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Individual variation in PROP status, fungiform papillae density and responsiveness to taste stimuli in a large population sample

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Abstract

Despite considerable research investigating the role of PROP bitterness perception and variation of fungiform papillae density (FPD) in food perception, this relationship remains controversial as well as the association between the two phenotypes. Data from 1119 subjects (38.6% male; 18-60 years) enrolled in the Italian Taste project were analysed. Responsiveness to the bitterness of PROP was assessed on the general Labelled Magnitude Scale. FPD was determined from manual counting on digital images of the tongue. Solutions of tastes, astringent and pungent sensations were prepared to be moderate/strong on a gLMS. Four foods had tastants added to produce four variations in target sensations from weak to strong (pear juice: citric acid, sourness, chocolate pudding: sucrose, sweetness; bean purée: sodium chloride, saltiness and tomato juice: capsaicin, pungency). Females gave ratings to PROP and showed FPD that were significantly higher than males. Both phenotype markers significantly decreased with age. No significant correlations were found between PROP ratings and FPD. FPD variation doesn't affect perceived intensity of solutions. Responsiveness to PROP positively correlated to perceived intensity of most stimuli in solution. A significant effect of FPD on perceived intensity of target sensation in foods was found in a few cases. Responsiveness to PROP positively affected all taste intensities in subjects with low FPD while there were no significant effects of PROP in high FPD subjects. These data highlight a complex interplay between PROP status and FPD and the need of a critical reconsideration of their role in food perception and acceptability.

key words: basic tastes; pungency; food stimuli; intensity; age; gender

INTRODUCTION

The perception of sensory qualities plays a pivotal role in our food choices (Sobal et al., 2014), through both innate and learned hedonic responses to those flavour qualities (Yeomans et al. 2006; Yeomans et al. 2008; Cox et al. 2016; Prescott, 2016). In turn, food sensory qualities act as anticipatory signals of food energy and nutrient content thus modulating satiety feeling and food intake (Dongen et al. 2012; Forde et al. 2013). However, substantial individual variations in chemosensory perceptions exist, and associations with diet-related differences have been highlighted (Duffy, 2007; Lease et al. 2016; Cox et al. 2016; Stevenson et al. 2016; Fogel and Blissett, 2017). Importantly, large scale studies, aimed at exploring the salient dimensions of food choice, have found that the variation in perceived intensity of prototypical taste solutions are significantly related to food preferences and intake (Cruickshanks et al. 2009).

Individual variations in fungiform papillae density (FPD: FP/cm^2) on the tongue and in response to the bitter taste of 6-n-propylthiouracil (PROP Status) are the most well researched phenotypic markers of responsiveness to oral stimulations. FPD varies widely among individuals, from 0.0 (Webb et al. 2015) to 233.0 FP/cm^2 (Zhang et al. 2009). Environmental and demographic factors are reported to affect FPD, with lower FPD being associated with smoking, high alcohol consumption and obesity (Fischer et al. 2013; Proserpio et al. 2016). Variations of FPD across genders remain unclear, with females either having (Duffy et al. 2010; Fischer et al. 2013) or not having (Hayes and Duffy, 2007; Masi et al. 2015) higher FPD than males. However, FPD is generally thought to increase from childhood to adulthood (Correa et al.2013), thereafter declining with age (Fischer et al. 2013; Pavlidis et al. 2013).

FPD can be used as a rough estimate of taste-bud density (Miller and Reedy,1990; Just et al 2006; Srur et al. 2010). Since taste buds carry taste receptor cells, FP are considered to be key anatomic structures responsible (along with circumvallate and foliate papillae) for taste perception. In addition, mechanoreceptors in the somatosensory system are located in trigeminal neurons that surround taste buds in FP (Whitehead et al. 1985; Whitehead and Kachele, 1994) and are responsible for perception of food textural attributes (Engelen and Van der Bilt, 2008). Free endings of the trigeminal nerves serving as receptors of chemesthetic (pungency; spiciness) agents are found in high abundance surrounding the taste buds, especially in the fungiform papillae (Saunders and Silver, 2016). All these anatomic features suggest that FP are the main anatomic structures for oral stimuli sensing and hence that FPD underlies the intensity of food sensory properties. Despite this, some recent large studies have suggested a lack of association between the perception of prototypical taste solution intensity and FPD (Fenney and Hayes, 2014; Fischer 2013; Webb et al. 2015). Conflicting results also exist in the literature examining relationships between FPD and perception of tactile sensations such as astringency (Bakke and Vickers, 2008; Linne et al. 2017), fat content (Nachtseim and Schlich, 2013) and lingual tactile acuity (Essick et al. 2003; Bangcuyo and Simons, 2017).

Phenotypic responses to PROP also vary considerably among individuals, from 'taste blindness' to PROP bitter taste (Non Taster: NT) to a wide range of perceived bitterness intensity (taster) (Bartoshuk, 2000). PROP tasters are further classified as medium (MT) and super tasters (ST), who perceive PROP as

87 moderately and extremely bitter, respectively (Bartoshuk, 1991). The polymorphisms in the gene *TAS2R38*
88 mainly explain the observed phenotypic variation, with individuals carrying the PAV allele perceiving greater
89 intensity from supra-threshold PROP solutions than carriers of the AVI allele (Duffy et al. 2004a).
90 Responsiveness to PROP bitterness is significantly affected by psychosocial variables (McAnally et al. 2007),
91 as well as gender and age. The percentage of tasters has been found to be consistently higher in females
92 than in males (Bartoshuk et al. 1994; Guo and Reed, 2001, Monteleone et al. 2017) and a decline in
93 responsiveness to PROP is typically observed with age (Guo and Reed, 2001, Tepper et al. 2014), especially
94 in females (Monteleone et al. 2017).
95
96 Several studies have demonstrated that responsiveness to PROP is positively associated with
97 responsiveness to chemosensory stimulation in standard solutions (Hayes et al. 2008; Yang et al. 2014;
98 Webb et al. 2015, Fischer et al. 2014; Melis et al. 2017; Prescott et al. 2001) and real food (Dinehart et
99 al. 2006; Zhao and Tepper, 2007; Bakke and Vickers, 2008, Bajec and Pickering, 2008; Masi et al. 2015,
100 Spinelli et al. 2018). Furthermore, PROP responsiveness was reported to increase discrimination among
101 foods and beverages with systematic variations in tastes, oral irritants (Prescott et al. 2004) and textures
102 (de Wijk et al. 2007).
103
104 Despite such findings, the mechanism behind the relationship between responsiveness to PROP and
105 perception of other chemosensory qualities is still unclear. Bartoshuk and co-workers found FPD correlated
106 with the bitterness of PROP (Bartoshuk et al. 1994), and further studies supported this observation (Tepper
107 and Nurse, 1997; Delwiche et al., 2001; Yackinous and Guinard, 2002; Essick et al. 2003; Duffy et al.
108 2004b; Hayes and Duffy, 2007; Yeomans et al. 2007; Bajec and Pickering, 2008; Hayes et al. 2010).
109 Moreover, the term Super Taster has been used by Bartoshuk (Bartoshuk et al. 1994) to indicate individuals
110 who perceived PROP as extremely bitter, with an increased taste and oral somatosensory responsiveness,
111 and who also had high FPD. Thus, a causal relationship between high FPD and the increased responsiveness
112 to oral stimuli, including PROP, has been hypothesized with some empirical justification.
113
114 More recently, the definition of Super Taster individuals has been reconsidered (Hayes and Keast, 2011;
115 Kalva et al., 2014). Moreover, large population studies have failed to find significant associations between
116 the PROP phenotype and FPD (Fisher et al. 2013; Garneau et al. 2014). FPD has been reported as significant
117 determinant of PROP bitterness in TAS2R38 homozygotes and not in heterozygotes (Hayes et al. 2008),
118 but this has not been confirmed in larger sample studies where FPD does not differ by diplotype (Fischer
119 et al. 2013; Garneau et al. 2014). To explain the mechanistic link between PROP responsiveness and
120 chemosensory acuity, complex and still controversial relationships between polymorphism of TAS2R38 and
121 gustin genes, and FP development and maintenance have been proposed (Padiglia et al. 2010; Calo et al.
122 2011; Melis et al. 2013; Barbarossa et al. 2015). However, other studies have failed to find such
123 associations (Feeney and Hayes, 2014; Bering et al. 2013; Barbarossa et al. 2015; Yang, 2015; Shen et
124 al. 2016; Shen et al. 2017).
125
126 Overall chemosensory responsiveness is affected by lingual nerve damage (Bartoshuk et al. 2012), chronic
127 pathologies and medications (Boltong and Keast, 2012; Pavlidis et al. 2014), eating disorders and dietary
128 restrictions (Bartoshuk et al. 2006; Stafford et al. 2013) and smoking habits (Venneman et al. 2008; Jacob
129 et al. 2014; Pavlidis et al. 2014). However, the impairment of orosensory function due to these factors is

not necessarily associated with modifications of FP number and morphology. Furthermore, environmental factors affect PROP phenotypic expression (Tepper et al. 2017). The lack of control of such factors has been suggested as a possible contributor to the non-replication of results (Piochi et al. 2018) and accounting for altered responses to oral stimulation as a confounding factor has been strongly recommended as a way of clarifying the relationship between oral phenotypes and chemosensory responses (Bartoshuk et al. 2012; Tepper et al. 2017).

In summary, the associations between phenotype marker of taste functioning and the intensity of oral sensations remain controversial. The mutual influences between responsiveness to PROP and FPD are still a matter of debate as well. However, phenotype measurements of oral responsiveness represent a valuable tool to investigate the relationship between chemosensory ability and food preference in representative population sample. For the most part, studies have used standard tastant solutions. Actual food tasting has been performed in a few studies, but no studies to date have explored the systematic variation of target sensations in real foods in large population samples. Therefore, the aim of this study was to investigate, in more than one thousand subjects, both phenotype measurements of taste sensitivity, and their effects on perception of food products systematically varying in tastes, pungency and astringency. Furthermore, to assess the impact of the marker phenotype variation on intensity independently for PROP responsiveness and FPD, relationships between phenotype and intensity were explored in subject groups varying for only one of the considered markers (*i.e.* PROP NT and ST groups were independently considered to assess the effect of FPD; Low and High FPD groups were independently considered to assess the effect of responsiveness to PROP). Age and gender differences were also explored.

MATERIALS and METHODS

1. Overview

The present data were collected as part of the larger "Italian Taste" study, which is aimed at investigating influences on food choice and preferences in a large population sample (Monteleone et al. 2017). This multi-session study consisted of a questionnaire session at home and one-on-one testing in a sensory laboratory across two days. Only a selection of these data will be presented here. For a complete overview of the test and further details on the definition of the procedures, see Monteleone et al. (2017).

2. Participants

Participants were recruited on a national basis by means of announcements published on research unit and social network websites, emails, pamphlet distribution and word of mouth. The data from 1225 participants were collected during 2015. In the present study, data from 1119 subjects who correctly used the general Labelled Magnitude Scale (gLMS) and provided valid FP count from tongue picture inspection are reported. At the time of recruitment, respondents were asked to complete an online questionnaire on socio-demographic, socio-economic, anthropometric and physical health characteristics (Monteleone et al. 2017). Gender, age, Body Mass Index, food allergies and intolerances, practice of restrictive diets, chronic diseases that imply long-term dietary restrictions, infections and traumas that would impair perceptive abilities and smoking habits are considered in the present work (Tab. 1).

3. Procedure

3.1 General

The procedure was approved by the Ethics Committee of Trieste University. Subjects took part in two sessions hold in two days according to the Italian Taste project data collection scheme (Monteleone et al. 2017). On day 1, participants signed the informed consent according to the principles of the Declaration of Helsinki and were introduced to the general organization of the day which includes the measurement of PROP responsiveness. Intensity of water solutions and food products were evaluated on day 2. Participants were first asked to rate the intensity in the seven water solutions. Subjects had 15 min break and then were presented with the four series of food products, each consisting in four samples varying for the intensity of the target sensations, for evaluations of tastes, astringency and burning intensities. The picture of the tongue for papillae counting was taken at the end of day 1 or day 2, according to individual availability.

3.2 Scale

Before PROP tasting, participants were introduced to the use of the general Labelled Magnitude Scale (gLMS) (Bartoshuk et al. 2004) with particular emphasis on the meaning of the descriptor "the strongest imaginable sensation of any kind". Verbal instructions were given that the top of the scale represented the most intense sensation that subjects could ever imagine experiencing and a variety of remembered sensations from different modalities including loudness, oral pain/irritation, tastes were recalled (Bajeck and Pickering 2008; Kalva et al. 2014; Webb et al. 2015). For orientation to the gLMS scale use, subjects rated intensities of the brightest light they had ever seen. The task was performed individually, the criteria to conclude that the subjects correctly used to scale was that ratings must have been higher than very strong and lower than the strongest imaginable. In case of ratings out of this range a short individual interview was carried out to understand the reason of the ratings and the scale use was explained again. In a limited number of cases subjects were unable to properly use the scale even after the second explanation, they were allowed to perform the test, but the relevant results excluded from further data analysis.

4.Taste function phenotype measurements

4.1 Fungiform Papillae Density

The anterior portion of the dorsal surface of the tongue was swabbed with household blue food coloring (F.lli Rebecchi, Italy), using a cotton-tipped applicator. This made the FP easily visible as red structures against the blue background of the stained tongue. Digital pictures of the tongue were recorded (Shahbake et al. 2005) using a digital microscope (MicroCapture, version 2.0 for 20x-400x) (Masi et al. 2015). For each participant, the clearest image was selected, and the number of FP was counted in two 0.6 cm diameter circles, one on right side and one on left side of tongue, 0.5 cm from the tip and 0.5 cm from the tongue midline. The number of FP was manually counted by two researchers independently according to the Denver Papillae Protocol (Nuessle et al. 2015). The presence of scorer effects was checked at local unit level by submitting to one-way ANOVA counts from the two independent scorers (Masi et al. 2015). Counts were considered valid if the scorer effect was not significant ($p>0.05$). The equivalence test (two-one sided test - TOAST) on raw data from all the units participating in data collection indicated that counts form different scorer were equivalent (90% confidence interval on the difference between the means; TOAST interval between -1 and 1; $\alpha=0.005$; $p<0.001$). The mean of FP number from valid counts was used for

each image and expressed as density (FP/cm^2: FPD). Limits of 25th and 75th percentiles were used as empirical cut-offs to classify subjects in low (L-FPD) and high (H-FPD) fungiform papillae density.

4.2 PROP taster status

A 3.2 mM PROP solution was prepared by dissolving 0.5447 g/L of 6-n-propyl-2-thiouracil (Sigma Aldrich, Saint Louis-Missouri, USA) into deionized water (Prescott et al. 2004). Subjects were presented with two samples (10 ml) coded with three-digit codes and were instructed to hold each sample in their mouth for 10 s, expectorate, and then wait 20 s before evaluating the intensity of bitterness using the gLMS. The average bitterness score across the two samples was used for each subject. The arbitrary cut-offs used in previous studies were used to categorize subjects as NT (PROP bitterness on gLMS<moderate-17) and ST (PROP bitterness on gLMS>very strong-53) (Hayes et al. 2010; Fischer et al. 2013).

5. Sensory stimuli

5.1 Aqueous solutions

Seven aqueous solutions corresponding to five tastes (bitterness, sourness, sweetness, saltiness and umami), astringent and pungent sensations were prepared to be moderate/strong on a gLMS (Bartoshuk et al. 2004). The concentration of the tastants (Sigma-Aldrich, Saint Louis-Missouri, USA) were: citric acid 4 g/kg (sourness); caffeine 3 g/kg (bitterness); sucrose 200 g/kg (sweetness); sodium chloride 15 g/kg (saltiness); monosodium glutamate 10 g/kg (umami); capsaicin 1.5 mg/Kg (pungent); and aluminium sulphate 0.8 g/kg (astringency). The concentration of the tastants were selected based on published psychophysical data (Hayes et al. 2010; Feeney et al. 2014; Masi et al. 2015) and preliminary trials conducted with one hundred untrained subjects recruited in five Italian sensory laboratories (unpublished data).

5.2 Food Products

Pear juice (PJ), chocolate pudding (CP), bean purée (BP) and tomato juice (TJ) were selected as the most appropriate food matrices for testing the responses to target sensations (Monteleone et al. 2017). Canned, bottled or powdered ingredients produced by large food companies were used to prepare the food products since their composition is constant, and they were easily available across the country without seasonality restrictions. Detailed recipes for food products preparation and handling were made available to all the labs participating in the project. The four foods each had four levels of tastants added to produce variations in target sensations from weak to strong. These are detailed in Table 2.

6. Sensory evaluations

Before sensory stimuli tasting, the gLMS was briefly introduced again. Aqueous solutions (10 mL) and food products (15 gr) were presented in 80cc plastic cups identified by a 3-digit code consisting of a random sequence of three numbers generated by the software used for data collection. Semi-solid food samples (chocolate pudding, bean purée, tomato juice) were presented with a tea-spoon. Subjects were presented with a set consisting of the seven water solutions, in random order for the five tastes and astringent solution, while the pungent capsaicin solution was always evaluated as the last sample to avoid carry-over effects due to the long duration of the pungency. The food product series was presented in independent sets, each consisting of four samples of the same product. The four samples of a food series were presented in random order. The presentation order of food series was always the same and was designed to avoid

258 carry-over effects across samples due to the long-lasting sensations of chocolate pudding and tomato juice
259 spiked with capsaicin. Pear juice was presented as first set followed, after a 10 min break, by chocolate
260 pudding. Subjects had a 15 min break and then were presented with the bean purée set followed, after 10
261 min break, by tomato juice.
262
263 During tasting, subjects were instructed to hold the whole water solution sample in their mouth for 3 s,
264 then expectorate, wait 3 s (5 s in the case of bitterness, umami, astringency and pungency) and evaluate
265 the intensity of relevant target sensation. For the food samples, subjects were instructed to hold the whole
266 pear juice sample in their mouth or to take a full spoon of chocolate pudding, bean purée and tomato juice
267 wait for 10 s, then swallow and evaluate the intensity of the sensations as detailed in Table 2. The order of
268 attribute evaluation was randomized for the tastes, while overall flavor was always evaluated last. In the
269 present paper, only results relevant to the target sensation of each food series are considered (pear juice:
270 sourness; chocolate pudding: sweetness; bean purée: saltiness; tomato juice: pungency).
271
272 The intensity of each sensation was rated on a gLMS from "not detectable" to "the strongest imaginable
273 sensation of any kind", including pain. After each sample, subjects rinsed their mouth with water for 30 s,
274 ate some plain crackers for 30 s and finally rinsed their mouth with water for a further 30 s. Evaluations
275 were performed in individual booths under white lights. Data were collected with the software *Fizz*
276 (ver.2.51. A86, Biosystèmes, Couternon, France).
277
278 **7. Data analysis**
279 Difference in age class distribution by gender was assessed by chi-square test (α=0.05). The normality
280 assumption of the FPD data was tested by the Shapiro-Wilk W test (α = 0.05) and by the Pearson skewness
281 test. The distributions of PROP bitterness ratings and FPD values in female and male populations were
282 compared with the Kolmogorov-Smirnov test (α = 0.05). Gender and age effects on FPD values and PROP
283 bitterness ratings were assessed by means of a 2-way ANOVA model (factors: Gender-2 levels; Age Class-
284 3 levels: C1, C2, C3) with interactions. The Pearson correlation coefficient was used to assess linear
285 correlations among PROP bitterness ratings, FPD values and intensity ratings in water solutions (9
286 variables). Significance criteria were set at α=0.05. The Bonferroni correction for multiple comparison was
287 applied, the critical value for each test was then calculated as 0.05/[9*(9-1)/2]=0.0014. Relationships
288 between ratings for PROP bitterness and FPD were assessed by linear regression.
289
290 The effect of variation of FPD and responsiveness to PROP on the intensity of the oral sensations was
291 assessed considering only the extreme groups of data distributions (FPD: 25th percentile low density- L and
292 75th percentile high density-H ; PROP: bitterness lower than 17=moderate on the gLMS - NT, higher than
293 53=very strong on the gLMS - ST) to avoid possible confounding effects due to the partial overlapping of
294 the intermediate group. The comparison between the extremes of data distribution (25th and 75th
295 percentile) is a common approach to investigate differences in perception due to phenotype marker
296 variations, making it more likely to highlight group differences. However, when the comparison is restricted
297 to the extreme groups, it is not possible to conclude if the observed differences are due to a continuous
298 variation within the undivided population or if only one of the extremes deviates from of the rest. Therefore,
299 caution is needed in inferring the trend of the observed differences to the population that also includes the
300 intermediate group.

A 3-way ANOVA model was used to assess the effect of FPD class (2 levels: H-FPD and L-FPD), age (3 levels: C1, C2 and C3) and gender and their two-way interactions on taste solution intensity in PROP NT and PROP ST groups, independently. Another 3-way ANOVA mixed model with repeated measures was used to assess the effects of FPD and tastant concentration (fixed factor: FPD- 2 levels H-FPD and L-FPD; repeated measure: tastant concentration - 4 levels: Conc1, Conc2, Conc3 and Conc4; random factor: subjects) and their interaction on intensity of target sensations in food samples in PROP NT and PROP ST groups, independently. A 3-way ANOVA mixed model with repeated measures was used to assess the effects of PROP status and tastant concentration (fixed factor: PROP status- 2 levels PROP NT and PROP ST; repeated measure: tastant concentration - 4 levels: Conc1, Conc2, Conc3 and Conc4; random factor: subjects) and their interaction on perceived intensity of target sensations in food samples in L-FPD and H-FPD groups, independently. A p-value of 0.05 was considered as threshold for statistical significance. The XLSTAT statistical software package version 19.02 (Addinsoft) was used for data analysis.

RESULTS

1. Participants

Characteristics of the population sample considered in the present work are reported in Table 1. The sample was 61.4% female with a mean age of 36.6 years (SD 13.1; 18-60 years old range). Three age classes were defined: C1 (18-30), C2 (31-45) and C3 (46-60). The age class distributions of the male and female groups were not significantly different (chi-square=1.86; chi-square critical value=5.99; p=0.39). Based on World Health Organization classification for BMI, 62.0% of participants were normal weight and 27.1% were overweight. Underweight or obese subjects represent a minority of the population (3.9 and 7.0%, respectively). Almost all participants reported no food allergies and intolerances (99.5%), chronic diseases requiring long-term diet restrictions (98.7%), infections and traumas that would impair perceptive abilities (93.4%), or dietary restrictions for other reasons (93.1%). Most respondents did not smoke or smoked only occasionally (75% and 11%, respectively). The sample can therefore be considered representative of the Italian healthy adult population, and it is reasonable to hypothesize that the associations of phenotype markers of taste responsiveness and intensity response to oral stimuli explored in the present paper are not affected by specific environmental insults as confounding factors.

2. Taste function phenotypic measures

2.1 Responsiveness to PROP

The distribution of the PROP bitterness ratings confirms that reported by Monteleone et al. (2017) on the same population but on a slightly larger sample (1149 subjects) and is not detailed here. The distribution of the PROP bitterness ratings followed a bimodal distribution, but with the female and male groups significantly differing (D=0.153; p<0.0001): on average, ratings were significantly higher in females (F=17.84; p<0.0001). Increasing age was negatively associated with PROP bitterness (F=3.59; p=0.028). Descriptive values of PROP bitterness score distributions are reported in Table 3 and are very close to the arbitrary cut off proposed to classify subjects as Non-Taster – NT (arbitrary cut-off gLMS < moderate, 17) and Super Taster - ST (arbitrary cut-off gLMS > very strong, 53) (Hayes et al. 2010; Fischer et al. 2013).

344 *2.2 Fungiform Papillae Density*
345 FPD across the whole population, as well as females and males, tended towards a normal distribution (W
346 ≥ 0.967; p ≤ 0.001) with data positively skewed. Gender and age significantly affected FPD values (gender:
347 F=7.93; p=0.005; age: F=62.43; p<0.0001), but the gender*age interaction was not significant (Figure
348 1). FPD distributions of female and male groups significantly differed (D=0.096; p=0.015), with females
349 showing a higher FPD mean value (22.3 FPD) than males (20.2 FPD). FPD mean values significantly
350 decreased with age (C1=26.2; C2=20.8; C3=16.7), a decline more evident in males than in females, with
351 males belonging to C2 age class showing FPD lower than females from the same age group and not different
352 from subjects belonging to C3 age class. Descriptive values of distributions are reported in Table 3. Mean
353 values are in good agreement with values reported in studies using analogous counting procedures on the
354 same portion of the tongue (Shahbake et al. 2005; Feeney and Hayes 2014; Webb et al. 2015), as well as
355 with values from more precise techniques such as contact endoscopy (Pavlidis et al. 2013).
356
357 **3. Aqueous solutions**
358
359 *3.1 Relationships between PROP bitterness ratings, FPD values and intensity ratings in aqueous solutions.*
360 The correlations among taste function phenotypic measures and intensity ratings in solutions were tested
361 (Table 4). PROP bitterness ratings were positively correlated to the intensity of bitterness, sourness,
362 sweetness, umami and pungency while no significant correlations were found between FPD values and any
363 taste or oral sensation intensity ratings. Intensity ratings of tastes, astringency and pungency were highly
364 positively correlated each other.
365
366 PROP bitterness ratings and FPD values were not significantly related whether considering the whole
367 population sample (r^2=0.000; F=0.23; p=0.629) (Figure2) or subjects grouped by gender and age (e.g
368 Female C1: r^2=0.000, F=0.05, p=0.824, n=290; Male C1: r^2=0.001, F=0.1, p=0.755, n=188).
369
370 *3.2 Effects of FPD on intensity of aqueous solutions in PROP NT and PROP ST group*
371 The effect of FPD variation in terms of class (low density-L: 25th percentile; high density-H: 75th percentile)
372 on intensity of taste solutions was further explored in PROP NT and PROP ST subject groups, independently
373 (Table 5). The PROP NT group rated the intensity of taste solutions from moderate to strong (range:17.57-
374 42.24). The FPD class did not significantly affect the mean taste intensity ratings, and although the mean
375 values from H-FPD tended to be higher than those from L-FPD group, this difference was only marginally
376 significant for pungency (p=0.06).
377
378 Mean intensity ratings did not significantly vary with gender and age, with the exception of pungency.
379 Females rated pungency significantly higher than did males (p<0.001), and mean intensity ratings from
380 subjects belonging to the C2 age class (31-45 years) were significantly higher than for the rest of population
381 (p=0.05). In PROP NT, significant FPD*Gender interactions for bitterness (p=0.05) and saltiness (p=0.01)
382 were found. Here, decreasing intensity was observed from H- to L-FPD in males, while no differences were
383 observed in females belonging to different FPD classes. Furthermore, a significant FPD*Age interaction was
384 found for bitterness (p<0.001), with a positive effect of FPD variation on intensity in C2 and C3 classes
385 while a negative effect was observed in the C1 age class.
386

387 PROP ST rated the intensities of taste solutions from moderate to very strong (range: 19.16 - 57.90).
388 However, the FPD class did not significantly affect the mean taste solution intensities, although mean values
389 from H-FPD tend to be lower than those from L-FPD. Mean intensity ratings did not significantly vary with
390 gender, with the exception of astringency, that females rated significantly lower than did males (p=0.00).
391 Age class did not influence intensity ratings in PROP ST. Interactions for FPD*Gender and FPD*Age were
392 never significant in PROP ST.
393
394 **4. Food stimuli**
395
396 *4.1 Effects of FPD class on perceived intensity of target sensations in PROP NT and PROP ST groups*

397 Subject groups considered for this analysis are showed in Fig 2: PROP NT subjects belong to groups I (L
398 FPD) and II (H FPD); PROP ST subjects belong to groups III (L FPD) and IV (H FPD). A 3-way ANOVA
399 mixed model with repeated measures on intensity of target sensations in food stimuli was computed to test
400 the effect of FPD (low-L and high-H density) in both PROP NT and PROP ST groups (Table 6, Figures 3 and
401 4).
402
403 In PROP NT, the intensity of target sensations significantly increases with tastant concentration from weak
404 to strong in all the stimuli series (p≤0.0001). FPD significantly affected the intensity of target sensations
405 only in pear juice (p=0.047), and no FPD*Concentration interactions were significant. Mean values from
406 H-FPD tended to be higher than those from L-FPD group but this difference reached significance as a
407 function of food and tastant concentration level only in a few cases (Figure 3 A-D). LSD post-hoc tests
408 indicated that H-FPD group scored sourness in pear juice and saltiness in bean purée higher than L-FPD
409 group in the sample added with the highest tastant concentration (Conc4). Pungency in sample Conc3 of
410 the tomato juice was rated higher by H-FPD than L-FPD group.
411
412 PROP ST also showed significant increases in target sensation intensity from weak to strong as a function
413 of the tastant concentration (p≤0.0001). FPD significantly affected only the intensity of saltiness in bean
414 purée (p=0.010), and the FPD*Concentration interaction was significant in bean purée only (p=0.010).
415 Mean values from H FPD tended to be lower than those from L FPD group, but this difference reached
416 significance level only in a few cases (Figure 4 A-D). LSD post-hoc tests indicated that H-FPD group rated
417 saltiness in bean purée and pungency in tomato juice lower than did the L-FPD group in the Conc4 sample.
418
419 *4.2 Effects of PROP status on perceived intensity of target sensations in L-FPD and H-FPD groups*

420 Subject groups considered for this analysis are showed in Fig 2: L-FDP subjects belong to groups I (NT)
421 and III (ST); H-FPD subjects belong to groups II (NT) and IV (ST). The effect of PROP status (NT and ST)
422 on the intensity of target sensations in foods was assessed in L-FPD and H-FPD groups (Table 7). Both in
423 L-FPD and H-FPD groups, the intensity of target sensations significantly increased with tastant
424 concentration from weak to strong in all stimuli series (p≤0.0001). In L-FPD group, the intensity of target
425 sensations was significantly affected by PROP status (p≤0.022), and the FPD*Concentration interactions
426 were always significant. In the L-FPD group, mean intensity values of PROP ST were higher than those of
427 PROP NT group, with this difference reaching significance at different tastant concentrations, depending on
428 the food (Figure 5 A-D). PROP ST rated sourness in pear juice and pungency in tomato juice as higher than
429 did PROP NT in all samples with tastant added (Conc2-Conc4), and rated saltiness higher than PROP NT in

430 bean purée samples Conc3 and Conc4, and sweetness in chocolate pudding sample Conc4. PROP status did
431 not affect the intensity of target sensations in H-FPD group, and the PROP*Concentration interactions were
432 never significant.
433
434 **DISCUSSION**
435 A great deal of research has been devoted to studying associations between PROP taste status, FPD and
436 responses to oral stimulation, but these relationships remain controversial. Conclusions based on large
437 scale studies tend to agree on the lack of simple causal relationships among these variables and instead
438 highlight a complex interplay among factors regulating oral responsiveness (Garneau et al. 2014; Fischer
439 et al. 2013; Monteleone et al. 2017). Demographics, genetics and other environmental factors may
440 influence phenotypic responses to oral stimulation, including PROP, and FP density thus acting as possible
441 confounders (Tepper et al. 2017; Piochi et al. 2018).
442
443 In the present study, aging was found to significantly lower both phenotype indices, with a stronger effect
444 on FPD than on responsiveness to PROP. In adults, age is negatively correlated with FPD (Segovia et al.
445 2002; Correa et al. 2013; Shen et al. 2016). Aging has been associated with lowered responsiveness to
446 PROP and it has been suggested that phenotypic expression of TAS2R38 gene varies with age (Mennella et
447 al. 2010). Furthermore, changes in distribution of PROP taster groups has been observed with an increased
448 percentage of PROP NT in older populations (age > 50 years) (Tepper et al., 2017).
449
450 In the present work, a significant gender effect was also found, with females rating PROP bitterness, and
451 showing FPD mean values, significantly higher than males. This gender effect was stronger on PROP
452 phenotype than on FPD value. Females are reported to be more sensitive to PROP than males, and more
453 likely to be tasters (Bartoshuk et al. 1994; Zhao et al, 2007). Furthermore, results from the same
454 population analysed in the present study, but on a slightly larger sample, confirmed significant changes in
455 distribution of PROP taster groups depending on gender and age (Monteleone et al. 2017). Our results also
456 confirm data on the higher number of FPD in females than in males (Bartoshuk et al. 1994; Tepper and
457 Nurse, 1997; Duffy et al. 2004b; Hayes et al. 2008; Fischer et al. 2013; Pavlidis et al. 2013). Here,
458 differences in FPD across gender were dependent from age class, and significant differences were found
459 only in C2 class (31-45 years). Furthermore, a regular decreasing of FPD was observed with age, an effect
460 more pronounced in males than in females thus confirming males more susceptible to FPD lowering with
461 age (Pavlidis et al. 2013). The data from the present study thus show the interplay of gender and age in
462 determining interindividual variations in phenotype markers of oral responsiveness.
463
464 Many studies examining oral responsiveness have used samples unbalanced for age and gender, and this
465 is likely to at least partially account for inconsistencies in the effect of these factors on FPD and PROP
466 responsiveness. Furthermore, the impact of age and gender on interindividual variation in phenotype
467 markers of oral responsiveness might also partially account for uncertainties regarding the relationship
468 between PROP responsiveness and FPD. Young females tend to show higher responsiveness to PROP and
469 higher FPD than older males. In unbalanced study populations, significant relationships between these two
470 factors can be observed that may be due to gender and age characteristics of the considered subject group,
471 inappropriately generalized to a population. Previous large scale studies on more than one thousand
472 individuals failed to find significant associations PROP phenotype/FPD (Fischer et al. 2013; Garneau et al.

2014). The results from the present study confirm the lack of simple linear relationship between PROP phenotype and FPD, both in the whole population and in samples selected by age and gender.

In the present study, the PROP phenotype was significantly associated with heightened responses to most of the basic tastes and pungent stimuli, thus supporting the notion that it is a reliable marker of orosensory responsiveness to sensory properties of both solutions and real foods. Prior studies have linked PROP bitterness to increased taste intensity of sucrose, citric acid, sodium chloride, quinine caffeine and monosodium glutamate solutions (Prescott et al. 2001; Hayes et al. 2008; Fischer et al. 2015; Webb et al. 2015). The BOSS study (Fischer et al. 2013) confirmed the intensity of PROP positively correlated to four basic tastes and pointed out that the strength of the relationships differed by TAS2R38 haplotype, being significantly stronger in the PAV homozygotes (Fischer et al. 2015). Other studies have found significant positive relationships between PROP bitterness and chemesthetic sensations (pungency from capsaicin and other oral irritants) (Prescott et al. 2000; Yang et al. 2014), as well as with tactile sensations (astringency from alum) (Bajec and Pickering, 2008). PROP responsiveness was reported to be associated with heightened intensity of bitterness in vegetables (Dinehart et al. 2006), taste, flavour and chemesthetic sensations in soft drink models (Prescott et al. 2004; Zhao and Tepper, 2007), bitterness, astringency and sourness in coffee (Masi et al. 2015), and roughness, bitterness and sweetness in bread (Bakke and Vickers, 2008).

Despite such findings, doubt has been cast upon the idea that a single phenotypic marker such as PROP tasting is insufficient to fully characterize the interindividual variability in response to oral stimulation (Hayes and Keast, 2011; Garneau et al. 2014). It may be that a general heightened or lowered response to oral stimuli, which includes PROP bitterness, and well as (other) taste, somatosensory and chemestethic qualities, generalized a hypo- or hyper-"geusia", can be used to classify subjects (Hayes and Keast, 2011; Puputti et al., 2017). The significant correlations found here between the intensity of basic tastes, astringency and pungency and PROP ratings (see tab.4) confirms the concept of a generalized common variation of intensity response to oral stimuli, since the perceived intensities of tastes, astringency and pungency are positively associated each other.

On the other hand, the present data provides little evidence that FPD variation is associated with variations in the intensity of oral stimuli, and this is consistent with a number of previous studies. Webb and co-workers did not find significant correlations between individual variations in FPD and the intensity of supra-threshold solutions of sucrose, NaCl, citric acid, caffeine and monosodium glutamate in whole mouth stimulation conditions (Webb et al. 2015). Similarly, using a larger sample (n=200), no relationships were found between FPD and the sweetness from either sucrose and acesulfame, saltiness from KCl, bitterness from quinine, burning from capsaicin and the perception of umami from MSG/IMP mixtures, either with whole mouth or regional tongue stimulation (Fenney and Hayes, 2014). The Beaver Dam Offspring study on more than two- thousand individuals reported no significant associations between sweetness (sucrose), sourness (citric acid) and bitterness (quinine) from regional supra-threshold stimulation and FPD, while a weak inverse correlation was found between saltiness from NaCl and FPD (Fischer et al. 2013). Similarly, FPD variation did not influence the intensity of the tactile sensation of astringency, in agreement with previous small data sets using real food (n=37; Bakke and Vickers, 2008) and standard stimuli (n=30; Linne et al. 2017).

516
517 The assumption of direct association of FPD with perceived intensity relies on the logic of spatial summation,
518 namey that, as the area of taste stimulation is increased (and hence the number of papillae and buds), the
519 taste intensity increases (Delwiche et al. 2001). Recent evidence on significant associations between
520 parameters describing electrophysiological records from the tongue after local stimulation with PROP
521 solutions and both perceived bitterness intensity and FPD confirm the spatial summation assumption (Sollai
522 et al. 2017). On the other hand, the lack of close relationships between taste bud and FP densities and the
523 influence of several environmental factors on FP response to oral stimuli weaken the direct association
524 FPD/perceived intensity (see Piochi et al. 2018, for a review). Coupling the quantitative measures of
525 peripheral taste function and the intensity responses from sensory evaluations would certainly help a
526 deeper understanding of the mechanisms underlying the perception of food stimuli and the relevant
527 interindividual variations.
528
529 Complex, and still controversial, associations have been reported between both PROP phenotype and
530 TAS2R38 polymorphism with polymorphism of rs2274333 gene (A/G) that controls the functionality of
531 gustin, the salivary trophic factor. Gustin plays a crucial role in taste function and has been proposed to
532 promote growth and development of taste buds (Henkin et al. 1999). Gustin genotypes were associated
533 with both fungiform papillae density and morphology (Melis et al. 2013). However, other studies have failed
534 to find such associations (Bering et al. 2014, Feeney and Hayes, 2014, Barbarossa et al. 2015, Yang, 2015,
535 Shen et al. 2016, Shen et al. 2017). Furthermore, the strength of positive relationships between the
536 intensity of PROP and basic tastes differed by TAS2R38 haplotype with stronger association found in
537 PAV/PAV than in the other diplotypes (Fischer et al. 2015). Thus, it is possible that interindividual variation
538 in TAS2R38 genotype and responsiveness to PROP might partially account for decoupling taste intensity
539 and FPD.
540
541 In the present study, the importance of FPD in taste sensing was explored in PROP NT and PROP ST groups,
542 independently. The results indicate that FPD variation has only a slight impact on orosensory perception.
543 In the PROP NT, FPD did not affect the intensity of taste solutions, and a significant positive effect was only
544 found for sourness in pear juice. The lack of a significant effect of FPD on intensity in taste solutions was
545 also confirmed in PROP ST. In this group, the only significant effect of FPD variation was found in bean
546 purée where L-FPD subjects perceived saltiness intensity higher than H-FPD group. Thus, if we assume that
547 these findings are not false positives, it appears that the contribution of FPD to intensity depends on the
548 stimulus considered, the target sensation intensity and PROP status. Some researchers found PROP NT
549 status associated with the recessive and less functional form of the gustin (GG) and AA genotype more
550 frequently carried by PROP Tasters (Padiglia et al. 2010, Calò et al. 2011, Melis et al. 2013). It may be
551 that PROP insensitive individuals that carry AVI haplotype cannot take advantage from the reinforced
552 perception capacity of FP associated with the PAV haplotype (such as for example gustin active form). In
553 this case FP responsiveness might basically depend from their number and the increased FPD also
554 correspond to a heightened intensity perception.
555
556 The negative impact of FPD on intensity perception in PROP ST was unexpected, even if other reports
557 documented such negative correlations for saltiness in populations not segmented by PROP status (Fischer
558 et al. 2013). The interaction of FPD/PROP status on perception of oral stimuli was further explored

559 considering subject groups belonging to the same FPD class (H and L FDP) but varying for PROP status.
560 PROP status strongly affected the intensity of food stimuli in L-FPD subject group, with PROP ST rating the
561 intensity of target sensations higher than did PROP NT. These results indirectly confirm the general positive
562 effect on chemosensory abilities contributed by PAV haplotype and associated effects. On the other hand,
563 being a PROP ST did not produce equivalent effects in subject groups with H-FPD. In this case, the high
564 number of FP possibly compensates for the perceptive system capacity less in AVI than in the PAV carrier
565 group. Tentatively, it can be speculated that the PROP ST status of H-FPD individuals results from the
566 combination of the high papillae number and the presence of the PAV haplotype, possibly in heterozygous
567 form, and thus with a partial expression of perceptive advantages associated with PROP sensitivity. This
568 hypothesis can also explain the differences observed between L and H FPD in PROP ST. L-FPD/PROP ST
569 subjects can represent the "real" supertaster characterized by a generalized hypergeusia possibly induced
570 by the association of gene polymorphisms (i.e. PAV/PAV and G/G) and perceptive system features
571 advantageous for orosensation. The ongoing gene analysis on this population will help to gain further insight
572 on the factors underlying the observed results.
573
574 In conclusion, the results of the present study depict a complex interplay of several factors affecting
575 phenotype markers of orosensory acuity, their relationships and their impact on the intensity of target
576 sensations. The fact that demographic factors influence FPD and PROP responsiveness lead to strong
577 recommendations for the strict control of population sample characteristics when using these phenotypes
578 as markers of food perception and preference, and once more highlight the risk of generalizing results from
579 small convenience samples. As well, care should be taken in stimulus selection since intensity responses
580 as a function of PROP/FPD appear to be significantly influenced by the context (model or real food) and by
581 the tastant concentration. However, PROP responsiveness appears to be confirmed as a reliable marker of
582 heightened response to oral stimuli broadly, and the concept of hypergeusia to describe a generalized
583 heightened response across oral stimuli. The mechanistic explanation for why PROP responsiveness
584 positively affects the response to stimuli that are not mediated by the TAS2R38 receptor deserves further
585 research efforts. As already concluded by other authors (Hayes et al 2008), additional insight should be
586 gained on associations between gene polymorphism impacting on perceptive system functioning, and the
587 role of peripheral sensing organs reconsidered.
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589
590
591 **Author contributions**
592 CD undertook the analyses and wrote the manuscript; CD and EM contributed to plan the analyses; CD,
593 EM, JP, MP, SS, LP discussed the interpretation of the results; EM, AB, CD, FG, LT, ML, EP, SP, SS,
594 collaborated in the design of the project Italian Taste; all authors helped with data collection, reviewed and
595 offered critical comments on the manuscript.
596
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601

602 **Conflict of interest**
603 Dr Prescott is director of TasteMatters Research & Consulting.
604 The authors declare to have no conflict of interest.
605
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900 **Table 1:** Characteristics of respondents

	Males (n=432) %	Females (n=687) %	Total (n=1119) %
Sex	38.6	61.4	100
Age (years)			
18-30	43.5	42.2	42.7
31-45	24.8	28.4	27.1
46-60	31.7	29.4	30.2
Body Mass Index (kg/m^2)			
Underweight (<18.50)	1.2	5.7	3.9
Normal range (18.50-24.99)	54.6	66.7	62.0
Overweight (25.00-29.99)	35.6	21.7	27.1
Obese (≥30.00)	8.6	5.9	7.0
Food Allergies/Intolerances			
Celiac disease	-	0.4	0.3
Lactose/Dairy	0.2	0.1	0.2
Others	0.2	-	0.01
Practice of restrictive diets			
Vegetarian	1.6	2.5	2.1
Vegan	-	-	-
Low-calorie	0.3	5.7	4.6
Others	-	0.3	0.2
Diseases			
Diabete	0.2	0.4	0.4
High blood pressure	0.5	-	0.2
High cholesterol level		0.4	0.3
Gastric pathologies	-	0.6	0.4
Infections and Head trauma			
Otitis (≥6 times in the life)	4.9	7.1	6.2
Sinusitis/Polyp	0.5	0.3	0.4
Nasal bone fracture	-	-	-
Smoking			
Not smoking (never tried or quit)	73	76	75
Occasionally	11 (*1.1/day)	11 (*0.5/day)	10.5
Regularly	16 (*10/day)	13 (*10/day)	14.5

901 **cigarette/day median value*

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Table 2. Food products: food, tastant, tastant concentration in four samples (Conc1-Conc4) to produce variations in target sensations (in bold) from weak to strong and rated sensations

Food	Tastant	Concentration g/Kg	Sensations
Pear Juice - PJ	Citric acid	Conc1:0.5 Conc2:2.0 Conc3:4.0 Conc4:8.0	**Sourness** Sweetness Overall Flavour
Chocolate Pudding - CP	Sucrose	Conc1:38 Conc2:83 Conc3:119 Conc4:233	**Sweetness** Bitterness Astringency Overall Flavour
Bean Purée - BC	Sodium chloride	Conc1:2.0 Conc2:6.1 Conc3:10.7 Conc4:18.8	**Saltiness** Umami Overall Flavour
Tomato Juice - TJ	Capsaicin	Conc1:$0.3*10^{-3}$ Conc2:$0.68*10^{-3}$ Conc3:$1.01*10^{-3}$ Conc4:$1.52*10^{-3}$	**Pungency** Sourness Sweetness Overall Flavour

Table 3: Descriptive values of PROP bitterness ratings and FPD distributions in the whole sample (all), female (F) and male (M) groups.

	PROP bitterness ratings			FPD values		
	All	F	M	All	F	M
Observations	1119	687	432	1119	687	432
1° Q	17.0	19.0	14.0	13.2	13.2	12.4
Median	38.0	42.5	32.0	20.3	22.0	18.5
3° Q	58.0	63.0	50.4	30.0	31.8	28.3
Mean	39.4	42.2	35.4	22.1	22.3	20.2
SD	27.0	27.7	25.2	12.5	12.6	12.3

Table 4. Correlations among taste function phenotypic measures and intensity ratings in water solutions: Pearson correlation matrix. Values in bold represent significant correlation (α=0.05); p critical value after Bonferroni correction significant for p≤0.0014.

Variables	FPD cm^2	PROP ratings	Sour	Bitter	Sweet	Salty	Umami	Astringent	Pungent
FPD cm^2	**1**								
PROP ratings	0.016	**1**							
Sour	-0.037	**0.089**	**1**						
Bitter	-0.030	**0.116**	**0.380**	**1**					
Sweet	-0.032	**0.122**	**0.424**	**0.334**	**1**				
Salty	-0.059	0.079	**0.442**	**0.333**	**0.462**	**1**			
Umami	0.007	**0.128**	**0.334**	**0.283**	**0.362**	**0.440**	**1**		
Astringent	-0.014	0.056	**0.386**	**0.334**	**0.302**	**0.309**	**0.282**	**1**	
Pungent	0.015	**0.199**	**0.349**	**0.340**	**0.302**	**0.333**	**0.256**	**0.195**	**1**

928 **Table 5.** 3-way ANOVA - Effects of FPD class (high -H and low-L density), Gender (female-F and male-M) and Age Class (C1: 18-30
929 years; C2: 31-45 years; C3: 46-60) on perceived intensity of water solutions in PROP NT and PROP ST groups: mean intensity, F and
930 p values

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			Sour	Bitter	Sweet	Salty	Umami	Astringent	Pungent
PROP NT									
FPD	mean	H	36.66	40.75	42.24	41.08	27.57	23.63	48.39
		L	31.19	34.88	36.89	34.37	23.92	19.28	41.32
	F		1.63	0.56	1.83	1.92	1.05	0.99	3.62
	p		0.20	0.45	0.18	0.17	0.31	0.32	0.06
Gender	mean	F	34.10	38.93	40.08	38.23	28.03	23.41	51.87
		M	33.74	36.70	39.05	37.22	23.46	19.49	37.85
	F		0.03	0.41	0.13	0.15	2.12	1.68	15.55
	p		0.87	0.52	0.72	0.70	0.15	0.20	**0.00**
Age	mean	C1	36.71	37.43	40.36	35.22	27.61	19.60	41.53
		C2	31.69	38.82	41.16	37.49	26.29	20.87	50.72
		C3	33.36	37.19	37.16	40.46	23.34	23.89	42.31
	F		0.71	0.65	0.33	1.00	0.44	0.94	3.16
	p		0.49	0.52	0.72	0.37	0.64	0.39	**0.05**
PROP ST									
FPD	mean	H	35.76	37.63	40.89	35.74	27.65	19.16	51.15
		L	40.03	40.19	45.04	42.19	31.06	25.94	57.90
	F		1.53	0.61	1.33	0.91	0.41	2.00	0.06
	p		0.22	0.44	0.25	0.34	0.53	0.16	0.81
Gender	mean	F	35.50	35.94	43.03	37.91	25.07	17.52	57.56
		M	40.29	41.89	42.90	40.01	33.64	27.58	51.49
	F		2.05	1.52	0.24	0.03	3.07	10.81	1.96
	p		0.15	0.22	0.63	0.86	0.08	**0.00**	0.16
Age	mean	C1	39.63	35.81	43.37	37.80	28.05	23.78	51.75

	C2	32.37	34.55	41.42	44.00	31.58	22.49	52.52
	C3	41.69	46.37	44.10	35.09	28.42	21.38	59.30
F		0.45	2.03	0.28	1.06	0.67	0.19	1.03
p		0.64	0.13	0.76	0.35	0.51	0.83	0.36

933 **Table 6.** 3-way ANOVA mixed model with repeated measures: Effects of FPD class and tastant concentration on responsiveness to target sensations of food
934 stimuli in PROP NT and PROP ST groups.
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	Sour - Pear Juice		Sweet - Chocolate Pudding		Salty - Bean Purée		Pungent - Tomato Juice	
	F	Pr > F	F	Pr > F	FF	Pr > F	F	Pr > F
PROP NT								
FPD	4.037	**0.047**	0.050	0.823	1.053	0.307	2.832	0.095
Concentration	187.571	**<0.0001**	213.739	**<0.0001**	305.022	**<0.0001**	147.600	**<0.0001**
FPD*Conc	2.055	0.106	1.525	0.208	1.969	0.118	1.941	0.122
PROP ST								
FPD	1.703	0.194	1.471	0.227	6.837	**0.010**	3.480	0.064
Concentration	275.522	**<0.0001**	269.599	**<0.0001**	454.908	**<0.0001**	219.401	**<0.0001**
FPD*Conc	0.329	0.805	0.902	0.440	3.844	**0.010**	2.573	**0.053**

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947 **Table 7.** 3-way ANOVA mixed model with repeated measures: Effects of PROP status and tastant concentration on responsiveness to target sensations of
948 food stimuli in L-FDP and H-FPD groups.
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	Sour - Pear Juice		Sweet - Chocolate Pudding		Salty - Bean Purée		Pungent - Tomato Juice	
	F	Pr > F	F	Pr > F	F	Pr > F	F	Pr > F
L-FPD								
PROP status	13.929	**0.000**	5.394	**0.022**	15.595	**0.000**	14.099	**0.000**
Concentration	222.846	**<0.0001**	211.161	**<0.0001**	355.692	**<0.0001**	193.137	**<0.0001**
PROP*Conc	3.317	**0.020**	3.400	**0.018**	10.567	**<0.0001**	6.670	**0.000**
H-FPD								
PROP status	0.017	0.896	1.913	0.169	0.295	0.588	0.300	0.585
Concentration	240.620	**<0.0001**	272.560	**<0.0001**	404.150	**<0.0001**	177.341	**<0.0001**
PROP*Conc	0.156	0.926	0.589	0.622	1.055	0.368	0.368	0.776

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Figure and Figure Legend

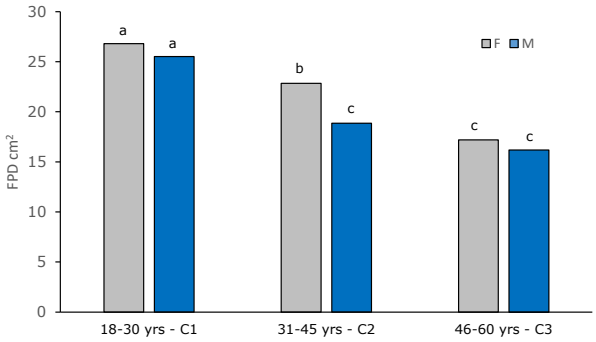

Figure 1. 2-Way ANOVA: gender (F-females; M-males) and age effect on FPD values. Different letters indicate significantly different values (p≤ 0.005).

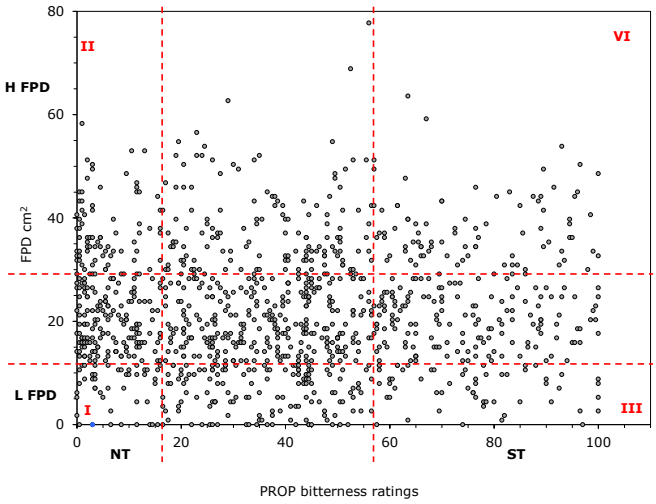

Figure 2. Individual variation in PROP bitterness ratings and FPD values. Dotted lines represent limits of PROP Status groups on x axe (cut-off: NT<17; ST>53) and FPD groups on y axe (cut off: LFPD 25th percentile; HFPD 75th percentile).

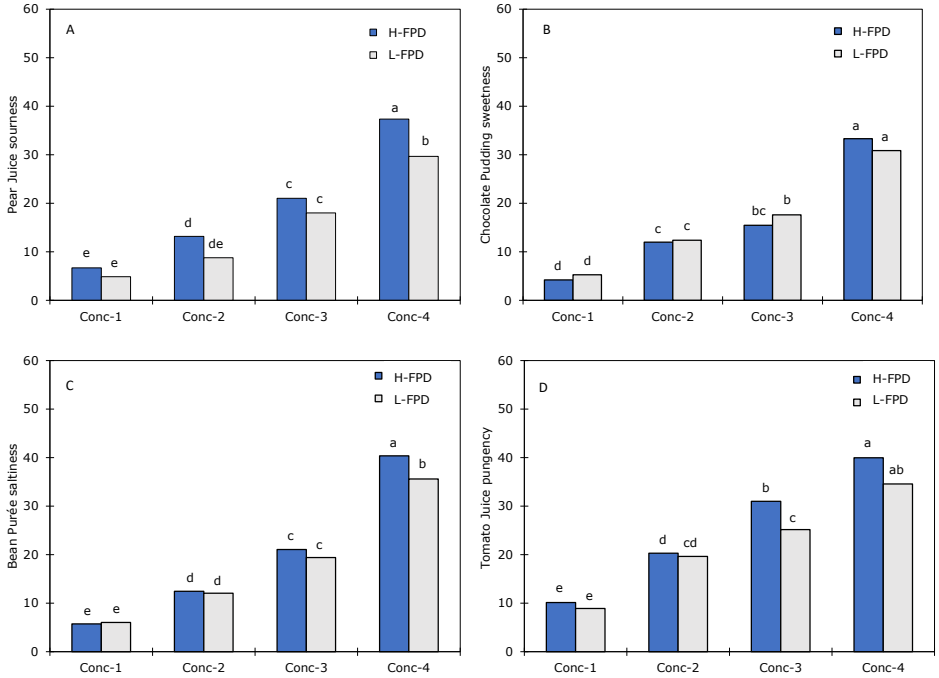

Figure 3. PROP NT subject group: Effect of FPD variation (high H-FPD and low L-FPD) and tastant concentration (conc-1 – conc-4) on perceived intensity of target sensation in food stimuli (A:pear juice; B: chocolate pudding; C:bean purée; D: tomato juice).

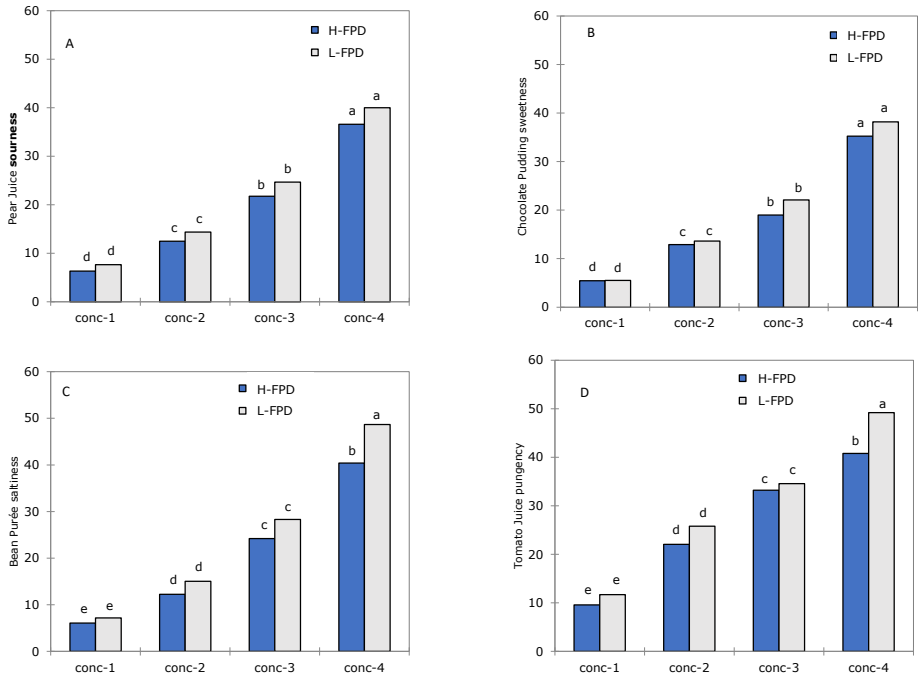

Figure 4. PROP ST subject group: Effect of FPD variation (high H-FPD and low L-FPD) and tastant concentration (conc-1 - conc-4) on perceived intensity of target sensation in food stimuli (A:pear juice; B: chocolate pudding; C:bean purée; D: tomato juice).

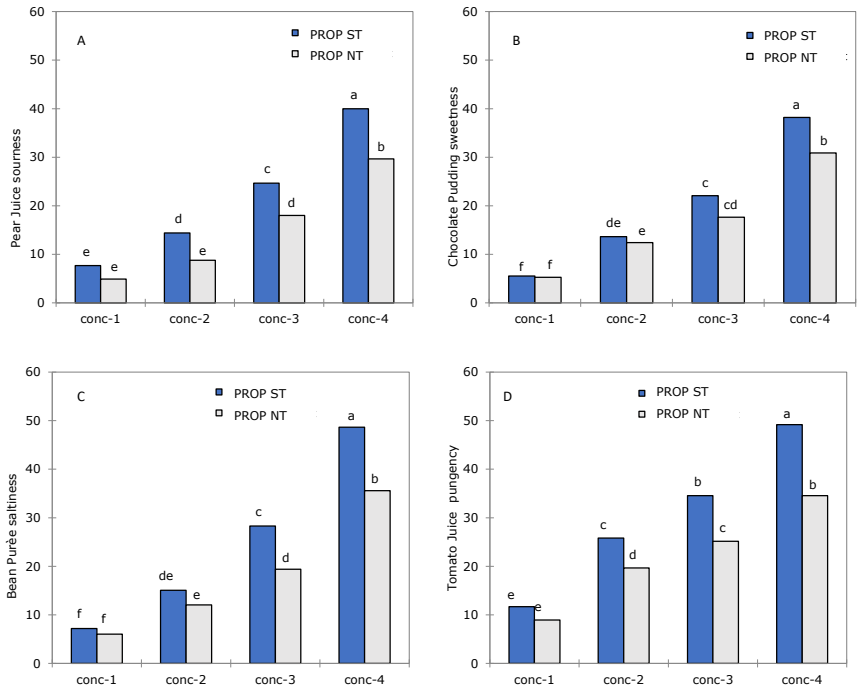

Figure 5. L-FPD subject group: Effect of PROP responsiveness variation (PROP NT and PROP ST) and tastant concentration (conc-1 – conc-4) on perceived intensity of target sensation in food stimuli (A:pear juice; B: chocolate pudding; C:bean purée; D: tomato juice).