UNIVERSITÀ
DEGLI STUDI
FIRENZE

DOTTORATO DI RICERCA IN

Gestione sostenibile delle risorse agrarie, forestali e alimentari

CICLO XXXIV

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Factors underlying individual differences in responses to oral tactile stimulation

Settore Scientifico Disciplinare AGR/15

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Anni 2018/2022

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Abstract

Food texture perception plays an important role in driving food acceptance, with overall consequences on individual's health status. Individual differences in food texture perception may be associated with oral somatosensory system sensitivity, with individuals higher in oral tactile sensitivity perceiving food texture-related sensations more intensely than individuals with lower oral tactile sensitivity. PROP bitterness responsiveness and fungiform papillae density (FPD) are common phenotypic marker of chemosensory responsiveness, including both taste and tactile stimuli. Recent studies suggest measures of oral tactile sensitivity, such as the perception of touch, spatial resolution and oral stereognosis, as specific phenotypic marker of individual variability in responsiveness to food texture related sensations. However, the association of these measures with the perception of texture related sensations and with other phenotypes of oral chemosensory responsiveness are relatively underexplored.

The perception of generally disliked food texture-related sensations, such as graininess and astringency, may affect the hedonic response to food products with varied texture properties. Individual differences in the perception of warning texture-related sensations may underline different pattern of food preference and choice, with higher responsive individuals showing liking responses for food characterized by astringency and graininess lower than less responsive individuals. The systematic exploration of individual differences in responsiveness to oral tactile stimulation and its possible consequences on hedonic responses would take advantage from the development of food model systems showing systematic variation in intensity of warning texture-related sensations.

The complex interplay between physiological and biological factors, such as gender, age and responsiveness to oral stimulation, and psycho-attitudinal traits have been reported to affect both the perceptual and the hedonic responses to food sensory properties including tastes, flavour and irritant sensations but these relationships are still relatively underexplored for texture.

Thus, based on these critical issues, the aims of the thesis were:

1) To develop measures capturing individual variation in tongue responses to mechanical stimulation, including gratings recognition threshold (GRT) and both point-pressure detection and discrimination thresholds. The relationships between oral tactile acuity measures and phenotypic markers of chemosensory responsiveness (PROP responsiveness and fungiform papillae density) were explored for a deeper understanding of factors associated with individual variability in responsiveness to oral tactile stimuli.

2) To study the relationships between concentration and intensity of generally disliked food texture-related sensations evoked by specific oral tactile stimuli (microcrystalline cellulose for graininess and tannic acid for astringency) in water solution and fruit juices. Model systems varying in intensity of graininess and

astringency were developed as a tool for the systematic exploration of individual differences in responsiveness to oral tactile stimulation.

3) To explore individual differences in graininess and astringency perception in water and pear juice model systems and associated factors including gender, oral tactile sensitivity measures, phenotypic markers of oral chemosensory acuity (PROP responsiveness and fungiform papillae density) and personality/psychological traits.

4) To investigate liking for pear juice model systems with varied intensity of tactile sensations (graininess, astringency and their combinations) and to identify consumer groups with specific liking/target sensation patterns and exploring associated factors including gender, responsiveness to taste and tactile sensations evoked by model system, oral tactile sensitivity measures, phenotypic markers of oral chemosensory acuity (PROP responsiveness and fungiform papillae density) and personality/psychological traits.

Data collection was organised in a pre-study and a main study. The pre-study aimed at: 1) testing the protocols for assessing oral tactile sensitivity using both gratings orientation test and point-pressure test; 2) assessing the relationships between concentration of selected tactile stimuli (microcrystalline cellulose and tannic acid) and intensity of target sensations (graininess and astringency) to identify tastants concentration range inducing significant variation of graininess and astringency intensity in both water solutions and fruit juices. The main study aimed at collecting variables (physiological, sensory, hedonic, and psychological) which might affect perception and preference for foods with varied texture properties. Sensory and liking tests were performed on water solutions and fruit juices prepared to induce different intensity of tactile sensations. Responsiveness to PROP and fungiform papillae density (FPD) were assessed. Individual tactile acuity was assessed by means of grating recognition threshold (GRT) and both point-pressure detection and discrimination thresholds. Questionnaire were used to profile participants for psychological traits (food neophobia, sensitivity to core-disgust, sensation seeking, sensitivity to punishment and reward, state and trait anxiety).

Thirty-seven women (age range 18-30 years) participated in the pre-study. One-hundred and forty-four subjects (50% women, age range 18-30 years) participated in the main study.

Results showed that gratings orientation thresholds (GRT) discriminated amongst participants, therefore it appeared a suitable tool to explore the individual variation in oral responsiveness to mechanical stimulation. On the other hand, point-pressure thresholds did not highlight individual differences in responsiveness to oral tactile stimulation. GRT and point-pressure sensitivity did not correlate, therefore supporting the hypothesis that these measures represent different tactile functions underlined by different receptor/neural mechanisms. A substantial independence was further observed between GRT and phenotype markers of chemosensory responsiveness (PROP bitterness responsiveness and fungiform papillae density total ad size

classes). Future studies should be aimed at oral tactile acuity methodologies optimization, for example exploring the use of narrower grids and the adoption of longer staircases, to capture the differences in tactile sensitivity among the most sensitive individuals.

Psychophysics curves were developed for microcrystalline cellulose (MCC) and tannic acid (TA) in both water solutions and fruit juices. Microcrystalline cellulose appeared a pure stimulus for graininess sensation, while tannic acid was confirmed to be able to evoke both a tactile sensation, astringency, and a taste sensation, bitterness and sourness. Tastant concentration levels were identified to induce systematic variation in the intensity of target sensations. Furthermore, physico-chemical and perceptual interactions between tactile stimuli and dispersion medium components were hypothesised to account for changes in model system sensory properties.

Groups varying for responsiveness to tactile (graininess and astringency) and taste (bitterness and sweetness) sensations in model systems were identified. Groups did not significantly vary in gender distribution, PROP bitterness responsiveness, fungiform papillae density total and size classes and grating recognition threshold. Significant differences between groups associated to psychological and personality traits, including sensitivity to punishment, sensitivity to disgust and state anxiety, suggesting that individuals higher responsive to warning tactile sensations showed “closed” personality type.

Differences in liking pattern were observed that did not relate to differences in the perception of tactile sensations, phenotype markers of chemosensory responsiveness, oral tactile acuity measures or to psychological and personality traits.

Keywords: food texture, graininess, astringency, food model systems, oral tactile acuity measures, PROP bitterness responsiveness, fungiform papillae density, responsiveness to taste and tactile sensations, liking

List of original publications

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

- Mani E., Ford R., Pierguidi L., Spinelli S., Ramsey I., Monteleone E., Dinnella C. **Exploring the association between oral tactile sensitivity measures and phenotypic markers of oral responsiveness.** Submitted to Journal of Texture Studies. (submitted)

List of abbreviations and glossary

MCC: Microcrystalline cellulose

TA: Tannic acid

MCC+TA: Microcrystalline cellulose + tannic acid

W: water solutions

PJ: pear juice samples

PcJ: Peach juice samples

VFH: Von Frey Hair monofilaments

GRT: gratings recognition threshold

PROP: 6-n-propyl-thiouracil

FPD: Fungiform papillae density

FN: Food neophobia

DS-SF: Disgust sensitivity-short form

SS: Sensation seeking

SP: Sensitivity to punishment

SR: Sensitivity to reward

"Tastant" was used to indicate sensory active molecules able to evoke taste and tactile sensations

"Responsiveness" was used for suprathreshold responses

"Sensitivity" was used for threshold responses

1. Introduction

1.1 Food texture

Food texture perception is defined as “the attribute of a substance resulting from a combination of physical properties and perceived by the senses of touch (including kinaesthesis and mouthfeel), sight and hearing” (Brennan, 1984). Physical properties are classified into mechanicals, geometrical and mouthfeel characteristics (Szczesniak, 1963). Mechanical properties are further subdivided into primary and secondary parameters; first ones refer to hardness, cohesiveness, viscosity, adhesiveness, and elasticity, while secondary parameters include fracturability, chewiness and the gumminess. Geometrical characteristics refer to particle size and shape and to particle shape and orientation, while mouthfeel characteristics refer to moisture content, fat content, oiliness and greasiness (Szczesniak, 1963). Moreover, the definition further highlights that food texture perception is a synthesis of information from several senses. Responses from somatosensory, visual, and auditory systems are integrated, and elaborated in central nervous system to form a global cognitive representation of food texture (Verhagen & Engelen, 2006; Rolls, 2020).

1.1.1 The role of oral processing on food texture perception and preference

Several factors are known to influence food texture perception, including food structure (Koç et al., 2013; Foegeding et al., 2017) and physiological and behavioural aspects of oral process (Engelen & Van Der Bilt, 2008; Chen, 2009). In turn, oral processing continuously changes food structure by muscle activities, jaw and tongue movements (Wilkinson, et al., 2000; Witt & Stokes, 2015) and by interaction of food components with saliva (Mosca & Chen, 2017; Laguna et al., 2021).

Oral processing is fundamental for food to be swallowed since it leads to bolus formation; oral processing let food to be mixed with saliva to form the bolus, which is a smooth mass of food particles mechanically broken-down. During food oral processing, the hardness and size of food particles rapidly decrease, whereas the adhesiveness and the cohesiveness of the bolus increase until the time of swallowing (Koç et al., 2014; Witt & Stokes, 2015). Furthermore, saliva moistens food structure and salivary mucins bind food into a coherent and slippery bolus that can be easily swallowed (Prinz & Lucas, 1997; Pedersen et al., 2018). Saliva is incorporated in food structure to form the bolus through processes of comminution, agglomeration, hydration and dilution (Witt & Stokes, 2015). Mechanisms of food-saliva interactions have been recently reviewed (Mosca & Chen, 2017) and the complex role of saliva characteristics in texture perception explored (Pedersen et al., 2018).

During oral processing, a cognitive representation of food texture is formed (Rolls, 2020). Physiological signals are highly complex and dynamic in nature due to continuous mechanical and biochemical changes in food structure (Brown & Braxton, 2000; Koç, et al., 2013). Food texture perception continuously evolves during food oral processing; therefore, food texture perception is considered a dynamic process, influenced

by continuous changes in food structures, oral processing behaviours and food-saliva interactions that lead to modification of sensory perception over time (Devezeaux de Lavergne, et al., 2015; Nguyen et al., 2017; Aguayo-Mendoza et al., 2021).

The dynamic aspect of food texture perception might be also explained by cross-modal interactions which occur during food oral processing. Both food structure and oral processing have been suggested to impact the release of tastants from food structure, and food texture perception might also depend from texture-taste interactions (Boisard et al., 2014). Different kind of interactions are known to occur in foods: - physico-chemical mechanisms which includes both chemical interactions and interactions between one components and the taste receptors/transduction mechanisms of another component; - perceptual mechanisms which refer to the cognitive effect of different qualities being perceived together in the mouth (Keast & Breslin, 2003; Thomas-Danguin et al., 2016). Effect of cross-modal interactions on sensory perception have been recently explored in yoghurt samples added with dairy proteins as texture enhancers showing that proteins induced variations of both texture and flavour perception due to both physico-chemical interactions between food components and to perceptual cross-modal interactions between texture and sensations (Lesme et al., 2020). Furthermore, cross-modal interactions (taste-odour-texture) have been recently suggested to be used as a strategy to promote healthier food consumption. The addition of vanilla and starch in milk desserts has been proposed as strategy to reduce sugar intake in children. The interaction between starch and sugar molecules was hypothesised to influence the diffusion of the latter by desserts, decreasing sweet perception without affecting the overall liking of products (Velázquez et al., 2020).

Oral processing represents the physiological basis for food texture perception. Recently, it has been suggested that individuals use different oral processing mechanisms to manipulate food in their mouth and these different oral manipulation strategies might lead to differences in food texture perception and preference (Brown & Braxton, 2000). Classification based on individual differences in mouth behaviours have been proposed based on results from custard and mayonnaise evaluations (Engelen, & van Doorn, 2000). Four groups were identified: Simple, Taster, Manipulator, and Tonguer. Simple group was characterised by placing the food on the tongue, raising the tongue to the palate, and then swallowing the food; Tasters made the same initial movement but also made a series of short sucking movements against the palate before swallowing. The behavior of Manipulators was more variable and consisted of a combination of chewing with the incisors and molars, while Tonguers used their tongue to push the food against the palate using back-and-forth and sideways movements. Recently, a new model has been proposed (Jeltema et al., 2015) and four major mouth behavior groups were defined: Crunchers, Chewers, Suckers and Smooshers. Crunchers and Chewers have been defined as those who liked to use their teeth to break down foods. Crunchers are more forceful in their bite and prefer foods that broke up (fractured) on biting. Chewers like foods that could be chewed longer (the length of time varied leading to the sub-classification of “short” Chewers and “long” Chewers) and do not fracture on biting. Suckers and Smooshers manipulate food between the tongue and

the mouth roof. Suckers like harder foods that can be sucked on for a long time (like hard candies and items that they can hold in their mouths), while Smooshers preferred soft foods, such as creamy candies or puddings that do not require much mouth activity but would spread throughout the mouth and could be held in the mouth for a long time. Texture perception and preference differed in the Mouth Behavior groups by means of products which most easily allowed a person to eat foods with their preferred Mouth Behavior were most liked and preferentially chosen, while foods that were rejected were difficult or impossible to eat using the preferred Mouth Behavior (Jeltema et al., 2016). However, recent evidences suggest that consumer differences in oral processing behaviours do not necessarily result in differences in food texture perception and liking (Santagiuliana et al., 2019). For example, perception and liking for yogurt samples added with particles were not affected by mouth behaviour (Liu et al., 2021a) and that texture liking ratings of 106 food texture attributes of a wide range of products were unrelated to mouth behaviour groups (Kim & Vickers, 2020).

1.1.2 The role of food texture on food intake

Food texture is a major driver of food liking and acceptance (Jaeger et al., 1998; Kalviainen et al., 2000). Liking for food texture arises from both physiological characteristics (i.e. oral processing behaviours) and learned influences (i.e. exposure, expectation) (Tuorila et al., 1998). It is the confirmation or disconfirmation of expectations that mostly determines acceptance or rejection of food products (Burgess, 2016). Furthermore, exposure to specific foods plays an important role in developing familiarity with and thus, preference for specific food textures (Piqueras-Fiszman & Spence, 2015; Santagiuliana et al., 2019).

Food texture has a great impact on both food intake and health status by affecting the way and the length of oral manipulation during consumption. The consumption of foods that can be quickly ingested may promote overweight and obesity on the long term (Schulze et al., 2004; Malik et al., 2010). The hypothesis is that quick oral processing of foods and the reduced residence time in the mouth may lead to an inadequate cephalic phase response. Cephalic phase response is the physiological response to sensory signals and informs the brain and the gut about the inflow of nutrients (de Graaf, 2012). Reduced oral residence time leads to inadequate sensory signaling which produces an inadequate satiation response. Food texture plays a fundamental role in determining how and how long food is manipulated in the mouth, thus it has a significant impact on satiation response (Hogenkamp et al., 2011), with products which require longer oral processing associating to lower ad libitum intake (de Wijk et al., 2008). Evidence reported that foods consumed with smaller bites, higher number of chews and longer oral exposure time, associated with higher expected satiation (Forde et al., 2013). Furthermore, slower eating rates, longer pauses between bites and longer oral exposure time determined higher expected satiation, greater post-meal fullness and greater satiety (Ferriday et al., 2016). Thicker porridge version which was slower consumed, with larger bite size, longer oral exposure time per bite and more chews per bite, was found to associate to a lower intake than a thinner porridge

version (McCrickerd et al., 2017). More viscous yogurts were reported to associate to an increase of expected satiation, and the addition of lyophilized pineapple cubes to yogurts were further found to increase the expected satiation of both low and high viscosity yogurts (Tarrega et al., 2016). Furthermore, a decrease in granola particles size added to yogurts, was found to increase the number of chews and decrease the eating rate and intake of products without affecting both the familiarity with and the liking for products (Mosca et al., 2019).

1.2 Food texture-related sensations

1.2.1. Graininess

Graininess is a texture-related sensation evoked by the presence of particles in foods. Graininess is classified as a "geometrical characteristic" of textural parameters, referring to particle size and shape (Szczesniak, 1963). Graininess perception can be affected by particles size, shape, and concentration (Tyle, 1993; Imai et al., 1999; Engelen et al., 2005). Graininess perception has been reported to increase with increasing particles concentration (Imai et al., 1995; Lopez et al., 2016; Aguayo-Mendoza et al., 2021). Small and hard particles have been reported to lead to an undesired graininess perception in products like cheese spread and cream (Modler et al., 1989; Sainani et al., 2004) while smooth and soft particles appear to prevent this negative response (Chojnicka-Paszun et al., 2012). Yogurts added with small/hard peach gel particles resulted less appreciated than yogurts added with medium/soft particles (Aguayo-Mendoza et al., 2020). Graininess has been reported to decrease with the increasing viscosity of the dispersion medium (Imai et al., 1995; Lopez et al., 2016) and it is perceived higher in liquid than in semi-solid or solid foods (Liu et al., 2016). The presence of particles in foods not only affects graininess perception but also creaminess, roughness, and dryness sensations (Cheftel & Duma, 2009; Kilcast & Clegg, 2002; Petersson et al., 2013). The addition of particles in vanilla custard desserts was found to increase roughness perception and significantly decreased the ratings of attributes associated to lubrication such as smoothness, creamy , fatty and slippery (Engelen et al., 2005). Graininess perception significantly affects the liking and the acceptability of foods (Tyle et al., 1990; Olarte Mantilla et al., 2020; Aguayo-Mendoza et al., 2020; Liu et al., 2021). Several papers described graininess as an undesired sensation that might decrease the overall liking of products (Engelen et al., 2005; Lopez et al., 2016). Therefore, strategies to compensate this sensation have been recently proposed such as the addition of macroparticles or fat to foods hypothesising to compensate the grainy negative perception by more positive ones or by shifting participants' attention induced by the perception of more dominant sensations (Santagiuliana et al., 2020). In a recent paper, individual differences in liking and intake of yoghurt samples varying in viscosity and particle size were explored. Two consumer groups were identified, one group showed a positive correlation between liking and intake, while a negative correlation was found in second group in which the less liked version were eat more due to textural changes in the matrix (Varela et al., 2021). Studies on individual differences in the ability to detect particles in yoghurt samples showed that consumers who

selected the attribute “particles” during the evaluation of yoghurts added with agar particles showed lower product acceptability (Olarte Mantilla et al., 2020) and that consumers able to identify particles preferred more cohesive yoghurt textures (fatty, spoonable, not separated) and were frequent consumers of yoghurt products in combination with cereal (Olarte Mantilla et al., 2022).

1.2.2 Astringency

Astringency is a dry mouthfeel sensation perceived through the activation of the oral somatosensory system (Thorngate & Noble, 1995). Different mechanisms have been proposed to explain the development of astringency sensation, but they all agree in associating astringency to a loss of lubrication of the salivary film which coat the oral cavity. Several events involving astringent compounds and saliva may cause alterations of oral surface properties, variations in both the rheological and the lubrication properties, and the activation of cell receptors. In particular, protein precipitation, breakage of the salivary pellicle, decrease in salivary lubrication and mechanical perception have been hypothesised as possible mechanisms underlying astringency sensation (Gibbins & Carpenter, 2013). Recently, it has been proposed that astringency may derived by an aggregation process of the mucosal pellicle as a consequence of the interaction between astringent compounds and salivary proteins which are anchored to the oral mucosa (Ployon et al., 2018). The aggregation of this thin layer of salivary proteins has been associated to an increase in the friction forces and to a loss of lubrication properties (Ployon et al., 2018). On the contrary, a protective role has been highlighted for proline-rich proteins (PRPs), which are the salivary proteins secreted by the parotid glands. PRPs may act as scavengers for astringent compounds preventing the aggregation of the mucosal pellicle (Ployon et al., 2018). The interaction between astringent compounds, namely phenol compounds, and PRPs is widely explored. It has been reported that astringency is directly correlated with the capacity of tannins to interact with PRPs, resulting in the formation of protein-tannin aggregates in the mouth (Monteleone et al., 2004; García-Estévez et al., 2018). The type of interaction, including hydrophobic ones and hydrogen bonds, may affect phenol compounds capacity to bind with salivary proteins. Tannin-salivary proteins aggregates are reported to disrupt the salivary film, increasing the oral friction and altering the oral mucosa, and to establish direct receptors interactions, leading to astringency perception (Rossetti et al., 2009). Furthermore, tannins-salivary proteins aggregates may crosslink, forming tannins bridges and protein dimers which may further aggregate, forming large complexes which precipitate (Charlton et al., 2002). Astringency is therefore affected by the oral production of salivary PRP and individual differences in saliva characteristics including flow rate, composition and haze-forming capacity, may influence astringency perception (Fleming et al., 2016; Melis et al., 2017; Dinnella et al., 2009; Dinnella et al., 2010). Physical properties of food texture may also affect astringency perception: addition of lubricants such as gums, polysaccharides and proteins have been reported to decrease astringency perception (Brannan et al., 2001; Colonna et al., 2004).

Astringency perception plays a key role in determining preference for and acceptability of various food products (Bajec, 2011; Dinnella et al, 2011; Yang & Lee, 2020; Louro et al., 2021). For example, high astringency intensity have been reported to lead to rejection of foods and beverages rich in phenol compounds (De Toffoli et al., 2019; Yang & Lee, 2020).

1.3. Individual differences in oral somatosensory system sensitivity

Food texture perception plays an important role in driving food acceptance (Jaeger et al., 1998; Kalviainen et al., 2000). Responsiveness of oral somatosensory system to stimuli evoking texture-related sensations differs amongst individuals (Bartoshuk, 1993) and, recently, great attention has been devoted to the identification of phenotypic markers of oral tactile responsiveness (Lukasewycz & Mennella, 2012; Aktar et al., 2015; Furukawa et al., 2019; Breen et al., 2019; Zhou et al., 2020).

1.3.1 Oral tactile sensitivity

Individual differences in oral somatosensory system sensitivity have been hypothesised to relate to oral mechanoreceptors functionality (Guinard & Mazzucchelli, 1996; Strassburg et al., 2009; Van Aken, 2010). Oral mechanoreceptors innervate the tongue, the periodontal ligament, the gingiva and the palate. They convey information on a wide range of mechanical sensory events, including touch, pressure, vibration and proprioception, thus playing an important role both in the manipulation and the perception of food (Trulsson & Johansson, 2002). They can be classified on the basis of sensory adaptation after continuous stimulation in fast adapting (FA), and in slowly adapting (SA) (Trulsson & Essick, 2010). Each of these neuron types responds to deformation or motion of cutaneous surface in a different way and the activation of different fibre types produces different qualities of tactile sensations, such as light touch, flutter, sustained pressure (Capra, 1995). Afferents terminating in Meissner's corpuscles and Merkel disk have small, well-defined receptive fields. Meissner' corpuscles respond to both movement and light touch, and transmit sensations of stroking and flutter, while Merkel disks are thought to convey sensations of light touch from texture and edge detection. Afferent nerve fibres terminating in Ruffini endings are slowly adapting and convey sensations of stretch, while those terminating in Pacinian corpuscles are rapidly adapting and are involved in the detection of vibration (Dargahi & Najarian, 2004; Neubarth et al., 2020). As the tongue is constantly in motion in relation to the surrounding tissues and to the food inside the mouth during eating, these receptors most likely work together in the mouth to create perceptions of food texture. However, until now the response of specific mechanoreceptors to food texture has not yet been assessed (Foegeding et al., 2011).

1.3.1.1 Types of oral tactile sensitivity

It has been hypothesised that food texture perception is associated to oral tactile sensitivity (Lukasewycz & Mennella, 2012; Nederkoorn et al., 2019; Zhou et al., 2021; Liu et al., 2021). Cutaneous tissues represent the main target of studies aimed at exploring sensitivity to tactile stimulation (Greenspan & Bolanowski, 1996)

and in the past different methods have been tested to assess the somatosensory system functionality after trigeminal nerve injury or age-related oral disorders (Zuniga, 1992). These methodologies have been developed based on the high sensitivity of fingertips. However, tongue and lips were found to have higher sensitivity than fingertips (Van Boven & Johnson, 1994), probably due to the absence of the epidermal barrier and their involvement in high-sensitivity behaviours such as eating and speaking (Miles et al., 2018). One of the critical points in the assessment of oral tactile sensitivity is that the currently available methods, developed for application in less sensitive tissues, could be affected by a floor effect and might fail in highlighting individual differences in somatosensory system responsiveness (Yackinous & Guinard, 2001; Santagiuliana et al., 2019; Breen et al., 2019).

Currently available tests and devices to assess oral tactile sensitivity convey information about specific properties of oral mechanoreceptors such as the spatial resolution (Ringel & Ewanowski, 1965; Johnson & Phillips, 1981; Van Boven & Johnson, 1994), the oral stereognosis (Johnson & Phillips, 1981; Essick et al., 1999; Shupe, et al., 2019) and the perception of touch (Weinstein, 1968; Aktar et al., 2015; Etter, et al., 2020) and reflect the complexity of oral somatosensory system functions involved in food texture perception (Figure 1).

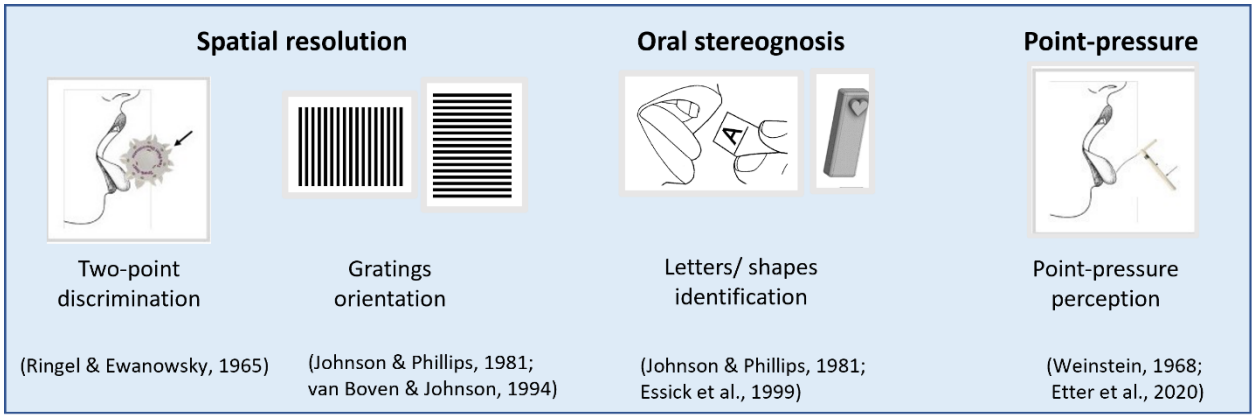


Figure 1. Currently available test and devices to assess oral tactile sensitivity (adapted by Aktar et al., 2015; Shupe et al., 2019)

Recent works have explored oral tactile sensitivity using the light-touch, or point-pressure method (Yackinous & Guinard, 2001; Breen et al., 2018; Etter et al., 2020; Zhou et al., 2020), while other studies have proposed the assessment of oral tactile sensitivity by measuring the oral stereognosis through the identification of letters and shapes (Essick, Chen, & Kelly, 1999; Essick et al., 2003; Lukasewycz & Mennella, 2012; Steel et al 2014; Bancguyo et al 2017) or the spatial resolution acuity through the identification of gratings orientation (Appiani et al., 2020). It was suggested that the stimuli used to test light-touch sensitivity mainly stimulates superficial receptors, while stimuli used to test the oral stereognosis or the spatial resolution might excite more deeply set receptors (Engelen & Van Der Bilt, 2008).

Point-pressure task is one of the most common methods to assess oral tactile sensitivity (Weinstein 1968); it is based on the perception of a point-like stimulus that is applied on cutaneous surfaces, for example on the tongue surface to assess lingual tactile acuity. Devices commonly used are monofilaments, particularly Von Frey Hair monofilaments or Semmes-Weinstein monofilaments, varying for their diameter and thus, for the applied force. The lower the diameter, the lower the force.

The letter-identification task was developed by Essick in 1999 (Essick et al., 1999). The test consisted in identifying letters of the alphabet with varied sizes embossed onto Teflon strips (Essick et al., 2003). The task measure lingual tactile acuity in terms of oral stereognosis which is the ability to recognize and discriminate forms (Jacobs et al., 1998). The letter-identification task is characterised by a cultural limit since letters of the Latin alphabet might not be equally familiar to subjects of different cultures. It has been recently suggested that the use of geometrical shapes like square, rectangle, triangle, star, hexagon, circle, half circle, diamond, cross, and heart rather than letters would be more appropriate, since they might be to be universally familiar (Shupe et al., 2018).

The grating orientation method consists in the recognition of the orientation of linear gratings applied vertically or horizontally on the tongue surface. This method has been developed from a psychophysics procedure known as two-point discrimination but compared to the latter it was able to provide lower thresholds (e.g. 1mm on the fingertips, opposed to 2-4mm) (Lederman, 2009).

Limits have been observed for all methods. Point-pressure sensitivity method stimulates a very small area of the tongue which cannot reflect the oral tactile sensitivity of the whole mouth (Zhou et al., 2021). Furthermore, the device used to assess oral point-pressure sensitivity consists of a kit of monofilaments developed to test skin sensitivity, which might not deliver to the lower force that tongue mechanoreceptors could perceive, thus, leading to a floor effect (Santagiuliana, Marigómez, et al., 2019). Spatial resolution acuity methods are recognised as a more complex task involving cognitive processes and affected by cultural factors such as the familiarity with the cues (Essick et al., 1999; Shupe et al., 2018; Cattaneo et al., 2019). To overcome intrinsic limitations belonging to each method and to have a more reliable measure of oral tactile sensitivity it has been recently suggested to take into account a variety of methods when exploring oral tactile acuity (Cattaneo et al., 2019; Appiani et al., 2020; Santagiuliana et al., 2019; Zhou et al., 2021).

1.3.1.2 Measures of oral tactile sensitivity

Measures used to estimate oral tactile sensitivity also vary; the main measures are thresholds and the estimation of R-index. Both discrimination and detection thresholds have been explored to assess oral tactile sensitivity, with Von Frey Hair monofilaments, by a two-alternative forced choice (2-AFC) task using a 3 down/1 up staircase method (Breen et al., 2019; Etter et al., 2020). Detection threshold is defined as the lowest force that each participant is able to perceive, while the discrimination threshold is defined as the lowest force that each participant is able to discriminate when administered with a stimulus one level higher.

Recent works have also reported measures of oral tactile sensitivity by the estimation of an R-Index. R-index

is a measure of the size of the discrimination level between stimuli in which a stimulus is asked to be recognised by a noise and the level of certainty of response is registered. The R-Index value takes into account the number of correct responses given on a total number of trials and the level of certainty, thus indicating the size of discrimination of the stimulus (O'Mahony, 1992; Lee et al., 2009).

1.3.1.3. Oral tactile sensitivity and food texture perception

Evidence on the association between oral tactile sensitivity and the perception of food texture-related sensations are relatively scarce and contradictory. High oral tactile sensitivity, measured as discrimination threshold in point-pressure test, has been positively associated to particle size discrimination in two chocolate samples (Breen et al., 2019). Positive association was also found with perception of hardness in biscuits and R-Index using 0.02 g VFH monofilament (Zhou et al., 2021), while no associations have been found between oral tactile sensitivity assessed through both point-pressure test and two-point discrimination test and firmness discrimination ability of soft-solid jelly samples (Aktar et al., 2015).

Recent works further investigated the association between oral tactile sensitivity and preference for foods with varied texture. No correlation were found between food texture preference and oral stereognosis acuity using letters in both children and their mothers (Lukasewycz & Mennella, 2012). Similarly, preference for and consumption of food with varied food texture were not affected by tongue tactile sensitivity estimated as R-Index using both point-pressure and gratings orientation tests, in children and in adults (Appiani et al., 2020). No association was found between oral tactile acuity assessed by Von Frey Hair monofilaments and liking for yogurt samples with different texture (Liu et al., 2021b).

1.3.2 PROP bitterness responsiveness

6-*n*-propylthiouracil (PROP) is a bitter-tasting compound. It is structurally characterised by a N-C=S moiety which is responsible for the bitter taste of PROP, phenylthiocarbamide (PTC) and all thiourea containing compounds. In foods, thioureas are commonly found in many vegetables, such as Cruciferae (Stoewsand, 1995). It has long been known that individuals differ on their ability to detect the bitterness of thioureas; particularly, some individuals are sensitive to PTC/PROP bitterness, while others are "tasteblind" (Fox, 1931). Phenotypic variation in the ability to taste PROP bitterness is in part genetically determined by the TAS2R38 gene; in particular, three single-nucleotide polymorphisms (SNPs) at amino acid 49 (proline or alanine), 262 (alanine or valine), and 296 (valine or isoleucine) combine to form the haplotype PAV (taster) and the haplotype AVI (non-taster)(Kim et al., 2003; Bufe et al., 2005) . Individuals can be classified according to their PROP responsiveness, as "non tasters" classified as those who do not perceive the PTC/PROP bitterness, and "tasters" classified as those who are able to perceive the PTC/PROP bitterness (Drewnowski & Rock, 1995). Tasters are further divided into "medium tasters", who show moderate responsiveness to PROP and "super tasters", highly responsive to PROP bitterness (Bartoshuk, 1993; Reed et al., 1995). Arbitrary cut-offs derived from several studies can be used to categorize subjects according to their PROP status as non taster (NT)

(PROP bitterness on gLMS < moderate, 17), medium taster (MT) (17 ≤ PROP bitterness ≤ 53) and super taster (ST) (PROP bitterness on gLMS > very strong, 53) (Hayes et al., 2010; Fischer et al., 2014). PROP bitterness sensitivity is known to significantly differ between women and men, with women showing higher PROP bitterness ratings than men (Bartoshuk, Duffy, & Miller, 1994; Monteleone et al., 2017). PROP status is a phenotypic marker of oral responsiveness, since many papers support the association between PROP bitterness responsiveness and the perceived intensity of a wide range of oral stimuli. PROP status associates to intensity of basic tastes, such as sour, bitter, sweet, umami in both water solutions (Drewnowski et al., 1997; Yeomans et al., 2007; Hayes & Duffy, 2007; Bajec & Pickering, 2008; Dinnella et al., 2018), and real foods (Prescott et al., 2004; Dinehart et al., 2006; de Wijk et al., 2007; Masi et al., 2015). Further evidences suggested an association between the PROP status and the intensity of chemical irritants (Spinelli et al., 2018; Prescott & Swain-Campbell, 2000; Piochi et al., 2021) and fat (Tepper & Nurse, 1997; Yackinous & Guinard, 2001; Nachtsheim & Schlich, 2013; Melis et al., 2015). PROP bitterness responsiveness was also found to positively associate to food texture-related sensations such as astringency (Melis et al., 2017; Pickering & Robert, 2006; Dinnella et al., 2018), creaminess (Kirkmeyer & Tepper, 2003) and roughness (Bakke & Vickers, 2008).

1.3.3 Fungiform papillae density

Fungiform papillae are the anatomical structures designated to oral stimuli detection and transduction since they house taste buds which respond to chemical stimulation, and are innervated by chorda tympani and trigeminal nerve fibres which respond to tactile stroking and temperature stimuli (Whitehead, Beeman, & Kinsella, 1985; Mistretta & Bradley, 2021). Fungiform papillae density (FPD) is considered a phenotype of taste sensitivity, based on the hypothesis that the greater the number of fungiform papillae, the greater the perception of taste stimuli intensity according to the spatial summation theory (the higher the number of stimulated receptors the higher the signal intensity) (Delwiche et al., 2001). There is controversial evidence of significant differences in FPD between women and men; some authors suggest women show higher FPD than men (Duffy et al., 2010; Fischer et al., 2014; Dinnella et al., 2018); others have not found a significant effect (Hayes & Duffy, 2007) .

Relationships between FPD and intensity of oral stimuli is controversial (see Piochi et al., 2018 for review). FPD has been associated to increased perception of sucrose, sodium chloride and citric acid (Zhang et al., 2009; Miller et al., 1990; Piochi et al., 2018). In other more recent studies, no association between FPD and the perception of the intensity of basic tastes including bitterness from PROP (Dinnella et al., 2018) and chemesthetic sensations like pungency were found. Positive associations were reported between FPD and sensitivity to fatty acid (Zhou et al., 2021) as well as the perception of texture related sensations such as creaminess (Hayes & Duffy, 2007) and the mouthfeel perception of biscuits, such as hardness and crunchiness (Zhou et al., 2021). On the other hand previous reports failed in finding such significant associations between

FPD and food texture attributes such as astringency in bread (Bakke & Vickers, 2008). Some evidences have suggested significant associations between FPD and food preference (Bakke & Vickers, 2011; Masi et al., 2015; Hayes et al., 2010).

1.4. Personality traits and food texture perception and preference

1.4.1 Food Neophobia

Food neophobia (FN) is defined as the reluctance to eat and/or avoidance for novel foods (Pliner & Pelchat, 1991). It primarily reflects the degree of reluctance to consume foods that are novel, particularly those from other food cultures, and this trait is currently considered one of the main barrier for the adoption of a varied and balanced diet (Pliner & Pelchat, 1991). During childhood food neophobia is considered an adaptive behaviour that prevent children from the ingestion of unfamiliar and potentially dangerous foods (Pliner, Pelchat, & Grabski, 1993). Highly neophobic children have been found to show both a lower preference for and intake of vegetables, fruits and protein-rich foods both in terms of variety and amount (Cooke, Wardle, & Gibson, 2003; Cooke, Carnell, & Wardle, 2006; Russell & Worsley, 2008). Food neophobia can persist in adulthood and associates with a reduced dietary variety, low vegetables, fruits and protein-rich foods intake as well as with a high numbers of disliked foods (Knaapila et al., 2011; Jaeger et al., 2017). Food neophobia was found to associate to food texture preference (Coulthard & Thakker, 2015; Coulthard & Sahota, 2016). Findings from behavioural studies suggested that children base their rejections to novel foods on both appearance (Dovey et al., 2012; Heath et al., 2014) and tactile processing (Coulthard & Thakker, 2015; Nederkoorn et al., 2015). Enjoyment of the feel of sticky foods was associated with reduced food neophobia (Coulthard & Thakker, 2015). Evidence on the relationship between food neophobia and texture perception in adults are still relatively scarce.

1.4.2 Disgust

Disgust is considered an adaptive food rejection response developed to help individuals to avoid the contact with poisons and pathogens (Rozin & Fallon, 1987; Curtis, 2011; Curtis, Barra, & Aunger, 2011).

Disgust in part occurs as a response to sensory perception of specific odour, texture, or visual cues that may lead to food product avoidance (Mataix-Cols et al., 2008; Kauer et al., 2015; Sherlock et al., 2016). Sensitivity to disgust has been strongly associated with higher perception of bitter taste (Schienle, et al., 2015; Rocha-Parra et al., 2021) and super-tasters were found to be more responsive to disgust triggers than tasters and non-tasters (Herz, 2011).

Rejection of foods, based on texture and tactile perception, has been well established in the literature (Egolf et al., 2018; Nederkoorn et al., 2019). Some foods naturally have texture properties, such as high viscosity, that can generate a disgust response even though they are safe to eat (Rozin, Millman, & Nemeroff, 1986). It has been hypothesised that food texture can contribute to disgust responses by reminding individuals of

food rot and decay (Martins & Pliner, 2006). People with high levels of disgust sensitivity were reported to be more likely to reject foods with specific texture property, such as chewy, slippery (Egolf et al., 2018).

1.4.3 Sensation seeking

Sensation seeking is the 'seeking of varied, novel, complex, and intense sensations and experiences, and the willingness to take physical, social, legal, and financial risks for the sake of such experience'. Sensation seeking has been positively correlated with the willingness to taste novel foods (Terasaki & Imada, 1988; Ludy & Mattes, 2012; Byrnes & Hayes, 2013). Sensation seekers, such as high-risk takers, were found to be much more likely to use alcohol, smoke, use other drugs, and be involved in deviant behaviours compared to low-risk takers (Donohew et al., 1990). Rozin and Schiller suggested a possible association between sensation seeking and chili preference (Rozin & Schiller, 1980) and both liking and intake of spicy foods (Byrnes & Hayes, 2013; Byrnes & Hayes, 2016). Sensation seeking has also been reported to be involved in the consumption of adverse foods, specifically those yielding specific post-ingestive effects, such as coffee, tea, or chocolate containing caffeine (Mattes, 1994) or methylxanthine (Evans et al., 2006). Recently, consumers higher in sensation seeking and bitterness perception were found to prefer pale ale beers rather than lager beers (Higgins et al., 2020). The role of sensation seeking, and the perception and consumption of specific food textures appeared to have been explored in a lesser extent, thus requiring more investigations.

1.4.4 Sensitivity to punishment and sensitivity to reward

Sensitivity to punishment and sensitivity to reward describe individual differences in reactivity and responsivity to the behavioural inhibition and activation systems, respectively (Gray & McNaughton, 2008). According to Gray's neuropsychological theory of personality, two basic brain systems control behaviour and emotions: the Behavioural Inhibition System (BIS) and the Behavioural Activation System (BAS) (Gray & McNaughton, 2008). The responsiveness of these systems has been measured using the Sensitivity to punishment and sensitivity to reward questionnaire (SPSRQ) (Torrubia et al., 2001).

Sensitivity to punishment was found to be negatively associated with liking of spicy foods (Byrnes & Hayes, 2013), while sensitivity to reward was found to be positively associated with chili intake, liking of spicy foods, and choice of pungent foods (Byrnes & Hayes, 2016). Recent studies have also highlighted an association between sensitivity to reward and unhealthy food behaviours, such as a preference for sweet and fatty foods, higher fat intake, higher alcohol consumption, and smoking frequency (Morris et al., 2016; Tapper et al., 2015). However, relationship between sensitivity to punishment and reward and the perception and consumption of specific food textures appeared to have been explored in a lesser extent, requiring more investigations.

1.4.5 State and trait anxiety

Trait anxiety refers to the dispositional and relatively stable tendency of an individual to experience anxiety, defined as a set of physical and psychological reactions, including unpleasant state of inner agitation, nervous behavior, somatic complaints, and rumination (Seligman, Walker, & Rosenhan, 2001).

The underlying mechanism by which anxiety contributes to the rejection of new foods is still unclear; however, it has been recently suggested that anxiety could have an additive effect in the disgust response (Brown and Harris, 2012): being forced to eat a food towards which one feels disgust may increase the disgust and the associated anxiety response (Lafraire et al., 2016). Anxiety has also been linked to food neophobia (Pliner & Hobden, 1992; Galloway et al., 2003). State anxiety may play a role in food choices by modulating the perceived intensity of tastes. Bitterness sensitivity has been reported to vary with self-reported anxiety, either positively or negatively (Thomas et al., 2014).

2. Aim of the thesis

Several evidence suggests a primary role of food texture perception in driving food preference, choice and intake, with overall consequences on individual's health status.

Individual variability in food texture perception may be partially explained by individual variability in the oral somatosensory system sensitivity. PROP bitterness responsiveness and fungiform papillae density (FPD) are common phenotypic marker of oral responsiveness, including both taste and tactile stimuli. However, specific phenotypic marker of oral somatosensory system sensitivity is currently not available and the relationships between measures of oral tactile acuity and phenotype markers of chemosensory responsiveness is relatively underexplored.

The hedonic response to food products with varied texture properties may be affected by the perception of generally disliked texture-related sensations, such as graininess or astringency. A negative association between intensity of tactile sensations and liking is likely to be supposed, and individual differences can be hypothesised based on individual variation in responsiveness to oral tactile sensations. The availability of foods and food model systems showing systematic variation in intensity of tactile sensations is relatively scarce as well there is a paucity of information on the effect of tactile tastant concentration, perception of texture-related sensations and their impact on liking. Moreover, the impact of the individual variation in the several dimensions involved in food perception and liking (socio-demographic, physiological and psycho-attitudinal) is relatively well studied for tastes but it is still relatively underexplored for texture.

Individual differences in food texture perception and preference may be underlined by both physiological and psychological factors. Personality traits including food neophobia, sensitivity to disgust, sensation seeking, sensitivity to punishment and reward and anxiety may influence the hedonic response to food characterised by warning food texter-related sensations.

Thus, based on these critical issues, the aims of the present thesis can be summarized as in the following:

1) To develop measures capturing individual variation in tongue responses to mechanical stimulation to be used as functional tools to explore individual responsiveness to the different properties of food texture. The relationships between oral tactile acuity measures and phenotypic markers of chemosensory responsiveness (PROP responsiveness and fungiform papillae density) will be explored for a deeper understanding of factors associated with individual variability in responsiveness to oral tactile stimuli.

2) To study the relationships between concentration and intensity of sensations evoked by specific oral tactile stimuli (microcrystalline cellulose for graininess and tannic acid for astringency) in water solution and fruit juices. Model systems varying in intensity of graininess and astringency will be developed as a tool for the systematic exploration of individual differences in responsiveness to oral tactile stimulation.

3) To explore individual differences in graininess and astringency perception in water and pear juice model systems and associated factors including gender, oral tactile sensitivity measures, phenotypic markers of oral

chemosensory acuity (PROP responsiveness and fungiform papillae density) and personality/psychological traits.

4) To investigate liking for pear juice model systems with varied intensity of tactile sensations (graininess, astringency and their combinations). Identifying consumer groups with specific liking/target sensation patterns and exploring associated factors including gender, responsiveness to taste and tactile sensations evoked by model system, oral tactile sensitivity measures, phenotypic markers of oral chemosensory acuity (PROP responsiveness and fungiform papillae density) and personality/psychological traits.

3. General material and methods

Data collection was organised in a pre-study and in a main experiment.

Pre-study. The aims of this data collection were twofold: 1) assessing the relationships between concentration of selected tactile stimuli (microcrystalline cellulose and tannic acid) and intensity of target sensations (graininess and astringency, respectively) to identify tastants concentration range to induce significant variation of intensity of graininess and astringency in water solution and fruit juices; 2) testing the protocols for assessing tactile acuity thresholds using both point-pressure test and gratings orientation test.

Main experiment. Variables including physiological, sensory, hedonic, and psychological ones, hypothesised to affect perception and liking for foods with varied texture properties were collected in the main study. Sensory and liking test were performed on water solutions and fruit juices prepared to induce different intensity of target sensations. Responsiveness to PROP and density of fungiform papillae (FPD) were determined. Individual tactile acuity was assessed as grating recognition threshold. Questionnaire were used to profile participants for psychological traits (food neophobia, sensitivity to disgust, sensation seeking, sensitivity to punishment and reward, state, and trait anxiety).

3.1 Participants

Participants were recruited on regional basis by announcements published on research unit websites, emails, pamphlet distribution and word of mouth. At the time of recruitment, respondents were asked to complete an online questionnaire on socio-demographic and physical health characteristics. Familiarity with and liking for fruit juices were collected on a five (1= I do not recognize it; 2= I recognize it, but have never tasted it; 3= I have tasted it but I don't eat it; 4= I occasionally eat it; 5= I regularly eat it; (Tuorila et al., 2001)) and nine point (1=dislike extremely; 5= neither like nor dislike; 9=extremely like) scales. Both pregnancy and breastfeeding, food allergies and history of perceptual disorders were exclusion criteria. The study was conducted in agreement with the European ethical requirements on research activities and personal data protection (General Data Protection Regulation, GDPR, UE 2016/679). At the time of recruitment, respondents signed the informed consent according to the principles of the Declaration of Helsinki. At the end of the study, participants were compensated for their time with a voucher.

Main study was conducted during 2020/2021, thus it was performed according to Italian government regulations to control for COVID-19 spread, which included: controlled access to the lab only in absence of Covid-19 symptoms, compliance with the minimum interpersonal distance of 1.8 m, wearing masks apart from whilst performing the test, environment and individual workstation sanitization after every use.

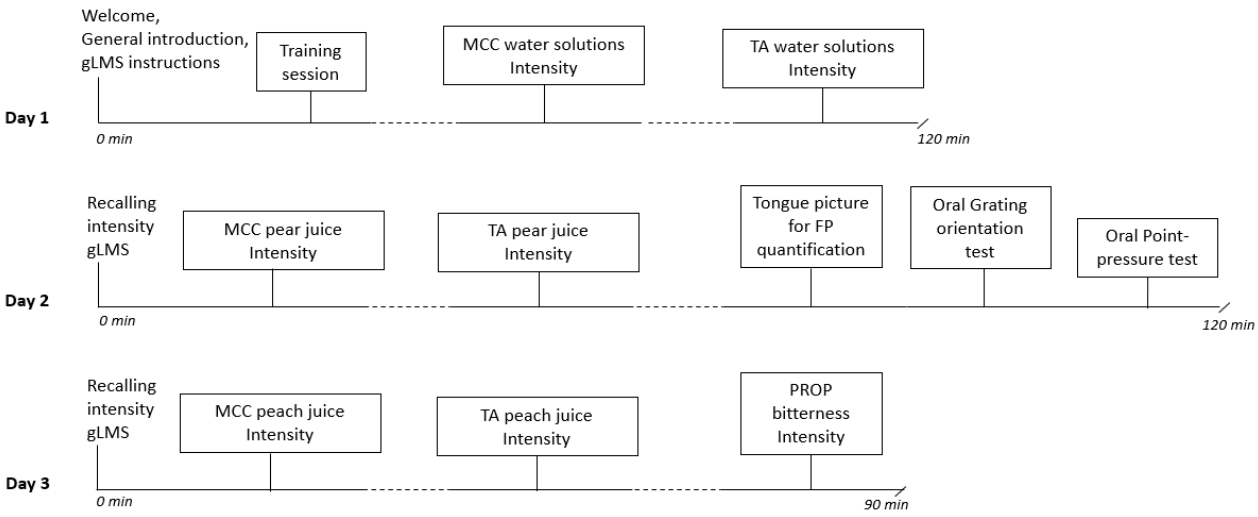
In both studies, participants, in the age range between 18 and 30 years, were selected to avoid age-related oral disorders or injury variations. Thirty-seven young women participated in the pre-study and one-hundred forty-four subjects participated in the main study (Table 1).

Table 1. Socio-demographic characteristics of participants recruited in the pre-study and in the main study

Participants		
	Number	Age ± SD
Pre-study		
Women	37	24.1 ± 1.4
Main study		
Women	70	23.7 ± 2.6
Men	74	22.8 ± 4
Total	144	23.3 ± 3.4

3.2 Pre-study: Overview of the experimental plan

Pre-study was organised in independent sessions for sensory evaluations, oral tactile acuity measures and phenotype markers of oral sensitivity assessment (Figure 2). Subjects participated in three sessions held in three consecutive days of a week with a time commitment of one and half or two hours/day. Training sessions and sensory evaluations of water solutions of microcrystalline cellulose and tannic acid were performed on day one; sensory evaluations of fruit juices were performed on days two and three. PROP responsiveness was assessed on day three after fruit juice samples evaluations. During the three days, density of fungiform papillae and measures of oral tactile sensitivity were performed according to time availability of participants.



MCC: Microcrystalline cellulose; TA: Tannic acid; FP: Fungiform papillae

Figure 2. Overview of pre-study data collection

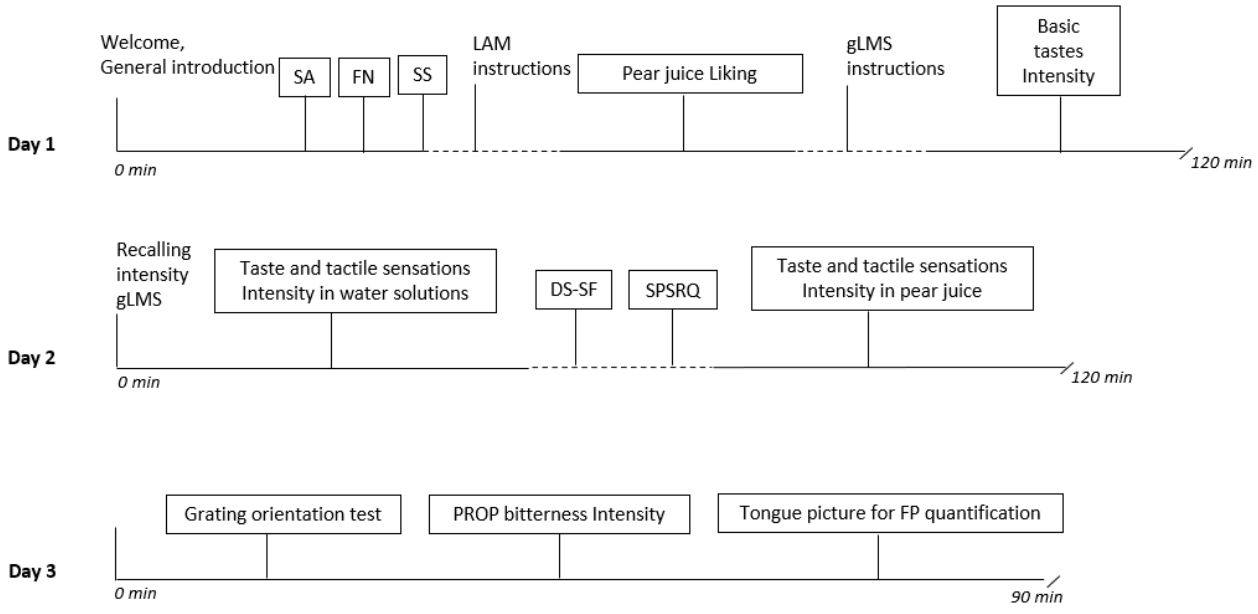
3.3 Main study: Overview of the experimental plan

Participants took part in three sessions on three different days (Figure 3).

On day 1, participants completed questionnaires on psychological traits, including the state anxiety (SA), the food neophobia (FN) and the sensation seeking (SS). Then, they were asked to evaluate liking for pear juice samples varying for graininess and astringency intensity. Finally, they were asked to evaluate the intensity of basic taste water solutions (sweet by sucrose, bitter by quinine, sour by citric acid).

In day 2 participants were asked to evaluate the intensity of tastes (sweet, sour and bitter) and tactile sensations (graininess and astringency) in water solutions and pear juice. They were also asked to complete questionnaire on psychological traits, sensitivity to disgust (DS-SF) and sensitivity to punishment and reward (SPSRQ).

Day 3 was devoted to the assessment of phenotypic markers of oral acuity including PROP bitterness responsiveness and fungiform papillae density. Grating orientation threshold (GRT) was assessed as measure of oral tactile sensitivity.



SA: State anxiety; FN: Food neophobia; SS: Sensation seeking; DS-SF: Disgust sensitivity-short form; SPSRQ: Sensitivity to punishment and reward questionnaire; FP: Fungiform papillae

Figure 3. Overview of main study data collection

3.4 Sensory evaluations

3.4.1 Sensory stimuli

Microcrystalline cellulose (MCC) (Merck, AVICEL(R) PH-101, PH EUR) was selected as stimulus to evoke graininess sensation (Santagiuliana et al., 2019). To develop MCC psychophysics curves eight concentration levels were chosen: 0.0, 0.8, 1.6, 2.4, 3.2, 4.8, 5.6, 6.4 g/100g (Furukawa et al., 2019).

Tannic acid (TA) (Tannic acid, Sigma-Aldrich) was selected as stimulus for astringency sensation. To develop TA psychophysics curve eight different concentration levels were selected: 0.0, 0.043, 0.06, 0.083, 0.116, 0.168, 0.228, 0.320 g/100g (Monteleone et al., 2017; Condelli et al., 2006).

MCC and TA psychophysics curves were developed in both water solutions and fruit juices. The criteria for the selection of the dispersion mediums were: 1) being drink products widely consumed and distributed in Italy; 2) being simple and reproducible to prepare (e.g., preferable ready-made products), to handle (e.g., to be consumed at room temperature) and homogeneous in composition and aspect to be easily portioned (e.g., liquids or semi solid). Two different commercial fruit juices were selected based on their difference in texture properties. Pear juice (PJ) (Yoga, Italy; pear (puree, juice and concentrate), antioxidant: ascorbic acid) was selected based on its natural graininess (Tarea et al., 2007) and peach juice (PcJ) (Yoga, Italy; peach puree 75%, grapes 25% (concentrated juice and juice), antioxidant: ascorbic acid) based on its natural smoothness. Based on MCC and TA psychophysics curves in water and pear juice four concentration levels were chosen for each tastant to induce the same range of variations in target sensations from weak to strong. Thus, four MCC and TA concentrations were selected in water solutions (W+MCC: 0.0, 1.6, 3.2, 5.6 g/100g; W+TA: 0.0, 0.116, 0.228, 0.32 g/100g:) and in pear juice (PJ+MCC: 0.0, 2.4, 4.8, 6.4 g/100g; PJ+TA: 0.0, 0.083, 0.168, 0.32 g/100g).

Two model systems were developed using both MCC and TA in the same solution (water or pear juice). Thus, four water solutions (W+MCC+TA) and pear juice samples (PJ+MCC+TA) were prepared with the following four tastant concentrations: water 1. MCC: 0.0 + TA: 0.0; 2. MCC: 1.6 + TA: 0.116; 3. MCC: 3.2 + TA: 0.228: 4. MCC: 5.6 + TA: 0.320; pear juice 1. MCC: 0.0 + TA: 0.0; 2. MCC: 2.4 + TA: 0.083; 3. MCC: 4.8 + TA: 0.168: 4. MCC: 6.4 + TA: 0.320 g/100g).

3.4.2 Training session

Before sensory evaluations, participants were introduced to the study and then participated in a training session. They were first introduced to the use of the general Labelled Magnitude Scale (gLMS) (Bartoshuk et al., 2004) with particular emphasis on the meaning of the descriptor “the strongest imaginable sensation of any kind.” Verbal instructions were given that the top of the scale represented the most intense sensation that subjects could ever imagine experiencing and a variety of remembered sensations from different modalities including loudness, oral pain/irritation, and tastes were recalled (Bajec & Pickering, 2008; Kalva et al., 2014; Webb et al., 2015; Dinnella et al., 2018). For participant alignment to the use of gLMS, subjects

individually rated intensities of the brightest light they had ever seen. Correct understanding of the scale was concluded if subjects rated between very strong and strongest imaginable. In case of ratings out of this range, a short individual interview was carried out to understand the ratings and scale use was explained again.

Subjects were then trained to recognize the following target sensations in water solutions prepared to be at "moderate/strong" intensity on gLMS scale: astringency (aluminium potassium sulphate 0.8 g/Kg), bitterness (caffeine 3.00 g/Kg), sourness (citric acid 4.00 g/Kg) and sweetness (sucrose 200 g/Kg) (Monteleone et al., 2017). They were also trained to recognize graininess sensation in water solution of microcrystalline cellulose prepared at a concentration of 3.2 g/100g (Furukawa et al., 2019).

3.4.3 Procedures

Samples (15mL) were presented in 80cc plastic cups identified by a 3-digit random code consisting of a random sequence of three numbers generated by the software used to collect data.

In the pre-study, water solutions of microcrystalline cellulose (W+MCC) and tannic acid (W+TA), pear juice samples added with MCC (PJ+MCC) and TA (PJ+TA), and peach juice samples added with MCC (PcJ+MCC) and TA (PcJ+TA) were presented as separated sets of eight samples each. Each set was divided in two subsets of four samples each. Samples within each set were always presented in random order across subjects. The presentation order of pear juice and peach juice samples was balanced across subjects, as well as the presentation order of MCC and TA series of samples (Figure 4).

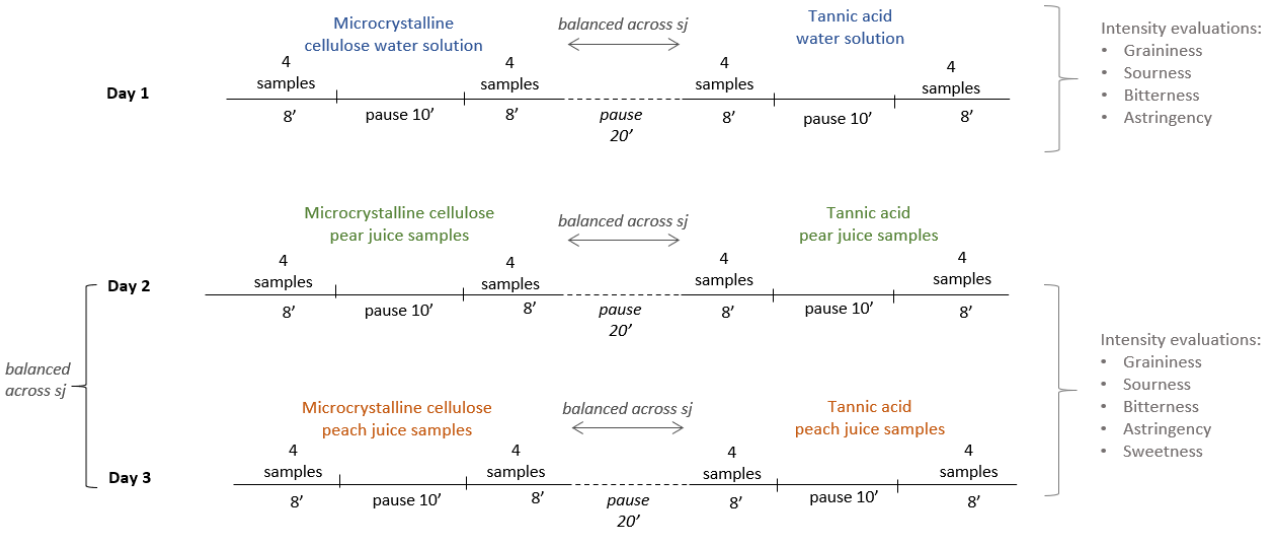


Figure 4. Order of set presentation in the pre-study

In the main study, water solutions of microcrystalline cellulose (W+MCC), tannic acid (W+TA) and microcrystalline cellulose plus tannic acid (W+MCC+TA), and pear juice samples added with MCC (PJ+MCC), TA (PJ+TA) and MCC+TA (PJ+MCC+TA), were presented as separated sets of four samples each. Samples

within each set were always presented in random order across subjects. The presentation order of MCC, TA and MCC+TA series of samples was balanced across subjects. (Figure 5). During each session participants had 20 minutes break between the sets and 10 minutes break between the subsets.

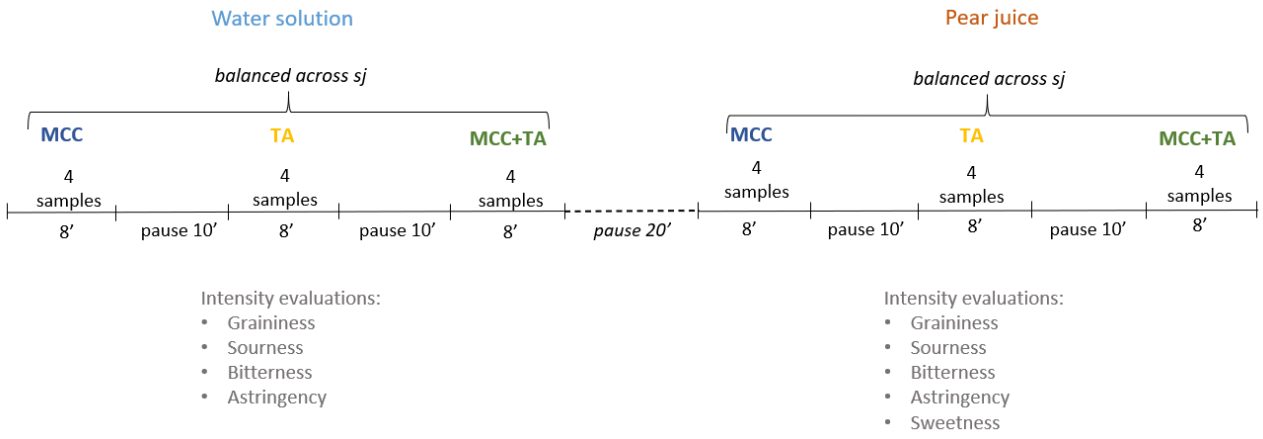


Figure 5. Order of set presentation in the main study

During tasting, subjects were instructed to hold the whole sample in the mouth and to pay their attention to graininess; after eight seconds, they were asked to evaluate the graininess intensity. Then, they were asked to expectorate and evaluate the intensity of the following sensations: residual graininess (graininess sensation which remains in the mouth after sample expectoration), sourness, bitterness and astringency. In pear and peach juices evaluations, subjects were also asked to evaluate sweetness intensity. Residual graininess was always evaluated first, then sourness, bitterness and sweetness were evaluated in randomized order. Astringency was always evaluated as the last sensation to allow for the full development of its intensity. The procedure used for the evaluation of each sample is schematized in (Figure 6). Similar conditions were adopted for the intensity evaluation in the mains study with the only exception of graininess in mouth which was not evaluated. After each sample, subjects rinsed their mouth with water for 30 seconds, had some plain cracker for 30 seconds and finally rinsed their mouth with water for a further 30 seconds. Evaluations were performed in individual booths under white light. Data were collected using software *Fizz* (ver.2.51. A86, Biosystèmes, Couternon).

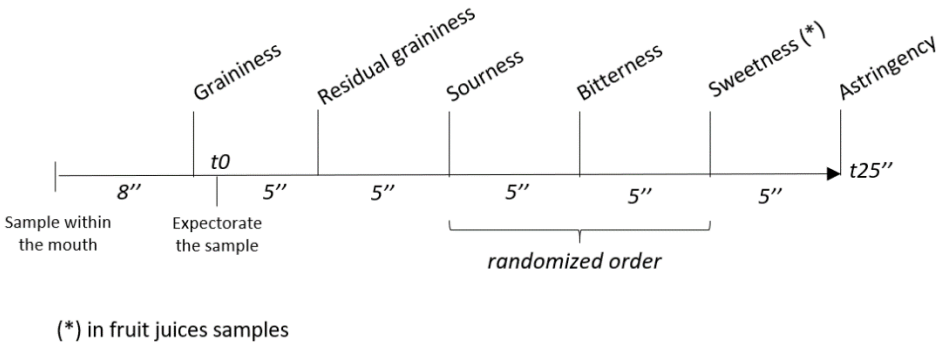


Figure 6. Order of sensations evaluation

3.5 Liking evaluation

Before starting the hedonic evaluation of pear juice samples, participants were introduced to the use of the Labelled Affective Magnitude scale (LAM; Schutz & Cardello, 2001; Cardello & Schutz, 2004) and familiarised with it. The scale anchors were spaced according to the values of Cardello and Schutz (2004), from *greatest imaginable dislike* (0) to *greatest imaginable like* (100), with *neither liked nor disliked* set at 50. Numerical labels were not reported on the scale. Participants were instructed to make a mark on the vertical line to indicate their degree of liking or disliking after tasting each sample, and to rate the sample relative to the greatest imaginable like/dislike for foods (Lawless & Heymann, 2010).

3.6 Oral tactile acuity measures

3.6.1 Test condition

The test was conducted individually by a trained operator in a quiet room. Firstly, the operator asked the participants to sit comfortably in front of him/her and explained what the test consisted of. Participants were then asked to relax their dorsal and neck muscles, show their tongue, and keep it relaxed. Participants were asked to close their eyes and to keep them closed during the test; a blindfold was not used during the test to avoid participants distraction due the feeling of being blindfolded (Etter et al., 2020). Participants were invited to ask for a pause during the test every time they felt they lost their oral lubrication and to refresh their mouth with water.

3.6.2 Threshold estimation

A 3-down/1-up staircase method was used for threshold estimation (Etter et al., 2020). The test started with the stimulus with the highest force which was applied on the tongue midline around 0.5 cm from the tip. Three correct answers to the same stimulus resulted in a presentation of the next lower stimulus level. One incorrect answer resulted in a presentation of the next higher stimulus level (Figure 7).

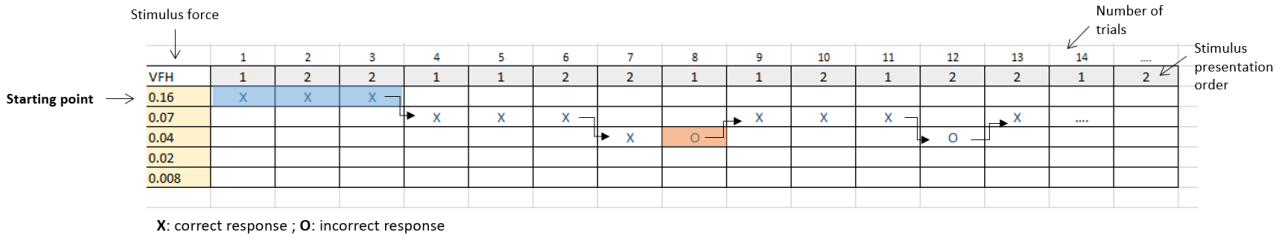


Figure 7. 3-down/1-up staircase method for threshold estimation

The test continued until the stopping point, defined as "the point when a participant has crossed over or received the test stimulus from the same target force a total of five times" (Etter et al., 2020). Examples of stopping point are shown in Figure 8.

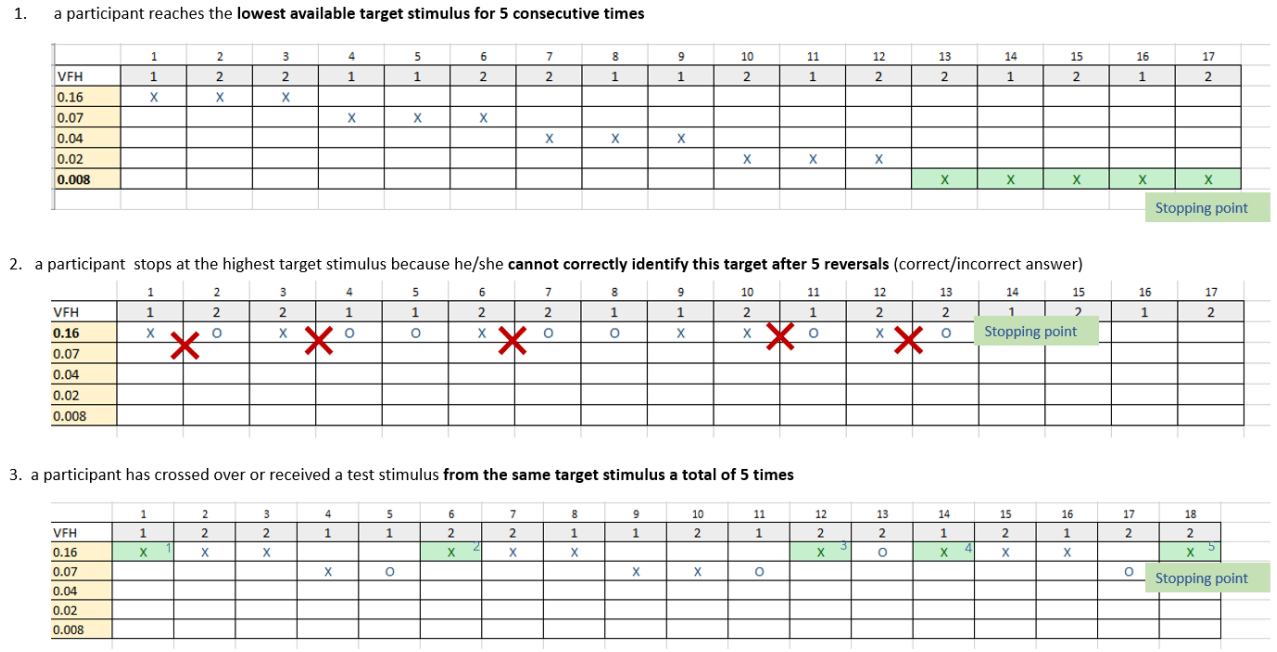


Figure 8. Examples of stopping point

Threshold for each participant was calculated as the geometric mean of all the stimulus forces included between the first time the participant received the stimulus of the stopping point and the last (Etter et al., 2020) (Figure 9).

Geometric mean of all the stimuli delivered between the **first** instance of the **stopping point** and the **last**.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
VFH	1	2	2	1	1	2	2	1	1	2	1	2	2	1	2	1	2	2
0.16	X	X	X			X	X	X				X	O	X	X	X		X
0.07				X	O				X	X	O						O	
0.04																		
0.02																		
0.008																		

First instance of the stopping point

Stopping point

Geometric mean $\mu g = \sqrt[N]{\prod_{i=1}^{N} Xi} = \sqrt[N]{x1 \cdot x2 \cdot x3 \dots xn}$

Ex.1

$\mu g = \sqrt[18]{(0{,}16 * 0{,}16 * 0{,}16 * 0{,}07 * 0{,}07 * 0{,}16 * 0{,}16 * 0{,}16 * 0{,}07 * 0{,}07 * 0{,}07 * 0{,}16 * 0{,}16 * 0{,}16 * 0{,}16 * 0{,}07 * 0{,}16)} = \mathbf{0{,}12}$ **THRESHOLD ESTIMATE**

Figure 9. Threshold estimation

3.6.3 Grating orientation test

3.6.3.1 Grating stimuli

Stimuli consisted in six square-wave gratings 1 cm x 1 cm x 0.5 cm tiles supported by a 2 cm rod. Gratings differed in their groove widths from 0.2, 0.25, 0.5, 0.75, 1.00 to 1.25 mm (Figure 10). Main study data collection occurred during the Covid-19 pandemic period; therefore, disposable grating kits were produced to ensure the safety of participants. Disposable stimuli were manufactured by a 3D stereolithography (3D-SLA) technology printing process, using a biomedical resin (Biomed Clear Resin, Formlabs, GmbH, Germany) to guarantee biocompatibility and nontoxicity requirements.

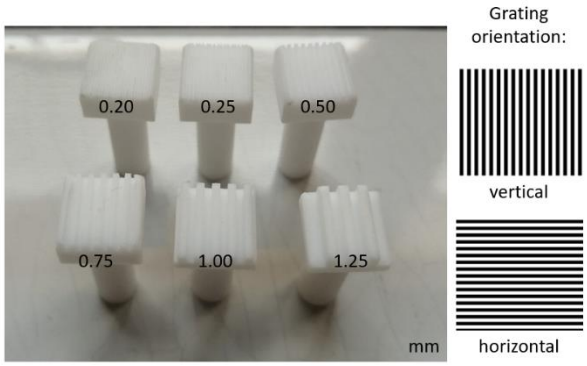


Figure 10. Grating stimuli

3.6.3.2 Grating orientation recognition threshold

Prior to the start of the test a simulation with the 1.25 mm grating was performed to make sure that participants correctly understood the task. Gratings were perpendicularly applied to the tongue surface. Participants were asked to identify the grating orientation (vertical or horizontal) (Figure 11).

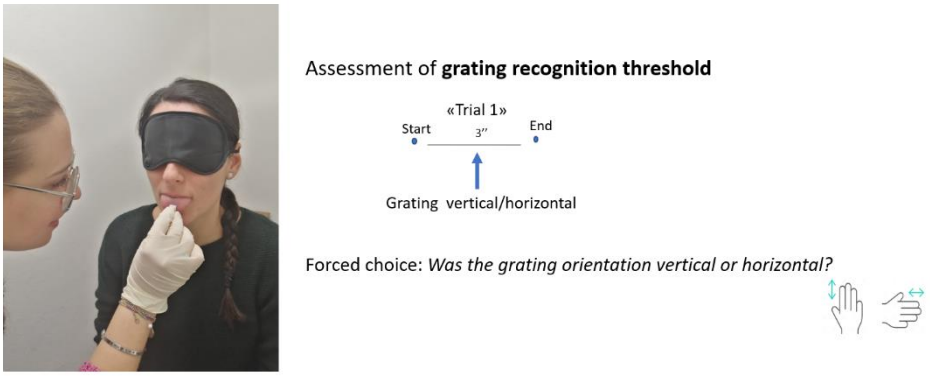


Figure 11. Grating recognition threshold assessment

3.6.4 Point pressure test

3.6.4.1 Stimuli

Stimuli consisted of commercially available aesthesiometers called Von Frey Hair monofilaments (Aesthesio-Tactile Sensory Evaluator, Ugo Basile, Italy), varying in diameter and in the force they delivered (Figure 12). Eight monofilaments were selected to perform a detection test: 1.0, 0.6, 0.4, 0.16, 0.07, 0.04, 0.02, 0.008 g (Breen et al., 2019).

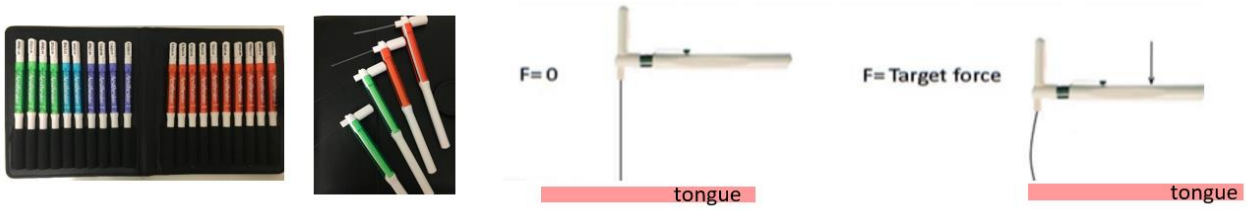


Figure 12. Von Frey Hair monofilament for point pressure test

3.6.4.2 Detection thresholds

A point-like area, 0.5 cm from the tongue tip on the anterior dorsal surface of the tongue at the midline, was highlighted using a cotton-tip applicator dipped in a blue food colouring. Each monofilament was applied perpendicularly to the tongue surface in the coloured area. Participants were asked to identify in which of the two trials they could feel the stimulus: one of the trials was a 'real' touch and the other a 'false' touch in a randomised order (Figure 13).

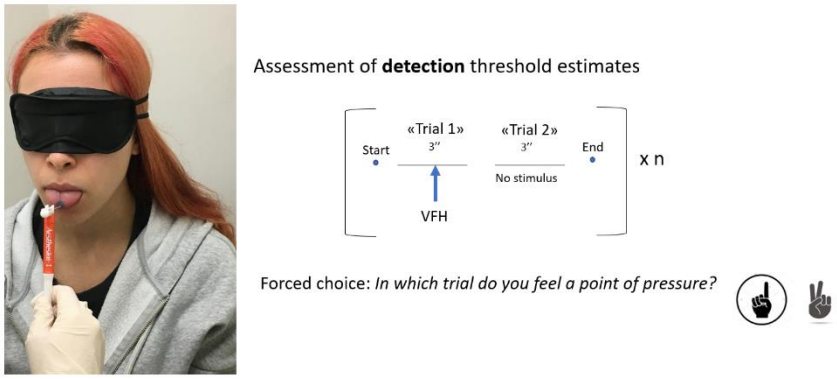


Figure 13. Point-pressure detection threshold assessment

3.6.4.3 Discrimination thresholds

Based on detection threshold estimates, five monofilaments were selected to perform the discrimination test: 0.16, 0.07, 0.04, 0.02, 0.008 grams. In each trial participants received a pair of stimuli of consecutive force levels (0.16 g was coupled with 0.07 g filament; 0.07 g was coupled with 0.04 g; 0.04 g was coupled with 0.02 g and 0.02 g was coupled with 0.008 g resulting in four pairs of stimuli) and they were asked to identify in which trial the strongest pressure was delivered (Figure 14). All the participants received the pairs of stimuli in the same order, starting from the couple 0.16 g / 0.07 g and with the subsequent pairs presented in a decreasing order. Three correct answers to the same couple (e.g., 0.16 g/ 0.07 g) resulted in a presentation of the next lower couple (e.g., 0.07 g / 0.04 g).

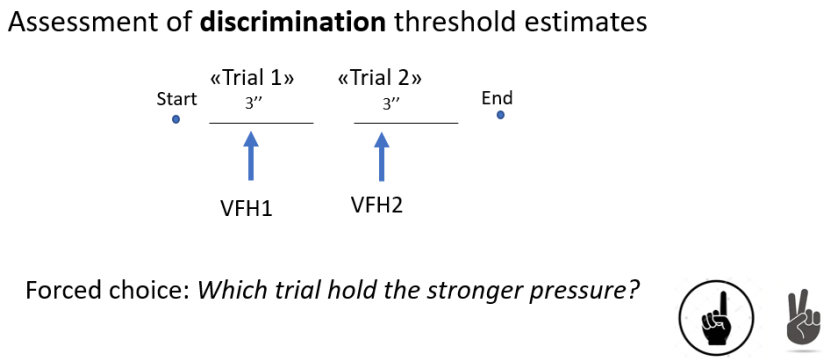


Figure 14. Point-pressure discrimination threshold assessment

3.7 Phenotypic markers of oral acuity

3.7.1 PROP bitterness ratings

Participants were trained to the use of the General Labelled Magnitude Scale (gLMS) (Bartoshuk et al., 2004) as described in 3.4.2. A 3.2 mM PROP solution was prepared by dissolving 0.5447 g/L of 6-n-propyl-2-thiouracil (Sigma-Aldrich, USA) into deionized water (Prescott et al., 2004). Samples were presented in

duplicate (10 mL), labelled with 3-digit codes (Masi et al., 2015). Participants were instructed to hold the sample in their mouth for 10 s, expectorate, and then wait 20 s before evaluating the bitterness intensity using the gLMS (Bartoshuk et al., 2004). After cleaning the palate with plain crackers, participants rinsed their mouths with water and waited for 90 seconds before being served a duplicate sample with a different 3-digit code. The average bitterness score of the two duplicate samples was calculated for each participant.

3.7.2 Fungiform papillae density (FPD)

Participants were instructed to swab the anterior portion of the dorsal surface of their tongue with blue food colouring, using a cotton-tipped applicator. During the procedure, the operator controlled that participant correctly coloured their tongue surface (intensity of blue, colour both tongue dorsal surface and sides). Digital pictures of the tongue were taken (Shahbake et al., 2005) using a digital microscope (Micro Capture, version 2.0 for ×20 to ×400) (Masi et al., 2015) and the clearest image was selected for each participant. A rectangular area of the tongue image (1.125 cm^2; image resolution: 96 dpi) orthogonal to the median line and located 0.5 cm from the tongue tip was selected for processing. Fungiform papillae density (FPD) was quantified through an automated procedure that counted the number of circular-like elements on the picture considering a diameter range 0.30-1.05 mm and included them in 11 diameter classes (Piochi et al., 2017) (Figure 15). Circular-like elements counted by the script in each diameter class (DC) were converted into FPD by dividing for the area. For each participant, the total FPD was computed as the sum of FPD in all size classes (Piochi et al., 2019).

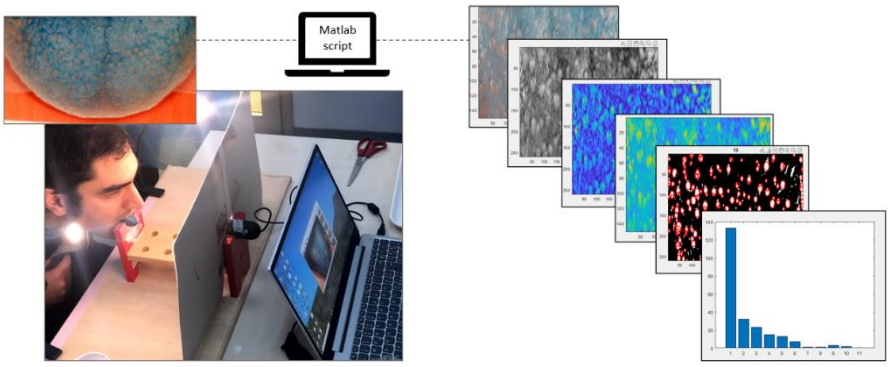


Figure 15. Procedure for fungiform papillae quantification (adapted by Piochi et al., 2017)

3.8 Measurement of psychological traits

Participants completed questionnaires to assess five psychological and personality traits: food neophobia, sensitivity to disgust, sensitivity to punishment and reward, sensation seeking and state and trait anxiety. Questionnaires showed satisfactory internal consistency with Cronbach's alpha ranging from 0.640 and 0.920. Cronbach's alpha for each trait and descriptive statistic of questionnaire scores are summarized in Table 2.

Table 2. Chronbach's alpha and descriptive statistics of personality traits (food neophobia, FN; sensitivity to disgust, DS-SF; sensation seeking, SS; sensitivity to punishment, SP; sensitivity to reward, SR, state and trait anxiety)

Statistic	FNS score	DS-SF score	SS score	SP score	SR score	State anxiety score	Trait anxiety score
Chronbach's alpha	0.920	0.640	0.898	0.819	0.670	0.904	0.897
Minimum	10.000	15.000	53.000	1.000	1.000	22.000	24.000
Maximum	70.000	38.000	137.000	22.000	15.000	71.000	64.000
1st Quartile	19.000	23.750	92.000	7.000	6.000	35.750	35.000
Median	24.500	27.000	99.500	11.000	7.000	41.500	42.000
3rd Quartile	33.000	31.000	109.250	14.000	10.000	47.000	47.000
Mean	26.847	27.368	99.361	10.556	7.799	41.500	41.771
Standard deviation (n-1)	11.453	5.238	15.235	4.920	3.205	8.806	8.702
Skewness (Pearson)	1.006	-0.038	-0.332	0.251	0.209	0.204	0.277
Standard error of the mean	0.954	0.437	1.270	0.410	0.267	0.734	0.725

3.8.1 Food neophobia

Food Neophobia (FN) was quantified using the 10-statement scale developed by Pliner & Hobden (1992), and validated in Italian by Laureati and colleagues (Laureati et al., 2018). Individual food neophobia scores were computed as the sum of ratings given to the 10 statements (using a 7-point Likert scale: *disagree strongly/agree strongly*). Items 1, 4, 6, 9, 10 were reversed. The individual scores ranged from 10 to 70, with higher scores corresponding to higher food neophobia.

Food neophobia scores did not follow a normal distribution ($W= 0.934$; $p< 0.0001$) (Figure 16A). FN scores ranged from 10 to 70 (Figure 16B). No significant differences were found by gender for FN mean values ($F= 0.378$; $p= 0.540$).

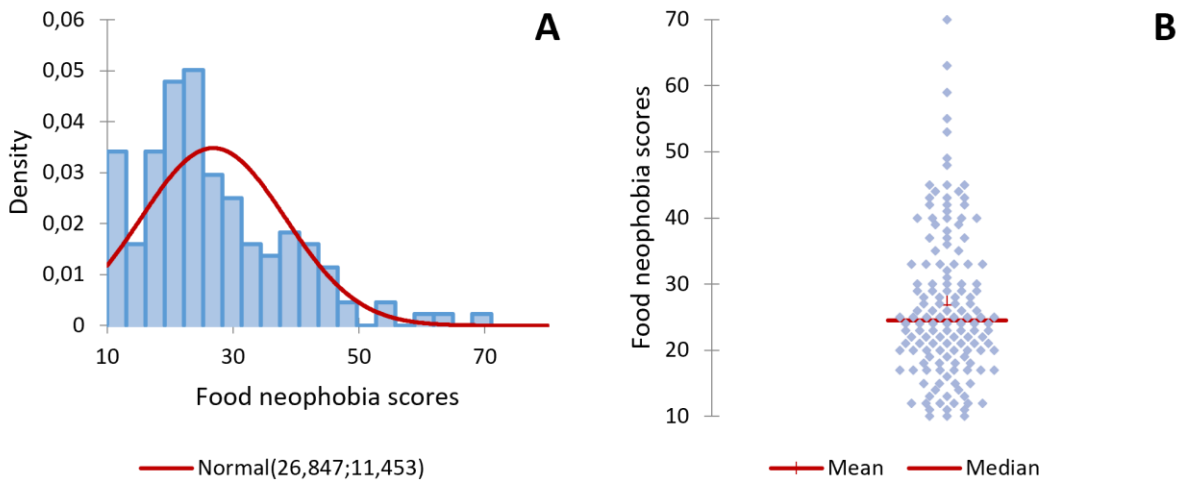


Figure 16. Distribution (A) and range of variation (B) of food neophobia score (n=144)

3.8.2 Sensitivity to disgust

Individual sensitivity to disgust was evaluated with the disgust sensitivity-Short Form (DS-SF) questionnaire which is a short form of the Disgust Scale (Haidt et al., 1994; Inbar et al., 2009; Olatunji et al., 2007) validated in Italian by (Spinelli et al., 2018). The responsivity to disgust was measured using 8 items divided into 2 parts. In the first part, the statements were rated using a scale from 1 (*strongly disagree/ very untrue about me*) to 5 (*strongly agree/very true about me*); for the second part, additional statements were rated from 1 (*not at all disgusting*) to 5 (*extremely disgusting*). Items 1 and 3 were reversed. The individual total score for sensitivity to disgust was given by the sum of scores and ranged from 8 to 40.

Sensitivity to disgust scores (DS-SF) followed a normal distribution (W= 0.985; p= 0.127) (Figure 17A). DS-SF scores ranged from 15 to 38 (Figure 17B). Significant differences were found by gender for DS-SF mean values, with women showing higher DS-SF scores than men (F= 19.060; p< 0.0001).

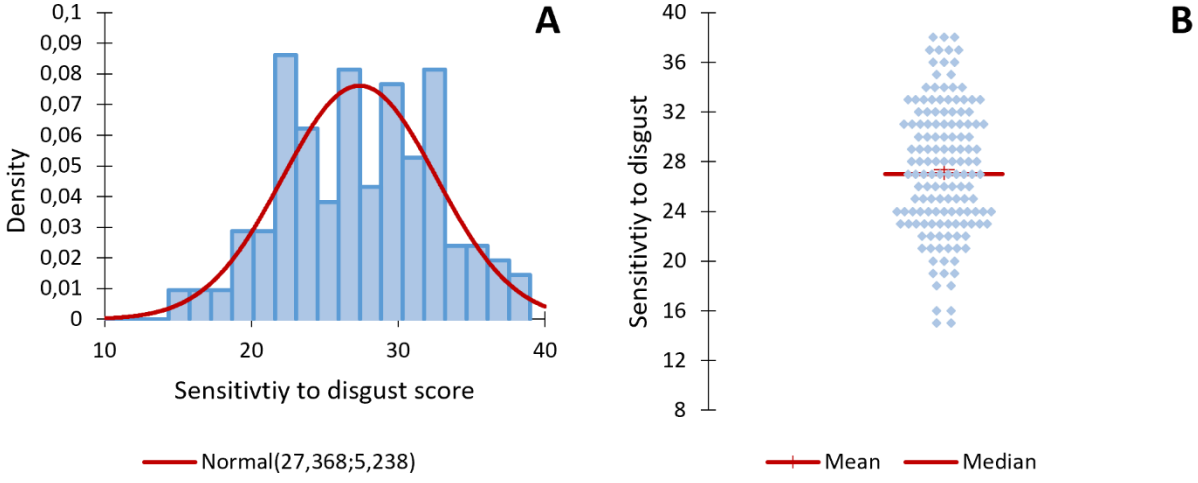


Figure 17.Distribution (A) and range of variation (B) of sensitivity to disgust scores (n=144)

3.8.3 Sensation Seeking

Sensation Seeking was quantified using the 4 subscales related to sensation seeking (SS1: Thrill and Adventure Seeking; SS2: Experience Seeking; SS3: Disinhibition; SS4: Boredom Susceptibility/Impulsivity) of the Zuckerman–Kuhlman–Aluja personality questionnaire (ZKA-PQ) (Aluja et al., 2010), validated in Italian by De Pascalis & Russo (2003). The questionnaire provides a total sensation seeking score (SS total) and four subscale scores. Individual scores were computed as the sum of ratings given to each subscale, using a 4-point Likert scale (*disagree strongly/agree strongly*). Items 12, 16, 20, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 40 were reversed. The SS total scores range from 40 to 160, with higher scores corresponding to higher sensation seeking.

Sensation seeking (SS) scores followed a normal distribution (W= 0.985; p= 0.132) (Figure 18A). SS scores ranged from 53 to 137 (Figure 18B). Significant differences were found by gender for SS mean values, with men showing higher SS scores than women (F= 5.039; p= 0.026).

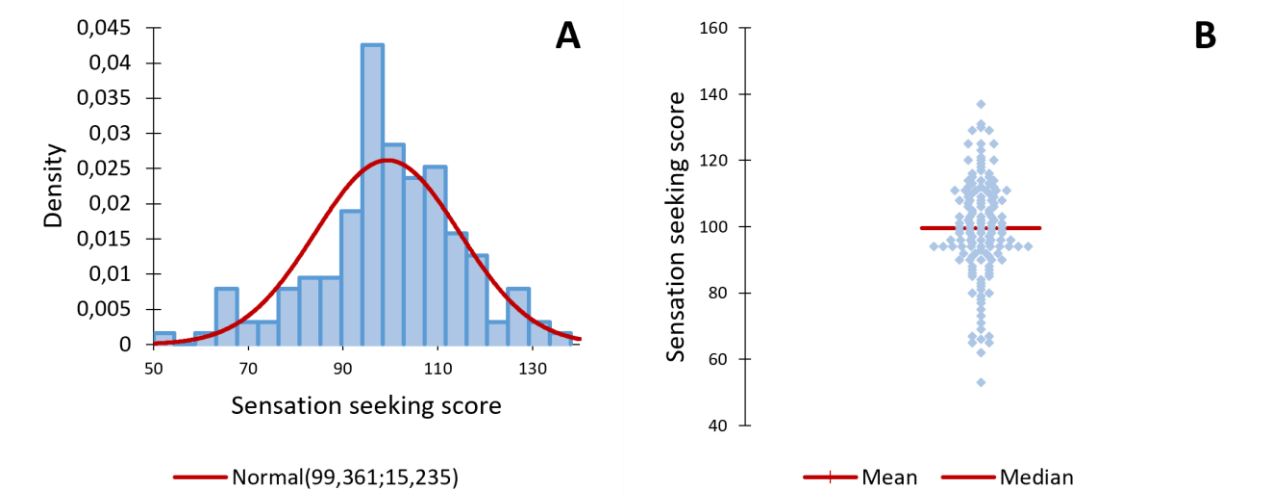


Figure 18. Distribution (A) and range of variation (B) of sensation seeking scores (n= 144)

3.8.4 Sensitivity to Punishment and Sensitivity to Reward

The SP scale is formed by a set of items reflecting situations which describe individual differences in reactivity and responsivity to the Behavioural Inhibition System (BIS). The SR scale was conceived as a single measure of the functioning of the Behavioural Activation System (BAS) dealing with specific rewards (i.e., money, gender, social power and approval, and praising). The SP and SR scales were scored with a *yes/no* format. For each subject, scores for each scale were obtained by adding all the yes answers. In the original version, the score for each scale ranges from 0 to 24. Items 4, 8, 16, 25, 32, 34, and 36 were discarded, based on the validation of the questionnaire in Italian (Spinelli et al., 2018); thus the scores range from 0 to 23 for SP and from 0 to 18 in SR, with higher scores reflecting, respectively, higher sensitivity to punishment and to reward.

Sensitivity to punishment (SP) scores did not follow a normal distribution (W= 0.997; p= 0.017) (Figure 19A). SP scores ranged from 1 to 22 (Figure 19B). Significant differences were found by gender, with women showing higher SP scores than men (F= 8.554; p= 0.004).

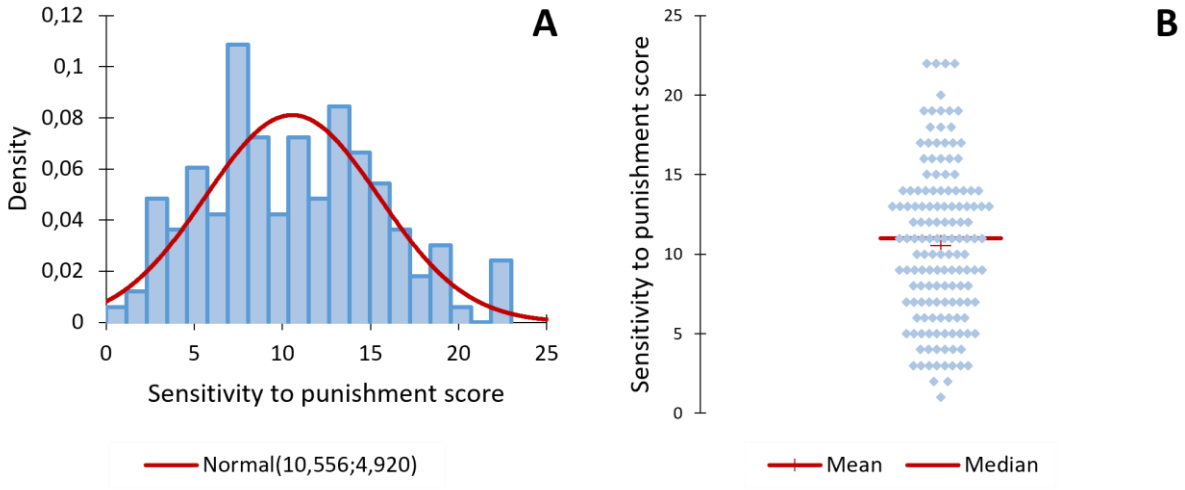


Figure 19. Distribution (A) and range of variation (B) of sensitivity to punishment (n= 144)

Sensitivity to reward (SR) scores did not follow a normal distribution (W= 0.976; p= 0.013) (Figure 20A). SP scores ranged from 1 to 15 (Figure 20B). No significant differences were found by gender for SR scores (F= 1.686; p= 0.196).

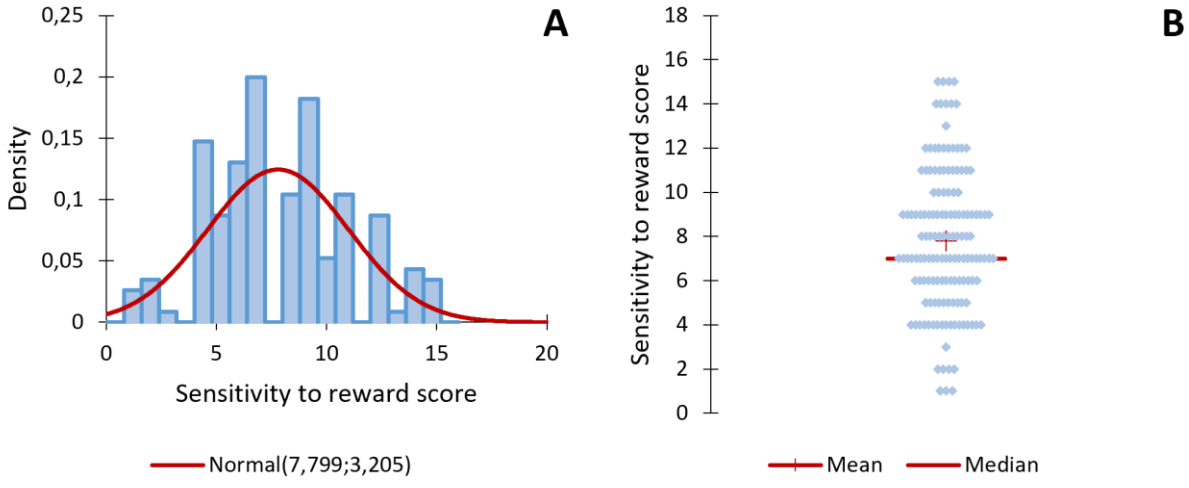


Figure 20. Distribution (A) and range of variation (B) of sensitivity to reward (n= 144)

3.8.5 State and Trait Anxiety

State and Trait anxiety was quantified using the State-Trait anxiety Inventory developed by Spielberger (1983) and validated in Italian by Pedrabissi & Santinello (1989).

In responding to the State anxiety items, subjects report the intensity of their feelings of anxiety ‘‘right now, at this moment’’ by rating them on 4-point Likert scale (*not at all/ somewhat/ moderately so/ very much so*). Responses to the Trait anxiety items require subjects to indicate how they generally feel by reporting how often they have experienced anxiety-related feelings and cognitions on a 4-point Likert scale *(almost never/ sometimes/ often/ almost always).*

Individual score was computed for each scale as the sum of ratings given to the 20 statements. Items 1, 2, 5, 8, 10, 11, 15, 16, 19, 20 (State anxiety) and items 1, 3, 6, 7, 10, 13, 14, 16, 19 (Trait anxiety) were reversed. Scores for both scales can vary from a minimum of 20 to a maximum of 80, with a higher score indicating a greater level of anxiety.

State anxiety scores followed a normal distribution (W= 0.990; p= 0.399) (Figure 21A). State anxiety scores ranged from 22 to 71 (Figure 21B). Significant differences were found by gender, with women showing scores significantly higher than men (F= 4.957; p= 0.028).

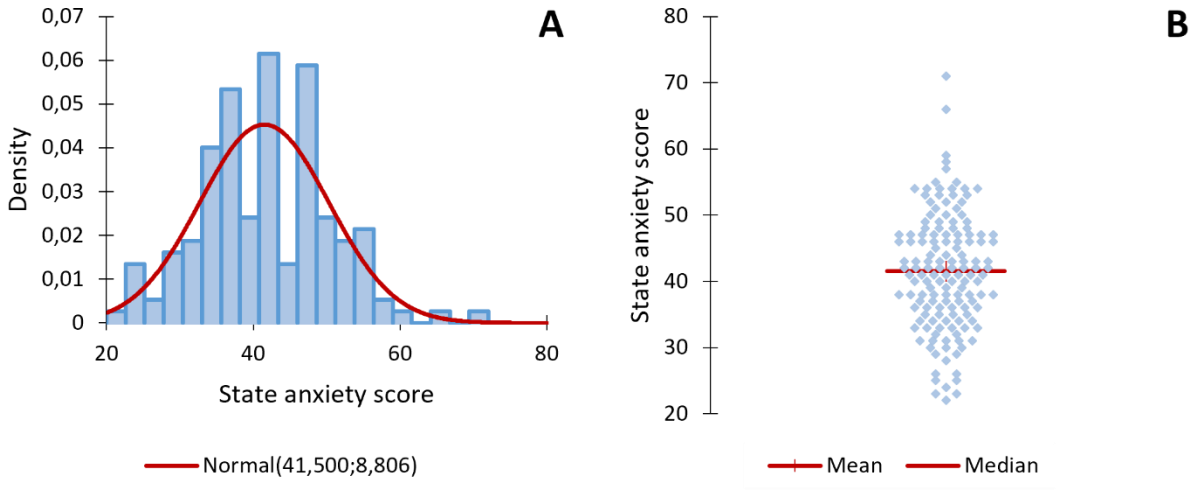


Figure 21. Distribution (A) and range of variation (B) of state anxiety score (n= 144)

Trait anxiety scores followed a normal distribution (W= 0.985; p= 0.120) (Figure 22A). Trait anxiety scores ranged from 24 to 64 (Figure 22B). Significant differences were found by gender, with women showing significantly higher scores than men (F= 6.341; p= 0.013).

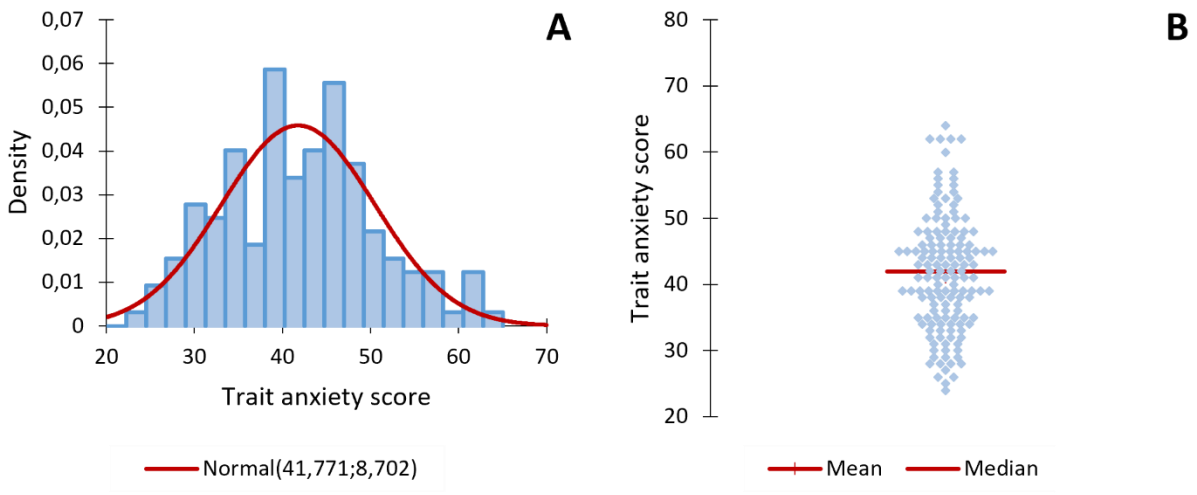


Figure 22. Distribution (A) and range of variation (B) of trait anxiety score (n= 144)

4. Results

4.1 Exploring the association between oral tactile sensitivity measures and phenotypic markers of oral responsiveness.

submitted to Journal of Textural studies (submitted copy attached)

4.1.1 Introduction

Food texture is one of the main drivers of food acceptance and is involved in physiological pathways, such as satiation and satiety mechanisms, thus playing a fundamental role in regulating the amount of food intake (James, 2018). Recent studies suggest that individual differences in food texture perception may be associated with oral somatosensory system sensitivity, with individuals higher in oral tactile sensitivity perceiving food texture-related sensations more intensely than individuals with lower oral tactile sensitivity (Zhou et al., 2021; Shupe et al., 2018, Olarte Mantilla, 2022). Consistent with this hypothesis, high oral tactile sensitivity was found to be positively associated with a greater particle perception and size discrimination in yogurt (Olarte Mantilla et al., 2022) and chocolate samples (Breen et al., 2019) and to a higher hardness perception in biscuits (Zhou et al., 2021). However, the literature regarding this is still relatively scarce and affected by many methodological differences in oral tactile acuity measures.

The current measures of oral tactile sensitivity convey information about specific functions of oral mechanoreceptors, such as the perception of touch (Weinstein, 1968), spatial resolution (Weinstein, 1968) and oral stereognosis (Lederman & Klatzky, 2009), indicating complexity of the oral somatosensory system. The most common methods are: the point-pressure method by Von Fryer monofilaments (Yackinous & Guinard, 2001; Breen et al., 2019; Etter et al., 2020; Zhou et al., 2020), the oral stereognosis assessment by the identification of letters and shapes (Essick et al., 1999; Essick et a., 2003; Lukasewycz & Mennella, 2012; Steel et al 2014; Bancguyo et al 2017) and the spatial resolution assessment by the identification of two point distance (Olarte Mantilla et al., 2022) or the identification of grating orientation (Johnson & Phillips, 1981; Wohlert, 1996).

Limitations were observed for all these methods. The point-pressure method stimulates a very small area of the tongue which does not reflect the oral tactile sensitivity of the whole mouth (Zhou et al., 2021). Furthermore, the device used to assess oral point-pressure sensitivity consists of a kit of monofilaments that deliver specific target forces, which might not correspond to the lower force that tongue mechanoreceptors could perceive, thus, leading to a floor effect (Santagiuliana et al., 2019). On the other hand, oral stereognosis and spatial resolution methods are recognised as a more complex task involving cognitive processes (recognition of the shape or the grid orientation), which are also affected by cultural factors such as familiarity with the tactile cues (i.e. alphabet letter) (Cattaneo et al., 2019; Essick et al., 1999; Shupe et al., 2018). It was suggested that the stimuli used to test touch sensitivity mainly stimulate superficial receptors,

while stimuli used to test the oral stereognosis or the spatial resolution might excite a set of deeper receptors (Engelen & Van Der Bilt, 2008). Therefore, to overcome intrinsic limitations belonging to each method and to have more reliable measure of oral tactile sensitivity it was recently suggested to consider a variety of methods when exploring oral tactile sensitivity (Cattaneo et al., 2019; Appiani et al., 2020; Santagiuliana et al., 2019; Zhou et al., 2021).

Associations have been previously reported between individual variation in food texture perception and common phenotypic markers of oral sensitivity, namely the responsiveness to the bitterness of 6-n-propylthiouracil (PROP status) (Melis & Barbarossa, 2017; Pickering & Robert, 2006; Kirkmeyer & Tepper, 2003; Bakke & Vickers, 2008) and the fungiform papillae density (FPD) (Hayes & Duffy, 2007; Zhou et al., 2021). Differences in PROP responsiveness, are mainly due to genetic variation in the TAS2R38 gene, which define two common haplotypes: PAV (considered the "taster haplotype") and AVI (considered the "non taster" haplotype)(U. kyung Kim et al., 2003). PROP bitterness responsiveness is largely known to be positively associated to the perception of basic tastes in water solutions and in real products (Dinnella et al., 2018; Masi et al., 2015; Tepper et al., 2017). PROP bitterness was found to be positively associated with the perception of tactile/texture-related sensations such as astringency (Melis et al., 2017; Pickering & Robert, 2006), creaminess (Kirkmeyer & Tepper, 2003) and roughness (Bakke & Vickers, 2008).

Fungiform papillae are the anatomical structures designated to oral stimuli detection and transduction since they house taste buds that respond to chemical stimulation, and are innervated by chorda tympani and trigeminal nerve fibers which respond to tactile stroking and temperature stimuli (Whitehead et al., 1985; Mistretta & Bradley, 2021). Fungiform papillae were found to vary significantly across individuals, from 0 to 200 papillae/cm^2 (Zhang et al., 2009; Fischer et al., 2013; Eldeghaidy et al., 2018). Fungiform papillae also vary in size and shape (Essick et al., 2003). Recently a classification of subjects based on their differences in both FP density and diameter was proposed (Piochi et al., 2019). Positive associations were reported between FPD and the perception of texture related sensations such as creaminess in milk (Hayes & Duffy, 2007) and hardness and crunchiness in biscuits (Zhou et al., 2021). On the other hand, other studies failed to find significant associations between FPD and food texture attributes, such as astringency in bread (Bakke & Vickers, 2008). Furthermore, FP size was hypothesised to associate with oral responsiveness (Melis et al., 2013) with uniform patterns of low density and small size FP related with higher responsiveness to tastes, astringency and pungency (Piochi et al., 2019).

Literature on the relationship between PROP status and FPD with measures of oral tactile sensitivity has been limited and has shown conflicting results. PROP responsiveness was found positively associated with oral spatial stereognosis (Essick et al., 2003) and point-pressure (Yackinous & Guinard, 2001a). Similar to PROP, positive associations were also reported between FPD and both oral spatial stereognosis (Essick et al., 2003; Bangcuyo, Christopher, & Simons, 2017) and point-pressure measures (Zhou et al., 2021). On the other hand,

no significant association between both PROP responsiveness and FPD with point pressure measures was reported (Cattaneo et al., 2019; Nachtsheim & Schlich, 2013).

Demographic factors are reported to affect responsiveness to PROP and FPD, on average PROP bitterness ratings and FPD are significantly higher in women than in men and increasing age is negatively associated to both phenotypic markers (Shahbake et al., 2005; Tepper et al., 2017; Mennella et al., 2010; Feeney & Hayes, 2014; Webb et al., 2015; Dinnella et al., 2018; Barragán et al., 2018). Little is still known about the effect of gender on oral tactile sensitivity measures, no significant differences by gender have been reported in a letter identification task (Bangcuyo et al., 2017), while adult women performed better than men in grating orientation test only for the greatest grating size (Appiani et al., 2020).

Thus, individual variations in food texture perception were related, even if with contrasting results, to both measures of oral tactile sensitivity and phenotypes of oral responsiveness. However, the associations between measures of oral tactile sensitivity and both PROP responsiveness and FPD are yet to be fully elucidated.

This study aims to explore the individual variability in oral tactile sensitivity considering both touch and spatial resolution, by means of the VHF point-pressure and grating orientation tests in a sample of young adults. The association between the two considered oral tactile sensitivity measures was assessed in a smaller subsample of women. The relationships of oral tactile sensitivity measures with both PROP bitterness responsiveness and fungiform papillae density and size were investigated with a possible gender effect also considered.

4.1.2 Data analysis

Descriptive statistics (mean and median values, first and third quartile limits) were used to describe GRT and VFH thresholds, PROP bitterness ratings and FPD. Shapiro-Wilk test was used to assess variable distribution (α=0.05). Distributions were compared by two-sample Kolmogorov-Smirnov (α=0.05). One-way ANOVA models were independently applied to test for gender effect on GRT, PROP bitterness ratings, FPD for each diameter class and total FPD. Correlations between GRT, PROP bitterness and FPD and between GRT and VFH discrimination thresholds were tested by Pearson correlation, with significance level fixed at $p \leq 0.05$. Agglomerative hierarchical clustering (AHC) was used to classify participants for their mean PROP bitterness rating, total FPD and GRT threshold. Euclidean distance was selected as proximity type and Ward's method was chosen as agglomeration method; data were centered, and automatic-entropy criterion was selected for truncation. One-way ANOVA was used to test the effect of each cluster on PROP bitterness ratings, total FPD, and GRT. The association between cluster and gender was investigated using chi-square tests. Fisher's exact test was run to test the significance by cell (significance level fixed at $p = 0.05$). All data analysis was conducted using XLSTAT (2020.2.1, Addinsoft, USA).

4.1.3 Results

4.1.3.1 Oral tactile acuity measures

GRT threshold followed a normal distribution (W = 0.983; p-value = 0.071) (Figure 23A). GRT thresholds ranged from 0.2 mm to 1.25 mm, thus covering the whole range of groove widths (Figure 23B). The median value was 0.68 and first and third quartile limits were 0.51 and 0.89 respectively. No significant differences were found by gender for GRT threshold distributions (D = 0.113; p-value = 0.750) or GRT mean values (F = 0.455; p-value = 0.501). The mean number of trials performed by each participant to complete the grating orientation test was 27.8. The minimum number of trials was 10, while the maximum was 40. The test required approximately 10-15 min to be completed. A significant negative correlation was found between GRT and the number of trials performed by each participant to complete the test (r = - 0.636; p-value < 0.0001).

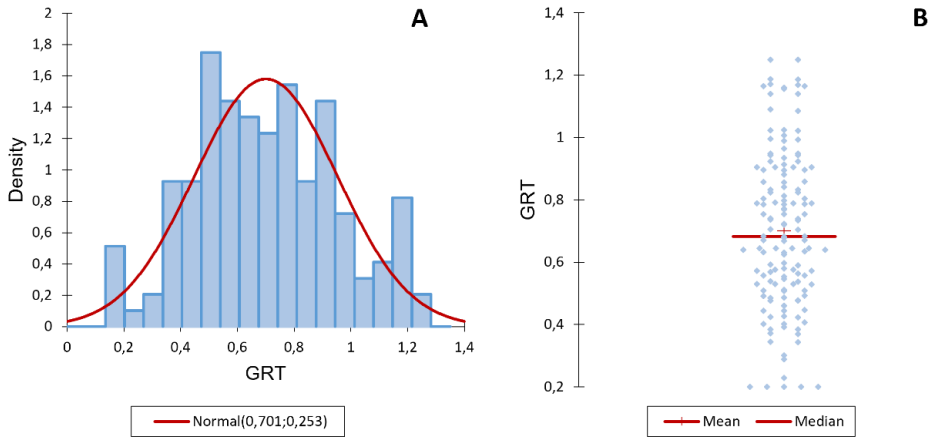


Figure 23. Distribution (A) and range of variation (B) of grating recognition threshold (GRT) (n= 144)

The detection threshold for oral point pressure sensitivity was 0.008 g for all participants therefore this measure was not included in any further analysis as it was unable to discriminate between participants. Discrimination thresholds did not follow a normal distribution (W = 0.584; p-value < 0.0001) (Figure 24A). Threshold values ranged from 0.008 g and 0.160 g (mean 0.037 g), with 72% of participants correctly identifying the lowest target stimulus (Figure 24B).

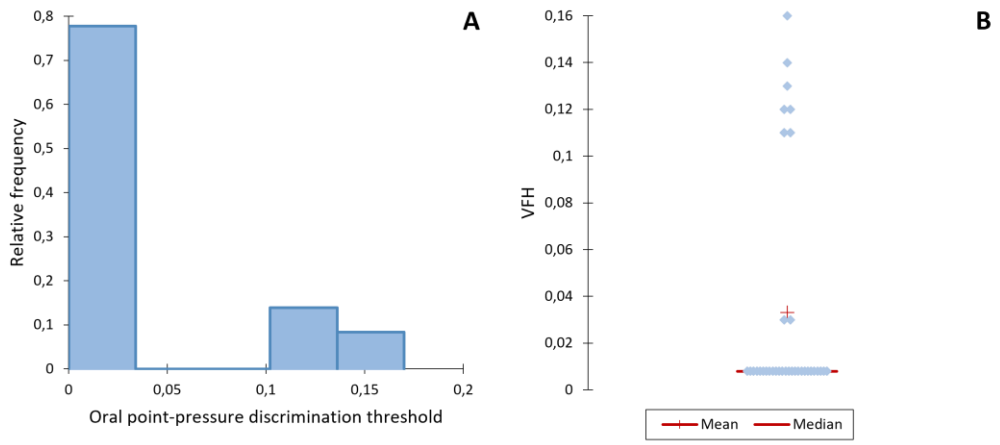


Figure 24. Distribution (A) and range of variation (B) of point-pressure discrimination threshold values (VFH) (n=37)

4.1.3.2 Oral acuity markers

The distribution of PROP bitterness ratings does not follow a normal distribution (W= 0.969; p-value= 0.002). The median value was 35.75 ("strong" on gLMS), while the first and third quartile limits were 20.25 and 50.96 respectively, very close to the arbitrary cut-offs used for subject classification in Non-Taster (NT) (< 17, weak on the gLMS) and Super Taster (ST) (> 53, very strong on the gLMS) groups (Hayes et al. 2010; Fischer et al. 2013). Thus, according to the limits of percentile distribution, participants were classified in Non-Tasters (NT: PROP bitterness < 20.25, n = 36), Medium Tasters (MT: PROP bitterness ≥ 20.25 and ≤ 50.96; n=78) and Super Tasters (ST: PROP bitterness < 50.96, n = 36) groups. Gender significantly affected PROP mean bitterness ratings (F = 14.377; p-value < 0.001), with women that were found to have a significantly higher PROP responsiveness (43.26 – between "strong" and "very strong" on the gLMS) in comparison to men (29.15 – between "moderate" and "strong" on the gLMS).

The distribution of total FPD values tended to follow a normal distribution (W= 0.981; p-value= 0.045). The median value was 138.85 FP/cm², and the first and third quartile limits were 84.9 and 189.7 FP/cm² respectively. Gender significantly affected both total FPD value and FPD in the diameter classes ranging from DC2 to DC10 with women showing significantly higher density values than men (Table 3).

Table 3 One-way ANOVA. Effect of gender on FPD classes and total FPD (mean, F and p values). Different letters indicate significant different values (p ≤ 0.041) (n= 144)

FPD	DC1	DC2	DC3	DC4	DC5	DC6	DC7	DC8	DC9	DC10	DC11	TOT
Women	76.196	24.966 a	17.761 a	14.649 a	9.117 a	6.084 a	2.729 a	2.070 a	1.500 a	0.520 a	0.223	155.787 a
Men	69.894	19.968 b	14.262 b	10.642 b	6.247 b	3.670 b	1.610 b	1.056 b	0.709 b	0.214 b	0.093	128.361 b
F	1.249	6.011	5.085	7.827	8.168	9.252	6.083	6.145	6.639	4.261	3.391	5.665
p	0.266	**0.015**	**0.026**	**0.006**	**0.005**	**0.003**	**0.015**	**0.014**	**0.011**	**0.041**	0.068	**0.019**

4.1.3.3 Relationship between oral tactile sensitivity measures, PROP bitterness ratings and fungiform papillae density

Linear correlation between oral tactile acuity measures, PROP bitterness ratings and fungiform papillae density were tested (Table 4). No significant linear correlations were found amongst PROP, total FPD, FPD in each of the 11 diameter classes and GRT measures in the whole sample ($r \leq 0.138$; p-value ≥ 0.099), in women ($r \leq -0.217$; p-value ≥ 0.071) and in men ($r \leq 0.160$; p-value > 0.173) with the only exception of a positive, but weak, linear correlation between PROP bitterness ratings and FPD DC10 in men ($r= 0.262$; p-value= 0.024). FPD TOT was significantly associated with FPD classes both in the whole sample and by gender ($r \leq 0.928$; $p \leq 0.05$). No linear correlation was found between GRT and point-pressure discrimination threshold (r=0.197; p-value= 0.257) in the smaller subsample of women.

Table 4. Correlation among grating orientation recognition threshold (GRT), PROP bitterness responsiveness (PROP) and fungiform papillae density total (FPD TOT) and diameter classes (FPD DC1-11). Values in bold represent significant correlation ($\alpha= 0.05$). p critical value significant for $p \leq 0.050$

Variables	GRT	PROP	FPD DC1	FPD DC2	FPD DC3	FPD DC4	FPD DC5	FPD DC6	FPD DC7	FPD DC8	FPD DC9	FPD DC10	FPD DC11	FPD TOT
Whole sample (n=144)														
GRT	-	0.001	0.036	0.111	0.000	0.098	0.046	0.054	0.065	0.047	0.052	0.005	0.074	0.060
PROP	0.001	-	0.115	0.083	0.001	0.061	0.096	0.058	0.052	0.138	0.045	0.106	0.103	0.041
FPD TOT	0.060	0.041	**0.904**	**0.914**	**0.886**	**0.869**	**0.777**	**0.655**	**0.624**	**0.482**	**0.455**	**0.333**	**0.424**	-
Women (n=70)														
GRT	-	0.054	0.133	0.124	0.017	0.029	0.052	0.046	0.049	0.065	0.015	0.070	0.074	0.077
PROP	0.054	-	0.217	0.205	0.059	0.023	0.061	0.026	0.075	0.115	0.025	0.016	0.157	-0.138
FPD TOT	0.077	0.138	**0.887**	**0.903**	**0.898**	**0.891**	**0.750**	**0.615**	**0.583**	**0.438**	**0.456**	**0.328**	**0.503**	-
Men (n=74)														
GRT	-	0.105	0.062	0.077	0.004	0.153	0.132	0.160	0.061	0.055	0.138	0.107	0.057	0.023
PROP	0.105	-	0.082	0.085	0.061	0.009	0.018	0.091	0.120	0.032	0.011	0.262	0.160	0.073
FPD TOT	0.023	0.073	**0.928**	**0.923**	**0.866**	**0.834**	**0.790**	**0.685**	**0.639**	**0.495**	**0.429**	**0.338**	**0.289**	-

4.1.3.4 Participant's clustering

To further explore possible associations among measures, participants were clustered according to GRT thresholds, PROP bitterness ratings and total FPD. Three clusters were identified: Cl1 (n= 67), Cl2 (n= 42) and Cl3 (n= 35). The cophenetic correlation of clustering was 0.646; the within-class variance was 28.92% and the between-class variance was 71.08%, indicating clear clusters. Clusters significantly differed for PROP bitterness responsiveness ($F= 4,716$; $p = 0.010$) with Cl2 showing significantly higher mean value than Cl1 and

Cl3 (Figure 25A). A significant difference was found among clusters for FPD (F= 285,19; p< 0.0001) with Cl1 showing the highest FPD value and Cl3 the lowest (Figure 25B). Clusters did not significantly differ for GRT thresholds (F= 0.596; p= 0.552) (Figure 25C).

Significant difference was found in distribution by gender among clusters with Cl3 showing a significant lower number of women compared to men (chi-square= 3,864; chi-square critical value= 5,991, p= 0.031) while no significant differences were observed in Cl1 and Cl2.

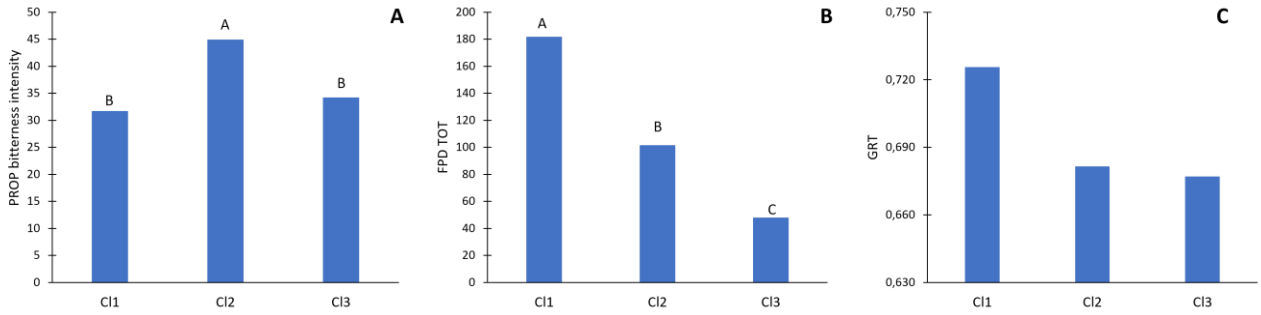


Figure 25 One-way ANOVA. Effect of cluster on PROP bitterness intensity (A), total fungiform papillae (FPD TOT) (B) and grating orientation recognition threshold (GRT) (C). Different letters represent significant different values (p≤ 0.010).

4.1.4 Discussion

One of the major aims of this study was to compare the variability across participants for oral tactile sensitivity by means of both the VFH point-pressure and grating orientation recognition (GRT) thresholds.

Results showed that all the participants were able to detect the lowest VFH force (0.008 g) and that 72% of them were able to discriminate at the lowest VFH monofilament level (0.02/0.008 g filament pair). Results from VFH point-pressure test confirmed previous studies showing that the majority of subjects were able to detect the lowest VFH force and indicated the strong "floor effect" of this method (Santagiuliana et al., 2019; Breen et al., 2019; Appiani, Rabitti, Methven, Cattaneo, & Laureati, 2020). The lowest available force provided by VFH monofilaments appeared higher compared to the sensitivity of the tongue mechanoreceptors. Thus, VFH detection and discrimination thresholds appear to be unsuitable in revealing individual variation in responsiveness to point-pressure on the tongue in a young adult population.

In comparison, results of the GRT showed greater variation compared to the point-pressure thresholds, with GRT values covering the range of all possible thresholds (from 0.2 to 1.25 mm), thus indicating an ability to measure individual variation in oral spatial resolution. Furthermore, some participants were able to correctly identify the orientation of the 0.2 mm grating, indicating that these participants might have an even greater spatial resolution acuity. Similar to our results, Appiani and colleagues showed higher individual variation in grating orientation than in point-pressure tests using the R-Index to express oral tactile acuity both in adults

and children (Appiani et al., 2020). GRT threshold was not significantly affected by gender. To the best of our knowledge, little is still known about the effect of gender on oral tactile acuity measures. Our results agree with a previous study in which no significant differences between men and women have been reported in a letter identification task (Bangcuyo et al., 2017).

VFH point-pressure discrimination and GRT thresholds were not significantly correlated, in agreement with recent results (Appiani et al., 2020), supporting the hypothesis that these measures represent different tactile functions underlined by different neural mechanisms (Johnson et al., 1981). It might be hypothesised that the application of localised pressures on the tongue by VFH only stimulates superficial mechanoreceptors and does not consider the response to the deeper mechanical pressures that also contribute to the oral tactile sensitivity (Engelen & Van Der Bilt, 2008). GRT is considered a more complex process involving both the stimulation of the deeper tongue mechanoreceptors and the cognitive processes required to identify grid orientation (Appiani et al., 2020). Thus, tactile sensitivity measured with gratings might be a function of both peripheral and central activities (Miles et al., 2018).

The number of trials and the time required to complete the test are important variables to take into account when assessing oral tactile sensitivity through these different approaches. In this study a significant negative correlation was found between GRT threshold and the number of trials required to complete the test, with high thresholds were associated with a lower number of trials. These results are related to the staircase method: all subjects started the test with the highest grating (1.25 mm), thus, subjects characterised by lower oral tactile sensitivity stopped the test at higher gratings levels in fewer numbers of trials; on the contrary, subjects characterised by a higher oral tactile sensitivity were tested for a higher number of gratings until their GRT threshold, meaning a higher number of trials were performed. It could be argued that more accurate data is collected with longer staircases which raises the question as to whether a fixed number of reversals should be conducted (see García-Pérez, 1998).

Available measures to assess oral tactile sensitivity have been widely used to explore the organisation of somatosensory system structures of the skin (mechanoreceptors and nervous fibers) and their activity in response to different kinds of tactile stimuli (Weinstein, 1968; Essick et al., 1999). However, the skin was later found to be less sensitive than the tongue, due to differences in cutaneous tissues characteristics (the oral cavity is characterised by glabrous cutaneous tissue). Furthermore, the type and functions of tongue mechanoreceptors and nerve fibers in oral tactile perception only partially reflect those of cutaneous system (Van Boven & Johnson, 1994; Miles et al., 2018). Further studies are still necessary to find more reliable measures to explore the individual variability in the oral tactile system sensitivity, including the development of sensitive devices able to test the limits of oral mechanoreceptor sensitivity. Our results confirm the hypothesis that the grating orientation test might be an effective and sensitive tool to map individual differences in oral spatial resolution acuity (Van Boven & Johnson, 1994; Appiani et al., 2020).

Recommendations for future work include the development of a grating below 0.2 mm to cover the variation in the most sensitive individuals.

Overall, the results from the present study are in line with already existing data on individual variation in PROP responsiveness and FPD and their association with gender, confirming the reliability of both oral acuity phenotype characterization. The distribution of PROP bitterness ratings found in this study was in agreement with previous results on larger population samples (Monteleone et al., 2017; Fischer et al., 2013), confirming the reliability of both oral acuity phenotype characterization measures. Descriptive values were close to the arbitrary cut-offs used to classify subjects in Non-Taster and Super-Taster groups (Hayes et al., 2010; Fischer et al., 2013). Gender was confirmed to be a predictor of PROP bitterness responsiveness, with women showing significantly higher sensitivity than men (Bartoshuk et al., 1994; Monteleone et al., 2017; Tepper et al., 2014). FPD mean values were similar to those reported in previous studies on young adult population (Piochi et al., 2019) and women were confirmed to have higher FPD than men (Fischer et al., 2013; Dinnella et al., 2018).

To the best of author's knowledge, the present study represents the first systematic investigation on the relationships between spatial resolution acuity measured by the grating orientation method and phenotypic markers of oral acuity. In this study the individual variation in GRT threshold was not significantly associated with responsiveness to PROP bitterness neither to FPD total and by diameter classes, in the whole sample and by gender. Previous studies have shown an association of both PROP responsiveness and FPD with oral stereognosis, with significant negative association between these measures in smaller samples of young women (letter identification task; n=52; aged 18-35) (Essick et al., 2003) and adults (letter identification task; n=48; aged 18-59) (Bangcuyo & Simons, 2017). The discrepancies between results could be due to the different methodologies used here and in previous works, both in phenotypes of oral acuity assessment (e.g., staircase method, R-Index) and in oral tactile acuity measures (e.g., letter identification task, point-pressure sensitivity), as well as due to a lower participant number.

Participants were clustered according to PROP responsiveness, total FPD and GRT values. Clusters were significantly different for PROP responsiveness and FPD but not for GRT. These results confirm the wide individual variation of PROP responsiveness and FPD in a population characterised by little variability in age (18-30) and balanced for gender. It could be further observed that young adults show in general a high sensitivity to PROP bitterness responsiveness with mean values of clusters ranging between "moderate-strong" and "strong-very strong" on the gLMS, according to previous study on large scale (E. Monteleone et al., 2017), thus supporting that this sensitivity might reflect a high sensitivity to other oral sensations due to high functionality of peripheral receptor systems and anatomical structures involved in taste and tactile perception. On the contrary, GRT did not discriminate participants among the three clusters, thus indicating a substantial independence among the phenotypic markers of oral acuity and the oral tactile sensitivity measures adopted in the present study.

4.1.5 Conclusion

Oral tactile responsiveness measures capturing individual variation in tongue responses to mechanical stimulation would represents an easily functional tool to explore individual sensitivity and the response to different properties of food texture. This tool could be helpful to better understand individual differences in texture perception related food preferences.

Point-pressure sensitivity thresholds did not appear useful to map individual variability in responsiveness to oral tactile stimulation. On the other hand, grating orientation thresholds discriminated amongst participants and appeared suitable in exploring the individual variation in oral responsiveness to mechanical stimulation and the cognitive processes behind it. Point-pressure sensitivity and grating orientation threshold did not correlate, supporting the hypothesis that these measures represent different tactile functions underlined by different receptor/neural mechanisms. This encourages future studies aimed at a deeper investigation of individual variability in sensitivity to different types of oral tactile stimuli. Finally, a substantial independence was observed between the phenotype markers of oral responsiveness and grating orientation test thresholds, but it is suggested that a larger scale study is required to confirm this. Furthermore, future studies should be aimed at the method optimization, for example exploring the use of narrower grids and the adoption of longer staircases, to capture the differences in tactile sensitivity among the most sensitive individuals.

4.2 Development of model systems for graininess and astringency sensations

4.2.1 Introduction

Food texture perception results by an integration of gustatory, auditory and somatosensory signals (Brennan, 1984) and can be described by many attributes referring to specific physical geometrical and mouthfeel food properties (Szczesniak, 1963).

Graininess is a "geometrical characteristic" of food texture parameters which is evoked by the presence of particles in foods (Szczesniak, 1963). Particles concentration, size and shape affect graininess perception (Tyle, 1993; Imai, et al., 1999; Engelen et al., 2005; Lopez et al., 2016; Aguayo-Mendoza et al., 2021). Increasing particles concentration, low-size and hard particles have been associated to an undesired graininess perception (Lopez et al., 2016; Santagiuliana et al., 2019) in different food products such as cheese (Modler et al., 1989; Sainani et al., 2004) and yogurt (Aguayo-Mendoza et al., 2020). In a recent work, the addition of bell pepper gel pieces to processed cheeses have been reported to affect the dynamic sensory perception of food products by decreasing both the dominance rate and duration of creaminess, smoothness, melting and dairy flavor and increasing graininess and bell pepper flavor (Aguayo-Mendoza et al., 2021). Physical properties of the dispersion medium may also affect particles perception with graininess highly perceived in liquids than in semi-solid or solid foods (Imai, et al., 1995; Lopez et al., 2016; Liu et al., 2016). In a recent work, cellulose particles have been reported to be highly detected in low-viscous/low-fat quark than in semi-solid/high-fat processed cheese (Santagiuliana et al., 2019). Particles of microscale dimension (e.g. microcrystalline cellulose) may induce grainy sensation in food products. Microcrystalline cellulose (MCC) is a porous, aggregate, white, odourless, impurity-free crystalline powder derived by cellulose after a removing process of its amorphous region (Hamid et al., 2014). MCC particles are composed of glucose units linked together by β-1,4-glycosidic linkages to form a linear polymer chain of shorter length compared to its precursor. Each glucose monomer has three free hydroxyl groups which define MCC chemical reactivity (Trache et al., 2014). Microcrystalline cellulose is widely used in food industries for its non-toxic (generally recognised as safe- GRAS), physiologically inert, renewable, and biodegradable properties (Nsor-Atindana et al., 2017). Its main application is as bulking agent in dairy products, baked foods, desserts, sausage, and frozen food in which it gives better consistency, mouthfeel, and other organoleptic properties (Schuh et al., 2013)(Thoorens et al., 2015). In beverages (e.g., cocoa beverages) MCC is reported to increase the stability of suspension, creaminess and particles suspensions (Yaginuma & Kijima, 2007). However, depending on particles concentration, size and shape, MCC may also induce an undesired graininess sensation (Santagiuliana, Broers, et al., 2019). It is therefore important taking into account possible variations in the sensory profile of products added with MCC particles to take advantage of its technological and functional properties without affecting food consumers acceptance.

Astringency is defined as a complex group of sensations involving dryness of the oral surface and tightening and puckering sensations of the mucosa and muscles around the mouth (Lee & Lawless, 1991). Astringency is a tactile sensation perceived through the activation of the oral somatosensory system (Thorngate & Noble, 1995). The mechanism of astringency perception involves formation of bonds between astringent compounds and salivary proteins (Monteleone et al., 2004). Salivary proteins such as proline-rich proteins and histatins are highly effective in binding astringent compounds such as tannins. Salivary mucins are responsible of oral lubrication and lead to the formation of insoluble complex after the interaction with astringent compounds. Precipitation leads to the activation of the oral somatosensory system as a result of the loss of lubrication of the oral cavity (Monteleone et al., 2004; Dinnella et al., 2011). Astringency is therefore affected by the oral production of salivary PRP and individual differences in saliva characteristics including flow rate, composition and haze-forming capacity, may influence astringency perception (Fleming, et al., 2016; Melis et al., 2017; Dinnella et al., 2009; Dinnella et al., 2010). Physical properties of food texture may also affect astringency perception: addition of lubricants such as gums, polysaccharides and proteins have been reported to decrease astringency perception (Brannan et al., 2001; Colonnae et al., 2004). The perception of astringency is a dynamic process that continuously changes or evolves to reach a maximum intensity almost after 15s or more. Lee and Lawless suggested that astringency could be confused with bitterness since certain compounds can induce both (Lee & Lawless, 1991). Furthermore, both astringency and bitterness perception by caffeine (bitter), quinine (astringent), and wine (astringent) were reported to develop similarly, slowly, and possess lingering aftertastes (Guinard, et al., 1986) even if bitterness did not result in changes in the perceived texture of the oral mucosa.

In order to explore individual differences in the perception of graininess and astringency perception, model systems representative of these sensations should be available. The development of a model system requires: 1) to identify a pure tastant, thus a chemical compound which is able to induce only the target sensation; 2) to identify different tastant concentration levels which are able to induce significant differences in the perceived intensity of the target sensations and 3) to identify dispersion medium within study the relationship between tastants concentration and the intensity of target sensations.

Therefore, the aims of this study were:

1. to develop model systems for graininess and astringency sensations
2. to explore the relationship between tastant concentrations and the intensity of target sensations
3. to explore variations in the intensity of target sensations induced by tastant when tastants were individually or together present in the model systems
4. to explore variations in the intensity of target sensations induced by different mediums in which sensations were evoked

4.2.2 Data analysis

One-way ANOVA model (factor: tastant concentration, eight levels) was used to assess the effect of single tastant concentration on the intensity of target sensations in both water solutions (W+MCC and W+TA), pear (PJ+MCC, PJ+TA,) and peach (PcJ+MCC, PcJ+TA) juices.

One-way ANOVA model (factor: tastants concentration, four levels) was used to assess the effect of the combination of tastants (MCC and TA) on the intensity of target sensations in both water solutions (W+MCC+TA) and pear juice (PJ+MCC+TA).

Two-way ANOVA model with interactions, was used to assess the effect of tastant concentration (four levels) and their combination (three levels: MCC, TA and MCC+TA) on the intensity of target sensations in water solutions and pear juice.

Two-way ANOVA model with interactions, was used to assess the effect of model system (two levels: water and pear juice) and tastant concentration (four levels) on the intensity of target sensations.

4.2.3 Results

4.2.3.1. Psychophysic curves of microcrystalline cellulose and tannin acid in water and fruit juices

MMC psychophysic curve in water (W+MCC) showed that intensity of graininess and residual graininess significantly increased with the increasing concentration of microcrystalline cellulose from "any sensation" to "strong/very strong" (graininess: F= 26.496, p< 0.0001; residual graininess: F= 25.596; p< 0.0001); intensity of astringency, bitterness and sourness didn't differ across MCC concentration levels (astringency: F= 1.438, p= 0.190; bitterness: F= 0.478, p= 0.850; sourness: F= 0.346, p= 0.932) (Figure 26).

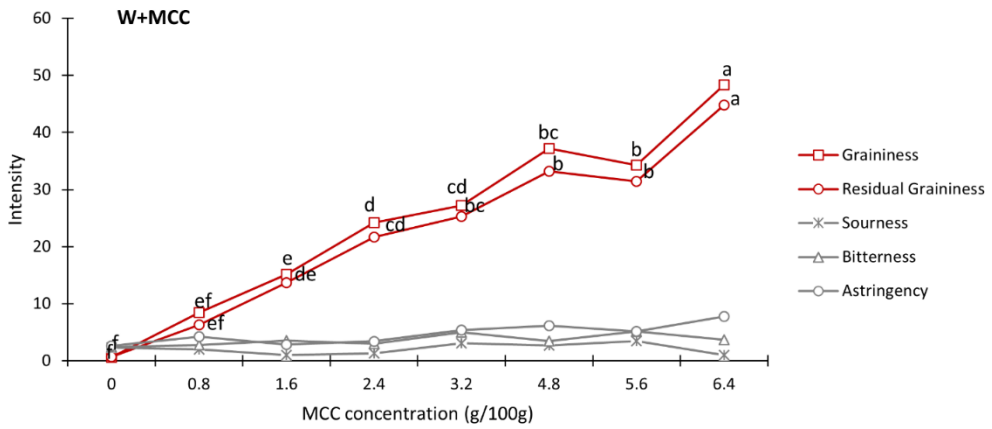


Figure 26. MCC psychophysic curve in water solution: effect of MCC concentration on the intensity of target sensations (n=37). Different letters represent significant different values (p≤ 0.0001)

TA psychophysic curve in water (W+TA) showed that intensity of both astringency and bitterness significantly increased with the increasing of tannic acid concentration (astringency: F= 16.413, p<0.0001; bitterness: F= 16.883; p<0.0001) from "barely detectable/weak" to "moderate/strong". A significant increasing of sourness

was observed from “any sensation/barely detectable” to “barely detectable/weak” (F= 3.665, p= 0.001) even if at a lower extent. Graininess and residual graininess intensity didn’t differ across tannic acid concentration levels (graininess: F= 0.662, p= 0.705; residual graininess: F= 0.940; p= 0.476) (Figure 27).

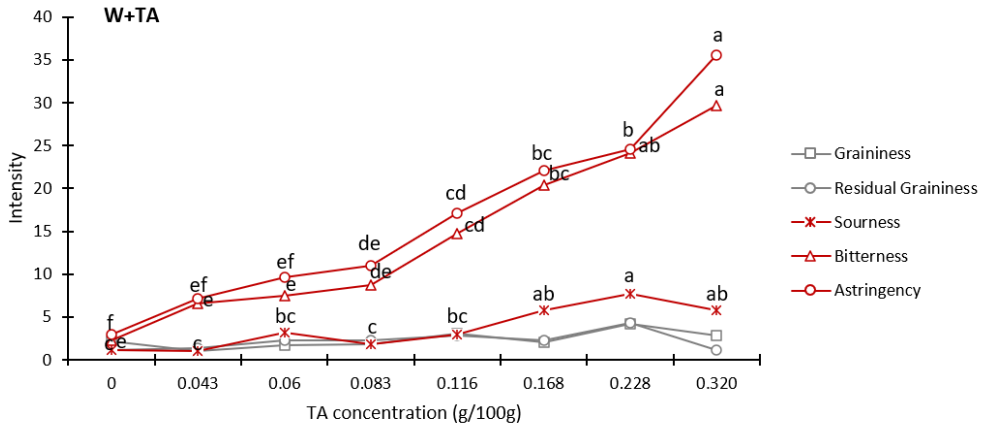


Figure 27. TA psychophysic curve in water solution: effect of TA concentration on the intensity of target sensations (n=37). Different letters represent significant different values (p≤ 0.001)

MMC psychophysic curve in pear juice (PJ+MCC) showed that intensity of graininess and residual graininess significantly increased with the increasing concentration of microcrystalline cellulose from “barely detectable/weak” to “moderate/strong” (graininess: F= 13.207, p< 0.0001; residual graininess: F= 13.808; p< 0.0001). Intensity of sweetness, astringency, bitterness, and sourness didn’t differ across MCC concentration levels (sweetness: F= 0.115, p= 0.997; astringency: F= 0.236, p= 0.976; bitterness: F= 1.218, p= 0.293; sourness: F= 0.822, p= 0.569) (Figure 28).

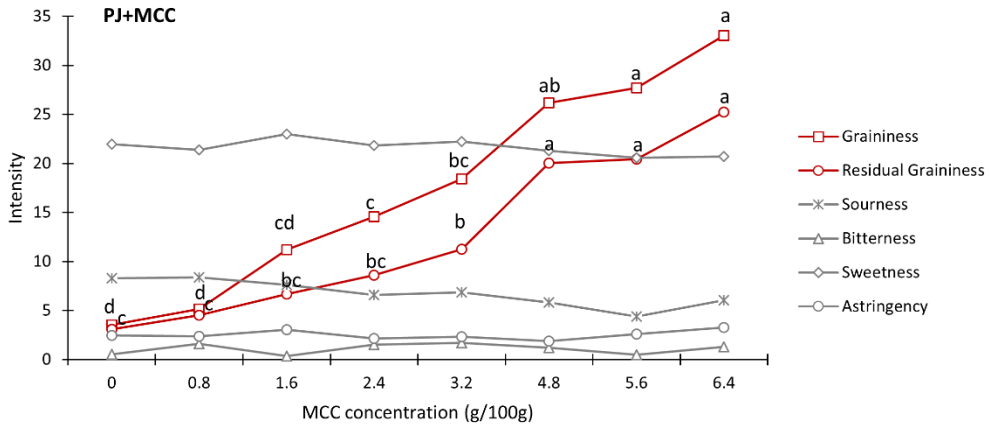


Figure 28. MCC psychophysics curve in pear juice: effect of MCC concentration on the intensity of target sensations (n=37). Different letters represent significant different values (p≤ 0.0001)

TA psychophysic curve in water (W+MCC) showed that intensity of both astringency and bitterness significantly increased with the increasing of tannic acid concentration (astringency: F= 12.865, p<0.0001; bitterness: F= 12.388; p<0.0001) from "barely detectable/weak" to "moderate/strong". A significant decreasing of sweetness was observed (F= 3.010, p= 0.005). Graininess, residual graininess and sourness intensity did not differ across tannic acid concentration levels (graininess: F= 0.402, p= 0.901; residual graininess: F= 0.497; p= 0.836; sourness: F= 0.344, p= 0.933) (Figure 29).

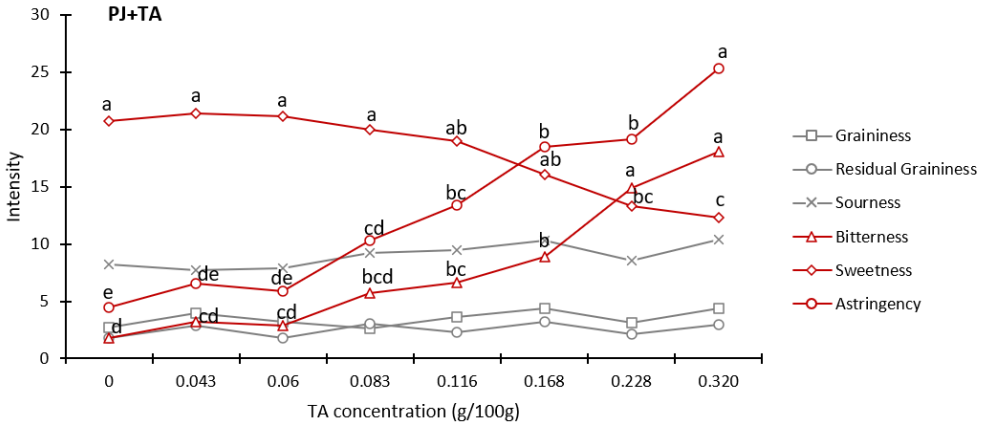


Figure 29. TA psychophysic curve in pear juice: effect of TA concentration on the intensity of target sensations (n=37). Different letters represent significant different values (p≤ 0.005)

MMC psychophysic curve in peach juice (PcJ+MCC) showed that model system, intensity of graininess and residual graininess significantly increased with the increasing concentration of microcrystalline cellulose from "barely detectable/weak" to "moderate/strong" (graininess: F= 23.722, p< 0.0001; residual graininess: F= 24.396; p< 0.0001); intensity of sweetness, astringency, bitterness, and sourness didn't differ across MCC concentration levels (sweetness: F= 0.222, p= 0.980; astringency: F= 0.672, p= 0.696; bitterness: F= 0.203, p= 0.985; sourness: F= 0.774, p= 0.609) (Figure 30).

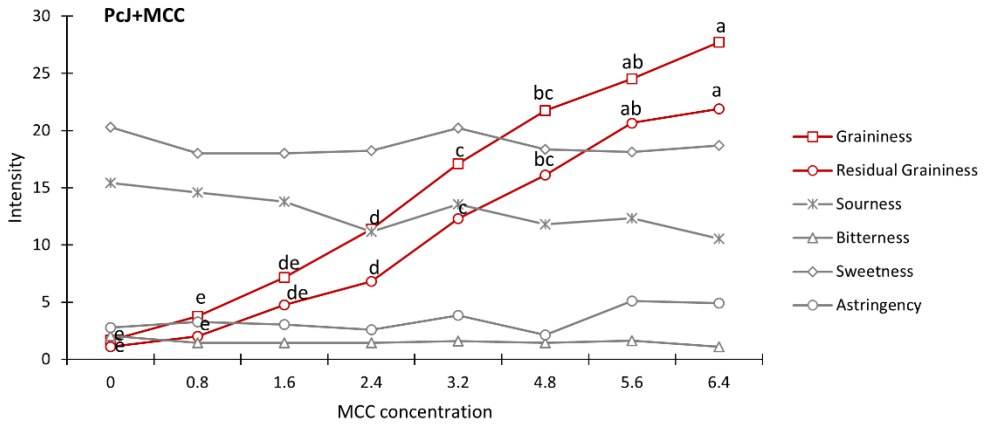


Figure 30. MCC psychophysics curve in peach juice: effect of MCC concentration on the intensity of target sensations (n=37). Different letters represent significant different values (p≤ 0.0001)

TA psychophysic curve in peach juice (PcJ+TA) showed that model system, intensity of both astringency and bitterness significantly increased with the increasing of tannic acid concentration (astringency: F= 10.942, p<0.0001; bitterness: F= 9.577; p<0.0001) from "barely detectable/weak" to "moderate/strong". Sweetness tended to decrease (F= 1.950, p= 0.062). Graininess, residual graininess, sourness intensity didn't differ across tannic acid concentration levels (graininess: F= 0.149, p= 0.994; residual graininess: F= 0.151; p= 0.994; sourness: F= 0.679, p= 0.990) (Figure 31).

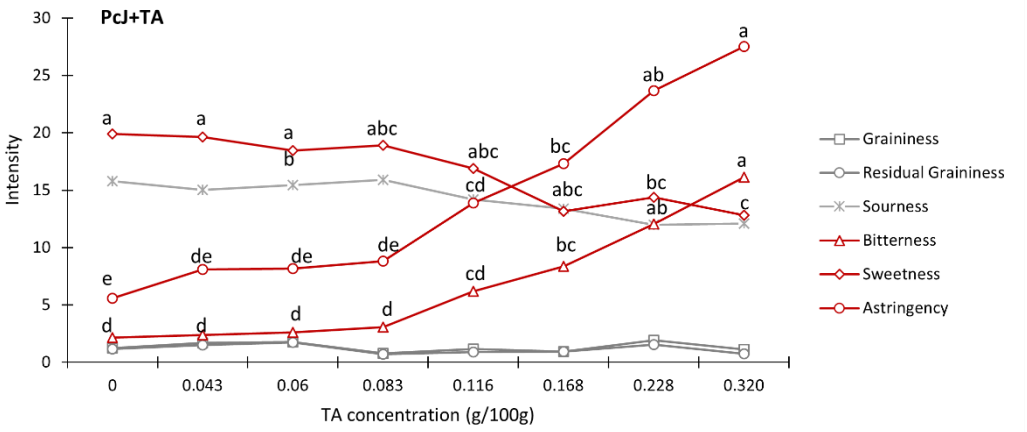


Figure 31. TA psychophysics curve in peach juice: effect of TA concentration on the intensity of target sensations (n=37). Different letters represent significant different values (p≤ 0.062)

The addition of tastants induced similar changes in sensory profile of fruit juices thus peach juice was not further considered.

4.2.3.2. Effect of concentration, tastant and their combination on target sensation intensity in water solution and pear juice model systems

Based on results from psychophysic curves, four concentration levels were selected for MCC and TA in water solution and in pear juice, which induce the same significant variations of graininess and astringency. Two further model systems in water and pear juice were prepared adding both MCC and TA at four isointense concentration levels. Tastant concentration and relevant intensities are summarized in Table 5.

Table 5. MCC and TA concentration levels which induce the same intensity variation of graininess and astringency (in italics) in water solutions and pear juice. MCC and TA concentrations selected for MCC+TA model systems in water solutions and pear juice (column)

	Concentration level	MCC concentration (g/100g)	Graininess Intensity	TA concentration (g/100g)	Astringency Intensity	MCC+TA concentrations
Water	1	0.0	*Any sensation*	0.0	*Any sensation*	0.0
	2	1.6	*Weak/Moderate*	0.116	*Moderate*	1.6 + 0.116
	3	3.2	*Moderate/Strong*	0.228	*Moderate/Strong*	3.2 + 0.228
	4	5.6	*Strong/Very strong*	0.320	*Strong*	5.6 + 0.320
Pear juice	1	0.0	*Any sensation*	0.0	*Any sensation*	0.0
	2	2.4	*Weak/Moderate*	0.083	*Weak/Moderate*	2.4 + 0.083
	3	4.8	*Moderate/Strong*	0.168	*Moderate*	4.8 + 0.168
	4	6.4	*Strong*	0.320	*Moderate/Strong*	6.4 + 0.320

4.2.3.2.1 Concentration effect

Model systems at the selected concentration levels were evaluated by a larger sample of 144 subjects. MCC and TA concentration significantly affected the intensity of target sensations in both water solutions and pear juice confirming previous results obtained with the eight concentration levels of tastants tested in the psychophysic curve (Table 6).

Table 6. One-way ANOVA. Effect of tastant concentration (four levels) on the intensity of target sensations in water solutions and pear juice (n=144). Different letters indicate significant different values (p≤ 0.014)

Water solution					Pear juice					
MCC concentration level	Graininess	Sourness	Bitterness	Astringency	MCC concentration level	Graininess	Sourness	Bitterness	Sweetness	Astringency
1	1.085 d	1.217 a	1.524 a	2.226 c	1	4.597 d	4.789 a	1.421 c	26.694 a	3.832 c
2	15.351 c	1.602 a	2.285 a	5.431 b	2	16.343 c	4.085 ab	1.846 bc	21.751 b	7.518 b
3	23.428 b	1.851 a	2.402 a	6.183 b	3	28.595 b	4.139 ab	2.547 ab	21.260 b	10.155 ab
4	37.372 a	1.408 a	2.410 a	9.594 a	4	37.163 a	3.081 b	3.122 a	19.363 b	12.279 a
F	124.038	0.579	1.147	13.704	*F*	110.970	1.335	3.587	8.535	14.560
p	**< 0.0001**	0.629	0.329	**< 0.0001**	*p*	**< 0.0001**	0.262	**0.014**	**< 0.0001**	**< 0.0001**
TA concentration level					TA concentration level					
1	0.522 b	1.603 c	1.601 d	2.295 d	1	4.215 b	4.161 c	2.370 d	25.780 a	4.729 d
2	2.491 a	5.647 b	13.107 c	16.017 c	2	5.451 ab	5.708 bc	5.792 c	22.633 b	8.991 c
3	2.331 a	8.919 a	25.210 b	26.242 b	3	6.638 a	8.134 b	13.742 b	15.983 c	18.856 b
4	3.449 a	11.214 a	29.684 a	31.840 a	4	6.501 a	11.734 a	24.251 a	10.981 d	29.392 a
F	4.664	93.365	15.622	93.440	*F*	2.237	76.720	11.648	41.723	88.664
p	**0.003**	**< 0.0001**	**< 0.0001**	**< 0.0001**	*p*	0.083	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**

The effect of combination of MCC and TA in water and pear juice model systems was studied.

In W+MCC+TA, graininess intensity significantly increased with the increasing of stimuli concentration ($p<0.0001$) from “any sensation” to “strong/very strong”. Significant astringency increases were also observed from “barely detectable” to “moderate/strong” ($p<0.0001$). Bitterness significantly increased from “barely detectable” to “weak/moderate” ($p<0.0001$), while sourness significantly increased but in a lower extent from “barely detectable” to “weak” ($p<0.0001$) (Figure 32).

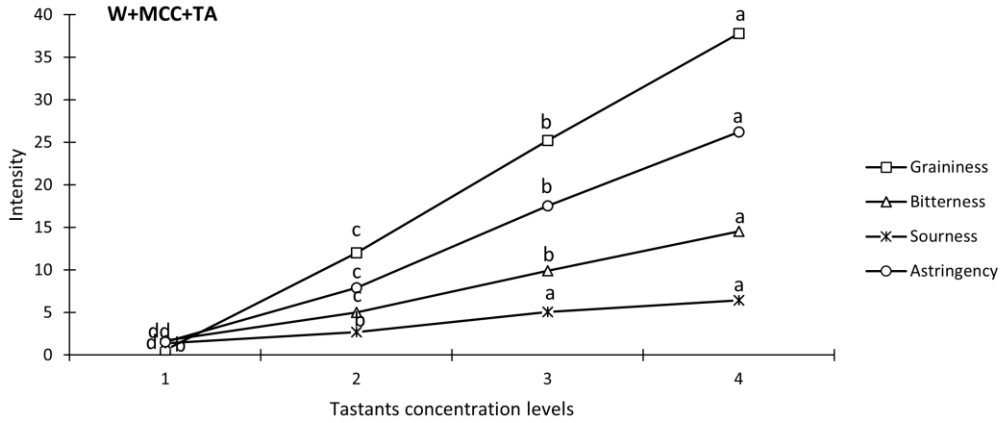


Figure 32. MCC+TA psychophysics curve in water solution: effect of both MCC and TA concentration on the intensity of target sensations (n=144). Different letters represent significant different values (p≤ 0.0001)

In PJ+MCC+TA, graininess intensity significantly increased with the increasing of stimuli concentration (p<0.0001) from "barely detectable/weak" to "moderate/strong". Significant astringency increases were also observed from "barely detectable/weak" to "moderate/strong" (p<0.0001). Bitterness significantly increased from "barely detectable" to "weak/moderate" (p<0.0001), while sourness significantly increased but in a lower extent in the range "barely detectable/weak" (p= 0.002). Sweetness significantly decreased in a "strong/moderate" range of intensities (p< 0.0001) (Figure 33).

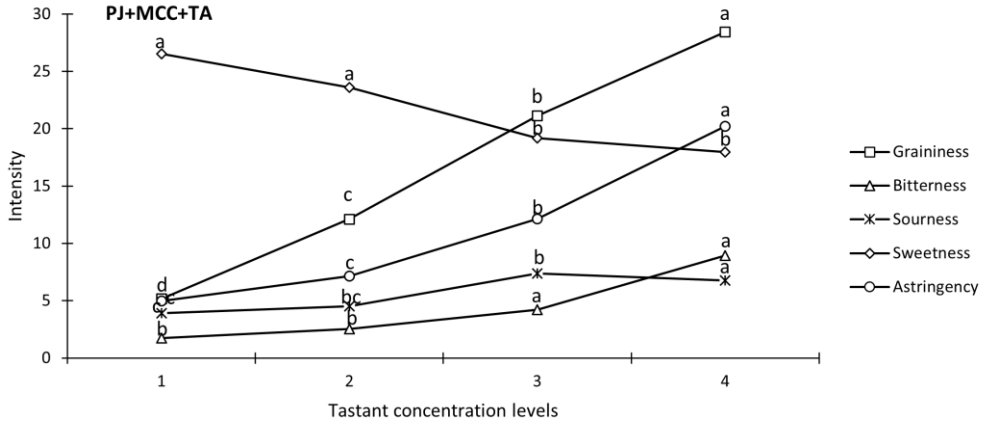


Figure 33. MCC+TA psychophysics curve in pear juice: effect of tastant concentration levels on the intensity of target sensations (n=144). Different letters represent significant different values (p≤ 0.002)

4.2.3.2.2 Effect of tastants and their combination

The effect of tastant used alone (MCC and TA) or in combination (MCC+TA) at different concentration levels on sensory properties of the model systems was explored. Graininess, sourness, bitterness and astringency

ratings from water (W+MCC, W+TA, W+MCC+TA) and pear juice (PJ+MCC, PJ+TA, PJ+MCC+TA) were independently compared. Furthermore, also sweetness ratings were considered in pear juice model systems. Both tastant and concentration and their interactions significantly affected the intensity of target sensations in water solutions and pear juice (Table 7).

Table 7. Two-way ANOVA: Effect of tastant concentration levels, tastants and interactions on the intensity of target sensations in water solutions and in pear juice (n=144). F and p values.

	Concentration level		Tastant		Concentration level*Tastant	
	F	*p*	F	*p*	F	*p*
Water solutions						
Graininess	259.000	**< 0.0001**	283.001	**< 0.0001**	52.841	**< 0.0001**
Sourness	22.892	**< 0.0001**	45.500	**< 0.0001**	6.654	**< 0.0001**
Bitterness	125.516	**< 0.0001**	260.254	**< 0.0001**	42.284	**< 0.0001**
Astringency	176.520	**< 0.0001**	132.131	**< 0.0001**	22.398	**< 0.0001**
Pear juice						
Graininess	170.796	**< 0.0001**	211.184	**< 0.0001**	36.147	**< 0.0001**
Sourness	9.233	**< 0.0001**	18.399	**< 0.0001**	6.659	**< 0.0001**
Bitterness	104.043	**< 0.0001**	159.652	**< 0.0001**	36.446	**< 0.0001**
Sweetness	52.756	**< 0.0001**	12.203	**< 0.0001**	4.243	**0.000**
Astringency	134.582	**< 0.0001**	45.431	**< 0.0001**	13.417	**< 0.0001**

Graininess was perceived significantly higher in both MCC and MCC+TA model systems compared to TA model systems ($p<0.0001$). Graininess mean intensity didn't significantly differ between MCC and MCC+TA in water solutions in the whole concentration range with the only exception of level 2 at which graininess was perceived significantly higher in W+MCC than in W+MCC+TA system. On the other hand, graininess was always significantly higher in PJ+MCC compared to PJ +MCC+TA (Figure 34).

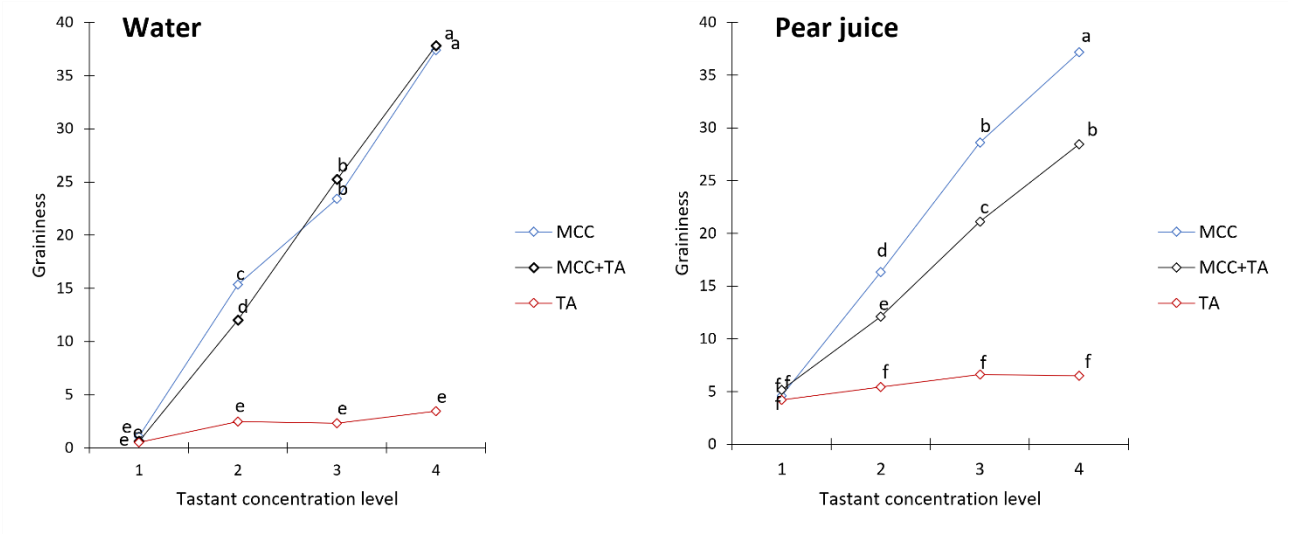


Figure 34. Effect of the interaction between tastants (three levels: MCC, MCC+TA, TA) and tastant concentration (four levels) on graininess intensity, in water solutions and in pear juice. Different letters represent significant different values ($p \leq 0.0001$) (n=144)

In water solution astringency was perceived at the highest intensity in W+TA model system at each TA concentration level with the only exception of level 1. In W+MCC+TA, astringency was perceived significantly lower than W+TA, while it was perceived significantly higher compared to astringency in W+MCC model system for tastants concentration levels 3 and 4. In general, astringency was perceived at the lowest intensity in W+MCC model system (Figure 35A).

In pear juice, astringency was perceived at the highest intensity in PJ+TA model system at the highest TA concentration levels (level 3 and 4). In PJ+MCC+TA, astringency was perceived significantly lower than PJ+TA, but higher than astringency perceived in PJ+MCC with the only exception of level 1 and 2. In general, astringency was perceived at the lowest intensity in PJ+MCC model system, except for levels 1 and 2 (Figure 35B).

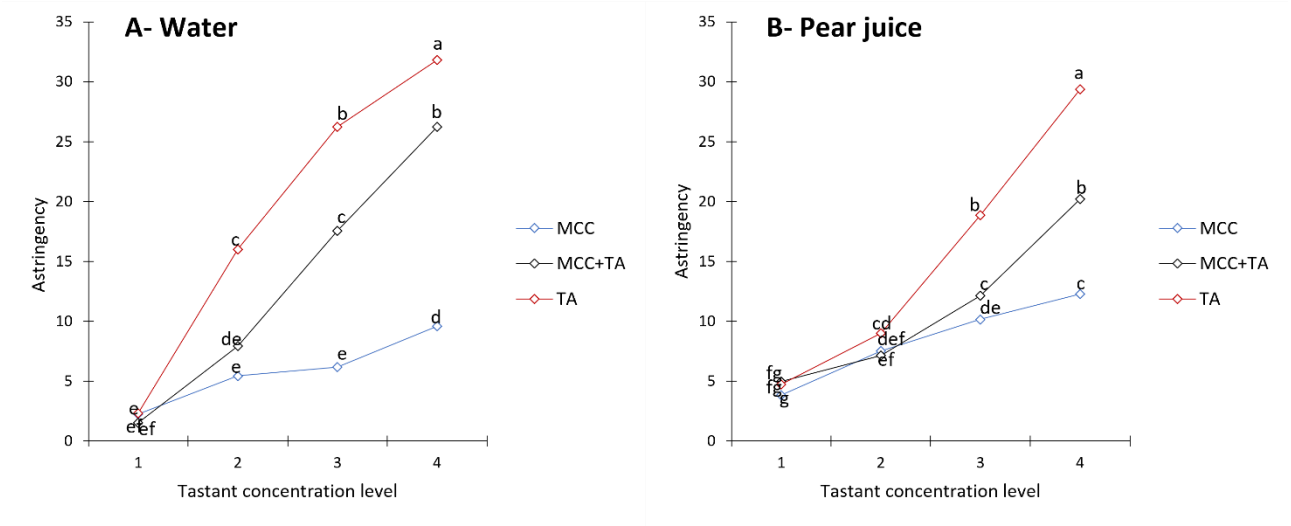


Figure 35. Effect of the interaction between tastants (three levels: MCC, MCC+TA, TA) and tastant concentration (four levels) on astringency intensity, in water solutions (A) and in pear juice (B). Different letters represent significant different values ($p \leq 0.001$) (n=144)

In water solution bitterness was perceived at the highest intensity in W+TA model system at each TA concentration level with the only exception of level 1. In W+MCC+TA, bitterness was perceived significantly lower than W+TA, except for level 1, while it was perceived significantly higher compared to bitterness in W+MCC model system at each tastants concentration levels, except for level 1. In general, bitterness was perceived at the lowest intensity in W+MCC model system (Figure 36A).

In pear juice, bitterness was perceived at the highest intensity in PJ+TA model system at each TA concentration level, with the only exception of level 1. Bitterness was perceived at the lowest intensity in both PJ+MCC+TA and PJ+MCC at each tastant concentration levels, except for level 4 in PJ+MCC+TA in which bitterness was perceived significantly higher (Figure 36B).

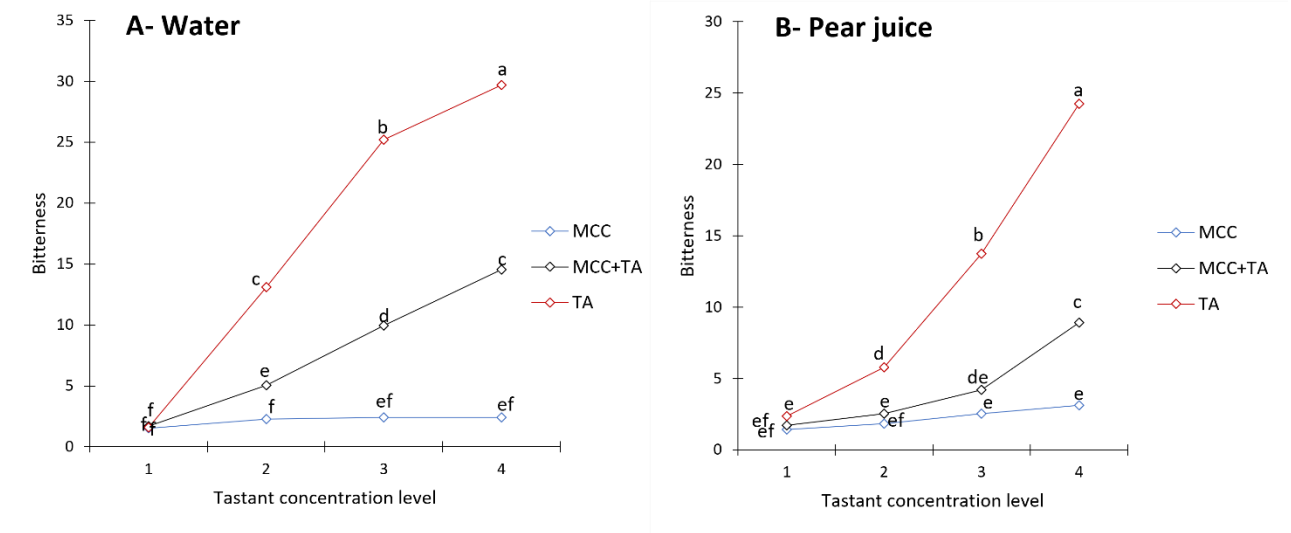


Figure 36. Effect of the interaction between tastants (three levels: MCC, MCC+TA, TA) and tastant concentration (four levels) on bitterness intensity, in water solutions (A) and in pear juice (B). Different letters represent significant different values ($p \leq 0.0001$) (n=144)

In water solution sourness was perceived at the highest intensity in W+TA model system at each TA concentration level with the only exception of level 1. In W+MCC+TA, sourness was perceived significantly lower than W+TA, except for level 1, while it was perceived significantly higher compared to sourness in W+MCC model system at each tastants concentration levels, except for levels 1 and 2. In general, sourness was perceived at the lowest intensity in W+MCC model system (Figure 37A).

In pear juice, sourness was perceived at the highest intensity in PJ+TA model system only at level 4, while it did not significantly differ for the other tastant concentration levels in PJ+TA and PJ+MCC+TA. In PJ+MCC, sourness was perceived at the lowest intensity at levels 3 and 4 (Figure 37B).

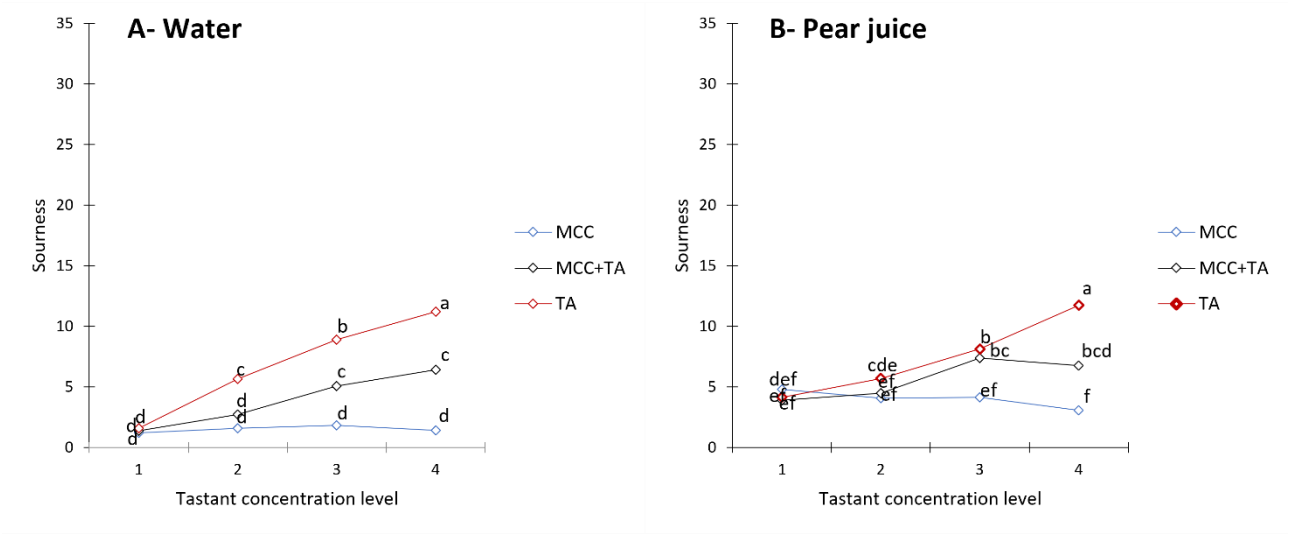


Figure 37. Effect of the interaction between tastants (three levels: MCC, MCC+TA, TA) and tastant concentration (four levels) on sourness intensity, in water solutions (A) and in pear juice (B). Different letters represent significant different values (p≤ 0.0001) (n=144)

In pear juice, sweetness was perceived significantly higher in MCC and MCC+TA than in TA (p<0.0001). Sweetness mean intensity significantly decreased with increases of stimuli concentration (p<0.0001). Significant sweetness decreases were observed for TA concentration levels 3 and 4 compared to MCC+TA and MCC (p=0.000) (Figure 38).

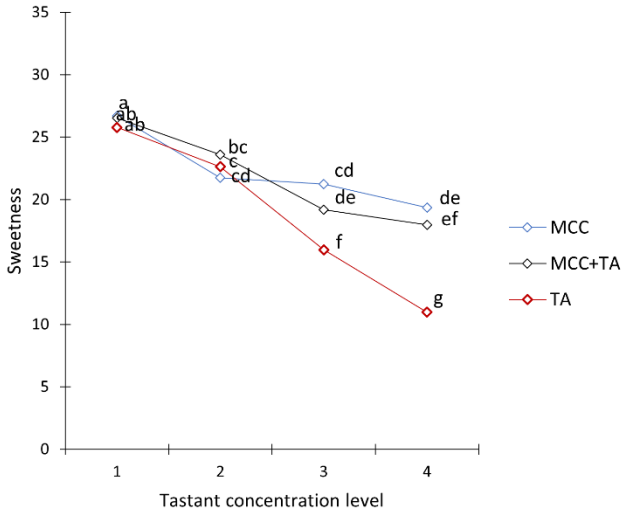


Figure 38. Effect of the interaction between tastants (three levels: MCC, MCC+TA, TA) and tastant concentration (four levels) on sweetness intensity in pear juice. Different letters represent significant different values (p≤ 0.000) (n=144)

4.2.3.3 Effect of the medium on the intensity of target sensations induced by MCC, TA and MCC+TA

In order to explore the effect of medium composition (water and pear juice) on the intensity of the different sensations induced by the same tastant, graininess, astringency, bitterness and sourness ratings in water and pear juice model systems were compared. All factors (medium, concentration and their interactions) significantly affected the intensity of graininess, astringency, bitterness and sourness induced by MCC, TA and MCC+TA with some exceptions (Table 8). Medium composition did not significantly affected bitterness induced by MCC and sourness induced by TA, MCC concentration did not affected sourness intensity. Significant interactions were found for bitterness and astringency induced by TA and graininess, astringency and bitterness induced by MCC+TA.

Table 8. Two-way ANOVA. Effect of tastant concentration levels, medium and interactions on the intensity of target sensations induced by MCC, TA and MCC+TA (n=144). F and p values.

	Concentration level		Medium		Concentration level*Medium	
	F	*p*	F	*p*	F	*p*
MCC						
Graininess	233.519	**< 0.0001**	6.077	**0.014**	1.600	0.188
Sourness	1.031	0.378	50.229	**< 0.0001**	1.254	0.289
Bitterness	3.991	**0.008**	0.078	0.780	0.746	0.525
Astringency	27.733	**< 0.0001**	16.973	**< 0.0001**	0.663	0.575
TA						
Graininess	5.784	**0.001**	55.466	**< 0.0001**	0.445	0.721
Bitterness	164.014	**< 0.0001**	46.667	**< 0.0001**	8.792	**< 0.0001**
Sourness	26.638	**< 0.0001**	0.676	0.411	0.983	0.400
Astringency	176.047	**< 0.0001**	16.433	**< 0.0001**	6.719	**0.000**
MCC+TA						
Graininess	211.345	**< 0.0001**	5.938	**0.015**	10.788	**< 0.0001**
Bitterness	60.342	**< 0.0001**	37.827	**< 0.0001**	6.003	**0.000**
Sourness	12.317	**< 0.0001**	9.802	**0.002**	0.789	0.500
Astringency	118.072	**< 0.0001**	7.425	**0.007**	7.559	**< 0.0001**

Graininess induced by MCC was not perceived significantly different between water solution and pear juice, with the only exception of level 3 at which graininess was perceived significantly higher in pear juice than in water solution. On the contrary, graininess mean intensity induced by MCC+TA was perceived higher in water solutions than in pear juice with the only exception of level two at which no significant differences were found (Figure 39).

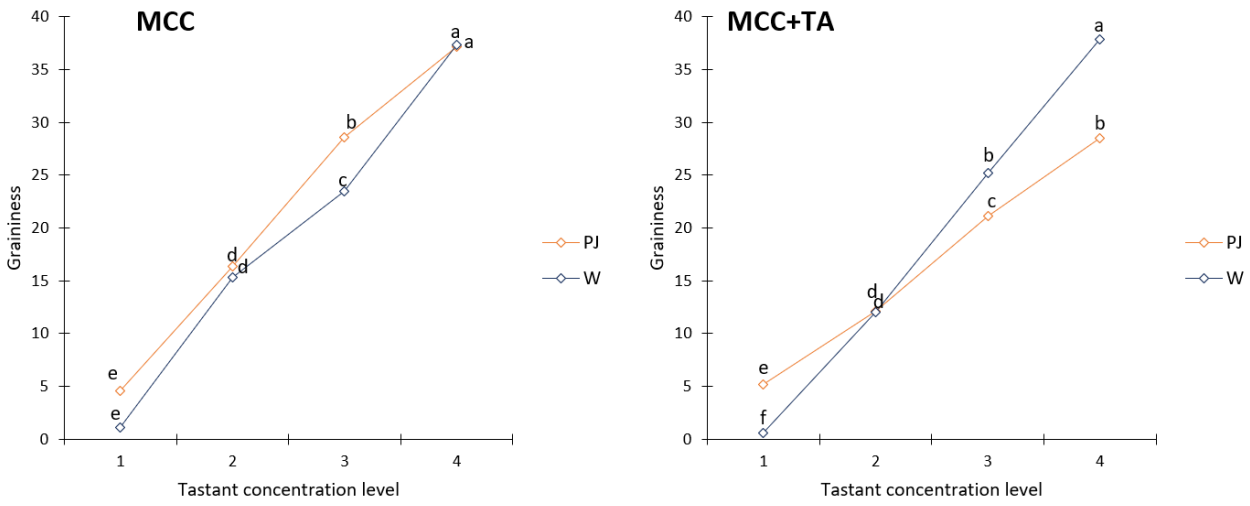


Figure 39. Effect of the interaction between matrix (two levels: water solution and pear juice) and tastant concentration (four levels) on graininess intensity, induced by MCC and MCC+TA. Different letters represent significant different values ($p \leq 0.0001$) (n=144)

Astringency induced by TA was perceived significantly higher in water solution than in pear juice, with the only exception of both level 1 and level 4 at which no significant differences were found. Astringency induced by MCC+TA was perceived significantly higher in water solution than in pear juice, with the exceptions of levels 1 at which astringency was perceived significantly higher in pear juice and of level 2 at which astringency did not significantly vary between water and pear juice (Figure 40).

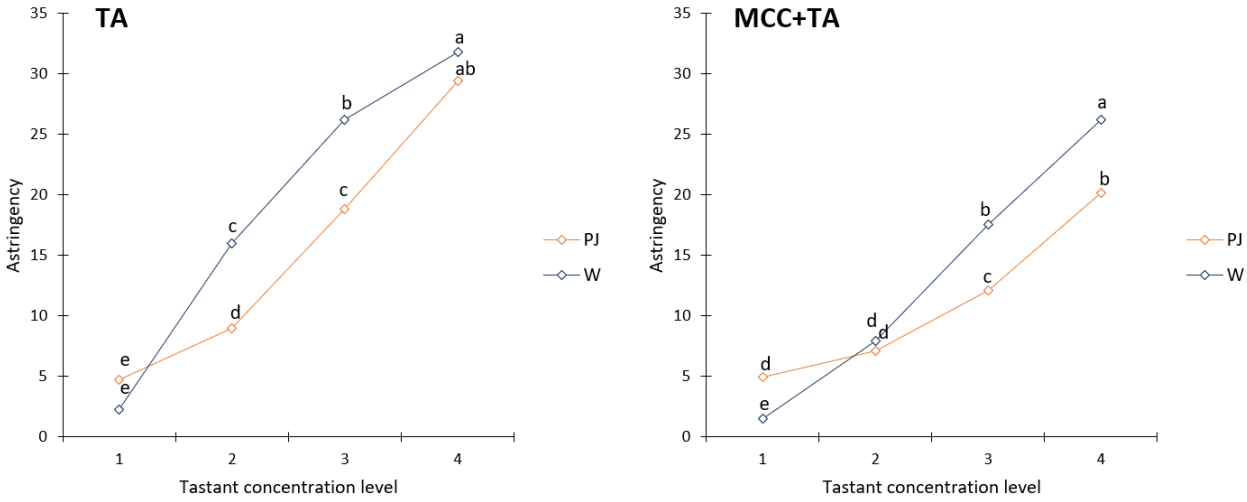


Figure 40. Effect of the interaction between matrix (two levels: water solution and pear juice) and tastant concentration (four levels) on astringency intensity, induced by TA and MCC+TA. Different letters represent significant different values (p≤ 0.0001) (n=144)

Bitterness was perceived significantly higher in water solution than in pear juice in both TA and MCC+TA model systems (p<0.0001) (Figure 41).

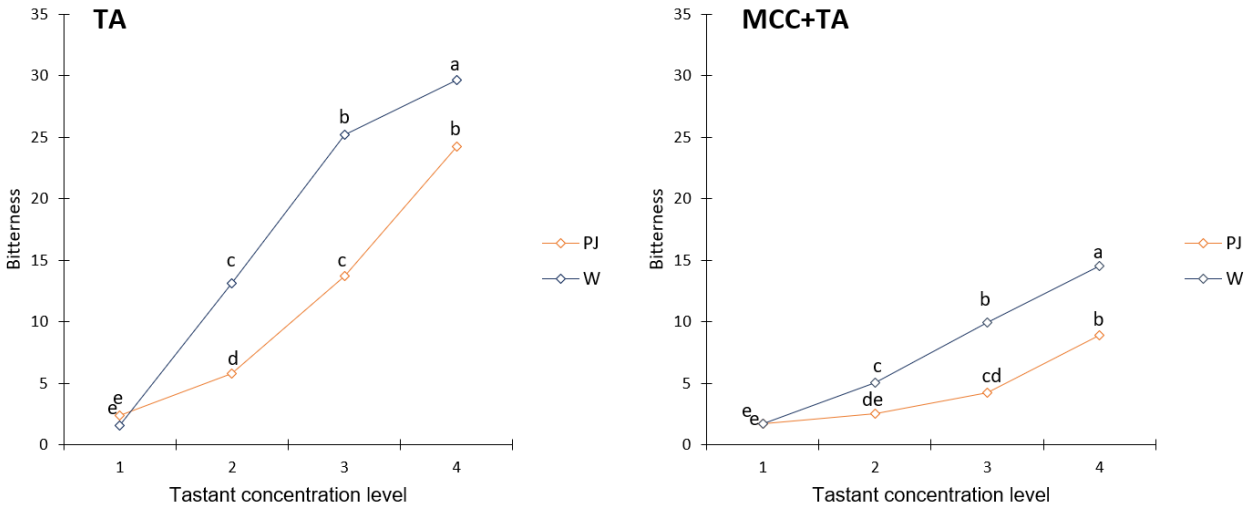


Figure 41. Effect of the interaction between matrix (two levels: water solution and pear juice) and tastant concentration (four levels) on bitterness intensity, induced by TA and MCC+TA. Different letters represent significant different values (p≤ 0.0001) (n=144)

4.2.4 Discussion

The first aim of this study was to develop model systems for specific texture-related sensations: graininess and astringency. Microcrystalline cellulose (MCC) was found to be a specific stimulus for graininess sensation in both water solution and in pear/peach juice, and no other tastes or tactile sensations were evoked by MCC. This result support previous evidences of MCC as graininess stimulus (Santagiuliana et al., 2019) and

add new information on the relationships between MCC concentration and graininess perception which covers intensity variation from weak to strong both in water and fruit juice models. On the other hand, tannic acid (TA) was found to induce both tactile (astringency) and taste (bitterness and sourness) sensations, their intensity significantly increased with increasing TA concentration, in both water solutions and in pear/peach juices. Results are in agreement with previous studies, in which tannic acid was added with increasing concentration levels to evoke astringency sensation in wine and water solutions (Condelli et al., 2006). Both astringency and bitterness sensations induced by tannic acid may be related to TA structure. TA is a mixture of polygalloyl glucose molecules, including penta-(digalloyl)-glucose, tetra-(digalloyl)-glucose and tri-(digalloyl)-gluocose etc. (Fischer, 2002). Astringency may be hypothesised to be evoked after the interaction of highly-dimension tannic acid molecules (e.g. penta-(digalloyl)-glucose molecules) with salivary proteins and their precipitation. On the other hand, bitterness may be derived by the interaction of lower-dimension tannic acid molecules (e.g., tri-(digalloyl)-glucose molecules and lower ones) with bitter taste receptors (Monteleone et al., 2004).

The identification of stimuli able to specifically induce food texture-related sensations and assessment of their psychophysic curves would be helpful to explore individual variability in food texture perception. Based on this consideration MCC appears an interesting candidate as reference stimulus to study responsiveness to geometrical features of food texture and TA can be used as stimulus aimed at exploring responsiveness to mouthfeel sensations such as astringency. However, in this latter case, possible sensory bias, such as halo dumping effect, due to the simultaneous perception of bitterness and sourness have to be taken into account (Clark & Lawless, 1994).

The sensory profile of model systems with both MCC and TA varied compared to the sensory profile of MCC or TA when they were independently added to water solution or pear juice. In general, graininess, astringency and bitterness increased with increasing tastants concentration, while in pear juice sweetness decreased. In water no significant differences in graininess intensity were observed when comparing MCC and MCC+TA solutions while a significant decreasing of astringency, bitterness and sourness were observed comparing TA with MCC+TA solution. The decreasing of sensations induced by TA when combined with MCC might be explained hypothesising that chemical interactions occur between MCC and TA when they were both present in the model systems thus leading to modification of the relevant sensations (Keast & Breslin, 2003; Thomas-Danguin, Guichard, & Salles, 2019). In particular, microcrystalline cellulose has been reported to have the property of forming “charged network” structure, which is able to adsorbs substances on its surface and prevents them from particle–particle associations and agglomerations (Dan et al., 2016). In products such as cocoa beverages, the presence of MCC improved both the creaminess and the stability of the suspension because MCC particles associated with other particles to form aggregated structures, including network structures (Araki et al., 1998; Yaginuma & Kijima, 2006a). Moreover, tannic acid has been found to possess the ability of adhering onto various kinds of substrates, including organic and inorganic ones, hydrophilic and

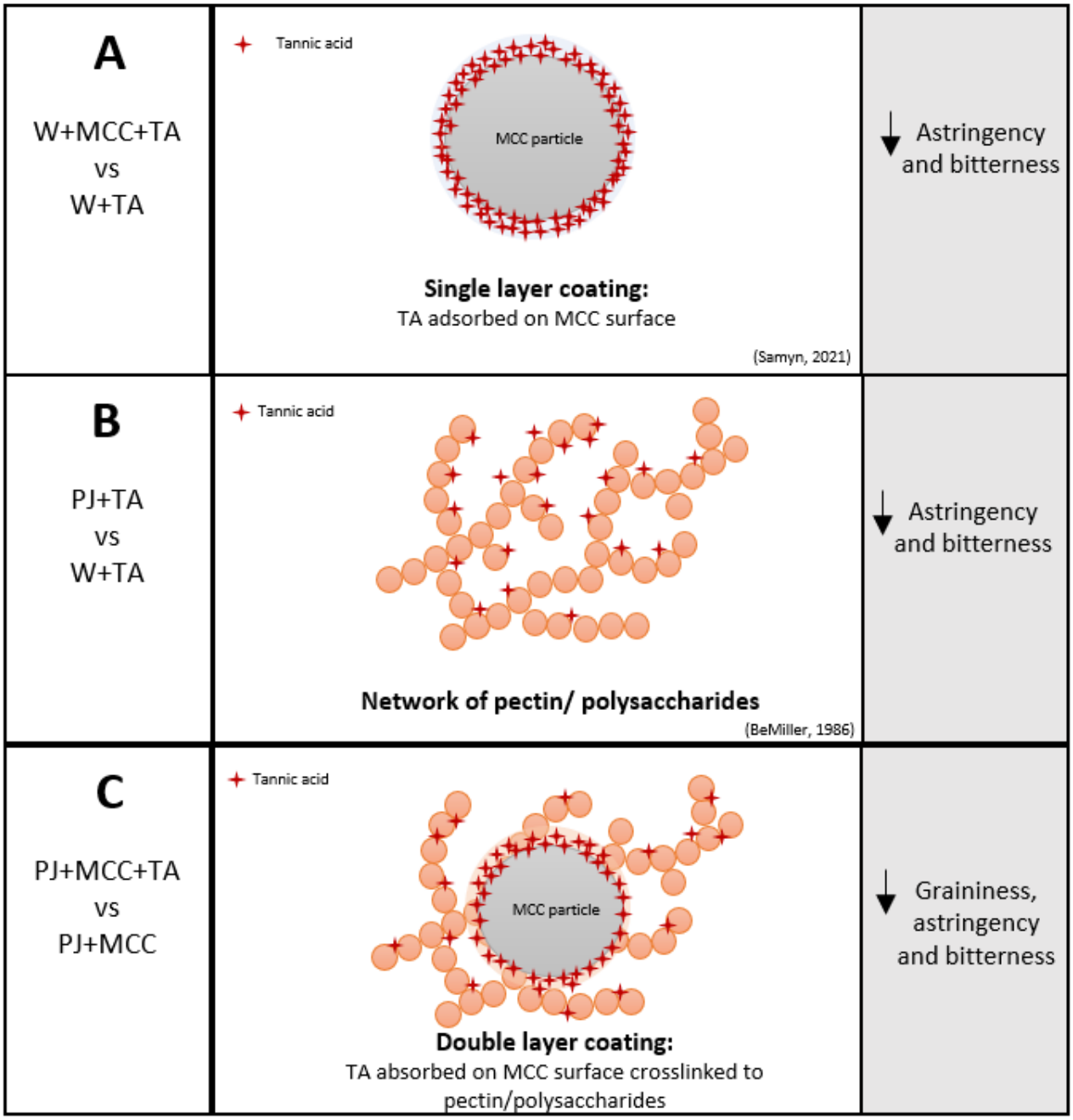
hydrophobic ones, particles and planar ones (Yan et al., 2020). It may be therefore hypothesised that tannic acid could be adsorbed, in some extent, on microcrystalline cellulose particles surface. Microcrystalline cellulose particles might act as sequestrant agent, preventing tannic acid components from interaction with taste receptors and salivary mucins thus lowering astringency, bitterness and sourness perception. Hydrophobic interactions between the hydrophobic regions of TA and the crystalline portion of MCC as well as hydrogen bonds between TA hydroxyl groups and those of MCC can be expected (Yan et al., 2020) (Nsor-Atindana et al., 2017). Moreover, since tannic acid possesses multiple phenolic groups it may act as a building unit of a crosslinked film on microcrystalline cellulose surface (Yan et al., 2020; Samyn, 2021). The mechanism of interaction between MCC particles and tannic acid is schematised in Table 9A.

In pear juice the effect of MCC+TA combination is more evident and intensity ratings of the sensations induced by MCC and TA when individually used were always higher than those rated when the stimuli are combined. Moreover, sweetness was perceived lower in TA model system than in MCC and MCC+TA pear juice systems. The effect of MCC and TA combination on perception of astringency, bitterness and sourness in pear juice can be explained based on tannic acid component/microcrystalline chemical interactions as previously described. The decreasing of sweetness suppression observed in MCC+TA pear juice in comparison with pear juice added only with TA can also be attributed to the sequestrant activity of MCC on TA components. The adsorption of TA components on MCC hinders their interaction with bitter receptors and thus the reduced bitterness perception induces the significant lowering of suppression on sweet taste.

In water solution, graininess mean intensity values in MCC model system were found to overlap with graininess mean intensity values in MCC+TA model system. On the other hand, graininess intensity in pear juice added with MCC was significantly lowered when also TA was present in the juice. A more complex system of interactions taking into account pear juice components should be hypothesized to explain these results. Pear juice components include pectin that are known to be characterised by gelation properties (BeMiller, 1986). Pear juice therefore results as a more viscous liquid than water. The different viscosity between these liquids might partially explain why graininess perception is perceived lower in pear juice than in water solution. Viscosity is reported to affect particles perception with higher viscosity of the dispersion medium leading to a lower particles perception (Imai et al., 1999). Furthermore, the decreasing of both astringency and bitterness induced by TA observed in fruit juice in comparison with water might be explained as a consequence of chemical interactions (i.e. hydrogen bonds) between tannic acid and pear juice components. Pear juice pectin and polysaccharides in general, may form networks which hind TA particles preventing their interaction with both taste receptors and salivary proteins thus reducing both bitterness and astringency perception (Table 9B). In fact, it is widely reported that polysaccharides may inhibit the interactions between salivary proteins and tannins through a competitive bounds formation process, reducing protein precipitation and, consequently, the perceived astringency (Troszyńska et al., 2010). Furthermore, cross linking interactions between tannic acid adsorbed on MCC surface and fruit juice

polysaccharides might also results in hydrogel layer formation on MCC surface (Samyn, 2021) which lowers the perception of particles, thus hindering graininess perception (Table 9C).

Table 9. Schematization of possible mechanisms of interaction between MCC particles and TA (A), pear juice polysaccharides and TA (B) and MCC particles, TA and pear juice polysaccharides (C). Effect on sensory perception of target sensations is shown in column grey.



4.2.5 Conclusion

Model systems for both graininess and astringency sensations were developed. Microcrystalline cellulose was defined as a pure stimulus for graininess sensation in both water solution and pear juice. On the other hand, tannic acid was confirmed to be able to evoke both astringency and bitterness sensations in agreement

with previous works. Stimuli concentration levels were identified in order to induce significant differences in the perceived intensity. Both chemical and perceptual interactions appear to occur when the tactile stimuli were both present in a same model system that can partially explain variations in the perception of target sensations. Furthermore, physico-chemical interactions appeared to occur between stimuli and dispersion medium components resulting in a different perceptive response. This study, therefore, highlights that the perceptual responses obtained in a specific model system cannot be generalised.

The developed model systems for specific texture-related sensations, namely astringency and graininess, appear promising tools for the systematic exploration of individual differences in responsiveness to oral tactile stimulation.

4.3 Individual differences in perception of and liking for model systems with varied intensity of tactile and taste sensations

4.3.1 Introduction

Food texture is a major driver of food liking and acceptance (Jaeger et al., 1998; Kalviainen et al., 2000; Jeltema et al., 2016; Varela et al., 2021). Astringency and graininess may result undesirable sensations to some consumers and may potentially lead to food rejection (Bajec, 2011; Dinnella et al., 2011; Lopez et al., 2016; Santagiuliana et al., 2019; Yang & Lee, 2020; Louro et al., 2021). The negative effect of high astringency intensity on liking for food and beverages rich in phenol compounds has been reported (De Toffoli et al., 2019; Yang & Lee, 2020). Graininess induced by the presence of cellulose microparticles negatively influenced liking for quark samples (Santagiuliana et al., 2019) and orange-flavoured multiparticulate formulations (Lopez et al., 2016).

Individual differences in the perception of food texture-related sensations may partially explain differences in food texture preference and acceptance. Individuals highly respondent to tactile sensations might be hypothesised to have lower liking for specific food textures than less responsive individuals. However, a complex relationship between liking and intake of yoghurt samples, added with granola particles and varying in texture, was recently highlighted, depending on individual differences in both food oral processing and texture perception (Varela et al., 2021).

Phenotypic markers of oral acuity, such as PROP bitterness responsiveness and fungiform papillae density are methodological tools useful to explore individual differences in both taste and tactile responsiveness. PROP responsiveness was found to be positively associated with the perception of tactile/texture-related sensations such as astringency (Melis et al., 2017; Pickering & Robert, 2006), creaminess (Kirkmeyer & Tepper, 2003) and roughness (Bakke & Vickers, 2008). Positive associations were also reported between fungiform papillae density (FPD) and the perception of creaminess in milk (Hayes & Duffy, 2007) and both hardness and crunchiness in biscuits (Zhou et al., 2021). On the other hand, other studies failed to find significant associations between FPD and food texture attributes, such as astringency in bread (Bakke & Vickers, 2008). Recently, specific measures have been proposed to explore individual variability in the oral tactile system sensitivity, including point-pressure test and grating orientation test (Breen et al., 2019; Etter et al., 2020; Appiani et al., 2020). Oral tactile sensitivity measured with a point-pressure test was found to positively associate to both hardness and crunchiness perception in biscuits (Zhou et al., 2021) and to dryness and particles detection in yogurt samples (Olarte Mantilla et al., 2022).

Personality and psychological traits may influence food preference and choice (Monteleone et al., 2017; Jaeger et al., 2017; Robino et al., 2016). Great attention has been focused on the relationship between personality traits and the liking and consumption of foods characterised by innately warning sensations (Byrnes & Hayes, 2013; Byrnes & Hayes, 2016; Spinelli et al., 2018; De Toffoli et al., 2019; Higgins, Bakke, &

Hayes, 2020). Evidence on the relationship between personality traits and food texture perception are still relatively scarce. Recently, children who prefer softer and non-particulate versions of foods were reported to be more neophobic (Cappellotto & Olsen, 2021). Furthermore, consumers showing an open personality type were reported to be more sensitive in detecting particles in yogurt samples, to prefer more cohesive yoghurt textures (e.g., fatty, spoonable, not-separated) and to have a history of consuming yoghurt products in combination with cereal (Olarte Mantilla et al., 2020). These findings support the hypothesis of a relationship between personality traits and food texture perception.

Exploring individual differences in the perception of specific texture-related sensations and their underlined factors may help to better understand specific pattern of food liking and intake, and to promote strategies to increase healthy product's consumption.

This chapter, therefore, is aimed at exploring individual differences in responsiveness to graininess and astringency perception in water and pear juice model systems prepared to induce systematic variation of these sensations and their possible relationship with hedonic responses. Furthermore, the effects of individual variability in phenotypic markers of chemosensory responsiveness (PROP bitterness responsiveness and fungiform papillae density), oral tactile acuity (grating recognition threshold) and psycho-attitudinal traits were explored as factors possible underlined differences in responsiveness and liking for water and pear juice model systems.

4.3.2 Data analysis

In order to explore individual variability in the intensity of target sensations in response to tastants variations, a delta intensity (Δ INT) of target sensations was computed. Δ INT was calculated by subtracting the intensity value corresponding to the lowest concentration level by the highest: Δ INT= (highest intensity level – level 4) − (lowest intensity level – level 1). Δ INT was calculated for all the target sensations (graininess, astringency, bitterness, and sweetness) in both water solutions (W) and pear juice samples (PJ); thus, each subject was described by 15 Δ INT values. Δ INT quantifies how large each subject is able to perceive the difference in the intensity between the highest concentration level of the tastant and the lowest. Furthermore, Δ INT shows how a subject is sensitive to a tastant variation: the highest the Δ INT, the highest the variation in the perceived intensity and therefore the highest the sensitivity to that tastant variation. Δ INT might not reflect how intensely a sensation is perceived: Δ INT might be the same for higher intensity values as well as for lower intensity values. Descriptive statistics were used to described Δ INT of target sensations.

A Principal Component Analysis (PCA) on Δ INT of target sensations was computed in order to explore differences and similarities between subject's responses to tastants variations. Based on coordinates on the first two principal components (PC1, PC2), subjects were grouped into 4 groups, representing different sensitivity patterns to taste, tactile and texture-related sensation variations, each corresponding to a quarter

of the Bi-plot. Differences in the composition among groups in terms of gender were assessed with Chi-square tests followed by Fisher's exact tests ($p < 0.05$).

Two-Way ANOVA model (fixed factors: group, concentration level; models with interactions) was used to assess the effect of groups on the intensity of target sensations in both water solutions and pear juice (graininess, bitterness, astringency and sweetness) in order to explore how Δ INT could reflect how intensely sensations were perceived.

One-way ANOVA model (fixed factor: group) was used to assess the effect of Δ INT groups on markers of oral acuity (PROP bitterness responsiveness and FPD and size classes) and oral tactile acuity measure (grating orientation recognition threshold) in order to explore differences in taste and somatosensory sensitivity between groups. Furthermore, one-way ANOVA model (fixed factor: group) was used to assess the effect of Δ INT groups on attitudes and personality traits (food neophobia, sensitivity to disgust, sensitivity to punishment and reward, sensation seeking, state and trait anxiety).

Liking scores of pear juice samples varying in microcrystalline cellulose concentration (PJ+MCC), tannic acid concentration (PJ+TA) and both microcrystalline cellulose and tannic acid concentration (PJ+MCC+TA) were described by a descriptive statistics analysis. One-way ANOVA models were independently applied to test liking scores of pear juice samples for stimuli concentration levels and for gender.

To explore individual differences and similarities in liking of pear juice samples, a Principal Component Analysis (PCA) was computed on liking scores of all subjects for all the pear juice samples. The intensity mean values of target sensations (graininess, astringent, bitter and sweet) were used as supplementary variables.

To test if liking of pear juice samples could be related to sensitivity for target sensations, only pear juice samples that were more liked (scored over 50 on LAM scale - "neither like nor dislike"), were considered for further analysis. A liking mean score was calculated on the scores of 144 subjects for all the products. A Principal Component Analysis (PCA) was computed again, based on liking scores of all subjects for PJ+MCC1, PJ+MCC2, PJ+TA1, PJ+TA2, PJ+MCC+TA1 and PJ+MCC+TA2. The intensity mean values of target sensations (graininess, astringent, bitter and sweet) were used as supplementary variables.

Based on coordinates on the first two principal components (PC1, PC2), subjects were grouped into 2 groups, corresponding to different liking response for pear juice samples varying in graininess, astringency, bitterness and sweetness sensations. Differences in the composition among groups in terms of gender were assessed with Chi-square tests followed by Fisher's exact tests ($p < 0.05$).

Two-Way ANOVA model (fixed factors: group, concentration level; models with interactions) was used to assess the effects of groups on the liking of pear juice samples in order to assess differences in liking response among groups.

Two-Way ANOVA model (fixed factors: group, concentration level; models with interactions) was also used to assess the effects of groups on the intensity of target sensations in both water solutions and pear juice (graininess, bitterness, astringency and sweetness).

One-way ANOVA model (fixed factor: group) was used to assess the effect of groups on markers of oral acuity (PROP bitterness responsiveness and FPD and size classes) and oral tactile acuity measure (grating orientation recognition threshold), as well as, on attitudes and personality traits.

4.3.3 Results

4.3.3.1 Individual differences in responsiveness to tactile and taste sensations in model systems

Individual responsiveness to sensations induced by model systems was expressed in terms of difference between intensity perceived at the highest concentration level of tastant (level 4) and intensity perceived in model systems without added tastant (level 1) (Δ INT). Δ INT values of target sensations covered a wide range of variation, indicating large individual variability in responsiveness to the increased tastant concentration (Table 10). Negative Δ INT values for sweetness were related to the decreasing of sweetness perception induced by the increasing of TA concentration.

Table 10. Descriptive statistics of Δ INT for target sensations in water and pear juice model systems

	Target sensations	Minimum	Maximum	1st Quartile	Median	3rd Quartile	Mean	Standard deviation (n-1)	Skewness (Pearson)	Standard error of the mean
Water										
MCC	Graininess	0.500	93.400	17.500	37.000	53.050	36.555	22.790	0.308	1.899
TA	Bitterness	0.000	90.600	13.600	27.900	40.450	28.083	19.053	0.615	1.588
	Astringency	0.000	96.800	17.200	26.500	38.350	29.544	18.592	0.916	1.549
MCC+TA	Graininess	0.000	96.000	18.500	36.900	52.375	37.218	22.508	0.274	1.876
	Bitterness	0.000	86.800	1.250	7.150	17.800	13.305	16.265	1.902	1.355
	Astringency	0.000	95.300	8.550	18.900	35.000	24.894	21.219	1.010	1.768
Pear juice										
MCC	Graininess	0.000	96.000	17.275	30.750	45.600	32.669	20.123	0.827	1.677
	Sweetness	-57.900	18.900	-15.225	-4.750	0.850	-7.331	13.410	-0.889	1.118
TA	Bitterness	0.000	83.600	5.650	18.600	34.900	22.067	18.655	0.863	1.555
	Sweetness	-69.000	27.700	-24.500	-13.100	-3.975	-14.799	15.529	-0.444	1.294
	Astringency	0.000	80.500	10.150	24.850	35.550	25.478	18.166	0.520	1.514
MCC+TA	Graininess	0.000	93.600	9.775	21.800	33.650	24.585	18.274	0.996	1.523
	Bitterness	0.000	66.000	0.200	3.000	12.150	8.022	10.825	2.091	0.902
	Sweetness	-67.900	21.900	-14.650	-5.050	-0.500	-8.640	13.614	-1.150	1.134
	Astringency	0.000	89.300	5.300	11.900	24.900	16.422	15.783	1.669	1.315

The Bi-plot of PCA on Δ INT values of target sensations is shown in Figure 42. The first two components in the PCA accounted for 39.44% of total variance, with PC1 explaining 27.86% and PC2 contributing 11.58%. Along PC1, on the right part of the Bi-plot, a positive correlation was observed for Δ INT of graininess, astringency and bitterness sensations. On the left part of the biplot, a positive association was observed for Δ INT of sweetness. Subjects positively positioned on PC1 can be considered highly responsive to the intensity variation in graininess, astringency and bitterness observed between the highest and the lowest tastant levels. On the contrary, subjects negatively positioned on PC1 can be considered less responsive to the increasing of graininess, astringency and bitterness sensations induced by tastant addition and positively associated to Δ INT sweetness values. Along PC2, differences between graininess and astringency/bitterness Δ INT were observed with subjects in the upper part of the map associated with highest Δ INT for graininess, while those in the bottom part were associated with highest Δ INT for astringency and bitterness.

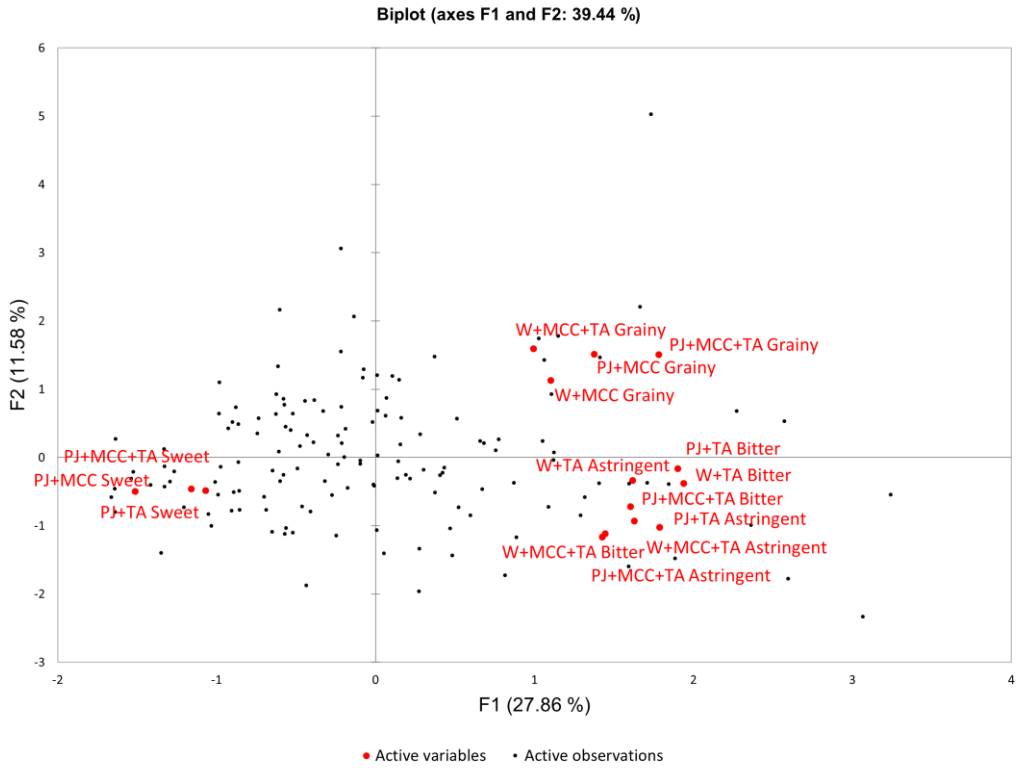


Figure 42. Bi-plot from PCA on Δ INT values of target sensations in both water solution and pear juice model systems (n=144)

Individual coordinates on PC1 and PC2 were computed to identify subject groups varying in responsiveness to graininess, astringency and bitterness (Endrizzi et al., 2014). This approach is also referred as interpretation-based on segmentation which allows subject segmentation based on primary interest (Nsæ et al., 2018). Four groups were identified, each corresponding to a quarter of the Bi-plot: quadrant upper right

ΔG+ (n= 27), quadrant bottom right ΔA/B+ (n= 35), quadrant bottom left ΔG- (n= 44) and quadrant upper left ΔA/B- (n= 38).

The effect of group, tastant concentration level and group*tastant concentration level interactions on the intensity of target sensations in both water solution and in pear juice model systems is summarised in Table 11. All factors (group, tastant concentration level and their interactions) significantly affected the intensity of graininess, astringency, bitterness and sweetness induced by MCC, TA and MCC+TA in both water solutions and pear juice with some exception. Group*tastant concentration level interaction did not significantly affect sweetness induced by both MCC and MCC+TA. Interactions group*tastant concentration level were always significant but were caused by very small intensity variations and did not show systematic relationships between factors, and were not further considered.

Table 11. Two-way ANOVA. Effect of group, tastant concentration level, and interactions on the intensity of target sensations induced by MCC, TA and MCC+TA in both water solutions (W) and pear juice (PJ) (n=144). F and p values.

		Group		Tastant concentration level		Group*tastant concentration level	
		F	*p*	F	*p*	F	*p*
Water solution (W)							
MCC	Graininess	17.133	**< 0.0001**	147.210	**< 0.0001**	3.743	**0.000**
TA	Bitterness	49.016	**< 0.0001**	131.585	**< 0.0001**	5.973	**< 0.0001**
	Astringency	40.602	**< 0.0001**	122.561	**< 0.0001**	4.107	**< 0.0001**
MCC+TA	Graininess	25.868	**< 0.0001**	180.808	**< 0.0001**	6.994	**< 0.0001**
	Bitterness	34.165	**< 0.0001**	46.322	**< 0.0001**	4.883	**< 0.0001**
	Astringency	39.409	**< 0.0001**	101.671	**< 0.0001**	7.157	**< 0.0001**
Pear juice (PJ)							
MCC	Graininess	34.775	**< 0.0001**	150.564	**< 0.0001**	5.964	**< 0.0001**
	Sweetness	19.108	**< 0.0001**	11.003	**< 0.0001**	1.229	0.274
TA	Bitterness	54.535	**< 0.0001**	118.295	**< 0.0001**	8.619	**< 0.0001**
	Sweetness	6.677	**0.000**	49.748	**< 0.0001**	3.311	**0.001**
	Astringency	28.555	**< 0.0001**	113.567	**< 0.0001**	6.080	**< 0.0001**
MCC+TA	Graininess	22.493	**< 0.0001**	101.098	**< 0.0001**	7.213	**< 0.0001**
	Bitterness	30.556	**< 0.0001**	32.356	**< 0.0001**	6.769	**< 0.0001**
	Sweetness	12.675	**< 0.0001**	15.688	**< 0.0001**	1.322	0.222
	Astringency	22.904	**< 0.0001**	51.898	**< 0.0001**	5.625	**< 0.0001**

Intensity ratings from different groups confirmed what was expected by subject positioning on the bi-plot and further highlighted similarity and differences among groups in responsiveness to sensations perceived in water and pear juice model systems (Figure 43). ΔG+ showed the highest responsiveness to graininess intensity in both water and pear juice added with MCC and MCC+TA, while ΔG- was the group with the lowest responsiveness to this sensation. Graininess ratings did not significantly differ between ΔG+ and ΔA/B+ both in water and pear juice added with MCC. ΔA/B+ was the most responsive group to astringency and bitterness in both in water and pear juice added with TA and MCC+TA. Astringency and bitterness were rated significantly lower by ΔG+ than by ΔA/B+, while ΔG- and ΔA/B- were the least responsive to these sensations. Sweetness in pear juice was rated significantly lower by ΔA/B- and ΔG- than by ΔA/B+ and ΔG+. These groups appeared less responsive to sweetness and to the decreasing of this sensation induced by TA addition as indicated by their association with high Δ INT for sweetness on the biplot.

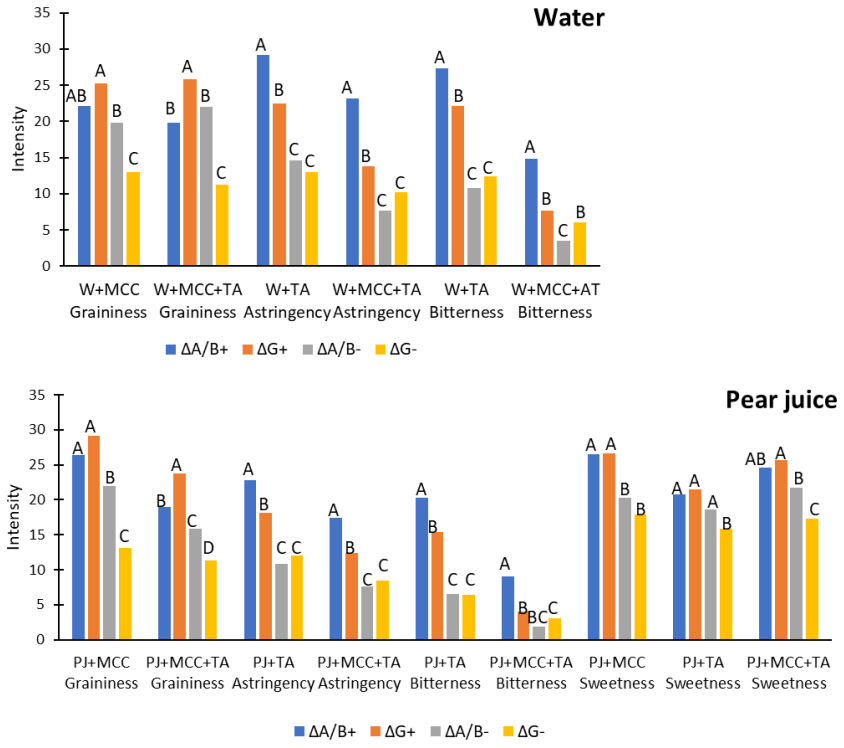


Figure 43. Effect of group on the intensity of target sensations in both water solutions and pear juice added with microcrystalline cellulose (MCC), tannic acid (TA) and both the tastants (MCC+TA). Different letters represent significant different values (p≤ 0.0001) (n=144)

4.3.3.2 Factors underlying individual differences in responsiveness to taste and tactile sensations

No significant differences were found in gender distribution by subject groups (group/gender: chi-square= 5.162; chi-square critical value= 7.815, p= 0.160). Groups did not significantly differ for preference for and familiarity with pear juice (preference: F= 1.122, p= 0.304; familiarity: F= 0.428, p= 0.733).

Possible association of individual variation in responsiveness to tactile and taste sensations perceived in water solution and pear juice model systems with chemosensory acuity markers (PROP responsiveness and FPD) and oral tactile acuity sensitivity (grating recognition threshold, GRT) were assessed (Table12).

Table 12. One-way ANOVA. Effect of group on phenotypic markers of chemosensory acuity (PROP bitterness responsiveness and fungiform papillae density, total and size classes) and on oral grating recognition threshold (GRT) (n=144). F and p values.

		PROP responsiveness	FPD DC1	FPD DC2	FPD DC3	FPD DC4	FPD DC5	FPD DC6	FPD DC7	FPD DC8	FPD DC9	FPD DC10	FPD DC11	FPD TOT	GRT
Group	F	2.373	0.358	0.120	0.469	0.312	0.386	0.480	0.777	0.296	0.273	0.177	0.447	0.152	0.139
	p	0.073	0.784	0.949	0.704	0.817	0.764	0.696	0.509	0.828	0.845	0.912	0.720	0.928	0.936

Groups did not significantly differ for FPD (total and diameter classes) (F> 0.120; p> 0.509) and for grating recognition threshold (F= 0.139; p= 0.936). Groups tended to significantly differ for PROP bitterness responsiveness (F= 2.372; p= 0.073), with ΔG+, ΔA/B+ and ΔG- resulting more responsive than ΔA/B- (Figure 44).

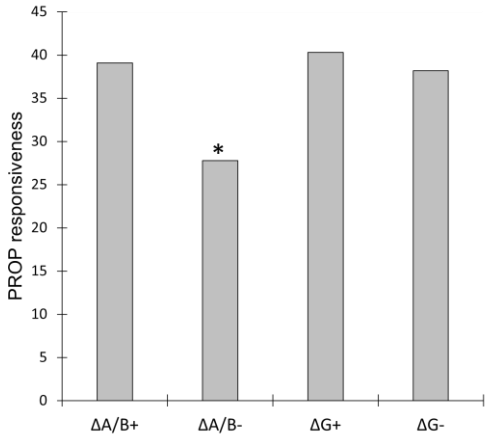


Figure 44. One-way ANOVA. Effect of group on PROP bitterness responsiveness (* indicates a p= 0.0073) (n=144)

Possible association of individual variation in responsiveness to tactile and taste sensations perceived in water solution and pear juice model systems with personality traits (food neophobia (FN), sensitivity to disgust (DS-SF), sensitivity to punishment (SP) and reward (SR), sensation seeking (SS) and state and trait anxiety) were assessed (Table 13).

Table 13. One-way ANOVA. Effect of group on personality traits (n=144). F and p values.

		FN	DS-SF	SP	SR	SS	State anxiety	Trait anxiety
Group	F	0.431	2.771	2.701	0.567	1.565	2.645	1.911
	p	0.731	**0.044**	**0.048**	0.638	0.201	0.052	0.131

Groups did not significantly differ for FN, SS, SR, trait anxiety scores (p> 0.196). Groups significantly differ for sensitivity to punishment (F= 2.701; p= 0.048) and for sensitivity to disgust (F= 2.771; p= 0.044), with ΔA/B- showing the lowest scores (Figure 45A and B, respectively). Groups tended to significantly vary for state anxiety scores, with ΔA/B- showing the lowest state anxiety score (F= 2.645; p= 0.052) (Figure 45C).

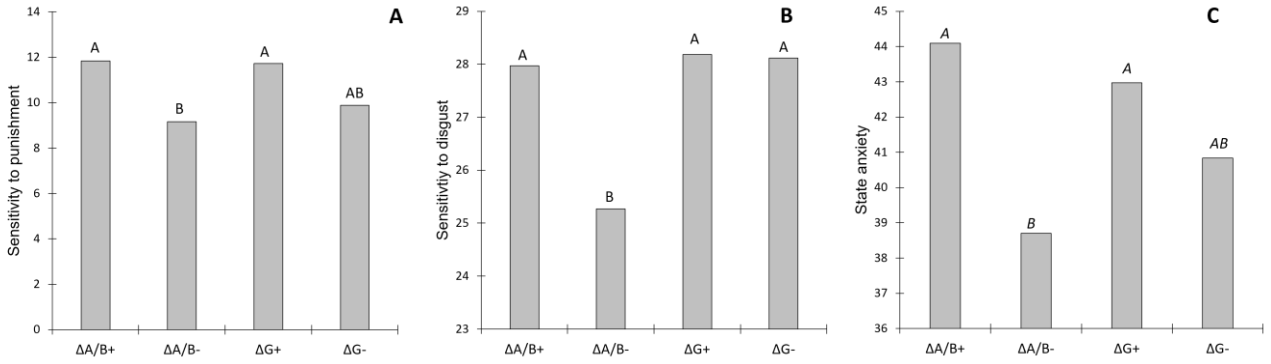


Figure 45. Effect of groups on sensitivity to punishment (A), sensitivity to disgust (B) and state anxiety scores (n=144). Different letters indicate significantly different values (p≤ 0.05), italic in plot C indicates p= 0.052. (n=144)

4.3.3.3 Effect of tastant concentration on mean liking ratings for pear juice model systems

The effect of tastant concentration on mean liking for pear juice added with MCC, TA and MCC+TA was assessed (Table 14).

Table 14. Effect of tastant concentration level on liking ratings for pear juice samples added with microcrystalline cellulose (PJ+MCC), tannic acid (PJ+TA) and both microcrystalline cellulose and tannic acid (PJ+MCC+TA) (n=144). F and p values.

		PJ+MCC Liking	PJ+TA Liking	PJ+MCC+TA Liking
Tastant concentration level	F	137.973	287.325	173.342
	p	**< 0.0001**	**< 0.0001**	**< 0.0001**

Tastant concentration levels negatively affected liking ratings (p< 0.0001). Liking for pear juice samples varying in microcrystalline cellulose concentration (PJ+MCC) significantly decreased with increasing MCC concentration, from “like moderately” to “dislike moderately” (Figure 46A). Liking for pear juice samples varying in tannic acid concentration (PJ+TA) significantly decreases with increasing TA concentration, from “like moderately” to “dislike very much” (Figure 46B). Liking for pear juice samples varying in both microcrystalline cellulose and tannic acid concentration (PJ+MCC+TA) significantly decreases, from “like moderately” to “dislike moderately” (Figure 46C).

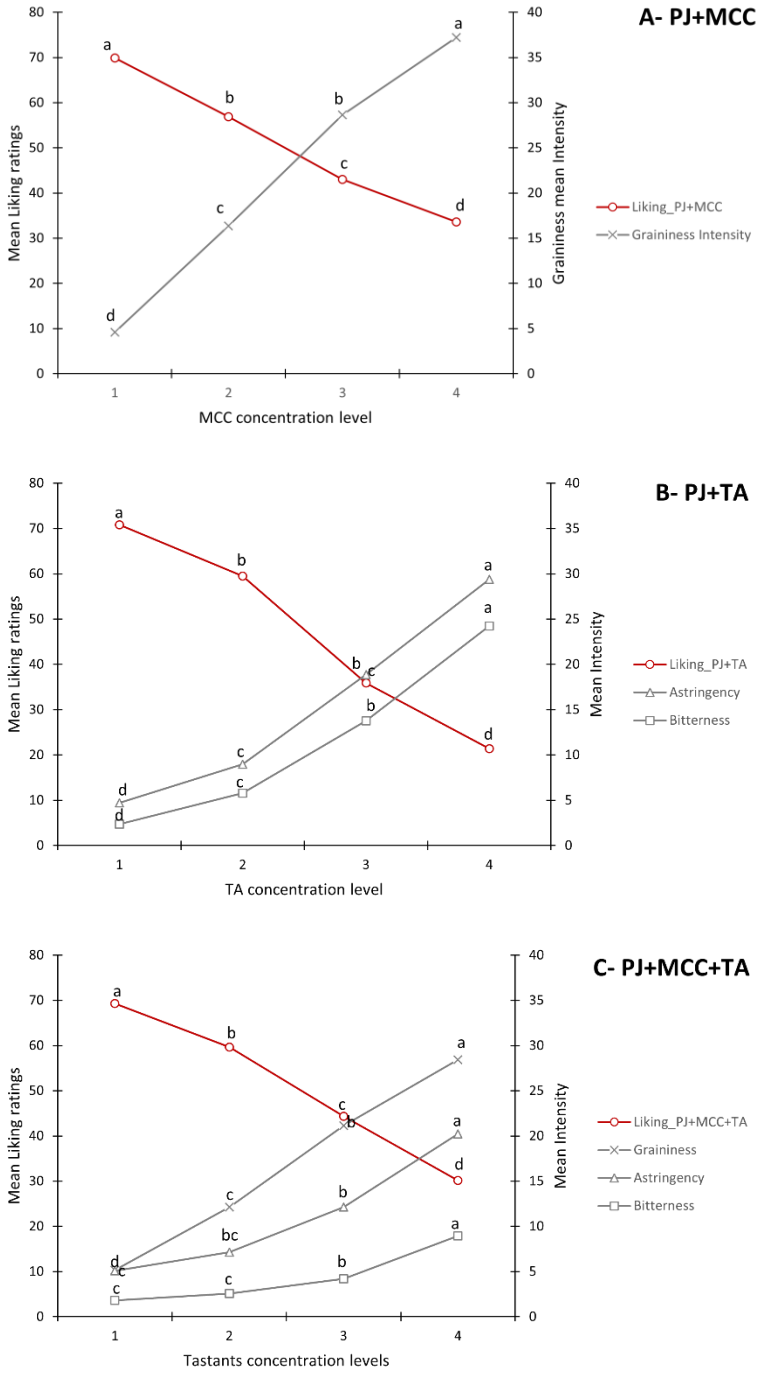


Figure 46. Effect of tastants concentration levels on liking ratings for pear juice samples added with microcrystalline cellulose (A), tannic acid (B) and both microcrystalline cellulose and tannic acid (C). Different letters represent significant different values ($p \leq 0.0001$).

4.3.3.4. Individual differences in liking for pear juice model systems with varied intensity of tactile and taste sensations

The Bi-plot from PCA computed on the liking scores from pear juice samples is shown in Figure 47. The first two components in the PCA accounted for 75.83% of total variance, with PC1 explaining 66.68% and PC2 contributing 9.15%. Most subjects were positioned on the right part of the biplot. Thus, liking for pear juice

samples was mainly driven by sweet sensation which was mainly associated with pear juices with the lower tastant concentration (concentration level 1 and 2). On the contrary, astringent and bitter sensations were negatively associated with liking for products characterized by higher TA and MCC+TA concentrations. Liking for products further varied along PC2 according to graininess intensity associated to juices added with the highest MCC concentrations. Subject positioned in the upper part of the biplot showed a negative association between graininess intensity and pear juice liking.

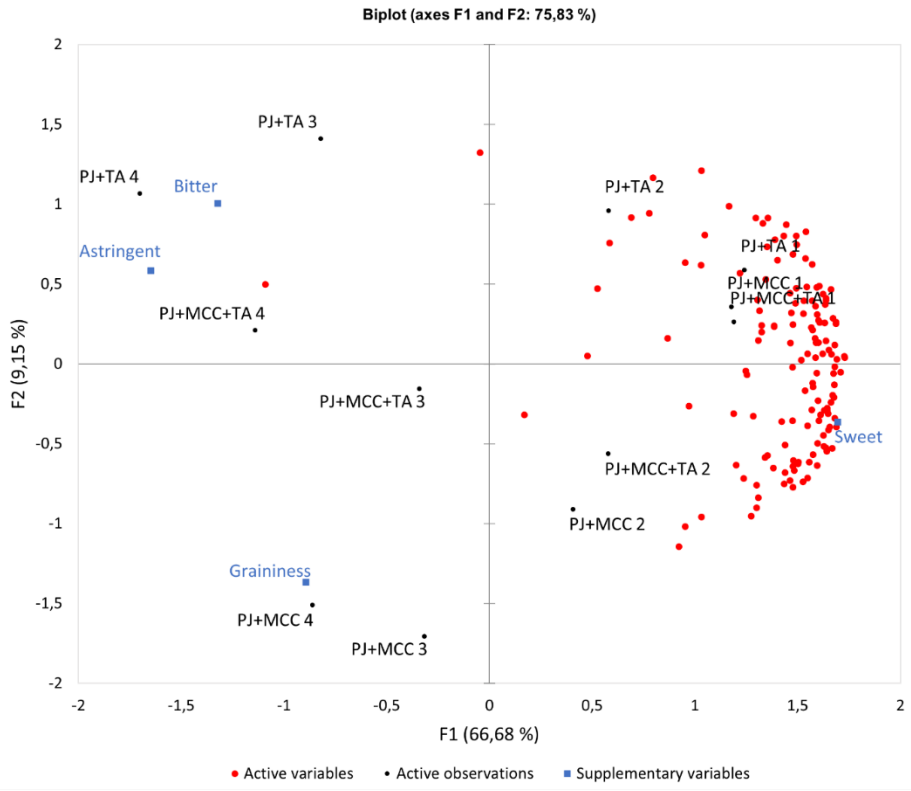


Figure 47. Bi-plot from PCA on liking ratings of pear juice samples added with microcrystalline cellulose (PJ+MCC), tannic acid (PJ+TA) and both microcrystalline cellulose and tannic acid (PJ+MCC+TA). Target sensations are reported as supplementary variables (n=144)

To further explore individual differences in liking due to variation in intensity of target sensations, mean liking scores for pear juices were compared to identify the most accepted samples (Figure 48). Results confirmed that pear juices added with level 1 and 2 of TA, MCC and MCC+TA were rated ≥50 on the LAM scale (neither like nor dislike) while juices added with level 3 and 4 of tastants were always rated lower.

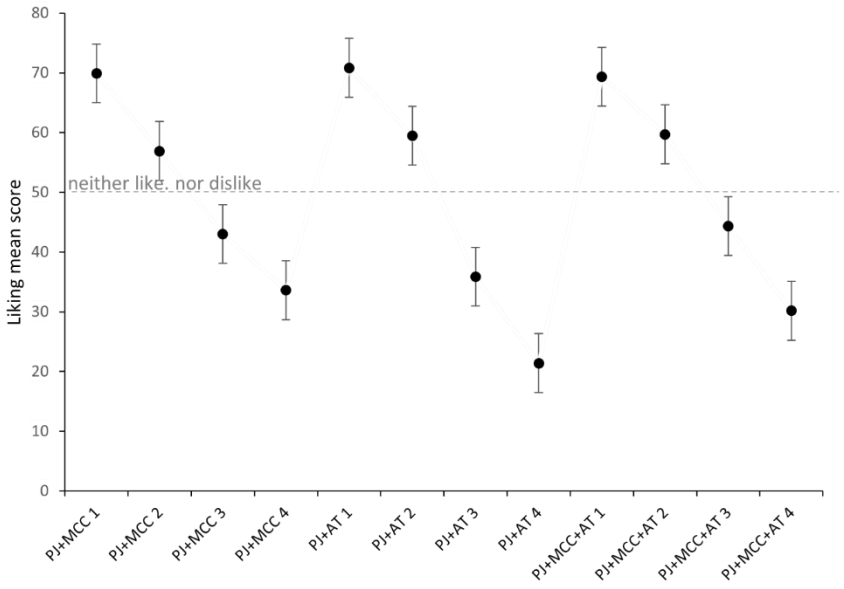


Figure 48. Mean liking scores for pear juice samples addedd with MCC, TA and MCC+TA. Bars represent the standard error.

The Bi-plot from PCA computed on the liking scores from PJ+MCC1, PJ+MCC2, PJ+TA1, PJ+TA2, PJ+MCC+TA1, PJ+MCC+TA2 samples is shown in Figure 49. The first two components in the PCA accounted for 62.52% of total variance, with PC1 explaining 39.92% and PC2 contributing 22.60%. Subjects positioning on the bi-plot space appeared more scattered compared to the map computed on liking scores from the whole pear juice sample set. Sweet intensity confirmed its positive association with graininess and astringency. However different patterns could be observed along the PC2. Based on coordinates on the second component (PC2), two groups were identified: A/B group consisting of subjects positioned in the upper right quadrant for which liking was positively driven by sweet intensity in opposition to bitterness and astringency (n= 68) and G group consisting of subject positioned in the bottom right quadrant in which liking was positively driven by sweet intensity in opposition to graininess (n= 57).

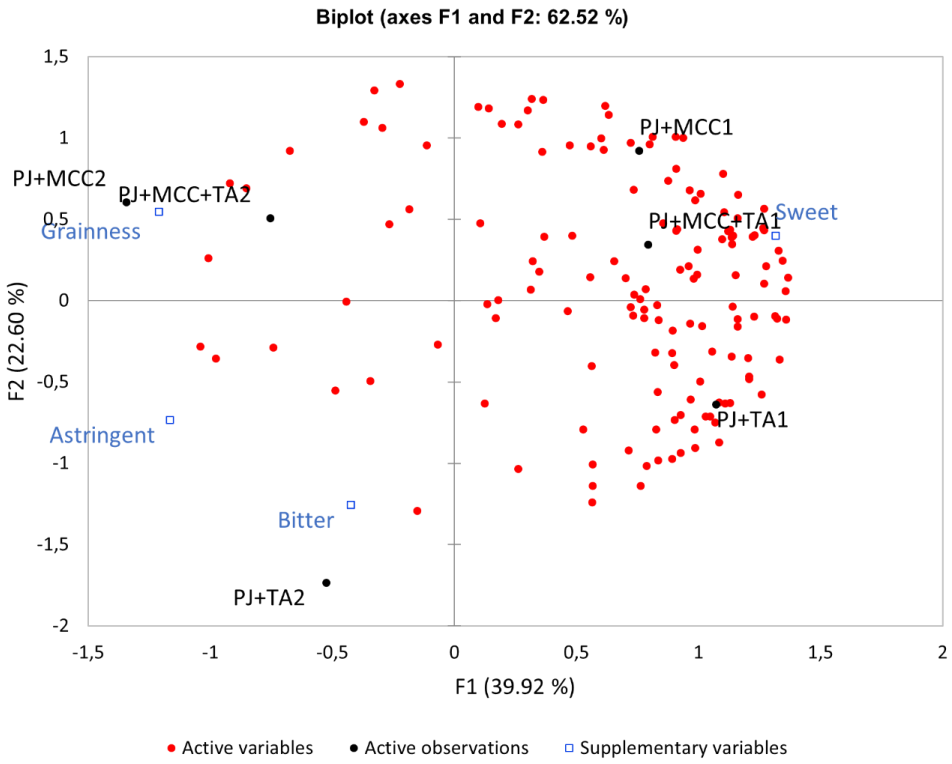


Figure 49. Bi-plot from PCA on liking ratings for pear juice samples added with microcrystalline cellulose (Pj+MCC), tannic acid (PJ+TA) and both microcrystalline cellulose and tannic acid (PJ+MCC+TA) (two tastant concentration levels). Target sensations are reported as supplementary variables (n=144)

Liking responses for G and A/B groups on liking for pear juices added with the four levels of tastant were assessed (Table 15).

Table 15. Two-way ANOVA. Effect of group, tastant concentration level and their interaction on liking ratings for pear juice samples added with microcrystalline cellulose (PJ+MCC), tannic acid (PJ+TA) and both microcrystalline cellulose and tannic acid (PJ+MCC+TA). (n=144). F and p values.

Liking	Group		Tastant concentration level		Group*tastant concentration level	
	F	*p*	F	*p*	F	*p*
PJ+MCC	14.175	**0.000**	146.701	**< 0.0001**	1.409	0.239
PJ+TA	31.090	**< 0.0001**	311.354	**< 0.0001**	5.912	**0.001**
PJ+MCC+TA	1.355	0.245	188.979	**< 0.0001**	3.288	**0.021**

Liking ratings from different groups confirmed what was expected from subject positioning on the bi-plot and further highlighted similarity and differences among groups in the liking for pear juice samples added with MCC, TA, or MCC+TA. G group showed the lowest liking for pear juice samples added with microcrystalline cellulose at MCC concentration levels 1 and 2 (lowest graininess intensity levels) (Figure 50A). On the contrary, A/B group showed the lowest liking for pear juice samples added with tannic acid for all TA concentration levels with the only exception of level 4 (highest astringency and bitterness intensity)

(Figure 50B). Group did not differ for liking for pear juice samples added with both MCC and TA at each tastants concentration levels, with the only exception of level 4 (highest graininess, astringency and bitterness intensity), at which A/B group showed the lowest liking ratings (Figure 50C).

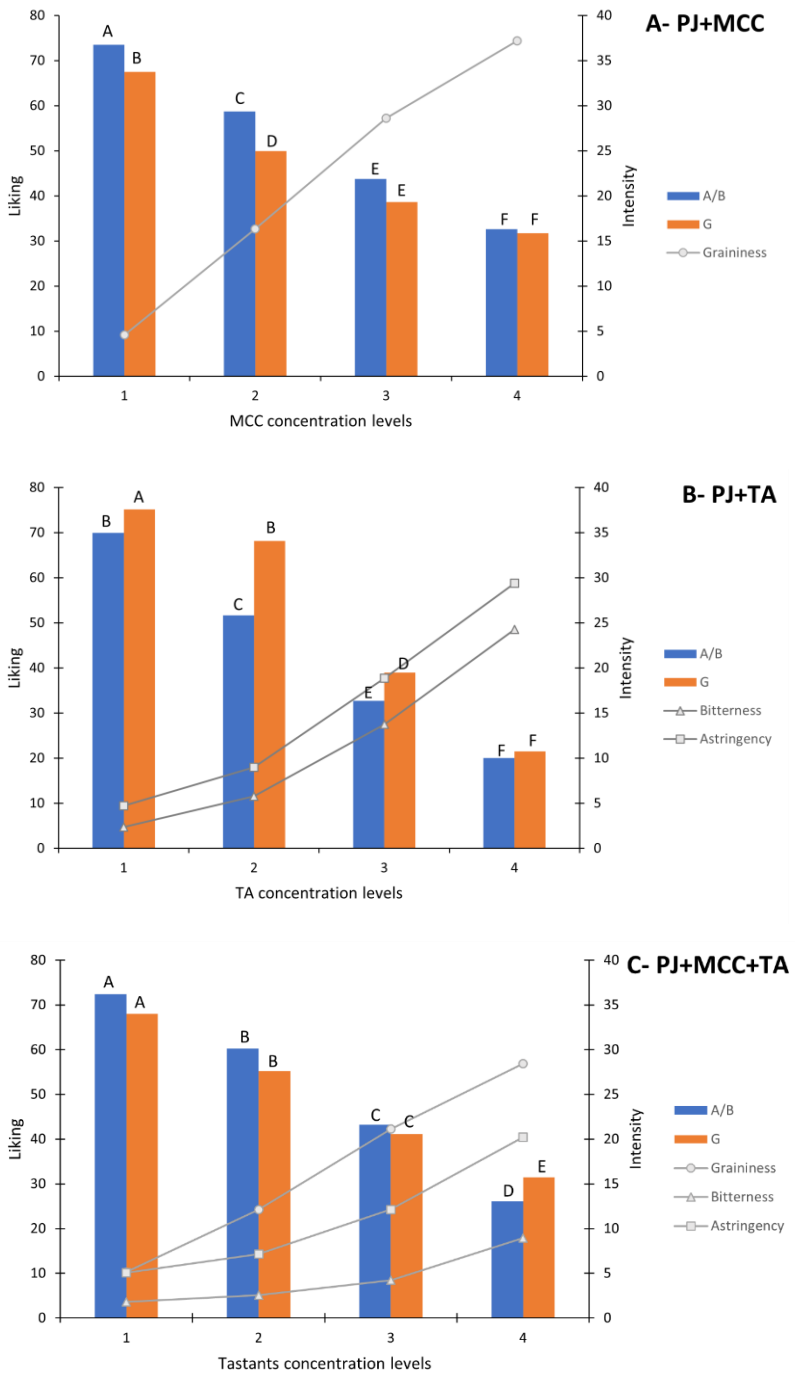


Figure 50. Effect of group on liking ratings for pear juice samples added with microcrystalline cellulose (A), tannic acid (B) and both microcrystalline cellulose and tannic acid (C) (n=144). Different letters represent significant different values ($p < 0.0001$)

4.3.3.5 Factors underlying differences in liking patterns

No significant difference was found in distribution by gender among groups (chi-square= 1.005; chi-square critical value= 3.841, p= 0.316).

Groups did not significantly differ for preference for and familiarity with pear juice (preference: mean G= 6.79, A/B= 7.09; F= 1.01, p= 0.32; familiarity: mean G= 4.02, A/B= 3.98; F= 0.079, p= 0.779)

The association between differences in liking patterns of G and A/B groups with variation in phenotypic markers of chemosensory responsiveness and in oral tactile acuity was investigated. G and A/B groups did not significantly vary for PROP bitterness responsiveness (F= 0.042, p= 0.839), fungiform papillae density total and size classes (F< 3.767, p> 0.055) and for grating recognition threshold (F= 1.410, p= 0.237).

Perception of tactile and taste sensations in water and pear juice samples of G and A/B groups were compared (Table 16). No significant differences were observed between G and A/B groups for graininess and astringency intensities in water solutions (F< 1.741, p> 0.188) and pear juices (F< 2.035, p> 0.154). The only exceptions were sweetness intensity in PJ+MCC samples (F= 8.717, p= 0.003), and bitterness perception in PJ+TA (F= 5.285, p= 0.022) in which G group showed the lowest mean intensity. Factors interactions were never significant (p> 0.216).

Table 16. Two-way ANOVA: Effect of group, tastants concentration levels and interactions on the intensity of target sensations in water solution and pear juice samples added with microcrystalline cellulose (MCC), tannic acid (TA) and both microcrystalline cellulose and tannic acid (MCC+TA) (n=144). F and p values.

Model system	Sensations	Group		Tastant concentration level		Group*Tastant concentration level	
		F	*p*	F	*p*	F	*p*
Water							
MCC	Graininess	1.741	0.188	114.051	**< 0.0001**	0.841	0.472
TA	Bitterness	2.040	0.154	81.367	**< 0.0001**	0.769	0.512
	Astringency	0.026	0.872	82.991	**< 0.0001**	0.290	0.833
MCC+TA	Graininess	0.369	0.544	131.632	**< 0.0001**	0.146	0.933
	Bitterness	0.459	0.498	31.504	**< 0.0001**	1.395	0.243
	Astringency	1.363	0.244	68.880	**< 0.0001**	0.774	0.509
Pear juice							
MCC	Graininess	0.026	0.872	98.964	**< 0.0001**	0.429	0.733
	Sweetness	8.717	**0.003**	6.612	**0.000**	0.197	0.898
TA	Bitterness	5.285	**0.022**	67.407	**< 0.0001**	0.603	0.613
	Sweetness	0.493	0.483	41.398	**< 0.0001**	1.490	0.216
	Astringency	1.314	0.252	85.270	**< 0.0001**	0.378	0.769
MCC+TA	Graininess	0.505	0.478	79.582	**< 0.0001**	1.324	0.266
	Bitterness	0.016	0.899	24.707	**< 0.0001**	0.554	0.646
	Sweetness	0.202	0.653	13.341	**< 0.0001**	0.223	0.880
	Astringency	2.035	0.154	49.750	**< 0.0001**	0.286	0.836

G and A/B groups did not differ for personality traits ($F < 2.132$, $p > 0.147$).

4.3.4 Discussion

The aim of the first part of this chapter was to explore individual differences in responsiveness to tactile sensations induced by microcrystalline cellulose (graininess) and by tannic acid (astringency), and to taste sensations (bitterness and sweetness) of model systems showing significant intensity variation due to tastant addition. Individual responsiveness to the intensity variation induced by the increasing of tastant concentration was expressed in terms of Δ INT (intensity perceived at the highest tastant concentration – intensity perceived at the lowest tastant concentration) (Piochi et al., 2021). Four subject groups were identified varying in Δ INT of target sensations: ΔG+ characterized by the highest responsiveness to graininess, ΔA+ characterized by the highest responsiveness to both astringency and bitterness, ΔG- and ΔA- with lowest responsiveness to graininess and astringency sensation increasing, respectively. Δ INT values represent the extent of variation in intensity perception due to the increasing of tastant concentration but do not necessarily associate to the absolute value of perceived intensity. Results indicated that graininess was rated the highest by ΔG+ and the lowest by ΔG-, thus indicating that the responsiveness to the variation level associated with intensity ratings with wider variation range corresponded to higher intensity perception. ΔA+ showed the highest astringency ratings while ΔA- showed ratings lower than ΔA+ but not significantly different from ΔG-. Individual variability in salivary protein composition in response to astringent stimulation (Dinnella et al., 2010; Dinnella et al., 2009) affect the perception of astringency intensity and possibly partially account for the more complex pattern of relationship between responsiveness to astringency variation and the perceived intensity of this sensations. ΔA+ also showed the highest intensity of bitter taste, followed by ΔG+ while ΔA- and ΔG- showed the lowest intensity perception. Results indicate a general different responsiveness of subject groups to both tactile and taste sensations with ΔG+ and ΔA+ resulting more responsive to both tactile and taste sensations and ΔA- and ΔG- less responsive to these oral stimuli. The association of increased responsiveness to different taste stimuli was already reported for tastes (Hayes & Keast, 2011; Puputti et al., 2018) and this work enlarge this concept to different perceptual modalities.

Individual variations in responsiveness to taste and tactile stimuli are widely explored through the assessment of PROP phenotype with individuals more responsive to PROP showing in general increased responsiveness to oral stimulation (Goldstein et al., 2007). Results from the present work showed that groups varying for responsiveness to tactile and taste sensations in model systems did not significantly vary in PROP bitterness responsiveness. Only a tendency was observed for ΔA- group in which the general low responsiveness to tactile and taste sensations tended to be associated to the lower responsiveness to PROP. The variation of responsiveness to tactile and taste sensations did not associate to fungiform papillae density and to grating recognition threshold. These results are at least in part supported by previous evidences

showing that roughness perception in bread samples positively associated to PROP responsiveness while no associations were found for FPD variations (n=37) (Bakke & Vickers, 2008). The detection of particles in dairy products was not affected by FPD, PROP responsiveness or oral tactile acuity variations (n=92) (Santagiuliana et al., 2019). On the other hand, in a recent work on 117 consumers, FPD and oral tactile sensitivity were found to associate to individual ability in detecting particles in yoghurt samples while no significant associations were found for PROP responsiveness (Olarte Mantilla et al., 2022). Evidence from literature report controversial results on the relationship between the perception of specific texture-related sensations and phenotypic markers of chemosensory acuity. This in part might be explained by the relatively low sample size considered and by the different methodology adopted for oral tactile acuity assessment. The adoption of larger population sample would help to deeper explore these relationships but oral tactile measurements in their present form are very labour-intensive for both sensory lab personnel and for assessors thus making challenging the collection of larger number of observations. Moreover, the currently available measures of oral tactile sensitivity have been adapted from measures for less sensitive body tissues (such as skin and fingers) and their capacity to cover individual variation in oral tactile acuity has not yet been fully demonstrated.

The aim of the second part of this chapter was to explore individual variability in the hedonic response to pear juice samples varying in microcrystalline cellulose concentration (PJ+MCC), tannic acid concentration (PJ+TA) and both microcrystalline cellulose and tannic acid concentration (PJ+MCC+TA). The increasing of tastant concentration resulted in a regular decreasing of mean liking ratings. MCC and TA induced generally disliked tactile and taste sensations such as graininess evoked by MCC, and astringency and bitterness evoked by TA. A larger decreasing in liking was observed for pear juice samples added with TA, while the addition of MCC induced pear juice dislike in a smaller extent. The larger negative effect of TA concentration on liking appeared to be related to the more evident modifications of pear juice sensory profile. In fact, TA concentration associated to the increasing of both astringency and bitterness and to sweetness suppression while MCC induced the simple graininess increasing. Thus, the combination of astringency/bitterness increasing appears more critical for pear juice acceptance than the increasing in graininess. Participants showed a common pattern, with pear juices added with the lower levels of tastants being more liked than juices added with the higher tastants amount. Sweet sensation was the main driver of products liking while astringent, bitter and, in a lower extent, graininess were drivers of products dislike. Differences in liking pattern were observed when only the two juices with the lower tastant content were considered. Two groups of subjects were identified, G group in which disliking was mainly driven by graininess increasing and A/B group in which both astringency and bitterness increasing were the main drivers of disliking. In fact, G group showed liking ratings for juice added with MCC lower than A/B group while A/B group liked less than G group pear juice samples containing TA. The observed differences in liking in the two groups did not relate to significant differences in the perception of tactile sensations. A/B group was more responsive to tastes thus,

its higher perception of bitter taste evoked by TA seems to be the most likely reason for the lower liking for pear juices added with TA. Differences in liking between G and A/B groups did not associate to significant variation in phenotype markers of chemosensory responsiveness or to oral tactile acuity measures. These results support recent evidences on the lack of straight relationship between liking for foods with varied texture properties and oral physiological parameters, including saliva flow rate, chewing efficiency, biting force, and particle size sensitivity (Kim & Vickers, 2019). Furthermore, differences in texture perception, oral processing behaviours and oral tactile sensitivity were found to be negligible in driving the liking response to yogurt samples varying in granularity (Liu et al., 2021). Different patterns of intake and liking for yogurts added with particles were found to associate to different eating styles (Varela et al., 2021). Therefore, results on factors affecting liking for varied food texture appeared to be controversial in identifying. Individual variations should be explored, taking into account individual differences in oral system sensitivity (both phenotypic markers of oral acuity and oral tactile acuity measures), oral processing behaviours, and sensory perception. Physiological factors, including saliva flow rate and composition, should be also explored as source of individual variation in both texture perception and liking response.

4.3.5 Conclusion

Individual differences in the perception of food texture-related sensations may underlined specific patterns of food texture preference.

Groups varying for responsiveness to tactile (graininess and astringency) and taste (bitterness and sweetness) sensations in model systems were identified. Groups did not significantly vary in gender distribution, PROP bitterness responsiveness, fungiform papillae density total and size classes and grating recognition threshold. Significant differences between groups associated to psychological and personality traits, including sensitivity to punishment, sensitivity to disgust and state anxiety, suggesting that individuals higher responsive to warning tactile sensations showed closed personality type. Differences in liking pattern were observed that did not relate to differences in the perception of tactile sensations, phenotype markers of chemosensory responsiveness, oral tactile acuity measures or to psychological and personality traits.

5.General conclusions

The development of oral tactile responsiveness measures capturing individual variation in tongue responses to mechanical stimulation would be helpful to better understand individual differences in texture perception related food preferences.

Results of the present thesis showed that point-pressure sensitivity thresholds do not appear a useful tool to map individual variability in responsiveness to oral tactile stimulation. On the other hand, grating recognition thresholds discriminated amongst participants and appeared suitable in exploring the individual variation in oral responsiveness to mechanical stimulation and the cognitive processes behind it. Point-pressure sensitivity and grating recognition threshold did not correlate, supporting the hypothesis that these measures represent different tactile functions underlined by different receptor/neural mechanisms. A substantial independence was observed between grating recognition thresholds and the phenotype markers of chemosensory responsiveness (PROP bitterness responsiveness and fungiform papillae density total ad size classes), but it is suggested that a larger scale study is required to confirm this. Future studies should be aimed at oral tactile acuity methodologies optimization, for example exploring the use of narrower gratings and the adoption of longer staircases, to capture the differences in tactile sensitivity among the most sensitive individuals.

The development of model systems for specific texture-related sensations, namely astringency and graininess, would be a strategy for the systematic exploration of individual differences in responsiveness to oral tactile stimulation.

In the present thesis psychophysics curves were developed for microcrystalline cellulose (MCC) and tannic acid (TA) in both water solutions and fruit juices. Microcrystalline cellulose appeared a pure stimulus for graininess sensation, while tannic acid was confirmed to be able to evoke both a tactile sensation, astringency, and taste sensations, bitterness and sourness. Tastant concentration levels were identified to induce systematic variation in the intensity of target sensations. Both chemical and perceptual interactions appear to occur when the tactile stimuli were both present in a same model system that can partially explain variations in the perception of target sensations. Furthermore, physical-chemical and perceptual interactions between tactile stimuli and dispersion medium components were hypothesised to account for changes in model system sensory properties.

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Exploring the association between oral tactile sensitivity measures and phenotypic markers of oral responsiveness

Journal:	*Journal of Texture Studies*
Manuscript ID	JTS-3383
Manuscript Type:	Research Article
Date Submitted by the Author:	22-Dec-2021
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Keywords:	grating orientation test, point-pressure test, PROP, fungiform papillae

Exploring the association between oral tactile sensitivity measures and phenotypic markers of oral responsiveness

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Abstract

Recent efforts have been devoted to identifying oral tactile sensitivity measures to explore individual variation in oral somatosensory system responsiveness and its role in food texture perception. This study investigated the individual variability in oral tactile sensitivity considering touch, by means of Von Frey Hair monofilaments (VFH) and spatial resolution, using the grating orientation test (GRT). The relationships of the two measures with 6-n-propylthiouracil (PROP) responsiveness and fungiform papillae density and size were investigated. One hundred and forty-four subjects (48.6% women, aged 18-30) participated in the study. VFH and GRT thresholds were assessed by three-down/one-up staircase method. Responsiveness to 3.2mM PROP was assessed on the general Labelled Magnitude Scale. Fungiform papillae density (FPD) and size were determined from automated counting. VFH thresholds appeared unsuitable to reveal individual variation in responsiveness to point-pressure on the tongue with all the participants detecting the lowest VFH force and 72% of them discriminating at the lowest monofilament level. The frequency of GRT thresholds approximated a normal distribution and covered the whole range of variation, thus indicating an ability to measure individual variation in oral tactile sensitivity. No significant linear correlations were found between any of the oral tactile sensitivity measures and PROP responsiveness, FPD total and size class. VHF and GRT thresholds were not significantly associated. Agglomerative hierarchical clustering was used to classify participants for their PROP responsiveness, total FPD and GRT threshold. Three clusters were identified, C1 (n=67), Cl2 (n=42) and Cl3 (n=35), differing for PROP responsiveness and FPD only.

key words: grating orientation test, point-pressure test, PROP, fungiform papillae

1. Introduction

Food texture is one of the main drivers of food acceptance and is involved in physiological pathways, such as satiation and satiety mechanisms, thus playing a fundamental role in regulating the amount of food intake (James, 2018). Recent studies suggest that individual differences in food texture perception may be associated with oral somatosensory system sensitivity, with individuals higher in oral tactile sensitivity perceiving food texture-related sensations more intensely than individuals with lower oral tactile sensitivity (Zhou et al., 2021; Shupe, Resmondo, & Luckett, 2018, Olarte, 2022). Consistent with this hypothesis, high oral tactile sensitivity was found to be positively associated with a greater particle perception and size discrimination in yogurt (Olarte Mantilla et al., 2022) and chocolate samples (Breen et al.,

2019) and to a higher hardness perception in biscuits (Zhou et al., 2021). However, the literature regarding this is still relatively scarce and affected by many methodological differences in oral tactile acuity measures.

The current measures of oral tactile sensitivity convey information about specific functions of oral mechanoreceptors, such as the perception of touch (Weinstein, 1968), spatial resolution (Weinstein, 1968) and oral stereognosis (Lederman & Klatzky, 2009), indicating complexity of the oral somatosensory system. The most common methods are: the point-pressure method by Von Fryer monofilaments (Yackinous & Guinard, 2001; Breen et al., 2019; Etter, Breen, Alcala, Ziegler, & Hayes, 2020; Zhou et al., 2020), the oral stereognosis assessment by the identification of letters and shapes (Essick, Chen, & Kelly, 1999; Essick, Chopra, Guest, & Mcglone, 2003; Lukasewycz & Mennella, 2012; Steel et al 2014; Bancguyo et al 2017) and the spatial resolution assessment by the identification of two point distance (Olarte Mantilla et al., 2022) or the identification of grating orientation (Johnson & Phillips, 1981; Wohlert, 1996).

Limitations were observed for all these methods. The point-pressure method stimulates a very small area of the tongue which does not reflect the oral tactile sensitivity of the whole mouth (Zhou et al., 2021). Furthermore, the device used to assess oral point-pressure sensitivity consists of a kit of monofilaments that deliver specific target forces, which might not correspond to the lower force that tongue mechanoreceptors could perceive, thus, leading to a floor effect (Santagiuliana et al., 2019). On the other hand, oral stereognosis and spatial resolution methods are recognised as a more complex task involving cognitive processes (recognition of the shape or the grid orientation), which are also affected by cultural factors such as familiarity with the tactile cues (i.e. alphabet letter) (Cattaneo, Liu, Bech, Pagliarini, & Bredie, 2019; Essick et al., 1999; Shupe et al., 2018). It was suggested that the stimuli used to test touch sensitivity mainly stimulate superficial receptors, while stimuli used to test the oral stereognosis or the spatial resolution might excite a set of deeper receptors (Engelen & Van Der Bilt, 2008). Therefore, to overcome intrinsic limitations belonging to each method and to have more reliable measure of oral tactile sensitivity it was recently suggested to consider a variety of methods when exploring oral tactile sensitivity (Cattaneo et al., 2019; Appiani, Rabitti, Methven, Cattaneo, & Laureati, 2020; Santagiuliana et al., 2019; Zhou et al., 2021).

Associations have been previously reported between individual variation in food texture perception and common phenotypic markers of oral sensitivity, namely the responsiveness to the bitterness of 6-n-propylthiouracil (PROP status) (Melis & Barbarossa, 2017; Pickering & Robert, 2006; Kirkmeyer & Tepper, 2003; Bakke & Vickers, 2008) and the fungiform papillae density (FPD) (Hayes & Duffy, 2007; Zhou et al., 2021). Differences in PROP responsiveness, are mainly due to genetic variation in the TAS2R38 gene, which define two common haplotypes: PAV (considered the "taster haplotype") and AVI (considered the "non taster" haplotype)(Kim et al., 2003). PROP bitterness responsiveness is largely known to be positively associated to the perception of basic tastes in water solutions and in real products (Dinnella et al., 2018; Masi, Dinnella, Monteleone, & Prescott, 2015; Tepper et al., 2017). PROP bitterness was found to be positively associated with the perception of tactile/texture-related sensations such as astringency (Melis et al., 2017; Pickering & Robert, 2006), creaminess (Kirkmeyer & Tepper, 2003) and roughness (Bakke & Vickers, 2008).

Fungiform papillae are the anatomical structures designated to oral stimuli detection and transduction since they house taste buds that respond to chemical stimulation, and are innervated by chorda tympani and trigeminal nerve fibers which respond to tactile stroking and temperature stimuli (Whitehead, Beeman, & Kinsella, 1985; Mistretta & Bradley, 2021). Fungiform papillae were found to vary significantly across individuals, from 0 to 200 papillae/cm^2 (Zhang et al., 2009; Fischer et al., 2013; Eldeghaidy et al., 2018). Fungiform papillae also vary in size and shape (Essick et al., 2003).

Recently a classification of subjects based on their differences in both FP density and diameter was proposed (Piochi et al., 2019). Positive associations were reported between FPD and the perception of texture related sensations such as creaminess in milk (Hayes & Duffy, 2007) and hardness and crunchiness in biscuits (Zhou et al., 2021). On the other hand, other studies failed to find significant associations between FPD and food texture attributes, such as astringency in bread (Bakke & Vickers, 2008). Furthermore, FP size was hypothesised to associate with oral responsiveness (Melis et al., 2013) with uniform patterns of low density and small size FP related with higher responsiveness to tastes, astringency and pungency (Piochi et al., 2019).

Literature on the relationship between PROP status and FPD with measures of oral tactile sensitivity has been limited and has shown conflicting results. PROP responsiveness was found positively associated with oral spatial stereognosis (Essick et al., 2003) and point-pressure (Yackinous & Guinard, 2001). Similar to PROP, positive associations were also reported between FPD and both oral spatial stereognosis (Essick et al., 2003; Bangcuyo, Christopher, & Simons, 2017) and point-pressure measures (Zhou et al., 2021). On the other hand, no significant association between both PROP responsiveness and FPD with point pressure measures was reported (Cattaneo et al., 2019; Nachtsheim & Schlich, 2013).

Demographic factors are reported to affect responsiveness to PROP and FPD, on average PROP bitterness ratings and FPD are significantly higher in women than in men and increasing age is negatively associated to both phenotypic markers (Shahbake, Hutchinson, Laing, & Jinks, 2005; Tepper et al., 2017; Mennella, Pepino, Duke, & Reed, 2010; Feeney & Hayes, 2014; Webb, Bolhuis, Cicerale, Hayes, & Keast, 2015; Dinnella et al., 2018; Barragán et al., 2018). Little is still known about the effect of gender on oral tactile sensitivity measures, no significant differences by gender have been reported in a letter identification task (Bangcuyo et al., 2017), while adult women performed better than men in grating orientation test only for the greatest grating size (Appiani et al., 2020).

Thus, individual variations in food texture perception were related, even if with contrasting results, to both measures of oral tactile sensitivity and phenotypes of oral responsiveness. However, the associations between measures of oral tactile sensitivity and both PROP responsiveness and FPD are yet to be fully elucidated.

This study aims to explore the individual variability in oral tactile sensitivity considering both touch and spatial resolution, by means of the VHF point-pressure and grating orientation tests in a sample of young adults. The association between the two considered oral tactile sensitivity measures was assessed in a smaller subsample of women. The relationships of oral tactile sensitivity measures with both PROP bitterness responsiveness and fungiform papillae density and size were investigated with a possible gender effect also considered.

2. Materials and Methods

2.1 Participants

One hundred and forty-four young adults (48.6% women, age range 18-30) participated in the study. The whole sample was tested for the gratings orientation test, PROP responsiveness and FP density and size. The point pressure test was conducted on a sub-sample of 37 women. Pregnancy and breastfeeding at the moment of the test, food allergies and history of perceptual disorders were exclusion criteria. The study was conducted in agreement with the European ethical requirements on research activities and personal data protection (General Data Protection Regulation, GDPR, UE

2016/679). At the time of recruitment, respondents signed the informed consent according to the principles of the Declaration of Helsinki. At the end of the study, participants were compensated for their time with a voucher.

Tests during 2020 were performed according to Italian government regulations to control for COVID-19 spread, which included: controlled access to the lab after only in absence of Covid-19 symptoms; compliance with the minimum interpersonal distance of 1.8 m; wearing masks apart from whilst performing the test; environment and individual workstation sanitization after every use.

2.2 Oral tactile acuity measures

2.2.1 Test condition

The test was conducted individually by a trained operator in a quiet room. Firstly, the operator asked the participants to sit comfortably in front of her whilst they and explained what the test consisted of. Participants were then asked to relax their dorsal and neck muscles, show their tongue and keep it relaxed. Participants were asked to close their eyes and to keep them closed during the test; a blindfold was not used during the test to avoid participants distraction due the feeling of being blindfolded (Etter et al., 2020). Participants were invited to ask for a pause during the test every time they felt they lost their oral lubrication and to refresh their mouth with water.

2.2.2 Grating orientation test

2.2.2.1 Grating stimuli

Data collection occurred during the Covid-19 pandemic period; therefore, disposable grating kits were produced to ensure the safety of participants. Disposable stimuli were manufactured by a 3D stereolithography (3D-SLA) technology printing process, using a biomedical resin (Biomed Clear Resin, Formlabs, GmbH, Germany) to guarantee biocompatibility and nontoxicity requirements. Stimuli were custom printed in 1 cm x 1 cm x 0.5 cm tiles supported by a 2 cm rod. Tile surface was characterised by square-wave grating elements and six different grids were produced maintaining differences in their groove widths: 0.2, 0.25, 0.5, 0.75, 1.00 and 1.25 mm (Fig. 1).

2.2.2.2 Grating orientation recognition threshold

Prior to the start of the test a simulation with the 1.25 mm grating was performed to make sure that participants correctly understood the task (to correctly recognise the horizontal/vertical orientation of gratings). A 3-down/1-up staircase method was used for threshold estimation (Etter et al., 2020). The test started with the stimulus with the highest groove width (1.25 mm) applied on the tongue midline around 0.5 cm from the tip. Three correct answers to the same stimulus resulted in a presentation of the next lower groove width level. One incorrect answer resulted in a presentation of the next higher groove width level. The test continued until the stopping point defined as “the point when a participant has crossed over or received the test stimulus from the same target force a total of five times” (Etter et al., 2020). GRT threshold for each participant was calculated as the geometric mean of all the grating’s groove width values included between the first time the participant received the stimulus of the stopping point and the last (Etter et al., 2020).

2.2.3 Point pressure test

2.2.3.1 Stimuli

Stimuli consisted of commercially available aesthesiometers called Von Frey Hair monofilaments (Aesthesio-Tactile Sensory Evaluator, Ugo Basile, Italy), varying in diameter and in the force they deliver. Eight monofilaments were selected to perform the detection test: 1.0, 0.6, 0.4, 0.16, 0.07, 0.04, 0.02, 0.008 g (Breen et al., 2019).

2.2.3.2 Detection thresholds

Oral point-pressure detection threshold was determined using a two-interval forced choice procedure with a 3-down/1-up approach (Breen, Etter, Ziegler, & Hayes, 2019). A point-like area, 0.5 cm from the tongue tip on the anterior dorsal surface of the tongue at the midline, was highlighted using a cotton-tip applicator dipped in a blue food colouring. Each monofilament was applied perpendicularly to the tongue surface in the coloured area. Participants were asked to identify in which of the two trials they could feel the stimulus: one of the trials was a 'real' touch and the other a 'false' touch in a randomised order. A 3-down/1-up staircase method was used for threshold estimation (Etter et al., 2020). The test started with 1.0 g monofilament. Three correct answers to the same stimulus resulted in a presentation of the next lower monofilament. One incorrect answer resulted in a presentation of the next larger monofilament. The test continued until the stopping point. Detection threshold estimates were calculated as the geometric mean of all the force values included between the first time the participant received the stimulus of the stopping point and the last (Etter et al., 2020).

2.2.3.3 Discrimination thresholds

Based on detection threshold estimates, five monofilaments were selected to perform the discrimination test: 0.16, 0.07, 0.04, 0.02, 0.008 grams. A two-interval forced choice procedure with a 3-down/1-up approach was followed (Breen et al., 2019). Each test consisted of several trials, the total number of which was dependent on the individual response. In each trial participants received a pair of stimuli of consecutive force levels (0.16 g was coupled with 0.07 g filament; 0.07 g was coupled with 0.04 g; 0.04 g was coupled with 0.02 g and 0.02 g was coupled with 0.008 g resulting in four pairs of stimuli) and they were asked to identify in which trial the strongest pressure was delivered. All the participants received the pairs of stimuli in the same order, starting from the couple 0.16 g / 0.07 g and with the subsequent pairs presented in a decreasing order. Three correct answers to the same couple (e.g., 0.16 g/ 0.07 g) resulted in a presentation of the next lower couple (e.g., 0.07 g / 0.04 g). One incorrect answer resulted in a presentation of the next higher couple. The test finished when participants correctly discriminated all the pairs of stimuli, thus resulting in a discrimination threshold of 0.008, or when they reached the stopping point, i.e., they crossed over or received the same pair a total of five times (Etter et al., 2020). Discrimination threshold estimates were calculated as the geometric mean of all the monofilaments force values included between the first time the participant received the pair of the stopping point and the last (Etter et al., 2020).

2.3 Oral responsiveness markers

2.3.1 PROP bitterness ratings

Participants were introduced to the use of the General Labelled Magnitude Scale (gLMS) (Bartoshuk et al., 2004) with particular emphasis on the meaning of the descriptor "the strongest imaginable sensation of any kind." Verbal instructions were given that the top of the scale represented the most intense sensation that subjects could ever

imagine experiencing and a variety of remembered sensations from different modalities including loudness, oral pain/irritation, and tastes were recalled (Bajec & Pickering, 2008; Kalva, Sims, Puentes, Snyder, & Bartoshuk, 2014; Webb, Bolhuis, Cicerale, Hayes, & Keast, 2015; Dinnella et al., 2018). For participant alignment to the use of gLMS, subjects individually rated intensities of the brightest light they had ever seen. Correct understanding of the scale was concluded if subjects rated between very strong and strongest imaginable. In case of ratings out of this range, a short individual interview was carried out to understand the ratings and scale use was explained again. A 3.2 mM PROP solution was prepared by dissolving 0.5447 g/L of 6-n-propyl-2-thiouracil (Sigma-Aldrich, USA) into deionized water (Prescott, Johnstone, & Francis, 2004). Samples were presented in duplicate (10 mL), labelled with 3-digit codes (Masi et al., 2015). Participants were instructed to hold the sample in their mouth for 10 s, expectorate, and then wait 20 s before evaluating the bitterness intensity using the gLMS (Bartoshuk et al., 2004). After cleaning the palate with plain crackers, participants rinsed their mouths with water and waited for 90 seconds before being served a duplicate sample with a different 3-digit code. The average bitterness score of the two duplicate samples was calculated for each participant.

2.3.2 Fungiform papillae density (FPD)

Participants were instructed to swab the anterior portion of the dorsal surface of their tongue with blue food colouring, using a cotton-tipped applicator. During the procedure, the operator controlled that participants correctly coloured their tongue surface (intensity of blue, colour both tongue dorsal surface and sides). Digital pictures of the tongue were taken (Shahbake et al., 2005) using a digital microscope (MicroCapture, version 2.0 for ×20 to ×400) (Masi et al., 2015) and the clearest image was selected for each participant. A rectangular area of the tongue image (1.125 cm^2; image resolution: 96 dpi) orthogonal to the median line and located 0.5 cm from the tongue tip was selected for processing. Fungiform papillae density was quantified through an automated procedure that counted the number of circular-like elements on the picture considering a diameter range 0.30-1.05 mm and included them in 11 diameter classes (Piochi et al., 2017). Circular-like elements counted by the script in each diameter class (DC) were converted into FPD by dividing for the area. For each participant, the total FPD was computed as the sum of FPD in all size classes (Piochi et al., 2019).

2.4. Data analysis

Descriptive statistics (mean and median values, first and third quartile limits) were used to describe GRT and VFH thresholds, PROP bitterness ratings and FPD. Shapiro-Wilk test was used to assess variable distribution (α=0.05). Distributions were compared by two-sample Kolmogorov-Smirnov (α=0.05). One-way ANOVA models were independently applied to test for gender effect on GRT, PROP bitterness ratings, FPD for each diameter class and total FPD. Correlations between GRT, PROP bitterness and FPD and between GRT and VFH discrimination thresholds were tested by Pearson correlation, with significance level fixed at $p \leq 0.05$. Agglomerative hierarchical clustering (AHC) was used to classify participants for their mean PROP bitterness rating, total FPD and GRT threshold. Euclidean distance was selected as proximity type and Ward's method was chosen as agglomeration method; data were centered, and automatic-entropy criterion was selected for truncation. One-way ANOVA was used to test the effect of each cluster on PROP bitterness ratings, total FPD, and GRT. The association between cluster and gender was investigated using chi-square tests. Fisher's exact test was run to test the significance by cell (significance level fixed at $p = 0.05$). All data analysis was conducted using XLSTAT (2020.2.1, Addinsoft, USA).

3. Results

3.1 Oral tactile acuity measures

GRT threshold followed a normal distribution (W = 0.983; p-value = 0.071) (Fig. 2A). GRT thresholds ranged from 0.2 mm to 1.25 mm, thus covering the whole range of groove widths (Fig. 2B). The median value was 0.68 and first and third quartile limits were 0.51 and 0.89 respectively. No significant differences were found by gender for GRT threshold distributions (D = 0.113; p-value = 0.750) or GRT mean values (F = 0.455; p-value = 0.501). The mean number of trials performed by each participant to complete the grating orientation test was 27.8. The minimum number of trials was 10, while the maximum was 40. The test required approximately 10-15 min to be completed. A significant negative correlation was found between GRT and the number of trials performed by each participant to complete the test (r = -0.636; p-value < 0.0001).

The detection threshold for oral point pressure sensitivity was 0.008 g for all participants therefore this measure was not included in any further analysis as it was unable to discriminate between participants. Discrimination thresholds did not follow a normal distribution (W = 0.584; p-value < 0.0001) (Fig. 3A). Threshold values ranged from 0.008 g and 0.160 g (mean 0.037 g), with 72% of participants correctly identifying the lowest target stimulus (Fig. 3B).

3.2 Oral acuity markers

The distribution of PROP bitterness ratings does not follow a normal distribution (W= 0.969; p-value= 0.002). The median value was 35.75 (“strong” on gLMS), while the first and third quartile limits were 20.25 and 50.96 respectively, very close to the arbitrary cut-offs used for subject classification in Non-Taster (NT) (< 17, weak on the gLMS) and Super Taster (ST) (> 53, very strong on the gLMS) groups (Hayes et al. 2010; Fischer et al. 2013). Thus, according to the limits of percentile distribution, participants were classified in Non-Tasters (NT: PROP bitterness < 20.25, n = 36), Medium Tasters (MT: PROP bitterness ≥ 20.25 and ≤ 50.96; n=78) and Super Tasters (ST: PROP bitterness < 50.96, n = 36) groups. Gender significantly affected PROP mean bitterness ratings (F = 14.377; p-value < 0.001), with women that were found to have a significantly higher PROP responsiveness (43.26 – between “strong” and “very strong” on the gLMS) in comparison to men (29.15 – between “moderate” and “strong” on the gLMS).

The distribution of total FPD values tended to follow a normal distribution (W= 0.981; p-value= 0.045). The median value was 138.85 FP/cm², and the first and third quartile limits were 84.9 and 189.7 FP/cm² respectively. Gender significantly affected both total FPD value and FPD in the diameter classes ranging from DC2 to DC10 with women showing significantly higher density values than men (Tab. 1).

3.3 Relationship between oral tactile sensitivity measures, PROP bitterness ratings and fungiform papillae density

Linear correlation between oral tactile acuity measures, PROP bitterness ratings and fungiform papillae density were tested (Tab. 2). No significant linear correlations were found amongst PROP, total FPD, FPD in each of the 11 diameter classes and GRT measures in the whole sample (r ≤ 0.138; p-value ≥ 0.099), in women (r ≤ - 0.217; p-value ≥ 0.071) and in men (r ≤ 0.160; p-value > 0.173) with the only exception of a positive, but weak, linear correlation between PROP bitterness ratings and FPD DC10 in men (r= 0.262; p-value= 0.024). FPD TOT was significantly associated with FPD classes both in the whole sample and by gender (r ≤ 0.928; p ≤ 0.05). No linear correlation was found between GRT and point-pressure discrimination threshold (r=0.197; p-value= 0.257) in the smaller subsample of women.

3.4 Participant's clustering

To further explore possible associations among measures, participants were clustered according to GRT thresholds, PROP bitterness ratings and total FPD. Three clusters were identified: Cl1 (n= 67), Cl2 (n= 42) and Cl3 (n= 35). The cophenetic correlation of clustering was 0.646; the within-class variance was 28.92% and the between-class variance was 71.08%, indicating clear clusters. Clusters did not significantly differ for GRT tresholds (F = 0.596; p-value= 0.552) (Fig. 4A). Clusters significantly differed for PROP bitterness responsiveness (F = 4,716; p-value = 0.010) with Cl2 showing significantly higher mean value than Cl1 and Cl3 (Fig. 4.B). A significant difference was found among clusters for FPD (F = 285,19; p-value< 0.0001) with Cl1 showing the highest FPD value and Cl3 the lowest (Fig. 4C). Significant difference was found in distribution by gender among clusters with Cl3 showing a significant lower number of women compared to men (chi-square = 3,864; chi-square critical value = 5,991, p = 0.031) while no significant differences were observed in Cl1 and Cl2.

4. Discussion

One of the major aims of this study was to compare the variability across participants for oral tactile sensitivy by means of both the VFH point-pressure and grating orientation recognition (GRT) thresholds.

Results showed that all the participants were able to detect the lowest VFH force (0.008 g) and that 72% of them were able to discriminate at the lowest VFH monofilament level (0.02/0.008 g filament pair). Results from VFH point-pressure test confirmed previous studies showing that the majority of subjects were able to detect the lowest VFH force and indicated the strong "floor effect" of this method (Santagiuliana et al., 2019; Breen et al., 2019; Appiani, Rabitti, Methven, Cattaneo, & Laureati, 2020). The lowest available force provided by VFH monofilaments appeared higher compared to the sensitivity of the tongue mechanoreceptors. Thus, VFH detection and discrimination thresholds appear to be unsuitable in revealing individual variation in responsiveness to point-pressure on the tongue in a young adult population.

In comparison, results of the GRT showed greater variation compared to the point-pressure thresholds, with GRT values covering the range of all possible thresholds (from 0.2 to 1.25 mm), thus indicating an ability to measure individual variation in oral spatial resolution. Furthermore, some participants were able to correctly identify the orientation of the 0.2 mm grating, indicating that these participants might have an even greater spatial resolution acuity. Similar to our results, Appiani and colleagues showed higher individual variation in grating orientation than in point-pressure tests using the R-Index to express oral tactile acuity both in adults and children (Appiani et al., 2020). GRT threshold was not significantly affected by gender. To the best of our knowledge, little is still known about the effect of gender on oral tactile acuity measures. Our results agree with a previous study in which no significant differences between men and women have been reported in a letter identification task (Bangcuyo et al., 2017).

VFH point-pressure discrimination and GRT thresholds were not significantly correlated, in agreement with recent results (Appiani et al., 2020), supporting the hypothesis that these measures represent different tactile functions underlined by different neural mechanisms (Johnson & Phillips, 1981). It might be hypothesised that the application of localised pressures on the tongue by VFH only stimulates superficial mechanoreceptors and does not consider the response to the deeper mechanical pressures that also contribute to the oral tactile sensitivity (Engelen & Van Der Bilt,

2008). GRT is considered a more complex process involving both the stimulation of the deeper tongue mechanoreceptors and the cognitive processes required to identify grid orientation (Appiani et al., 2020). Thus, tactile sensitivity measured with gratings might be a function of both peripheral and central activities (Miles, Simaeys, Whitecotton, & Simons, 2018).

The number of trials and the time required to complete the test are important variables to take into account when assessing oral tactile sensitivity through these different approaches. In this study a significant negative correlation was found between GRT threshold and the number of trials required to complete the test, with high thresholds were associated with a lower number of trials. These results are related to the staircase method: all subjects started the test with the highest grating (1.25 mm), thus, subjects characterised by lower oral tactile sensitivity stopped the test at higher gratings levels in fewer numbers of trials; on the contrary, subjects characterised by a higher oral tactile sensitivity were tested for a higher number of gratings until their GRT threshold, meaning a higher number of trials were performed. It could be argued that more accurate data is collected with longer staircases which raises the question as to whether a fixed number of reversals should be conducted (see García-Pérez, 1998).

Available measures to assess oral tactile sensitivity have been widely used to explore the organisation of somatosensory system structures of the skin (mechanoreceptors and nervous fibers) and their activity in response to different kinds of tactile stimuli (Weinstein, 1968; Essick et al., 1999). However, the skin was later found to be less sensitive than the tongue, due to differences in cutaneous tissues characteristics (the oral cavity is characterised by glabrous cutaneous tissue). Furthermore, the type and functions of tongue mechanoreceptors and nerve fibers in oral tactile perception only partially reflect those of cutaneous system (Van Boven & Johnson, 1994; Miles et al., 2018). Further studies are still necessary to find more reliable measures to explore the individual variability in the oral tactile system sensitivity, including the development of sensitive devices able to test the limits of oral mechanoreceptor sensitivity. Our results confirm the hypothesis that the grating orientation test might be an effective and sensitive tool to map individual differences in oral spatial resolution acuity (Van Boven & Johnson, 1994; Appiani et al., 2020). Recommendations for future work include the development of a grating below 0.2 mm to cover the variation in the most sensitive individuals.

Overall, the results from the present study are in line with already existing data on individual variation in PROP responsiveness and FPD and their association with gender, confirming the reliability of both oral acuity phenotype characterization. The distribution of PROP bitterness ratings found in this study was in agreement with previous results on larger population samples (Monteleone et al., 2017; Fischer et al., 2013), confirming the reliability of both oral acuity phenotype characterization measures. Descriptive values were close to the arbitrary cut-offs used to classify subjects in Non-Taster and Super-Taster groups (Hayes, Sullivan, & Duffy, 2010; Fischer et al., 2013). Gender was confirmed to be a predictor of PROP bitterness responsiveness, with women showing significantly higher sensitivity than men (Linda M. Bartoshuk, Duffy, & Miller, 1994; Monteleone et al., 2017; Tepper, Banni, Melis, Crnjar, & Barbarossa, 2014). FPD mean values were similar to those reported in previous studies on young adult population (Piochi et al., 2019) and women were confirmed to have higher FPD than men (Fischer et al., 2013; Dinnella et al., 2018).

To the best of author's knowledge, the present study represents the first systematic investigation on the relationships between spatial resolution acuity measured by the grating orientation method and phenotypic markers of oral acuity.

In this study the individual variation in GRT threshold was not significantly associated with responsiveness to PROP bitterness neither to FPD total and by diameter classes, in the whole sample and by gender. Previous studies have shown an association of both PROP responsiveness and FPD with oral stereognosis, with significant negative association between these measures in smaller samples of young women (letter identification task; n=52; aged 18-35) (Essick et al., 2003) and adults (letter identification task; n=48; aged 18-59) (Bangcuyo & Simons, 2017). The discrepancies between results could be due to the different methodologies used here and in previous works, both in phenotypes of oral acuity assessment (e.g., staircase method, R-Index) and in oral tactile acuity measures (e.g., letter identification task, point-pressure sensitivity), as well as due to a lower participant number.

Participants were clustered according to PROP responsiveness, total FPD and GRT values. Clusters were significantly different for PROP responsiveness and FPD but not for GRT. These results confirm the wide individual variation of PROP responsiveness and FPD in a population characterised by little variability in age (18-30) and balanced for gender. It could be further observed that young adults show in general a high sensitivity to PROP bitterness responsiveness with mean values of clusters ranging between "moderate-strong" and "strong-very strong" on the gLMS, according to previous study on large scale (Monteleone et al., 2017), thus supporting that this sensitivity might reflect a high sensitivity to other oral sensations due to high functionality of peripheral receptor systems and anatomical structures involved in taste and tactile perception. On the contrary, GRT did not discriminate participants among the three clusters, thus indicating a substantial independence among the phenotypic markers of oral acuity and the oral tactile sensitivity measures adopted in the present study.

5. Conclusion

Oral tactile responsiveness measures capturing individual variation in tongue responses to mechanical stimulation would represents an easily functional tool to explore individual sensitivity and the response to different properties of food texture. This tool could be helpful to better understand individual differences in texture perception related food preferences.

Point-pressure sensitivity thresholds did not appear useful to map individual variability in responsiveness to oral tactile stimulation. On the other hand, grating orientation thresholds discriminated amongst participants and appeared suitable in exploring the individual variation in oral responsiveness to mechanical stimulation and the cognitive processes behind it. Point-pressure sensitivity and grating orientation threshold did not correlate, supporting the hypothesis that these measures represent different tactile functions underlined by different receptor/neural mechanisms. This encourages future studies aimed at a deeper investigation of individual variability in sensitivity to different types of oral tactile stimuli. Finally, a substantial independence was observed between the phenotype markers of oral responsiveness and grating orientation test thresholds, but it is suggested that a larger scale study is required to confirm this. Furthermore, future studies should be aimed at the method optimization, for example exploring the use of narrower grids and the adoption of longer staircases, to capture the differences in tactile sensitivity among the most sensitive individuals.

Author Contributions: Conceptualization, C.D.; methodology, C.D., El.M.; formal analysis, C.D., El.M., L.P, Er.M, S.S.; investigation, C.D., El.M.; data curation, E.M.; writing— original draft preparation, El.M., C.D. RF, I.R.; writing—review

and editing, S.S., L.P., E.M.; supervision, C.D. All authors have read and agreed to the published version of the manuscript.

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Figure captions:

1. Fig. 1. Grating's 3D-printing models
2. Fig. 2 Distribution (A) and the range of variation (B) of grating orientation recognition threshold values (GRT) (n=144)
3. Fig. 3 Distribution (A) and the range of variation (B) of point-pressure discrimination threshold values (VFH) (n=37)
4. Fig4. 1-way ANOVA. Effect of cluster on PROP bitterness intensity. total fungiform papillae (FPD TOT) and grating orientation recognition threshold (GRT)

Research data are not shared.

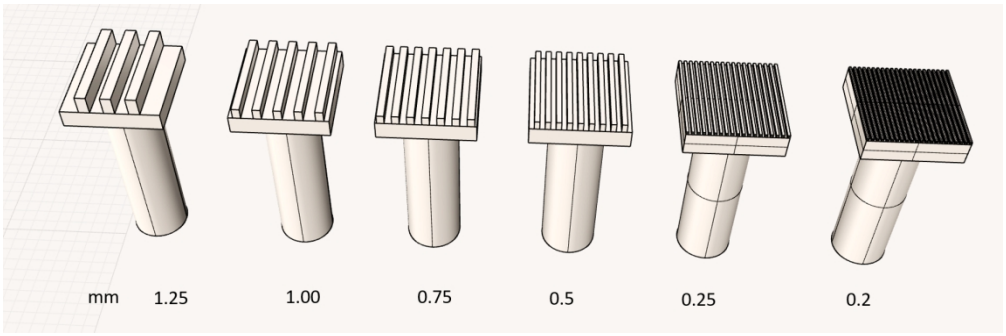

Fig. 1. Grating's 3D-printing models

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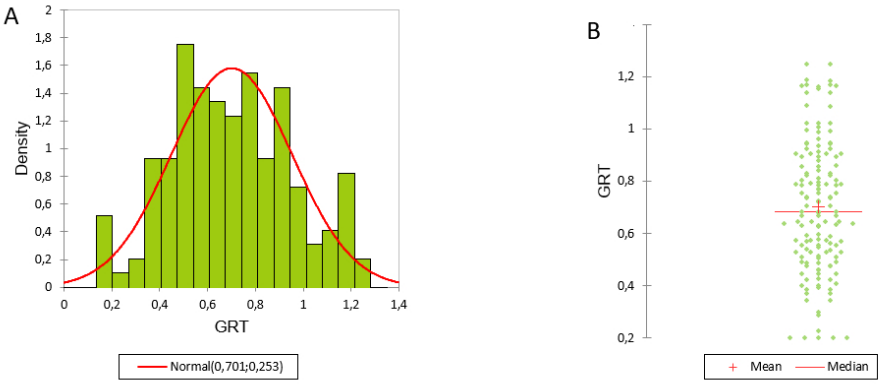

Fig. 2 Distribution (A) and the range of variation (B) of grating orientation recognition threshold values (GRT) (n=144)

680x300mm (38 x 38 DPI)

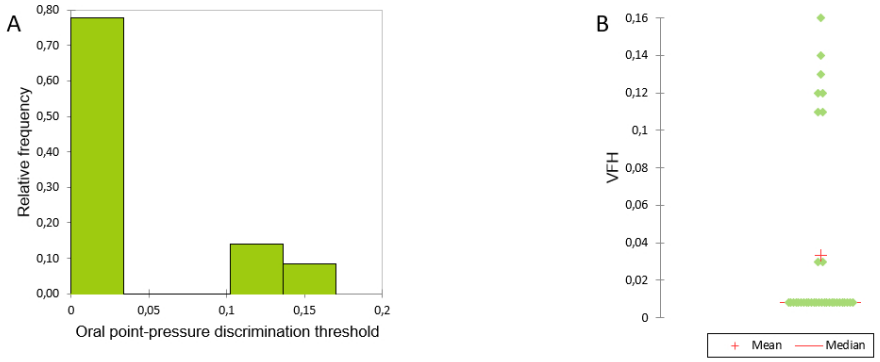

Fig. 3 Distribution (A) and the range of variation (B) of point-pressure discrimination threshold values (VFH) (n=37)

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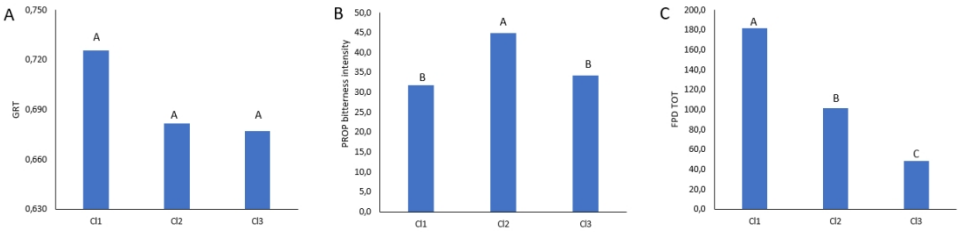

Fig4. 1-way ANOVA. Effect of cluster on PROP bitterness intensity. total fungiform papillae (FPD TOT) and grating orientation recognition threshold (GRT)

958x274mm (38 x 38 DPI)

Tab. 1. 1-way ANOVA. Effect of gender on FPD classes and total FPD mean values. Different letters indicate significant difference ($p \leq 0.041$)

FPD	DC1	DC2	DC3	DC4	DC5	DC6	DC7	DC8	DC9	DC10	DC11	TOT
Women	76.196	24.966 a	17.761 a	14.649 a	9.117 a	6.084 a	2.729 a	2.070 a	1.500 a	0.520 a	0.223	155.787 a
Men	69.894	19.968 b	14.262 b	10.642 b	6.247 b	3.670 b	1.610 b	1.056 b	0.709 b	0.214 b	0.093	128.361 b
F-value	1.249	6.011	5.085	7.827	8.168	9.252	6.083	6.145	6.639	4.261	3.391	5.665
P-value	0.266	**0.015**	**0.026**	**0.006**	**0.005**	**0.003**	**0.015**	**0.014**	**0.011**	**0.041**	0.068	**0.019**

Tab. 2. Correlation among grating orientation recognition threshold (GRT). PROP bitterness mean ratings (PROP). fungiform papillae density total (FPD TOT) and diameter classes (FPD DC1-11). Values in bold represent significant correlation (α= 0.05). P critical value significant for p≤ 0.050.

Variables	GRT	PROP	FPD DC1	FPD DC2	FPD DC3	FPD DC4	FPD DC5	FPD DC6	FPD DC7	FPD DC8	FPD DC9	FPD DC10	FPD DC11	FPD TOT
Whole sample (n=144)														
GRT	-	-0.001	0.036	0.111	0.000	0.098	0.046	0.054	0.065	-0.047	0.052	-0.005	0.074	0.060
PROP	-0.001	-	-0.115	-0.083	0.001	0.061	0.096	0.058	0.052	0.138	0.045	0.106	-0.103	-0.041
FPD TOT	0.060	-0.041	**0.904**	**0.914**	**0.886**	**0.869**	**0.777**	**0.655**	**0.624**	**0.482**	**0.455**	**0.333**	**0.424**	-
Women (n=70)														
GRT	-	0.054	0.133	0.124	-0.017	0.029	-0.052	-0.046	0.049	-0.065	-0.015	-0.070	0.074	0.077
PROP	0.054	-	-0.217	-0.205	-0.059	-0.023	0.061	0.026	0.075	0.115	-0.025	-0.016	-0.157	-0.138
FPD TOT	0.077	-0.138	**0.887**	**0.903**	**0.898**	**0.891**	**0.750**	**0.615**	**0.583**	**0.438**	**0.456**	**0.328**	**0.503**	-
Men (n=74)														
GRT	-	-0.105	-0.062	0.077	-0.004	0.153	0.132	0.160	0.061	-0.055	0.138	0.107	0.057	0.023
PROP	-0.105	-	-0.082	-0.085	-0.061	0.009	-0.018	-0.091	-0.120	0.032	-0.011	**0.262**	-0.160	-0.073
FPD TOT	0.023	-0.073	**0.928**	**0.923**	**0.866**	**0.834**	**0.790**	**0.685**	**0.639**	**0.495**	**0.429**	**0.338**	**0.289**	-