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Analysis of seven salad rocket (*Eruca sativa*) accessions: The relationships between sensory attributes and volatile and non-volatile compounds

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ABSTRACT

Sensory and chemical analyses were performed on accessions of rocket (*Eruca sativa*) to determine phytochemical influences on sensory attributes. A trained panel was used to evaluate leaves, and chemical data were obtained for polyatomic ions, amino acids, sugars and organic acids. These chemical data (and data of glucosinolates, flavonols and headspace volatiles previously reported) were used in Principal Component Analysis (PCA) to determine variables statistically important to sensory traits. Significant differences were observed between samples for polyatomic ion and amino acid concentrations. PCA revealed strong, positive correlations between glucosinolates, isothiocyanates and sulfur compounds with bitterness, mustard, peppery, warming and initial heat mouthfeel traits. The ratio between glucosinolates and sugars inferred reduced perception of bitter aftereffects. We highlight the diversity of *E. sativa* accessions from a sensory and phytochemical standpoint, and the potential for breeders to create varieties that are nutritionally and sensorially superior to existing ones.

1. Introduction

Rocket and other members of the *Brassicaceae* plant family have been consistently shown to contribute beneficial, health-promoting phytochemicals to the human diet (Holst & Williamson, 2004). Consumption of such vegetables, that contain glucosinolates (GSLs) and flavonols in particular, is associated with a reduced risk of numerous cancers (Higdon, Delage, Williams, & Dashwood, 2007) and improved cardiovascular health (Podsedek, 2007). In this study we consider several phytochemical attributes that may also contribute to sensory traits of rocket, as well as influence nutritional 'quality'.

Glucosinolates react with myrosinase enzymes (thioglucoside glucohydrolase, EC 3.2.1.147) to form several classes of compound which have potential benefits to human health (Saha et al., 2012). These products (particularly isothiocyanates; ITCs, thiocyanates, nitriles and sulphates) are thought to be primarily responsible for the array of sensory perceptions that humans detect in *Brassicaceae* vegetables. ITCs can result in bitter taste perception due to thiourea moieties, such as those found in synthetic bitter compounds like 6-*n*-propylthiouracil (PROP; Lipchock & Mennella, 2013). ITCs are also known to contribute to the hot and burning perceptions on the tongue (Cartea, Velasco, Obregon, Padilla, & de Haro, 2008), as well as pungent aromas. Thiocyanates are thought to infer bitter taste (Drewnowski & Gomez-Carneros, 2000), and sulphates the sulfurous, 'rotten cabbage' aromas and flavours often experienced (Pasini, Verardo, Cerretani, Caboni, & D'Antuono, 2011). A previous study (Pasini et al., 2011) indicated that the individual glucosinolate and flavonol compounds in rocket contributed towards different sensory perceptions. The GSLs progoitrin/epiprogoitrin and dimeric-mercaptobutyl glucosinolate (DMB) were significantly associated with bitter taste, and total GSL content with perceived pungency. This study did not quantify the two forms of glucosativin separately however, (Cataldi, Rubino, Lelario, & Bufo, 2007), and it is unknown whether they infer differing sensory properties.

Flavonols are also thought to contribute towards the taste and aroma of *Brassicaceae* plants. Research is somewhat lacking in this area for the *Brassicaceae*, but studies conducted in other plants/foods (such as *Ribes rubrum*, redcurrant juice) have found that flavonols are generally associated with astringent and bitter sensations (Schwarz & Hofmann, 2007).

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The effect of polyatomic ion (PI) content and concentration on rocket sensory profiles has not been previously considered. PIs are covalently bonded atoms that act as single units or become dissociated from larger molecules, and can be created when small molecules become negatively charged. For example, hydrogen sulphate (HSO_4^-) is the polyatomic anion of sulfuric acid (H_2SO_4). Rocket is known to accumulate high nitrate (NO_3^-) concentrations (Jakše, Hacin, & Kacjan Maršić, 2013) but it is not known how this, and other PIs such as chlorides, phosphates and sulphates impact upon sensory attributes in the plant.

Free amino acids (AAs) are ubiquitous compounds found within foodstuffs and living organisms, and vary in relative concentration/abundance. They are known to contribute to sensory perceptions in foods, but to date no study has considered this in rocket. Some compounds such as glutamic acid infer savoury (umami) attributes in fruits such as tomato (Jinap & Hajeb, 2010) for example; whereas others may taste sweet (alanine), sour (asparagine), or bitter (leucine; Kirimura, Shimizu, Kimizuka, Ninomiya, & Katsuya, 1969). In this way, it is thought that they modify or enhance the flavours and tastes of food. The effects of sugars and organic acids (OAs) on taste/aroma/flavours has not been previously determined in rocket. It is widely known that sweetness reduces the perception of bitterness, but the degree to which this effect occurs in rocket leaves is poorly understood. OAs typically infer sour taste, and the relative abundances in crops such as tomato are known to infer changes to flavour (Jinap & Hajeb, 2010).

The rocket species *Eruca sativa* is commonly known as 'salad' or 'cultivated' rocket, and is notable for having hot, peppery and bitter attributes (Pasini et al., 2011). In this study a sensory profile of seven *E. sativa* accessions was developed, using a trained sensory panel, to objectively quantify an agreed vocabulary of various sensory traits. The data were analysed in conjunction with chemical analyses of rocket, cultivated in controlled environment conditions, to determine which specific variables have an impact significantly on sensory properties. We hypothesised that the increased relative concentrations and abundances of the major GSL/ITC compounds alongside the concentration of PIs, free sugars, free AAs and OAs would be key influencing factors in the pungency and bitterness of the accessions.

2. Materials and methods

2.1. Plant material

For the source of each of the seven accessions used in this paper, and the exact controlled environment conditions under which plants were grown, see Bell, Oruna-Concha, and Wagstaff (2015). 20 accessions were analysed by this previous study, and the seven selected here represent a diverse range of GSL and flavonol profiles. Another factor for consideration was the availability of seed that could be provided by Elsoms Seeds Ltd. (Spalding, UK). SR2, SR5, SR6, SR12, SR14 and SR19 are accessions sourced from European germplasm collections, and SR3 is a commercially available cultivar sold by Elsoms Seeds Ltd.

Each accession was germinated in a Fitotron controlled environment room (Weiss-Technik UK, Loughborough, UK) after being sown in a random sequence (using random number allocation in Microsoft Excel; Microsoft Corp., Redmond, WA, USA). Growth of plants was staggered over seven days to ensure that all leaves were of the same age (30 days) on each of the sensory assessment days. Plants were harvested each morning of the study (~10.00 am). After transport, samples were washed with cold water to remove any soil detritus and prepared under food grade conditions. Leaves were stored in a fridge (~4 °C) until ready to be served to assessors (between 12.30 pm and 2.00 pm). Leaves were selected at random from zip-loc storage bags when preparing samples for presentation on plates.

For chemical analyses, the leaves of four plants were harvested together and collectively treated as one replicate. There were three 'blocks' of four plants for each accession, resulting in a total of three replicates per accession ($n = 3$); therefore a total of 12 plants were used as representative samples of each population. Leaves were harvested in an identical fashion as outlined above, but placed immediately into a −80 °C freezer after transport. Samples were lyophilized in batches and ground into a fine powder using a miniature coffee grinder.

2.2. Sensory analysis

Sets of sensory descriptors for rocket were established using an expert panel of eleven sensory assessors (see Table 1 for definitions of terms used). Panelists were selected and trained in accordance with ISO standards for sensory analysis (ISO 8586:2012) and are subject to performance monitoring (ISO 11132:2012). All panelists had a minimum of 6 months experience in sensory evaluation, and some up to eight years of experience.

Samples were presented in a random, coded fashion over the course of five, half-hour sessions on consecutive days. Assessors discussed, with the aid of a facilitator, the various sensory attributes associated with the appearance, odour, mouthfeel, taste, flavour and aftereffects of leaf samples. Reference standards were used where appropriate to ensure agreement of the descriptive terms chosen. For example, for mustard attributes, assessors used a jar of Colman's Mustard (Colman's, Norwich, UK) as a reference. Once a consensus set of descriptors was established, a formal sensory assessment was conducted.

Sensory descriptors were entered into Compusense software (version 5.2; Guelph, ON, Canada) and assessors were asked to score each attribute on anchored unstructured line scales (15 cm, scaled 0–100), with each anchor corresponding to the agreed extremes of each attribute definition. Each accession was presented and assessed twice by each of the 11 panelists, and averaged. Odour, taste, flavour and aftereffects were assessed as an overall representation of the two leaves presented per accession ($n = 22$). Due to the variability of leaf morphology within gene bank accessions, the test was designed to ask assessors about the sensory characteristics of two leaves separately for appearance and mouthfeel descriptors ($n = 44$), which were then averaged.

Stem colour was the only attribute assessed using a multiple-choice question (categories: white/green or pink/red/purple). *E. sativa* accessions show gradations of colouring in the leaf stem and it is thought to be a desirable commercial trait. Colour can range from being absent, to pink, to red, to purple. If colour was present, assessors selected 'pink/red/purple' and were asked to score the degree of this coloration on a standard, anchored line scale. Assessors were presented with a size chart encompassing the extremes of rocket salad leaf sizes in order to standardise responses. This indicated into which range on the line scale they should enter their response based on the leaf area (three size examples were given).

Evaluation sessions were carried out under artificial daylight conditions in an air-conditioned room (~22 °C), in isolated sensory booths within the Sensory Science Centre at the Department of Food & Nutritional Sciences, University of Reading, UK. Freshly harvested plant samples were presented twice to each assessor in a balanced order over five days (approximately two to three hours after harvest). Two random leaves from each accession were placed on a single plate with a randomly assigned, three-digit code. Panelists were provided with water (room temperature) and frozen natural yoghurt (Yeo Valley Farms (Production) Ltd., Bristol, UK) for palate cleansing between samples. Warm water was also provided

Table 1
Definitions for sensory attributes associated with 7 *Eruca sativa* accessions.

Attribute	Agreed definition
Appearance	
Leaf shape	Variability of leaf shape between the two presented; none – completely different
Depth of colour	Shade of green; light green – dark green
Leaf size	Small, medium or large in reference to a scale provided to assessors
Hairiness	Extent of visibility of hairs on leaf petiole and underside of lamina
'Purple' stem	Presence of pink, red or purple within the stem, petiole or midrib of leaves
Odour	
Sulfur	Aroma associated with eggs
Green	Aroma(s) associated with cut grass and freshness
Stalky	Dry aroma associated with dried leaves or grasses
Pepper	Pungent aroma associated with ground peppercorns
Earthy	Resembling or suggestive of earth or soil
Burnt rubber	An aroma reminiscent of burning rubber
Pungent	A sharp aroma; associated with perceived strength
Sweet	A pleasant, sugary aroma
Aromatic	A pleasant aroma associated with herbaceous oils
Mustard	Potent aroma associated with crushed mustard seeds or condiment mustard
Mouthfeel	
Initial heat	The initial burst of 'hotness' on the tongue momentarily after placing into the mouth and chewing
Spikiness	Sensation associated with the sharpness of any leaf hairs that may be present on samples
Crispiness	Brittle sensation on the teeth or tongue when chewing or biting leaves
Chewiness	Degree of ease with which leaves are chewed and swallowed
Toughness	Degree of ease with which leaf stems can be broken by the teeth
Moistness	Associated with the water content of the leaf samples ingested
Salivating	Degree to which samples induced the production of saliva in the mouth upon chewing
Astringent	Degree to which samples induced drying and/or the sensation of shrinkage of the tongue and soft palate
Tingliness	The sensation produced upon the tongue; associated with slight prickling or stinging
Warming	The sensation of increased temperature within the mouth while chewing; prolonged and separate from "initial heat"
Taste	
Sweet	Pleasant taste associated with sugary foods
Sour	Acidic sensation associated with vinegar
Bitter	Sharp, unpleasant or pungent taste upon the tongue
Savoury	Taste associated with slightly salty or spicy food
Flavor	
Green	Flavor associated with cut grass and freshness
Stalky	Flavor associated with dry, fibrous leaves
Peppery	Flavor associated with ground peppercorns
Mustard	Flavor associated with the potency of crushed mustard seeds or condiment mustard
Sulfur	A flavour associated with consumption of eggs
Earthy	A flavour resembling or suggestive of earth or soil
Aftereffects	
Bitter	A persistence of bitter taste after swallowing leaf samples
Sweet	A persistence of pleasant, sugary taste
Acid	Persistence of a sharp, unpleasant taste upon the tongue; reminiscent of vinegar
Savoury	Persistence of a salty or slightly spicy flavour upon the tongue
Peppery	Persistence of the flavour of peppercorns
Mustard	Persistence of the flavour of mustard seed/condiment mustard
Green	Persistence of a grassy, fresh flavour
Earthy	Persistence of flavours resembling or suggestive of earth or soil
Warming	A persistence of the sensation of heat/temperature within the mouth after swallowing

for assessors to wash their fingers between samples, to avoid carry-over of aromas to subsequent samples. No more than four samples were presented in any one session to avoid palate/trigeminal fatigue. There was a one-minute time delay between the finishing of one sample and the presenting of the next.

2.3. Reagents and chemicals

All solvents and chemicals used were obtained from Sigma-Aldrich (Gillingham, UK) unless otherwise stated. The EZ:faast Free (Physiological) Amino Acid Analysis by GC–MS kit was obtained from Phenomenex (Macclesfield, UK).

2.4. Glucosinolate and flavonol analysis

GSLs and flavonols were extracted and analysed by LC–MS and presented in Bell et al. (2015). Briefly, lyophilized leaves were milled, and extracted using 70% methanol at 70 °C. Crude extracts were filtered and diluted ($n = 3$) before being run on a HPLC and ion trap mass spectrometer (MS/MS) with an isocratic gradient of 95% water (0.1% ammonium formate) and 5% acetonitrile over a 40 min run. GSLs and flavonols were quantified separately at two different wavelengths and quantified by two different external standards (GSLs: sinigrin hydrate; flavonols: isorhamnetin).

2.5. Polyatomic ion analysis by ion chromatograph

Lyophilized rocket powder for each accession ($n = 3$) was re-dried after transport (to the Dipartimento di Scienze Agro-Ambientali e Territoriali, University of Bari, Italy) at 65 °C, and subsequently re-milled with a micrometric mill (IKA, Germany). 0.5 g of the material was placed in a bottle of 100 ml, to which 50 ml of a solution composed of Na_2CO_3 (3.5 mM) + $NaHCO_3$ (1.0 mM) was added. The bottle was shaken for 20 min (145 rpm). Before inserting the solution into the ion chromatograph (IC), the supernatant was filtered using a 0.22 μm filter to remove any residual organic matter. A Dionex DX-120 Chromatograph (Dionex Corporation, Sunnyvale, CA, USA) was used to measure chloride, nitrate, phosphate and sulphate anions by comparison to a multi-anion standard (Dionex, Milan, Italy).

2.6. Volatile organic chemical analysis

Volatile organic chemicals (VOCs) were extracted from the headspace of leaves, with data previously presented in Bell, Spadafora, Müller, Wagstaff, and Rogers (2016), using Thermal Desorption Gas Chromatography Time-Of-Flight Mass Spectrometer (TD-GC-TOF-MS). See this paper for detailed methodology.

Briefly, rocket leaves of each accession (70 g) were placed into sealed bags and manually disrupted to release volatiles into the headspace ($n = 3$). Samples were collected using a hand-pump device attached to a portable thermal desorption tube, which was inserted through a port in the bag. Tubes were desorbed using a TD100 thermal desorption system (Markes International Ltd., Llantrisant, Wales, UK) and samples analysed using a BenchTOF-dx mass spectrometer (Almsco International, Cincinnati, OH, USA).

2.7. Free amino acid analysis

Lyophilized rocket powder (50.0 mg; $n = 3$) was added to 0.5 ml of 25% acetonitrile in 0.01 M hydrochloric acid. Samples were vortexed for five minutes and left to settle for one hour at room temperature ($\sim$22 °C), and then centrifuged. The supernatant of each sample was removed and filtered with 0.22 μm filter discs with a low protein binding Durapore polyvinylidene fluoride (PVDF) membrane (Millex; EMD Millipore, Billerica, MA, USA).

A diluted aliquot of the filtrate (10 μl sample, 90 μl H_2O) was derivatized using the EZ:faast Free (Physiological) Amino Acid

Analysis by GC–MS kit. GC–MS analysis of the derivatized samples was carried out using an Agilent 7890A/5795C GC–MS instrument as described by Elmore, Koutsidis, Dodson, Mottram, and Wedzicha (2005). Samples were quantified using an internal standard of norvaline.

2.8. Free sugars and organic acids analyses

Lyophilized rocket powder (0.4 g; $n = 3$) was suspended in 10 ml of 0.01 M hydrochloric acid (except SR19 where dried material of two replicates was depleted; $n = 1$). Each sample was stirred for 30 min at room temperature (~22 °C), and the mixture was set aside to settle for 30 min. An aliquot of the supernatant (1.5 ml) was centrifuged for 30 min. The supernatant of the resulting extract was filtered with a Millex Millipore sterile syringe driven filter unit (0.22 μm) and analysed by capillary electrophoresis (CE). An external standard method for sugars (glucose, fructose, sucrose, and galactose) and OAs (malic acid and citric acid; ranging from 0.5 to 10 mg.g^{-1}) was used for the quantification of the analytes of interest.

The CE method used was adapted from Lignou, Parker, Oruna-Concha, and Mottram (2013) and Soga and Ross (1999). Briefly, a HP3D CE with DAD and Agilent ChemStation software (Santa Clara, CA, USA) was used to run sugars and OAs within the same chromatographic run. Electrophoretic separation was performed at a constant pressure of 50 mbar, with a six second injection of sample, followed by a four second injection of buffer. A G1600-61311 capillary (Agilent, Stockport, UK) was used which measured 75 μm id, 64.5 cm in length, with an effective length of 56 cm, maintained at 15 °C. An anion buffer was used for sample separation and the column was preconditioned for four minutes with buffer before each run.

2.9. Statistical analysis

2.9.1. ANOVA

To analyze the sensory profiling data, two-way analysis of variance (ANOVA; with accessions and assessors as treatment effects, and these main effects tested against their interaction) was carried out in Senpaq (Qi Statistics Ltd., Reading, UK). ANOVA was conducted using a 95% confidence interval and a tolerance of 0.0001%. A post hoc Tukey's HSD test was used for multiple pairwise comparisons. This was chosen for the higher level of stringency than other pairwise comparison tests, such as Fisher's LSD Test.

The quantitative data for each compound identified in the CE, IC and GC analyses (sugars, OAs, PIs, AAs) were analysed independently by one-way ANOVA using XL Stat (Addinsoft, Paris, France). Significant differences between varieties were determined using Tukey's HSD test to generate pairwise comparisons.

2.9.2. Principal Component Analysis

The means for the sensory data were taken (as described in Section 2.2.) and used in Principal Component Analysis (PCA, Pearson n-1; XL Stat) to extract principal components (PCs). Sensory relationships were determined by coefficient analysis. Phytochemical data obtained from PIs, free sugars, organic acids, and free AAs were collated with data from Bell et al. (2015) for GSLs and flavonols, and data from Bell et al. (2016) for headspace VOCs. These were regressed onto the sensory PCA as supplementary data, and a correlation matrix was constructed to determine significant relationships. Sensory variables with statistically significant correlations were identified at levels of $P < 0.05$, <0.01 and <0.001.

3. Results and discussion

3.1. Sensory attributes

3.1.1. Appearance

A summary table of sensory attribute scores can be found in Table 2, along with pairwise comparison statistical significances and the typical appearance of each cultivar can be seen in Supplementary Fig. S1.

Leaf sizes varied greatly across each accession; SR14 and SR12 had very large leaves, whereas SR5 and SR19 were significantly smaller by comparison. The range of sizes could potentially give breeders traits to select within gene bank populations, where new, novel types can be identified. Significant differences were also found for, depth of colour, leaf shape, hairiness and the prevalence of 'purple stem' (Table 2; $P < 0.05$). SR2 had a significantly higher degree of colouration in the stem than both SR5 and SR19, potentially making this a desirable accession to select this trait from.

3.1.2. Odour

There was a significant difference overall between samples for sulfur odour (Table 2; $P < 0.05$). No other odour attributes were significantly different between accessions. The strength of sulfur traits may play a key role in this differentiation between rocket accessions and consumer preferences (Pasini et al., 2011), though consumer studies of rocket cultivars are lacking in the literature.

3.1.3. Mouthfeel

Significant differences between accessions for mouthfeel attributes were found for initial heat, spikiness, chewiness, tingliness and warming (Table 2; $P < 0.05$). Accessions SR5 and SR19 were significantly different from SR2, SR3, SR6 and SR12 for initial heat, and also significantly higher in terms of tingling than SR3. SR5 was significantly different from SR2, SR3, SR6 and SR12 for warming mouthfeel. These data suggest a genetic component for inferring differing degrees of pungency between accessions, as SR5 in particular is scored highly in these traits.

SR14 was significantly chewier than SR3, and spikier than SR3 and SR19. The presence of hairs on leaves is not thought to be a desirable characteristic for consumers, and would need to be bred out of any potential future varieties (Bell & Wagstaff, 2014).

3.1.4. Taste, flavour & aftereffects

There were significant differences in peppery, mustard flavour and sulfur between accessions (Table 2; $P < 0.05$). Peppery flavour in SR19 and SR5 was significantly higher than in SR3; and mustard and sulfur flavour in SR5 was significantly higher than in SR12 and SR3, respectively. Acid, peppery, mustard and warming aftereffects were significantly different between some cultivars ($P < 0.05$), though no statistically significant differences were found for taste attributes. These data suggest that pungency/warming effects are more important for discriminating between cultivars than bitterness as has been suggested in a previous study (Pasini et al., 2011).

3.2. Phytochemical Analyses

3.2.1. Previous phytochemical analyses

The analysis of GSLs, flavonols and headspace VOCs is presented in Bell et al. (2015) and Bell et al. (2016). The data for the seven accessions used here are summarised in Supplementary Table S1. The material used in these analyses was grown under identical conditions to those presented in this paper, and the data were combined with new analyses of PIs, AAs, OAs and sugars.

Table 2
Average values of sensory traits of *Eruca sativa* accessions rated by 11 trained panel assessors.

Sensory trait	Accession							Significance (*P* values)	
	SR2	SR3	SR5	SR6	SR12	SR14	SR19	Sample	Sample*assessor
Appearance									
Depth of leaf colour (A)	57.3^{a}	59.5^{a}	60.8^{ab}	61.0^{ab}	60.9^{ab}	63.5^{ab}	68.1^{b}	0.0111^{*}	0.9228
Leaf shape (A)	40.5^{a}	41.8^{ab}	37.4^{a}	42.9^{ab}	57.7^{bc}	63.9^{c}	66.7^{c}	$<0.0001^{*}$	0.3526
Leaf size (A)	51.3^{bc}	40.8^{abc}	31.4^{a}	38.9^{ab}	55.9^{c}	56.3^{c}	33.8^{a}	$<0.0001^{*}$	0.9933
Hairiness (A)	10.6^{bc}	1.7^{ab}	1.2^{a}	0.2^{a}	4.1^{abc}	11.7^{c}	1.0^{a}	0.0002^{*}	0.0001^{*}
Purple stem (A)	36.0^{b}	31.0^{ab}	17.7^{a}	32.1^{ab}	25.3^{ab}	30.8^{ab}	13.7^{a}	0.2387	0.0009^{*}
Odour									
Sulfur (O)	11.8^{ab}	15.4^{ab}	19.9^{b}	7.3^{a}	9.0^{a}	13.8^{ab}	11.3^{ab}	0.0198^{*}	0.0088^{*}
Green (O)	33.4	37.8	34.6	34.4	34.1	40.0	39.2	0.2663	0.7788
Stalky (O)	21.4	27.4	29.0	24.6	24.0	25.5	28.0	0.4152	0.2568
Pepper (O)	12.9	12.1	15.3	13.6	12.9	15.6	17.2	0.2489	0.4200
Earthy (O)	10.5	11.0	7.9	12.6	10.7	13.6	10.6	0.4603	0.1956
Burnt rubber (O)	6.6	5.5	11.3	4.3	4.3	5.9	7.2	0.4274	0.0193^{*}
Pungent (O)	14.8	19.5	20.8	13.3	11.6	12.9	16.9	0.1334	0.1200
Sweet (O)	12.0	12.4	10.0	11.1	14.5	12.2	14.4	0.4247	0.0219^{*}
Aromatic (O)	4.1	6.4	7.0	5.9	6.0	9.2	5.1	0.3226	0.9454
Mustard (O)	9.2	12.2	16.5	12.1	7.9	11.6	11.3	0.0958	0.2066
Mouthfeel									
Initial heat (MF)	21.3^{a}	20.8^{a}	37.8^{c}	21.8^{a}	21.5^{a}	24.6^{ab}	31.4^{bc}	$<0.0001^{*}$	0.5755
Spikiness (MF)	2.9^{ab}	0.2^{a}	2.3^{ab}	1.4^{ab}	2.1^{ab}	4.3^{b}	1.1^{a}	0.1153	0.1393
Crispiness (MF)	14.2	15.4	16.3	17.4	15.8	17.4	18.7	0.5049	0.0114^{*}
Chewiness (MF)	20.3^{ab}	17.9^{a}	23.1^{ab}	23.5^{ab}	21.2^{ab}	25.7^{b}	19.4^{ab}	0.0381^{*}	0.0273^{*}
Toughness (MF)	16.4	15.4	17.6	20.3	17.2	20.7	15.0	0.0418^{*}	0.3210
Moistness (MF)	23.6	24.9	21.3	22.9	24.3	22.3	24.5	0.5499	0.3206
Salivating (MF)	19.5	18.0	17.2	19.0	17.2	19.9	21.9	0.3921	0.1493
Astringent (MF)	17.9	15.8	19.5	15.9	19.1	15.1	14.6	0.2518	0.0004^{*}
Tingling (MF)	10.8^{abc}	8.5^{a}	18.8^{c}	9.7^{ab}	9.5^{ab}	13.0^{abc}	16.8^{bc}	0.0010^{*}	0.1998
Warming (MF)	16.4^{a}	13.7^{a}	26.0^{b}	14.0^{a}	14.1^{a}	18.3^{ab}	22.4^{ab}	0.0008^{*}	0.0847
Taste									
Sweet (T)	9.6	9.5	7.1	11.6	12.7	10.1	13.5	0.2740	0.4906
Sour (T)	6.5	6.1	8.5	5.1	5.0	5.4	6.7	0.4743	0.1067
Bitter (T)	20.1	23.2	29.2	23.4	25.3	21.7	24.6	0.1682	0.5437
Savoury (T)	11.6	15.7	17.9	14.0	12.3	16.7	15.1	0.0361^{*}	0.4433
Flavour									
Green (F)	31.0	32.2	27.9	31.4	29.1	36.0	35.2	0.2599	0.5475
Stalky (F)	19.7	25.5	29.1	19.6	21.3	21.8	24.5	0.0613	0.6718
Peppery (F)	16.5^{abc}	12.8^{a}	21.2^{bc}	14.9^{ab}	14.2^{ab}	16.9^{abc}	22.6^{c}	0.0009^{*}	0.7834
Mustard (F)	16.9^{abc}	15.4^{ab}	27.3^{c}	19.3^{abc}	11.6^{a}	16.7^{abc}	24.8^{bc}	0.0003^{*}	0.8755
Sulfur (F)	8.2^{a}	10.5^{a}	20.7^{b}	10.8^{ab}	8.5^{a}	11.4^{ab}	11.6^{ab}	0.0076^{*}	0.0697
Earthy (F)	8.6	6.8	10.2	8.2	10.7	10.0	7.8	0.5436	0.8714
Aftereffects									
Bitter (AE)	20.5	19.7	24.9	23.3	21.3	21.6	23.5	0.4921	0.7345
Sweet (AE)	6.8	5.9	3.6	7.8	8.4	6.7	6.2	0.4044	0.0375^{*}
Acid (AE)	4.6^{ab}	4.2^{ab}	9.2^{b}	3.2^{a}	2.5^{a}	4.0^{ab}	4.7^{ab}	0.0175^{*}	0.0544
Savoury (AE)	10.0	12.0	13.0	12.1	12.2	13.9	12.3	0.5462	0.8850
Peppery (AE)	15.9^{ab}	12.6^{a}	20.4^{b}	14.4^{ab}	14.5^{ab}	16.5^{ab}	19.3^{ab}	0.0116^{*}	0.8395
Mustard (AE)	13.0^{a}	12.3^{a}	21.4^{b}	15.1^{ab}	12.0^{a}	14.2^{ab}	19.9^{ab}	0.0016^{*}	0.8292
Green (AE)	21.5	24.9	20.9	21.8	21.1	23.6	26.6	0.2629	0.7435
Earthy (AE)	7.5	7.7	7.5	7.4	10.6	10.6	7.1	0.3296	0.9384
Warming (AE)	19.6^{abc}	13.8^{a}	26.2^{c}	16.4^{ab}	13.7^{a}	22.5^{bc}	23.9^{bc}	$<0.0001^{*}$	0.9869

Abbreviations: A, appearance; O, odour; MF, mouthfeel; T, taste; F, flavour; AE, aftereffects. Significantly different values indicated by superscript letters within rows (ANOVA Tukey's HSD test, $P \leqslant 0.05$). An absence of letters indicates no significant difference was observed. *Denotes significance (P = <0.05) for the sample and sample*assessor interactions. For multi-leaf attributes (A & MF) $n = 44$, for all other attributes $n = 22$.

3.2.2. Polyatomic ions

Table 3 summarises the concentrations of four PI groups found in the rocket accessions: chlorides, nitrates, phosphates and sulphates. The PI content of the seven cultivars varied significantly. Nitrate concentrations are relatively low for all accessions compared to previous reports, but this is not unusual as large variations in cultivar accumulations are known to occur across growing methods, cultivars, and environments (Cavaiuolo & Ferrante, 2014).

Chloride concentration was lowest in accession SR12 (9.5 g.kg^{-1} DW) and highest in SR5 (16.6 g.kg^{-1} DW) and this was a significant difference ($P < 0.05$). SR5 is also high in phosphate concentration (15.2 g.kg^{-1} DW) and is significantly different from SR3, SR6 and SR14. SR19 accumulated significantly more phosphate than any of the other accessions tested. SR5 is conversely very low in nitrate concentration (10.0 g.kg^{-1} DW) – almost five times less than SR19 (48.5 g.kg^{-1} DW). SR19 was also relatively high in phosphate (20.7 g.kg^{-1} DW) and sulphate (17.7 g.kg^{-1} DW), making it distinct in terms of PI concentrations.

3.2.3. Free amino acids

Table 3 shows the AA concentrations found in each of the seven accessions. In total 11 free AAs were detected and quantified, however only serine and glutamine showed significant differences between cultivars. The commercial cultivar SR3 had a high serine concentration of 167 µg.g^{-1} DW, which was significantly greater

Table 3
Polyatomic ion (g.kg^{-1} DW), amino acid (µg.g^{-1} DW), sugar (mg.g^{-1} DW) and organic acid (mg.g^{-1} DW) concentration for seven accessions of *Eruca sativa* ($n = 3$) with standard errors (±).

	Accession						
	SR2	SR3	SR5	SR6	SR12	SR14	SR19*
Polyatomic ions (g.kg^{-1} DW)							
Chloride	12.6 ± 3.0^{ab}	10.3 ± 0.1^{ab}	16.6 ± 0.9^{b}	13.1 ± 0.7^{ab}	9.5 ± 1.2^{a}	11.1 ± 2.0^{ab}	14.0 ± 1.1^{ab}
Nitrate	26.9 ± 7.2^{ab}	24.2 ± 1.0^{a}	10.0 ± 0.8^{a}	21.6 ± 7.1^{a}	15.4 ± 6.7^{a}	13.0 ± 1.3^{a}	48.5 ± 5.1^{b}
Phosphate	14.6 ± 0.4^{bc}	9.8 ± 1.3^{ab}	15.2 ± 0.7^{c}	9.4 ± 0.4^{a}	13.4 ± 1.7^{abc}	9.6 ± 0.1^{ab}	20.7 ± 0.6^{d}
Sulphate	12.8 ± 2.8^{ab}	10.5 ± 1.0^{a}	10.8 ± 1.3^{a}	10.7 ± 0.6^{a}	12.5 ± 1.2^{ab}	12.3 ± 1.4^{ab}	17.7 ± 0.4^{b}
Amino acids (µg.g^{-1} DW)							
Alanine	65.1 ± 8.5	59.6 ± 4.5	39.1 ± 5.3	61.9 ± 5.2	63.8 ± 13.4	46.8 ± 6.8	52.5 ± 8.1
Valine	11.1 ± 6.4	7.2 ± 5.9	nd	11.9 ± 5.0	6.0 ± 4.9	nd	12.0 ± 4.9
Leucine	nd	3.5 ± 2.9	nd	4.0 ± 3.3	2.6 ± 2.1	nd	nd
Threonine	21.8 ± 2.2	24.1 ± 1.3	12.9 ± 1.1	25.0 ± 1.1	19.7 ± 3.9	13.7 ± 1.1	22.3 ± 3.6
Serine	63.0 ± 31.5^{a}	167.2 ± 20.5^{b}	70.7 ± 14.4^{ab}	81.9 ± 36.4^{ab}	113.6 ± 43.5^{ab}	84.5 ± 10.7^{ab}	117.3 ± 33.2^{ab}
Proline	73.7 ± 7.4	41.3 ± 5.2	67.1 ± 21.2	50.5 ± 5.5	75.4 ± 16.7	69.9 ± 35.0	25.8 ± 3.8
Asparagine	4.3 ± 2.5	nd	nd	nd	nd	nd	3.2 ± 2.6
Aspartic acid	155.8 ± 16.4	155.8 ± 17.5	93.9 ± 4.0	162.8 ± 5.9	117.3 ± 22.2	80.5 ± 16.4	138.2 ± 22.5
Glutamic acid	143.1 ± 69.5	158.2 ± 8.8	120.2 ± 6.7	222.9 ± 34.0	177.1 ± 61.6	146.9 ± 15.9	148.0 ± 26.1
Glutamine	90.8 ± 12.3^{c}	83.0 ± 5.6^{bc}	29.2 ± 1.5^{a}	77.2 ± 9.4^{abc}	49.3 ± 13.0^{abc}	35.8 ± 4.3^{ab}	65.1 ± 8.5^{abc}
Lysine	nd	2.1 ± 1.7	nd	nd	nd	nd	nd
Total AAs	628.9 ± 107.1	701.9 ± 31.9	433.0 ± 45.0	698.2 ± 44.6	624.8 ± 128.8	478.2 ± 81.5	584.4 ± 97.8
Sugars (mg.g^{-1} DW)							
Fructose	3.7 ± 0.6	3.6 ± 0.1	2.8 ± 0.3	2.0 ± 0.2	3.2 ± 0.6	4.9 ± 0.9	1.9
Glucose	16.2 ± 1.2	16.7 ± 1.2	23.7 ± 0.5	14.7 ± 2.5	19.6 ± 0.9	21.1 ± 0.9	9.1
Galactose	3.9 ± 1.0	3.5 ± 1.5	4.6 ± 1.0	2.6 ± 0.8	4.1 ± 0.3	6.6 ± 0.5	0.7
Sucrose	2.1 ± 0.6	3.8 ± 1.8	2.7 ± 0.7	1.4 ± 0.3	1.7 ± 0.3	5.3 ± 1.9	3.1
Total sugars	25.9 ± 2.4	27.6 ± 3.4	33.8 ± 0.7	20.8 ± 4.9	28.6 ± 0.3	37.9 ± 3.2	14.8
Organic acids (mg.g^{-1} DW)							
Malic acid	83.5 ± 24.1	67.7 ± 0.2	62.9 ± 4.3	54.4 ± 9.1	46.8 ± 0.7	47.2 ± 0.1	59.0
Citric acid	19.1 ± 1.2	30.9 ± 5.0	14.0 ± 0.8	10.4 ± 7.3	20.0 ± 2.0	28.3 ± 0.4	22.8
Total OAs	102.7 ± 25.4	98.5 ± 4.8	77.0 ± 3.5	64.8 ± 16.4	66.8 ± 2.7	75.5 ± 0.5	81.9

Significant differences (ANOVA Tukey's HSD test, $P < 0.05$). Different letters in each row indicates a significant difference; an absence of letters indicates no significant difference. *= $n = 1$ for sugar and organic acid analyses. nd = not detected.

than SR2 (63 µg.g^{-1} DW). SR2 conversely had statistically higher glutamine concentration (91 µg.g^{-1} DW) than SR5 (29 µg.g^{-1} DW) and SR14 (36 µg.g^{-1} DW). Aspartic acid and glutamic acid were the most abundant AAs detected overall, and were present in every accession. Valine, leucine, asparagine and lysine were not observed in several accessions, with concentrations very low where they were detected.

3.2.4. Free sugars & organic acids

Table 3 displays the free sugar content of each accession tested. No significant differences were found in the ANOVA, with the possible exception of SR19. Unfortunately leaf material of this accession was limited, and only one biological replicate could be analysed (not included in ANOVA).

Concentrations of free OAs are also presented in Table 3, and as with sugars, no significant differences between each accession were observed. This is perhaps due to the very large variation within some samples, particularly SR2, which had large variation in malic acid concentration.

3.3. Principal Component Analysis

3.3.1. Sensory attributes

PCA extracted six components, all of which had Eigenvalues >1.0; however the majority of information was contained in the first three PCs (78.6%; Supplementary Table S2). On this basis PC1, PC2 and PC3 are presented. The majority of explained variation is found in PC1 (43.49%) and this component separates traits associated with pungency and bitterness, and coupled with the correlation matrix data (Supplementary Table S3), many of these traits share significant relationships. PC2 identifies a dimension characterised by green and sweetness characters, as well as some appearance and mouthfeel traits. The information contained within PC3 is related to earthy attributes and aromatic odour, but also visual and morphological characteristics such as leaf size, toughness, chewiness and spikiness.

These separations are easily identifiable within the biplots presented in Fig. 1. SR5 is distinctive in Fig. 1a, characterised by a high degree of association with pungent attributes, acid aftereffects and bitterness. SR19 is also separate from the main cluster (lower left), but separates along PC2 in terms of the distinct difference in appearance from the other cultivars. SR14, SR6, SR12, SR3 and SR2 are broadly similar in these dimensions, and are characterised by a comparatively low bitterness, and lower scoring mustard, pepper, sulfur and initial heat mouthfeel attributes. This is coupled with an increase in relative perceptions of sweetness attributes, moistness mouthfeel and larger leaf shapes. In Fig. 1b this pattern is broadly repeated, however SR19 separates along the negative axis of PC3 due to low scores for leaf hairiness, purple stem, spikiness, and earthy/aromatic attributes. The distinctiveness of SR5 and SR19 was also repeated in components PC4, PC5 and PC6 (plots not presented).

The purple stem attribute was correlated highest in PC5 (plot not presented), and in the Pearson's correlation analysis was inversely and significantly correlated to traits such as bitter taste, peppery flavour, mustard aftereffects, initial heat, tingly and warming aftereffects (all $P < 0.05$). This may suggest that stem colouration could be used as a visual cue for determining pungency/bitterness of leaves, although this would need to be assessed in more focused experiments.

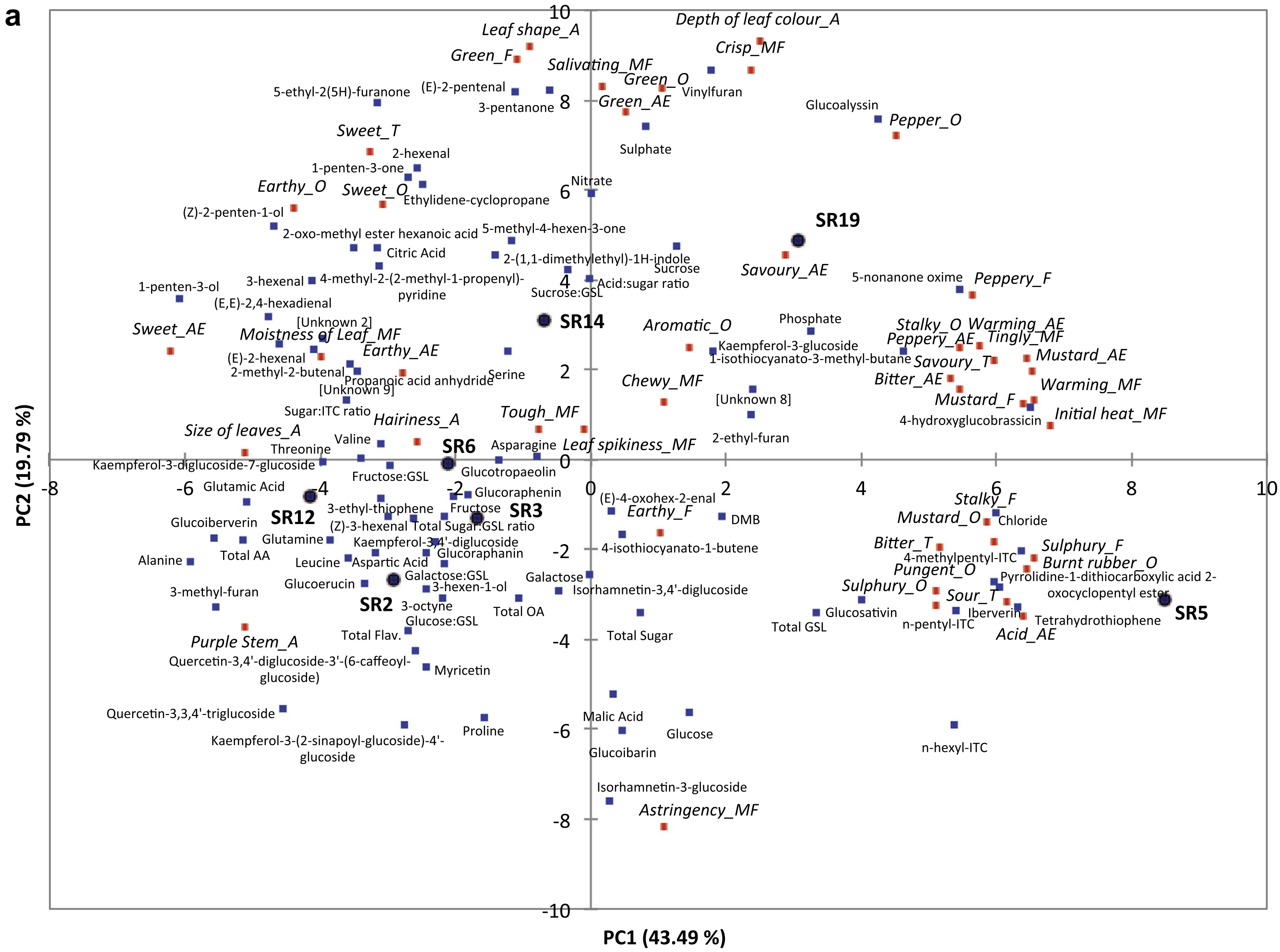

Fig. 1. Principal component analysis biplots of sensory descriptive trait scores and regressed supplementary phytochemical data. PC1 vs. PC2 (a) accounts for 63.28% of the explained variation. PC1 vs. PC3 (b) accounts for 58.81% of the explained variation. Dark blue circles with bold labels indicate scores values for each respective accession. Red circles with italic labels indicate measured sensory attributes. Blue squares indicate supplementary data points of each phytochemical analysis: GSLs, flavonols, PIs, headspace VOCs, AAs, free sugars, organic acids, sugar-GSL ratios, sugar-ITC percentage abundance ratio and the sugar-OA ratio. Abbreviations: A, appearance; O, odour; MF, mouthfeel; T, taste; F, flavour; AE, aftereffects.

Many of the sensory attributes assessed share highly significant correlations (Supplementary Table S3). Briefly, the odour attribute of burnt rubber shares several significant relationships with traits such as sulfury flavour ($P < 0.01$), initial heat mouthfeel ($P < 0.01$) and warming mouthfeel ($P < 0.01$). Many of the perceptions associated with these types of pungent attributes are correlated and co-locate within the PCA in PC1. Peppery flavour is also significantly correlated with tingly mouthfeel ($P < 0.001$), and mustard flavour/aftereffects with initial heat ($P < 0.01$; $P < 0.001$, respectively).

3.3.2. Phytochemical data

3.3.2.1. General. The regressed phytochemical data is presented in Fig. 1, superimposed upon the sensory PCA, and illustrates the relationships found with these data across the three most informative principal components. Significant correlations (Pearson *n*-1) between phytochemicals and sensory attributes are also summarised in Supplementary Table S3, and the regressed factor loadings of each variable are presented in Supplementary Table S4.

3.3.2.2. Glucosinolates. Eleven GSL compounds were detected in the seven rocket accessions by Bell et al. (2015). These were 4-hydroxyglucobrassicin, glucotropaeolin, glucoraphanin, glucoiberverin, glucosativin, DMB, glucoalyssin, glucoerucin, glucoraphenin, diglucothiobeinin and glucoibarin. For the purposes of the analysis, data for diglucothiobeinin were not included, as it was only detected in one of the accessions analysed.

The major GSL of rocket, glucosativin (2.7–7.7 mg.g^{-1} DW; Bell et al., 2015), was significantly and positively correlated to earthy flavour ($P < 0.05$; Table S3) and was most positively correlated along PC3 (Fig. 1b). Unlike other studies where glucosativin and its dimer (DMB) have been linked with bitterness, there was no significant relationship found here. DMB was most highly correlated along PC1 and positioned between earthy and pungent attributes but no significant correlations were observed.

Total GSL concentration separated along PC3 and PC1 (Fig. 1b), and shared a significant correlation with bitter aftereffects ($P < 0.05$), and negatively with the perceived moistness mouthfeel of leaves ($P < 0.05$). These two correlations suggest an overall tendency for rocket GSLs to have a bitter component associated with them post-swallowing, and the intensity to be inverted to the levels of moisture.

SR6 had high concentrations of total GSLs (10.0 mg.g^{-1} DW; Bell et al., 2015), but the sensory profile of this accession is more similar to SR2, and is associated with sweet/green attributes (PC1 vs. PC3; Fig. 1b). This indicates that individual GSLs may be more influential on sensory properties than the total concentration. SR6 was characterised by relatively high concentrations of glucoerucin (1.3 mg.g^{-1} DW), for example. The absence of any sig-

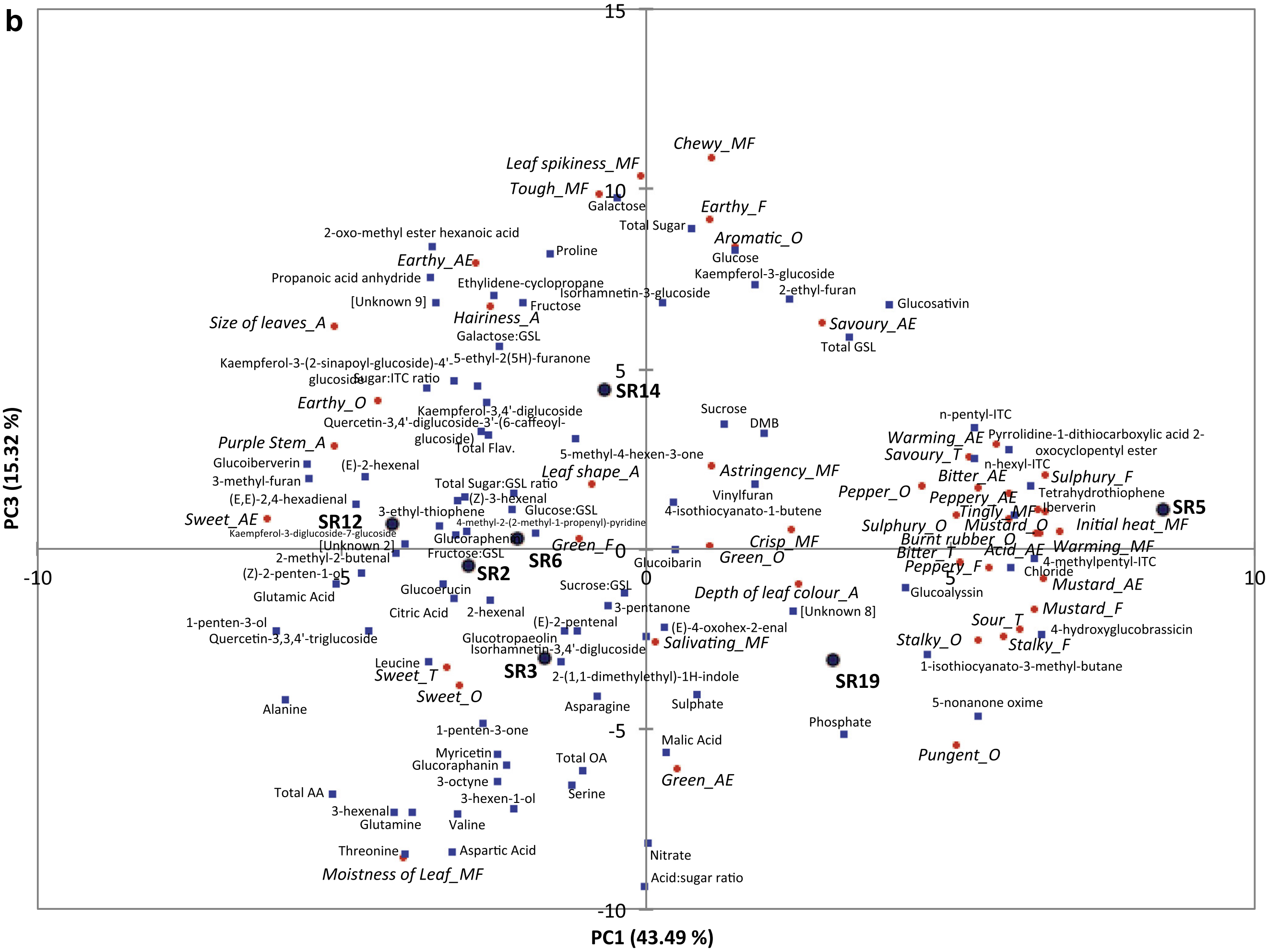

Fig. 1 (*continued*)

nificant correlations with this GSL and glucoraphanin are also of potential importance. We hypothesise that they do not directly contribute to the sensory profile of rocket, and could be increased through selective breeding to produce more nutritionally dense rocket varieties without affecting flavour.

Minor rocket GSLs such as 4-hydroxyglucobrassicin and glucoalyssin were reported in very low concentrations by Bell et al. (2015) yet produced strong correlations with PC1 and PC2, respectively. This may be indicative of the role minor GSLs play in sensory perceptions of rocket, and what creates distinctive flavours between cultivars. 4-hydroxyglucobrassicin for example was only detected in SR5 and SR19, and when these supplementary data were regressed onto the sensory principal components the presence of this compound is significantly correlated with bitter aftereffects ($P < 0.05$), pepper flavour and aftereffects ($P < 0.01$), mustard flavour ($P < 0.01$) and aftereffects ($P < 0.001$), the initial heat of leaves ($P < 0.001$), and tingly and warming mouthfeels ($P < 0.01$). It was observed that the presence of glucoalyssin had significant correlations with pepper odour ($P < 0.01$), pepper flavour ($P < 0.05$), and mustard aftereffects ($P < 0.05$).

Although concentrations/presence differs across accessions, it is not possible to know definitively if they are the cause of sensory differences without isolated standards. It is likely however that a higher diversity of ‘minor’ GSLs is associated with distinctive sensory attributes, such as pepperiness in SR19, and hotness/pungency in SR5, rather than total GSL concentration. D’Antuono, Elementi, and Neri (2009) similarly found that 4-hydroxyglucobrassicin was highly associated with “pleasant” taste and pungency. It may be that minor GSLs and their hydrolysis products contribute more to these effects than has been previously realized.

3.3.2.3. Flavonols. Eleven flavonol compounds were identified and quantified in the rocket accessions tested by Bell et al. (2015). These were myricetin, kaempferol-3-glucoside, quercetin-3-glucoside, isorhamnetin-3-glucoside, kaempferol-3,4′-diglucoside, isorhamnetin-3,4′-diglucoside, kaempferol-3-diglucoside-7-glucoside, quercetin-3,3,4′-triglucoside, kaempferol-3-(2-sinapoyl-glucoside)-4′-glucoside, quercetin-3,4′-diglucoside-3′-(6-caffeoyl-glucoside) and quercetin-3,4′-diglucoside-3′-(6-sinapoyl-glucoside). For the purposes of the analysis quercetin-3-glucoside and quercetin-3,4′-diglucoside-3′-(6-sinapoyl-glucoside) data were omitted as they were each only detected in one accession. Some of these compounds were highly correlated along PC3 (Fig. 1b) and were generally associated with mouthfeel traits, and negatively with stalky and intense sensory attributes.

Flavonols and other polyphenols have been strongly linked with astringent sensory perceptions in studies of drink products, such as red current juice (Schwarz & Hofmann, 2007), red wine (Hufnagel & Hofmann, 2008), berry juice (Laaksonen, Ahola, & Sandell, 2013) and black tea (Scharbert, Holzmann, & Hofmann, 2004). Isorhamnetin-3-glucoside was the only compound where a significant correlation was observed with astringent mouthfeel ($P < 0.05$). This compound was significantly, negatively correlated with the perception of salivating mouthfeel ($P < 0.05$), implying a possible link with perceptions of moisture on the palate.

3.3.2.4. Polyatomic ions. Chloride and phosphate separated highly along PC1, and this was largely due to the relatively high concentrations present in both SR5 and SR19. Nitrate and sulphate however were highly correlated with PC2 (Fig. 1a), opposite to SR5, which is characterised by low nitrate concentration.

In a previous study by Hufnagel and Hofmann (2008) on red wine fractions, both chloride and phosphate were linked with astringency and sourness. In this study, only chloride was positively correlated with sour taste ($P < 0.05$). One hypothesis for this association (which is usually caused by acids), might be that chloride ions react with thiol groups of some ITCs to produce hydrochloric acid (La Quèrè, Gierezynski, Langlois, & Sèmon, 2006), and thus create H+ ions which would be perceived as sour on the tongue. Such reactions may also influence volatile formations (La Quèrè et al., 2006) and infer differing sensory properties according to relative abundances. Scores for sourness were low uniformly across accessions and non-significant in the ANOVA, but it is interesting to note SR5 was scored highest overall, as well as for acid aftereffects with which chloride ion concentration was also significantly correlated with ($P < 0.05$). Unfortunately studies of this kind are absent for rocket and other leafy vegetables.

Accession SR5 had the highest concentration of chloride, and SR19 the highest concentration of phosphate, and is again a distinguishing attribute in terms of sensory properties. Numerous significant correlations were observed between chloride ions and traditional rocket traits (Table S3); particularly of note is mustard flavour ($P < 0.001$) and aftereffects ($P < 0.01$). These correlations are of course not proof that they are the causative agents; however there does seem to be a relationship in these samples between sensory attributes and chloride concentrations.

The other three PIs also had significant correlations ($P < 0.05$; Table S3). Phosphate was significantly correlated to peppery flavour and both sulphate and nitrate produced significances relating to salivating mouthfeel. Nitrate levels have been linked to differences in spinach flavour (Maga, Moore, & Oshima, 1976), however information regarding direct and specific effects of these ions in leafy vegetables is sparse within the literature.

3.3.2.5. VOCs. ITCs, sulfur volatiles and an oxime showed large separation along PC1, indicating that there is a strong relationship between their relative abundances and the hot, peppery, mustard and warming attributes present in accessions, such as SR5 and SR19. Alcohols, aldehydes and ketone compounds separated along PC2 indicating a high degree of association with odour and some taste, flavour and mouthfeel attributes (Supplementary Table S4), such as stalky, sweet and green. A smaller number of compounds separated to a high degree on PC3, and included some furans, acids, a thiophene and a cyclopropane. This associates them with earthy and savoury attributes, as well as with accessions that were typically described as being chewy or tough. The relative distribution of these compounds with sensory attributes is presented in the PCA biplots (Fig. 1). 3-ethyl-1,5-octadiene, *O*-methyloximebutanal and oxalic acid diallyl ester were removed from the analysis as they were only detected in one accession, respectively (Bell et al., 2016).

4-methylpentyl-ITC and iberverin showed significant correlations with bitter taste ($P < 0.05$; $P < 0.01$, respectively) and aftereffects (both $P < 0.05$). *n*-pentyl-ITC and 1-isothiocyanato-3-methylbutane also correlated strongly with bitter taste ($P < 0.01$; $P < 0.05$, respectively). Bitterness in ITC-containing compounds is well documented within the literature (Behrens, Gunn, Ramos, Meyerhof, & Wooding, 2013) and our data are in agreement with other studies in this regard.

Three other compounds that are not ITCs were also correlated with bitter taste: pyrrolidine-1-dithiocarboxylic acid 2-oxocyclopentyl ester ($P < 0.05$), tetrahydrothiophene ($P < 0.05$), and an unidentified compound (Unknown 8; $P < 0.05$). Only one significant negative correlation was found with bitter taste, which was 3-methyl-furan ($P < 0.05$). This latter compound was significantly positive in correlation with the purple stem attribute ($P < 0.001$). Purple stem was inversely related to 5-nonanone oxime ($P < 0.05$) and 1-isothiocyanato-3-methyl-butane ($P < 0.001$). This may again provide a possible visual cue for leaf pepperiness, bitterness and overall pungency.*n*-hexyl-ITC and iberverin are correlated significantly with aroma perceptions such as mustard ($P < 0.05$) and sulfur ($P < 0.05$; Table S3), indicating that these compounds contribute heavily to rocket odour properties, despite their low relative abundance within the VOC bouquet. 4-methylpentyl-ITC, *n*-hexyl-ITC, *n*-pentyl-ITC and iberverin all correlated with burnt rubber aroma at the $P < 0.01$ significance level. These along with pyrrolidine-1-dithiocarboxylic acid 2-oxocyclopentyl ester ($P < 0.01$) were higher in relative abundance in SR5, which is separated along PC1 with sulfur and mustard odours/flavours, as well as with high GSL concentrations and relative ITC abundances.

5-nonanone oxime was significantly correlated with several attributes usually attributed to ITCs, and is correlated strongly with SR19 and PC1 (Fig. 1). Significant correlations included pepperiness (odour, $P < 0.05$; flavour, $P < 0.01$; and after effects, $P < 0.05$), initial heat ($P < 0.05$), tingliness ($P < 0.05$), warming aftereffects ($P < 0.05$), and mustard flavour and aftereffects (both $P < 0.05$; Table S3). These results infer that the sensations commonly associated with rocket are perhaps not wholly due to direct products of the GSL-myrosinase reaction, and that other VOCs may have a role.

Tetrahydrothiophene is a pungent chemical odourant (Swanston, 2000) and is likely an ITC derivative. It has significant correlations with burnt rubber odour ($P < 0.01$), initial heat ($P < 0.01$), warming ($P < 0.05$), tingliness ($P < 0.05$), sour taste ($P < 0.05$), bitter taste ($P < 0.05$), bitter aftereffects ($P < 0.05$), acid aftereffects ($P < 0.01$) and peppery aftereffects ($P < 0.05$; Table S3). In agreement with Jirovetz, Smith, and Buchbauer (2002), we found this compound to be significantly correlated to mustard odour, flavour and after effects (all $P < 0.05$), as well as sulfur flavour ($P < 0.01$). This compound has been linked with unpleasant odours, allium-like smells and 'cabbage' odour (Jirovetz et al., 2002), and our results suggest that it is an important component in the volatile mixture produced by rocket leaves.

At the low end of PC1, and opposite to the pungency/pepperiness of SR5 and SR19 are the 'green leaf volatiles', produced in higher relative abundances by accessions such as SR2. The initial heat of leaves ($P < 0.05$), and the aroma sensations of mustard ($P < 0.05$) and burnt rubber ($P < 0.01$) were negatively correlated with 1-penten-3-ol. This is an unexpected result as in previous studies 1-penten-3-ol has been linked with burnt and pungent attributes (Berger, Drawert, & Kollmannsberger, 1989; Buttery, Teranishi, Ling, & Turnbaugh, 1990), which is not consistent with our data.

Ketones are VOCs thought to play an active part in plant defense (Jimenez, Lanza, Antinolo, & Albaladejo, 2009) and as such it is unsurprising that as these compounds are released they contribute to the sensory profile of rocket. They are known to have pleasant odours, and 3-pentanone was significantly correlated with green odour, flavour (both $P < 0.01$) and aftereffects ($P < 0.05$). 3-pentanone has been previously described as having an 'ether' odour (Berger et al., 1989).

Several alcohol, ketone, indole and aldehyde compounds were significantly correlated with sweet attributes, and separated highly along PC2. These include 2-(1,1-dimethylethyl)-1H-indole, 1-penten-3-ol, 1-penten-3-one, 2-hexenal, (Z)-2-penten-1-ol and (E)-2-pentenal (Table S4). In previous studies 3-hexenal has been linked with green, stalky and aromatic attributes (Carrapiso, Jurado, Timón, & García, 2002), but no significant correlations with these was observed in our data. Green flavour was low in SR5, as was relative abundance of 3-hexenal. This may suggest a tentative link between relative abundances of 'green-leaf' VOCs and the perception of pungency caused by sulfur-containing VOCs such as ITCs. From a plant defense point-of-view, this may be an evolutionary strategy to favour one biosynthetic pathway over another and *vice versa*. Differing genetic regulation of GSL synthesis/ITC formation and the octadecanoid pathway for 'green-leaf' VOCs in different cultivars may be responsible for the balance between ITC/sulfur volatile formation and 'green-leaf' volatiles (Ahuja, Rohloff, & Bones, 2010). The relative abundances of VOCs between these two pathways are likely to be a determining factor in rocket sensory properties.

3.3.2.6. Free amino acids. AA concentrations were primarily separated along PC4 (plot not presented), with the exception of proline on PC3. In Fig. 1b AAs are co-located with sweetness attributes, and negatively associated with pungency. AA compounds are known to infer a variety of tastes, and sometimes flavours. Sweet tasting AAs include: alanine, threonine, serine, proline and glutamine; sour/umami tasting include: aspartic acid and glutamic acid; and bitter tasting include: valine and leucine (Nishimura & Kato, 1988; Solms, 1969).

No significant correlations with sweet attributes were observed for alanine, threonine, serine, proline or glutamine, however a general trend was observed for these AAs to correlate in the same spatial orientation of sweetness. Glutamic acid was significantly correlated with sweet aftereffects ($P < 0.05$), which is unexpected, as this AA has been previously described as having umami properties. As an observational trend, high AA concentrations are negatively correlated with strong and pungent rocket attributes in PC1, and concentrations are typically higher in the 'milder' accessions such as SR2 and SR3.

Glutamic acid and aspartic acid did not correlate with savoury (umami) or sour tastes (Kirimura et al., 1969). Aspartic acid was significantly inversely correlated with savoury aftereffects within the model ($P < 0.05$). The AAs known to be bitter (valine and leucine) showed no significant correlations with this attribute. This is unsurprising as these compounds were of very low concentration and were not detected at all in some samples.

3.3.2.7. Sugars. Few significant correlations were found for sugar concentration in the sensory PCA. The spatial positions of the free sugars can be seen in Fig. 1b, in the lower half of the plot, and are associated generally with sweetness attributes along PC3. After this determination, the sugar-GSL ratio was calculated and added as supplementary data and regressed onto the sensory PCA. Previous studies have suggested that the role of sugar-GSL ratios may influence the perception of bitterness (Jones, Faragher, & Winkler, 2006). The correlation matrix revealed no significant correlations with bitter taste, however three significant negative correlations were observed for total sugar-GSL, glucose-GSL, and fructose-GSL ratios with bitter aftereffects (all $P < 0.05$). This indicates that a greater ratio infers a reduction in bitterness after the swallowing of leaves, but does not in turn correspond to a significant increase in sweetness attributes.

3.3.2.8. Organic acids. OAs have been linked with sourness (Tandon, Baldwin, & Shewfelt, 2000) and astringency (Hufnagel & Hofmann, 2008) in previous sensory analyses of other foods. Here we saw no such associations, which was unexpected considering the relatively high accumulations of OAs in rocket leaves compared with other *Brassicaceae*. It is possible that an acid-sugar ratio should be considered, however only one significant negative correlation between this ratio and a sensory attribute (earthy flavour; $P < 0.05$) was observed (Table S3). Studies on apples have shown that the acid-sugar ratio affects sweetness and sourness (Kühn & Thybo, 2001) but in a crop such as rocket with so many bitter and pungent volatiles, it is difficult to separate and identify if such a ratio is truly affecting perceptions.

4. Conclusion

In this study six promising gene bank cultivars of rocket and one commercial comparator (SR3) were used to objectively elucidate the relationships between sensory characteristics and phytochemical content, as well as aspects of appearance. No study of rocket salad has previously encompassed such a wide range of analytical methods and chemical analyses in combination with sensory evaluation. It marks a significant step forward in understanding how compounds interact and influence perceptions. Whilst only a relatively few samples were tested here for practical reasons, it is recommended that in future other cultivars/accessions of rocket should be used to expand upon and elucidate the relationships identified.

There was a large amount of morphological variation between accessions, and this also seems to be the case for some sensory attributes, as these varied significantly for pungent traits such as sulfur, initial heat on the tongue, tingliness, warming sensations, pepperiness, mustard flavour and some of the associated aftereffects. It should be remembered that these accessions are not commercial products, but are effectively wild (with the exception of SR3). That being said, no truly domesticated rocket varieties currently exist because of the relatively short time in which humans have actively bred the species compared to other crops (Bell & Wagstaff, 2014). It is important for breeders to have a wide range of traits to select for within germplasm collections, but this also makes producing a commercially viable end product much more difficult. Bell et al. (2015) highlighted the diversity of GSL and flavonol accumulations in both commercial and germplasm accessions, which vary to a large degree regardless of the source or commercial availability.

Unlike previous sensory studies on rocket, bitterness was not a significantly variable attribute in these particular accessions, but the concentrations of bitter-causing compounds (such as ITCs) are highly variable. This indicates that the sugar-GSL ratio, and perhaps the relative abundances of 'green-leaf' VOCs and ITCs, plays an important role in rocket taste perceptions, and could be utilised and modified by plant breeders in creating new varieties. These relationships would benefit from more in-depth investigation in future studies.

Several ITC compounds were significantly correlated to the well-known hot and pungent rocket attributes, and some VOCs and AAs are negatively associated with these perceptions. ITCs typically constitute <9.0% of the overall VOC headspace bouquet (Bell et al., 2016), suggesting that even in low relative abundances within the headspace of leaves, they have a very large impact upon sensory attributes. Selecting and breeding rocket plants with higher ITC headspace volatile abundance, by even a relatively small amount, may have large effects on the sensory properties of leaves.

The results presented indicate the possibility of elevating health beneficial compounds such as glucoraphanin and glucoerucin without any perceptible or negative changes in sensory attributes. In this study, no significant correlations were observed for these GSLs with any sensory attribute. High glucoraphanin content has been selectively bred for in *Beneforté* broccoli, for example, with no apparent adverse effects on consumer acceptance (Traka et al., 2013). Low concentration GSLs such as 4-hydroxyglucobrassicin also seem to infer, or are related to, an increased perception of pungent attributes. Therefore selecting for 'minor' rocket GSL constituents and ITCs could feasibly lead to the creation of "hot rocket" varieties. This has been attempted commercially through conventional breeding methods, but varieties marketed as such are often unstable across growing environments and have problems with reliable seed production due to a lack of true domestication (Bell & Wagstaff, 2014).

A consumer study of these same seven rocket salad accessions has been conducted, the results of which will be subsequently published. Future work will also consider the impact of the industrial supply chain on phytochemical constituents, and the implications this might have for sensory attributes.

Conflict of interest

The authors declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.foodchem.2016.09.076.

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