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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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Abstract

Among factors influencing food preferences and choices, individual differences in taste perception play a key role in defining eating behaviour. In particular, sour and bitter responsiveness could be associated with the acceptance and the consumption of phenol-rich plant-based foods recommended for a healthy diet. The aim of this study was to investigate, in a large population sample, the associations among sour and bitter responsiveness and liking, familiarity and choice for plant-based foods characterized by these target tastes. Adults aged 18 to 60 years (n = 1198; 58% women) were tested for their sour and bitter responsiveness both in water solutions and in food models (pear juice-based beverages modified in citric acid content to induce different levels of sourness: 0.5, 2.0, 4.0 and 8.0 g/kg; chocolate pudding samples modified in sucrose content to induce different levels of bitterness: 38, 83, 119, 233 g/kg). Familiarity, stated liking and choice for fruit juices and vegetables varying for sour/bitter taste (high in bitter/sour taste: e.g. grapefruit juice and cauliflower; low in bitter/sour taste: e.g. zucchini and pineapple juice) were measured. Results showed a significant positive correlation between bitter and sour taste perception in water solutions and model foods, as

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

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

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

1. Introduction

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

Despite the positive impact that the vegetable and fruit consumption plays on subjects' health, there is evidence reporting that plant-based diet represents also a more environmentally sustainable choice compared with animal-based diet. Previous research highlighted that, assuming a constant daily calorie intake, the meat-based food system requires more water, land and energy than the plant-based food system (Pimentel & Pimentel, 2003; FAO, 2017). More recently, this assumption has been also corroborated by other research showing that plant-based diets require fewer natural resources and have less impact on the environment compared with diets rich in animal-based products (Ruini et al., 2015; Davis et al., 2016). In particular, the results obtained by Ruini et al. (2015) suggested that the Mediterranean diet may lead to a lower environmental impact compared to diets that are heavily based on daily meat consumption. The actual approaches applied to make the global food system sustainable, such as food waste reduction, are inadequate given the global population growth and the

72 lack of natural non-renewable resources (Béné et al., 2020). “Going back” to plant-based diets seems
73 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 et 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 al., 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**

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

120 The study was conducted in agreement with the Italian ethical requirements on research activities and
121 personal data protection (D.L. 30.6.03 n. 196) and in adherence with the principles laid down the
122 Declaration of Helsinki. The protocol was approved by the Ethics Committee of Trieste University
123 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
133 sour and bitter perception in food models (Monteleone et al., 2017). Ingredients and products
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. Oetker, 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
143 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
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
157 varied level of expected bitterness and sourness according to results from a preliminary study
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 ± SD 14.1) and fruit juices (188 respondents, 75.4% women; age range 19–68;
162 mean age 40.1 ± 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...”).

166 Familiarity for the selected items was measured using a 5-point labelled scale (1 = I do not recognize
167 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
168 eat it; 5 = I regularly eat it; Tuorila et al., 2001) while stated liking was assessed using the 9-point
169 hedonic scale (1: extremely disliked; 9: extremely liked, Peryam & Pilgrim, 1957). If the participant
170 had never tasted the food in question, he/she could choose the answer “I have never tasted it”. The
171 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
175 asparagus; 3: chard vs chicory) and two fruit juice pairs (1: multivitamin juice - made with carrots,
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,
187 appropriate food and beverages examples were recalled and discussed (chicory, black coffee and
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
203 brightest light they had ever seen on a paper ballot. The criterion to conclude that the subjects
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)

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

211

212 **2.4.2. Evaluation session**

213 Subjects were instructed to hold the whole tastant solution in their mouth for 3 s, then expectorate,
214 wait few seconds and evaluate the perceived intensity. Tastant solutions (10 mL) were presented in
215 80 cc plastic cups identified by a 3-digit code in random order. Food samples (15 g) were presented
216 in 80 cc plastic cups identified by a 3-digit code. Pear juice and dark chocolate pudding samples were
217 presented in independent sets each consisting of four samples presented in random order. Pear juice
218 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**

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

237 Subjects were divided into three age groups: group 1=18–30 years (45%), group 2=31–45 years
238 (28%) and group 3=46–60 years (27%). The age distribution of men and women was not significantly
239 different according to chi-square test ($\alpha = 0.05$). The normality assumption of continuous data was
240 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. Correlations between taste responsiveness in water solutions and food models were examined using 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 water solutions by means of Hierarchical Cluster Analysis. Two familiarity scores were computed for each subject as the sum of ratings given to high bitter/sour items (*FAM_High bitter/sour*) and to low bitter/sour items (*FAM_Low bitter/sour*) of the food familiarity questionnaire (range from 1 to 5). Two liking scores were computed for each subject as mean of the liking ratings for to high bitter/sour items (*LIK_High bitter/sour*) and to low bitter/sour items (*LIK_Low bitter/sour*) of the food preference questionnaire (range from 1 to 9). Options within the pairs of the Food Choice Questionnaire were coded as "0" if the low bitter/sour option was chosen and "1" if the high bitter/sour option was selected. For each subject, a choice index (*CHO_Index*) was then calculated as the sum of the choices of the bitter/sour option (range from 0 to 5). Differences 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 Team, 2020) was used for this latter graphical representation. Partial eta squared (η^2 values: 0.01 small; 0.06 medium; 0.13 large; Cohen, 1988) was applied to evaluate the effect size. All the analyses were performed using IBM SPSS Statistics for Windows, Version 24.0 (IBM Corp., Armonk, NY, USA), with the exception of the CATA data that were analysed using XLSTAT 19.4.1 (Addinsoft).

3. Results

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**.

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.

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

281

282 3.2. Taste perception in water solutions and food models

283 No significant gender effects on sour and bitter perception in water solutions were found. Only weak
284 tendencies have been highlighted for sour and bitter perception according to age ($F_{(2,1192)}=2.72$,
285 $p=0.06$, $\eta^2= 0.005$; $F_{(2,1192)}=2.21$, $p=0.11$, $\eta^2= 0.004$, respectively), with the youngest group of

286 subjects (18-30 years old) that tended to be more responsive compared with subjects aged 31-45 and
 287 46-60 years.

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

294 Age was associated with the perceived intensity of both sourness in pear juice and bitterness in
 295 chocolate but to a lesser extent ($F_{(2,4768)}=12.67$; $p<0.0001$; $\eta^2= 0.005$; $F_{(2,4768)}=19.19$; $p<0.0001$; $\eta^2=$
 296 0.008 , respectively).

297 In both model foods the interaction age*samples (**Figure 1a-b**) showed a significant but very
 298 small/small effect on sour and bitter responsiveness ($F_{(6,4768)}=3.66$, $p<0.001$; $\eta^2= 0.005$; $F_{(6,4768)}=9.20$,
 299 $p<0.0001$, $\eta^2= 0.01$ respectively.). An age effect was found on intensity ratings only in samples where
 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

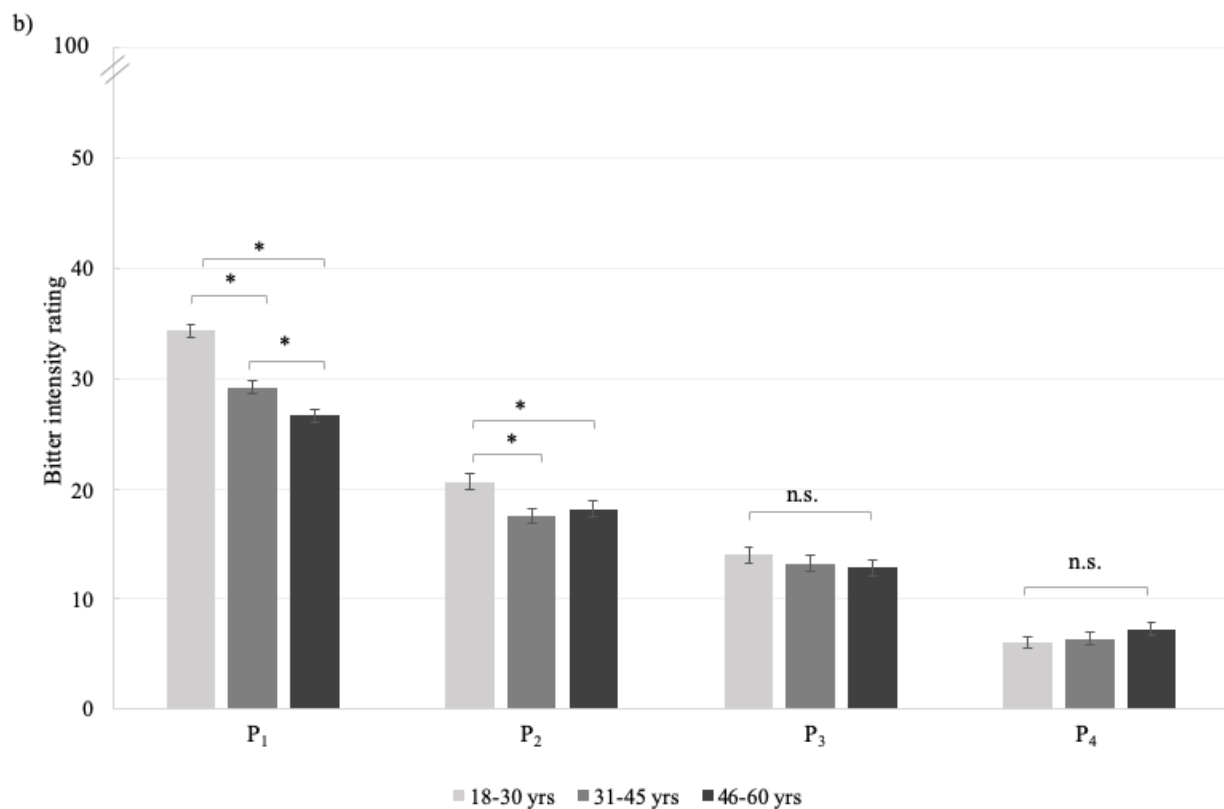
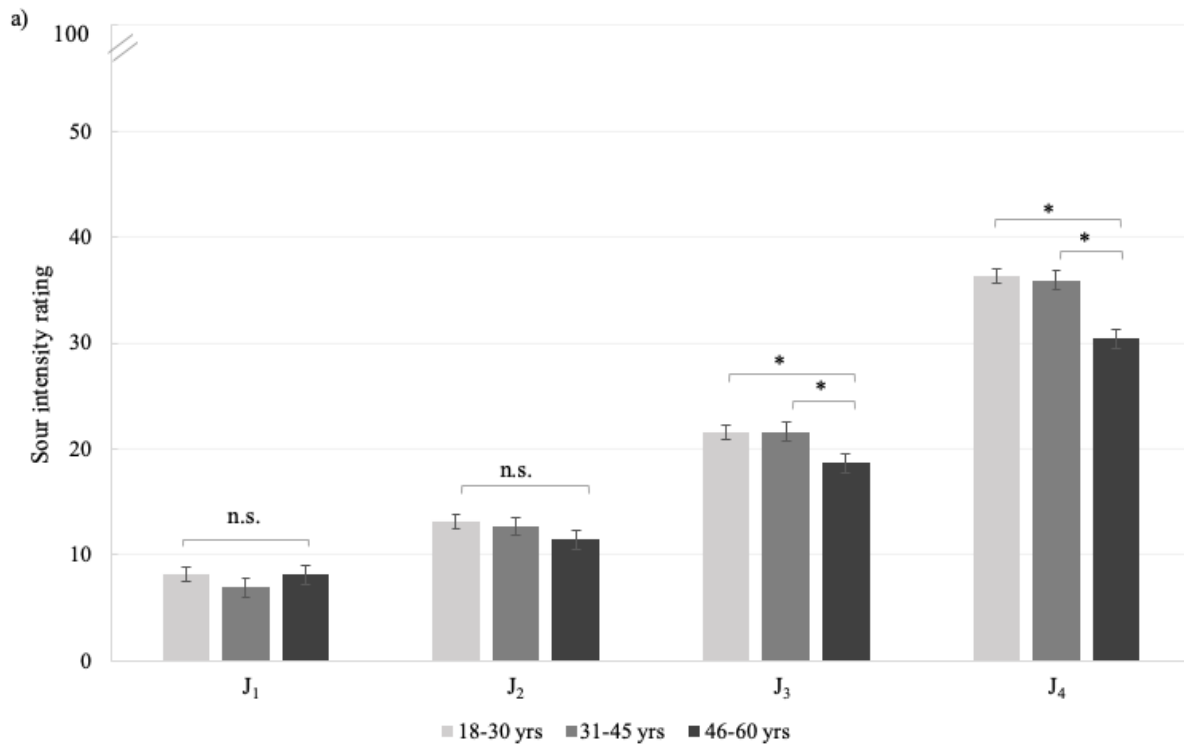


Figure 1a-b. Sour (a) and bitter (b) mean intensity ratings (\pm SEM) by samples (pear juice samples: J₁ - J₄; chocolate pudding samples: P₁ - P₄) and age groups (18-30; 31-45; 46-60 years old). * $p < 0.05$; n.s. not significant

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

316 significant lower intensity ratings (18.4 ± 0.6) compared to women (20.2 ± 0.5), while no gender
317 differences were found in the other age groups (group 1 and group 3). The interaction gender*sample
318 was significant ($F_{(3,4768)}=3.02$, $p<0.05$; $\eta^2= 0.002$) only on bitter intensity ratings. Gender-related
319 differences have been found only for sample P₁ which was perceived as more bitter by women (31.0
320 ± 0.5) compared to men (29.1 ± 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
333 **Table 2.** Pearson correlation coefficients among taste perception (S= sour, B=bitter) in water solution
334 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
335 J₄=8.0 g/kg; Chocolate pudding with increasing sugar: P₁=38 g/kg; P₂=83 g/kg; P₃=119 g/kg and
336 P₄=233 g/kg)
337

	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**

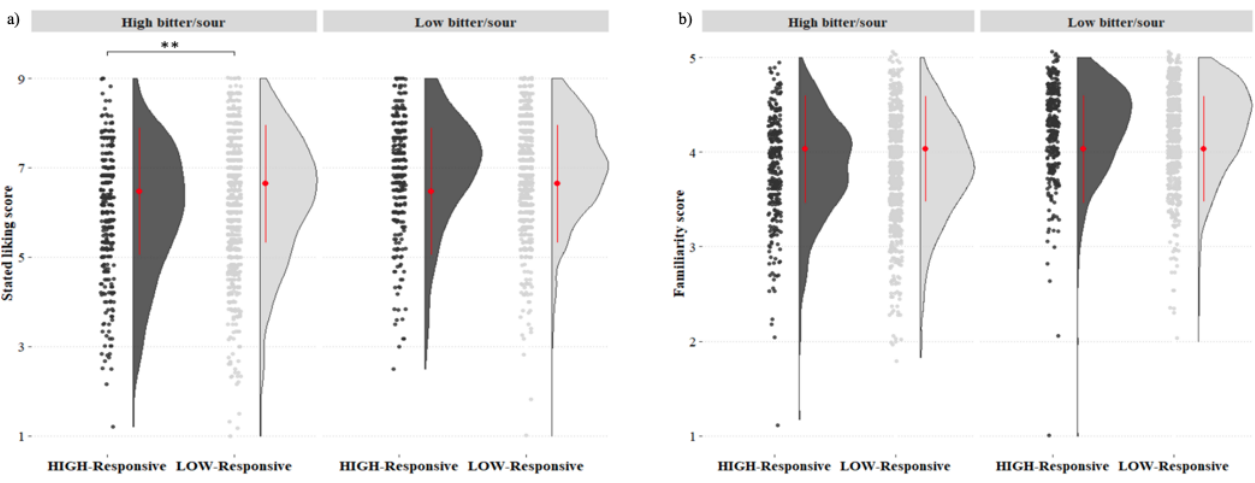
341 Sour and bitter intensity in water were used as a general index to classify subjects according to their
342 responsiveness to target tastes. Two clusters were identified showing significant differences in sour
343 ($F_{(1,1196)}=1456.46$; $p<0.000$; $\eta^2=0.55$) and bitterness perception ($F_{(1,1196)}=418.71$;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

344 In particular, Cluster 1 (*HIGH_Responsive*; n=309) showed higher responsiveness to both the target
 345 tastes (sour: 60.2 ± 0.8 ; bitter: 49.5 ± 1.0) compared to Cluster 2 (*LOW_Responsive*, n= 889; sour:
 346 25.0 ± 0.5 ; bitter: 25.5 ± 0.6). According to χ^2 test, age and gender distributions were not significantly
 347 different between clusters ($p>0.05$).

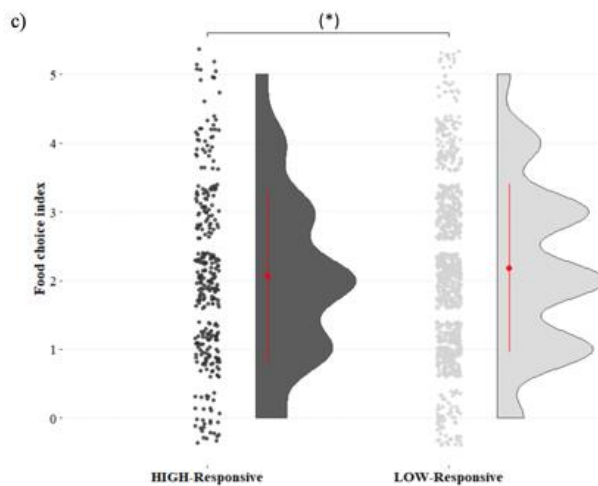
348
 349 **3.4. Associations among sour/bitter responsiveness and familiarity with, liking for and choice of**
 350 **plant-based foods**

351 Clusters significantly differed in liking scores for *High bitter/sour* vegetables and fruit juices (F
 352 $(1:1193)=10.19$; $p<0.001$; $\eta^2=0.06$) (**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 ± 0.08) compared to less responsive subjects (*LOW_Responsive*, 6.3 ± 0.05). No
 355 significant differences between clusters were observed for liking scores for *Low bitter/sour* group (F
 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).

363



364



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). * $p < 0.05$; (*) $p < 0.10$.

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

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

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

Looking to age effects on bitter and sour responsiveness older subjects (46-60 years old) tended to give lower intensity rating scores in water solutions compared to younger subjects. This tendency was found to become significant, although the effect size was always small, considering bitter and sour perception in food models. These results are supported by previous evidence reporting a decline in the gustatory function, mainly investigated using aqueous solutions, in the older population that could be due to several factors, including physiological changes such as a taste receptor cells dysfunction (Methven et al., 2012). Even if evidence about the extent and type of taste loss with aging, sour and bitter tastes seem to be the most affected taste with increasing age (Sergi et al., 2017).

The present findings are in line with previous results by Hansen and colleagues (2006) who reported an inverse association between age and the bitter taste of caffeine. Interestingly, the results of our study revealed a systematic decrease in sour/bitter perception in food models with increasing age but only at the highest concentration of the target tastes. Indeed, an age effect was found only in pear juice samples with higher citric acid concentrations, and in the more bitter chocolate pudding samples. Accordingly, recent data by a large sample of Caucasian European subjects demonstrated a significant

426 decrease in taste perception for all five basic tastes, measured in water solutions, with increasing age,
427 and this association was found to be stronger for the higher concentrations especially for bitter and
428 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.

434 Responsiveness to the two target tastes was associated with food liking for the selected food items
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.

465

466 **5. Conclusions**

467 In conclusion, the large sample size as well as the several variables considered in the present study
468 help to deepen the knowledge about the role of sour and bitter taste perception associated with
469 consumers' eating behaviour. The present results suggest that the ability to perceive these taste
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.

475

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480

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484

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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.

492

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