Rapid Extraction Method of Quantitating the Lachrymatory Factor of Onion Using Gas Chromatography

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A rapid extraction method has been developed for the quantitation of (*Z*,*E*)-propanethial *S*-oxide, the lachrymatory factor (LF), and other flavor chemicals in onion (*Allium cepa*) using gas chromatography. This method involves crushing the onion and extracting the resulting juice with a solution of methylene chloride and an internal standard. The resulting extract is concentrated and injected onto a gas chromatograph. This method is shown to be simple, fast, and reproducible. Results show that onion juice contains 1−22 μmol of the LF/mL.

Keywords: *Onion; Allium cepa; onion lachrymatory factor; gas chromatography*

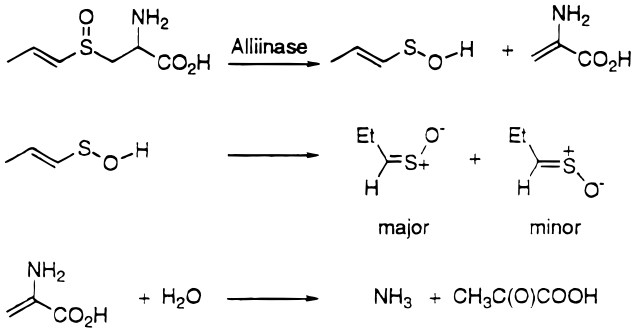
INTRODUCTION

(*Z*,*E*)-Propanethial *S*-oxide, the lachrymatory factor (LF) in onion, has long been known to be a primary product of the action of the enzyme alliinase (EC 4.4.1.4) on the main onion flavor precursor, (+)-*S*-(*E*)-1-propenyl-L-cysteine *S*-oxide by the reaction shown in Scheme 1 (Block, 1992). In addition, two other flavor precursors, *S*-methylcysteine *S*-oxide and *S*-*n*-propylcysteine *S*-oxide, yield additional flavor compounds. The initial reaction yields 1-propenesulfenic acid and 2-aminopropenoic acid. The 1-propenesulfenic acid then undergoes a rapid [1,4] rearrangement to yield the LF, with the *Z*-isomer comprising about 95% of the total. The 2-aminopropenoic acid reacts much more slowly to form ammonia and pyruvic acid.

A similar reaction occurs with the other flavor precursors whereby the sulfenic acid, ammonia, and pyruvic acid are formed. The three sulfenic acids can then condense to form mixed thiosulfinates and water. The exact thiosulfinate formed depends upon the identity of the two sulfenic acids forming the thiosulfinate. Although 1-propenesulfenic acid does form thiosulfinates, the majority of 1-propenesulfenic acid goes to form the LF. It should also be noted that 1-propenyl 1-propenethiosulfinate is unknown because it undergoes a rapid rearrangement to form *cis*- and *trans*-zwiebelanes (Bayer et al., 1989).

There is presently no reliable method to quantify the LF and the flavor chemicals produced from an onion. One method of quantitating the LF is by the use of spectrophotometric methods. An extraction and spectroscopic method has been shown to quantitate the LF using its absorbance at 254 nm (Freeman and Wenham, 1975). However, this method has an obvious flaw in that a multitude of components which will extract into hexane absorb at this wavelength. Little effort has been made to separate the LF from other components. A similar method utilizes the reaction of the LF with a glycine−formaldehyde reagent to yield a pink color which can be quantitated using thin layer chromatography (Tewari and Bandyopadhyay, 1975). These methods quantify only the LF and do not analyze for other flavor chemicals.

Scheme 1



Another method for quantifying the LF present in a sample is gas chromatography (GC). The advantage of a chromatographic method is its ability to separate compounds from a mixture. Prior to Eric Block's pioneering work in onion chemistry, a number of groups analyzed onion for the LF and other components using gas chromatographic methods. However, all of this early work was performed with GC methods in which the injection ports were heated to a high temperature. Block and co-workers have shown that under these conditions many of the onion chemicals rearrange and decompose (Block et al., 1992). Therefore, all gas chromatographic work on onions using heated injection ports should be carefully considered.

Block and co-workers were able to identify the LF and other flavor chemicals using a rather involved procedure of extractions, evaporation, and finally GC or GC−MS analysis (Block et al., 1992; Randle et al., 1994). While substantial amounts of the LF were observed in onion extracts, their results were highly variable, and reported LF levels are considerably lower than those of the present study. These results are not surprising given the fact that their method was optimized to analyze for thiosulfinates.

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Through use of the present method, it is possible to extract the LF and other flavor chemicals from onion juice and obtain GC results in less than an hour. Samples are analyzed as quickly as possible and maintained at the lowest possible temperature to prevent sample degradation. The method is reproducible, and our results correspond to those obtained by other researchers.

EXPERIMENTAL PROCEDURES

Materials. Onion samples were obtained from either local grocery stores or local onion growers. Locally grown onions were of the Granex type grown under standard field conditions. The store-bought onions were typical yellow storage onions. HPLC grade methylene chloride was obtained from Fisher Scientific and was used as received. All other chemicals were obtained from Aldrich Chemical Co. and were of reagent grade or superior. No further purification was performed on these chemicals.

The method of Block was used to synthesize the LF (Block et al., 1996). The authenticity of the compound was confirmed by GC−MS, NMR, retention time on the GC, and lachrymatory effects on the researchers. The mass spectrum of the sample showed a parent ion peak at *m*/*z* 90. An isotope pattern consistent with a sulfur compound was found. Major fragment peaks also occurred at *m*/*z* 85, 73, and 59. All of these data are in agreement with those for the LF (Block et al., 1992).

Thiosulfinates were synthesized by the oxidation of disulfides with peracetic acid. The presence of the thiosulfinate was confirmed by NMR and GC analysis. Retention times of synthetic methyl methanethiosulfinate, methyl propanethiosulfinate, propyl methanethiosulfinate, and propyl propanethiosulfinate matched those of native thiosulfinates from onions.

Equipment. An onion crusher was constructed by a local machine shop. This crusher contained a cylinder into which the sample was placed. A piston was then pushed down upon the sample using a manual lever. Screens were placed in the bottom of the cylinder to separate the onion solids from the juice. The juice was directed out of the cylinder with a slot in the bottom of the cylinder into a waiting beaker.

A Hewlett-Packard 5890 Series II gas chromatograph was employed with a cold on-column injection port, a 5 m × 0.54 mm i.d. OV-1 column, and 99.999% He carrier gas at 1.0 psi. The GC oven temperature was set isothermal at 60 °C for 1 min and then increased at 5 °C/min to 200 °C. The carrier gas flow rate was 8.5 mL/min. The injector temperature was maintained at 3 °C greater than the oven temperature (oven tracking). The detector was a flame ionization detector (FID) maintained at a temperature of 250 °C. The GC response to the LF and thiosulfinates was determined by comparing the NMR and GC peak areas of the compound and *p*-cymene for the same solution.

Mass spectra were collected using a VG Trio 303 mass spectrometer equipped with a Hewlett-Packard 5890 gas chromatograph with electron impact analysis at 70 eV. The GC used a cold on-column injector. A head pressure of 6 psi was maintained with 99.999% He on a 30 m × 0.30 mm OV-1 column. The temperature program used an initial temperature of 30 °C for 1 min followed by a temperature ramp of 5 °C/min to 200 °C.

Procedure. An onion sample consisting of several onion bulbs or wedges (approximately 50 mL) is placed in the crusher. The sample is then crushed and the juice collected in a beaker (approximately 25 mL). Time is then allowed for the alliinase to react with the flavor precursors at room temperature, about 24 °C. Five milliliters of juice is then extracted a single time with 4 mL of methylene chloride and 1 mL of 0.0100% (v/v) *p*-cymene in methylene chloride. The extract is then centrifuged, and the lower organic layer is concentrated by blowing it down to approximately 0.5 mL with compressed air or nitrogen. The concentrated sample is immediately placed in an ice bath, and a 1.0 μL sample is injected onto the GC within an hour. The crusher was thoroughly cleaned between each sample to prevent contamination.

Pyruvic acid concentrations of all onion samples were determined using the method of Randle and Bussard (1993).

RESULTS AND DISCUSSION

The present method has been found to be a rapid and reproducible procedure for quantitating the LF and thiosulfinates produced from an onion. The GC analysis time can be shortened to as little as 35 min per analysis including time for reequilibration of the GC. The LF comes off of the column in approximately 1 min, while the remaining thiosulfinates and other flavor compounds take considerably longer.

Figure 1A shows a typical chromatogram of an onion extract. Figure 1B is an expanded view of the resolution between the LF and the solvent peak for a different but similar onion sample. The LF (peak 1) is found in the tail of the solvent peak but is sufficiently resolved for good quantitation. The resolution between the LF and the closest interfering peak is calculated to be 4.9. Peaks 5 and 7 are believed to be (*Z*,*E*)-1-propenyl methanethiosulfinate and methyl 1-propenethiosulfinate by comparison of our results with those of Block and co-workers (Block et al., 1992). Peaks 9, 10, and 11 are believed to be *cis*-zwiebelane, *trans*-zwiebelane, and propyl 1-propenethiosulfinate, again based on Block's work. Relative GC responses per mole for all confirmed compounds in this study are given in Table 1.

It should be noted that our GC conditions are considerably harsher than those used by Block et al. (1992). In Block's work an injector temperature of 0 °C was used. In our study we varied the initial temperature of the injector (and column), the column length, and the carrier gas flow rate. In the study of the percent recovery of methyl methanethiosulfinate, it was found that the best results were obtained using a short column, a higher temperature (60 °C), and a high carrier gas flow rate. Results with a long column and/or a low carrier gas flow rate showed drastic reductions in the amount of methyl methanethiosulfinate observed. Apparently, the longer methyl methanethiosulfinate is on the column, the more it breaks down. It is believed that the LF and other thiosulfinates would have a similar response to chromatographic conditions as methyl methanethiosulfinate. Under the present conditions no appreciable compound breakdown is observed. A lower initial temperature could have been chosen so that better resolution between the LF and the solvent peak could be obtained. However, it was felt that at 60 °C the resolution between the LF and the solvent peak was not severely compromised, the recovery of methyl methanethiosulfinate was greatly increased, and the analysis time was considerably shortened.

p-Cymene was chosen as an internal standard for a number of reasons. First, the retention time did not coincide with any other peaks found in onion extracts. *p*-Cymene is insoluble in water and is found only in the organic phase. *p*-Cymene is also a stable compound, and solutions of internal standard can be prepared well in advance and stored. Finally, chromatography of *p*-cymene yields a sharp, well-defined peak with good detector response.

The results obtained from the analysis of identical samples indicate that the method is quite reproducible. When a single onion is split into six wedges and each

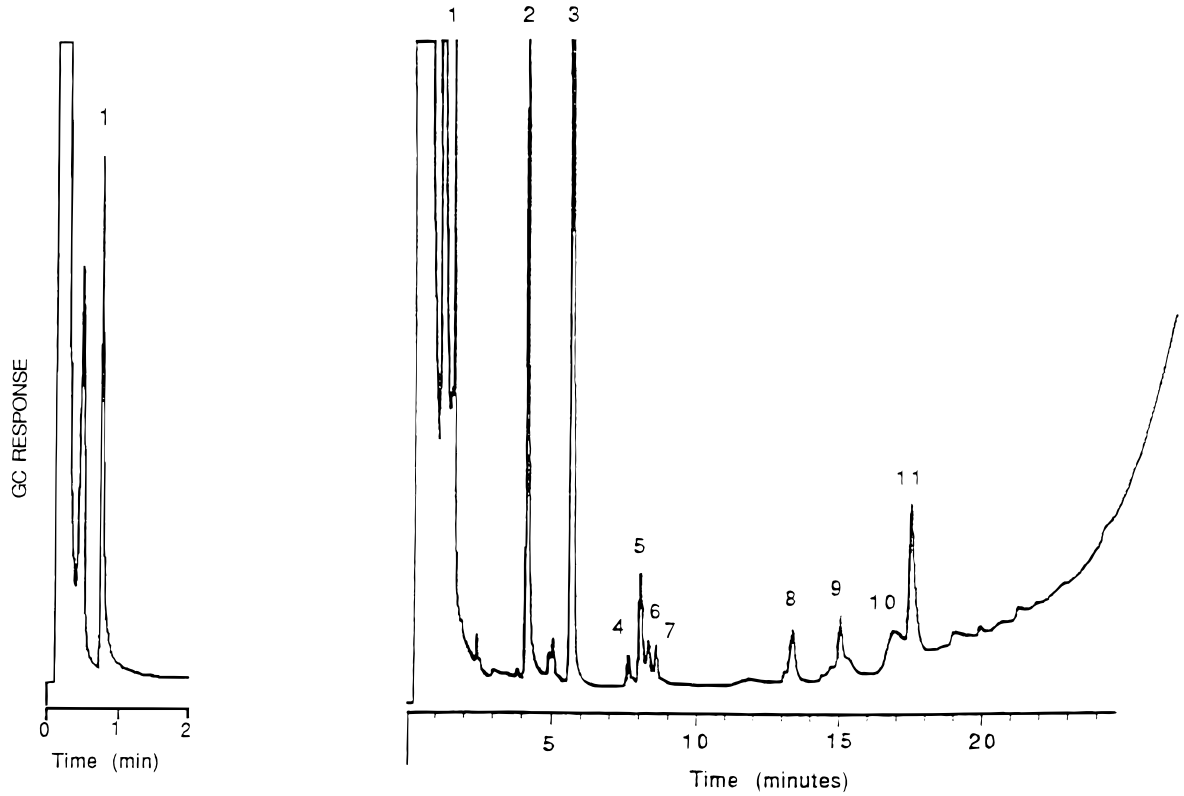

Figure 1. Typical chromatogram of an onion extract: (A, right) entire GC run; (B, left) expanded view of the first 2 min of the chromatogram at a much higher attenuation. The identified peaks are LF (1), methyl methanethiosulfinate (2), *p*-cymene (3), methyl propanethiosulfinate (4), propyl methanethiosulfinate (6), and propyl propanethiosulfinate (8). A 5 m × 0.53 mm wide-bore capillary column was used: program from 60 °C for 1.00 min at a rate of 5 °C/min to 200 °C using a flame ionization detector.

Table 1. Relative GC-FID Responses

compd no.	chemical	rel molar detector response
1	(*Z*,*E*)-propanethial *S*-oxide	1.00
2	methyl methanethiosulfinate	0.57
3	*p*-cymene	8.60
4	methyl *n*-propanethiosulfinate	1.46
6	*n*-propyl methanethiosulfinate	1.46
8	*n*-propyl *n*-propanethiosulfinate	3.36

wedge is analyzed individually, the LF concentrations only had a 7% relative standard deviation. Once the onion juice is extracted, it is most important that the extract be analyzed immediately. We have observed that even in an ice bath the concentration of the LF decreases slowly over a period of hours. Block has stated that the LF is stable only at temperatures less than −20 °C (Block et al., 1996). The concentrations of thiosulfinates will also change due to cross-reactions.

A calibration curve of the linearity of the method is shown in Figure 2. In this experiment a single onion was divided into wedges and each wedge analyzed separately with different amounts of juice extracted from each wedge. It is assumed in this experiment that each wedge would yield an identical concentration of the LF. The sample was diluted with water if needed to obtain a total of 5 mL of aqueous solution to be extracted. This calibration curve is very linear with a value of $r^2 = 0.9988$. The limit of detection for the method is estimated to be 0.1 μmol of LF/mL of onion juice. A nonzero intercept is obtained in the graph, indicating that at low concentrations the LF is lost due to poor extraction efficiency or volatilization. Work done using LF in any form, synthetic or native, must be done rapidly because the high volatility of LF in aqueous

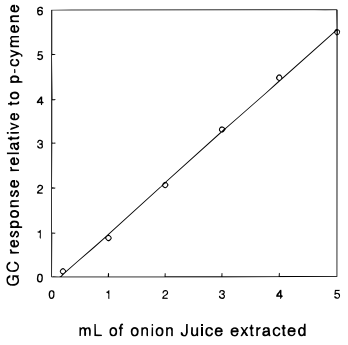

Figure 2. Linearity of the method when differing amounts of onion juice are extracted. The volume of onion juice was varied, but water was added so that the total amount of aqueous solution in the extraction remained the same.

solutions creates a solution that is constantly decreasing in concentration.

Initially in our method we allowed 10 min for the alliinase to react with the flavor precursors at room temperature. In this work the concentration of the LF found in locally grown Granex onions ranged from 1 to 4 μmol of the LF/mL of onion juice. In yellow onions the concentration of the LF was slightly higher.

From results with a 10 min wait time it is readily apparent that less LF was collected than pyruvic acid from the same onion sample. It was found that only 5 μmol of LF/mL of onion juice was obtained for a sample yielding 12 μmol of pyruvic acid/mL of onion juice. A study was therefore performed to determine the effect of reaction time upon the concentration of the LF. These results are shown in Figure 3. From these results it is apparent a maximum of 2 min should be allowed for the LF to be formed. Beyond this length of time the production of the LF from the enzymatic reaction is

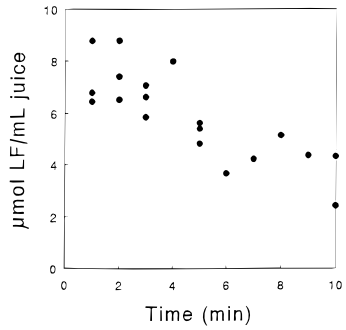

Figure 3. Effect of time allowed for flavor development.

complete and the LF is lost due to volatilization, hydrolysis, or reduction. The extent of loss caused by each of these processes has not been determined. However, once the LF is extracted into the methylene chloride, the loss of the LF is greatly reduced. Recent work has found alliinase totally consumes all of the *S*-1-propenylcysteine *S*-oxide within 30 s (Randle and Lancaster, 1993). However, pyruvic acid is not fully developed for at least 6 min. Therefore, some of the LF was lost when a 10 min wait time was used. One additional reason for the 2 min wait time was that the maximum amount of methyl methanethiosulfinate was detected using a 2 min wait time.

The values obtained for the LF concentration in onion juice are considerably higher than the values reported by earlier workers. Our numbers show that mature onions have 2−10 μmol of the LF/mL. Growing onions generally were within this range, although occasional samples reached 22 μmol of the LF/mL. Block and coworkers reported that onion juice had 0.94 μmol of the LF/mL (Block et al., 1992). Block's procedure entailed a lengthy extraction and sample preparation which optimized for analysis of thiosulfinates and zwiebelanes. It is possible that in his procedure the LF was volatilized, thus resulting in variable amounts of the LF being observed. Our numbers also correspond well with values reported recently for the flavor precursors by Randle and Lancaster (1993). They report that the onion they studied had up to 20 μmol of flavor precursors/g of onion.

CONCLUSIONS

The new method of onion analysis presented here is fast, accurate, and reproducible and requires no background samples. The LF and a number of thiosulfinates have been analyzed using this method. Future work will concentrate on being able to deduce the distribution of the flavor precursors in the onions on the basis of the observed concentration of thiosulfinates, zwiebelanes, and the LF.

ABBREVIATIONS USED

GC, gas chromatography; LF, onion lachrymatory factor, (*Z*)-propanethial *S*-oxide; GC−MS, coupled gas chromatography−mass spectrometry; NMR, nuclear magnetic resonance.

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