THE PENNSYLVANIA STATE UNIVERSITY
SCHREYER HONORS COLLEGE

DEPARTMENT OF FOOD SCIENCE

SALIVARY PROTEIN LEVELS AS A PREDICTOR OF PERCEIVED ASTRINGENCY FROM ETHANOL IN MODEL SYSTEMS

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ABSTRACT

Astringency is a complex sensation caused by multiple compounds which evoke sensory qualities including but not limited to drying/roughing, puckering or drawing in the mouth. Traditionally, astringency perception and its sensory properties have been studied mechanistically in relation to polyphenolic stimuli. Less is understood about ethanol's contribution to astringency, which is surprising considering that it is a known drying agent and the second largest component in alcoholic beverages by mass. The purpose of this investigation is to determine if an ethanol stimulus prompts astringency perception, and if it is similar mechanistically to better studied astringents like polyphenols and alums. The study was split into two sessions. Visit 1 consisted of intensity ratings for drying/roughing, burning, bitter and sweet sensations using a general Labeled Magnitude Scale (gLMS) with two concentrations of ethanol (16 % and 32% (v/v)) (n = 56). Visit 2 involved saliva collection, including a tannic acid challenge; subsequent chemical analysis was used to quantify salivary protein to determine salivary protein difference (SP-D) values before and after the tannic acid challenge. Associations between astringency ratings and SP-D values were modeled via ANOVA using a two-group and three-group classification scheme. Astringency perception significantly increased over time unlike burn or bitter sensations. Mean astringency ratings from the three-group model were not significantly different from each other. However, the two-group model found significant differences at 32% ethanol but not at 16%. These data suggest that differences in perceived astringency from ethanol is not strongly explained by SP-D values, in contrast to polyphenols and alums in water.

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Chapter 1

Introduction

Astringency is a multifaceted sensation that can be studied from numerous perspectives. From a sensory science perspective, astringency has been defined as, "the complex sensations due to shrinking, drawing or puckering of the epithelium as a result of exposure to substances such as alums or tannins," (ASTM, 2004). Medically, an astringent compound is considered as, "a drug that causes cells to shrink by precipitating proteins from their surfaces," (CMD, 2007). Foods that elicit astringent sensations are typically those that contain a variety of polyphenolic compounds, such as alcoholic beverages like red wines, teas, coffee and unripe fruit (Joslyn and Goldstein, 1964; Courregelongue et al., 1999; Bate-Smith, 1954; Arnold et al., 1980). Salts and multivalent metallic cations (alum), dehydrating agents (ethanol or acetone) and mineral and organic acids also cause astringent sensations (Joselyn and Goldstein, 1964).

There are five widely accepted prototypical tastes: sweet, sour, bitter, salty and umami/savory. Each is experienced through specialized taste receptor cells found in taste buds on the tongue and other oral surfaces. The most well understood mechanisms are G-coupled protein receptor cells (GPCRs). Taste receptor cells with specific GPCRs allow individuals to experience sweet, bitter and umami. Less is known about the ion channels that mediate salty and sour sensations.

Unlike the classic five tastes, oral astringency is traditionally thought to be a purely tactile process (Bate-Smith et al. 1954; Breslin et al., 1993). Early investigations used hydrogen bonding and hydrophobic interactions to explain astringent perceptions, concluding that the

relationship between polyphenols and protein-rich proteins (PRPs) in the mouth depends on the polarity of the polyphenol. Hydrogen bonding will be prominent in the presence of polar polyphenols while hydrophobic interactions will have a greater role if the majority of polyphenols are nonpolar (Hagerman et al., 1998).

Decades of investigation have led to the current model, known as the "de-lubrication hypothesis". In this model, PRPs interact and bind when exposed to polyphenols in astringent foods in three phases. First, polyphenols interact with PRPs present in human saliva, wrapping around the polyphenol and altering their shape to create protein-phenol complexes. Second, multiple protein-phenol complexes bind with each other, forming polyphenol bridges and protein dimers. Lastly, the dimers aggregate and precipitate out of the salvia which triggers the astringent sensation (Luck et al., 1994; Prince and Lucas, 2000; Jöbstl et al., 2004).

Saliva characteristics, such as PRP concentration, can vary drastically across individuals, influencing sensations like astringency. One method to measure relevant differences in saliva composition is the salivary protein differences value (SP-D value). This *in vitro* measurement calculates the difference in total salivary protein before (S1) and after (S2) exposure to an astringent stimulus, typically tannic acid. More negative values (S2-S1) identify individuals who undergo less protein replenishment in the mouth after exposure to the stimulus, while values near and above zero indicate individuals who are capable of replenishing their proteins to baseline levels (Fleming et al., 2016). Previous work by Fleming et al. (2016) explored associations between SP-D values of individuals and astringency ratings for tannic acid and alum solutions. Her study assessed individual astringent responses from the stimuli relative to SP-D values, creating three groups of high, medium and low responders. She observed that high responders (individuals with low SP-D values) gave significantly higher astringency ratings than medium or

low responders for both tannic acid and alum solutions, showing that intensity ratings for astringent stimuli through measurement of SP-D values can be generalized to both polyphenol compounds and multivalent salts (Fleming et al., 2016).

Although ethanol is widely accepted as a dehydrating agent in chemistry and is the second largest component of alcoholic beverages (after water), minimal work has been performed to understand its role in relation to astringency perception. As noted above, Fleming et al. (2016) found SP-D values can predict astringency ratings for polyphenols and multivalent salts; however, it is not known if this *in vitro* measurement can also predict differences in the astringency of ethanol.

The following study aimed to investigate astringency perception after exposure to ethanol stimuli (16% and 32% v/v) in the context of SP-D measurements. Understanding the role of ethanol in astringency perception through SP-D values has the potential to extend research by Fleming et al. (2016) and Dinnella et al. (2010) and explain a common mechanism for diverse astringent compounds.

Chapter 2

Materials and Methods

2.1 Overview

Data were collected in two sessions. In session one, participants completed an orientation with test stimuli before rating ethanol solutions. In session two, saliva samples were collected before and after exposure to a tannic acid solution.

2.2 Participants

Individuals from the Penn State University campus and surrounding area were recruited for their interest in participating in a taste study through email, and were prescreened for eligibility via an online screening questionnaire. Criteria for eligibility involved the following: between 21 -64 years old; no reasons preventing them from consuming alcohol; not pregnant or breastfeeding; no known defects of smell or taste; no lip, cheek, or tongue piercings, nonsmoker (had not smoked in the last 30 days); no food allergies or sensitivities; no history of choking or difficulty swallowing; and no history of dry mouth (e.g., Sjögren’s Syndrome). All tests were completed at the Sensory Evaluation Center in the Erickson Food Science Building at Penn State.

2.3 Session 1 Orientation Procedure: Astringency Intensity Testing

Participants were given a brief orientation before both sessions. During session 1, individuals were introduced to the study in small groups before entering isolated sensory booths. The orientation included an overview of the gLMS scale with practice exercises using references of sweet (100g/L sucrose from Domino), astringent (0.9 g/L alum from McCormick), bitter (0.05 g/L quinine monohydrochloride dihydrate from SAFC Supply Solutions #6119-47-7), and burn (0.4 ppm of capsaicin from Sigma Aldrich #404-86-4). The concentrations for sweet, astringent and bitter were based on a previous study (Fleming et al., 2016). The capsaicin concentration was selected from results of prior studies to obtain burn intensity ratings on the gLMS scale between moderate and strong (Nolden & Hayes, 2017).

2.4 Session 1 Stimuli and Presentation

Aqueous solutions of ethanol (16% v/v and 32% v/v) were presented in triplicate in 15 mL aliquots at room temperature. Concentrations were based on relative alcoholic contents in common alcoholic beverages (e.g., wine and hard liquor). Two water blanks were also included into the sample set, for a total of 8 stimuli, each labelled with a random 3-digit blinding code. Sampling instructions were: "Please pour the entire contents of sample [xxx] into your mouth and swish the solution for 10 seconds. Expectorate the sample into the covered spit cup provided after 10 seconds.". Sample presentation order was counterbalanced using a Williams design. Participants rinsed their mouths with room temperature reverse osmosis (RO) water before rating the first sample, and between subsequent samples. A minimum inter-stimulus interval (ISI) of 2

minutes was enforced between samples, although individuals were encouraged to take as much time as needed until they no longer experienced sensations from the previous sample.

2.5 Scaling Methods

Intensity ratings were made on a general labeled magnitude scale (gLMS) (Bartoshuk et al., 2003). This scale is derived from the Labeled Magnitude Scale (Green et al 1993, 1996) and is labeled at 0 (no sensation); 1.4 (barely detectable); 6 (weak); 17 (moderate); 35 (strong); 52 (very strong); 100 (strongest imaginable sensation of any kind) (Hayes et al. 2013; Fleming et al., 2014). All data were collected via Compusense Cloud, Academic Consortium (Guelph, ONT). All procedures were approved by the local Institutional Review Board (IRB; protocol # 00011070).

2.6 Session 2 Orientation Procedure: Saliva Collection and Analysis

At session 2, participants were oriented again in small groups before entering isolated sensory booths. The orientation involved an overview of the saliva collection method and its materials.

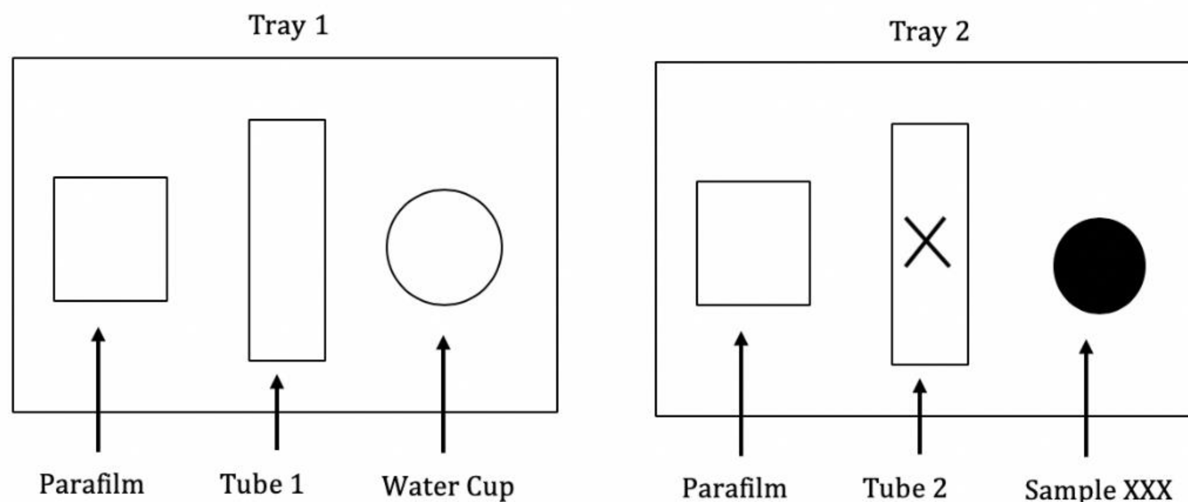

Figure 1. Tray presentation of the saliva collection method during Session 2. Whole mouth saliva was collected before (Tray 1) and after (Tray 2) tannic acid stimulation. Tray 2 was given to participants after the 20-minute break. Each tube 2 was marked with a black "X" to clearly distinguish it from tube 1.

2.7 Saliva Collection

During the second session, two samples of whole mouth saliva were collected from each participant. Figure 1 depicts the tray presentation for the session. Participants were given each tray separately. Saliva collection was done by first having participants rinse their mouths with RO water and chew on parafilm (5 cm x 5 cm) for 10 minutes to systematically evoke saliva (first saliva collection, "S1"). After a 20-minute break, individuals received an aqueous solution of tannic acid (3.0 g/L) from Spectrum Chemical (#1401-55-4). This tannic acid concentration was used in prior studies to evoke protein precipitation (Fleming et al., 2016 and Dinnella et al., 2010). Instructions were to pour the entire contents (15 mL) of the sample into the mouth and rinse with the solution for 10 seconds before expectorating. Individuals rinsed their mouths with

RO water before chewing a second piece of Parafilm for another 10 minutes (second saliva collection, "S2"). Participants rated the dry/roughing sensation of the aqueous solution in between the first and second saliva collections. No attempts were made to control for intake of tea or coffee, or any other polyphenol-containing food and/or beverage prior to testing. Additionally, no attempt was made to control for hydration among individuals. Only RO water was provided before and after the tannic acid stimulus.

Saliva samples were frozen immediately after collection in a -80°C freezer, and held for ~45 days. For chemical analysis, samples were thawed in a water bath set at 37°C for 30 minutes and centrifuged at 4,700 g for 10 minutes. The pellet was discarded and the supernatant was recovered. Total protein for each sample (S2-S1) was determined in triplicate using the bicinchoninic acid (BCA) protein assay and Bovine Serum Albumin as a reference for the standard curve (Pierce Biotechnology). Samples were read on a Clariostar microplate reader at 562 nm (BMG Labtech).

2.8 Data Analysis

All analyses were conducted in MiniTab (Version 18, MiniTab Inc., State College, PA). Six participants' intensity ratings were extremely high on the gLMS scale, suggesting improper understanding and use of the scale. These data were removed from the analysis, resulting in a final n of 56. Separate ANOVA models assessing the effect of ethanol concentration and replicate were used for ratings of: dry/rough, bitter, and burn. To analyze the *in vitro* measurement, SP-D values were categorized in two ways. First, the values were split by the first and third quartile values, which separated individuals into three groups: Low (LR), Medium

(MR) and High responders (HR), in line with prior work by Dinnella et al. (2010) and Fleming et al. (2016). Separately, an alternative grouping approach was explored, where the SP-D distribution was split in terms of Negative (n = 66) and Positive (n = 102) values. These values exceed the number of participants, as they are based on SP-D values collected in triplicate (i.e., 3 x 56 = 168). Significant main effects in the mixed model ANOVA were analyzed with Tukey's Honestly Significant Difference ($\alpha = 0.05$), where the overall group means for each SP-D group were assessed.

Chapter 3

Results

Session 1: Intensity ratings from ethanol solutions

In the ANOVA model for drying/roughing ratings, replicate was significant [F (2,275) = 3.75; p = 0.025]; as shown in Figure 2, the third replicate was perceived as significantly more dry/rough than the first. This is consistent with prior work showing that astringency can compound over time (Guinard et al., 1986; Lymann and Green, 1990).

Concentration was also significant [F (1,275) =31.20; p <0.001], as higher ethanol concentration received increased dry/rough ratings (See Appendix A, Table 3). This was expected, as higher concentrations of ethanol cause more intense sensations (e.g., Nolden and Hayes, 2017).

The concentration by replicate interaction was not significant [F (2, 275) = 0.60; p = 0.549], suggesting increased perceived astringency between the first and third replicates was not dependent on the ethanol concentration. Complete ANOVA tables and Tukey HSD values are provided in Appendix A, Tables 1-3.

Separate ANOVA models were run for burn and bitterness ratings. Ethanol concentration was significant for both burn [F (1, 275) = 72.07, p = 0.000] and bitterness [F (1, 275) = 46.22, p = 0.000]. However, unlike astringency, burn and bitterness did not have a significant replicate effect (Appendix A, Tables 5 and 8); that is, astringency from ethanol compounds with repeated exposure, while burn and bitterness do not.

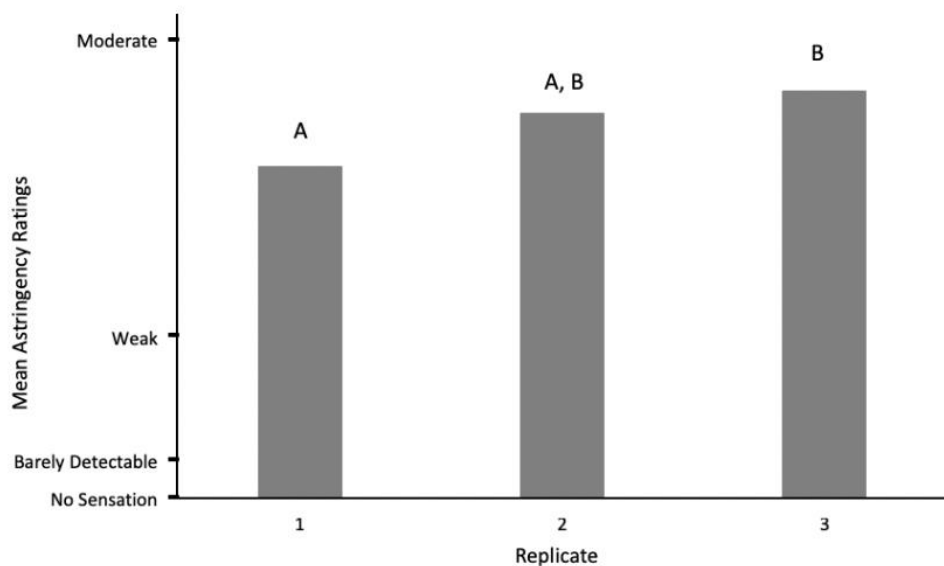

Figure 2. Mean astringency ratings of ethanol solutions across replicate trials.

Session 2: Astringency perception and SP-D value

The potential influence of SP-D value on astringency perception was assessed in two ways. The first approach, based on prior work by Dinnella (2010) and Fleming (Fleming et al., 2016), splits SP-D values into three groups: High, Medium and Low. These splits were based on the descriptive statistics of the SP-D values, using the 25th and 75th percentiles. The second grouping method split SP-D values on the basis of whether the observed SP-D value was negative or positive, forming two groups.

For the three-group model, mean astringency differed significantly by concentration (F (1, 318) = 49.65; p = 0.002], but not by SP-D group [F (2, 318) = 0.61; p = 0.541]. There was no significant interaction between concentration and SP-D group (F (2, 318) = 0.08; p = 0.924) (Appendix B, Table 10). These results suggest that astringency intensity from ethanol increased with increased concentration, but was not dependent on a three-way split of SP-D values into High, Medium and Low groups.

In contrast, the two-group model, which spit individuals into either the Negative or Positive group, obtained different results. Again, the concentration effect was significant [$F(1, 324) = 10.91$; $p = 0.001$]. SP-D group was also significant as a main effect [$F(1, 324) = 10.90$; $p = 0.001$], demonstrating that concentration and group each independently impact astringency ratings. As expected, higher concentrations of ethanol produced increased intensity ratings. The significant group effect revealed that individuals classified in the Negative SP-D group gave higher intensity ratings than those in the Positive group, regardless of ethanol concentration. No significant interaction was observed between concentration and group [$F(1, 324) = 0.45$; $p = 0.500$], suggesting that the effect of group does not depend on concentration. Across both ethanol concentrations, the Negative group gave higher astringency ratings than the Positive group. As seen in Figure 3a, the mean astringency rating for the Negative group was moderate while the Positive group gave a mean rating in between weak and moderate.

The two-group approach was applied to the burn intensity ratings as well. Concentration was significant as expected [$F(1, 324) = 123.74$; $p = 0.000$]; as above, higher ethanol concentrations produced higher burn intensities, Unexpectedly, the group effect was significant [$F(1, 324) = 12.07$; $p = 0.001$]. Similar to the astringency results, the Negative SP-D group gave higher burn ratings than the Positive group. The concentration by group interaction was not significant [$F(1, 324) = 0.23$; $p = 0.629$]. That is, the Negative group had higher burn ratings than the Positive group at both concentration levels. Figure 3b depicts these findings; both groups' averaged ratings were between moderate and strong, yet the Negative group was closer to strong and the Positive group to moderate.

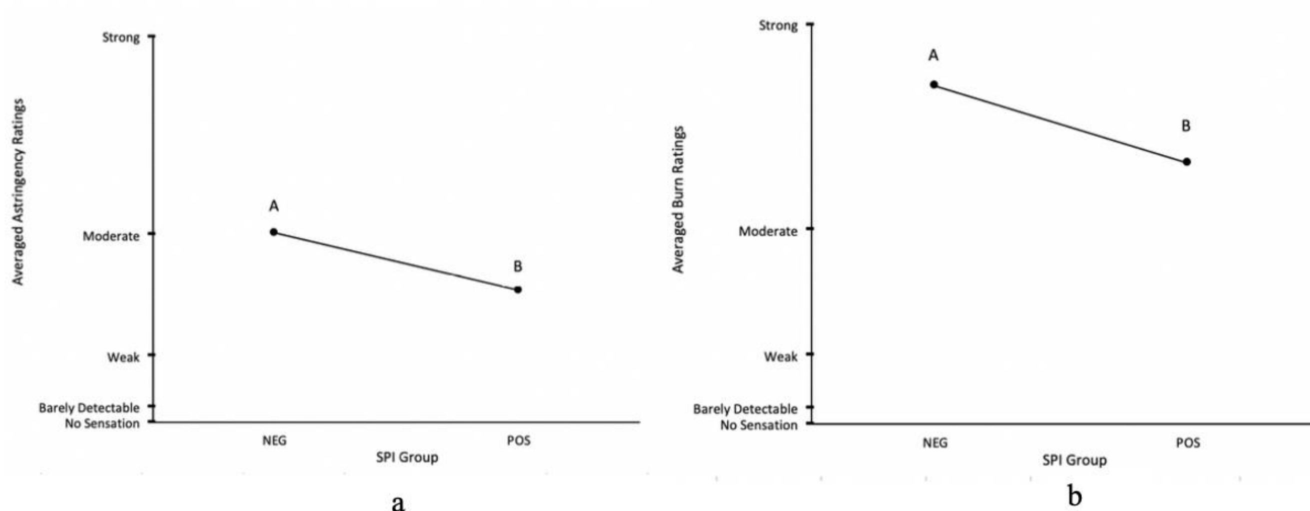

Figure 3. Mean astringency (a) and burn (b) ratings for the Negative and Positive SP-D groups, collapsing across both ethanol concentrations.

Last, it should be noted that for bitterness, concentration differed significantly [$F(1, 324) = 14.35$; $p < 0.001$], but not SP-D group [$F(1, 324) = 0.79$; $p = 0.374$] (Appendix B, Table 15). Higher concentrations of ethanol were perceived to be more bitter (Appendix A, Table 9); however as shown in Figure 4, this did not differ meaningfully across group, with both rating it just below moderate.

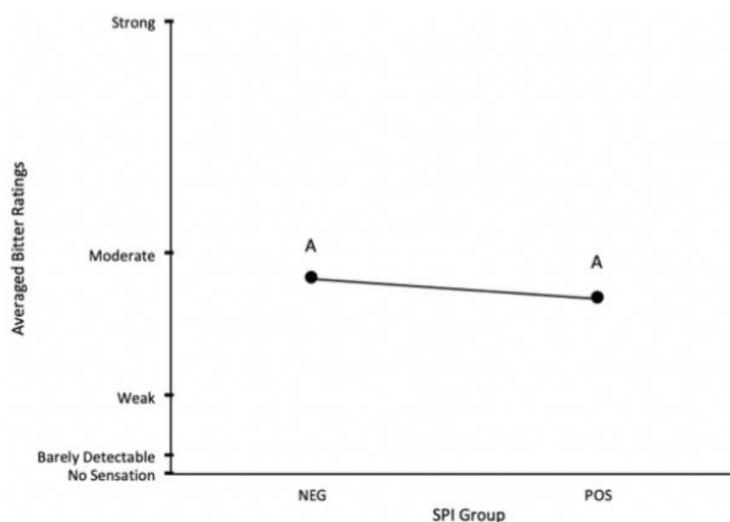

Figure 4. Mean bitterness ratings across the Negative and Positive SP-D groups, collapsing across ethanol concentration.

Chapter 4

Discussion

My data indicate that oral astringency from ethanol increases across repeated exposure, as the third replicate was significantly rated more astringent than the first, but this progression was not seen with the burn or bitter sensations. This finding is consistent with prior work showing astringency can compound across repeated exposure (Guinard et al., 1986; Lymann and Green, 1990).

Contrary to my original hypothesis, the oral astringency from ethanol was not strongly predicted by SP-D value, suggesting that the de-lubrication hypothesis does not fully explain this sensation when given ethanol as a stimulus. The three-group model of Low, Medium and High responders did not show significant differences in oral astringency, in contrast to prior work on tannic acid and alum. Specifically, Fleming and colleagues found that High responders (individuals that had more negative SP-D values) gave significantly higher mean astringency ratings to tannic acid and alum compared to the Medium and Low response groups (Fleming et al., 2016). Moreover, Dinnella et al. (2010) showed that reaction to oral stimulation was different between High, Medium and Low responders. Those with less oral stimulation (HR group) experienced greater astringency build-up after repeated exposure to tannic acid than the MR or LR groups.

Similar to the three-group model, results from the two-group model did not strongly support the de-lubrication hypothesis. Nonetheless, small but significant differences in intensity ratings between Positive and Negative SP-D value groups were observed. Astringent sensations were perceived to be significantly more intense for individuals in the Negative group than the Positive group. Although these differences were not large, this was expected and followed a

similar pattern to the work of Fleming and colleagues (2016), where High Responders (negative SP-D values) gave higher intensity ratings. Interestingly, the same pattern was found for burn intensity, supporting the possibility that astringency from ethanol could be observed via a different mechanism from that of polyphenols and multivalent salts. Alternatively, these results could be evidence of a smearing bias, where individuals could not adequately distinguish between burning and dry/rough due to lack of training (Bennett and Hayes, 2012).

Recent work by Schöbel et al. (2014) challenges the de-lubrication hypothesis, showing that specific structures of astringent stimuli with one or more galloyl moieties cause astringency by activating trigeminal ganglia neurons (TGs) and possibly G-protein coupled receptors. Even though ethanol was not included in Schöbel's study, it is worthwhile to consider that ethanol's structure could be triggering one or more pathways that elicit both astringency and burn, which are perceived at a higher intensity by individuals with negative SP-D values.

Furthermore, while bitterness increased with concentration, both SP-D groups rated the sensation at about the same intensity, suggesting that the degree of protein precipitation did not influence bitterness ratings. These relationships between bitterness, burn and astringency from ethanol further support the possibility of a common mechanism for astringent and burning perception that is separate from the pathway for bitterness.

Taking note of the possible mechanisms for oral astringency and the significant but weakly predictive evidence here, this suggests ethanol may cause astringency via multiple mechanisms, or in general could not have as great of an effect on the sensation as other stimuli like multivalent salts and tannins. Indeed, prior work on relationships between salivary proteins and wine components have found that higher concentrations of ethanol have an inverse effect on astringency perception, where overall perception decreases as the alcohol content increases.

McRae et al. (2015) concluded that the ethanol concentration in wine to some degree inhibits the dominant chemical interactions between tannins and salivary proteins. Hydrophobic interactions were dominant at ethanol concentrations lower than 15%, while above this number (40% ethanol) hydrogen bonding was prominent. Hydrogen bonds at 40% ethanol concentration led to weaker interactions between tannins and salivary proteins, decreasing protein precipitation and astringency perception (McRae et al., 2015). Rinalidi et al. also found similar characteristics of ethanol in Merlot wines and model solutions. An addition of 2% (v/v) ethanol to samples decreased salivary protein precipitation. The investigators determined that ethanol disrupts bonding between the tannins and salivary proteins decreasing precipitation, as found in the McRae (2015) study, but in addition modifies the proteins by altering their conformation and lowering binding capability (Rinaldi et al., 2012).

Future work should consider some of the limitations of this experiment. First, the number of subjects excluding outliers was relatively small (n = 56). A larger sample size would provide more confidence in the generally null results. Second, the experiment did not control for consumption of astringent foods and/or beverages right before testing. Recent prior exposure to astringent stimuli (e.g., coffee or tea) could have potentially altered salivary protein concentrations, influencing perceived sensations and or SP-D values, although this would likely add noise rather than systematically biasing my data. Finally, salivary flow of the individuals was not taken into consideration here and could be a confounding factor in this study. Differences in salivary flow have been shown to impact the intensities of stimuli. Boulton et al. (1994) found that salivary flow rate impacted the intensity and duration of bitterness and astringency. Low flow individuals experienced astringency for 30 minutes longer than high or medium flow individuals (Boulton et al., 1994). Moreover, in an investigation of relationships

between salivary flow rate, haze development and tannic acid astringency, Horne et al. (2002) found that increased haze development correlated to individuals with high salivary flow rates. These individuals also gave lower astringency ratings. The inverse relationship points to increased protection from astringents for individuals with high salivary flow (Horne et al., 2002). Controlling for salivary flow would provide a better understanding of how individuals differ in basal salivary protein concentrations, and how this variation could drive astringency intensity.

Chapter 5

Summary and Conclusions

This study supports the nuance of astringency perception and the mechanisms that are involved. Prior to this experiment, the ability of the de-lubrication hypothesis to explain ethanol induced astringency had not been researched. Here, SP-D values were found to influence astringency perception from ethanol somewhat, but not to the same degree as other astringents – e.g., tannic acid or alum – studied previously. This suggests a combination of mechanisms may be involved that may overlap with other perceived sensations. Through this work, the complexity of astringency perception was confirmed, suggest a need for additional research to gain better understanding of this sensation.

Appendix A

ANOVA analyses for Intensity Ratings

Table 1. Analysis of Variance for Astringency Intensity Ratings

Source	DF	Adj SS	Adj MS	F-value	P-value
Replicate	2	478.1	239.04	3.75	0.025
Concentration	1	1987.5	1987.5	31.20	0.000
Concentration*Replicate	2	76.5	38.27	0.60	0.549
Panelist	55	46602.7	847.32	13.30	0.000
Error	275	17517.7	63.70		
Total	335	66662.6			

Table 2. Tukey's Analysis at 95% confidence of significant Replicate effects for astringency intensity ratings.

Replicate	N	Mean	Grouping	
3	112	15.1768	A	
2	112	14.3580	A	B
1	112	12.3384		B

Means that do not share a letter are significantly different.

Table 3. Tukey's Analysis at 95% confidence of significant Concentration effects for astringency intensity ratings.

Concentration	N	Mean	Grouping	
32	168	16.3899	A	
16	168	11.5256		B

Means that do not share a letter are significantly different.

Table 4. Analysis of Variance for Burn Intensity Ratings

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Panelist	55	66008	1200.1	8.50	0.000
Replicate	2	39	19.5	0.14	0.871
Concentration	1	38400	38400.22	72.07	0.000
Concentration*Replicate	2	282	140.8	1.00	0.370
Error	275	38814	141.1		
Total	335	143543			

Table 5. Tukey's Analysis at 95% confidence of significant Replicate effects for burn intensity ratings.

Replicate	N	Mean	Grouping
1	112	25.8205	A
2	112	25.2491	A
3	112	25.0071	A

Means that do not share a letter are significantly different.

Table 6. Tukey's Analysis at 95% confidence of significant Concentration effects for burn intensity ratings.

Concentration	N	Mean	Grouping	
32	168	36.0494	A	
16	168	14.6685		B

Means that do not share a letter are significantly different.

Table 7. Analysis of Variance for Bitter Intensity Ratings

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Panelist	55	54399.6	989.08	14.25	0.000
Replicate	2	221.3	110.65	1.59	0.205
Concentration	1	3208.5	3208.53	46.22	0.000
Concentration*Replicate	2	184.7	92.35	1.33	0.266
Error	275	19088.7	69.41		
Total	335	77102.8			

Table 8. Tukey's Analysis at 95% confidence of significant Replicate effects for bitter intensity ratings

Replicate	N	Mean	Grouping
1	112	14.8080	A
2	112	14.4018	A
3	112	12.9196	A

Means that do not share a letter are significantly different.

Table 9. Tukey's Analysis at 95% confidence of significant Concentration effects for bitter intensity ratings.

Concentration	N	Mean	Grouping	
32	168	17.1333	A	
16	168	10.9530		B

Means that do not share a letter are significantly different.

Appendix B

ANOVA analyses of SP-D values

Table 10. Mixed model ANOVA of astringency intensity ratings using the 3-group (High, Medium, Low) approach.

Source	DF	Adj SS	Adj M	F-Value	P-Value
Concentration	1	1929.3	1929.3	49.65	0.002
Replicate	2	399.8	199.90	1.00	0.369
Concentration*Replicate	2	57.6	28.82	0.14	0.866
Responders	2	245.8	122.92	0.61	0.541
Concentration*Responders	2	31.5	15.77	0.08	0.924
Replicate*Responders	4	23.2	5.80	0.03	0.998
Concentration*Replicate*Responders	4	222.8	55.69	0.28	0.892
Error	318	63597.11	99.99		
Total	335	66662.0			

Table 11. Mixed model ANOVA of astringency intensity ratings using the 2-group (Negative and Positive) approach.

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Concentration	1	2073.8	2073.82	10.91	0.001
Replicate	2	417.4	208.69	1.10	0.335
Concentration*Replicate	2	152.6	76.28	0.40	0.670
SPI Group	1	2071.8	2071.79	10.90	0.001
Concentration*SPI Group	1	86.5	86.49	0.45	0.500
Replicate*SPI Group	2	22.8	11.39	0.06	0.942
Concentration*Rep*SPI Group	2	349.1	174.55	0.92	0.400
Error	324	61590.2	190.09		
Total	335	66662.6			

Table 12. Tukey's Analysis at 95% confidence of significant SPI group effects with the 2-group ANOVA model for astringency ratings.

Concentration	N	Mean	Grouping
NEG	132	17.0447	A
POS	204	11.9603	B

Means that do not share a letter are significantly different.

Table 13. Mixed model ANOVA of burn intensity ratings using the 2-group model (Negative and Positive) approach.

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Concentration	1	37959	37958.6	123.74	0.000
Replicate	2	0	0.1	0.00	1.000
Concentration*Replicate	2	210	104.8	0.34	0.711
SPI Group	1	3702	3702.4	12.07	0.001
Concentration*SPI Group	1	255	255.0	0.83	0.363
Replicate*SPI Group	2	738	369.1	1.20	0.302
Concentration*Rep*SPI Group	2	735	367.3	1.20	0.303
Error	324	99392	306.8		
Total	335	143543			

Table 14. Tukey's Analysis at 95% confidence of significant SPI group effects with the 2-group ANOVA model for burn ratings.

Concentration	N	Mean	Grouping	
NEG	132	29.4856	A	
POS	204	22.6887		B

Means that do not share a letter are significantly different.

Table 15. Mixed model ANOVA of bitter intensity ratings using the 2-group model (Negative and Positive) approach.

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Concentration	1	3235.8	3235.78	14.35	0.000
Replicate	2	190.9	95.44	0.42	0.655
Concentration*Replicate	2	236.6	118.28	0.52	0.592
SPI Group	1	178.5	178.49	0.79	0.374
Concentration*SPI Group	1	52.7	52.71	0.23	0.629
Replicate*SPI Group	2	11.2	5.59	0.02	0.976
Concentration*Rep*SPI Group	2	173.5	86.76	0.38	0.681
Error	324	73072.3	225.53		
Total	335	77102.8			

Table 16. Tukey's Analysis at 95% confidence of significant SPI group effects with the 2-group ANOVA model for bitter ratings.

Concentration	N	Mean	Grouping
NEG	132	14.9492	A
POS	204	13.4569	A

Means that do not share a letter are significantly different.

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ACADEMIC VITA

Katherine M. Mobley

EDUCATION

The Pennsylvania State University; University Park, PA May 2019
Bachelor of Science - Food Science; Minor - Spanish
Schreyer Honors College
Advisor: Dr. John Hayes, Ph.D.
Thesis Title: Total Salivary Protein Levels used as an Indicator for Astringency Perception from Ethanol Stimuli
Salivary protein precipitation measurements coupled with sensory analysis were applied to determine oral astringent perception from ethanol stimuli.

CLIC International House; Seville, Spain Sept. 2014 - May 2015
Professional working proficiency in speaking, reading, and writing Spanish

WORK & RESEARCH EXPERIENCE

Ocean Beauty Seafoods: Quality Assurance
Quality Assurance Supervisor and Manager in Training; Alaska Region Summer 2017, 2018

- Led team of seven employees to maintain and monitor quality of seafood products such as salmon, salmon roe, salmon ancillary products, halibut, sablefish, black cod and rockfish. (2018)
- Conducted customer and third-party audits for BRC (British Retail Consortium) certification. (2018)
- Reviewed quality assurance paperwork and critical control points for compliance with HACCP plans.
- Managed Quality Assurance Program for salmon and salmon roe processing facility in remote, Native American community; Population: 190. (2017)

Quality Assurance Technician; Alaska Locations Summer 2014, 2015

- Implemented Good Manufacturing Practices within processing plant for salmon and salmon byproducts.
- Performed daily sanitations checks to ensure operations were compliant with ADEC and FDA regulations.
- Graded, inspected and documented data for canned and frozen, headed and gutted, salmon species.

Food Science Department; Penn State University
Student Researcher under Dr. Helene Hopfer, Ph.D. Spring 2018

- Individual research to characterize lipids of avocado seeds using official chemical methods.

Penn State Agricultural Research and Extension Center; Manheim, PA
Research Technician Summer 2016

- Conducted field research experiments and variety trials on strawberries, tomatoes, onions and peppers.
- Collected and analyzed data to assess crop productivity and quality.

Penn State Sensory Evaluation Center; Penn State University
Food Science Sensory Technician
2015-Present

- Prepared and served samples for various sensory tests and trained descriptive panels.
- Introductory working knowledge of Compusense Software.

CERTIFICATIONS & RECOGNITIONS

- Seafood HACCP Certification from Association of Food and Drug Officials (AFDO) *Completed 2018*
- Penn State Department of Food Science Ice Cream Short Course *Completed 2018*
- Girl Scout Gold Award Recipient - Highest Girl Scout leadership achievement
Received 2012

ACTIVITIES

Penn State Campus Girl Scouts Volunteer Organization

- Co-President and Vice President Fall 2016- 2018

Penn State Navigators

- Small Group Leader 2017-Present

Chemical Methods of Food Analysis Teaching Assistant Spring 2018