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The influence of phytochemical composition and resulting sensory attributes on preference for salad rocket (*Eruca sativa*) accessions by consumers of varying TAS2R38 diplotype

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ABSTRACT

Seven accessions of *Eruca sativa* ("salad rocket") were subjected to a randomised consumer assessment. Liking of appearance and taste attributes were analysed, as well as perceptions of bitterness, hotness, pepperiness and sweetness. Consumers were genotyped for TAS2R38 status to determine if liking is influenced by perception of bitter compounds such as glucosinolates (GSLs) and isothiocyanates (ITCs). Responses were combined with previously published data relating to phytochemical content and sensory data in Principal Component Analysis to determine compounds influencing liking/perceptions. Hotness, not bitterness, is the main attribute on which consumers base their liking of rocket. Some consumers rejected rocket based on GSL/ITC concentrations, whereas some preferred hotness. Bitter perception did not significantly influence liking of accessions, despite PAV/PAV 'supertasters' scoring higher for this attribute. High sugar-GSL/ITC ratios significantly reduce perceptions of hotness and bitterness for some consumers. Importantly the GSL glucoraphanin does not impart significant influence on liking or perception traits.

1. Introduction

Eruca sativa ("salad" rocket) and other species of rocket are popular leafy vegetables consumed all over the world as part of salads or as a garnish (Bennett, Carvalho, Mellon, Eagles, & Rosa, 2007). Previous research has largely focused on the diversity of phytochemical content and post-harvest quality. Studies have investigated the impacts of modified atmosphere and general sensory trends in rocket (Amodio, Derossi, Mastrandrea, & Colelli, 2015; D'Antuono, Elementi, & Neri, 2009; Lokke, Seefeldt, & Edelenbos, 2012; Martinez-Sanchez, Marin, Llorach, Ferreres, & Gil, 2006; Pasini, Verardo, Cerretani, Caboni, & D'Antuono, 2011), however these made certain assumptions regarding what is the 'ideal' or 'preferred' rocket sensory profile of consumers. Few have taken into account the genetic and phytochemical variability of rocket varieties, and none have accounted for the genetic variability of consumers. Harvest, post-harvest and shelf life processes affect salad 'quality' (Amodio et al., 2015), but no study has tested consumers to determine the reasons for their liking/disliking of rocket. This is needed in addition to the quantification of sensory traits to plan and implement breeding and marketing strategies.

Studies by D'Antuono et al. (2009) and Pasini et al. (2011) have combined aspects from both sensory and consumer studies on *Eruca sativa* and *Diplotaxis tenuifolia*. While no scores for liking of traits were given, some subjective descriptive terms were used, such as "typical rocket salad flavour". Both studies used six untrained individuals but the minimum for profiling is eight trained assessors (Carpenter, Lyon, & Hasdell, 2012), and the minimum for a consumer study is 30 (Hough et al., 2006).

Based on these previous studies of preserving appearance and analysing sensory traits (Lokke et al., 2012; Pasini et al., 2011), it is difficult to propose modification of supply chains/breeding programs without knowing the effects of phytochemicals on consumer acceptance. It has yet to be determined which attributes consumers like, and if they are able to discriminate between varieties on the basis of quantifiable traits. Previous studies have been successful at identifying 'bad' sensory traits, such as leaf browning and off-odours (Lokke et al., 2012), as these are uniformly rejected. There has been less focus on identifying positive traits preferred by the consumer.

The reasons given why consumers like the taste and flavour of rocket salad are anecdotal. High levels of bitterness are quoted as

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being a negative aspect of consumer acceptance, but this is not universal (Hayes & Keast, 2011). Across Brassicaceae crops, it is has been demonstrated that bitter tastes contribute negatively to acceptance of products, and this could be part of a protective mechanism to prevent ingestion of harmful compounds, particularly at a young age (Tepper et al., 2009).

Bitterness is cited as the main taste attribute of rocket that consumers reject. It is an extremely complex taste sensation, with 25 putative G-protein-coupled TAS2R receptors existing in humans (Le Nevé, Foltz, Daniel, & Gouka, 2010). Glucosinolates (GSLs) and isothiocyanates (ITCs) have been linked with the gene *hTAS2R38* (Meyerhof et al., 2010) and the thiocyanate moiety (-N-C═S) confers the perception of bitterness, and shows a bimodal distribution of two haplotypes: sensitive and insensitive (Tepper, 2008). Due to genetic recombination, three common diplotypes are present within the human population: PAV homozygotes ('supertasters'), heterozygotes ('medium-tasters'), and AVI homozygotes ('non-tasters'; Hayes, Bartoshuk, Kidd, & Duffy, 2008).

The *hTAS2R38* gene is known to confer varying bitter-tasting sensitivity for certain bitter compounds depending on the diplotype of the person (Wooding et al., 2004). Pasini et al. (2011) suggested that bitterness and pungency in rocket leaves has an association with the GSLs progoitrin/epiprogoitrin and dimeric-4-mercaptobutyl-GSL (DMB). Individuals who have the PAV/PAV 'supertaster' conformation theoretically perceive bitter compounds such as these and their myrosinase derivatives with greater intensity. Some consumers find these tastes overpowering or repulsive and avoid consuming Brassicaceae vegetables (Garcia-Bailo, Toguri, Eny, & El-Sohemy, 2009). By contrast, perceptions of sweetness in other foods increase liking, and for some people, hotness is also a desirable characteristic; e.g. in hot peppers. Hotness is a trigeminal sensation, and consumers vary in their sensitivity according to the number of papillae they possess, and the abundance of associated trigeminal neurons (Reed & Knaapila, 2010). It should be noted that hotness is distinct from pepperiness; in the context of this study, pepperiness refers to the flavour associated with ground peppercorns.

We hypothesised those individuals with PAV/PAV diplotype would score samples more intensely for bitter taste, and negatively for liking of rocket taste than those with PAV/AVI or AVI/AVI diplotypes. This study questioned which of seven *E. sativa* cultivars people preferred based on phytochemical composition and visual and textural characteristics. Data were combined with sensory analysis and phytochemical analyses presented in Bell, Oruna-Concha, and Wagstaff (2015), Bell, Spadafora, Müller, Wagstaff, and Rogers (2016), and Bell, Methven, Signore, Oruna-Concha, and Wagstaff (2017) to determine which sensory attributes are most important for consumers in deciding if they like or dislike rocket. We also tested the hypothesis that sweetness, hotness and pepperiness are positive attributes in rocket consumer acceptance.

The study aims were to (a) determine which sensory attributes contribute most to consumer liking of rocket, (b) determine if TAS2R38 diplotype status influences consumer liking, and (c) determine which specific phytochemical components influence liking and disliking of rocket.

2. Materials and methods

2.1. Plant material

Plant material was grown and harvested under identical conditions to those presented in Bell et al. (2017). SR2, SR5, SR6, SR12, SR14 and SR19 were sourced from European germplasm collections: The Centre for Genetic Resources (CGN; Wageningen, The Netherlands), The Leibniz-Institut für Pflanzengenetik und Kulturpflanzenforschung (IPK; Gatersleben, Germany), and The University of Warwick Genetic Resources Unit (Wellesbourne, UK). SR3 is a commercially available cultivar sold by Elsoms Seeds Ltd. (Spalding, UK).

2.2. Untrained consumer assessments

The untrained consumer study consisted of 91 consenting individuals, who were recruited from in and around the University of Reading (Reading, UK). Recruitment stipulated individuals must be over 18 years of age and be non-smokers. Anchored unstructured line scales were used to determine assessors' liking of overall appearance, leaf shape, mouthfeel and taste (extremely dislike – like extremely). Individual perception of selected sensory attributes (bitterness, hotness, sweetness and pepperiness) were rated using labeled magnitude scales (LMS). Scales ascended from 'not detectable', 'weak', 'moderate', 'strong', 'very strong' to 'strongest imaginable', where spacing between descriptors increased logarithmically. These values were then converted into antilog values and normalised for statistical analyses (Bartoshuk et al., 2003).

Consumers were asked the likelihood of purchasing each of the samples if they were available in supermarkets (5 point category scale; 1 = low purchase intent, 5 = high purchase intent). The questionnaire was designed, and data acquired, using Compusense software (version 5.2; Guelph, ON, Canada). After the testing was complete, consumers were asked to complete a demographic questionnaire and answer questions regarding their usual rocket consumption (n = 90; 1 person declined to answer).

Assessments were conducted in a similar manner to the trained sensory panel presented in Bell et al. (2017) over six weekdays. There were two main differences: consumers were presented with each accession only once, and were asked to assess the two leaves presented for each accession in combination rather than separately. Samples (random coded) were presented in a balanced design over two days (four samples at first visit, three samples at second) to avoid palate and trigeminal fatigue. On the second visit, volunteers were asked to provide a buccal swab sample (in duplicate) using C.E.P. ejectable buccal swabs (Fitzco International Ltd., Plymouth, UK).

2.3. DNA extraction

Buccal DNA samples taken from consenting participants were extracted using an Omega Bio-Tek E.Z.N.A. Forensic DNA Kit (Norcross, GA, USA). 550 µl of phosphate buffered saline (PBS) and 25 µl of protease solution was added to each sample, a further 550 µl of bacterial lysis buffer, then vortexed (30 s). Samples were incubated for 30 min at 60 °C in a heat block with occasional mixing. Samples were subsequently centrifuged (14,000g), then 550 µl of 100% ethanol (Sigma, Poole, UK) was added, vortexed and centrifuged again. 700 µl of sample was passed through a Hi-Bind DNA mini column and centrifuged for 1 min and repeated. 500 µl of isopropanol buffer was added to columns and centrifuged for 1 min. 700 µl of DNA wash buffer (diluted with 100% ethanol) was applied to columns and centrifuged, then repeated. Columns were dried by centrifugation for 2 min. DNA was eluted into sterile micro centrifuge tubes by adding 200 µl of preheated elution buffer (70 °C) and left for 3 min at room temperature (~22 °C). Samples were centrifuged for 1 min and then the elution step was repeated. DNA was quantified using a NanoDrop ND 1000 spectrophotometer (Thermo Scientific, Wilmington, DE, USA) and was subsequently stored at −20 °C until analysis.