Original Article

Investigating Mixture Interactions of Astringent Stimuli Using the Isobole Approach

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Abstract

Astringents (alum, malic acid, tannic acid) representing 3 broad classes (multivalent salts, organic acids, and polyphenols) were characterized alone, and as 2- and 3-component mixtures using isoboles. In experiment 1, participants rated 7 attributes ("astringency," the sub-qualities "drying," "roughing," and "puckering," and the side tastes "bitterness," "sourness," and "sweetness") using direct scaling. Quality specific power functions were calculated for each stimulus. In experiment 2, the same participants characterized 2- and 3-component mixtures. Multiple factor analysis (MFA) and hierarchical clustering on attribute ratings across stimuli indicate "astringency" is highly related to "bitterness" as well as "puckering," and the subqualities "drying" and "roughing" are somewhat redundant. Moreover, power functions were used to calculate indices of interaction (I) for each attribute/mixture combination. For "astringency," there was evidence of antagonism, regardless of the type of mixture. Conversely, for subqualities, the pattern of interaction depended on the mixture type. Alum/tannic acid and tannic acid/malic acid mixtures showed evidence of synergy for "drying" and "roughing"; alum/malic acid mixtures showed evidence of antagonism for "drying," "roughing," and "puckering." Collectively, these data clarify some semantic ambiguity regarding astringency and its subqualities, as well as the nature of interactions of among different types of astringents. Present data are not inconsistent with the idea that astringency arises from multiple mechanisms, although it remains to be determined whether the synergy observed here might reflect simultaneous activation of these multiple mechanisms.

Key words: antagonism, isobole method, organic acids, polyphenols, salts, synergy

Introduction

Oral astringency is a complex percept, not merely a single sensation; rather, astringent compounds are characterized by subqualities like drying, roughing, and puckering which may or may not be interchangeable, as well as the side tastes bitterness, sourness, and sweetness, which may modulate the overall sensation(s) of astringency (Lee and Lawless 1991; Lawless and Corrigan 1994). Lee and Lawless (1991)

first proposed a model of astringent subqualities in a study exploring the time-course of astringent sensations. Their work included nondirective small group discussions, wherein participants chose various terms to describe tastes and sensations of a range of astringent stimuli. These terms included both the astringent subqualities and side tastes mentioned previously. Accordingly, the potential for both astringent subqualities and side tastes to modulate the percept need to be considered when characterizing astringency; however the manner in which

these terms potentially relate to one another and to astringency itself remains unclear. Here, we address this knowledge gap.

Better understanding of the psychophysical responses mixtures of astringents elicit is also warranted, because astringent compounds rarely occur in isolation. For example, many fruit-derived products contain both polyphenols and acids—both of which are capable of eliciting astringency (Lawless et al. 1994). Previously, mixtures of astringent compounds have been shown to exhibit varied interactions that depend on the components of the mixture. Specifically, mixtures of alum and citric acid showed evidence of suppression, in that the 50/50 mixture of an intensity-matched pair of these components was less intense than either component alone, at least for the attributes astringency, drying, roughing, and puckering (Lawless et al. 1994). Conversely, the response to mixtures of catechin and gallic acid was additive using the same criteria. In subsequent work exploring the effect of acid on the perception of alum and phenolic compounds, Peleg et al. (1998) found the astringency of alum was reduced with the addition of equi-sour levels of lactic, citric, or hydrochloric acid. Conversely, when these acids were added to phenolic compounds (i.e., grape seed tannins, tannic acid, catechin, and gallic acid), the astringency of the mixture increased as compared with either component alone. Although these studies provide substantial empirical evidence for marked differences between the effects of acid addition to phenolic as opposed to multivalent astringent compounds, they are limited for 2 reasons. First, these studies did not utilize contemporary best practices for direct scaling of intensity, as category scales (Lawless et al. 1994) and unstructured line scales (Peleg et al. 1998) were used to collect psychophysical responses. Furthermore, the researchers tacitly assumed a linear dose response curve for the components in concluding synergism or antagonism in the mixture. These limitations are discussed in more detail later.

Direct scaling is often used to quantify the intensity of specific attributes across stimuli. Previously, a number of methods have been used to quantitatively characterize astringent compounds including category scaling (Lee and Lawless 1991; Lawless et al. 1994, 1996; Thomas and Lawless 1995; Monteleone et al. 2004; Fia et al. 2009; Lee and Vickers 2010), visual analogue scales (VAS; Breslin et al. 1993; Horne et al. 2002), and the labeled magnitude scale (LMS) (Dinnella et al. 2009, 2010, 2011). While debate exists regarding the relative strengths and limitations of these scaling techniques, current dogma suggests intensity ratings across groups or individuals can only be compared if they are made “relative to a stimulus that is perceived to be equally intense to all subjects” (Bartoshuk et al. 2003). To address this issue, the general Labeled Magnitude Scale (gLMS) was developed. Similar to the LMS, the gLMS is a semantically labeled scale that is generalized outside a specific sensory modality via a top anchor of strongest (imaginable) sensation of any kind, as well as a multimodal orientation procedure. Here, a gLMS was used to quantify the intensity of specific attributes within a generalized context, which purportedly increases the validity of comparisons of intensity ratings across groups or individuals.

Various methods have been proposed to describe interactions of mixtures in chemical stimuli (see Sühnel 1993 for a review). As noted by Lawless (1998), psychophysically “the demonstration of synergy is not straightforward, and depends on the model one adopts” for the comparison. Here, the isobole method was used to determine the nature of interactions of astringent compounds. Historically, the isobole approach has been employed to predict the effect of combinations of drugs that produce similar measureable effects, and determine if the combination demonstrates hyperadditivity (synergism) or hypoadditivity (antagonism or suppression). This is usually accomplished by comparing the observed effect of combinations to the linear isobole, that is, that set of dose pairs that give a specified effect

magnitude, plotted on an isobologram (eq. 1). A similar approach has been proposed to evaluate combinations of stimuli in olfactory and taste mixtures (Sühnel 1993), although this approach has rarely been used by chemosensory researchers.

$$\frac{c_a}{C_A} + \frac{c_b}{C_B} = 1 \quad (1)$$

where “ c_a ” and “ c_b ” denote the concentrations of components “A” and “B” used in the mixture, and “ C_A ” and “ C_B ” denote the concentrations of “A” and “B” that would individually produce the same intensity as the mixture. If 2 components act independently then equation (1) is satisfied. When components do interact to alter the effect, the sum ($c_a/C_A + c_b/C_B$) is less than 1 in the case of synergy and greater than 1 in the case of antagonism.

In their investigation of interactions of astringent compounds, Lawless et al. (1994) employed a “concentration-based” comparison (Lawless 1998), where 2 intensity-matched chemical stimuli were mixed 50/50 and the resulting perceived intensity was compared with that of the original components or to their average intensity if the intensity didn’t quite match. Synergy was defined as a response greater than expected and suppression as a response less than expected. Lawless et al. (1994) concluded “this type of comparison is consistent with the isobole criterion.” However, this assumption is valid only when both components have linear dose–effect relationships (Berenbaum 1985).

Critically, the use of the common linear isobole described by equation (1) as a reference for establishing synergy or antagonism carries with it some implicit assumptions: 1) each component shows dose dependence increasing monotonically, 2) the components act through mechanisms known or presumed to be noncompetitive, and 3) the components have a constant relative potency. The interested reader is referred to (Tallarida 2006) for additional detail. The last of these assumptions requires that the dose–response curves have equivalent maxima and their log dose–response curves are parallel—that is, the “Hill coefficients” that describe their dose–response relations are equal. Nonlinear isoboles result if the first of these conditions is not met, and in the second case, the result is 2 nonlinear but symmetric isoboles (Tallarida 2006). Berenbaum (1985) demonstrated that the isobole approach works for combinations of inactive components that otherwise do not produce the qualitative effect but increase or decrease the effect produced by active components. Although Berenbaum (1985) was cautious about extending the isobole approach to components with nonmonotonic dose–response functions, Sühnel (1993) did so by use of so-called “zero interaction response surfaces.” This was accomplished by rearranging the dose–response relationship to yield the concentration, substituting the corresponding equations into the denominator of the isobole equation (e.g., for C_A and C_B in eq. 1), and replacing the effects E_A and E_B with a single value of E_{AB} . Sühnel (1993) illustrates this approach for a number of different dose–response functions.

Deviations from the zero interaction response surface can be quantified using the index of interaction (I), proposed by Sühnel (Sühnel 1993), which is defined by equation (2) in the next section. An I value less than 1 indicates synergism, greater than 1 indicates antagonism, and an I value near unity (1) implies no interaction. As noted by Sühnel, the isobole approach is a mathematical model that is independent of the mechanism of interaction (Sühnel, 1993). Given that the mechanism(s) underlying oral astringency are not well-understood (Bajec and Pickering 2008; Schobel et al. 2014), we think this is the most conservative approach when modeling interactions of astringent mixtures.

The aims of the present study were primarily empirical: first, to determine the semantic relationships between astringency and the

associated subqualities (“drying,” “roughing,” and “puckering”) and side tastes (“bitterness,” “sourness,” and “sweetness”); second, to determine mixture interactions of astringent compounds using the isobole approach for these attributes.

Materials and methods

Participants

Reportedly healthy individuals ($n = 67$; 15 men) with a mean age of 32.5 years (± 12.5 SD) were recruited from the Pennsylvania State University campus and surrounding area (State College, PA) via email after prescreening for eligibility and their willingness to participate in a taste study. Criteria for eligibility included: between 18 and 64 years old; not pregnant or breastfeeding; no known defects of smell or taste; no lip, cheek, or tongue piercings; nonsmoker (had not smoked in last 30 days); no food allergies or sensitivities; no history of choking or difficulty swallowing; no history of dry mouth or other salivary problems (e.g., Sjögren’s syndrome).

Given that individual variation in saliva characteristics have been shown to modulate one’s sensitivity to the astringency of polyphenolic compounds (Fischer et al. 1994; Horne et al. 2002; Monteleone et al. 2004; Fleming et al. 2016), it was important from an experimental design standpoint that the same individuals participated in all 4 sessions. Accordingly, individuals attended a total of 4 sessions on 4 separate days: experiment 1 consisted of 3 sessions, whereas experiment 2 was a single session. Experiment 1 was used to estimate the dose–response functions for each stimulus (i.e., unmixed) whereas experiment 2 was used to assess perceptual responses to mixtures of the various stimuli. Participants implicitly gave consent via their actions, and this was documented electronically. Specifically, after logging into the data collection system in the testing booth, the first screen they viewed contained standard informed consent language, including the purpose of the study, and their ability to withdraw at anytime. They were then given a yes/no question to document consent, and the experiment only proceeded if they selected “yes”. All participants were paid a token incentive in cash for their time. All procedures were approved by the Pennsylvania State University Institutional Review Board, and the study complied with the Declaration of Helsinki for Medical Research involving Human Subjects.

Stimuli and presentation

All stimuli were presented at room temperature in 10 mL aliquots labeled with random 3 digit blinding codes. Sampling procedures were kept consistent between experiments 1 and 2 and were as follows: “Please pour the entire contents of sample [XXX] into your

mouth and swish the solution for 5 s as if using mouthwash. Spit the sample into the covered spit cup provided after the 5 s” where XXX was the random blinding code. Presentation order of stimuli was counterbalanced across participants using a Williams design. Participants rinsed with room temperature reverse osmosis (RO) water prior to the first stimulus and between each subsequent stimulus. A minimum interstimulus interval (ISI) of 2 min was enforced between samples via software. Compusense *five* software, version 5.2 (Guelph, Ontario, Canada) was used for data collection. All tests were completed at the Sensory Evaluation Center in the Department of Food Science at the Pennsylvania State University.

All stimuli were served in aqueous solutions made with RO water. Appropriate concentrations of the stimuli were determined based on intensity matching experiments described previously (Fleming et al. 2015) as well as extensive bench-top testing. In experiment 1, 4 concentrations of each astringent stimulus (i.e., alum, malic acid, and tannic acid) were presented to participants in duplicate (Table 1) over the course of 3 sessions. Within a session, participants were presented with only 1 of the 3 astringent stimuli (i.e., in a single session, a participant received 8 malic acid stimuli (4 concentrations in duplicate), and then received 8 tannic acid stimuli on another day, and 8 alum stimuli on yet another). The order in which participants received each of the 3 astringent stimuli (i.e., which session) was counterbalanced using a Latin Square design in order to minimize first order effects.

In experiment 2 (the fourth and final session), 3 astringent compounds and their concentration-matched mixtures were presented to participants (Table 1). That is, the alum/tannic acid mixture included 2.75 g of alum and 2.0 g tannic acid in a liter of water. This is small but critical point in the design, as simply mixing 1 part of the alum solution with 1 part of the tannic acid solution would dilute each in the final mixture, resulting in a solution that only contains ~1.38 g of alum and 1 g of tannic acid per liter. A concentration-matched mixture of alum, tannic acid, and malic acid was also included, bringing the total number of stimuli to 7.

Participants indicated the intensity of specific attributes (“astringency,” “drying,” “roughing,” “puckering,” “bitterness,” “sourness,” and “sweetness”) on a gLMS. This semantically labeled scale has 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). Due to the common confusion between bitterness and astringency, as well as the nuance of the astringent subqualities, definitions for some of the attributes were provided to participants. Given our interest in exploring how various astringent compounds are characterized, “astringency” was defined for participants using verbal (written) examples of foods rather than by a reference stimulus, as we did not want to bias ratings for this attribute towards whichever compound would have acted as the

Table 1. Stimuli used in experiments 1 and 2

Stimulus	Concentration (g/L)						
	Experiment 1				Experiment 2		
					Alum	MA	TA
Alum	1.50	3.00	4.50	6.00	2.75	—	—
Malic acid (MA)	1.25	2.50	3.75	5.00	—	2.20	—
Tannic acid (TA)	1.25	2.50	3.75	5.00	—	—	2.00
Alum/tannic acid	—	—	—	—	2.75	—	2.00
Alum/malic acid	—	—	—	—	2.75	2.20	—
Malic acid/tannic acid	—	—	—	—	—	2.20	2.00
Alum/malic acid/tannic acid	—	—	—	—	2.75	2.20	2.00

physical exemplar. In other words, we did not want to present alum as the reference for “astringency” only to then ask participants to rate the astringency of alum.

Here, we defined “astringency” as “the common sensations experienced in the mouth when consuming any of the following foods: tea/coffee without milk, cranberry juice, unripe fruit, red wine, and/or dark chocolate.” Consistent with prior work by Corrigan and Lawless (1994), “drying” was defined as “a lack of lubrication, or friction between the surfaces of the mouth;” “roughing” as “a physical bumpiness of the tissues, not unlike coarse sandpaper;” “puckering” as “a tightening or drawing sensation that can be felt in the cheeks and the muscles of the face.” These definitions were provided on the computer screen prior to each stimulus, as well as on sheets of paper that were always visible in the individual sensory testing booths. Although making their ratings, participants could also access these definitions at any time by pressing a “request directions” button in Compusense with his or her mouse.

Data analyses

Perceived intensity scores for each attribute were plotted as a function of concentration (g/L) on log-log axes. Power functions were selected a priori to allow for nonlinear dose response functions, and fits were obtained using group mean intensities as a function of stimulus concentration (Supplementary Table 1). Across all stimuli, intensity ratings for each attribute were analyzed using a one-way analysis of variance (ANOVA) using Minitab (Minitab statistical software, Version 17.1 Minitab Inc.). When overall *F*-tests were significant, Tukey’s HSD was used to determine significant differences between stimuli. Agglomerative hierarchical cluster analysis with Ward’s minimum variance for the linkage criteria was also performed via Minitab. Multiple factor analysis (MFA) was performed using R version 2.14.1 (R Project for Statistical Computing) using the FactoMineR library (Lê et al. 2008).

The index of interaction (*I*) was calculated for each attribute and mixture using the generalized isobole equation (eq. 2) (Sühnel 1993). In this equation, “*c_i*” denotes the concentration of component “*i*” used in the mixture, and “*C_i*” denotes the concentration of “*i*” that would individually produce the same intensity as the mixture calculated using the corresponding dose response function. “*I*” is the index of interaction; *I* greater than 1 indicates antagonism; less than 1 synergy; and equal to 1 no interaction. Inferential statistics were not applied to the *I* values reported here, as doing so would require estimating *I* values at the level of the individual, rather than at the group level. We rejected this approach as being too computationally intensive, as it would require estimating dose response functions for each stimulus and each percept at the individual level. However, we acknowledge that variation in *I* should exist, and accounted for it by considering the range 0.9 < *I* < 1.1 to be the zero-interaction criterion.

Equation 2: Isobole equation for a combination of *n* components

$$\sum_{i=1}^n \frac{c_i}{C_i} = I \quad (2)$$

Results

Dose–response curves and power functions for the attributes “astringency,” “drying,” “roughing,” “puckering,” “sweetness,” “sourness,” and “bitterness” were determined for malic acid, tannic acid, and alum (Figures 1–3). As expected, an increase in the concentration of an astringent compound was accompanied by an increase in the perceived intensity (gLMS) for all attributes (with the exception of “sweetness” for tannic acid), which is reflected

Figure 1. Perceived “astringency” of malic acid, tannic acid, and alum from experiment 1. See text for the verbal description of “astringency” that was provided to participants.

by the positive exponent shown in each individual power function. Although the time course of astringent compounds has been shown previously (Lee and Lawless 1991; Lawless et al. 1994), this is the first time the dose–response functions of astringent compounds have been reported for these attributes across a range of concentrations.

Perceived intensities for the attributes “astringency,” “drying,” “roughing,” “puckering,” were obtained using a gLMS in experiment 2 for the various mixtures and their unmixed components; arithmetic means and standard errors are shown in Figure 4. Within an attribute, means that do not share a common letter are significantly different from one another ($P < 0.05$). As shown in Figure 4, no significant differences in mean “astringency” ratings were found between stimuli, except between the alum/malic acid and tannic acid/malic acid mixtures, suggesting our prior efforts to match the intensity of the individual stimuli were generally successful.

Conversely, the mean ratings for “drying,” “roughing,” and “puckering” varied significantly and substantially between the various mixtures, as shown in the right side of Figure 4. Generally, the terms “drying” and “roughing” were rated in a somewhat similar manner, as these 2 attributes shared an analogous pattern in mean ratings across all the mixtures. For example, the alum/tannic acid mixture, the tannic acid/malic acid mixture, and the 3-way mixture had high ratings for both “drying” and “roughing,” whereas the alum/malic acid mixture was relatively low in these attributes. Additionally, the alum/malic acid mixture was rated significantly lower in all astringent subqualities (Figure 4, right side) as compared with the tannic acid/malic acid mixture, consistent with previous studies (Lawless et al. 1994; Peleg et al. 1998). In some cases, mean ratings for the astringent subqualities were greater than mean “astringency” ratings, consistent with prior reports (Lee and Lawless 1991; Lawless et al. 1994); this suggests astringency is not merely some sort of “weighted sum” of the various subqualities.

Mean ratings for “bitterness,” “sourness,” and “sweetness” were also determined in experiment 2; these data (arithmetic means and standard errors) are summarized in Figure 5. Within an attribute,

means that do not share a common letter are significantly different from one another ($P < 0.05$). Mean “sweetness” ratings did not differ significantly except for tannic acid, which was rated significantly lower in this attribute when compared with alum or the 2 binary alum mixtures; this is likely due to the bitterness associated with tannic acid (Figure 5, far right). Although the sweetness of alum has been described previously (Breslin et al. 1993), none of the mean “sweetness” ratings exceeded “weak” on the gLMS (Figure 3, panel A), indicating that this attribute is relatively less salient as compared with other attributes measured. All stimuli containing malic acid were rated significantly greater in “sourness” as compared with all other stimuli (Figure 5, middle), consistent with previous reports (Hartwig and McDaniel 1995; Thomas and Lawless 1995). Likewise, a similar tendency regarding the side taste bitterness was also observed for the mixtures containing tannic acid: they tended to be rated higher in “bitterness” compared with other stimuli, consistent with previous reports (Robichaud and Noble 1990; Lee and Lawless 1991;

Figure 2. Dose–response functions for astringent subqualities from malic acid, tannic acid, and alum, using group means on a gLMS from experiment 1. Panel A shows “drying,” panel B shows “roughing,” and panel C shows “puckering.” See text for the specific definitions of these attributes that were provided to participants.

Drewnowski and Gomez-Carneros 2000). Although simply comparing the mean intensity between individual components and their mixtures for each attribute is helpful in showing general trends, the nature of the interaction (i.e., synergistic, antagonistic, or no effect) cannot be determined through simple ANOVAs.

The MFA and dendrogram for the agglomerative-linkage cluster analysis using Ward’s minimum variance as the linkage criteria are shown in Figures 6 and 7. These data reinforce the perceptual similarity between roughing and drying that was alluded to above. Specifically, although these terms separate slightly on dimension 1 of the MFA plot, they are close to each other in dimension 2, and far away from puckering. Likewise, this is also reflected in the dendrogram, where roughing and drying show the shortest linkage distance.

Table 2 shows the index of interaction (I) for mixtures used in experiment 2; also see Supplementary Table 2. Power functions for each stimulus and attribute combination (Figures 1–3) were used to calculate the “ C_i ” terms in equation (2). Generally, there was consistent evidence of antagonism (suppression) for astringency in all the mixtures. Conversely, for the 3 subqualities, the pattern of interaction depended on the specific mixture. The tannic acid/alum and tannic acid/malic acid mixtures both showed evidence of synergy for both “drying” and “roughing,” whereas the malic acid/alum mixture showed evidence of antagonism for “drying,” “roughing,” and “puckering.”

Discussion

In the present study, semantic relationships between “astringency” and its associated subqualities (“drying,” “roughing,” and “puckering”) and side tastes (“bitterness,” “sourness,” and “sweetness”) and mixture interactions were investigated using direct intensity scaling and the isobole approach. Taken together, these analyses help to further elucidate the underlying complexities of the percept: specifically, the nature of the various mixture interactions as well as how the words commonly used to describe astringency relate to one another.

Figure 3. Dose–response functions for side tastes from malic acid, tannic acid, and alum, using group means on a gLMS from experiment 1. Panel A shows “sweetness,” panel B shows “sourness,” and panel C shows “bitterness.”

component “ i ” ($\min_i, \max_i$) includes the concentrations of individual components that would individually produce the same intensity as the mixture, that is, C_i (eq. 2). [Berenbaum \(1985\)](#) states that this condition is met if:

Equation 3

$$\sum_{i=1}^n \frac{x_i}{\max_i} \leq 1 \text{ and } \sum_{i=1}^n \frac{x_i}{\min_i} \geq 1. \quad (3)$$

In the event that the maximum (minimum) doses tested are not equally effective, $\max_i$ ($\min_i$) are replaced with $\max_j$ ($\min_j$), where $\max_j$ is the least effective and $\min_j$ the most effective of the components.

For example, the dose–response curves in [Figures 1–3](#) cover the approximate concentration range of 1–5 g/L, therefore $\max_j$ (in the case of “astringency” for malic acid) = 5 g/L ([Table 1](#)), and $\min_j$ (in the case of “astringency” for tannic acid) = 1.25 g/L ([Table 1](#)). Therefore, for the mixture of malic and tannic acid in [Table 2](#), $(2.2/5 + 2/5) = 0.84 < 1$, and $(2.2/1.25 + 2/1.25) = 3.36 > 1$, so the condition is met. If this condition were not met, as is the case with the 3 component mixture (where $2.75/5 + 2.2/5 + 2/5 = 1.39 > 1$), it is impossible to calculate the effect of a zero interactive combination for comparison, but testing combinations outside this range may still be informative ([Berenbaum 1985](#)). In this instance, where “the x_i ’s are too large, antagonism may be indicated unequivocally if the observed effect of the combination is less than the minimum effect of any $\max_j$.” In our example, the minimum effect, that is, “astringency,” of any $\max_j$ is that of 5 g/L malic acid equal to 13.6, whereas the “astringency” of the 3-component mixture was 15.5. Therefore, we cannot unequivocally assert antagonism, despite the value of I in [Table 2](#), but given that all the 2-component mixtures showed antagonism it is likely that the 3-component mixture would as well.

Here, all mixtures showed evidence of antagonism for “astringency,” whereas various interactions were observed for “drying,” “roughing,” and “puckering” depending on the components present in the mixture. Specifically, mixtures containing alum and malic acid

The isobole approach compares the concentration of each component present in the mixture to that of the theoretical concentration corresponding to the observed intensity of the mixture, C_i (eq. 2). The concentration C_i is calculated from an empirically derived dose–response function from the same participants. These ratios are then summed to calculate the index of interaction (I), which corresponds to synergism (here $I < 0.9$), antagonism (here $I > 1.1$), or no interaction (here $0.9 < I < 1.1$). The indices of interaction for “astringency,” “drying,” “roughing,” and “puckering” for each mixture are shown in [Table 2](#), and are based on the corresponding dose–response functions from [Figures 1–3](#). While [Sühnel \(1993\)](#) states that the isobole approach “does not require any assumptions about the shapes of relations between doses or concentrations and effects or responses of single agents” or “of the underlying mechanism,” citing [Berenbaum \(1985\)](#), this is an over simplification. Calculation of the expected effect requires that the range for each

Figure 4. Ratings of perceived “astringency” of malic acid, tannic acid, and alum and their 1:1 mixtures from experiment 2 (left), as well as the astringent subqualities “drying,” “roughing,” and “puckering” (right). Within an attribute, 1-way ANOVAs were conducted between mean intensity ratings; bars which do not share a letter within the same attribute are significantly different at $\alpha = 0.05$.

Figure 5. Ratings of side tastes (“sweetness,” “sourness,” and “bitterness”) from malic acid, tannic acid, and alum and their 1:1 mixtures (experiment 2). One-way ANOVAs were conducted between mean intensity ratings for each attribute; bars which do not share a letter within the same attribute are significant at $\alpha = 0.05$.

showed evidence for antagonism in “astringency,” “drying,” “roughing,” and “puckering.” Conversely, the alum/tannic acid and tannic acid/malic acid mixtures showed evidence of synergism in the attributes “drying” and “roughing” and no interaction for “puckering.” This finding for tannic acid and malic acid mixtures is not entirely unexpected, as previous studies have shown that the astringency of phenolic compounds increases in the presence of added acid (i.e., lower pH) (Guinard et al. 1986; Fischer and Noble 1994; Kallithraka et al. 1997).

Overall, the tendency for mixtures containing an organic acid and either a polyphenol (e.g., tannic acid) or alum to exhibit marked differences in perceived astringency has been shown in previous studies examining interactions of astringent compounds. Lawless et

Figure 6. MFA plot for attributes used in experiments 1 and 2.

al. examined 2-component mixtures of astringent compounds and their perceived intensity over time for the attributes “astringency,” “drying,” “roughing,” and “puckering.” In their study, a suppression of “astringency,” “roughing,” and “drying,” but not “puckering” was observed for alum/gallic acid mixtures but not for alum/catechin or catechin/gallic acid mixtures; however, this was not formally modeled in a way that accounts for the observed dose–response function, as we have done here. Subsequently, Peleg et al. (1998) explored the influence of various acids on the astringency of both alum and phenolic compounds. Although “astringency” was defined with an alum reference in that study, rather than with written food examples as was done here, similar effects were observed. The astringency of phenolic compounds increased with the addition of citric acid, whereas the astringency of alum was reduced regardless of the type of acid (i.e., lactic, citric, or hydrochloric acid). Those authors speculated that these differences were a result of the chemical modifications

Figure 7. A dendrogram of qualities generated from hierarchical clustering using Ward's method.

Table 2. Indices of interaction (I) terms for astringency and astringent subqualities for alum, malic acid, and tannic acid mixtures

Stimuli in mixture			Index of interaction (I)			
Alum	Malic acid	Tannic acid	Astringency	Drying	Roughing	Puckering
x	x	—	3.67 ↓	>5 ↓	>5 ↓	2.40 ↓
—	x	x	1.27 ↓	0.30 ↑	0.32 ↑	0.95 ↔
x	—	x	1.75 ↓	0.28 ↑	0.35 ↑	0.98 ↔
x	x	x	2.44 ↓	0.78 ↑	1.02 ↔	1.35 ↓

I < 0.9 indicates synergy (↑); I > 1.1 indicates antagonism (↓); I values between 1.1 and 0.9 were interpreted as zero interaction (↔).

affecting binding of astringents with oral proteins rather than cognitive differences (such as those responsible for mixture suppression). Specifically, chelation of the aluminum ion in alum by acids may reduce its availability to interact with salivary proteins. In contrast, the increased astringency from acidification of phenolic compounds was speculated to result from pH-driven increase in the affinity of the phenols for binding with proteins. Accordingly, because of these differences, they concluded that alum and phenolic astringents cannot be used interchangeably for psychophysical studies (Peleg et al. 1998).

To investigate the semantic relationships between “astringency,” the subqualities “drying,” “roughing,” and “puckering” and side tastes “bitterness,” “sourness,” and “sweetness,” we also performed MFA and hierarchical cluster analysis. We found “astringency” is highly related to “Bitterness” as well as to the astringent subqualities (Figure 6). The term “puckering” was situated approximately between “astringency” and “sourness”. This is generally consistent with Bertino and Lawless (1993) who found similar clusters and groupings of mouthfeel attributes. Critically however, they did this in a verbal sorting task using words on index cards, rather than tasted stimuli as was done here. As expected, we also found “sweetness” and “sourness” plotted further from “astringency” relative to “bitterness,” indicating these attributes were less related to astringency than the other attributes measured. The dendrogram generated via hierarchical clustering shows “sweetness” had the largest linkage distance (i.e., was the most different) from all other attributes measured. “Drying” and “roughing” clustered together whereas “bitterness” clustered with “astringency” and “puckering.” Although previous studies have shown that astringent subqualities are not totally interchangeable (Lee and Lawless 1991), our data

suggest that the terms “drying” and “roughing” may be somewhat redundant. It is tempting to speculate that this semantic redundancy reflects a shared mechanism that is distinct from the mechanism for puckering, but more work with a broader range of stimuli would be required to confirm this.

Collectively, the MFA plot and dendrogram suggest that the term “astringency” is highly related to “puckering” and “bitterness” while being less related to “drying” and “roughing.” If correct, this may reflect distinct mechanisms for these clusters of percepts, which would also explain the differences in the interaction identified in Table 2. The inclusion of a side taste like bitterness so close to astringency and puckering is likely not a result of a shared mechanism; rather, it could reflect a shared affective response or may be an artifact of the stimuli tested (e.g., tannic acid).

It is very tempting to interpret the present results in the context of recent reports on the biological mechanisms that underlie astringency. Today, present dogma within the field suggests astringency arises from delubrication secondary to precipitation of salivary proteins, a view that is supported by numerous studies from many research groups (see Bajec and Pickering 2008) for a review). However, emerging evidence (Schobel et al. 2014) suggests there may be a second mechanism which involves direct action on G-protein coupled signaling in murine trigeminal neurons for some astringents, including epigallocatechin gallate (EGCG) and high molecular weight wine tannins. In the same report, Schobel and colleagues also demonstrated temporarily blocking trigeminal nerve input in humans via anesthesia substantially reduces ratings of an astringent stimulus, but this response is not reduced when the chorda tympani nerve (which carries taste signals) is temporarily anesthetized or permanently cut following middle ear surgery (Schobel et al. 2014).

Finally, those authors also showed numerous astringents containing at least one galloyl moiety, including EGCG and tannic acid, were capable of eliciting robust dose dependent responses from murine trigeminal neurons in calcium imaging assays. Collectively, they interpreted these findings as evidence that structurally diverse astringents that contain at least one galloyl moiety are able to directly act on a specialized mechanism in the trigeminal neuron. Elsewhere, it has been shown that acids and multivalent salts (like alum) are capable of precipitating salivary mucins (Lee et al. 2012).

Conceivably then, the synergistic responses observed here for roughing and drying for the tannic acid/alum mixture and the tannic acid/malic acid may reflect superadditive responses arising from stimulation of multiple, independent mechanisms: one based on receptor activation and one based on delubrication. That is, tannic acid may directly activate a receptor that responds to molecules containing a galloyl ring whereas malic acid and alum primarily act via delubrication, and parallel activation of these 2 systems may evoke stronger sensations of drying/roughing than either one alone. However, this speculation should be interpreted cautiously until it can be directly tested empirically. If correct, we would expect that alum/catechin gallate mixtures should synergize with respect to drying/roughing whereas alum/catechin mixtures should not, as catechin gallate has a galloyl ring whereas catechin does not.

Conclusions

Just as the attribute “creamy” may be driven by an integration of sensations such as viscosity, lubricity, and dairy-type aromas, astringency may be driven by an integration of the subqualities “drying,” “roughing,” and “puckering” as well as the associated side tastes “bitter,” “sour,” and possibly “sweet” (Lawless and Corrigan 1994). However, past and current data also indicate that overall astringency is not merely a sum of various subqualities and side tastes, as ratings for some (i.e., drying and roughing) may greatly exceed astringency ratings in some mixtures. Here, we propose the complex of sensations associated with “astringency” is most closely linked to the subquality “puckering” and the side taste “bitterness,” whereas drying and roughing may form a distinct percept. Based on cluster analysis, we also find that “drying” and “roughing” were somewhat redundant, suggesting it may be appropriate to combine these terms in future studies. Moreover, marked differences were observed in the mixture interactions between alum and malic acid compared with those of tannic and malic acid in astringent subqualities, but not in overall astringency as it was defined here. In summary, here we observed complex interactions across difference astringents and percepts; additional work is needed to determine whether these interactions reflect multiple biological mechanisms, perceptual interactions that occur centrally, or possibly some combination of both.

Supplementary material

Supplementary material can be found at <http://www.chemse.oxford-journals.org/>

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Conflict of interest

E.E.F. is presently employed by Mars Chocolate North America, who has no other interest in this work. J.E.H. and G.R.Z. have received speaking and consulting fees from nonprofit organizations, federal agencies, and corporate clients in the food industry. Additionally, the Sensory Evaluation Center at Penn State routinely conducts taste tests for the food industry to facilitate experiential learning for students. None of these organizations have had any role in study conception, design or interpretation, or the decision to publish these data.

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