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Saliva Characteristics and Individual Sensitivity to Phenolic Astringent Stimuli

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Abstract

Astringency sensation is due to interactions between salivary proteins and phenols and is based on an increased-friction mechanism. Modifications to the profile of salivary proteins and their concentration could affect tannin/protein reactions and hence the intensity of perceived astringency. Salivary characteristics of 65 subjects were compared after abstention from phenol-containing food and immediately after ingestion of tannic acid. The effect of stimulation on saliva characteristics was expressed in terms of *D* value, computed as the arithmetic difference between values found in saliva samples from the 2 conditions. Based on *D* values, subjects were clustered in two groups. Cluster 1 (Cl1, 53 cases) was characterized by low *D* values thus indicating that the basal saliva condition was quickly restored in these subjects. Cluster 2 (Cl2) was composed of 12 subjects whose basal salivary condition was not quickly restored, particularly in terms of salivary protein concentration and profile and saliva haze-forming capacity. Sensory data showed that subjects capable of maintaining constant saliva characteristics were less sensitive to astringent stimuli than subjects in which the same stimulations induced significant saliva modifications. The results suggest that a large proportion of the population are able to maintain their salivary protein concentration and simultaneously intercept and inactivate dietary tannins.

Key words: haze-forming capacity, phenol/protein insoluble aggregates, PRPs, salivary proteins, sensory evaluations, tannic acid

Introduction

Phenolic compounds represent one of the most widespread classes of plant secondary metabolites that include various groups with different chemical structures and properties. They are widely present in food and beverages, and their antioxidant activity probably accounts for the role of phenols (Phs) in preventing diseases related to oxidative stress. In fact, experimental evidence indicates that one group of Phs, flavonoids, may provide protection against cardiovascular disease and possibly also have an anticarcinogenic effect (Alvarez et al. 2006; Hooper and Cassidy 2006; Prior et al. 2006; Gardner et al. 2007). Tannins, a related group of high molecular weight Phs, are also antioxidant, but various harmful effects have been reported for these compounds. These include inhibition of digestive enzymes, formation of relatively less digestible complexes with dietary proteins, depressed growth in rats, altered food consumption, and acute hepatotoxicity (Chung et al. 1998; Mueller-Harvey 2006).

A number of postingestive countermeasures are used by mammalian herbivores in order to mitigate the negative effects caused by tannins that include secretion of tannin-binding proteins, increased gastrointestinal mucus production, degradation of tannins by microorganisms in the gut, and activation of detoxifying enzymes (Dearing et al. 2005). Avoiding the ingestion of food too high in Ph content is the simplest preingestive countermeasure against dietary tannins. These compounds have a strong astringent effect, and it has been proposed that the sensation of astringency represents a sensory warning cue that would discourage animals from ingesting foods too high in tannin content.

The sensation of astringency on the human palate has been defined as a complex group of sensations involving dryness of the oral surface and tightening and puckering sensations of the mucosa and muscles around the mouth (Lee and Lawless 1991; Gawel et al. 2000). Studies addressing acceptance of astringency indicate that this is perceived as a negative attribute

and is cited as a reason for consumers rejecting some plant food products (Lesschaeve and Noble 2005).

A distinctive characteristic of tannins is the ability to bind proteins. Dietary tannins form complexes with salivary proteins (SPs) resulting in their aggregation and/or precipitation (Kallithraka et al. 1998). Proline-rich proteins (PRPs) and histatins are effective precipitators of tannins, and an important tannin-binding activity has also been demonstrated for salivary glycoprotein with strong lubricating activity such as mucin, glycosylated PRP, and amylase (Asquit et al. 1987; de Freitas and Mateus 2001; Bennick 2002; Monteleone et al. 2004; Nayak and Carpenter 2008). A friction-based mechanism for astringency development due to precipitation of SPs has been suggested (Prinz and Lucas 2000; de Wijk and Prinz 2006). A 3-stage model of the interaction between Phs and proteins has been proposed (Charlton et al. 2002) and recently further developed (Jobstl et al. 2004; Poncet-Legrand et al. 2006). The model consists of proteins binding to polyphenols followed by the formation of polyphenol-coated protein dimers. In the third phase, protein–polyphenol complexes interact to form large aggregates that can grow to colloidal size, at which point they scatter light and, larger still, leading to sediment formation. A schematic representation of the phases occurring in binding and insolubilization of tannins by proteins is shown in Figure 1.

Development of haze in saliva–tannic acid mixtures over a range of tannic acid concentrations known to elicit astringent sensations has been observed (Horne et al. 2002). Haze development in saliva–tannic acid mixtures was found to be positively correlated with sensory responses when tannic acid concentration was the source of variation (Condelli et al. 2006). Therefore, the formation of insoluble aggregates of tannin–SP represents the key step in the development of astringency. The formation in the oral cavity of tannin–SP aggregates and the consequent perception of astringency could be considered a sort of "alarm system" that signals the presence of potentially harmful compounds in the oral cavity. This hypothesis implies that the binding of tannins to proteins produces a precipitate that itself causes astringency, and in this case, haze development would parallel the intensity of perceived astringency. Alternatively, there is evidence to suggest that SPs, indeed PRPs, act as a "defense system" against dietary tannins (Mehansho et al. 1987; Haslam 1989; Baxter et al. 1997). Tannin-binding salivary proteins (TBSPs) are able to readily bind tannins in the oral cavity. TBSPs are supposed to prevent the tannins from interacting with other proteins, such as digestive enzymes and food proteins, thus exerting a protective action against their negative effects on nutritional uptake (Bennick 2002; Shimada 2006). PRP–tannin complexes remain stable under conditions similar to those in the digestive tract, thus decreasing the transport of tannins across intestinal wall and their absorption into the organism (Skopec et al. 2004; Cai and Bennick 2006; Cai et al. 2006). Furthermore, PRPs synthesis is induced by the presence of dietary tannins and β agonists even if, as reviewed by Shimada, this response varies among species (Shimada 2006). PRPs are produced at high levels in humans, and it is interesting to note that mastication induces stimulation of the parotid glands thus increasing PRPs concentration in saliva during food ingestion (Dodds et al. 2005).

On the basis of the cited data, an increased formation of insoluble protein–tannin complexes in the oral cavity would not necessarily correspond to an increase of the perceived astringency. In fact, it is possible to speculate that increased level of TBSPs would correspond to a better protection against dietary tannins and to a higher perception threshold for astringency. Based on the "defense" theory, SPs having

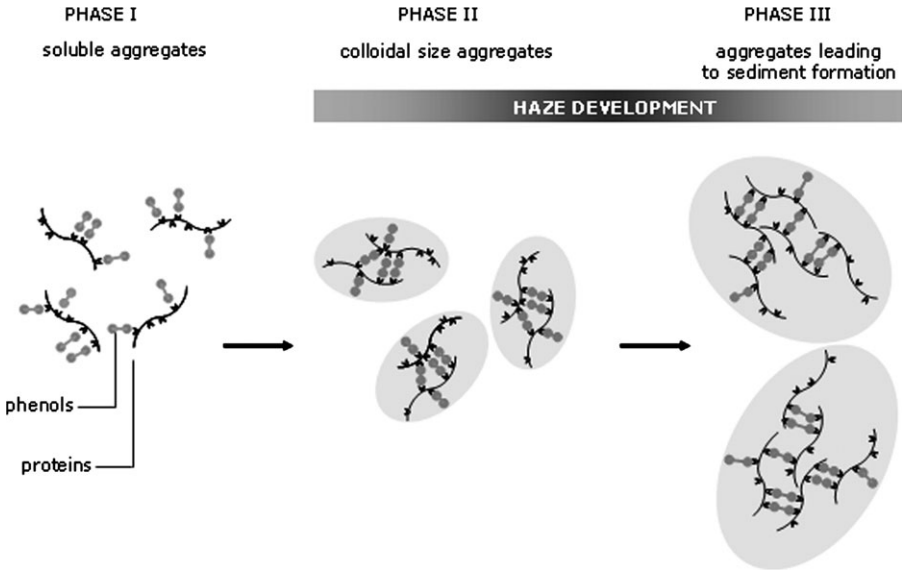

Figure 1 Scheme of polyphenol/protein interactions.

high tannin-binding activity as well as strong haze-forming capacity (HFC), such as PRPs and histatins, would play only a secondary role in the mouth feeling of astringency. On the other hand, salivary glycoproteins, indeed those involved in the mucosal pellicle formation (mucins, cystatins, amylase) could be responsible for elicitation of the sensory warning cue. A 2-step SP/dietary tannin interaction can be hypothesized, the first one involving proteins with the highest tannin-binding affinity (PRPs and hystatins) that exert a sequestering and protecting role and a second step based on glycoprotein/tannin interactions with the consequent oral cavity delubrication and the astringency generation. The inhibiting effect of soluble SPs on astringency perception has recently been demonstrated by Nayak and Carpenter (2008).

Studies across individuals, aimed to examine the relationship between saliva HFC when reacting in vitro with tannic acid and astringency ratings, indicated that subjects with a high level of saliva HFC rated astringency at a significantly lower level than subjects with low saliva HFC. These results are consistent with a pattern of ''low-responding subjects'' better protected against the deleterious effect of Phs and consequently less sensitive toward astringent stimuli (Horne et al. 2002; Condelli et al. 2006). Also other salivary characteristics such as flow rate and Ph content have been reported to modulate the intensity of astringency induced by phenolic compounds (Fischer et al. 1994; Horne et al. 2002; Siebert and Chassy 2003; Condelli et al. 2006). The subject group with high salivary flow rate perceived astringency intensity at a significantly lower level than the low flow rate group, and the analysis of time–intensity curves showed a longer time of recovering from astringency for the latter group (Fischer et al. 1994).

The physical–chemical characteristics of saliva are not constant and vary within a person over time and between individuals. Salivary flow rate is modulated by a number of factors such as circadian rhythm, ageing, and chemical and mechanical stimulation (Ghezzi et al. 2000; Engelen et al. 2003; Bourdiol et al. 2004; Dawes 2005). The type of taste stimuli and the intensity of chewing strongly affect salivary gland functionality thus inducing modifications of saliva composition (Dawes 1984; Dawes et al. 2000; Rayment et al. 2001). The major contributor to unstimulated flow is the submandibular gland, which produces less serous, mucin-rich saliva that provides lubrication to oral tissues. Whereas parotid flow increases dramatically during stimulation producing fluid with high PRPs concentration to protect against extrinsic insults. Diet modifications, such as a liquid versus a solid diet, also affect salivary flow and composition. Saliva was found to contain polyphenols after consumption of a polyphenol-rich beverage, and these compounds have been found to persist a long time after ingestion (Siebert and Chassy 2003).

Based on the hypothesis that SPs with different secretion patterns could lead to SPs/Phs aggregates with astringency-inducing or -inhibiting roles, the main objective of this work was to examine their role as an ''alarm'' or ''defense'' system against dietary tannins. Moreover the relations between individual physiological differences in saliva and sensitivity to (phenolic) astringent stimuli were investigated. The experimental plan was designed on the basis that phenolic astringent stimuli induce modifications to oral cavity conditions in terms of protein profile, Ph content, and lubrication (Prinz and Lucas 2000; Kallithraka et al. 2001; Siebert and Chassy 2003). Therefore, the characteristics of whole saliva induced by chewing a tasteless piece of parafilm and the astringency perception of 65 subjects were measured in 2 different conditions:

1. after resting: after 10-h abstention from Ph-containing food and beverage and 2-h abstention from mechanical (chewing) and gustatory salivation stimuli
2. after stimulation: 30 min after a mechanical stimulation (chewing a parafilm square) and 2.0 min after ingestion of a liquid Ph-containing sample.

Materials and methods

Subjects

Sixty-five subjects, 29 males and 36 females, aged from 22 to 27 years were recruited from the University of Firenze students. All subjects had no history of oral perception ability disorders. They were paid for their participation in the study. The Ethic Committee of the Dipartimento di Biotecnologie Agrarie, Università di Firenze, approved the protocol. Written informed consent was obtained from each subject after a full explanation of the experiment.

Sensory procedure

Training

Prior to their participation in the experiment, the subjects were trained to recognize and rate the perceived intensity of the following different sensations: sourness, bitterness, and astringency using the following standard solutions—citric acid: 0.25, 0.38, 0.50 g/l; quinine monohydrochloride dihydrate 0.025, 0.037, 0.050 g/l; aluminium potassium sulphate: 0.3, 0.6, 0.9 g/l. During training sessions, the subjects were asked to rate the perceived intensity on a Labeled Magnitude Scale (LMS) with the lower bound of the scale labeled as ''barely detectable'' and the upper bound as ''strongest imaginable'' oral sensations, including pain. Subjects participated in a total of 4 training sessions.

Evaluation

Tannic acid (Sigma-Aldrich, St Louis, MO) solutions at different concentrations in 1% ethanol (v/v) were used for astringency stimulation. Samples were presented at room

temperature. In order to evaluate the target stimuli, subjects were asked to hold each sample in their mouth for 10 s, spit it out, wait for further 20 s, and rate the intensity of astringency, bitterness, and sourness on an LMS. Across subjects, the order of attribute evaluation was balanced in order to minimize a possible ''proximity'' effect. Between sample evaluation, subjects were asked to rinse their mouths with distilled water for 40 s, to have some plain crackers for 40 s, and finally to rinse their mouths with water for a further 40 s. Samples presentation was evenly balanced to control both order and carryover effect on sensory responses (MacFie et al.1989). The evaluations were performed in individual booths under red lights, and subjects were asked to wear a nose clip in order to eliminate both visual and odor clues.

Saliva measurements

Salivary flow

Whole salivary flow evaluation was performed according to the procedure described by Gaviao et al. 2004. Subjects mechanically evoked saliva by chewing on a square of parafilm (3 × 3 cm). They spat saliva into a weighed container for 5 min. Then, a rest of 5 min was given before a further saliva collection. The entire procedure was repeated 3 times for a total saliva collection time of 25 min. The collected saliva was weighed on an analytical balance.

Haze-forming capacity

Saliva samples were put in an ultrasonic water bath at the maximum output for 5 min at 37 °C. The pellet eventually still present in the salivary sample was discarded, whereas the clear upper phase was recovered and analyzed.

Sample reactivity with tannic acid solution was evaluated in terms of HFC as described by Horne et al. 2002. An amount of 0.6 ml of saliva was mixed with 2.4 ml of tannic acid solution (0.23 g/l) in ethanol 1%. The mixture was allowed to stand for 1 min at 37 °C, and the turbidity was determined in a HACH 2100N Laboratory Turbidimeter (Hach Co, Loveland, CO) and expressed in nephelometric turbidity unit (NTU). The optical system was fitted with a tungsten-filament lamp with 3 detectors: a 90°scattered light detector, a forward-scatter light detector, and a transmitted light detector. The instrument was calibrated prior to the experiment with formazin primary standards prepared from a 4000 NTU stock solution (Hach Co) and high performance liquid cromatography (HPLC) grade deionized water. The instrument's calibration was periodically verified during the experiments using Gelex secondary turbidimetry standards (Hach Co). Each determination was performed in triplicate.

Protein content

SP content of the clear saliva samples was determined by the Biuret method (Kallithraka et al. 2001) in triplicate on each sample, and bovine serum albumin was used as reference protein.

Phenol content

Salivary phenolic content (Phs) was determined by using a modified Folin–Ciocalteau assay (Siebert and Chassy 2003). Clear salivary samples (0.25 ml) were mixed with 1.25 ml of diluted Folin–Ciocalteau reagent (1:10 v/v) and left to react for 15 min at 45 °C. Sample absorbance at 760 nm was determined, phenolic content calculated, and expressed as gallic acid concentration (mg/ml saliva).

Data set collection

The experiment required the collection of 2 saliva characterization sets and 2 sensory data sets. The experimental plan is schematized in Figure 2.

The day before the experiment, participants received instructions about food and beverages to avoid for at least 8 h before the session starts. They were also instructed not to smoke or have food, chewing gum, or candies for 2 h before the session started. Moreover a Ph-free menu for breakfast was suggested.

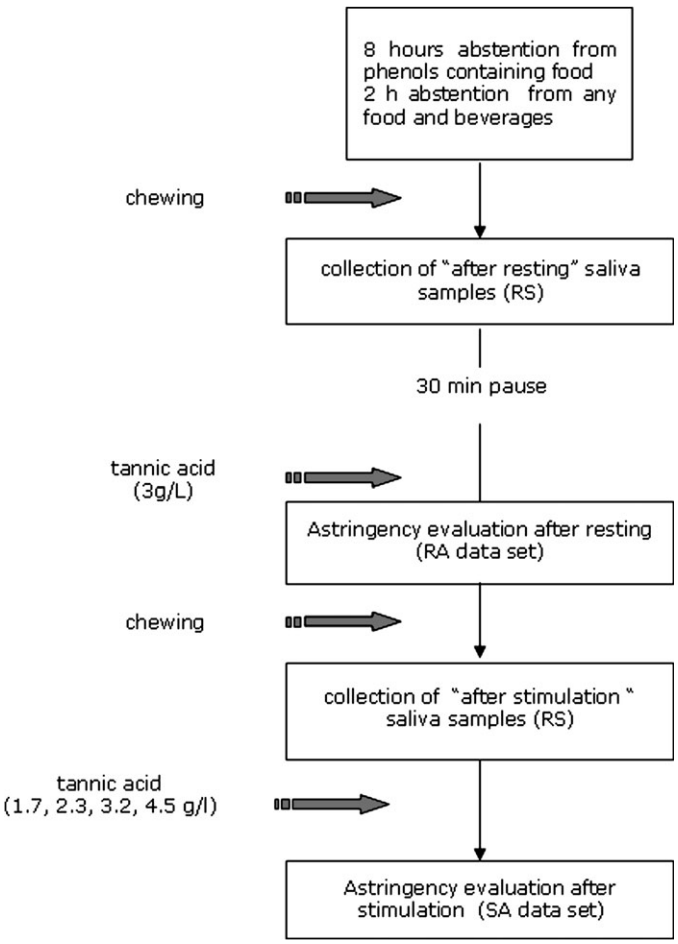

Figure 2 Experimental plan for data set collection.

The session for data set collection was held in individual booths, started at 9.00 AM with an average duration of 90 min.

Subjects received water to wash their mouth (20 ml water, 20 s, 6 times), then were instructed to mechanically evoke saliva by chewing a parafilm as described above. These saliva samples were indicated as "after resting" because they were obtained after 10-h abstention from Ph-containing food and 2-h abstention from the main salivation stimuli (resting sample [RS]). After a 30-min break, subjects received a tannic acid solution (3.0 g/l) and were asked to taste and rate the perceived astringency. Thus, the "astringency after resting" data set (resting astringency [RA]) was obtained.

Subjects were again asked to wash their mouth and mechanically stimulate and collect saliva as previously described. These saliva samples were indicated as "after stimulation" saliva samples (stimulated sample [SS]). Subjects received 4 tannic acid samples (1.7, 2.3, 3.20, 4.5 g/l) and were asked to taste and rate the perceived astringency of each. Thus, the "astringency after stimulation" data set (stimulated astringency [SA]) was obtained.

Results

Saliva characteristics in resting and stimulated conditions

Characteristics of saliva evoked by chewing were compared after resting conditions, that is, after 10-h abstention of Ph-containing food and 2-h abstention from the main salivation stimuli and after stimulated conditions, that is, 30 min after mechanical stimulation and immediately after ingestion of a Ph-containing liquid sample. Table 1 reports the correlation among salivary characteristics measured after resting and stimulated conditions. Flow rate was negatively correlated to HFC and SP concentration in both resting and stimulated conditions. SP concentration was positively correlated to both HFC and salivary Ph concentration with the highest r values found in the stimulated saliva data. Table 2 shows that characteristics of saliva after a resting period (RS) were significantly different from those measured after stimulation (SS). Both SPs and HFC were significantly lower in SS than in RS samples (SPs: $t_{64,1.67} = 4.39$, $P = 0.00$; HFC: $t_{64,1.67} = 3.36$, $P = 0.00$). As expected, salivary Ph concentration significantly increased after tasting astringent phenolic stimuli ($t_{64,1.67} = -5.82$, $P = 0.00$). A significant decrease was also found when comparing flow rate values measured after resting with those after stimulation (flow: $t_{64,1.67} = 2.13$, $P = 0.04$). The effect of stimulation on saliva characteristics was expressed in terms of D, computed as the arithmetic difference between values found in SS and RS samples for each salivary variable. Considerable variation was found between subjects as indicated by the wide range between minimum and maximum D values and showed by frequency diagrams (Figure 3). Consequently, the correlations among D values of salivary characteristics were computed, and the r values were not higher than 0.35 (Table 3).

Table 1 Correlations among salivary measures

	Resting			Stimulated		
	Flow	SPs	Phs	Flow	SPs	Phs
Flow						
SPs	−0.40*			−0.50*		
Phs	−0.17	0.59*		−0.43*	0.82*	
HFC	−0.37*	0.64*	0.43*	−0.41*	0.63*	0.60*

*Significant ($P > 0.05$). $n = 65$, degrees of freedom = 63, critical r value = 0.27.

Subjects grouping

A k-means cluster analysis was performed on D values, and 2 subject groups were obtained. One group was composed by a total of 53 subjects (Cl1) and the other by a total of 12 subjects (Cl2). Salivary characteristics of the 2 clusters are reported in Figure 4. A faint decrease in both flow ($t_{52,1.67} = 2.39$, $P = 0.02$) and SPs concentration ($t_{52,1.67} = 2.73$, $P = 0.00$) and an expected increase in Phs ($t_{52,1.67} = -6.33$, $P = 0.00$) were found after stimulation in Cl1. No significant modification of HFC was found ($t_{52,1.67} = 1.08$, $P = 0.28$). On the other hand, a strong modification of both SPs concentration and HFC mean values were found in

Table 2 Mean SP concentration, HFC, Phs, flow rate, and relevant D values of 65 subjects determined after resting (RS) and after stimulation (SS)

	SPs (mg/ml)			HFC (NTU)			Phs (mg/ml)			Flow rate (mg/min)		
	RS	SS	D	RS	SS	D	RS	SS	D	RS	SS	D
Mean	2.52	2.27	−0.25	4.42	3.48	−0.94	0.084	0.096	0.012	1.41	1.34	−0.07
SE	0.09	0.09	0.06	0.38	0.37	0. 28	0.002	0.003	0.002	0.07	0.06	0.03
SD	0.79	0.74	0.46	3.10	2.98	2.27	0.020	0.024	0.017	0.54	0.53	0.26
Min	1.18	1.19	−1.25	0.38	0.48	−11.53	0.051	0.057	−0.035	0.38	0.30	−1.02
Max	4.29	5.05	1.42	15.44	21.07	6.83	0.153	0.157	0.059	3.03	3.04	0.83

SE, standard error; SD, standard deviation; min, minimum; max, maximum values.

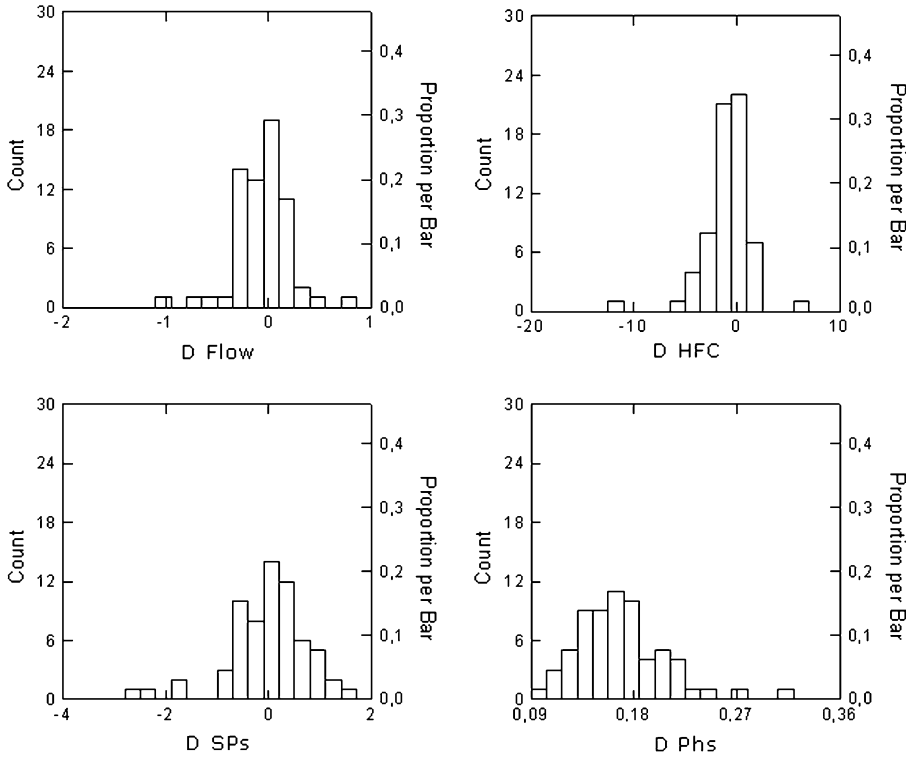

Figure 3 Frequency distribution diagrams of *D* values computed on salivary characteristics. Flow: flow rate, g/min, HFC (NTU), SPs: mg/ml, Phs: mg/ml.

Cl2, and both these values were significantly lower in SS than in RS samples ($t_{11,1.79} = 5.29$, $P = 0.00$; $t_{11,1.79} = 5.72$, $P = 0.00$, respectively). Neither Phs concentration ($t_{11,1.79} = -0.76$, $P = 0.46$) nor flow rate ($t_{11,1.79} = -0.39$, $P = 0.70$) mean values were significantly modified by stimulation in Cl2. The SPs profile, both after resting and stimulated conditions, was analyzed by sodium-dodecyl-sulphate polyacrylamide gel electrophoresis (SDS–PAGE) on 4 saliva samples collected from each cluster (Figure 5). No differences were found when the SPs electrophoresis patterns of RS and SS Cl1 samples were compared. However, modifications of SPs electrophoresis profile were observed comparing lanes relevant to RS and SS of Cl2 samples in the area comprised between 45 and 66 kDa.

In general, the salivary characteristics mean values and electrophoresis profiles showed that the subjects from Cl1 were able to maintain constant characteristics of their SPs after stimulation whereas they are greatly modified in subjects from Cl2.

Salivary characteristics and astringency sensitivity

A further part of this work investigated the sensitivity to phenolic compounds of the 2 clusters. The astringency intensity of a tannic acid sample (3 g/l) was rated 30 min after the RS sample collections; thus, the "astringency after resting" data set (RA) was obtained. A *t*-test (separate variance) was performed on RA ratings from the 2 subject clusters. Subjects from Cl1 perceived the astringency induced by tannic acid solution at a significantly lower intensity than subjects from Cl2 (ratings 36.8 and 52.2, respectively, $t_{16,2.11} = 2.51$, $P = 0.023$). The difference in sensitivity for phenolic astringent stimuli between the 2 clusters was confirmed by the "astringency after stimulation" data set (SA).

Table 3 Correlations among salivary characteristic *D* values

	Flow	SPs	Phs
Flow			
SPs	−0.16		
Phs	−0.22	0.25*	
HFC	−0.26*	0.34*	0.35*

*Significant ($P > 0.05$). $n = 65$, degrees of freedom = 63, critical *r* value = 0.25.

SA data were submitted to a 2-way split-plot repeated-measure analysis of variance (ANOVA) to estimate the cluster effect (low- and high-responding subjects); the tannic acid concentration (4 levels) effect and the interaction cluster × concentration effect. Results indicated that the 2 clusters differed significantly for the intensity of perceived astringency ($F_{1,63} = 7$, $P = 0.01$). Mean astringency ratings from Cl2 subjects were significantly higher than those from Cl1 subjects (Figure 6). As expected, a significant effect of samples was found ($P = 0.00$). No significant effect was found for sample × cluster interactions ($P = 0.40$). The 2-way split-plot

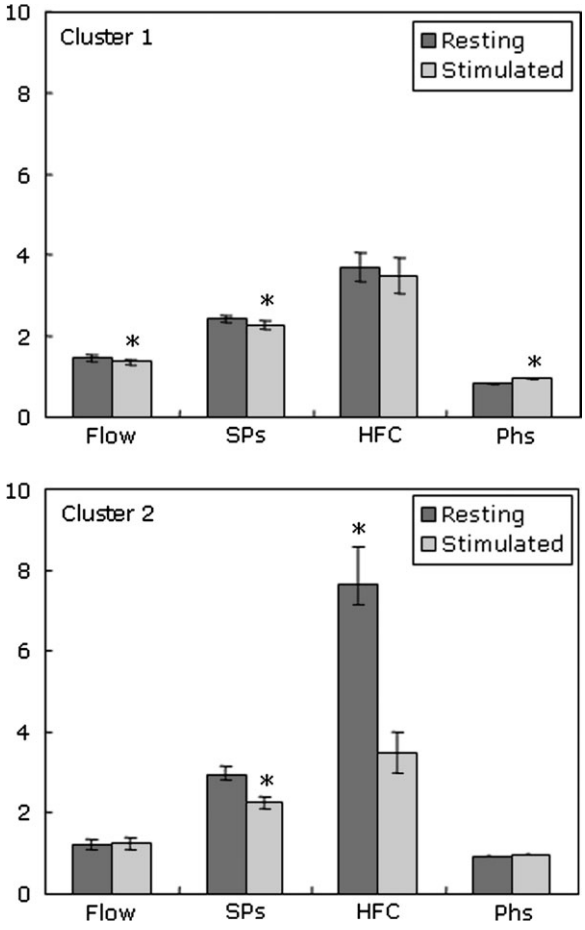

Figure 4 Mean flow rate (mg/min), SP concentration (mg/ml), HFC (NTU), Ph content (mg/ml10^{-1}), determined in subject clusters after resting and after stimulation. *Significantly different $P \leq 0.05$.

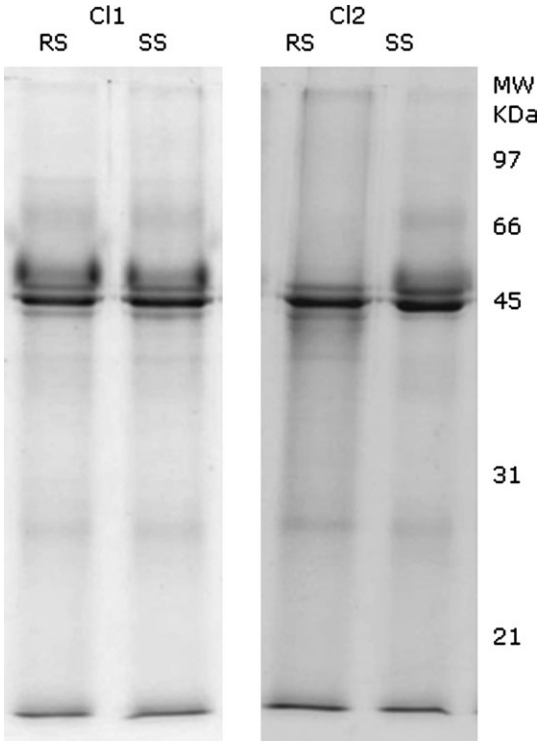

Figure 5 SPs molecular patterns after resting (RS) and stimulated (SS) conditions of subjects form Cl1 and Cl2 in a representative SDS-PAGE stained with Coomassie brilliant blue R250.

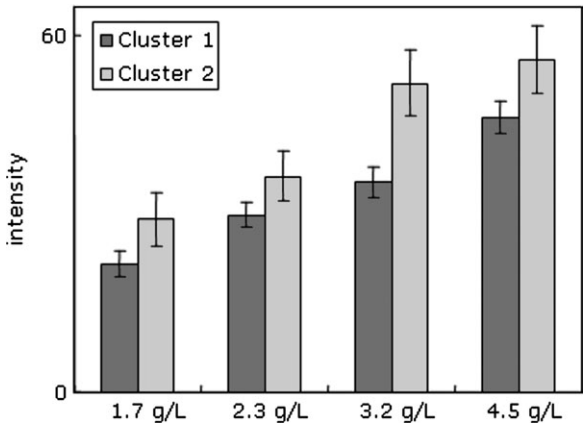

Figure 6 Astringency after stimulation data set: cluster effect on mean ratings from 4 concentration of tannic acid. Bars represent standard error.

repeated-measure ANOVA model was used to estimate the cluster effect on bitterness and sourness ratings too. A significant sample effect was found for both tastes ($F_{\text{bitterness } 3,189} = 3.9$, $P = 0.00$; $F_{\text{sourness } 3,189} = 2.84$, $P = 0.04$). However, no significant cluster effect was found for either ($P \geq 0.3$). These results indicate that low- and high-responding subjects differ from each other only in their astringency sensitivity.

Discussion

The experimental plan

The experimental plan was drawn taking in to consideration different requirements. These were related to the collection of saliva samples representative of the whole oral environment in different stimulation conditions.

Chewing tasteless parafilm stimulates both parotid and submandibular/sublingual glands that contribute 60% and 40%, respectively, to the whole salivary flow (Bourdiol et al. 2004). Therefore, this is a well-accepted procedure for collecting saliva representative for the entire SP secretion pattern (Schwartz et al. 1995; Hirtz et al. 2005) and can be considered an appropriate procedure for saliva collection aimed to investigate the role of the whole SP profile on astringency perception.

Saliva characteristics are deeply modified depending on type and duration of stimuli. However, correlation has been found between these parameters determined in different conditions. Significant correlations ($r = 0.87$) were observed between tannic acid and mechanically stimulated flow rate (Horne et al. 2002). Analogous results were obtained

correlating natural food ingestion, and parafilm chewing induced flow rate (Gaviao et al. 2004). A significant SPs concentration decreasing was observed during continuous saliva stimulation with both parafilm (Rayment et al. 2001) and gustatory stimuli (Dawes 1984). Therefore, characteristics of ''after stimulation'' saliva samples might be considered as representative of modifications induced by either chemical (tannic acid) or mechanical (chewing) stimulation.

The 6-n-propylthiouracil (PROP) taster status might represent a further individual trait influencing individual differences in astringency sensitivity. In fact, an increased oral sensitivity and a greater tactile sensitivity of super PROP tasters have been reported. On the other hand, although numerous studies have investigated the relation between PROP taster status and astringency perception, a direct interaction has not yet been established (Bajec and Pickering 2008). Moreover, it has been recently demonstrated that the PROP status is neither good predictor of general taste sensitivity nor of texture attributes perception (Lim et al. 2008). A reframing of the concept of supertasters referred to those people with heightened sensation for all tastes, not only the bitterness of one class of chemical compound has been suggested (Reed 2008). Based on this data, the PROP taster status of subjects involved in this study was not considered.

Salivary pH variation was not considered as a source of individual variability in the adopted experimental plan. In fact, perceptual phenomena should take place at the pH of the ingested stimulus due to the low amount of saliva in respect to the average stimulus volume. The possible effect of salivary pH on astringency perception seems to be unlikely given the nonsignificant variation found when comparing the pH value of expectorants with that of the relevant stimuli before ingestion (Siebert and Euzen 2008).

Saliva characteristics

The salivary characteristics determined both after resting and after stimulation were significantly correlated each other, with the only exception being Phs versus flow in the ''after resting'' samples. A dilution effect can explain the significant negative correlation found between flow rate and other salivary characteristics in both conditions. Previous findings of a positive effect of both protein and polyphenol concentrations on haze development in in vitro experiments (Carvalho et al. 2004; Monteleone et al. 2004; Pascal et al. 2007) can account for the positive correlations found between SPs concentration, Phs content, and HFC values in both RS and SS samples. The relatively low r values describing the correlation between SPs and HFC indicate that factors other than reactant concentration, such as SPs profile, are involved in SPs/Phs reactivity modulation. In fact, the in vitro molecular model clearly indicates the importance of compositional and structural features in modulating protein/Ph aggregate formation. The persistence of Phs in the mouth due to their interaction with SPs (Siebert and Chassy 2003) can account for the observed increase of r values for Phs versus SPs and Phs versus HFC found in SS samples when compared with RS samples.

A comparison between RS and SS samples shows that the salivary characteristics induced by mastication after a prolonged resting period are different from those measured after a shorter resting period and stimulation with a tannic acid solution. The mean values for all the salivary characteristics were negatively affected by stimulation with the only exception of Phs that obviously increases as a consequence of tannic acid tasting. Saliva collected after the resting period can be considered representative of a basal composition. In fact, it can be hypothesized that resting samples represent in the most part secretion from preformed vesicles because de novo synthesis and processing of saliva through the secretory apparatus requires approximately 30 min and the duration of saliva collection was 25 min (Becerra et al. 2003). D values can be considered to be an index of the subjects' ability to react to stimulation and restore the basal saliva composition, where low D values correspond to a high ability in restoring saliva composition. The large variation between minimum and maximum D values for all the salivary characteristics indicates a strong individual variability in the capacity to react to stimulation; this is consistent with previous findings on individual differences in responsiveness to chemical and mechanical oral stimulation (Oberg et al. 1982; Dawes 1984; Guinard et al. 1998; Gaviao et al. 2004).

Effect of salivary characteristics on astringency sensitivity

Based on D values, subjects were clustered in 2 groups. The majority (53 cases) was grouped in Cl1, and the basal salivary conditions are quickly restored in these subjects as indicated by the small differences between salivary characteristics in RS and SS samples. A small proportion of subjects (12 cases) were grouped in Cl2, and some of their basal salivary conditions are not quickly restored, particularly in terms of HFC values and SPs concentration (Figure 4). The electrophoresis patterns show a modification in the PRP profile; a molecular weight ranging from 40 to 70 kDa has been described by others for both basic and glycosylated PRPs in analogous run conditions (Francis et al. 2000; de Freitas and Mateus 2001). Moreover, a PRPs profile modification induced by the ingestion of astringent stimuli has also been proposed on the basis of SP HPLC analyses (11). Lu and Bennick (1998) studied purified SP fractions and demonstrated different capabilities among PRP classes to form insoluble aggregates when reacting with Phs. In view of these findings, a variation in PRP composition in RS and SS samples could be related to the difference between the 2 clusters of subjects described in this study.

Saliva is the medium in which the reaction dietary tannin/SP takes place. Modifications to the reaction conditions such

as the type of and concentration of SPs can affect the kinetics of the tannin/protein interactions, the consequent development of insoluble aggregates and then the intensity of perceived astringency. The sensory data showed that subjects (Cl1) capable of maintaining constant saliva characteristics after both mechanical and chemical stimulation were less sensitive to astringent stimuli than subjects (Cl2) in which the same stimulations induced significant saliva modifications. The capacity of Cl1 subjects to maintain constant salivary characteristics, in terms of both concentration and composition, may parallel their capacity to intercept and inactivate Phs; these subjects are then better protected and less sensitive to astringent stimuli. The higher sensitivity to astringent stimuli of Cl2 subjects seems to be related to a decrease of saliva Ph-binding capacity in SS conditions compared with basal conditions, and a modification of PRPs profile seems to be involved in this.

A number of data indicate the importance of physiological individual variation of saliva characteristics in modulating the sensitivity to phenolic astringent stimuli even if unequivocal relationships between salivary parameters and perceived intensity are still not found. The importance of flow rate in modulating astringency perception through a number of possible mechanisms is well documented (Fischer et al. 1994; Ishikawa and Noble 1995; Guinard et al. 1998; Peleg et al. 1999; Horne et al. 2002; Condelli et al. 2006). However, the conflicting results obtained in different experimental conditions (whole vs. parotid flow, mechanical vs. gustatory stimulation, type and concentration of gustatory stimuli) clearly indicate that salivary volume can not account by itself for differences in astringency perception. Different rates of oral cavity relubrication (Bajec and Pickering 2008) as well as modification of protein salivary profile induced by the adopted experimental conditions could account for the differences in sensitivity found in subject groups differing for salivary flow rate. The effect of SP concentration and composition on astringency sensitivity has also been suggested considering the effect of SPs reactivity toward dietary tannins measured in terms of HFC. The results of the present study provide further support for the generally well-accepted central role of SPs in the development of astringency sensation. Moreover, the oral environment appears to be a complex and dynamic reaction medium in which the protein/tannin interactions take place. These latter cannot be considered a simple alert mechanism based on the perception of an increased astringency (friction) but instead may be considered to be a more sophisticated system aimed at defending the organism by inactivating these potentially dangerous compounds.

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References

Alvarez P, Alvarado C, Mathieu F, Jimenez L, De la Fuente M. 2006. Diet supplementation for 5 weeks with polyphenol-rich cereals improves several functions and the redox state of mouse leucocytes. Eur J Nutrition. 45(8):428–438.

Asquit TN, Uhling J, Mehansho H, Putman L, Carlson DM, Butler L. 1987. Binding of condensed tannins to salivary prolin rich glycoproteins: the role of carbohydrate. J Agric Food Chem. 35:331–334.

Bajec MR, Pickering GJ. 2008. Astringency: mechanisms and perception. Crit Rev Food Sci Nutr. 48:858–875.

Baxter NJ, Lilley TH, Haslam E, Williamson MP. 1997. Multiple interactions between polyphenols and salivary proline-rich protein repeat result in complexation and precipitation. Biochemistry. 36:5566–5577.

Becerra L, Soares RV, Bruno LS, Siqueira CC, Oppenheim FG, Offner GD, Troxler RF. 2003. Patterns of secretion of mucins and non-mucin glycoproteins in human submandibular/sublingual secretion. Arch Oral Biol. 48(2):147–154.

Bennick A. 2002. Interaction of plant biopolyphenols with salivary proteins. Crit Rev Oral Biol Med. 13(2):184–196.

Bourdiol P, Mioche L, Monier S. 2004. Effect of age on salivary flow obtained under feeding and non-feeding conditions. J Oral Rehabil. 31(5):445–452.

Cai KH, Bennick A. 2006. Effect of salivary proteins on the transport of tannin and quercetin across intestinal epithelial cells in culture. Biochem Pharmacol. 72(8):974–980.

Cai KH, Hagerman AE, Minto RE, Bennick A. 2006. Decreased polyphenol transport across cultured intestinal cells by a salivary proline-rich protein. Biochem Pharmacol. 71(11):1570–1580.

Carvalho E, Mateus N, de Freitas V. 2004. Flow nephelometric analysis of protein-tannin interactions. Anal Chim Acta. 513:97–101.

Charlton AJ, Baxter NJ, Lokman Khan M, Moir AJG, Haslam E, Davies AP, Williamson MP. 2002. Polyphenol/peptide binding and precipitation. J Agric Food Chem. 50:1593–1601.

Chung KT, Wei CI, Johnson MG. 1998. Are tannins a double-edged sword in biology and health? Trends Food Sci Technol. 9(4):168–175.

Condelli N, Dinnella C, Cerone A, Monteleone E, Bertuccioli M. 2006. Prediction of perceived astringency induced by phenolic compounds II: criteria for panel selection and preliminary application on wine sample. Food Qual Prefer. 17:96–107.

Dawes C. 1984. Stimulus effects on protein and electrolyte concentrations in parotid saliva. J Physiol. 346:579–588.

Dawes C. 2005. The unstimulated salivary flow rate after prolonged gum chewing. Arch Oral Biol. 50(6):561–563.

Dawes C, O'Connor AM, Aspen JM. 2000. The effect on human salivary flow rate of the temperature of a gustatory stimulus. Arch Oral Biol. 45(11):957–961.

Dearing MD, Foley WJ, McLean S. 2005. The influence of plant secondary metabolites on the nutritional ecology of herbivorous terrestrial vertebrates. Annu Rev Ecol Evol Syst. 36:169–189.

de Freitas V, Mateus N. 2001. Nephelometric study of salivary protein-tannin aggregates. J Sci Food Agric. 82(2):113–119.

de Wijk RA, Prinz JF. 2006. Mechanisms underlying the role of friction in oral texture. J Texture Stud. 37:413–427.

Dodds MWJ, Johnson DA, Yeh CK. 2005. Health benefits of saliva: a review. J Dentistry. 33(3):223–233.

Engelen L, de Wijk RA, Prinz JF, van der Bilt A, Bosman F. 2003. The relation between saliva flow after different stimulations and the perception of flavor and texture attributes in custard desserts. Physiol Behav. 78(1):165–169.

Fischer U, Boulton RB, Noble A. 1994. Physiological factors contributing to the variability of sensory assessments: relationship between salivary flow rate and temporal perception of gustatory stimuli. Food Qual Prefer. 5:55–64.

Francis CA, Hector MP, Proctor GB. 2000. Precipitation of specific proteins by freeze-thawing of human saliva. Arch Oral Biol. 445:601–606.

Gardner EJ, Ruxton CHS, Leeds A. 2007. Black tea—helpful or harmful? A review of the evidence. Eur J Clin Nutr. 61(1):3–18.

Gaviao MBD, Engelen L, van der Bilt A. 2004. Chewing behavior and salivary secretion. Eur J Oral Sci. 112(1):19–24.

Gawel R, Oberholster A, Francis I. 2000. A 'mouth-feel wheel': terminology for communicating the mouth-feel characteristics of red wine. Aust J Grape Wine Res. 6:203–207.

Ghezzi EM, Lange LA, Ship JA. 2000. Determination of variation of stimulated salivary flow rates. J Dent Res. 79(11):1874–1878.

Guinard JX, Zoumas-Morse C, Walchak C. 1998. Relation between parotid saliva flow and composition and the perception of gustatory and trigeminal stimuli in food. Physiol Behav. 63:109–118.

Haslam E. 1989. Plant polyphenols. Cambridge, UK: Cambridge press.

Hirtz C, Chevalier D, Ceteno D, Egea JC, Rossignol M, Sommerer N, de Perriere D. 2005. Complexity of the whole saliva proteome. J Physiol Biochem. 61(3):469–480.

Hooper L, Cassidy A. 2006. A review of the health care potential of bioactive compounds. J Sci Food Agric. 86(12):1805–1813.

Horne J, Hayes J, Lawless HT. 2002. Turbidity as a measure of salivary proteins reactions with astringent substances. Chem Senses. 27:653–659.

Ishikawa T, Noble AC. 1995. Temporal perception of astringency and sweetness in red wine. Food Qual Pref. 27–33.

Jobstl E, O'Connell J, Fairclough JPA, Williamson MP. 2004. Molecular model for astringency produced by polyphenol/protein interactions. Biomacromolecules. 5(3):942–949.

Kallithraka S, Bakker J, Clifford MN. 1998. Evidence that salivary proteins are involved in astringency. J Sens Stud. 13(1):29–43.

Kallithraka S, Bakker J, Clifford MN, Vallis L. 2001. Correlations between saliva protein composition and some T-I parameters of astringency. Food Qual Prefer. 12:145–152.

Lee CB, Lawless HT. 1991. Time-course of astringent materials. Chem Senses. 16:225–238.

Lesschaeve I, Noble AC. 2005. Polyphenols: factors influencing their sensory properties and their effects on food and beverage preference. Am J Clin Nutr. 81(1):330s–335s.

Lim J, Urban L, Green BG. 2008. Measures of individual differences in taste and creaminess perception. Chem Senses. 33:493–501.

Lu Y, Bennick A. 1998. Interaction of tannin with human salivary proline-rich proteins. Arch Oral Biol. 43(9):717–728.

MacFie HJ, Bratchell N, Greenhoff K, Vallis LV. 1989. Designs to balance the effect of order of presentation and first-order carry-over effects in hall tests. J Sens Stud. 4(2):129–148.

Mehansho H, Butler LG, Carlson DM. 1987. Dietary tannins and salivary proline-rich proteins: interactions, induction and defense mechanisms. Annu Rev Nutr. 7:423–440.

Monteleone E, Condelli N, Dinnella C, Bertuccioli M. 2004. Prediction of perceived astringency induced by phenolic compounds. Food Qual Prefer. 15:761–769.

Mueller-Harvey I. 2006. Unravelling the conundrum of tannins in animal nutrition and health. J Sci Food Agric. 86(13):2010–2037.

Nayak A, Carpentier GH. 2008. A physiological model for tea induced astringency. Physiol & Behav. 95:290–294.

Oberg SG, Izutsu KT, Truelove EL. 1982. Human parotid saliva protein composition: dependence on physiological factors. Am J Physiol. 5:G231–G236.

Pascal C, Poncet-Legrand C, Imberty A, Gautier C, Sarni-Manchado P, Chenier V, Vernhet A. 2007. Interactions between a non glycosylated human proline-rich protein and flavan-3-ols are affected by protein concentration and polyphenol/protein ratio. J Agric Food Chem. 55:4895–4901.

Peleg H, Gacon K, Schlich P, Noble AC. 1999. Bitterness and astringency of flavan-3-ol monomers, dimers and trimers. J. Sci. Food Agric. 79:1123–1128.

Poncet-Legrand C, Edelmann A, Putaux JL, Cartalade D, Sarni-Manchado P, Vernhet A. 2006. Poly(L-proline) interactions with flavan-3-ols units: influence of the molecular structure and the polyphenol/protein ratio. Food Hydrocolloids. 20:687–697.

Prinz JF, Lucas PW. 2000. Saliva tannin interaction. J Oral Rehabil. 27:991–994.

Prior RL, Wu XL, Gu LW. 2006. Flavonoid metabolism and challenges to understanding mechanisms of health effects. J Sci Food Agric. 86(15):2487–2491.

Rayment SA, Liu B, Soares RV, Offner GD, Oppenheim FG, Troxler RF. 2001. The effects of duration and intensity of stimulation on total protein and mucin concentrations in resting and stimulated whole saliva. J Dent Res. 6:1584–1587.

Reed D. 2008. Birth of a new breed of supertaster. Chem Senses. 33(6):489–491.

Schwartz SS, Zhu WX, Sreebny LM. 1995. Sodium dodecyl sulphate-polyacrylamide gel electrophoresis of human whole saliva. Arch Oral Biol. 40(10):949–958.

Shimada T. 2006. Salivary proteins as a defense against dietary tannins. J Chem Ecol. 32(6):1149–1163.

Siebert KJ, Chassy AW. 2003. An alternate mechanism fort the astringent sensation of acids. Food Qual Prefer. 15:13–18.

Siebert KJ, Euzen C. 2008. The relationship between expectorant pH and astringency perception. J Sens Stud. 23:222–233.

Skopec MM, Hagerman AE, Karasov WH. 2004. Do salivary proline-rich proteins counteract dietary hydrolyzable tannin in laboratory rats? J Chem Ecol. 30(9):1679–1692.

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