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Aroma analysis of fresh and preserved onions and leek by dual solid-phase microextraction–liquid extraction and gas chromatography–mass spectrometry

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Abstract

The lachrymatory factor (thiopropanal-*S*-oxide) was directly analysed on fresh onion (*Allium cepa*) juice by solid-phase microextraction (polyacrylate fibre) using a fast routine GC–MS method on a 10 m×0.32 mm I.D. (4 μm thick polydimethylsiloxane film) column with splitless mode injection. The identification and quantification of thiosulphinates and zwiebelanes were obtained on the same juice extracted by diethyl ether after 80 min maceration using the same GC–MS method. Selected ion recording enhanced the differentiation possibilities and the detection limits. This dual method was used to evaluate flavour differences between onion and shallot varieties as it provides accurate profiles of all initially formed compounds. Moreover, this method allowed us to compare qualitatively and quantitatively transformed products: frozen, freeze-dried powders and sterilised products. Excepting the lachrymatory factor, frozen onion compounds were similar compared to those of fresh onion sample. Conversely, the other transformed samples have lost most of the initially formed compounds and produced mainly di- and trisulphides corresponding to the degradation of thiosulphinates and zwiebelanes. These dramatic changes can explain the very different flavours of these manufactured products compared to fresh material.

Keywords: Lachrymatory factor; *Allium* spp.; Food analysis; Onions; Organosulphur compounds; Thiosulphinates; Zwiebelanes

1. Introduction

The aroma and flavour of onion (*A. cepa*) and other *Allium* species are characterised by a variety of sulphur compounds. They are produced after the rupture of the cell structure, when precursor compounds, S-alk(en)yl cysteine sulphoxides, come into contact with alliinase. This enzyme cleaves the precursors to produce alk(en)yl sulphenic acids. These intermediate alk(en)yl sulphenic acids rearrange rapidly to form thermally unstable thiosulphinates (Ti) (Table 1). In the case of onions, the prominent 1-propenylsulphenic acid rearranges to give mostly (*Z,E*) thiopropanal-*S*-oxide, the lachrymatory factor (LF) and zwiebelanes, isomers of di(1-propenyl)Ti [1,2] (Table 1).

Qualitative and quantitative analysis of *Allium* organosulphur compounds have an additional significance because health benefits from the consumption of fresh *Allium* species and numerous health food products is well documented [3]. However, it seems that the composition of the volatiles found is depen-

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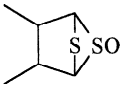
Table 1
Abbreviations and structures of thiosulphinates and zwiebelanes found in *A. cepa* and *A. porrum*

Compound	Abbreviation	Structure
1	LF[a] (*Z*)	$CH_3–CH_2–CH=SO$
2	LF (*E*)	$CH_3–CH_2–CH=SO$
3	2-Methyl-2-pentenal	$CH_3–C(CH_3)=CH–CH_2–CH_3$
4	$TiMe_2$	$CH_3–SO–S–CH_3$
5	TiMePr	$CH_3–SO–S–CH_2–CH_2–CH_3$
6	TiMePe	$CH_3–SO–S–CH=CH–CH_3$
7	TiPrMe	$CH_3–CH_2–CH_2–SO–S–CH_3$
8	TiPeMe	$CH_3–CH=CH–SO–S–CH_3$
9	$TiPr_2$	$CH_3–CH_2–CH_2–SO–S–CH_2–CH_2–CH_3$
10	TiPrPe	$CH_3–CH_2–CH_2–SO–S–CH=CH–CH_3$
11	*cis*-Zwiebelane[b]	
12	*trans*-Zwiebelane	
13	Zwiebelane isomer	
14	TiPePr	$CH_3–CH=CH–SO–S–CH_2–CH_2–CH_3$

[a] Lachrymatory factor.
[b] 5-Oxo-2,3-dimethyl-5,6-dithiabicyclohexane.

dent on the isolation and analytical methods involved [4–8] and that the individual effect of each Ti may be rather different, especially for cardiovascular activity [9].

Recently, we proposed a dual analysis method by GC–MS, using a short column with a thick coating. Using the same onion juice sample, we performed (i) an analysis of the LF by solid-phase microextraction (SPME) with a maceration time of 10 min, and (ii) a complementary analysis by solvent partition following a maceration time of 80 min for the Ti and zwiebelane detection [10,11]. This maceration time enables alliinase to react with the precursors at room temperature.

In this study, firstly, we used this dual method to compare qualitatively and quantitatively fresh *Allium* species, including onions, shallots and leeks, to evaluate flavour differences between varieties as it provides accurate profiles of all the initially formed compounds. A selected ion recording (SIR) method was optimized to enhance the differentiation possibilities and the detection limits because when peak overlap occurred, computer processing made possible the deconvolution of peaks. Then, we checked the efficiency of this analysis method by comparison of different transformed onion products.

2. Experimental

2.1. Plant material and transformed products

Cultivated onions and transformed products were provided by Coop d'Or-STL (Auxonne, France) or purchased in a local market. They consisted of cloves of cultivated *A. cepa* including pink and grey shallots. Transformed products were obtained from three *A. cepa* cultivars: white onion (Southport), red onion (Simiane), yellow onion (Auxonne). These plants were tested frozen, freeze-dried and sterilised. Another *Allium* species, *A. porrum* (leek), was included in this study as a control because it emits the same compounds as onions in different proportions.

2.2. Instrumentation and methods

2.2.1. GC–MS analysis

GC–MS analysis was carried out on a benchtop Perkin-Elmer Turbomass system with a split-splitless injector and a fused-silica capillary column (10 m× 0.32 mm) with a 4 μm methylsilicone coating. The carrier gas was helium (99.999%) and the column temperature program was 5 °C/min from 70 to

250 °C. The injection port temperature was 200 °C. The transfer line and the source temperature were maintained at 150 °C. Total ion chromatograms and mass spectra were recorded in the electron impact ionization mode at 70 eV. Data were treated with both full-scan and SIR methods.

The compounds were identified by matching their mass spectra to our previous reports [2,6] and to the US National Institute of Standards and Technology (NIST) spectrum library.

2.2.2. Sampling

Onion bulbs (10 g) were rapidly crushed using an electrical crusher. After 10 min, headspace volatiles were extracted for 2 min and transferred to the injector by SPME (polyacrylate fibre 85 μm). After 80 min, the juice was filtered and saturated with sodium chloride. Volatiles were extracted using 10% of the juice volume of diethyl ether followed by a centrifugation (5 min, 8000 *g*). This step was conducted at 4 °C to avoid the loss of the volatiles. The organic phase was dried on sodium sulphate and injected in splitless mode into the GC system.

2.2.3. Analysis of data

We calculated the distribution of the thioalk(en)yl moieties (methyl, propyl and 1-propenyl) of Ti and zwiebelanes for the fresh samples to characterise the different *Allium* spp. tested. Then, the examination of the ratios of the particular compounds well known for their impact on flavour and taste allowed us to carry out this study.

3. Results and discussion

3.1. Study of fresh onions

In onion headspace, LF and zwiebelanes, arising from the rearrangement of 1-propenylsulphenic acid, predominate largely. LF gives the onion cultivars their ‘‘piquant’’ taste but only zwiebelanes and Ti produce their various characteristic flavours, each having its typical sensory characteristics. For instance, Block et al. [1] said that *trans*-zwiebelane has a green or raw onion taste and a sweet sulphur taste with a detection threshold of 0.1 ppm. The *cis*-zwiebelane has a sweet or brown ‘‘sauté’’ taste with liver and hydrogen sulphide notes with a detection threshold of 0.5 ppm. So, to compare different onion cultivars we need to detect and to quantify all the Ti and zwiebelanes. As previously reported, results obtained with direct SPME after 10 min of crushing of the fresh onion showed the presence of lachrymatory factor (*Z*) (**1**) and its isomer (**2**) [11]. Solvent extraction after 80 min (Fig. 1A) showed all the suspected Ti: TiMePr (**5**), TiMePe (**6**), TiPrMe (**7**), TiPeMe (**8**) and TiPrPe (**10**). Also three zwiebelane isomers (*cis*, *trans* and an unidentified one) were detected (**11**, **12** and **13**). This profile was not suitable for the quantification of the Ti and zwiebelanes because these compounds were not well separated and some of them (e.g. *cis*-zwiebelane and TiPrPe) were merged. So, in order to improve the poor selectivity attributed to the short length of the column, the chromatographic data were processed using the selected ion recording method (Fig. 1B). With this method, we were able to resolve the peaks of all the Ti and zwiebelanes and measure their areas with a better accuracy than previously published methods, i.e. GC–flame ionization detection (FID) [1]. Then, we calculated the alk(en)yl moiety distribution in percent (Table 2). In all *Allium* samples, the 1-propenyl moiety predominated (more than 50%) and the methyl moiety was constant for all plants (on average 12%). The propyl moiety appeared to be more suitable for a comparative study between *Allium* species. The shallots, in particular the pink shallots, differed from the onions by their propyl content. The leek contained less 1-propenyl

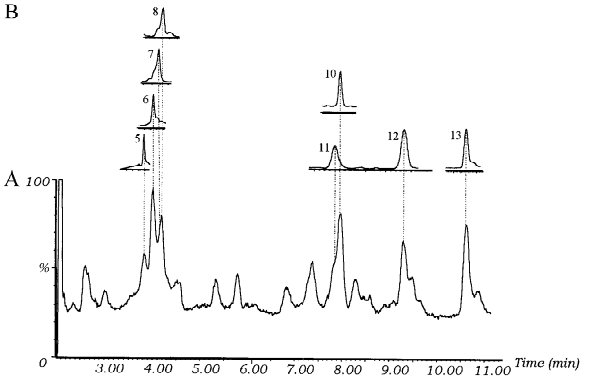


Fig. 1. Chemical analysis of volatiles of fresh onion by solvent extraction. (A) Full scan; (B) selected ion recording.

Table 2
Thioalk(en)yl moieties distribution (%) on Ti and zwiebelanes for fresh *Allium*

Sample	Methyl	1-Propenyl	Propyl
A. cepa			
Onion	15	81	4
Pink shallot	11	62	27
Grey shallot	10	81	9
A. porrum			
Leek	12	56	33

and more propyl compared to other *Allium* spp. tested.

With this SIR method, we were able to compare directly specific compounds which have a particular interesting biological activity. For example, onions were characterised by a high level of TiMePr (22%) and zwiebelane isomer (37.3%) when compared to pink and grey shallots (on average 3% and 12.8%). The pink shallots contained more TiPrMe (17%) than grey shallots (10%) or onion (5%). Conversely, *trans*-zwiebelane is one of the most significant compounds in grey shallots (38.1% versus 33.8% for pink shallot and 29% for onion). Concerning all the organosulphur volatiles, grey and pink shallots had similar profiles which were different from the onion volatiles distribution even though shallots and onions were both *A. cepa*. In fact, some authors claimed now that grey shallot, and this one, may be another species, *A. oschaninii*.

So, our dual method with a SIR data process permits a real inter- and intra-species comparison between different *Allium* spp.

3.2. Study of onion transformed products

These samples presented notable differences in their chemical composition, so a simple full-scan data processing is sufficient for this part of the study. The chromatograms of volatiles obtained after SPME and solvent extraction of frozen, freeze-dried and sterilised yellow onion (Fig. 2) were typical of those obtained from all transformed onions analysed whatever the variety (white, red and yellow).

Direct SPME results (Fig. 2BI) of the three cultivars of frozen onions were characterised by the presence of the 2-methyl-2-pentenal (**3**), the main degradation compound of the LF, and three disulphides (dipropyl disulphide, propyl 1-propenyl disulphide and di(1-propenyl) disulphide). The three chromatograms of freeze-dried onions were similar (Fig. 2BII). Compared to the frozen onion, we noted a drastic diminution of disulphides with a significant increase of the trisulphides, particularly the propyl 1-propenyltrisulphide. It was interesting to note the presence of methyl propyl tetrasulphide and methyl

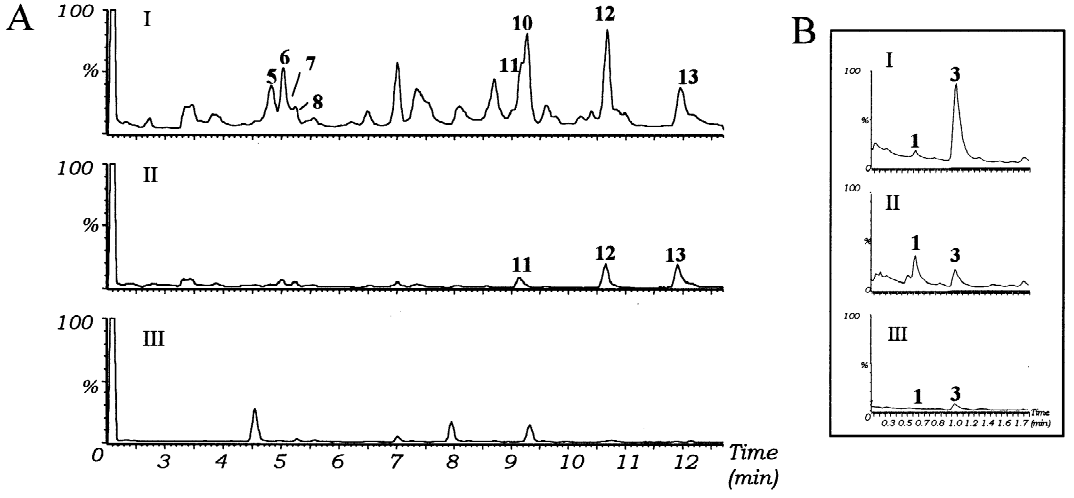


Fig. 2. Analyses of the volatiles of transformed products (I, frozen; II, freeze-dried; III, sterilised). (A) Solvent extraction; (B) SPME transfer.

(1-propenyl)tetrasulphide. The chromatograms of sterilised onions (Fig. 2BIII) were different from the two others. The main compounds were dipropyl disulphide, methyl propyl trisulphide and dipropyl trisulphide. Compounds with the 1-propenyl moiety completely disappeared.

Interestingly, the solvent extraction permitted the comparison of frozen and freeze-dried samples. The results showed the presence of all suspected Ti and zwiebelanes: TiMePr, TiMePe, TiPrMe, TiPeMe, $TiPr_2$, TiPrPe and the three zwiebelane isomers both in various proportions and in absolute quantities. The main compounds obtained with the freeze-dried onions were TiPrPe and all the zwiebelanes. The chromatographic profiles of the sterilised onions obtained by a solvent extraction (Fig. 2AIII) were similar to those obtained by direct SPME with no detection of Ti or zwiebelanes.

4. Conclusion

In conclusion, our dual method of volatiles analysis by SPME–solvent extraction–GC–MS eventually complemented with a selected ion recording data process, is suitable to perform inter- or intra-specific comparison in *Allium* species. This method can lead to interesting applications such as flavour quality and health benefit labelling of food, selection of cultivars and determination of optimal harvesting and conservation times. The consumer's choice of fruits and vegetables is generally determined by appearance attributes such as color, size and defects. Those external features are no guarantee for a good internal sensorial and health quality which from the consumer's point of view ought to be more important. For these reasons, the establishment of methods to check functional qualities on an objective chemical basis is important.

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