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Texture and trigeminal sensations: new approaches to measure the human sensitivity

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INDEX

Thesis overview	1
Chapter 1: Introduction.....	3
Chapter 2: How sensory sensitivity to graininess could be measured?	24
Chapter 3: Food hardness sensitivity: young and male are more sensitive than old and female subjects.....	45
Chapter 4: Oral sensitivity to viscosity and food neophobia drive food preferences and choice.....	66
Chapter 5: Is it still still water? Detection thresholds of sparkling sensations are not influenced by consumption behaviour and preferences for carbonated beverages	89
Chapter 6: Summary, conclusions and future perspectives	114

Thesis overview

Food preference and choice are affected by many interacting factors in humans (Monteleone et al., 2017). These variables are related to the food (taste, odour, texture etc.), to the consumer (biological, physiological, psychological and attitudinal factors) and to the external context (Koster, 2009).

Among the consumer variables, sensory sensitivity is intended as personal characteristic related to individual differences in detection and reaction to sensory information (Dunn, 1999). It plays a pivotal role in food preference and choice. Individual sensitivity to taste, and also to other oral sensations, shows substantial variability between individuals, and many studies showed how these differences significantly change food preference and consumption (Tuorila, 2007; Caton et al., 2014; Törnwall et al. 2014; Pirastu et al., 2012, 2016). Indeed, several methods have been validated to measure the taste sensitivity, such as PROP test (Bartoshuk, Duffy, & Miller, 1994; Zhao, Kirkmeyer, & Tepper, 2003), thresholds tests (Martinez-Cordero, Malacara-Hernandez, & Martinez-Cordero, 2015), and perceived intensity measurements (Jayasinghe et al., 2017). Across the years, these methods have been used to study the relationships between taste sensitivity and food preference and choice (Kaminski, Henderson, and Drewnowski, 2000; Coulthard and Blissett 2009; Tepper et al., 2009; Törnwall et al., 2013; Masi, Dinnella, Monteleone, and Prescott, 2015; Monteleone et al., 2017).

The same thing applies to odour sensitivity and its impact on food preferences. Examples of validated methods to measure the odour sensitivity presenting high reliability are the "Sniffin' Sticks" test (Hummel, Sekinger, Wolf, Pauli, and Kobal, 1997), Smell Threshold TestTM (Doty 2000), the European Test of Olfactory Capabilities (ETOC) (Thomas-Danguin et al., 2003), the Italian Olfactory Identification Test (IOIT) (Maremmani et al., 2012). These methods have been largely used to investigate the role of odour sensitivity on food preferences (Demattè et al., 2013; Guido et al., 2016; IJpma et al., 2016; Schloss, Goldberger, Palmer, & Levitan, 2015).

However, besides tastes and odour, texture and trigeminal sensitivities, and how they could affect food rejection or preferences and choice, is poorly investigated. The reason why little is known about texture and trigeminal sensations is a missing standardized procedure to measure the individual sensitivities, which uses real food products or food ingredients. Indeed, the methods that have been proposed across the years, to study the texture sensitivity, do not involve food products, as it will be explained in the following paragraphs.

So, with these premises, the aim of this PhD thesis was to develop a new approach to measure the individual sensory sensitivities to specific key attributes, varied in different levels of intensity, by conducting four cases study. Simultaneously, the effect of individual sensitivities on food preference and

choice was also investigated.

The first case study aimed to develop the method to measure the texture sensitivity, using the graininess as key-texture attribute. To this purpose, cocoa-based creams with different levels of graininess were instrumentally characterized and evaluated by the consumers in terms of perceived graininess and liking. Data showed that using the instrumental characterization to predict the individual sensitivity allows clustering people according to their ability to discriminate different levels of graininess.

The second case study aimed to validate the approach used in the first one, using a larger sample of consumers and measuring the sensitivity to the hardness, as key-texture attribute. To this purpose, different jellies were developed by changing the concentration of the gelling agent, in order to obtain different levels of hardness. Results confirmed that the developed statistical methodology can be used to measure the sensitivity to any texture attribute that can be first analysed by means of instruments. Also, individual characteristics, such as gender and age, showed to play an important role in hardness perception and food liking.

The third case study aimed to use the approach developed in the first case study and validated in the second one, to explore the role of the sensitivity to viscosity on food choice. According to the aim, several pastry choco-creams were made by using different solid/liquid ratios, in order to obtain different viscosities. After clustering people in three groups of sensitivity, a food choice index was calculated from the Food Choice questionnaire developed on the basis of texture dichotomies. Data showed that, first, once again, the proposed method is reproducible and can be used to measure the sensitivity to viscosity as well. Secondly, different sensitivities led to different preferences and different texture choices.

Finally, the last case study aimed to explore the sparkling sensation, used as key-trigeminal attribute, in order to determine how detection thresholds for and perception of sparkling sensations in carbonated mineral water are affected by familiarity with carbonated beverages and individual consumer characteristics. Data collected from consumers showed that detection thresholds of sparkling sensations are independent of consumption behaviour and preferences for carbonated beverages. On the other hand, liking and perception of sparkling intensity of carbonated mineral water were significantly affected by the consumption frequency of sparkling water.

All in all data obtained in this thesis demonstrated that differences in texture and trigeminal perception exist, the proposed methods are valid to measure them and the statistical approach is useful to cluster the people with different sensitivities. Also, individual characteristics, such as gender, age and consumption behaviour, play an important role in individual sensitivities and food liking.

Chapter 1

Introduction

1. Introduction

1.1. Texture perception

Food texture has historically been considered those properties that are not covered in the classical definitions for taste and flavor compounds. This includes the mechanical properties evaluated from the force–deformation relationships, tactile sensations such as adhesion, in addition to visual and auditory stimuli (Lawless & Heymann, 1998). The pivotal contribute of Alina Szczesniak allowed to define the texture as a multi-parameter attribute, related to the structure of the foods and detected by several senses. Furthermore, texture is the most complex sensory property because it involves the senses of vision, hearing, touch and kinaesthetic (Szczesniak, 2002).

The complexity of this perception is because, in some cases, only one of those senses is needed to perceive the related texture, but in other cases, the texture is perceived by a combination of two or more senses. For example, the roughness of a fruit, such as orange, can be perceived by both visual and tactile senses; the crispness of a potato chip is both a tactile and auditory textural perception (Vickers, 1987).

The complexity related to the texture is even more emphasized if one considers that texture perception is a dynamic process in which the physicochemical properties of the food are continuously altered by chewing, salivation, and, potentially, body temperature. The melting behavior of some foods in the mouth is an easy example to explain the dynamicity of the texture. Hyde and Witherly (1993) proposed an “ice cream effect”, to describe the phase change of the ice cream in the mouth, due to the manipulation and the increased temperature in the oral cavity.

Even though, as stated above, texture can be perceived by several senses, the oral–tactile texture is probably the main object of the studies conducted on texture perception. According to what reported by van Vliet et al. (2009) the oral–tactile texture involves ingestion by the lips, biting by the front teeth, chewing of hard foods by the molars, wetting with saliva and enzymatic breakdown, deformation of semi-solid foods between the tongue and hard palate, manipulation of the food into a bolus by the tongue and swallowing. The oral behavior, especially regarding solid foods, can be explained by the eating rates (g/s), the number of chewing cycles and the time of consumption (s). Across the years, the oral behavior and its effect on the texture perception have been largely described (Lucas et al., 2002; Bourne, 2004; van der Bilt et al., 2006; Xu et al., 2008; Chen, 2009; Lenfant et al., 2009). The food oral processing not only is dependent on the rheological and mechanical properties of foods (Hiemae, 2004; Chen & Stokes, 2012), but it can also be different among the individuals, and consumer characteristics such as age, gender, ethnicity and oral health strongly affect and contribute to oral behavior (Ketel et al., 2019).

In particular, with age, oral sensitivity decreases (Mioche et al., 2005) as well as other physiological measures like fungiform papillae density (Calhoun et al., 1992; Bangcuyo & Simons, 2017), and more in general, bite force, oral capacity and jaw muscle activity (Percival, Challacombe, & Marsh, 1994; Yeh, Johnson, & Dodds, 1998; Youmans, Youmans, & Stierwalt, 2009; Alsanei and Chen, 2014; Laguna, Sarkar, Artigas, & Chen, 2015). Moreover, dental health is determinant on the oral sensitivity when natural teeth are replaced by prosthetic (Kremer et al., 2007), because the nerves involved in carrying the feedback to the brain disappear (Trulsson, 2005; Trulsson & Johansson, 2002).

Also, considering the eating rates (g/s), the number of the chewing cycles and the time of consumption (s) as the variables that can explain the oral sensitivity, males seem to be more sensitive than females. Indeed, males show shorter chewing cycle durations (Nagasawa et al., 1997; Youssef, Throckmorton, Ellis, & Sinn, 1997), shorter meal durations, lower number of chews, and higher eating rates (g/s) (Hill & McCutcheon, 1984; Park & Shin, 2015) than females.

What is more, differences in eating processing lead to different saliva production, which consequently, is differently incorporated into the food bolus (Yven et al., 2012), obtaining changes of the texture of the food (Mioche et al. 2003; Pereira et al. 2006; Van Der Bilt, Engelen, Abbink, and Pereira, 2007). As a further consequence, the texture is differently perceived according to different characteristics of the food bolus (de Wijk, Terpstra, Janssen, & Prinz, 2006; Young, Cheong, Hedderley, Morgenstern, & James, 2013). Looking further into, texture perception is also affected by saliva composition (Engelen et al., 2007; Salles et al., 2010).

In addition to these individual variables, food texture can be differently perceived according to the preferred mouth behaviour (Devezeaux de Lavergne et al., 2015; Jeltama, Beckley, and Vahalik, 2014, 2015, 2016). It is true, indeed, that consumers follow individual strategies when masticating (Yven et al., 2012).

Also, supplementary subjective factors, such as memory, expectation and emotional state, and context variables such as the social background and the time of day, are an important determinant in texture perception (Tanaka 1986; Engelen and Van der Bilt, 2007).

Finally, as it can be intuitive, the oral perception of the texture, strongly depends on the kind of product which is handled in the mouth. Ingredients, production techniques and temperature of tasting, affect the texture perception (Engelen and Bilt, 2007).

What stated above suggests that there are several factors, both product and subject-related, that can affect, directly or indirectly, the texture perception. Moreover, many of the factors influence each other, which makes the whole concept even more complex (Fig. 1).

Therefore, in contrast to odor and taste, the absence of specific receptors dedicated to the texture, makes the measurement of the texture sensitivity at very least tricky.

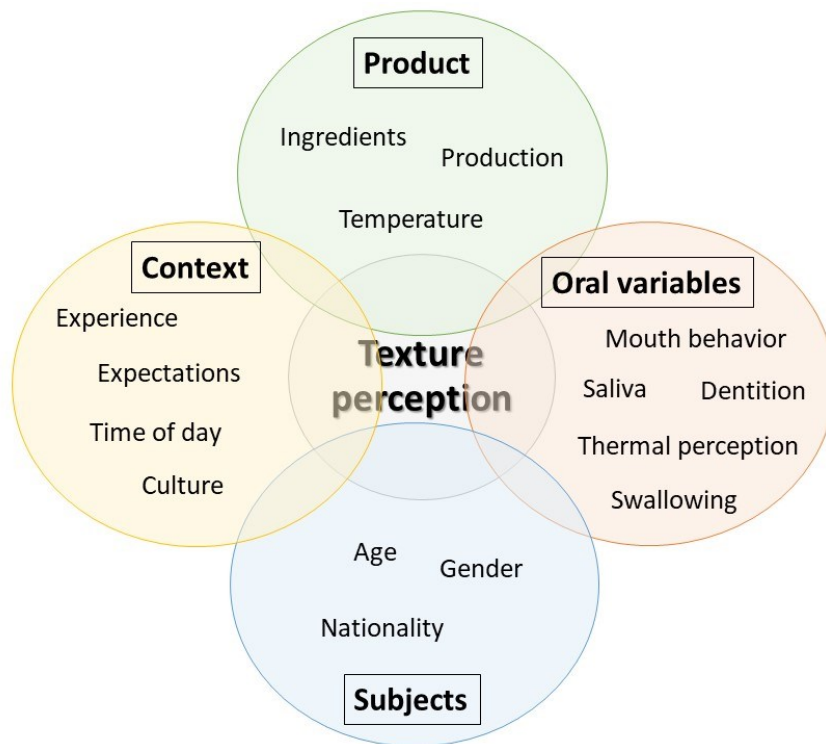


Figure 1. Diagram of factors influencing food texture perception.

1.2 Texture and food preference

The main reason why people choose a food instead of another is the personal liking.

Besides taste, smell and appearance, texture plays a pivotal role on food preference (Moskowitz & Krieger, 1995; Szczesniak, 1991; Scott & Downey, 2007).

On this base, across the years, several pieces of research have been conducted on describing and measuring different texture attributes and studying the relationships with the food liking. The vast majority of these studies statistically correlated the sensory results collected by trained panels, with consumer liking (such as biscuits (Booth, Earl, & Mobini, 2003), candies (Kälviäinen, Schlich, & Tuorila, 2000), cheese (Murray & Delahunty, 2000), liquid dairy (Richardson-Harmon et al., 2000) stirred yogurt (Kora, Latrille, Souchon, & Martin, 2003), caramel (Steiner, Foegeding, & Drake, 2003), almond (Vickers, Peck, Labuza, & Huang, 2014), meat (Peñaranda et al., 2017), prebiotic white chocolate (Ferreira, Azevedo, Luccas, & Andr, 2017), and many others.

By generally speaking, food that is tough, gummy, or slimy is disliked more often than food that is crispy or crunchy (Szczesniak, 2002).

In the study conducted by (Sow & Grongnet, 2010), the relationship between texture attributes and consumer preferences for five chicken meat in Guinea was found. In particular, results showed that consumers' preferences were mostly influenced by the chicken texture attributes such as tender and hard than odour and flavour attributes.

In addition to meat, texture attributes strongly affect also the liking of vegetables. Indeed, Oltman, Yates, & Drake (2016) showed that firmness, slicing, wetness and juiciness were the main drivers for tomatoes acceptance.

A similar study was conducted by Bord, Guerinon, & Lebecque (2017). They evaluated, by descriptive analysis, raw and heated cheeses, in order to describe the sensory properties and to identify the sensory keys drivers of liking and disliking which could explain the consumers' preferences. From the consumer test results, three clusters of consumers were identified with distinctive preference profiles. In particular, the second cluster, 50% of consumers, liked only heated cheeses, for which texture was determined as the main driver of liking.

Just as texture can be a driver for food appreciation, it can also lead the food rejection (Drewnowski, 1997) and be one of the strongest drivers of food aversion (Scott & Downey, 2007).

In the study conducted by Scott & Downey (2007), they identified a list of foods that people are more or less averse to eating, and secondly, they grouped these foods in terms of their characteristics. As result, they obtained three categories of food aversions: factor 1 included food items identified as Vegetables (7 items); factor 2 included six items identified as foods related to Texture/Appearance (including liver, brussels sprouts, fish and oysters, tofu and soggy bread); factor three included four items: three types of meat and mayonnaise; so it was identified as Meat/Fat foods. Furthermore, the results suggested that people tend to be averse to specific texture of certain foods (e.g. oysters and liver).

So, if on the one hand a significant amount of research has been done to describe the texture, to group consumers based on their texture preferences and to identify which textural attributes may drive liking, on the other hand, little is known about what can drive the differences in texture rejection or preference. Brown and Braxton (2000), for example, found that individuals use different mechanisms for the oral breakdown of food, so different groups of individuals would experience the samples differently. What is more, they found a correlation between chewing force and preference. Therefore, they suggested that individual differences in the ability to manipulate and manage the product in the mouth may be a key driver of liking and personal preferences.

Mouth behaviour, intended as how we manipulate food in our mouth, influences food texture preferences. Jeltema and colleagues (2015, 2016), clustered individuals according to their way to manipulate food.

Specifically, they formed four groups: crunchers, chewers, suckers and smooshers.

They demonstrated that the Mouth Behavior groups show food preference differences and that there were food textures that fit “best” with each mouth behaviour. To better explain, taking chocolate as an example, the authors found that smooshers liked (and would have chosen) chocolate that melts fast, instead, crunchers liked (and would have chosen) chocolate that contains nuts, and so on.

This research demonstrated that people differ in the ways they like to manipulate food in their mouths and these preferences drive texture preference and food choice.

In addition to mouth behavior, other individual variables also play a relevant role in texture preferences. It has been shown, indeed, that ageing drives the individual texture preferences. In the study conducted by Kälviäinen, Salovaara, & Tourila (2002), elderly cohort showed a higher degree of liking for muesli products compared to the young group. The year after, Kälviäinen, Roininen, & Tuorila (2003) showed that liking of texturally modified yoghurts was rated as lower by elderly subjects, compared to the young group.

Also, studying the different textural preferences for carrots, adults liked difficult textures such as rough, crispy, crunchy and hard much more than the elderly subjects (Roininen, Fillion, Kilcast, & Lahteenmaki, 2003).

Even more evident, the results of the study conducted by Lukasewycz & Mennella (2012) showed that ageing was strongly related to food texture preferences: mothers preferred harder foods and those containing more particles than did children, and also, children’s preferences became more adult-like with increasing age.

Also, psychological traits have been demonstrated influencing food preferences and choice (Monteleone et al., 2017; Spinelli et al., 2018; Törnwall et al., 2014). Regarding texture, the enjoyment of different texture was related to food neophobia in young children (Coulthard & Sahota, 2016; Coulthard & Thakker, 2015) and to picky eating in adults (Nederkoorn, Houben, & Havermans, 2019).

However, although it is reasonable to believe that texture properties are key determinants of the acceptability of foods and beverages, there is limited understanding of whether preferences for textural characteristics are innate or learned.

Preferences for texture and mouthfeel seem to vary greatly among individuals, but little is known about the sensitivity of texture and mouthfeel perception.

1.3 Texture sensitivity

Despite the evidence of individual differences in oral behaviour described in the previous paragraph, and the belief that texture plays an important role in food acceptance (Baxter & Schroder, 1997; Szczesniak, 1971; Ton Nu, MacLeod, & Barthelemy, 1996), little is known about individual texture sensitivity. The paucity of knowledge is probably due to a lack of a standardized methodology to study texture sensitivity. Across the years, different methods have been developed and used to measure individual texture sensitivity, mainly focused on tactile and oral perception.

Berry and Mahood (1966) introduced the oral stereognosis test, developing different standardised test procedures (e.g. shape, size, number, material). After this pioneering work, oral stereognosis has been largely used by several authors as an approach to measure the ability to recognise and discriminate forms (see review by Jacobs, Serhal, & van Steenberghe (1998) and Lederman and Klatzky 2009). In addition to stereognosis test, Calhoun and colleagues (1992), also tested vibration detection by a 256-Hz tuning fork, applied on the lower lip, hard enough to vibrate but not so hard to be hearable. The task consisted of differentiating between simple pressure and perceived vibration.

Also, Johnson & Phillips (1981) used size and weight discrimination tests to measure tactile spatial resolution. The approach included four tasks: a two-point discrimination test (see also Engelen, 2004), which tested the ability of subjects to discriminate between steel pins with flat ends singly or double embedded; a gap detection test which tested the ability of subjects to discriminate between stimuli with and without central gaps; a grating resolution test where subjects were asked to judge whether two gratings with the same period were presented with the same alignment, or with orthogonal alignments; a letter recognition test where subjects were asked to identify 26 raised capital letters of the English alphabet by touching them without lateral movements.

To overcome the limitations associated with the two-point discrimination task, Essick, Chen, & Kelly (1999) developed an oral form recognition test based on the ability of the subjects to identify different-sizes embossed letters of the alphabet, explored with the tongue tip. This test is a simple up-down tracking procedure and it allows to obtain a threshold height value, assumed as a descriptor of the individual texture sensitivity. Across the years, several researchers have used this approach to investigate further insight into oral tactile acuity and its relationship with taste sensitivity, age and food preferences (Bangcuyo & Simons, 2017; Essick et al., 1999; Essick, Chopra, Guest, & Mcglone, 2003; Lukasewycz & Mennella, 2012; Steele, James, Hori, Polacco, & Yee, 2014).

Moreover, more recently, Linne & Simons (2017) used the forced-choice, up-down staircase method for surface roughness from stainless steel coupons to determine the individual sensitivities to lingual tactile

roughness, intended as texture sensitivity.

Another wide approach used to determine the oral and tactile sensitivity is the measurement of tactile point pressure sensitivity through Semmes–Weinstein monofilaments (SWM), the modern version of von Frey hairs (VFH). SWM are small force-calibrated monofilaments which apply a specific amount of pressure and they provide a fast way of estimating somato-sensations (Etter, Miller, & Ballard, 2017). This tool has been largely used by food researchers which demonstrated that pressure sensitivity varies across people (Aktar, Chen, Ettelaie, & Holmes, 2015a; Costa et al., 2011; Pigg, Baad-Hansen, Svensson, Drangsholt, & List, 2010; Yackinous & Guinard, 2001). In particular, in the recent study, Breen and colleagues (2019) tested whether there was a relationship between oral touch sensitivity and the perception of particle size in chocolate. They measured the individual differences in oral point-pressure sensitivity, intended as detection and discrimination thresholds for oral point pressure determined with Von Frey Hairs. They found a significant relationship between differences in oral somatosensory function at the tongue tip and texture perception of chocolate. These shreds of evidence suggest that, as with smell and taste, phenotypic differences in oral somatosensory can influence the perception of real foods.

The methods that have been proposed across the years, and mentioned above, have some limitations.

First, it is obvious that differences and even some contradictions could be noted when comparing the results of different approaches. This is partially due to the different types of receptors involved in the oral perception, and also to the direct recording from sensory afferents. Stereognostic ability and oral point-pressure testing, indeed, do not reflect the real perception of the food texture.

Furthermore, those methods do not involve real foods, and they do not measure the sensitivity to specific texture attributes.

Food scientists are aware of this gap, but despite this, no real food has been properly used to measure the texture sensitivity.

Across the years, some authors tried to solve this issue, approaching different methods and using different –model/real - foods. Steele and colleagues (2014) indeed, measured the viscosity discrimination acuity using five non-Newtonian xanthan gum-thickened liquids in the nectar- and honey-thick range. In this work, assessors were asked to identify which liquid of the three was perceived to be different in thickness from other two, performing a triangle test. Their approach allowed the calculation of the minimum difference in xanthan gum concentration accurately detected, which may indirectly reflect the sensitivity to the viscosity. However, although they used non-Newtonian liquids, those products can be considered as model food and not as real food. Thus, the individual sensitivity they measured, cannot be extended

to the real perception of the food.

The year after, Aktar and colleagues (2015b), to investigate the correlation between the touch sensitivity and the capability of viscosity discrimination among individuals (using finger and tongue sensory perception), used a series of syrup solutions differing in a wide range of viscosities. The approach consisted of a two-alternative forced-choice (2-AFC) test where assessors were asked to report if they perceived the viscosities as the same or different, by both fingertip and tongue. They concluded that the human capacity for viscosity discrimination varies substantially among individuals. However, although the syrups are common food ingredients, they are solutions with almost Newtonian behavior; the majority of the foods are non-Newtonian products, thus their findings are not really generalizable.

More recently, the study conducted by Breen, Etter, Ziegler, and Hayes (2019) explored the grittiness perception, using chocolate as a model food. However, they described the subjects as high and low sensitive in grittiness perception, on the base of discrimination thresholds for oral point pressure determined with Von Frey Hairs. Consequently, they found a relationship between oral pressure point threshold estimated and the discrimination of particle size in chocolates, measured by Just Noticeable Difference.

Also, Forde and Delahunty (2002) investigated the ability to perceive changings in the intensity of texture between young and older age cohorts, asking to evaluate semi-solid and solid foods. They found that older consumers showed a poorer ability to discriminate between foods evaluated, when measuring attribute intensity using a 100-mm line scale. They concluded that the lack of discrimination was most likely caused by a reduced sensory ability.

Although they found differences in perceived intensity of texture attributes between different age groups, this study does not provide an effective method to cluster people according to their sensitivity.

On this background, it seems evident that a lack of methodology to measure the texture sensitivity exists and that a standardized technique is needed to cover all the gaps highlighted above.

1.4 Trigeminal sensations

Trigeminal sensations are touch-position and pain-temperature sensations perceived by mechanoreceptors and nociceptors which activate the trigeminal nerve system. Trigeminal sensations arise as a result of intense chemical (e.g., chili powder), mechanical (e.g., cutting, crushing), or thermal (heat and cold sensations) stimulation of sensory nerve cells. They include also the non-heat related irritations (e.g. given by horseradish, mustard and wasabi), the tear-inducing stimuli (e.g. from onions), and also irritation from carbon dioxide. Trigeminal sensations, also called chemesthetic sensations in an

analogy to “somesthesia” or the tactile and thermal sensations perceived by the human body surface (Green and Lawless, 1991; Lawless and Lee, 1994), trigger a variety of physiological and behavioural responses and usually result in a subjective experience of pain in humans (Darian-Smith, 1973). This kind of sensations does not fit neatly into the traditional classes of tastes and smells. Old researches found three times as many trigeminal fibers in the fungiform papillae than the facial (taste) nerve fibers innervating taste buds (Farbman and Hellekant, 1978). Several trigeminal sensations are controlled by the trigeminal nerves and the number of the trigeminal tracts is impressive. Also, going deeper, trigeminal fibers ascend around the taste bud forming a chalice-like structure (Whitehead et al., 1985), possibly enhancing their access to the external environment (Fig. 2).

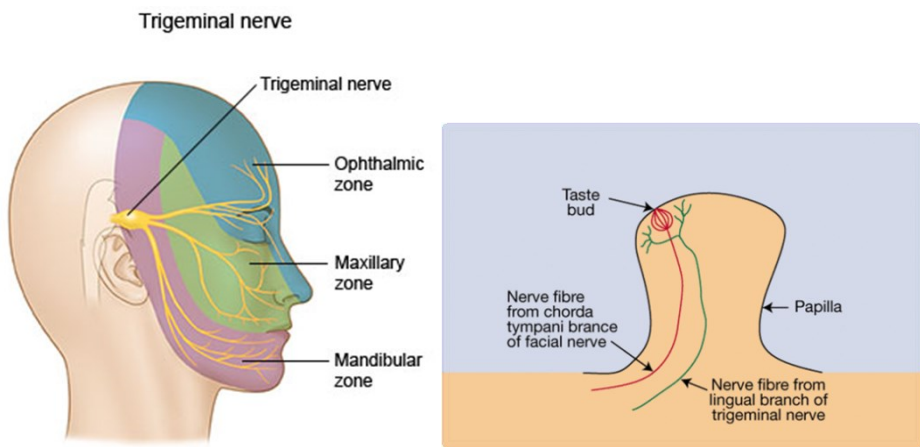


Figure 2. Representation of trigeminal apparatus.

A largely studied group of trigeminal sensations arises from pepper compounds such as capsaicin from chili peppers, piperine from black pepper, and the ginger compounds such as zingerone. Stimulation given by those compounds, at concentrations above threshold may last 10 min or longer (Lawless, 1984) and, for this reason, they are ideal for the application of time-intensity methods (Cliff & Heymann, 1992; Esti, Contini, Moneta, & Sinesio, 2009; Reinbach, Toft, & Møller, 2009). Also, although the burning sensation is painful, spicy foods are worldwide consumed and appreciated by many individuals.

The same goes for the sparkling sensation given by carbon dioxide. CO₂, indeed, is a potent irritant in the nasal cavity, as are many organic compounds (Cain and Murphy, 1980; Cometto-Muñiz and Cain, 1984; Cometto-Muñiz and Hernandez, 1990), but, despite this, carbonated beverages business - soda, beer, sparkling water and wine, etc. – amounts to huge sales around the world.

Menthol is a trigeminal stimulus which leads to cool sensations, interacting with thermal stimulation in a complex way. The sensory properties of menthol are complex, inducing a number of cooling, warming, aromatic, and other sensory effects depending upon the isomer, concentration, and temporal parameters

(Gwartney and Heymann, 1995, 1996) and the conditions of stimulation (Green, 1985, 1986). Despite the cooling sensation can be painful at high concentrations, the menthol has an important commercial significance in confections, oral health care, and tobacco products (Patel et al., 2007).

Therefore, even though the evident painful and aversive sensations given by different trigeminal attributes, they are common to many foods and drinks and appreciated by many consumers. Numerous researches have been conducted to explain the reasons linked to the consumption of foods/drinks that elicit irritation sensations. People may like specific kind of food because of cultural reasons (Rozin & Schiller, 1980; Stevens, 1990), frequency of consumption (Logue and Smith, 1986), individual genetic properties (Duffy, 2007; Duffy & Bartoshuk, 2000) and also oral anatomy (Bartoshuk, 1993; Miller & Reedy, 1990). Also, it has been reported that individual can learn to like aversive sensations, such as the burn of capsaicin, with the exposure to gradually increasing levels (Logue & Smith, 1986; Rozin & Schiller, 1980).

Moreover, it is also well known that sensory sensitivity plays a pivotal role in food preference and choice (Kaminski et al., 2000; Coulthard et al., 2008; Monteleone et al., 2017). In turn, it has been specifically shown that taste sensitivity can be affected by individual physiological factors, such as gender, age, health status and nutritional status and by the interaction between these variables (Mojet et al., 2001).

However, although several studies have been conducted on the individual sensitivity to different trigeminal sensations (Wright et al., 2003; Condelli et al., 2006; Le Calvè et al., 2008; Ludy & Mattes, 2012), and on how they can be correlated with liking and consumption (Rozin & Rozin, 1981; Stevenson & Yeomans, 1993; Stevenson & Prescott, 1994; Byrnes & Hayes, 2015), very little is known about the influence of people characteristics (socio-demographic, psychological and consuming habits) on individual sensitivity to specific trigeminal properties.

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Chapter 2

How sensory sensitivity to graininess could be measured?

Published paper

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Abstract

Considering the lack of the literature and the need for developing a valid method to measure the texture sensitivity, in this study, we investigated the individual sensitivity to discriminate among different levels of graininess. With this purpose, five samples of cocoa-based creams were prepared, by changing the refining time. Samples were first characterized in terms of particles size distribution, by means of laser diffraction. Then, 59 subjects evaluated the cream sample graininess intensity, by using gLM scales, and their liking, by using LAM scales.

The graininess scores of each subject were fitted with a power model, already observed with the instrumental results, estimating both the power law exponent and the R^2 coefficient, and using them as clustering parameters. Subjects were then clustered into three groups: high sensitivity; moderate sensitivity; low sensitivity.

First, as it was hypothesized, results showed a significant difference between the three groups in terms of perceived graininess. Second, even though results showed a significant difference between the three groups in terms of perceived graininess, only little differences were found in terms of liking scores. Indeed, all the samples were equally liked for both the moderate and low sensitivity groups, whereas a significant trend was observed for the highly sensitive subjects who liked more the most refined samples. No significant relationships were found with age, and only a little trend was observed with gender: females seemed to be more sensitive than males.

Keywords: Graininess; sensitivity; cocoa-based creams, refining process.

Practical applications

Texture attributes discrimination ability, as for example sensitivity to graininess, could affect food rejection or preferences and choice, but it is poorly investigated.

This exploratory study, proposes a method to cluster consumers, based on their sensitivity. A statistical methodology has been developed to discriminate among consumer sensitivity levels.

The results provide useful information about graininess sensitivity suggesting that the used methodologies could be applied to other texture properties resulting in a valid tool for the industry in the development and optimization of tailored new products.

2.1. Introduction

Sensory sensitivity is a personal and intrinsic characteristic, related to individual differences in the detection of, and reaction to, sensory information, and it involves physiological markers (Dunn, 1999). This means that information from the taste, touch, vision and smell senses are differently perceived, and consequently evaluated, by different subjects.

Many studies have highlighted that sensory sensitivity plays a pivotal role in food preference and choice. Several authors found that individual sensory differences affect both preference and consumption of food (Cardello, 1996; Tuorila, 2007). For instance, the study conducted by Coulthard & Blisset (2009) showed that children with high sensitivity to taste and smell liked less fruits and vegetables, and they were also more reluctant towards new foods, than less sensitive ones.

Taste sensitivity and food preferences were also related one to each other in the study of Kaminski and colleagues (2000), which showed that the most sensitive people perceived the Brussels sprouts as bitterer and less acceptable than the lowest sensitive ones. However, more in general, the most part of published studies investigated extensively on taste sensitivity compared to the other human sense sensitivity, probably as several validated methods were already available, such as PROP test (Bartoshuk et al. 1994; Zhao et al., 2003), thresholds tests (Martinez-Cordero et al., 2015), and perceived intensity measurements (Jayasinghe et al., 2017). Texture sensitivity and the way it could affect food rejection or preferences and choice are poorly investigated. Texture is a multi-parameter attribute and it involves several senses (Szczesniak, 2002). Mechanoreceptors at oral surfaces are designed for sensing and detecting any physical/mechanical stimulus exerted on the tissue surface and they are directly responsible for food texture sensation (Chen, 2014). Generally, humans are incredibly sensitive to texture. Touch is the primary sense we use to determine it, but kinesthetics (the sense of movement and position), sound and sight are also involved. Actually, the texture attributes are unique characteristics, which contribute to the overall enjoyment of the food, for example, attributes such as “crunchiness”, “crispiness” and “thickness” are terms commonly used which drive the consumers to the acceptance of the food (Forde et al., 2002).

Consequently, a huge amount of papers described texture attributes as key drivers for the liking of several foods, such as strawberry candies (Kalviainen et al., 2000), hazelnut spreads (Di Monaco et al., 2008); chicken meat (Sow et al., 2010), almonds (Vickers 2014), fresh tomatoes (Oltman et al., 2016), white chocolate (Ferreira et al., 2017), cheese (Bord et al., 2017) and many others.

Some texture attributes can positively affect the overall perception, e.g. the crunchiness of chips, others, such as the graininess, negatively do. Chocolate with a particle size above 35µm is usually perceived as gritty or coarse in the mouth, and it is not accepted by consumers. In ice cream, ice

crystals larger than 40 μ m can be perceived, reducing the consumer's acceptability (Servais, Jones, & Roberts, 2002; Birkett, 2009; Petković, Pajin, & Tomić, 2013).

Notwithstanding the importance of texture on food preferences is well documented, there is a limited understanding of individual texture sensitivity, and whether preferences for texture and mouthfeel characteristics are innate or learned.

Preferences for texture and mouthfeel greatly varied among individuals (Jeltema et al., 2014). Jeltema, Beckley, and Vahalik (2016) results have shown that consumers can be segmented by their mouth behaviors and those groups of individuals show differences in food texture preferences. The studies conducted by Jeltema and colleagues (2014, 2016) confirmed what Brown and Braxton (2000) already found: the individuals use different mechanisms for the oral breakdown of food and the differences in the ability to manipulate the product is a key driver of liking and preference. Nevertheless, little is known about the individual texture sensitivity and mouthfeel perception. Food scientists are aware of both the variations in texture perception among different individuals and the gap between the instrumental measured texture and human perceived texture, even though the full understanding of the causes behind these variations still remains a challenge (Chen and Rosenthal, 2015).

The scarcity of data may be due to a partial knowledge on how food structure is converted into texture perception during oral processing and manipulation, and, also, to a lack of a useful methodology to measure the texture sensitivity. The few already available studies focused the attention on tactile sensitivity measured by fingers (Nederkoorn et al., 2015; Aktar et al., 2016) or by the tongue (Lukasewycz and Mennella, 2012; Aktar et al., 2015a), or by the comparison between these two (Aktar et al., 2015b). Furthermore, even fewer studies have been conducted using real food to measure texture sensitivity (Steele et al., 2014). In particular, a very recent study conducted by Breen and colleagues (2019) explored the grittiness perception, using chocolate as a model food. However, they described the subjects as high and low sensitive in grittiness perception, on the base of discrimination thresholds for oral point pressure determined with Von Frey Hairs. Consequently, they found a relationship between oral pressure point threshold estimates and the discrimination of particle size in chocolate, measured by Just Noticeable Difference. Therefore, to the best of author' knowledge, no real food has been properly used to measure the texture sensitivity.

The first aim of this study was to develop a method to measure individual sensitivity to the graininess. Thus, five samples of cocoa-based creams were prepared, by only changing the refining time in order to obtain five different levels of graininess. In foods such as chocolate, ice cream, cream soup, sauces, and spreadable creams, graininess is an undesirable texture characteristic (Imai et al, 1999). Since people can be differently sensitive to the graininess, also their acceptable levels of graininess may be

different. Therefore, food designers and developers should consider that people have different sensitivities and use that information to develop tailored products for specific target groups. Starting from this background, the second aim of this study was to verify how the graininess sensitivity affected the cocoa-based cream acceptability of the consumers. The relationships between individual variables, such as age and gender, and graininess sensitivity were also investigated.

2.2. Materials and Methods

2.2.1. Materials

The following ingredients were used to produce the cocoa-based creams: powder mix (63.66%, including sugar, icing sugar, cocoa powder, milk powder, and others), sunflower oil (27.76%), hazelnuts paste (8.00%) and soy lecithin (0.58%). All the ingredients, except for the sunflower oil, were provided by MAC3 Company (San Clemente, Ravenna, Italy).

The cream was made by using a stirred ball mill (Model Micron 20, Selmi s.r.l., Modena, Italy), equipped with 60kg of stainless-steel balls, 12 mm in diameter (Miele et al., Accepted Manuscript) and a recycling pump, allowing the production of 10kg of cream batches.

The refining operation was carried out at a starting temperature of 25°C (at a speed rate of 72 rpm), and at five different times (20, 30, 35, 45 and 70 minutes) about 1kg of refined cream was withdrawn and stored in glass containers at room temperature before analysis.

2.2.2. Particle size measurement

A laser diffraction particle size analyzer equipped with Hydro 3000 dispersion unit (Mastersizer, Malvern Instruments, Worcestershire, UK) was used. About 0.1 g of cream were analyzed at room temperature and several indices of the particle size distribution based on the volume of particles were estimated, including the D_{10} , D_{50} , and D_{90} , corresponding to the percentages 10%, 50%, and 90% of particles under the reported particle size, respectively (Fidaleo et al., 2017). For each cream, two different replicates were analyzed and for each replicate, 20 measurements were performed.

2.2.3. Sensory evaluation

59 subjects (females=33, age range=18-60 years old, age median=28 years old, young adults \leq 28, 27, old adults $>$ 28, 32) evaluated the five creams and scored their liking by using Labelled Affective Magnitude scale (LAM), a 100mm vertical line, from 0= “greatest imaginable dislike” to 100= “greatest imaginable like” and with anchor words spaced according to the spacing’s provided by Schutz & Cardello (2001). In addition, subjects were asked to score the perceived graininess intensity by using the generalized Labelled Magnitude scale (gLM), a 100mm vertical line, from 0=“no sensation” to 100= “the strongest imaginable sensation of any kind” and intermediate anchors as provided by Bartoshuk et al. (2004). The subjects were instructed to the use of gLM scale following

published procedures (Monteleone et al. 2017). In particular, they had to treat the “strongest imaginable sensation” as the most intense sensation they could image/remember in any sensory modality.

All the involved subjects were Italian people and naïve with sensory evaluation. The tests were run in separate booths in the sensory laboratory of the Department of Agricultural Sciences (University of Naples, Italy). 5 grams of each sample were served on plastic teaspoons in a monadic, randomized and balanced order, identified by three-digit random codes. The subjects were asked to use the following common procedure to evaluate the graininess intensity: “put all the sample in the mouth, manipulate it between the tongue and the palate and evaluate the overall graininess (by estimating both granule dimensions and their abundance). The subjects were provided with a cup of still water to rinse their mouth before testing the next sample.

Data were collected by means of “Fizz Acquisition” software (Biosystèmes, Couternon, France).

2.2.4. Statistical analysis

The particle size indices were analyzed by means of one-way analysis of variance (ANOVA), and multiple comparison test (Duncan’s test) was used to statistically compare the samples ($p \leq 0.05$). Repeated measures ANOVA and multiple comparison test (Duncan’s test) were used to evaluate if differences among the sample means (used as repeated factor) were statistically significant ($p \leq 0.05$), in terms of both perceived graininess and liking. Next, subjects were clustered in three groups: low sensitive (LS), moderate sensitive (MS) and highly sensitive (HS). For each group (LS, MS, HS) a second repeated measures ANOVA was used to verify the effect of the different graininess levels on both sensory liking and perceived graininess intensity.

In order to verify whether those groups differed in the use of the LAM scale, the individual liking score range (Δ liking= scores given to the most refined sample minus that given to the less refined one) was submitted to a one-way ANOVA and multiple comparison test (Duncan’s test).

Finally, chi square test was run to analyze the relationship between graininess sensitivity and age groups (young adults vs old adults). The same approach was used to analyze the relationship with gender as well.

The XLSTAT statistical software package version 2016.02 (Addinsoft) was used for data analysis.

2.3. Results and Discussion

2.3.1. Particle size distribution

As showed in figure 2.1, refined creams presented an almost unimodal particle size distribution (PSD) in accordance with Bolenz, Holm, and Langkrär (2014), with a left small shoulder that increased with the refining time, according to Miele and colleagues (Accepted Manuscript). By increasing the

refining time, the width of the curves progressively decreased as the peak became higher and moved towards smaller size values. The PSDs were well summarized by D_{10} , D_{50} and D_{90} percentiles, reported in table 2.1 as well as Duncan's test results ($p \leq 0.05$).

As expected, the refining time significantly and negatively affected the particle size, result also consistent with Fidaleo and colleagues (2017). Furthermore, the samples were significantly different one from each other, in terms of all the parameters extrapolated from the PSDs: D_{10} , D_{50} , and D_{90} .

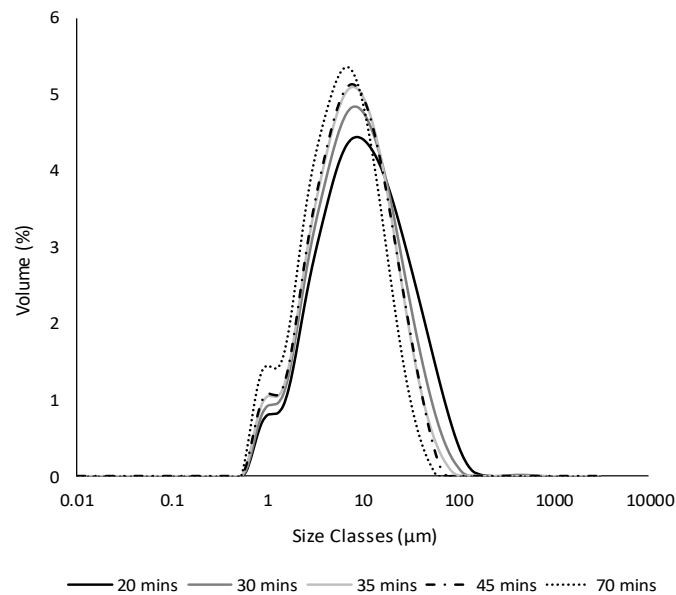


Figure 2.1. Particle size distributions of the creams after 20, 30, 35, 45 and 70 min of refining.

Table 2.1. Particle size distribution parameters of cocoa-based creams at different refining times.

Refining time (min)	D_{10} (μm)	D_{50} (μm)	D_{90} (μm)
20	2.51 ± 0.01^e	10.2 ± 0.10^e	42 ± 1^e
30	2.30 ± 0.02^d	8.8 ± 0.13^d	32 ± 2^d
35	2.17 ± 0.01^c	7.94 ± 0.05^c	26.4 ± 0.4^c
45	2.14 ± 0.01^b	7.78 ± 0.04^b	25.6 ± 0.3^b
70	1.72 ± 0.01^a	6.32 ± 0.03^a	19.5 ± 0.2^a

In each column, the values followed by different letters were significantly different at $p \leq 0.05$.

Fidaleo and colleagues (2017) showed that D_{90} values well explained the perceived graininess by a trained panel. Accordingly, in this study, the D_{90} values were used as representative parameters of the particle size distribution of the analyzed creams. The trend of the D_{90} values versus the refining time was described with a power law equation ($R^2=0.964$), as shown in figure 2.2.

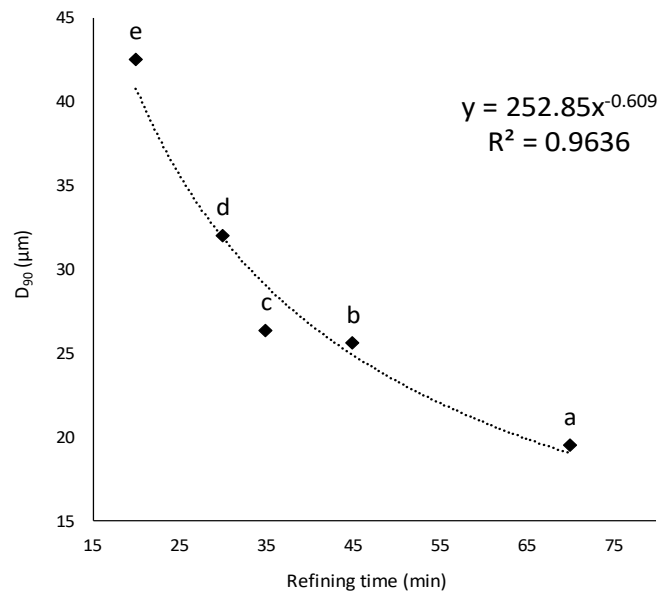


Figure 2.2. Trend of D₉₀ values, in function of the refining time. The points marked with different letters were significantly different ($p \leq 0.05$).

2.3.2. Sensory evaluation

59 subjects scored the graininess intensity and the liking by using a gLM and LAM scale, respectively. By averaging both perceived graininess intensity and the liking scores, small differences came out among the sample, as shown in table 2.2. Regarding the perceived graininess, subjects moved on the scale around the moderate intensity (score ≈ 16) and there were no significant differences between the first two samples, but with the increasing of refining time, the average graininess intensity significantly decreased. Regarding the liking scores, subjects moved on the scale between like slightly (score ≈ 56) and like moderately (score ≈ 68), and even though a little upward trend was observed with the increasing of the refining time, only the first and the second samples were significant different from the last one. The latter result is partially in contrast with the literature. Indeed, according to Birkett (2009), for products like chocolate creams, particle size should be in a range between 15 and 25 μm to ensure the right palatability. Products presenting particle size higher than 25 μm are perceived as grainy and rough, resulting in lower acceptability. We assumed that this was due to the different graininess sensitivity of the subjects.

Table 2.2 Perceived graininess and liking means scored by 59 consumers.

Refining time (min)	Graininess (gLMs)	Liking (LAMs)
20	21 \pm 14 ^c	62 \pm 13 ^a
30	23 \pm 18 ^c	62 \pm 19 ^a
35	17 \pm 15 ^{bc}	65 \pm 15 ^{ab}

45	15±13 ^{ab}	67±13 ^{ab}
70	12±12 ^a	69±10 ^b

In each column, the values followed by different letters were significantly different (Duncan test, $p \leq 0.05$).

To cluster the subjects according to their graininess sensitivity we assumed that in principle sensory scores should follow the same trend exhibited by D_{90} with varying the refining time, i.e. a decreasing power law relationship. According to this hypothesis, the graininess scores of each subject were fitted with a power model, estimating both the power law exponent (which represented the rate of decreasing of the power law equation) and the R^2 coefficient, and using them as clustering parameters. This means that subjects whose scores correlated to a power law equation with high R^2 coefficients had a discrimination capability of graininess similar to the instrumental test. Moreover, among these subjects, those who also had a lowest power law exponent were the most sensitive ones. Accordingly, the low sensitive subjects were those to whom corresponded low R^2 and exponent of the power law correlation equation close to zero.

Next, a descriptive analysis was performed on the exponents and the R^2 coefficients obtained for each subject (Table 2.3). The subjects were then clustered based on the quartile's distribution of both the exponents and the R^2 values into three groups: high sensitivity ($n=17$); moderate sensitivity ($n=30$); low sensitivity ($n=12$).

Table 2.3. Quartiles distribution of power function exponents and the R^2 .

Quartiles	Power function coefficients	R^2
Minimum	-3.666	0.002
Maximum	1.187	0.916
Mean	-0.781	0.334
1st Quartile	-1.440	0.060
Median	-0.550	0.272
3rd Quartile	0.105	0.529

So, as a result, the subjects of the high sensitivity group had to have an exponent lower than the first quartile (25% of distribution equal to -1.44), and at the same time, a R^2 value higher than the third quartile (75% of distribution equal to 0.53). On the other hand, subjects who showed an exponent higher than the third quartile (75% of distribution equal to 0.11), and at the same time, a R^2 value lower than the first quartile (25% of distribution equal to 0.06), were clustered in the low sensitivity group. Consequently, the middle 50% of distribution of both the exponent and R^2 was classified as moderate sensitivity group. Figure 2.3 shows the perceived graininess vs refining time response given

by one representative subject belonging to each group. The subject which belonged to the high sensitivity group provided scores that well correlated with a rapidly decreasing power law equation (a). The scores given by the subject which belonged to the low sensitivity group did not correlate to a power law equation and were independent on refining time (b). Finally, the one which belonged to the moderate sensitivity group provided scores that lightly correlated to a power law equation which slowly decreased with increasing the refining time (c).

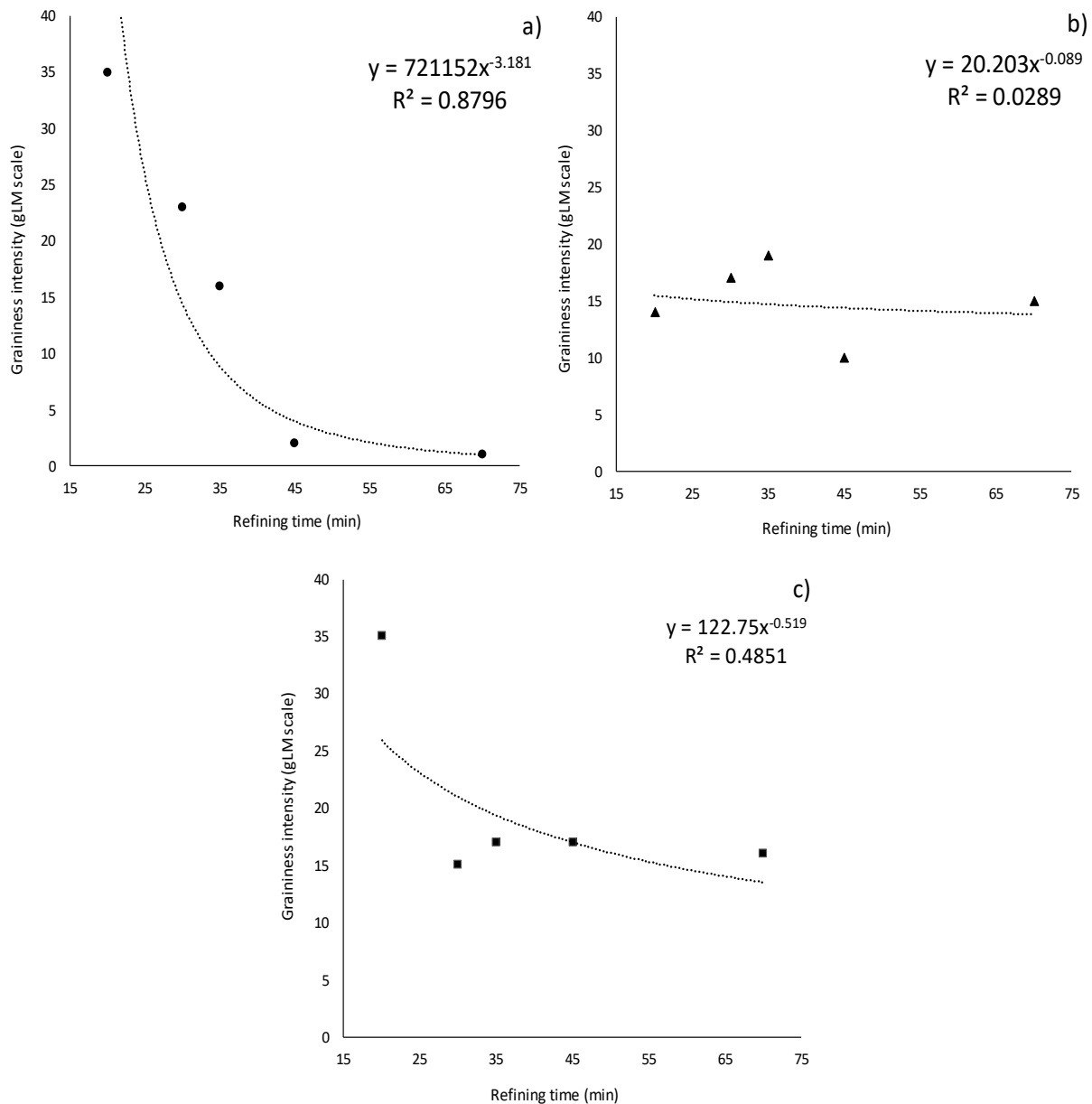


Figure 2.3. Trend of the perceived graininess intensity for one subject belonging each sensitivity group in function of time. High sensitivity group (HS) (a); low sensitivity group (LS) (b); moderate sensitivity group (MS) (c).

The scores given by each sensitivity group were submitted to repeated measures ANOVA and the graininess intensity values were plotted against D_{90} index in figure 2.4. The scores given by the

subjects which belonged to the high sensitivity group were statistically different ($p \ll 0.001$) with varying the D_{90} index and well described how the graininess intensity varied from a score around 25 (between strong and moderate on gLM scale) to a score around 2 (less than weak on gLM scale) with reducing the particle size. By contrast, the low sensitivity subjects were not able to discriminate among the sample and all the score were not statistically different and independent on the particle size ($p=0.78$). Moreover, for these subjects all the samples were moderately grainy perceived. Concerning the moderately sensitive subjects, they were able only to differentiate the most refined sample (scored as moderate) from the less two refined ones (scored between moderate and strong), whereas the other two samples were perceived similar one to each other ($p=0.017$).

The above discussion supports the starting hypothesis that not all the subjects are able to discriminate in the same way the graininess. Actually, some of them well perceive differences in the particles size, scaling the graininess intensity of the sample from less than weak to more than moderate, demonstrating their sensitivity, other subjects score always the samples on the middle region of the scale, due to their low sensitivity. These results suggest that the proposed approach may be a useful procedure to differentiate among the subject behavior.

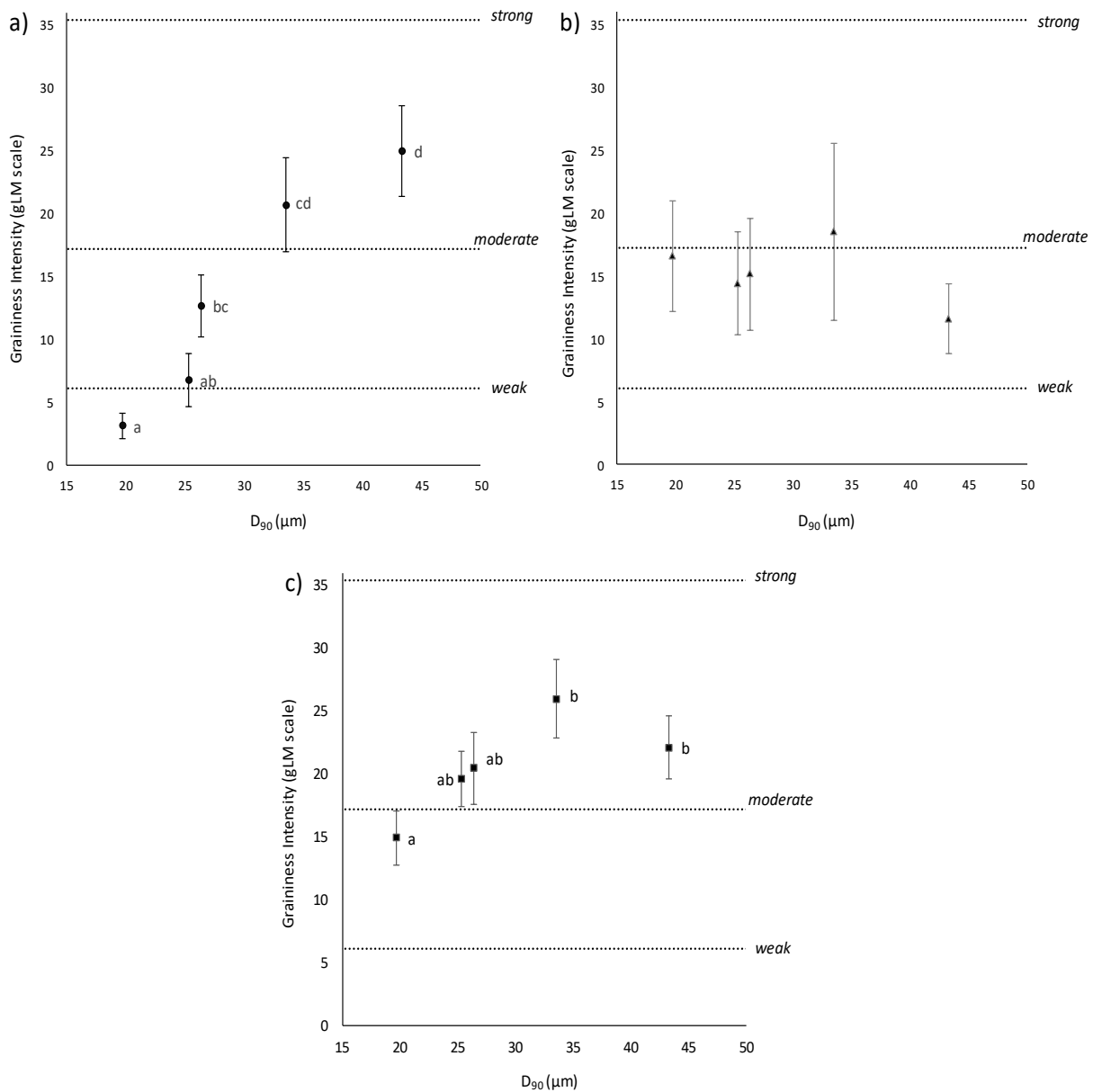


Figure 2.4. Perceived graininess scored by different sensitivity groups: High sensitivity group (HS) (●); low sensitivity group (LS) (▲); moderate sensitivity group (MS) (■). In each group, the points marked with different letters were significantly different ($p \leq 0.05$).

Based on the results presented in table 2.2, which showed small differences among the samples in terms of the liking, the second aim of this study was to verify whether graininess sensitivity of the subjects affected the liking. Figure 2.5 shows the average liking scores given by the subjects which belong to the three different sensitive groups. The highly sensitive subjects (a) liked significantly more the two most refined creams than the other three samples ($p < 0.001$). Moreover, they differently liked the samples, by moving on the scale from very much (score ≈ 78) to slightly (score ≈ 56). The liking scores given by both lowly (b) and moderately (c) sensitive subjects ranged from slightly and

moderately, with a wide variability. However, regarding the moderately sensitive subjects, even if no effect was found, a little downward trend was observed between the liking scores and the D_{90} values. These results seem to suggest that subjects differently used the LAM scale. To statistically support this statement, the individual liking score range (Δ liking) was calculated and submitted to a one-way ANOVA, by using the sensitivity group as independent variable. Actually, highly sensitive subjects (Δ liking= 15 ± 3) used a wider portion of the scale than both the moderate sensitive group (Δ liking= 6 ± 3) and the low sensitive one (Δ liking= 2 ± 2). This result supports what has been noticed. As graininess was the only attribute that varied among the samples, (Fidaleo et al., 2017), subjects who were not able to perceive the difference in term of graininess, equally liked the samples. Instead, subjects who were able to discriminate among the particle size in the samples liked more the most refined one, which being less grainy was more accepted by consumers, as reported in the study conducted by Petković, Pajin, & Tomić (2013).

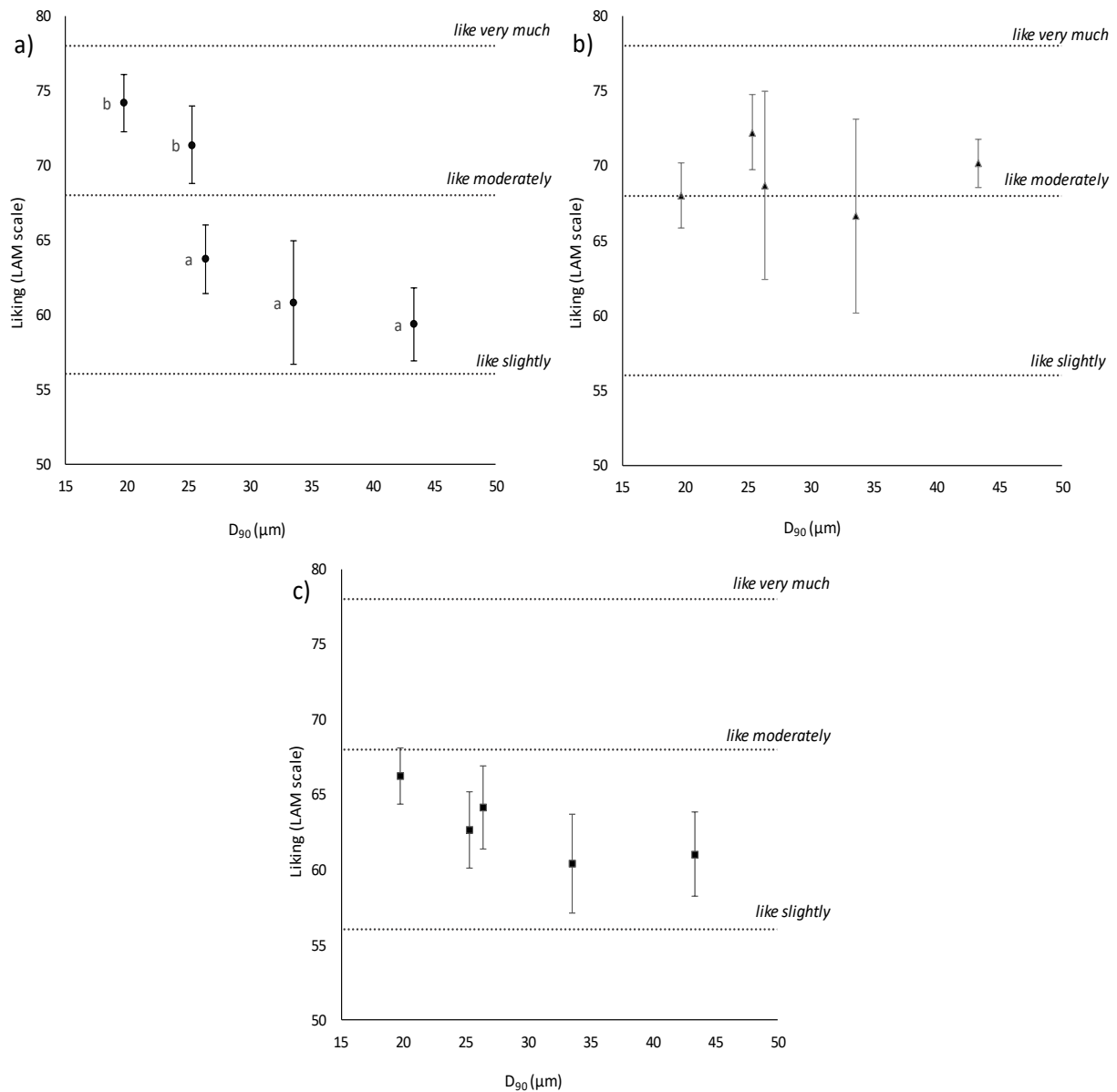


Figure 2.5. Liking scored by different sensitivity groups: High sensitivity group (HS) (●); low sensitivity group (LS) (▲); moderate sensitivity group (MS) (■). In each group, the points marked with different letters were significantly different ($p \leq 0.05$).

Finally, concerning the influence of age and gender on the graininess sensitivity from the chi square test results, no significant relationships were found with age ($\chi^2=2.99$, $p=0.224$), that is in contrast with the founding of Laguna and colleagues (2016), who described a significant decline in touch sensory abilities with the increasing of age. Moreover, only a small influence was found for gender ($\chi^2=5.71$, $p=0.057$). In particular, females seemed to be more sensitive than males. In fact, 54% of males were included in the low sensitivity group.

To the best of the authors' knowledge, the methodology used in this study has not been adopted by other authors and thus, a direct comparison of our results with other results reported in the literature

should be performed with caution. However, our results are consistent with the recent study conducted by Breen and colleagues (2019) who measured the ability of consumers to discriminate between different particle sizes in chocolate. Although they measured the individual sensitivity by means of oral point-pressure, they also found a significant relationship between the different oral sensitivity groups and the texture perception of dark chocolate. In particular, their high sensitive group was able to identify chocolate with larger particle size as being grittier, while the low sensitive group performed the experiments by chance.

Also, our results are congruent with the study recently conducted by Furukawa and colleagues (2019). They measured the graininess recognition threshold by using an up-down staircase method on nine different aqueous suspensions of microcrystalline cellulose, clustering people in different groups according to their sensory acuity, proving an oral sensory variety. Our results are also consistent with the study conducted by Nederkoorn, Houbena, and Havermans (2019) which showed that texture sensitivity is related to the appreciation of mouthfeel. However, to prove that, they measured the fingers-tactile sensitivity which in turn was related to the pickiness in eating. It is noteworthy that, Aktar and colleagues (2015) demonstrated that the tongue is even more sensitive to touch than fingers, supporting, even more, our results. They suggested also that people capability to discriminate viscosity is related to the experience and it was little influenced by the tactile sensitivity. On the other hand, the study of Engelen and van der Bilt (2008) demonstrated that some oral physiological parameters such as: oral sensitivity, tongue movements, temperature and saliva composition, were relevant for texture evaluation of semisolid foods, since they well correlated with various perceived texture attributes, and therefore differences in texture perception among individuals could be attributed to variations in oral physiology.

Our results are in contrast with the results of Lukasewycz and Mennella (2012) which, measuring the lingual tactile acuity and the food texture preferences among children and adults, concluded that lingual acuity and preferences are not correlated. However, to measure the lingual sensitivity they used a modified Essick letter identification task, that consists in recognizing letters of the alphabet embossed on rectangular Teflon strips and they have not proved that this method is representative of what happens when one manipulates a real food. In addition, Kremer and colleagues (2005) demonstrated that differences in texture perception exist between different groups of people. Indeed, they measured the intensity of the perception of some texture attribute such as creaminess, flouriness, and roughness and verified if the texture perception influenced the overall liking, using real soups changing in thickness levels. They found that elderly people perceived the soups as less creamy than the young people, but no differences came out for the other texture attributes. Also, they did not find any relationships with the overall liking.

It is well known that texture perception may be influenced by the culture, experience and external context (Engelen and van der Bilt, 2008), and, in addition, previous studies have shown that variation in oral processing behavior can affect the sensory perception of foods (Devezeaux de Lavergne et al., 2015), and the preferences as well (Jeltema, Beckley, and Vahalik, 2014, 2015). For this reasons all the people involved in our experiment were untrained Italian subjects and a standardized technique was used to evaluate the samples, as described in a previous section. Still, there are some difficulties in comparing our result to the literature. First, because the food system we used is different from that used in other works based on other food systems or even not food samples. Second, because of the new statistical approach we used to cluster the subjects. Therefore, some differences in outcomes can be due both to differences in the used food product and to the approach we proposed. Finally, the small number of involved subjects has to be considered as a critical limitation of this study, especially in terms of hedonic results.

2.4. Conclusions

There is a lack in the literature about methods for measuring the sensitivity to the graininess as key texture attribute. In order to develop a suitable method for evaluating the graininess sensitivity, five samples of cacao-based creams were prepared by means of a stirred ball mill using different refining times. Instrumental data showed that different refining times resulted in different levels of graininess, via the D_{90} index of the PSD, and that this index was correlated to refining time by a power law equation. By assuming that sensory graininess scores must correlate to refining time in the same way the D_{90} index does, the R^2 values and the estimated exponents of the power law equation, derived by the best fit of the data relative to each subject, were used to cluster the subjects into three groups having different graininess sensitivity.

The graininess sensitivity also lightly affected the liking scores. Indeed, although all the samples were equally liked for both the moderate and low sensitivity groups, a significant trend was observed for the highly sensitive subjects.

However, no significant relationships were found with other variables. This was probably due to the small number of subjects involved in the study.

In conclusion, texture sensitivity knowledge is crucial to highlight or avoid specific textural attributes for a specific target of consumers; consequently, it will be useful for the food industry to develop tailored foods. The present approach could be used to investigate other texture attributes. Indeed, we showed that if a sensory characteristic can be measured by means of an instrumental analysis, first, a relationship between this characteristic and a process/ingredient variable has to be found. Then, the equation coming out could be used to cluster subjects for their sensitivity to that sensory property. This approach is easy to perform and can be applied by means of a user-friendly software, such as

excel. Finally, more studies should be performed to investigate also on other individual variables hypothetically related to texture perception, such as BMI, prop status, as well.

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Chapter 3

Food hardness sensitivity: young and male are more sensitive than old and female subjects

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Abstract

Hardness is a key texture attribute which plays an important role in food preferences, however, relatively little is known about the sensitivity to perceive the hardness by different people, since there are no validate methods useful to measure it.

This study aimed to validate our previous research based on developing a method to measure the graininess sensitivity by clustering people according to their ability. The role of age and gender on hardness sensitivity was also investigated.

Four formulations of jellies were prepared by mixing orange juice, sugar and agar-agar, in different concentrations, in order to obtain different levels of hardness. First, mechanical measurements by means of a texture analyser were performed in order to characterize the samples. Then, 248 consumers (females=112; age median = 28 years old) evaluated first jellies liking by using the LAM scale and secondly the hardness intensity by using the GLM scale. The subjects were then clustered based on their hardness sensitivity into three groups: high sensitivity; moderate sensitivity; low sensitivity. Repeated measures ANOVA model was used to analyse the data.

Jellies with 3, 5, 7 and 9% of agar-agar significantly varied in terms of hardness. Moreover, the hardness values (N) followed a linear model with an R^2 equal to 0.98. The hardness intensity trend for each consumer was also fitted by a linear equation. Then, consumers were clustered based on both the angular coefficient and R squared value into three groups (highly sensitive; average sensitive; lowly sensitive).

Repeated measures ANOVA results showed a significant difference between the three groups in terms of perceived hardness ($p < 0.0001$), demonstrating how the used criteria can be valid for clustering the subjects. Different hardness sensitivity slightly affected liking scores ($p < 0.05$). Age and gender significantly affect the hardness sensitivity; in particular, the highly sensitive group was more represented by young and adult subjects than old ones ($p = 0.032$) and by more males than females ($p = 0.027$).

We conclude that differences in hardness perception exist and the approach we used is valid to measure them. Also, individual characteristics, such as gender and age, play an important role on hardness perception and food liking. Future researches need to be conducted in order to investigate other texture attributes and include other variables, such as psychological traits in the model.

Keywords: texture sensitivity; individual variables; jellies; TPA.

3.1. Introduction

Food preferences and choice are influenced by many interacting factors (Monteleone et al., 2017), but a pivotal role is played by the personal liking. Besides taste, smell and appearance, texture is a prominent aspect on which liking of food is based (Szczesniak, 1991; Moskowitz & Krieger, 1995). Texture also plays a decisive role in the aversion to some types of food, like oysters (Scott & Downey, 2007), or grainy chocolate creams (Petković, Pajin, & Tomić, 2013). In general, food that is tough, gummy, or slimy is disliked more often than food that is crispy or crunchy (Szczesniak, 2002).

Also, in some foods, the perceived texture is a key indicator of food quality and it is more influential on consumer choice than flavour and colour (Lawless & Heymann, 2010). The context plays a key role in preferences, for example at breakfast time, generally, soft products are more preferred than products with a harder consistency, while an opposite behaviour is observed at dinner time (Szczesniak, 2002).

Of course, what makes a product texturally different are the ingredients. For instance, the type of thickener and of course the quantity, strongly affect the perceived consistency of candies, because different thickener agents produce different texture characteristics (Kälviäinen et al, 2000).

Therefore, the interaction effect between the formulation and the perception of texture attributes is quite obvious and intuitive. Also, variations in texture can be obtained by changing some process variables. Different refining times, for instance, can lead to different levels of graininess, in products like fat-based creams (Miele, Borriello, Fidaleo, Masi, & Cavella, 2020). More in general, preparation of foods can influence their crunchiness, sliminess, softness and easiness to handle, and this, in turn, influences the liking of foods (Zeinstra, Koelen, Kok, & De Graaf, 2010). The study conducted by Zeinstra and colleagues (2010) demonstrated that preparation methods increasing crunchiness were liked better, whereas preparation increasing granular texture was disliked.

On the other hand, studies conducted by Jeltema, Beckley, and Vahalik (2014, 2016) have shown that consumers can be clustered by the way they manipulate food in their mouths (Mouth Behavior) and these groups of individuals show differences in food texture preferences. Furthermore, if on the one hand there are general preferences for specific textures (Szczesniak, 2002), on the other hand, this effect could be larger for people who are more sensitive to specific texture attributes and have less tolerance for specific textures. This hypothesis is based on what happens with taste and odour sensitivity (Cardello, 1996; Kaminski, Henderson, and Drewnowski, 2000; Tuorila, 2007; Coulthard and Blissett, 2009).

With this background, different studies have been conducted on texture sensitivity and on whether it may affect food liking (Finkelstein & Schiffman, 1999; Coulthard & Sahota, 2016). The differences in liking are usually explained by differences in detection thresholds, but it is known, for instance,

that altered processing of tactile stimulation also play a role (Ide, Yaguchi, Sano, Fukatsu, & Wada, 2018). However, since texture perception is multisensory in nature, involving touch (both manual and oral) sight and hearing, (Szczesniak, 2002; Nishinari, Kohyama, Kumagai, Funami, & Bourne, 2013), the evaluation of individual texture sensitivity can be tricky. Over the years, different methods have been developed and used to measure the sensitivity, such as oral form recognition (Essick, Chen, & Kelly, 1999), size and weight discrimination tests (Johnson & Phillips, 1981), stereognosis (Jacobs, Serhal, & van Steenberghe, 1998), two-point discrimination (Engelen et al., 2004), force perception (Pigg, Baad-Hansen, Svensson, Drangsholt, & List, 2010), and other physiological measures (Bangcuayo & Simons, 2017; Calhoun et al., 1992; Linne & Simons, 2017).

Although the proposed approaches showed interesting results, they do not fully reflect the real perception of the texture and do not allow to measure sensory sensitivity to it, as it relates to real products. Some recent studies have been conducted using model food to measure texture sensitivity (Aktar et al., 2017; Shupe et al., 2018; Steele, 2018; Breen, Etter, Ziegler, and Hayes 2019) and even fewer studies have been performed using real food (Steele, James, Hori, Polacco, & Yee, 2014; Pellegrino et al., 2019). Recently, our last study (Puleo et al., 2019) showed a new method to measure the human sensitivity to graininess, using cocoa-based creams as real tested food. The subjects were asked to evaluate five samples with different levels of the target sensation. To cluster the subjects according to their graininess sensitivity it was assumed that, in principle, sensory scores of highly sensitive subjects should have followed the same trend observed from the instrumental results. The authors of that study were able to cluster people according to their sensitivity, and they concluded that the proposed approach could be used to investigate other texture attributes.

The aim of this work was to validate the proposed method (Puleo et al. 2019), using a larger sample of subjects and investigating another texture attribute: the hardness. Thus, four jellies were prepared, by only changing the concentration of agar-agar, in order to obtain four different levels of hardness. In foods such as jellies, the hardness is an important key attribute for the consumer preference (Kälviäinen et al., 2000; Saint-Eve et al., 2011). Since people can be differently sensitive to the hardness (Nederkoorn 2015), also their acceptable levels of hardness could be different.

Starting from this background, the second aim of this study was to verify how the hardness sensitivity affected the jellies liking of the consumers. The relationships between individual variables, such as age and gender, and hardness sensitivity were also investigated.

3.2. Materials and methods

3.2.1 Materials

Agar-agar powder was supplied by ACEF (Italy). Agar powder was added to red-orange juice (Valfrutta, San Lazzaro Di Savena, Bologna, Italy) which was heated up to 95°C. The solution was stirred for 10 min, poured into Petri plates and cooled down at room temperature. Once cooled, a circular mould (diameter=16mm) was used to make barrel-shaped samples (height=10mm). Finally, the jellies were covered with non-hygroscopic icing sugar in order to mask potential changes in colour. Four agar-agar concentrations were used (3, 5, 7 and 9%).

3.2.2 Texture Profile Analysis

Texture profile analysis (TPA) test, which is based on the imitation of mastication or chewing process, was performed with double-compression cycles (Texture Analyser - Food Technology Corporation TMS-Pro). TPA was performed using a 75-mm diameter plate and with load-bearing cells (50 N, 500 N) chosen according to the attainable maximum load on samples during test execution. Data were acquired through Texture Lab Pro Software. Tests were performed with a crosshead speed of 40 mm/min, imposing 40% as maximum deformation for the first cycle, with preventive confirmation of breakup within such a range of the examined samples. For each sample, ten replications were performed and the values of the first maximum peak (hardness, N) were extrapolated from the curve.

3.2.3 Sensory evaluation

A total of 248 subjects (females = 112, age-range = 18–60 years old, age median = 28 years old, young ≤ 25 , n= 68; 25<adults>41, n= 115; old >41, n= 65) evaluated the four jellies and scored their liking by using Labeled Affective Magnitude scale (LAM), a 100 mm vertical line, from 0 = “greatest imaginable dislike” to 100 = “greatest imaginable like” and with anchor words spaced according to the spacing's provided by Schutz and Cardello (2001). In addition, subjects were asked to score the perceived hardness intensity by using the generalized Labeled Magnitude scale (gLM), a 100 mm vertical line, from 0 = “no sensation” to 100= “the strongest imaginable sensation of any kind” and intermediate anchors as provided by Bartoshuk et al. (2004). The subjects were instructed to the use of gLM scale following published procedures (Monteleone et al., 2017). In particular, they had to treat the “strongest imaginable sensation” as the most intense sensation they could image/remember in any sensory modality.

All the involved subjects were Italian people recruited by means of social network, flyers and word of mouth. The tests were run in separate booths in the sensory laboratory of the Department of Agricultural Sciences (University of Naples, Italy). Four samples were served on the plastic plate in a monadic, randomized and balanced order, identified by three-digit random codes under red light.

The subjects were asked to use a common procedure to evaluate the hardness intensity, consisting of compressing the samples between molars and evaluate the perceived hardness explained as the force required to compress the sample (Sensory Analysis Methodology – Texture profile: ISO 11036). The subjects were provided with a cup of still water to rinse their mouth before testing the next sample. Data were collected by means of “Fizz Acquisition” software (Biosystèmes, Couternon, France).

3.2.4 Data analysis

The instrumental hardness values were analysed by means of one-way analysis of variance (ANOVA), and multiple comparison test (Duncan's test) was used to statistically compare the samples ($p \leq 0.05$). Repeated measures ANOVA and multiple comparison test (Duncan's test) were used to evaluate if differences among the sample means (used as a repeated factor) were statistically significant ($p \leq 0.05$), in terms of both perceived hardness and liking. Next, subjects were clustered in three groups: lowly sensitive (LS), moderately sensitive (MS) and highly sensitive (HS), according to the approach proposed by Puleo et al. (2019). For each group (LS, MS, HS) a second repeated-measures ANOVA was used to verify the effect of the different hardness levels on both sensory liking and perceived hardness intensity.

Finally, chi-square test was run to analyse the relationship between hardness sensitivity and age groups (young vs adult vs old subjects). The same approach was used to analyse the relationship with gender as well. Also, age groups and gender were used as independent variables in repeated measures ANOVA, to verify the effect of these individual variables on perceived hardness intensity and sensory liking.

The XLSTAT statistical software package version 2016.02 (Addinsoft) was used for data analysis.

3.3. Results and discussion

3.3.1 TPA results

The TPA curves of the four jellies are shown in figure 3.1.

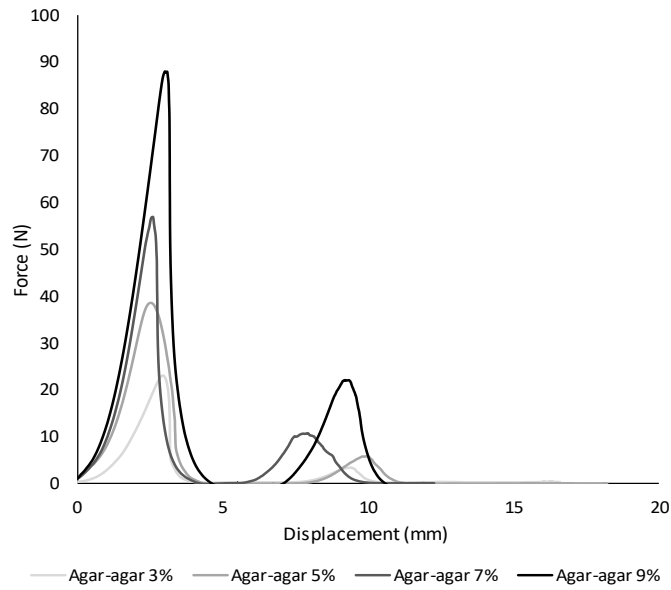


Figure 3.1. TPA curves (average of ten replications) of the four jellies differing for agar-agar concentration (3, 5, 7 and 9%).

As showed in the figure, by increasing the concentration of the gelling agent, both the first and second peak became higher, in accordance with (Muñtoz, Pangborn, & Noble, 1986). The values of the first peak of each sample were extrapolated from each curve and reported as values of instrumental hardness (Table 3.1). Maximum peak values were found useful in modelling the human perception of hardness in the mouth (Di Monaco, Cavella, & Masi 2008; Drake & Gerard, 1999; Martens & Thybo, 2000). Accordingly, in this study, the values of the maximum peaks were used as representative parameters of the hardness of the tested jellies.

Table 3.1. Maximum peak values (N) of jellies at different agar-agar concentrations (%).

Agar-agar concentration (%)	Maximum peak (N)	Standard deviation	Significant groups
3	24.207	1.402	A
5	40.105	3.216	B
7	62.048	3.124	C
9	91.509	4.515	D

These values are higher than those described by Cappa, Lavelli, & Mariotti (2015) which reported low peak force values (below 1.62N). However, they extrapolated the value of hardness from the results of a puncture test, conducted by a conical probe, at a speed of 120 mm/min, up to 35 mm of distance from the contact point. The hardness instrumentally measured is strongly depended on the probe, the crosshead speed and the ratio of deformation (Pons & Fiszman, 1996). Indeed, our values resulted also higher than those reported by Henry, Katz, Pilgrim, & May (1971), which reported

values between 0.18 and 1.20N, by conducting a double compression at a speed of 127 mm/min, up to 25% of deformation.

By generally speaking, the values of the maximum peak are well correlated to the sensory attribute of hardness (Corollaro et al., 2014; Costa et al., 2011; Di Monaco, Cavella, & Masi, 2008). Accordingly, in this study, the values of the maximum peaks were used as representative parameters of the hardness of the tested jellies. The trend of the maximum peaks was described with a linear equation ($R^2=0.98$), as shown in Figure 3.2. In figure 3.2 the significant differences between the samples are also showed (Duncan's test, $p<0.0001$).

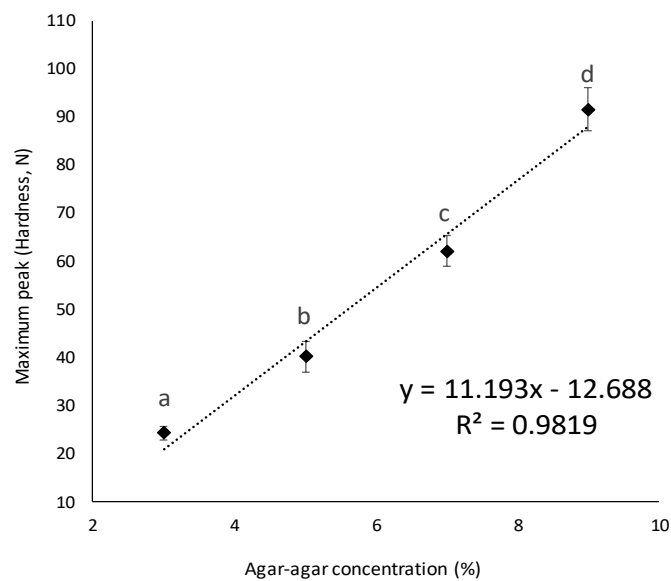


Figure 3.2. Trend of maximum peak values (hardness, N), in function of the concentration of gelling agent (agar-agar). The points marked with different letters were significantly different ($p \leq .05$).

3.3.2 Sensory evaluation

A total of 248 subjects scored the liking and the hardness intensity by using a LAM and gLM scale, respectively. By averaging both liking and perceived hardness scores, significant differences came out among the samples, as shown in Table 3.2.

Table 3.2. Liking and perceived hardness scores (mean value \pm standard deviation) given by 248 subjects.

Agar-agar concentration (%)	Liking (LAM scale)	Perceived hardness (gLM scale)
3	50 \pm 14 ^c	8 \pm 8 ^a
5	47 \pm 14 ^b	14 \pm 11 ^b
7	43 \pm 15 ^a	20 \pm 14 ^c
9	43 \pm 15 ^a	23 \pm 15 ^d

In each column, the values followed by different letters were significantly different (Duncan test, $p \leq 0.05$).

Regarding the liking scores, subjects moved on the scale between dislike slightly (score ≈ 43) and neither like nor dislike (score ≈ 50), thus a slight downward trend was observed with the increase of the concentration of the gelling agent. Typically, a jelly is constituted by low concentrations of gelling agent (Barrangou, Drake, & Daubert, 2006; Cappa et al., 2015) and the effect of previous experiences on texture preferences it is well documented (Baron & Penfield, 1993; Monteleone, Frewer, Wakeling, & Mela, 1998; Pliner, 1982). Therefore, the observed liking decreasing with the increasing of the gelling agent concentration is probably due to the unfamiliarity of the most concentrated samples. Indeed, our results are in agreement with the study conducted by Kälviäinen, Schlich, & Tuorila (2000), which demonstrated that specifically in the case of candy, consumers' preferences were explained by the liking of commercial candies with a similar texture.

Regarding the perceived hardness, subjects moved on the scale around the weak and between moderate and strong intensity (score ≈ 8 and ≈ 23 , respectively), significantly discriminating among all the samples.

To cluster the subjects according to their hardness sensitivity, we followed the method proposed by Puleo et al. (2019). Therefore, we assumed that the sensory scores should follow the same trend exhibited by the instrumentally measured hardness, with the increasing of the gelling agent concentration, that is, an upward linear relationship.

According to Puleo et al. (2019), the hardness scores of each subject were fitted with a linear equation, estimating both the angular coefficient (which represented the rate of increase of the linear equation) and the R^2 coefficient, and using them as clustering parameters.

This means that subjects whose scores correlated to a linear equation with both high R^2 coefficient and high angular coefficient had a very good sensitivity to hardness differences among samples, similar to the instrumental method (TPA). Accordingly, the lowly sensitive subjects were those to whom corresponded low R^2 coefficients and angular coefficient of the linear equation close to zero. Next, a descriptive analysis was performed on both the angular and the R^2 coefficients obtained for each subject (Table 3.3). The subjects were then clustered based on the quartile's distribution of both the angular and the R^2 values into three groups: high sensitivity ($n = 77$); moderate sensitivity ($n = 86$); low sensitivity ($n = 85$).

Therefore, the subjects in the high sensitivity group had to have an angular coefficient and, at the same time, an R^2 value greater than the third quartile (75% of distribution, equal to 7.80 and 0.85, respectively). On the other hand, subjects described by a linear equation with angular coefficients and, at the same time, R^2 values, lower than the first quartiles (25% of distribution, equal to 1.40 and 0.20, respectively) were grouped in the low sensitivity group. Consequently, the middle 50% of the distribution of both the angular coefficients and R^2 was classified as moderate sensitivity group.

Table 3.3. Quartiles distribution of angular coefficients and the R².

Quartiles	Angular coefficient	R ²
1 st Quartile	1.40	0.20
Median	4.50	0.58
3 rd Quartile	7.80	0.85
Maximum	34.60	0.99
Minimum	-13.10	0.00

The scores given by each sensitivity group were submitted to repeated measures ANOVA and the hardness intensity values were plotted against the instrumentally measured hardness in Figure 3.3 (a,b,c).

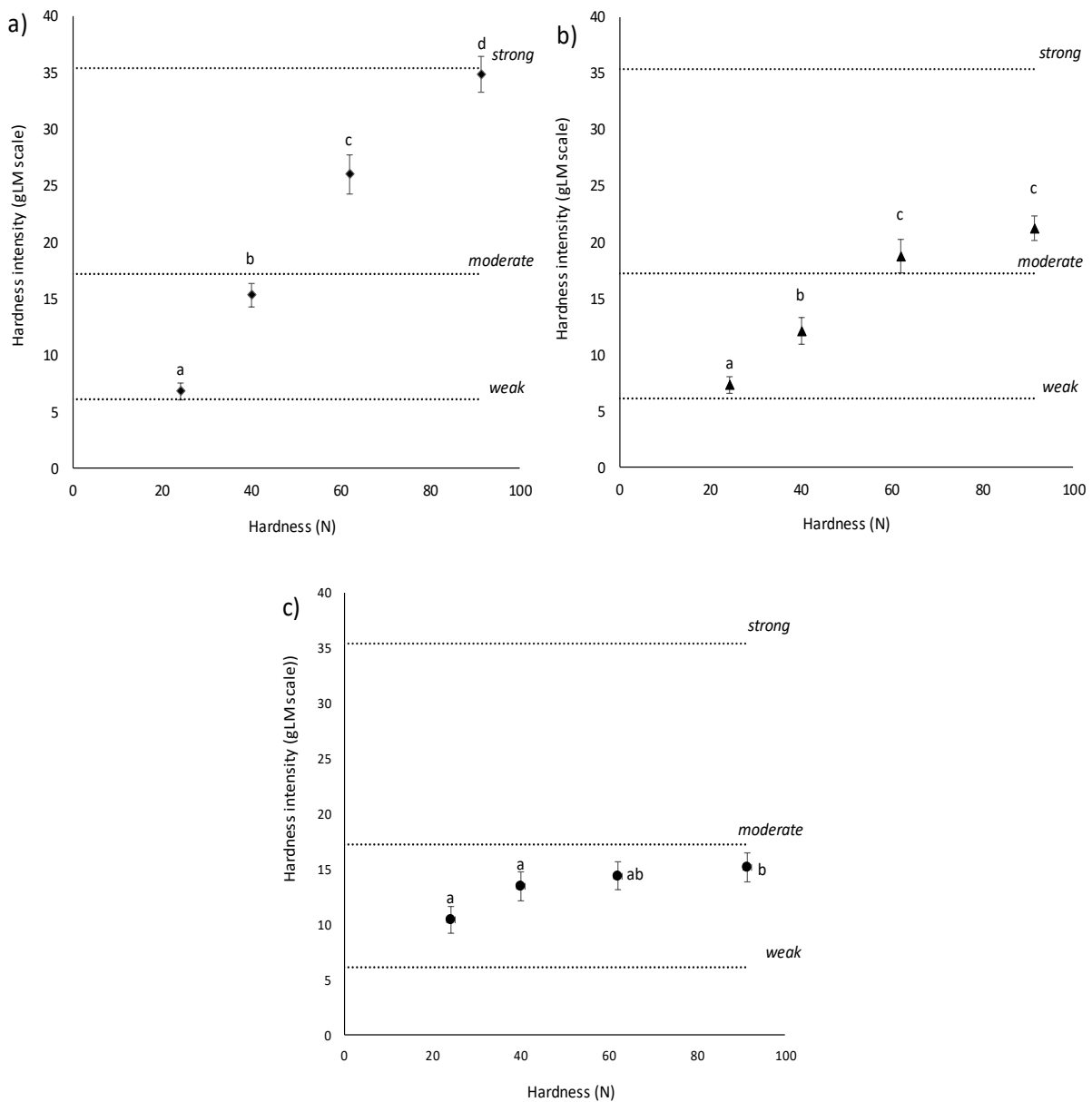


Figure 3.3. Perceived hardness scored by different sensitivity groups. (a) High sensitivity group (◆ HS); (b) moderate sensitivity group (▲ MS); (c) low sensitivity group (● LS). In each group, the points marked with different letters were significantly different ($p \leq .05$).

The scores given by the subjects which belonged to the high sensitivity group (figure 3.3a) were statistically different ($p < 0.0001$) with varying the hardness (N) and well described how the hardness intensity varied from a score around 6 (weak on gLM scale) to a score around 34 (strong on gLM scale). By contrast, the lowly sensitive subjects (figure 3.3c) were able to discriminate only among the less and the most concentrated sample ($p = 0.043$). Moreover, for these subjects, all the samples were scored between weak and moderately hard. Concerning the moderately sensitive subjects (figure 3.3b), they were able to discriminate between all the samples ($p < 0.0001$), except for the two most concentrated ones ($p = 0.17$) which were both perceived as little bit more than moderately hard.

The observed differences regarding the use of the scale demonstrated that subjects are differently sensitive to the hardness attribute. Indeed, in particular, the lowly sensitive subjects scored the samples always on a little region of the scale, properly because of their inability to perceive the differences.

The results are absolutely in agreement with our previous findings (Puleo et al., 2019). Indeed, in our precedent study, we measured the graininess sensitivity and we concluded that the proposed approach could have been used to investigate also other texture attributes. Nevertheless, in this present study, the relationship between the instrumentally measured hardness and the concentration of gelling agent was linear, in contrast with our previous study (Chapter 2), where the relationship between graininess and refining time was the power-law type. It is true indeed, that usually, the correlation between the perception of a sensory property and its governing driver (different ingredient or a different process variable, etc.) is not linear-like. Therefore, different ranges of gelling agent concentration, probably, could be explained by other mathematical models.

Despite this, the results described above demonstrated that if a sensory attribute can be instrumentally measured, no matter what kind of relationship exists between this attribute and the ingredient/process variable, it will explain the individual sensitivities.

Of course, by generally speaking, differences in texture sensitivity may be due to poor dentition (Roininen, Fillion, Kilcast, & Lahteenmaki, 2003) social factors, medications, physical and physiological issues (Donini, Savina, & Cannella, 2003) and oral processing behaviour (Devezeaux de Lavergne, Derks, Ketel, de Wijk, & Stieger, 2015). However, to set aside all these affecting variables, all the people involved in our experiment were healthy Italian subjects, without dental issues, and a standardized technique was explained to them before the test, as described in a previous section.

Therewith, to the best of the authors' knowledge, really few studies have been conducted on individual sensitivity to the hardness, and moreover, the methodology used in this study has not been adopted by other authors. However, significant differences in hardness sensitivity, mostly related to the age, were also significant in the study conducted by Shupe, Resmondo, & Luckett, (2018), where the subjects were asked to discriminate between foam samples of varying hardness.

By looking at the results presented in Table 3.1, small differences among the samples in terms of the liking came out. Furthermore, the second aim of this study was to verify whether the hardness sensitivity of the subjects could affect the liking. Figure 3.4 shows the average liking scores given by the subjects belonging to the three different sensitivity groups.

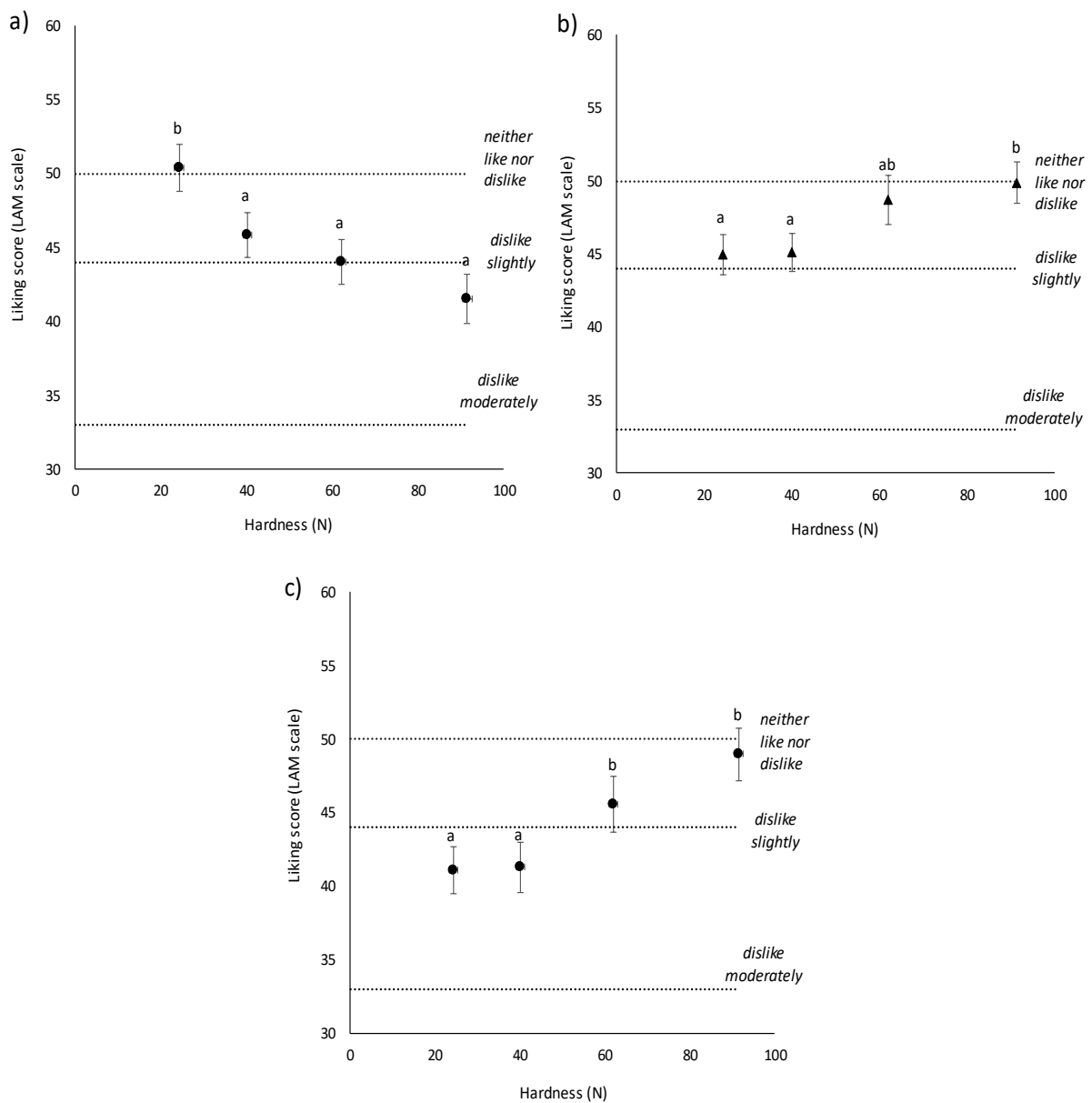


Figure 3.4 Liking scored by different sensitivity groups. (a) High sensitivity group (◆ HS); (b) moderate sensitivity group (▲ MS); (c) low sensitivity group (● LS). In each group, the points marked with different letters were significantly different ($p \leq 0.05$).

The highly sensitive subjects (a) liked significantly more the softer sample (3% agar-agar) than the other three samples ($p \ll 0.001$), which in turn were equally liked. Instead, the lowly and moderately sensitive subjects liked significantly more the harder version (9% agar-agar) than the other three samples ($p \ll 0.001$). This result may confirm what explained above. Typically, a jelly is constituted by low concentrations of gelling agent (Barrangau et al., 2006; Cappa, Lavelli, Mariotti, 2015), therefore the highly sensitive subjects are more able than others to isolate the familiar sample from the other ones, recognizing the unfamiliar hardness. Moreover, repeated-measures ANOVA analysis was run to verify the effect of the individual sensitivity on the liking for each tested sample (Table 3.4). The lowly sensitive subjects gave lower scores than moderately and highly sensitive ones to all the samples, however, these differences were not significant.

Table 3.4. Liking scores (mean value \pm standard deviation) given by different sensitivity groups: high sensitivity group (HS); low sensitivity group (LS); moderate sensitivity group (MS).

Sensitivity group	Agar-agar 3%	Agar-agar 5%	Agar-agar 7%	Agar-agar 9%
HS	50 \pm 14	46 \pm 13	44 \pm 13	41 \pm 15
MS	50 \pm 13	49 \pm 12	45 \pm 15	45 \pm 13
LS	50 \pm 15	46 \pm 16	41 \pm 17	41 \pm 16
Pr > F	0.800	0.266	0.257	0.174

Finally, to study the effect of the individual variables, the chi-square test was run to analyse the relationship between hardness sensitivity and three age groups (young people, adults, old people). The same approach was used to analyse the relationship with gender as well.

Therefore, regarding the age effect, subjects were clustered in three groups of age, according to cut-offs based on the age quartiles distribution: young ≤ 25 , $n=68$; 25<adults>41, $n=115$; old >41 , $n=65$. Young, adult and old subjects significantly differed in hardness sensitivity group distribution ($\chi^2 = 10.5$; $p = 0.032$). Highly sensitive group was significantly more represented by young (39%) and adult (43%) than by the old subjects (18%). While moderate sensitive young subjects (17%) formed a significant less abundant group than moderate sensitive adult (50%) and old (33%). This result is in accordance with the study conducted by Conroy, O' Sullivan, Hamill, & Kerry (2017). They highlighted that textural sensitivity was affected by the increasing of age. In particular, in their study, subjects over 70 years old were not able to discriminate different levels of tenderness in the meat. Our results are also in accordance with the previous study conducted by Forde & Delahunty (2002) which showed that older consumers were less able to discriminate between texture stimuli of liquid, semisolid e solid foods, indicating a loss of sensory function. More in general, different studies

demonstrated how the loss of textural acuity may be due to poor dentition (Roininen et al., 2003), and how sensory decline may be related to the ageing process (Morley, 2001).

Moreover, to study the effect of age on liking, repeated-measures ANOVA was run using the age groups as independent variables. Results showed that old subjects liked significantly more the first two samples ($p < 0.05$) than the adult and young subjects, while there were no significant differences between the age groups for the other two samples (Table 3.5).

Table 3.5. Liking (means \pm standard deviation) scored by three age groups, young, adult and old subjects.

Age groups	Agar-agar 3%	Agar-agar 5%	Agar-agar 7%	Agar-agar 9%
Young (n=68)	50 \pm 2 ^{ab}	46 \pm 2 ^{ab}	42 \pm 2 ^a	42 \pm 2 ^a
Adult (n=115)	48 \pm 1 ^a	44 \pm 1 ^a	42 \pm 1 ^a	41 \pm 1 ^a
Old (n=65)	53 \pm 2 ^b	52 \pm 2 ^b	47 \pm 2 ^a	46 \pm 2 ^a

In each column, the values followed by different letters were significantly different (Duncan test, $p \leq 0.05$).

As it is explained in the paragraph of TPA results, the first two samples were softer than the others. Thus, the preference for softer versions of food by old subjects has been already demonstrated in some studies. In particular, our results are in accordance with the study conducted by Conroy et al. (2017), where the oldest age cohort (71-75 years old) significantly and positively correlated the most tender sample to the overall acceptability. Moreover, other studies described the effect of age on texture preferences. For instance, textural changes in muesli products evaluation had a higher impact on the liking of elderly assessors when compared to younger subjects (Kälviäinen, Salovaara, & Tourila, 2002). In another paper, studying the different textural preferences for carrots, it was shown that young adults liked tricky textures such as rough, crispy, crunchy and hard to a far greater level than the elderly subjects (Roininen et al., 2003).

Regarding the effect of gender on the hardness sensitivity, the results of the chi-square test showed that males and females significantly differed in hardness sensitivity group distribution ($\chi^2 = 5.9$; $p = 0.012$). Indeed, the high sensitivity group was significantly more represented by males (65%) than females (35%). On the contrary, of course, the low sensitive group was significantly more represented by females (58%) than males (42%). In the moderate sensitivity group, females and males were equally distributed.

In this case also, a direct comparison with other studies cannot be done, because the approach used in this study has not been adopted by other authors. However, by comparing our results with studies that used different methodologies, the last found effect is in accordance with the results of Youmans, Youmans, & Stierwalt (2009), where a gender difference in tongue acuity was showed. In particular,

they measured the texture sensitivity as the strength of the tongue and concluded that women had a reduced tongue strength reserve compared to men. Also, it is worth emphasising that individuals presenting different mouth behaviour (different ways and times to consume the food) perceive the texture of products differently (Jeltema, Beckley, & Vahalik, 2015). Furthermore, if intended as related to the consumption time, bite-size and eating rate, the texture sensitivity resulted affected by the gender variable in the recent study conducted by Ketel et al., (2019). Indeed, males resulted in having a larger average bite size for liquid, semi-solid and solid foods and higher eating rate for solid foods than females.

Finally, repeated measures ANOVA conducted to study the effect of gender on liking, did not show any significant differences between males and females ($p>0.05$).

In general, the results presented in this paper confirmed our previous findings (Puleo et al., 2019), validating, in this way, the proposed approach. People have an individual ability to perceive the differences in texture which translates into different texture sensitivity. In our previous paper, indeed, we were able to cluster subjects according to their graininess sensitivity. By means of the same approach, in this study, we were able to cluster subjects according to their hardness sensitivity. This kind of sensitivity leads to differences in texture preferences which is a crucial notion to determine food product acceptance, especially in view of developing new tailored food products, such as for elderly people. It is a fact, indeed, that hardness sensitivity was affected by age and that different age groups preferred different levels of hardness.

3.4. Conclusions

In order to validate our approach to cluster subjects according to their texture sensitivity, and to investigate the perception of another texture attribute, four jellies were developed by changing the level of hardness. Instrumental data showed that different concentration of the gelling agent resulted in different levels of hardness, via the maximum peak (hardness, N) of the TPA curve, and that this index was correlated to the agar-agar concentration by a linear equation. By assuming that sensory hardness scores had to be correlated to the agar-agar concentration in the same way of instrumental hardness does, the R^2 and the angular coefficients of the linear equation, derived by the fitting of the sensory data of each subject, were used as clustering parameters. Indeed, subjects were clustered into three groups having different hardness sensitivity. Belonging to a group instead of another, lead to different preferences for different levels of hardness.

Hardness sensitivity resulted affected by age and gender, indicating that, on one side, it can decrease during the ageing process, and on the other side, it may be innate and depending on the gender.

In conclusion, once again we demonstrated that this approach is easy to perform and valid to cluster people according to their sensitivity. For this reason, other studies can be performed by using this

method, to investigate other texture attributes and other individual variables, such as taste sensitivity and psychological traits.

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Chapter 4

Oral sensitivity to viscosity and food neophobia drive food preferences and choice

Paper in preparation.

Abstract

Food preferences and choice are affected by individual variables, some of which related to sensory sensitivity and psychological traits. How texture sensitivity could lead to different food preferences is quite doubting, especially because a unique standardized procedure to measure the texture sensitivity does not exist. Also, it is known that psychological traits, such as food neophobia, may drive the food preferences, but whether texture sensitivity varies according to the degree of food neophobia is unclear.

The aim of the present study was two-fold. First, the role of sensitivity to viscosity on food liking and choice was investigated. Secondly, the relationship between sensitivity to viscosity and food neophobia, and the role of this latter on food liking, were also investigated.

Five chocolate creams were prepared by changing the solid concentration (C1, 31%, C2, 33%, C3, 36%, C4, 39%, C5, 43% (w/v), in order to obtain different levels of viscosity. First, rheological measurements were performed in order to characterize the samples. Then, 176 consumers (females = 118, age median = 25 years old) were asked to fill in the Food Neophobia scale (FNS) and the Food Choice questionnaires (FCq). In particular, the FCq was developed to evaluate preferences within a pair of food items similar for flavour but different for textures (soft vs hard option and liquid vs dense option). Secondly, participants evaluated first creams liking by using the LAM scale and secondly the viscosity intensity by using the GLM scale. The subjects were then clustered based on their sensitivity into three groups: high sensitivity; moderate sensitivity; low sensitivity. Also, considering the data collected from the FCq, two choice indexes were calculated as a sum of the choices for the soft (SCI) and liquid options (LCI), respectively. Subjects were clustered in two groups of preference according to their SCI and LCI and the effect of individual viscosity sensitivity on the food choice was investigated. Finally, considering the data collected from the FNS questionnaire, participants were clustered into two groups according to their level of food neophobia, based on the total scores calculated.

The sensitivity to viscosity significantly affected the liking of chocolate creams ($p < 0.05$) and the solid food choice ($p = 0.01$). Moreover, liking of chocolate creams was also affected by the individual level of neophobia ($p = 0.01$), that in turn, was not correlated to the viscosity sensitivity.

The presented results confirm that texture sensitivity and food neophobia affect what a person likes and drives what a person chooses to eat. These findings can be helpful for the food industries in the view to develop new food products.

Keywords: Texture sensitivity; food liking; psychological traits.

4.1. Introduction

Food preferences and choice are affected by many interacting factors (Monteleone et al., 2017).

Tastes, odours, flavour and other sensory properties are crucial components of food preference and choice (Boesveldt et al., 2018; Monteleone et al., 2017; Rozin, 1982). Food texture also plays a pivotal role in how foods and beverages are perceived (Bourne, 2002; Schiffman, 1977) and whether food is liked or disliked (Scott & Downey, 2007). While research largely explored how individual differences in the genetics and physiology, related to taste and odour perception, interact with food experiences to contribute to food likes and dislikes (Birch, 1999; Kim, Breslin, Reed, & Drayna, 2004; Mennella, Pepino, Duke, & Reed, 2011; Mennella, Pepino, & Reed, 2005), very few studies have examined texture perception sensitivity and how it affects food preference, despite the belief of its importance in food choice (Szczesniak, 2002). As with the other senses, individual differences in how textures are perceived may contribute to how texture preferences develop.

Despite the well-known individual differences in oral sensitivity (Heft & Robinson, 2010; Wohlert, 1996; Zuniga & Essick, 1992) and the awareness that texture influences food acceptance during life (Baxter & Schröder, 1997; Nu, MacLeod, & Barthelemy, 1996; Szczesniak, 1987), it is doubting how different texture sensitivities may lead to different food preferences. As largely explained in the previous chapters, one of the main issues related to the paucity of data is due to a lack of a standardized methodology to study oral sensitivity to texture. Secondly, the approaches proposed across the years do not investigate specific texture attributes, but rather the overall - oral/non oral - tactile acuity, using tools that do not reflect the real perception of the food texture (Bangcuyo & Simons, 2017; Etter, Miller, & Ballard, 2017; Linne & Simons, 2017; Steele, 2018; Steele, James, Hori, Polacco, & Yee, 2014). The reason why no study has used real food products to investigate the texture sensitivity is the fact that texture is a multi-parameter attribute, related to the structure of the foods and detected by several senses (Szczesniak, 2002), and when it is modified in different levels of intensity, the other properties of food (taste, flavour and odour) get differently perceived, as they are also affected by the structure of the food (Lawless & Heymann, 1998). Therefore, the main trouble related to the use of a real food product is the difficulty in modifying the texture intensity levels of the product, without changing any other sensory property. The modification of also other sensory properties would influence the sensory perception and consequently it could affect the food preferences.

Aware of the gap related to the methods to measure the texture sensitivity and of the main troubles affecting the use of real food products, Puleo and colleagues (2019) proposed a new approach to measure the graininess sensitivity, using five samples of chocolate creams, changing in only their graininess levels. The authors were able to cluster subjects involved in the study in three different groups of sensitivity and concluded that their approach could be used to investigate other texture

attributes. Indeed, they showed that if a texture property can be instrumentally measured, then the same relationship existing between this characteristic and a process/ingredient variable can be used to cluster subjects for their sensitivity to that sensory property. However, although they succeeded in measuring the graininess sensitivity, and found also an effect on the individual likings, they did not investigate whether the individual sensitivities could drive the food choice. Also, they did not explore any possible existing relationships between the individual sensitivities and other individual variables, such as psychological behaviour, which, in turn, has been demonstrated having an important influence on texture perception and liking (Nederkoorn, Houben, & Havermans, 2019).

With these initial considerations, the first aim of this study was to use the method proposed by Puleo and colleagues (2019) to measure the sensitivity to viscosity, using chocolate creams as target real food, and verify whether and how different levels of sensitivity could affect food liking and choice. The viscosity was chosen as key texture attribute because considered one of the most dominant sensory characteristics of semisolid foods (Szczesniak, 1987). Indeed, across the years, several studies investigated the viscosity perception, and different methods to state the individual sensitivities have been proposed (Engelen & Bilt, 2007; Smith, Logemann, Burghardt, Zecker, & Rademaker, 2006; Steele, James, Hori, Polacco, & Yee, 2014). However, although those authors were able to measure the individual sensitivity to the viscosity, the results cannot be generalized, because obtained by means of different methods, therefore it is not possible drawing a unique conclusion (see Chapter 1). Moreover, even less studies have investigated the relationships between individual variables, such as gender and age, and individual viscosity sensitivity, and what is more, the results seem to be contrasting. In particular, Steele and colleagues (2014), failed in finding a relationship between the viscosity sensitivity and age and gender. On the contrary, Forde & Dalahunty (2002), observed an age effect on the capability to discriminate different levels of thickness. Also, to the best of the knowledge, no studies have been conducted on the relationship between viscosity sensitivity and food liking and choice.

Moreover, as stated above, food preference and choice are influenced by several factors. Psychological traits seem to be important predictors in food preferences, and indeed, the relationships between several psychological domains and different sensory properties have been largely investigated (Byrnes & Hayes, 2013; Monteleone et al., 2017; Spinelli et al., 2018; Törnwall et al., 2014). In particular, in the last two decades, food neophobia, intended as the reluctance to try unknown foods, has been extensively investigated by taking into account a number of different personal factors going from food preferences to food choice, from active chemosensory exploration of the world (sniffing and tasting) to physiological responses associated with alertness (for a further review, see Dovey, Staples, Gibson, & Halford, 2008). Spinelli and colleagues (2018) found a

significant effect of neophobia level and perception of burning sensation and acid taste. They observed that neophobic individuals scored the intensity of pungency and acid taste higher than neophilic ones. Thus, this personality trait was associated with a different perception of that key sensations. Also, starting from the observation that neophobic children mainly refuse fruit and vegetables rather than other food categories (Wardle and Cooke, 2008), Coulthard and Blissett (2009) hypothesized that the rationale behind could be a higher sensitivity to taste, and to bitterness in particular. On the other hand, neophobic subjects tend to use smaller sniff-magnitudes than non-neophobics, as measured during an odor detection task (Raudenbush et al., 1998), and this may be interpreted as an index of an attempt made by neophobics to avoid any possible bad odor-related experiences (Prescott et al., 2010). Following this logic, the study conducted by Demattè et al. (2013) revealed that neophobic people were significantly worse in the odor identification task than neophilic participants.

Regarding texture, the enjoyment of different texture was related to food neophobia in young children (Coulthard & Thakker, 2015; Coulthard & Sahota, 2016) and to picky eating in adults (Nederkoorn, Houben, & Havermans, 2019).

However, quite surprisingly, there has been very little research carried out to ascertain whether texture responsiveness varies according to the degree of food neophobia, and whether individual differences in perception may contribute to influencing food preference and choice among neophobic and neophilic subjects

With these other considerations, in the present study we also analysed the correlation between the sensitivity to viscosity and the food neophobia trait, and investigated the role of food neophobia on liking.

4.2. Materials and methods

4.2.1. Samples preparation

Chocolate mix powder (Paneangeli, Cameo S.p.A., Brescia, Italy) and completely skimmed milk (Berna, Parmalat S.p.A., Milan, Italy) were purchased from a local supermarket and used to prepare samples differing in viscosity. Chocolate powder mix and skimmed milk were mixed by means of an electric whisk, at room temperature, for 2 minutes, until a homogenous mix was obtained. A trained panel of 10 assessors (six females, age average = 23 years old) was involved in focus group evaluations (10 hours) to select chocolate creams which differed for viscosity, but not for other properties (taste, flavour, colour) (preliminary results data not reported for sake of brevity). Five samples differing for solid concentrations were selected (C1, 31%; C2, 33%; C3, 36%; C4, 39%; C5,

43% (w/v)). Samples were stored in glass containers at refrigerated temperature (4°C) and served at room temperature.

4.2.2. Rheological properties: Stress Overshoot

The rheological properties of the samples were determined by a Modular Advanced Rheometer System (Haake MARS, Thermo Scientific, Waltham, USA), equipped with a vane tool geometry (diameter 22 mm, length 16 mm, distance 8.5 mm). Transient tests were carried out and to this end, the stress (τ , Pa) was measured as a function of time (60s), keeping the shear rate constant ($\dot{\gamma} = 10\text{s}^{-1}$). The flow curves were carried out at 30.5°C, as an arithmetic average of room and mouth temperature, according to the method proposed by Dickie & Kokini (1983). Three replications for each sample were performed. Results were used to produce a shear stress growth function and to collect the stress overshoot values (Prentice, 1992).

4.2.3. Consumer evaluation overview

The consumer evaluation consisted in two steps. First, at the time of recruitment, participants were requested to complete an online questionnaire, where age and gender were collected, together with the responds of Food Neophobia Scale and the Food Choice Questionnaire. Secondly, participants were asked to attend one consumer session in individual booths, to evaluate liking and perceived viscosity of five samples of chocolate creams. Further details are explained below.

4.2.3.1. Participants

A total of 176 Italian subjects (females = 118, age-range = 18–70 years old, age median = 25 years old, young ≤ 23 , 52; 23 < adults > 30, 79; old ≥ 30 , 45) were recruited using social media, word of mouth and emails.

Participants gave written informed consent according to the principles of the Declaration of Helsinki.

4.2.3.2. Online questionnaire

Participants were asked to fill in an online survey (Google form) at home on the time of recruitment. The online questionnaire included the Food Neophobia Scale (FNS) and the Food Choice Questionnaire (FCQ).

The trait of food neophobia was measured using the 10-items questionnaire developed by Pliner & Hobden (1992). The individual FNS scores were computed as the sum of ratings given to the ten statements, after the neophilic items had been reversed; the scores thus ranged from 10 to 70, with higher scores reflecting higher food neophobia levels. Based on the calculated total score, subjects were clustered into two sub-groups representing low and high scores, using the median value as cut-off. Participants with the median score were not considered to evaluate the effects of food neophobia on the other variables. Secondly, in order to correctly explore the role of the food neophobia on the

viscosity sensitivity, subjects were also clustered in three groups of neophobia, according to the quartile distributions of the individual total scores.

The Food Choice Questionnaire was developed in order to evaluate preferences within a pair (similar in flavour but especially different for texture) of items developed on the base of texture dichotomies belonging to two texture domains: a liquid texture domain, containing 5 pairs of items, differing for viscosity (liquid/dense); a solid texture domain, containing 5 pairs of items, differing for hardness (soft/hard). In table 4.1 all the pairs are showed.

For each pair, respondents were asked to indicate which food they would choose in a normal eating situation, without diet restrictions.

The presentation order of the food items, within and between each pair, was randomised across participants. A choice index (SCI=Solid Choice Index; LCI=Liquid Choice Index) for each domain was calculated as a sum of the choice of the liquid options and of the soft options, assigning to each one a value of 1 (texture indexes range=0–5), with higher scores reflecting higher choice for the dense and hard options. For each domain, based on the calculated CIs, subjects were clustered into two sub-groups representing low and high scores, using the median values as cut-off. Participants with the median score were not considered.

Table 4.1. Texture dichotomies belonging to two texture domains used in the FCQ.

Liquid texture domain	Liquid option	Dense option
	Milk	Yogurt
	Coffee	Coffee cream
	Fruit juice	Fruit centrifuge
	Fruit smoothie	Frappé
	Vegetable broth	Vegetable creamed soup
Solid texture domain	Soft option	Hard option
	Banana	Apple
	Ice cream in cup	Ice lolly
	Sandwich bread	Crackers
	Soft chocolate snack	Chocolate bar
	Plumcake	Cookies

4.2.3.3. Sensory evaluation

Subjects evaluated the five chocolate creams and scored their liking by using Labeled Affective Magnitude scale (LAM), a 100mm vertical line, from 0 = “greatest imaginable dislike” to 100 = “greatest imaginable like” and with anchor words spaced according to the spacing's provided by

Schutz & Cardell (2001). In addition, subjects were asked to score the perceived viscosity intensity by using the generalized Labeled Magnitude scale (gLM), a 100mm vertical line, from 0 = “no sensation” to 100= “the strongest imaginable sensation of any kind” and intermediate anchors as provided by Bartoshuk et al. (2004). The subjects were instructed to the use of gLM scale following published procedures (Monteleone et al., 2017).

Five samples were served to the subjects on the plastic teaspoons, in a monadic, randomized and balanced order, identified by three-digit random codes.

The subjects were asked to use a common procedure to evaluate the viscosity intensity, consisting of applying shearing with the tongue against the palate, for few seconds, when subjects feel that a judgment could be made (Aktar, Chen, Ettelaie & Holmes, 2015).

The subjects were provided with a cup of still water to rinse their mouth before testing the next sample. Data were collected by means of “Fizz Acquisition” software (Biosystèmes, Couternon, France).

4.2.4 Data analysis

The values of stress overshoot were analyzed by means of one-way analysis of variance (ANOVA), and multiple comparison test (Duncan's test) was used to statistically compare the samples ($p \leq 0.05$). Repeated measures ANOVA and multiple comparison test (Duncan's test) were used to evaluate if differences among the samples (used as a repeated factor) were statistically significant ($p \leq 0.05$), in terms of both perceived viscosity and liking. Next, subjects were clustered in three groups: lowly sensitive (LS), moderately sensitive (MS) and highly sensitive (HS), according to the approach proposed by Puleo, Miele, Cavella, Masi, & Di Monaco (2019). For each group (LS, MS, HS) a second repeated-measures ANOVA was used to verify the effect of the different viscosity levels on both sensory liking and perceived viscosity intensity.

Moreover, chi-square test was run to analyse the relationship between viscosity sensitivity and age groups (young vs adult vs old subjects) and gender. The same approach was used to analyse the relationship with gender as well.

Finally, chi-Square test was used to determine the effects of viscosity sensitivity on the solid and liquid food choices (two dichotomized form, see above). The same approach was used to investigate the association between viscosity sensitivity (three sensitivity groups, see above), and neophobia traits (two and three dichotomized form, see above), At the end, a last repeated-measures ANOVA was used to verify the effect of the two neophobia levels on sensory liking.

The XLSTAT statistical software package version 2016.02 (Addinsoft) was used for data analysis.

4.3. Results and Discussion

4.3.1. Rheological properties: Stress Overshoot

As explained in the material and methods section, shear stress was measured as a function of time at the estimated shear rate of the mouth, according to Dickie and Konini (1983).

During start-up flow, a shear rate is suddenly imposed on a viscoelastic fluid held previously at rest. Shear stress produced by this transient deformation displays an initial overshoot, at short times, before reaching a steady-state value at long enough times; hence, the phenomenon is commonly referred to as stress overshoot. Results were described as shear stress overshoot functions, as suggested by Leider and Bird (1974). Figure 4.1 shows the stress overshoot average curves for each tested sample.

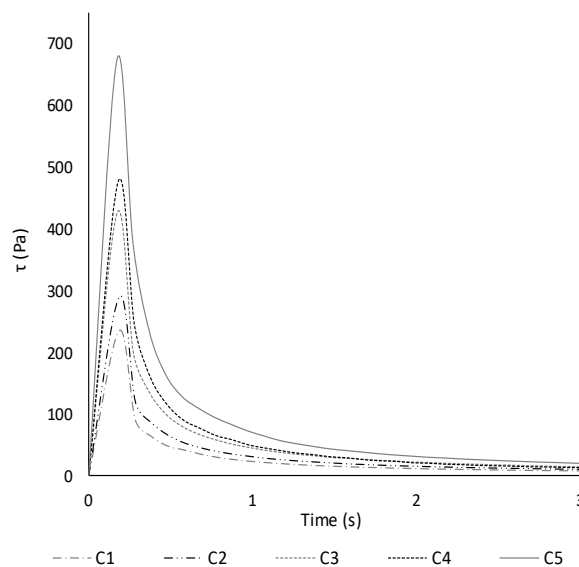


Figure 4.1. Stress overshoot curves (average of three replications) of chocolate creams differing for solid concentrations (C1, 31%, C2, 33%, C3, 36%, C4, 39%, C5, 43%, (w/v)).

As expected, by increasing the solid concentration, the maximum shear stress value (stress overshoot, Pa) increased accordingly. Indeed, the higher solid concentration is, the higher stress overshoot is obtained (da Silva, Arellano, & Martini, 2019; Elliot & Ganz, 1978; Lee & Song, 2011; Ahuja, Lu & Pontanin, 2019; Karyappa & Hashimoto, 2019). Also, the values of the stress overshoot of each sample were extrapolated from each curve and reported as the representative parameter of the viscosity of the tested creams (Figure 4.2).

Stress overshoot data were found useful in modelling the human perception of fluid thickness in the mouth (Dickie and Konini, 1983). The trend of the maximum stress overshoot was described with a linear equation ($R^2=0.95$), as shown in Figure 4.2. In figure 4.2 the significant differences between the samples are also showed (Duncan's test, $p<0.0001$). The linear correlation observed is in accordance with the study conducted by Pangborn, Gibbs, & Tassan, (1978).

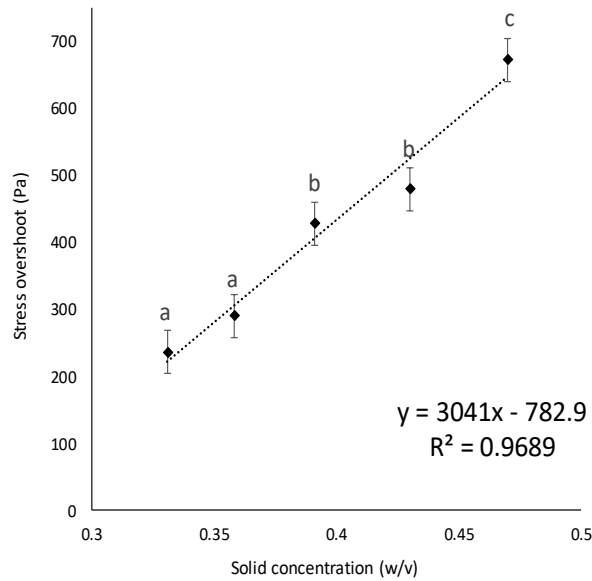


Figure 4.2. Trend of maximum stress overshoot values (Pa), in function of the solid concentration of the tested creams (w/v). The points marked with different letters were significantly different ($p \leq 0.05$).

4.3.2. Sensory evaluation

A total of 176 subjects scored the liking and the viscosity intensity by using the LAM and gLM scale, respectively. By averaging both liking and perceived viscosity scores, significant differences came out among the samples, as shown in Table 4.2.

Table 4.2. Liking and perceived viscosity scores (mean value \pm standard error) given by 176 subjects.

Sample code	Solid concentration (%w/v)	Liking (LAM scale)	Perceived viscosity (gLM scale)
C1	31	60 \pm 1 ^a	22 \pm 1 ^a
C2	33	60.5 \pm 0.9 ^{ab}	21 \pm 1 ^a
C3	36	63.5 \pm 0.9 ^c	28 \pm 1 ^b
C4	39	63.1 \pm 0.9 ^{bc}	40 \pm 1 ^c
C5	43	62.6 \pm 0.9 ^{abc}	37 \pm 2 ^c

In each column, the values followed by different letters were significantly different (Duncan test, $p \leq 0.05$).

Regarding the liking scores, subjects moved on the scale between the labels *slightly liked* (score \approx 59) and *liked* (score \approx 65), with higher values reflecting the intermediate concentrations.

The concentration suggested by the company of the chocolate powder mix to prepare the creams at home is the third one (C3, 36% w/v). The effect of previous experiences on texture preferences is well documented (Baron & Penfield, 1993; Michon, O'Sullivan, Sheehan, Delahunty, & Kerry, 2010; 1993; Monteleone, Frewer, Wakeling, & Mela, 1998; Pliner, 1982). Therefore, the highest scores associated with the intermediate concentration are probably due to the familiarity of a known viscosity. Indeed, our results are in agreement with the study conducted by Richardson-Harman et

al., (2000) that showed how the liking of a range of liquid dairy-products was affected by unfamiliar viscosities due to different fat contents. More in general, familiar texture strongly affects the consumers' preference, as demonstrated in the study conducted by Kälviäinen, Roininen, & Tuorila (2000). Indeed, they demonstrated that consumers' preferences for candies were explained by the liking of commercial candies with a similar texture.

Regarding the perceived viscosity, subjects moved on the scale around the moderate and strong (score ≈ 16 and ≈ 33 , respectively) and between strong and very strong intensity (score ≈ 33 and ≈ 50 , respectively), significantly discriminating among the evaluated samples. By looking at the results (Table 4.2), subjects equally perceived the viscosity of the first two samples (C1, 31% and C2, 33% w/v), as well as of the last two (C4, 39% and C5, 43% w/v). The viscosity of the intermediate sample (36%w/v) was perceived as significantly different, compared to the perceived viscosity of the other samples. As already stressed above, the familiarity with well-known sensory property influences the consumers' sensory perception (Nielsen, Bech-Larsen, & Grunert, 1998). In particular, the study conducted by Kim, Jombart, Valentin, & Kim (2013), showed that Korean consumers were more able to discriminate among green teas than the French consumers, who were not familiar with that kind of beverage. Two years after, the same authors demonstrated that even trained panels were affected by familiarity and liking when asked to sensory describe different samples of tea (Kim, Valentin, & Kim, 2015). Also, Camacho et al. (2015) determined the Just Noticeable Differences (JNDs) of oral thickness perception and the Weber fraction (K) of Newtonian model stimuli (maltodextrin solutions), using the method of constant stimuli with 5 reference stimuli ranging in viscosity from 10 to 100mPas. They found that JND and K for the reference stimulus with a viscosity of 50mPas reflected a higher value corresponding to a lower sensitivity of the panel towards the comparison of the different viscosities. This was due to the fact that the samples set containing the reference with a viscosity of 50mPas was the first to be evaluated, during the sensory sessions. Therefore, the subjects involved in their study might have had more difficulties on discriminating between the stimuli presented in the 1st session compared with following sessions since the subject might have been not sufficiently familiarized with the kind of samples to taste.

To cluster the subjects according to their viscosity sensitivity, the method proposed by Puleo et al. (2019) was used. Therefore, it was assumed that sensory scores should follow the same trend exhibited by instrumentally measured viscosity, with the increasing of the solid concentration, that was, an upward linear relationship. Thus, according to Puleo et al. (2019), the viscosity scores of each subject were fitted with a linear equation, estimating both the angular coefficient (which represented the rate of increase of the linear equation) and the R^2 coefficient, and using them as clustering parameters.

Therefore, subjects whose scores correlated to a linear equation with both high R^2 coefficient and high angular coefficient (values greater than the third quartile, 75% of distribution) were clustered into the high sensitivity group. Accordingly, the low sensitivity group was represented by the subjects to whom corresponded low R^2 coefficients (values lower than the first quartiles, 25% of distribution), and angular coefficient of the linear equation close to zero.

Next, a descriptive analysis was performed on both the angular and the R^2 coefficients, obtained for each subject (Table 4.3). The subjects were then clustered based on the quartile's distribution of both the angular and the R^2 values into three groups: high sensitivity ($n = 45$); moderate sensitivity ($n = 83$); low sensitivity ($n = 48$).

Table 4.3. Quartiles distribution of angular coefficients and the R^2 .

Quartiles	Angular coefficient	R^2
1 st Quartile	113.0	0.40
Median	176.4	0.67
3 rd Quartile	294.8	0.89
Maximum	776.0	0.99
Minimum	1.99	0.001

First, according to Steele and colleagues (2014) no significant effect of age ($p=0.297$) and gender ($p=0.78$) on viscosity sensitivity was found. Therefore, data were analysed without considering any interaction between those variables.

The scores given by each sensitivity group were submitted to repeated measures ANOVA and the significant differences between and within groups were estimated (multiple comparison test, Duncan's test). In table 4.4, liking and perceived viscosity scored by different sensitivity groups are showed.

Table 4.4. Perceived viscosity and liking scores (mean value \pm standard error) given by different sensitivity groups: high sensitivity group (HS); low sensitivity group (LS); moderate sensitivity group (MS).

Sensitivity groups	Perceived viscosity (gLM scale)					Significance within groups
	C1 31% w/v	C2 33% w/v	C3 36% w/v	C4 39% w/v	C5 43% w/v	
LS ($n = 48$)	29 \pm 2 ^b	26 \pm 2 ^b	29 \pm 2	28 \pm 3 ^a	23 \pm 3 ^a	0.12 ^{n.s.}
MS ($n = 83$)	20 \pm 2 ^{aA}	19 \pm 2 ^{aA}	28 \pm 2 ^B	36 \pm 3 ^{bC}	40 \pm 2 ^{bC}	<0.0001
HS ($n = 45$)	17.7 \pm 3 ^{aA}	18 \pm 2 ^{aA}	25 \pm 2 ^B	36 \pm 2 ^{abC}	45 \pm 3 ^{bC}	<0.0001
Significance between groups	0.003	0.01	0.5 ^{n.s.}	0.03	0.0001	-

Sensitivity groups	Liking (LAM scale)					Significance within groups
	C1	C2	C3	C4	C5	
	31% w/v	33% w/v	36% w/v	39% w/v	43% w/v	
LS (n = 48)	60±2	62±2	61±2 ^a	63±2	60±2	0.4 ^{n.s.}
MS (n = 83)	61±2 ^{AB}	60±1 ^A	63±1 ^{abAB}	62±1 ^{AB}	65±1 ^B	0.02
HS (n = 45)	59±2 ^A	60±2 ^A	67±2 ^{bc}	66±2 ^{BC}	62±2 ^{AB}	0.003
Significance between groups	0.89 ^{n.s.}	0.62 ^{n.s.}	0.04	0.24 ^{n.s.}	0.10 ^{n.s.}	-

For each line (upper case) and for each column (lower case), at different letters correspond significantly different values (Duncan test, $p \leq 0.05$).

As it can be observed in table 4.4, regarding the perceived viscosity, the lowly sensitive subjects were not able to perceive the differences in terms of viscosity, while both moderately and highly sensitive subjects significantly discriminated between the samples, giving upward scores as solid concentration increased. The results are absolutely in agreement with previous researches (Puleo et al., 2019). Puleo and colleagues (2019), indeed, clustered people according to their graininess sensitivity, basing their approach on the correlation between the instrumentally measured graininess and the refining time. Also, even though by means of other approaches, individual differences in viscosity sensitivity were found, across the years, by several authors (Aktar, Chen, Ettelaie, & Holmes, 2015; Smith, Logemann, Burghardt, Zecker, & Rademaker, 2006; Steele, 2018; Steele et al., 2014).

However, the first aim of this research was to investigate the effect of viscosity sensitivity on liking of chocolate creams. We started with the assumption that along with the taste sensitivity (Cardello, 1996; Drewnowski, 1997; Tuorila, 2007), also the texture (in this case viscosity) sensitivity can lead to different food preferences. Therefore, regarding the liking, by looking at the differences within each group, lowly sensitive subjects equally liked the five samples, while significant differences were observed within the moderately and highly sensitive subjects. In particular, the most preferred sample by the highly sensitive subjects was the third one (36% w/v).

As viscosity was the only attribute that varied among the samples, subjects who were not able to perceive the difference in term of viscosity, equally liked the samples.

Instead, subjects who were able to well discriminate among the different solid concentrations in the samples, liked more the ideal one, which, being recognized as familiar, was more accepted by consumers.

By looking at the differences between the groups, the viscosity sensitivity affected the liking of only this sample (36% w/v). In particular, the highly sensitive subjects evaluated with higher scores this sample, compared to the lowly and moderately sensitive subjects. As explained above, the intermediate sample is made by ideal solid concentration, therefore this result can suggest that the

highly sensitive subjects were able to identify the familiar sample and their relative high scores reflected their sensitivity.

However, these results seem to be in contrast with the finding of Kremer, Bult, Mojet, & Kroeze, (2007) and Kremer, Mojet, & Kroeze (2005). Indeed, these last two studies showed how the individuals who differed for texture perception, exhibit no clear difference in liking scores when evaluating custards and soups, differing for creaminess.

The second aim of the research was to investigate the role of viscosity sensitivity on food choice. Thus, a choice index for each domain (liquid and solid food) was calculated, for all the participants, as a sum of the choices of the liquid options and of the soft options, assigning to each one a value of 1 (texture indices range=0–5), with higher scores reflecting higher choice of the dense and hard options, respectively. The distribution of liquid (a) and solid (b) choice indexes are showed in Figure 4.3.

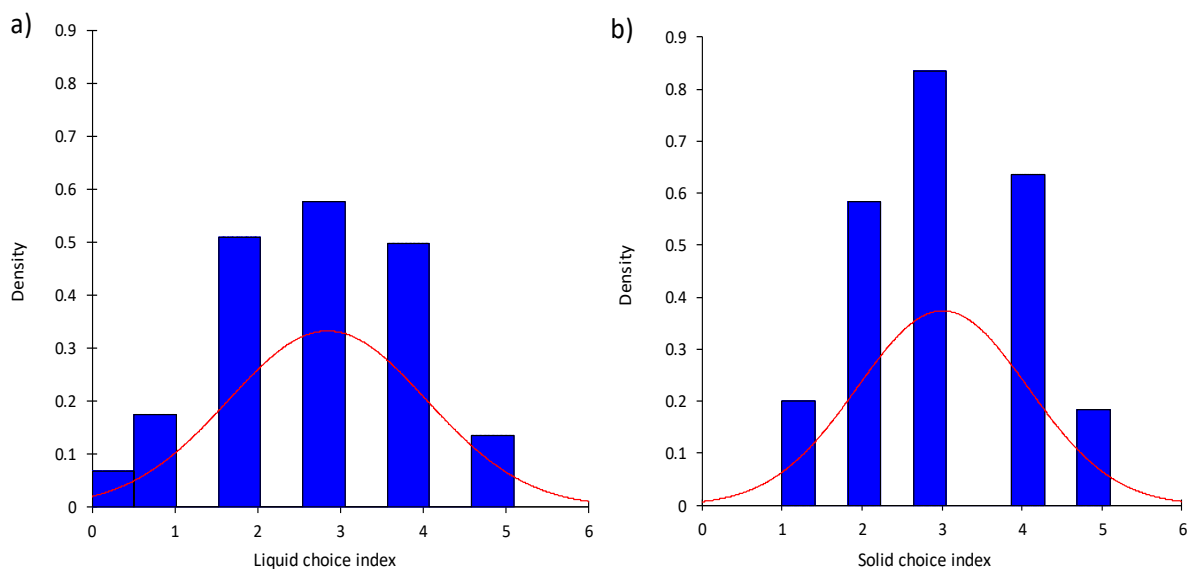


Figure 4.3. Choice index for the liquid domain (a) and the solid domain (b).

From the distribution of the LCI and SCI, median values were extrapolated in order to cluster subjects in two groups of preference. Regarding the liquid domain, subjects with a LCI less than the median value (median LCI = 3) were clustered as subjects who preferred liquid version of the proposed foods; subjects with a LCI higher than the median value, on the other hand, were clustered as subjects who preferred the dense version of the proposed foods. In the same way, regarding the solid domain, subjects with a SCI less than the median value (median SCI = 2) were clustered as subjects who preferred soft version of the proposed foods; subjects with a SCI higher than the median value, on the other hand, were clustered as subjects who preferred the hard version of the proposed foods.

The relationship between viscosity sensitivity and the individual food choice was tested by running a Chi-Square test, using the three groups of sensitivity and the two groups of preference, as variables. Considering the liquid domain, no significant relationships came out ($\chi^2=0.75$; $p=0.69$). It means that the choice of the two options was equally distributed among the three groups of sensitivity. On the other hand, the solid choice was strongly affected by the viscosity sensitivity ($\chi^2=6.9$; $p=0.03$) (Figure 4.4). In particular, highly sensitive subjects tendentially chose soft versions of the proposed options, while the choices of the moderately and lowly sensitive subjects were equally distributed among the soft and the hard option.

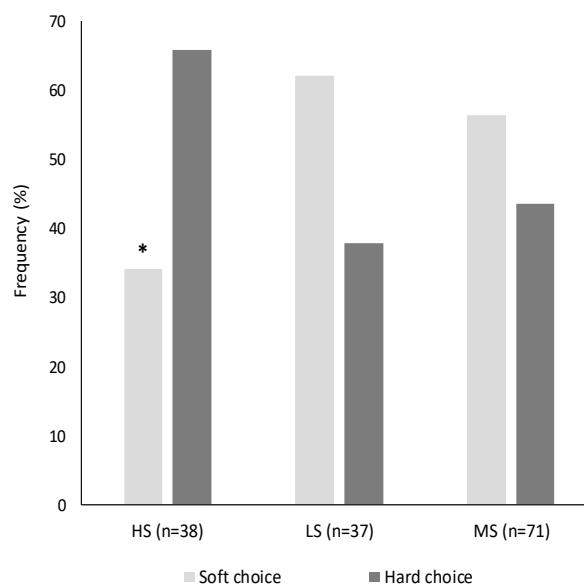


Figure 4.3. Choice frequency (%) for soft and hard option, given by lowly (LS, n=37), moderately (MS, n=71) and highly (HS, n=38) sensitive subjects. The bar marked with a star was significantly different ($p \leq 0.05$).

To discuss these shreds of evidence, a direct comparison with other researches is not possible, because, to the best of the knowledge, the methodology used in this study has not been adopted by other authors. However, some considerations can be done, on the studies reported in the literature. Actually, it is well known that texture sensitivity is influenced by many interacting factors (see Chapter 1), and among them, mouth behaviour plays an important role in the individual differences in sensitivity. Indeed, Jeltema, Beckley, & Vahalik (2015, 2016) clustering individuals according to their mouth behaviour, demonstrated that the Mouth Behaviour groups showed differences in food preference and choice, and that there were food textures that fit “best” with each mouth behaviour. To better explain, taking chocolate as an example, the authors found that smoothers liked (and would have chosen) chocolate that melts fast, instead, crunchers liked (and would have chosen) chocolate that contains nuts, and so on. Their findings demonstrated that individuals differ in the way they manipulate food in their mouths and these differences led to specific food choices. However, in the present study, all the subjects were instructed to follow a standardized procedure to taste the samples,

thus they were not allowed to as-they-like manipulate the samples in their mouth. Also, Jeltema and colleagues started with the assumption that different mouth behaviours were due to different mouth sensitivities, such as saliva flow rate, bite force etc. (see chapter 1). Nevertheless, very recently, the studies conducted by Franks et al. (2019) and Kim & Vickers (2019) strongly contrasted the previous results published by Jeltema and colleagues, demonstrating that mouth behaviour type does not link to mastication behaviour and saliva flow rate observed when consumers masticate foods. Thus, our results suggested that an innate sensitivity exists, which is independent of personal mouth behaviour. However, the individual differences in sensitivity we found, resulted influencing the food choice and preference. This last result seems to be in contrast with the findings of Lukasewycz & Mennella (2012). Indeed, they measured the lingual acuity using a modified letter-identification task and a forced-choice questionnaire assessed to measure the preferences for foods similar in flavour but different in texture. They involved children and their mothers and concluded that age, but not lingual acuity influenced the food choices.

Despite the contrasting discussions reported above, the results of the present research may suggest some speculations. By generally speaking, considering the proposed approach to cluster subjects according to their viscosity sensitivity, the moderately sensitive group represent the average population and, therefore, it reflects the behaviour of the average consumer. The highly and lowly sensitive groups, instead, are respectively the right and left tail of the viscosity sensitivity distribution, representing the outlier consumers. Thus, understanding how texture sensitivity can drive the food choices is necessary for the food companies, with a view to developing new tailored food products for specific target of consumers.

The third aim of this research was to explore the relationship between viscosity sensitivity and individual neophobia traits. This association was tested by means of the chi-square test, considering the three levels of viscosity sensitivity (LS, MS and HS) and first, two levels of neophobia (neophilic and neophobic subjects), obtained using the median value of the calculated total score as cut-off, and secondly, three levels of neophobia according to the quartile distributions of the individual total scores. The obtained results were the same both considering two and three levels of neophobia, therefore the following results are described taking into account only the two-levels approach, because easier to explain, and already used in other studies (Demattè et al., 2013; Spinelli et al., 2018) Neophilic (n=71) and neophobic (n=75) subjects did not significantly differ in viscosity sensitivity group distribution ($\chi^2 = 3.16$; $p=0.21$).

Food neophobia is considered an adaptive, evolutionary response, which prevents from the ingestion of poisonous substances more commonly found in fruits and vegetables (i.e., bitter, sour, and astringent compounds) (Pliner & Salvy, 2006). Therefore, it could have been reasonable to

hypothesize that neophobic subjects were more sensitive in the sensory perception, detecting also little changes of the food properties.

However, the present result is in accordance with the study of Lukasewycz and Mennella (2012). They measured in children and adults whether lingual tactile acuity – the ability to identify raised alphabetical letters with the tips of their tongues – was related to food neophobia. No such a relation was found, which suggests that neophobic subjects are not more sensitive in texture perception. If extended to sensitivity to other stimuli, the present results are in accordance also with the study conducted by Törnwall et al. (2014), where neophobic and neophilic subjects did not differ in their PROP responsiveness.

It is however possible that, although their perceptual abilities are not different, their appreciation of different levels of texture stimuli could be. With this assumption, repeated one-way ANOVA was run, using the two neophobia levels as fixed variables, to investigate the differences in liking within the groups. There were no significant differences among the liking scores given by neophilic subjects ($F_{4,280}=1.35$; $p=0.25$). This result suggests that neophilic subjects equally liked all the tasted samples and did not discriminate between them in terms of liking. On the other hand, neophobic subjects' scores were significantly different among the samples ($F_{4,296}=3.3$; $p=0.01$), giving higher scores to the intermediate concentrations. This last evidence can be explained by the fact that high levels of neophobia, basically, reflect a rejection of unfamiliar foods (Jaeger, Rasmussen, & Prescott, 2017). Also, Tuorila, Lähteenmäki, Pohjalainen, & Lotti, (2001) speculated that subjects having high levels of food neophobia are possibly not only those who are afraid of new foods; they may also be individuals who have little interest in foods.

On these reasonable hypotheses, the present results find accordance with other studies which showed such a relationship between food neophobia levels and food familiarity (Laureati et al., 2018; Spinelli et al., 2018). Those studies, indeed, demonstrated that neophobic subjects liked significantly less the unfamiliar food, than the neophilic ones.

The discussions of this last part deserve to have further considerations. Although no significant relationship was found between viscosity sensitivity and food neophobia, the fact that neophobic subjects discriminated between the samples in terms of liking may suggest the existence of a different perception. Thus, since the viscosity was the only thing that changed among the five chocolate creams, it seems that neophobic subjects were able to perceive those differences, in contrast to what observed with the neophilic subjects. In other words, neophobic subjects seemed to show a higher viscosity acuity than neophilic ones. As a final remark, it can be highlighted that the actual product prepared in this study is a rather familiar product in Italy, thus it would be interesting to replicate the

study in order to verify whether the relationship between texture sensitivity and food neophobia would be stronger when using novel and unfamiliar foods.

4.4. Conclusion

This study was conducted to better explore the role of texture sensitivity on food preference and choice, which is rather dubious. In order to study the effect of viscosity sensitivity on liking of chocolate creams and food choice, the method proposed by Puleo and colleagues (2019) was first used to cluster people according to their viscosity sensitivity. To this aim, five chocolate creams were prepared by changing the solid concentration in order to obtain different levels of viscosity. Instrumental data showed that different solid concentrations resulted in different levels of viscosity, via the stress overshoot index, and that this index was correlated to solid concentration by a linear equation. By assuming that sensory viscosity scores must correlate to solid concentration in the same way the stress overshoot index does, the R^2 values and the estimated angular coefficient of the linear equation, derived by the best fit of the data relative to each subject, were used to cluster the subjects into three groups having different graininess sensitivity. The viscosity sensitivity significantly affected the liking of chocolate creams and the solid food choice. Moreover, liking of chocolate creams was also affected by the individual level of neophobia, that in turn, was not correlated to the viscosity sensitivity.

The presented findings confirm that texture sensitivity and food neophobia both affect what a person likes and drives what a person chooses to eat. In the view of developing new food products, the possibility to have a picture of subjects, clustered for different sensitivities, is two-fold helpful for the food industries. In this way, indeed, the food industries could both look for products designed to the average consumers, and for tailored food products targeted to the outliers.

Finally, the authors are aware that currently there are different ways to phenotype people according to their sensitivities, such as PROP status, thermal status, sweetness liking etc. Therefore, future researches could investigate the relationships between all these individual sensitivities, trying to find a greatest common divisor to explain the human sensory sensitivity.

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Chapter 5

Is it still still water?

Detection thresholds of sparkling sensations are not influenced by consumption behaviour and preferences for carbonated beverages

Submitted paper

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Abstract

Little is known about how trigeminal stimulation sensitivity is affected by consumption habits and consumer characteristics. The aim of this study was to determine how detection thresholds for and perception of sparkling sensations in carbonated mineral water are affected by familiarity with carbonated beverages and individual consumer characteristics. One hundred subjects differing in sparkling water consumption behaviour (non-consumers, moderate consumers, regular consumers) participated. First, sparkling sensation detection thresholds were determined using the method of best estimate threshold (BET) with CO₂ concentrations ranging from 0.03 to 1.05 g/L. Secondly, intensity of sparkling sensation and liking of five sparkling waters (CO₂ concentrations ranging from 0.21 to 4.92 g/L) were assessed. To characterize consumers, consumption frequency of sparkling beverages, PROP taster status, demographic information, health interest, eating behaviour and sensitivity to punishment and reward were determined. Average detection threshold of sparkling sensation (BET) was 0.44 g/L CO₂ concentration. BET of sparkling sensation was not affected by consumption frequency of sparkling water and was not related to PROP taster status and consumer psychological variables. Liking and perception of sparkling intensity of carbonated mineral water were significantly affected by consumption frequency of sparkling water. Non-consumers liked sparkling water significantly more than moderate or regular consumers, probably because non-consumers did not drink sparkling water for cost reasons. Sparkling sensations were perceived significantly more intensive by non-consumers compared to moderate and regular consumers. We conclude that detection thresholds of sparkling sensations are independent of consumption behaviour of and preferences for carbonated beverages.

Keywords: Trigeminal perceptions; consumer evaluation; individual sensitivity; food familiarity

5.1 Introduction

Consumers differently elaborate the sensory information perceived by taste, touch, vision and smell (Dunn, 1999) leading to differences in enjoyment of the eating experience. Many studies highlighted that sensory sensitivity plays a pivotal role in food preference and choice (Kaminski *et al.*, 2000; Coulthard *et al.*, 2008; Monteleone *et al.*, 2017). Little is known about how food consumption habits, food familiarity and attitude influence sensory sensitivity.

Taste sensitivity can be improved through training, so that taste thresholds decrease with training (Meilgaard *et al.*, 1999; Mojet *et al.*, 2001). Engen (1960) found increments in taste sensitivity with experience suggesting that it is a learning effect. It has been shown that taste sensitivity, so taste thresholds, can be affected by individual physiological factors, such as gender, age, health status and nutritional status and by the interaction between these variables (Mojet *et al.*, 2001). Regarding the influence of age and gender on taste sensitivity, many studies are contradictory. Sweet taste sensitivity was affected by age in the studies of Schiffman (1993) and Mojet *et al.* (2001), but not in the studies of Hyde and Feller (1981) and Nordin *et al.* (2003). A significant decrease in sour sensitivity with increasing age was reported by Bartoshuk *et al.* (1986) and Yamauchi *et al.* (2002) whereas others reported no effect of age on sour sensitivity (Weiffenbach, Baum, Burghauser, 1982; Cowart, 1989). Yamauchi and colleagues (2002) demonstrated an effect of gender on detection thresholds for bitterness but not for sweetness, saltiness and sourness. In particular, female subjects had significantly lower thresholds compared to males for sour taste. Wardwell *et al.* (2009) observed a gender effect on bitter recognition thresholds in elderly but not in young adults.

Duffy (2007) reported that bitterness sensitivity may decrease with age and during menopause, while during pregnancy, bitterness sensitivity rises to its peak during the first trimester and is lowest in the third trimester. Nolden (2019) reported reduced sweet taste sensitivity associated with a reduced appetite and food intake in individuals undergoing treatment for cancer (Vignini, 2019). Moreover, previous studies have addressed a link between taste sensitivity and BMI (Simchen 2016; Vignini 2019). However, results are contradictory. Simchen (2016) found a higher sweetness sensitivity in consumer with an inclination of developing obesity and diabetes. On the other hand, Vignini (2019) found no differences between healthy and obese individuals regarding sweetness sensitivity. Moreover, a decrease in sour taste sensitivity has been shown in subjects with BMI >28 kg/m² (Vignini, 2019). Cox (1999) found that obese adults had a higher preference for salty foods, compared to normal-weight adults suggesting an alteration in salt sensitivity might have led to changes in preference.

On the other hand, it is well known that the sense of taste is governed by specialized organs on the tongue and soft palate containing specific receptors and organized roughly according to their

sensitivity to salt, sour, sweet, and bitter tastes (Lawless and Heymann, 2010). Furthermore, given this human genetic inheritance several studies pointed out that taste sensitivity might be innate (McCorkindal, 1992; Duffy & Bartoshuk, 2000). Moreover, other biological variables including genetic factors (Allen, McGeary, & Hayes, 2014; Kim et al., 2004; Perry et al., 2007; Tærnwall, Silventoinen, Kaprio, & Tuorila, 2012) or differences in oral anatomy (Bartoshuk, 1993; Miller & Reedy, 1990) do not cause differences in taste sensitivity, suggesting that sensory sensitivity is innate. The vast majority of studies focused on the influence of individual factors such as mood, physiology, health status etc. on taste sensitivity. To the best of our knowledge, little is known about the influence of these individual factors on sensitivity of trigeminal sensations.

Trigeminal sensations are touch-position and pain-temperature sensations perceived by mechanoreceptors and nociceptors which activate the trigeminal nerve system. Trigeminal sensations arise as a result of intense chemical (e.g., chili powder), mechanical (e.g., cutting, crushing), or thermal (heat and cold sensations) stimulation of sensory nerve cells, that trigger a variety of physiological and behavioural responses and usually result in a subjective experience of pain in humans (Darian-Smith, 1973). Sparkling sensations are a well-known example of trigeminal stimulation. Carbon dioxide (CO₂) is commonly used in beverages to provoke sparkling sensations. During consumption of carbonated beverages, dissolved CO₂ acts on both trigeminal (Dessirier et al., 2000; Kleeman et al., 2009; Meusel et al., 2010) and gustatory receptors, via the conversion of dissolved CO₂ to carbonic acid by carbonic anhydrase (Symoneaux et al., 2015). In addition, CO₂ provides tactile stimulation of mechanoreceptors in the oral cavity through the bursting of bubbles (Chandrashekar et al., 2009; Dunkel and Hofmann, 2010). As expected, perceived sparkling intensity increased with increasing CO₂ concentration (Wise et al., 2013). Sparkling sensations caused by carbonation are pleasurable and desirable in many beverages for many consumers even though they can be irritating or painful (Dessirier et al., 2000).

Carbonated mineral water is an ideal beverage to study carbonation and sparkling sensations since it only contains water, minerals and CO₂. The detection threshold of sparkling sensations caused by CO₂ was previously found to be 0.26g/L (Le Calvè et al., 2008). An increase of CO₂ concentration by 2g/L resulted in an increase in perceived sparkling intensity (Le Calvè et al., 2008). The study focused on the determination of CO₂ average thresholds in carbonated water and did not address underlying causes for individual differences in thresholds. Wright and colleagues (2003) quantified the detection threshold of CO₂ in Swiss-style yogurt (0.263g/L). Relationships between trigeminal thresholds and individual characteristics were not explored.

Several studies explored other trigeminal stimuli, such as pungency caused by capsaicin, and its relationship with individual consumer characteristics. Familiarity with spicy foods has been

demonstrated to correlate with pungency sensitivity, and the preference for spicy foods. Ludy & Mattes (2012) demonstrated that spicy food consumers perceived spicy foods as more palatable than non-spicy food consumers. Spicy food consumers were also better able to discriminate different levels of pungency than non-consumers. Inter-individual differences in pungency preferences have been suggested to be related to personality traits. Consuming spicy foods, for example, may appeal to thrill-seekers, who enjoy the body's feeling of imminent danger. Correlations between sensation-seeking behavior and the consumption of "unusual spices" have been reported, suggesting that sensation seekers appreciate dangerous sensations from foods, such as the perceived pungency, in addition to taste and texture (Byrnes and Hayes, 2016).

Little is known about how sensitivity of sparkling sensations is related to consumption habits for sparkling beverages and individual consumer characteristics. We hypothesize that psychological traits and frequency of consumption of sparkling waters affects sparkling sensitivity. We hypothesize that a higher consumption of carbonated water leads to an increase in thresholds to perceive a sparkling sensation. Those factors could be crucial in understanding consumer preference for carbonated beverages. As sensation-seeking behavior affects consumption of pungent foods (Byrnes and Hayes, 2016), it could also affect preference for carbonated beverages. We hypothesize that more punishment and reward sensitive individuals are more sensitive to trigeminal sparkling sensations. Also, if consumption of carbonated beverages may affect threshold as previously hypothesized, then measuring attitude towards healthy food could give insight into consumption of sparkling water, and consequently, carbonation threshold. This last hypothesis is due to the fact that subjects might not consume sparkling water for health reasons, since it is known that the consumption of sparkling water could contribute to dental erosion (Reddy et al., 2016).

The aim of this study was to determine how detection thresholds for and perception of sparkling sensations in carbonated mineral water are affected by familiarity with carbonated beverages and individual consumer characteristics. First, sparkling sensation detection thresholds were determined using the method of best estimate threshold in consumers differing in consumption behaviour of sparkling water. Secondly, intensity of sparkling sensation and liking of different sparkling waters varying in CO₂ concentration were assessed in the same consumers. Consumers were characterized by demographic information, consumption frequency of sparkling water, PROP taster status, health interest, eating behaviour and sensitivity to punishment and reward. Relationships between liking and perceived sparkling intensity of carbonated mineral water with the consumer characteristics were explored.

5.2. Materials and methods

An overview of the study design and approach is provided in table 5.1 and described in detail in the following paragraphs.

Table 5.1. Overview of the study design and approach.

Online survey	Variables	Options
Socio-demographics	Age	Years
	Gender	Male/Female
	Nationality	Country
Anthropometric	Weight (self-reported)	Kg
	Height (self-reported)	cm
Physical health	How often do you smoke?	Never; 1 time/month; 1 time-2 times/week; 3 times-6 times /week; every day
	Drugs consumption	For blood pressure/or arthritic pain/for digestion/ diabetes/ antidepressant/ hormonal therapies/ to sleep/ to be mentally focused/ I do not regularly use any of the drugs listed above
Familiarity and liking	How often do you consume sparkling water?	Never; 1 time/month; 1 time-2 times/ week; 3 times-6 times/week; / 1 time/day and more)
	How much do you like sparkling water?	9-point hedonic scale (1=extreme dislike; 9=extreme like)
	What is the reason for you to drink sparkling water?	Taste/ texture/ price/ convenience/ cultural identity/ I do not drink sparkling water
	What is the reason for you to not drink sparkling water?	Taste/ texture/ price/ health/ never tried before/ other
Dutch Eating Behavior Questionnaire (DEBQ)	33 items – 3 domains:	5-point scale:
	- Restrained eating	- never (1)
	- Emotional eating	- seldom (2)
	- External eating	- sometimes (3) - often (4) - very often (5)
First session	Samples/Items	Test/Question format
Sparkling Detection Threshold	Seven samples (CO ₂ content: 0.03,0.06, 0.10, 0.13, 0.26, 0.52, 1.05 g/L)	Standard ascending ASTM-E670 method: Seven three-alternative forced choice tests (3AFC)
Health and Taste Attitudes Scale (HTAS)	38 items – 6 domains: 3 health-related domains: - General Health Interest (GHI)	7-point Likert scale (1 = disagree strongly; 7 = agree strongly)

	- Light Products Interest (LPI)	
	- Natural Products Interest (NPI)	
	3 taste-related domains:	
	- Craving for Sweet Foods (CSF)	
	- Food as a Reward (FR)	
	- Pleasure (P)	
Private Body Consciousness (PBC)	5 items	5-point scale (1 = extremely uncharacteristic; 5 = extremely characteristic)
PROP test (Repetition I)	3.2 mM PROP solution. Two replicates	Generalized Labeled Magnitude Scale (0–100), gLMS
Second session	Samples/Items	Test/Question format
Liking test	Five samples (CO ₂ content: 0.21, 1.05, 1.68, 2.9, 4.2 g/L)	Labeled Affective Magnitude Scale (0–100), LAMs
Sparkling intensity test	Five samples (CO ₂ content: 0.21, 1.05, 1.68, 2.9, 4.2 g/L)	Generalized Labelled Magnitude Scale (0–100), gLMS
Sensitivity to Punishment and Sensitivity to Reward Questionnaire (SPSRQ)	48 items – 2 subscales: - Sensitivity to punishment (SP) - Sensitivity to reward (SR)	Yes/No
PROP test (Repetition II)	3.2 mM PROP solution. Two replicates	Generalized Labelled Magnitude Scale (0–100), gLMS

5.2.1 Subjects

The study was performed at Wageningen University (The Netherlands) with n= 100 participants (females = 58; males = 42; average age = 25±4 yrs). Participants were recruited by announcements posted around Wageningen University and student buildings, using social media, word of mouth and emails. Consumers differing in consumption behaviour of sparkling water were included. Participants gave written informed consent according to the principles of the Declaration of Helsinki. Participants were requested to complete an online questionnaire and to attend two sessions of 30 minutes each in meeting rooms with separators (see table 5.1). All tests were performed on paper and then digitalized.

5.2.2 Sample preparation

Commercially available sparkling mineral water (CO₂ concentration: 4.2g/L, SPA Intense, Barisart, Mineral Sparkling Water) was mixed in different ratios with commercially available still mineral water (SPA Reine, Pure mineral water) to obtain mineral waters differing in CO₂ concentration as shown in table 5.2. Samples were stored in closed plastic bottles at 4°C and served chilled at around 10°C in plastic cups containing 10 mL. The time between the mixing of waters and serving to the participants was controlled and always less than 30 min to minimize loss of CO₂.

Table 5.2: Composition and CO₂ concentration of all sparkling waters.

Detection threshold tests				Liking and sparkling intensity tests			
Sample code	CO ₂ [g/L]	Sparkling water (%)	Still water (%)	Sample code	CO ₂ [g/L]	Sparkling water (%)	Still water (%)
T1	0.03	0.8	99.2	S1	0.21	5	95
T2	0.06	1.5	98.5	S2	1.05	25	75
T3	0.10	2.4	97.6	S3	1.68	40	60
T4	0.13	3.2	96.8	S4	2.90	70	30
T5	0.26	6.2	93.8	S5	4.20	100	0
T6	0.52	12.4	87.6	-	-	-	-
T7	1.05	25.0	75.0	-	-	-	-

5.2.3 Online survey

Participants were asked to fill in an online survey at home on the day preceding the first session (table 5.1). Personal information was collected on sociodemographic (age, gender and nationality), anthropometric (weight and height) and physical health measures (tobacco smoking habit and drugs consumption). The online questionnaire included both multiple-choice questions (select one or select multiple) and open-ended questions. Information about frequency of consumption of sparkling water was collected. Responses were recorded on a 5-point category scale (frequency of consumption of sparkling water: never, 1 time/month, 1 time–2 times/week, 3–6 times/week, 1 time/day or more). Frequency of consumption of sparkling water was expressed as yearly frequency and log-transformed to reduce skew. Participants were clustered in three groups based on frequency of consumption of sparkling water: non-consumers (never), moderate consumers (1 time/month) and regular consumers

(> 1 time–2 times/week). The Dutch Eating Behaviour Questionnaire (DEBQ) was used to assess restrained, emotional and external eating behaviours.

5.2.4 First session

During the first session of 30 min, participants performed sparkling sensation threshold tests and filled in the Health and Taste Attitude Scales questionnaire (HTAS), the Private Body Consciousness questionnaire (PBC) and the PROP status test (see table 5.1).

5.2.4.1 Threshold measurements

The standard ASTM-E670 method of the Best Estimate Threshold (BET) was used. Seven three-alternative forced choice (3AFC) tests were performed with ascending CO₂ concentrations. For each 3AFC test, participants had to identify the odd sample among three. The test was conducted in three replications by n=100 participants. Seven concentrations of CO₂ were tested (table 2): 0.03, 0.06, 0.10, 0.13, 0.26, 0.52 and 1.05 g/L CO₂. Samples were marked with random three-digit codes and evaluated following a balanced randomized design (AAB, ABA, BAA) within the series. The 3AFC combinations were balanced so that half of the participants evaluated 3AFC's in which still water was included twice and sparkling water once as the odd sample, whereas the other half of participants evaluated 3AFC's in which sparkling water was included twice and still water once as the odd sample.

5.2.4.2 Health and Taste Attitudes Scale (HTAS)

The HTAS was used to assess the orientation of the participants towards health and hedonic aspects of food (Roininen, Lahteenmaki & Tuorila, 1999). The HTAS was used with 7-point category scales from “disagree strongly” to “agree strongly”. For each participant a mean value was determined for each domain after recodification of negatively worded items. The items and domains are summarised in Table 5.1.

5.2.4.3 Private Body Consciousness (PBC)

Private Body Consciousness (PBC) defined as the disposition to focus on internal bodily sensations (awareness of internal sensations) was quantified using the 5-item methodology developed by Miller, Murphy & Buss (1981), (table 5.1). The individual score was computed as the sum of the ratings given to five statements using a 5-point scale anchored from extremely uncharacteristic to extremely characteristic. The PBC score ranges from 5 to 25 with higher scores reflecting higher PBC levels.

5.2.4.4 Taste responsiveness to PROP

A 3.2mM 6-n-propyl-2thiouracil (European Pharmacopoeia Reference Standard, Sigma Aldrich, Milano, IT) solution (PROP) was prepared by dissolving 0.545 g/L of PROP in deionized water (Prescott et al., 2004). Participants received 2 identical samples (10 mL) coded with 3-digit codes. They were instructed to hold each sample for 10 s in the oral cavity, then spit it out, wait for 20s and

then evaluate the bitterness intensity using the Generalized Labeled Magnitude scale (GLMs). Before the evaluation, the use of the gLMs (0-100) was explained to all participants (Bartoshuk et al., 2004). Subjects had a 90 s break to avoid carry-over effects. During the break, subjects rinsed their mouth with distilled water for 30 s, ate plain crackers for 30 s, and finally rinsed their mouths with water for 30 s. PROP scores were categorized into three groups (Non-taster, Medium taster and Super taster) with cut-offs by the first and third quartile. The PROP taste test were performed as a duplicate measurement in all subjects (n=100) at the end of the second session (table 5.1).

5.2.5 Second session

During the second session of 30 min, participants evaluated liking and perceived sparkling intensity of sparkling waters (table 5.2). Participants were asked to fill in the Sensitivity to Reward and Sensitivity to Punishment questionnaire and to perform a replication of the PROP status test (table 5.1).

5.2.5.1 Liking and sparkling intensity of sparkling waters

Liking and perceived sparkling intensity of five sparkling waters (0.21, 1.05, 1.68, 2.9 and 4.2 g/L CO₂) were assessed using the Labelled Affective Magnitude scale (LAMs) and Generalized Labelled Magnitude scale (gLMs), respectively (table 5.1). Between samples participants had to rinse their mouth with still water. Carbonated waters (serving size 10 mL per cup) were served at around 10°C in three digit coded cups. The presentation order was randomized over subjects.

5.2.5.2 Sensitivity to punishment and reward questionnaire (SPSRQ)

The Sensitivity to punishment and reward questionnaire (SPSRQ) developed by Torrubia et al. (2001) quantifies the responsiveness of Behavioural Inhibition System (BIS) and Behavioural Activation System (BAS). The SP scale sets situations that describe individual differences in reactivity to BIS. The SR scale measures the functioning of the BAS dealing with certain rewards, such as money, gender, social power and approval or praise. The SP and SR scales were answered with yes/no. The scores ranged from 0 to 24 for each scale. The higher scores reflected higher sensitivity to punishment and to reward (Spinelli et al., 2018).

5.2.6. Statistical data analysis

The distribution of the variables frequency of consumption, nationality, DEBq, HTAS, SR, SP, PROP status were analysed by descriptive statistical tools. The internal consistency of each domain of the psychological questionnaires was measured by Cronbach's alpha.

Regarding the data coming from the first session, first, the BET of each participant was calculated as the geometric mean of both, the value of the concentration at which the participant first answered correctly, and all higher concentrations were also correct, and the highest concentration missed. The

geometric mean was applied to determine the group carbonation detection threshold (BET). Secondly, Pearson's correlations were calculated to determine relationships between consumption frequency, psychological and behavioural traits, PROP test responses with individual detection thresholds for sparkling sensation. One-way ANOVA was run to analyse the effect of gender and nationality on sparkling detection thresholds.

The effect of all the individual characteristics on the sparkling detection thresholds was also investigated by means of the chi-square test. Personality traits ratings were first categorized using the characteristic values of the percentile distribution (first and third quartiles), and then three segments or levels of interest/sensitive (Low, moderate and high) were considered. In the same way, PROP scores were categorized into three groups (Non-taster, Medium taster and Super taster) with cut-offs by the first and third quartile. Finally, participants were clustered in two groups according to their detection threshold for sparkling sensations considering the group geometric mean as cut-off.

Regarding the data coming from the second session repeated measures ANOVA model was used to analyse the differences between samples for liking and perceived sparkling intensity, and to verify the effect of PROP status and consumption frequency on both liking and perceived sparkling intensity. Pearson's correlations were calculated to determine relationships between psychological and behavioural traits and liking and perceived sparkling intensity. All data analyses were conducted using XLSTAT (Version 2016.1.01, Addinsoft, Andernach, Germany). A significance level of $p < 0.05$ was chosen.

5.3. Results

Characteristics of the $n=100$ participants in the study are reported in Table 5.3.

Table 5.3. Individual characteristics of $n=100$ participants.

Variables	Males (n=43)	Females (n=57)	Total (n=100)
<i>Age (years)</i>	25±3	24±4	25±4
<i>Nationality</i>			
Chinese	14	16	30
Dutch	7	15	22
Indonesian	3	7	10
Italian	7	3	10
Others	12	16	28
<i>Body mass index (kg/m²)</i>			
Under weight (<18.50)	0	4	4
Normal range (18.50–24.99)	32	47	79
Overweight (25.00–29.99)	10	4	14
Obese (>30.00)	1	2	3

<i>Sparkling water consumption</i>			
Non-consumers (never)	8	18	26
Medium consumers (1 time/month)	24	27	51
Frequent consumers (> 1-2 times/week)	11	12	23
<i>Smoking</i>			
Never	34	52	86
Once a month	2	2	4
1-2 times/week	2	0	2
3-6 times/week	1	1	2
Everyday	4	2	6
<i>Prop status</i>			
Non taster	11	15	26
Medium taster	19	29	48
Super taster	13	13	26

According to the consumption frequency of sparkling water, participants were clustered in three groups: non consumers (never, n=26), moderate consumers (1 time/month, n=51) and regular consumers (> 1 time–2 times/week, n=23).

Distribution of PROP ratings among participants showed a normal distribution. The upper limit of the first quartile and lower limit of the third quartile were 26 and 61 on gLMs, respectively.

Regarding the psychological traits, the internal consistency of each domain for each questionnaire was validated by Cronbach's alpha, as shown in Table 5.4 together with the scores range. Concerning the internal consistency of each questionnaire, only Using Food as a Pleasure and PBC revealed a low internal validity (Cronbach's $\alpha = 0.55$ and 0.56 respectively). For this reason, Using Food as a Pleasure domain and PBC questionnaire results were excluded from the data elaboration.

Table 5.4. Descriptive statistics and Cronbach's alpha (α) for each questionnaire.

Questionnaires/Domains	Theoretical range	Min	Max	Mean	SD	α
HTAS						
General Health Interest (GHI)	8-56	14	55	34.82	8.15	0.84
Light Product Interest (LPI)	6-42	6	40	23.54	5.76	0.81
Natural Product Interest (NPI)	6-42	7	37	21.81	6.88	0.80
Craving for Sweet Foods (CSF)	6-42	12	41	27.20	6.65	0.80
Using Food as a Reward (UFR)	6-42	13	42	29.51	5.67	0.73
Using Food as a Pleasure (UFP)	6-42	16	41	28.77	5.17	0.55
SPSRQ						
Sensitivity to Punishment (SP)	0-24	0	23	11.35	5.36	0.84

Sensitivity to Reward (SR)	0-24	1	22	10.52	4.10	0.73
DEBQ						
Restrained eating	10-50	11	50	25.95	8.48	0.90
Emotional eating	13-65	13	59	32.59	10.9	0.93
External eating	10-50	16	46	32.90	5.83	0.80
PBC	5-25	10	22	15.94	3.12	0.56

5.3.1 Relationships between individual sparkling detection thresholds (sparkling sensitivity) and individual variables

The individual detection thresholds for sparkling sensation ranged from 0.02 g/L to 1.48 g/L CO₂. The BET group geometric mean detection threshold for sparkling sensation was 0.44 g/L CO₂ with a 95% level of confidence. No significant correlations were observed between detection threshold for sparkling sensation (sparkling sensitivity) and any of the individual variables (consumption behaviour (Figure 5.1), PROP status, GHI, LPI, NPI, Craving for sweet food, Using food as reward, Restrained eating, Emotional eating, External eating, SP, SR, gender). In particular, detection thresholds were independent of consumption behaviour ($r=-0.164$, $p=0.101$, see figure 1), PROP status ($r=-0.153$, $p=0.127$), GHI ($r=0.032$, $p=0.751$), LPI ($r=-0.016$, $p=0.877$), NPI ($r=0.129$, $p=0.197$), Craving for sweet food ($r=-0.038$, $p=0.708$), Using food as reward ($r=-0.043$, $p=0.671$), Restrained eating ($r=-0.020$, $p=0.230$), Emotional eating ($r=-0.186$, $p=0.063$), External eating ($r=-0.180$, $p=0.072$), SP ($r=0.068$, $p=0.500$), SR ($r=-0.088$, $p=0.379$).

No significant differences were observed between males and females in terms of sparkling detection thresholds ($p=0.862$).

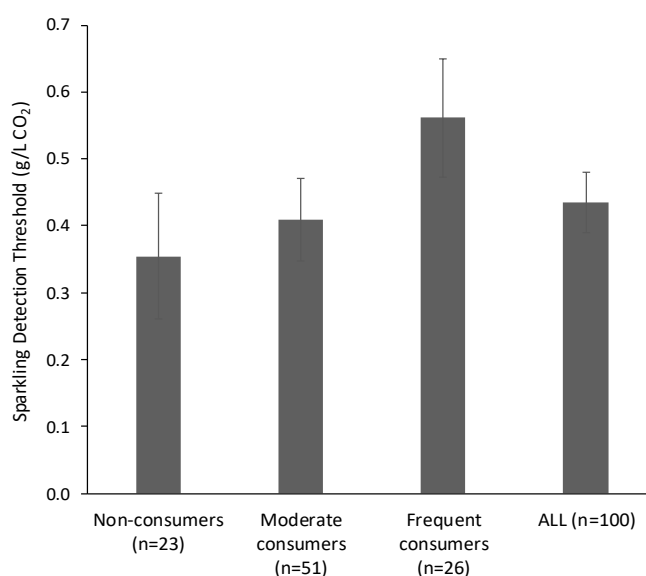


Figure 5.1. Sparkling detection threshold (g/L CO₂) of non-consumers (never), moderate consumers (1 time per month) and frequent consumers (at least 1-2 times per week) of sparkling water. The average value (BET) calculated for all participants (n=100) is also shown. Error bars indicate standard error.

To investigate the effect of nationality on detection threshold 28 participants from different nationalities were excluded from the dataset, since there was only one participant per nationality. Four groups were compared (Chinese, n=30; Dutch, n=22; Indonesian, n=10; Italian, n=10). A significant effect of nationality on detection threshold of sparkling sensations (sparkling sensitivity) was observed. Italian consumers had a higher detection threshold compared to Dutch (p=0.035) and Chinese (p=0.043) consumers (table 5.5).

Table 5.5. Sparkling detection threshold of four nationality groups.

Nationality	Sparkling DT	Standard error	Lower bound	Upper bound	Groups	
			(95%)	(95%)		
Italian	0.742	0.130	0.482	1.003	A	
Indonesian	0.524	0.130	0.264	0.784	A	B
Chinese	0.372	0.075	0.222	0.522		B
Dutch	0.305	0.088	0.130	0.481		B

To further analyse the data, different chi-square tests were performed using participants clustered in different groups according to all the individual variables, including the sparkling detection thresholds. Participants (n=100) were clustered in two sensitivity groups using the average detection threshold (BET) as cut-off. No significant correlations were observed between the investigated variables (table 5.6). Furthermore, the nationality differences observed earlier, were not significant anymore.

Table 5.6. Effect of individual factors on sparkling detection thresholds - χ^2 analysis on frequencies.

Individual factors	χ^2 observed	χ^2 critical	p-value
Gender	0.003	3.841	0.955
Nationality	0.927	7.815	0.819
Frequency of consumption	3.968	5.991	0.138
PROP status	0.403	5.991	0.818
GHI	0.003	5.991	0.999
LPI	0.232	5.991	0.890
NPI	0.926	5.991	0.629
CSF	1.715	5.991	0.424
UFR	4.855	5.991	0.088
SP	0.417	5.991	0.812
SR	0.646	5.991	0.724
Restrained eating	3.483	5.991	0.175

Emotional eating	0.305	5.991	0.859
External eating	2.818	5.991	0.244

5.3.2 Liking and perception of sparkling intensity of carbonated mineral waters

Liking and perceived sparkling intensity of carbonated mineral waters ranging in CO₂ concentration from 0.21 to 4.2 g/L were determined using LAM and gLM scales, respectively (Fig. 5.2 a, b). Liking of the carbonated mineral water with the lowest CO₂ concentration (0.21 g/L) was significantly higher than liking of all other samples ($p = 0.001$). Regarding perceived sparkling intensity, all mineral waters were perceived significantly different from each other ($p < 0.0001$) and, as expected, the scores followed an upward trend with increasing intensity with increasing CO₂ concentration.

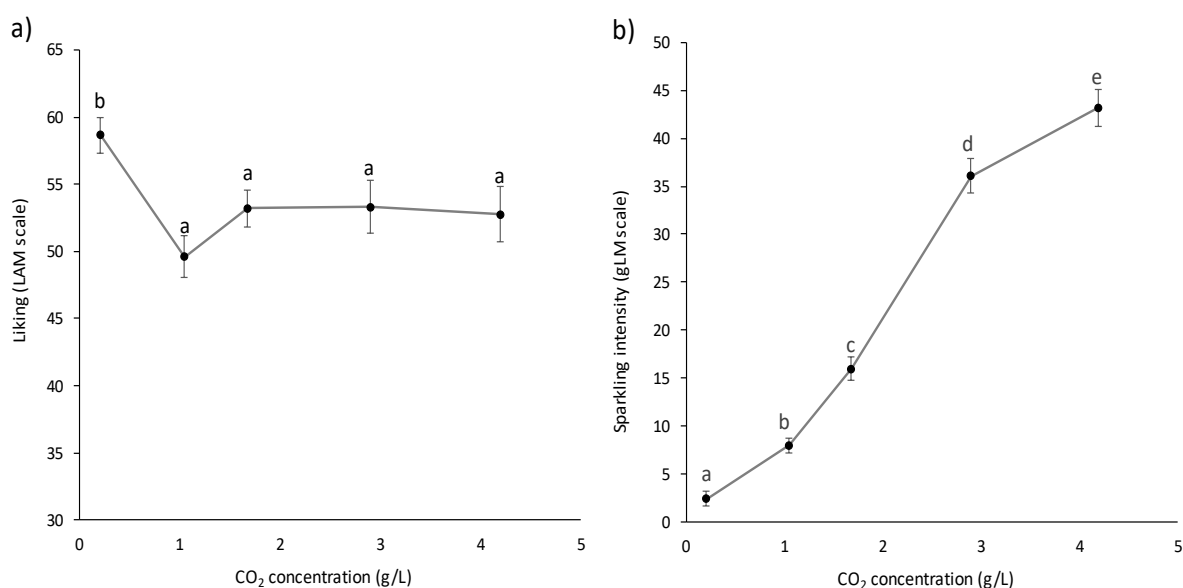


Figure 5.2. Liking (a) and sparkling intensity (b) (means \pm standard error) scored by $n=100$ participants of mineral waters differing in CO₂ concentration.

The effect of frequency of consumption on liking and perceived sparkling intensity was also investigated considering three groups of frequency of consumption (Figure 5.3 a, b). Non-consumers liked the mineral water with the lowest CO₂ concentration (S1, corresponding 0.21 g/L CO₂) significantly less than moderate and frequent consumers. Liking of mineral waters with CO₂ concentrations of 1.68, 2.9 and 4.2 g/L CO₂ (samples S3, S4 and S5), by non-consumers was significantly higher than liking of those waters by moderate and regular consumers.

Regarding the perceived sparkling intensity, non-consumers perceived the sparkling sensation of the highly carbonated waters (S4 and S5, corresponding to 2.9 g/L and 4.2 g/L CO₂) as significantly more intensive than the water with lower CO₂ concentrations.

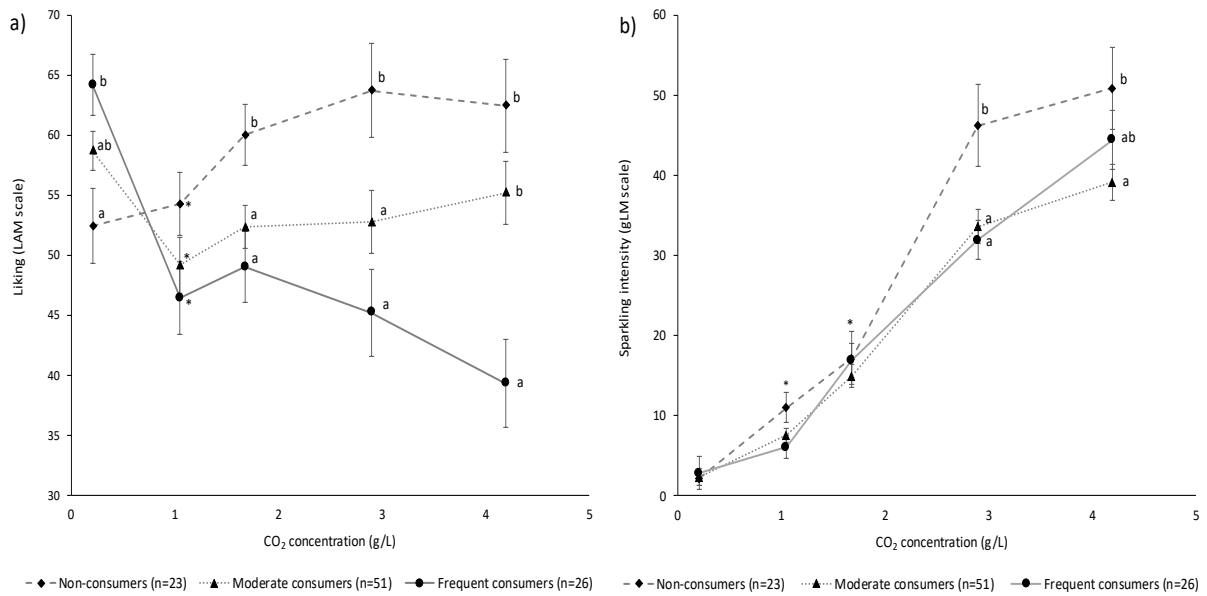


Figure 5.3. Liking (a) and perceived sparkling intensity (b) (mean \pm standard error) scored by non-consumers, (never), moderate consumers (1 time per month) and frequent consumers (at least 1-2 times per week) of sparkling water. For each concentration, at different letters correspond significantly different values ($p < 0.05$) from Duncan's test.

*The differences between groups for those concentrations are not significant.

5.3.3 Relationships between liking and sparkling intensity with individual characteristics

No relationships were found between liking of carbonated mineral waters and gender ($p=0.753$) or PROP status ($p=0.989$). No relationships were found between perceived sparkling intensity of carbonated mineral waters and gender ($p=0.839$) or PROP status ($p=0.904$).

Pearson's correlations were calculated to determine relationships between psychological and behavioural traits and liking and perceived sparkling intensity. The sensitivity to reward was significantly and negatively related ($r=-0.202$, $p=0.043$) to liking of the least carbonated sparkling water (S1, 0.021 g/L CO₂), and significantly and positively related ($r=0.219$, $p=0.028$) to the liking of the most carbonated sparkling water (S5, 4.2 g/L). No significant correlations were observed between liking and the other psychological and behavioural traits.

Emotional eating was significantly and positively correlated with perceived sparkling intensity of the most carbonated water ($r=0.206$, $p=0.038$). Restrained eating was significantly and positively correlated with the perceived sparkling intensity of the two most carbonated waters (S4, 2.9 g/L with $r=0.234$ and $p=0.019$; S5 4.2 g/L with $r=0.249$ and $p=0.012$).

5.4. Discussion

The aim of this study was to determine how detection thresholds for and perception of sparkling sensation in carbonated mineral water are affected by consumption frequency of sparkling water and individual consumer characteristics. The BET geometric mean detection threshold of dissolved CO₂

in water was 0.44 g/L. This threshold is comparable to the threshold previously reported by Le Calvé et al. (2008) (0.26 g/L).

Differences in the chosen concentrations of sparkling waters and differences in the mineral composition might cause differences in detection thresholds of sparkling sensations.

The present study aimed to understand how sparkling sensation detection thresholds are influenced by individual consumer characteristics. To the best of our knowledge, no studies have been reported that investigated sparkling sensations and its relationships with individual consumer variables. In particular, the influence of consumption behaviour of sparkling water on sparkling detection thresholds has not been investigated. We did not find significant correlations between sparkling detection thresholds and consumption frequency of sparkling water. This is in agreement with Pangborn and Pecore (1982) who demonstrated no significant correlations between salt intake and detection thresholds for NaCl. Recently, Low and colleagues (2016) reported that no strong correlations between sweetness detection thresholds and mean total energy intake and percentage of energy from total fat, protein, carbohydrate, sugar, starch, and fibre. Furthermore, no significant differences between ethanol detection thresholds and beer consumption were found by Mattes and Di Meglio (2001).

In contrast, detection thresholds of oleic acid (fat sensitivity) were influenced by dietary patterns (low vs. high fat diet) (Stewart and Keast (2012)). Whether significant correlations between detection thresholds and consumption behaviour are found or not may depend on the sample size that is investigated. Interestingly, most studies exploring associations between detection thresholds of various sensations and consumption behaviour used relatively small sample size with typically $n \leq 60$ subjects.

Sparkling detection thresholds were not significantly different between non-PROP tasters, medium and super PROP tasters. The ability to taste PROP has been linked to the bitter receptor gene hTAS2R38 (Duffy et al. 2004). Relationships between PROP taster status and detection thresholds for sweetness (sucrose) and bitterness (quinine-HCl) have been demonstrated (Hong et al., 2004). However, Keast and Roper (2007) found no correlations between detection thresholds for bitterness (caffeine, quinine HCl) and PROP taster status. Different results reported by different studies and lack of correlations of taste threshold and PROP taster status may be due to multiple factors. Recent advances in the knowledge of the peripheral organization of the taste system strongly indicated that taste receptor cells are quality specific (Mueller et al. 2005; Huang et al. 2006). Furthermore, whilst the relationship between PROP status and taste detection thresholds is complex and conflicting, it is reasonable to think that a bond with the sparkling sensitivity may be even more intricate. The results from this study do not diminish that complexity especially because the sparkling sensation is

perceived by both trigeminal (Dessirier et al., 2000; Kleeman et al., 2009; Meusel et al., 2010) and gustatory receptors (Symoneaux et al., 2015).

Moreover, in this study we verified how sparkling detection thresholds are related with several psychological traits of the consumer. Attention to specific sensory stimuli could be modulated by personal factors including the emotional state. Our results showed no relationships between sparkling detection thresholds and any measured psychological trait. In particular, the sensitivity to punishment and to reward, measured with the SPSR questionnaire, was not correlated with the sparkling sensation sensitivity. We speculate that consumers who are sensitive to signals of punishment, frustrative non-reward and to signals of incentives may have an unconditioned system of protection for aversive and painful stimuli, as, by generally speaking, the sparkling sensation might be (Dessirier et al., 2000), and consequently might have high sensory sensitivity for those sensations. Our results are in contrast with the study of Croy and colleagues (2011), who found a significant positive correlation between trigeminal chemosensory detection sensitivity of intranasal gaseous CO₂ and neuroticism, which was positively related to sensitivity to punishment and reward (Torrubia et al., 2000). However, the CO₂ perception starts to be painful at concentrations around 0.83g/L (Hummel et al., 2011). In our study, only 13 people showed a sparkling detection threshold higher than 0.83g/L. Therefore, the reason why we did not find any relationship with the sensitivity to punishment and to reward may be due to the fact that the majority of people did not perceive the sparkling sensation as painful.

Our study demonstrates that eating attitudes quantified by the DEBQ did not predict sparkling sensation thresholds. Those findings are in contrast with the study conducted by Stafford et al. (2013), which showed that dietary restraint and eating attitudes were associated with poorer odour sensitivity. We also did not find any correlations between sparkling sensation sensitivity and attitude for healthy foods measured assessed by the HTAS questionnaire. A limitation of the current study was that a rather homogeneous group of subjects was used. All participants were young, healthy adult students, so the population was highly educated and very aware of food and health.

The second part of the study aimed to verify how frequency of consumption of sparkling water and individual consumer characteristics affect liking of sparkling waters and perceived sparkling intensity. We found an effect of the frequency of consumption of sparkling water on both liking and perceived sparkling intensity. In particular, non-consumers of sparkling water liked the sparkling waters more than moderate and regular consumers. This result is probably caused by the fact that many of the non-consumers reported to not consume sparkling water for cost reasons and not because they dislike the sensory properties of it (data not shown). They prefer tap water over sparkling water since it is cheaper. Since the non-consumers were not used to drink sparkling water, they might have perceived the possibility to consume sparkling water as very pleasant and therefore scored liking

higher than the medium and frequent consumers. Intensity of sparkling sensation was also perceived as higher in non-consumers than the medium and frequent consumers. This finding was expected, since the intensity scores given by consumers who are used to drink sparkling water are based on well-known sensations.

Our results did not show significant relationships between liking and sparkling intensity and PROP status. This last result is in contrast with other studies which showed significant relationships between PROP status and perception of other sensations. PROP status has been positively correlated with suprathreshold sweetness, bitterness (Bajec and Pickering 2008; Bartoshuk, Duffy, and Miller 1994; Fischer et al. 2014), saltiness, sourness, (Bajec and Pickering 2008; Fischer et al. 2014), astringency, and metallic intensity (Bajec and Pickering 2008) in aqueous solutions. Also, several studies have established associations between PROP responsiveness and perception of oro-sensations elicited by foods and beverages (Akella, Henderson, and Drewnowski 1997; Bell and Tepper 2006; Lanier, Hayes and Duffy 2005).

Finally, we found a few relationships between liking of sparkling water and sensitivity to reward. In particular, sensitivity to reward was positively related with the liking of the most sparkling sample. It is interesting that liking of sparkling water shows correlation with a personality construct thought to measure sensitivity to rewards such as money, sex, and social status. This finding seems to be in agreement with the results of Byrnes and Hayes (2012), which showed similar results considering the burn sensation provoked by capsaicin. Indeed, that study showed a positive trend between liking of spicy foods and sensitivity to reward.

5.5 Conclusions

Sparkling sensation sensitivity expressed as detection threshold of sparkling sensation (BET) in carbonated mineral water was 0.44 g/L CO₂ concentration. BET of sparkling sensation was not affected by frequency of consumption of sparkling water and not related to PROP taster status and consumer psychological variables. These results may suggest that the human sensitivity to sparkling sensation is innate and not influenced by any individual characteristics and consumption behaviour of carbonated beverages.

Liking and perception of sparkling intensity of carbonated mineral water were affected by frequency of consumption of sparkling water. Non-consumers liked sparkling water more than moderate or regular consumers, probably because mainly for cost reasons the non-consumers did not drink sparkling water. Sparkling sensations were perceived more intensive by non-consumers compared to moderate and regular consumers.

The relationships presented in this study showed that liking for sparkling water is determined by an individual's sensitivity to reward. The few significant relationships presented here, while indicative that personality variables are related with food choice and liking, are only qualitative associations. In the future, further exploration into the source of these differences is necessary to better understand the drivers of beverage choice with chemesthetic compounds.

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Chapter 6

Summary, conclusions and future perspectives

Sensory sensitivity, intended as personal characteristic related to individual differences in detection and reaction to sensory stimuli, plays a pivotal role in food preference and choice.

As largely described in Chapter 1, how the sensitivity to texture and trigeminal sensations may drive the individual food preferences and choice is quite doubting.

The reason why the existing evidence is not solid is a missing standardized procedure to measure the sensitivity to those sensory manifestations. Across the years, many methods have been proposed to investigate the individual acuity to the texture and all the troubles related to those approaches are discussed in Chapter 1, and thoroughly investigated in the other chapters of this PhD thesis. To summarize, the first main issue is due to the fact that results present in the literature cannot be generalized, because coming from different methods. Secondly, the majority of the approaches used to investigate the texture sensitivity has not been performed using real food products, therefore they do not reflect the real perception of the food.

The main aim of this PhD thesis, spread over four study cases, was to develop a new approach to measure the individual sensitivities to specific key sensory attributes, by creating real foods varied in different levels of the target stimulus intensity and by using a mathematical procedure to cluster people according to their sensitivity. Simultaneously, as a secondary aim, the effect of individual sensitivity on food preference and choice was also investigated as far as the relationship with other individual variables

The presented results confirm, first, that differences in texture and trigeminal perception exist among people and that proposed approach was valid to measure them. Also, individual characteristics, such as gender, age, psychological traits and food behaviour play an important role in both sensitivity and food liking and choice.

In particular, the main conclusions of each study case are following reported.

The first study case (Chapter 2) aimed to propose the method to measure the texture sensitivity, using the graininess as key-texture attribute. To this purpose, five cocoa-based creams with different levels of graininess were instrumentally characterized and evaluated by 59 consumers in terms of perceived graininess and liking. On the base of the relationship between the refining time and the D_{90} – parameter used to instrumentally describe the graininess – people were clustered in three groups of sensitivity, according to their ability to discriminate different levels of graininess. The measured graininess sensitivity resulted affecting the individual texture perception, and also affected the personal liking. Indeed, a significant liking trend was observed for the highly sensitive subjects, who tendentially preferred the most refined samples, which in turn were perceived less grainy. In this study, individual characteristics, such as gender and age, did not influence the graininess sensitivity and did not affect the liking. This result was probably due to the small number of subjects involved

in the study. However, data collected in this first explorative study showed that using the instrumental characterization to predict the individual sensitivity allowed clustering people in different levels of sensitivity, and that, according to what stated in Chapter 1, texture sensitivity may drive the food liking.

On the basis of the results of Chapter 2, the second study case (Chapter 3) aimed to validate the proposed method, investigating another texture attribute - the hardness - and using a larger sample of subjects (n=248). To this purpose, four jellies were developed by changing the concentration of the gelling agent, in order to obtain different levels of hardness. Following Chapter 2, subjects were clustered in three groups of sensitivity according to the relationship observed between the instrumentally measured hardness and the concentration of the gelling agent. Results confirmed that the developed statistical methodology could be used to measure the sensitivity to any texture attribute that can be first analysed by means of instruments. Indeed, subjects with different level of hardness sensitivity differed in hardness perception and liking expressed for the jellies. Also, the individual characteristics of gender and age, showed to play an important role in hardness perception, especially demonstrating that ageing is – as largely explained in Chapter 1 – an important variable affecting the texture sensitivity.

With the belief that the proposed method resulted effective to investigate the texture sensitivity and applicable to any texture property, the third study case (Chapter 4) aimed to use the approach developed in the first study case and validated in the second one, to explore the role of the sensitivity to viscosity in food liking and choice.

Since there are other variables that can drive the food preferences and choice, such as the psychological traits, the role of food neophobia in liking was also investigated. According to the aim, five chocolate creams were made by using different solid concentrations (w/v), in order to obtain different viscosities. Subjects were asked to evaluate the liking and the perceived viscosity of the creams and to fill in a Food Choice questionnaire - developed on the base of texture dichotomies - and the Food Neophobia questionnaire. Data showed that, first, once again, the proposed method was reproducible and can be used to measure the sensitivity to viscosity as well. Secondly, different levels of sensitivity led to different preferences and different texture choices. Finally, according to several pieces of research present in literature, food neophobia appeared to affect personal liking.

Finally, the last study case (Chapter 5) aimed to explore the sparkling sensation, used as key-trigeminal attribute, in order to determine how detection thresholds for and perception of sparkling sensations in carbonated mineral water are affected by familiarity with carbonated beverages and individual consumer characteristics. Detection thresholds of sparkling sensations, measured in 100

consumers, are independent of consumption behaviour and preferences for carbonated beverages. Thus, different levels of familiarity for sparkling water did not affect the sensitivity to the sparkling sensation, which in turn was not influenced by gender, age or psychological traits. These findings suggested that the sensitivity for this trigeminal sensation may be innate and does not change during life. On the other hand, liking and perception of the sparkling intensity of carbonated mineral water were significantly affected by the consumption frequency of sparkling water, demonstrating that consumption behaviour, and thus the familiarity, is an important predictor of the individual preferences.

In conclusion, to a large extent, the present PhD covered an important gap present in the literature. The presented results demonstrated that used approach can be transferred to investigate other key texture properties. Also, with a view to further validate the method, other individual variables that can be related to individual sensitivity could be investigated, such as taste and odor sensitivity, and other psychological traits.

If the sensitivity to the texture and trigeminal sensations may be affected by individual characteristics or is innate, is still doubting. On the other hand, however, the individual sensitivities, psychological traits and consumption behaviour, showed a significant effect on food liking and choices. Those last remarks drive some final speculation.

In view of developing new food products, the possibility to have a picture of subjects, clustered for different sensitivities, is two-fold helpful for the food industries. Indeed, the food industries could look both for products designed to the average consumers, i.e. those subjects having a moderate acuity in perception, and for tailored food products targeted to the outliers, i.e. those subjects showing a low and high acuity. Therefore, this knowledge is crucial to emphasize or avoid specific textural attributes for specific targets of consumers.