Salivary proteomics in ingestive behaviour research: advances, potentialities and limitations

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Abstract

Human saliva proteomics gained interest in the last two decades, mostly due to the non-invasive nature of this fluid collection and to its potential for the diagnosis of different oral and systemic pathologies. Curiously, despite saliva being the fluid that first contacts with food, the interest in the relationship between its composition and ingestive behavior increased recently. The relevance of saliva protein composition in food acceptance and preferences is evidenced by the observation that individuals who differ in oral food perception present particularities in salivary proteome: individuals with different sensitivities for astringency diverge in the levels of several salivary proteins; the same is true concerning the perception of basic tastes, namely bitter and sweet. Even aroma perception depends on saliva protein composition. Interestingly, some of the proteins observed to differ according to oral food perception are proteins that present variations with Body Mass Index (BMI). Besides this potential role of saliva in driving food choices, this fluid may have also potential as a source of biomarkers of ingestion. Although less explored, until now, there are evidences of changes in saliva protein composition related with the type of diet: diets rich in polyphenols induce modifications in saliva composition, in animal models; high-fat diets consumption by rats were observed to change their levels of salivary alpha-amylase. These different points will be reviewed in the present article.

Keywords: Biomarkers of ingestion; Oral food perception; Salivary proteomics

Abbreviations: HPLC – High-performance liquid chromatography; PROP – 6-n-propylthiouracil; CA-VI – carbonic anhydrase VI; PRPs – Proline-rich proteins.

1. Introduction

Saliva has been considerably studied for its use in diagnosis, because this is a fluid that contains many of the molecules present in blood, in amounts proportional to the ones present in this last, but with the advantage, over plasma, of allowing non-invasive and simple collection. As such, repeated sampling is possible, without the need of special trained people or expensive equipment.

Salivary proteins constitute one of the main groups of salivary molecules with potential as biomarkers. For that reason the interest in salivary proteomics emerged, with more than 2400 non-redundant salivary proteins [1]. Generally, saliva proteomics studies rely on methodologies for separation and identification of proteins such as 2-D electrophoresis, capillary electrophoresis and high-performance liquid chromatography (HPLC), in combination with mass spectrometry. Several reviews report the main methodological approaches used in the area of salivary proteomics (e.g. [2–4]).

A considerable number of studies has been made with the aim of comparing healthy individuals with individuals suffering from different oral and systemic diseases. For example: i) in the search for breast (reviewed in [5]), gastric [6], or oral (e.g. [7]) cancer salivary biomarkers; ii) in the understanding and diagnosis of periodontal disease (e.g. [8]); iii) in neurology and psychiatry [9], among other clinical

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areas. But saliva is the fluid that bathes oral cavity tissues and that first contacts with food and, as such, it is somehow expected that saliva can be related with food sensorial perception, ingestion and digestion. However, the use of proteomics to investigate and understand ingestion has been few explored.

This article reviews the research carried out in the field of saliva and ingestive behavior, namely: i) the relationship between salivary proteome and oral food perception (astringency and basic tastes); ii) the way saliva can reflect ingestive strategies and dietary behavior; iii) the potential of saliva as an objective and non-invasive source of biomarkers of ingestion.

2. Salivary proteomics and oral food perception

2.1. Astringency perception

The relevance of salivary proteome in oral food perception started to be studied in the context of the involvement of salivary proteins in astringency development. Tannins are a group of polyphenols with different structural characteristics, but with the common feature of complexing and precipitating proteins what is responsible for the sensation of astringency perceived in foods containing these compounds. In 1954, Bate-Smith [10] proposed that astringency results from the interaction of tannins, present in foods and beverages, with salivary proteins. Since then, and despite some controversy about the exact mechanisms involved, it is accepted that salivary proteins have a major role in astringency development and intensity. Different authors presented evidences that the compounds perceived as more astringent are the ones that precipitate salivary proteins to a higher degree [11,12]. Moreover, differences in astringency perception among different individuals were reported to be linked to different salivary protein profiles. Dinnella and colleagues [13] observed that saliva composition is related to the intensity with which astringency is perceived and that there are differences in salivary responses between individuals high- and low-sensitive to this oral sensation. Recently, we confirmed that individuals presenting different tannic acid detection thresholds differ from each other in their salivary response to stimulation with this compound, and that the proteins whose levels change are salivary proteins with affinity to form insoluble complexes with these polyphenols [14].

2.2. Basic tastes perception

Salivary proteomics has been also related with basic taste perception. Bitter taste responsiveness, evaluated through 6-n-propylthiouracil (PROP) test (i.e. evaluation of the relationship between the intensity perceived of three solutions of PROP and the intensity of three solutions of NaCl) has been related with salivary proteome. S-type cystatins, prolactin-inducible protein, carbonic anhydrase VI (CA-VI) [15,16] and some proline-rich proteins (PRPs) [17] have been observed in different levels in tasters and non-tasters. Moreover, in tasters and non-tasters the changes in saliva composition induced by stimulation with bitter compounds are different: in tasters both S-type cystatins and CA-VI decrease after stimulation, whereas in non-tasters S-type cystatins levels increase [16].

Recently we provided evidences that sweet taste sensitivity is also related with salivary proteome [18]. Salivary amylase is one of the proteins whose levels and enzymatic activity are negatively correlated with sensitivity to this taste, i.e., there is a tendency for the individuals with higher amounts of this protein to have lower sensitivity for sucrose sweet taste. Besides that, some salivary proteins that are related with bitter taste perception appeared to be also related with sweet taste sensitivity, namely cystatins, CA-VI and prolactin-inducible protein [18].

Differences in the salivary proteome of individuals with different sensitivities for salty taste have also been reported. The variations were particularly at level of salivary proteases and protease inhibitors, suggesting that the action of proteases may influence transepithelial sodium transport mediated by epithelial sodium channels [19].

Concerning sour taste, although the relationship between salivary proteome and sensitivity for this basic taste appears to be less explored, changes in protein composition induced by acid stimulation has been reported [20,21]. It would be interesting to investigate how these changes are associated with salivary pH changes and/or with oral perception of this basic taste.

2.3. Retronasal aroma perception

The retronasal perception of the volatiles released during food mastication greatly influences global food perception and may constitute, together with olfaction, one of the main drivers of food preference and acceptance. Aroma release can be affected by factors that are inherent to food products, such as the chemical nature of the volatiles or the physicochemical characteristics of the food, or factors external to food products, such as the physiological characteristics of individuals. Among these last, saliva composition appears to have potential in modulating aroma perception (reviewed in [22]). Despite that the relation between the salivary proteome and aroma perception has been little studied, compared to the relation between this fluid and taste perception, it has been suggested that salivary proteins may affect the release of volatile molecules and their access to receptors: one study reported that the differences in wine aroma perception, between normal weight and obese individuals, are probably related with their different salivary protein composition [23]. Moreover, saliva was also observed to affect olive oil aroma release [24]. From the salivary proteins potentially involved in aroma perception, α-amylase and mucins have been reported to decrease the release of ketones and esters [25].
The effect of saliva in aroma perception appears to be dependent on the characteristics of food matrix. Whereas in foods like gelatine, the addition of saliva resulted in the enhancement of hydrophobic volatiles diffusion and in the decrease in hydrophilic compounds release [26], in cheese this fluid had an opposite effect, with an increase in the release of the hydrophilic compound ethyl propanoate [27].

Another interesting example of how salivary proteins may influence food aroma release is what happens in products rich in polyphenols. Phenolic compounds have been observed to interact with volatiles, modulating their release and the consequent aroma of olive oils [24] or wines [28]. Since salivary PRPs have the ability of binding food polyphenols, it has been suggested that the changes in aroma release from these products, when saliva is added, are the result of polyphenol-salivary protein interaction [24,28].

3. Saliva and food intake related diseases

The interest in studying salivary proteomics under the theme of ingestive behavior is highlighted by the observation that saliva composition differs in individuals with some nutrition-related diseases. If, on one hand, the knowledge of these changes may be of interest for the identification of non-invasive biomarkers of some of these diseases, on the other hand, it may be relevant to better understand metabolism and disease development. By linking some of the potential differences in salivary proteome with the knowledge about salivary proteins influence in oral food perception, as described in earlier section, it is possible to speculate about the factors involved in the unhealthy nutritional choices that result in the diseases.

Diabetes has been one of the metabolic diseases main studied in terms of salivary changes, with several authors reporting changes both at salivary gland level [29] and in salivary proteome [30]. Undernutrition in children has also been reported to associate with changes in salivary proteome [31]. Less well studied are eating diseases, but for anorexia changes in salivary biochemistry has been also found [32].

Previous studies have supplied us with evidence that salivary proteome from obese individuals differ from normal-weight ones [16,33]. Some of the differences are in the amounts of proteins associated with bitter and sweet tastes sensitivities, namely CA-VI, cystatins and salivary amylase. The simultaneous observation that some of the differences in obese salivary proteome are no longer observed in individuals that lose weight after bariatric surgery lead us to hypothesize that weight loss may induce changes oral medium composition, which affect taste perception and, consequently influence food acceptance. This is somehow understandable, since bariatric surgery results in alterations in the levels of hormones, such as leptin, and for this last, an association between blood [34] or salivary levels [35] and sweet taste perception is known.

Maybe the changes in saliva composition observed under nutrition-related diseases are one of the more puzzling issues, where it is necessary to investigate if the salivary differences are responsible for changes in food choices that promote disease or if are the disease that results in salivary changes. A long-term follow-up of the biochemistry of individuals, from healthy to disease conditions, would be valuable to elucidate this question. Saliva biobanks are not common, but start to emerge [36] and this appears to be a promising way to investigate saliva relationship with ingestive behavior.

4. Can saliva provide non-invasive biomarkers of food intake?

One of the main challenges in nutritional studies is to find objective biomarkers of ingestion. The assessment of food intake is a complicated task for several reasons, including the type of instruments available, which greatly rely on memory and/or subjective assessment. Currently, the most used dietary biomarkers are sodium, nitrogen, sucrose and fructose in 24h urine samples or the doubly water technique [37].

Studies in animals present evidence that salivary proteomics differs among species according to feeding strategies [38]. For a particular type of polyphenols, namely tannins, it is known that consumption elicits changes in the levels of some salivary proteins, among which salivary PRPs have been considerably studied (reviewed in [39]). Even closely related species, such as the ruminant species sheep and goats, present dissimilarities that can be related to their different feeding strategies [40] and change their salivary proteome in a different way when subjected to variations in diet composition [41].

In previous studies, with rodents we did observe variations in salivary glands histology and salivary protein composition by the increase in food’s tannin levels [42,43]. Interestingly, the variations were not the same when different structural types of tannins were consumed [44]: although salivary amylase increased after both hydrolysable (tannic acid) and condensed (quebracho) tannin consumption, the protein aldehyde reductase was only identified in saliva of animals supplemented with the condensed tannin [44]. This may lead to the hypothesis of salivary responses that are specific of diet composition.

In children, Morzel and colleagues [45] observed an association between dietary habits and saliva composition. Although the preliminary nature of this study, it reinforces the potential that salivary proteins may have as biological non-invasive markers of food intake. From our point of view, this is an area deserving attention, where more research needs to be done. However, this may be not an easy task, due to the plasticity of saliva, whose composition can be changed both due to short-term and long-term factors. The fluctuations in saliva composition induced by hygiene aspects or pathological conditions can even increase the difficulties, with the need of carefully controlled experiments.
5. Concluding Remarks

Despite the direct contact of saliva with oral structures and food constituents, the focus of salivary proteomics has been mainly in the search for disease biomarkers. However, the potential of saliva in the area of ingestive behavior deserves attention, since it may increase the understanding about food perception and choices and can be even a good source of ingestion biomarkers. By integration of several omics approaches it would be possible to increase the knowledge about this fluid, in the future, helping to understand its importance and its role in food ingestion.

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