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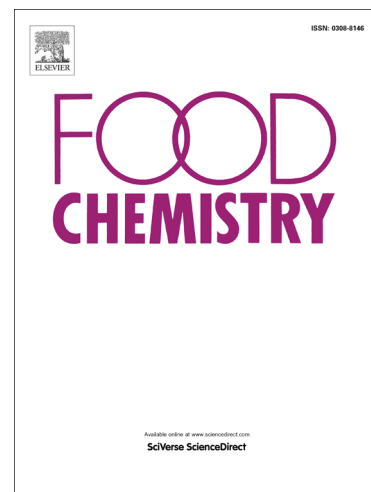
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1 **The influence of phytochemical composition and resulting sensory attributes**
2 **on preference for salad rocket (*Eruca sativa*) accessions by consumers of**
3 **varying TAS2R38 diplotype**

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12

13 **Abstract**

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

26 reduce perceptions of hotness and bitterness for some consumers. Importantly the
27 GSL glucoraphanin does not impart significant influence on liking or perception traits.

28

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

31

32 **1. Introduction**

33 *Eruca sativa* ("salad" rocket) and other species of rocket are popular leafy
34 vegetables consumed all over the world as part of salads or as a garnish (Bennett,
35 Carvalho, Mellon, Eagles, & Rosa, 2007). Previous research has largely focused on
36 the diversity of phytochemical content and post-harvest quality. Studies have
37 investigated the impacts of modified atmosphere and general sensory trends in
38 rocket (Amodio, Derossi, Mastrandrea, & Colelli, 2015; D'Antuono, Elementi, & Neri,
39 2009; Lokke, Seefeldt, & Edelenbos, 2012; Martinez-Sanchez, Marin, Llorach,
40 Ferreres, & Gil, 2006; Pasini, Verardo, Cerretani, Caboni & D'Antuono, 2011),
41 however these made certain assumptions regarding what is the 'ideal' or 'preferred'
42 rocket sensory profile of consumers. Few have taken into account the genetic and
43 phytochemical variability of rocket varieties, and none have accounted for the
44 genetic variability of consumers. Harvest, post-harvest and shelf life processes affect
45 salad 'quality' (Amodio et al. 2015), but no study has tested consumers to determine
46 the reasons for their liking/disliking of rocket. This is needed in addition to the
47 quantification of sensory traits to plan and implement breeding and marketing
48 strategies.

49 Studies by D'Antuono et al. (2009) and Pasini et al. (2011) have combined
50 aspects from both sensory and consumer studies on *Eruca sativa* and *Diplotaxis*

51 *tenuifolia*. While no scores for liking of traits were given, some subjective descriptive
52 terms were used, such as “typical rocket salad flavour”. Both studies used six
53 untrained individuals but the minimum for profiling is eight trained assessors
54 (Carpenter, Lyon, & Hasdell, 2012), and the minimum for a consumer study is 30
55 (Hough et al. 2006).

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

65 The reasons given why consumers like the taste and flavour of rocket salad
66 are anecdotal. High levels of bitterness are quoted as being a negative aspect of
67 consumer acceptance, but this is not universal (Hayes & Keast, 2011). Across
68 Brassicaceae crops, it has been demonstrated that bitter tastes contribute
69 negatively to acceptance of products, and this could be part of a protective
70 mechanism to prevent ingestion of harmful compounds, particularly at a young age
71 (Tepper et al., 2009).

72 Bitterness is cited as the main taste attribute of rocket that consumers reject.
73 It is an extremely complex taste sensation, with 25 putative G-protein-coupled
74 TAS2R receptors existing in humans (Le Nevé, Foltz, Daniel, & Gouka, 2010).
75 Glucosinolates (GSLs) and isothiocyanates (ITCs) have been linked with the gene

76 *hTAS2R38* (Meyerhof et al. 2010) and the thiocyanate moiety (-N-C=S) confers the
77 perception of bitterness, and shows a bimodal distribution of two haplotypes:
78 sensitive and insensitive (Tepper, 2008). Due to genetic recombination, three
79 common diplotypes are present within the human population: PAV homozygotes
80 ('supertasters'), heterozygotes ('medium-tasters'), and AVI homozygotes ('non-
81 tasters'; Hayes, Bartoshuk, Kidd, & Duffy, 2008).

82 The *hTAS2R38* gene is known to confer varying bitter-tasting sensitivity for
83 certain bitter compounds depending on the diplotype of the person (Wooding et al.,
84 2004). Pasini et al. (2011) suggested that bitterness and pungency in rocket leaves
85 has an association with the GSLs progoitrin/epiprogoitrin and dimeric-4-
86 mercaptobutyl-GSL (DMB). Individuals who have the PAV/PAV 'supertaster'
87 conformation theoretically perceive bitter compounds such as these and their
88 myrosinase derivatives with greater intensity. Some consumers find these tastes
89 overpowering or repulsive and avoid consuming Brassicaceae vegetables (Garcia-
90 Bailo, Toguri, Eny, & El-Sohemy, 2009). By contrast, perceptions of sweetness in
91 other foods increase liking, and for some people, hotness is also a desirable
92 characteristic; e.g. in hot peppers. Hotness is a trigeminal sensation, and consumers
93 vary in their sensitivity according to the number of papillae they possess, and the
94 abundance of associated trigeminal neurons (Reed & Knaapila, 2010). It should be
95 noted that hotness is distinct from pepperiness; in the context of this study,
96 pepperiness refers to the flavour associated with ground peppercorns.

97 We hypothesised those individuals with PAV/PAV diplotype would score
98 samples more intensely for bitter taste, and negatively for liking of rocket taste than
99 those with PAV/AVI or AVI/AVI diplotypes. This study questioned which of seven *E.*
100 *sativa* cultivars people preferred based on phytochemical composition and visual and

101 textural characteristics. Data were combined with sensory analysis and
102 phytochemical analyses presented in Bell, Oruna-Concha, & Wagstaff (2015), Bell,
103 Spadafora, Müller, Wagstaff, & Rogers (2016), and Bell, Methven, Signore, Oruna-
104 Concha, & Wagstaff (2017) to determine which sensory attributes are most important
105 for consumers in deciding if they like or dislike rocket. We also tested the hypothesis
106 that sweetness, hotness and pepperiness are positive attributes in rocket consumer
107 acceptance.

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

112

113 **2. Materials and methods**

114 *2.1. Plant material*

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

122

123 *2.2. Untrained consumer assessments*

124 The untrained consumer study consisted of 91 consenting individuals, who
125 were recruited from in and around the University of Reading (Reading, UK).

126 Recruitment stipulated individuals must be over 18 years of age and be non-
127 smokers. Anchored unstructured line scales were used to determine assessors'
128 liking of overall appearance, leaf shape, mouthfeel and taste (extremely dislike – like
129 extremely). Individual perception of selected sensory attributes (bitterness, hotness,
130 sweetness and pepperiness) were rated using labeled magnitude scales (LMS).
131 Scales ascended from 'not detectable', 'weak', 'moderate', 'strong', 'very strong' to
132 'strongest imaginable', where spacing between descriptors increased logarithmically.
133 These values were then converted into antilog values and normalised for statistical
134 analyses (Bartoshuk et al. 2003).

135 Consumers were asked the likelihood of purchasing each of the samples if
136 they were available in supermarkets (5 point category scale; 1 = low purchase intent,
137 5 = high purchase intent). The questionnaire was designed, and data acquired, using
138 Compusense software (version 5.2; Guelph, ON, Canada). After the testing was
139 complete, consumers were asked to complete a demographic questionnaire and
140 answer questions regarding their usual rocket consumption ($n = 90$; 1 person
141 declined to answer).

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

151

152 *2.3. DNA extraction*

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

169

170 *2.4. SNP genotyping*

171 SNP genotyping kits were obtained from Life Technologies Ltd. (Paisley, UK)
172 according to the three most common alleles of the *hTAS2R38* gene: A49P
173 (rs713598), A262V (rs1726866) and V296I (rs10246939). A reaction mixture of
174 TaqMan Genotyping Mastermix (Life Technologies Ltd.) and primers was prepared
175 as follows: 12.5 µl Mastermix, 1.25 µl primer, 6.25 µl d.H₂O and 5 µl of human DNA

176 template (25 µl total per reaction). 3 non-template controls were used on each
177 genotyping plate. Analysis was performed on a 7300 Real Time PCR system
178 (Applied Biosystems Inc., Foster City, CA, USA). PCR run parameters were as
179 follows: 0 minutes at 55°C, 10 minutes at 95°C, 15 seconds at 92°C and 1 minute at
180 60°C. Alleles were automatically 'called' by RT-PCR software according to
181 fluorescence probes. Genotype was determined by the presence/absence of the
182 corresponding alleles; the diplotype of 69 individuals was successfully determined.
183 The remaining 21 individuals either: 1) did not consent to having a sample taken ($n =$
184 1), 2) did not yield sufficient DNA for analysis ($n = 2$), or 3) failed to attend the
185 second study visit ($n = 19$). The expected frequencies of diplotypes were determined
186 by comparison to observations by Mennella, Pepino, Duke, & Reed (2010).

187

188 *2.5. Phytochemical analyses*

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

196

197 *2.6. Statistical analyses*

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

201 Individual consumer TAS2R38 diplotypes were input as a nested effect in a separate
202 ANOVA, testing genotype*sample interaction. All ANOVA were conducted using a
203 95% confidence interval and a tolerance of 0.0001%, and post-hoc Tukey's HSD test
204 was used for multiple pairwise comparisons. Observed TAS2R38 diplotype
205 frequencies were compared with expected frequencies (Mennella et al. 2010) by
206 Pearson's chi-squared test. Any influence of bitter perception (normalised scores) on
207 taste liking was tested by Pearson's correlation.

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

214 Taste liking data were used to extract principal components (PCs; Pearson n -
215 1). Phytochemical data were fitted as supplementary variables, as well as the ratios
216 between sugars and GSLs, sugars and ITCs, and organic acids and sugars (see Bell
217 et al. 2017), and cluster means. A correlation matrix was constructed as part of the
218 analysis to determine significant correlations between variables ($P < 0.05$, $P < 0.01$ and
219 $P < 0.001$). Internal preference maps were produced using PCA of consumer data
220 (firstly taste liking, secondly appearance liking), with sensory profiling data and AHC
221 class centroids regressed as supplementary variables. The taste liking preference
222 map also used AHC class centroids relating to mouthfeel liking as well as taste
223 liking, and taste perception (normalised bitterness, sweetness, hotness and
224 pepperiness) and purchase intent as supplementary variables. All analysis was
225 carried out using XLStat (Version 12.0, Addinsoft, Paris, France).

226

227 **3. Results and discussion**228 *3.1. Consumer demographics and usual rocket consumption*

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

235 Participants were asked to answer one question about their usual rocket
236 consumption: '*How often do you consume rocket when it is available?*' 36 people
237 (40.0%) stated they sometimes eat rocket when available. 11 (12.2%) stated they
238 never eat rocket, and only 4 (4.4%) said they always consume rocket when
239 available. These responses indicate that the typical consumer makes conscious
240 decisions about the rocket they consume, and there are sensory attributes on which
241 they base these decisions. Rocket from diverse growing regions are currently all
242 used the same way for each salad product sold on the market. Due to this blanket
243 approach to the species, and the inherent sensory diversity present between
244 varieties/growing regions, consistency within products is not guaranteed. For the
245 consumer this could affect the likelihood of re-purchase, and affect how often they
246 choose to consume rocket.

247

248 *3.2. Consumer preference, perceptions and purchase intent*249 *3.2.1. General*

250 The response of consumers for each perception and preference modality
251 tested is presented in Table 2. Each of the attributes assessed by consumers were
252 consistently divided into three clusters in each respective AHC analysis. The
253 average scores of all consumers are summarised, as well as the results of ANOVA
254 Tukey HSD test pairwise comparisons. Within the text, clusters where a significant
255 difference was observed (Tukey HSD test, $P < 0.05$) are denoted by *. Clusters with
256 <20 individuals, but contained significant differences between consumer scores, are
257 denoted by ^.

258

259 3.2.2. Appearance liking

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

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

273 In terms of colour liking consumers discriminated significantly, again favouring
274 SR19 over SR3 and SR12. Cluster analysis identified some consumers (C3*; $n = 22$,

275 32.8%) liked the dark green leaf colour of SR19 significantly more than the lighter
276 coloured SR3, SR6, SR12 and SR14.

277 The liking of leaf shape was also significantly different between accessions.
278 SR19 scored significantly higher than SR3 across all consumers. C3* individuals (n
279 = 23, 34.3%) showed a high degree of preference for SR19 over SR2, SR3, SR5,
280 SR6 and SR14, but C1 (n = 20, 29.9%) and C2 (n = 24, 35.8%) did not show any
281 significant preference. C1 uniformly scored lower than average for all accessions,
282 whereas C2 scored much higher for their leaf shape. These data indicate some
283 people discriminate based on leaf shape, favouring a “wild” rocket-type leaf, but over
284 two thirds show no significant preference.

285

286 *3.2.3. Mouthfeel liking*

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

291

292 *3.2.4. Taste liking*

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

299 of all seven samples (scoring >63.4), yet still differentiated significantly between
300 them.

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

305

306 3.2.5. Bitterness perception

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

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

318 Neither SR12 nor SR14 contain especially high concentrations of GSLs (Bell
319 et al. 2015) or volatile ITCs (Bell et al. 2016). Following the assumption these
320 compounds are generally responsible for bitterness in rocket, one would expect SR5
321 to be perceived as the most bitter as it has been found to contain 11.5 mg.g⁻¹ dw in
322 total GSL concentration, and observed to have a high percentage of volatile ITCs
323 within the headspace. This suggests other compounds present within leaves

324 contribute to bitterness to a greater degree than has been previously realised. The
325 counter-hypothesis is the bitterness caused by GSL-related compounds are masked
326 to some degree, either by sugars, amino acids, or green-leaf VOCs (Bell et al. 2017).

327

328 *3.2.6. Hotness perception*

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

337 Hotness was the only attribute measured in which all clusters discriminated
338 significantly between accessions. C2* was the largest cluster ($n = 34$, 50.7%) and
339 mirrored the consumer average, perceiving SR19 to be hotter than all of the other
340 accessions. The smaller clusters did not follow this trend – in particular C3*[^] ($n = 19$;
341 28.4%) perceived SR5 to be hotter than SR2 and SR14, and C1*[^] ($n = 14$, 20.9%)
342 found SR12 to be the hottest and significantly different from SR2, SR6, SR14 and
343 SR19. The apparent differences in perceptions between each of the clusters infers a
344 genetic component is responsible, but further study of papillae numbers and specific
345 genes involved would be required to draw any meaningful conclusions. As observed
346 for attributes associated with heat in Bell et al. (2017; initial heat, tingliness,
347 warming) the hotness attribute measured here has a significant degree of variability.
348 This suggests heat is a key characteristic in determining the liking of rocket, rather

349 than bitterness, as has been observed in other crops (Schonhof, Krumbein, &
350 Brückner, 2004).

351

352 *3.2.7. Sweetness perception*

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

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

367

368 *3.2.8. Pepperiness perception*

369 SR19 was again scored significantly higher than SR12 for pepperiness
370 overall, and higher than SR2 and SR12 in C1* ($n = 44$; 65.7%). C3*^ ($n = 18$; 26.9%)
371 scores were by comparison higher than the average, but SR2 was perceived as
372 being more peppery than SR14. The differences between the two main clusters (C1*
373 and C3*^) suggest a subset of people perceive this attribute more intensely. Further

374 study is needed in this area, as no previous data have been published in relation to
375 rocket and consumer perceptions/liking of this trait.

376

377 *3.2.9. Purchase intent*

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

389

390 *3.3. Effects of TAS2R38 diplotype*

391 *3.3.1. Taste liking and bitterness perception*

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

396 TAS2R38 genotype had a significant effect on bitterness perception ($P < 0.02$)
397 (Figure 1a), and the effect of consumer genotype on bitterness scores was $P < 0.02$
398 (ANOVA sum of squares analysis). This suggests a significant effect on bitter

399 perceptions, but in the ANOVA there were no significant differences between
400 genotypes within a specific rocket accession. The effect of diplotype is not as
401 pronounced as was originally hypothesised, but a general trend for 'non-tasters' to
402 score bitterness of rocket lower than 'medium' or 'supertasters' is apparent.

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

411 The disparity between bitter perceptions and taste liking suggests TAS2R38
412 diplotype is only one of (potentially) many factors influencing an individual's
413 preference. A correlation test was performed independently of diplotype status on
414 the total cohort data, comparing taste liking with bitterness perception. This test
415 showed a significant negative relationship between the two attributes ($r = -0.227$,
416 $P < 0.0001$) and infers as bitter perception increases taste-liking decreases.

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

424

425 *3.3.2. TAS2R38 diplotype frequencies between agglomerative hierarchical clusters*

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

433 The frequencies of each diplotype in each cluster were compared to total
434 expected population frequencies (Mennella et al. 2010; Table 3) by chi-squared
435 tests. No significant differences were found between the observed and expected
436 frequencies in either cluster (C1*: $P = 0.918$; C2: $P = 0.564$). There was no
437 significant difference in diplotype frequencies between the two clusters either ($P =$
438 0.919), further suggesting TAS2R38 status is not a singularly determining factor in
439 consumer preference of rocket. The basis for preference is likely due to learned
440 responses and/or other sensory factors as mentioned in the previous section (Shen
441 et al. 2016).

442

443 *3.5. Principal Component Analysis*444 *3.5.1. Correlations between consumer preference & perceptions*

445 Two biplots from the PCA are presented in Figure 2 and PCs were extracted
446 on the basis of consumer taste liking scores. A total of six components were
447 generated, all with Eigenvalues >1.0 , but only the first five contained $>10\%$ of the
448 explained variation. PC1 explained the largest amount of variance (24.9%) and

449 predominantly separated SR12 from all other products. The other dimensions (PCs 2
450 to 5) all gave differing separations of the remaining accessions. PCs 1 vs. 4, and 1
451 vs. 5 have been selected for discussion as they represented the highest correlations
452 with the supplementary AHC centroid scores and phytochemical variables according
453 to their respective loadings scores; they are most informative for the purposes of this
454 discussion. Cumulatively, these PCs illustrate 53.7% of the total variation within the
455 data. For respective cluster scores for each accession refer to Table 2.

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

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

474

475 *3.5.2. Correlations between consumer preference, perceptions & phytochemical*
476 *content*

477 *3.5.2.1. General*

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

480

481 *3.5.2.2. Glucosinolates*

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

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

496 Another health beneficial GSL is erucin, which separates along PC5, and
497 significantly with sweetness perception C2*[^] ($P < 0.01$). Glucoraphenin is also
498 significantly correlated with this attribute (PC5; $P < 0.05$), but is only found in small

499 concentrations in SR2 and SR6 (Bell et al. 2015). These compounds are unlikely to
500 be causing sweetness, but are more abundant in sweet-tasting accessions (Bell et
501 al. 2015; 2017). Future rocket breeding should perhaps be selective for individual
502 health beneficial GSLs such as glucoraphanin and glucoerucin, as suggested by
503 Ishida et al. (2014).

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

512

513 3.5.2.3. Flavonols

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

519 Another significant positive correlation observed was for bitter perception C1*,
520 the largest bitter perception cluster, and kaempferol-3-(2-sinapoyl-glucoside)-4'-
521 glucoside ($P<0.05$). It is unusual for a flavonol to have bitter taste, though in the
522 complex matrix of the rocket leaf, consumers could have interpreted astringency as
523 bitterness. It is likely field-grown rocket would have produced higher concentrations

524 of flavonols due to higher light intensities than controlled environment (Bell et al.
525 2015; Jin et al., 2009), and therefore might have produced stronger effects within the
526 data. Further study is needed to properly determine the extent that flavonol
527 glycosides influence taste attributes in rocket.

528

529 3.5.2.4. Polyatomic ions

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

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

541

542 3.5.2.5. VOCs

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

545 An unexpected association with sweetness perception C3* was observed with
546 3-methyl-furan (**C27**; $P < 0.01$), and a corresponding negative correlations with
547 hotness perception C3* and pepper perception C1* (both $P < 0.05$). Bell et al. (2017)
548 observed that this compound was significantly inversely correlated with bitter

549 perception, but no corresponding association with sweetness. C3* was the largest
550 cluster for sweetness perception, and the high degree of separation along PC5
551 (Figure 2b) means the compound could be utilised as a chemical marker for non-
552 pungent, sweeter varieties of *E. sativa*. The compound was also significantly
553 correlated with increased purchase intent C3 (who generally would not buy rocket),
554 and inversely correlated for purchase intent C2*[^] (who discriminated for the hot
555 accession SR19). This suggests hotness is preferable for one group of consumers,
556 but is rejected by another.

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

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

572 Pepper perception C1* (discriminated for SR19) was negatively correlated
573 with 3-methyl-furan (**C27**), as with hotness perception C3*[^] (Figure 2b). Pepperiness

574 perception C3*[^] shared negative correlations with several volatiles, such as 2-
575 hexenal (**C7**), (E)-2-pentenal (**C10**), 5-ethyl-2(5H)-furanone (**C12**) and ethylidene-
576 cyclopropane (**C24**; all $P < 0.05$). The green-leaf VOCs **C7** and **C10** were noted by
577 Bell et al. (2017) for being linked with sweeter-tasting cultivars, and detracting from
578 the sensations of bitterness and pungency. **C12** has previously been observed in
579 tomato as a degradation product of (Z)-3-hexenal (**C16**; Buttery & Takeoka, 2004).
580 The presence of these compounds within the headspace of rocket has important
581 implications for consumer perceptions of pungent traits.

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

593

594 3.5.2.6. Free amino acids

595 High free AA concentrations detracted from the perception of pungent
596 compounds such as ITCs in Bell et al. (2017). In this study only one significant
597 negative correlation was observed between pepper perception C1* and proline

598 concentration. Proline is spatially distant at the bottom of the plot (Figure 5a),
599 separating negatively along PC4 from the peppery accession SR19.

600 Threonine correlated significantly with purchase intent C1 ($P<0.05$) and is
601 known to have sweet taste (Nelson et al. 2002). AAs correlated along PC5 (Figure
602 2b) and are more highly associated with the milder accessions SR2 and SR6. This
603 indicates amino acid content is generally in opposition to hotness, but further study is
604 needed to determine the full extent of the effects. Repeat experiments with other
605 cultivars of rocket would help to confirm or reject this hypothesis.

606

607 *3.5.2.7. Free sugars, organic acids and compound ratios*

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

618 The sucrose-GSL ratio negatively correlated with bitterness perception C2[^].
619 This ratio is almost directly opposite to SR12 (Figure 2b), separating strongly along
620 PC1. SR12 was noted for high perceptions of bitterness (Table 2), and these data
621 infer, for a proportion of the cohort (20.9%), the effect was an important determining
622 factor in their responses. As this was not seen in the other clusters, other factors

623 such as TAS2R receptor status and fungiform papillae density could impact the
624 effect sugar-GSL ratios have upon perceived bitterness.

625

626 *3.6. Internal preference map PCA*

627 *3.6.1. Sensory perceptions*

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

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

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

645 Taste liking C1* was negatively correlated with earthy flavour attributes
646 identified by the trained assessors ($P<0.05$). This was also seen for purchase intent
647 C1 ($P<0.01$), where scores were generally high for all accessions, but lower where

648 earthy flavour was more prominent (SR12; Figure 3a). Taste liking C2 by comparison
649 was negatively correlated with mustard odour ($P<0.05$). Purchase intent C3 was
650 negatively correlated with bitter taste ($P<0.05$) and further implies a uniform dislike of
651 rocket because of their perceptions of bitterness and hotness.

652

653 3.6.2. Appearance liking

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

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

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

684

685 **4. Conclusions**

686 This study has for the first time conducted a consumer analysis of *E. sativa*
687 accessions in conjunction with sensory, phytochemical and human genotype
688 analyses. The hypothesis all consumers reject bitter tasting cultivars is not fully
689 supported by the data presented, even when human TAS2R38 diplotype of
690 consumers is considered. Genotype effects are significant in determining the degree
691 to which a person will rate the bitterness of rocket and their liking of taste; but when
692 considered with sample effects, pairwise comparisons did not reveal significant
693 differences with any specific cultivar tested. 'Supertaster' (PAV/PAV) individuals
694 generally scored higher for bitterness and lower for taste liking, whereas AVI/AVI
695 individual were the opposite of this (with the exception of the commercial cultivar,
696 SR3). When these data are viewed in combination with AHCs and phytochemical

697 correlations, it seems the predominant basis of acceptance/rejection is actually more
698 related to the perceived hotness of leaves, rather than bitterness.

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

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

716 High concentrations of specific phytochemicals that typically contribute
717 towards hot and bitter sensations are not acceptable to some consumers. Breeding
718 varieties for high total GSL/ITC content is an unsophisticated approach that does not
719 account for these differences in consumer preference. Some preferred the hot ITC
720 and sulfur compounds that are produced from and associated with the GSL-

721 myrosinase reaction (as in SR19), but a substantial proportion rejected accessions
722 because of low sugar-ITC ratios.

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

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

745

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756

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848

849 **Figure legends**

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

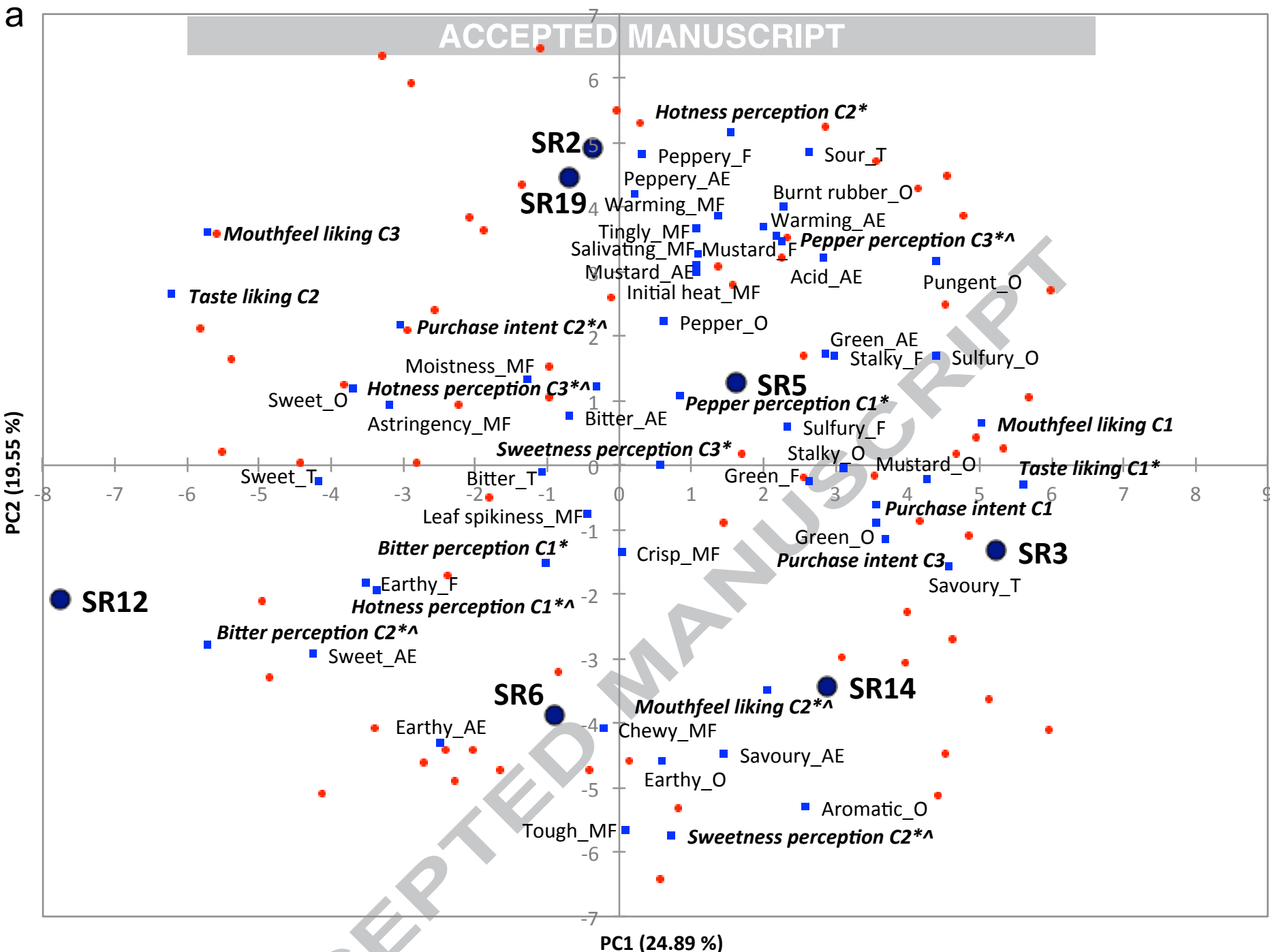
856 **Figure 2.** PCA biplot of consumer taste liking with phytochemical and AHC analysis
857 (in bold italic; refer to Table 2) data regressed as supplementary variables. * =
858 Significant differences observed with ANOVA ($P < 0.05$). ^ = AHC cluster with <20
859 individuals. PC1 vs. PC4 (a) represents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

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

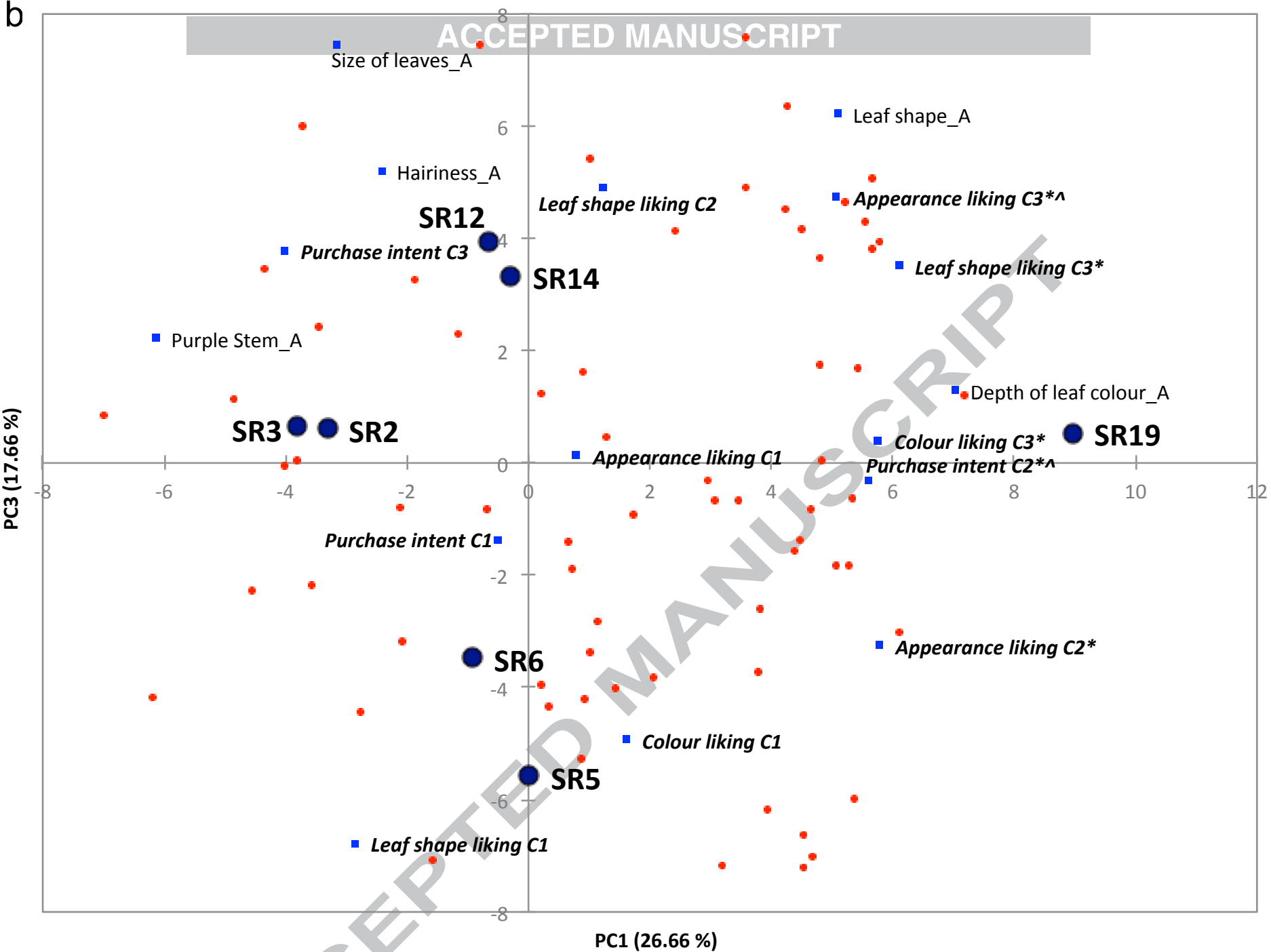
867 2017) PC1 vs. PC2 (a) represents 44.4% of variation within the data, and PC1 vs.
868 PC3 (b) represents 44.3% of variation within the data. Red circles = individual
869 consumer responses; blue squares = supplementary variables; dark blue circles =
870 rocket accession factor scores. Sensory variable suffix abbreviations: A =
871 appearance; O = odour; T = taste; F = flavour; MF = mouthfeel; AE = aftereffects.

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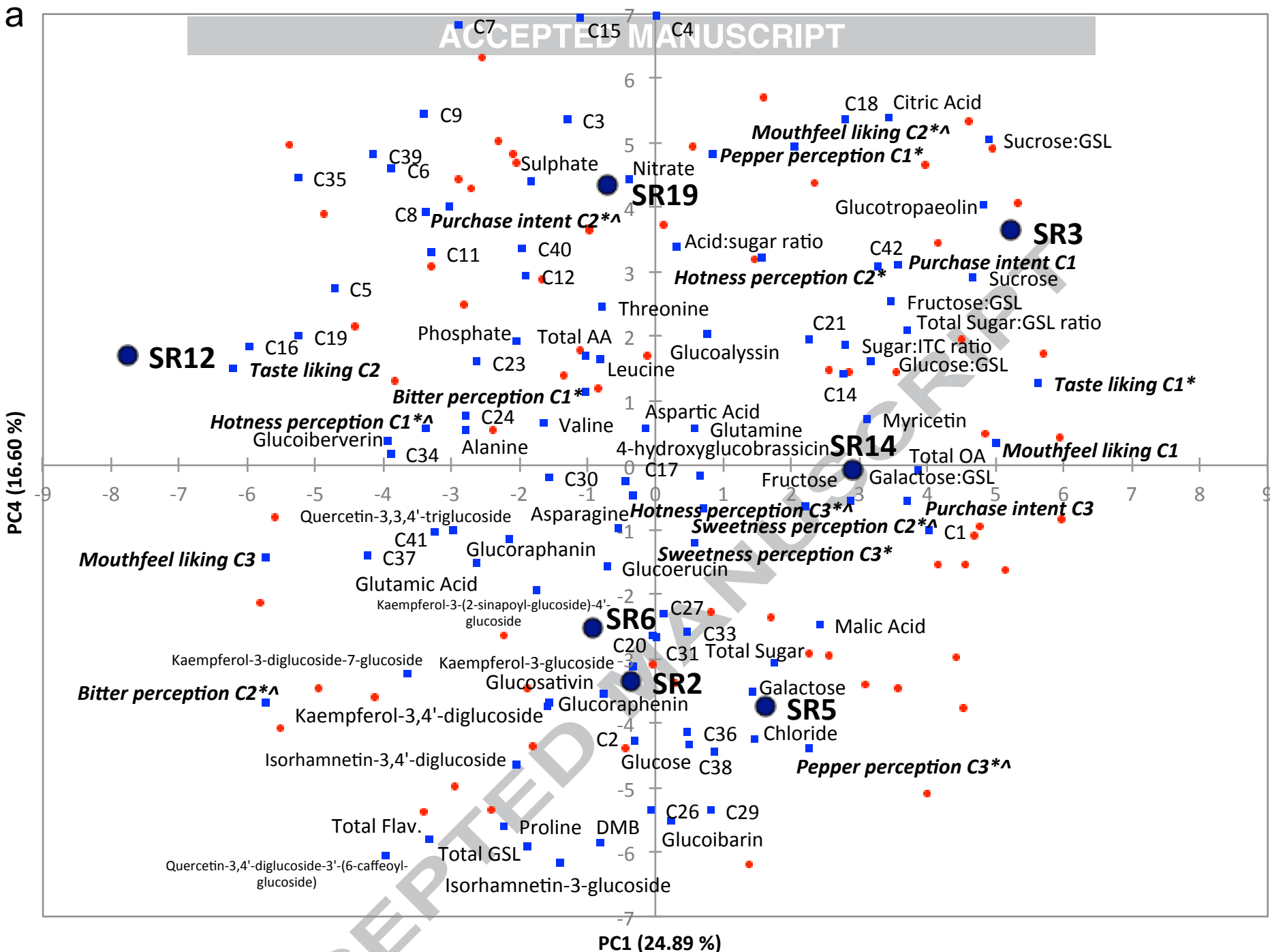
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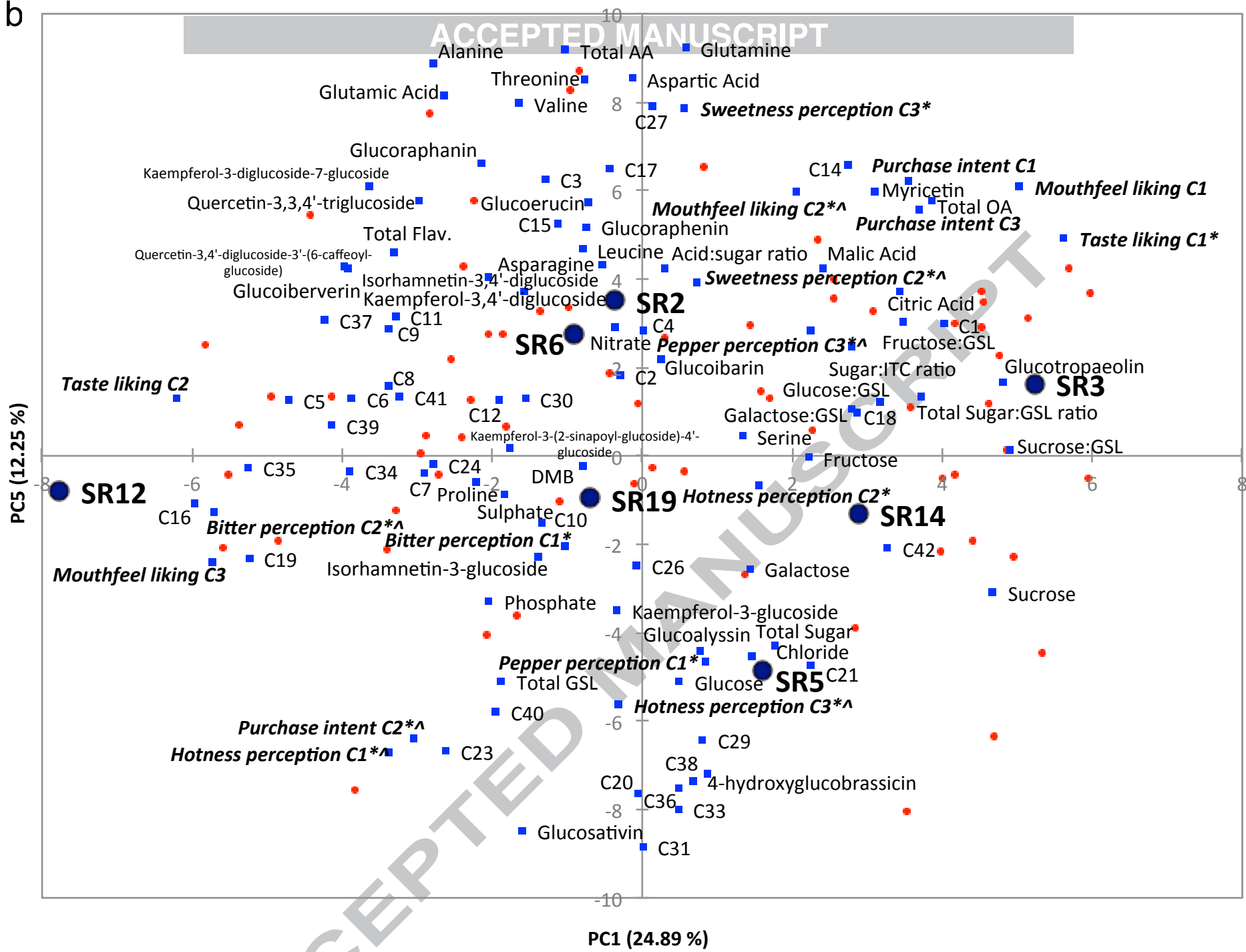
b



a

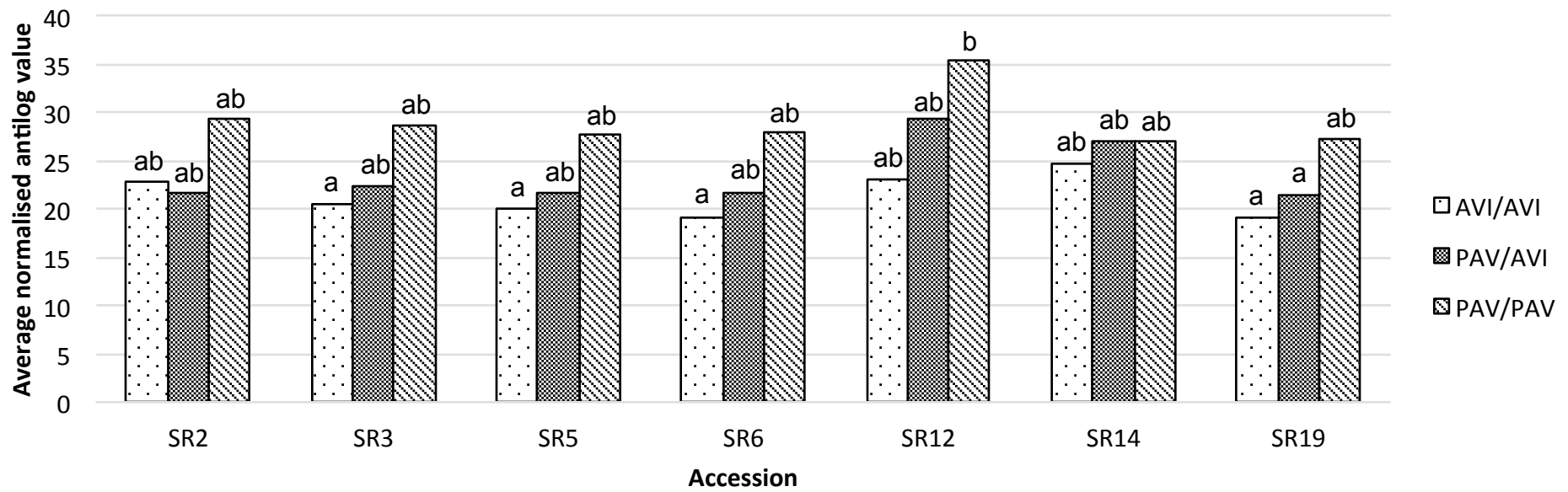


b



Bitterness perception

a



Taste liking

b

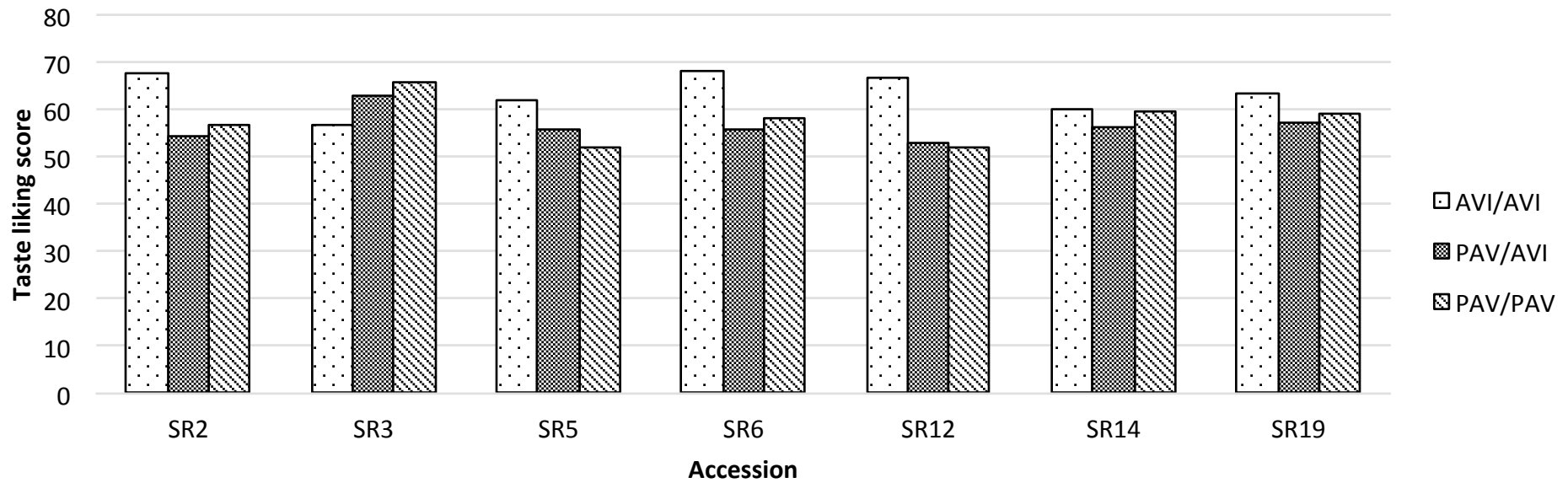


Table 1. Summary of study participant demographics ($n = 90$) and level of usual rocket consumption

Question	Number of individuals (%)
<i>Age range</i>	
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%)
<i>Ethnicity</i>	
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%)
<i>Gender</i>	
Male	21 (23.3%)
Female	69 (76.7%)
<i>Rocket consumption</i>	
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 %
<i>Total cohort</i>		
PAV/AVI	35 (52.2%)	51.1%
PAV/PAV	16 (23.9%)	24.3%
AVI/AVI	18 (26.9%)	24.6%
<i>Taste liking C1*</i>		
PAV/AVI	12 (48.0%)	51.1%
PAV/PAV	6 (24.0%)	24.3%
AVI/AVI	7 (28.0%)	24.6%
<i>Taste liking C2</i>		
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