

1 Title: Exploring the relationships between taste phenotypes, genotypes, ethnicity,
2 gender and taste perception using Chi-square and regression tree analysis

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9

10 Abstract

11 It is well known that perceived taste intensity varies greatly among individuals, and
12 that several factors including taste phenotypes (PROP Taster Status (PTS), Sweet
13 Liking Status (SLS), Thermal Taster Status (TTS)), ethnicity and gender, contribute to
14 variation in taste responsiveness, although such factors are usually investigated in
15 isolation. This study aimed to investigate the association between different taste
16 pheno/genotypes, explore whether these taste phenotypes associated with ethnicity
17 (Caucasian vs Asian) and gender, and determine the relative effects of the different
18 factors on perceived taste intensity. As analysis of this type of data with ANOVA can
19 be difficult due to confounding factors, interactions, and small sample sizes in
20 subcategories, the use of regression tree analysis as an alternative approach was
21 investigated. To that end, two-hundred and twenty-three volunteers were phenotyped
22 for their PTS, SLS and TTS and genotyped for TAS2R38 –rs713598 and gustin –
23 rs2274333. They also rated their perceived intensity of five basic taste and metallic
24 solutions on a gLMS scale. No significant association between the three taste
25 phenotypes were found indicating PTS, SLS and TTS are independent taste
26 phenotypes. However, the results indicated that Asians were not only more likely to
27 be PROP supertasters, but also more likely to be thermal tasters or Low Sweet Likers,
28 compared to Caucasians. Gender was also significantly associated with SLS, where
29 males were more likely to be High Sweet Likers. For perceived taste intensity,
30 traditional ANOVA analysis proved to be challenging. The alternative approach, using
31 regression trees, was shown to be an effective tool to provide a visualised framework
32 to demonstrate the multiple interactions in this dataset. For example, ethnicity was the
33 most influencing factor for perceived sour and metallic taste intensity, where Asians
34 had heightened response compared to Caucasians. The regression tree analysis also
35 highlighted that the PTS effect was dependent on ethnicity for sour taste, and PTS
36 and TTS effect was dependent on ethnicity for metallic taste. This study is the first
37 study to use regression tree analysis to explore variation in taste intensity ratings, and
38 demonstrated it can be an effective tool to handle and interpret complex sensory
39 datasets.

40 1. Introduction

41 Taste perception occurs when certain compounds released from food dissolve in
42 saliva and interact with taste receptor cells within taste buds located in the oral cavity.
43 Most mammals are able to detect five different modalities of taste: sweet, bitter, sour,
44 salty and umami (Chaudhari and Roper, 2010) whilst some other sensations have also
45 been purported to be potential tastes including fatty acid, metallic, kokumi and calcium
46 (Bachmanov *et al.*, 2014, Dipatrizio, 2014, Ohsu *et al.*, 2010, Bartoshuk, 1978).

47 Perceived intensity of taste and other oral sensations has been shown to vary greatly
48 among individuals and may be one of the most important determinants of food
49 preference and consumption affecting nutritional and health status (Stewart *et al.*,
50 2010, Tepper *et al.*, 2014, Ullrich *et al.*, 2004). Many factors have been shown to affect
51 perceived taste intensity perception such as health status (Overberg *et al.*, 2012,
52 Berteretche *et al.*, 2004), age (Bilash *et al.*, 1959, Mojet *et al.*, 2001, Monteleone *et al.*,
53 2017, Vignini *et al.*, 2019), gender (Hirokawa *et al.*, 2006, Michon *et al.*, 2009,
54 Bartoshuk *et al.*, 1994, Monteleone *et al.*, 2017, Vignini *et al.*, 2019), ethnicity (Williams
55 *et al.*, 2016, Bowser *et al.*, 2019), genetics (Kim *et al.*, 2003b, Chen *et al.*, 2009) and
56 taste phenotypes (Yang *et al.*, 2014, Bajec and Pickering, 2008, Dinnella *et al.*, 2018,
57 Yang *et al.*, 2019). Findings, however, are often conflicting across different studies.
58 For example, some studies have found that women tend to be more sensitive to taste
59 compared to men (Hirokawa *et al.*, 2006), whilst other studies failed to reveal a
60 significant effect (Chang *et al.*, 2006, Melis *et al.*, 2013). In general, some significant
61 effects of ethnicity on perceived taste intensity have been observed (Williams *et al.*,
62 2016, Bowser *et al.*, 2019), but there is a lack of research comparing Asian and
63 Caucasian populations. It is worth noting that some of the earlier studies used a
64 relatively small sample size, which could contribute to conflicting findings.

65 Naes *et al.*, (2018) outlined some key areas as to why individual differences are
66 important in sensory and consumer science, highlighting the various geno and
67 phenotypes impacting sensitivity to sensory perception. So far, many studies have
68 investigated the effect of different taste phenotypes such as PROP Taster Status (PTS)
69 (Bell and Song, 2005, Yang *et al.*, 2014), Sweet Liking Status (SLS) (Kim *et al.*, 2014,
70 Yeomans *et al.*, 2007) and Thermal Taster Status (TTS) (Bajec and Pickering, 2008,
71 Skinner *et al.*, 2018) on perceived taste intensity. PROP taster status (PTS) has been
72 studied extensively since it was first reported by Fox (1932) and individuals can be

73 classified into three groups: PROP supertasters (pST) – perceiving PROP as
74 extremely bitter, medium-tasters (pMT) who perceive PROP as moderately bitter, and
75 nontasters (pNT) who perceive PROP as tasteless (Lim *et al.*, 2008). TAS2R38, a
76 bitter taste receptor genotype, identified by Kim *et al.* (2003a), is known to influence
77 PROP tasting ability (Drayna *et al.*, 2003). Polymorphisms in TAS2R38 have been
78 shown to account for up to 85% of variation in PROP tasting ability (Wooding *et al.*,
79 2004), with PAV/PAV genotypes perceiving PROP as most bitter. Melis *et al.* (2013)
80 further suggested that gustin (rs2274333) also contributed to perceived PROP
81 intensity ratings but a later study failed to find such findings (Feeney and Hayes, 2014).

82 A number of studies have found PROP bitterness perception is positively correlated
83 with perceived intensity of other tastants and trigeminal stimuli including bitter, sweet,
84 salty, sour, fat, astringent, metallic, and temperature (Yang *et al.*, 2014, Bajec and
85 Pickering, 2008, Bartoshuk *et al.*, 1998, Tepper and Nurse, 1997, Dinnella *et al.*, 2018).
86 The distribution of pNT is known to vary greatly across ethnicity and was summarised
87 by Guo and Reed (2001) who showed that the percentage of pNT was between 2 to
88 37% in Africans, 7 to 37% in Europeans, 2 to 67% in Indians, and 5 to 23% in Chinese.
89 Robino *et al.* (2014) has also reported differences in ethnicity along the Silk Road.
90 Some studies found that females perceived PROP intensity higher than males
91 (Bartoshuk *et al.*, 1994, Shen *et al.*, 2016, Robino *et al.*, 2014, Monteleone *et al.*, 2017).

92 Sweet Liking Status (SLS) refers to individual variation in preferred sweetness in
93 sugary solutions, which was first reported by Pangborn (1970b). Researchers have
94 grouped individuals as sweet likers who increasingly prefer increasing levels of
95 sweetness; and sweet dislikers who prefer lower levels of sweetness and show
96 increasing dislike as sweetness increases (Methven *et al.*, 2016, Kim *et al.*, 2014).
97 Different Sweet Liking Status classification methods have been used in different
98 studies (Garneau *et al.*, 2018, Holt *et al.*, 2000, Kim *et al.*, 2014, Pangborn, 1970a,
99 Yang *et al.*, 2019). A recent study has adopted cluster analysis followed with validation
100 test (Pearson's correlation analysis) as a way to standardise the classification method
101 and re-named individuals as High Sweet Likers – prefer sweeter solutions, Medium
102 Sweet Likers – prefer mid sweet solutions, Low Sweet Likers - prefer low sweet
103 solutions (Yang *et al.*, 2019). Some studies have found sweet dislikers perceive
104 sweetness intensity as more intense than sweet likers (Drewnowski *et al.*, 1997, Looy
105 *et al.*, 1992, Peterson *et al.*, 1999), which was suggested to be one of the reasons why

106 sweet dislikers' overall liking declined as sweetness increased. However, other studies
107 failed to find such differences (Kim *et al.*, 2014) or found the effect is subjective to
108 sweetness levels (Methven *et al.*, 2016, Yang *et al.*, 2019). Interestingly, Yeomans *et*
109 *al.* (2009) found that PROP supertasters were more likely to be sweet dislikers,
110 indicating these two taste phenotypes are associated with each other. However, a
111 recent study failed to find such association (Yang *et al.*, 2019).

112 Thermal taster status (TTS) was first reported by Cruz and Green (2000), whereby
113 part of the population perceive a taste when their tongue is warmed or cooled (Cruz
114 and Green 2000), and are named thermal tasters (TT). Sweetness, bitterness, metallic
115 and sourness are most often reported by TT during warming or cooling (Yang *et al.*,
116 2014, Skinner *et al.*, 2018, Bajec and Pickering, 2008, Cruz and Green, 2000). Those
117 not perceiving a taste sensation from temperature stimulation are called thermal non-
118 tasters (TnT) (Green and George, 2004). Between 20 to 50% of the tested populations
119 have been found to be TT (Green and George, 2004b, Bajec and Pickering, 2008,
120 Yang *et al.*, 2014, Skinner *et al.*, 2018). Several studies have also found that TT have
121 heightened intensity responsiveness to some taste and trigeminal stimuli (Bajec and
122 Pickering, 2008, Green and George, 2004b). However, Yang *et al.* (2014) and Skinner
123 (2017) failed to find a significant TTS impact on individual perceived taste
124 responsiveness.

125 Looking at literature, the findings remain unclear, as different factors have been found
126 to contribute to perceived taste intensity, whereas others failed to find an effect. Some
127 positive findings could be due to the small sample sizes used in earlier studies (e.g. in
128 Bartoshuk *et al.*, (1994), 10 NT and 9 ST were evaluated). Most studies have
129 investigated the effect of a taste phenotype in isolation (Bartoshuk *et al.*, 1988,
130 Methven *et al.*, 2016, Green and George, 2004a). Similarly, the impact of ethnicity and
131 gender on taste perception have also been investigated (Williams *et al.*, 2016,
132 Bartoshuk *et al.*, 1994, Michon *et al.*, 2009) but again mainly as individual factors.
133 However, individuals are combinations of different phenotypes and genotypes,
134 ethnicity and gender, and there is a need to understand the impact of these
135 combinations. Unbalanced numbers in factors can contribute to inconsistency in
136 results, however, due to the nature of this type of study and population-based
137 convenience samples, it is difficult to balance all the factors of interest across
138 participants.

139 Furthermore, these factors interplay with each other, for example, Yang *et al.* (2014)
140 found that the effect of TTS is more apparent in MT than the other two PROP
141 phenotypes, and Dinnella *et al.*, (2018) reported a much more pronounced age effect
142 on fungiform papilla density in males than females. This indicates a complex interplay
143 among different factors.

144 Due to the complex nature of these type of studies, data is always associated with
145 unbalanced groups. Analysis of Variance (ANOVA) is not usually an appropriate
146 technique for understanding the separate effects in survey data (where there is limited
147 option to control the characteristics of subjects in the test), ANOVA requires data with
148 a structure design to allow estimation of the separate effects on a response and so is
149 of limited applicability in this context (Sheskin, 2011). Other statistical techniques thus
150 need to be explored to better understand this type of results.

151 Regression trees are a type of decision tree, and a data mining approach (Breiman *et al.*
152 *al.*, 1984, Bozkir and Sezer, 2011). There are many different types of decision trees,
153 including 'classification trees' which predict outcomes for categorical data and
154 regression trees which predict outcomes in numerical data. Regression trees use
155 algorithms to produce predictive graphical models to identify subgroups in the data
156 with differing levels of response between groups and homogeneity of response within
157 groups. They have not been widely used in the field of sensory and consumer science.
158 However, they have been commonly used in medical and health care applications over
159 the past three decades (Podgorelec *et al.*, 2002a, Tsien *et al.*, 1998) with applications
160 to support early diagnosis (Jabbar *et al.*, 2014, Tsien *et al.*, 1998) and to predict risk
161 factors in some diseases (Dimopoulos *et al.*, 2018). Recently, regression trees were
162 shown to be a useful technique to understand factors contributing to wine consumption
163 using 21 variables for analysis from a survey (e.g. demographic information, price
164 importance, location etc.) (Jovanović Mimir, 2017). The main advantage of a
165 regression tree is that it shows hierarchical and graphical representations of
166 interactions between variables (Loh, 2011, Machuca *et al.*, 2017), which helps
167 researchers to visualise the structure of interdependence of the data in a graphical
168 tree format. As survey data is often an unstructured dataset, regression trees can be
169 useful to understand individual characterisation in physiological and demographic
170 measures. However, regression trees can suffer the risk of overfitting in very large
171 datasets as small splits will be statistically significant. However, CHAID attempts to

172 stop overfitting from the beginning, as the tree only splits when there is significant
173 association (Shanthi, 2019). In some cases, analysts control the size of the splits by
174 specifying in the CHAID algorithm the smallest acceptable size of a subgroup
175 (Haughton & Oulabi, 1997). The CHAID technique is appropriate for datasets of
176 varying sizes (n=41 to 2000) (Arroyo *et al.*, 2018, Álvarez-Álvarez *et al.*, 2011, Díaz-
177 Pérez and Bethencourt-Cejas, 2016, Antipov and Pokryshevskaya, 2009).

178 The overall aim of this study was to investigate the interrelationships between taste
179 phenotypes, genotypes, ethnicity, gender and taste perception, through the
180 application of regression tree analysis. The objectives were to i) determine the
181 association between three different taste phenotypes (PTS, TTS, SLS), and explore
182 whether these taste phenotypes were associated with gender and ethnicity
183 (specifically Caucasian and Asian); and ii) determine the relative impact of these
184 factors on taste perception.

185 2. Materials and Methods

186 This study was approved by the University of Nottingham Medical School Research
187 Ethics Committee. All participants signed and gave informed consent before taking
188 part in the study. All data were collected using Compusense Cloud (Compusense,
189 Canada) at the Sensory Science Centre, University of Nottingham. Participants were
190 invited to attend two sessions over two separate testing days.

191 2.1. Subjects and sessions

192 Two hundred and twenty-three volunteers (160F, 63M; age range 18 – 65 years old;
193 156 Caucasians, 67 Asians) participated in this study. Subject characteristics were
194 shown in Table 1. In this study, participants were divided into Asian and Caucasian
195 based on understanding that there is more phenotypic homogeneity among Asian
196 subgroups in comparison to Caucasian (Leow, 2017). Participants who were smokers
197 or self-reported took medication known to affect taste and aroma perception were
198 excluded from this study.

199 The first session involved gLMS scale training, phenotyping for PROP Taster Status
200 and Sweet Liking Status together with taste intensity perception measurements. The
201 second session ran on a one to one basis to facilitate ease of Thermal Taster Status
202 phenotyping and buccal swab collection for genotyping.

203 2.2. gLMS training

204 In order to ensure participants understood the nature of the gLMS scale and facilitate
205 its correct use for intensity rating, a gLMS scale reference sheet was given to each
206 participant. To emphasise the general nature of the top of the scale, participants were
207 asked to think of the strongest sensation of any kind they had experienced previously,
208 or the strongest sensation they could imagine happening to them and write these down
209 at the top of the gLMS scale. Following Bartoshuk *et al.* (2002), they were also asked
210 to rate the intensities of 15 remembered sensations relative to their own strongest
211 sensation. Reference sheets were always provided in subsequent experiments, and
212 participants were encouraged to refer to their reference sheet for guidance when using
213 the gLMS scale.

214 2.3. Taste Phenotyping methods

215 2.3.1. PROP Taster Status determination

216 A 0.32mM 6-n-Propylthiouracil (PROP) (Sigma Aldrich, UK) solution was prepared by
217 dissolving PROP in water on a low heat stirring plate. Each subject was instructed to
218 roll a saturated cotton bud, which had previously been dipped in the PROP solution
219 (22 ± 2 °C), across the tip of the tongue for approximately 3s. Participants were
220 informed that the bitterness may take a few seconds to reach its maximum and were
221 instructed to rate its maximum intensity using the gLMS scale. After a two min break,
222 the procedure was repeated to collect duplicate ratings (Lim *et al.*, 2008).

223 Participants who rated bitterness intensity below barely detectable on the gLMS were
224 classified as pNT. Those who rated the bitterness intensity above barely detectable,
225 but below moderate were classified as pMT. Those who rated the intensity above
226 moderate were classified as pST (Lim, Urban *et al.* 2008).

227 2.3.2. Sweet Liking Status determination

228 Four sucrose solutions (17g/L, 78 g/L, 168/L and 397g/L) and a water sample were
229 served monadically in ascending sweetness concentration to each participant
230 (Yeomans *et al.*, 2007). All samples were prepared the day before tasting, stored in a
231 fridge (5 ± 2 °C) and brought out from the fridge at least an hour before testing to serve
232 at ambient temperature (20 ± 2 °C). 10 ml of each sample labelled with a random 3-
233 digit code was served. A two-minute break was given between samples and
234 participants were instructed to rinse their mouth with water (Evian, Danone, France)

235 to cleanse their palate. Participants were asked to rate how much they liked each
236 sample on a Labelled Affective Magnitude (LAM) Scale (Schutz and Cardello, 2001).

237 Sweet Liking Status classification was based on Yang *et al.* (2019). This used
238 agglomerative hierarchical clustering (AHC) analysis of overall liking of the four
239 sucrose solutions, followed by Pearson correlation tests between each individual's
240 results and cluster means to validate cluster grouping. Regrouping was applied until
241 the correlation coefficient reached above 0.6 within each cluster group, generating
242 categories of High Sweet Likers, Medium Sweet Likers and Low Sweet Likers. Those
243 participants whose correlation coefficients were lower than 0.6 in any of the three
244 groups were categorised into an Unclassified group

245 2.3.3. Thermal Taster Status determination

246 A circular advanced thermal stimulator peltier thermode (Medoc, Israel) was used to
247 heat and cool the anterior tip of the tongue. Two temperature trials (warming and
248 cooling) were used. For the warming trial, the thermode temperature began at 35°C,
249 was cooled to 15°C and then re-warmed to 40°C and held there for 1s. For the cooling
250 trial, the thermode temperature began at 35°C, was cooled to 5°C and held there for
251 10s (Bajec and Pickering, 2008). Two replicates of each temperature trial were
252 conducted. The temperature ramp for all trials was 1°C/s. Warming trials always
253 preceded cooling trials to avoid possible adaptation from the intense, sustained cold
254 stimulation. A break of two minutes was given before proceeding to the next trial to
255 allow the tongue temperature/sensation to return to normal.

256 Participants were asked to record the taste quality and intensity perceived during
257 temperature stimulation, only if they perceived any. In order to ensure accuracy
258 regarding taste quality reported by TT, a set of taste solutions, the same sample set
259 used for the intensity ratings (Section 2.5) were given to participants in Session 1 to
260 ensure participants were familiar with different taste qualities. TT were defined as
261 those who perceived a taste sensation from both replicates at either warming or
262 cooling trials, whereas TnT were defined as those who did not perceive any 'tastes'
263 throughout the temperature trials (Green & George, 2004). This also left a group of

264 individuals with inconsistent responses (e.g. only perceive taste from a single
265 temperature trial), who were deemed the Uncategorized group.

266 2.4. Taste Genotyping determination

267 Isohelix Buccal swab kits (Cell Projects, Kent, UK) were used for collecting buccal cells
268 from the inside of the cheek for DNA extraction. Buccal swab samples were collected
269 by instructing participants to rub the sterile swab firmly against the inside of their cheek
270 for one minute. The swab head was placed inside the associated tube together with a
271 'Dri-capsule' on top and stored at ambient temperature ($20 \pm 2^\circ\text{C}$). A label with the
272 participant's study number was placed on the tube for identification and anonymity.
273 Samples were sent to LGC Genomics (Herts, UK) for genotyping. Single nucleotide
274 polymorphisms for TAS2R38 – rs713598 and gustin – rs2274333 were genotyped
275 using the Kompetitive allele specific PCR (KASP) method (He *et al.*, 2014).

276 2.5. Perceived taste intensity rating measurement

277 Suprathreshold taste solutions were prepared at the following concentrations: sweet -
278 glucose (117.32g/L), sour - citric acid (1.5g/L), salt- sodium chloride (10g/L), bitter -
279 quinine (0.017g/L), umami/savoury - monosodium glutamate (20g/L) and metallic -
280 ferrous sulphate (0.83g/L) (Sigma Aldrich, UK). All taste samples were prepared using
281 deionised water the day before tasting and stored in a fridge ($5 \pm 2^\circ\text{C}$). All samples
282 were brought out from the fridge at least an hour before tasting, served at ambient
283 temperature ($20 \pm 2^\circ\text{C}$). 10 ml of each sample labelled with random three-digit codes
284 was presented, in duplicate, randomised across participants. Participants were given
285 a one-minute break between stimuli, and water (Evian, Danone, France) as a palate
286 cleanser. Participants were asked to rate the perceived intensity of each tastant on the
287 gLMS scale. The reference gLMS scale was provided during this session and
288 participants were encouraged to refer back to their own reference scale during rating.

289 2.6. Data Analysis

290 All statistical analyses were performed using XLSTAT version 2018. 07 (Addinsoft,
291 Paris, France) at an α -risk of 0.05.

292 Chi-square analysis was used to examine associations among taste phenotypes,
293 genotypes, ethnicity and gender. One-factor ANOVA was applied on PROP intensity
294 across PROP taster status, for sweet liking status, two-factor ANOVA (sweet liking

295 status and sucrose concentration) was applied on overall liking of the four sucrose
296 solutions to confirm robustness of phenotype classifications.

297 For perceived taste intensities, the average between the two replicates was used for
298 statistical analyses.

299 Initially it was planned to run a five factor ANOVA in PTS, TTS, SLS, Ethnicity and
300 Gender to determine if the factors studied impacted on taste perception. However,
301 significant associations were found between ethnicity and taste phenotypes, which
302 created confound effects, thus five-factor ANOVA is inappropriate. Consequently, the
303 data was split into the two separate ethnicity groups, and four-factor ANOVA models
304 were fitted for each ethnicity group (Caucasian vs Asian) separately. However due to
305 low sample size in some subcategories (e.g. only 1 subject in Asian HSL and PROP
306 NT group), including interactions in ANOVA model became problematic, so ANOVA
307 could not be used to investigate possible interactions.

308 Due to the complex nature of the data and interactions in the data, a regression tree
309 analysis was explored to investigate whether it would be a useful tool to highlight the
310 relative impact of the factors studied. Regression tree can break down a dataset into
311 smaller subsets and use a simple algorithm to build a tree structure to demonstrate
312 the subsets in branches. In this case, subsets of taste perception behaviour. It
313 therefore provides a framework to quantify the values of outcomes and the
314 probabilities of achieving them. Stopping rules in the regression tree can be set by
315 user to allow the tree to grow to an optimum size. The interpretation of the results is
316 generally summarised in a tree, which is easier to understand and interpret (Bozkir
317 and Sezer, 2011). Consequently, a regression tree was generated from the intensity
318 ratings for each taste modality, with the five factors as independent variables. The
319 CHAID option was applied with a minimum 5% of participants as the node size. The
320 CHAID technique depends on a significance test at each branch, allowing the
321 branching to stop when splits are not significant.

322 3. Results

323 3.1. Phenotype distribution

324 3.1.1. PROP Taster Status

325 In this study, seventy four participants (33%) were classified as pST, 102 participants
326 (46%) were classified as pMT, and 47 participants (21%) were classified as pNT. As

327 expected, pST rated PROP bitterness significantly higher than pMT and pNT; and pMT
328 rated significantly higher than pNT ($p < 0.0001$) (Figure 1).

329 3.1.2. Sweet Liking Status

330 Ninety-four participants (42%) were classified as High Sweet Likers (HSL), and 38
331 participants (17%) were classified as Medium Sweet Likers (MSL), 59 participants
332 (26%) were classified as Low Sweet Likers (LSL) and 32 participants (14%) were
333 classified as Unclassified due to their inconsistent responses.

334 The ANOVA data showed there is a significant Sweet Liking Status group difference
335 ($p < 0.0001$), where in general, HSL rated liking of sucrose solutions significantly higher
336 than the other three groups, and MSL and UN gave significant higher liking scores
337 than LSL, indicating LSL did not like the sweet solutions as much as the other three
338 SLS groups. As expected, A significant SLS*Concentration interaction was also
339 observed ($p < 0.0001$), where HSL significantly liked the sweeter sucrose solutions (78
340 to 397 g/L) over the low sweetened sucrose solution (0 to 17g/L) (Figure 2A). MSL
341 liked most the medium sweetened samples (78g/L and 168g/L) compared to low sweet
342 (0-17g/l) and high sweet (397g/l) samples (Figure 2B). LSL significantly disliked the
343 high sweetened solutions (168g/L and 397 g/L) than less sweetened solutions (0 to
344 78g/L) ($p < 0.05$) (Figure 2C). No clear trend was observed for UN group (Figure 2D).

345 3.1.3. Thermal Taster Status

346 In this study, 86 participants (39%) were classified as TT, and 109 participants (49%)
347 were classified as TnT, the remaining 28 participants (12%) were classified as
348 Uncategorised due to their inconsistent responses. The most reported taste
349 sensations during the warming trial were sweet (35%), metallic (16%) and bitter (12%).
350 For the cooling trial, the most reported taste sensations were bitter (28%), metallic
351 (19%), sweet (16%), sour (15%) and minty (11%). Other sensations such as salty and
352 spicy were also reported during both warming and cooling trials (Table 2).

353 3.2. Associations between taste phenotypes, genotypes, ethnicity and 354 gender

355 3.2.1. Associations between taste phenotypes and genotypes

356 As expected, a significant association was observed from Chi-square analysis
357 between PTS phenotype and TAS2R38 genotype ($p < 0.0001$). Participants who
358 carried the C:C genotype were more likely to be pST (67%) and less likely to be pNT

359 (6%); whereas participants who carried the G:G genotype were more likely to be pNT
360 (51%) and less likely to be pST (3%) (Table 3).

361 Interestingly, the association between PTS phenotype and gustin genotype
362 (rs2274333) approached significance ($p=0.09$), where a higher proportion of pST (53%)
363 carried the G:G genotype compared to pNT (14%) (Table 4).

364 3.2.2. Associations between taste phenotypes/genotypes and ethnicity
365 Not unexpectedly, a significant association between PTS phenotype and ethnicity was
366 observed ($p<0.0001$), where 55% Asians were pST and 9% were pNT, compared to
367 24% of Caucasians being pST and 26% pNT (Table 5). This data was supported with
368 the significant association between TAS2R38 (rs713598) and ethnic group ($p<0.0001$),
369 where a higher proportion of Asians (43%) carried PROP tasting genotype (C:C) than
370 Caucasians (17%). In addition, a higher proportion of Caucasians (17%) carried the
371 non-PROP tasting G:G genotype compared to Asians (6%) (Table 6).

372 Furthermore a significant ethnicity^gustin association was observed ($p<0.0001$).
373 Asians (38%) were more likely to carry G:G genotype compared to Caucasians (7%),
374 as shown in Table 7.

375 The association between SLS and ethnicity was very close to significance ($p=0.07$),
376 with 31% of Asians classified as HSL, compared to a higher proportion of Caucasians
377 (47%). In addition, 24% and 33% of Asians were classified as MSL and LSL
378 respectively, whereas lower proportions were observed in Caucasians (14% MSL and
379 24% LSL) (Table 8).

380 Notably, an original and interesting finding in this study was that a significant
381 TTS^ethnicity association was observed ($p=0.001$), where 51% Asians were classified
382 as TT, reporting a taste sensation during either warming or cooling trials and only 30%
383 of Asians were TnT. Instead, a much lower proportion of TT (33%) and a much higher
384 proportion of TnT (57%) were observed in Caucasians (Table 9).

385 3.2.3. Associations between taste phenotypes/genotypes and gender

386 No significant association between PTS and gender ($p=0.8$) was observed in this study.
387 A significant SLS^gender association was found ($p=0.03$) in this study, where 52% of
388 males were classified as HSL, compared to a lower proportion in females (38%); and
389 31% of females were classified as LSL, compared to 14% of males (Table 10).

3.2.4. Associations between different taste phenotypes

390
391 Importantly, no significant associations were found among any of the three taste
392 phenotypes ($p>0.05$), indicating these three taste phenotypes are likely to be
393 independent phenotypes.

3.3. Perceived Taste Intensity Rating

394
395 This study investigated the effect of the different taste phenotypes (PTS, SLS and
396 TTS), ethnicity and gender on perceived intensity of taste solutions. Four-factor
397 ANOVA within the Caucasian group showed that limited effects were observed for all
398 the factors examined ($p>0.05$), apart from a significant gender effect on bitterness
399 ($p=0.04$), as shown in Figure 3. Females perceived the bitter intensity significantly
400 higher than males (Figure 3d). Within the Asian group, interestingly, a significant PTS
401 effect was found for salty and metallic taste ($p<0.05$), and an approaching significant
402 effect was found for sour taste ($p=0.09$). As shown in Figure 4a, pST rated saltiness
403 and sourness significantly higher than pNT, where pMT was not significantly different
404 to pST or pNT. However, for metallic taste, it was for both pST and pNT that rated it
405 significantly higher than pMT. An additional TTS effect on metallic taste also
406 approached significance ($p=0.07$), where TT rated metallic taste as significantly higher
407 than Unclassified group.

408 No significant SLS effect was found for any of the tastes ($p>0.05$) measured here. In
409 addition, no significant gender effect was found for any of the individual tastes ($p>0.05$).

3.4. Regression Tree Analysis

410
411 ANOVA demonstrated that it is difficult to decouple the complexity of the data,
412 especially investigating interactions in a complex and relatively small dataset. As an
413 alternative approach, regression tree analysis was explored to interpret the data. For
414 each individual taste intensity, a regression tree was generated with the five factors as
415 independent variables, with a minimum 5% of participants in the node size. No
416 regression tree was generated for sweet, salty, bitter and umami taste.

417 For sour taste, regression tree analysis resulted in seven terminal nodes (see Figure
418 5). The first split, and therefore most influencing factor, was for ethnicity: It shows that
419 Asians had significantly higher predicted sour intensity ratings (1.56 on gLMS scale,
420 30% of total participants) than Caucasians (1.5 on gLMS scale, 70% total participants).

421 Among the Asian group, PROP Taster Status comes into play where pST (1.59 on
422 gLMS scale, 16.6% of total participants) had significantly higher predicted sour taste
423 intensity rating than pMT & pNT group (1.51 on gLMS scale, 13.5% of the total
424 participants). Whereas among the Caucasian group, PROP Taster Status also comes
425 into play, however, pST and pMT were grouped together (1.51 on gLMS scale, 51.6%
426 of total participants), and had a significant higher predicted sourness intensity than
427 pNT (1.45 on gLMS scale, 18.4% of total participants).

428 For metallic taste, a regression tree was also generated, resulting in 7 terminal nodes,
429 as shown in Figure 6. Interestingly, ethnicity again was the first split, where Asians
430 (1.3 on gLMS scale, 30% of total participants) had significantly higher predicted
431 metallic intensity ratings than Caucasians (1.15 on gLMS scale, 70% of the total
432 participants). No further split was observed under the Caucasian group. However, for
433 the Asian group, a further split was observed for PROP Taster Status, where pMT
434 (1.09 on gLMS scale, 10.8% of total participants) had significant lower predicted
435 metallic intensity than pST & pNT group (1.41 on gLMS scale, 19.3% of total
436 participants). No further split was observed for Asian pMT group, but Asian pST/pNT
437 was further differentiated by Thermal Taster Status phenotype. TT (1.48 on gLMS
438 scale, 10.8% of total participants) perceived the metallic taste as significantly higher
439 than TnT/Unclassified group (1.32 on gLMS scale, 8.5% of total participants).

440 4. Discussion

441 4.1. Phenotype distribution and associations

442 PTS distribution (33% pST, 16% pMT and 21% pNT) in the current study is in general
443 agreement with distributions observed in previous studies (Lim et al., 2008, Guo and
444 Reed, 2001). Although a slightly higher pST proportion was observed, this was
445 because higher proportion of Asians (55%) are pST compared to Caucasians (24%).
446 This finding agrees with previous studies (Guo and Reed, 2001, Risso et al., 2016,
447 Rankin et al., 2004) that approximately 25% of the population in Western countries
448 were pST and pNT respectively. In this study, 24% pST and 26% pNT in Caucasians
449 were observed. As expected, a much lower proportion of pNT (9%) was found in Asian
450 participants, which agrees with Guo and Reed (2001)'s finding that only 13.7% of
451 Chinese were pNT.

452 As expected, TAS2R38 genotype is significantly associated with PTS phenotype
453 ($p < 0.0001$), which supported previous findings (Kim et al., 2003b, Prodi et al., 2004)
454 that bitter receptor gene TAS2R38 play a key role in PROP tasting ability. Interestingly,
455 an approaching significant association between PTS phenotype and gustin genotype
456 (rs2274333) was found, where pST carried more G:G genotype. This finding disagrees
457 with previous findings (Barbarossa, et al., 2015, Melis et al., 2013), where an opposite
458 trend was found that pST were more likely to carry the A:A genotype of gustin
459 (rs2274333). Further investigation on ethnicity[^]gustin in the current study revealed that
460 Asians were more likely to carry G:G genotype. The approaching significant
461 PTS[^]Gustin association is undoubtedly due to the fact that Asians are more likely to
462 be pST and carry G:G genotypes. By analysing the association between PTS and
463 gustin genotype within each ethnic group, no significant associations ($p > 0.05$) were
464 found. This is in agreement with another investigation conducted by Feeney and
465 Hayes (2014) that no significant association between PTS and gustin genotype was
466 found. The findings here highlight the importance of characterising recruited
467 participants to better understand the relationship between taste phenotype and
468 genotype, due to international immigration over the last 20 years, recruited participants
469 in Western countries are becoming more ethnically diverse (Abel and Sander, 2014).

470 In addition, this study did not find any significant association between PTS and gender
471 ($p = 0.8$). However, a range of previous research have reported that females were more
472 likely to be supertasters (Bartoshuk et al., 1994, Shen et al., 2016, Robino et al., 2014),
473 but there are also some studies like ours who failed to find such association (Chang
474 et al., 2006, Garneau et al., 2014).

475 SLS distribution (42% HSL, 17% MSL, and 26% LSL) supported recent published
476 research where a slightly lower proportion (34%) of the participants were classified as
477 HSL, and slightly higher proportion (35%) of the participants were classified as LSL
478 (Yang et al., 2019). This could be due to the fact that previous studies used 5 sucrose
479 solutions (6g/L to 360g/L) following Methven et al. (2016)'s approach, whereas the
480 current study used the four sucrose solutions (17g/L to 397g/L) following Yeomans et
481 al. (2007)'s approach. Interestingly, an approaching significant SLS[^]ethnicity ($p = 0.07$)
482 was found, where higher proportion of Caucasians are HSL, and higher proportion of
483 Asians are LSL. This finding builds on the same trend observed in a recent published
484 study by the same research group (Yang et al., 2019). An older study supported the

485 current finding, where US students of European descent gave much higher
486 pleasantness ratings for sweeter cookies compared to Taiwanese students (Bertino et
487 al., 1983). However, it is worth noting that in the current small Asian cohort (n=67),
488 mainly as international students studying at University, 81% were Chinese (n=54), 9%
489 were Indian (n=6), 4% were Pakistan (n=3) and 7% were other Asian background
490 (n=5). Although variation within Asian ethnic groups on food preference and intake has
491 been previously reported (Abdullah et al., 2016), one of the limitations of this study is
492 that ethnic group within Asian participants were not further analysis due to small
493 sample size in subcategories. A larger sample size would be needed to further
494 investigate Asian subethnic groups.

495 Interestingly, this study found that males were more likely to be HSL than females.
496 This finding supports previous work by Turner-McGrievy et al. (2013) where a higher
497 proportion of males were found to be sweet likers. A recent study failed to find a
498 significant association between SLS and gender, but a similar trend was observed
499 (Yang et al., 2019). Several studies have reported that women are associated with
500 greater sweet cravings, especially for chocolate (Zellner et al., 1999, Roininen et al.,
501 2001). In general, women are reported to have experienced more frequent craving in
502 everyday life (Lafay et al., 2001, Hallam et al., 2016), which is believed to relate to
503 hormonal changes (Dye et al., 1995, Asarian and Geary, 2013). However, gender has
504 been found to significantly affect food choices, where females have a higher general
505 health interest than males (Roininen et al., 2001), indicating although females had
506 greater cravings for sweets, they are more conscious with sweet consumption due to
507 health concerns. In this study, HSL referred to participants who prefer high sweetened
508 solutions, whereas LSL prefer low sweetened solution. The fact that higher proportion
509 of females were LSL could be related to females' general health interest. The
510 relationship between Sweet Liking Status phenotype and sweet craving is yet to be
511 investigated. One of the limitations for this study is that gender and ethnicity were not
512 balanced, further studies with more balanced numbers of gender and ethnic groups to
513 further explore these relationships are needed.

514 TTS distribution (39% TT and 49% TnT) agrees with previous literature, where
515 between 20 to 50% of the population have been classified as TT (Bajec and Pickering,
516 2008, Yang et al., 2014, Skinner et al., 2018, Green and George, 2004a). The most
517 reported taste sensations reported in this study agrees with published evidence

518 (Skinner et al., 2018, Yang et al., 2014), and again highlighted sweetness was most
519 popular reported taste sensation during warming, and bitter and sour tastes were the
520 most popular perceived tastes during cooling. Interestingly, metallic taste was once
521 again reported as one of the most frequent reported taste responses perceived from
522 both warming and cooling trial since its first reported by Yang et al. (2014), which
523 warrants further investigation. The reasons why a wider range of TT proportion were
524 reported in previous studies (between 20 to 50%) (Bajec and Pickering, 2008, Yang
525 et al., 2014, Skinner et al., 2018, Green and George, 2004a), were believed to be the
526 variation in classification methods used across different studies. However, this study
527 has suggested that apart from the classification methods, ethnically diverse tested
528 population also contribute to such variation. It is well known that ethnic diversity in
529 terms of food preference and eating habit exist (Green et al., 2003, De Castro, 2007),
530 and it would be interesting to investigate the role of Thermal Taster Status in shaping
531 the diversity of ethnically diverse diets. Yang (2015) has already reported that TT
532 significantly disliked a strawberry flavoured drink served at extreme temperatures (e.g.
533 warm, cold and frozen) more than TnT, but no studies to date have looked at whether
534 differences in food choices across ethnicities could be linked to Thermal Taster Status.
535 In addition, no significant association between TTS and gender was observed ($p=0.6$),
536 supporting Yang et al. (2014)'s previous finding.

537 Although this study has revealed that Asians were more likely to be pST, TT and LSL,
538 it also revealed these three taste phenotypes are likely to be independent phenotypes.
539 Consequently, if an Asian participant is a pST, it does not necessarily mean that
540 he/she is also a TT or LSL. More research as to whether these latter phenotypes have
541 genetic drivers is warranted. The association between taste geno and phenotypes with
542 ethnicity is very interesting, however, the dataset is small in this study (only 67 in Asian
543 population), and research with larger datasets is required to confirm such findings.

544 4.2. Perceived Intensity rating

545 Due to confounding effect between ethnicity and taste phenotypes, including ethnicity
546 in ANOVA is inappropriate. Thus a four-factor ANOVA within each ethnic group was
547 conducted. ANOVA revealed limited effects across different taste phenotypes and
548 gender, apart from a significant gender effect on bitterness in Caucasians, and a
549 significant PTS effect for salty, sour and metallic tastes ($p<0.05$) in Asians. PTS effect

550 for salty taste follows previous findings, where pST and pMT have a general
551 heightened taste responsiveness (Yang et al., 2014, Bajec and Pickering, 2008,
552 Bartoshuk et al., 1998). An additional TTS effect on metallic taste approached
553 significance ($p=0.07$) in Asians. In the current study and previous studies (Yang et al.,
554 2014, Skinner et al., 2018), TT perceived metallic taste as one of the most commonly
555 reported taste sensation during temperature trials, whether the heightened metallic
556 response is linked to their ability to perceive a metallic taste from temperature is
557 currently unknown and warrants further investigation. However, it is worth noting that
558 this effect is only apparent in the Asian group not in the Caucasian group. Conflicting
559 results have been observed in the literature for the effect of Thermal Taster Status in
560 taste sensitivity, where some studies reported that TT have heightened taste response
561 (Bajec and Pickering, 2008, Pickering et al., 2010), whereas others did not (Yang et
562 al., 2014, Bajec and Pickering, 2010).

563 In general, no significant SLS effect was found for any of the taste qualities ($p>0.05$)
564 measured in this study. Previous research has found that the impact of SLS is
565 dependent on sweetness concentration, where an effect was found at lower sugar
566 solutions (3% and 6% sucrose solutions), but such an effect was diminished at intense
567 solutions (above 12% sucrose solution) (Yang et al., 2019). The sweet sample used
568 in this study is glucose (117.32g/L) which is quite intense, which might suppress SLS
569 effect.

570 In general, the effects observed across Asians and Caucasians were not the same,
571 especially for PROP Taster Status. For example, a significant PROP Taster Status
572 effect was found for salty, sour and metallic in Asians, but no such effect was found
573 for Caucasians. This indicates interactions exist between Ethnicity and PROP Taster
574 Status. Previous studies have found significant PTS*gender (Bajec and Pickering,
575 2008), PTS*TTS (Yang et al., 2014) and ethnicity*gender (Williams et al., 2016)
576 interaction in taste sensitivity. However, the data here is rather complex due to
577 confounded effects and small sample sizes in subcategories, which makes
578 investigating interactions using ANOVA models impossible.

579 4.3. Regression tree

580 Conventional ANOVA demonstrated it can be difficult to investigate the complex and
581 relatively small dataset with interactions, thus the alternative approach – regression

582 tree analysis was used to explore the datasets. Interestingly, regression tree was only
583 generated for sour and metallic tastes, but not for sweet, salty, bitter and umami tastes,
584 indicating the effects were not powerful enough to explain the variation in these taste
585 intensities. For sour taste, the first split was ethnicity, with Asians had higher predicted
586 intensity than Caucasians. Within each ethnic group, PTS comes into play, where pST
587 had higher predicted rating than pMT and pNT in Asians, but both pST and pMT
588 combined had higher predicted rating than pNT (Figure 5). From ANOVA output, only
589 significant PTS effect in Asian group was found but not in Caucasian group, indicating
590 regression tree could provide additional information than just traditional ANOVA.
591 Regression tree analysis also provides a visualised relationship tree that makes it
592 easier to understand and interpret the data, and suggested there is a significant
593 interaction between ethnicity and PROP Taster Status, where ANOVA could not
594 deliver due to the complex nature of the data.

595 For metallic taste, ethnicity again was the first split, with Asians had higher predicted
596 intensity ratings than Caucasians. Interestingly, no further split was found under
597 Caucasians. But a further PTS and TTS splits were observed under Asians, where
598 pMT had significantly lower predicted metallic ratings than both pST and pNT. This
599 agrees with ANOVA output that pMT rated lowest (Figure 4a). A further TTS split was
600 observed under Asian pST/pNT group, where TT had a significantly higher predicted
601 intensity rating than TnT/Unclassified. This data was supported with the ANOVA
602 output that TT perceived metallic as strongest (Figure 4b). Looking at the regression
603 tree output, three-way interactions (ethnicity*PROP Taster Status*Thermal Taster
604 Status) are likely to occur, however, ANOVA was not able to demonstrate such
605 complex relationship due to confounded effects and small sample size.

606 One of the limitations in this study is that the subject number of Caucasians and Asians
607 was not equal, as well as the subject numbers in different taste phenotypes, however,
608 it is not realistic to have a balanced design for this type of study. This study found that
609 regression tree could be a useful statistical tool to analyse complicated datasets that
610 have a slightly unbalanced design. However, larger sample size in the Asian group
611 would still be needed to further validate the finding in this study, especially the
612 differences between Asians and Caucasians.

613 The impact of PTS, SLS, TTS, ethnicity and gender were not fully examined in any
614 previous research, the data here suggests that the relationship is rather complex, and
615 multiple interactions are likely to occur. Regression tree analysis provided additional
616 information and can be used as a different approach to look at the data compared to
617 traditional ANOVA analysis. There are multiple statistical techniques available for
618 every data analysis, other techniques such as Partial Least squares discriminant
619 analysis could also be used to analyse this kind of datasets, however one of the
620 advantages of regression tree is that it can visualise the relationship between the
621 variables and related categorical predictors within a tree structure (Gandomi et al.,
622 2013, Miller et al., 2014). We speculate that regression tree analysis provides a useful
623 output of the dataset structure to aid interpretation. Thus, regression trees may
624 therefore be a useful tool to use in the field of sensory and consumer science to better
625 understand and visualise complex datasets.

626 5. Conclusion

627 Although this study was performed on a relatively small sample size, the findings
628 continue to support previous observations that pST were more likely to carry the PROP
629 tasting genes (TAS2R38) and that males were more likely to be High Sweet Likers
630 than females. However, it also brought new insights concerning ethnic differences
631 across different taste pheno and genotypes, where a significant association was
632 observed between ethnicity and taste genotypes. This highlighted the importance of
633 characterising recruited participants. For the first time it is shown that Asians were not
634 only more likely to be PROP supertasters, but also more likely to be thermal tasters or
635 Low Sweet Likers. However, interestingly, no significant association across these
636 three taste phenotypes was found, indicating these three taste phenotypes are
637 independent.

638 In terms of perceived taste intensity, due to confounding effect between factors, five-
639 factor ANOVA was inappropriate. Although 4-factor ANOVA was conducted under
640 different ethnicity groups, interactions could not be included in the ANOVA model due
641 to confounding effect and small sample size, which makes investigating interactions
642 impossible using ANOVA. An alternative approach, regression tree was hence
643 explored and was shown to highlight additional information beyond traditional ANOVA
644 analysis. For example, regression tree has demonstrated two-way interaction in sour
645 taste, and three-way interaction in metallic taste in a visualised framework, which

646 makes data interpretation much easier and efficient. It also enabled clear visualisation
647 at the impact of different factors studied, for example, it is clear that Asian pST TT
648 perceive metallic taste at more intense level than pMT Caucasians in general.
649 Additional and larger studies are needed to further validate the technique for this type
650 of data, but this study has highlighted that regression tree analysis is a promising
651 technique to handle complicated and multifactorial dataset for the sensory and
652 consumer science.

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654

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