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Original Research Paper

Rocket salad aroma is affected by sampling method, species and degree of leaf damage

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Abstract: Rocket salad is a valuable commercial product often sold as a bagged ready to eat salad. Two different species from the Brassicaceae family are sold as rocket salad: *Diplotaxis tenuifolia* and *Eruca sativa*. Both are aromatic, and previous studies have shown that their aroma is composed of a complex mixture of volatile organic compounds (VOCs) from several different chemical families. Of particular interest are isothiocyanates that are generated from the metabolism of glucosinolates produced by Brassicaceae species. Both types of rocket salad have a limited shelf-life and there has been interest in assessing whether analysis of VOCs could be used to help set 'use by dates' assess quality changes, and report on issues within the supply chain. However, different methods have been used to sample and analyse the VOC profiles. Here we compare the profiles from *D. tenuifolia* using two VOC sampling methods (solid phase microextraction, SPME) and sampling onto thermal desorption (TD) tubes. We also compare the VOC profiles sampled onto TD tubes from both species from leaves subjected to different levels of damage: intact, chopped and blended. We find that both the method of VOC sampling and the level of leaf damage have important effects on the VOC profile. In the comparison, fewer VOCs are detected when sampling with SPME compared to TD tubes. Overall, 41 different VOCs are detected in the leaf damage experiment but many fewer are detected in intact leaves. While the two rocket species are distinct based on the VOC profile of chopped leaves, they are not discriminated by VOC profiles of blended or intact leaves. When species are considered separately VOC profile discriminates level of damage. Very few isothiocyanates are detected in intact or chopped leaves, presumably due to the requirement for leaf damage to activate their production from glucosinolates. To conclude, level of leaf damage will strongly influence VOC profiles from rocket leaves, and medium damage seems to elicit the most discriminatory profiles.

Keywords: aroma; postharvest; rocket salad; volatile organic compounds

1. Introduction

Rocket salad is a popular ingredient of mixed salads as well as being valued alone for its peppery flavour. Two species are called rocket salad: *Diplotaxis tenuifolia*, sometimes referred to as wild rocket, and *Eruca sativa* also referred to as cultivated rocket (Bell and Wagstaff, 2019). Aroma and taste of both *D. tenuifolia* and *E. sativa* leaves as well as phytochemical content varies across cultivars (Pasini et al., 2011; Bell et al., 2016).

Both species of rocket salad are used in pre-washed ready to eat salad bags which have been increasingly popular with consumers. Both species also have a good postharvest shelf life of around 15 days, however, to maintain freshness it is necessary to store the leaves at low temperature, typically 2-5 °C (Hall et al., 2013). Indeed, salad leaves are subjected to a range of different stresses post-harvest including wounding during harvest and washing, dehydration of the leaves, low light levels or darkness, and changes in the gaseous environment as well as the potential growth of microflora. These stresses

can affect the metabolism of the leaves impacting also their nutritional value (Spadafora et al., 2016).

Shelf-life duration in salads is typically assessed in the industry through visual inspection of the leaves for damage and colour as well as wilting and general appearance, although other tests can be applied such as antioxidant activity, vitamin C content and total phenolic content (e.g. Preti and Vinci, 2016). However, it is difficult to assess internal quality or effects of suboptimal environmental factors before or after harvest. Biochemical analyses can be performed, sampling the batches of salad and assessing parameters such as vitamin C content and antioxidant status (Spadafora et al., 2016). It is also possible to develop markers for quality based on changes in gene expression, which in turn could be adapted into an antibody test (Cavaiuolo et al., 2015). However, all these tests are destructive as well as being relatively time consuming and requiring specialized equipment and trained personnel.

Plants produce over 1000 volatile organic compounds (VOCs) belonging to several distinct chemical families (Dudareva et al., 2013). The overall bouquet is distinctive for the organ and species and is of relevance to the ecology of the species acting as signaling cues for attracting pollinators or frugivores or acting as a deterrent for herbivores. The aroma bouquet is also very sensitive to environmental factors and therefore can act as a useful indicator of internal metabolic changes.

A number of studies in rocket salad of both species have shown that there is variability in the VOCs across varieties of a species and that the changes in VOC profile are related to postharvest storage (Bell et al., 2016; Spadafora et al., 2016). Indeed in *D. tenuifolia* changes in VOCs can be detected throughout shelf life and change in response to short post-harvest stress treatments (Spadafora et al. 2019). The changes in VOCs can also be correlated with changes in nutritionally relevant compounds such as vitamin C.

The major classes of VOCs detected to date in rocket salad include isothiocyanates, aldehydes, alcohols, nitrogen compounds, sulphur compounds, ketones and esters. Of particular interest are isothiocyanates which derive from the enzymatic breakdown of glucosinolates are considered to have nutritional relevance and may contribute to protection against cancers (Bell and Wagstaff, 2019; Sundaram et al., 2022). However, these compounds can have adverse effects in animal feed (Bischoff, 2021).

Over 40 different VOCs have been detected in the aroma of rocket salad dependent on the variety and also on the method of detection (Spadafora et al., 2016, 2019; Luca et al., 2016). The two main methods adopted for VOC collection are solid phase microextraction (SPME) in which the VOCs are adsorbed onto a coated fibre, and thermal desorption (TD) in which the VOCs are adsorbed onto appropriate adsorbant materials contained in metal tubes (Materić et al., 2015). SPME is very sensitive and widely used on food samples (Lancioni et al., 2022) but can easily be saturated by highly abundant compounds (e.g. Mascrez & Purcaro 2020) furthermore fibre holder design and delicate nature of the fibre itself make it difficult to collect samples for remote assay (Dugheri et al., 2022). Originally designed for workplace exposure monitoring, high-capacity and robust tools such as TD have been explored in the field of food analysis (e.g. Amaro et al., 2018; Muto et al., 2020). In this system the VOCs are collected onto an adsorbent packed within metal tubes. Adsorbent packings can be tailored to the range of VOCs that need to be detected. The tubes are robust and can be easily sealed for transport making them an attractive option for remote sampling. In either case VOCs are then analysed by gas chromatography mass spectrometry, with different mass spectrometers providing different levels of sensitivity and discrimination.

Irrespective of the collection and analysis method, the first step is to expose the adsorbant to the VOCs. In intact rocket leaves, VOCs are synthesized within the cell in specific subcellular compartments dependent on the VOC family (Dudareva et al., 2013). However, when leaves are wounded the range of VOCs may change as cells are damaged making it possible for substrates and enzymes to come into contact. In particular, this allows the production of isothiocyanates as the glucosinolates and myrosinase needed for their production are housed in different cells (Wittstock et al., 2016).

In this study we assessed both the effects on VOC profile of different sampling techniques (SPME or TD) from intact leaves, and the effect of different levels of leaf disruption in the two major rocket species: *Diplotaxis tenuifolia* and *Eruca sativa*. We show that the profiles are different and can relate the major differences to specific VOCs that change in relative abundance in relation to collection method, rocket species and level of leaf damage.

2. Materials and Methods

2.1. Plant Growth and VOC collection

Both *Eruca sativa* and *Diplotaxis tenuifolia* L. var frastagliata were grown hydroponically for approximately 30 days in a growth chamber at 25 °C and 12 h light as previously described (Cavaiuolo et al., 2017). Mature (serrated) leaves were harvested by cutting from the plant with a sharp blade. Intact leaves (65 g) were placed in a nalophene bag (TJM Ltd.) for VOC collection. For SPME analysis a grey fibre (50/30 µm divinylbenzene/carboxene/PDMS composite fibre on 2 cm fused silica; Sigma Aldrich) was exposed to the headspace for 1 h at 20 °C. For TD collection, the leaves in the bag were equilibrated for 1 h at 20 °C, then 1000 mL of headspace was collected using an EasyVoc manual pump (Markes International) onto TD tubes (SafeLok™, Tenax TA and Sulficarb, Markes International Ltd. Llantrisant, UK). Leaves (65g) were chopped by cutting into several pieces with a sharp blade, or blended using a mortar and pestle. VOCs were collected as above onto TD tubes. Samples were collected in triplicate from biological replicates and control headspace samples were collected from empty bags.

2.2. VOC analysis and data handling

VOCs were desorbed from the tubes using a TD100 thermal desorption system (Markes International Ltd. Bridgend, UK) and separated on a GC (7890A; Agilent Technologies, Inc. Santa Clara, CA, U.S.A.) as described in Spadafora et al. (2016). Mass spectra were recorded from m/z30-350 on a time-of-flight mass spectrometer (BenchTOF-dx, Markes International Ltd, Bridgend, UK). A retention standard was loaded directly onto TD tubes and analysed by GC-MS as above (1 µl of C8-C20 alkane standard; Sigma Aldrich, St. Louis, Mo., U.S.A.).

Data analysis of the VOC data was firstly performed using MSD ChemStation software (E.02.01.1177; Agilent Technologies, Inc, Santa Clara, CA, U.S.A.). Then deconvolution and integration was performed using AMDIS (NIST11) by creating a custom retention-indexed mass spectral library (including only compounds that scored >80% in both forward and backward fit). For all the analyses, compounds which were abundant in the control headspace samples (>10% of the minimum value for this compound in any of the samples) or not represented in all three replicates of at least one experimental sample were not included in the analysis. Known contaminants (Supp. Table 1) were also removed. Compounds are identified putatively based on the mass spectra (>80%) and retention index (RI +/- 15).

The data for each VOC were normalized against the total area for the sample and the values square rooted to reduce the weight of large components. The data were then analysed using R software version 3.5.2 and 4.2.3. The 'vegan' (Oksanen et al., 2013) and 'BiodiversityR' (Kindt & Coe, 2005) packages were used for PerMANOVA and Canonical Analysis of Principal Coordinates (CAP) (Anderson & Willis, 2003). CAP reduces data to principal co-ordinates, constructs a linear discriminant model based on these principal co-ordinates and tests the classification model by checking predicted output with actual output. This is reported as % correct classification. Ordination plots used a 95% confidence interval. Random Forest used the 'randomForest' package in R and was applied to assess the VOCs that were most critical for discrimination. This is shown by the 'Mean Decrease Accuracy' highlighting the key compounds that contribute significantly to the model's discriminatory power.

3. Results

3.1. Comparison of SPME and TD on intact leaves.

A total of 31 VOCs were detected using one of the two methods, with 16 detected using SPME and 28 using TD from intact *Diplotaxis tenuifolia* leaves (Table 1).

Table 1. VOCs detected in the comparison between TD and SPME from *D. tenuifolia* intact

Compounds	SPME	TD	RI
1-Isothiocyanato-4-methylpentane	Y	Y	1179.1
1-Isothiocyanatohexane	Y	Y	1218.1
1-Isothiocyanatopentane	N	Y	1112.6
Methyl thiocyanate	Y	Y	708.9
Methyl 2-oxohexanoate	Y	Y	1064.0
Nonanal	Y	N	1104
2-Pentenal,	N	Y	741.3
2-Hexenal	Y	Y	847.5
3-Hexenal	Y	Y	799.6
2,4-Hexadienal	N	Y	918.6
1-Penten-3-ol	Y	Y	671.1
2-Penten-1-ol	Y	Y	764.4
2-Hexen-1-ol	Y	Y	864.4
3-Hexen-1-ol	Y	Y	853.9
2-Butoxyethanol	N	Y	909.8
3-Hexen-1-yl acetate	Y	Y	1020.3
Ethyl Acetate	N	Y	611
1-Penten-3-one	N	Y	677.2
3-Pentanone	Y	Y	689.2
1-Phenylethanone	N	Y	1089.9
6-Methylhept-5-en-2-one	N	Y	1003.1
5-Ethyl-2(5H)-furanone	Y	N	968.4
2-Ethylfuran	Y	Y	694.1
2-Vinylfuran	N	Y	721.3
(4S)-4-Isopropenyl-1-methylcyclohexene	N	Y	1054.8
1,3,5,7-Tetraazatricyclo[3.3.1.13,7]decane	N	Y	1265.0
Benzaldehyde	N	Y	990
3-(2-Propen-1-ylidene)cyclobutene	N	Y	777.7
Alkane 1	N	Y	1664.4
Hexadecane, 4-methyl-	N	Y	1659.5
Pentalene, octahydro-	Y	N	856.1
Total number of VOCs: 31	16	28	

3.2. Effect of leaf damage on the VOC profiles of rocket leaves of both species.

Overall, 41 different compounds were detected in the headspace of all the rocket salad samples. These comprised esters (3), alcohols (5), aldehydes (4) alkanes (5), alkenes (4), one amine, two aromatic compounds, furans (3), isothiocyanates (5) one thiocyanate, ketones (4), one terpene and three compounds that could not be putatively identified (Table 4).

The extent of leaf damage had a dramatic effect on the number of VOCs detected (Table 3 and

Table 4). More different compounds were detected in the blended *E. sativa* leaves than in the *D. tenuifolia* leaves.

When all the samples were analysed together, PerMANOVA showed discrimination by sample ($p = 0.001$, $R^2 = 0.67$). Samples were also discriminated by degree of damage ($P = 0.001$; $R^2 = 0.28$) but not by species with no interactions between damage and species. CAP discriminated 66.7% of samples correctly overall, individual classifications are shown in Table 2 and indicate that the VOC profile from intact *E. sativa* leaves and *D. tenuifolia* blended leaves were the least discriminated. An ordination plot of the CAP results (Figure 1) shows an overlap of samples from blended leaves or intact leaves from the two species indicating that the VOC profile is similar and not suitable to discriminate the species. By contrast, profiles of less damaged leaves were distinct.

Table 2. CAP classification of individual samples

Species	Damage	Classification
D.tenuifolia	blended	(n=3) correct: 33.3 %
D.tenuifolia	chopped	(n=3) correct: 100 %
D.tenuifolia	intact	(n=3) correct: 100 %
E. sativa	blended	(n=3) correct: 100 %
E. sativa	chopped	(n=3) correct: 66.6 %
E. sativa	intact	(n=3) correct: 0 %

Table 3. Number of different VOCs in each treatment comparing extent of leaf damage

Treatment	*Diplotaxis tenuifolia*[1]	*Eruca sativa*[1]
Blended	27	36
Chopped	2	11
Intact	3	5

[1]A compound was scored as present if present in at least 2 out of the 3 replicates for each sample.

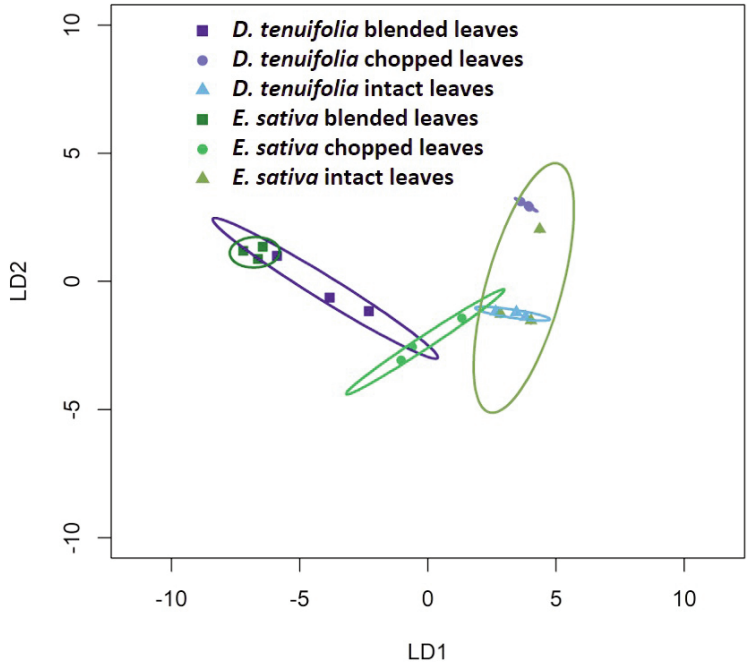

Figure 1. Discrimination of all samples using CAP, based on the whole VOC profile. The plot uses the first two linear discriminants (LD) and each ellipse represents the 95% confidence interval based on standard deviation.

Table 4. VOCs detected across all the rocket salad samples

IUPAC name	family	mean RI[1]	mean RA[2]	Dt B[3]	Dt C[3]	Dt I[3]	Es B[3]	Es C[3]	Es I[3]
3-Hexen-1-yl acetate	ester	1020.3	15.78	Y			Y	Y	
Ethyl Acetate	ester	611	0.11	Y			Y		
1-Penten-3-ol	alcohol	671.1	2.22	Y			Y		
2-Hexen-1-ol	alcohol	864.4	0.95	Y			Y		
2-Penten-1-ol	alcohol	764.4	0.35	Y			Y		
3-Hexen-1-ol	alcohol	853.9	8.05	Y			Y	Y	
2-Butoxyethanol	alcohol	909.8	0.48	Y			Y		
2-Hexenal	aldehyde	847.5	2.47	Y			Y		
2-Pentenal	aldehyde	741.3	0.25	Y			Y		
2,4-Hexadienal	aldehyde	918.6	0.61	Y			Y		
3-Hexenal	aldehyde	799.6	5.52	Y			Y		
1,2-dimethylcyclopropane	alkane	547.6	0.20				Y	Y	
Nonane	alkane	903.0	0.59					Y	
Tetradecane	alkane	1400.9	18.98	Y	Y		Y		
Octadecane	alkane	1801.6	13.61			Y		Y	Y
Octahydropentalene	alkane	855.15	2.00				Y		
1-Nonene	alkene	897.3	4.19			Y			Y
3-Ethyl-1,5-octadiene	alkene	959.0	0.20				Y	Y	
3-(2-Propen-1-ylidene)cyclobutene	alkene	777.7	4.51	Y			Y	Y	Y
Ethylidenecyclopropane	alkene	555.8	0.10				Y		
1,3,5,7-Tetraazatricyclo[3.3.1.13,7]decane	amine	1265.0	6.46	Y	Y	Y		Y	Y
1-Phenylethanone	aromatic	1089.9	0.15	Y			Y		
1-Methyl-4-[(2-methyl-3-butyn-2-yl)oxy]benzene	aromatic	1427.9	0.09				Y		
5-Ethyl-2(5H)-furanone	furan	968.4	3.31	Y					
2-Ethylfuran	furan	694.1	0.40	Y			Y		
2-Vinylfuran	furan	721.3	0.10				Y		
4-Isothiocyanato-1-butene	isothiocyanate	1008.0	0.09	Y			Y		
1-Isothiocyanato-4-methylpentane	isothiocyanate	1179.1	1.11	Y			Y	Y	
1-Isothiocyanato-3-methylbutane	isothiocyanate	1077.5	0.09				Y		
1-Isothiocyanatohexane	isothiocyanate	1218.1	0.18	Y			Y		
1-Isothiocyanatopentane	isothiocyanate	1112.6	0.22	Y			Y		
Methyl thiocyanate	thiocyanate	708.9	0.11	Y			Y		
1-Penten-3-one	ketone	677.2	1.75	Y			Y		
3-Isopropyl-7a-methyl-1,4,5,6,7,7 a-hexahydro-2H-inden-2-one	ketone	1523.2	0.21				Y		
3-Pentanone	ketone	689.2	1.77	Y			Y	Y	
6-Methylhept-5-en-2-one	ketone	1003.1	0.25	Y			Y		
Methyl 2-oxohexanoate	ester	1064.0	1.47				Y		
(4S)-4-Isopropenyl-1-methylcyclohexene	terpene	1054.8	0.77	Y			Y	Y	Y
Unknown 1	unknown	600.3	0.09				Y		
Unknown 2	unknown	691.4	0.12				Y		
Unknown 3	unknown	830.4	0.08	Y			Y		

[1] retention index; [2] relative abundance [3] Presence in *Diplotaxis tenuifolia* (Dt) and *Eruca sativa* (Et): B, blended, C, chopped, I, intact leaves

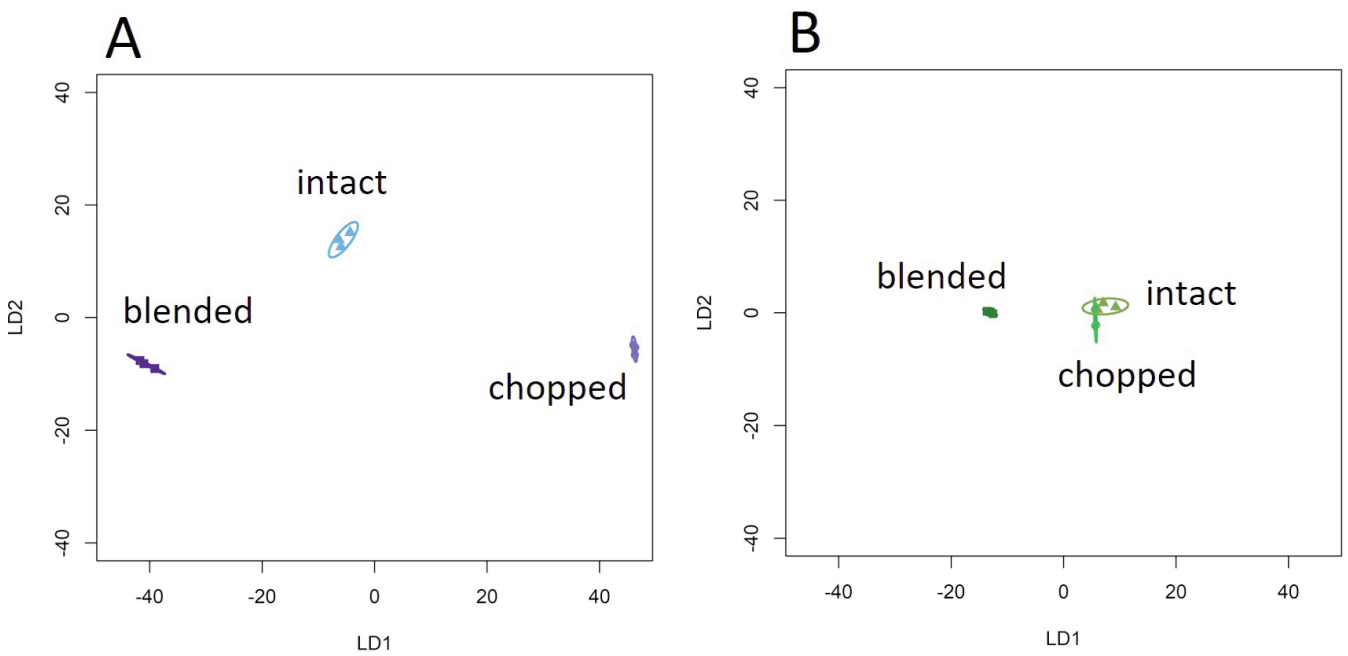

Figure 2. Discrimination of VOC profiles across leaf damage treatments and rocket salad species. CAP based on all the VOCs in the profile for each species: (A) *Diplotaxis tenuifolia* (B) *Eruca sativa* leaves where the headspace was sampled from blended, chopped or intact leaves. The plots use the first two linear discriminants (LD) and each ellipse represents the 95% confidence interval based on standard deviation.

When the two species were assessed separately, PerMANOVA showed a significant discrimination of VOC profiles based on damage level ($p = 0.05$, $R^2 = 0.75$ for *D. tenuifolia* and $p = 0.007$, $R^2 = 0.57$ for *E. sativa*). Using Canonical Analysis of Principal Coordinates (CAP) it was possible to discriminate between the three damage treatments for *D. tenuifolia* (with 100% correct classification overall and for each sample) but in *E. sativa* the chopped and intact profiles were not distinct (67% correct classification overall with 100% for blended, 66.7% for chopped and only 33.3% for intact leaves), also shown in an ordination plot (Figures 2A and B).

3.3. Differences in the isothiocyanate profiles elicited by the leaf damage treatments in the two species.

Given the dietary importance of these compounds, the five isothiocyanates and one thiocyanate detected were assessed separately to see if they alone were able to discriminate between levels of damage taking both species of rocket salad together. PerMANOVA analysis shows a significant effect of damage on the isothiocyanate/thiocyanate profile ($p=0.001$, $R^2 = 0.71$). CAP was able to discriminate blended from the intact or chopped leaves (Figure 3A) with a 67% correct classification. Although it was not possible to discriminate *D. tenuifolia* from *E. sativa* based on the isothiocyanate/thiocyanate profile, even when only blended leaves were considered. Random Forest was used to identify the isothiocyanates/thiocyanate compounds that exhibited the most significant influence on the difference between blended *D. tenuifolia* and *E. sativa* leaves. Two compounds putatively identified as methyl thiocyanate and 1-isothiocyanatopentane are the top discriminators (Figure 3B).

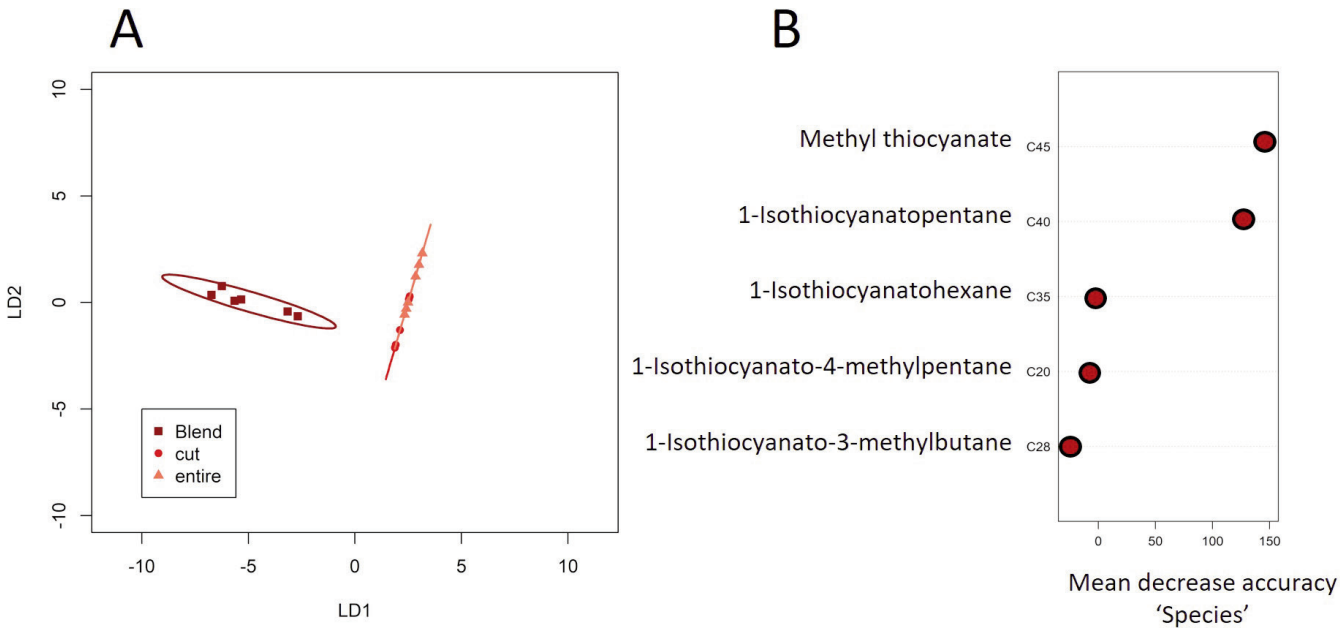

Figure 3. Discrimination of VOC profiles across leaf damage treatments and rocket salad species. CAP based only on the 6 isothiocyanate and thiocyanate VOCs (A) CAP to discriminate across leaf damage treatments (B) Random Forest mean decrease accuracy identifying the most important isothiocyanates that discriminate between blended leaves of the two rocket salad species. The plots use the first two linear discriminants (LD) and each ellipse represents the 95% confidence interval based on standard deviation.

4. Discussion

TD detected almost double the number of different VOCs compared to SPME. This is in line with other studies. Both Spadafora et al. (2016) and Bell et al., (2016) collected headspace VOCs from crushed leaves of *Diplotaxis tenuifolia* and *Eruca sativa* . and detected 43 and 42 different VOCs, respectively. In contrast, Luca et al. (2016) collected VOCs from intact leaves using SPME and detected only 23 VOCs. Although it is difficult to compare directly these different studies due to the damage state of the leaves, it seems a reasonable conclusion that SPME detects fewer VOCs.

To assess the effect on VOC detection of leaf damage we compared rocket salad leaves that had been blended, chopped or sampled intact. Surprisingly few VOCs were detected from the intact leaves, despite the use of TD. Indeed many fewer VOCs were detected compared to Luca et al. (2016). This may have been because the material used by Luca et al. (2016) had been mechanically harvested and packed on a commercial packaging line. This may have inflicted some damage, compared to the laboratory experiment here where leaves were cut individually. However, in this experiment even chopping the leaves into sections elicited small numbers of VOCs in the headspace. The *Brassicaceae,* that include both rocket salad species, lack specialized secretory tissues, unlike some other aromatic plants (Caissard et al., 2004). This means that in rocket salads there is no specific mechanism for release of VOCs from intact leaves. However, VOC emission can be elicited not only by wounding but also by other damaging treatments such as ozone, heat and high light that may result in membrane damage (Loreto et al., 2006), and emission can also be distant from the site of wounding. Thus, growth in the field and leaf crushing as may occur in a commercial setting as was used by Luca et al. (2016) compared to the growth in growth chambers here, may elicit a greater emission of VOCs, explaining the differences in VOC number found here and in the previous study (Luca et al., 2016).

The families of VOCs emitted from the blended leaves of both species were similar to those reported previously using TD sampling and the same GC-MS instrumentation (Spadafora et al., 2016). However, here more isothiocyanates were detected from the blended leaves. This might be because

blending is more effective than the crushing, used in previous studies, at bringing together the glucosinolates and the enzymes required for their conversion to isothiocyanates. This is probably due to the increased cell breakage in blended leaves. This allows the myrosinases stored in the vacuoles of idioblasts, specialized phloem parenchyma cells, to access glucosinolates, stored in the vacuoles of S-cells (Wittstock et al., 2016).

Using CAP it was possible to discriminate completely between the three leaf damage states in *D. tenuifolia* but not in *E. sativa* where the VOC profiles from chopped and intact leaves remained indistinct even when the profiles of each species were considered separately, and there was low discrimination (only 33.3%) for the intact leaves. This indicates that the release of VOCs due to less damage (chopping) changes the profile more significantly in *D. tenuifolia* than in *E. sativa* compared to intact leaves. Moreover, while it was possible to discriminate between the two species using VOCs collected from chopped leaves, this was not possible from intact or blended leaves. This result therefore suggests that an intermediate level of damage between intact and blended may be the most appropriate for assessing the profiles of these two different species of rocket salad for identification purposes. However, further cultivars of each species would need to be assessed together, as the VOC profiles vary (Bell et al., 2016).

Five isothiocyanates and one thiocyanate were detected from the blended leaves of *E. sativa* and four isothiocyanates and one thiocyanate from *D. tenuifolia.* The numbers of these VOCs detected are in line with other studies (Bell et al., 2016; Spadafora et al. 2016; 2018). All four isothiocyanates and the thiocyanate detected in *D. tenuifolia* had previously been detected in this species (e.g. Spadafora et al., 2016). The one which was present in *E. sativa* but not *D. tenuifolia* had been previously detected in *E. sativa* (Bell et al., 2016), but was absent from several studies on *D. tenuifolia* (e.g. Spadafora et al., 2016, 2018, 2019) and may therefore be more prevalent in *Eruca spp*. Methyl thiocyanate and 1-isothiocyanatopentane were most discriminatory between leaves that were blended and leaves that were intact or subjected to less damage and thus may require more leaf damage to be generated from stored glucosinolates.

5. Conclusions

Overall, the data show that the rocket salad species, level of leaf damage and method of adsorption are all important in determining the VOC profile. Surprisingly it was not possible to discriminate between species from blended or intact leaves, while it was possible from chopped leaves indicating that this degree of damage elicits the most informative VOC profile. Thus, the degree of leaf damage may explain varying results across studies and also some of the variability seen within studies, and needs careful attention during VOC sampling. Leaf damage had a strong effect on isothiocyanate content as might be expected from the way these compounds are generated in the leaf.

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