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1	The role of sour and bitter perception in liking, familiarity and choice
2	for phenol-rich plant-based foods
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25	Abstract
26	Among factors influencing food preferences and choices, individual differences in taste perception
27	play a key role in defining eating behaviour. In particular, sour and bitter responsiveness could be
28	associated with the acceptance and the consumption of phenol-rich plant-based foods recommended
29	for a healthy diet. The aim of this study was to investigate, in a large population sample, the
30	associations among sour and bitter responsiveness and liking, familiarity and choice for plant-based
31	foods characterized by these target tastes. Adults aged 18 to 60 years ($n = 1198$; 58% women) were
32	tested for their sour and bitter responsiveness both in water solutions and in food models (pear juice-
33	based beverages modified in citric acid content to induce different levels of sourness: 0.5, 2.0, 4.0
34	and 8.0 g/kg; chocolate pudding samples modified in sucrose content to induce different levels of
35	bitterness: 38, 83, 119, 233 g/kg). Familiarity, stated liking and choice for fruit juices and vegetables

- 36 varying for sour/bitter taste (high in bitter/sour taste: e.g. grapefruit juice and cauliflower; low in
- 37 bitter/sour taste: e.g. zucchini and pineapple juice) were measured. Results showed a significant
- 38 positive correlation between bitter and sour taste perception in water solutions and model foods, as

39 well as a positive correlation between the perceived intensity of the two taste stimuli. Subjects 40 characterized by high responsiveness to the two target stimuli were found to give lower liking scores 41 to foods characterized by sour/bitter tastes and tended to choose less sour/bitter foods compared to 42 less responsive subjects.

43 Thus, food choice for phenol rich plant-based products could be associated with a reduced 44 responsiveness to bitter and sour tastes and a consequent higher acceptance of food products 45 characterized by these taste qualities.

46

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

48

49 **1. Introduction**

50 It is widely reported that following a balanced diet is one of the key factors to prevent several non-51 communicable diseases, such as cardiovascular diseases and some types of cancer. An adequate 52 intake of fruit and vegetables is reportedly associated with a reduced risk of all-cause mortality (Aune 53 et al., 2017) as well as pivotal to ensure the recommended daily intake of micronutrients, such as 54 vitamins and minerals (Hartley et al., 2013).

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

61 Despite the positive impact that the vegetable and fruit consumption plays on subjects' health, there 62 is evidence reporting that plant-based diet represents also a more environmentally sustainable choice 63 compared with animal-based diet. Previous research highlighted that, assuming a constant daily 64 calorie intake, the meat-based food system requires more water, land and energy than the plant-based 65 food system (Pimentel & Pimentel, 2003; FAO, 2017). More recently, this assumption has been also 66 corroborated by other research showing that plant-based diets require fewer natural resources and 67 have less impact on the environment compared with diets rich in animal-based products (Ruini et al., 68 2015; Davis et al., 2016). In particular, the results obtained by Ruini et al. (2015) suggested that the 69 Mediterranean diet may lead to a lower environmental impact compared to diets that are heavily based 70 on daily meat consumption. The actual approaches applied to make the global food system 71 sustainable, such as food waste reduction, are inadequate given the global population growth and the lack of natural non-renewable resources (Béné et al., 2020). "Going back" to plant-based diets seems
to be an important alternative for a more sustainable future (Sabate & Soret 2014).

74 Although it is clear that a diet rich in fruit and vegetables has several positive aspects, adults often 75 fail to reach the recommend daily intake (Appleton et al., 2016), since the consumption of these 76 products has to face with consumer sensory perception, which is determinant in defining food 77 preference and choices. Plant-based foods are characterized by specific sensory attributes, such as 78 bitterness and sourness (Dinnella et al., 2016), due to the presence of polyphenols, isoflavones and 79 other natural compounds, that are responsible of low acceptability possibly leading to a reduced 80 consumption. Sourness and bitterness are innately disliked (Steiner, 1979; Ventura & Mennella, 81 2011) and could represent 'warning sensations' that negatively impact on consumers responses 82 (Laureati et al., 2018)

83 The individual variation in taste perception has been largely investigated as responsiveness to the 84 bitter compound 6-n-propylthiouracil (PROP), which is considered as a marker for taste 85 responsiveness, as well as for responsiveness to chemesthetic sensations (e.g. capsaicin; Spinelli et 86 al., 2018; Nolden et al., 2020) that may influence food preferences and eating behaviours (Tepper et 87 al., 2014). More recently, a general taste responsiveness score was proposed to identify subject groups 88 differing for responsiveness to basic tastes (Puputti et al., 2018). However, to date, little attention has 89 been paid to interindividual variations in sour perception and its possible role in defining food 90 preference and choices. Food choice represents an important measure to investigate and describe 91 actual food behaviours beyond food liking (Spinelli et al., 2020). Indeed, there is more to food choice 92 than sensory acceptance per se, as confirmed for example by market failure of new food formulations 93 that previously overcome consumers' hedonic test (Gutjar et al., 2015).

94 The majority of the studies used standard solutions with varied stimuli concentrations to measure the 95 intensity of perception of a basic taste (see for a review: Cox et al., 2016), while few studies used 96 actual food (Dinehart et al., 2006, Lanier at al., 2005), and foods as models added with varied 97 concentrations of a tastant (Tornwall et al., 2014). However, the sensory experience of eating is 98 complex, and each component may influence food perception, choice and consequent intake 99 (Boesveldt et a., 2018). In fact, food sensory experience is the result of multisensory interactions with 100 all senses, which play together in defining what is liked or disliked (Delwiche, 2004; Small & 101 Prescott, 2005; Hoppu et al., 2020). Thus, responsiveness to tastes in water do not necessarily 102 associates to their perception in food and to related hedonic responses. The extent to which taste 103 responsiveness is associated with food preferences and food consumption has yet to be fully 104 understood and few studies investigated this relationship in representative population samples (Cox 105 et al., 2016).

106 The aims of the present study were to: 1) investigate sour and bitter perception in water solutions and

- 107 food models in a large population sample; 2) evaluate how taste responsiveness to these two target
- 108 tastes could be associated with food choices, familiarity with and liking for selected phenol rich plant-
- 109 based foods.
- 110

111 **2. Material and method**

112 2.1 Participants

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

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

124

125 **2.2. Sensory stimuli**

126 Tastant solutions

127 Citric acid and caffeine (Sigma-Aldrich) were used to elicit sourness and bitterness perception. Two 128 solutions were prepared by dissolving 4 g/kg of citric acid and 3 g/kg of caffeine in water. These 129 concentrations were chosen based on previously published data (Monteleone et al., 2017).

130

131 Food models

132 Pear juice (J) and dark chocolate pudding (P) were selected as appropriate food matrices for testing sour and bitter perception in food models (Monteleone et al., 2017). Ingredients and products 133 134 distributed by large food companies were used in order to obtain a constant composition and to avoid 135 problems associated with products seasonality. Pudding base formulation was prepared by mixing a 136 commercial pudding powder (ingredients: starch, low-fat cocoa, dextrose, salt, aromas; Cameo 137 S.p.A., Dr. Oekter, Bielefeld, Germany) with 40 g of cocoa powder and 1L of water at 40°C. This 138 mixture was heated in microwave at 900W for 6 min and then at 450W for 4 min. The heating was 139 stopped every 2 min to mix the pudding. A commercial pear juice (ingredients: water, Williams pear

140 puree 50%, sugar, flavourings, acidifier: acid citric; antioxidant: ascorbic acid; Santal, Parmalat 141 S.p.A., Milan, Italy) was used for the base juice formulation. Four pear juice and four dark chocolate 142 pudding samples were prepared by adding, respectively, increased concentrations of citric acid (pear juice: $J_1=0.5$ g/kg; $J_2=2.0$ g/kg; $J_3=4.0$ g/kg and $J_4=8.0$ g/kg) and sucrose (chocolate pudding: $P_1=38$ 143 144 g/kg; P₂=83 g/kg; P₃=119 g/kg and P₄=233 g/kg) to base formulations. Tastants concentrations were 145 selected to elicit a variation in the strength of target sensations from weak to strong. Both food models 146 were preliminarily described by a focus group of trained subjects. Pear juice was characterized by 147 sweetness, sourness and pear flavour; chocolate pudding by sweetness, bitterness, chocolate flavour 148 and to a lesser extent by astringency.

149

150 **2.3. Questionnaires**

151 Food familiarity and stated liking

152 Familiarity with and stated liking for phenol-rich vegetables were measured using a selection of the 153 IT-Food Familiarity Questionnaire (IT-FFQ) and of the IT-Food Preference Questionnaire (IT-FPQ), 154 developed within the Italian Taste (IT) project (Monteleone et al., 2017). The selection included ten 155 vegetables (carrots salad, zucchini, lettuce and valerian salad, chard, broccoli, asparagus, radish, 156 artichoke, chicory, radicchio and rocket salad) and two fruit juices (grapefruit and pineapple) with varied level of expected bitterness and sourness according to results from a preliminary study 157 158 conducted at the University of Florence. A Check-All-That-Apply (CATA) questionnaire was used 159 to describe sensory properties of IT-FFQ and IT-FPQ items (De Toffoli et al., 2019). Here only results 160 of "bitterness" and "sourness" attributes in vegetables (201 respondents, 77.7% women; age range 161 18–70; mean age $40.3 \pm$ SD 14.1) and fruit juices (188 respondents, 75.4% women; age range 19–68; 162 mean age 40.1 \pm SD 14.3) were reported. To check for the correct use of terms to describe sensory 163 properties, a semantic categorisation task was applied; participants to the CATA test were asked prior 164 to the test to provide the best example coming to their mind of a "sour" and of a "bitter" food, 165 respectively (e.g. "Sour as...").

Familiarity for the selected items was measured using a 5-point labelled scale (1 = I do not recognize 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 eat it; 5 = I regularly eat it; Tuorila et al., 2001) while stated liking was assessed using the 9-point hedonic scale (1: extremely disliked; 9: extremely liked, Peryam & Pilgrim, 1957). If the participant had never tasted the food in question, he/she could choose the answer "I have never tasted it". The presentation order of the items was randomized across participants.

- 172
- 173 Food choice

174 Three vegetables pairs (1: lettuce and valerian salad vs radicchio and rocket salad; 2: zucchini vs asparagus; 3: chard vs chicory) and two fruit juice pairs (1: multivitamin juice - made with carrots, 175 176 oranges and lemons - vs orange juice; 2: pineapple juice vs grapefruit juice) were selected from the 177 IT-Food Choice Questionnaire (Monteleone et al., 2017) so that the options in each pair significantly 178 differed for bitterness and sourness. For each pair, respondents were asked to indicate which option 179 they would choose in a main meal either lunch or dinner (for vegetables) or breakfast (for fruit juices). 180 The presentation order of the pairs of food items within each meal occasion (breakfast, lunch and 181 dinner) was randomized across participants.

182

183 **2.4. Sensory evaluations**

184 **2.4.1** Training session to the evaluation of taste stimuli and to the use of the scales

185 Subjects participated in a training session immediately before the evaluation session. In the first part 186 of the training session, subjects were familiarized with the target sensations. For each sensation, appropriate food and beverages examples were recalled and discussed (chicory, black coffee and 187 188 tonic water were used to recall bitter taste; fresh lemon juice was used as an example of sourness). 189 Participants were encouraged to join the discussion giving their own examples of food and beverages 190 characterized by the target sensations and the appropriateness of their examples provided was 191 collectively discussed. This part of the training session ended with a verbal agreement on the meaning 192 of the target sensations. In the second part of the training session, participants were instructed to the 193 use of the general Labelled Magnitude Scale (gLMS; 0: no sensation; 100: the strongest imaginable 194 sensation of any kind; Bartoshuk et al., 2004) following published standard procedures (Green et al., 195 1993; Bartoshuk, 2000).

196 Subjects were extensively instructed to treat the "strongest imaginable sensation" as the most intense 197 sensation they could ever imagine experiencing. To familiarize the participants with the scale 198 anchors, they were asked to recall a variety of remembered sensations from different modalities 199 (Bajec & Pickering, 2008; Kalva et al., 2014; Webb et al., 2015). Examples of oral (e.g. the cold of a 200 cube of ice in the mouth; the pungency from hot chili pepper) and non-oral sensations (e.g. the noise 201 of a plane that is flying low, the pain felt when shutting a finger in a door) were proposed to encourage 202 the discussion. To practice on the use of the gLMS, subjects were asked to rate the intensity of the brightest light they had ever seen on a paper ballot. The criterion to conclude that the subjects 203 204 correctly used the scale was that their ratings were higher than "very strong" and lower than "the 205 strongest imaginable sensation of any kind". Ratings out of this range were individually discussed 206 and the correct use of the scale clarified (Dinnella et al., 2018). Despite an extensive training was 207 performed with the subjects involved, a measure from an independent modality (e.g., sound, or sight)

to corroborate the correct use of the scale was performed but not recorded in the present study.
However, a similar approach using recalled sensations has been used in many studies (Parkinson et al., 2016; Duffy et al., 2019; Yang et al., 2019).

211

212 **2.4.2. Evaluation session**

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

219 Subjects were instructed to hold the whole pear juice sample in their mouth or to take a full spoon of 220 chocolate pudding, then swallow and evaluate relevant sensory qualities according to the food model 221 considered. For pear juice, participants were asked to evaluate the intensity of sourness, sweetness, 222 and the overall flavour of pear juice. Conversely, the intensity of sweetness, astringency, and the 223 overall flavour of chocolate pudding were chosen to evaluate the perception of the chocolate pudding. 224 Only sourness in pear juices and bitterness in chocolate puddings were here considered for data 225 analysis. The intensity of each sensation was rated on a gLMS and after each sample, subjects rinsed 226 their mouth with water for 30 s, ate some plain crackers for 30 s, and finally rinsed their mouth with 227 water for a further 30 s. Evaluations were performed in individual booths under white lights. After 228 the tasting session, participants filled in the questionnaires. Data were collected with the software 229 Fizz (ver. 2.51. A86, Biosystèmes).

230

231 2.5. Data analysis

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

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

tested by Skewness and Kurtosis.

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

247 Correlations between taste responsiveness in water solutions and food models were examined using 248 Pearson's correlation coefficient with a minimum significance level defined as p<0.05.

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

251 Two familiarity scores were computed for each subject as the sum of ratings given to high bitter/sour 252 items (FAM High bitter/sour) and to low bitter/sour items (FAM Low bitter/sour) of the food 253 familiarity questionnaire (range from 1 to 5). Two liking scores were computed for each subject as 254 mean of the liking ratings for to high bitter/sour items (*LIK_High bitter/sour*) and to low bitter/sour 255 items (LIK_High bitter/sour) of the food preference questionnaire (range from 1 to 9). Options within 256 the pairs of the Food Choice Questionnaire were coded as "0" if the low bitter/sour option was chosen 257 and "1" if the high bitter/sour option was selected. For each subject, a choice index (CHO_Index) 258 was then calculated as the sum of the choices of the bitter/sour option (range from 0 to 5). Differences 259 in familiarity, liking and choice scores between the clusters with different taste responsiveness were evaluated by means of separate ANOVAs and then displayed using rain cloud plots. R 4.0.2 (R Core 260 Team, 2020) was used for this latter graphical representation. Partial eta squared (η^2 values: 0.01 261 small; 0.06 medium; 0.13 large; Cohen, 1988) was applied to evaluate the effect size. All the analyses 262 263 were performed using IBM SPSS Statistics for Windows, Version 24.0 (IBM Corp., Armonk, NY, 264 USA), with the exception of the CATA data that were analysed using XLSTAT 19.4.1 (Addinsoft).

265

266 **3. Results**

267 3.1. Differences for expected bitterness and sourness among questionnaire items

Results of the semantic categorisation task showed that the number of subjects who provided as example a term that was ambiguous or not correct was negligible (3.3% in the case of bitterness, 1.6% in the case of sourness), thus indicating that the subjects understood the concept of sour and bitter taste. Cochran's Q test results obtained in the preliminary study applying CATA methodology are reported in **Table 1**.

- 273
- 274

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

279

280

Food item		Bitter	Sour
Vegetables			
	Low bitter/sour		
	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}
	High bitter/sour		
	Asparagus	35 ^{de}	13 ^{bc}
	Radish	46 ^e	22 °
	Artichoke	63 ^f	15 ^{bc}
	Chicory	82 ^g	19 ^c
	Radicchio and rocket salad	82 ^g	20 °
	p-value	<0.0001	<0.0001
Fruit juices			
	Low bitter/sour		
	Multivitamin juice	12 ^a	55 ^a
	Pineapple	11 ^a	45 ^a
	High bitter/sour		
	Orange juice	29 ^b	70 ^b
	Grapefruit	75 ^c	75 ^b
	p-value	<0.0001	<0.0001

281

282 **3.2.** Taste perception in water solutions and food models

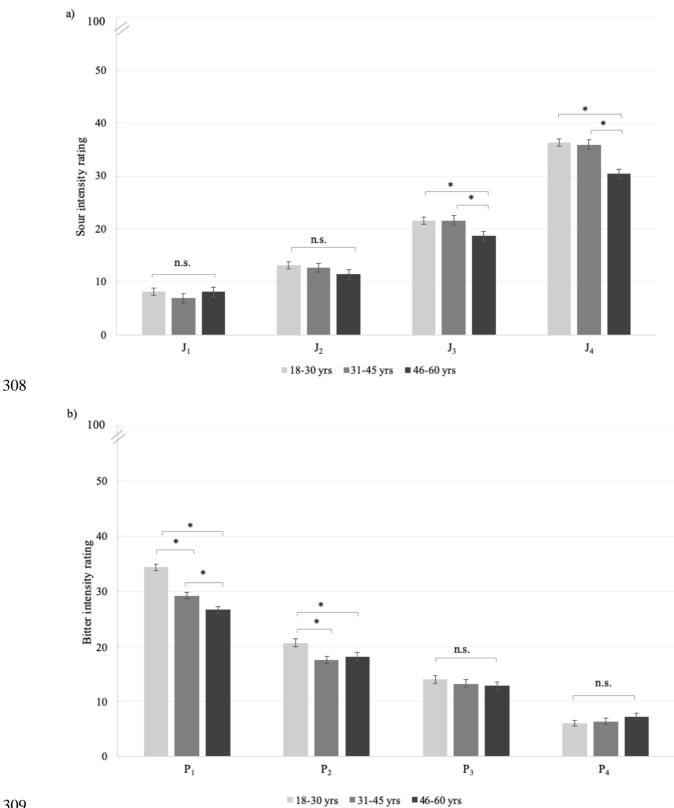
No significant gender effects on sour and bitter perception in water solutions were found. Only weak tendencies have been highlighted for sour and bitter perception according to age ($F_{(2,1192)}=2.72$, p=0.06, $\eta^2=0.005$; $F_{(2,1192)}=2.21$, p=0.11, $\eta^2=0.004$, respectively), with the youngest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

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

Age was associated with the perceived intensity of both sourness in pear juice and bitterness in 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; $\eta^2=0.008$, respectively).

In both model foods the interaction age*samples (Figure 1a-b) showed a significant but very 297 small/small effect on sour and bitter responsiveness ($F_{(6,4768)}=3.66, p<0.001; \eta^2=0.005; F_{(6,4768)}=9.20, \eta^2=0.005; F_{(6,4768)}=9.005; F_{(6,4768)$ 298 p < 0.0001, $\eta^2 = 0.01$ respectively.). An age effect was found on intensity ratings only in samples where 299 300 the intensity of target sensations was rated at moderate level or higher. Samples J₃ and J₄ were rated 301 lower in sourness by subjects aged 46 to 60 years than younger (18-30 and 31-45 years), which did 302 not significantly differ from each other. Bitterness intensity decreased with increasing age in sample 303 P₁ and it was rated higher by subjects aged 18-31 years than older (31-45 and 46-60 years), which 304 did not significantly differ from each other. The lack of significant differences due to age in sample 305 J₁ and J₂ and P₃ and P₄ is possibly due to a floor effect induced by the low intensity level of the target 306 sensations in these samples (ranging from weak to less than moderate).

307





310 **Figure 1a-b.** Sour (a) and bitter (b) mean intensity ratings (\pm SEM) by samples (pear juice samples: $J_1 - J_4$; chocolate pudding samples: $P_1 - P_4$) and age groups (18-30; 31-45; 46-60 years old). * p < 0.05; 311 312 n.s. not significant 313

- The interaction age*gender showed a significant but very small effect ($F_{(2,4768)}$ =4.06, p<0.05; η^2 = 314
- 315 0.002) only on sour intensity ratings. In particular, among subjects of 31-45 years, men gave

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

321 Pearson correlations coefficients (Table 2) highlighted a significant positive correlation among sour 322 intensity perceived in water solution and in pear juice samples. The correlation became stronger with 323 the increasing amount of citric acid in the pear juice. A significant positive correlation was also found 324 between bitter intensity perceived in water solution and in chocolate pudding samples. The correlation 325 became weaker with the increasing amount of sucrose as the intensity of the bitterness decreased. 326 Moreover, bitter and sour perception were always weakly but positively correlated to each other both 327 in water solution and food models. For example, the sourness perception in samples J₄ with the higher 328 amount of citric acid was significantly and positively correlated with the bitterness perception in the 329 chocolate pudding sample with the lower amount of sugar P₁ (most bitter). Pearson correlations 330 performed with consumers split according to the three-age groups revealed similar results (see 331 supplementary material).

332

Table 2. Pearson correlation coefficients among taste perception (S= sour, B=bitter) in water solution and model foods (pear juice with increasing citric acid: $J_1=0.5$ g/kg; $J_2=2.0$ g/kg; $J_3=4.0$ g/kg and $J_4=8.0$ g/kg; Chocolate pudding with increasing sugar: $P_1=38$ g/kg; $P_2=83$ g/kg; $P_3=119$ g/kg and $P_4=233$ g/kg)

	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

338 All values are significant at p < 0.01

339

340 **3.3** Consumers segmentation according to sour and bitter taste responsiveness

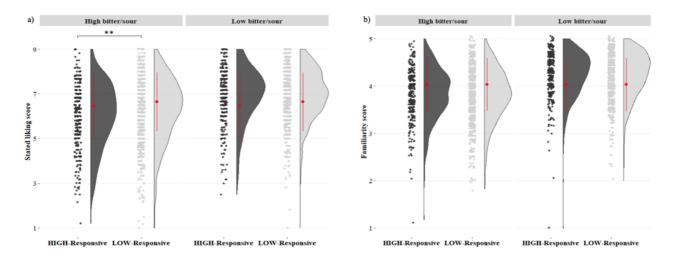
Sour and bitter intensity in water were used as a general index to classify subjects according to their responsiveness to target tastes. Two clusters were identified showing significant differences in sour $(F_{(1,1196)}=1456.46; p<0.000; \eta^2=0.55)$ and bitterness perception $(F_{(1,1196)}=418.71; p<0.000; \eta^2=0.26)$. In particular, Cluster 1 (*HIGH_Responsive*; n=309) showed higher responsiveness to both the target tastes (sour: 60.2 ± 0.8 ; bitter: 49.5 ± 1.0) compared to Cluster 2 (*LOW_Responsive*, n= 889; sour: 25.0 ± 0.5 ; bitter: 25.5 ± 0.6). According to χ^2 test, age and gender distributions were not significantly different between clusters (*p*>0.05).

348

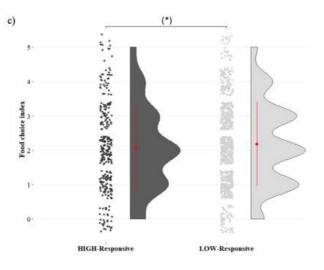
349 3.4. Associations among sour/bitter responsiveness and familiarity with, liking for and choice of 350 plant-based foods

Clusters significantly differed in liking scores for High bitter/sour vegetables and fruit juices (F 351 352 (1:1193)=10.19; p<0.001; η 2=0.06) (Figure 2a). Consumers more responsive to these target tastes 353 (HIGH_Responsive) gave significant lower liking scores to High bitter/sour vegetables and fruit 354 juices (6.0 \pm 0.08) compared to less responsive subjects (LOW_Responsive, 6.3 \pm 0.05). No significant differences between clusters were observed for liking scores for Low bitter/sour group (F 355 356 (1:1193)=0.52; p=0.47). Familiarity scores for both High and Low bitter/sour items were not 357 significantly different by cluster (*High bitter/sour*: F (1:1188)=0.02; p=0.89; Low bitter/sour: 358 $F_{(1:1188)}=0.67$; p=0.80) (Figure 2b). Clusters tended to differ in food choice score (p<0.10) with 359 *HIGH Responsive* subjects showing a lower choice for *High bitter/sour* food (2.0 ± 0.07) compared 360 to LOW_Responsive subjects (2.2 ± 0.04) (Figure 2c). Results split according to the three-age groups 361 revealed that the differences in eating behavioural variables by clusters were mainly associated with 362 subjects aged 18-30 years (see supplementary material).





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

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373 **4. Discussion**

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

377 The present results highlighted a weak but significant positive correlation between the perception of 378 sour and bitter tastes in water solutions. In this vein, Cattaneo and colleagues (2019), have recently 379 reported a positive correlation between sour and bitter thresholds in a small group of healthy adults. 380 Moreover, clusters based on tastant solution perception (more sensitive, semi-sensitive, and less 381 sensitive tasters) have been identified by Puputti et al., 2018 involving a large population sample. 382 The authors highlighted that the membership in a taste cluster could be partially forecasted by the 383 sensitivity to other taste modalities. This correlation among tastes mediated by different mechanisms, 384 G-coupled protein receptors for bitter and ion channels for sour (Drayna, 2005), could be explained 385 by a dichotomy in taste coding for pleasant compounds, such as sweet and savoury, versus those 386 perceived as dangerous, such as sour and bitter stimuli (Hladik et al., 2002). It could be questioned 387 that the correlations here highlighted could be due to the well-established sour-bitter confusion 388 (Robinson, 1970; Gregson & Baker, 1973). However, prior to tasting, extensive instructions were 389 provided by the experimenters to the subjects to avoid this misperception. Moreover, in this study 390 sourness and bitterness were evaluated in different food samples (the former on pear juices and the 391 latter on chocolate puddings). It is also worth considering that sourness was evaluated for a pure

392 stimulus in water and for a fruit juice added with citric acid. The intensity of sourness in fruit juice 393 significantly increases with citric acid concentration (see fig. 1a) thus it is reasonable to assume that 394 ratings refer to sour taste and not to bitter taste. Bitterness was rated in a water solution of a pure 395 stimulus and in chocolate added with increasing amount of sugar. Bitterness regularly decreases as 396 effect of suppression by sweetness (see fig. 1b). All these considerations make unlikely the confusion 397 between the two sensations.

398 The present results depicted also a positive correlation between sour/bitter perception in water 399 solutions and in food matrices with correlations becoming stronger in samples characterized by higher 400 intensity of the two target tastes. High responsive subjects to bitter taste seems also to be high 401 responsive to sour, both in water and in food models. Several studies have investigated how taste 402 sensitivity varies among individuals and how this is related to food consumption and subsequent 403 consumer health status (see for a review: Cox et a., 2016). Several authors focussed their attention to 404 sweet and salty perception that could be directly associated with the consumption of food rich in 405 calories and fats. Similarly, bitter perception and food liking represents a widely investigated field of 406 research, while less attention has been paid to sour taste. Moreover, research has been conducted 407 using solution-based approaches to measure hedonic responses (e.g. Drewnowski et al., 1985; Salbe 408 et al., 2004); this can help in modelling perceptual mechanisms but fails to represent the daily 409 experience with foods. Taste responsiveness measured using real foods could provide instead deeper 410 information on food preferences and choice even if fewer studies using this approach are available 411 (e.g. Dinehart et al., 2006; Tornwall et al., 2014; Proserpio et al 2016; Dinnella et al., 2018).

412 Looking to age effects on bitter and sour responsiveness older subjects (46-60 years old) tended to 413 give lower intensity rating scores in water solutions compared to younger subjects. This tendency 414 was found to become significant, although the effect size was always small, considering bitter and 415 sour perception in food models. These results are supported by previous evidence reporting a decline 416 in the gustatory function, mainly investigated using aqueous solutions, in the older population that 417 could be due to several factors, including physiological changes such as a taste receptor cells 418 dysfunction (Methven et al., 2012). Even if evidence about the extent and type of taste loss with 419 aging, sour and bitter tastes seem to be the most affected taste with increasing age (Sergi et al., 2017). 420 The present findings are in line with previous results by Hansen and colleagues (2006) who reported 421 an inverse association between age and the bitter taste of caffeine. Interestingly, the results of our 422 study revealed a systematic decrease in sour/bitter perception in food models with increasing age but 423 only at the highest concentration of the target tastes. Indeed, an age effect was found only in pear 424 juice samples with higher citric acid concentrations, and in the more bitter chocolate pudding samples. 425 Accordingly, recent data by a large sample of Caucasian European subjects demonstrated a significant decrease in taste perception for all five basic tastes, measured in water solutions, with increasing age,
and this association was found to be stronger for the higher concentrations especially for bitter and
sour (Barragán et al., 2018).

429 No differences in taste perception by women and men in both water solutions and model foods have 430 been here highlighted. The relationship between taste perception and gender yield to mixed literature 431 results (Fischer et al., 2013; Shen et al., 2016, Dinnella et al., 2018) that could be due to several 432 factors, such as the methodology applied to measure taste responsiveness, the food matrix used to 433 elicit different taste perceptions as well as the sample size of subjects involved.

Responsiveness to the two target tastes was associated with food liking for the selected food items 434 435 only in the most responsive consumers. These subjects expressed lower liking for vegetables and fruit 436 juices characterized by high sour/bitter tastes compared to least responsive subjects. Cox et al., (2012) 437 depicted that sensory perception tended to predict liking and intentions to consume brassica 438 vegetables. For example, broccoli hedonics as well as intentions to consume these vegetables were 439 predicted by bitterness perception. Contrarily, recent findings on a large sample size of Finnish adults 440 failed to find a relationship between bitter sensitivity and either vegetable liking or consumption 441 (Puputti et al., 2019). Our results are in line with previous findings showing that perceived bitterness, 442 correlated also with sour taste, of brussels sprouts, kale and asparagus is negatively associated with 443 vegetable preferences (Dinehart et al., 2006) and with findings showing that liking was inversely and 444 significantly associated with perceived bitterness in beverages (grapefruit juice, beer, and scotch; 445 Lanier et al., 2005). Literature data on fruit and vegetable preferences with respect to taste 446 responsiveness is controversial and it has been predominantly investigated through PROP (e.g. Duffy 447 et al., 2010, Bell and Tepper, 2006; Armstrong and Mattes 2008; Kaminski et al., 2000) as general 448 marker of taste responsiveness, as well as chemesthetic sensations (e.g. capsaicin; Nolden et al., 449 2020).

450 No significant differences among subjects with different taste responsiveness on preference for low 451 bitter/sour foods was found, suggesting that the differences in preference were related to taste stimuli 452 usually associated to warning sensations and something that could be potentially toxic, non-edible as 453 well as unripe fruits and spoiled foods (Laureati et al., 2018). Looking also to the familiarity data, no 454 differences in the scores provided by the two clusters of consumers to the food items considered have 455 been shown. This lack of difference between clusters can be explained by the fact that all the food 456 items included in the questionnaires are usually part of the Mediterranean diet, that is widely adopted 457 in Italy (Predieri et al., 2020).

458 Interestingly, the two clusters tended to differ in the choice for vegetables and fruit juices 459 characterized by intense sour/bitter tastes. In particular, low bitter/sour responsive subjects seem to 460 choose more specific sour/bitter plant-based foods (e.g. chicory and grapefruit juice) compared to the 461 high responsive subjects. These results, even if the differences highlighted are small, corroborated 462 the previous liking findings suggesting that subjects less responsive to sour and bitter taste choose 463 and prefer fruit and vegetables described by these taste qualities. Thus, it could be hypothesized that 464 these subjects may have a diet richer in healthier components, such as phenols.

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466 **5. Conclusions**

In conclusion, the large sample size as well as the several variables considered in the present study 467 468 help to deepen the knowledge about the role of sour and bitter taste perception associated with consumers' eating behaviour. The present results suggest that the ability to perceive these taste 469 470 qualities, tested both in water solutions and real foods, is associated with food acceptability, and to a 471 lesser extent with food choice, for specific foods characterized by components that could have a 472 positive health effect. Dietary intake should be further envisaged to understand if the relationship 473 found among sour/bitter taste and food preferences also reflects differences in actual food 474 consumption.

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484

485 **Conflicts of Interest:** The authors declare no conflict of interest.

486

487 Author contributions

488 CP undertook the analyses and wrote the original draft of the manuscript; CP, EP, SS, CD and EM 489 contributed to plan the analyses; SS and CD contributed to enrich the analysis and to revise the 490 original draft; CP, EP, SS, CD and EM discussed the interpretation of the results; all authors helped 491 with data collection, reviewed and offered critical comments on the manuscript.

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