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Human saliva protein profile: Influence of food ingestion

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ABSTRACT

Saliva is a mixture composed by the secretions of the major and minor salivary glands, together with the crevicular fluid, bacteria and cellular debris. Due to saliva being a complex and dynamic fluid, the protein profile may qualitatively vary under different conditions. So, in this work, the saliva protein composition on different days, throughout the day and in fasting and fed states was evaluated by high-performance liquid chromatography (HPLC) analysis. The results show that the saliva protein amount has the maximum peak at 2 p.m. and at this hour no differences on saliva protein composition were observed on different days. Nevertheless, gPRPs and aPRPs vary significantly throughout the day and after food ingestion in the early afternoon. However, feeding effect seems to be more pronounced in the morning after a fasting period. This fact suggests that besides the influence of food ingestion, saliva protein composition is also influenced by circadian rhythms. This work allows one to comprehend how the different families of salivary proteins (SP) may vary throughout the day and with the influence of food ingestion, which could be a helpful tool in several studies, such as, astringency perception and biological studies.

1. Introduction

Polyphenols, often referred as phenolic compounds, are present in several plant-derived foods and beverages such as fruits, vegetables, beer, red wine, coffee and chocolate. These compounds contribute directly to several organoleptic characteristics such as color and flavor. Regarding flavor, polyphenols are related to astringency, a sensory attribute defined as a complex group of sensations involving dryness, tightening and shrinking of the oral surface, and puckering sensations of the oral cavity (Bajec & Pickering, 2008; Lee & Lawless, 1991; Soares et al., 2011). Astringency has been proposed as the result of the interaction of salivary proteins (SP) and a complex group of polyphenols called tannins (Bate-Smith, 1954). Actually, it has been generally accepted and supported in the literature that astringency is due to the tannin-induced interaction and/or precipitation of salivary proline-rich proteins (PRPs) in the oral cavity (Dinnella, Recchia, Fia, Bertuccioli, & Monteleone, 2009, Dinnella, Recchia, Vincenzi, Tuorila, & Monteleone, 2010; Obreque-Slier, Pena-Neira, & Lopez-Solis, 2010; Schwarz & Hofmann, 2008). For instance, Soares, Mateus, and de Freitas (2012) reported the higher relative affinity of acidic PRPs (aPRPs) and statherin to interact and to precipitate with two classes of tannins (hydrolysable and condensed tannins).

In general, saliva consists in a mixture of proteins, electrolytes and small organic compounds. Whole saliva represents a mixture of the secretions of the major (submandibular, sublingual and parotid) and minor salivary glands, together with the crevicular fluid, bacteria and cellular debris. Saliva secretion is controlled by the autonomic nervous system via signal transduction systems that couple receptor stimulation to ion transport and protein secretory mechanisms (Dodds, Johnson, & Yeh, 2005). The saliva secreted at rest is often called unstimulated secretion, while saliva secreted in response to a strong stimulus is designated stimulated saliva. It has been reported that the contribution of the different salivary glands to the whole saliva in resting and stimulated conditions is different (Ekström, Khosravani, Castagnola, & Messana, 2012). The major contributors to unstimulated saliva are the submandibular and submaxillary glands, whereas parotid contribution increases dramatically during stimulation, producing a fluid with high PRPs concentration to protect against extrinsic agents (Dinnella et al., 2009; Ekström et al., 2012).

The secretions from the different glands have been shown to differ considerably and to be affected by circadian rhythms, diet, age, gender, several disease states and pharmacological agents and different forms of stimulation (Dawes, 1972, 1975; Dodds et al., 2005; Mandel, 1974). Circadian rhythms are endogenous self-sustained oscillations with 24-hour periods that regulate diverse physiological and metabolic processes through complex gene regulation by “clock” transcription factors (Zheng, Seon, McHugh, Papagerakis, & Papagerakis, 2012). It was reported elsewhere that saliva protein concentration follows a diurnal pattern that is higher in the afternoon than in the morning (Dawes, 1975; Ferguson & Botchway, 1980).

Food ingestion is a strong stimulus for the secretion of saliva and a number of sensory receptors are activated in response to food intake: gustatory receptors, mechanoreceptors, nociceptors and olfactory receptors. The last group responds to volatile molecules of the nasal and the retronasal airflow (the latter arising from the oral cavity or

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the pharynx) (Denker et al., 2006). When volatiles are released from food into the saliva phase, interactions may occur between volatile compounds and proteins (e.g. mucins). This interaction results in chemical and biochemical reactions which could affect the volatile concentration and the retronasal aroma perception (Denker et al., 2006; Friel & Taylor, 2001).

Regarding SP, they have been grouped into 6 structurally related major classes namely, histatins, basic PRPs (bPRPs), acidic PRPs (aPRPs), glycosylated PRPs (gPRPs), statherin and cystatins. All these peptides (except bPRPs) have relevant biological functions in saliva associated with maintenance of ionic calcium concentration (aPRPs and statherin), antimicrobial action (histatins and cystatins) or protection of oral tissues against degradation by proteolytic activity (cystatins) (Bennick, Cannon, & Madapallimattam, 1981; Bennick, Kells, & Madapallimattam, 1983; Helmerhorst & Oppenheim, 2007; Oppenheim et al., 1988; Schlesinger, Hay, & Levine, 1989; Shomers, Tabak, Levine, Mandel, & Ellison, 1982). For bPRPs, it has been suggested that one of their functions is to bind tannins, preventing their toxic effects in the gastrointestinal tract (Asquith et al., 1987).

Despite all the knowledge referred previously about SP, there is little information on how the saliva protein composition can be modified by different conditions (Hardt et al., 2005), such as the time of the day and with the influence of food ingestion. The specific study of the variations presented by PRPs and statherin is correlated to their biological properties in the maintenance of a good oral health and in sensory analysis. Changes in saliva protein composition can be associated with disease susceptibility, disease state, or both (Hardt et al., 2005). So, assessing the effect of the conditions previously cited could be an helpful tool in several areas.

In this work it was aimed to study the saliva protein composition from different subjects by high performance liquid chromatography (HPLC) on different days, throughout the day and the influence of food ingestion.

2. Material and methods

2.1. Reagents

All reagents used were of analytical grade or better. Acetonitrile (ACN) was purchased from Panreac Quimica. Trifluoroacetic acid (TFA) was purchased from Fluka Biochemica.

2.2. Human saliva collection, treatment and analysis

Human saliva was collected from healthy and nonsmoking volunteers. The experiences were conducted with a total of 10 subjects, aged from 21 to 31 years. Each volunteer contributed to a maximum of 4 independent samples ($15 \leq n \leq 21$). All participants were instructed to avoid food and beverage for at least 1 h before the different sessions started. Other specific instructions will be referred in the following sections according to the experimental procedure. The scheme of the experimental approach used in this work was represented in Fig. 1. The recording procedure was explained in detail to the subjects, who provided written consent prior to participation. The study was conducted according to the Declaration of Helsinki and was approved by the Ethics Committee of Medical School of University of Porto (EK84032011).

All subjects accumulate passively saliva in their mouths and these samples were collected into a plastic container. It was added TFA solution (10% aqueous TFA) to 900 μL of collected saliva (1:90 v/v) and the solution was centrifuged at 8000 *g* for 5 min. After centrifugation, the supernatant (acidic saliva, AS) was separated from the precipitate and 90 μL of each solution was injected on a HPLC Lachrom system (Merck Hitachi, L-7100) equipped with a Vydac C8 column (Grace Davison Discovery Sciences), with 5 μm particle diameter (column dimensions 150 × 2.1 mm); detection was carried out at 214 nm, using a UV–Vis detector (L-7420). The HPLC solvents were 0.2% aqueous TFA (eluent A) and 0.2% TFA in ACN/water 80/20 (v/v) (eluent B). The gradient applied was linear from 10 to 40% (eluent B) in 60 min, at a flow rate of 0.30 $mL \cdot min^{-1}$. After the program, the column was washed with 100% eluent B for 20 min in order to elute S-type cystatins and other late-eluting proteins. After washing, the column was stabilized with the initial conditions (Messana et al., 2004; Soares et al., 2011).

2.3. Saliva protein profile from different subjects

2.3.1. Daily saliva protein profile

The saliva protein profile was assessed on four different non-contiguous days by analysis of saliva collected at 2 p.m. AS samples were analyzed in triplicate by HPLC.

2.3.2. Saliva protein profile throughout the day and influence of food ingestion (fasting state vs. fed state)

In order to investigate if saliva protein composition exhibit differences throughout the day, saliva was collected at 10 a.m., 12 a.m., 2 p.m. and 4 p.m. Meals were performed until 9 a.m. and between 12 and 1 p.m.

After observing an increase of proteins area at 2 p.m. (see Results and Discussion section), it became important to analyze whether this increase is dependent of the food ingestion time. So, it was asked to the subjects to delay their meals to be between 2 p.m. and 3 p.m.

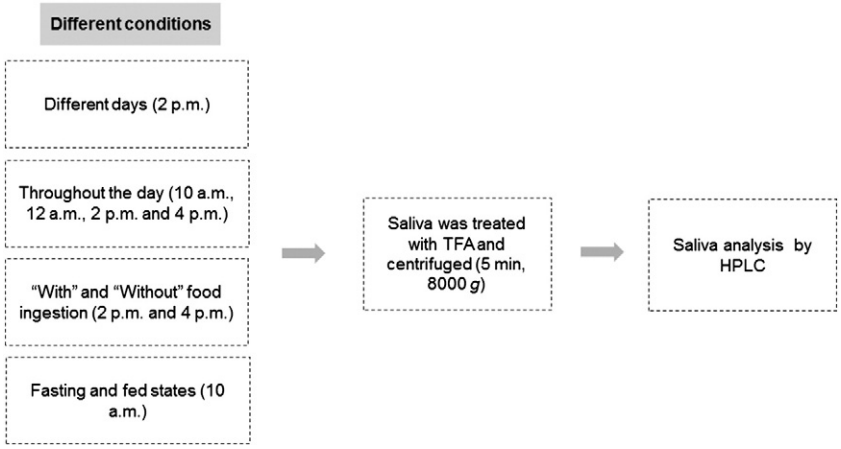

Fig. 1. Scheme of the experimental approach used to study saliva protein composition in different conditions.