The role of saliva in food sensory perception: relevant knowledge to design healthy foods

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Food choices and consumption are determined by a range of factors that contribute to aversion or pleasure and guide to final intake. Among these, the sensorial characteristics of food have a major and decisive role in choice behaviour. Although some of the mechanisms involved in oral food perception, namely in taste and astringency perception, are considerable known, many questions remains, particularly in what concerns variations among individuals in their sensitivity for food sensorial aspects. The understanding of the mechanisms leading to different responses for the same sensorial stimulus is particularly important to understand food choices.

Bitter has been the basic taste most studied for variations among individuals in perception and in how this influences food behaviour and nutritional status. The observation, at several years ago, that some individuals are very sensitive to the bitterness of the compounds phenyl thiocarbamide (PTC) or 6-n-propylthyouracil (PROP), whereas others are almost insensitive, triggered the emergence of diverse studies about the motif for that, resulting in the identification of gene polymorphisms for the bitter taste receptor TAS2R38. Subsequently to that, polymorphisms for other receptors and taste qualities have been identified. Even so, these genetic variations are not able to explain the total diversity in taste/oral sensations responses. In recent years, it has begun to become apparent that saliva has a relevant role in taste recognition mechanisms. Apart from astringency, which is well known to depend on salivary proteins to develop and being perceived, basic tastes started to be related with saliva composition. Some salivary proteins, among which carbonic anhydrase VI, cystatins, amylase and others, have been observed to relate with taste perception. However, saliva secretion changes with taste stimulation and according dietary habits. Moreover, body weight condition, metabolic status or diverse pathologies are responsible for changes in saliva composition. Being this fluid important in modulating oral food perception, to know individuals' saliva composition becomes of interest for modulating or directing choices. Based on the literature and recent scientific results, the role of saliva in food sensory perception will be discussed according to these two angles. The question of the high between-subject variability in view of saliva properties and its consequence on perception will be emphasized.

Keywords: Saliva, oral sensory perception, healthy foods

1. Introduction - Determinants of food choices

All of us need to eat, but each of us have different preferences and make different choices whenever possible. These different choices result in different dietary styles, which can have influence in health. The understanding of the factors influencing ingestive behaviour is fundamental for avoidance and resolution of numerous nutritional problems, as well as for food industry development and promotion of products. Consumer choices are influenced by diverse factors, among which biological and sensory attributes play a key role. Besides these, food choices and intake also depend on psychological and social factors, including beliefs, habits, values and past experiences [1]. Aspects such as age, gender, individual's personality, different levels of knowledge and experience with regard to food related issues may induce different types of behaviours relative to food [2]. Moreover, an individual's thought about food will influence sensorial perception of that food. For example, familiar brands can modulate individual's taste perception [3]. As well, an apparently unrelated cue, such as the sound, may change the way food is perceived in the mouth. This is observed by famous chefs, such as Heston Blumethal, in its daily contact with his restaurant consumers, who report that the salty taste of a seafood dish is higher when eaten at the same time that the sound of sea is listen. The study of the effect of sound in consumer food perception has gained interest in the last years [4].

The accessibility of resources is another important aspect when choosing food. The higher consumption of fish by the populations that lived near sea or vegetables from rural individuals are good examples of intake based on products availability. This is not so evident nowadays, in developed countries, where supermarkets contain products from a diversity of places. Even so, it is still possible to observe some different food habits from rural populations, comparatively to urban ones. Low financial resources, transportation constraint and architectural barriers, may also prevent people from having access to preferred food products and determine the purchase of more or less suitable alternatives, which in turn may influence habitual preferences [5].

Concerning biological factors, sensory-affective responses to food are a major influence on food preferences and choices [6]. Palatability links sensorial and physiological cues with the emotional perception of foods and is a major factor in food acceptance and choices. Food sensorial characteristics such as taste, texture, smell and appearance

influences palatability and, as such, the perception of food sensorial properties is one of the main determinants of food consumption.

Among the sensorial characteristics, taste and smell are chemical senses of well-known influence in food perception and choices. Inter-individual differences in taste perception have been studied and may explain some of the differences in food acceptance and choices.

This chapter presents a review about how oral perception relates with food acceptance and choices. Particular attention will be given to saliva due to the recent evidences of its influence in food sensorial perception, namely in taste and astringency perception. The way this may influence sustainable and healthy choices will be discussed.

2. Oral food perception

2.1 Taste system: anatomy and physiology

At the moment, five basic tastes are accepted: sweet, sour, bitter, salty and umami. In the recent years more sensations have been proposed as basic tastes, namely the taste of fat [7] and the taste of carbohydrates [8]. Taste perception is important to ensure the acquisition of nutrients and minerals and to avoid the intake of potentially nocive substances. Aversion caused by sourness and bitter taste, for example, prevents mammals to ingest injurious food substances, whereas sweetness of sugars guides mammals to ingest energy [9].

Non-volatile food constituents, dissolved in saliva, are detected by receptors present in taste cells, which are embedded in structures called taste buds, located in tongue, soft palate, epiglottis, larynx, and pharynx. Taste buds consist in groups of 50-100 cells, which are divided in four types of cells (types I to IV), which have been described based on their ultrastructural and cytological characteristics: type I cells appear to be supporting cells; type II are the taste receptor cells; type III are cells with characteristics that are “intermediate” between type I and type II cells and form synapses with afferent nerve fibres; type IV cells are basal cells, which are thought to have the capacity to differentiate and replace the cells in taste buds (reviewed in [10]).

Food molecules, responsible for taste, contact with taste cells through the taste pore located in the apical region of taste buds. Most of the information about taste starts with the contact of these molecules with the microvilli of these cells, leading to intra-cellular signal transduction. Moreover, the transduction machinery also involves ion channels on both the apical and basolateral membrane. When chemical stimuli interacts with taste cells, voltage-gated Na+, K+ and Ca2+ channels, located on the basolateral membrane of these cells, produce depolarizing potentials. This raise Ca2+ levels, leading to synaptic vesicle fusion and synaptic transmission [11]. The information goes from taste buds to the brain via afferent fibres from branches of three cranial nerves: VII, IX and X cranial nerves. Taste information is transmitted into the brain stem (nucleus tractus solitarius - NTS), from this to the ventroposteromedial nucleus of thalamus and from this to insular/opercular cortex (primary gustatory cortex) and orbitofrontal cortex (secondary gustatory cortex).

2.2 Factors influencing taste sensitivity

Although sweetness is universally accepted and preferred and bitterness avoided, the concentration at which these senses result in such responses varies according to the molecule that induce each of them, to the matrix in which that molecule is present (and the relative amount of different compounds that constitute that matrix) and to individual’s characteristics.

Taste sensitivity is by definition the ability with which each individual perceives the different tastes. The inter-individual variability in perception of basic tastes began to be noted for bitter taste. The notion that this taste is not sensed in the same way by all individuals arose in the thirties, of the last century, more or less "by chance." When weighing the compound phenylthiocarbamide (PTC) in the laboratory, some powders from this reagent were released, with some investigators "complaining" of bitterness, while others did not feel or felt with very little intensity of such sensation [12]. From then on, variations in bitterness perception started to be studied in detail, particularly for PTC and the other compound of the same family, 6-n-propyl-thiouracil (PROP). Several studies have been performed to elucidate the mechanisms underlying variation in bitterness. Three main factors have been proposed: i) specific genetic variation (polymorphisms present in taste receptors) [13]; ii) generic genetic variation (eg, taste bud density) [14]; iii) and environmental factors, such as eating habits [15] or even saliva composition [16–18].

The taste receptor that responds to PTC and PROP compounds is encoded by the TAS2R38 gene, for which polymorphisms have been identified and related to bitter taste perception [19]. These single nucleotide polymorphisms (SNPs) result in the substitution of three amino acids (Pro49Ala, Ala262Val and Val296Ile), giving rise to two common haplotypes: PAV, dominant variation, and AVI, recessive variation. Homozygous or heterozygous individuals for the haplotype PAV perceive the bitterness of PROP at low concentrations and are considered tasters (homozygous being super tasters and heterozygous medium-tasters), while individuals homozygous for the AVI haplotype do not perceive PROP bitterness or perceive it only when in high concentrations, being classified as non-tasters [13].

Some studies exist also for other basic tastes, where genetic factors have been reported as influencing sensitivity. Variation in the TAS1R2 gene, which codifies one component of the G-protein–coupled receptors TAS1R2-TAS1R3, has been linked to sweet taste sensitivity and food choices, although such relationship being dependent on Body Mass Index (BMI) [20]. For sour and salty taste, receptors are less well characterized. Even so, Dias and colleagues [21] reported single nucleotide polymorphisms in the putative salt taste receptors, ENaC and TRPV1, which were associated with differences in salt taste perception. The association between polymorphisms in taste receptors and taste perception and eating behavior has been recently reviewed [22].

Although genetic changes in the membrane receptor are an important factor, they do not fully explain the variations observed in taste sensitivity. According to Genick and colleagues [23], approximately 30% of the observed phenotypic variation in bitter taste response should be explained by other factors, such as changes in the characteristics of the oral environment involving receptors. In this context, saliva composition started to be taken into account, and nowadays there are evidences that this fluid is related to bitter (e.g. [16,24]) and sweet (Rodrigues et al., Salivary proteome and glucose levels are related with sweet taste sensitivity in young adults, *submitted*) taste sensitivities. More detail about this issue will be presented in the next point.

3. Saliva and oral food perception

3.1 Saliva composition and functions

Whole saliva, the fluid that continually baths the mucosa of the oral cavity, oropharynx and larynx is a complex mixture deriving from the secretion of salivary glands and gingival crevicular fluid, containing oral bacteria, food remainders and desquamated epithelial and blood cells [25]. Total saliva is composed by a variety of electrolytes, including sodium, potassium, calcium, magnesium, bicarbonate and phosphates, by proteins such as immunoglobulins, enzymes (e.g amylase and lysozyme), mucins (glycoproteins involved in the protection and prevention of oral epithelium), microbial enzymes and nitrogenous products such as urea and ammonia. The relative proportion of these different components varies with several factors, among which changes in salivary flow [25].

Saliva is an exocrine secretion of specialized cells grouped in salivary glands, distributed in the oral cavity. According to their size and contribution for the total amount of saliva, the salivary glands are classified as "major" and "minor". There are three pairs of major salivary glands (which exist as bilateral pairs): parotid, submandibular and sublingual glands. Of the major salivary glands, the parotid is the largest one and contributes with the greatest flow (as much as 60% of the total) when stimulated by taste or chewing [26]. However, this gland contributes with only a small amount to resting salivary flow. It secretes a serous secretion that contains no mucins but is rich in amylase and proline-rich proteins (PRPs) [27]. In addition to the major salivary glands, there are hundreds of minor salivary glands located in the submucosa throughout the oral cavity. Most of these glands produce a mucous secretion, in small volumes (<1 µl min^{-1} per gland) with mucin-rich content. Although only contributing approximately 10% of salivary flow, the minor glands are important in maintaining a mucin-rich layer adjacent to the mucosa [27].

There are two main types of salivary secretion: serous and mucous secretion. The first is a fluid secretion rich in water, with enzymes and diverse other proteins. It plays an important role in ingestion and chewing of food. Serous fluid is produced and secreted by the serous acinar cells of the parotid glands and some minor salivary glands, such as von Ebner's glands in tongue. Mucous secretion is rich in glycoproteins such as mucins, acting predominantly in lubrication, bolus formation and deglutition. This type of secretion is derived from mucosal cells. Glands such as the sublingual glands and several minor salivary glands are mostly (or even totally) constituted by this type of cells [28]. It is also possible to consider a third type of secretion, mixed between the two mentioned above, as is the case of the secretion coming from the submandibular glands, which are mixed glands, constituted both by serous and mucous cells.

Five main functions of saliva have been proposed: (1) lubrication and protection, (2) buffering and antibacterial activity, (3) maintenance of tooth integrity, (4) tissue repair and (5) taste and digestion [25]. The major lubricating components of saliva are mucins. Chewing, speech and swallowing are processes aided by the lubricating effect of these glycoproteins. Mucins also perform antibacterial activity through the selective modulation of microorganism adhesion to oral tissues, contributing to protection of oral cavity. The buffering capacity of saliva is mainly due to its composition in bicarbonates, phosphates and proteins. Moreover, saliva contains a spectrum of proteins with antibacterial properties, such as histatin and lysozyme, which can hydrolyse the cell wall of some bacteria [27]. The maintenance of tooth integrity is another of the functions performed by saliva. This fluid is saturated with calcium and phosphate ions, ensuring the ionic exchange directed to the tooth surface.

With regard to oral perception, saliva also plays an important role. This fluid is required to dissolve the substances which are then transported to the taste receiving/detecting sites (taste-receiving cells located in the taste buds) [29]. Moreover, salivary constituents may interact with food constituents, promoting modifications or changing its assessment to the structures responsible or sensorial detection, such will be subsequently detailed.

3.2 The role of saliva in oral perception and eating behavior

Several studies, in animals and humans, present evidences that saliva is involved in eating behaviour (e.g. [18,30–32]). The link between saliva composition and oral perception is increasingly reported. The involvement of salivary proteins in the perception of food has been most studied in the context of its effect on the development of astringency. Although the mechanisms involved in astringency development are not fully elucidates, the participation of salivary proteins is well accepted. One of the main accepted theories for explaining astringency is based in a two-phase model: a first phase, in which polyphenols (or other astringent molecules) bind to the proteins present in the "liquid part" of saliva; a second phase where the astringent molecules that did not bind in phase 1 can interact with the adsorbed glycoprotein layer, in the oral mucosa, reducing lubrication of the oral cavity and developing astringency. Different salivary proteins have been linked to the astringency caused by polyphenols, due to their affinity for these compounds. Among these, PRPs [33], histatins [34], cystatins [35] and α-amylase [36] have been observed to bind polyphenols. Salivary PRPs constitute the main family of salivary proteins that are associated with astringency. These proteins, due to the richness in the amino acid proline, have an open structure that allows them to bind tannins with high affinity, forming stable complexes [37]. Mucins also play a role in astringency, although there are some controversies in this regard. Studies developed by McColl and colleagues [38] present evidence that mucins have a reduced lubrication effect when mixed with tannins. Recently, it has been observed that astringent dietary components may influence the lubricating properties of the protective mucous barrier in the oral cavity, by affecting the arrangement of the salivary mucins MUC5 (gel forming) and MUC7 (non-gel-forming) [39].

Other salivary proteins have also been reported as potentially involved in oral sensorial perception, namely at taste level. Lipocalin 1 protein, originated from the Von Ebner glands [40], shows homology with transporters of hydrophobic molecules, and it has been proposed that they can assist in the concentration and transport of molecules to the taste receptor cells [41]. Carbonic anhydrase VI (CA-VI) is the salivary protein most reported as associated with bitter taste perception. Besides parotid, the Von Ebner glands, which are located near tongue circumvallate papillae, secretes this protein. The close proximity of von Ebner's glands with these taste papilla, which have high density of bitter taste receptors, lead to the suggestion that their secretion, including CA-VI, could affect directly bitter taste. CA-VI have been pointed as implicated in the growth and renewal of taste buds, since the levels of this protein have been observed to be reduced in individuals with taste buds anatomical abnormalities [42]. Moreover, this salivary protein has been suggested to have anti-apoptotic action on taste buds [43]. Padiglia and colleagues [44] observed that different polymorphisms in the gene that codifies salivary CA-VI are related with the ability of this protein to bind zinc, and consequently with the functionality of this protein. Other proteins, such as metalloproteinases [45] and epidermal growth factor [46] have been related with taste perception, what can be done by the assistance, by these proteins, in maintaining the morphological integrity of taste buds.

The impact of salivary proteins on taste perception may also reside in their direct physico-chemical interaction with taste molecules, modifying their accessibility to receptors in taste cells. Salivary histatin 5 was reported to bind quinine and, for that reason, individuals with higher amounts of these proteins present lower sensitivity for quinine bitter taste [47]. The amino acids arginine and lysine were reported to interact with the bitter compound PROP, changing its perception [48].

Salivary proteomic studies allowed to identify a number of salivary proteins related to taste sensitivity. Cystatins [16,24], CA-VI [24] and PRPs [17] are proteins present in different amounts in individuals with different levels of bitter taste response. Whereas these proteins interact with taste molecules or have only indirect association with taste perception remains to be elucidated. One suggestion that emerged from our latest studies is that the charge of the protein may be relevant for taste perception. We did find that, more than individual proteins, were the protein forms with isoelectric point (pI) lower than salivary pH that related with PROP bitter taste responsiveness [24]. But this needs to be explored in future studies.

The influence of saliva in sweet taste perception has been less explored, comparatively to bitter taste. Our recent study (Rodrigues et al., Salivary proteome and glucose levels are related with sweet taste sensitivity in young adults, *submitted*), comparing saliva composition among young adults with different sensitivities for sucrose sweetness, presented evidences that some salivary proteins may be related with sweet taste sensitivity. These are the cases of cystatins, CA-VI and salivary α-amylase. Salivary α-amylase plays its major role in the initial digestion process by hydrolyzing α-1,4 glycosidic linkage between glucose units in the starch polysaccharide chain. The possible involvement of this protein in the perception of sensory properties of foods has already been suggested by several authors, namely by the influence on the perceived viscosity of starch [49] and the texture of semi-solid foods [50]. In the study of Rodrigues and colleagues (Rodrigues et al., Salivary proteome and glucose levels are related with sweet taste sensitivity in young adults, *submitted*), it was in individuals with higher amounts of salivary α-amylase that sucrose was perceived as less sweet. Although this has been observed to depend on sex, such negative relationship between sweetness perception and salivary α-amylase levels can be hypothesized as a result of higher constant amounts of taste substances in the oral cavity of individuals with higher amounts of this salivary protein. Salivary α-amylase has been associated with food intake and body weight in other studies: rodents with higher susceptibility for obesity

development, when subjected to high-fat diet, presented higher levels of this salivary protein, comparatively to obesity resistant animals [51]; salivary α-amylase was observed to be increased in saliva from obese women, comparatively to regular-weight pairs [52].

4. Implications of oral food perception in healthy food design and choices

Several studies in human nutrition have suggested that the PROP phenotype may serve as a general marker for oral sensations and food preferences, thus influencing dietary behavior and nutritional status [53]. Individual differences in the ability to discriminate and perceive bitter taste has aroused interest in assessing the relationship between this ability and food choices. Although some studies did not observe differences between sensitive and low sensitive individuals in food consumption, neither in adults [54] nor in children [55], others have proposed that PROP supertasters have higher sensitivity to various oral stimuli, compared to non-tasters, including bitter-tasting compounds such as dark chocolate, coffee, soy-based products and green tea [56], sweet foods, oral cavity irritants and high-fat foods [57]. Other studies did show that individuals who perceive PROP with high intensity have decreased acceptance of cruciferous vegetables, bitter fruits, spicy foods, and alcoholic beverages [58,59]. Children low sensitive to PROP bitter taste presented high intake of bitter-tasting vegetables [60], whereas children sensitive to this compound had less acceptance of foods such as spinach [61] and broccoli [62].

Given the nutritional importance of dietary lipids, the relationships between PROP status and fat perception and liking have been widely investigated. Most studies reported that PROP non-tasters had low ability to distinguish fat content and creaminess in certain fatty foods. Besides, PROP non-tasters showed higher preferences for dietary fat (such as full-fat milk, high-fat salad dressings and sweet-fat dairy mixtures) and consumed more servings of discretionary fats and high-energy foods per day than did tasters (reviewed in [53]).

4.1 Relationship between body mass index (BMI) and gustatory sensitivity

The influence of oral food perception in Body Mass Index (BMI) is naturally hypothesized due to the influence that parameters such perception has in food choices, as it has been mentioned so far. As such, studies aimed to understand the factors that influence and determine obesity have related taste perception with BMI. Most of the studies emphasize not only the relationship between sweet taste perception and obesity, but also the relationship between the perception of bitter and other tastes and this condition. Several studies suggested that obese individuals perceive sweet taste with lower intensity than normal-weight individuals and show higher preference for it [63]. However, this relationship is not consensual and other authors have not observed differences in the perception of sweet taste in individuals with different BMI [64]. Joseph and colleagues [65], in a study with children, did not observe relationship between BMI and sucrose detection thresholds, however, the authors did observe that children who presented higher weight and waist width had lower sucrose thresholds, i.e., perceived sweetness at lower concentration of tastant.

The relationship between oral sensorial perception and BMI had also been reported for fat. It was stated that obese women, despite preferring foods low in sweetness, prefer higher fat levels, comparatively to normal weight women [66].

Concerning bitter taste, and similarly to the other tastes, the way its perception level influences food choices and BMI is not consensual. Tepper and Ullrich [67] observed an inverse association between PROP phenotype and the BMI, in which adult individuals who were less sensitive to this compound had a higher BMI. On the other hand, the same group of authors [68], showed that girls sensitive to PROP had a higher BMI percentile.

4.2 Oral perception and foods design

Deep knowledge about the inter-relation between oral food perception and saliva may be used for design of healthy foods that may be appreciated by consumers. Food healthiness is one of the main driver in the creation and marketing of new food products [69–71]. The introduction of new ingredients with potential health benefits is a strategy for increasing nutritional value of foods [72]. Also the production processes used for new healthy products, which may optimise the amount of particular nutrients and to decrease the formation of potentially harmful compounds is an important strategy, for health promotion. Nevertheless, the achievement of such nutritional characteristics is many times at expense of food palatability. This, in turn, results in poor acceptability. Moreover, foods targeted at specific population groups as children [73,74] elderly or athletes [71] and intended for the prevention of specific pathological conditions [70,72] or with specific dietary needs [75] can be designed.

As such, the understanding of the factors influencing oral food perception is essential for the design of new food products, with optimised food structures and flavours, without compromising sensorial attributes that enhance the physiological regulatory mechanisms controlling appetite and energy intake having obvious benefits for weight management and help to achieve a balanced diet [76,77].

Different strategies have been used in the design of healthy foods. For example increasing the protein content of designed foods can be used as a strategy for enhancing that food satiating ability [78]. However, this strategy may affect food palatability, contributing with increased astringency or an inhomogeneous texture. As it was referred in previous

points, saliva may influence such sensorial attributes and from this point of view the evaluation of saliva composition may be useful for a better understanding and for acting at the level of changing the dynamics of in-mouth lubrication and the physical mechanisms underlying texture and mouthfeel perception [78].

Besides taste, food texture plays a key role in satiety [76]. The understanding of oral food processing in relation to the microstructure of the foods and its breakdown can lead to the development of several approaches to reduce the salt and sugar content of semi- and soft-solid foods without altering sensorial characteristics [79]. Textured foods require mastication which will slow the rate of consumption and will enhance the time of orosensory exposure [76]. During consumption the texture of foods changes continuously by chewing, shearing, mixing, heating or cooling and salivation [79], influencing perception.

The usefulness of a deep knowledge about the role of saliva in food sensory perception can be also illustrated to design healthy foods for elderly. Ageing modifies various aspects of oral physiology such as dental status, bite force, muscle fatigue and saliva composition [80] and production [81]. On the other hand a decrease in salivary flow (hyposalivation), leading to a complaint of dry mouth (xerostomia), mainly as a consequence of systemic diseases and medications is common in old people [82]. These two aspects lead to the loss of sensory abilities, resulting in changed perception of food. In turn, this may have the consequence of decreasing the pleasure felt when eating, resulting in less acceptance of eating in a healthy way. As such, old persons need foods that require little or no chewing, that are easily swallowed and that have attractive sensory characteristics [83]. Additionally, the information on optimal volume of bolus and saliva secretion for swallowing should be considered when manipulating the rheological properties of food for elderly (for detail, see [84]).

5. Conclusions

In conclusion, the influence of saliva in oral food perception and the consequent relationship between inter-individual differences in the composition of this fluid and inter-individual differences in sensorial perception can be relevant in food science. As such, it is important to start including the knowledge about this oral fluid in the strategies adopted for food design and for promoting healthy dietary habits, which will have obvious benefits for food industry. This knowledge can also be useful for this sector at the level of sensorial panel training and sensorial evaluation for new food products development.

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