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 moderately and extremely bitter, respectively (Bartoshuk, 1991). The polymorphisms in the gene *TAS2R38* mainly explain the observed phenotypic variation, with individuals carrying the PAV allele perceiving greater intensity from supra-threshold PROP solutions than carriers of the AVI allele (Duffy et al. 2004a). Responsiveness to PROP bitterness is significantly affected by psychosocial variables (McAnally et al. 2007), as well as gender and age. The percentage of tasters has been found to be consistently higher in females than in males (Bartoshuk et al. 1994; Guo and Reed, 2001, Monteleone et al. 2017) and a decline in responsiveness to PROP is typically observed with age (Guo and Reed, 2001, Tepper et al. 2014), especially in females (Monteleone et al. 2017).

Several studies have demonstrated that responsiveness to PROP is positively associated with responsiveness to chemosensory stimulation in standard solutions (Hayes et al. 2008; Yang et al. 2014; Webb et al. 2015, Fischer et al. 2014; Melis et al. 2017; Prescott et al. 2001) and real food (Dinehart et al. 2006; Zhao and Tepper, 2007; Bakke and Vickers, 2008, Bajec and Pickering, 2008; Masi et al. 2015, Spinelli et al. 2018). Furthermore, PROP responsiveness was reported to increase discrimination among foods and beverages with systematic variations in tastes, oral irritants (Prescott et al. 2004) and textures (de Wijk et al. 2007).

Despite such findings, the mechanism behind the relationship between responsiveness to PROP and perception of other chemosensory qualities is still unclear. Bartoshuk and co-workers found FPD correlated with the bitterness of PROP (Bartoshuk et al. 1994), and further studies supported this observation (Tepper and Nurse, 1997; Delwiche et al., 2001; Yackinous and Guinard, 2002; Essick et al. 2003; Duffy et al. 2004b; Hayes and Duffy, 2007; Yeomans et al. 2007; Bajec and Pickering, 2008; Hayes et al. 2010). Moreover, the term Super Taster has been used by Bartoshuk (Bartoshuk et al. 1994) to indicate individuals who perceived PROP as extremely bitter, with an increased taste and oral somatosensory responsiveness, and who also had high FPD. Thus, a causal relationship between high FPD and the increased responsiveness to oral stimuli, including PROP, has been hypothesized with some empirical justification.

More recently, the definition of Super Taster individuals has been reconsidered (Hayes and Keast, 2011; Kalva et al., 2014). Moreover, large population studies have failed to find significant associations between the PROP phenotype and FPD (Fisher et al. 2013; Garneau et al. 2014). FPD has been reported as significant determinant of PROP bitterness in TAS2R38 homozygotes and not in heterozygotes (Hayes et al. 2008), but this has not been confirmed in larger sample studies where FPD does not differ by diplotype (Fischer et al. 2013; Garneau et al. 2014). To explain the mechanistic link between PROP responsiveness and chemosensory acuity, complex and still controversial relationships between polymorphism of TAS2R38 and gustin genes, and FP development and maintenance have been proposed (Padiglia et al. 2010; Calo et al. 2011; Melis et al. 2013; Barbarossa et al. 2015). However, other studies have failed to find such associations (Feeney and Hayes, 2014; Bering et al. 2013; Barbarossa et al. 2015; Yang, 2015; Shen et al. 2016; Shen et al. 2017).

Overall chemosensory responsiveness is affected by lingual nerve damage (Bartoshuk et al. 2012), chronic pathologies and medications (Boltong and Keast, 2012; Pavlidis et al. 2014), eating disorders and dietary restrictions (Bartoshuk et al. 2006; Stafford et al. 2013) and smoking habits (Venneman et al. 2008; Jacob 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 carry-over effects across samples due to the long-lasting sensations of chocolate pudding and tomato juice spiked with capsaicin. Pear juice was presented as first set followed, after a 10 min break, by chocolate pudding. Subjects had a 15 min break and then were presented with the bean purée set followed, after 10 min break, by tomato juice.

During tasting, subjects were instructed to hold the whole water solution sample in their mouth for 3 s, then expectorate, wait 3 s (5 s in the case of bitterness, umami, astringency and pungency) and evaluate the intensity of relevant target sensation. For the food samples, subjects were instructed to hold the whole pear juice sample in their mouth or to take a full spoon of chocolate pudding, bean purée and tomato juice wait for 10 s, then swallow and evaluate the intensity of the sensations as detailed in Table 2. The order of attribute evaluation was randomized for the tastes, while overall flavor was always evaluated last. In the present paper, only results relevant to the target sensation of each food series are considered (pear juice: sourness; chocolate pudding: sweetness; bean purée: saltiness; tomato juice: pungency).

The intensity of each sensation was rated on a gLMS from "not detectable" to "the strongest imaginable sensation of any kind", including pain. After each sample, subjects rinsed their mouth with water for 30 s, ate some plain crackers for 30 s and finally rinsed their mouth with water for a further 30 s. Evaluations were performed in individual booths under white lights. Data were collected with the software *Fizz* (ver.2.51. A86, Biosystèmes, Couternon, France).

7. Data analysis

Difference in age class distribution by gender was assessed by chi-square test (α=0.05). The normality assumption of the FPD data was tested by the Shapiro-Wilk W test ($\alpha = 0.05$) and by the Pearson skewness test. The distributions of PROP bitterness ratings and FPD values in female and male populations were compared with the Kolmogorov-Smirnov test ($\alpha = 0.05$). Gender and age effects on FPD values and PROP bitterness ratings were assessed by means of a 2-way ANOVA model (factors: Gender-2 levels; Age Class-3 levels: C1, C2, C3) with interactions. The Pearson correlation coefficient was used to assess linear correlations among PROP bitterness ratings, FPD values and intensity ratings in water solutions (9 variables). Significance criteria were set at α=0.05. The Bonferroni correction for multiple comparison was applied, the critical value for each test was then calculated as $0.05/[9*(9-1)/2]=0.0014$. Relationships between ratings for PROP bitterness and FPD were assessed by linear regression.

The effect of variation of FPD and responsiveness to PROP on the intensity of the oral sensations was assessed considering only the extreme groups of data distributions (FPD: 25th percentile low density- L and 75th percentile high density-H ; PROP: bitterness lower than 17=moderate on the gLMS - NT, higher than 53=very strong on the gLMS - ST) to avoid possible confounding effects due to the partial overlapping of the intermediate group. The comparison between the extremes of data distribution (25th and 75th percentile) is a common approach to investigate differences in perception due to phenotype marker variations, making it more likely to highlight group differences. However, when the comparison is restricted to the extreme groups, it is not possible to conclude if the observed differences are due to a continuous variation within the undivided population or if only one of the extremes deviates from of the rest. Therefore, caution is needed in inferring the trend of the observed differences to the population that also includes the 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).

2.2 Fungiform Papillae Density

FPD across the whole population, as well as females and males, tended towards a normal distribution (W ≥ 0.967; p ≤ 0.001) with data positively skewed. Gender and age significantly affected FPD values (gender: F=7.93; p=0.005; age: F=62.43; p<0.0001), but the gender*age interaction was not significant (Figure 1). FPD distributions of female and male groups significantly differed (D=0.096; p=0.015), with females showing a higher FPD mean value (22.3 FPD) than males (20.2 FPD). FPD mean values significantly decreased with age (C1=26.2; C2=20.8; C3=16.7), a decline more evident in males than in females, with males belonging to C2 age class showing FPD lower than females from the same age group and not different from subjects belonging to C3 age class. Descriptive values of distributions are reported in Table 3. Mean values are in good agreement with values reported in studies using analogous counting procedures on the same portion of the tongue (Shahbake et al. 2005; Feeney and Hayes 2014; Webb et al. 2015), as well as with values from more precise techniques such as contact endoscopy (Pavlidis et al. 2013).

3. Aqueous solutions

3.1 Relationships between PROP bitterness ratings, FPD values and intensity ratings in aqueous solutions.

The correlations among taste function phenotypic measures and intensity ratings in solutions were tested (Table 4). PROP bitterness ratings were positively correlated to the intensity of bitterness, sourness, sweetness, umami and pungency while no significant correlations were found between FPD values and any taste or oral sensation intensity ratings. Intensity ratings of tastes, astringency and pungency were highly positively correlated each other.

PROP bitterness ratings and FPD values were not significantly related whether considering the whole population sample (r^2=0.000; F=0.23; p=0.629) (Figure2) or subjects grouped by gender and age (e.g 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).

3.2 Effects of FPD on intensity of aqueous solutions in PROP NT and PROP ST group

The effect of FPD variation in terms of class (low density-L: 25th percentile; high density-H: 75th percentile) on intensity of taste solutions was further explored in PROP NT and PROP ST subject groups, independently (Table 5). The PROP NT group rated the intensity of taste solutions from moderate to strong (range:17.57-42.24). The FPD class did not significantly affect the mean taste intensity ratings, and although the mean values from H-FPD tended to be higher than those from L-FPD group, this difference was only marginally significant for pungency (p=0.06).

Mean intensity ratings did not significantly vary with gender and age, with the exception of pungency. Females rated pungency significantly higher than did males (p<0.001), and mean intensity ratings from subjects belonging to the C2 age class (31-45 years) were significantly higher than for the rest of population (p=0.05). In PROP NT, significant FPD*Gender interactions for bitterness (p=0.05) and saltiness (p=0.01) were found. Here, decreasing intensity was observed from H- to L-FPD in males, while no differences were observed in females belonging to different FPD classes. Furthermore, a significant FPD*Age interaction was found for bitterness (p<0.001), with a positive effect of FPD variation on intensity in C2 and C3 classes while a negative effect was observed in the C1 age class.

PROP ST rated the intensities of taste solutions from moderate to very strong (range: 19.16 - 57.90). However, the FPD class did not significantly affect the mean taste solution intensities, although mean values from H-FPD tend to be lower than those from L-FPD. Mean intensity ratings did not significantly vary with gender, with the exception of astringency, that females rated significantly lower than did males (p=0.00). Age class did not influence intensity ratings in PROP ST. Interactions for FPD*Gender and FPD*Age were never significant in PROP ST.

4. Food stimuli

4.1 Effects of FPD class on perceived intensity of target sensations in PROP NT and PROP ST groups

Subject groups considered for this analysis are showed in Fig 2: PROP NT subjects belong to groups I (L FPD) and II (H FPD); PROP ST subjects belong to groups III (L FPD) and IV (H FPD). A 3-way ANOVA mixed model with repeated measures on intensity of target sensations in food stimuli was computed to test the effect of FPD (low-L and high-H density) in both PROP NT and PROP ST groups (Table 6, Figures 3 and 4).

In PROP NT, the intensity of target sensations significantly increases with tastant concentration from weak to strong in all the stimuli series (p≤0.0001). FPD significantly affected the intensity of target sensations only in pear juice (p=0.047), and no FPD*Concentration interactions were significant. Mean values from H-FPD tended to be higher than those from L-FPD group but this difference reached significance as a function of food and tastant concentration level only in a few cases (Figure 3 A-D). LSD post-hoc tests indicated that H-FPD group scored sourness in pear juice and saltiness in bean purée higher than L-FPD group in the sample added with the highest tastant concentration (Conc4). Pungency in sample Conc3 of the tomato juice was rated higher by H-FPD than L-FPD group.

PROP ST also showed significant increases in target sensation intensity from weak to strong as a function of the tastant concentration (p≤0.0001). FPD significantly affected only the intensity of saltiness in bean purée (p=0.010), and the FPD*Concentration interaction was significant in bean purée only (p=0.010). Mean values from H FPD tended to be lower than those from L FPD group, but this difference reached significance level only in a few cases (Figure 4 A-D). LSD post-hoc tests indicated that H-FPD group rated saltiness in bean purée and pungency in tomato juice lower than did the L-FPD group in the Conc4 sample.

4.2 Effects of PROP status on perceived intensity of target sensations in L-FPD and H-FPD groups

Subject groups considered for this analysis are showed in Fig 2: L-FDP subjects belong to groups I (NT) and III (ST); H-FPD subjects belong to groups II (NT) and IV (ST). The effect of PROP status (NT and ST) on the intensity of target sensations in foods was assessed in L-FPD and H-FPD groups (Table 7). Both in L-FPD and H-FPD groups, the intensity of target sensations significantly increased with tastant concentration from weak to strong in all stimuli series (p≤0.0001). In L-FPD group, the intensity of target sensations was significantly affected by PROP status (p≤0.022), and the FPD*Concentration interactions were always significant. In the L-FPD group, mean intensity values of PROP ST were higher than those of PROP NT group, with this difference reaching significance at different tastant concentrations, depending on the food (Figure 5 A-D). PROP ST rated sourness in pear juice and pungency in tomato juice as higher than did PROP NT in all samples with tastant added (Conc2-Conc4), and rated saltiness higher than PROP NT in bean purée samples Conc3 and Conc4, and sweetness in chocolate pudding sample Conc4. PROP status did not affect the intensity of target sensations in H-FPD group, and the PROP*Concentration interactions were never significant.

DISCUSSION

A great deal of research has been devoted to studying associations between PROP taste status, FPD and responses to oral stimulation, but these relationships remain controversial. Conclusions based on large scale studies tend to agree on the lack of simple causal relationships among these variables and instead highlight a complex interplay among factors regulating oral responsiveness (Garneau et al. 2014; Fischer et al. 2013; Monteleone et al. 2017). Demographics, genetics and other environmental factors may influence phenotypic responses to oral stimulation, including PROP, and FP density thus acting as possible confounders (Tepper et al. 2017; Piochi et al. 2018).

In the present study, aging was found to significantly lower both phenotype indices, with a stronger effect on FPD than on responsiveness to PROP. In adults, age is negatively correlated with FPD (Segovia et al. 2002; Correa et al. 2013; Shen et al. 2016). Aging has been associated with lowered responsiveness to PROP and it has been suggested that phenotypic expression of TAS2R38 gene varies with age (Mennella et al. 2010). Furthermore, changes in distribution of PROP taster groups has been observed with an increased percentage of PROP NT in older populations (age > 50 years) (Tepper et al., 2017).

In the present work, a significant gender effect was also found, with females rating PROP bitterness, and showing FPD mean values, significantly higher than males. This gender effect was stronger on PROP phenotype than on FPD value. Females are reported to be more sensitive to PROP than males, and more likely to be tasters (Bartoshuk et al. 1994; Zhao et al, 2007). Furthermore, results from the same population analysed in the present study, but on a slightly larger sample, confirmed significant changes in distribution of PROP taster groups depending on gender and age (Monteleone et al. 2017). Our results also confirm data on the higher number of FPD in females than in males (Bartoshuk et al. 1994; Tepper and Nurse, 1997; Duffy et al. 2004b; Hayes et al. 2008; Fischer et al. 2013; Pavlidis et al. 2013). Here, differences in FPD across gender were dependent from age class, and significant differences were found only in C2 class (31-45 years). Furthermore, a regular decreasing of FPD was observed with age, an effect more pronounced in males than in females thus confirming males more susceptible to FPD lowering with age (Pavlidis et al. 2013). The data from the present study thus show the interplay of gender and age in determining interindividual variations in phenotype markers of oral responsiveness.

Many studies examining oral responsiveness have used samples unbalanced for age and gender, and this is likely to at least partially account for inconsistencies in the effect of these factors on FPD and PROP responsiveness. Furthermore, the impact of age and gender on interindividual variation in phenotype markers of oral responsiveness might also partially account for uncertainties regarding the relationship between PROP responsiveness and FPD. Young females tend to show higher responsiveness to PROP and higher FPD than older males. In unbalanced study populations, significant relationships between these two factors can be observed that may be due to gender and age characteristics of the considered subject group, inappropriately generalized to a population. Previous large scale studies on more than one thousand 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).

The assumption of direct association of FPD with perceived intensity relies on the logic of spatial summation, namey that, as the area of taste stimulation is increased (and hence the number of papillae and buds), the taste intensity increases (Delwiche et al. 2001). Recent evidence on significant associations between parameters describing electrophysiological records from the tongue after local stimulation with PROP solutions and both perceived bitterness intensity and FPD confirm the spatial summation assumption (Sollai et al. 2017). On the other hand, the lack of close relationships between taste bud and FP densities and the influence of several environmental factors on FP response to oral stimuli weaken the direct association FPD/perceived intensity (see Piochi et al. 2018, for a review). Coupling the quantitative measures of peripheral taste function and the intensity responses from sensory evaluations would certainly help a deeper understanding of the mechanisms underlying the perception of food stimuli and the relevant interindividual variations.

Complex, and still controversial, associations have been reported between both PROP phenotype and TAS2R38 polymorphism with polymorphism of rs2274333 gene (A/G) that controls the functionality of gustin, the salivary trophic factor. Gustin plays a crucial role in taste function and has been proposed to promote growth and development of taste buds (Henkin et al. 1999). Gustin genotypes were associated with both fungiform papillae density and morphology (Melis et al. 2013). However, other studies have failed to find such associations (Bering et al. 2014, Feeney and Hayes, 2014, Barbarossa et al. 2015, Yang, 2015, Shen et al. 2016, Shen et al. 2017). Furthermore, the strength of positive relationships between the intensity of PROP and basic tastes differed by TAS2R38 haplotype with stronger association found in PAV/PAV than in the other diplotypes (Fischer et al. 2015). Thus, it is possible that interindividual variation in TAS2R38 genotype and responsiveness to PROP might partially account for decoupling taste intensity and FPD.

In the present study, the importance of FPD in taste sensing was explored in PROP NT and PROP ST groups, independently. The results indicate that FPD variation has only a slight impact on orosensory perception. In the PROP NT, FPD did not affect the intensity of taste solutions, and a significant positive effect was only found for sourness in pear juice. The lack of a significant effect of FPD on intensity in taste solutions was also confirmed in PROP ST. In this group, the only significant effect of FPD variation was found in bean purée where L-FPD subjects perceived saltiness intensity higher than H-FPD group. Thus, if we assume that these findings are not false positives, it appears that the contribution of FPD to intensity depends on the stimulus considered, the target sensation intensity and PROP status. Some researchers found PROP NT status associated with the recessive and less functional form of the gustin (GG) and AA genotype more frequently carried by PROP Tasters (Padiglia et al. 2010, Calò et al. 2011, Melis et al. 2013). It may be that PROP insensitive individuals that carry AVI haplotype cannot take advantage from the reinforced perception capacity of FP associated with the PAV haplotype (such as for example gustin active form). In this case FP responsiveness might basically depend from their number and the increased FPD also correspond to a heightened intensity perception.

The negative impact of FPD on intensity perception in PROP ST was unexpected, even if other reports documented such negative correlations for saltiness in populations not segmented by PROP status (Fischer et al. 2013). The interaction of FPD/PROP status on perception of oral stimuli was further explored considering subject groups belonging to the same FPD class (H and L FDP) but varying for PROP status. PROP status strongly affected the intensity of food stimuli in L-FPD subject group, with PROP ST rating the intensity of target sensations higher than did PROP NT. These results indirectly confirm the general positive effect on chemosensory abilities contributed by PAV haplotype and associated effects. On the other hand, being a PROP ST did not produce equivalent effects in subject groups with H-FPD. In this case, the high number of FP possibly compensates for the perceptive system capacity less in AVI than in the PAV carrier group. Tentatively, it can be speculated that the PROP ST status of H-FPD individuals results from the combination of the high papillae number and the presence of the PAV haplotype, possibly in heterozygous form, and thus with a partial expression of perceptive advantages associated with PROP sensitivity. This hypothesis can also explain the differences observed between L and H FPD in PROP ST. L-FPD/PROP ST subjects can represent the "real" supertaster characterized by a generalized hypergeusia possibly induced by the association of gene polymorphisms (i.e. PAV/PAV and G/G) and perceptive system features advantageous for orosensation. The ongoing gene analysis on this population will help to gain further insight on the factors underlying the observed results.

In conclusion, the results of the present study depict a complex interplay of several factors affecting phenotype markers of orosensory acuity, their relationships and their impact on the intensity of target sensations. The fact that demographic factors influence FPD and PROP responsiveness lead to strong recommendations for the strict control of population sample characteristics when using these phenotypes as markers of food perception and preference, and once more highlight the risk of generalizing results from small convenience samples. As well, care should be taken in stimulus selection since intensity responses as a function of PROP/FPD appear to be significantly influenced by the context (model or real food) and by the tastant concentration. However, PROP responsiveness appears to be confirmed as a reliable marker of heightened response to oral stimuli broadly, and the concept of hypergeusia to describe a generalized heightened response across oral stimuli. The mechanistic explanation for why PROP responsiveness positively affects the response to stimuli that are not mediated by the TAS2R38 receptor deserves further research efforts. As already concluded by other authors (Hayes et al 2008), additional insight should be gained on associations between gene polymorphism impacting on perceptive system functioning, and the role of peripheral sensing organs reconsidered.

Author contributions

CD undertook the analyses and wrote the manuscript; CD and EM contributed to plan the analyses; CD, EM, JP, MP, SS, LP discussed the interpretation of the results; EM, AB, CD, FG, LT, ML, EP, SP, SS, collaborated in the design of the project Italian Taste; all authors helped with data collection, reviewed and offered critical comments on the manuscript.

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Conflict of interest

Dr Prescott is director of TasteMatters Research & Consulting.

The authors declare to have no conflict of interest.

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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

**cigarette/day median value*

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

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 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 p values

			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

Table 6. 3-way ANOVA mixed model with repeated measures: Effects of FPD class and tastant concentration on responsiveness to target sensations of food stimuli in PROP NT and PROP ST groups.

	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**

Table 7. 3-way ANOVA mixed model with repeated measures: Effects of PROP status and tastant concentration on responsiveness to target sensations of food stimuli in L-FDP and H-FPD groups.

	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

Figure and Figure Legend

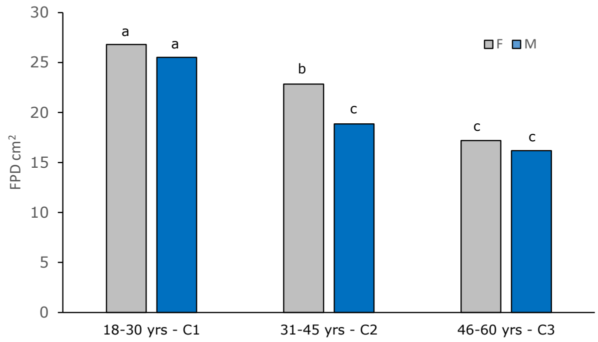

Figure 1. 2-Way ANOVA: gender (F-females; M-males) and age effect on FPD values. Different letters indicate significantly different values ($p \leq 0.005$).

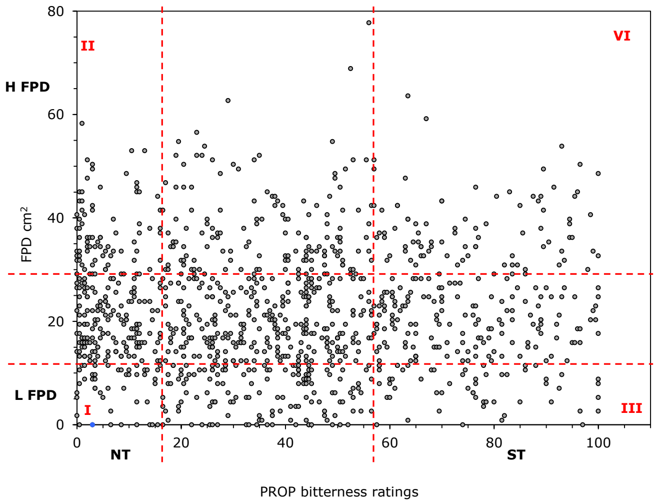

Figure 2. Individual variation in PROP bitterness ratings and FPD values. Dotted lines represent limits of PROP Status groups on x axe (cut-off: NT<17; ST>53) and FPD groups on y axe (cut off: LFPD 25th percentile; HFPD 75th percentile).

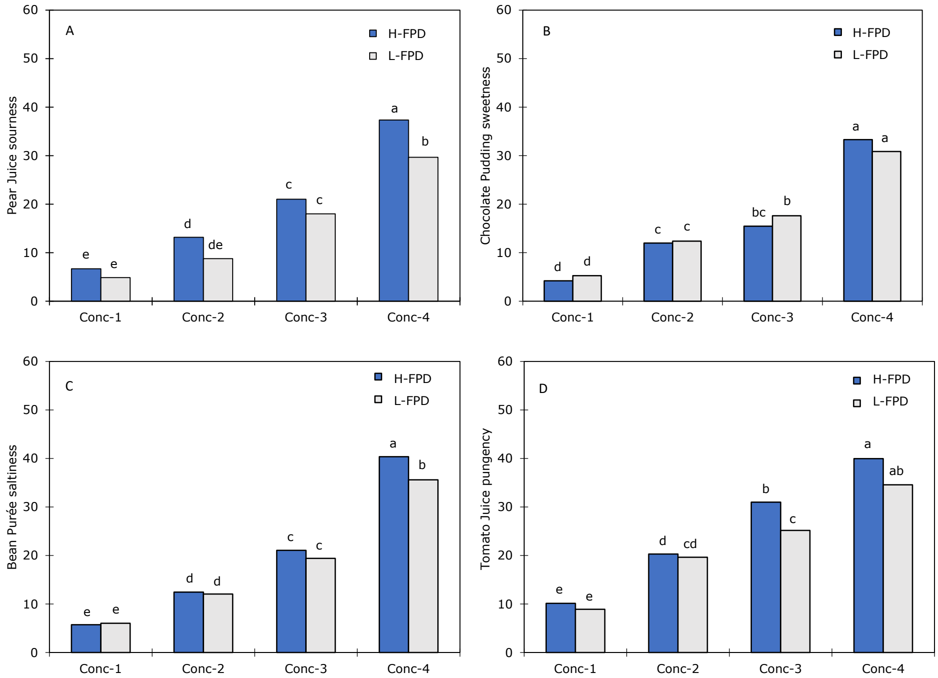

Figure 3. PROP NT subject group: Effect of FPD variation (high H-FPD and low L-FPD) and tastant concentration (conc-1 – conc-4) on perceived intensity of target sensation in food stimuli (A:pear juice; B: chocolate pudding; C:bean purée; D: tomato juice).

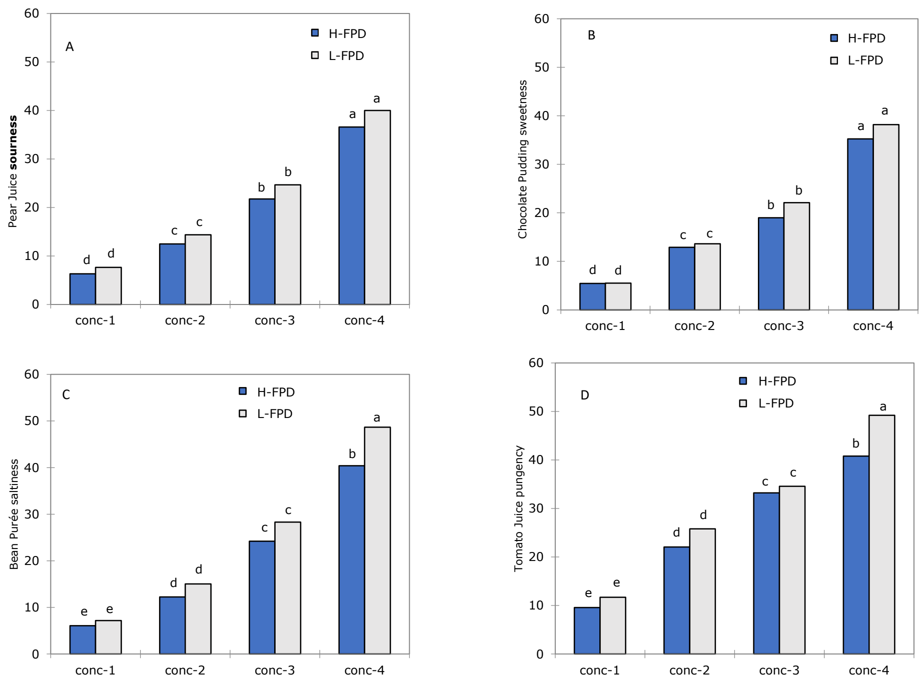

Figure 4. PROP ST subject group: Effect of FPD variation (high H-FPD and low L-FPD) and tastant concentration (conc-1 - conc-4) on perceived intensity of target sensation in food stimuli (A:pear juice; B: chocolate pudding; C:bean purée; D: tomato juice).

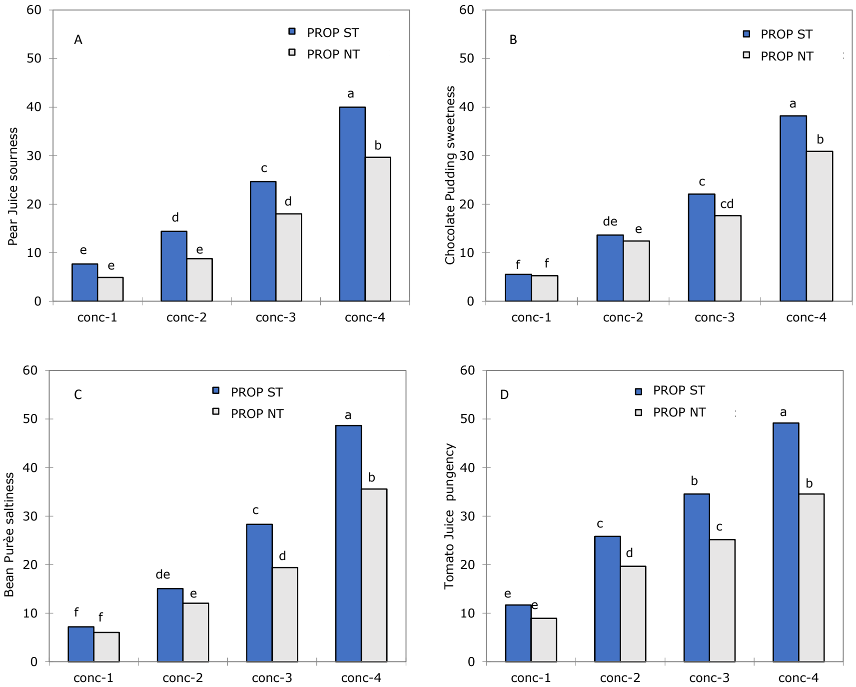

Figure 5. L-FPD subject group: Effect of PROP responsiveness variation (PROP NT and PROP ST) and tastant concentration (conc-1 – conc-4) on perceived intensity of target sensation in food stimuli (A:pear juice; B: chocolate pudding; C:bean purée; D: tomato juice).