UNIVERSITÀ
DEGLI STUDI
FIRENZE

Università degli Studi di Firenze

DOTTORATO IN GESTIONE SOSTENIBILE DELLE RISORSE AGRARIE, FORESTALI E ALIMENTARI

CICLO XXX

Coordinatore Prof.ssa Susanna Nocentini

Maria Piochi

Matricola DT16144

Indices of individual variation in taste responsiveness

Settore Scientifico Disciplinare AGR/15

Coordinatore:
Prof.ssa Susanna Nocentini

Tutor:
Prof. Erminio Monteleone

Dottoranda:
Dr.ssa Maria Piochi

Co-Tutor:
Dr.ssa Caterina Dinnella

Anni 2014/2017

Index

Acknowledgments

This study was carried out during 2014-2017 at the Department of Agricultural, Food and Forestry System Management, University of Florence, Italy and at the University of Gastronomic Sciences, Pollenzo, Italy. The PhD scholarship was economically supported by the University of Gastronomic Sciences (Pollenzo (CN), Italy). Therefore, I express my gratitude to the whole University of Gastronomic Sciences for having supported the project. In particular, my thanks go to Professor Piercarlo Grimaldi and Professor Andrea Pieroni for having recognised the importance of the research. I am honoured to have had the chance to work in such an inspiring, creative, and multidisciplinary environment as the University of Pollenzo.

I want to sincerely thank my supervisor Prof. Erminio Monteleone for his guidance, his support, the opportunities and for the inspiration he gave me during these years. He drove me to approach the research with creativity and curiosity.

Warm thanks go to my great co-supervisor Dr. Caterina Dinnella for her guidance and her great knowledge. Beyond that, I thank her for the time she spent in revising papers and for the effort in teaching me the meaning of "synthesis".

Special thanks go to Dr. Luisa Torri for her great professional support, the knowledge she has transferred to me, for her human support and for all the beautiful experiences we have shared during these years.

I am also very grateful to Prof. John Prescott (TasteMatters Research & Consulting, Sydney, Australia), visiting professor at the Department of Agricultural, Food and Forestry System Management, University of Florence, for providing me additional constructive criticism that helped to put my work into a larger perspective.

Special thanks go to Dr. Valérie Lengard Almli and Dr. Jens Petter Wold (Nofima, Ås, Norway) for the great collaborations that we have had.

Sincere thanks go to Dr. Agnes Giberau of the Institut Paul Bocuse (Lyon, France) for having welcomed me into her team and supervised me during my stay abroad.

I thank past and present colleagues for the shared experiences: Camilla, Danny, Isabella, Renzo, Lapo, Alessandra (University of Florence) and Cristina (University of Gastronomic Sciences in Pollenzo).

Finally, I want to thank my family for the unique support they gave me during these years.

Abstract

A complexity of factors influences food choices and behaviours.

Among these factors, the individual variability in oral responsiveness (the diversity among individuals in perceived intensity of oral sensations) plays an important role.

The great difference among individuals in responsiveness to tastes and somatosensory sensations is partly due to physiological variations in chemoreceptor systems.

In the present thesis, we refer to oral responsiveness, intended as the perceived intensity of the fundamental tastes and burning from capsaicin.

Taste responsiveness is associated to food preferences and diet. Since, via reduced/increased sensibility, taste responsiveness modulates our response to food preferences and ultimately diet, the understanding of individual variability can determinately contribute to explaining food behaviours.

Indices exist to estimate taste responsiveness. Among these indices, the Fungiform Papillae Density (FPD = papillae/cm^2) and the responsiveness to 6-n-propylthiouracil (PROP) are among the most studied. The Fungiform Papillae (FP) are anatomical structures designated to the oral stimuli detection and transduction, due to their innervation with the chorda thympani nerve (taste) and trigeminal nerve (somatosensory). PROP status (being or not being responsive to bitterness of PROP, and to which degree; henceforth abbreviated PST) is correlated to responsiveness to a high number of compounds naturally found in foods responsible for a variety of taste sensations. Therefore, PST is considered a general marker for taste responsiveness.

Uncertainty has recently emerged from literature on whether or not FPD can be considered a reliable indicator for taste responsiveness. While early studies found that subjects with higher number of FP had higher tactile acuity and increased responsiveness to tastes, recent large-scale studies failed to confirm the positive relationship concerning tastes.

The disagreement on the relationship between FP and taste responsiveness may originate from 1. Individual variability in papillae functionality, 2. Characteristics of the population considered, and 3. Methodological issues related to the approaches used to estimate FPD and sensory response. A particularly critical source of variability is the type of stimuli used. So far, the role of FP in taste responsiveness has not been systematically studied both in standard solutions and complex food matrices.

Instead, responsiveness to PROP seems a solid indicator of taste responsiveness. Also, the

relationship between these two common indices is still very controversial. Finally, while for PROP status determination several approaches have been optimised, in the FP determination some critical methodological aspects must be still approached.

Based on these critical issues, the aim of the present thesis was to study the role of lingual fungiform papillae in respect to tastes and PROP responsiveness. The specific objectives were:

I. to explore the relationship between FPD and perceived intensity of tastes and burning from capsaicin, evaluated in water solutions and in complex food matrices;

II. to investigate the relationship between FPD and PROP responsiveness;

III. to improve methodological tools to measure the fungiform papillae on the tongue.

In the present thesis 408 subjects (38% males, aged from 18 to 65 years) were involved, recruited in two Italian cities. The effects of FPD and PST were separately tested on the perceived intensity of tastes and burning from capsaicin, both in water solutions and in complex food matrices (Study I). The nature of the relationship between FPD and PROP was explored considering PROP responsiveness estimated by whole mouth stimulation (One-solution test) (Study II). A manual count of papillae may obscure the relationship between FPD and PROP, so to further eradicate any bias a new approach for the automated quantification of FP on the human tongue was proposed (Piochi et al., 2017) (study III).

Key conclusions of the thesis are that 1. FPD variation does not affect taste responsiveness in water solutions and food stimuli; 2. PROP phenotype is confirmed to be a reliable predictor of taste responsiveness, with super-tasters individual perceiving heightened intensity both in solution and food matrices; 3. FPD and PROP phenotypes do not show a straight significant association. Several factors may account for this. These factors mainly related to the FP functionality: the simple detection of the number of FP is not informative of FP functionality (such as the taste pore density - relevant for both taste sensations and PROP – or the presence of damaged nerves), and to the combination of genetic factors (some of which are still unknown), which may additionally complicate this relationship. 4. Advancement in FPD estimation is proposed by developing a new automated FP counting methodology that overcomes noise related to manual count. The method opens interesting scenarios in studying how the variation in fungiform papillae shape and dimension (diameter size) effect their functionality.

Key words:

Taste responsiveness, fungiform papillae, 6-n-Propylthiouracil, PROP, individual variation

List of original publications

The present PhD project contributed to the following original scientific publications (published in international journals available on Scopus and WoS database):

- **Piochi**, M., Monteleone, E., Torri, L., Masi, C., AlmliLangard, V., Wold, J.P., &Dinnella, C. (2017). Comparing manual counting to automated image analysis for the assessment of fungiform papillae density on human tongue. Chemical Senses, 42, 553-561, doi:10.1093/chemse/bjx035

- Monteleone, E., Spinelli, S., Dinnella, C., …. E., **Piochi**, et al. (2017). Exploring influences on food choice in a large population sample: the Italian Taste Project. Food Quality and Preference, 59, 123-140

- **Piochi**, M., Dinnella, C., Prescott, J., Monteleone, E. Associations between human fungiform papillae and responsiveness to oral stimuli: effects of individual variability, population characteristics, and methods for papillae quantification. Chemical Senses, submitted

- C. Dinnella, E. Monteleone, J. Prescott, M. **Piochi**, S. Spinelli, M. Laureati, E. Pagliarini, L. Torri, F. Gasperi, P. Gasparini. Individual variation in PROP status, fungiform papillae density and responsiveness to taste stimuli in a large population sample. In preparation

1. Introduction

1.1 The importance of the taste responsiveness in food preference

Individuals differ greatly in responsiveness to sensory stimuli due in part to physiological variations in chemoreceptor systems. In fact, people do not show the same responsiveness to tastes (Doty, Bagla, Morgenson, & Mirza, 2001; Keast & Roper, 2007; Miller & Reedy, 1990) and somatosensory sensations (Hayes & Duffy, 2007; Prescott & Swain-Campbell, 2000).

Oral responsiveness is defined as the perceived intensity of oral sensations, including tastes and somatosensory sensations and flavour. In the present thesis, we referred to oral responsiveness as the perceived intensity of fundamental tastes, including spiciness from capsaicin.

Since taste responsiveness can modulate our food preference, via reduced/increased sensibility (Hayes, Sullivan, & Duffy, 2010), the understanding of individual variability is important in order to explain food behaviours.

Taste responsiveness is associated with food preferences and diet (Cox, Hendrie, & Carty, 2016; Fogel & Blissett, 2017; Monteleone et al., 2017; Stevenson et al., 2016; Törnwall et al., 2014). For example, more "adventurous" people (those who like more spicy and sour foods) were shown to be more tolerant to capsaicin burn (Törnwall et al., 2014) and this attitude implicated a higher preference for fruit and vegetables (Törnwall et al., 2014).

Different sensitivity to bitter taste can represent a barrier to the consumption of certain foods or bring rejection to bitter foods (Drewnowski & Gomez-Carneros, 2000; Monteleone et al., 2017). A recent study on Western-diet highlighted that responsiveness to PROP bitterness highly positively correlated with dietary intakes of saturated fat and added sugar (Stevenson et al., 2016), thus indicating that adult responsive to PROP tended to feature more highly in an unhealthier diet.

Responsiveness to bitterness and consumption of fruit and vegetables has been particularly studied in children. Despite some inconsistency (Feeney, Brien, Scannell, Markey, & Gibney, 2014), intake of vegetables is higher in PROP non-tasters children (Bell & Tepper, 2006). Moreover, the number of fungiform papillae was correlated with vegetable intake among students (Duffy et al., 2010).

The number of fungiform papillae on the tongue, in the presence of supportive home food environment, may be beneficial for fruit and vegetables intake in children with a high number of papillae but not in children with a low number of papillae (Fogel & Blissett, 2017). The number of fungiform papillae was also found to be inversely associated to alcohol intake in adults (Fischer et al., 2013).

A recent review on sensitivity, hedonics and preference for tastes and fat, concluded that no clear evidence suggested a negative relationship between fat, sweet, salty and bitter taste sensitivities and weight status, but that (due to the complexity of the topic) further investigations are required particularly on the role of taste responsiveness (Cox et al., 2016).

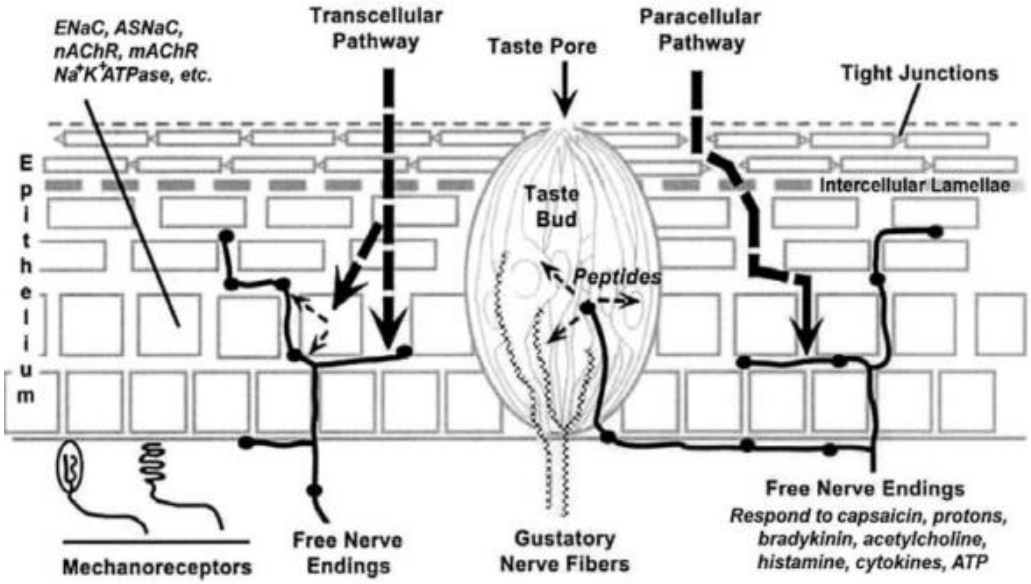
Markers for the taste responsiveness exist. The most studied are the fungiform papillae density (FPD= papillae/cm^2) and the responsiveness to 6-n-propylthiouracil (PROP).

1.2 Fungiform papillae

1.2.1 Fungiform Papillae Density (FPD) as taste responsiveness index

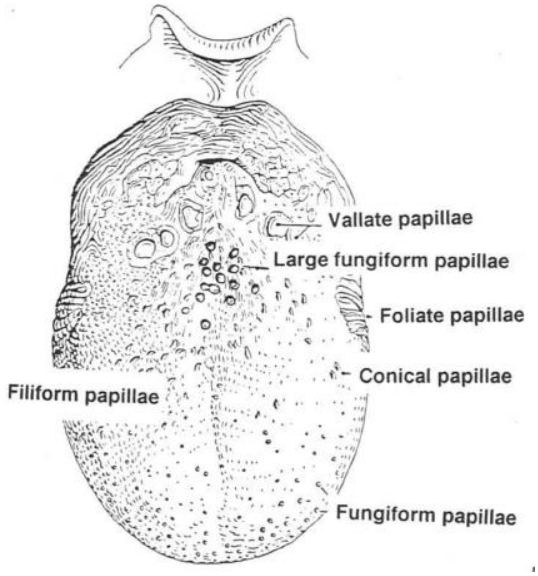
The number of FP on the tongue is considered an index for taste responsiveness. The fungiform papillae (FP) are anatomical structures involved in the detection and transduction of oral stimuli. FP carry taste buds, the peripheral structures for taste sensing on the mammalian tongue (Feng, Huang, & Wang, 2014). Taste buds cells transmit signals to gustatory nerves (chorda tympani nerve - cn. VII)(Farbman & Mbiene, 1991), which innervate the base of taste cells (Figure 1). The epithelium of FP is also innervated by different trigeminal fiber types (mechanoreceptors and free nerve endings) (Fig. 1). These innervations by trigeminal nerve (cn. V) transduce somatosensory and irritant sensations (Prescott & Tepper, 2004; Whitehead, Beeman, & Kinsella, 1985).

Fig. 1 Schematic of the innervation of taste buds and surrounding epithelium (adapted from (Prescott & Tepper, 2004)).



FP have been selected to derive markers of taste sensitivity over papillae foliate and circumvallate, because of their relative abundance, their location on the anterior part of the tongue and their association with taste buds' density (Miller & Reedy, 1990; Miller & Reedy, 1990). FP are distributed all over the anterior two-thirds of the tongue according to a stereotyped pattern (Jung, Akita, & Kim, 2004), with the highest density found on the tongue tip close to the midline (Sollars & Bernstein, 2000; Tepper & Nurse, 1997)(Figure 2).

Fig. 2 Human tongue showing different regions of papillae (adapted from (Miller & Bartoshuk, 1991).

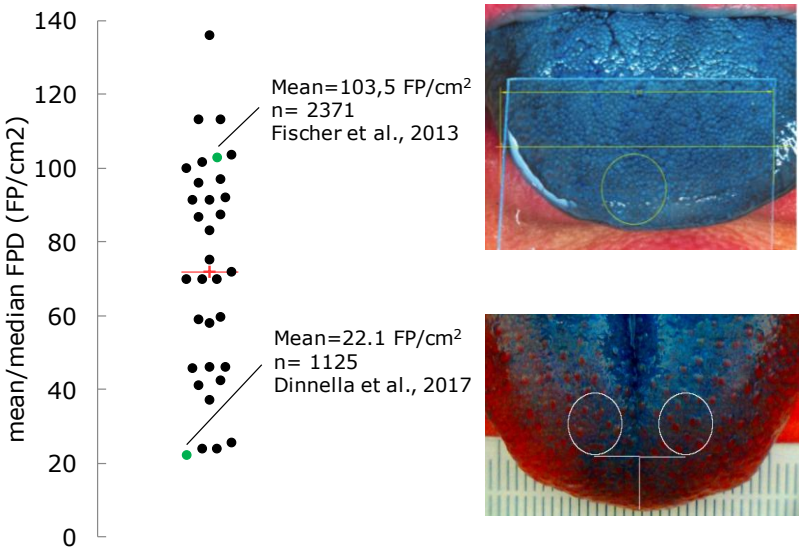


For the detection of fundamental tastes, tastants are bonded to different receptors in taste receptor cells (TRCs) (Lindemann, 2001), and the bond initiates signaling pathways leading to taste perception (Ishimaru & Matsunami, 2009). The transduction cascades activate synapses and cause the excitation of the nerve fibres which carry the signal to the brain stem, where central taste processing begins, ultimately eliciting adaptive responses (Lindemann, 2001). Since TRC are renewed at a constant rate – approximately every 8-12 days (Feng et al., 2014) – and nerve fibres must reconnect them, the maintenance of gustatory innervation and the functioning of the regulation of this process (Meng, Ohman-gault, Ma, & Krimm, 2015) may have an important role in oral responsiveness. Moreover, mechanical and electrical stimulation of individual FP has been shown to produce taste sensations (Cardello, 1981).

Taste intensity is proportional to the number of stimulated FP (Delwiche, Buletic, & Breslin, 2001; Smith, 1971). The number of FP is generally converted into fungiform papillae density (fungiform papilla/cm^2 =FPD). A great variability in FPD was found across different studies was observed. The FPD varies from 0 to over 200 papilla/cm^2 across studies (Fischer et al., 2013; Zhang et al., 2009).

As an example, the mean/median values obtained from 33 studies ranged from 22.0 FP/cm^2 to 136.0 FP/cm^2 are shown in Figure 3.

Fig. 3 Distribution of mean/median values of FPD found in 33 studies. In green are shown two studies with a high number of observations (> 1000) and their relative area of count. Mean (cross) and median (line) (adapted from Piochi et. al, submitted).



1.2.2 Factors affecting the fungiform papillae index

The composition of the population in terms of gender and age affects the FPD.

For age, FP are formed early in gestation (Witt & Reutter, 1997) and their number evolves during the first few years of life. The number of papillae ceases to increase from around 9–10 years of age, and the distribution and growth of papillae become stabilised at around 11–12 years (Correa, Hutchinson, Laing, & Jinks, 2013). Most studies report that age is negatively correlated with FPD in adults, considering different age-spans (years): 21-84 years (Fischer et al., 2013), 10-80 years (Pavlos Pavlidis, Gouveris, Anogeianaki, Koutsonikolas, & Koblenz, 2013), 18-55 years (Shen, Kennedy, & Methven, 2016), and children (7-12 years) vs adults (20-24 years) (Correa et al., 2013).

For gender, several studies reported a significantly higher number of FP in women compared to men (Bartoshuk, Duffy, & Miller, 1994; Duffy, Peterson, & Bartoshuk, 2004; Fischer et al., 2013; Hayes, Bartoshuk, Kidd, & Duffy, 2008; Pavlidis et al., 2013; Tepper & Nurse, 1997). One large study (2371 subjects; 49% males) confirmed higher FPD in females (108.4 papillae/cm^2) than to males (97.9 papillae/cm^2), even after adjusting for the effects of age (Fischer et al., 2013). Women also showed a greater variability in taste buds number compared to men (Prutkin et al., 2000). Other studies

failed to find a significant effect of gender on FPD (Bajec & Pickering, 2008; Bakke & Vickers, 2008; Bakke, Vickers, Marquart, & Sjoberg, 2007; Correa et al., 2013; Feeney & Hayes, 2014a; Just, Pau, Witt, & Hummel, 2006; Masi, Dinnella, Monteleone, & Prescott, 2015; Nachtsheim & Schlich, 2014; Yackinous & Guinard, 2002; Yeomans, Tepper, Rietzschel, & Prescott, 2007). However, some of these studies had unbalanced male/female ratios (males≤ 30%) (Bakke & Vickers, 2008, 2011; Nachtsheim & Schlich, 2014) or had a relatively low number of subjects (n≤ 60) (Just et al., 2006; Yeomans et al., 2007).

Papillae functionality is intended as the effective functioning of a single FP. The concept of FP functionality includes the individual variability existing in healthy papillae and the degree of deviation from normal functioning of papillae due to a pathological situation.

The functionality of healthy FP relates to the number of taste pores. Taste pores density (number of taste pores/cm^2 =TPD), determined by the number of papillae in a tongue region and by the number of taste pores per papillae (Miller & Reedy, 1990), greatly varies across individuals up to 14-fold among subjects from 36 to 511 pores/cm^2(Miller, 1986; Miller & Reedy, 1990). Fungiform papillae contain from 0 to 22 taste pores, with an average of 3.75±1.4 (s.d., n=10) (Miller & Reedy, 1990). Estimates of the actual percentage of FP that contain pores also vary considerably across individuals, ranging from 8% (Reedy et al., 1993) to 68% (Cheng & Robinson, 1991). Not all FP are responsive to tastes. One study found that 42% of single stimulated FP were unresponsive to any taste stimuli (sucrose, quinine sulfate, NaCl, HCl) (Cardello, 1978), probably due to the absence of taste pores in these FP.

The deviation from normal functioning of papillae is due to a pathological situation. Pathologies of various kinds can impact on FP morphology and functioning. Negoro and colleagues proposed a classification of FP based on the shape and on the nature of the associated blood vessels (Negoro, Umemoto, Fukazawa, Terada, & Sakagami, 2004). Normal tasters (subjects who correctly recognised fundamental taste quality of solutions) showed round-shaped papillae and clear blood vessels, while subjects with taste disorders (reduced ability to recognised taste due to different causes – idiopathic, surgery or drug assumption) showed atrophic, irregular, and tapering fungiform papillae (Negoro et al., 2004). A flattening of FP was observed in many patients with decreased secretory function in both salivary glands (Tanaka, 2009). Patients with salivary gland dysfunction also had abnormal morphology of the papillae of the tongue (Tanaka, 2009). FPD is reduced in patients with diabetes mellitus (Pavlidis, Gouveris, Kekes, & Maurer, 2014). Reduced number of FP was reported in young

adolescent females with eating disorders (Wockel, Jacob, Holtmann, Poustka, & Wo, 2008). Otitis media, tonsillectomy, and head and neck radiation treatment can all damage nerves associated with oral stimulus detection (see (Bartoshuk, Catalanotto, Hoffman, Logan, & Snyder, 2012), thus altering taste sensation without affecting tongue anatomy. Nerve damages may strong obscure potential relationship between FPS and oral responsiveness (Snyder et al., 2014).

The variability in techniques used to quantify FP and the techniques adopted to estimate the sensory response, may contribute to obscuring the nature of the relationship between FDP and taste responsiveness across studies. The differences in techniques for FP quantification include the equipment, the area and location of the tongue to count, and the procedures to validate the count. Types of equipment used to detect FP may be divided into: filming technique (video-microscope contact endoscopy, confocal laser scanning microscopy), digital photography (digital camera and digital microscope), and direct techniques (Table 1).

Tab. 1 Advantages and limitations of techniques used for fungiform papillae detection (adapted from Piochi et. al, submitted).

Technique	Advantages	Limitations	References
Video microscopy	high quality images taste pores observation & quantification check of data quality in real time	equipment cost not portable for field use high operator training for image acquisition time needed to obtain high quality images high respondent burden time-consuming counting	Miller & Reedy, 1988; Miller & Reedy, 1990a; Miller & Reedy, 1990b; Segovia et al., 2002
Contact endoscopy	high quality images observation of blood vessel networking	equipment cost not portable for field use high operator training for image acquisition time needed to obtain high quality images high respondent burden time-consuming counting	Negoro et al., 2004
Digital photography	high quality images relatively inexpensive equipment portable time-saving image acquisition low respondent burden	no taste pores observation or quantification *a posteriori* check of data quality time-consuming counting high operator training for FP identification	Shahbake et al., 2005
Digital microscopy	acceptable quality images inexpensive equipment portable time-saving image acquisition low respondent burden	no taste pores observation or quantification *a posteriori* check of data quality time-consuming counting high operator training for FP identification	Temple et al., 2002; Masi et al., 2015; Shen et al., 2016

Direct count with handheld cell counter	relatively inexpensive equipment portable time-saving immediate count	no taste pores observation or quantification no possibility *of a posteriori* data check operator training	Tepper and Nurse, 1997; Yackinous and Guinard, 2001, 2002
Direct count with tailor-made devices	relatively inexpensive equipment relatively time-saving portable immediate count	low quality of scanned images no magnification possibility no taste pores observation or quantification *a posteriori* check of data quality operator training for FP identification	Delwiche et al., 2001

Video-microscopy was the first non-invasive technique used to quantify FP in living humans. It is an excellent tool, due to the high quality of images and the possibility of quantifying taste pores, however, its use is limited to the research laboratory due to its high cost, the fact of not being portable, and of being quite demanding for subjects because of its long duration (approximately 60-120 minutes). Therefore, subjects are required to keep their tongue motionless (I. J. Miller & Reedy, 1988; Segovia, Hutchinson, Laing, & Jinks, 2002). Digital cameras were introduced as a reliable tool for video microscopy (Shahbake, Hutchinson, Laing, & Jinks, 2005). Due to their advantages, digital cameras have become widely used. Digital images can be easily modified using software (e.g. Adobe Photoshop, ImageJ, FotoFiltre) that enhances FP resolution and greatly helps in selecting areas for counting. In general, methods which provide middle-low resolution images are enough if the aim of the study is an arbitrary classification of subjects into FPD classes (i.e: low, medium, high FPD). Conversely, if reliable information concerning TPD or vascular innervation is required, the use of filming techniques is necessary.

Methods for FP counting are manual. FP are generally counted in restricted areas (from 0.09 to 1.0 cm^2) of the tongue, based on the significant correlations between FP numbers counted in small regions located in the first 3 cm of the anterior tongue and the total number of FP on the tongue. Explained variance of these models spans from 13 to 70 %, depending on the region considered (Shahbake et al., 2005). A circular area of 0.283 cm^2 has been most frequently used, irrespective of method. However, since FP are unevenly distributed all over the anterior two-thirds of the tongue (Jung et al., 2004), the choice of the exact localisation and the extension of count area for FP count can meaningfully impact the final FPD values.

In manual count, FP are identified and counted by trained operators. The commonly accepted criteria to identify FP on digital images derives from the work of Miller and Ready (Miller & Reedy, 1990). Accordingly, FP are identified as pink/uncoloured round, elevated spots on the blue background, with

a diameter of about 0.5 mm. However, several deviations from the expected shape and colour contrast have been reported (Kullaa-Mikkonen & Sorvari, 1985; Masi, Dinnella, Monteleone, & Prescott, 2015; Melis et al., 2013; Segovia, Hutchinson, Laing, & Jinks, 2002; Shahbake, Hutchinson, Laing, & Jinks, 2005). The Denver Papillae Protocol has been developed, which defines and prioritises the characteristics of FP according to 1. shape, 2. colour, 3. size and 4. elevation. The protocol uses a dichotomous key and suggests a maximum 10% difference between scores from two independent counters to consider the count and the derived consensus FPD value valid (Nuessle, Garneau, Sloan, & Santorico, 2015). Trained operators should be blind to other types of subject data such as PROP status, taste responsiveness, or genetic data (Delwiche et al., 2001; Feeney & Hayes, 2014b).

When using whole mouth stimulation, taste responsiveness reflects stimulation of taste buds in multiple regions of the tongue, soft palate, larynx and pharynx (Miller & Bartoshuk, 1991), while in regional stimulation the effect of tongue locus on taste stimuli perception strongly reflects the characteristics of the particular receptor population present in each locus (Collings, 1974). Since taste pore distributions vary highly across versus regional stimulation (Miller & Bartoshuk, 1991), whole mouth and regional stimulation may provide different responses when evaluating the relationship between FPD/ taste responsiveness. However, most studies congruently failed to find significant relationship between FPD-taste when comparing regional vs whole mouth stimulation, for: quinine bitterness (Feeney & Hayes, 2014b), NaCl saltiness (Duffy, Peterson, et al., 2004), citric acid sourness (Duffy, Peterson, et al., 2004), and umami from a mixture of monosodium glutamate and inosine monophosphate (Feeney & Hayes, 2014b).

It is uncertain whether the approaches used to collect the sensory responses (detection threshold, suprathreshold scaling) may influence the FPD index. The relationship between FPD and sweetness from sucrose was not significantly associated in supra-threshold scaling in males (Fischer et al., 2013) but positively associated when using thresholds (Proserpio, Laureati, Bertoli, Battezzati, & Pagliarini, 2016). Conversely, other studies found congruent results for bitterness of caffeine (Masi et al., 2015; Proserpio et al., 2016) or saltiness of NaCl (Doty et al., 2001; Hayes et al., 2010), despite having adopted different techniques (detection threshold vs suprathreshold).

When evaluating complex stimuli (real food matrices, modified foods), instead of simple standard solutions, the relationship between FPD and taste intensities tends to disappear. FP showed a positive

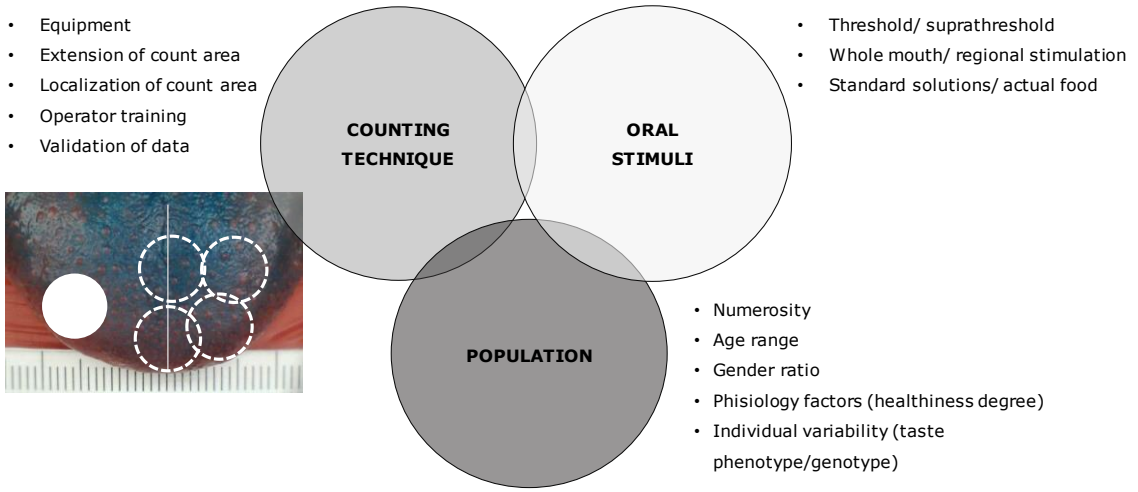
relationship with saltiness in aqueous solutions, while FPD did not explain intensity differences in saltiness in chicken broth (Hayes et al., 2010). Masi and colleagues found a positive relationship between FPD and caffeine bitterness in aqueous solutions, but not in bitterness in real coffee containing caffeine (Masi et al., 2015). However, studies exploring the effect of the type of stimulus (standard solutions vs complex food matrices) are too few to draw a general conclusion.

1.2.3 Relationships between Fungiform Papillae index and taste responsiveness

Disagreement related to the effects of FP on oral responsiveness has recently emerged from literature analysis. Some studies have demonstrated a positive relationship between FPD and taste sensitivity, finding that subjects with higher FPDs perceived greater intensity from supra-threshold taste stimuli (Bartoshuk, 2000; Delwiche et al., 2001; Miller & Reedy, 1990; Smith, 1971; Stein, Laing, & Hutchinson, 1994; Yackinous & Guinard, 2002). Similarly, positive correlations between FPD and trigeminally-mediated oral somatosensations were found, such as creaminess (Hayes & Duffy, 2007; Nachtsheim & Schlich, 2013; Proserpio et al., 2016), roughness (Bakke & Vickers, 2008), alcohol burn (Duffy, Davidson, et al., 2004; Duffy, Peterson, et al., 2004) and tongue spatial resolution acuity (Bangcuyo & Simons, 2017; Essick, Chopra, Guest, & McGlone, 2003). However, recent large-scale studies (observations > 1100) have failed to find a relationship between FPD and responsiveness to taste (Dinnella, et al., 2016; Fischer et al., 2013). This lack of relationship was also found for somatosensory sensations, such as for burning from capsaicin (Feeney & Hayes, 2014b) and astringency in coffee (Masi et al., 2015), strongly questioning the role of FPD as an effective marker for oral responsiveness. Therefore, despite these and other studies over the past few decades, the relationship between FPD and taste responsiveness remains unclear.

Disagreement observed in literature may arise from variations in from one or more of the following domains: the papillae functionality, the population characteristics, and methodological issues related to the approaches used to estimate FPD and the sensory response. Factors contributing to the variability in studies on FPD and taste responsiveness are graphically summarised in Figure 4.

Fig 4. Factors contributing to variability among studies on the relationship between fungiform papillae density and taste responsiveness (adapted from Piochi et. al, submitted).

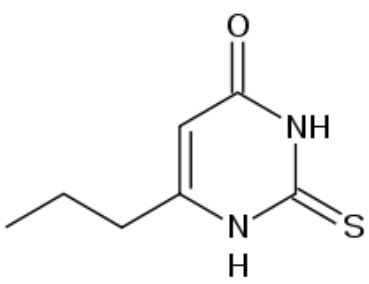


1.3 PROP responsiveness

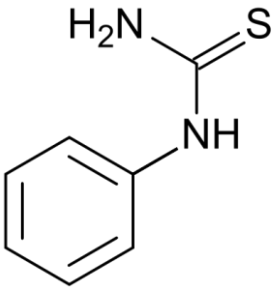
1.3.1 PROP status

Molecules of 6-n-Propylthiouracil (PROP) and phenylthiocarbamide (PTC) (Figure 5) are members of a class of bitter-tasting compounds known as thioureas, containing N-C=S, which is responsible for their bitter taste.

Fig. 5. Molecules of 6-n-Propylthiouracil (PROP) (A); and phenylthiocarbamide (PTC) (B)



A 6-n-Propylthiouracil (PROP)



B phenylthiocarbamide (PTC)

Taste blindeness to phenylthiocarbamide (the fact that this compound tastes bitter to some people but not to others) has been accidentally discovered by Fox in 1931 (Fox, 1931).

Being or not being responsive to PROP is referred to as "PROP status" (PST). The responsiveness to PROP can by phenotipically determined or genetically determined. In the present thesis, the PROP status refers to the responsiveness determined phenotipically.

Researchers who studied blindness to N-C=S compounds gradually substituted PROP to PTC, because the latter has an unpleasant sulfurous odor, according to Fischer's studies in the '60s.

Originally, the taste blindness to PTC and PROP was thought to be inherited as a Mendelian recessive

trait, with nontasters having two recessive alleles (tt) and tasters having at least one dominant allele (Tt or TT) (Kalmus, 1958).

Further studies found that the gene responsible for variation in PTC/PROP sensitivity (gene TAS2R38) is located on human chromosome 7 (Kim et al., 2003). This receptor gene has three polymorphism sites (A49P, V262A, I296V) which give rise to two common haplotypes: PAV, the dominant (taster) variant and AVI, the recessive (non-taster) one. Heterozygous individuals (PAV/AVI diplotype) are PTC and PROP tasters, although their suprathreshold responses are to some extent lower than those of PAV homozygotes (Kim et al., 2003). Interestingly, the heterozygotes group (PAV/AVI) shows the widest range of bitter perception (Lipchock, Mennella, Spielman, & Reed, 2013). Rare haplotypes (AAV, AAI, PVI, and PAI) have also been observed (Wooding et al., 2004).

Researchers observed that the phenotype linked to this gene is of high evolutionary interest because the ability to taste PTC is correlated with the ability to taste other bitter substances, many of which are toxic (Wooding et al., 2004). It has been recently proposed that other bitter receptors may be involved in PROP sensitivity (Hayes et al., 2008).

In general, subjects who do not perceive PROP as bitter are generally identified as non-taster (NT) while subjects perceiving bitter taste associated to PROP solutions are generally called medium-taster (MT) or super-taster (ST), depending on the intensity of the perceived sensation (Bartoshuk et al., 1992). The PST can be determined by various approaches. For example, subjects can be classified based on their perceived bitterness ratings using specific cut-off values (Goldstein, Daun, & Tepper, 2005; Hayes et al., 2010; Tepper, Christensen, & Cao, 2001), or according to quartile (Duffy et al., 2010) or tertile (Duffy, Davidson, et al., 2004) values of PROP ratings obtained in the investigated population.

A certain variability has been highlighted in frequencies of non-tasters and tasters among races and populations over the world (NT from 7% to 40%) (Guo & Reed, 2001). Considering gender, several studies found significant perceived bitterness intensity in females compared to males (Choi, 2014; Shen et al., 2016), but this evidence has been not always confirmed (Masi et al., 2015).

The phenotypic sensibility to PROP can be estimated with various methods.

These methods are generically grouped into approaches that involve a whole mouth stimulation (Bartoshuk et al., 2004; Prescott, Soo, Campbell, & Roberts, 2004; Tepper et al., 2001) and techniques which involve a regional stimulation of the tongue (Delwiche et al., 2001; Fischer et al.,

2013; Robino et al., 2016; Webb, Bolhuis, Cicerale, Hayes, & Keast, 2015; Zhao, Kirkmeyer, & Tepper, 2003). The former techniques are based on a sip-and-split procedure which stimulate the whole oral cavity. The latter approaches are based on a stimulation of localised areas of the tongue using filter paper impregnated with a PROP solution.

Whole mouth stimulation methods have evolved from thresholds techniques (Bartoshuk et al., 1994; Duffy, Peterson, et al., 2004) to suprathreshold technique (Bartoshuk et al., 2004). In thresholds techniques, increased concentrations of PROP (from 0.001 to 3.2 mM) were used to estimate the lowest concentration detected by each subject. PROP threshold scores ranged from 0.0015 to 2.18 mM (Duffy, Peterson, et al., 2004). A low detection threshold for a given compound indicated a high responsiveness for that substance.

Thesholds techniques evolved into magnitude estimation applied to cross-modality matching (quantification of a stimulus compared to one other stimulus belonging to a different sensory modality). In magnitude estimation, subjects are typically asked to compare the intensity of two different stimuli, such that one stimulus perceived to be twice as strong as another is assigned a number twice as large and so on. The magnitude of PROP intensity has been compared to sound intensity (Marks et al., 1988), brightness (Stevens and Marks, 1965) and to the intensity of NaCl (Bartoshuk et al., 1994). The procedure that utilises NaCl as a reference standard was extensively studied. However, concerns were raised about using NaCl as appropriate standard, since differences among PROP tasters groups were underestaimated when using NaCl, compared to results found with sound (Gent & Bartoshuk, 1983). Moreover, Prutkin raised the question about the validity of using NaCl as a standard in PROP tasting studies, since the saltiness of NaCl increased as a function of increasing bitterness to PROP with the LMS (Prutkin et al., 2000), suggesting that these measures are not independent.

Therefore, approches further evolved into suprathreshod techniques. Suprathreshold techniques consist of the evaluation of the perceived intensity of concentrated PROP solutions. For example, methods providing three PROP solutions -0.032, 0.32 and 3.2 mM (Tepper et al., 2001), or one solution evaluated in duplicate - 3.2 mM (Prescott et al., 2004), have been developed. Suprathresholds techniques have gradually substituted thresholds and magnitude estimation, since they clearly separate subjects based on their responsiveness but require a lower number of samples to be tasted. Therefore, they are less fatiguing for subjects and less labor-intensive for operators.

Finally, regional stimulation is used to estimate PROP responsiveness on specific areas of the tongue, using filter paper disks impregnated with varied concentration of PROP ranging from 0.005 M (Delwiche et al., 2001) to 1.0 M (Fischer et al., 2013). The concentration of 0.050 M is the most frequently used (Feeney et al., 2014; Goldstein et al., 2005; Robino et al., 2016; Tepper et al., 2001; Webb et al., 2015; Zhao et al., 2003).

1.3.2 PROP phenotype and taste responsiveness

The importance of PROP is linked to the fact that subjects responsive to PROP were found to be more responsive than non-taster to many other compounds naturally found in foods, such as caffeine, quinine, and urea (Hall et al., 1975; Leach and Noble 1986; Bartoshuk et al. 1988; Mela 1989), sucrose (Bartoshuk, 1979; Gent and Bartoshuk, 1983), sodium chloride (Bartoshuk et al., 1998) and other compunds. Therefore, although not all studies agree (Delwiche et al., 2001; Horne, Lawless, Speirs, & Sposato, 2002), subjects sensible to PROP generally perceive greater intensity than non-tasters from a wide variety of compounds - see Tepper and Prescott for review (Prescott et al., 2004; Tepper et al., 2001), suggesting that PROP responsiveness is an index of a broader sensitivity to taste sensations in general.

For this reason, PROP status has been studied in relation to complex aspects linked to food behaviours, such as food preferences (Glanville, & Kaplan; 1965), rejection of bitter-tasting fruits and vegetables (Drewnowski, Henderson, & Shore, 1997), energy intake (Tepper, Neilland, Ullrich, Koelliker, & Belzer, 2011), macronutrients consumption (Kamphuis & Westerterp-Plantenga, 2003), eating attitude (Oftedal & Tepper, 2013), alcohol consumption (Pelchat, & Danowski, 1992;Kranzler et al., 1998), weight control and Body Mass Index (Tepper & Ullrich, 2002) and, very recently, to personality traits (Robino et al., 2016). All these studies showed characteristic behaviours of super-taster compared to subjects non sensible to PROP, suggesting that oral phenotype has an impact on food behaviours.

1.4 The relationship between FPD and PROP

The relationship between FPD and PROP has been extensively studied.

One of the first studies investigating the relationship between PROP bitterness ratings and FPD, found a positive relationship (Bartoshuk et al., 1994). Authors found that the density of taste receptors on the anterior tongue (fungiform papillae, taste buds) correlated significantly with perceived bitterness of PROP (Bartoshuk et al., 1994). This study highly contributed the spread of the concept of

supertaster characterised by a high PROP responsiveness and a high number of FP.

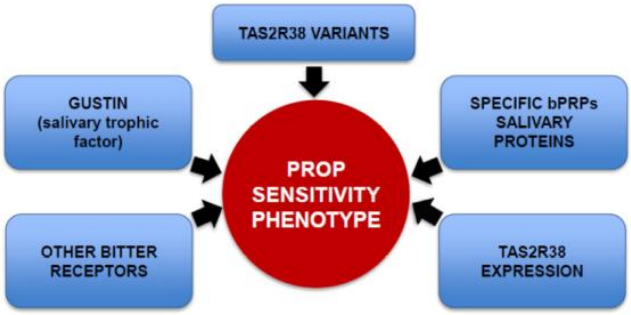
After that, many studies found a positive relationship between PROP bitterness and FPD (Bajec & Pickering, 2008; Bartoshuk et al., 1994; Delwiche et al., 2001; Duffy et al., 2010; Essick et al., 2003; Hayes & Duffy, 2007; Hayes et al., 2010; Tepper & Nurse, 1997; Yackinous & Guinard, 2002; Yeomans et al., 2007). However, the magnitude of this association has shown considerable variation, ranging from relatively high (Pearson Coefficient values $r>0.8$) (Delwiche et al., 2001; Essick et al., 2003), to moderate ($r\leq 0.5$) (Duffy et al., 2010; Hayes et al., 2010) and low ($r\leq 0.3$) (Duffy, Peterson, et al., 2004; Nachtsheim & Schlich, 2013, 2014). Moreover, in line with studies of other tastes and FPD, several recent studies have failed to find a significant FPD/PROP bitterness relationship (Bakke & Vickers, 2011; Barbarossa Tomassini et al., 2015; Dinnella et al., 2016; Feeney & Hayes, 2014a; Fischer et al., 2013; Garneau et al., 2014; Masi et al., 2015).

The concept of "supertasting" has been recently reviewed (Hayes & Keast, 2011). Among the key elements of this review, the authors highlighted the fact that since individuals may have elevated response to specific bitter tastants independently of other compounds, the significance of speaking of specific "*compound*-supertaster" (i.e. "*PROP*-supertaster", "*quinine*-supertaster", etc.) could be useless (Hayes & Keast, 2011). Therefore, a single oral marker, be it phenotypic or genotypic, appears insufficient to fully characterise orosensory response (Hayes & Keast, 2011). Moreover, the authors left open the relationship between PROP responsiveness and papillae density (Hayes & Keast, 2011), wishing for further clarifications.

Recent investigations conducted on a wide number of observations found that PROP bitterness is not in any way predicted by papillae density (Garneau et al., 2014) and that PROP taster status, TAS2R38 haplotype and perceived taste intensity were not related to density (Fischer et al., 2013).

Uncertainty on the nature of this relationship is partially due to the methods used to estimate FP (their number and their functionality), and in part it is due to complex factors affecting PROP responsiveness. Tepper and colleagues recently reviewed the principal genetic factors that contribute to PROP phenotype (Tepper, Banni, Melis, Crnjar, & Barbarossa, 2014), higlighting the complexity and the numerosityof these factors (Figure 6).

Fig. 6 Principal genetic factors contributing to PROP taste sensitivity phenotype (adapted from (Tepper et al., 2014)).



Recent evidences rather suggest that either FPD and PST are related to each by a more complex relationship or the relationship does not exist.

2. Aim of the thesis

Uncertainty has recently emerged from literature on whether to consider the Fungiform Papillae Density (FPD = papillae/cm^2) as a reliable indicator for taste responsiveness, even though fungiform papillae (FP) are anatomically designated to the detection and transduction of tastes and somatosensory stimuli.

The disagreement across studies on the relationships between FP and taste responsiveness may originate from: 1. Individual variability in papillae functionality; 2. Methodological issues related to the approaches used to estimate FPD; 3. the characteristics of the population considered.

A particularly critical source of variability is the type of stimuli used. So far, FPD and taste responsiveness has not been systematically studied both in standard solutions and complex food matrices.

Instead, sensitivity to PROP seems a solid indicator of taste responsiveness.

Despite early finding, the relationship between these two common indices of taste responsiveness (FPD and PROP status) is still very controversial. Therefore, it deserves further investigations. Moreover, while for PROP status determination several approaches have been optimised, for the FP determination some critical methodological aspects must still be faced.

Based on these critical issues, the aim of the present thesis is to study the role of lingual fungiform papillae in respect to tastes and PROP responsiveness. Detailed objectives of this research were:

I. to study the relationship between fungiform papillae density and perceived intensity of taste sensations and burning from capsaicin, in water solutions and in complex food matrices;

II. to investigate the associations between the fungiform papillae density and PROP status;

III. to improve methodological tools to measure the fungiform papillae on the tongue.

To fulfil these objectives, the following hypothesis were tested, respectively in three studies:

Study I

Ia. FPD does not directly affect the responsiveness to tastes or burning from capsaicin, both in water solutions and in complex food matrices (subjects with a higher number of FP do not show incresed responsiveness to tastes and burning from capsaicin, both in water solutions and in complex food matrices);

Ib. PST positively affect the responsiveness to tastes or burning from capsaicin, both in water solutions and in complex food matrices (subjects highly respondent to PROP showed the highest responsiveness to tastes and burning from capsaicin).

Study II

IIa. A significant linear positive relationship does not exist between these two indices.

Study III

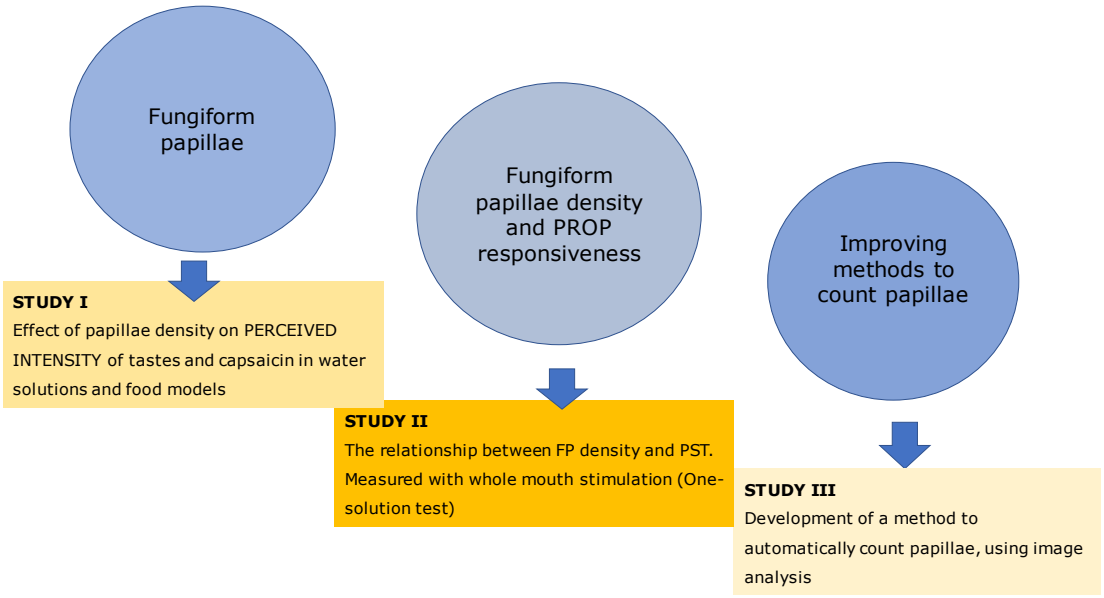
IIIa. To develop a new approach for the automated quantification of FP on human tongue (Piochi et al., 2017), in order to exclude bias in FP count originated from manual count.

3. General material and methods

3.1 Overview of the experimental plan

The present research was developed with the Italian Taste Project (Monteleone et al., 2017), during the years 2015-2017.The study followed the general conceptual plan showed in Figure 7.

Fig.7 Conceptual plan of the PhD study.



3.2 Subjects

A total of 408 subjects were involved during the PhD project. The composition of the population is shown in Table 2.The majority (n=313) attended the whole procedure of the "Italian Taste" project (IT), which envisaged the collection of a wide range of data, including the acquisition of pictures of the tongues and PROP responsiveness (Monteleone et al., 2017).

Tab 2. Overview of subjects' characteristics and data collection.

				Gender (%)		Age (%)		
City	Location	subjects (n)	Data set	F	M	E1 (18-30)	E2 (31-45)	E3 (46-65)
Bra	University of Gastronomic Sciences	176	whole *	60	40	32	38	30
Florence	University of Florence	137	whole *	58	42	52.0	23.0	25.0
Turin	Central Location test	94	reduced	70	30	66.0	13.0	21.0
			Tot:	62	38	47	27	26

* referred to the IT Project (Monteleone et al., 2017)

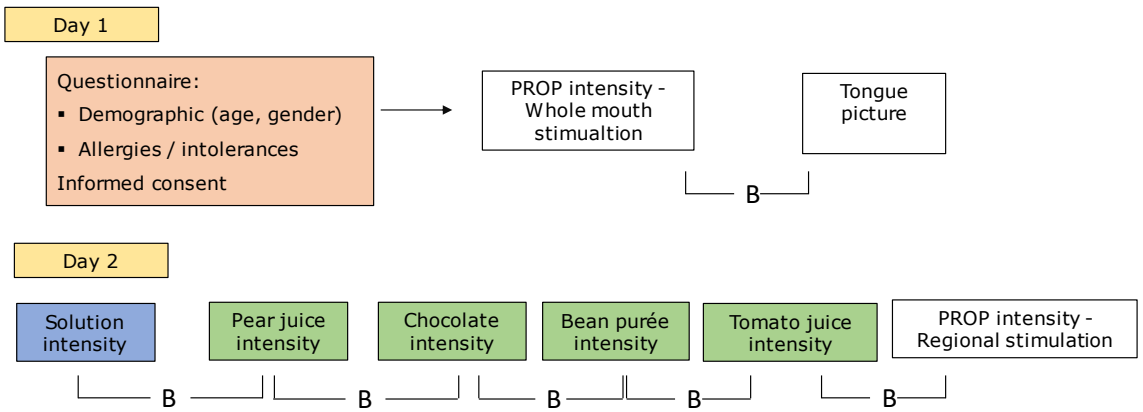
The whole procedure of the IT project was approved by the Ethical Committee of the IRCCS Burlo Garofolo Children Hospital of Trieste (Italy). Data were collected in two different laboratories in Italy (University of Florence – center Italy; University of Gastronomic Sciences in Bra – Nothern Italy). Participants were recruited using social media (Facebook), fliers, articles published on local journals, using the mailing list of the two laboratories, and announcements published on the Italian Taste project website (www.it-taste.it), the SISS website (www.scienzesensoriali.it). Tests took place during years 2015 and 2016. Exclusion criteria were not being born in Italy or having lived at least 20 years in Italy. Subjects had no history of disorders of oral perception. Written informed consent was obtained from each subject prior to the experiment. Procedures were identical between the two laboratories. A small subset of the data (94 subjects) was collected in Turin, in occasion of a Central Location test. Efforts were done to balance subjects for age and gender. Concerning age, subjects were divided into 3 classes: e1 (ranging from 18 to 30 years), e2 (ranging from 31 to 45 years), and e3 (ranging from 46 to 65 years).

3.3 General procedures

The whole data acquisition included the procedures indicated by Monteleone et al regarding the "Italian Taste" Project (Monteleone et al., 2017). Identical procedures were adopted in the two laboratories. Participants preliminarily completed some questionnaires at home and came to the laboratory twice in two subsequent days (Figure 8).

Evaluations were performed in individual booths under white lights. Data were collected with the software Fizz (ver.2.47.B, Biosystemes, Couternon, France). Breaks (10–15 min) were observed between tests.

Fig. 8. Overview of data set collection.



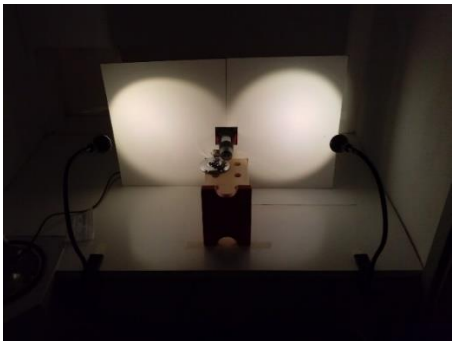
Legend: B = break of 10-15 minutes.

3.4 Manual quantification of Fungiform Papillae Density on tongue

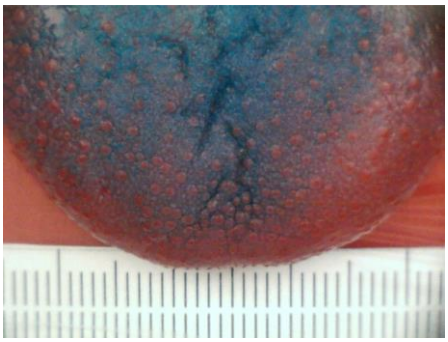
Participants were asked to rinse their mouth before the beginning of the test. Participants dried their tongue with paper before shooting the photos. Subjects were seated with the tongue held by a holder. Pictures were taken in a completely dark room, using a standardised setting (Figure 9-A). The anterior portion of the dorsal surface of each tongue was swabbed with household blue food colouring, using a cotton-tipped applicator, in order to make fungiform papillae (FP) easily visible as red structures against the blue background of the stained tongue.

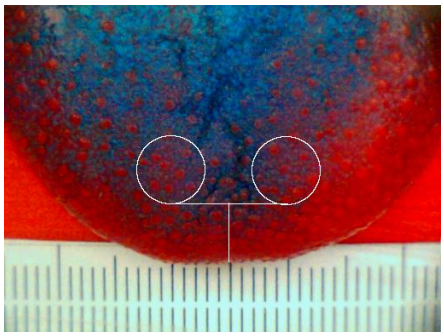
Fig. 9. Set-up of standardised tongue-holder (9-A); Example of raw tongue's picture and modified picture (9-B).

A



B





At least 5 digital pictures of the tongue were recorded using a digital microscope (MicroCapture, version 2.0 for 20x-400x) (Masi et al., 2015) for each subject. The best image was selected for each participant, based on the following criteria: 1. The image must be in focus; 2. The image must be clear (subjects must keep the tongue motionless); 3. The tongue must be centered in the framing. Pictures captured both the anterior part of the tongue and a ruler fixed behind the tongue which provided a spatial calibration. The whole picture acquisition had a duration of around 5-10 minutes per subject. Tongue images were modified with ImageJ (Color Inspector 3D plugin: saturation= x2.49, brightness=-23.0) to make the visual count easier (Fig. 9-B). Two operators, blind to any

data concerning subjects, trained according to the Denver Protocol (Nuessle et al., 2015) and with 1-year experience, independently counted FP in two 0.6 cm diameter circles, one on the right side and one on the left side of tongue, 0.5cm from the tip and 0.5cm from the tongue midline. The counts from the two operators were submitted to two-way fixed ANOVA (factors: operator, tongue side). Counts were considered valid if the operator effect was not significant ($p>0.05$). The mean FP number from valid counts was used for each image and expressed as density (papillae/cm^2- FPD).

3.5 PROP responsiveness: One-solution test

PROP status was evaluated with a double procedures on separate days: the one solution procedure (Prescott et al., 2004) on the first day and a regional stimulation procedure on the second day. For the one solution procedure, briefly, a 3.2mM PROP solution was prepared by dissolving 0.5447 g/L of 6-n-propyl-2-thiouracil (Sigma Aldrich, Milano, IT) into deionised water. Participants received 2 identical samples (10 ml) coded with a three-digit code. Subjects were instructed to hold each sample (10 ml) in their mouth for 10 seconds, then expectorate, wait 20 seconds and evaluate the intensity of bitterness using the gLMS (Bartoshuk et al., 2004). A 90-seconds break was enforced after the first sample to control carry-over effect. During the break, subjects rinsed their mouths with distilled water for 30 seconds, had some plain crackers for 30 seconds, and finally rinsed their mouths. The 3.2 Mm PROP solution was prepared the day before the test and kept in the fridge overnight.

3.6 General Data Analysis

Descriptive statistics were preliminarily applied to study all variables. Correlations between variables were explored with Pearson coefficient. Chi-squared tests were applied to compare the distribution of observations between two variables. One-way, two-way or three-way ANOVA models were computed to assess the effect of one or more factors on the response variables. A 0.05 was generally selected as the level of significance for all the statistical tests (if lower, p is specified). All data analyses were performed with XLStat 2016.05 (Addinsoft). Prediction models (PLSR) were computed with The Unscrambler ® (ver. 10.4 – © 2016 CAMO Software AS, Oslo Norway). Details of data analyses are reported within each relevant corresponding section.

4. Study I. The effects of Fungiform Papillae Density (FPD) and PROP in taste responsiveness

This study aimed to explore the distributions of the two indices in a wide population and to assess whether these two indices confirmed their role in being considered reliable indicators for taste responsiveness. The effects of Fungiform Papillae Density (FPD) and PROP status (PST) were separately tested on the perceived intensity of tastes and burning from capsaicin, both in water solutions and in complex food matrices.

4.1 Material and methods

4.1.1 Subjects

408 observations were used to explore the distributions of FPD and PROP intensity (Table 3 -A).

A subset of 309 subjects joined the part of taste responsiveness. Due to incomplete dataset, 12 subjects were excluded. Therefore, data of 297 participants were finally used to assess the effect of FPD and PROP on taste responsiveness (Table 3-B). Participants were characterised for FPD and PST, and provided intensity ratings for all sensory stimuli in water solutions and complex food models.

Tab. 3. Population used to explore the distribution of FPD and PROP responsiveness.

A

Gender	Obs	Age class	N	%
F	252	e1	116	46
		e2	63	25
		e3	73	29
M	156	e1	75	48
		e2	46	29
		e3	35	22
			408	

B

Gender	Obs	Age class	N	%
F	176	e1	69	39
		e2	49	28
		e3	58	33
M	121	e1	51	42
		e2	42	35
		e3	28	23
			297	

Legend: Obs= Observations; M=males, F=females; e1= 18-30 years, e2=31-45 years, e3= 46-65 years.

4.1.2 Sensory evaluations

Participants evaluated six water solutions and four series of products. Samples were used to rate the perceived intensity of some target sensations, modulated by spiking relevant compounds. Concentrations of tastants were selected in order to obtain intensities equivalent to moderate/strong on a generalised Labelled Magnitude Scale (gLMS) (Bartoshuk et al., 2004), based on pilot tests conducted in Pollenzo and Firenze in the first year of PhD. For the evaluation of intensity, subjects were trained to the use of gLMS (0: no sensation-100: the strongest imaginable sensation of any

kind) following a standard procedure (Bartoshuk, 2000; Green et al., 1996). Subjects were instructed to treat the ‘‘strongest imaginable sensation” as the most intense sensation they can imagine that involves remembered/imagined sensations in any sensory modality.

4.1.2.1 Water solutions

Six water solutions were evaluated. Concentrations in solutions were: sourness: 4.0 g/kg of citric acid, bitterness 3.0 g/kg caffeine, sweetness 200.0 g/kg sucrose, saltiness: 15.0 g/kg sodium chloride, umami 10.0 g/kg monosodium glutamate, burning: capsaicin 1.5 mg/kg (Monteleone et al., 2017). Water solutions (10 mL) were presented in 80cc plastic cups identified by a 3-digit code. Subjects were presented with a tray with six water solutions. The presentation order of water solutions was randomised across subjects, except for the solution of capsaicin which was served as the last sample for all subjects. Subjects were instructed to hold the whole water solution sample in their mouth for 3 seconds, then expectorate and evaluate the intensity of relevant target sensation on gLMS. After each sample, subjects rinsed their mouths with distilled water for 30 seconds had some plain crackers for 30 seconds and rinsed their mouths with water for a further 30 seconds.

4.1.2.2 Food Models

Four series of products were tasted: pear juice (PJ), chocolate pudding (CP), bean purée (BP), and tomato juice (TJ). Food products were spiked with a relevant tastants concentration for each matrix, in order to modulate the perceived intensity of taste target sensations (Table 4). Intensities of additional sensations (other than the target sensation) were rated, which could vary, due to sensory interactions that can take place in foods (Green, Lim, Osterhoff, Blacher, & Nachtigal, 2010).

Tab.4. Concentrations in food models of target stimuli.

Product	Tastant	Sensations
pearjuice (PJ)	citric acid 0.5; 2.0; 4.0; 8.0 g/kg	▪ *sourness* ▪ sweetness ▪ overall flavour
chocolate pudding (CP)	sucrose 38; 83; 119; 233 g/kg	▪ *sweetness* ▪ Bitterness ▪ Astringency ▪ overall flavour
beanpurée (BP)	sodiumchloride 2.0; 6.1;10.7; 18.8 g/kg	▪ *saltiness* ▪ umami ▪ overall flavour
tomato juice (TJ)	capsaicin 0.3; 0.68; 1.01; 1.52 mg/kg	▪ *burning* ▪ overall flavour

Legend: The target sensation is shown in *Italic* type for each product category. Additional sensations (sensations explored without that a corresponding stimulus was varied) are shown in normal characters.

The four types of products were presented in independent sets, each consisting of four samples of the same product. The order of the products was the same for all subjects but the presentation order of the levels within each product was randomised across subjects. The presentation order of the four product types was established to avoid perceptive interferences across samples due to the long-lasting sensations (respectively the bitterness of chocolate pudding and the burning of capsaicin of tomato juice).

4.1.3 Data Analysis

Descriptive statistics were computed for both FPD values and PROP bitterness ratings. Values of fungiform papillae counts given by two trained operators on the right and left side of the tongue were submitted to two-way fixed ANOVA (factors: operator, side of the tongue; with interaction). Counts were considered valid if the operator effect was not significant ($p>0.05$). If so, the mean FP number from valid counts was used for each image and expressed as density (FP/cm^2- FPD). Normality of FPD distribution was tested with Shapiro-Wilk test ($W=0.98$, $\alpha= 0.1$). Mean of FPD and standard error are provided in the text for FPD.

In PROP One-solution test, the effect of the replicate on bitterness intensity ratings was assessed with 1-w ANOVA (factor: replicate). If no effect of the replicate was found, the mean value of bitterness for two solutions was used for each subject. The effects of age (class e1=18-30 years; class e2=31-45years; class e3=46-65 years) and gender (M, F) were separately assessed on FPD and on PROP intensity bitterness ratings with 2w-ANOVA models (fixed factors: age classes, gender; with interaction).

Three-way ANOVA models were separately assessed for each solution (Fixed factors: groups for PST/ groups of FPD, age groups, gender; with 3-way interactions) to separately analyse the effect of each index, of gender and of age on intensity ratings of tastes and burning from capsaicin in water solutions. To estimate the effect of the stimulus modification in each food matrix (PJ, CP, BP, TJ), 1-way ANOVA models (factor: product) were separately conducted on perceived intensity of the target sensation. To estimate respectively the effects of FPD (3 levels= LP, MP, HP) and of PST (3 levels=NT, MT, ST) on the perceived intensity of the target sensations, 1-way ANOVA models (factor: PST group/ FPD group) were then separately computed for each product in each food matrix.

4.2 Results

4.2.1 Structure of the dataset: FPD and PROP indices

4.2.1.1 Distribution of fungiform papillae density

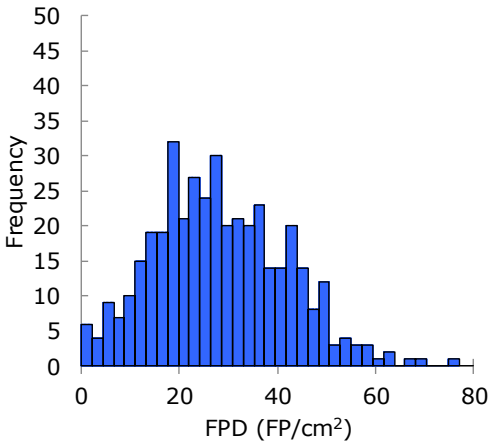
For all participants, the best tongue's picture was selected, and the FP were counted by the two independent operators. No significant effects of the side (right, left) of the tongue (F=0.073, p=0.78) or of the operator were found (F=0.006, p=0.93), thereby indicating that the number of FP between the two sides of the tongue was comparable and the count procedure was validated. The mean of the two counters was used for each subject. The mean number of FP in the 0.6 diameter circles was converted into density (FP/cm^2). Descriptive statistics and the distribution of FPD found in the population are shown in Table 5.

Tab. 5. Descriptive statistics and distribution of FPD found in the population (n=408).

A

	FPD (FP/cm^2)
Observations (n)	408
Min	0.0
Max	76.0
1° Quartile	18.6
Median	26.5
3° Quartile	37.1
Mean	28.2

B



Values of 0.0 FP/cm^2 in FPD must be interpreted as a subject having no FP in the considered area, not necessarily as the subject being completely lacking in FP. The distribution of FPD tended to be normal according to the Shapiro-Wilk test (W=0.98, p=0.02, α= 0.1) (Table 5-B). Based on values for first and third quartile of FPD distribution, subjects were divided into three groups of: low FPD subjects (LP <18.6 FP/cm^2), medium FPD (MP from 18.6 to 37.1 FP/cm^2) and high FPD (HP>37.1 FP/cm^2) subjects.

4.2.1.2 Distribution of PROP sensitivity evaluated from One-solution test

From One-solution test, no significant effect of the replicate was found (p> 0.05) on PROP intensity of two solutions, therefore the mean of the two solutions was used for each subject.

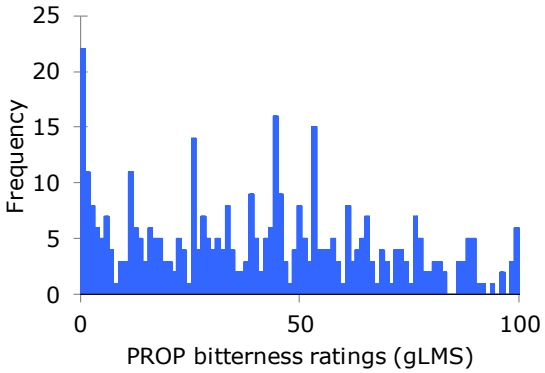
Descriptive statistics and the distribution of PROP bitterness ratings found in the population are shown in Table 6.

Tab. 6 Descriptive statistics and distribution of PROP bitterness ratings.

A

	PROP One-solution
Observations (n)	408
Min	0
Max	100
1° Quartile	17
Median	41
3° Quartile	61
Mean	41

B



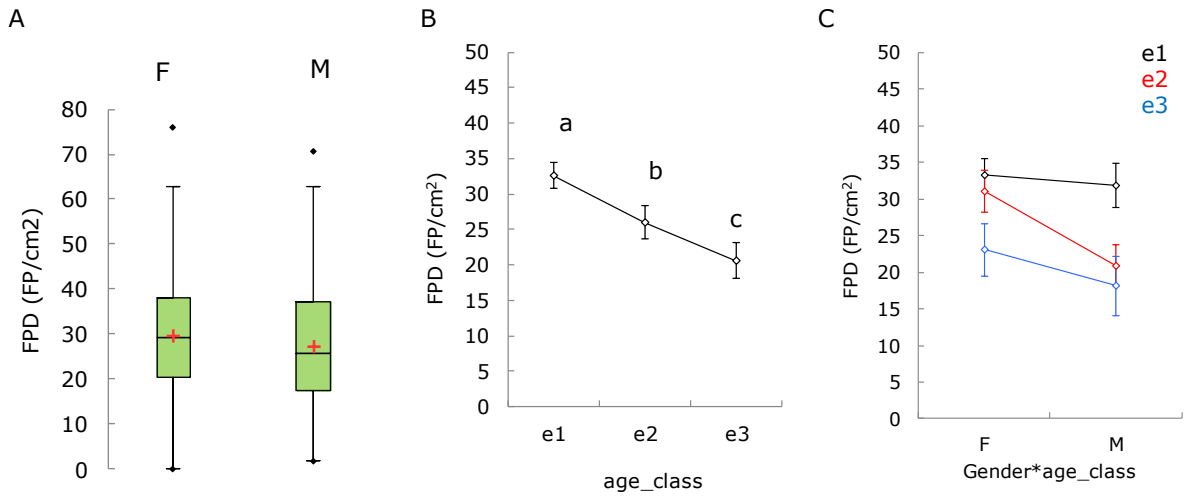
PROP bitterness ratings showed a typical bimodal distribution (Bartoshuk et al., 2003). The upper limit of the first quartile (17) and the lower limit of the third quartile (61) measured on the gLMS were used to classified subjects into Non-Taster (NT<17), Medium-Taster (MT), and Super-Taster (ST>61).

4.2.2 Effects of age and gender on FPD and PROP

4.2.2.1 Effects of age and gender on fungiform papillae density

A significant effect of gender was found on FPD ($F=17.29$, $p<0.0001$), with women showing significantly higher FPD values (29.1±0.8 FP/cm^2) than men (23.6±1.0 FP/cm^2). Moreover, a significant strong effect of age was found ($F=30.84$, $p<0.001$). FPD linearly decreased with age (Figure 10). The mean FPD values for the three age classes were: 32.6±0.9 FP/cm^2 (18-30 years), 26.0±1.2 FP/cm^2 (31-45 years), 20.6±1.2 FP/cm^2 (46-65 years). A significant interaction gender*age was found ($F=4.11$, $p=0.02$), indicating a more accentuated decrease among 31-45 years men compared to women.

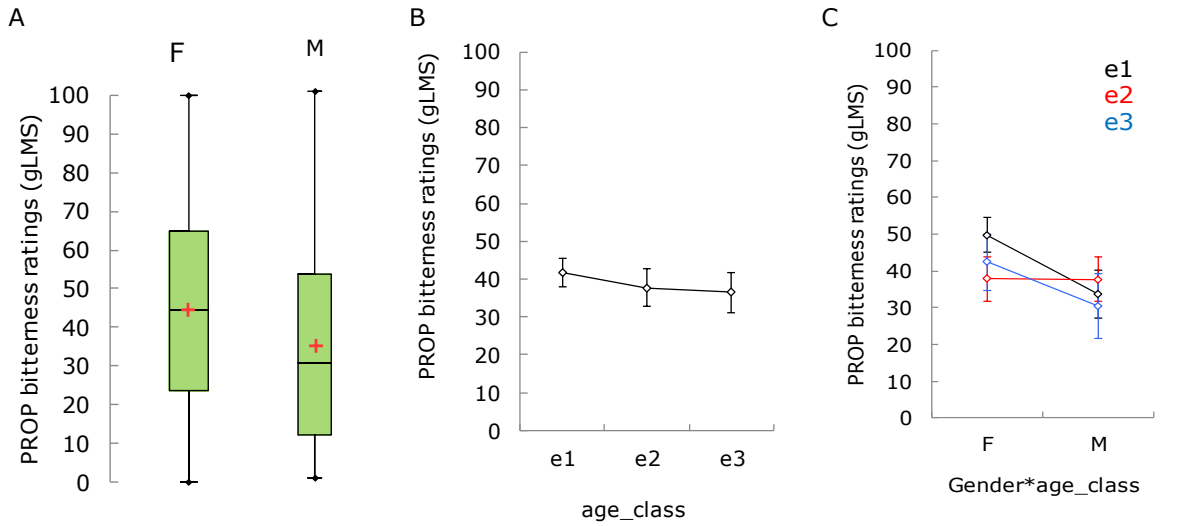
Fig. 10. Box-plot for FPD in females and males (A); effect of age on FPD (B); effect of the interaction gender*age on FPD (C).



4.2.2.2 Effects of age and gender on PROP responsiveness

Two-way ANOVA to estimate the effect of gender and age class revealed a significant effect of gender (F=10.97, p=0.001) on perceived PROP bitterness, with females perceiving at higher intensity (43.4±1.7) than men (34.0±2.2). No effect of age (F=1.50, p=0.22) was found (Figure11), despite a tendency showing a slight decrease in PROP bitterness ratings with age. A significant effect of the interaction gender*age (F=3.09, p=0.05) was found. Among the youngest age class (18-30 years), women showed significantly higher (49.8±2.5) mean bitterness ratings than men (33.7±3.1).

Fig. 11. Box-plot for PROP in females and males (A); effect of age on PROP (B); effect of the interaction gender*age on PROP (C).

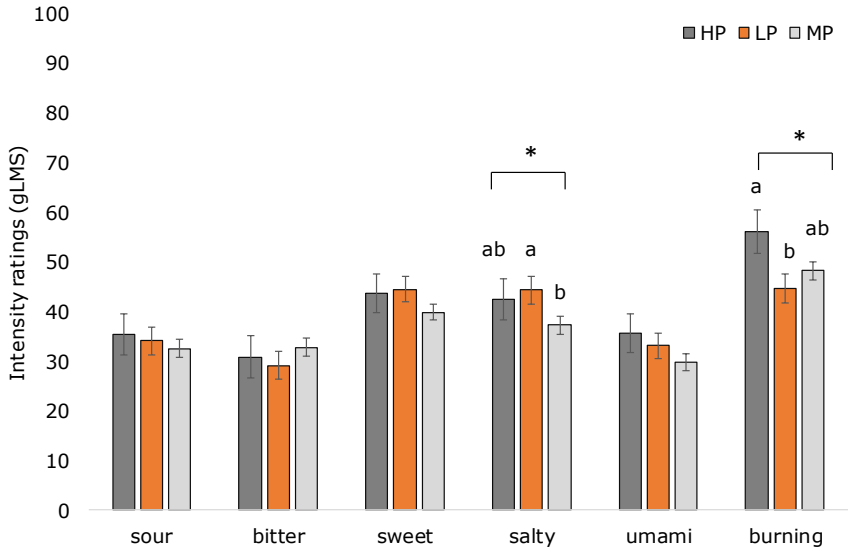


4.2.3 Effect of FPD and PST on taste responsiveness evaluated in water solutions

4.2.3.1 Effect of FPD on water solutions

The effects of FPD group (Low=LP, Medium=MP, High=HP), of age and of gender were assessed on the intensity ratings of solutions. The effect of FPD on perceived intensity of the 6 water solutions is shown in Figure 12.

Fig. 12. Effect of FPD on perceived intensity of six water solutions.



Legend: LP=Low, MP=Medium, HP=High papillae density. Different letters indicate significantly different means based on LSD Fisher test for a significant level of p≤0.10.

FPD did not have a significant effect on perceived intensity of sour (F=0.25), bitter (F=0.60), sweet (F=1.28), and umami (F=1.20). The increase in FPD only tended to have a negative effect on the intensity ratings of saltiness (F=2.56, p=0.08) and a positive effect on burning (F=2.36, p=0.10). Gender did not have a significant effect on perceived intensity for any of the water solutions.

For saltiness, MP tended to rate at a lower intensity (37.3±1.8) than the other two groups, LP (44.4±2.8) and HP (42.5±4.2).

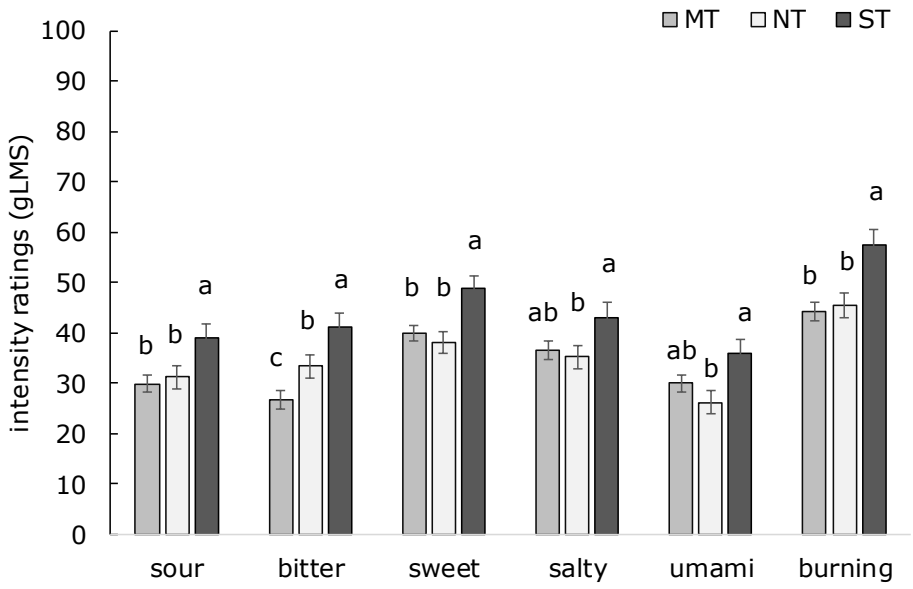
Burning from capsaicin tended (p=0.10) to be positively and linearly associated with FPD. HP subjects rated burning as higher (56.1±4.4), than MP (48.2±1.9) and then LP (44.8±2.9), with MP having not significantly different scores from the extremes groups. A strong significant effect of the age class (F=7.75, p=0.001) was found on intensity of burning, which linearly increased with age. More mature subjects (e3) rated burning significantly higher (59.6±4.1) than e2 (48.6±2.8) and e1 (40.9±2.5). The exact test of Fisher ($X^2_{23.68}$=17.11, df=14, p=0.25) revealed that in the oldest group

(e3) the number of heavy-spicy users (*'I consume chili and spicy food 5-6 times per week'* and *'I consume chili and spicy food2 or more times a day'*) was lower than expected. Since the frequency of consumption of spicy foods has been associated with the reduction in the reported burn of sampled capsaicin (John Prescott & Stevenson, 1995), the higher ratings found among e3 may have been influenced by the reduced frequency of spicy food consumption declared by this group.

4.2.3.2 Effect of PROP status on water solutions

The effect of PST on perceived intensity of water solutions was in the expected direction. Three-way ANOVA models (factors: PST groups, age class, gender) revealed a significant effect ($p<0.10$) of PST on intensity ratings for sour ($F=3.91$), bitter ($F=9.86$), sweet ($F=5.47$), salty ($F=2.38$), umami ($F=3.66$), and burning ($F=7.62$) (Figure 13). ST gave the significantly highest intensity ratings for all water solutions. Mean ratings given by MT and by NT did not significantly differ for all sensations, except for bitter taste of caffeine, which was rated as significantly less bitter by NT than MT.

Fig. 13. Effect of PST on perceived intensity of 6 water solutions.



Legend: Different letters indicate significantly different means ($p<0.10$) based on LSD Fisher test. ST= super-taster, MT= medium-taster, NT=non-taster.

Age only had a significant effect on perceived intensity of burning ($F=5.58$, $p<0.001$). Gender had a significant effect on umami ($F=3.9$, $p=0.05$) and burning ($F=5.59$, $p=0.02$).

For saltiness, a significant interaction PST*age class was found ($F=2.59$, $p=0.04$). Among the over-

45, the decrease in intensity for saltiness was more accentuated in non-tasters compared to other groups. No significant effects of age class or gender were found from 3-w ANOVA on the intensities of the other sensations.

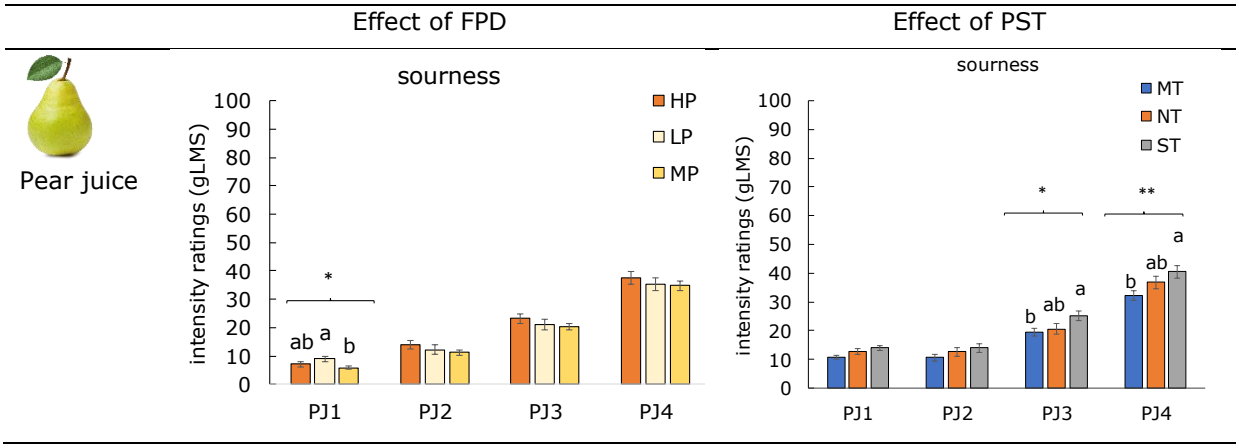
For burning, females provided higher burning scores (52.4±1.8) than males (45.7±2.2). The capsaicin solution was perceived as significantly less intense by the youngest group (e1: 42.9±2.1) compared to older groups (e2: 50.1±2.3; e3: 54.1±2.9).

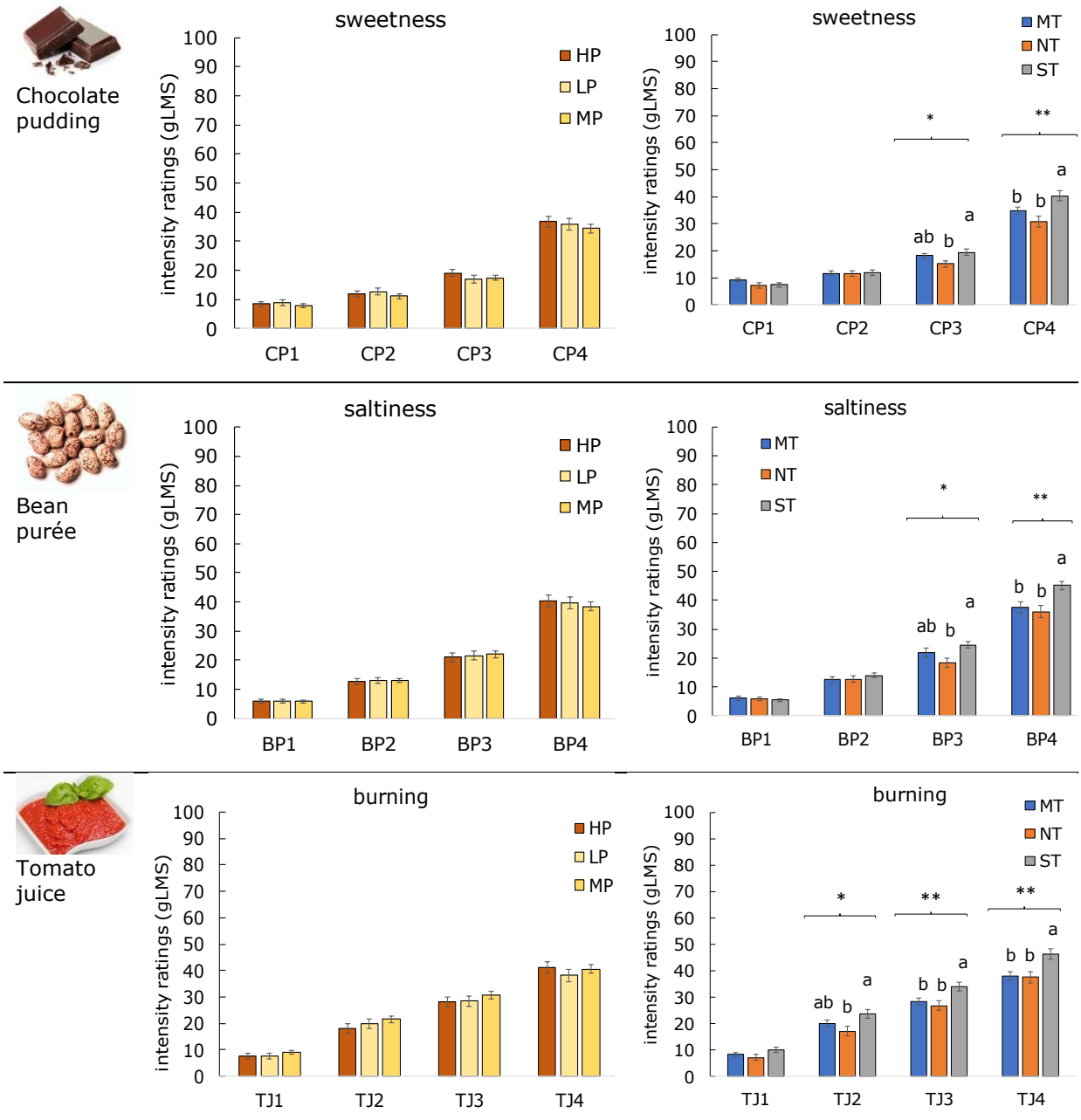
These results showed that supertasters rated intensity of tastes and burning significantly higher than other groups, confirming the hypothesis Ib in water solutions.

4.2.4 Effects of FPD and PST on taste responsiveness in real foods

From 1-w ANOVA models conducted on the four matrices (factors: product) a strong effect ($p<0.0001$) of sample was always found for the target sensations: sourness in pear juice ($F=217.2$), sweetness in chocolate pudding ($F=346.0$), saltiness in bean purée ($F=430.0$) and burning in tomato juice (TJ) ($F=235.0$). The effect was in the expected direction for all matrices (a clear linear increase of the intensity of the target sensation), confirming that the developed products were effective in reproducing a gradual increase of the target sensation. The effects of FPD and PST were separately estimated on each food matrix are shown below (Figure 14).

Fig. 14. Effects of FPD and PST separately estimated on intensities of prototypical food matrices.





Legend: Different letters indicate significantly different means: * p<0.05, **p<0.01 based on LSD Fisher test. LP=low FPD, MP=medium FPD, HP= high FPD. ST= super-taster, MT= medium-taster, NT=non-taster.

A significant effect of FPD (F=3.19, p=0.04) was only found for perceived intensity of sourness on the pear juice sample spiked with the lowest citric acid concentration (PJ1). The effect was not clear because LP rated the sample PJ1 as significantly higher (9.0±1.0) than MP (5.9±0.7), with HP showing intermediate ratings (7.1±1.0) not significantly different from LP and MP.

Instead, PST showed a significant effect on perceived intensity for most samples in all food matrices. As the concentration of the target stimulus increased, the effects of PST were visible either starting from the third (pear juice, chocolate pudding, bean purée) or the second concentration (tomato juice). For all target sensations, ST had the highest ratings.

For samples PJ3 and PJ4, sourness rated by ST was significantly higher than ratings given by MT, but not significantly different from NT.

In chocolate pudding, as the amount of sucrose increased, ST rated the intensity of sweetness significantly higher than NT (in CP3) and MT/NT (in CP4). The same trend was observed for saltiness in bean purée. Similarly, in tomato juice this effect was already visible in TJ2.

These results confirmed the hypothesis that subjects highly respondent to PROP have the highest responsiveness to tastes and burning from capsaicin in real foods.

No clear effect of FPD was observed for perceived intensity of tastes and burning from capsaicin in real foods, as expected.

4.3 Discussion

Findings on distribution of FPD and PROP responsiveness showed good agreement with data available in literature, therefore proving that data are reliable.

The mean value of FPD found in the present study was in line with values found in other studies which used the same extension area for count (Webb et al., 2015).

Women were found to have significantly higher FPD than men, in agreement with other results reported in literature (Fischer et al., 2013; Pavlidis et al., 2013; Tepper & Nurse, 1997).

FPD linearly decreased with age, congruently with evidences found in literature (Correa et al., 2013; Dinnella et al., 2016; Pavlidis et al., 2013; Segovia et al., 2002; Shen et al., 2016).

A significant interaction age*gender on FPD was found, indicating a more accentuated decrease in papillae number occurring among 31-45 years men compared to women. One other study, confirmed a decrease in FPD earlier in men (>50 years) than in women (>60 years) (Pavlidis et al., 2013).

PROP bitterness ratings showed a typical bimodal distribution (Bartoshuk et al., 2003). Females were found to rate at higher intensity PROP bitterness, in line with an earlier study, showing that women are supertasters more frequently than men (Bartoshuk et al., 1994). PROP bitterness was not significantly affected by age, despite bitterness ratings tending to decrease with age. This goes in the same direction of one other study, which showed that age was not a significant predictor of the bitterness of 3.2mMPROP (Hayes et al., 2008).

The values found for PROP bitterness quartiles were very close to the ones with the arbitrary cut-offs used in previous studies to classify subjects as non-tasters and super-tasters (Fischer et al., 2013; Hayes et al., 2010).

In general, FPD did not have a significant effect on taste responsiveness.

No significant effects were found for FPD and perceived intensity of sour, bitter, sweet and umami. This is congruent with studies, finding that FP number did not directly correlate to the intensity of sourness from citric acid by whole mouth stimulation (Duffy, Peterson, et al., 2004; Feeney & Hayes, 2014b), bitterness from caffeine (Feeney & Hayes, 2014b), sweetness from sucrose (Feeney & Hayes, 2014b; Webb et al., 2015) and umami taste from glutamate (Feeney & Hayes, 2014b).

In water solutions, the increase in FPD only tended to have a negative effect on saltiness intensity and a positive effect on spiciness. Previous studies have reported an inverse relationship between saltiness perception and FPD in water solution (Fischer et al., 2013) and complex stimuli (Hayes et al., 2010). The former study was relevantly conducted on a large number of observations (>2300) (Fischer et al., 2013). We found a slight negative effect of FPD on saltiness in water solution, similarly to other authors (Fischer et al., 2013), but no effect on saltiness perceived in complex matrices (bean purée spiked with NaCl solution) similarly to Hayes and colleagues (Hayes et al., 2010).

The tendency of a positive effect of FPD on spiciness could be tentatively explained as follows. Since capsaicin activated nociceptor TRPV1 (Caterina et al., 1997), which is part of the trigeminal stimulation that innervates the epithelium of oral papillae, subjects with higher FPD may have a higher degree of innervation and therefore be more sensitive to irritant substances. However, a few studies have documented the relationship between FP density and perceived oral burn (Feeney and Hayes, 2014b), but did not find any relationship between FP number and intensity of whole mouth capsaicin solution (Feeney and Hayes, 2014b). As found for saltiness, the slight effect of FPD observed in water solutions was not confirmed in the complex matrix tomato juice (spiked with capsaicin solution).

Taken together, these results confirmed the absence of a direct relationship between FPD and taste and spiciness.

Considering the effect of PST on oral responsiveness, ST gave the significantly highest intensity ratings for all water solutions and the result was confirmed also in complex matrices. In food matrices, these effects were more evident particularly at the higher concentrations of the individual tastants (for pear juice, chocolate pudding, bean purée, tomato juice).

Several studies showed that subjects respondent to PROP gave higher taste intensities than non-tasters, for a variety of bitter compounds, sweet compounds such sucrose, for NaCl saltiness and citric acid sourness (see (Prescott et al., 2004) for a review). We found a superiority of STs

discriminative ability in intensity ratings over NT, particularly for sweetness, saltiness, and spiciness, but not for sourness.

ST rated sourness at the highest intensity in water solution and in pear juice than MT, with NT not significantly different. This is partially in agreement with a study where ST gave higher ratings of sourness of a drink added with citric acid, even if in that study ST significantly differed from us (Prescott et al., 2004).

In the present study, ST clearly rated higher than NT in intensity of sweetness in solutions and in chocolate pudding. Similarly, perceived sweetness of sucrose was found more intense to tasters than to non-tasters (Gent et al., 1983). And, when shifting from water to milk mixtures, the increase in sweetness intensity was greatest for those subjects tasting PROP as most bitter (Hayes & Duffy, 2007). Moreover, ST have been rating a range of sensations in red wines at higher degree than NT, such as saltiness (Pickering & Robert, 2006).

Spiciness has been also rated higher in PROP taster than non-taster (Karrer et al., 1992), congruently with the present results.

Therefore, the present study fully confirmed that PST is an effective index for oral sensibility.

4.4 Conclusions

A strong effect of gender was found on both FPD and PST, with women having higher FPD and showing higher responsiveness to PROP. Age had a significant effect only for FPD, with a clear decreasing occurring with the aging process.

No clear effect of FPD was observed for perceived intensity of tastes and burning from capsaicin both in water solutions and in real foods, therefore caution is advised in using the FPD as index.

Instead, the effect of PST was clear and in the expected direction for all taste sensations and burning both in water solutions and in complex matrices (ST> other groups). Therefore, PST was confirmed to be an effective index for oral sensibility.

5. Study II. The relationship between fungiform papillae density (FPD) and PROP responsiveness evaluated by whole mouth stimulation

5.1 Subjects and Experimental plan

A totality of 408 subjects were involved in this part of the study (Table 7).

Tab. 7. Data set used in the study.

Gender	Obs	Age class	N	%
F	252	e1	116	46
		e2	63	25
		e3	73	29
M	156	e1	75	48
		e2	46	29
		e3	35	22
			408	

The FPD was determined from photos acquired with digital microscope (paragraph 3.4) and responsiveness to PROP was determined using One-solution test (paragraph 3.5).

5.2 Results

The relationships between FPD and PROP responsiveness were explored with Pearson coefficient, on the totality of subjects and within groups homogeneous for age and gender (Table 8).

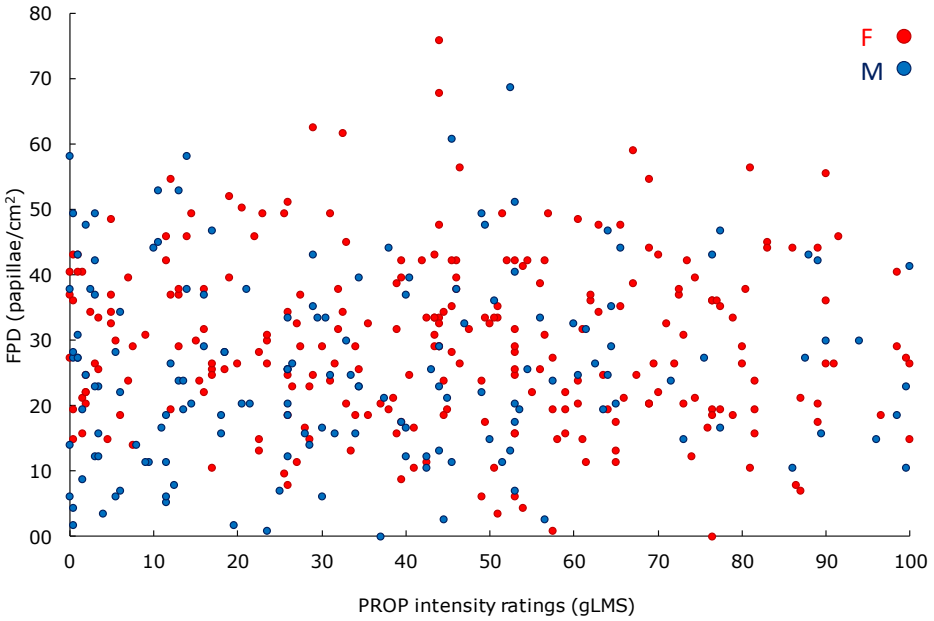
Tab. 8. Pearson correlation coefficients (r) for the relationships between FPD and PROP responsiveness measured by One-solution test, considering age and gender.

	Tot	e1	e2	e3	F	M
Obj (n)	408	191	109	108	252	156
r	0.01	-0.02	-0.01	0	-0.067	0.061
p	0.77	0.74	0.91	0.97	0.291	0.448

Legend: Obj= observations; F=females, M=males; e1=18-30 years, e2=31-45 years, e3=46-65 years.

No significant correlations were found between FPD and PROP either for the totality of subjects, or in all groups homogeneous for gender (F; M) and for age (e1; e2; e3). The relationship between FPD and PROP intensity measured by One-solution test in males and females is shown below (Figure 15).

Fig. 15. Correlation between FPD and PROP ratings found in females (red) and males (blue).



Correlations between FPD and PROP bitterness intensity ratings in groups different for PST is shown below (Table 9). No significant relationships were found in any of the groups.

Tab. 9. Pearson correlation coefficients (r) for the relationships between FPD and PROP responsiveness measured by One-solution test, considering

	NT	MT	ST
Obs (n)	101	202	105
r	0.10	0.03	-0.08
p-value	0.32	0.67	0.44

A 1-way ANOVA (factor: PST group, three levels: NT, MT, ST) confirmed that groups different for PST did not significantly differ in terms of FPD: 28.8±1.3 FP/cm^2 (ST), 28.5±1.3 FP/cm^2 (MT), and 27.7±1.0 FP/cm^2 (NT).

5.3 Discussion

In the present study, FPD and PROP intensity ratings were not positively correlated, therefore an increase in number of FP was not associated to an increase in PROP responsiveness.

This is congruent with other studies which adopted the same approach to estimate PROP responsiveness

with One-test solution (Bakke & Vickers, 2011; Dinnella, C. et al., 2016; Masi et al., 2015), and also with other studies using other approaches, such as the regional stimulation (Fischer et al., 2013; Garneau et al., 2014).

The absence of relationship was confirmed in the totality of subjects and in groups homogeneous for demographic factors (all having a consistent number of observations, > 100), therefore suggesting that the lack of relationship does not depend on age and gender.

We tentatively explain the lack of relationship FPD-PROP as follows.

The lack of relationship mainly relates to two domains: the first concerns the FP functionality and the second relates to genetic factors.

In general, due to the high variability of taste buds in papillae and the complex neurophysiological mechanisms taking place both at peripheral and central level, the number of papillae in itself may not necessarily reflect differences in perceived intensity of PROP sensation (and tastes), as it does not strictly provide important information related to the functionality (such as the taste pores number or the presence of eventual damaged nerves).

An important issue in estimating the relationship between FPD and PROP (and tastes), is that the density of papillae alone does not directly predict the taste pore density. In fact, a positive relationship between FP number and taste pores per FP has been only demonstrated on small sample size (I. Miller & Reedy, 1990; Segovia et al., 2002). In the present study, the technique used to quantify FP (portable digital microscope) did not allow the taste pores quantification, similar to other types of equipment such as digital cameras (Fischer et al., 2013; Shahbake et al., 2005) or devices for direct count (Delwiche et al., 2001; Tepper & Nurse, 1997). As most recent studies use techniques which do not allow the taste pores quantification, it is unclear how reliable FPD is as a proxy for stimulation of taste receptors.

Moreover, in the few studies investigating individual variability in taste pores (Miller, 1986; Miller & Reedy, 1990; Segovia et al., 2002), a high variability was found. Taste pores density varies across individuals up to 14-fold among subjects, from 36 to 511 pores/cm^2 (Miller, 1986; Miller & Reedy, 1990), and some FP do not contain taste pores (Miller & Reedy, 1990; Segovia et al., 2002). This variability has been confirmed both in children (pores/FP ranged from 0 to 28) and adults (from 0 to 22) (Segovia et al., 2002).

Therefore, the assumption that all FP carry taste pores and in a comparable number can be partially misleading. Due to the difficulty in quantifying taste pores, no study has investigated yet the relationship between FPD and taste pores density on large scale.

Another critical issue affecting FP functionality is the nerves damage. For example, otitis media can damage the chorda tympani nerve (CN VII), tonsillectomy and head and neck radiation treatment can damage the glossopharyngeal nerve (CN IX), and these damages induce complex and unexpected intensifications of oral sensations (see (Bartoshuk et al., 2012) for a review). Therefore, FP functionality can be affected, without compromising the FP number.

Moreover, the following considerations can be made for the genetic factors affecting FPD and PROP responsiveness.

Several genetic factors contribute to the definition of both PROP responsiveness and FPD. The combination of these multiple factors may represent a confounding source of variation in studies which do not consider genotypes, such as the present one.

It was recently highlighted that several genetic factors contribute to PROP taste sensitivity phenotype (Tepper et al., 2014). These factors include: the salivary trophic factor gustin, a protein that provides the mechanistic explanation for why PROP super-tasters are more responsive to stimuli that are not mediated via the TAS2R38 bitter receptor; TAS2R38 variants with their different affinities for the PROP stimulus; specific salivary peptides belonging to the basic proline-rich protein family (bPRP), which could facilitate binding of PROP with its receptor site; the involvement of other bitter receptors which may be associated with supertasting and PROP bitterness; and greater mRNA expression associated with the PAV allele of the TAS2R38 receptor which correlates with greater PROP bitterness perception (Tepper et al., 2014).

For lingual papillae, studies on the genetic pathways have identified several transcription factors involved in the differentiation of basal progenitor cells into taste receptor cells or keratinocytes in mammalians (Nishiguchi et al., 2016).

In humans, the gustin (CA6) gene polymorphism, seems one of the most important for FP number and their maintenance (Melis et al., 2013).

Gustin is a zinc-dependent salivary protein, previously described as trophic factor for taste pores, whose

gene (rs2274333) alone predicts 16% of the variance of FPD (Barbarossa Tomassini et al., 2015). Polymorphism in the gustin gene (A/G) gives rise to three forms: homozygous dominant A/A (native form of the protein), heterozygote A/G (intermediate form) and homozygous recessive G/G (the less functioning structure) (Padiglia et al., 2010).Individuals G/G show lower FPD and A/A individuals higher FPD(Barbarossa Tomassini et al., 2015; Melis et al., 2013). The link between TAS2R38 and gustin is that genotype A/A and allele A are more frequent in TASR38 homozygous (PAV/PAV) while genotype G/G and allele G are more frequent in non-taster AVI/AVI (Barbarossa Tomassini et al., 2015; Calò et al., 2011; Padiglia et al., 2010).

The PROP bitterness/FPD relationship has been found to differ across genotypes (Hayes et al., 2008), with FPD being a strong determinant of PROP bitterness only in the 2 homozygous groups (PAV/PAV; AVI/AVI) but not in the heterozygotes subjects (PAV/AVI). Since PROP medium-tasters (PAV/AVI) carry the most frequent genotype across different populations (Feeney et al., 2014; Fischer et al., 2013), and they are more likely to be heterozygous for both PAV haplotype and A/G genotype (PAV/AVI-AG) showing intermediate papillae densities (Barbarossa Tomassini et al., 2015), it is possible that in the present study (which did not consider genotypes) we have had a higher prevalence of gustin heterozygotes subjects with an intermediate FPD. Therefore, we failed to find significant relationship between FPD and PROP responsiveness.

However, while positive associations between TAS2R38 genotype and FPD have been later supported by findings that PAV/PAV subjects had higher papillae densities than PAV/AVI and AVI/AVI individuals (Melis et al., 2013), other studies have found no significant differences in FPD between the TAS2R38 haplotype groups (Duffy et al., 2004a; Fischer et al., 2013; Feeney et al., 2014; Garneau et al., 2014; Barbarossa Tomassini et al., 2015). Again, this suggests that complex genetic factors (some of which unknown) may interact and obscure the relationship FPD-PROP responsiveness.

All considered, the determination of these indices still has some unexplored elements.

We conclude that a linear relationship between FPD and PROP bitterness intensity shouldn’t be expected in studies which consider these indices phenotypically.

We also highlight that, while PROP responsiveness has been extensively studied in terms of methods to estimate it and genetic factors contributing to it, the FP have been less investigated in terms of genetic

factors, approaches to correctly quantify FP, and relationships between the detection of the anatomical structure and its functioning.

5.4 Conclusions

Results show that no linear relationship exists between FPD and PROP responsiveness evaluated phenotypically by whole mouth stimulation.

The measurement of FPD may represent a source of variation potentially influencing the nature of the relationship FPD/PROP. Therefore, it should be further explored.

6. Study III. Development of a new automated approach to quantify fungiform papillae on human tongue

Based on findings of Study II, the measurement of FPD may represent a remaining source of variation potentially influencing the nature of the relationship FPD-PROP. In fact, manual count for FP quantification suffers from some bias.

To exclude that bias associated to manual count obscure the relationship FPD-PROP, a new approach for the automated quantification of FP on human tongue was proposed.

The method is presented in the published paper attached below, at the end of this chapter (Piochi et al., 2017).

The technique used a script developed with Matlab software, which counted circular-like elements on the tongue (papillae) on modified images for different diameter sizes.

6.1 Supplemental discussion

Despite not solving the problem of the direct relationship between FPD-PROP, the method highlighted a last interesting point. The dimension of FP (diameter size), not only the number, may have a potentially important effect on taste perception.

In fact, keeping the number of FP constant, we found that subjects with a smaller FP diameter tended to perceive higher salty taste than those with larger size.

This result need to be further confirmed in a larger size population, but a small FP diameter has been positively related to tongue tactile acuity and PROP responsiveness (Essick et al., 2003).

More generally, the use of automated analysis with the possibility to estimate the size distribution may help to clarify the associations between FP and oral functionality.

The diameter of fungiform papillae varied 1.9-fold (from 0.51 to 0.97 mm) in one study of healthy adults (Essick et al., 2003). Another study gave an estimation of the range of 0.42–1.15 mm in adults and 0.35-0.91 mm in children (Segovia et al., 2002).

Therefore, a great individual variation exists not only in FP number but also in FP morphology.

It was shown that FP can be characterised by a certain degree of distortion, measured as spatial distortion in FP diameters measured in four dimensions (at 0, 45, 90 and 135°) (Melis et al., 2013).

Interestingly, the distortion and the percentage of distorted papillae depended on gustin genotype (Melis et al., 2013). GG gustin genotypes showed higher percentage of distorted papillae and FP with significantly larger diameters, than the other genotypes (AG, AA) (Melis et al., 2013).

These variations in morphology affected PROP responsiveness, so that subjects with more distorted FP (characterised by GG gustin genotype) were less responsive to PROP (had significantly higher thresholds) (Melis et al., 2013).

Thus, the variation in papillae functionality according to their size might be a further important point to explain the association between FPD and the perception of oral sensations in general.

Hypothetically, the FP shape could be indirectly related to the taste pores density or to the degree of innervation, therefore influencing the functionality.

6.2 Conclusions

A novel procedure was developed to automatically count fungiform papillae based on the automated analysis of tongue pictures. FPD predicted from automated analysis output showed good agreement with data from manual count. Due to the high reliability to output from manual count, the inexpensive and portable equipment, and reduced time required to process the images, the proposed method appears a reliable and easy substitute for manual counting when the purpose is subject classification according to FPD variation.

Importantly, patterns of FP number (density) and morphology (diameter size) were found, which seemed associated to diversity in taste response for salty.

These results open interesting scenarios in the study of the functionality of FP in relationship to shape and dimension.

Chemical Senses, 2017, Vol 42, 553–561
doi:10.1093/chemse/bjx035
Original Article
Advance Access publication June 1, 2017

Original Article

Comparing Manual Counting to Automated Image Analysis for the Assessment of Fungiform Papillae Density on Human Tongue

Maria Piochi[1], Erminio Monteleone[1], Luisa Torri[2], Camilla Masi[1], Valérie Lengard Almli[3], Jens Petter Wold[3] and Caterina Dinnella[1]

[1]GESAAF, University of Florence, Via Donizetti, 6, 50144 Florence, Italy, [2]University of Gastronomic Sciences, Piazza Vittorio Emanuele 9, 12060 Bra, CN, Italy and [3]NOFIMA, Postboks 210, NO-1431 Ås, Norway

Correspondence to be sent to: Maria Piochi, University of Florence, Via Donizetti, 6, 50144 Florence, Italy. e-mail: maria.piochi@unifi.it

Editorial Decision 23 May 2017.

Abstract

The density of fungiform papillae (FPD) on the human tongue is currently taken as index for responsiveness to oral chemosensory stimuli. Visual analysis of digital tongue picture and manual counting by trained operators represents the most popular technique for FPD assessment. Methodological issues mainly due to operator bias are considered among factors accounting for the uncertainty about the relationships between FPD and responsiveness to chemosensory stimuli. The present study describes a novel automated method to count fungiform papillae (FP) from image analysis of tongue pictures. The method was applied to tongue pictures from 133 subjects. Taking the manual count as reference method, a partial least squares regression model was developed to predict FPD from tongue automated analysis output. FPD from manual and automated count showed the same normal distribution and comparable descriptive statistic values. Consistent subject classifications as low and high FPD were obtained according to the median values from manual and automated count. The same results on the effect of FPD variation on taste perception were obtained both using predicted and counted values. The proposed method overcomes count uncertainties due to researcher bias in manual counting and is suited for large population studies. Additional information is provided such as FP size class distribution which would help for a better understanding of the relationships between FPD variation and taste functions.

Key words: density, individual differences, prediction, size, taste intensity

Introduction

The fungiform papillae (FP) are the anatomical structures involved in the detection and transduction of oral stimuli. Together with foli- ate and circumvallate papillae, FP are considered gustatory papillae since they carry taste receptors (Engelen 2012).

FP are innervated by the chorda tympani (responsible for taste signals) and by the trigeminal nerve (associated to the somatosensory perception) (Whitehead et al. 1985; Prescott et al. 2004). Due to these double innervations, FP has been taken as a relevant oral responsiveness marker. Human subjects show large variations in FP density (FP/cm^2 – FPD), from 0.0 (Webb et al. 2015) to 233.0 (Zhang et al. 2009). The fundamental assumption is that, the higher is the FPD, the more intense is the signal sent to the central system and the higher is the perceived intensity. Taste bud density varies among humans from 374 to 135 pores/cm^2 and not all FP bear taste buds (Miller and Reedy 1990b; Segovia et al. 2002). Thus, even if significant associations have been reported between taste pores and FP densities (Miller and Reedy 1990a, 1990b), the higher FPD

values might not necessarily correspond to the more intense stimulation. Several studies confirmed the positive relationship between FPD and responses to taste (Miller and Reedy 1990b; Bartoshuk 2000; Delwiche et al. 2001; Yackinous and Guinard 2002; Hayes et al. 2008) and somatosensations (Duffy et al. 2004a, 2004b; Hayes and Duffy 2007; Nachtsheim and Schlich 2013). On the other hand, more recent studies failed to find a relationship between FPD and responsiveness to oral stimuli (Fischer et al. 2013).

Issues related to the methodology for FP identification and counting have been invoked among reasons responsible for controversial relationships found between FPD and oral responsiveness to chemosensory stimulation (Nuessle et al. 2015; Sanyal et al. 2016). Visual inspection of digital pictures of blue stained tongue, followed by manual counting by trained operators, represents the most popular technique for FPD assessment since when digital camera was validated as suitable substitute for videomicroscopy (Shahbake et al. 2005). The use of digital camera does not allow the taste bud detection, thus impairments in the identification of gustatory FP (carrying taste pores) and not gustatory FP (without taste pores) can occur and this might partially account for uncertainty of relationships between FPD assessed by visual digital picture inspection and taste responsiveness.

According to Miller and Ready (1990a) description, FP are identified as round, elevated, and pink or stained lighter structures on the blue tongue background. However, FP identification suffers from researcher bias since often papillae can fail to meet every criterion and operators subjectively prioritize the importance of different characteristics leading to FP identification (Nuessle et al. 2015). Thus, highly variable counts can result from the same tongue image analyzed by different operators. A guideline called Denver Papillae Protocol has been developed to help in FP identification and to improve scoring consistency between operators (Nuessle et al. 2015). Bias related to the manual FP count can be even more severe in large population studies when thousands of pictures must be visually analyzed and several operators, even working in different locations, are in charge for counting. The adoption of a shared standardized protocol to help in FP identification, together with a quite intensive operator training, can reduce but not fully remove the operator bias in FP count (Garneau et al. 2014).

Another limitation of manual counts relates to dimension and location of the considered tongue area. In fact, to simplify and speed the count, only restricted areas of the tongue picture are visually analyzed and relevant counts used to infer the overall FPD value. FP is unevenly distributed all over the anterior two-third of the tongue (Jung et al. 2004). Wide differences between distribution of papillae of individuals have been reported, with some having high density on the tip whereas others exhibit more even distribution across the anterior area (Miller 1986). Furthermore, the correlations among counts performed in small different area of the anterior part of the tongue are highly variable (Shahbake et al. 2005). All these aspects add variability in FPD visual estimation thus further impairing the investigation of relationships between FPD and taste function.

Automated image analysis could be a very useful tool to standardize FP count and to improve the consistency of data. Recently, 2 studies have been conducted to automatically count FP on human tongue (Sanyal et al. 2016; Valencia et al. 2016), demonstrating the increasing interest towards this issue. However, these methods have some limitations related to the need of manual intervention, to the restriction of tongue area suitable for the analysis (Valencia et al. 2016) and the relatively small number of pictures considered to test the correlation between automated and manual count (Sanyal et al. 2016).

This article presents a novel automated procedure for FPD estimation based on the analysis of digital pictures taken with a digital microscope. The relationships between automated method response and manual counting were investigated. A multivariate model was proposed for FPD prediction from automated analysis outputs. The effect of the variation of FPD from manual and automated count on the perceived intensities of supra-threshold taste solutions was explored.

Advantages are the complete automation of the procedure and the analysis of large portions of the tongue, thus overcoming the main factors responsible for bias in manual count; the device for picture acquisition is portable and inexpensive and the time required to process the images and estimate FPD is strongly reduced, thus the method is suited to handle the large size sample from population studies aimed at investigating relationships between FPD and oral responsiveness. Finally, the proposed image analysis procedure adds information on FP size distribution that was not previously available with manual counting method.

Materials and methods

Subjects

One hundred thirty-three subjects (33% males; aged from 18 to 65 years, mean age = 32) were recruited in 2 sensory analysis laboratories in Italy (University of Florence; University of Gastronomic Science in Pollenzo). Participants were part of the extended "Italian Taste" project, which envisaged the collection of a wide range of data, including pictures of their tongues (Monteleone et al. 2017). The whole procedure of the "Italian Taste" project was approved by the Ethical Committee of the IRCCS Burlo Garofolo Children Hospital of Trieste (Italy). The present study complies with the Declaration of Helsinki for Medical Research involving human subjects. Subjects had no history of disorders of oral perception. Written informed consent was obtained from each subject prior the experiment.

Acquisition of tongue images

Participants were asked to rinse their mouth before the beginning of the test. Subjects were seated with the tongue held by a holder. The anterior portion of the dorsal surface of the tongue was swabbed with household blue food coloring (F.lli Rebecchi), using a cotton-tipped applicator. Pictures of the tongue were recorded using a portable USB digital microscope (2.0 mega pixels image sensor, MicroCapture version 2.0 bundle software, ×20 to ×400 magnification ratio) (Masi et al. 2015). Pictures captured both the anterior part of the tongue and a ruler fixed behind the tongue which provided a spatial calibration. The picture acquisition had duration of around 5–10 min per subject. From each picture a rectangle (400 × 200 pixels, area = 1.125 cm^2), orthogonal to the median line and located 0.5 cm from the tongue tip, was selected. The selection was saved as image in JPG format (96 dpi) using the ImageJ software (ver. 1.50i, National Institutes of Health, USA). The selected area was chosen as representative of FPD on the whole tongue (Shahbake et al. 2005; Correa et al. 2013).

Manual count

Tongue images were modified with ImageJ (Color Inspector 3D plugin: saturation= ×2.49, brightness = −23.0) to make the visual count easier. Two operators, blind to any data concerning subjects, trained according to the Denver Protocol (Nuessle et al. 2015)

and with 1-year experience, independently counted FP. The counts from the 2 operators were submitted to 1-way fixed ANOVA. Counts were considered valid if the operator effect was not significant ($P > 0.05$). The mean FP number from valid counts was used for each image and expressed as density (FP/cm^2 – FPD).

Automated count

A script was developed with the software Matlab (Mathsworks, ver. R2015a) (Appendix) based on the procedure used by Kraggerud et al. (2009). The script analyzed the image of each subject (Figure 1a) in 3 automated steps: 1) correction of the background variation and graphical emphasis of the elevated structures providing an image with black background and white spots (Figure 1b); 2) identification of circular-like elements among the white spots (Figure 1c); 3) computing the frequency of circular-like elements in classes with varied diameter size (DS) (Figure 1d). The script was set up to return 11 classes in the range from 8 to 28 pixels (0.30–1.05 mm: DS 1 = 0.30–0.36, DS 2 = 0.37–0.43, DS 3 = 0.44–0.49, DS 4 = 0.50–0.56, DS 5 = 0.57–0.63, DS 6 = 0.64–0.70, DS 7 = 0.71–0.77, DS 8 = 0.78–0.84, DS 9 = 0.85–0.91, DS 10 = 0.92–0.98, DS 11 = 0.99–1.05). The 11 DS classes covered a diameter's range slightly larger than the average variation of FP size (Segovia et al. 2002).

Sensory evaluations

Five water solutions, corresponding to 5 basic tastes, were rated for intensity. The concentration of the tastants was selected in order to obtain solutions equivalent to moderate/strong on a generalized labelled magnitude scale—gLMS (sourness: 4.0 g/kg of citric acid, bitterness 3.0 g/kg caffeine, sweetness 200.0 g/kg sucrose, saltiness: 15.0 g/kg sodium chloride, umami 10.0 g/kg monosodium glutamate) (Monteleone et al. 2017). Subjects were trained to the use of gLMS (0: *no sensation* to 100: *the strongest imaginable sensation of any kind*) following published standard procedure (Green et al. 1996; Bartoshuk 2000). Subjects are instructed to treat the "strongest imaginable sensation" as the most intense sensation they can imagine that involves remembered/imagined sensations in any sensory modality. Water solutions (10 mL) were presented in 80 cc plastic cups identified by a 3-digit code. Subjects were presented with a set consisting of the 5 water solutions. The presentation order of water solutions was randomized across subjects. Subjects were instructed to hold the whole water solution sample in their mouth for 10 s, then expectorate and evaluate the intensity of relevant target sensation on gLMS. After each sample, subjects rinsed their mouths with distilled water for 30 s had some plain crackers for 30 s and rinsed their mouths with water for a further 30 s. Evaluations were performed in individual booths under white lights. Data were collected with the software Fizz (ver.2.47.B, Biosystemes).

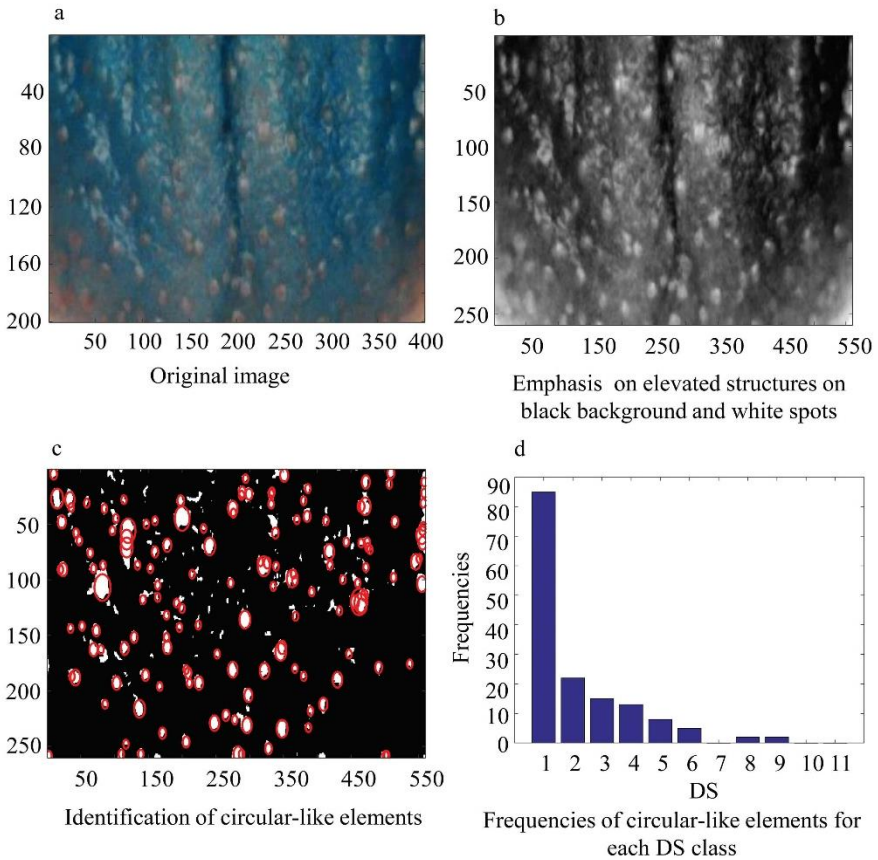


Figure 1. Scheme of automated analysis steps operated by Matlab script.

Data analysis

The normality assumption of the FPD distributions from manual count (FPDm) and predicted from automated image analysis (FPDp) was tested by the Shapiro–Wilk W test ($\alpha = 0.05$) and by Pearson skewness test. The 2 distributions were compared with Kolmogorov–Smirnov test ($\alpha = 0.05$).

ANCOVA using Type III sum of square was performed to assess gender and age effects on FPDm and FPDp, independently (significant for $P \leq 0.05$).

Principal component analysis (PCA) was computed on frequencies of the 11 DS of each image. FPDm was included as supplementary variable. A visually oriented approach, based on the inspection of correlation loading plot, was used for grouping images and Y-axis was set as limit (Næs et al. 2010). The distribution along the PC2 of images on the left and on the right of the map was described by the box plots of their coordinate on the PC2.

A partial least squares regression (PLSR) model (full cross validation, Kernel algorithm, 100 interactions) was applied to predict the FPD from the image analysis output, using the DS classes as explanatory variables (X) and the FPD from manual count as dependent variable (Y). In order to test the model, the image data set was split into a calibration ($n = 100$) and a prediction ($n = 33$) set. The observations for the prediction set were systematically selected to fully cover the FPDm variation across images. Three outliers were removed from the original calibration set, due their high residuals (2 observations) or high leverage value (1 sample). The model was full cross validated on 97 samples and then applied to the prediction set.

Images were split in low (L) and high (H) FPD according to the median of the FPDm and FPDp data sets. Two group of subjects were identified in each data set: L-FPDm (≤FPDm median value) and H-FPDm (>FPDm median value); L-FPDp (≤FPDp median value) and H-FPDp (>FPDp median value).

Unpaired *t*-tests (significant for $P \leq 0.05$) were used to compare intensity ratings from low-FPDm to low-FPDp, and from high-FPDm to high-FPDp, for each stimulus.

ANCOVA models using Type III sum of square with FPD variation as main factor (2 levels: H and L) and age as covariate were applied on intensity ratings, for each stimulus independently (significant for $P \leq 0.05$).

H-FPDp subjects were categorized as mainly associated to DS with smaller diameter (DS 1–4) and mainly associated to DS with larger diameter (DS 7–11) based on the characteristic values of the percentile distribution of their coordinate values on PC2 (small size ≤first tertile; large size ≥ second tertile). Unpaired *t*-tests (significant for $P \leq 0.05$) were used to compare intensity ratings from small size to large size subjects.

All data analysis was performed with XLStat 2016.05 (Addinsoft). PLSR model was computed using The Unscrambler® (ver. 10.4 – © 2016 CAMO Software AS).

Results

Manual count

The manual count had an error of 2.3 FPD, measured as mean of standard deviations given by the 2 operators for each image. The distribution of FPDm across the 133 subjects tended to a normal distribution ($W = 0.968$; $P = 0.004$) with data skewed to the right (Figure 2a).

Descriptive statistic of FPDm is reported in Table 1, with a mean value of 37.2 and limits of the percentile distribution for first and

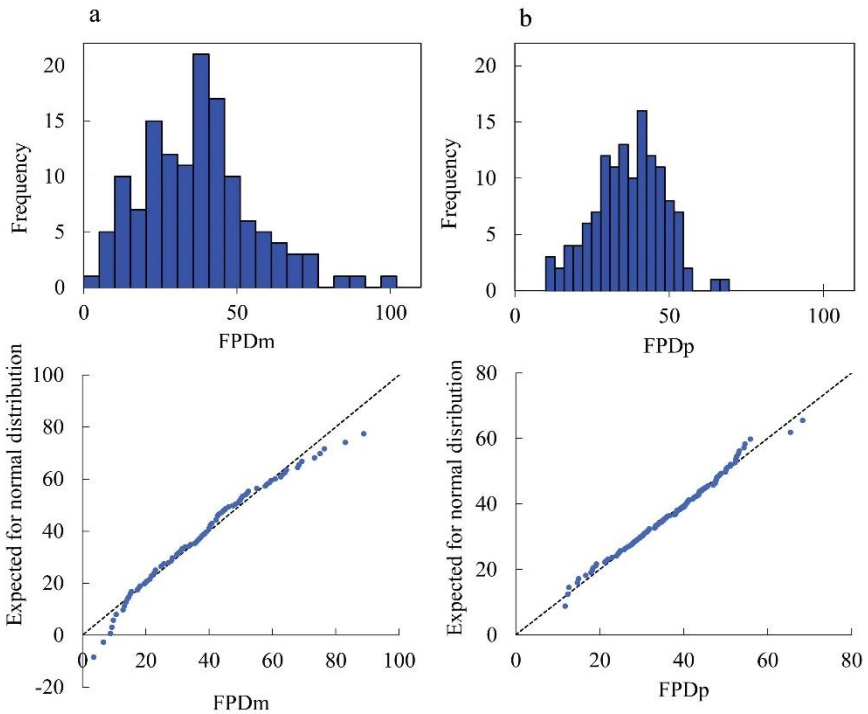


Figure 2. Distribution and q-q plots of papillae density from manual count (FPDm) and predicted from automated analysis outputs (FPDp).

third quartile of 23.1 and 46.2, respectively. No significant effect of gender on FPDm was found ($F = 1.13$; $P = 0.29$); FPDm significantly decreased with aging ($F = 16.53$, $P < 0.0001$). No significant interaction gender × age were found ($F = 1.49$; $P = 0.22$).

Image analysis output

Similarities and differences among images in frequencies of DS classes are visualized in the correlation loading plot from PCA (Figure 3). The first 2 principal components accounted for 66.9% of the total variability (PC1 contributing with 46.5%). Tongue images were evenly spread across the bi-dimensional space. Image positioning along the first component was positively associated to the increase of frequencies of all DS classes. PC2 contributed to separate images according to the size of the classes. Images positioned on the bottom of the bi-dimensional space were mainly associated to the smaller size DS classes (DS 1–5, 0.30–0.63 mm) while images positioned on the top of the map were associated to the larger size DS classes (DS 7–11, 0.71–1.05 mm).

The projection of FPDm on the map indicated a positive association to PC1, thus tongue images positioned on the left were characterized by a lower FPDm than images positioned on the right. The map visual inspection indicated that images positioned on the right were more spread along the PC2 than images on the left, thus indicating a wider diameter variation (Figure 4).

Four image groups were tentatively identified according to their position on the map (Figure 5): group 1 (left-top) negatively related to both FPDm and frequencies of DS classes and mainly associated to DS classes with the large diameter, group 2 (right-top) positively associated to both FPDm and frequencies of DS classes and mainly associated to DS classes with large diameter; group 3 (right-bottom) positively associated to both FPDm and frequencies of DS classes and mainly associated to DS classes with small diameter; group 4 (left-bottom) negatively associated to FPDm and frequencies of DS classes and mainly associated to DS classes with small diameter.

Table 1. Descriptive statistics of papillae density from manual count (FPDm) and predicted from automated image analysis output (FPDp)

Descriptive statistics	FPDm	FPDp
Observations (n)	133	130
Min	3.56	11.8
Max	101.33	68.4
1° quartile	23.11	29.6
Median	37.33	38.1
3° quartile	46.22	44.9
Mean	37.25	37.1
Standard deviation (n – 1)	17.96	11.1

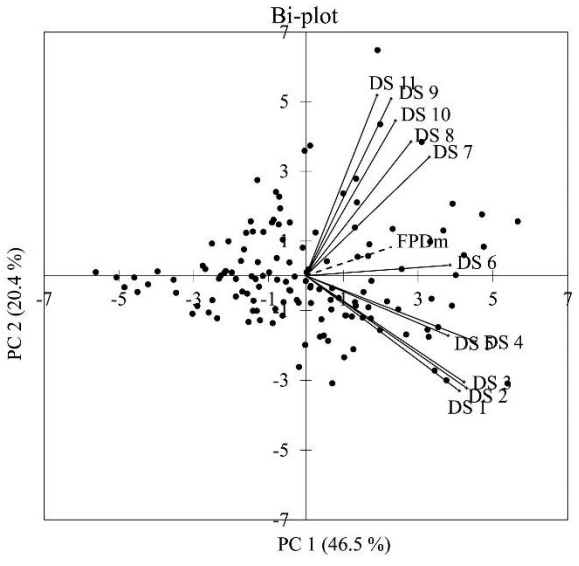


Figure 3. Bi-plot from PCA on frequency values of DS classes (DS 1–11) from 133 observations. Papillae density from manual count (FPDm) is plotted as supplementary variable (dotted line).

Prediction of FPD from automated analysis output

The PLSR was full-cross validated. The calibration (RMSEC) and cross-validation (RMSECV) errors were 12.4 and 13.9 FPD, respectively. Calibration and validation R values were 0.7 and 0.6, respectively. The first PLSR component explained 46% of the X variables (DS frequencies) and 31% of the Y variable (FPDm). The second PLSR component explained 8% of the X variables and 14% of the Y variable. The first PLSR dimension separated observations based on the frequencies of DS classes. The opposition of DS 5–7 versus DS 1–4 was responsible for sample separation along the second dimension. The regression of predicted versus manually counted FPD for the validation of the training model is shown in Figure 6.

To test the model's predictive ability, the model was run on the prediction set, showing an error of prediction (RMSEP) of 13.9 FPD, in line with that found in cross-validation.

The distribution of predicted FPD (FPDp) across the 130 subjects followed a normal distribution ($W = 0.99$; $P = 0.46$) (Figure 2b). Descriptive statistic of FPDp is reported in Table 1, with a mean value of 37.1 and limits of the percentile distribution for first and third quartile of 29.6 and 44.9, respectively. No significant differences were found between distributions from manual and automated count ($D = 0.15$; $P = 0.12$). No significant effect of gender on FPDp was found ($F = 1.99$; $P = 0.16$); FPDp significantly decreased with

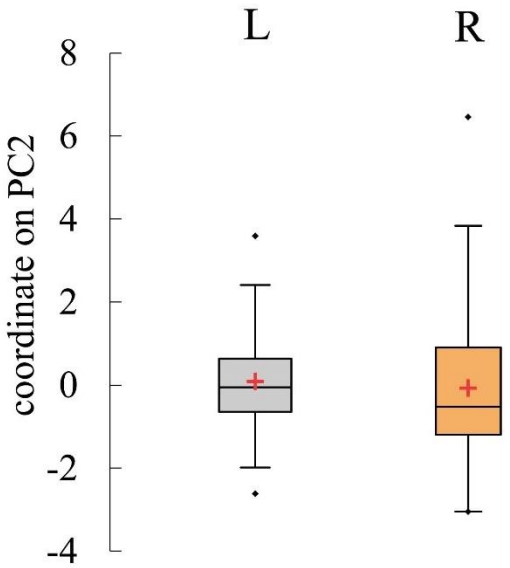


Figure 4. Box plots of coordinate on PC2 of images positioned on the left (L) and on the right (R) of the PCA. Median (line) and mean (cross) values.

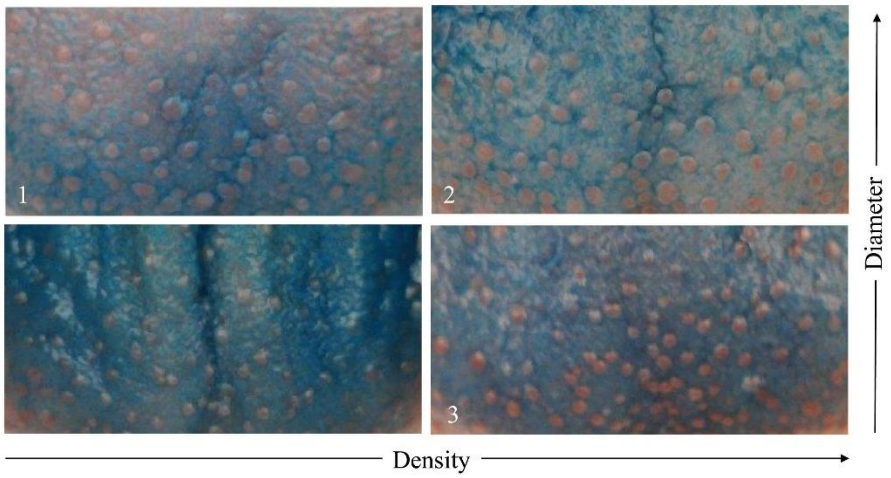


Figure 5. Images representative of 4 groups with varied FP density and diameter, according to the positioning on PCA: group 1 low density and large diameter; group 2 high density and large diameter; group 3 high density and small diameter; group 4 low density and small diameter. Arrows indicate the increase of the observed characteristics.

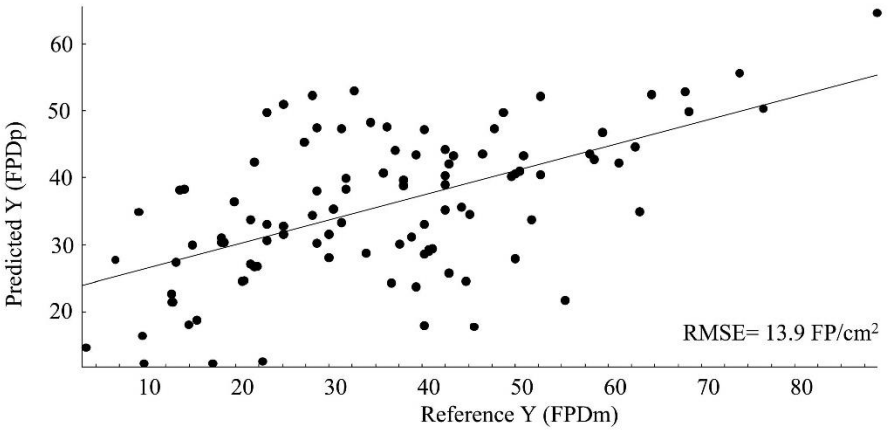


Figure 6. Relationships between FPD from manual count (FPDm) and predicted by PLSR model from automated analysis output (FPDp). Model was build using 11 DS classes as explanatory variables (X) and the FPDm as dependent variable (Y). RMSE = root mean square error.

aging ($F = 5.52$, $P < 0.02$). No significant interaction gender × age was found ($F = 2.28$; $P = 0.13$).

Comparison between counted and predicted FPD as indicators for taste functions

Taste solutions were all rated almost at strong intensity on the gLMS (mean value and standard error: sourness 31.2 ± 1.7; bitterness 31.1 ± 1.8; sweetness 40.1 ± 1.5; saltiness 35.6 ± 1.8; umami 30.0 ± 1.8).

Ratings by subjects grouped as L and H according to the median of manually counted (L-FPDm from 3.6 to 37.3, $n = 68$; H-FPDm from 38.0 to 101.3, $n = 65$) and predicted FPD (L-FPDp from 11.8 to 38.1, $n = 66$; H-FPDp from 39.0 to 68.4, $n = 64$) were independently compared. No significant intensity differences were found comparing L-FPDm to L-FPDp ($P \geq 0.63$) and H-FPDm to H-FPDp ($P \geq 0.54$).

The effect of FPD variation on perceived taste intensity was assessed comparing ratings from L and H groups. A significant effect of FPD variation was found for saltiness ratings. L-FPD rated saltiness higher than H-FPD (L vs. H FPDm: $F = 4.50$; $P = 0.03$; L vs. H FPDp: $F = 6.46$; $P = 0.01$). No significant effect of FPD variation was found on perceived intensity of sourness, bitterness, sweetness, and umami ($P \geq 0.218$). Age did not significantly influence taste ratings ($P \geq 0.140$).

The effect of variation in FP size on the perceived taste intensity was assessed within H-FPDp group. H-FPDp subjects with small size FP (coordinate value on PC2 ≤ −0.884; $n = 16$) tended to rated intensity of taste solutions significantly higher than subjects with large size FP (coordinate value on PC2 ≥ 0.418; $n = 17$) ($t_{163;197} = 1.85$; $P = 0.06$).

Discussion

In the present study, a novel automated procedure for FPD estimation based on the analysis of tongue pictures taken with a digital microscope is described. Results from automated image analysis were compared to those from manual count taken as reference.

The FPDm distribution across observations tended to a normal distribution (Segovia et al. 2002; Zhang et al. 2009; Webb et al. 2015). The mean was similar to values reported in studies using analogous counting procedures on the same portion of the tongue (Segovia et al. 2002; Shahbake et al. 2005; Correa et al. 2013; Feeney and Hayes

2014a; Webb et al. 2015). Aging confirms as negative predictor of papillae density (Correa et al. 2013; Fischer et al. 2013; Pavlidis et al. 2013). No effect of sex on FPD was found, in agreement with studies performed on similar sample size and females/males ratio (Bajec and Pickering 2008; Feeney and Hayes 2014a). In general, results from manual count were in line with existing findings, thus supporting the reliability of the data set taken as reference.

The script used to analyze images identifies circular elements in a diameter ranging from 0.30 to 1.05 mm and covers the expected variation of FP diameter on tongue of adults (Essick et al. 2003). PCA confirmed the positive association between the number of circular elements and the papillae density assessed by manual count. The association to classes of circular elements with varied diameters contributed to discriminate among tongue images. The variation of diameter size was more evident in images associated to high than low papillae density. Automated analysis outputs allowed a tentative visual image classification based on the variation of both density and size of FP.

Automated image analysis output was significantly related to papillae density variation. The predictive model explained 60% of variance among images.

The images used to build the predictive model can be considered as representative of field experimental data set since no inclusion criteria were adopted for the picture clarity and uniformity of tongue blue coloring. The only condition was that the 2 operators independently agreed on the papillae count. Thus, despite a prediction error of 13.9 FPD, the reliability of the model is considered encouraging.

In general, results from predicted papillae density matched those from manual count. The influence of the population demographics (age and sex) on the variation of papillae density predicted by the model was coherent with findings observed on data from manual count. Predicted values showed a normal distribution as expected for the variation of papillae density across adult individuals and superimposed the distribution of data from manual count. Median, mean values and limits of percentile distribution are widely used to categorize subjects as low and high papillae density in studies aimed to investigate the relationships between papillae density and taste functions (Hayes and Duffy 2008; Bakke and Vickers 2011; Masi et al. 2015). Descriptive statistics values of FPDm and FPDp were in good agreement thus providing very similar subject segmentation according to FPD variation. The consistency in subject classification was further highlighted by the same mean ratings for taste solutions observed in subject groups classified as low or high papillae density according to the median value of counted and predicted FPD. The same results on the effect of FPD variation on taste perception were obtained both using predicted and counted values. FPD variation failed to explain perceived intensity of bitterness, sourness, sweetness, and umami in line with recent studies (Fischer et al. 2013). Only the perception of saltiness intensity was significantly affected by the variation of papillae density. Subjects categorized as high FPD rated saltiness lower than subjects categorized as low FPD both using the median of counted and predicted density. The influence of papillae density on the perceived intensity of saltiness from sodium chloride is still controversial. FP associated to heightened saltiness perception on the tongue tip (Miller and Reedy 1990b; Doty et al. 2001) but may not explain whole mouth saltiness (Hayes et al. 2008). Hayes et al. (2010) already reported an inverse relationship between saltiness perception and papillae density in complex stimuli (Hayes et al. 2010). Intensity ratings from whole-mouth and regional stimulation are significantly correlated even if at varying extent for different prototypical tastes (Feeney and Hayes 2014b). The lack of uniformity in the procedures adopted for stimulation can be seen as a further reason for uncertainty of association between FPD and taste responsiveness in the existing literature. However, the variation of responsiveness to different tastes across different regions of the tongue is still controversial and other indices of oral responsiveness (e.g., thermal taste) appear to be involved in regional responsiveness (Cruz and Green 2000). Intensity responses from whole-mouth stimulation are considered reliable proxy of the average individual oral responsiveness and still appear the most appropriate and ecological stimulation procedure in studies aimed at investigating association between food perception and preference (Törnwall et al. 2012; Monteleone et al. 2017). Investigating the relationships between FPD variation and taste functioning is behind the aim of the present study. The study rather focuses on the comparison between methods. The proposed automated image analysis of tongue pictures appears a reliable substitute for manual counting when the purpose is subject classification according the papillae density.

It is worthy to note that the proposed automated analysis allowed an explorative analysis on the role of papillae size in taste function. High papillae density seemed to be associated to a wider size variation. Subjects with small size papillae perceived higher taste intensity than large size subjects. This result need to be further confirmed in a larger size population. The variation of papillae functionality according to diameter supports the hypothesis that size other than density is a relevant feature for oral chemosensory acuity. Small papillae diameter has been positively related to tongue tactile acuity (Essick et al. 2003), PROP responsiveness and gustin expression (Melis et al. 2013). Thus, the variation in papillae functionality according to their size might be a further bias impairing investigations on the association between papillae density and perceived taste intensity. The use of automated analysis with the possibility to estimate the size distribution may help to clarify these associations.

Some considerations can be done considering strengths and weaknesses of the presented method. The distortion degree has previously been suggested as potentially having an effect on taste function (Melis et al. 2013) and could further contribute to explain the association between FP density and taste perception. Other proposed methods for automated papillae detection make this measure available (Sanyal et al. 2016) while the script adopted in the present study did not. The possibility to include the detection of distortion degree in circular-like elements detection deserves further investigations. Moreover, the script may be further developed to handle unstained tongues, in order to eliminate this step which is somewhat annoying for subjects and to avoid technical issues due to the lack of background uniformity (Valencia et al. 2016). The number of observations higher than in the previous studies on methods alternative to manual counting (Sanyal et al. 2016; Valencia et al. 2016) represents one of strength points of the present study. Another positive aspect is that the area to be analyzed can be easily changed (extended/reduced or moved) allowing to investigate different areas and improving reliability of the count as representative of the whole tongue. The developed approach is well suited for large field experiments, even involving different teams in different locations, for the following reasons: 1) the device for pictures acquisition is really inexpensive and can be afforded even by relatively small laboratories, 2) the script is not limited in the number of pictures that can be handled, 3) apart from the selection of the area to be analyzed, the whole procedure is completely automated and takes a few seconds per picture, 4) image analysis can be easily centralized with a core team appointed for the image analysis, without overworking as in the case of manual count where several operators are needed. Further future applications could combine outputs from the proposed technique to in vivo methods (e.g., video microscopy and confocal endomicroscopy) that allow the identification of taste pores or gustatory organs, to gain knowledge on associations between papillae morphological characteristics (e.g., size and relevant distributions) and taste functionality.

Conclusions

The present article describes a novel procedure to count FP based on the automated analysis of tongue pictures. FPD predicted from automated analysis output are in good agreement with data from manual count. The proposed method appears a reliable and easy to handle substitute for manual counting when the purpose is subject classification according to FPD variation. The method fits the requirements of field researches aimed to investigate the relationships between FPD and taste functions in large size population studies. Furthermore, the new method makes available the estimation of the number of papillae for different diameter classes. Future research on larger sample would address the relevance of papillae size on taste functions.

Appendix

The Matlab script (1) and the additional *FindCircleFast* function (2) adopted in the present study are provided below. Both scripts are necessary to properly run the analysis. Scripts must be put in the same folder of images. To run the script, open it in Matlab and press run. The script will automatically stop at the end of operation and provide a table with the frequencies of all RS for all subjects under the section "SizeHist." Frequencies values can be directly exported and used for the analysis.

1. Matlab script

```
Dr=dir('C:\..... \*.jpg');
[ant,dummy]=size(Dr);
texture=zeros(ant,200);
SizesHist=zeros(ant,11);
FileNames=struct2cell(Dr);
FileNames=FileNames(1,1:end);
Sizes=zeros(ant,2);
%%
i_fig = 1;
for K= 1:ant
  filename=[Dr(K).name];
  a=imread(filename,'jpg');
%a=imread('43 (2) contrast.jpg','jpg');
figure(i_fig), i_fig = i_fig + 1;
imagesc(a)
   title(filename)
   figure(i_fig), i_fig = i_fig + 1; imagesc(a(:,:,1));
   a=a(:,:,1);
   D= imresize(a, [260 560]);
   figure(i_fig), i_fig = i_fig + 1; imagesc(D);colormap('gray')

   D=double(D(:,:,1));

   background = imopen(D,strel('disk',15));
D2 = imsubtract(D,background);
   title(filename)
figure(i_fig), i_fig = i_fig + 1;
imagesc(D2)
   title(filename)
eval(['Im', num2str(K),'=D2;']);
D3=D2/max(max(D2));
   D3BW = im2bw(D3,0.3);
   title(filename)
   figure(i_fig), i_fig = i_fig + 1;
imagesc(D3BW)
eval(['ImBW', num2str(K),'=D3BW;']);
S=svd(D2);
   [L,d]=size(S);
   figure(i_fig), i_fig = i_fig + 1;hold on
title(filename)
   plot(log(S))
   texture(K,1:L)=log(S);
   [totVol, radHist] = findCirclesFast(D3BW, K);
title(filename)
   figure(i_fig), i_fig = i_fig + 1;bar(radHist)
   title(filename)
SizesHist(K,:)=radHist;
   pause(1)
end
figure(6);hold off
```

2. FindCircleFast function:

```
function [totVol, radHist] = findCircles(img, imgName)

% Correlation threshold for identification of holes
corrThres = 0.51;
rMin=4;rMax=14;
[M,N] = size(img);
corrMat = zeros(rMax,M,N);

% Calculate correlation images for each radius
for r = rMin:rMax
   circle = getnhood(strel('disk', r, 0));
c = normxcorr2(circle, img);
corrMat(r,:,:) = c(r+1:end-r,r+1:end-r);
end

% Find pixels and corresponding radii with highest correlation
[maxCorr, maxRadius] = max(corrMat,[],1);
maxCorr = squeeze(maxCorr);
maxRadius = squeeze(maxRadius);

% Threshold max-correlation image and identify centroids
maxCorr(maxCorr<corrThres) = 0;
L = bwlabel(maxCorr);
s = regionprops(L, 'Centroid', 'Area');
if (numel(s) == 0)
errordlg('Beklager, ingen hull funnet')
totVol = 0;
radHist = zeros(1,rMax-rMin+1);
return
end
centroids = round(cat(1, s.Centroid));

% Calculate total hole-volume and distribution of hole-sizes
radii = maxRadius(sub2ind(size(maxRadius), centroids(:,2),
centroids(:,1)));
totVol = sum(4/3*pi*radii.^3) / 1000;
radHist = hist(radii, rMin:rMax);

% Optional plotting for debugging purposes
%if (opts.debugplot)
   figure(11)
imagesc(img), colormap(gray)
   hold on
% plot(centroids(:,1), centroids(:,2), 'b*');
fori = 1:size(centroids,1)
drawCircle(centroids(i,1), centroids(i,2), radii(i), 20, 'r');
```

```
end
    hold off
    title(imgName,'Interpreter','None')
%end
end

function h = drawCircle(x, y, r, nseg, S)

theta = 0: (2 * pi / nseg): (2 * pi);
pline_x = r * cos(theta) + x;
pline_y = r * sin(theta) + y;

h = plot(pline_x, pline_y, S,'LineWidth', 2);
end
```

References

Bajec MR, Pickering GJ. 2008. Thermal taste, PROP responsiveness, and perception of oral sensations. *Physiol Behav*. 95:581–590.

Bakke A, Vickers Z. 2011. Effects of bitterness, roughness, PROP taster status, and fungiform papillae density on bread acceptance. *Food Qual Prefer*. 22:317–325.

Bartoshuk LM. 2000. Comparing sensory experiences across individuals: recent psychophysical advances illuminate genetic variation in taste perception. *Chem Senses*. 25:447–460.

Correa M, Hutchinson I, Laing DG, Jinks AL. 2013. Changes in fungiform papillae density during development in humans. *Chem Senses*. 38:519–527.

Cruz A, Green BG. 2000. Thermal stimulation of taste. *Nature*. 403:889–892.

Delwiche JF, Buletic Z, Breslin PA. 2001. Relationship of papillae number to bitter intensity of quinine and PROP within and between individuals. *Physiol Behav*. 74:329–337.

Doty RL, Bagla R, Morgenson M, Mirza N. 2001. NaCl thresholds: relationship to anterior tongue locus, area of stimulation, and number of fungiform papillae. *Physiol Behav*. 72:373–378.

Duffy VB, Davidson AC, Kidd JR, Kidd KK, Speed WC, Pakstis AJ, Reed DR, Snyder DJ, Bartoshuk LM. 2004a. Bitter receptor gene (TAS2R38), 6-n-propylthiouracil (PROP) bitterness and alcohol intake. *Alcohol Clin Exp Res*. 28:1629–1637.

Duffy VB, Peterson JM, Bartoshuk LM. 2004b. Associations between taste genetics, oral sensation and alcohol intake. *Physiol Behav*. 82:435–445.

Engelen L. 2012. Oral receptors. In: Chen J, Engelen L; Wiley-Blackwell, editors. Food oral processing: fundamentals of eating and sensory perception. Chichester (UK): John Wiley and Sons. p. 15–43.

Essick GK, Chopra A, Guest S, McGlone F. 2003. Lingual tactile acuity, taste perception, and the density and diameter of fungiform papillae in female subjects. *Physiol Behav*. 80:289–302.

Feeney EL, Hayes JE. 2014a. Exploring associations between taste perception, oral anatomy and polymorphisms in the carbonic anhydrase (gustin) gene CA6. *Physiol Behav*. 128:148–154.

Feeney EL, Hayes JE. 2014b. Regional differences in suprathreshold intensity for bitter and umami stimuli. *Chemosens Percept*. 7:147–157.

Fischer ME, Cruickshanks KJ, Schubert CR, Pinto A, Klein R, Pankratz N, Pankow JS, Huang GH. 2013. Factors related to fungiform papillae density: the beaver dam offspring study. *Chem Senses*. 38:669–677.

Garneau NL, Nuessle TM, Sloan MM, Santorico SA, Coughlin BC, Hayes JE. 2014. Crowdsourcing taste research: genetic and phenotypic predictors of bitter taste perception as a model. *Front Integr Neurosci*. 8:33.

Green BG, Dalton P, Cowart B, Shaffer G, Rankin K, Higgins J. 1996. Evaluating the "Labeled Magnitude Scale" for measuring sensations of taste and smell. *Chem Senses*. 21:323–334.

Hayes JE, Bartoshuk LM, Kidd JR, Duffy VB. 2008. Supertasting and PROP bitterness depends on more than the TAS2R38 gene. *Chem Senses*. 33:255–265.

Hayes JE, Duffy VB. 2007. Revisiting sugar-fat mixtures: sweetness and creaminess vary with phenotypic markers of oral sensation. *Chem Senses*. 32:225–236.

Hayes JE, Duffy VB. 2008. Oral sensory phenotype identifies level of sugar and fat required for maximal liking. *Physiol Behav*. 95:77–87.

Hayes JE, Sullivan BS, Duffy VB. 2010. Explaining variability in sodium intake through oral sensory phenotype, salt sensation and liking. *Physiol Behav*. 100:369–380.

Jung HS, Akita K, Kim JY. 2004. Spacing patterns on tongue surface-gustatory papilla. *Int J Dev Biol*. 48:157–161.

Kraggerud H, Wold JP, Høy M, Abrahamsen RK. 2009. X-ray images for the control of eye formation in cheese. *Int J Dairy Technol*. 62:147–153.

Masi C, Dinnella C, Monteleone E, Prescott J. 2015. The impact of individual variations in taste sensitivity on coffee perceptions and preferences. *Physiol Behav*. 138:219–226.

Melis M, Atzori E, Cabras S, Zonza A, Calò C, Muroni P, Nieddu M, Padiglia A, Sogos V, Tepper BJ, *et al.* 2013. The gustin (CA6) gene polymorphism, rs2274333 (A/G), as a mechanistic link between PROP tasting and fungiform taste papilla density and maintenance. *PLoS One*. 8:e74151.

Miller I, Reedy F. 1990a. Quantification of fungiform papillae and taste pores in living human subjects. *Chem Senses*. 15:281–294.

Miller IJ. 1986. Variation in human fungiform taste bud densities among regions and subjects. *Anat Rec*. 216(4):474–482.

Miller I, Reedy F. 1990a. Quantification of fungiform papillae and taste pores in living human subjects. *Chem Senses*. 15:281–294.

Miller IJ Jr, Reedy FE Jr. 1990b. Variations in human taste bud density and taste intensity perception. *Physiol Behav*. 47:1213–1219.

Monteleone E, Spinelli S, Dinnella C, Endrizzi I, Laureati M, Pagliarini E, Sinesio F, Gasperi F, Torri L, Aprea E, *et al.* 2017. Exploring influences on food choice in a large population sample: the Italian Taste Project. *Food Qual Prefer*. 59:123–140.

Nachtsheim R, Schlich E. 2013. The influence of 6-n-propylthiouracil bitterness, fungiform papilla count and saliva flow on the perception of pressure and fat. *Food Qual Prefer*. 29:137–145.

Næs T, Brockhoff PB, Tomic O. 2010. *Statistics for sensory and consumer science*. Chichester (UK): John Wiley and Sons.

Nuessle TM, Garneau NL, Sloan MM, Santorico SA. 2015. Denver papillae protocol for objective analysis of fungiform papillae. *J Vis Exp*. 100:e52860. doi:10.3791/52860.

Pavlidis P, Gouveris H, Anogeianaki A, Koutsonikolas D, Anogianakis G, Kekes G. 2013. Age-related changes in electrogustometry thresholds, tongue tip vascularization, density, and form of the fungiform papillae in humans. *Chem Senses*. 38:35–43.

Prescott J, Bartoshuk LM, Prutkin J. 2004. PROP tasting and the perception of non-taste oral sensations. In: Prescott J, Tepper B, editors. *Genetic variation in taste sensitivity*. New York: Marcel Dekker. p. 89–104.

Sanyal S, O'Brien SM, Hayes JE, Feeney EL. 2016. TongueSim: development of an automated method for rapid assessment of fungiform papillae density for taste research. *Chem Senses*. 41(4):357–365.

Segovia C, Hutchinson I, Laing DG, Jinks AL. 2002. A quantitative study of fungiform papillae and taste pore density in adults and children. *Brain Res Dev Brain Res*. 138:135–146.

Shahbake M, Hutchinson I, Laing DG, Jinks AL. 2005. Rapid quantitative assessment of fungiform papillae density in the human tongue. *Brain Res*. 1052:196–201.

Törnwall O, Silventoinen K, Kaprio J, Tuorila H. 2012. Why do some like it hot? Genetic and environmental contributions to the pleasantness of oral pungency. *Physiol Behav*. 107:381–389.

Valencia E, Ríos HV, Verdalet I, Hernández J, Juárez S, Herrera R, Silva ER. 2016. Automatic counting of fungiform papillae by shape using cross-correlation. *Comput Biol Med*. 76:168–172.

Webb J, Bolhuis DP, Cicerale S, Hayes JE, Keast R. 2015. The relationships between common measurements of taste function. *Chemosens Percept*. 8:11–18.

Whitehead MC, Beeman CS, Kinsella BA. 1985. Distribution of taste and general sensory nerve endings in fungiform papillae of the hamster. *Am J Anat*. 173:185–201.

Yackinous CA, Guinard JX. 2002. Relation between PROP (6-n-propylthiouracil) taster status, taste anatomy and dietary intake measures for young men and women. *Appetite*. 38:201–209.

Zhang GH, Zhang HY, Wang XF, Zhan YH, Deng SP, Qin YM. 2009. The relationship between fungiform papillae density and detection threshold for sucrose in the young males. *Chem Senses*. 34:93–99.

8. General Conclusions

Individuals differ greatly in responsiveness to sensory stimuli and this is in part due to differences in oral anatomy.

Since indices exist to estimate taste responsiveness, the present thesis focuses on one of the most controversial indices: the density of Fungiform Papillae (FPD = papillae/cm^2) on human tongue.

In fact, it is still unclear whether the number of Fungiform Papillae (FP) directly relies to the intensity of taste sensations. Moreover, the relationship between FPD and responsiveness to PROP (another fundamental indicator of taste responsiveness) is poorly understood.

Results of the present thesis showed that the FPD index was not correlated to the perceived intensities of tastes or burning from capsaicin, both in water solutions and food matrices. Thus, subjects with a higher number of FP did not show incresed oral responsiveness considering both types of stimuli.

It is suggested that, due to the high variability of taste buds in papillae and the complex neurophysiological mechanisms taking place both at peripheral and central level, the number of papillae in itself does not necessarily reflect differences in perceived intensity of taste.

The quantification of the anatomical structure may not directly reflect the papillae functionality (including the quantification of the taste pores density - relevant for both taste sensations and PROP – or the presence of damaged nerves), especially when using detection techniques which do not imply the assessment of FP functionality (such as digital cameras, or portable microscopes).

Instead, the responsiveness to PROP was confirmed to be an effective index for taste responsiveness and burning from capsaicin, both in water solutions and complex matrices. Subjects highly responsive to PROP (super-tasters) exihibited the highest responsiveness to all oral stimuli, in agreement with most studies available in literature.

Therefore, the PROP status confirmed its role as a strong determinant of taste responsivess.

A linear positive relationship between these two indices (FPD and PROP responsiveness) was not found in the present study, and this result held considered groups of individuals homogeneous for age and gender. Therefore, it is suggested that no positive relationship between these two indices should be expected when both indicators are estimated by phenotypes.

As suggested for responsiveness to tastes, other important factors related to the papillae functionality may have a relevant role in explaining the nature of the relationship FPD-PROP.
Moreover, the combination of some genetic factors contributing to FPD and PROP genotypes (some of which still unknown) may further obscure the nature of this relationship.

A new automated method to count FP was successfully developed in the present thesis, which was reliable to the manual count. In the new approach, papillae with different diameter sizes were quantified. The procedure overcomes important critical issues of a manual count, such as being time-consuming and suffering from bias in prioritising criteria when trained operators count manually.
Moreover, the method highlighted the potential effect of the variation in papillae diameter size in taste responses. In fact, keeping constant the number of FP, subjects with smaller FP diameter perceived at higher intensity tastes, suggesting that the morphology (not only the number) of FP may have an important role in taste responsiveness.
Results open interesting scenarios in the study of papillae patterns, which include both FP number and their size.

In general, since taste responsiveness strongly modulates our food preference and ultimately diet (via reduced/increased sensibility), the understanding of individual variability in taste indices can determinately contribute to explain food behaviours in sensory studies.

References

Bartoshuk L.M., Fast K, Karrer TA et al. (1992). PROP super tasters and the perception of sweetness and bitterness. *Chemical Senses*, 17, 594 (abstract).

Bartoshuk, L.M. (1979). Bitter taste of saccharin: related to the genetic ability to taste the bitter substance 6-n-propylthiouracil (PROP). *Science*, 205, 934–935.

Bartoshuk, L.M., Duffy, V.M., Lucchina, L.A., Prutkin, J., & Fast, K. (1998). PROP (6-n-propylthiouracil) supertasters and the saltiness of NaCl. *Ann. NYAcad. Sci*. 855, 793–796.

Bartoshuk, L.M., Rifkin, B., Marks, L., & Hooper, J. (1988). Bitterness of KCl and benzoate: Related to genetic status for sensitivity to PTC/PROP. *Chemical Senses*, 13, 517–528.

Bajec, M. R., & Pickering, G. J. (2008). Thermal taste, PROP responsiveness, and perception of oral sensations. *Physiology and Behavior*, *95*(4), 581–590. http://doi.org/10.1016/j.physbeh.2008.08.009

Bakke, A., & Vickers, Z. (2008). Relationships between fungiform papillae density, PROP sensitivity and bread roughness perception. *Journal of Texture Studies*, *39*, 569–581.

Bakke, A., & Vickers, Z. (2011). Effects of bitterness, roughness, PROP taster status, and fungiform papillae density on bread acceptance. *Food Quality and Preference*, *22*(4), 317–325. http://doi.org/10.1016/j.foodqual.2010.11.006

Bakke, A., Vickers, Z., Marquart, L., & Sjoberg, S. (2007). Consumer Acceptance of Refined and Whole Wheat Breads. *Whole Grains and Health*, 255–262. http://doi.org/10.1002/9780470277607.ch20

Bangcuyo, R. G., & Simons, C. T. (2017). Lingual tactile sensitivity: effect of age group, sex, and fungiform papillae density. *Experimental Brain Research*, *235*(9), 2679–2688. http://doi.org/10.1007/s00221-017-5003-7

Barbarossa Tomassini, I., Melis, M., Mattes, M. Z., Calò, C., Muroni, P., Crnjar, R., & Tepper, B. J. (2015). The gustin (CA6) gene polymorphism , rs2274333 (A/G), is associated with fungiform papilla density , whereas PROP bitterness is mostly due to TAS2R38 in an ethnically-mixed population. *Physiology & Behavior*, *138*, 6–12. http://doi.org/10.1016/j.physbeh.2014.09.011

Bartoshuk, L. M. (2000). Comparing sensory experiences across individuals: recent psychophysical advances illuminate genetic variation in taste perception. *Chemical Senses*, *25*(4), 447–460. http://doi.org/10.1093/chemse/25.4.447

Bartoshuk, L. M., Catalanotto, F., Hoffman, H., Logan, H., & Snyder, D. J. (2012). Taste damage (otitis media, tonsillectomy and head and neck cancer), oral sensations and BMI. *Physiology and Behavior*, *107*(4), 516–526. http://doi.org/10.1016/j.physbeh.2012.06.013

Bartoshuk, L. M., Duffy, V. B., Fast, K., Green, B. G., Prutkin, J., & Snyder, D. J. (2003). Labeled scales (e.g., category, Likert, VAS) and invalid across-group comparisons: What we have learned from genetic variation in taste. *Food Quality and Preference*, *14*(2), 125–138. http://doi.org/10.1016/S0950-3293(02)00077-0

Bartoshuk, L. M., Duffy, V. B., Green, B. G., Hoffman, H. J., Ko, C. W., Lucchina, L. A., … Weiffenbach, J. M. (2004). Valid across-group comparisons with labeled scales: The gLMS versus magnitude matching. *Physiology and Behavior*, *82*(1), 109–114. http://doi.org/10.1016/j.physbeh.2004.02.033

Bartoshuk, L. M., Duffy, V. B., & Miller, I. J. (1994). PTC/PROP tasting: Anatomy, psychophysics, and sex effects. *Physiology and Behavior*, *56*(6), 1165–1171. http://doi.org/10.1016/0031-9384(94)90361-1

Bell, K. I., & Tepper, B. J. (2006). Short-term vegetable intake by young children classified by.

Calò, C., Padiglia, A., Zonza, A., Corrias, L., Contu, P., Tepper, B. J., & Tomassini, I. (2011). Physiology & Behavior Polymorphisms in TAS2R38 and the taste bud trophic factor , gustin gene co-operate in modulating PROP taste phenotype. *Physiology & Behavior*, *104*(5), 1065–1071. http://doi.org/10.1016/j.physbeh.2011.06.013

Cardello, A. V. (1978). Chemical stimulation of single human fungiform taste papillae: Sensitivity profiles and locus of stimulation. *Sensory Processes*, *2*, 173–190.

Cardello, A. V. (1981). Comparison of taste qualities elicited by tactile, electrical, and chemical stimulation of single human taste papillae. *Perception & Psychophysics*, *29*(2), 163–9. http://doi.org/10.3758/BF03207280

Caterina, M. J., Schumacher, M. a, Tominaga, M., Rosen, T. a, Levine, J. D., & Julius, D. (1997). The capsaicin receptor: a heat-activated ion channel in the pain pathway. *Nature*, *389*(6653), 816–824. http://doi.org/10.1038/39807

Choi, S. E. (2014). Racial differences between African Americans and Asian Americans in the effect of 6-n-propylthiouracil taste intensity and food liking on body mass index. *Journal of the Academy of Nutrition and Dietetics*, *114*(6), 938–944. http://doi.org/10.1016/j.jand.2013.11.015

Collings, V. B. (1974). Human taste response as a function of locus of stimulation on the tongue and soft palate. *Perception & Psychophysics*, *16*(1), 169–174. http://doi.org/10.3758/BF03203270

Correa, M., Hutchinson, I., Laing, D. G., & Jinks, A. L. (2013). Changes in Fungiform Papillae Density During Development in Humans. *Chemical Senses*, *38*(6), 519–527. http://doi.org/10.1093/chemse/bjt022

Cox, D. N., Hendrie, G. A., & Carty, D. (2016). Sensitivity, hedonics and preferences for basic tastes and fat amongst adults and children of differing weight status: A comprehensive review. *Food Quality and Preference*, *48*, 359–367. http://doi.org/10.1016/j.foodqual.2015.01.006

Delwiche, J. F., Buletic, Z., & Breslin, P. a. (2001). Relationship of papillae number to bitter intensity of quinine and PROP within and between individuals. *Physiology & Behavior*, *74*(3), 329–37.

Dinnella, C., Monteleone, E., Gasperi, F., Endrizzi, I., Sinesio, F., Spinelli, S., … S., P. (2016). Exploring the effect of individual differences in taste sensitivity, perception and psychological traits on food preferences among Italians: The Italian Taste project. *Oral Presentation, Eurosense 2016*.

Doty, R. L., Bagla, R., Morgenson, M., & Mirza, N. (2001). NaCl thresholds: relationship to anterior tongue locus, area of stimulation, and number of fungiform papillae. *Physiology and Behavior*, *72*, 373–378. http://doi.org/10.1016/S0031-9384(02)00672-8

Drewnowski, A., & Gomez-Carneros, C. (2000). Bitter taste, phyonutrients, and the consumer: a review. *Am J Clin Nutr*, *72*(22), 1424–1435.

Duffy, V. B., Davidson, A. C., Kidd, J. R., Kidd, K. K., Speed, W. C., Pakstis, A. J., … Bartoshuk, L. M. (2004). Bitter receptor gene (TAS2R38), 6-n-propylthiouracil (PROP) bitterness and alcohol intake. *Alcoholism, Clinical and Experimental Research*, *28*(11), 1629–1637. http://doi.org/Doi 10.1097/01.Alc.0000145789.55183.D4

Duffy, V. B., Hayes, J. E., Davidson, A. C., Kidd, J. R., Kidd, K. K., & Bartoshuk, L. M. (2010). Vegetable intake in college-aged adults is explained by oral sensory phenotypes and TAS2R38 genotype. *Chemosensory Perception*, *3*(3–4), 137–148. http://doi.org/10.1007/s12078-010-9079-8

Duffy, V. B., Peterson, J. M., & Bartoshuk, L. M. (2004). Associations between taste genetics, oral sensation and alcohol intake. *Physiology and Behavior*, *82*(2–3), 435–445. http://doi.org/10.1016/j.physbeh.2004.04.060

Essick, G. K., Chopra, A., Guest, S., & McGlone, F. (2003). Lingual tactile acuity, taste perception, and the density and diameter of fungiform papillae in female subjects. *Physiology and Behavior*, *80*(2–3), 289–302. http://doi.org/10.1016/j.physbeh.2003.08.007

Farbman, A. I., & Mbiene, J. P. (1991). Early development and innervation of taste bud-bearing papillae on the rat tongue. *The Journal of Comparative Neurology*, *304*, 172–186.

Feeney, E. L., Brien, S. A. O., Scannell, A. G. M., Markey, A., & Gibney, E. R. (2014). Genetic and environmental influences on liking and reported intakes of vegetables in Irish children. *Food Quality and Preference*, *32*, 253–263. http://doi.org/10.1016/j.foodqual.2013.09.009

Feeney, E. L., & Hayes, J. E. (2014a). Exploring associations between taste perception, oral anatomy and polymorphisms in the carbonic anhydrase (gustin) gene CA6. *Physiology and Behavior*, *128*, 148–154. http://doi.org/10.1016/j.physbeh.2014.02.013

Feeney, E. L., & Hayes, J. E. (2014b). Regional Differences in Suprathreshold Intensity for Bitter and Umami Stimuli. *Chemosensory Perception*, (February 2016), 147–157. http://doi.org/10.1007/s12078-014-9166-3

Feng, P., Huang, L., & Wang, H. (2014). Taste bud homeostasis in health, disease, and aging. *Chemical Senses*, *39*(1), 3–16. http://doi.org/10.1093/chemse/bjt059

Fischer, M. E., Cruickshanks, K. J., Schubert, C. R., Pinto, A., Klein, R., Pankratz, N., … Huang, G. H. (2013). Factors related to fungiform papillae density: The beaver dam offspring study. *Chemical Senses*, *38*(8), 669–677. http://doi.org/10.1093/chemse/bjt033

Fogel, A., & Blissett, J. (2017). Past exposure to fruit and vegetable variety moderates the link between fungiform papillae density and current variety of FV consumed by children. *Physiology & Behavior*. http://doi.org/10.1016/j.physbeh.2017.04.015

Garneau, N. L., Nuessle, T. M., Sloan, M. M., Santorico, S. a, Coughlin, B. C., & Hayes, J. E. (2014). Crowdsourcing taste research: genetic and phenotypic predictors of bitter taste perception as a model. *Front Integr Neurosci*, *8*(May), 33. http://doi.org/10.3389/fnint.2014.00033

Goldstein, G. L., Daun, H., & Tepper, B. J. (2005). Adiposity in Middle-aged Women is Associated 6-n-Propylthiouracil, *13*(6), 1017–1023.

Green, B. G., Dalton, P., Cowart, B., Shaffer, G., Rankin, K., & Higgins, J. (1996). Evaluating the "labeled magnitude scale" for measuring sensations of taste and smell. *Chemical Senses*, *21*(3), 323–334. http://doi.org/10.1093/chemse/21.3.323

Green, B. G., Lim, J., Osterhoff, F., Blacher, K., & Nachtigal, D. (2010). Physiology & Behavior Taste mixture interactions : Suppression , additivity , and the predominance of sweetness. *Physiology & Behavior*, *101*(5), 731–737. http://doi.org/10.1016/j.physbeh.2010.08.013

Guo, S. W., & Reed, D. R. (2001). The genetics of phenylthiocarbamide perception. *Annals of Human Biology*, *28*(2), 111–142. http://doi.org/10.1080/03014460151056310

Hayes, J. E., Bartoshuk, L. M., Kidd, J. R., & Duffy, V. B. (2008). Supertasting and PROP bitterness depends on more than the TAS2R38 gene. *Chemical Senses*, *33*(3), 255–265. http://doi.org/10.1093/chemse/bjm084

Hayes, J. E., & Duffy, V. B. (2007). Revisiting sugar-fat mixtures: Sweetness and creaminess vary with phenotypic markers of oral sensation. *Chemical Senses*, *32*(3), 225–236. http://doi.org/10.1093/chemse/bjl050

Hayes, J. E., & Keast, R. S. J. (2011). Two decades of supertasting: Where do we stand? *Physiology and*

Behavior, *104*(5), 1072–1074. http://doi.org/10.1016/j.physbeh.2011.08.003

Hayes, J. E., Sullivan, B. S., & Duffy, V. B. (2010). Explaining variability in sodium intake through oral sensory phenotype, salt sensation and liking. *Physiology & Behavior*, *100*(4), 369–380. http://doi.org/10.1016/j.physbeh.2010.03.017

Horne, J., Lawless, H. T., Speirs, W., & Sposato, D. (2002). Bitter taste of saccharin and acesulfame-K. *Chemical Senses*, *27*, 31–38.

Ishimaru, Y., & Matsunami, H. (2009). Transient Receptor Potential (TRP) Channels and Taste Sensation. *Journal of Dental Research*, *88*(3), 212–218. http://doi.org/10.1177/0022034508330212

Jung, H. S., Akita, K., & Kim, J. Y. (2004). Spacing patterns on tongue surface-gustatory papilla. *International Journal of Developmental Biology*, *48*(2–3), 157–161. http://doi.org/10.1387/ijdb.15272380

Just, T., Pau, H. W., Witt, M., & Hummel, T. (2006). Contact endoscopic comparison of morphology of human fungiform papillae of healthy subjects and patients with transected chorda tympani nerve. *The Laryngoscope*, *116*(7), 1216–22. http://doi.org/10.1097/01.mlg.0000224509.61099.29

Kamphuis, M. M. J. W., & Westerterp-Plantenga, M. S. (2003). PROP sensitivity affects macronutrient selection. *Physiology and Behavior*, *79*(2), 167–172. http://doi.org/10.1016/S0031-9384(03)00063-5

Keast, R. S. J., & Roper, J. (2007). A complex relationship among chemical concentration, detection threshold, and suprathreshold intensity of bitter compounds. *Chemical Senses*, *32*(3), 245–53. http://doi.org/10.1093/chemse/bjl052

Kim, U., Jorgenson, E., Coon, H., Leppert, M., Risch, N., Drayna, D., & Kim EricCoon, HilaryLeppert, MarkRisch, NeilDrayna, Dennis, U. (2003). Positional cloning of the human quantitative trait locus underlying taste sensitivity to phenylthiocarbamide. *Science (New York, N.Y.)*, *299*(5610), 1221–1225. http://doi.org/10.1126/science.1080190

Lindemann, B. (2001). Receptors and transduction in taste. *Nature*, *413*(6852), 219–225. http://doi.org/10.1038/35093032

Lipchock, S. V., Mennella, J. A., Spielman, A. I., & Reed, D. R. (2013). Human bitter perception correlates with bitter receptor messenger RNA expression in taste cells 1 – 3. *The American Journal of Clinical Nutrition*, *98*(4), 1136–1143. http://doi.org/10.3945/ajcn.113.066688.INTRODUCTION

Masi, C., Dinnella, C., Monteleone, E., & Prescott, J. (2015). The impact of individual variations in taste sensitivity on coffee perceptions and preferences. *Physiology & Behavior*, *138*, 219–226. http://doi.org/10.1016/j.physbeh.2014.10.031

Melis, M., Atzori, E., Cabras, S., Zonza, A., Calò, C., Muroni, P., … Tomassini Barbarossa, I. (2013). The Gustin (CA6) Gene Polymorphism, rs2274333 (A/G), as a Mechanistic Link between PROP Tasting and Fungiform Taste Papilla Density and Maintenance. *PLoS ONE*, *8*(9), 1–15. http://doi.org/10.1371/journal.pone.0074151

Meng, L., Ohman-gault, L., Ma, L., & Krimm, R. F. (2015). Taste Bud-Derived BDNF Is Required to Maintain Normal Amounts of Innervation to Adult Taste. *eNeuro*, *2*(December), 1–20.

Miller, I. J., & Bartoshuk, L. M. (1991). Taste Perception, Taste Bud Dustribution, and Spatial Relationships. In G. TV, L. M. Bartoshuk, R. L. Doty, & Snow JB. (Eds.), *Smell and Taste in Health and Disease* (Raven Pres, pp. 205–233). New York.

Miller, I. J. (1986). Variation in Human Fungiform Taste Bud Densities Among Regions and Subjects. *The Anatomical Record*, *216*, 474–482. http://doi.org/10.1017/CBO9781107415324.004

Miller, I. J., & Reedy, F. E. (1990). Variations in human taste bud density and taste intensity perception. *Physiology and Behavior*. http://doi.org/10.1016/0031-9384(90)90374-D

Miller, I. J., & Reedy, F. J. (1988). Human taste pore quantification with videomicroscopy. *Chemical Senses*, *13*, 719. http://doi.org/10.1017/CBO9781107415324.004

Miller, I.J., & Reedy, F. (1990). Quantification of fungiform papillae and taste pores in living human subjects. *Chemical Senses*, *15*(3), 281–294. http://doi.org/10.1017/CBO9781107415324.004

Monteleone, E., Spinelli, S., Dinnella, C., Endrizzi, I., Laureati, M., Pagliarini, E., … Tesini, F. (2017). Exploring influences on food choice in a large population sample: the Italian Taste Project. *Food Quality and Preference*. http://doi.org/10.1016/j.foodqual.2017.02.013

Nachtsheim, R., & Schlich, E. (2013). The influence of 6-n-propylthiouracil bitterness, fungiform papilla count and saliva flow on the perception of pressure and fat. *Food Quality and Preference*, *29*(2), 137–145. http://doi.org/10.1016/j.foodqual.2013.03.011

Nachtsheim, R., & Schlich, E. (2014). The influence of oral phenotypic markers and fat perception on fat intake during a breakfast buffet and in a 4-day food record, *32*, 173–183. http://doi.org/10.1016/j.foodqual.2013.10.009

Negoro, A., Umemoto, M., Fukazawa, K., Terada, T., & Sakagami, M. (2004). Observation of tongue papillae by video microscopy and contact endoscopy to investigate their correlation with taste function, *31*, 255–259. http://doi.org/10.1016/j.anl.2004.01.009

Nishiguchi, Y., Ohmoto, M., Koki, J., Enomoto, T., Kominami, R., Matsumoto, I., & Hirota, J. (2016). Bcl11b/Ctip2 is required for development of lingual papillae in mice. *Developmental Biology*, *416*(1), 98–110. http://doi.org/10.1016/j.ydbio.2016.06.001

Nuessle, T. M., Garneau, N. L., Sloan, M. M., & Santorico, S. a. (2015). Denver Papillae Protocol for Objective Analysis of Fungiform Papillae. *Journal of Visualized Experiments*, (100). http://doi.org/10.3791/52860

Oftedal, K. N., & Tepper, B. J. (2013). Influence of the PROP bitter taste phenotype and eating attitudes on energy intake and weight status in pre-adolescents: A 6-year follow-up study. *Physiology and Behavior*, *118*, 103–111. http://doi.org/10.1016/j.physbeh.2013.05.016

Padiglia, A., Zonza, A., Atzori, E., Chillotti, C., Cal??, C., Tepper, B. J., & Barbarossa, I. T. (2010). Sensitivity to 6-n-propylthiouracil is associated with gustin (carbonic anhydrase VI) gene polymorphism, salivary zinc, and body mass index in humans. *American Journal of Clinical Nutrition*, *92*(3), 539–545. http://doi.org/10.3945/ajcn.2010.29418

Pavlidis, P., Gouveris, H., Anogeianaki, A., Koutsonikolas, D., & Koblenz, K. K. (2013). Age-related Changes in Electrogustometry Thresholds, Tongue Tip Vascularization, Density, and Form of the Fungiform Papillae in Humans. *Chemical Senses*, *38*, 35–43. http://doi.org/10.1093/chemse/bjs076

Pavlidis, P., Gouveris, H., Kekes, G., & Maurer, J. (2014). Electrogustometry thresholds, tongue tip vascularization, and density and morphology of the fungiform papillae in diabetes. *B-ENT*, *10*(4), 271–278.

Pickering, G. J., & Robert, G. (2006). Perception of Mouthfeel Sensations Elicited By Red Wine Are Associated With Sensitivity To 6-N-Propylthiouracil. *Journal of Sensory Studies*, *21*(3), 249–265. http://doi.org/10.1111/j.1745-459X.2006.00065.x

Piochi, M., Monteleone, E., Torri, L., Masi, C., Almli, V. L., Wold, J. P., & Dinnella, C. (2017). Comparing manual counting to automated image analysis for the assessment of fungiform papillae density on human tongue. *Chemical Senses*, *42*(7), 553–561. http://doi.org/10.1093/chemse/bjx035

Prescott, J., Soo, J., Campbell, H., & Roberts, C. (2004). Responses of PROP taster groups to variations in sensory qualities within foods and beverages. *Physiology and Behavior*, *82*(2–3), 459–469. http://doi.org/10.1016/j.physbeh.2004.04.009

Prescott, J., & Stevenson, R. J. (1995). Effects of oral chemical irritation on tastes and flavors in frequent and infrequent users of chili. *Physiology and Behavior*, *58*(6), 1117–1127. http://doi.org/10.1016/0031-9384(95)02052-7

Prescott, J., & Swain-Campbell, N. (2000). Responses to repeated oral irritation by capsaicin, cinnamaldehyde and ethanol in PROP tasters and non-tasters. *Chemical Senses*, *25*(3), 239–246. http://doi.org/10.1093/chemse/25.3.239

Prescott, J., & Tepper, B. J. (2004). *Genetic variation in taste sensitivity*, New York, Basel, Ed.: Marcel Ekker Inc. ISBN: 0-8247-4087-4

Proserpio, C., Laureati, M., Bertoli, S., Battezzati, A., & Pagliarini, E. (2016). Determinants of Obesity in Italian Adults : The Role of Taste Sensitivity , Food Liking , and Food Neophobia. *Chemical Senses*, *41*, 169–176. http://doi.org/10.1093/chemse/bjv072

Prutkin, J., Duffy, V. B., Etter, L., Fast, K., Gardner, E., Lucchina, L. A., … Bartoshuk, L. M. (2000). Genetic variation and inferences about perceived taste intensity in mice and men. *Physiology and Behavior*, *69*(1), 161–173. http://doi.org/10.1016/S0031-9384(00)00199-2

Reedy, F. E. J., Bartoshuk, L. M., Millerm, I. J., Duffy, V. B., Lucchina, L., & Yanigasawa, K. (1993). Relationships among papillae, taste pores and 6-n-propylthiouracil (PROP) supra threshold taste sensitivity. *Chemical Senses*, *18*, 618–619.

Robino, A., Mezzavilla, M., Pirastu, N., La Bianca, M., Gasparini, P., Carlino, D., & Beverly J., T. (2016). Understanding the role of personality and alexithymia in food preferences and PROP taste perception. *Physiology & Behavior*, *157*, 72–78. http://doi.org/10.1016/j.physbeh.2016.01.022

Segovia, C., Hutchinson, I., Laing, D. G., & Jinks, A. L. (2002). A quantitative study of fungiform papillae and taste pore density in adults and children. *Developmental Brain Research*, *138*(2), 135–146. http://doi.org/10.1016/S0165-3806(02)00463-7

Shahbake, M., Hutchinson, I., Laing, D. G., & Jinks, A. L. (2005). Rapid quantitative assessment of fungiform papillae density in the human tongue. *Brain Research*, *1052*(2), 196–201. http://doi.org/10.1016/j.brainres.2005.06.031

Shen, Y., Kennedy, O. B., & Methven, L. (2016). Exploring the effects of genotypical and phenotypical variations in bitter taste sensitivity on perception , liking and intake of brassica vegetables in the UK. *Food Quality and Preference*, *50*, 71–81. http://doi.org/10.1016/j.foodqual.2016.01.005

Smith, D. V. (1971). Taste Intensity as a Function of Area and Concentration: differentiation between compounds. *Journal of Experimental Psychology*, 163–171.

Snyder, D. J., Bartoshuk, L. M., Grushka, M., Stamps, J. J., Colquhoun, T. A., Schwieterman, L., … Dotson, C. S. (2014). Oral sensory pathology moderates the relationship between fungiform papillae density and taste intensity Derek J. Snyder, Linda M. Bartoshuk, Miriam Grushka, Jennifer J. Stamps, Thomas A. Colquhoun, Michael L. Schwieterman, Asli Z. Odabasi, Charles A. Sim. *Poster*, *AChemS Ass*.

Sollars, S. I., & Bernstein, I. L. (2000). Neonatal chorda tympani transection permanently disrupts fungiform taste bud and papilla structure in the rat. *Physiology and Behavior*, *69*(4–5), 439–444. http://doi.org/10.1016/S0031-9384(99)00259-0

Stein, N., Laing, D. G., & Hutchinson, I. (1994). Topographical differences in sweetness sensitivity in the

peripheral gustatory system of adults and children. *Developmental Brain Research*, *82*(1–2), 286–292. http://doi.org/10.1016/0165-3806(94)90170-8

Stevenson, R. J., Boakes, R. A., Oaten, M. J., Yeomans, M. R., Mahmut, M., & Francis, H. M. (2016). Chemosensory abilities in consumers of a western-style diet. *Chemical Senses*, *41*(6), 505–513. http://doi.org/10.1093/chemse/bjw053

Tanaka, M. (2009). Secretory Function of the Salivary Gland in Patients with Taste Disorders or Xerostomia: Correlation with Zinc Deficiency. *Acta Oto-Laryngologica*, *122*, 134–141.

Tepper, B. J., & Nurse, R. R. J. (1997). Fat perception is related to PROP taster status. *Physiology and Behavior*, *61*(6), 949–954. http://doi.org/10.1016/S0031-9384(96)00608-7

Tepper, B. J., Banni, S., Melis, M., Crnjar, R., & Barbarossa, I. T. (2014). Genetic sensitivity to the bitter taste of 6-n-propylthiouracil (PROP) and its association with physiological mechanisms controlling Body Mass Index (BMI). *Nutrients*, *6*(9), 3363–3381. http://doi.org/10.3390/nu6093363

Tepper, B. J., Christensen, C. M., & Cao, J. (2001). Development of brief methods to classify individuals by PROP taster status. *Physiology and Behavior*, *73*(4), 571–577. http://doi.org/10.1016/S0031-9384(01)00500-5

Tepper, B. J., Neilland, M., Ullrich, N. V., Koelliker, Y., & Belzer, L. M. (2011). Greater energy intake from a buffet meal in lean, young women is associated with the 6-n-propylthiouracil (PROP) non-taster phenotype. *Appetite*, *56*(1), 104–110. http://doi.org/10.1016/j.appet.2010.11.144

Tepper, B. J., & Ullrich, N. (2002). Influence of genetic taste sensitivity to 6-< i> n</i>-propylthiouracil (PROP), dietary restraint and disinhibition on body mass index in middle-aged women. *Physiology & Behavior*, *75*, 305–312.

Törnwall, O., Silventoinen, K., Hiekkalinna, T., Perola, M., Tuorila, H., & Kaprio, J. (2014). Identifying flavor preference subgroups. Genetic basis and related eating behavior traits. *Appetite*, *75*, 1–10. http://doi.org/10.1016/j.appet.2013.11.020

Webb, J., Bolhuis, D. P., Cicerale, S., Hayes, J. E., & Keast, R. (2015). The Relationships Between Common Measurements of Taste Function. *Chemical Perception*, 11–18. http://doi.org/10.1007/s12078-015-9183-x

Whitehead, M. C., Beeman, C. S., & Kinsella, B. A. (1985). Distribution of taste and general sensory nerve endings in fungiform papillae of the hamster. *American Journal of Anatomy*, *173*(3), 185–201. http://doi.org/10.1002/aja.1001730304

Witt, M., & Reutter, K. (1997). Scanning Electron Microscopical Studies of Developing Gustatory Papillae in Humans. *Chemical Senses*, *8*, 601–612.

Wockel, L., Jacob, A., Holtmann, M., Poustka, F., & Wo, L. (2008). Reduced number of taste papillae in patients with eating disorders, 537–544. http://doi.org/10.1007/s00702-007-0845-y

Wooding, S., Kim, U.-K., Bamshad, M. J., Larsen, J., Jorde, L. B., & Drayna, D. (2004). Natural selection and molecular evolution in PTC, a bitter-taste receptor gene. *American Journal of Human Genetics*, *74*(4), 637–46. http://doi.org/10.1086/383092

Yackinous, C. a, & Guinard, J.-X. (2002). Relation between PROP (6-n-propylthiouracil) taster status, taste anatomy and dietary intake measures for young men and women. *Appetite*, *38*(3), 201–209. http://doi.org/10.1006/appe.2001.0481

Yeomans, M. R., Tepper, B. J., Rietzschel, J., & Prescott, J. (2007). Human hedonic responses to sweetness: Role of taste genetics and anatomy. *Physiology & Behavior*, *91*(2–3), 264–273.

http://doi.org/10.1016/j.physbeh.2007.03.011

Zhang, G. H., Zhang, H. Y., Wang, X. F., Zhan, Y. H., Deng, S. P., & Qin, Y. M. (2009). The relationship between fungiform papillae density and detection threshold for sucrose in the young males. *Chemical Senses*, *34*(1), 93–99. http://doi.org/10.1093/chemse/bjn059

Zhao, L., Kirkmeyer, S. V., & Tepper, B. J. (2003). A paper screening test to assess genetic taste sensitivity to 6-n-propylthiouracil. *Physiology and Behavior*, *78*, 625–633. http://doi.org/10.1016/S0031-9384(03)00057-X