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Use of TD-GC–TOF-MS to assess volatile composition during post-harvest storage in seven accessions of rocket salad (*Eruca sativa*)

Luke Bell [a,1,*], Natasha D. Spadafora [b,1], Carsten T. Müller [b], Carol Wagstaff [a,c], Hilary J. Rogers [b]

[a] Department of Food and Nutritional Sciences, University of Reading, Whiteknights, PO Box 226, Reading, Berkshire RG6 6AP, UK
[b] Cardiff School of Biosciences, Cardiff University, Main Building, PO Box 915, Cardiff, CF10 3TL, UK
[c] Centre for Food Security, University of Reading, Whiteknights, Reading, Berkshire RG6 6AP, UK

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Iberverin (PubChem CID: 62351)
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1-Penten-3-ol (PubChem CID: 12020)
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2-Methyl-2-butenal (PubChem CID: 5321950)
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ABSTRACT

An important step in breeding for nutritionally enhanced varieties is determining the effects of the post-harvest supply chain on phytochemicals and the changes in VOCs produced over time. TD-GC–TOF-MS was used and a technique for the extraction of VOCs from the headspace using portable tubes is described. Forty-two compounds were detected; 39 were identified by comparison to NIST libraries. Thirty-five compounds had not been previously reported in *Eruca sativa*. Seven accessions were assessed for changes in headspace VOCs over 7 days. Relative amounts of VOCs across 3 time points were significantly different – isothiocyanate-containing molecules being abundant on 'Day 0'. Each accession showed differences in proportions/types of volatiles produced on each day. PCA revealed a separation of VOC profiles according to the day of sampling. Changes in VOC profiles over time could provide a tool for assessment of shelf life.

1. Introduction

Rocket (or arugula, rucola, roquette) species are of increasing commercial importance across the world. Leaves of the crop are usually sold in mixed salad bags or whole bags, and in some niche markets as gourmet microleaves. The nutritional and sensory quality of leaves throughout the supply chain is of major concern to producers and supermarkets, as they will ultimately be accepted or rejected, by the consumer, based on these attributes. Much of the current rocket supply chain is designed to preserve predominantly visual and morphological traits of leaves (such as stem browning/yellowing) before they reach the consumer. Very little research has been conducted to determine the phytochemical and volatile organic compound (VOC) losses incurred post-harvest (Verkerk, Dekker, & Jongen, 2001).

* Corresponding author.
E-mail address: luke.bell@pgr.reading.ac.uk (L. Bell).
[1] These authors contributed equally to the work.

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Rocket varieties are genetically very diverse, and morphological uniformity can be an issue for plant breeders and growers alike. One plant can have very different leaf shapes from another, even within the same variety (Egea-Gilabert et al., 2009). Rocket species are generally preferential outbreeders, making production of uniform breeding lines difficult. This variability has been shown to extend to concentrations of phytochemicals (Bell, Oruna-Concha, & Wagstaff, 2015), where significant differences in glucosinolate (GSL) and flavonols have been detected amongst accessions. An important step in breeding for nutritionally enhanced varieties is determining the effects of the post-harvest supply chain on phytochemicals and the changes in volatile degradation products produced over time. The concentrations and/or relative abundances of both of these, which include isothiocyanates, (ITCs) may also vary greatly, depending on the levels of physical damage during processing of the leaves. The VOC bouquet is the term used to describe the collection of volatiles within the headspace of a plant or other foodstuff, often giving rise to aromas. These aromas will affect the sensory attributes perceived by the consumer when the product is eaten, and they influence re-purchase (Ragaert, Verbeke, Devlieghere, & Debevere, 2004). This may have consequences on consumers' nutritional intake, and hence long-term health.

VOCs found in rocket comprise ITCs, alkanes, aliphatic alcohols, carbonyl compounds, fatty acids, esters, phenols and C_{13}-norisoprenoids (Blazevic & Mastelic, 2008). However, comparison of the relative abundance amongst cultivars has not been established, as earlier studies only utilized one commercially bought, bagged variety, and leaves from a wild population (Blazevic & Mastelic, 2008; Jirovetz, Smith, & Buchbauer, 2002). Given the very different sample sources, differences between the two studies may be representative of environmental stresses, as well as genetic variation (Varming et al., 2004). These include exposure to fungal diseases, wounding (pre- and post-harvest), and variations in temperature and humidity during growth and while in controlled environment conditions – all of which can lead to changes in phytochemical content and VOCs produced (Schouten et al., 2009).

In this study rocket salad was grown under controlled environment conditions, thus reducing environmental stress responses, enabling effects of post-harvest storage on VOC profiles to be assessed. Thermal desorption gas chromatography time-of-flight mass spectrometry (TD-GC–TOF-MS) was used to determine changes in VOCs during storage of seven different accessions, demonstrating that collection of headspace volatiles onto thermal desorption tubes is a rapid and robust method for assessing post-harvest changes and identifying differences in these changes amongst accessions.

2. Materials and methods

2.1. Plant material

Six *Eruca sativa* accessions were obtained from European gene banks, four from the Leibniz-Institut für Pflanzengenetik und Kulturpflanzenforschung (IPK Gatersleben, Germany), one from the Centre for Genetic Resources in the Netherlands (CGN, Wageningen, The Netherlands) and one from The University of Warwick Crop Centre Genetic Resources Unit (Wellesbourne, UK; formerly Warwick HRI). The Elsoms Seeds variety SR3 was used as a commercial comparator. Accessions have been coded to protect commercially sensitive information.

2.2. Growing conditions and simulated shelf life

Each accession was germinated under controlled environmental conditions (Fitotron, Weiss-Technik UK, Loughborough, UK). Long-day lighting was used (16 h light, 8 h dark) at an intensity of 200 μmol m^{-2} s^{-1}. Day temperatures were set at 20 °C and night temperatures at 14 °C. Seedlings were grown for 10 days in seedling trays and then transplanted to larger trays. Subsequently, plants were grown for a further 20 days and then leaves were harvested at 30 days. Leaves were collected in batches of 70 g in three experimental replicates per accession. Amount of leaf material was chosen based on preliminary experiments, where abundant yields of VOCs were obtained.

2.3. Sample collection

Whole leaves were placed into a multi-purpose roasting bag (25 cm × 38 cm, TJM Ltd.) and sealed, using an elastic band and an Eppendorf tube with the end cut off, which served as a sampling port for the TD tubes (see supplementary material for diagram). Leaves were then disrupted manually within the bags for 10 s by crushing the leaves between the hands and making a vigorous rubbing motion. Care was taken not to perforate the bags and inadvertently release VOCs. Three replicates were taken for each sample, including three 'blank' samples of atmosphere within empty bags to rule out any possible contaminating VOCs. These were prepared and sampled in an identical fashion, with the exception that no leaves were contained in the bag.

2.4. Post-harvest storage simulation

Harvested rocket leaves were stored in a dark, controlled temperature room at 4 °C, to simulate industrial storage conditions for 7 days. Bags were only removed from the environment while samples were taken (room temperature, ~22 °C).

2.5. TD-GC–MS-TOF analysis

All tubes were desorbed by a TD100 thermal desorption system (Markes International Ltd., Llantrisant, Wales, UK), using the following settings for tube desorption: 5 min at 100 °C, followed by 5 min at 280 °C, trap flow of 40 ml/min and trap desorption and transfer: 20 °C/s to 300 °C, split flow of 20 ml/min into GC (7890A; Agilent Technologies, Inc., Stockport, UK). VOCs were separated over 60 min, 0.32 mm ID, 0.5 μm film thickness Rxi-5ms (Restek) at 2 ml continuous flow of helium, using the following temperature programme: initial temperature 40 °C for 2 min, 5 °C/min to 240 °C, final hold 5 min. The BenchTOF-dx mass spectrometer (Almsco International, Cincinnati, OH, USA) was operated in EI mode at an ion source temperature of 275 °C and a mass range of 35–350 *m/z*. A retention time standard (C8–C20, Sigma Aldrich, Gillingham, UK) was prepared by injection of 1 μl of the standard mixture directly onto a thermal desorption tube, and analysed under the same conditions as the samples.

Data from GC–MS measurements were processed with MSD ChemStation software (E.02.01.1177; Agilent Technologies, Inc., Stockport, UK) and deconvoluted and integrated with AMDIS (NIST 2011), using a custom retention-indexed mass spectral library. MS spectra from deconvolution were searched against the NIST 2011 library (Mallard, Sparkman, & Sparkman, 2008) and only compounds scoring >80% in forward and backward fit were included into the custom library. Putative identifications were based on match of mass spectra (>80%) and retention index (RI ± 15) (Beaulieu & Grimm, 2001).

Compounds abundant in controls or in only one of the three replicates were excluded from statistical analyses. Areas of remaining compounds were normalized to total area of chromatograms prior to averaging within samples.