. H. Y.; Goldman, I. L. Relationship of white rot resistance to pyruvate and *S*-alk(en)yl-L-cysteine sulfoxides in onion roots. *Acta Hortic.* **2004**, *637*, 195–201.
(30) Freeman, G. G. Distribution of flavor components in onion (*A. cepa* L.), leek (*A. porrum*) and garlic (*A. sativum*). *J. Sci. Food Agric.* **1975**, *26*, 471–478.
(31) Lancaster, J. E.; McCallion, B. J; Shaw, M. L. The dynamics of the flavor precursors, the *S*-alk(en)yl-L-cysteine sulfoxides, during leaf blade and scale development in the onion (*A. cepa*). *Physiol. Plant* **1986**, *66*, 293–297.
(32) Bacon, J. R.; Moates, G. K.; Ng, A.; Rhodes, M. J. C.; Smith, A. C.; Waldron, K. W. Quantitative analysis of flavor precursors and pyruvate levels in different tissues and cultivars of onion (*Allium cepa*). *Food Chem.* **1999**, *64*, 257–261.
(33) Brewster, J. L. *Onions and Other Vegetable Alliums: Crop Production Science in Horticulture, No. 3*; CAB International: Wallingford, CT, 1994; p 220.
(34) Block, E.; Putnam, D.; Zhao, S. H. *Allium* chemistry: GC-MS analysis of thiosulfinates and related compounds from onion, leek, scallion, chive and Chinese chive. *J. Agric. Food Chem.* **1992**, *40*, 2431–2438.
(35) Kubec, R.; Seokwon, K.; McKeon, D. M.; Musah, R. A. Isolation of *S*-n-butylcysteine sulfoxide and six n-butyl-containing thiosulfinates from Allium siculum. *J. Nat. Prod.* **2002**, *65* (7), 960–964.
(36) Kubec, R.; Svobodová, M.; Velíšek, J. Distribution of *S*-alk(en)ylcysteine sulfoxides in some *Allium* species. Identification of a new flavor precursor: *S*-ethyl-L-cysteine sulfoxide (ethiin). *J. Agric. Food Chem.* **2000**, *48*, 428–433.
(37) Keusgen, M.; Schultz, H.; Glodek, J.; Krest, I.; Krüger, H.; Herchert, N.; Keller, J. Characterization of some *Allium* hybrids by aroma percursors, aroma profiles and alliinase activity. *J. Agric. Food Chem.* **2002**, *50* (10), 2884–2890.
(38) Rhee, Y.; Hlousek-Radojcic, A.; Ponsamuel, J.; Liu, D.; Post-Beittenmiller, D. Epicuticular wax accumulation and fatty acid elongation activities induced during leaf development of leeks. *Plant Physiol.* **1998**, *116*, 901–911.
(39) Prince, C. L.; Shutter, M. L. Altering flavor profiles in onion (*Allium cepa* L.) root cultures through directed biosynthesis. *Biotechnol. Prog.* **1997**, *13*, 506–510.
(40) Turnbull, A.; Galpin, I. J.; Collin, H. A. Comparison of the onion plant (*Allium cepa*) and onion tissue culture III. Feeding of ^{14}C labeled precursors of the onion flavor precursor compounds. *New Phytol.* **1980**, *85*, 483–487.
(41) Turnbull, A.; Galpin, I. J.; Smith, J. L.; Collin, H. A. Comparison of the onion plant (*Allium cepa*) and onion tissue culture IV. Effect of shoot and root morphogenesis on flavor precursor synthesis in onion tissue culture. *New Phytol.* **1981**, *87*, 257–268.
(42) Ohsumi, C.; Hayashi, T.; Sano, K. Formation of alliin in the culture tissue of *Allium sativum*. Oxidation of *S*-allyl-L-cysteine. *Phytochemistry* **1993**, *33* (1), 107–111.
(43) Shen, C.; Xiao, H.; Parkin, K. L. *In vitro* stability and chemical reactivity of thiosulfinates. *J. Agric. Food Chem.* **2002**, *50*, 2644–2651.
(44) Randle, W. M. From the beginning, it was all about flavor. *Proceedings of the Third International Symposium on Edible Alliaceae: Alliums 2000*, Athens, GA, Oct 30–Nov 3, 2000; pp 86–89.
(45) Hanum, T.; Sinha, N. K.; Cash, J. N. Characteristics of gamma-glutamyl transpeptidase and alliinase of onion and their effects on the enhancement of pyruvate formation in onion macerates. *J. Food Biochem.* **1995**, *19*, 51–65.
(46) Boyhan, G. E.; Schmidt, N. E.; Woods, F. M.; Himelrick, D. G.; Randle, W. M. Adaptation of a spectrophotometric assay for pungency in onion to a microplate reader. *J. Food Qual.* **1999**, *22*, 225–233.

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