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Perspective

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Saliva and flavor perception: perspectives

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ABSTRACT

This paper reports the main trends and perspectives related to the current understanding of the relationships between saliva and flavor perception. Saliva is a key factor in flavor perception and controls the transport of flavor molecules to their receptors, their adsorption onto the mouth surfaces (i.e., oral mucosa), their metabolism by enzymatic modification and the friction force in the oral cavity. The proteins in free saliva or in the mucosal pellicle contribute to flavor perception by interacting with or metabolizing flavor compounds. Most of these reactions were observed when using fresh whole saliva; however, they were absent or less frequently observed when using artificial saliva or depleted/frozen whole saliva. There is a need to better understand the role of protein aggregates in flavor perception. Within humans, there is great inter-individual variation in salivary composition, which has been related to differences in flavor perception. However, the relative role of salivary proteins and the microbiota should be deeply investigated together with the impact of their composition on individual perception during life. Finally, future results must also consider cross-modal interactions at the brain level.

KEYWORDS

Saliva, flavor compounds, flavor perception, metabolism, astringency, taste, salivary proteins

INTRODUCTION

The aim of this perspective paper is to highlight future trends in the effect of saliva on flavor perception based on previous review articles on the impact of saliva on aroma and flavor perception [1, 2]. The paper focuses on flavor, which includes the stimulation of taste buds, the olfactory organ and trigeminal receptors within the oral cavity by chemicals, according to the American Society for Testing and Materials (ASTM). Therefore, it does not cover the role of saliva on food structure breakdown, bolus formation or texture perception, as reviewed by Mosca and Chen and Guichard et al. [3, 4].

Flavor is the main sensation perceived during eating. It fulfils a crucial function for the organism by tasting and evaluating the quality of ingested food, which leads to further acceptance or rejection of the food. Flavor is composed of several sensory modalities: taste, retro-olfaction and trigeminal sensation. Flavor perception results from the activation of receptors located in the mouth and in the nose (Figure 1A). Depending on their structure and properties, flavor compounds bind to chemoreceptors in the mouth (taste and trigeminal receptors) and in the nose (olfactory receptors) or increase the friction force at the surface of the oral mucosa, leading to the activation of mechanoreceptors. The pattern of flavor compounds activating the receptors generates a specific nervous signal processed both locally and centrally in the brain, allowing for an immediate categorization and recognition of the sensory image of a particular food.

As food is never in direct contact with flavor receptors, and as flavor active molecules must be released and transported for perception, this sensory image depends on the initial composition of the food, the release of flavor compounds in the mouth, their transport up to the receptors and their adsorption onto mucosal surfaces [5]. Taste receptors are embedded in taste buds, which present a small orifice, allowing for communication with the oral cavity and the entrance of saliva (Figure 1C). Saliva bathes

taste receptors present in the mouth. Therefore, the affinity of flavor compounds for saliva (i.e., the dissolution) and salivary components (i.e., molecular interaction, enzymatic degradation) will impact their access to receptors. Regarding trigeminal receptors, they are embedded in the epithelium of the oral mucosa and are not directly bath by saliva. Therefore, their access is under the compound property to diffuse through both saliva, from food to the surface of the mucosa, and the epithelium of the oral mucosa [6] (Figure 1E). Moreover, a thin layer of salivary proteins, called the mucosal pellicle, covers the epithelium and thus controls also the access of the epithelium of the oral mucosa (Figure 1E). Trigeminal sensations also result from the activation of mechanoreceptors, which are also located in the mucosa. They respond to mechanical pressure or distortion of the friction force in the mouth, which depends on the lubrication of the oral cavity [7]. Saliva and some salivary proteins, such as mucins, glycosylated proline-rich proteins or salivary proteins present in the mucosal pellicle, fulfill important roles in the lubrication of the mouth. Olfactory receptors are located in the nasal cavity, and the perception of aroma compounds therefore requires their release into the mouth and their transport to the receptors via the retro-nasal cavity. During eating, aroma compounds are released directly from food to the air but also from food to saliva, which quickly impregnates the food matrix, and then to the air phase. Partition coefficients between saliva and air control the quantity of aroma released into the air phase. Moreover, aroma compounds are likely to adsorb onto the mucosal pellicle [8].

Saliva controls flavor release, the transport of flavor molecules to their receptors, their adsorption onto the mouth surfaces (i.e., oral mucosa), their metabolism by enzymatic modification and the friction force in the oral cavity and therefore appears as a key parameter in flavor perception.

Saliva, composition and secretion

Saliva is a complex mixture resulting from secretion of the major (submandibular, sublingual and parotid) (90%) and minor (10%) salivary glands together with the crevicular fluid. Salivary flow and composition depend on the physiological conditions (rest or stimulation, nature of stimulation), which impact the contribution of the different glands, each one secreting a fluid with a different composition. The flow and composition of the fluids secreted by the different glands also rely on endogenous (circadian rhythms, age, gender, several disease states) or exogenous factors (diet and pharmacological agents) [9]. Saliva is mainly composed of water, salts and proteins, but it also contains microorganisms, cellular debris and food residues. The concentration of salivary proteins is generally between 1 and 2 mg/mL [9]. Regarding protein composition, almost 3000 proteins and peptides have been identified in saliva [10, 11]. These numerous proteins fulfil several functions in saliva, such as protection against microorganisms, mineralization of teeth, lubrication of the oral cavity, scavenging of harmful molecules, initiating digestion, and transport of flavor molecules. Many of these proteins are free in saliva, while some are specifically anchored onto in-mouth surfaces. As a result, a thin film of salivary proteins, called the mucosal pellicle, covers the oral mucosa [12]. A previous study tried to reconstitute the mucosal pellicle on a synthetic surface and found that the pellicle is composed of two layers of proteins. The first layer is composed of proteins anchored onto the synthetic surface. These proteins facilitate the binding of other salivary proteins that form the second layer [13]. Recently, another group reconstituted the mucosal pellicle onto the surface of the TR146 epithelial buccal cell line and showed that the expression of a transmembrane mucin, MUC1, facilitates the anchoring of salivary proteins [12] (Figure 1D). Therefore, different mechanisms occur

during the formation of the mucosal pellicle, involving non-specific adhesions of some proteins onto hydrophobic surfaces, while other proteins are anchored through specific protein-protein non-covalent interactions. The mucosal pellicle controls the physicochemical properties of the mucosa surface and fulfils a role in the protection and lubrication of the oral cavity.

Impact of saliva on trigeminal sensation

Astringency is probably the sensation for which the interactions between astringent compounds and saliva have been the most extensively studied. Astringency is a trigeminal sensation that is generally classified as tactile and is described as a drying-out, roughening and puckery sensation felt in the mouth. It is generally felt during the consumption of plant–based foods, such as red wines, teas as well as some fruits. Tannins are the main molecules at the origin of this sensation. They are plant secondary molecules and belong to the structural class of polyphenols. They are known for their ability to bind and precipitate proteins. However, the molecular mechanisms by which tannins generate the astringency sensation remain unclear and could result from the activation of either trigeminal chemoreceptors or trigeminal mechanoreceptors. Regarding the involvement of chemoreceptors, it has been reported that tannins and/or their products of oxidation can activate different types of rat trigeminal transient receptor potential (TRP) in two different cell lines [14, 15]. However, to our knowledge, the activation of trigeminal TRPs by astringent compounds has not been reported in either humans or in human cell lines. Moreover, trigeminal free nerve endings are mainly located below the multilayered squamous epithelium of the buccal mucosa, and nerve endings approaching the surface are actually rare [6] (Figure 1 F). If hydrophobic

molecules can diffuse through the multilayered squamous epithelium of murine buccal mucosa [6], it is unlikely that tannins can access the TRPs as they have been reported to bind to cell membranes [16]. The tactile origin of astringency requires the activation of mechanoreceptors. This activation is likely due to modification of the force friction at the surface of the oral mucosa. Two main hypotheses have been proposed to explain such changes. One hypothesis postulates that the precipitation of salivary proteins reduces the lubricating properties of saliva, leading to an increase of the friction force in the oral cavity [17] (Figure 1 F). Salivary proline-rich proteins (PRPs) are particularly prone to interact with tannins [18] and therefore could be predominantly involved. In agreement with this hypothesis, it has been observed that the aggregation threshold of a PRP by the tannin epigallocatechin gallate (EgCG) is close to its astringency threshold [17]. The second hypothesis proposes that this sensation is due to the direct interaction of tannins with the mucosal pellicle, leading to the loss of its lubricating properties and an increase of the friction force at the surface of the oral mucosa [19] (Figure 1 F). In this second hypothesis, PRPs play a protective role and prevent the sensation of astringency through binding and scavenging of tannins as they can wrap around tannins after structural rearrangement [20]. This hypothesis is supported by the observations that saliva is not required to induce the perception of astringency of tea in human subjects after mouth rinsing with water solution, while the presence of saliva in the mouth decreases the perceived astringency [21]. Moreover, it has been shown that the aggregation of the mucosal pellicle by tannins leads to an increase of the friction force, while the presence of the PRP, and the IB5 protein, precludes this aggregation [22]. Future research is required to link this observation to the threshold of astringency sensation. It could be particularly interesting to compare the ability of different salivary proteins to protect the mucosal pellicle. Indeed, PRPs are not the only proteins capable of

148 interacting with tannins. For instance, histatins constitute another major group of
149 tannin-binding salivary proteins (TBSPs). It has been hypothesized that the level of
150 secretion of TBSPs in the saliva of mammals is linked with the tannin content of their
151 natural diet [23]. Animals with a diet high in tannins have developed high levels of TBSPs,
152 while those with low tannin contents produce little or no TBSPs. However, the presence
153 of TBSPs may have originated from different evolutionary processes; therefore, there is
154 a need to characterize TPSPs in each species. This information should help to gain a
155 better understanding of the impact of salivary protein composition on astringency
156 sensitivity. Furthermore, new methodologies are needed to measure *in vivo* friction
157 forces and to link them with the perception of astringency and the composition of saliva.
158 This information would enable validation of the tactile dimension of astringency
159 sensation. However, this does not rule out the possibility of the involvement of
160 chemoreceptors. It is of importance to clarify which tannins receptors are activated and
161 how saliva composition can modulate their activation, as protein-tannin affinity and
162 precipitation depend on protein structure [20, 24, 25]. Thus, the composition of saliva is
163 likely to affect the amount of tannins available to interact with receptors. Indeed,
164 microbial and salivary secreted enzymes may also contribute to the biotransformation
165 of tannins to facilitate their elimination.
166
167 **Impact of saliva on taste perception**
168 To our knowledge, Henle is the first author to introduce the importance of saliva and its
169 composition in taste perception [26] through the concept that taste threshold depends on
170 the taste receptor milieu and thus on the basal concentration of tastants contained in
171 saliva. Some salivary compounds can continuously stimulate taste receptors, leading to
172 an adaptive mechanism impacting taste sensitivity [27]. For example, the taste detection

threshold for NaCl is slightly above the salivary sodium concentrations to prevent continuous stimulation of the taste receptor [27]. Therefore, interindividual variability in salt perception is, at least in part, due to different salt concentrations in individuals' saliva.

A study recently investigated if such an adaptive mechanism linking the basal concentration of tastant in saliva and the sensitivity occurs in fat perception [28]. Indeed, it has been reported that saliva has lipolytic activity, leading to the hydrolysis of triglycerides and the release of free fatty acids [29] that could be detected by fat receptors (e.g., CD36, GPCR120). Therefore, it has been suggested that fat could be the sixth taste. Authors reported a correlation between the basal concentration of free fatty acids in saliva and the lipolytic activity of saliva [28]. Further research is needed to determine if this basal concentration in saliva is correlated with sensitivity to fat. While rats secrete a salivary lipase, its presence in humans has not been reported. Salivary lipolytic activity could result from several lipases [30], although their involvement still needs to be confirmed.

It has also been hypothesized that saliva can dilute taste stimuli and decrease taste intensity, especially when high salivation occurs during mastication. A negative correlation was shown between salt perception and salivary flux [31], no correlation was found for bitterness [31] or sweetness [31], and contradictory results have been reported for sourness [31]. It should be noted that the dilution effect of saliva on perception is difficult to evaluate, as human taste receptors easily adapt to taste stimulation [27].

In addition to dilution, the buffering capacity of saliva, which can decrease the amount of H^+ ions present in saliva, has also been reported to decrease the response to acid stimulation [27], although that the concentration of organic acids, and therefore the titratable acidity, contribute also to sourness [32, 33].

198 Interactions between tastants and salivary proteins are also likely to occur and to affect
199 taste perception [34]. Several studies have explored the relationship between salivary
200 composition and taste perception [27]. In recent decades and since the advent of
201 proteomics, this field of research has been the subject of growing interest. Interestingly,
202 two research groups recently suggested that the proteolytic activity of human saliva
203 plays a role in the perception of bitter, fatty, and salty stimuli [29, 34, 35]. Thus, a study on
204 the interindividual variation in the sensitivity to bitterness suggested that enhanced in-
205 mouth proteolysis is a key perireceptor factor associated with higher gustatory
206 sensitivity [35]. The authors hypothesized that the mucosal pellicle forms a barrier that
207 controls the accessibility of tastants to the receptors. A thinner or looser pellicle due to
208 higher proteolytic activity would then be associated with a facilitated tastant•taste
209 receptor interaction [35]. Regarding salt perception, two hypotheses have been proposed
210 to explain the link between sensitivity and proteolytic activity [34]. The first hypothesis
211 proposes that trypsin may facilitate transepithelial sodium transport through the
212 endoprotease-catalyzed cleavage of the amiloride-sensitive epithelial sodium channel's
213 γ-subunit [34]. The second hypothesis postulates that the degradation of salivary proteins
214 leads to the release of salt-taste enhancing peptides [34]. In favor of the second hypothesis,
215 a tetrapeptide PLWR, resulting from trypsin activity, was found to elicit salty taste-
216 enhancing activity [34]. Further research must be conducted to determine to what extent
217 the endoprotease-catalyzed salt taste enhancement is due to an in vivo release of salt
218 taste-modulating peptides or facilitated transepithelial sodium transport [34]. The
219 abundance of lysozyme C and lipocalin-1 (LCN1) was reported to be indicative of non-
220 sensitive subjects [34], while the molecular mechanism is unclear.
221 Lipocalin-1 has also been suggested to be involved in fat perception (Figure 1H). Free
222 fatty acids are not soluble in aqueous media, such as saliva; therefore, they need to be

carried to reach their receptors. A potential candidate to transport free fatty acids is LCN1, which can also transport hydrophobic bitter compounds, such as quinine [27]. LCN1 presents a hydrophobic pocket, allowing the binding and transport of bitter compounds, including fatty acids [36]. However, no homologous proteins have been identified in the van Ebner's Glands of mice, guinea pig or cows [27]. The function of this protein, which is secreted in human saliva close to the taste receptors, remains to be clarified. Another hypothesis is that this protein could play a scavenging function of the tastants. Such a function could allow evasion of an adaptive mechanism and could maintain a higher sensitivity of taste perception.

Impact of saliva on aroma perception

To be perceived, aroma compounds must be released into the air phase. Most studies have focused on aroma release, and few relate directly to saliva composition and aroma perception.

Taylor and co-workers were the first to introduce the concept that different mechanisms can impact aroma release in the mouth [8] (Figure 1G). The first approach to probe the impact of saliva on aroma release was to use artificial saliva composed of salt and mucin [37] with or without α-amylase [38]. Decreased release of aroma compounds depending on their structure was observed [37, 38]. This observation was attributed to non-covalent interactions occurring between mucin and aroma compounds [37]. While one study failed to identify the type of non-covalent interactions, the number of binding site or the protein domain involved [37], another study probing the effect of mucin and α-amylase on the release of linear ketones and esters suggested the involvement of hydrophobic effects with both proteins [39]. The use of artificial saliva was justified by a study reporting no significant difference between aroma release in the presence of human and artificial

saliva [37]. The artificial saliva spread largely into *in vitro* approaches aiming at probing the impact of saliva on aroma release. However, other studies revealed that saliva can metabolize aroma compounds [39-41]. Important inter-individual variability in the impact of saliva on aroma release [41, 42] and aroma perception [43] has also been reported. These two observations demonstrate that artificial saliva, composed of only mucin and alpha-amylase, does not properly mimic human saliva. Therefore, the results obtained with artificial saliva should be taken with precaution, and future research should use human rather than artificial saliva.

Another important aspect concerns the treatment of human saliva and its conservation. Many studies using human saliva centrifuge saliva in order to remove aggregates, food debris and microorganisms. Muñoz-González *et al.* compared the impact of whole saliva and centrifuged saliva on aroma release [41]. They found that centrifugation tends to reduce saliva's effect and its inter-individual variability. Observations of a difference between whole saliva and centrifuged saliva suggest that some salivary compounds are lost during this treatment. Indeed, centrifugation leads to the separation of particles depending on their size and density. The larger the size and the larger the density of the particles, the faster they separate from the supernatant and form a precipitate (pellet) during centrifugation. Thus, the centrifugation of saliva leads to the loss of some salivary objects, such as microorganisms and protein aggregates. Indeed, Oppenheim described the presence of large aggregates composed of different salivary proteins in saliva [44]. These aggregates could contribute to the loss of salivary effects observed by Munoz *et al.* Therefore, it is important to gain a deeper understanding of the structure of these objects and their impact on flavor release.

Moreover, in the mouth, saliva is continuously renewed, while this process is stopped after the saliva is collected. Consequently, when saliva is outside the mouth, the

physicochemical parameters, such as pH, are likely to change, altering the structure and the activity of some enzymes. For example, the glutathione transferase P1 (GSTP1) present in human saliva is inactivated by hypothiocyanite when the saliva is not renewed. Hypothiocyanite is an antimicrobial molecule naturally occurring in saliva [45]. Consequently, different parameters, such as salivary flow or the level of secretion of hypothiocyanite, regulate GSTP1's enzymatic activity. Moreover, GST enzymes were previously shown to metabolize aroma compounds in rats [46]. Furthermore, the activity of human salivary GSTs (GSTA1, GSTP1, GSTMu1 and GSTMu2) toward aroma compounds has yet to be investigated.

It is likely that freezing leads to the denaturation of proteins and the formation of non-native protein aggregates, while centrifugation depletes saliva from its larger objects. Therefore, saliva treatments (e.g., centrifugation, freezing) are likely to affect the structure, quantity, properties and enzymatic activity of salivary proteins and have to be discussed when used in regard to the obtained results. Moreover, it is important to develop future studies using fresh and whole saliva as much as possible rather than depleted and frozen saliva.

As there are great interindividual differences in saliva composition between humans, investigations have to be done to gain a deeper understanding of the effect of this interindividual variability on aroma release and perception, taking into account both the composition and properties (e.g., the total antioxidant capacity) of saliva and the potential role of the microbiota. Indeed, two different studies found a correlation between the total anti-oxidant capacity of saliva and the decrease in aroma release [41, 42]. One of these studies reported that salivary enzymatic activity was at the origin of the observed decrease [41]. This finding suggests that the salivary enzymatic degradation of

298 aroma compounds is under the control of the redox of saliva. Beside enzymatic activity,
299 redox reactions control in-mouth lipid oxidation, which generates volatile compounds,
300 such as aldehyde or esters [47]. Interindividual variability in this reaction was also
301 observed [47]. Lipid oxidation is also under the control of the metallic ion content of saliva,
302 while the addition of antioxidants decreases the reaction [47]. Therefore, different
303 reactions (i.e., enzymatic and chemical) led to the formation of aroma compounds, which
304 were not present in the initial composition of the food. Following these pioneer studies,
305 further investigations should be performed to determine the impact of these
306 mechanisms on flavor perception and the regulatory role of the total antioxidant
307 capacity of saliva. This information could help in the development of new food products
308 containing active antioxidant compounds, which could modulate these different
309 reactions and ultimately the perceived flavor.
310
311 A part of the salivary protein is specifically anchored onto the surface of the oral
312 mucosa, forming a biological structure called the mucosal pellicle. It contains salivary
313 proteins, such as amylase, IgA, cystatins, carbonic anhydrase IV, secretory components
314 or mucins [12]. Among these salivary proteins, MUC5B has been identified as a major
315 component [12]. Despite the suggestion that this structure can impact aroma release,
316 particularly, aroma persistence [8, 48], there are surprisingly no data to validate this
317 hypothesis. A model of the oral mucosa taking into account the mucosal pellicle was
318 recently developed. This model revealed that epithelial cells are able to metabolize
319 aroma compounds, while the role of the mucosal pellicle was clear [49]. Nevertheless, it
320 appears that its hydrated nature plays an important role in aroma release.
321

To conclude, eating is a dynamic process during which flavor compounds are released, dissolved, metabolized and transported following different kinetics as a function of their structure. The dynamics of these mechanisms are largely impacted by the composition of saliva and affect the dynamics of receptor activation. Moreover, integrative brain processes, such as adaptation and cross modal interactions, occur at the same time, which renders it difficult to find a direct correlation between flavor release and perception. As an example, during cheese consumption, a low salt content in saliva will increase salt perception and, as a consequence, the perception of salt-congruent aroma compounds, and a high lipolytic activity will increase fat perception and, as a consequence, the perception of fat-congruent aroma compounds [43]. Thus, there is a need to conduct multidisciplinary studies combining in-mouth processes and multisensory integration.

It is also important to develop new methodologies deciphering the respective contributions of these different mechanisms and their origins (salivary proteins or microbiota). These studies should be accompanied by further studies on the interindividual variability of saliva properties (e.g., antioxidant capacity) and the impact on aroma compounds. These studies will help to understand the role of saliva in inter-individual perception variability and also how changes in saliva composition modulate individual perception during life.

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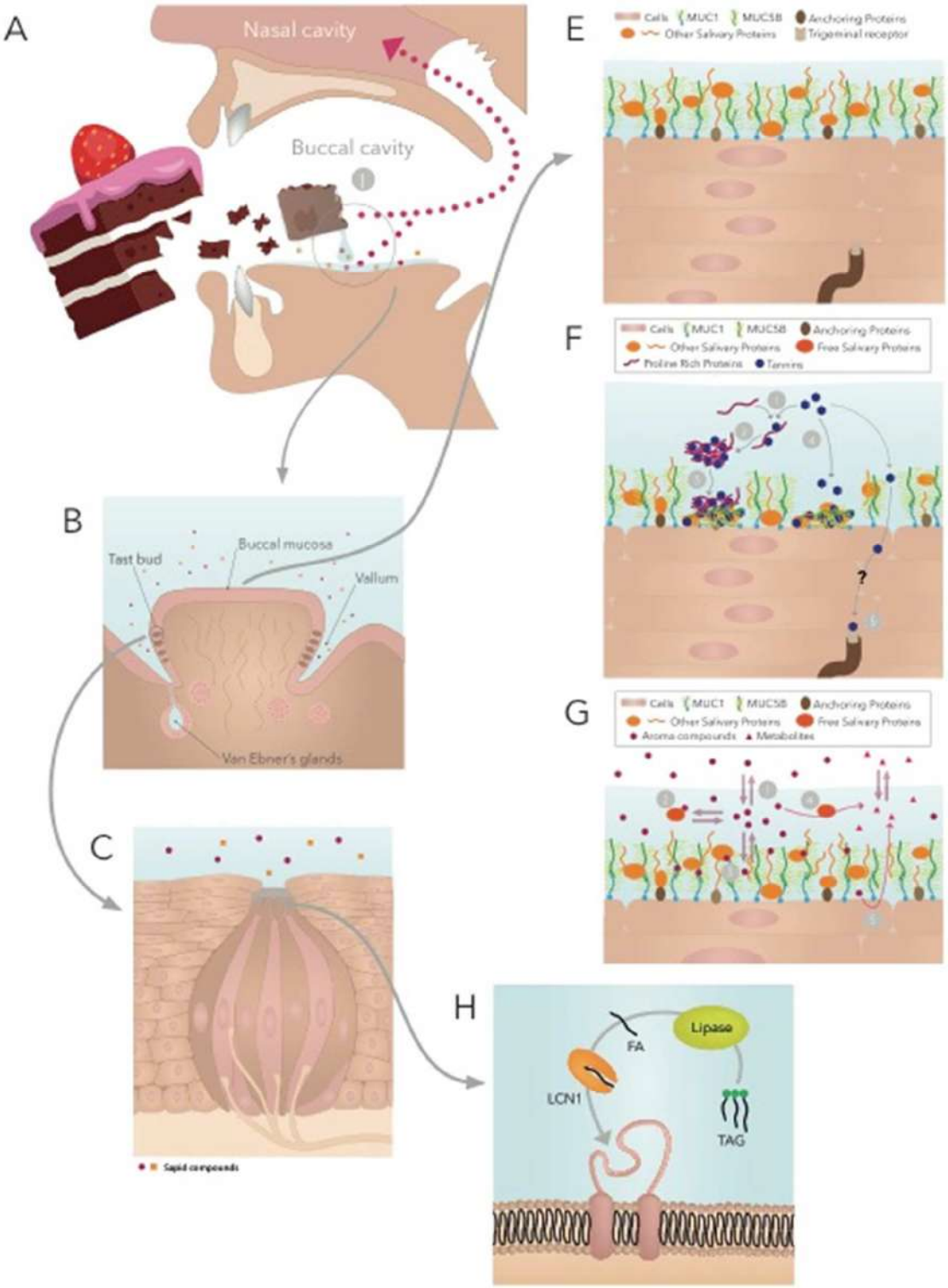
Legends:

Figure 1: A. Global view of oral mechanisms involving saliva; B. Structure of circumvallate papillae; C. Structure of taste bud; D. Structure of the epithelium of the oral mucosa including the mucosal pellicle; F. Hypotheses on astringency mechanisms; G. Hypotheses on the impact of saliva on aroma release; H. Hypothesis on the mechanisms involved in fat perception.

505 Figure 1



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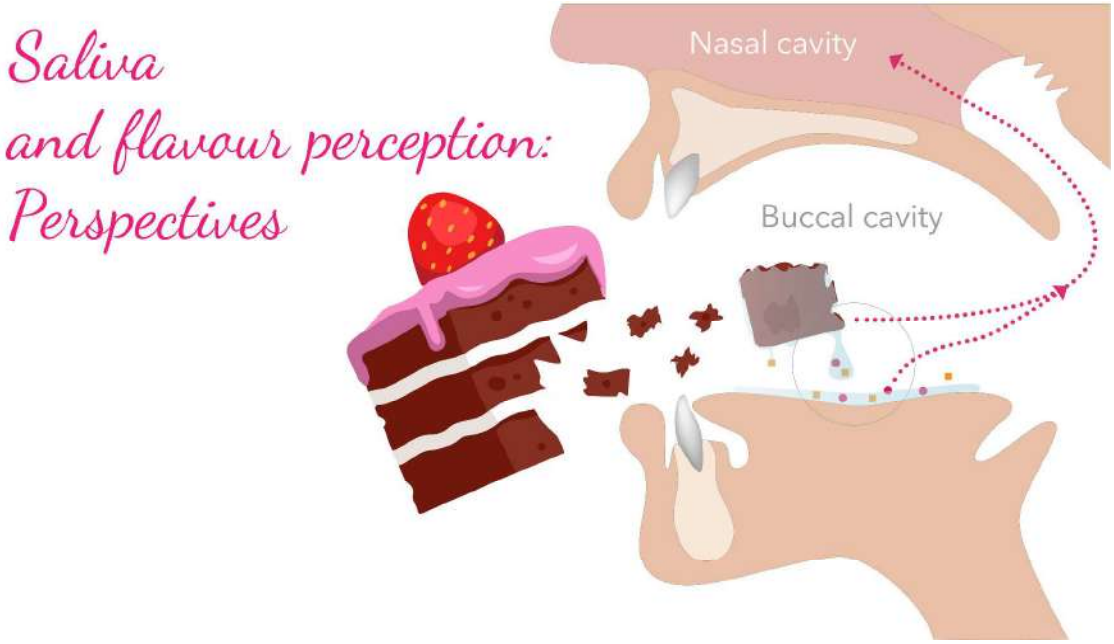
508 TOC Graphic for table of contents



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