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The role of sour and bitter perception in liking, familiarity and choice for phenol-rich plant-based foods

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5

6 **The role of sour and bitter perception in liking, familiarity and choice**
7 **for phenol-rich plant-based foods**

8

9 Pagliarini, E¹, Proserpio C^{1*}, Spinelli S², Lavelli V¹, Laureati M¹, Arena E³, Di Monaco R⁴, Menghi,
10 L^{5,6}, Gallina Toschi T⁷, Braghieri A⁸, Torri L⁹, Monteleone E², Dinnella C²

11

12 ¹Department of Food, Environmental and Nutritional Sciences (DeFENS), University of Milan,
13 20133 Milan, Italy

14 ²Department of Agricultural, Food, Environment and Forestry (DAGRI), University of Florence,
15 50144 Florence, Italy

16 ³Dipartimento di Agricoltura, Alimentazione e Ambiente (Di3A), University of Catania, 95123
17 Catania, Italy

18 ⁴Department of Agriculture Sciences, University of Naples Federico II, 80055 Naples, Italy

19 ⁵Center Agriculture Food Environment (C3A) University of Trento - Edmund Mach Foundation,
20 38010 San Michele all'Adige (TN), Italy

21 ⁶University of Southern Denmark – Department of Technology and Innovation; Campusvej 55 –
22 Odense – 5230 – Denmark

23 ⁷Department of Agricultural and Food Sciences, Alma Mater Studiorum, University of Bologna,
24 40126 Bologna, Italy

25 ⁸School of Agricultural, Forestry and Environmental Sciences (SAFE), University of Basilicata,
26 85100 Potenza, Italy

27 ⁹University of Gastronomic Sciences, Piazza Vittorio Emanuele II, 9, 12042 Pollenzo, Italy

28

29 *Correspondence: cristina.proserpio@unimi.it

30 **Abstract**

31 Among factors influencing food preferences and choices, individual differences in taste perception
32 play a key role in defining eating behaviour. In particular, sour and bitter responsiveness could be
33 associated with the acceptance and the consumption of phenol-rich plant-based foods recommended
34 for a healthy diet. The aim of this study was to investigate, in a large population sample, the
35 associations among sour and bitter responsiveness and liking, familiarity and choice for plant-based
36 foods characterized by these target tastes. Adults aged 18 to 60 years (n = 1198; 58% women) were
37 tested for their sour and bitter responsiveness both in water solutions and in food models (pear juice-
38 based beverages modified in citric acid content to induce different levels of sourness: 0.5, 2.0, 4.0
39 and 8.0 g/kg; chocolate pudding samples modified in sucrose content to induce different levels of

40 bitterness: 38, 83, 119, 233 g/kg). Familiarity, stated liking and choice for fruit juices and vegetables
41 varying for sour/bitter taste (high in bitter/sour taste: e.g. grapefruit juice and cauliflower; low in
42 bitter/sour taste: e.g. zucchini and pineapple juice) were measured. Results showed a significant
43 positive correlation between bitter and sour taste perception in water solutions and model foods, as
44 well as a positive correlation between the perceived intensity of the two taste stimuli. Subjects
45 characterized by high responsiveness to the two target stimuli were found to give lower liking scores
46 to foods characterized by sour/bitter tastes and tended to choose less sour/bitter foods compared to
47 less responsive subjects.

48 Thus, food choice for phenol rich plant-based products could be associated with a reduced
49 responsiveness to bitter and sour tastes and a consequent higher acceptance of food products
50 characterized by these taste qualities.

51

52 **Keywords:** taste perception, food preferences, food choice, plant-based diet, food familiarity

53

54 **1. Introduction**

55 It is widely reported that following a balanced diet is one of the key factors to prevent several non-
56 communicable diseases, such as cardiovascular diseases and some types of cancer. An adequate
57 intake of fruit and vegetables is reportedly associated with a reduced risk of all-cause mortality (Aune
58 et al., 2017) as well as pivotal to ensure the recommended daily intake of micronutrients, such as
59 vitamins and minerals (Hartley et al.,2013).

60 Plant-based foods are rich in dietary fibre and several non-nutrient substances including sterols,
61 flavonoids and other antioxidant compounds showing positive health outcomes (Buttriss & Stokes,
62 2008), which could help to prevent weight gain and reduce the risk of obesity (Mytton et al., 2014).
63 Among antioxidant compounds, phenols present in plant-based foods show several pro-healthy
64 activities, including antimicrobial, anti-inflammatory, and chemo-preventive properties (Servili et al.,
65 2014, De Toffoli et al., 2019).

66 Despite the positive impact that the vegetable and fruit consumption plays on subjects' health, there
67 is evidence reporting that plant-based diet represents also a more environmentally sustainable choice
68 compared with animal-based diet. Previous research highlighted that, assuming a constant daily
69 calorie intake, the meat-based food system requires more water, land and energy than the plant-based
70 food system (Pimentel & Pimentel, 2003; FAO, 2017). More recently, this assumption has been also
71 corroborated by other research showing that plant-based diets require fewer natural resources and
72 have less impact on the environment compared with diets rich in animal-based products (Ruini et al.,
73 2015; Davis et al., 2016). In particular, the results obtained by Ruini et al. (2015) suggested that the

74 Mediterranean diet may lead to a lower environmental impact compared to diets that are heavily based
75 on daily meat consumption. The actual approaches applied to make the global food system
76 sustainable, such as food waste reduction, are inadequate given the global population growth and the
77 lack of natural non-renewable resources (Béné et al., 2020). “Going back” to plant-based diets seems
78 to be an important alternative for a more sustainable future (Sabate & Soret 2014).

79 Although it is clear that a diet rich in fruit and vegetables has several positive aspects, adults often
80 fail to reach the recommend daily intake (Appleton et al., 2016), since the consumption of these
81 products has to face with consumer sensory perception, which is determinant in defining food
82 preference and choices. Plant-based foods are characterized by specific sensory attributes, such as
83 bitterness and sourness (Dinnella et al., 2016), due to the presence of polyphenols, isoflavones and
84 other natural compounds, that are responsible of low acceptability possibly leading to a reduced
85 consumption. Sourness and bitterness are innately disliked (Steiner, 1979; Ventura & Mennella,
86 2011) and could represent ‘warning sensations’ that negatively impact on consumers responses
87 (Laureati et al., 2018)

88 The individual variation in taste perception has been largely investigated as responsiveness to the
89 bitter compound 6-n-propylthiouracil (PROP), which is considered as a marker for taste
90 responsiveness, as well as for responsiveness to chemesthetic sensations (e.g. capsaicin; Spinelli et
91 al., 2018; Nolden et al., 2020) that may influence food preferences and eating behaviours (Tepper et
92 al., 2014). More recently, a general taste responsiveness score was proposed to identify subject groups
93 differing for responsiveness to basic tastes (Puputti et al., 2018). However, to date, little attention has
94 been paid to interindividual variations in sour perception and its possible role in defining food
95 preference and choices. Food choice represents an important measure to investigate and describe
96 actual food behaviours beyond food liking (Spinelli et al., 2020). Indeed, there is more to food choice
97 than sensory acceptance per se, as confirmed for example by market failure of new food formulations
98 that previously overcome consumers’ hedonic test (Gutjar et al., 2015).

99 The majority of the studies used standard solutions with varied stimuli concentrations to measure the
100 intensity of perception of a basic taste (see for a review: Cox et al., 2016), while few studies used
101 actual food (Dinehart et al., 2006, Lanier et al., 2005), and foods as models added with varied
102 concentrations of a tastant (Tornwall et al., 2014). However, the sensory experience of eating is
103 complex, and each component may influence food perception, choice and consequent intake
104 (Boesveldt et al., 2018). In fact, food sensory experience is the result of multisensory interactions with
105 all senses, which play together in defining what is liked or disliked (Delwiche, 2004; Small &
106 Prescott, 2005; Hoppu et al., 2020). Thus, responsiveness to tastes in water do not necessarily
107 associates to their perception in food and to related hedonic responses. The extent to which taste

108 responsiveness is associated with food preferences and food consumption has yet to be fully
109 understood and few studies investigated this relationship in representative population samples (Cox
110 et al., 2016).

111 The aims of the present study were to: 1) investigate sour and bitter perception in water solutions and
112 food models in a large population sample; 2) evaluate how taste responsiveness to these two target
113 tastes could be associated with food choices, familiarity with and liking for selected phenol rich plant-
114 based foods.

115

116 **2. Material and method**

117 **2.1 Participants**

118 One thousand one hundred and ninety-eight subjects (women = 58%; age range: 18–60 years; mean
119 men age: 35.9 ± 12.8 and women age: 35.2 ± 13.0) from different cities from Northern, Central and
120 Southern Italy were recruited in the study. Eight research units took part in data collection.
121 Participants were recruited by means of participant universities and research centers' websites,
122 announcements on social networks, article in national newspapers, mailing lists, pamphlet
123 distribution, and word of mouth. Exclusion criteria were pregnancy, breastfeeding, not being born in
124 Italy or having lived less than 20 years in Italy.

125 The study was conducted in agreement with the Italian ethical requirements on research activities and
126 personal data protection (D.L. 30.6.03 n. 196) and in adherence with the principles laid down the
127 Declaration of Helsinki. The protocol was approved by the Ethics Committee of Trieste University
128 and participants gave their written informed consent at the beginning of the study.

129

130 **2.2. Sensory stimuli**

131 *Tastant solutions*

132 Citric acid and caffeine (Sigma-Aldrich) were used to elicit sourness and bitterness perception. Two
133 solutions were prepared by dissolving 4 g/kg of citric acid and 3 g/kg of caffeine in water. These
134 concentrations were chosen based on previously published data (Monteleone et al., 2017).

135

136 *Food models*

137 Pear juice (J) and dark chocolate pudding (P) were selected as appropriate food matrices for testing
138 sour and bitter perception in food models (Monteleone et al., 2017). Ingredients and products
139 distributed by large food companies were used in order to obtain a constant composition and to avoid
140 problems associated with products seasonality. Pudding base formulation was prepared by mixing a
141 commercial pudding powder (ingredients: starch, low-fat cocoa, dextrose, salt, aromas; Cameo

142 S.p.A., Dr. Oetker, Bielefeld, Germany) with 40 g of cocoa powder and 1L of water at 40°C. This
143 mixture was heated in microwave at 900W for 6 min and then at 450W for 4 min. The heating was
144 stopped every 2 min to mix the pudding. A commercial pear juice (ingredients: water, Williams pear
145 puree 50%, sugar, flavourings, acidifier: acid citric; antioxidant: ascorbic acid; Santal, Parmalat
146 S.p.A., Milan, Italy) was used for the base juice formulation. Four pear juice and four dark chocolate
147 pudding samples were prepared by adding, respectively, increased concentrations of citric acid (pear
148 juice: J₁=0.5 g/kg; J₂=2.0 g/kg; J₃=4.0 g/kg and J₄=8.0 g/kg) and sucrose (chocolate pudding: P₁=38
149 g/kg; P₂=83 g/kg; P₃=119 g/kg and P₄=233 g/kg) to base formulations. Tastants concentrations were
150 selected to elicit a variation in the strength of target sensations from weak to strong. Both food models
151 were preliminarily described by a focus group of trained subjects. Pear juice was characterized by
152 sweetness, sourness and pear flavour; chocolate pudding by sweetness, bitterness, chocolate flavour
153 and to a lesser extent by astringency.

154

155 **2.3. Questionnaires**

156 *Food familiarity and stated liking*

157 Familiarity with and stated liking for phenol-rich vegetables were measured using a selection of the
158 IT-Food Familiarity Questionnaire (IT-FFQ) and of the IT-Food Preference Questionnaire (IT-FPQ),
159 developed within the Italian Taste (IT) project (Monteleone et al., 2017). The selection included ten
160 vegetables (carrots salad, zucchini, lettuce and valerian salad, chard, broccoli, asparagus, radish,
161 artichoke, chicory, radicchio and rocket salad) and two fruit juices (grapefruit and pineapple) with
162 varied level of expected bitterness and sourness according to results from a preliminary study
163 conducted at the University of Florence. A Check-All-That-Apply (CATA) questionnaire was used
164 to describe sensory properties of IT-FFQ and IT-FPQ items (De Toffoli et al., 2019). Here only results
165 of “bitterness” and “sourness” attributes in vegetables (201 respondents, 77.7% women; age range
166 18–70; mean age 40.3 ± SD 14.1) and fruit juices (188 respondents, 75.4% women; age range 19–68;
167 mean age 40.1 ± SD 14.3) were reported. To check for the correct use of terms to describe sensory
168 properties, a semantic categorisation task was applied; participants to the CATA test were asked prior
169 to the test to provide the best example coming to their mind of a “sour” and of a “bitter” food,
170 respectively (e.g. “Sour as...”).

171 Familiarity for the selected items was measured using a 5-point labelled scale (1 = I do not recognize
172 it; 2 = I recognize it, but I have never tasted it; 3 = I have tasted it, but I don’t eat it; 4=I occasionally
173 eat it; 5 = I regularly eat it; Tuorila et al., 2001) while stated liking was assessed using the 9-point
174 hedonic scale (1: extremely disliked; 9: extremely liked, Peryam & Pilgrim, 1957). If the participant

175 had never tasted the food in question, he/she could choose the answer “I have never tasted it”. The
176 presentation order of the items was randomized across participants.

177

178 *Food choice*

179 Three vegetables pairs (1: lettuce and valerian salad vs radicchio and rocket salad; 2: zucchini vs
180 asparagus; 3: chard vs chicory) and two fruit juice pairs (1: multivitamin juice - made with carrots,
181 oranges and lemons - vs orange juice; 2: pineapple juice vs grapefruit juice) were selected from the
182 IT-Food Choice Questionnaire (Monteleone et al., 2017) so that the options in each pair significantly
183 differed for bitterness and sourness. For each pair, respondents were asked to indicate which option
184 they would choose in a main meal either lunch or dinner (for vegetables) or breakfast (for fruit juices).
185 The presentation order of the pairs of food items within each meal occasion (breakfast, lunch and
186 dinner) was randomized across participants.

187

188 **2.4. Sensory evaluations**

189 **2.4.1 Training session to the evaluation of taste stimuli and to the use of the scales**

190 Subjects participated in a training session immediately before the evaluation session. In the first part
191 of the training session, subjects were familiarized with the target sensations. For each sensation,
192 appropriate food and beverages examples were recalled and discussed (chicory, black coffee and
193 tonic water were used to recall bitter taste; fresh lemon juice was used as an example of sourness).
194 Participants were encouraged to join the discussion giving their own examples of food and beverages
195 characterized by the target sensations and the appropriateness of their examples provided was
196 collectively discussed. This part of the training session ended with a verbal agreement on the meaning
197 of the target sensations. In the second part of the training session, participants were instructed to the
198 use of the general Labelled Magnitude Scale (gLMS; 0: no sensation; 100: the strongest imaginable
199 sensation of any kind; Bartoshuk et al., 2004) following published standard procedures (Green et al.,
200 1993; Bartoshuk, 2000).

201 Subjects were extensively instructed to treat the “strongest imaginable sensation” as the most intense
202 sensation they could ever imagine experiencing. To familiarize the participants with the scale
203 anchors, they were asked to recall a variety of remembered sensations from different modalities
204 (Bajec & Pickering, 2008; Kalva et al., 2014; Webb et al., 2015). Examples of oral (e.g. the cold of a
205 cube of ice in the mouth; the pungency from hot chili pepper) and non-oral sensations (e.g. the noise
206 of a plane that is flying low, the pain felt when shutting a finger in a door) were proposed to encourage
207 the discussion. To practice on the use of the gLMS, subjects were asked to rate the intensity of the
208 brightest light they had ever seen on a paper ballot. The criterion to conclude that the subjects

209 correctly used the scale was that their ratings were higher than “very strong” and lower than “the
210 strongest imaginable sensation of any kind”. Ratings out of this range were individually discussed
211 and the correct use of the scale clarified (Dinnella et al., 2018). Despite an extensive training was
212 performed with the subjects involved, a measure from an independent modality (e.g., sound, or sight)
213 to corroborate the correct use of the scale was performed but not recorded in the present study.
214 However, a similar approach using recalled sensations has been used in many studies (Parkinson et
215 al., 2016; Duffy et al., 2019; Yang et al., 2019).

216

217 **2.4.2. Evaluation session**

218 Subjects were instructed to hold the whole tastant solution in their mouth for 3 s, then expectorate,
219 wait few seconds and evaluate the perceived intensity. Tastant solutions (10 mL) were presented in
220 80 cc plastic cups identified by a 3-digit code in random order. Food samples (15 g) were presented
221 in 80 cc plastic cups identified by a 3-digit code. Pear juice and dark chocolate pudding samples were
222 presented in independent sets each consisting of four samples presented in random order. Pear juice
223 was presented as first set followed, after a 10 min break, by chocolate pudding.

224 Subjects were instructed to hold the whole pear juice sample in their mouth or to take a full spoon of
225 chocolate pudding, then swallow and evaluate relevant sensory qualities according to the food model
226 considered. For pear juice, participants were asked to evaluate the intensity of sourness, sweetness,
227 and the overall flavour of pear juice. Conversely, the intensity of sweetness, astringency, and the
228 overall flavour of chocolate pudding were chosen to evaluate the perception of the chocolate pudding.
229 Only sourness in pear juices and bitterness in chocolate puddings were here considered for data
230 analysis. The intensity of each sensation was rated on a gLMS and after each sample, subjects rinsed
231 their mouth with water for 30 s, ate some plain crackers for 30 s, and finally rinsed their mouth with
232 water for a further 30 s. Evaluations were performed in individual booths under white lights. After
233 the tasting session, participants filled in the questionnaires. Data were collected with the software
234 Fizz (ver. 2.51. A86, Biosystèmes).

235

236 **2.5. Data analysis**

237 Cochran’s Q test was applied to data from CATA questionnaire to check for significant differences
238 in sour/bitter citation among vegetables and fruit juices. Depending on the level of expected
239 bitterness/sourness expressed by participants, vegetables and fruit juices were assigned to either the
240 “*High bitter/sour*” or to the “*Low bitter/sour*” group. McNemar’s *post hoc* test was performed as
241 multiple comparison test.

242 Subjects were divided into three age groups: group 1=18–30 years (45%), group 2=31–45 years
243 (28%) and group 3=46–60 years (27%). The age distribution of men and women was not significantly
244 different according to chi-square test ($\alpha = 0.05$). The normality assumption of continuous data was
245 tested by Skewness and Kurtosis.

246 Responsiveness to sour and bitter tastes in water solutions was investigated by means of Two-way
247 ANOVA models considering gender (women and men), age (group 1, group 2 and group 3) as well
248 as their interaction as factors. Participants' responsiveness to the target tastes in pear juice and
249 chocolate pudding samples was assessed by separate ANOVAs considering gender, age, samples
250 (four levels) and their second/third order interactions as factors. When a significant difference
251 ($p < 0.05$) was found, the LSD *post hoc* test was performed as multiple comparison test.

252 Correlations between taste responsiveness in water solutions and food models were examined using
253 Pearson's correlation coefficient with a minimum significance level defined as $p < 0.05$.

254 Subjects were segmented according to their responsiveness to both sour and bitter tastes in water
255 solutions by means of Hierarchical Cluster Analysis.

256 Two familiarity scores were computed for each subject as the sum of ratings given to high bitter/sour
257 items (*FAM_High bitter/sour*) and to low bitter/sour items (*FAM_Low bitter/sour*) of the food
258 familiarity questionnaire (range from 1 to 5). Two liking scores were computed for each subject as
259 mean of the liking ratings for to high bitter/sour items (*LIK_High bitter/sour*) and to low bitter/sour
260 items (*LIK_Low bitter/sour*) of the food preference questionnaire (range from 1 to 9). Options within
261 the pairs of the Food Choice Questionnaire were coded as "0" if the low bitter/sour option was chosen
262 and "1" if the high bitter/sour option was selected. For each subject, a choice index (*CHO_Index*)
263 was then calculated as the sum of the choices of the bitter/sour option (range from 0 to 5). Differences
264 in familiarity, liking and choice scores between the clusters with different taste responsiveness were
265 evaluated by means of separate ANOVAs and then displayed using rain cloud plots. R 4.0.2 (R Core
266 Team, 2020) was used for this latter graphical representation. Partial eta squared (η^2 values: 0.01
267 small; 0.06 medium; 0.13 large; Cohen, 1988) was applied to evaluate the effect size. All the analyses
268 were performed using IBM SPSS Statistics for Windows, Version 24.0 (IBM Corp., Armonk, NY,
269 USA), with the exception of the CATA data that were analysed using XLSTAT 19.4.1 (Addinsoft).

270

271 **3. Results**

272 ***3.1. Differences for expected bitterness and sourness among questionnaire items***

273 Results of the semantic categorisation task showed that the number of subjects who provided as
274 example a term that was ambiguous or not correct was negligible (3.3% in the case of bitterness, 1.6%
275 in the case of sourness), thus indicating that the subjects understood the concept of sour and bitter

276 taste. Cochran's Q test results obtained in the preliminary study applying CATA methodology are
 277 reported in **Table 1**.

278

279

280 **Table 1.** Percentage of participants who selected the terms “bitter” and “sour” in the CATA
 281 experiment for selected vegetables and fruit juices and their consequent classification in *Low* and
 282 *High bitter/sour*. Different letters by columns within each food products category (vegetables and
 283 fruit juices), indicate significant differences ($p < 0.05$) according to McNemar's test.

284

285

Food item	Bitter	Sour
<i>Vegetables</i>		
<i>Low bitter/sour</i>		
Carrots salad	3 ^a	6 ^{ab}
Zucchini	12 ^b	4 ^a
Lettuce and valerian salad	19 ^{bc}	6 ^{ab}
Broccoli	24 ^{cd}	6 ^{ab}
Chard	27 ^{cd}	6 ^{ab}
<i>High bitter/sour</i>		
Asparagus	35 ^{de}	13 ^{bc}
Radish	46 ^e	22 ^c
Artichoke	63 ^f	15 ^{bc}
Chicory	82 ^g	19 ^c
Radicchio and rocket salad	82 ^g	20 ^c
<i>p-value</i>	<0.0001	<0.0001
<i>Fruit juices</i>		
<i>Low bitter/sour</i>		
Multivitamin juice	12 ^a	55 ^a
Pineapple	11 ^a	45 ^a
<i>High bitter/sour</i>		
Orange juice	29 ^b	70 ^b
Grapefruit	75 ^c	75 ^b
<i>p-value</i>	<0.0001	<0.0001

286

287 **3.2. Taste perception in water solutions and food models**

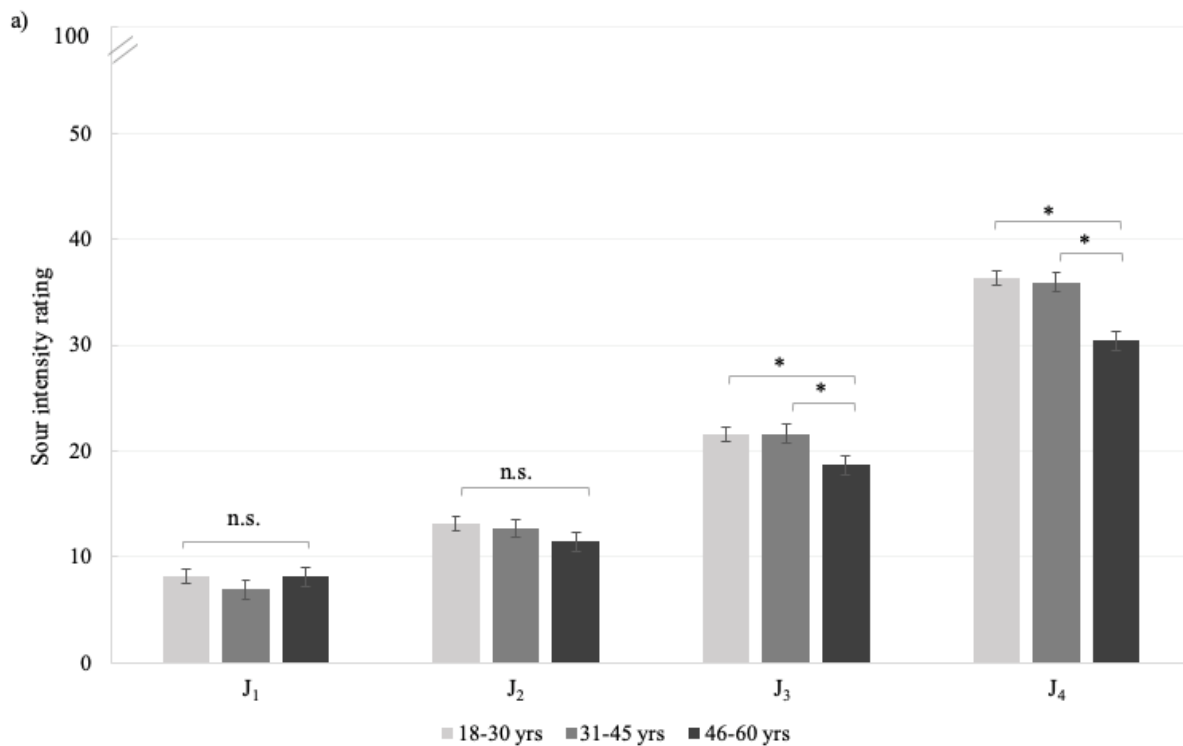
288 No significant gender effects on sour and bitter perception in water solutions were found. Only weak
289 tendencies have been highlighted for sour and bitter perception according to age ($F_{(2,1192)}=2.72$,
290 $p=0.06$, $\eta^2= 0.005$; $F_{(2,1192)}=2.21$, $p=0.11$, $\eta^2= 0.004$, respectively), with the youngest group of
291 subjects (18-30 years old) that tended to be more responsive compared with subjects aged 31-45 and
292 46-60 years.

293 Considering the pear juice and chocolate samples, results revealed a significant effect of the main
294 factor sample ($F_{(3,4768)}=674.90$; $p<0.000$; $\eta^2= 0.29$; $F_{(3,4768)}=647.73$; $p<0.0001$; $\eta^2= 0.29$;
295 respectively). Sour intensity ratings systematically increased from J₁ (7.7 ± 0.4) to J₄ (34.2 ± 0.4) in
296 pear juice samples and bitterness systematically decreased from P₁ (30.0 ± 0.4) to P₄ (6.6 ± 0.4) in
297 chocolate pudding samples. The main factor gender was not significant for sourness and bitterness in
298 model foods.

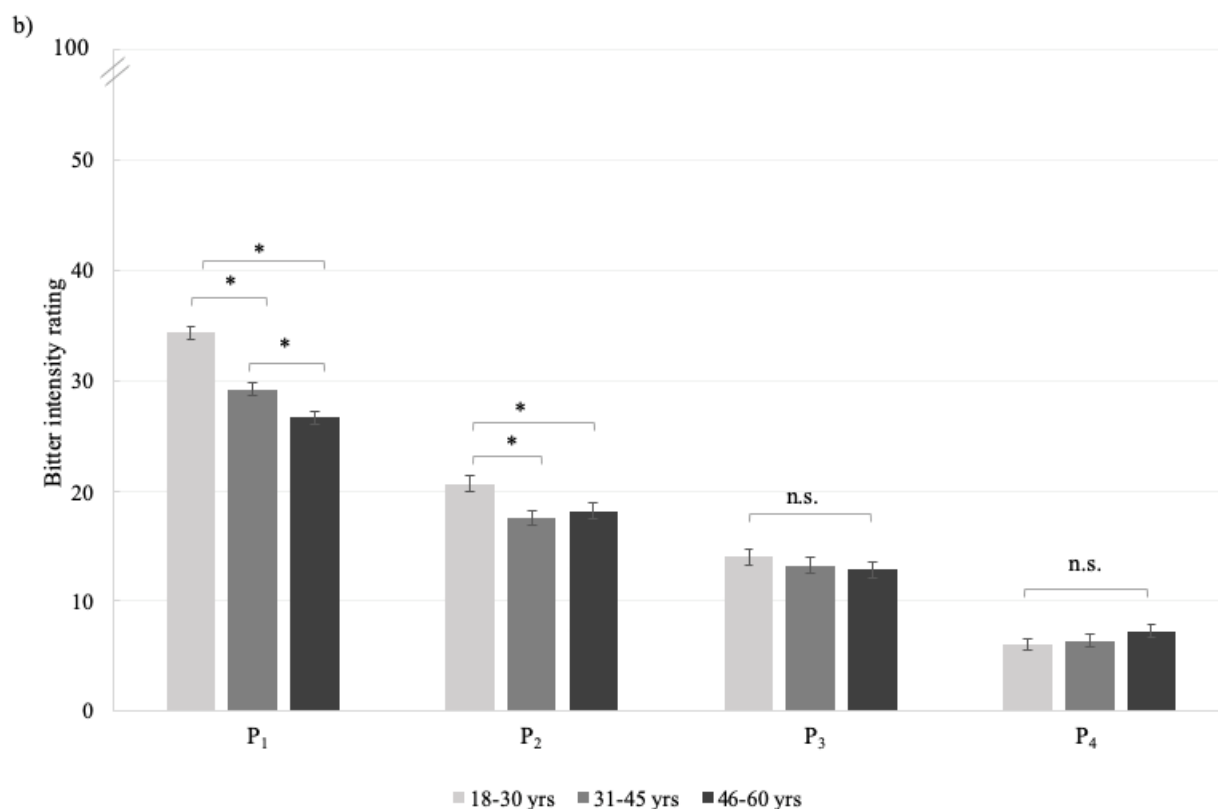
299 Age was associated with the perceived intensity of both sourness in pear juice and bitterness in
300 chocolate but to a lesser extent ($F_{(2,4768)}=12.67$; $p<0.0001$; $\eta^2= 0.005$; $F_{(2,4768)}=19.19$; $p<0.0001$; $\eta^2=$
301 0.008 , respectively).

302 In both model foods the interaction age*samples (**Figure 1a-b**) showed a significant but very
303 small/small effect on sour and bitter responsiveness ($F_{(6,4768)}=3.66$, $p<0.001$; $\eta^2= 0.005$; $F_{(6,4768)}=9.20$,
304 $p<0.0001$, $\eta^2= 0.01$ respectively.). An age effect was found on intensity ratings only in samples where
305 the intensity of target sensations was rated at moderate level or higher. Samples J₃ and J₄ were rated
306 lower in sourness by subjects aged 46 to 60 years than younger (18-30 and 31-45 years), which did
307 not significantly differ from each other. Bitterness intensity decreased with increasing age in sample
308 P₁ and it was rated higher by subjects aged 18-31 years than older (31-45 and 46-60 years), which
309 did not significantly differ from each other. The lack of significant differences due to age in sample
310 J₁ and J₂ and P₃ and P₄ is possibly due to a floor effect induced by the low intensity level of the target
311 sensations in these samples (ranging from weak to less than moderate).

312



313



314

315 **Figure 1a-b.** Sour (a) and bitter (b) mean intensity ratings (\pm SEM) by samples (pear juice samples:
 316 J₁ - J₄; chocolate pudding samples: P₁ - P₄) and age groups (18-30; 31-45; 46-60 years old). * $p < 0.05$;
 317 n.s. not significant
 318

319 The interaction age*gender showed a significant but very small effect ($F_{(2,4768)}=4.06$, $p < 0.05$; $\eta^2=$
 320 0.002) only on sour intensity ratings. In particular, among subjects of 31-45 years, men gave

321 significant lower intensity ratings (18.4 ± 0.6) compared to women (20.2 ± 0.5), while no gender
 322 differences were found in the other age groups (group 1 and group 3). The interaction gender*sample
 323 was significant ($F_{(3,4768)}=3.02$, $p<0.05$; $\eta^2= 0.002$) only on bitter intensity ratings. Gender-related
 324 differences have been found only for sample P₁ which was perceived as more bitter by women (31.0
 325 ± 0.5) compared to men (29.1 ± 0.6). The other interactions were not significant.

326 Pearson correlations coefficients (**Table 2**) highlighted a significant positive correlation among sour
 327 intensity perceived in water solution and in pear juice samples. The correlation became stronger with
 328 the increasing amount of citric acid in the pear juice. A significant positive correlation was also found
 329 between bitter intensity perceived in water solution and in chocolate pudding samples. The correlation
 330 became weaker with the increasing amount of sucrose as the intensity of the bitterness decreased.
 331 Moreover, bitter and sour perception were always weakly but positively correlated to each other both
 332 in water solution and food models. For example, the sourness perception in samples J₄ with the higher
 333 amount of citric acid was significantly and positively correlated with the bitterness perception in the
 334 chocolate pudding sample with the lower amount of sugar P₁ (most bitter). Pearson correlations
 335 performed with consumers split according to the three-age groups revealed similar results (see
 336 supplementary material).

337

338 **Table 2.** Pearson correlation coefficients among taste perception (S= sour, B=bitter) in water solution
 339 and model foods (pear juice with increasing citric acid: J₁=0.5 g/kg; J₂=2.0 g/kg; J₃=4.0 g/kg and
 340 J₄=8.0 g/kg; Chocolate pudding with increasing sugar: P₁=38 g/kg; P₂=83 g/kg; P₃=119 g/kg and
 341 P₄=233 g/kg)

342

	S_ water	S_ J ₁	S_ J ₂	S_ J ₃	S_ J ₄	B_ water	B_ P ₁	B_ P ₂	B_ P ₃	B_ P ₄
S_ water	1									
S_ J ₁	.17	1								
S_ J ₂	.24	.51	1							
S_ J ₃	.30	.38	.54	1						
S_ J ₄	.35	.26	.47	.63	1					
B_ water	.36	.12	.19	.19	.27	1				
B_ P ₁	.31	.19	.24	.30	.42	.37	1			
B_ P ₂	.24	.22	.26	.26	.33	.29	.58	1		
B_ P ₃	.22	.26	.28	.31	.29	.25	.45	.53	1	
B_ P ₄	.14	.28	.26	.15	.17	.15	.19	.35	.41	1

343 All values are significant at $p < 0.01$

344

345 **3.3 Consumers segmentation according to sour and bitter taste responsiveness**

346 Sour and bitter intensity in water were used as a general index to classify subjects according to their
 347 responsiveness to target tastes. Two clusters were identified showing significant differences in sour
 348 ($F_{(1,1196)}=1456.46$; $p<0.000$; $\eta^2=0.55$) and bitterness perception ($F_{(1,1196)}=418.71$; $p<0.000$; $\eta^2=0.26$).

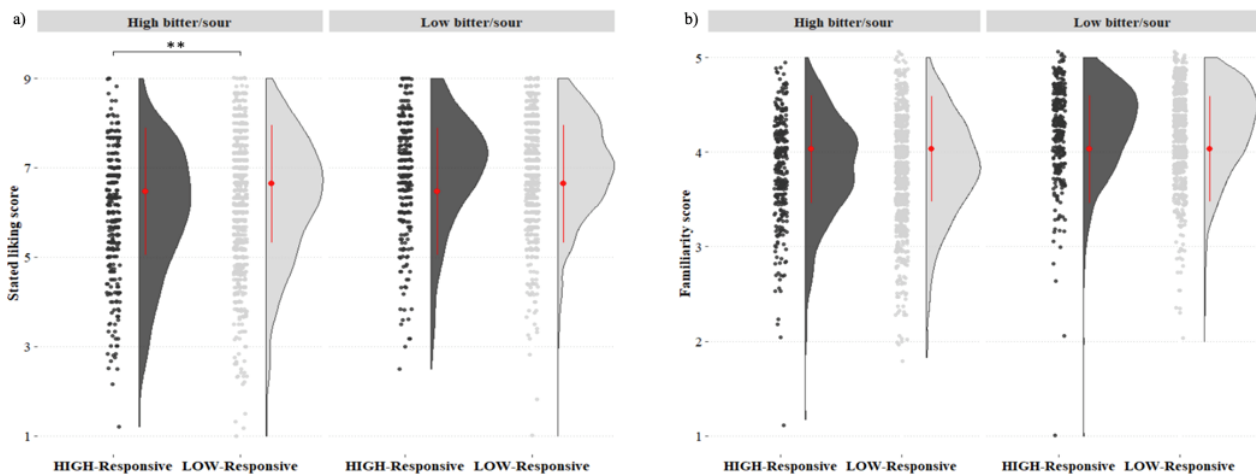
349 In particular, Cluster 1 (*HIGH_Responsive*; n=309) showed higher responsiveness to both the target
 350 tastes (sour: 60.2 ± 0.8 ; bitter: 49.5 ± 1.0) compared to Cluster 2 (*LOW_Responsive*, n= 889; sour:
 351 25.0 ± 0.5 ; bitter: 25.5 ± 0.6). According to χ^2 test, age and gender distributions were not significantly
 352 different between clusters ($p > 0.05$).

353

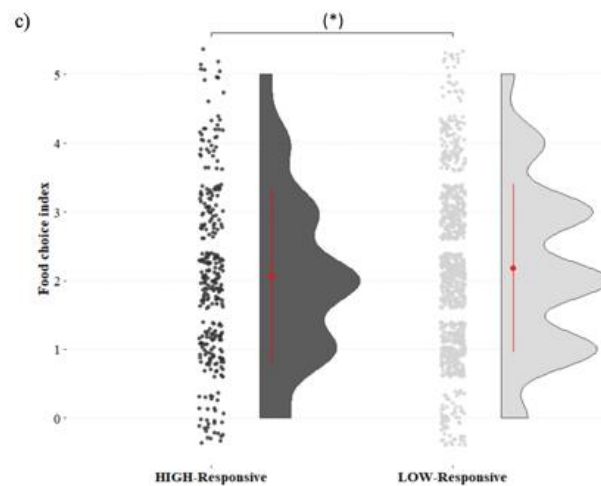
354 **3.4. Associations among sour/bitter responsiveness and familiarity with, liking for and choice of** 355 **plant-based foods**

356 Clusters significantly differed in liking scores for *High bitter/sour* vegetables and fruit juices (F
 357 $(1:1193) = 10.19$; $p < 0.001$; $\eta^2 = 0.06$) (**Figure 2a**). Consumers more responsive to these target tastes
 358 (*HIGH_Responsive*) gave significant lower liking scores to *High bitter/sour* vegetables and fruit
 359 juices (6.0 ± 0.08) compared to less responsive subjects (*LOW_Responsive*, 6.3 ± 0.05). No
 360 significant differences between clusters were observed for liking scores for *Low bitter/sour* group (F
 361 $(1:1193) = 0.52$; $p = 0.47$). Familiarity scores for both *High* and *Low bitter/sour* items were not
 362 significantly different by cluster (*High bitter/sour*: F $(1:1188) = 0.02$; $p = 0.89$; *Low bitter/sour*:
 363 F $(1:1188) = 0.67$; $p = 0.80$) (**Figure 2b**). Clusters tended to differ in food choice score ($p < 0.10$) with
 364 *HIGH_Responsive* subjects showing a lower choice for *High bitter/sour* food (2.0 ± 0.07) compared
 365 to *LOW_Responsive* subjects (2.2 ± 0.04) (**Figure 2c**). Results split according to the three-age groups
 366 revealed that the differences in eating behavioural variables by clusters were mainly associated with
 367 subjects aged 18-30 years (see supplementary material).

368



369



370

371 **Figures 2a-c.** Raincloud plot showing the differences on food stated liking scores (a), familiarity
 372 scores (b) and food choice index (c) for *High* and *Low sour/bitter* foods as a function of *HIGH-*
 373 *Responsive* and *LOW-Responsive* clusters. The plots provide a representation of data distribution (the
 374 ‘cloud’), individual raw observations (the ‘rain’), the mean (red filled circle) \pm SD (perpendicular). *
 375 $p < 0.05$; (*) $p < 0.10$.

376

377

378 4. Discussion

379 Sour and bitter perception in water solutions and food matrices were evaluated in a large population
 380 sample to investigate if responsiveness to these target tastes was associated with food choices,
 381 familiarity with and liking for specific phenol rich plant-based foods (vegetables and fruit juices).

382 The present results highlighted a weak but significant positive correlation between the perception of
 383 sour and bitter tastes in water solutions. In this vein, Cattaneo and colleagues (2019), have recently
 384 reported a positive correlation between sour and bitter thresholds in a small group of healthy adults.
 385 Moreover, clusters based on tastant solution perception (more sensitive, semi-sensitive, and less
 386 sensitive tasters) have been identified by Puputti et al., 2018 involving a large population sample.
 387 The authors highlighted that the membership in a taste cluster could be partially forecasted by the
 388 sensitivity to other taste modalities. This correlation among tastes mediated by different mechanisms,
 389 G-coupled protein receptors for bitter and ion channels for sour (Drayna, 2005), could be explained
 390 by a dichotomy in taste coding for pleasant compounds, such as sweet and savoury, versus those
 391 perceived as dangerous, such as sour and bitter stimuli (Hladik et al., 2002). It could be questioned
 392 that the correlations here highlighted could be due to the well-established sour-bitter confusion
 393 (Robinson, 1970; Gregson & Baker, 1973). However, prior to tasting, extensive instructions were
 394 provided by the experimenters to the subjects to avoid this misperception. Moreover, in this study
 395 sourness and bitterness were evaluated in different food samples (the former on pear juices and the
 396 latter on chocolate puddings). It is also worth considering that sourness was evaluated for a pure

397 stimulus in water and for a fruit juice added with citric acid. The intensity of sourness in fruit juice
398 significantly increases with citric acid concentration (see fig. 1a) thus it is reasonable to assume that
399 ratings refer to sour taste and not to bitter taste. Bitterness was rated in a water solution of a pure
400 stimulus and in chocolate added with increasing amount of sugar. Bitterness regularly decreases as
401 effect of suppression by sweetness (see fig. 1b). All these considerations make unlikely the confusion
402 between the two sensations.

403 The present results depicted also a positive correlation between sour/bitter perception in water
404 solutions and in food matrices with correlations becoming stronger in samples characterized by higher
405 intensity of the two target tastes. High responsive subjects to bitter taste seems also to be high
406 responsive to sour, both in water and in food models. Several studies have investigated how taste
407 sensitivity varies among individuals and how this is related to food consumption and subsequent
408 consumer health status (see for a review: Cox et al., 2016). Several authors focussed their attention to
409 sweet and salty perception that could be directly associated with the consumption of food rich in
410 calories and fats. Similarly, bitter perception and food liking represents a widely investigated field of
411 research, while less attention has been paid to sour taste. Moreover, research has been conducted
412 using solution-based approaches to measure hedonic responses (e.g. Drewnowski et al., 1985; Salbe
413 et al., 2004); this can help in modelling perceptual mechanisms but fails to represent the daily
414 experience with foods. Taste responsiveness measured using real foods could provide instead deeper
415 information on food preferences and choice even if fewer studies using this approach are available
416 (e.g. Dinehart et al., 2006; Tornwall et al., 2014; Proserpio et al 2016; Dinnella et al., 2018).

417 Looking to age effects on bitter and sour responsiveness older subjects (46-60 years old) tended to
418 give lower intensity rating scores in water solutions compared to younger subjects. This tendency
419 was found to become significant, although the effect size was always small, considering bitter and
420 sour perception in food models. These results are supported by previous evidence reporting a decline
421 in the gustatory function, mainly investigated using aqueous solutions, in the older population that
422 could be due to several factors, including physiological changes such as a taste receptor cells
423 dysfunction (Methven et al., 2012). Even if evidence about the extent and type of taste loss with
424 aging, sour and bitter tastes seem to be the most affected taste with increasing age (Sergi et al., 2017).
425 The present findings are in line with previous results by Hansen and colleagues (2006) who reported
426 an inverse association between age and the bitter taste of caffeine. Interestingly, the results of our
427 study revealed a systematic decrease in sour/bitter perception in food models with increasing age but
428 only at the highest concentration of the target tastes. Indeed, an age effect was found only in pear
429 juice samples with higher citric acid concentrations, and in the more bitter chocolate pudding samples.
430 Accordingly, recent data by a large sample of Caucasian European subjects demonstrated a significant

431 decrease in taste perception for all five basic tastes, measured in water solutions, with increasing age,
432 and this association was found to be stronger for the higher concentrations especially for bitter and
433 sour (Barragán et al., 2018).

434 No differences in taste perception by women and men in both water solutions and model foods have
435 been here highlighted. The relationship between taste perception and gender yield to mixed literature
436 results (Fischer et al., 2013; Shen et al., 2016, Dinnella et al., 2018) that could be due to several
437 factors, such as the methodology applied to measure taste responsiveness, the food matrix used to
438 elicit different taste perceptions as well as the sample size of subjects involved.

439 Responsiveness to the two target tastes was associated with food liking for the selected food items
440 only in the most responsive consumers. These subjects expressed lower liking for vegetables and fruit
441 juices characterized by high sour/bitter tastes compared to least responsive subjects. Cox et al., (2012)
442 depicted that sensory perception tended to predict liking and intentions to consume brassica
443 vegetables. For example, broccoli hedonics as well as intentions to consume these vegetables were
444 predicted by bitterness perception. Contrarily, recent findings on a large sample size of Finnish adults
445 failed to find a relationship between bitter sensitivity and either vegetable liking or consumption
446 (Puputti et al., 2019). Our results are in line with previous findings showing that perceived bitterness,
447 correlated also with sour taste, of brussels sprouts, kale and asparagus is negatively associated with
448 vegetable preferences (Dinehart et al., 2006) and with findings showing that liking was inversely and
449 significantly associated with perceived bitterness in beverages (grapefruit juice, beer, and scotch;
450 Lanier et al., 2005). Literature data on fruit and vegetable preferences with respect to taste
451 responsiveness is controversial and it has been predominantly investigated through PROP (e.g. Duffy
452 et al., 2010, Bell and Tepper, 2006; Armstrong and Mattes 2008; Kaminski et al., 2000) as general
453 marker of taste responsiveness, as well as chemesthetic sensations (e.g. capsaicin; Nolden et al.,
454 2020).

455 No significant differences among subjects with different taste responsiveness on preference for low
456 bitter/sour foods was found, suggesting that the differences in preference were related to taste stimuli
457 usually associated to warning sensations and something that could be potentially toxic, non-edible as
458 well as unripe fruits and spoiled foods (Laureati et al., 2018). Looking also to the familiarity data, no
459 differences in the scores provided by the two clusters of consumers to the food items considered have
460 been shown. This lack of difference between clusters can be explained by the fact that all the food
461 items included in the questionnaires are usually part of the Mediterranean diet, that is widely adopted
462 in Italy (Predieri et al., 2020).

463 Interestingly, the two clusters tended to differ in the choice for vegetables and fruit juices
464 characterized by intense sour/bitter tastes. In particular, low bitter/sour responsive subjects seem to

465 choose more specific sour/bitter plant-based foods (e.g. chicory and grapefruit juice) compared to the
466 high responsive subjects. These results, even if the differences highlighted are small, corroborated
467 the previous liking findings suggesting that subjects less responsive to sour and bitter taste choose
468 and prefer fruit and vegetables described by these taste qualities. Thus, it could be hypothesized that
469 these subjects may have a diet richer in healthier components, such as phenols.

470

471 **5. Conclusions**

472 In conclusion, the large sample size as well as the several variables considered in the present study
473 help to deepen the knowledge about the role of sour and bitter taste perception associated with
474 consumers' eating behaviour. The present results suggest that the ability to perceive these taste
475 qualities, tested both in water solutions and real foods, is associated with food acceptability, and to a
476 lesser extent with food choice, for specific foods characterized by components that could have a
477 positive health effect. Dietary intake should be further envisaged to understand if the relationship
478 found among sour/bitter taste and food preferences also reflects differences in actual food
479 consumption.

480

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485

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489

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491

492 **Author contributions**

493 CP undertook the analyses and wrote the original draft of the manuscript; CP, EP, SS, CD and EM
494 contributed to plan the analyses; SS and CD contributed to enrich the analysis and to revise the
495 original draft; CP, EP, SS, CD and EM discussed the interpretation of the results; all authors helped
496 with data collection, reviewed and offered critical comments on the manuscript.

497

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