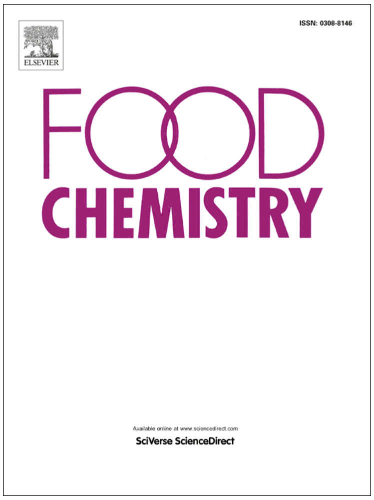

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The influence of phytochemical composition and resulting sensory attributes on preference for salad rocket (*Eruca sativa*) accessions by consumers of varying TAS2R38 diplotype

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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.

Keywords: Glucosinolates; Isothiocyanates; Brassicaceae; Health-beneficial compounds; Leafy vegetables; Bitter taste perception; Pungency; Taste

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 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, & Wagstaff (2015), Bell, Spadafora, Müller, Wagstaff, & Rogers (2016), and Bell, Methven, Signore, Oruna-Concha, & 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). 550µl of phosphate buffered saline (PBS) and 25µl of protease solution was added to each sample, a further 550µl of bacterial lysis buffer, then vortexed (30 s). Samples were incubated for 30 minutes at 60°C in a heat block with occasional mixing. Samples were subsequently centrifuged (14,000 x *g*), 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 minute and repeated. 500 µl of isopropanol buffer was added to columns and centrifuged for 1 minute. 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 minutes. DNA was eluted into sterile micro centrifuge tubes by adding 200 µl of preheated elution buffer (70°C) and left for 3 minutes at room temperature (~22°C). Samples were centrifuged for 1 minute 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.

2.4. SNP genotyping

SNP genotyping kits were obtained from Life Technologies Ltd. (Paisley, UK) according to the three most common alleles of the *hTAS2R38* gene: A49P (rs713598), A262V (rs1726866) and V296I (rs10246939). A reaction mixture of TaqMan Genotyping Mastermix (Life Technologies Ltd.) and primers was prepared as follows: 12.5 µl Mastermix, 1.25 µl primer, 6.25 µl d.H_2O and 5 µl of human DNA template (25 µl total per reaction). 3 non-template controls were used on each genotyping plate. Analysis was performed on a 7300 Real Time PCR system (Applied Biosystems Inc., Foster City, CA, USA). PCR run parameters were as follows: 0 minutes at 55°C, 10 minutes at 95°C, 15 seconds at 92°C and 1 minute at 60°C. Alleles were automatically 'called' by RT-PCR software according to fluorescence probes. Genotype was determined by the presence/absence of the corresponding alleles; the diplotype of 69 individuals was successfully determined. The remaining 21 individuals either: 1) did not consent to having a sample taken (*n* = 1), 2) did not yield sufficient DNA for analysis (*n* = 2), or 3) failed to attend the second study visit (*n* = 19). The expected frequencies of diplotypes were determined by comparison to observations by Mennella, Pepino, Duke, & Reed (2010).

2.5. Phytochemical analyses

Point-of-harvest GSL, flavonol, polyatomic ion (PI), headspace volatile organic compound (VOC), free amino acid (AA), free sugar and free organic acid (OA) data from previous studies were incorporated into a statistical analysis to determine significant correlations with consumer preferences and perceptions. These data can be found in Bell et al. (2015; 2016; 2017). All leaves were harvested 30 days after sowing and grown under identical controlled environment conditions (Hall, Jobling, & Rogers, 2012).

2.6. Statistical analyses

To ensure an unbiased data set, only consumers who attended both tasting sessions were included in statistical analyses (*n* = 67). Preference and perception data underwent analysis of variance (ANOVA) with accessions as a treatment effect.

Individual consumer TAS2R38 diplotypes were input as a nested effect in a separate ANOVA, testing genotype*sample interaction. All ANOVA were conducted using a 95% confidence interval and a tolerance of 0.0001%, and post-hoc Tukey's HSD test was used for multiple pairwise comparisons. Observed TAS2R38 diplotype frequencies were compared with expected frequencies (Mennella et al. 2010) by Pearson's chi-squared test. Any influence of bitter perception (normalised scores) on taste liking was tested by Pearson's correlation.

Agglomerative Hierarchical Cluster (AHC) analysis was used to identify liking and perception clusters; dissimilarity was determined by Euclidean distance, agglomeration using Ward's Method (automatic truncation). ANOVA was then carried out separately for each cluster. All clusters containing ≥20 people, plus clusters of ≤19 with significant discrimination between samples were included in subsequent Principal Component Analysis (PCA) analysis.

Taste liking data were used to extract principal components (PCs; Pearson *n*-1). Phytochemical data were fitted as supplementary variables, as well as the ratios between sugars and GSLs, sugars and ITCs, and organic acids and sugars (see Bell et al. 2017), and cluster means. A correlation matrix was constructed as part of the analysis to determine significant correlations between variables ($P<0.05$, $P<0.01$ and $P<0.001$). Internal preference maps were produced using PCA of consumer data (firstly taste liking, secondly appearance liking), with sensory profiling data and AHC class centroids regressed as supplementary variables. The taste liking preference map also used AHC class centroids relating to mouthfeel liking as well as taste liking, and taste perception (normalised bitterness, sweetness, hotness and pepperiness) and purchase intent as supplementary variables. All analysis was carried out using XLStat (Version 12.0, Addinsoft, Paris, France).

3. Results and discussion

3.1. Consumer demographics and usual rocket consumption

Table 1 presents the summarised demographic data for this study. 77.7% of the participants were between the ages of 18 and 35. Recruitment around the University of Reading, led to high numbers of female participants (*n* = 69; 76.7%), and Asian and African (*n* = 24; 22.2% and 4.4% respectively) participants volunteering for the study. 72.2% of those who took part described themselves as having White ethnicity.

Participants were asked to answer one question about their usual rocket consumption: '*How often do you consume rocket when it is available?*' 36 people (40.0%) stated they sometimes eat rocket when available. 11 (12.2%) stated they never eat rocket, and only 4 (4.4%) said they always consume rocket when available. These responses indicate that the typical consumer makes conscious decisions about the rocket they consume, and there are sensory attributes on which they base these decisions. Rocket from diverse growing regions are currently all used the same way for each salad product sold on the market. Due to this blanket approach to the species, and the inherent sensory diversity present between varieties/growing regions, consistency within products is not guaranteed. For the consumer this could affect the likelihood of re-purchase, and affect how often they choose to consume rocket.

3.2. Consumer preference, perceptions and purchase intent

3.2.1. General

The response of consumers for each perception and preference modality tested is presented in Table 2. Each of the attributes assessed by consumers were consistently divided into three clusters in each respective AHC analysis. The average scores of all consumers are summarised, as well as the results of ANOVA Tukey HSD test pairwise comparisons. Within the text, clusters where a significant difference was observed (Tukey HSD test, $P<0.05$) are denoted by *. Clusters with <20 individuals, but contained significant differences between consumer scores, are denoted by ^.

3.2.2. Appearance liking

Appearance liking scores differed significantly between some accessions (Figure S1). The appearance of SR19 was liked significantly more than SR3 (commercial cultivar) and SR14. SR19 closely resembles the leaf morphology of *Diplotaxis tenuifolia* ("wild" rocket), even though it is *E. sativa*. This demonstrates consumers have generally come to like and accept this leaf appearance, as it is the type they are most familiar with. SR3 and SR14 typically have much broader, less serrated leaf profiles.

From AHC analysis, appearance liking Cluster 2* (C2; n = 38, 56.7%) was the largest, and consumers differentiated their liking of appearance; generally these scores were lower than the total average. SR19 was again the most liked, and was significantly different from the commercial cultivar SR3. Appearance liking C3*^ was composed of only six individuals (9.0%), but showed a propensity for higher than average scores, and discriminated significantly between SR19, SR3 and SR6.

In terms of colour liking consumers discriminated significantly, again favouring SR19 over SR3 and SR12. Cluster analysis identified some consumers (C3*; n = 22, 32.8%) liked the dark green leaf colour of SR19 significantly more than the lighter coloured SR3, SR6, SR12 and SR14.

The liking of leaf shape was also significantly different between accessions. SR19 scored significantly higher than SR3 across all consumers. C3* individuals (*n* = 23, 34.3%) showed a high degree of preference for SR19 over SR2, SR3, SR5, SR6 and SR14, but C1 (*n* = 20, 29.9%) and C2 (*n* = 24, 35.8%) did not show any significant preference. C1 uniformly scored lower than average for all accessions, whereas C2 scored much higher for their leaf shape. These data indicate some people discriminate based on leaf shape, favouring a "wild" rocket-type leaf, but over two thirds show no significant preference.

3.2.3. Mouthfeel liking

The smallest cluster (C2*^; *n* = 7, 10.4%) showed a significant preference for SR3 over SR2, SR5 and SR19. Generally this attribute is comparatively unimportant with regards to most consumers' preferences, with only a minority discriminating in their liking of these accessions.

3.2.4. Taste liking

Considering the whole consumer group there was no significant difference in the liking of taste between samples, and this was reflected in the largest cluster (C2, *n* = 36; 53.7%). The minority cluster (C3^, *n* = 6; 9.0%) disliked the taste of most rocket samples (scoring <50). For C1* (*n* = 25; 37.3%) there was a significant difference between accessions where the taste of the commercial sample (SR3) was liked significantly higher than for SR12. These people were generally very accepting of all seven samples (scoring >63.4), yet still differentiated significantly between them.

These data suggest over half of the people tested are indifferent to the taste of the tested cultivars, whereas a proportion of people like all rocket, but especially the milder cultivar (SR3). A small percentage of people conversely reject rocket taste to a large degree, and they do not discriminate for this modality.

3.2.5. Bitterness perception

The perception of bitterness has long been held as a defining criterion of whether individuals accept or reject *Brassicaceae* vegetables. The role diplotype of the TAS2R38 taste receptor plays in this response will be explored in following sections, but irrespective of genetics, consumers could differentiate bitterness significantly between some cultivars.

SR12 was perceived as more bitter than SR6 and SR19. Bitter perception C1* was the largest cluster (*n* = 49, 73.1%) and scores were low compared to the average. These people found SR14 to be significantly more bitter than SR6, whereas C2*^ (*n* = 14; 20.9%) conformed to the significance observed in the total average scores (Table 2). These individuals scored higher by comparison to the average and to C1*, but not as high as the minority cluster C3^ (*n* = 4, 6.0%).

Neither SR12 nor SR14 contain especially high concentrations of GSLs (Bell et al. 2015) or volatile ITCs (Bell et al. 2016). Following the assumption these compounds are generally responsible for bitterness in rocket, one would expect SR5 to be perceived as the most bitter as it has been found to contain 11.5 mg.g^{-1} dw in total GSL concentration, and observed to have a high percentage of volatile ITCs within the headspace. This suggests other compounds present within leaves contribute to bitterness to a greater degree than has been previously realised. The counter-hypothesis is the bitterness caused by GSL-related compounds are masked to some degree, either by sugars, amino acids, or green-leaf VOCs (Bell et al. 2017).

3.2.6. Hotness perception

The perception and level of hotness has been used anecdotally to characterise the 'ideal' rocket leaf, and was defined in Bell et al. (2017) as the initial burst of heat experienced momentarily after mastication. As a whole cohort, consumers perceived SR19 to be the hottest and significantly different from SR2, SR3, SR6, SR12 and SR14. SR19 was shown to contain lower concentrations of GSLs than all of these accessions (with the exception of SR3; Bell et al. 2015), and as with bitterness, indicates other compounds influence the perception of hotness, such as the sugar-ITC ratio (see *3.5.2.7.*).

Hotness was the only attribute measured in which all clusters discriminated significantly between accessions. C2* was the largest cluster (*n* = 34, 50.7%) and mirrored the consumer average, perceiving SR19 to be hotter than all of the other accessions. The smaller clusters did not follow this trend – in particular C3*^ (*n* = 19; 28.4%) perceived SR5 to be hotter than SR2 and SR14, and C1*^ (*n* = 14, 20.9%) found SR12 to be the hottest and significantly different from SR2, SR6, SR14 and SR19. The apparent differences in perceptions between each of the clusters infers a genetic component is responsible, but further study of papillae numbers and specific genes involved would be required to draw any meaningful conclusions. As observed for attributes associated with heat in Bell et al. (2017; initial heat, tingliness, warming) the hotness attribute measured here has a significant degree of variability. This suggests heat is a key characteristic in determining the liking of rocket, rather than bitterness, as has been observed in other crops (Schonhof, Krumbein, & Brückner, 2004).

3.2.7. Sweetness perception

Several significant differences were observed for sweetness perception on average and in the AHC analyses. Overall, the consumers found SR6 to be sweeter tasting than SR5 and SR19, which have been previously noted for high levels of hotness (Bell et al. 2017).

C3* was the largest cluster for this attribute (*n* = 40; 59.7%) and scores were generally much lower than the average, and those of C1^ (*n* = 19; 28.4%) and C2*^ (*n* = 8, 11.9%). C3* found SR2 to be significantly sweeter than SR5 and SR19, and C2*^ found SR6 to be significantly sweeter than all the other accessions. C1^ individuals displayed no discrimination between samples, despite their scores being higher than the average. These data suggest the pungent compounds found in accessions such as SR5 and SR19 mask sweetness perception, which in turn mask bitterness. To develop new varieties of rocket that are more acceptable to the consumer, hotness, sweetness and bitterness must be considered together, not in isolation.

3.2.8. Pepperiness perception

SR19 was again scored significantly higher than SR12 for pepperiness overall, and higher than SR2 and SR12 in C1* (*n* = 44; 65.7%). C3*^ (*n* = 18; 26.9%) scores were by comparison higher than the average, but SR2 was perceived as being more peppery than SR14. The differences between the two main clusters (C1* and C3*^) suggest a subset of people perceive this attribute more intensely. Further study is needed in this area, as no previous data have been published in relation to rocket and consumer perceptions/liking of this trait.

3.2.9. Purchase intent

Overall there were no significant differences found for purchase intent, or for C1 (*n* = 31, 46.3%) and C3 (*n* = 21, 31.3%). C1 scores were generally higher than average, indicating the largest proportion of the cohort would consider buying most of the accessions were they all commercially available. C3 by comparison had lower than average scores, and would likely not buy any of the rocket accessions. Significant differences were observed for the smallest cluster, C2*^ (*n* = 15, 22.4%). These individuals would be significantly more likely to purchase SR19 than SR2, SR6 or SR14. These varieties are typically milder and sweeter, according to the cohort averages. The basis of preference is likely to be a combination of appearance and perception traits, with SR19 consistently being scored favorably for liking of appearance, hotness and pepperiness.

3.3. Effects of TAS2R38 diplotype

3.3.1. Taste liking and bitterness perception

Table 3 presents the numbers of each observed diplotype within the study. There was no significant difference between the observed and expected frequencies (Mennella et al. 2010; chi squared, P = 0.95). Figure 1 shows their respective average responses for perceived intensities of bitterness (a) and liking of taste (b).

TAS2R38 genotype had a significant effect on bitterness perception ($P<0.02$) (Figure 1a), and the effect of consumer genotype on bitterness scores was $P<0.02$ (ANOVA sum of squares analysis). This suggests a significant effect on bitter perceptions, but in the ANOVA there were no significant differences between genotypes within a specific rocket accession. The effect of diplotype is not as pronounced as was originally hypothesised, but a general trend for ‘non-tasters’ to score bitterness of rocket lower than ‘medium’ or ‘supertasters’ is apparent.

The effect of consumer genotype was significant for liking of taste ($P<0.004$; ANOVA sum of squares analysis) however pairwise comparison scores (Figure 1b) were not significant when the interaction with the sample was taken into account. AVI/AVI individuals generally scored higher for liking in some accessions of rocket, however this pattern was reversed in accessions where bitter scores were low (SR3). In this instance, SR3 has been noted for high concentrations of AAs (Bell et al. 2017), and for PAV/PAV ‘supertasters’ the relatively low concentration of GSLs and volatile VOCs infer higher liking.

The disparity between bitter perceptions and taste liking suggests TAS2R38 diplotype is only one of (potentially) many factors influencing an individual’s preference. A correlation test was performed independently of diplotype status on the total cohort data, comparing taste liking with bitterness perception. This test showed a significant negative relationship between the two attributes (r = -0.227, $P<0.0001$) and infers as bitter perception increases taste-liking decreases.

A similar observation was made by Shen, Kennedy, & Methven (2016) for perceptions of bitterness and liking in raw broccoli and white cabbage. Influences on liking according to TAS2R38 diplotype were observed, but this determination alone was not an accurate predictor of whether an individual would like or dislike *Brassica*-type vegetables. Other factors, such as consumer demographics, fungiform papillae density, familiarity with the food, and the conformation of other TAS2R taste receptors may also influence liking and preference in rocket.

3.3.2. TAS2R38 diplotype frequencies between agglomerative hierarchical clusters

The individuals in the two largest clusters for taste liking (C1* and C2) were scrutinised to see if the respective TAS2R38 diplotype frequencies therein conformed to the expected population frequency. As previously stated, C1* individuals tended be more discriminating of accessions (preferring SR3 overall) and C2 were indifferent. We hypothesised the frequency of PAV/PAV individuals would be higher in C1*, which would account for their preference of a non-bitter accession of rocket.

The frequencies of each diplotype in each cluster were compared to total expected population frequencies (Mennella et al. 2010; Table 3) by chi-squared tests. No significant differences were found between the observed and expected frequencies in either cluster (C1*: *P* = 0.918; C2: *P* = 0.564). There was no significant difference in diplotype frequencies between the two clusters either (*P* = 0.919), further suggesting TAS2R38 status is not a singularly determining factor in consumer preference of rocket. The basis for preference is likely due to learned responses and/or other sensory factors as mentioned in the previous section (Shen et al. 2016).

3.5. Principal Component Analysis

3.5.1. Correlations between consumer preference & perceptions

Two biplots from the PCA are presented in Figure 2 and PCs were extracted on the basis of consumer taste liking scores. A total of six components were generated, all with Eigenvalues >1.0, but only the first five contained >10% of the explained variation. PC1 explained the largest amount of variance (24.9%) and predominantly separated SR12 from all other products. The other dimensions (PCs 2 to 5) all gave differing separations of the remaining accessions. PCs 1 vs. 4, and 1 vs. 5 have been selected for discussion as they represented the highest correlations with the supplementary AHC centroid scores and phytochemical variables according to their respective loadings scores; they are most informative for the purposes of this discussion. Cumulatively, these PCs illustrate 53.7% of the total variation within the data. For respective cluster scores for each accession refer to Table 2.

Mouthfeel liking C1 and taste liking C1* correlated highest along PC1 (Figure 2). These clusters locate closely with SR3 and purchase intent C1, indicating a preference of the commercial cultivar for some consumers. The bitterness of accessions such as SR12, to the extreme left of PC1 and away from SR3, indicates this preference is in part due to bitterness being perceived more intensely between accessions.

Sweetness perception C3* correlated most strongly with PC5, as did purchase intent C1. These attributes again co-locate near SR3 and SR2, further indicating bitterness and hotness are not desirable traits for a subset of the cohort. Similarly pepper perception C1* correlates most strongly along PC4. In the top right corner of Figure 2a, this attribute is associated with SR3 and SR19, and this suggests some individuals favor mild, peppery cultivars most. The individuals correlating highest along PC4 generally co-locate with SR19 and purchase intent C2*^ (Figure 2a). Combined with the relatively low perceptions of bitterness, these data indicate SR19 would be well suited to develop into a commercial product. Individuals showing a high degree of preference for SR19 would therefore be more likely to purchase rocket if it had more heat and pepperiness, and a low level of bitterness.

3.5.2. Correlations between consumer preference, perceptions & phytochemical content

3.5.2.1. General

A summary table of all phytochemical-AHC correlation coefficients and significances is presented in supplementary Table S1.

3.5.2.2. Glucosinolates

In the PCA biplot presented in Figure 2, concentrations of GSLs yielded significant correlations with consumer preference and perception AHC centroids. Glucosativin was significantly inversely correlated with scores for purchase intent C1 and mouthfeel liking C1 (both $P<0.05$). Individuals in these clusters were non-discriminatory but gave higher than average scores for each accession. Glucosativin is the most abundant GSL in these samples, and a high abundance infers reduced liking.

Glucoraphanin concentration has no significant positive or negative effects on consumer preferences or perceptions, indicating it and its hydrolysis products do not have an inherent taste. The compound separates strongly on PC5 (Figure 2b), and towards the upper left, away from the positions of perception clusters. The broccoli variety *Beneforté* has been bred for very high concentrations of glucoraphanin/sulforaphane, and no significant impacts on taste or flavour have been reported (Traka et al. 2013).

Another health beneficial GSL is erucin, which separates along PC5, and significantly with sweetness perception C2*^ ($P<0.01$). Glucoraphenin is also significantly correlated with this attribute (PC5; $P<0.05$), but is only found in small concentrations in SR2 and SR6 (Bell et al. 2015). These compounds are unlikely to be causing sweetness, but are more abundant in sweet-tasting accessions (Bell et al. 2015; 2017). Future rocket breeding should perhaps be selective for individual health beneficial GSLs such as glucoraphanin and glucoerucin, as suggested by Ishida et al. (2014).

Glucoalyssin was significantly correlated with pepper perception C1* and hotness perception C2* scores ($P<0.01$ and $P<0.05$, respectively). 4-hydroxyglucobrassicin was positively correlated with scores from hotness perception cluster C3*^ and negatively with sweetness perception C3* (both $P<0.05$). These observations were also made by Bell et al. (2017) and indicate ‘minor’ GSLs of rocket contribute significantly to taste and flavour perceptions. Just as glucoraphanin is selected to produce health beneficial properties in plants, minor GSLs could also be selected to produce enhanced sensory properties.

3.5.2.3. Flavonols

Negative correlations were observed for isorhamnetin-3-glucoside with hotness perception C2*, and quercetin-3,3,4’-triglucoside and kaempferol-3-(2-sinapoyl-glucoside)-4’-glucoside with pepper perception C1* (all $P<0.05$). The reduction in perceptions implies an increased abundance of these flavonols is associated with reduced pungency.

Another significant positive correlation observed was for bitter perception C1*, the largest bitter perception cluster, and kaempferol-3-(2-sinapoyl-glucoside)-4’-glucoside ($P<0.05$). It is unusual for a flavonol to have bitter taste, though in the complex matrix of the rocket leaf, consumers could have interpreted astringency as bitterness. It is likely field-grown rocket would have produced higher concentrations of flavonols due to higher light intensities than controlled environment (Bell et al. 2015; Jin et al., 2009), and therefore might have produced stronger effects within the data. Further study is needed to properly determine the extent that flavonol glycosides influence taste attributes in rocket.

3.5.2.4. Polyatomic ions

Nitrate and sulfate were both correlated with the largest hotness perception cluster (Figure 2, C2*; both *P*<0.05). In Figure 2a, these are closely associated with SR19, which is likely responsible for the significant correlations.

Nitrate and sulfate assimilation pathways are known to be integral to GSL and amino acid metabolism within leaves (Hirai et al. 2004). By comparison to the other cultivars, GSL concentration was not high in SR19 (Bell et al. 2015), which suggests total GSL content alone is not a good indicator of hotness of rocket. The diversity of GSLs and VOCs, and the relative concentrations of accumulated PIs and free sugars likely interact to determine the heat perceived. Future studies should therefore explore and take these aspects into consideration when conducting sensory and phytochemical analyses of rocket.

3.5.2.5. VOCs

C numbers in bold within the text refer to VOCs labeled in Figure 2; see Table S1 for a list of compounds and their corresponding abbreviations.

An unexpected association with sweetness perception C3* was observed with 3-methyl-furan (**C27**; *P*<0.01), and a corresponding negative correlations with hotness perception C3*^ and pepper perception C1* (both *P*<0.05). Bell et al. (2017) observed that this compound was significantly inversely correlated with bitter perception, but no corresponding association with sweetness. C3* was the largest cluster for sweetness perception, and the high degree of separation along PC5 (Figure 2b) means the compound could be utilised as a chemical marker for non-pungent, sweeter varieties of *E. sativa*. The compound was also significantly correlated with increased purchase intent C3 (who generally would not buy rocket), and inversely correlated for purchase intent C2*^ (who discriminated for the hot accession SR19). This suggests hotness is preferable for one group of consumers, but is rejected by another.

Sweetness perception C3* also shared corresponding significant negative correlations with 4-methylpentyl-ITC (**C20**), 1-isothiocyanato-3-methylbutane (**C23**), iberverin (**C33**), pyrrolidine-1-dithiocarboxylic acid 2-oxocyclopentyl ester (**C36**) and an unknown compound (**C40;** all *P*<0.05). Individually, very little is known about the aroma characteristics of these compounds, but ITCs and their derivatives are generally known for sulfurous, pungent and unpleasant attributes (Engel, Baty, Le Corre, Souchon, & Martin, 2002). These data suggest higher abundance has a powerful masking effect on sweetness. This is particularly evident in Figure 2b where these compounds are clustered near to SR5 and SR19, which are both noted for their hotness (Table 2).

The same compounds were positively correlated with hotness perception C2* and C3*^ (**C20, C23, C36**, *P*<0.05; **C33**, *P*<0.01). Additionally, 5-nonanone oxime (**C21**) and tetrahydrothiophene (**C38**; both *P*<0.05) were also associated with these clusters. The later compound in particular has been previously associated with hotness and pungency in rocket (Bell et al. 2017).

Pepper perception C1* (discriminated for SR19) was negatively correlated with 3-methyl-furan (**C27**), as with hotness perception C3*^ (Figure 2b). Pepperiness perception C3*^ shared negative correlations with several volatiles, such as 2-hexenal (**C7**), (E)-2-pentenal (**C10**), 5-ethyl-2(5H)-furanone (**C12**) and ethylidene-cyclopropane (**C24**; all $P<0.05$). The green-leaf VOCs **C7** and **C10** were noted by Bell et al. (2017) for being linked with sweeter-tasting cultivars, and detracting from the sensations of bitterness and pungency. **C12** has previously been observed in tomato as a degradation product of (Z)-3-hexenal (**C16**; Buttery & Takeoka, 2004). The presence of these compounds within the headspace of rocket has important implications for consumer perceptions of pungent traits.

The dichotomy between those individuals who prefer hotter accessions and those who prefer milder can be seen in highly significant correlations with the ITC **C23**. Purchase intent cluster C2*^ (who discriminated for SR19) are positively correlated with this compound ($P<0.01$) and purchase intent cluster C3 (who had uniformly low scores for purchase intent) is the inverse of this ($P<0.01$). This implies part of the reason why the latter individuals (31.3%) scored the accessions so low is because of the abundance of ITCs. Taking into account the fact that glucoraphanin shared no significant correlations with sensory perceptions, it is desirable to breed rocket with reduced pungency and maintain health beneficial components. This would cater to the previously undefined demographic of consumers who reject rocket because of the hotness of leaves.

3.5.2.6. Free amino acids

High free AA concentrations detracted from the perception of pungent compounds such as ITCs in Bell et al. (2017). In this study only one significant negative correlation was observed between pepper perception C1* and proline concentration. Proline is spatially distant at the bottom of the plot (Figure 5a), separating negatively along PC4 from the peppery accession SR19.

Threonine correlated significantly with purchase intent C1 (*P*<0.05) and is known to have sweet taste (Nelson et al. 2002). AAs correlated along PC5 (Figure 2b) and are more highly associated with the milder accessions SR2 and SR6. This indicates amino acid content is generally in opposition to hotness, but further study is needed to determine the full extent of the effects. Repeat experiments with other cultivars of rocket would help to confirm or reject this hypothesis.

3.5.2.7. Free sugars, organic acids and compound ratios

Fructose concentration was positively correlated with purchase intent C3 (*P*<0.05), further suggesting these individuals would prefer rocket sweeter and less hot. Correlations with sugar-GSL and sugar-ITC ratios were more numerous. Purchase intent C3 (where scores were uniformly low) was correlated with high fructose-GSL, galactose-GSL and sugar-ITC ratios (all *P*<0.05). This suggests the ratios between sugars and GSLs/ITCs are more important in determining consumer acceptance than the concentrations of each compound individually. The sugar-ITC ratio had a negative correlation with hotness perception C3*^ (*P*<0.05), inferring higher sugar content masks hotness for a proportion of consumers, but not all, as no corresponding correlations were observed for C1*^ or C2*.

The sucrose-GSL ratio negatively correlated with bitterness perception C2*^. This ratio is almost directly opposite to SR12 (Figure 2b), separating strongly along PC1. SR12 was noted for high perceptions of bitterness (Table 2), and these data infer, for a proportion of the cohort (20.9%), the effect was an important determining factor in their responses. As this was not seen in the other clusters, other factors such as TAS2R receptor status and fungiform papillae density could impact the effect sugar-GSL ratios have upon perceived bitterness.

3.6. Internal preference map PCA

3.6.1. Sensory perceptions

Figure 3a presents a preference map of consumer taste liking scores, where sensory panel data for all attributes (taken from Bell et al. 2017; except appearance traits; see following section) and AHC centroids for mouthfeel liking, taste liking, perceptions and purchase intent have been regressed as supplementary variables. A summary table of relevant correlations is presented in Table S2.

Six PCs were extracted from the consumer liking data, with all having Eigenvalues >1.0. PCs 1 – 5 contained >10% of explained variation, respectively, but PC1 and PC2 discriminated most strongly for consumer responses, AHC centroid scores and sensory attribute scores. As such these two components were selected for presentation and 44.4% of the total variation is explained.

Of note are several correlations between sweet perception C3* and sensory analysis scores. Centroid scores for this cluster (which were discriminatory, but generally low) were inversely correlated with attributes such as stalky odour ($P<0.05$), bitter taste ($P<0.01$), bitter aftereffects ($P<0.05$) mustard aftereffects ($P<0.05$) and initial heat mouthfeel ($P<0.05$). These correlations suggest perceptions of sweetness for these individuals are low predominantly because of the pungency, heat and bitterness of leaves (such as in SR5 and SR19) masking the taste.

Taste liking C1* was negatively correlated with earthy flavour attributes identified by the trained assessors ($P<0.05$). This was also seen for purchase intent C1 ($P<0.01$), where scores were generally high for all accessions, but lower where earthy flavour was more prominent (SR12; Figure 3a). Taste liking C2 by comparison was negatively correlated with mustard odour (*P*<0.05). Purchase intent C3 was negatively correlated with bitter taste (*P*<0.05) and further implies a uniform dislike of rocket because of their perceptions of bitterness and hotness.

3.6.2. Appearance liking

Figure 3b illustrates a preference map of consumer appearance liking scores, where sensory data for appearance traits (Bell et al. 2017), and AHC centroids for appearance liking traits and purchase intent have been regressed onto the PCA. A summary table of relevant correlations is presented in Table S3. Six PCs were extracted from the data, with all scoring >1.0 Eigenvalues and >10% explained variability, respectively. PCs 1 and 3 discriminated the supplementary variables to the highest degree, and were selected for presentation (44.3% of data variation is explained).

A disparity between leaf shape clusters was observed. Leaf shape liking C1 was negatively correlated with leaf shape uniformity scores from the sensory analysis (*P*<0.01), whereas leaf shape liking C3* was positively correlated (*P*<0.05). C3* individuals, who discriminated for SR19 and the traditional rocket shape, prefer this type of leaf and the relative uniformity of the accession. C1 individuals did not discriminate significantly, but tended towards liking the shape of the broad-leaved accessions. A proportion of people therefore find the novel leaf types unobjectionable, but another proportion prefers the more familiar “wild” type. This dichotomy in preference can be observed in Figure 3b where these clusters are in opposing quadrants of the biplot, and associated with SR19 in the upper right of the plot, and SR5 and SR6 in the lower left.

Correlations along PC1 indicate many consumers overall preferred the appearance of SR19. The high concentration of data points to the right is indicative of this, and the shape, colour, serrated and dark green leaf type of this accession has likely driven this trend in the consumers. There is an indication of a general and substantial preference of this accession over the less familiar, round-shaped leaves overall. SR2, SR3, SR12 and SR14 are associated with attributes such as leaf hairiness and purple stem. It is perhaps unsurprising that hairiness is an undesirable attribute, but the purple stem has previously been thought of as a unique selling point for varieties, such as in the variety *Dragon's Tongue* (Tozer Seeds). This trait was significantly and inversely correlated to purchase intent C2*^ (*P*<0.01), indicating a proportion of individuals found this trait to be undesirable.

4. Conclusions

This study has for the first time conducted a consumer analysis of *E. sativa* accessions in conjunction with sensory, phytochemical and human genotype analyses. The hypothesis all consumers reject bitter tasting cultivars is not fully supported by the data presented, even when human TAS2R38 diplotype of consumers is considered. Genotype effects are significant in determining the degree to which a person will rate the bitterness of rocket and their liking of taste; but when considered with sample effects, pairwise comparisons did not reveal significant differences with any specific cultivar tested. 'Supertaster' (PAV/PAV) individuals generally scored higher for bitterness and lower for taste liking, whereas AVI/AVI individual were the opposite of this (with the exception of the commercial cultivar, SR3). When these data are viewed in combination with AHCs and phytochemical correlations, it seems the predominant basis of acceptance/rejection is actually more related to the perceived hotness of leaves, rather than bitterness.

Distinct clusters of consumer have been identified that show preferences for different accessions on the basis of phytochemical content and sensory properties, such as for and against ITCs and potent sulfur-containing VOCs. Our second hypothesis that hotness, pepperiness and sweetness were positive traits was therefore not wholly accurate. Consumers preferred peppery cultivars like SR19, but a substantial proportion of people within the study preferred the 'milder' cultivar SR3. Many of the consumers were indifferent to any of the accessions, and roughly a third would generally not purchase these cultivars.

The results run in opposition to the general dogma that a) rocket varieties should all be hot, but not bitter, and b) consumers either like or dislike varieties on this basis. The present study has shown this is an oversimplification of reality, and reduced hotness is a desirable sensory trait for a subset of consumers. Some of the consumers analysed preferred the hotness, pepperiness and appearance of SR19, perhaps making it the most accepted "all-round" accession tested in this study. By comparison, SR12 was perceived negatively due to its high levels of bitterness, and SR5 was not favored because of its high levels of hotness and low levels of sweetness.

High concentrations of specific phytochemicals that typically contribute towards hot and bitter sensations are not acceptable to some consumers. Breeding varieties for high total GSL/ITC content is an unsophisticated approach that does not account for these differences in consumer preference. Some preferred the hot ITC and sulfur compounds that are produced from and associated with the GSL-myrosinase reaction (as in SR19), but a substantial proportion rejected accessions because of low sugar-ITC ratios.

It is also important to note the health beneficial GSL glucoraphanin had no significant effect on consumer perceptions and preferences. This adds weight to our hypothesis that specific GSLs can be increased through breeding without having a negative impact on sensory attributes (Bell et al. 2017). With regular consumption of rocket and sulforaphane (the ITC of glucoraphanin) consumers could potentially improve their long-term health and reduce the risk of developing chronic diseases, such as cardiovascular disease and some forms of cancer (Traka et al. 2013).

The results of this study illustrate consumers of rocket leaves are able to differentiate between accessions, and are much more sophisticated in their evaluation of leaves than has been previously realised. Not all consumers of rocket are alike, and as such desire products that match their tastes. Plant breeders and processors must attempt to amalgamate positive visual, sensory and phytochemical traits in rocket to expand the market to individuals who at present are not specifically catered for. This can be achieved in the short term by selection of varieties that can produce a known and consistent standard of expected 'quality', and are well suited to specific growing regions or climates. In the long term, new varieties must be produced that account for the diverse preferences of consumers, such as those who prefer sweet and 'milder' leaves, and those who prefer hot and peppery leaves. These products must also be marketed appropriately; just as different types of apples are known for their differing sweet and sour tastes, rocket types could also be subdivided according to sensory properties and their intended consumer demographic.

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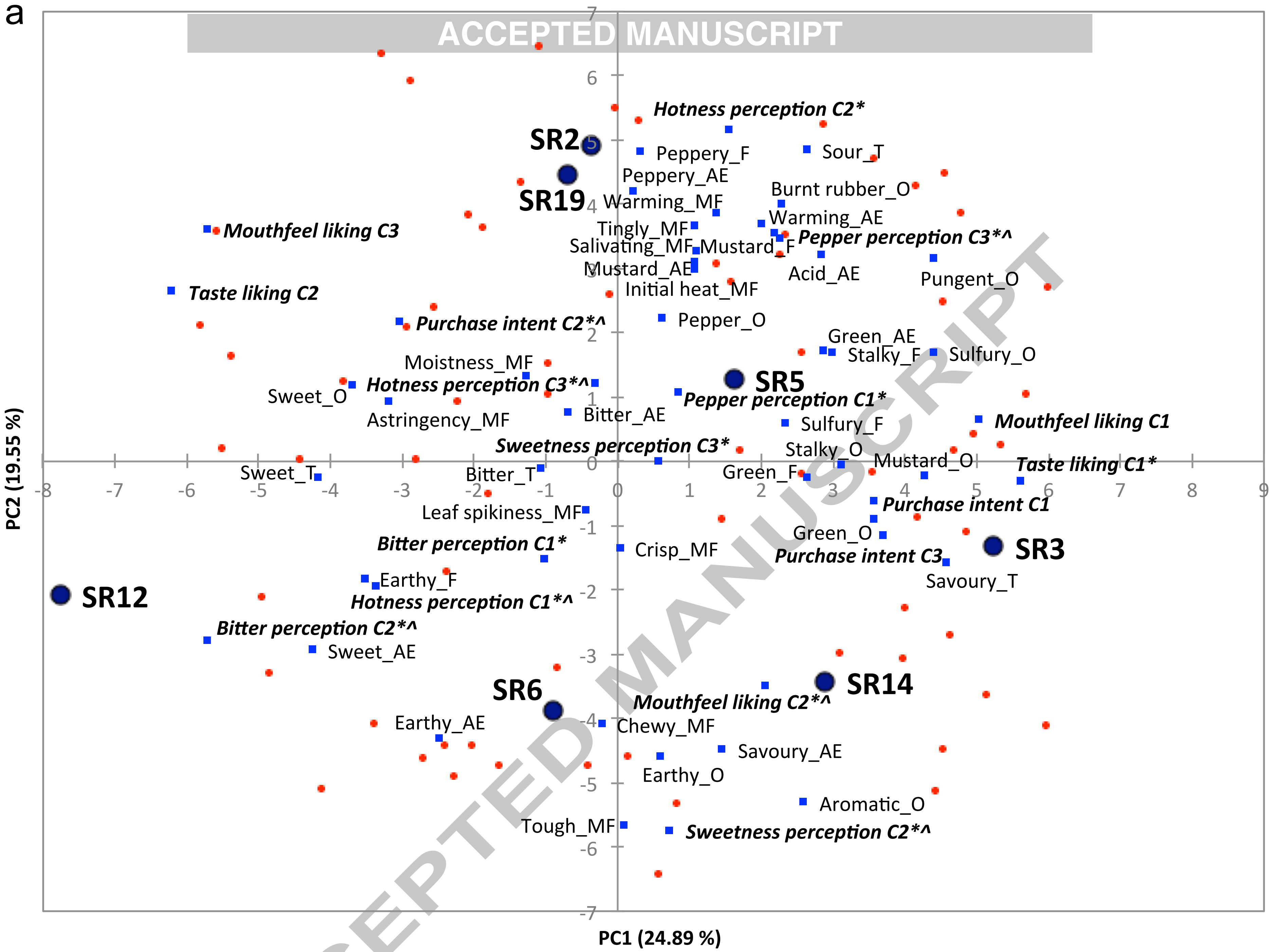
Figure legends

Figure 1. Consumer scores for bitterness perception (a) and taste liking (b) for seven accessions of *Eruca sativa* according to TAS2R38 taste receptor diplotype. Perception scores are given as normalised antilog values (a); differences in letters at the top of each bar indicate significant differences of ANOVA pairwise comparisons within and between accessions (P<0.05). An absence of letters indicates no significant differences were observed. See inset for diplotype colour coding.

Figure 2. PCA biplot of consumer taste liking with phytochemical and AHC analysis (in bold italic; refer to Table 2) data regressed as supplementary variables. * = Significant differences observed with ANOVA (P<0.05). ^ = AHC cluster with <20 individuals. PC1 vs. PC4 (a) represents 41.5% of variation within the data, and PC1 vs. PC5 (b) represents 37.1% of variation within the data. Red circles = individual consumer responses; blue squares = supplementary variables; dark blue circles = rocket accession factor scores. VOC compound abbreviations (C#) are summarised in supplementary Table S1, but can also be found in Bell et al. (2016).

Figure 3. Internal preference map PCA biplot of consumer taste liking (a) and consumer appearance liking (b) with AHC analysis (in bold italic; refer to Table 2) and sensory data regressed as supplementary variables (obtained from Bell et al.

2017) PC1 vs. PC2 (a) represents 44.4% of variation within the data, and PC1 vs. PC3 (b) represents 44.3% of variation within the data. Red circles = individual consumer responses; blue squares = supplementary variables; dark blue circles = rocket accession factor scores. Sensory variable suffix abbreviations: A = appearance; O = odour; T = taste; F = flavour; MF = mouthfeel; AE = aftereffects.


a
Hotness perception C2*
SR2
Peppery_F
Sour_T
Peppery_AE
SR19
Warming_MF
Burnt rubber_O
Warming_AE
Mouthfeel liking C3
Tingly_MF
Salivating_MF
Mustard_F
Pepper perception C3*^
Mustard_AE
Acid_AE
Pungent_O
Taste liking C2
Initial heat_MF
Purchase intent C2*^
Pepper_O
Green_AE
Stalky_F
Sulfury_O
Moistness_MF
Hotness perception C3*^
SR5
Sweet_O
Pepper perception C1*
Astringency_MF
Bitter_AE
Mouthfeel liking C1
Sulfury_F
Sweetness perception C3*
Stalky_O
Mustard_O
Taste liking C1*
Sweet_T
Bitter_T
Green_F
Purchase intent C1
Leaf spikiness_MF
Green_O
Bitter perception C1*
Crisp_MF
Purchase intent C3
SR3
Savoury_T
Earthy_F
SR12
Hotness perception C1*^
Bitter perception C2*^
Sweet_AE
SR14
SR6
Mouthfeel liking C2*^
Earthy_AE
Chewy_MF
Savoury_AE
Earthy_O
Aromatic_O
Tough_MF
Sweetness perception C2*^
7
6
5
4
3
2
1
0
-1
-2
-3
-4
-5
-6
-7
-8
-7
-6
-5
-4
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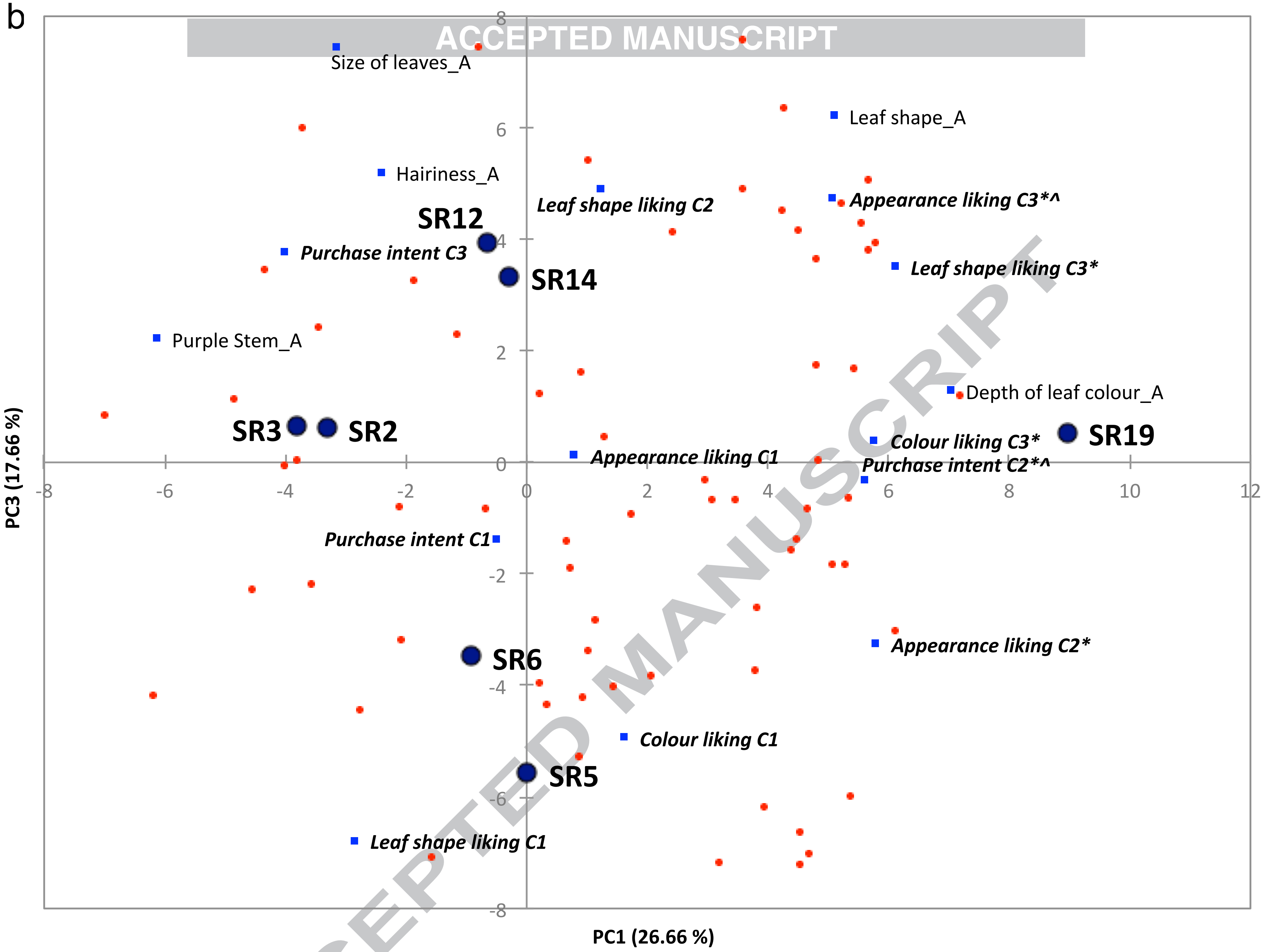
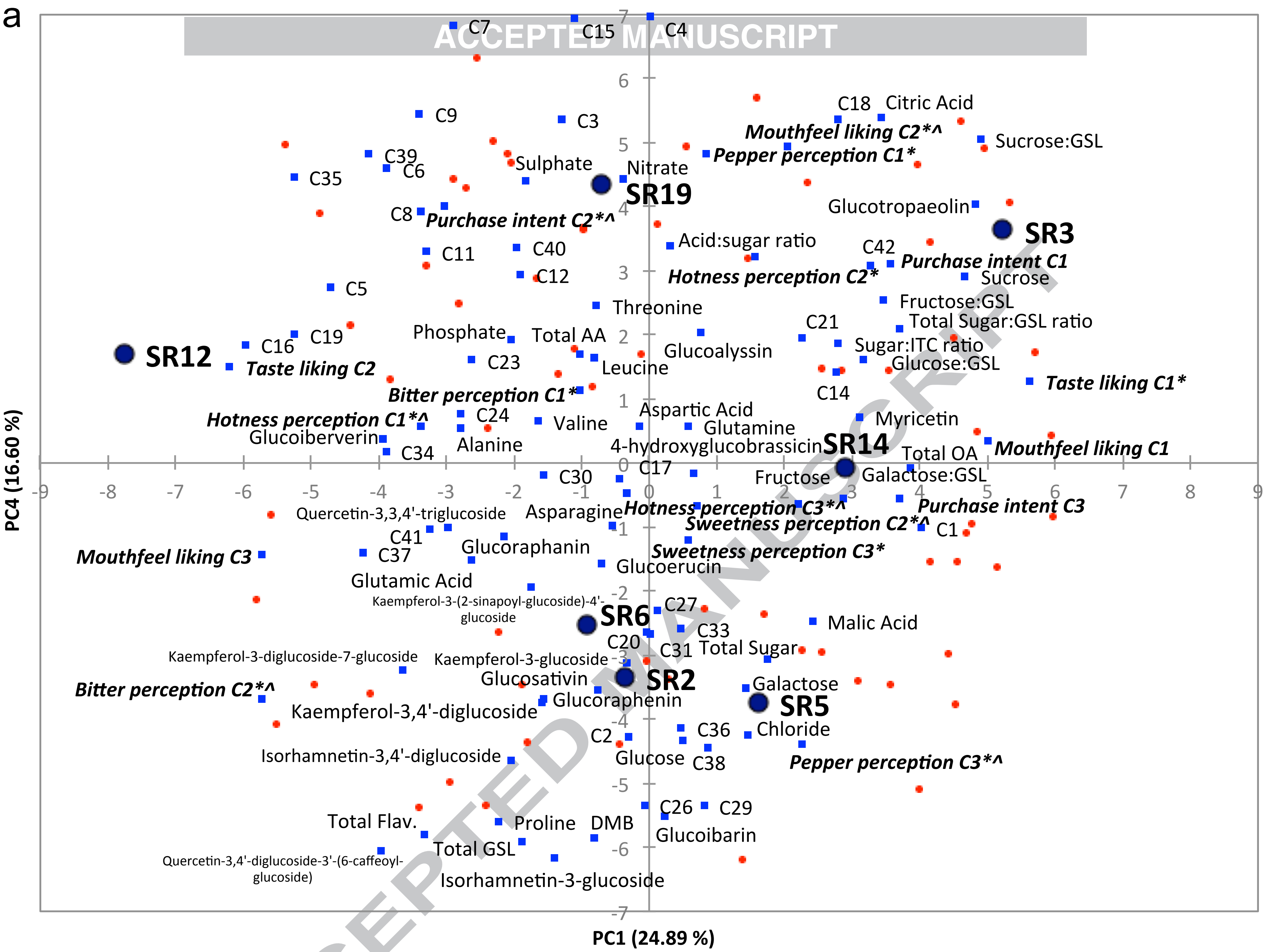
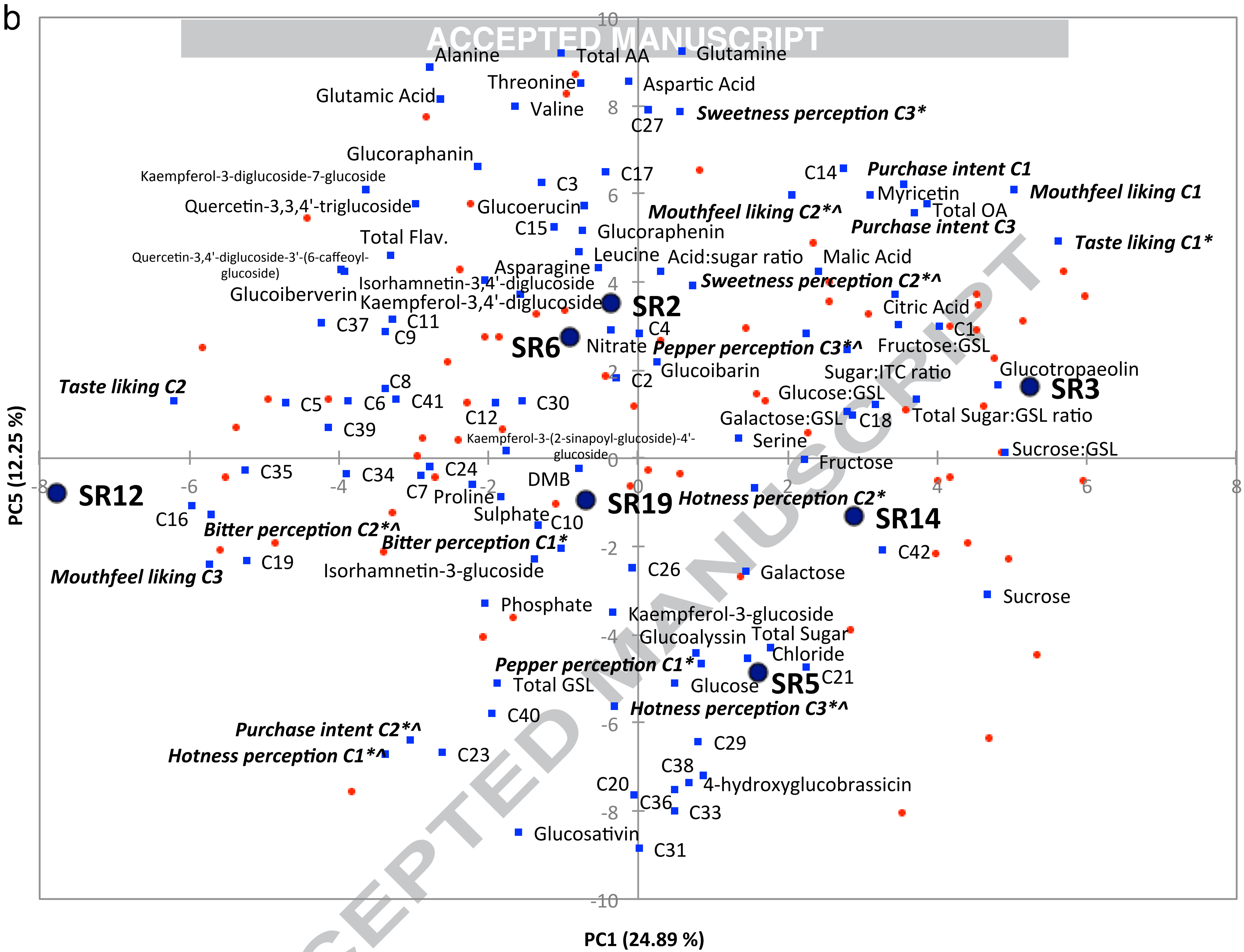
1
2
3
4
5
6
7
8
9
PC2 (19.55 %)
PC1 (24.89 %)


b
Size of leaves_A
Leaf shape_A
Hairiness_A
Leaf shape liking C2
Appearance liking C3*^
SR12
Purchase intent C3
SR14
Leaf shape liking C3*
Purple Stem_A
Depth of leaf colour_A
SR3
SR2
SR19
Colour liking C3*
Appearance liking C1
Purchase intent C2*^
Purchase intent C1
Appearance liking C2*
SR6
Colour liking C1
SR5
Leaf shape liking C1
PC3 (17.66 %)
PC1 (26.66 %)
8
6
4
2
0
-2
-4
-6
-8
-8
-6
-4
-2
0
2
4
6
8
10
12


a
C7
C15
C4
C18
Citric Acid
C9
C3
Mouthfeel liking C2*^
Sucrose:GSL
C39
C6
Sulphate
Pepper perception C1*
C35
Nitrate
SR19
C8
Purchase intent C2*^
Glucotropaeolin
SR3
C11
C40
Acid:sugar ratio
C42
Purchase intent C1
C12
Hotness perception C2*
Sucrose
C5
Threonine
Fructose:GSL
C21
Total Sugar:GSL ratio
C19
Phosphate
Total AA
C16
Sugar:ITC ratio
SR12
Glucoalyssin
Taste liking C2
C23
Leucine
Glucose:GSL
Taste liking C1*
C14
Bitter perception C1*
Aspartic Acid
Myricetin
Hotness perception C1*^
C24
Valine
Glutamine
Glucoiberverin
Alanine
4-hydroxyglucobrassicin
SR14
Total OA
Mouthfeel liking C1
C34
C17
C30
Fructose
Galactose:GSL
Quercetin-3,3,4'-triglucoside
Asparagine
Hotness perception C3*^
Purchase intent C3
C41
Sweetness perception C2*^
C1
Mouthfeel liking C3
C37
Glucoraphanin
Sweetness perception C3*
Glucoerucin
Glutamic Acid
Kaempferol-3-(2-sinapoyl-glucoside)-4'-glucoside
C27
SR6
Malic Acid
C33
C20
C31
Total Sugar
Kaempferol-3-diglucoside-7-glucoside
Kaempferol-3-glucoside
Glucosativin
SR2
Galactose
Bitter perception C2*^
SR5
Glucoraphenin
Kaempferol-3,4'-diglucoside
C36
Chloride
C2
Glucose
C38
Pepper perception C3*^
Isorhamnetin-3,4'-diglucoside
C26
C29
Total Flav.
Proline
DMB
Glucoibarin
Total GSL
Quercetin-3,4'-diglucoside-3'-(6-caffeoyl-glucoside)
Isorhamnetin-3-glucoside
7
6
5
4
3
2
1
0
-1
-2
-3
-4
-5
-6
-7
-9
-8
-7
-6
-5
-4
-3
-2
-1
0
1
2
3
4
5
6
7
8
9
PC4 (16.60 %)
PC1 (24.89 %)


b
Alanine
Total AA
Glutamine
Threonine
Aspartic Acid
Glutamic Acid
Valine
Sweetness perception C3*
C27
Glucoraphanin
Kaempferol-3-diglucoside-7-glucoside
C3
C17
C14
Purchase intent C1
Quercetin-3,3,4'-triglucoside
Glucoerucin
Myricetin
Mouthfeel liking C1
Mouthfeel liking C2*^
Total OA
C15
Glucoraphenin
Purchase intent C3
Total Flav.
Taste liking C1*
Quercetin-3,4'-diglucoside-3'-(6-caffeoyl-glucoside)
Leucine
Acid:sugar ratio
Malic Acid
Asparagine
Isorhamnetin-3,4'-diglucoside
Sweetness perception C2*^
Glucoiberverin
Kaempferol-3,4'-diglucoside
SR2
Citric Acid
C37
C11
C9
C4
C1
SR6
Nitrate
Pepper perception C3*^
Fructose:GSL
Glucoibarin
Glucotropaeolin
Taste liking C2
C8
C2
Sugar:ITC ratio
SR3
Glucose:GSL
C5
C6
C41
C30
Galactose:GSL
C18
Total Sugar:GSL ratio
C12
C39
Kaempferol-3-(2-sinapoyl-glucoside)-4'-glucoside
Serine
Sucrose:GSL
C35
C34
C24
Fructose
SR12
DMB
C7
Proline
SR19
Hotness perception C2*
C16
Sulphate
C10
SR14
Bitter perception C2*^
Bitter perception C1*
C42
C19
Isorhamnetin-3-glucoside
C26
Galactose
Mouthfeel liking C3
Sucrose
Phosphate
Kaempferol-3-glucoside
Glucoalyssin
Total Sugar
Chloride
Pepper perception C1*
C21
Total GSL
Glucose
SR5
C40
Hotness perception C3*^
Purchase intent C2*^
Hotness perception C1*^
C23
C29
C38
C20
4-hydroxyglucobrassicin
C36
C33
Glucosativin
C31
10
8
6
4
2
0
-2
-4
-6
-8
-10
-8
-6
-4
-2
0
2
4
6
8
PC5 (12.25 %)
PC1 (24.89 %)

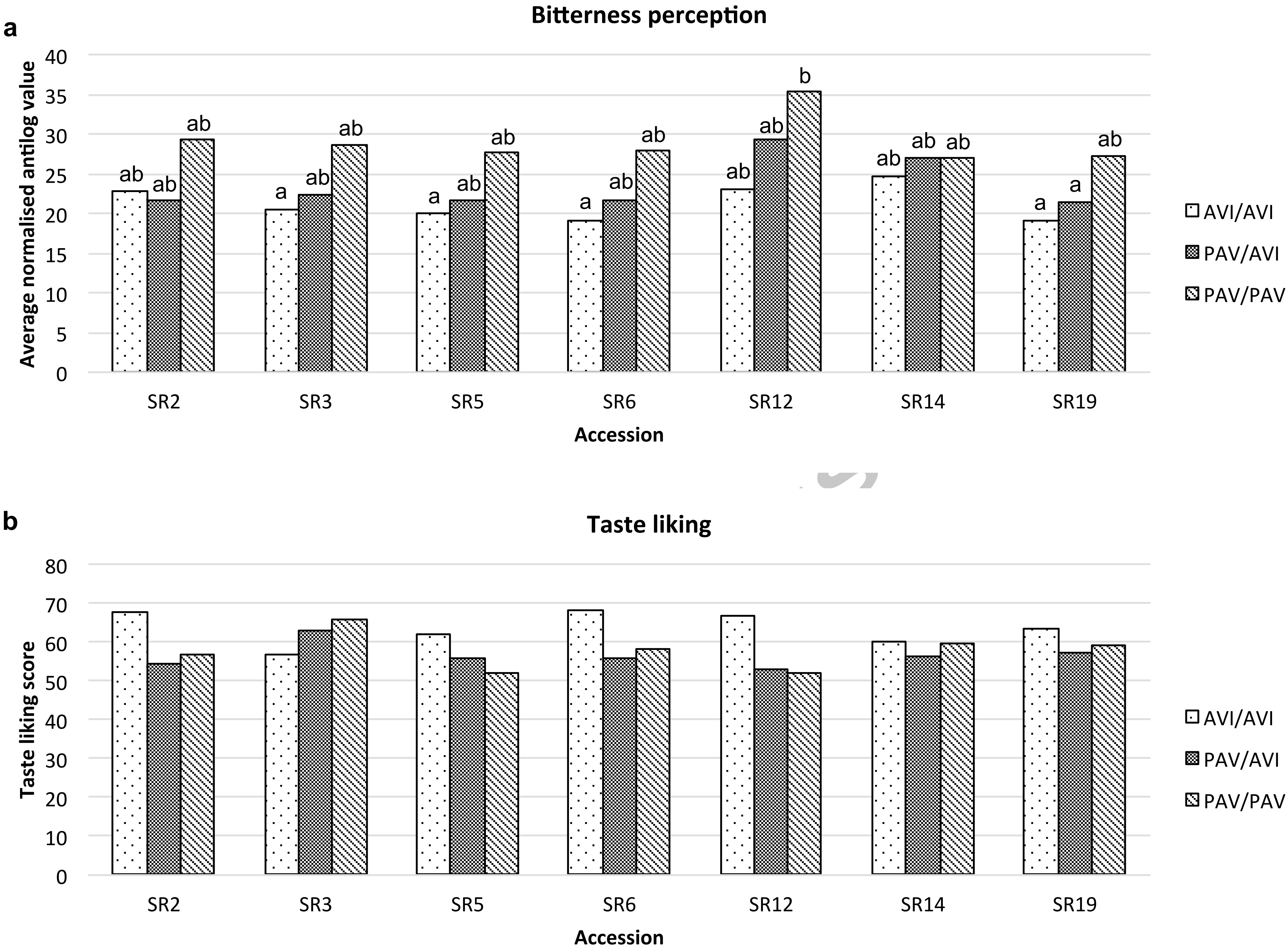

a
Bitterness perception
Average normalised antilog value
0
5
10
15
20
25
30
35
40
ab
ab
ab
a
ab
ab
a
ab
ab
a
ab
ab
ab
ab
b
ab
ab
ab
a
a
ab
SR2
SR3
SR5
SR6
SR12
SR14
SR19
Accession
AVI/AVI
PAV/AVI
PAV/PAV
b
Taste liking
Taste liking score
0
10
20
30
40
50
60
70
80
SR2
SR3
SR5
SR6
SR12
SR14
SR19
Accession
AVI/AVI
PAV/AVI
PAV/PAV

Table 1. Summary of study participant demographics (*n* = 90) and level of usual rocket consumption

Question	Number of individuals (%)
Age range	
18-25	40 (44.4%)
26-35	30 (33.3%)
36-45	15 (16.7%)
46-55	4 (4.4%)
56-65	1 (1.1%)
Ethnicity	
White European	26 (28.9%)
White British	37 (41.1%)
White Irish	2 (2.2%)
Asian Chinese	17 (18.9%)
White/Black Asian	1 (1.1%)
Black African	4 (4.4%)
Asian Bangladeshi	1 (1.1%)
Asian Indian	1 (1.1%)
Declined to answer	1 (1.1%)
Gender	
Male	21 (23.3%)
Female	69 (76.7%)
Rocket consumption	
Question: How often do you consume rocket when it is available?	
Never	11 (12.2%)
Rarely	19 (21.1%)
Sometimes	36 (40.0%)
Usually	20 (22.2%)
Always	4 (4.4%)

Table 2. Summary table of average consumer responses (*n* = 67), and class centroid values (determined by agglomerative hierarchical cluster analysis) for preference ('liking') and normalised antilog perception traits in seven accessions of rocket salad.

Trait	Mean score / AHC cluster means	No. in cluster (%)	SR2	SR3	SR5	SR6	SR12	SR14	SR19	*P*-value (sample effect)
Appearance liking	All		61.2^{ab}	57.5^{a}	62.8^{ab}	61.5^{ab}	62.5^{ab}	57.6^{a}	68.8^{b}	0.001
	Cluster 1	23 (34.3%)	64.5^{ns}	71.3^{ns}	64.2^{ns}	74.8^{ns}	73.3^{ns}	62.5^{ns}	70.5^{ns}	0.044
	Cluster 2	38 (56.7%)	55.1^{abc}	46.2^{a}	58.5^{bc}	51.2^{ab}	51.4^{ab}	48.7^{ab}	63.1^{c}	<0.0001
	Cluster 3	6 (9.0%)	87.2^{ab}	76.2^{a}	84.9^{ab}	76.0^{a}	91.3^{ab}	94.5^{ab}	98.3^{b}	0.011
Liking of colour	All		69.2^{ab}	63.8^{a}	68.5^{ab}	65.8^{ab}	64.6^{a}	65.2^{ab}	71.7^{b}	0.003
	Cluster 1	26 (38.8%)	71.8^{ns}	61.5^{ns}	68.7^{ns}	68.8^{ns}	64.5^{ns}	61.1^{ns}	68.7^{ns}	0.092
	Cluster 2	19 (28.4%)	81.8^{ns}	80.7^{ns}	83.7^{ns}	82.7^{ns}	81.1^{ns}	84.9^{ns}	84.9^{ns}	0.761
	Cluster 3	22 (32.8%)	55.5^{ab}	51.8^{a}	55.0^{ab}	47.5^{a}	50.4^{a}	53.1^{a}	63.9^{b}	0.001
Liking of shape	All		63.0^{ab}	58.3^{a}	59.6^{ab}	60.7^{ab}	63.3^{ab}	60.1^{ab}	68.6^{b}	0.026
	Cluster 1	20 (29.9%)	58.4^{ns}	51.2^{ns}	58.8^{ns}	53.5^{ns}	47.9^{ns}	44.4^{ns}	47.7^{ns}	0.096
	Cluster 2	24 (35.8%)	74.5^{ns}	75.7^{ns}	72.3^{ns}	66.4^{ns}	73.0^{ns}	74.3^{ns}	75.5^{ns}	0.511
	Cluster 3	23 (34.3%)	55.1^{abc}	46.3^{a}	46.9^{ab}	61.0^{bc}	66.7^{cd}	58.9^{abc}	79.4^{d}	<0.0001
Liking of mouthfeel	All		61.3^{ns}	62.7^{ns}	57.4^{ns}	61.6^{ns}	59.8^{ns}	60.3^{ns}	61.2^{ns}	0.586
	Cluster 1	28 (41.8%)	73.7^{ns}	75.1^{ns}	70.0^{ns}	74.6^{ns}	66.9^{ns}	72.5^{ns}	73.0^{ns}	0.453
	Cluster 2	7 (10.4%)	37.1^{a}	71.7^{b}	19.0^{a}	49.7^{ab}	43.6^{ab}	45.6^{ab}	39.2^{a}	0.001
	Cluster 3	32 (47.8%)	55.7^{ns}	49.8^{ns}	54.7^{ns}	52.9^{ns}	57.0^{ns}	52.9^{ns}	55.7^{ns}	0.429
Liking of taste	All		58.5^{ns}	62.2^{ns}	55.9^{ns}	59.2^{ns}	56.1^{ns}	58.1^{ns}	59.2^{ns}	0.420
	Cluster 1	25 (37.3%)	72.2^{ab}	80.1^{b}	69.4^{ab}	74.6^{ab}	63.5^{a}	70.7^{ab}	71.4^{ab}	0.079
	Cluster 2	36 (53.7%)	55.7^{ns}	51.8^{ns}	52.5^{ns}	53.4^{ns}	57.6^{ns}	53.1^{ns}	55.8^{ns}	0.685
	Cluster 3	6 (9.0%)	17.8^{ns}	49.9^{ns}	20.5^{ns}	30.0^{ns}	17.0^{ns}	35.3^{ns}	28.5^{ns}	0.074

Perception of bitterness	All		24.2^{ab}	22.7^{ab}	22.7^{ab}	21.8^{a}	27.1^{b}	25.8^{ab}	21.2^{a}	0.004
	Cluster 1	49 (73.1%)	19.9^{ab}	19.3^{ab}	18.6^{ab}	16.3^{a}	21.8^{ab}	22.5^{b}	17.8^{ab}	0.028
	Cluster 2	14 (20.9%)	30.4^{ab}	24.5^{a}	31.8^{ab}	33.1^{ab}	38.4^{b}	29.8^{ab}	26.0^{a}	0.002
	Cluster 3	4 (6.0%)	54.0^{ns}	57.0^{ns}	40.4^{ns}	50.0^{ns}	52.1^{ns}	53.0^{ns}	45.1^{ns}	0.371
Perception of hotness	All		16.0^{a}	16.3^{a}	18.9^{ab}	16.0^{a}	16.3^{a}	16.3^{a}	21.3^{b}	<0.0001
	Cluster 1	14 (20.9%)	9.4^{a}	12.9^{abc}	17.4^{bc}	11.8^{ab}	18.8^{c}	11.5^{ab}	12.1^{ab}	<0.0001
	Cluster 2	34 (50.7%)	17.5^{b}	14.8^{ab}	14.9^{ab}	13.8^{ab}	12.5^{a}	17.5^{b}	23.6^{c}	<0.0001
	Cluster 3	19 (28.4%)	18.3^{ab}	21.3^{abc}	27.1^{c}	23.0^{abc}	21.3^{abc}	17.6^{a}	24.0^{bc}	<0.0001
Perception of sweetness	All		12.5^{bc}	12.3^{bc}	8.6^{ab}	13.6^{c}	10.4^{abc}	11.5^{abc}	7.1^{a}	0.001
	Cluster 1	19 (28.4%)	23.3^{ns}	21.5^{ns}	19.6^{ns}	20.1^{ns}	19.8^{ns}	19.7^{ns}	12.2^{ns}	0.281
	Cluster 2	8 (11.9%)	3.9^{a}	17.6^{a}	7.2^{a}	35.8^{b}	10.1^{a}	14.3^{a}	7.9^{a}	<0.0001
	Cluster 3	40 (59.7%)	9.0^{b}	6.9^{ab}	3.7^{a}	6.1^{ab}	6.1^{ab}	7.0^{ab}	4.5^{a}	0.002
Perception of pepperiness	All		20.1^{ab}	21.5^{ab}	22.5^{ab}	21.4^{ab}	18.9^{a}	19.2^{ab}	23.2^{b}	0.011
	Cluster 1	44 (65.7%)	16.2^{a}	19.2^{ab}	19.9^{ab}	19.3^{ab}	18.4^{a}	19.4^{ab}	23.5^{b}	0.001
	Cluster 2	5 (7.5%)	5.8^{ns}	8.2^{ns}	9.4^{ns}	5.9^{ns}	6.3^{ns}	6.1^{ns}	7.7^{ns}	0.934
	Cluster 3	18 (26.9%)	33.6^{c}	30.8^{abc}	32.6^{bc}	23.7^{ab}	23.7^{ab}	22.2^{a}	26.7^{abc}	0.001
Purchase intent	All		3.1^{ns}	3.3^{ns}	3.0^{ns}	3.1^{ns}	3.0^{ns}	3.1^{ns}	3.3^{ns}	0.449
	Cluster 1	31 (46.3%)	3.6^{ns}	4.0^{ns}	3.5^{ns}	3.9^{ns}	3.4^{ns}	3.5^{ns}	3.8^{ns}	0.070
	Cluster 2	15 (22.4%)	2.2^{a}	2.6^{abc}	3.3^{abc}	2.5^{ab}	3.4^{bc}	2.4^{ab}	3.7^{c}	<0.0001
	Cluster 3	21 (31.3%)	2.8^{ns}	2.7^{ns}	2.0^{ns}	2.4^{ns}	2.1^{ns}	2.9^{ns}	2.1^{ns}	0.009

Differences in superscript letters within rows indicate significances according to ANOVA with Tukey's HSD test (*P*<0.05). ns = not significant.

Table 3. Summary of consumer TAS2R38 diplotype numbers (*n* = 69). Observed vs. expected numbers and percentages for the whole cohort and AHC taste liking clusters C1* (*n* = 25) and C2 (*n* = 36).

Diplotype	Observed number (%)	Expected %
Total cohort		
PAV/AVI	35 (52.2%)	51.1%
PAV/PAV	16 (23.9%)	24.3%
AVI/AVI	18 (26.9%)	24.6%
*Taste liking C1**		
PAV/AVI	12 (48.0%)	51.1%
PAV/PAV	6 (24.0%)	24.3%
AVI/AVI	7 (28.0%)	24.6%
Taste liking C2		
PAV/AVI	16 (47.1%)	51.1%
PAV/PAV	7 (20.6%)	24.3%
AVI/AVI	11 (32.4%)	24.6%
Undetermined$^{\$}$	2	-

Expected numbers determined by comparison to observations in Mennella et al. (2010), but not including the frequency of rare diplotypes. Chi-squared tests found no significant differences with expected frequencies (Total cohort, *P* = 0.95; C1*, *P* = 0.918; C2, *P* = 0.564). Chi-squared found no statistically significant differences between the observed frequencies in cluster C1* and C2 (*P* = 0.919).

* = Significant differences observed between scores (ANOVA, *P*<0.05; refer to Table 2).

$^{\$}$ = Individuals present in taste liking cluster C2 but declined to provide a DNA sample; not included in % determination