

**AVF/CCGPRVE Annual REPORT**

**Project Title:** Investigation of mechanisms for perception of astringency

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**Cooperators:** Kevin Scott (SRA) and Dr. Inger Baek (post doc), Ann Colonna and Helen Keplinger, MS students, Dept. Viticulture and Enology; Dr. Charles Plopper, Dept Vet Med, UC Vet School.

**Objectives and Experiments conducted to accomplish objectives.**

Overall objective: To understand how the following factors affect perception of astringency

- A. The effect of consumption pattern, salivary flow rate, and saliva composition on perception of astringency
- B. Comparison of different rinses and times between sips on reducing carry-over effect
- C. The morphological effect of tannin on epithelial tissue

**A. Experiments.**

**1. The effect of consumption pattern, salivary flow rate and saliva composition on astringency**

A. Single sip study.

Sixteen judges rated intensity of two levels of alum (0.4 and 0.8 g/L) and 21 judges rated tannic acid (1.1 and 1.9 g/L) in water. Judges sipped the sample and rated astringency until the sensation was gone. While the judges were rating astringency, artificial saliva (warmed to body temperature) was introduced into the oral cavity at three different flow rates (0.5, 1.5, 5 ml/min) for 20s. Two types of artificial saliva were used: "control" and "protein" (P2) which contained 2g/L of gelatin. Both control and protein salivas had a salt composition<sup>1</sup> resembling that of real saliva. Between samples, judges rinsed with 6g/L gelatin for 30s, then with distilled water for 30s, and then waited 30s.

B. Multiple sip study.

IB1 Tannic acid

Astringency of 1.65 g/L tannic acid was evaluated by nineteen trained judges. Judges took 4 sips at 25 second intervals while rating astringency continuously. Each sample was expectorated 8 sec after being sipped. Between samples, judges rinsed with 6g/l gelatin for 15s, then with distilled water for 15s, then again 15s with 6g/L gelatin and 15s with water followed by 30s wait. Either "control" and a "protein" (P3) saliva which here contained 3g/L of gelatin was introduced for 105 seconds (from the time the judges sipped the first sample until 30 seconds after the 4th sip) at very low (0.7 ml/min) and high (8.5 ml/min) flow rates.

(IB2) Tannic acid

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<sup>1</sup>) 0.234g NaCl, 0.746g KCl, 0.504g NaHCO<sub>3</sub>, 0.601g KHCO<sub>3</sub>, 0.0685g CaCl<sub>2</sub>, 0.102g MgCl<sub>2</sub>, 0.0327g KH<sub>2</sub>PO<sub>4</sub> and 0.0418g K<sub>2</sub>HPO<sub>4</sub> in 1L distilled water (adjusted to pH 7.0).

Astringency of tannic acid (1.65g/L) was rated as described above, except the salivas were introduced at only one flow rate: 5 ml/min

(IB3) Red Wine

Astringency of red wine was evaluated by trained judges, using the same 4 sip protocol as described above, except the flow rates were 0.7 and 5.0 ml/Min. The experiment is still underway.

### **Experiment 2. The effect of different rinses and times between sips**

#### **A. Rinses**

Judges sipped a red wine, expectorated at 10 seconds, and at 20 sec sipped a rinse, which was then expectorated 10 seconds later. The judges rated astringency continuously throughout this protocol until the sensation ceased. Between wines, the judges rinsed extensively with water for 20 s two times. The rinses which were compared were solutions of ovalbumin (4 g/L), gelatin (6 g/L), PVP (4g/L), pectin (1g/L) and water.

B. The effect of different intervals between sips is presently under study.

C. The effectiveness of either rinses or timing on wines varying greatly in astringency is proposed for next year.

### **Experiment 3. The physiological (morphological) effect of tannin on epithelial tissue.**

One definition of astringency which is often quoted is the " complex quality perceived due to the complex of sensations caused by **shrinking, drawing or puckering** of the skin or mucosal surfaces" (ASTM, 1982). This definition implies that a constriction of the oral epithelium upon contact with astringent substances causes the perception of astringency which has never been demonstrated. To characterize the morphological change in the oral (buccal) epithelium resulting from interaction with astringents, a control rinse or a rinse of grape seed tannin was applied for one and five minutes to buccal mucosa of anesthetized mice. The mouths were then rinsed with PBS and treated with 1% PFA to begin oral fixation in situ.(All rinses were saved to count sloughed off cells). After one hour, the tissue was excised, the buccal mucosa dissected out, affixed to a cover slip, and the tissue was then post-fixed in 1% PFA for 30 minutes to ensure complete fixation. Once fixed the tissue was rinsed, stained with YOPRO, a nuclear fluorophore, and Alexa 568, an actin marker to examine the morphological effect by assessing internuclear distances and perhaps shortening of the actin fibers. The tissue was examined via confocal microscopy, which can take snapshots at determined distance intervals and stack them into a 3 dimensional projection. The collected rinses were fixed, centrifuged, resuspended, and counted.

#### **4. Data analysis.**

Parameters (time to maximum intensity, maximum intensity etc) extracted from the smoothed raw time-intensity curves, were analyzed by mixed model analysis of variance where judges were treated as a random variable.

### **V. Summary of Major Research Accomplishments and Results**

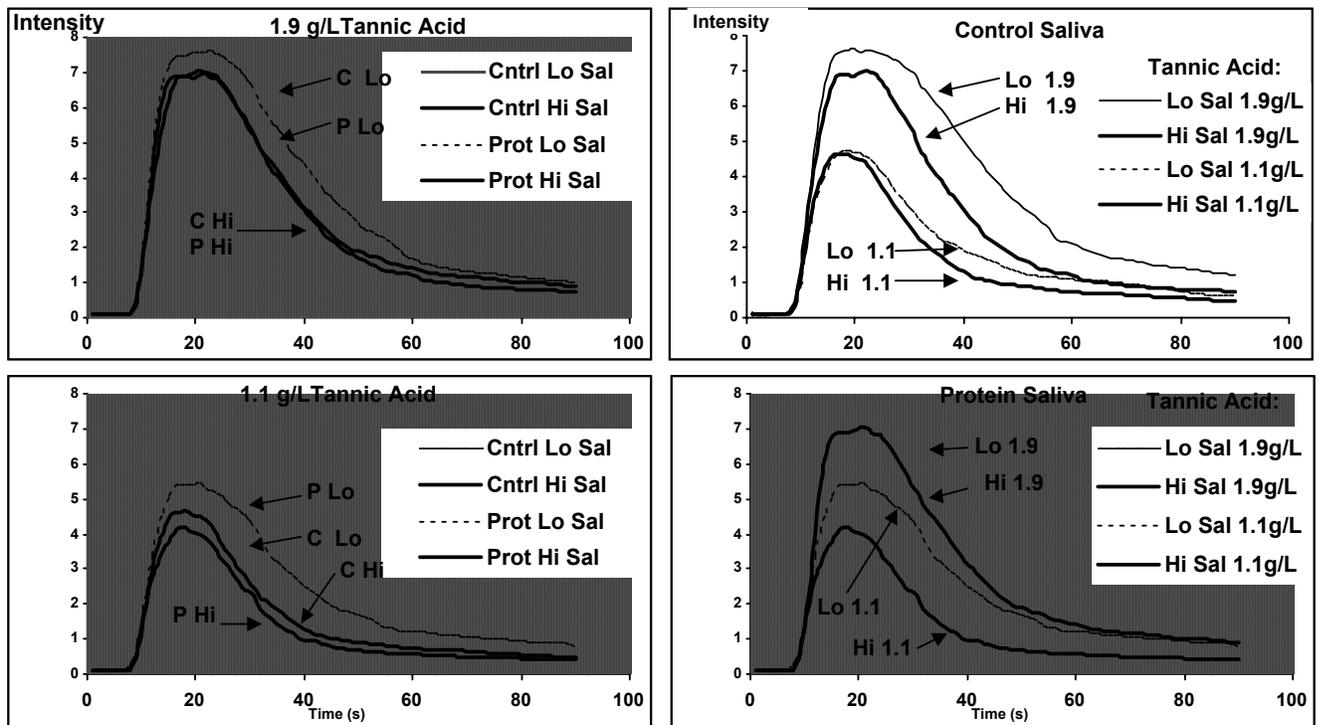
#### **1. The effect of consumption pattern, salivary flow rate and saliva composition**

Expt. 1A. One Sip Alum and Tannic Acid.

For both compounds the results were similar, thus only results for tannic acid are presented. For clarity, only the data for the lowest and highest rate of salivary flow are given. In Figure 1, the astringency intensity curves are shown for both levels of tannic acid in four panels. To compare the effect of flow rate and type of saliva, on the left of Figure 1, the curves for astringency of 1.9 g/L (top) and 1.1 g/L tannic acid (bottom) are shown. For both concentrations of tannic acid, astringency was rated lower at higher flow rates for both types of saliva. For both concentrations of tannic acid, there was no significant difference in astringency intensity between the types of saliva although astringency was slightly higher for the protein containing saliva at the low flow rate only (bottom left).

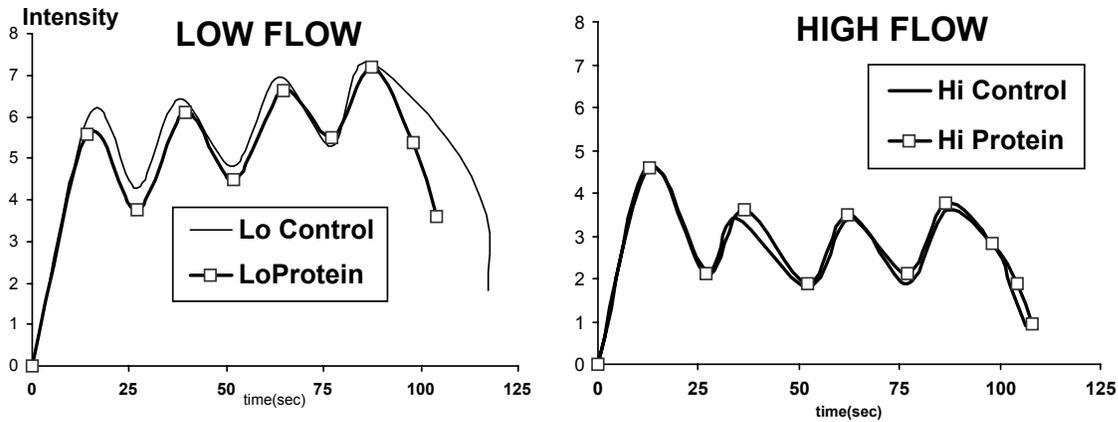
To examine the effect of flow rate and of concentration of tannic acid, curves are shown on the right side of Figure 1 for the control saliva (top) and the protein saliva (bottom). For both, the intensity of the higher concentration of tannic acid was rated higher than the lower level. For both saliva types, astringency was lower at the highest flow rate (5.0 ml/Min) than for the lowest flow rate (0.5 ml). However, for the lowest concentration of tannic acid (top right, Fig. 1), the difference between flow rates was not significant for the control saliva.

Figure 1. Expt. 1A. Effect of type of saliva (Protein vs Control) and salivary flow rate (0.7ml and 5.0 ml/min) on astringency of tannic acid (1.1 and 1.9 g/L) (14 Judges x 3 reps)



Expt. 1B Four sips Tannic Acid

Figure 2. Expt. 1B. Effect of salivary flow rate and type of saliva on astringency of tannic acid. (18 Judges x 2 reps).



In Figure 2, the effect of type of saliva and salivary flow rate on astringency are shown for 1.65 g/L tannic acid. For both the low flow (left) and high flow rates (right), there was no significant difference in astringency intensity between the types of saliva. However, the intensity of astringency from first sip to last was higher for the low flow (left) than the high flow (right) rates. At the lower flow rate (left), the maximum intensity of each sip increased with the number of sips as did the lowest value of intensity reached between sips. However, for the very high flow rate of 8.5 ml/min (right), the maximum astringency decreased from first sip to last, suggesting that at this flow rate both dilution and flushing out of the tannic acid occurred.

Between each "sample" or different condition (type and flow of saliva), after the judge finished rating the sample, he/she rinsed 15 s with 6 g/L gelatin then 15 s with water, then repeated these two rinses and then waited an additional 30 s. However, despite this extensive rinse protocol BETWEEN the different conditions, as shown in Figure 3, there still was carry over. Regardless of what sample/condition was presented, the first one was always lowest in intensity, and the fourth wine highest. This further justifies continuing research to develop a protocol for tasting astringent wines.

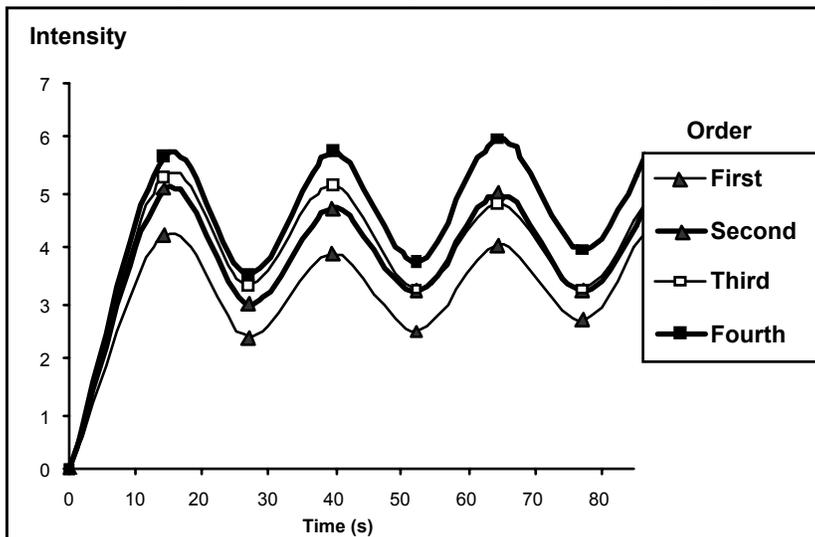


Figure 3. Expt. 1B. Effect of order of presentation on intensity of astringency of tannic acid  
**Experiment 2. Effect of rinses**

For this study, judges sipped red wine, spit at 10 seconds, and at 20 seconds rinsed for another 10 seconds. In Figure 4 below the average time intensity curves are shown for astringency of the wine during the different rinses. Pectin was the most effective rinse, followed by PVP and Gelatin.

PVP and Gelatin were equally effective in reducing astringency, but less so than Pectin. Water and ovalbumin were least effective. Ovalbumin was not expected to bind tannins based on its amino acid composition and it was no different from water in its effectiveness.

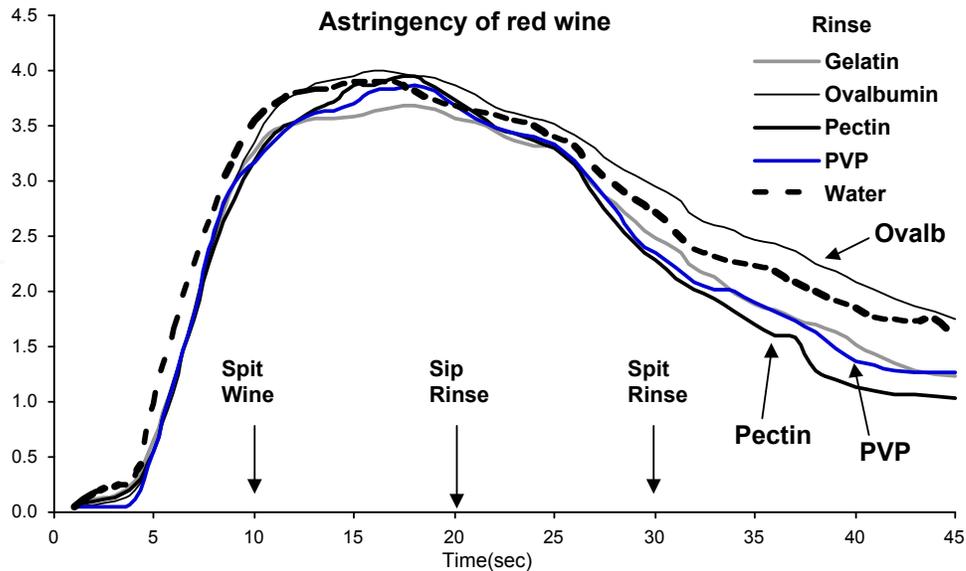


Figure. 4. Effect of type of rinse on persistence of astringency of red wine (21 judges x 2 reps).

To show the actual intensity values after rinsing, In Figure 4, the astringency intensity at 35, 40 and 44 seconds (which are respectively 5, 10 and 14 seconds after the rinse was expectorated) are plotted in Figure 4. The intensity at 35 sec was significantly reduced only by pectin. At 44 sec

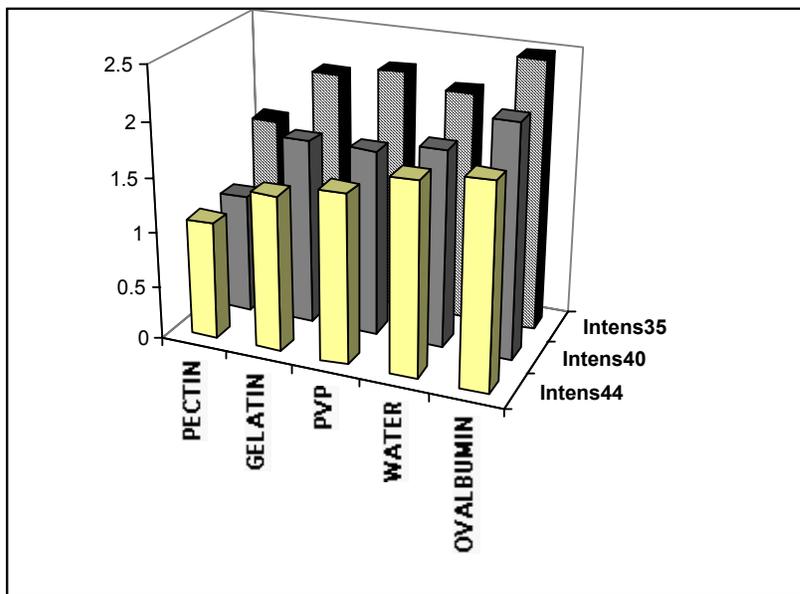


Figure 5. Intensity of astringency of red wine at 35, 40 and 44 seconds.

PVP and Gelatin reduced astringency significantly below water or ovalbumin rinses, but were less effective than pectin. Astringency was significantly lowest after the pectin rinse.

**Expt. 3. The morphological effect of tannin on epithelial tissue**

Inspection of the confocal microscopic view of buccal mucosa showed the green nuclei of surface squamous cells and underlying cuboidal cells which were "lit up" by yopro, a nuclear chromophore. (See appendix). The near absence of red suggested that no actin was involved. The epithelial tissues did not appear to "constrict or pucker" as no obvious difference in internuclei distance was seen (data analysis in process). This suggests that tannin did not cause a constrictive morphological change in epithelial mucosa in the mouth, but, perhaps more mucosal cells were sloughed off. To measure this, the number of sloughed off cells were counted in the rinse of the tissue after application of the GST. As shown in Figure 6, there appeared to be more sloughed off cells after the mouse tissue was rinsed with GST than water. However, this was not a significant effect. The large difference between mice may reflect difficulty in resuspending the oral mucosal cells.

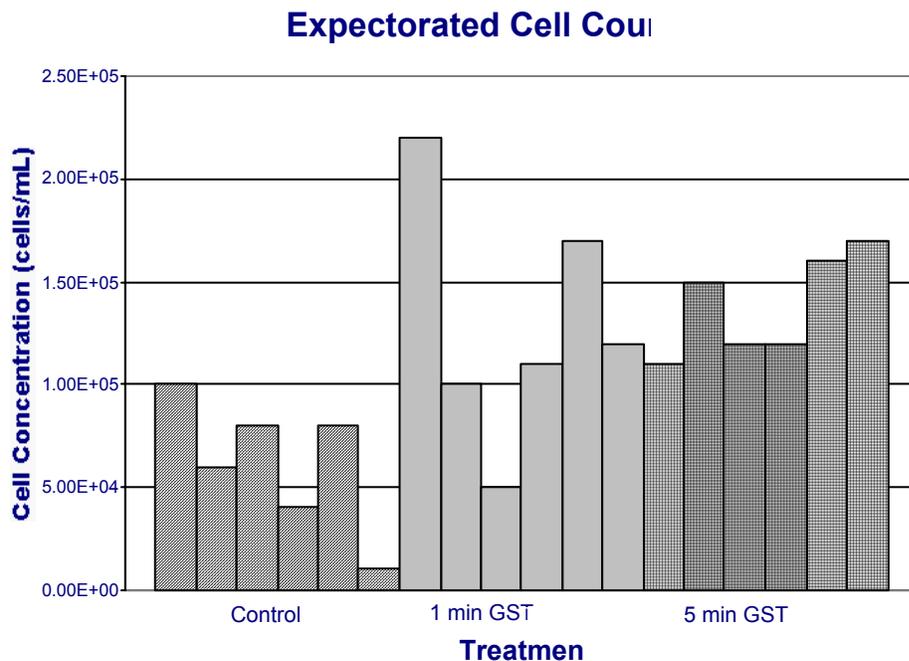


Figure 6. Cell count in oral rinses of epithelial tissue of individual mice after water rinse (control), 2 g/L grape seed tannin (GST) for one min and for 5 min.

**Outside presentations of research:**

Noble, A.C. , Astringency (and Bitterness) of Flavonoid Phenols. In Symposium on Chemistry of Taste, at Annual Meeting Am. Chem. Soc. San Francisco, March 2000

Baek, I. Sensory Analysis of Astringency. Seminar in Copehagen, Denmark. December 2000

**Publications:**

Noble, A.C. Astringency (and Bitterness) of Flavonoid Phenols. Chemistry of Taste, ACS. Ed. Given, P. and Paredes, D. in press.2000

Brossaud, F., V. Cheynier, and A C Noble. Bitterness and astringency of grape and wine polyphenols Austr. J. Grape and Wine Research in press. 2000.

**SUMMARY FOR AVF/CCGPRVE Annual REPORT**

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### Summary

The mechanism by which astringency is perceived is unknown. Two different mechanisms have been proposed.

- 1: The ASTM defines astringency as the puckering or constricting of the oral epithelial tissue, suggesting that a morphological change occurs when an astringent system is evaluated.
- 2: In contrast, others hypothesize that the rough feeling of astringency occurs when salivary proteins bind to tannins, oral lubrication decreases and friction (roughness) is perceived.

Preliminarily, from our study of the morphological changes in the epithelial mucosa, it appears that astringency does not result from "constriction of the oral tissue". No obvious differences in distances between epithelial cells were observed after application of astringents. However, it is possible that the loose cells on the surface slough off after binding with tannins, perhaps resulting in perception of astringency, but this has not been substantiated.

Consistent with the second hypothesis, in previous studies, it has been shown that individuals with low flow rates of saliva perceive astringency more intensely and longer than high-flow subjects. To confirm this effect of salivary flow rate independent of variation in use of the intensity rating scale, artificial saliva was introduced to the judge's mouth at different flow rates. Astringency was rated lower at high flow rates of application than when lower rates of application were used. In both cases, comparison of individuals with different flow rates and with the application of artificial saliva at varying flow rates, the presumption has been that astringency decreases at a higher flow rate due to restoration of lubrication. However, no difference in astringency was found between application of artificial saliva with protein vs. an artificial saliva containing no protein. These results suggest that higher salivary flow rates may dilute or more thoroughly flush the mouth, and suggest that further research into the mechanism of astringency is needed.

The persistence of astringency has been recognized but only recently has the carry-over effect been documented in which each sip of wine influences the astringency perceived in subsequent sips or wines. For meaningful evaluations of astringent red wines during winemaking, blending or in competitions a tasting protocol to remove or reduce the buildup of astringency must be developed. To do this, astringency was rated continuously while red wines were sipped, spit, and judges rinsed with one of 5 solutions. A pectin rinse reduced astringency intensity significantly more than the other rinses. We are presently investigating the most effective way to prevent the increase in intensity for wines varying in astringency level and to define the minimum time to recommend between wines.

Funds. Expended.