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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