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Determination of fresh onion (*Allium cepa* L.) volatiles by solid phase microextraction combined with gas chromatography-mass spectrometry

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Abstract Solid-phase microextraction (SPME) was used for the extraction of the volatiles of yellow onions (*Allium cepa* L.), where the primary volatiles are produced enzymatically after rupture of the plant cells. The SPME-GC analysis of successive samples at timed intervals provided information comparable with that obtained previously by headspace techniques; however, SPME was more convenient and faster to perform. Moreover, the SPME-GC-MS system employed permitted easy monitoring of the fast changes in the volatile composition. Because ambient temperatures were used in the analysis, the method described should produce only minor artefacts during the absorption and desorption steps of SPME. The most important compound found in the headspace of sliced onions by SPME-GC-MS after 1 min emission of volatiles was thiopropanal S-oxide. Also, some dipropenyl disulphides and propenyl propyl disulphides were identified. After 30 min, most of the thiopropanal-S-oxide disappeared, and other sulphur and non-sulphur compounds appeared in the GC chromatograms. The major constituents were diprop(en)yl disulphides. Similar constituents were also found in the water-slurred onions by headspace and direct SPME.

Key words Onions · Solid-phase microextraction · Volatiles

Names are necessary to report factually on available data; however, the USDA neither guarantees or warrants the standard of the product, and the use of the name by USDA implies no approval of the product to the exclusion of others that may also be suitable.

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Introduction

The aroma and flavour of onions and other *Allium* species are characterized by a variety of sulphur compounds. These are produced after the rupture of the cell structure, when the precursor compounds, S-alk(en)yl cysteine sulphoxides [where alk(en)yl is alkyl and/or alkenyl] come into contact with the alliinase enzyme. This enzyme cleaves the precursors to produce alkenyl sulphenic acids. In the case of onions, 1-propenyl sulphenic acids rearrange to give thiopropanal-S-oxide, the lachrymatory factor [1, 2]. Both alk(en)ylsulphenic acids and thiopropanal S-oxide, are highly reactive, and a variety of different sulphur-containing volatiles and aldehydes are formed. However, it seems that the composition of the volatiles found is dependent on the isolation and analytical methods involved [3–8]. To date, there have been two main approaches to studying onion aroma composition. These have included studies utilizing headspace techniques to analyse the volatiles emitted from onion and other *Allium* species after rupture of the cell structure [3–5]. Others have studied water slurries of onions (and other species) from which volatiles are isolated by means of liquid solvent extraction [6, 7] or supercritical fluid extraction [8–10]. An excellent review of the above approaches can be found in Ref. [6].

Solid-phase microextraction (SPME) is a relatively new analyte isolation technique developed by Pawliszyn and coworkers [11–13]. In this technique, a piece of fused silica fibre coated with a polymeric film coating is introduced into a headspace of the sample (solid or liquid), or immersed in a water solution of the sample. The latter technique is called direct SPME [12]. A relatively short absorption time is needed to trap most analytes, but the final equilibration of the trapped analytes is dependent upon their partition coefficients between the different phases involved. The analytes are then desorbed from the fibre phase in a heated GC injector, or a modified HPLC injector, onto a chromatographic column [11–13].

Applying SPME to the study of onion volatiles allows the application of these two approaches, because SPME fibres can be used to sample both headspace volatiles and the analytes dissolved in water. Headspace SPME can trap those compounds which are released into the headspace from the sample matrix. By immersing the fibre in a water solution, less volatile and/or more hydrophilic compounds can be analysed. Moreover, the same analytical conditions in the GC analyses could be used with both sampling methods, allowing a comparison to be made between the results from both sampling methods. The objective of this study was to use SPME for the analysis of highly reactive compounds, such as sulphur-containing volatiles in onions, thereby ascertaining the efficacy of SPME systems. This involved SPME sampling of fresh cut onion slices, at timed intervals, to qualitatively follow the generation and disappearance of the aroma constituents. Two major advantages of SPME are its simplicity and its time efficiency. Since no time is wasted from sampling to injection, SPME can monitor onion flavours at much smaller time intervals and allow the study of onion flavours at the earliest possible moment (immediately the onion has been cut).

Materials and methods

Samples. Yellow onions (*Allium cepa* L.) were purchased from a local grocery store (Peoria, Ill.).

Sample preparation. The samples used for SPME were sliced onions and onions sliced into water (1:5, w/w). It should be noted that the manner in which the onions are cut affects the rate at which compounds are produced and the identity of these compounds. In all of the experiments, onions were sliced by knife into similarly thin slices in order to get the sample into a headspace bottle (volume ~10 ml) in a short time interval (30 s), while maintaining uniform onion sample structure and weight. The amount of onion in each sample was about 1.5 g.

Sampling. The changing composition of volatiles desorbed from sliced onions was followed by successive SPME sampling at timed intervals from the headspace of fresh slices (HS) or slices immersed in water (HS-W), or directly sampling the water by SPME (D-W). The fibres used in this study were poly(dimethyl)siloxane (PDMS) with a 100 μm film thickness (Supelco, Bellafonte, Pa.). They were conditioned prior to sampling at 150 °C for 0.5–1 h. A sampling (absorption) time of 1 min was chosen after these preliminary studies. The fibre was desorbed at the GC injector for 5 min, a relatively long time compared to those used in previous SPME studies, due to the low desorption temperature (35 °C) employed. This temperature was chosen as a compromise between minimizing the carry-over of less volatile constituents, and the broadening of the peaks at the front of the chromatogram.

SPME-GC-MS. A Varian Star 3600 CX GC, equipped with a model 8200 CX SPME autosampler (Varian, Palo Alto, Calif.), was used to effect the absorption and desorption of the SPME fibre automatically. During the desorption step the injector split valve was closed; otherwise the split was open. The GC injector was held at 35 °C, and the column temperature program was to increase from 15 °C (held for 5 min) to 120 °C (held for 20 min) at 5 °C/min. A DB-5MS column, 30 m length, 0.25 mm i.d., 0.25 μm film thickness (J & W Scientific, Folsom, Calif.) was used with helium as the carrier gas. The compounds were detected and identified with a Varian Saturn 4D GC/MS system; the temperature of the transfer line followed the column temperature (up to 80 °C), while the temperature of the MS manifold was kept at 100 °C. The compounds were identified by comparing their mass spectra to a NIST spectrum library and to the previously published spectra obtained from *Allium* samples[3, 4, 7, 14–16].

Results and discussion

When the headspace is sampled by SPME, only compounds which are volatile at the chosen analysis conditions can be trapped onto the fibre [11]. The peak areas obtained by SPME-GC-MS method do not necessarily reflect the true proportions of the components in the headspace or in the sample, because different classes of compounds and even individual compounds with different structures exhibit different volatilities as well as other differing physical properties, which can affect partition coefficients. However, trends in the appearance and disappearance of individual compounds in successive samples can be studied.

In this study, sampling time was defined as the time elapsed from first cutting of the onions to the starting time of the SPME absorption. As an example, the chromatogram in Fig. 1A was obtained from an onion sample taken 1 min after first cutting the onion into halves. The earliest previously reported analysis of the onion volatiles was after 15 min reaction time, obtained by a dynamic headspace technique [4, 16]. Hence, SPME is a much more rapid and sensitive method for trapping and concentrating analytes from a headspace. The relative abundances of headspace components are shown in Table 1.

At 1 min after cutting the onions, the most abundant compound was thiopropanal S-oxide, or its tautomers, e.g. 1-propenylsulphenic acid, having a structure C_3H_6SO (mol.wt. = 90). Thiopropanal S-oxide is thus shown to be the primary volatile product of the alliinase activity in onions [1,5], although it is often lost via the solvent evaporation step in liquid solvent extraction methods [6]. A small amount of diprop(en)yl disulphides were trapped in the fibre over this time interval, too. These disulphides had molecular weights of 148, 146 and 146 respectively (elution order in Fig. 1A) corresponding to 1-propenyl propyl disulphide and two isomers of dipropenyl disulphide, respectively. According to the previously published spectra, the latter two were probably stereoisomers of di-1-propenyl disulphides [3, 14–16].

In previously published studies, the volatiles of onions were usually extracted 30 min after the rupture of the onion cell structure [3, 5–8]. The reason for that was to give adequate time for the enzymatic and chemical reactions to occur. As a result, di- and trimers of the basic structure of R-S(O), where R represents methyl or prop(en)yl groups, were found. As can be seen in Fig. 1B, in addition to thiopropanal S-oxide and diprop(en)yl disulphides, which were found at the beginning of the reaction, two aldehydes, dimethyl thiophenes

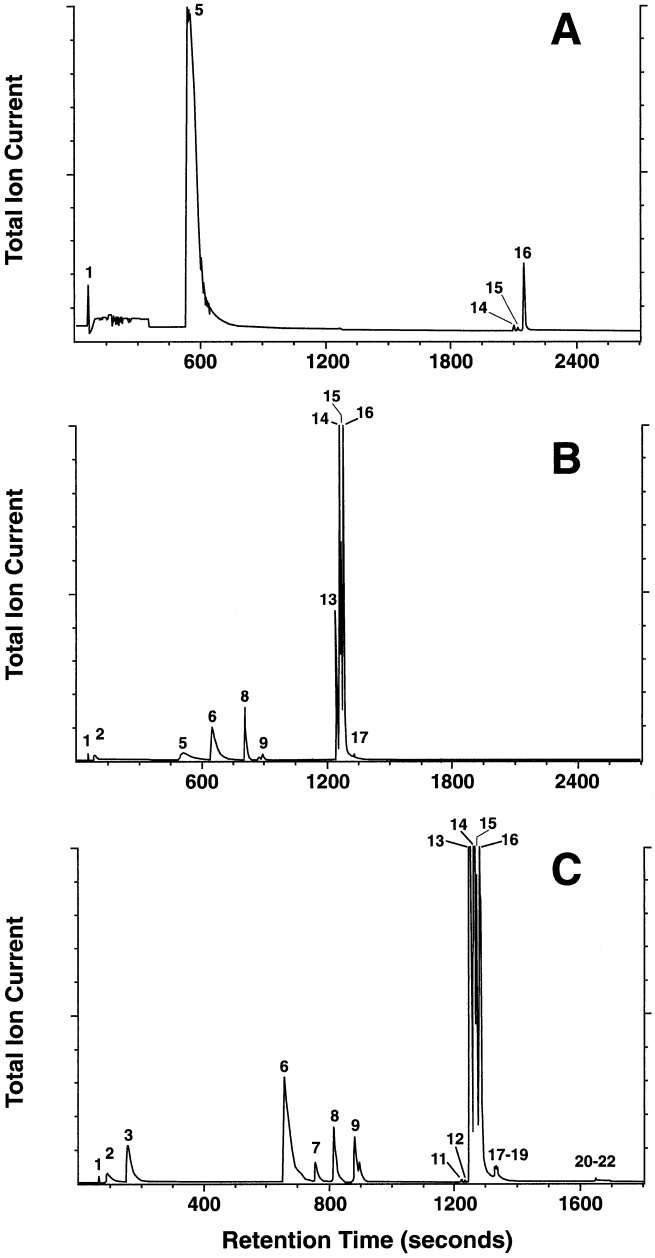

Fig. 1A–C Headspace SPME of fresh onions analysed by GC-MS: **A** 1 min; **B** 30 min; **C** 166 min. For analysis conditions, see Methods section. For peak identification, see Table 1

and methyl prop(en)yl di- and and trisulphides were also isolated by SPME from the headspace of onion slices at 30 min. The relative abundances of those compounds are shown in the Table 1. These same compounds had previously been found in onions by headspace GC analyses [3, 4, 16]. In contrast, thiosulphinates { R-(O)S-R or R-S-S(O)-R } and zwiebelanes have been reported in the solvent and SFE extracts of onion-water mixtures [6–8] or in headspace trappings [5], analysed by HPLC and/or GC. It has been suggested that the polysulphides found in headspace studies have been formed by the thermally induced reactions of thiosulphinates during GC analysis [7]. This transformation has been minimised in this study by using SPME, since an ambient absorption and a 35 °C desorption (injector) temperature were employed. Under these conditions, only extremely thermally labile compounds would degrade prior to introduction into the GC column. Hence, the appearance of diprop(en)yl disulphides in the mass spectrum could be due to the emission of the compounds into the headspace by onions, and/or their formation via rearrangement in the gas phase from the lachrymatory factor and sulphenic acids at temperatures of 35 °C or lower. In a previous study, SPME was used to evaluate the volatile composition of the trapped headspace concentrate [5]. The results were comparable with those in the present study with the exception that thiosulphonates were also found. Even at 30 min thiosulphonates were not found in this our study.

The further development of the volatile pattern in the headspace of onions was followed although many of the detected components may have limited impact on the flavour of fresh onions. As an example, the profile obtained at 166 min is shown in Fig. 1C. After 1 h, thiopropanal S-oxide can be found as a minor constituent, due to the appearance of degradation products of enzymatic or chemical reactions, such as 2-methyl-2-pentenal and C_3H_nS, which start to appear while the thiopropanal S-oxide moiety disappears. The most abundant compounds, however, are different diprop(en)yl disulphides, which as a group of compounds constitute over 80% of the total area detected in the GC chromatogram. Interestingly, the composition within this group can change, too. The first components described above are also found later, but after about 90 min the most abundant compound is dipropyl disulphide. The relative amount of dipropyl disulphide increases with the continuing reduction in the amount of thiopropanal S-oxide. Methyl prop(en)yl disulphides are found after the large amounts of diprop(en)yl disulphides are already present in the headspace. This is consistent with the biochemical transformation, described, for example by Block et al. [6]. The alliinase enzyme has been shown to prefer prop(en)yl containing precursors; thus volatiles containing methyl groups appear later in the headspace [4]. However, methyl prop(en)yl trisulphides were found in the headspace before their diprop(en)yl counterparts. After additional time has elapsed (about 2.5 h) methyl prop(en)yl and diprop(en)yl tetrasulphides were found. The diprop(en)yl tetrasulphides are higher molecular weight compounds (mol.wt. $\approx$ 180) and are difficult to detect under the experimental conditions used in this study, which is why they occur in the headspace long after the appearance of their methyl prop(en)yl counterparts.

Ferary and Auger (1996) suggested that the disulphides they observed are degradation products from thiosulphinates [5]. The degradation of thiosulphinates may occur in the 100 °C injector, where gas-phase reactions frequently to occur. However, Block et al. [6] sug-

Table 1 The compounds found in onion headspace by SPME-GC-MS. *Me* Methyl, *Pr* propyl, *Pe, Pe(n)* propenyl, (n) indicates position of double bond, – not found, *na* not applied, *tr* 0.01–0.1%

Peak[a]	Mol. wt.	Abbreviation	Name	Relative abundance		
				1 min	30 min	170 min
1	40–44		"Air peak"	na	na	na
2	56–58	C3HnO	Prop(en)yl aldehydes	–	0.7	0.4
3	76	Pr/Pe-SH	Prop(en)ylthiols	–	–	–
4	76		Prop(en)ylthiols	–	–	1.6
5a	94	Me-SS-Me	Dimethyl disulphide	–	–	–
5	90	C3H6SO	Thiopropanal S-oxide	97	3.2	–
6	98	2-Me-2-pentenal	2-Methyl-2-pentenal	–	6.8	5.9
7	112	di-Me-thiophene	Dimethyl thiophene	–	0.1	0.5
8	112	di-Me-thiophene	Dimethyl thiophene	–	3.2	1.4
9	122	Me-SS-Pr/Pe	Methyl prop(en)yl disulphides	0.1	0.1	1.3
10	126	Me-SSS-Me	Dimethyl trisulphide	–	–	–
11	148	Pr-SS-Pe[a]	Propenyl propyl disulphide	–	–	tr
12	146	Pe-SS-Pe[a]	Propenyl propenyl disulphide	–	tr	tr
13	150	Pr-SS-Pr	Dipropyl disulphide	–	5.5	44
14	148	Pe(1)-SS-Pr	1-Propenyl propyl disulphide	0.2	26	27
15	146	Pe(1)-SS-Pe(1)	Di-1-propenyl disulphide	0.1	9.2	4.1
16	146	Pe(1)-SS-Pe(1)	Di-1-propenyl disulphide	3.1	45	13
17	154	Me-SSS-Pr	Methyl propyl trisulphide	–	0.1	0.2
18	152	Me-SSS-Pe	Methyl propenyl trisulphide	–	–	–
19	152	Me-SSS-Pe	Methyl propenyl trisulphide	–	–	–
20	182	Pr-SSS-Pr[b]	Dipropyl trisulphide	–	–	tr
21	180	Pe-SSS-Pr[b]	Propenyl propyl trisulphide	–	–	tr
22	180	Pe-SSS-Pr[b]	Propenyl propyl trisulphide	–	–	tr

[a] Peak numbering refers to Fig. 1
[b] Tentative identification

gest that although the thiosulphinates are thermally labile, the *Allium* thiosulphinates, except those containing allylic groups, will not be altered in a MS transfer line at 100 °C. Di-1-propenyl disulphide has been reported to rearrange at 85 °C temperatures to form various cyclic compounds including dimethyl thiophenes [7]. In our study, two or more isomers of dimethyl thiophenes were found (see Table 1). The dimethyl thiophenes elute at much lower temperatures than diprop(en)yl disulphides, which eluted at about 80 °C (when the programmed-temperature GC run was used). However, in this case, if dimethyl thiophenes are formed from diprop(en)yl disulphides, they would have to be produced at much lower temperatures.

The results of previous studies suggest that the headspace of fresh sliced onions includes different compounds from those in the extracts of water slurred onions. It may be that water stabilizes the primary reaction products, leading to different structures for sulphur-containing compounds than those found in the gas phase. For this reason, onion slices immersed in water were also studied by headspace and direct SPME . The results from different samples are shown in Table 2, where the groups consist of structurally similar compounds.

Qualitatively, similar compounds were found in headspace of onion slices (HS) and water-containing samples (HS-W). The total amount of headspace vola-

Table 2 Proportions of different structural groups at 30 min in different SPME samples. *HS* Headspace of onion slices, *HS-W* Headspace SPME of onions in water, *D-W* direct SPME of onions in water

Group identity	Peaks[a]	Relative area		
		HS	HS-W	D-W
C_3H_nO	2	0.7	0.2	–
Pr/PeSH	3– 4	–	–	–
Thiopropanal-S-oxide	5	3.2	2.2	14
2-Me-2-pentenal	6	6.7	–	–
Dimethyl thiophenes	7–8	3.3	0.1	–
Me-S-S-Pr/Pe (isomers)	9	0.7	2.1	1.0
Me-SSS-Me	10			
Pr/Pe-S-S-Pr/Pe (isomers)	11–16	85	95	85
Me-SSS-Pr/Pe	17–19	0.1	–	–
Pr/Pe-SSS-Pr/Pe (isomers)	20–22	–	–	–
Total area count		2.9E+07	9.2E+06	1.9E+06

[a] Numbers refer to Table 1

tiles was found to be lower in the water-containing samples than those derived from onion slices, as seen by the smaller total ion current in the MS. This is consistent with previously reported SPME studies [11–13], that a smaller amount of volatiles is obtained from the HS-W than from non-aqueous systems. The total peak area was found to be even smaller when the fibre was immersed in a water-onion mixture (D-W). Both of these reductions in peak areas could be due to the high volatility of the analytes [12], which is moderated by the presence of water. The low response with direct SPME was probably due to low solubility of these compounds in water, or their low diffusion coefficients in water and partition coefficients from water onto a fibre surface [12, 13]. Furthermore, the highly volatile components may be less abundant in the water phase rather than in the headspace.

The direct comparisons between water-containing and fresh onion samples can become complicated due to differences in analyte partitioning coefficients [17]. However, ratios of peak areas in each sample can provide some useful information. The only compound having a larger area in direct SPME than in headspace SPME was thiopropanal S-oxide (the ratio being 1.4). However, headspace SPME of fresh onions resulted in a 4-fold increase in the area of this compound, when compared to the water-containing sample, which suggests it is released from onions into air more rapidly than from onions into water. Thiopropanal S-oxide seems to be stabilized by higher solubility and lower volatility, which may be due to hydrogen bonding. The absence of aldehydes in the water-containing samples also supports this explanation. The C_3– and C_6– aldehydes and dimethyl thiophenes occurred in the headspace of water samples in small amounts and were not found by direct SPME at 30 min. The absence of dimethyl thiophenes in water samples has been verified in previous studies with onion-water mixtures [5]. Somewhat unexpected were methyl prop(en)yl disulphides, which were found in equal amounts by headspace SPME in both water-containing and plain onion samples. By direct SPME (D-W) the area was 10% of that obtained by headspace SPME. Prop(en)yl disulphides were found by headspace SPME (both water immersed and plain onions) in larger amounts than by direct SPME. This is probably due to the relatively high volatility of these compounds [17]. However, there is a large variation in the individual compounds comprising this group of compounds, e.g. one isomer of di-1-propenyl disulphide is not found by direct SPME of water-containing sample (at 30 min), while the other isomer seems to be more pronounced in plain onion slices than in a water-containing sample (data not shown). Also, dipropyl disulphide is found in larger quantities in water-containing samples than in plain onion slices.

In conclusion, SPME is found to be a very time efficient and sensitive technique for monitoring enzymatic and/or chemically produced onion volatiles and their release from a sample matrix, especially at the earliest possible moment after the rupture of onion cells. The aroma constituents produced initially have an impact on the fresh aroma of sliced onions. The main constituent found by SPME technique at early stages was thiopropanal S-oxide. The polysulphides detected have low aroma thresholds, and thus probably have a impact on the aroma of onions even at very low concentrations. As noted above, at 30 min the production of thiopropanal S-oxide has maximized, thus allowing the production of the other volatile constituents; hence the 30 min mark is used to characterize the aroma profile. After additional time had elapsed, the compounds detected did not exhibit the fresh onion aroma any more.

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