
Oral food perception and polyphenol-rich foods acceptance - the importance of knowing individuals saliva characteristics for promoting consumption

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Abstract

Polyphenols are widely present in fruits, vegetables, cereals and beverages. Their study gained scientific interest because of their beneficial effects on health. Although there is currently no official dietary recommendation for polyphenol intake, health professionals recommend the consumption of 5-8 daily portions of fruits and vegetables. This is not always achieved and, despite possible causes associated to practical schedule difficulties, the aversive bitter and astringent sensations associated to polyphenols may also lead to avoidance. As such, a better understanding on mechanisms responsible for differences among people, in polyphenol oral perception, is needed for promoting healthier choices. Saliva has been linked to polyphenol consumption. We have previously observed, in animal models, changes in salivary proteome induced by tannin-enriched diets. Moreover, differences in astringency perception were attributed to differences in salivary protein composition. In a recent experiment, we observed differences among individuals with dissimilar tannic-acid perception: people with high sensitivity for the oral sensations elicited by tannins have higher amounts of salivary cystatins and lower capacity to maintain their levels after tannic-acid ingestion. Additionally, and similarly to previous studies, salivary amylase was observed to be involved in tannin perception. In this presentation, oral cavity characteristics influencing the perception of polyphenol-containing foods will be discussed.

Introduction

Epidemiological studies suggest that polyphenols are associated to health benefits, including reduced risk prevention of cardiovascular diseases and some types of cancers, or even by acting as

anti-microbial agents (Ullah & Khan, 2008). These compounds are mainly present in fruits and vegetables. Despite these evidences and recommendations from health professionals, many people does not fulfill the requirements. One of the possible causes of polyphenol based

foods avoidance (at least by some people) may be the aversive oral sensations of astringency, bitterness and sourness greatly associated to these compounds (Duffy et al., 2016). The intensity with which astringency is perceived has been tentatively related to the intake of polyphenol-rich foods (Dinnella *et al.*, 2011). However, inter-individual differences in oral perception are known and this may be important for understanding differences among individuals in dietary preferences and dietary choices.

Astringency development depends on the interaction of salivary proteins with astringent molecules. Different authors, including our team, have already observed variations in salivary protein composition according to the levels of tannins ingested (*e.g.* Lamy *et al.*, 2010). Italian researchers had previously reported differences between individuals with high vs low responsiveness to the astringent stimuli tannic acid, and suggested individual physiological variations of parotid gland function as a possible factor involved in differences in sensitivity to astringency (Dinnella *et al.*, 2010). The binding and precipitation of salivary proteins by polyphenols is known, but is more debatable if the levels and the types of proteins with polyphenol precipitation capacity are the same in individuals with different polyphenol oral acuity.

The objective of the present study was to identify the salivary proteins mainly related to astringency perception in low and high astringency sensitivity and to compare these two sensitivity groups for the type and amount of salivary proteins with polyphenol binding capacity.

Materials and methods

Individuals and sensorial tests

Thirty-one adults (13 males and 18 females), with ages ranging from 27 to 59 yrs. old, were tested with different concentrations of tannic acid (0.013, 0.027, 0.053, 0.106, 0.212, 0.425, 0.850, 1.70 g/mL). Individuals were recruited from the University of Evora and all kept the compromise of not taking food or drinks, beside water, in the

two hours previous to session. Tests and saliva collection were performed in the morning, between 10:30 and 11:30 a.m. The sensory analysis was performed according recommendations of ISO 6658-2005. Solutions were tested from the lowest to the higher concentration and saliva collection (non-stimulated samples) was performed before and after the complete tests. People were asked to say if each cup contains water or something different from water. Detection threshold was considered as the lower concentration perceived as different from water. All subjects read and signed an informed consent form.

Saliva analysis and Electrophoretic (SDS-PAGE) profile

Saliva flow rate was determined by measuring the saliva volumes collected during the 4 minutes collection and divided by that time. Saliva collected before and after sensorial tests was assayed for protein concentration using Bradford procedure and proteins from that samples were separated according to their molecular masses, by Sodium-dodecyl sulphate polyacrylamide gel electrophoresis (SDS –PAGE), using 12% acrilamide gels (mini-gels). Sample preparation and running was performed as described elsewhere (Rodrigues *et al.*, 2015). After running, gels were scanned and images analysed using GelAnalyzer software and statistical analysis (SPSS).

Tannic acid – protein binding assay

Six saliva samples from tannic acid high sensitive and 6 samples from low sensitive individuals were incubated with a solution of tannic acid (1.70 g/mL), 30 minutes, 37°C. Other tubes contained saliva samples incubated with distilled water instead of tannic acid solution (control). After that samples were centrifuged at 15000g, 15 min, RT. Supernatant and precipitate were collected for different tubes and the salivary proteins of both fractions were separated by SDS-PAGE, and analyzed as described before.

Results and discussion

Comparing groups for the saliva collected before tannic acid sensorial tests, only protein band B (Figure 1) presented different expression levels: lower expression in low sensitive individuals compared to sensitive ones (8.53 ± 0.18 vs 9.86 ± 0.4 % vol, respectively; $P=0.007$).

However, the main differences between individuals with different sensitivity for tannic acid, in terms of saliva composition, is not observed at each time point but rather in terms of response to tannic acid stimulation. Comparing the saliva from the period before sensorial tests with saliva collected after tannic acid stimulation, it was observed a decrease in total protein concentration in all sensitivity groups. Nevertheless, in terms of saliva flow rate, increases were only observed in high-sensitive individuals (0.66 ± 0.1 mL/min before vs 0.79 ± 0.11 mL/min after; $P=0.006$). Increases in saliva flow secretion in response to aversive stimuli, such as acids, is greatly reported (Guinard *et al.*, 1997) and it possibly represents a defence strategy for faster elimination of the aversive stimuli from the mouth. For our experiment, we can hypothesize that the most sensitive individuals are the ones for which tannic acid produces higher aversion (due to its astringency, bitterness and sourness) and as such, these individuals respond with higher intensity in terms of flow secretion.

Three protein bands (Figure 1), identified as containing 4 different proteins, changed differently in high and low sensitive individuals in response to tannic acid stimulation: band I (cystatins) have higher decreases in high-sensitive, comparatively to low-sensitive individuals. Bands G (not-identified) and H (Ig K chain C region + Zymogen granule homolog 16) change their expression levels in response to tannic acid stimulation only in low-sensitive individuals, with band G increasing its expression and band H decreasing (Supplementary Table 1).

Among the proteins that respond to tannic acid differently in high- and low-sensitive individuals, 3 are salivary proteins that precipitate tannic acid,

as we observed in the protein profile of the precipitates, after in-vitro saliva incubation with tannic acid (Figure 2). Salivary α -amylase has been already referred to precipitate tannins (Mateus *et al.*, 2004). Concerning salivary cystatins, their presence in the precipitate formed after saliva-tannic acid interaction has been already reported for a primate species (Mau *et al.*, 2011). Moreover, in a recent study from our lab, we also observed decreases in salivary cystatins expression, in individuals with high sensitive to bitter taste, after bitterness stimulation (*not published*). As such, further studies are needed to see if the present results are mainly due to astringent or bitter properties of tannic acid.

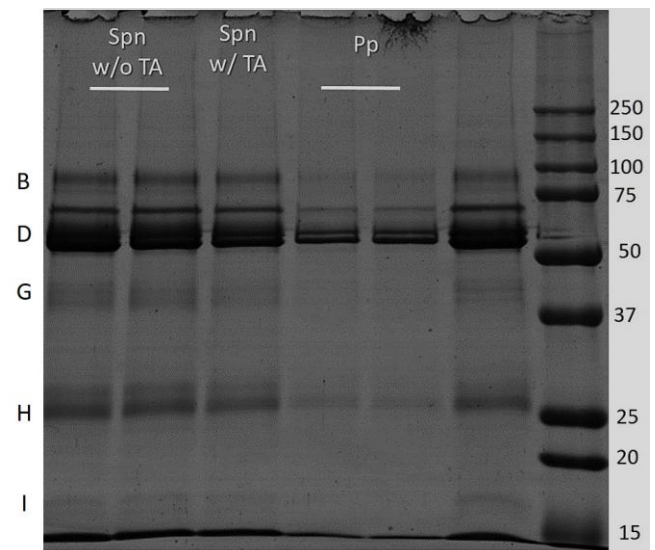


Figure 1. SDS-PAGE profiles of saliva with and without incubation with tannic acid (Spn w/o TA – supernatant resultant from the incubation of saliva with water; Spn w/TA – supernatant resultant from the incubation of saliva with tannic acid; Pp – precipitate resultant from the incubation of saliva with tannic acid).

Upper letters represent bands for which differences were observed among tannic acid sensitivity groups.

Acknowledgements

This work is funded by FEDER funds through the Operational Programme for Competitiveness Factors - COMPETE and by National Funds through FCT - Foundation for Science and Technology under the Strategic Project pest-

C/AGR/UI0115/2011 and PEst-OE/AGR/UI0115/2014. Authors acknowledge also the financial support from the Portuguese Science Foundation (FCT) in the form of Elsa Lamy FCT investigator contract IF/01778/2013. The authors acknowledge also financial support provided by Programa Operacional Regional do Alentejo (InAlentejo) ALENT-07-062-FEDER-001871

References

Dinnella C, Recchia A, Vincenzi S, Tuorila H, Monteleone E. 2010. Temporary modification of salivary protein profile and individual responses to repeated phenolic astringent stimuli. *Chemical senses* 35(1), 75-85.

Dinnella C, Recchia A, Tuorila H, Monteleone E. 2011. Individual astringency responsiveness affects the acceptance of phenol-rich foods. *Appetite* 56(3), 633-642.

Duffy VB, Rawal S, Park J, Brand MH, Sharafi M, Bolling BW. Characterizing and improving the sensory and hedonic responses to polyphenol-rich aronia berry juice. *Appetite*. 2016,107:116-125.

Guinard JX, Zoumas-Morse C, Walchak C. 1997. Relation between parotid saliva flow and composition and the perception of gustatory and trigeminal stimuli in foods. *Physiology & behaviour* 63(1), 109-118.

Lamy E, Graça G, da Costa G, Franco C, e Silva FC, Baptista ES, Coelho AV. 2010. Changes in mouse whole saliva soluble proteome induced by tannin-enriched diet. *Proteome Science* 8:65.

Mateus N, Pinto R, Ruão P. de Freitas V. 2004. Influence of the addition of grape seed procyanidins to Port wines in the resulting reactivity with human salivary proteins. *Food Chemistry* 84(2), 195-200.

Mau M. de Almeida A M, Coelho AV, Südekum KH. 2011. First identification of tannin-binding proteins in saliva of *Papio hamadryas* using MS/MS mass spectrometry. *American Journal of Primatology* 73(9), 896-902.

Rodrigues L, Mouta R, Costa AR, Pereira A, Capela e Silva F, Amado F, Antunes CM, Lamy E. 2015. Effects of high-fat diet on salivary α -amylase, serum parameters and food consumption in rats. *Archives of Oral Biology* 60(6), 854-862.

Ullah MF, Khan MW. 2008. Food as medicine: potential therapeutic tendencies of plant derived polyphenolic compounds. *Asian Pacific Organization for Cancer Prevention* 9(2), 187-196.

Supplementary Table 1 – Variations in the expression levels of salivary protein bands with tannic acid oral stimulation

	High sensitive			Low sensitive		
	<i>Before</i>	<i>After</i>	<i>P</i>	<i>Before</i>	<i>After</i>	<i>P</i>
Band I	13.0±1.0	7.9±0.5	0.021	12.8±1.1	8.5±0.8	0.027
Band G	18.8±1.8	21.5±2.5	0.276	19.1±0.8	21.8±1.1	0.043
Band H	14.5±1.3	13.7±1.1	0.585	14.3±0.6	12.3±0.6	0.018