Physiology & Behavior 163 (2016) 56–63

Contents lists available at ScienceDirect

Physiology & Behavior

journal homepage: www.elsevier.com/locate/phb

Salivary protein levels as a predictor of perceived astringency in model systems and solid foods

Erin E. Fleming [a,b], Gregory R. Ziegler [b], John E. Hayes [a,b,*]

[a] Sensory Evaluation Center, College of Agricultural Sciences, The Pennsylvania State University, University Park, PA, USA
[b] Department of Food Science, College of Agricultural Sciences, The Pennsylvania State University, University Park, PA, USA

HIGHLIGHTS

- Multiple mechanisms have been proposed to explain oral astringency.
- Depletion of salivary proteins has been associated with differential astringency.
- Here, protein had a modest relationship with astringency from tannic acid and alum.
- No relationship between astringency and protein levels was seen in solid chocolate.
- These data are consistent with multiple mechanisms for astringency.

ARTICLE INFO

Article history:
Received 16 June 2015
Received in revised form 1 April 2016
Accepted 24 April 2016
Available online 27 April 2016

Keywords:
Astringency
Salivary proteins
In vitro methods
Individual differences

ABSTRACT

Salivary protein difference value (SP D-value) is a quantitative measure of salivary protein replenishment, which reportedly relates to individual differences in perceived astringency. This in vitro measure is calculated as the difference in total salivary protein before (S1) and after (S2) stimulation with tannic acid, with a greater absolute value (S2–S1) indicating less protein replenishment. Others report that this measure predicts perceived astringency and liking of liquid model systems and beverages containing added polyphenols. Whether this relationship generalizes to astringent compounds other than polyphenols, or to solid foods is unknown. Here, the associations between SP D-values and perceived astringency and overall liking/disliking for alum and tannic acid (experiment 1) as well as solid chocolate-flavored compound coating with added tannic acid or grape seed extract (GSE) (experiment 2) were examined. In both experiments, participants ($n = 84$ and 81, respectively) indicated perceived intensity of astringency, bitterness, sweetness, and sourness, and degree of liking of either aqueous solutions, or solid chocolate-flavored compound coating with added astringents. Data were analyzed via linear regression, and as discrete groups for comparison to prior work. Three discrete groups were formed based on first and third quartile splits of the SP D-value distribution: low (LR), medium (MR), and high responding (HR) individuals. In experiment 1, significantly higher mean astringency ratings were observed for the HR as compared to the LR/MR groups for alum and tannic acid, confirming and extending prior work. In experiment 2, significantly higher mean astringency ratings were also observed for HR as compared to LR groups in solid chocolate-flavored compound containing added tannic acid or GSE. Significant differences in liking were found between HR and LR groups for alum and tannic acid in water, but no significant differences in liking were observed for chocolate-flavored compound samples. A significant linear relationship between SP D-values and perceived astringency was observed for both alum and tannic acid (p's < 0.001), although the variance explained was relatively low ($R^2 = 0.33$ and 0.29, respectively). In the solid chocolate-flavored compound spiked with either tannic acid or GSE, the relationship was not significant ($p = 0.17$ and 0.30; $R^2 = 0.03$ and 0.02, respectively). Due to the weak associations overall, and the lack of significant differences in perception of astringency between the MR and LR groups, we conclude that SP D-values are not a strong predictor of astringency, especially in solid, high-fat foods. Additional research investigating alternative methods for quantifying individual differences in astringency, as well as exploring the underlying complexities of this percept appears warranted.

1. Introduction

The American Society for Testing and Materials [1] defines astringency as "the complex of sensations due to shrinking, drawing or

* Corresponding author at: Department of Food Science, Pennsylvania State University, 220 Rodney A. Erickson Food Science Building, University Park, PA 16802, USA
E-mail address: jeh40@psu.edu (J.E. Hayes).

http://dx.doi.org/10.1016/j.physbeh.2016.04.043
0031-9384/

puckering of the epithelium as a result of exposure to substances such as alums or tannins." This complex percept has traditionally been associated with negative consumer reactions at high intensities [2]. Common astringent stimuli in the diet are polyphenolic compounds such as those found in red wine, tea, chocolate, and a variety of fruits and nuts. In addition to polyphenols, a number of other classes of compounds can elicit oral astringency, including organic and inorganic acids (e.g. malic or hydrochloric acid), dehydrating agents (e.g. ethanol), and multivalent salts (e.g. ammonium potassium sulfate, or 'alum'). For more information, see the review by Bajec and Pickering [3],

While it is widely accepted that the prototypical tastes are elicited via G-protein coupled receptors (sweet, bitter, and umami), or ion channels (sour, and salty), the mechanism(s) through which oral astringency is elicited is (are) not fully understood [4–6]. The most commonly cited mechanism for astringent polyphenols – salivary protein precipitation followed by decreased lubricity – is supported by the observation that proline-rich proteins (PRPs) which make up the majority of proteins found in human saliva interact with and are precipitated by tannins [7]. However, recent work by Schöbel et al. [8] proposes an alternative hypothesis involving specific chemosensors for astringency distinct from delubrication.

Sensory responses vary greatly between individuals, which likely influence eating behaviors [9,10]. Individual differences in saliva characteristics appear to modulate the perception of astringency induced by phenolic stimuli [11–13]. To quantify such individual differences, various in vitro methods have been developed, including approaches based on haze development [11,12,14,15], direct qualitative and quantitative measures of salivary proteins [9,16–19], colorimetry [20], and voltammetry [21].

Associations between changes in salivary proteins and the perception of oral astringency are well documented, but poorly understood mechanistically. Dinnella and colleagues [17] showed that individuals capable of maintaining constant saliva characteristics (i.e. concentration and composition of salivary proteins) are less perceptually responsive than individuals who exhibited significant saliva modifications when challenged with a phenolic astringent. Specifically, they describe an in vitro measure, salivary protein difference value (SP D-value), as the difference in total salivary protein before (S1) and after (S2) stimulation with tannic acid, where a more negative value of S2 minus S1 indicates lesser protein replenishment. That is, individuals with a SP D-value near zero are able to return their saliva to basal conditions relatively quickly after exposure to a model astringent stimulus (tannic acid), while individuals with a negative SP D-value do not. Critically, differences in observed SP D-value were associated with differences in perceived astringency; a relatively stable salivary protein profile (as measured by S2–S1 values near 0) characterizes individuals with lower sensitivity to astringency, while individuals with reduced replenishment of salivary proteins (i.e. more negative SP D-values) report greater oral astringency when asked to rate the intensity of astringent stimuli.

Relationships between SP D-values and real foods were subsequently explored by Dinnella and colleagues [18], who investigated perceptual and hedonic responses to fruit and vegetable juices with added tannic acid. Using first and third quartile splits of the SP D-value distribution, they divided participants into high, medium, and low responding (HR, MR, LR) groups. Generally, added tannic acid induced a greater increase in perceived astringency in the HR group, compared to the MR and LR groups. They also observed a drop in liking for apple juice with added tannic acid in the HR group, with smaller effects in MR and LR groups. These data suggest that SP D-values may predict both intensity and hedonic response to astringent stimuli, at least in beverages. However, the extent to which this measure generalizes to astringent compounds other than tannins, and to complex foods, especially solid foods, remains unclear.

According to Peleg [22], "astringency is a complex phenomenon: it elicits a range of sensations, different types of compounds evoke it, and several mechanisms have been suggested to explain it." As Peleg asserted, astringency is not a simple percept. A diverse set of compounds are capable of eliciting this cluster of sensations, including polyphenols, dehydrating agents, multivalent salts, and organic acids. Alum — a term which collectively refers to both a specific compound (i.e. potassium aluminum sulfate or ammonium aluminum sulfate) as well as any double sulfate salt — is one of the most extensively cited exemplars of an astringent stimulus, and it is widely used for training participants on the sensation of astringency [18,23–25]. Both alum and tannic acid have previously been shown to precipitate PRPs (in addition to mucins for alum) from saliva [26]. However, a gap in the literature remains, as potential relationships between SP D-values and the astringency from non-phenolic astringents, like alum, have not yet been quantified. Determining the extent to which an in vitro measure like SP D-value generalizes to other classes of astringents may provide insight into whether diverse classes of astringent compounds share a common mechanism.

Previously, the relevance of SP D-values has been explored in beverages like fruit and vegetable juices [18]. While this is a critical step in determining whether SP D-values generalize beyond model systems to real foods, a variety of other foods, including solid foods, also elicit astringency. Solid chocolate is a plant-based food containing a substantial amount of polyphenols embedded in a sugar and fat matrix. Although the consumption of high-cacao-content chocolate has been associated with positive health benefits ascribed to polyphenols (see Visioli [27] for a recent review), these compounds also impart bitterness and astringency when present at the concentrations found in dark chocolate [28]. Thus, potential relationships between SP D-value and the perception of astringency in solid dark chocolate may provide insight into the importance of purported individual differences in astringency perception from polyphenols when they are present in a solid food matrix.

Here, we investigated associations between SP D-values and perceived astringency and liking for aqueous solutions of alum and tannic acid, as well as for solid dark chocolate-flavored compound coating with added astringents (tannic acid and grape seed extract). Collectively, the application of SP D-values to non-polyphenol astringents may help determine the role of salivary protein replenishment in the mechanism(s) underlying oral astringency, and in the context of a solid food, the potential relevance to ingestive behavior and food choice.

2. Materials and methods

2.1. Overview

We report data from two separate experiments: aqueous tannic acid and alum solutions were presented in experiment 1 ($n = 84$), and solid chocolate compound was presented in experiment 2 ($n = 81$). In each experiment, participants completed an orientation before rating test stimuli in a first session and saliva samples were collected in a second session, which occurred within 1 week. All other procedures were kept consistent. Participants were not eligible to participate in both experiments.

2.2. Scaling methods

Intensity ratings were made on a general labeled magnitude scale (gLMS) [29]. Derived from the labeled magnitude scale [30], the gLMS is a semantically-labeled scale with labels located at 0 (no sensation); 1.4 (barely detectable); 6 (weak); 17 (moderate); 35 (strong); 52 (very strong); and 100 (strongest imaginable sensation of any kind). Affective ratings were made on a generalized bi-polar hedonic scale (e.g. [31]) with −100 ('strongest disliking of any kind') on the left, +100 ('strongest liking of any kind'), on the right, and 0 ('neither like nor dislike'), at the midpoint. Scales were presented via Compusense *five* software, v5.2 (Guelph, ONT), and all procedures were approved by the local Institutional Review Board.