

Wine pH Prevails over Buffering Capacity of Human Saliva

Elías Obreque-Slier,[†] Valeria Espínola-Espínola,[†] and Remigio López-Solís^{*,‡}

[†]Department of Agro-Industry and Enology, Faculty of Agronomical Sciences, University of Chile, P.O. Box 1004, Santiago, Chile

[‡]Program of Cellular and Molecular Biology, Faculty of Medicine–ICBM, University of Chile, Independencia 1027, Santiago, Chile

ABSTRACT: Wine is an acidic beverage; its pH (2.9–3.8) is critically important to its organoleptic properties. During degustation, wine interacts with <1 mL of mouth saliva, the pH of which is near 7.0. This is buffered predominantly by the carbonate/bicarbonate pair ($pK_a = 6.1$). Few data are available on whether the buffering capacity of saliva may alter the pH of wine and thus its sensorial properties. In this study both in vitro and in vivo approaches were conducted to measure pH in mixtures of representative red and white wines with human saliva. Continuous additions of microvolumes of either wine to a definite volume (3 mL) of saliva in vitro resulted in a progressive and steep decline in the pH of the wine/saliva mixture. Thus, a few microliters of either wine (<0.27 mL) was sufficient to reduce the pH of saliva by 1 pH unit. Further additions of wine to saliva lowered the pH to that of the corresponding wine. In the in vivo assay, definite volumes (1.5–18 mL) of either wine were mixed for 15 s with the mouth saliva of individual healthy subjects before pH determination in the expectorated wine/saliva mixtures. Compared to saliva, pronounced decreases in pH were observed, thus approaching the pH of wine even with the smallest volume of wine in the assay. Altogether, these results demonstrate that the buffering capacity of wine prevails over that of saliva and that during degustation the pH of the wine/saliva mixture in the mouth is, at least temporarily, that of the corresponding wine.

KEYWORDS: wine, pH, saliva, buffering capacity, degustation, titratable acidity

■ INTRODUCTION

Wine is produced by fermentation of *Vitis vinifera* L. grape juice. Such beverage is a complex mixture of water, alcohols, sugars, phenolics, a number of aromatic compounds, minerals, and organic esters and acids. Organic acids derived from both grape pulp and fermentation activities are mainly responsible for wine acidity.¹ Tartaric, malic, citric, succinic, lactic, and acetic acids represent the most common organic acids present in wine. Both the concentration of organic acids and the malic acid to tartaric acid ratio are the main determinants of grape and grape must pH, which is in the range from 2.8 to 3.8. In winemaking, pH is critical to microbiological stability, growth of fermenting yeasts and fermenting bacteria, color stability, and organoleptic properties. In addition, organic acids provide wines with a buffering capacity in direct relationship with their contents. Such capacity is maximal in wines having a pH close to the pK_a of its main acid component, for example, tartaric acid.² During degustation, wine is mixed with mouth saliva (also called “whole saliva” or “mixed saliva”). Saliva is a highly complex liquid, a colorless, odorless, tasteless, and viscous mixture of exocrine secretions, the main components of which are water (99.5%), protein (0.3%), and inorganic compounds (0.2%).^{3,4} The protein fraction of saliva is mainly composed of glycoproteins, enzymes, immunoglobulins, and a wide variety of polypeptides, such as cystatins, statherins, histatins, and proline-rich proteins.^{3–5} The inorganic fraction of saliva is composed of various concentrations of electrolytes, such as sodium and potassium cations and chloride and bicarbonate anions.⁶ The main functions of saliva are lubrication, oral health protection, antimicrobial activity, food digestion, and oral sensoriality.^{3,7,8} A main scarcely studied role of saliva is its buffering capacity to stabilize the oral pH and to counteract changes in pH produced by foods.⁹ The pH of saliva is in the range from 6.2 to 7.4.⁶ The

buffering capacity of saliva would be basically dependent on bicarbonate, histidine-rich low-molecular-weight polypeptides, and phosphate ions.³ Buffering capacity would be beneficial for protecting oral tissues against acids and alkalis derived from foods, beverages, and bacterial plaque.

On these grounds, there is not much information in the literature about the pH of the wine/saliva mixture in the mouth during wine tasting or, in other words, whether the pH of the mixture of neutral buffered saliva and acidic buffered wine is different from either the salivary pH, the wine pH, or both. Thus far, not much information is available regarding either the buffering capacity of saliva to neutralize wine acidity or the buffering capacity of wine to counteract the buffering power of saliva. This study aimed at determining pH in mixtures of saliva and two representative commercial red and white wines both under conditions mimicking wine tasting (mixing in the mouth) and under in vitro conditions (mixing in a test tube).

■ MATERIALS AND METHODS

Subjects. Two healthy females (23 and 25 years old) and one healthy male (28 years old) without history of smoking, alcoholism, or medication consumption, with no evidence of disease during the 60 days prior to the study and displaying both normal saliva flow (>1 mL/min) and normal salivary protein profile were included under the terms of a signed informed consent.¹⁰ To minimize confounding variables, all three volunteers consumed a balanced diet for 3 days before beginning the experiments.

Received: July 5, 2016

Revised: October 7, 2016

Accepted: October 11, 2016

Published: October 11, 2016

Saliva Collection. Following a mouth rinse with water, samples of whole saliva were passively collected (with no use of stimulants). To this end, saliva accumulated in the mouth for 1 min was transferred to a glass vessel maintained in ice. The procedure was repeated up to three times, and saliva was pooled. Collections were routinely conducted between 9:00 and 11:00 a.m. to minimize eventual diurnal variations in salivary composition.¹¹ Saliva was conserved in an ice bath during the experiments.

Wines. Two commercially available wines were used, namely, a Cabernet Sauvignon (Vineyard Misiones de Rengo, Cachapoal Valley, Libertador Bernardo O'Higgins Region, Chile, year 2009, serial no. L-0039) and a Sauvignon blanc (Vineyard Misiones de Rengo, year 2010, serial no. L-0288). Each wine was characterized by the following chemical analyses: total phenols by absorptiometry at 280 nm,¹² total tannins by the methyl cellulose procedure,¹³ total anthocyanins by the bisulfite decoloration method,¹² color intensity (absorbances at 420 + 520 + 620 nm for red wines and at 420 nm for white wines),¹⁴ total acidity by alcalimetry with 0.1 N NaOH and phenolphthalein indicator for white wine and bromothymol blue indicator for red wine), pH by potentiometry using a combination glass electrode at 20–22 °C,¹² ethanol content by densimetry,¹² reducing sugar content by the Fehling Causse–Bonnans liquor method,¹² and contents of organic acids (tartaric, malic, citric, and lactic acids) by HPLC-DAD analysis.¹⁵ Physicochemical characteristics of both wines are summarized in Table 1.

Table 1. Physicochemical Properties of Red and White Wines in the Study^a

	red wine	white wine
total phenols (g GAE/L)	1.7 ± 0.0	0.1 ± 0.0
total tannins (g procyanidin/L)	1.4 ± 0.3	0.1 ± 0.0
total anthocyanins (mg malvidin/L)	309.4 ± 19.4	
color intensity	9.4 ± 0.1	0.1 ± 0.0
titratable acidity (g tartaric acid/L)	4.8 ± 0.2	6.6 ± 0.1
pH	3.8 ± 0.0	3.4 ± 0.0
ethanol (% v/v)	13.8 ± 0.0	12.2 ± 0.3
reducing sugars (g glucose/L)	4.3 ± 0.4	2.8 ± 0.1
tartaric acid (mg/L)	3514.9 ± 20.2	3986.7 ± 102.8
malic acid (mg/L)	0.0 ± 0.0	3641.9 ± 115.3
citric acid (mg/L)	0.0 ± 0.0	1089.1 ± 92.4
lactic acid (mg/L)	1271.5 ± 7.9	140.9 ± 9.5

^aFigures represent means ± standard deviation (triplicates). GAE, gallic acid equivalents.

pH after Wine/Saliva Interaction: In Vitro Assay. A 3 mL aliquot of saliva was placed in a glass beaker with constant mechanical stirring and a setting for continuous pH reading and scoring. After initial reading of the pH, successive aliquots of wine were added at 15 s intervals. Aliquots of wine added to saliva were increased gradually according to the following schedule: 5 μ L from 0 to 200 μ L of added wine, 20 μ L from 200 to 300 μ L, 50 μ L from 300 to 600 μ L, 100 μ L from 600 μ L to 3 mL, 1 mL from 3 to 9 mL and, finally, 3 mL from 9 to 18 mL of total added wine. For each subject, the assays were carried out in triplicate with independent samples of saliva and using both wines.

pH after Wine/Saliva Interaction: In Vivo Assay. An aliquot of wine (red or white) was placed in the mouth of each volunteer ($n = 3$). After mixing thoroughly during 15 s, the wine/saliva mixture was transferred from the mouth to a glass vessel for pH determination. The procedure was conducted in triplicate at 5 min intervals with aliquots of wine in the range from 1.5 to 18.0 mL (1.5, 2.0, 2.5, 3.0, 6.0, 9.0, 15.0, and 18.0 mL). In those intervals, the volunteer rinsed his/her mouth with 10 mL of water.

Statistics. Infostat 9.0 software and the Kruskal–Wallis non-parametric test were used to analyze data from the in vitro assay for pH. If significant differences between treatments were observed, a multiple-comparison test for differences between means was

conducted. On the other hand, Minitab 16 software and ANOVA were used to analyze results from the in vivo assay for pH. In the case of significant differences between treatments, Tukey's test was applied with a 5% margin of error.

RESULTS

Changes in pH following Mixing of Wine with Saliva:

In Vitro Assay. At the start of the assay, the pH of saliva was about 7.22, and the pH values of the red and white wines were 3.84 ± 0.0 and 3.38 ± 0.0 , respectively. As shown in Table 2, addition of wine (red or white) to a definite amount of saliva (3 mL) resulted in a progressive decrease in pH of the wine/saliva mixture. Both pH curves were identical (Figure 1). For the first part of the assay with either wine (up to about 1800 μ L) the pH fell steeply, then the fall of the pH slowed (to about 4000 μ L) and, finally (>6000 μ L), it reached the pH of the corresponding wine. With no exception, the pH of the wine/saliva mixtures was lower for corresponding volumes of white wine compared to red wine. A closer analysis of pH progression in the wine/saliva mixtures as either wine was being added showed that the pH fall was significantly more pronounced at the beginning of wine additions (Figure 2). Accordingly, additions of red wine to 3 mL of saliva provoked pH decreases of 0.45 pH unit/100 μ L wine in the range of 100–500 μ L of wine compared to decreases of 0.16, 0.05, and 0.025 pH unit/100 μ L wine in the ranges of 600–900, 1000–1300, and 1400–1800 μ L of wine, respectively. Likewise, the initial successive additions of the white wine to saliva resulted in pH decreases of 0.52 pH unit/100 μ L wine in the range of 100–500 μ L of wine compared to decreases of 0.11, 0.05, and 0.03 pH unit/100 μ L wine in the same respective ranges indicated above (Figure 2 and Table 2). As indicated before, the pH decrease was practically zero when the total volume of wine added to 3 mL of saliva was 6 mL or more.

Changes in pH following Mixing of Wine with Saliva:

In Vivo Assay. Growing aliquots of wine (range 1.5–18 mL) were thoroughly mixed with the total volume of saliva present in mouth just before the mixture was returned to a glass vessel for measuring the pH. At the start of the assay, the pH of saliva was 7.2 ± 0.2 and the pH values of wines were 3.9 ± 0.0 (red) and 3.4 ± 0.0 (white). The smallest aliquots of either red or white wine in the assay, that is, 1.5 mL, were sufficient to provoke a sharp and significant fall in the pH of the wine/saliva mixture (about 3 pH units) (Figure 3 and Table 3). Mixing higher volumes of either wine (in the range of 2–18 mL) with mouth saliva produced small additional pH decreases. By mixing 3 mL (or more) of red wine or 6 mL (or more) of white wine with mouth saliva, the pH of the wine/saliva mixture achieved its minimal value, which was the one of the corresponding plain wine. Again, under the same experimental conditions in the assay, the observed pH in the various wine/saliva mixtures was significantly lower for white wine compared to red wine.

DISCUSSION

On the basis of both in vitro and in vivo pH determinations, we have now provided evidence that during wine tasting, that is, when wine comes in contact for a short time with saliva in the mouth, saliva is unable to neutralize or modify the wine pH, at least temporarily. The in vitro assay consisted in continuous pH monitoring along with the stepwise addition of aliquots of wine to a known volume of saliva, usually 3.0 mL for convenience. Thus, the starting pH in the assay was that of saliva, around

Table 2. pH of Wine/Saliva Mixtures Produced in Vitro by Continuous Addition of Definite Volumes of Red or White Wine (μL) to 3 mL of Saliva^a

T	volume wine	pH sal–RW	pH sal–WW	T	volume wine	pH sal–RW	pH sal–WW
1	0 ^b	7.25 ± 0.2	7.19 ± 0.1	44	260	6.29 ± 0.4	5.33 ± 0.3
2	5	7.29 ± 0.2	7.18 ± 0.2	45	280	6.16 ± 0.4	5.23 ± 0.3
3	10	7.28 ± 0.2	7.06 ± 0.2	46	300	6.05 ± 0.3	5.11 ± 0.3
4	15	7.27 ± 0.2	7.02 ± 0.3	47	350	5.84 ± 0.3	4.95 ± 0.2
5	20	7.24 ± 0.2	7.14 ± 0.1	48	400	5.63 ± 0.3	4.82 ± 0.2
6	25	7.23 ± 0.2	7.12 ± 0.1	49	450	5.46 ± 0.3	4.70 ± 0.2
7	30	7.21 ± 0.2	7.11 ± 0.1	50	500	5.29 ± 0.2	4.60 ± 0.2
8	35	7.23 ± 0.2	7.05 ± 0.2	51	550	5.17 ± 0.2	4.51 ± 0.2
9	40	7.21 ± 0.2	7.06 ± 0.1	52	600	5.06 ± 0.1	4.43 ± 0.2
10	45	7.21 ± 0.2	7.05 ± 0.1	53	700	4.89 ± 0.1	4.33 ± 0.2
11	50	7.20 ± 0.2	7.00 ± 0.1	54	800	4.74 ± 0.0	4.25 ± 0.1
12	55	7.19 ± 0.2	6.97 ± 0.1	55	900	4.62 ± 0.1	4.15 ± 0.1
13	60	7.16 ± 0.2	6.92 ± 0.1	56	1000	4.54 ± 0.0	4.09 ± 0.1
14	65	7.17 ± 0.2	6.90 ± 0.1	57	1100	4.47 ± 0.0	4.03 ± 0.1
15	70	7.16 ± 0.3	6.88 ± 0.1	58	1200	4.41 ± 0.0	3.97 ± 0.1
16	75	7.14 ± 0.3	6.85 ± 0.1	59	1300	4.36 ± 0.0	3.94 ± 0.1
17	80	7.14 ± 0.3	6.84 ± 0.1	60	1400	4.32 ± 0.0	3.89 ± 0.1
18	85	7.12 ± 0.3	6.80 ± 0.1	61	1500	4.29 ± 0.0	3.85 ± 0.1
19	90	7.11 ± 0.3	6.73 ± 0.2	62	1600	4.25 ± 0.0	3.82 ± 0.1
20	95	7.09 ± 0.3	6.72 ± 0.2	63	1700	4.23 ± 0.0	3.78 ± 0.1
21	100	7.07 ± 0.3	6.69 ± 0.2	64	1800	4.21 ± 0.0	3.77 ± 0.1
22	105	7.04 ± 0.3	6.63 ± 0.2	65	1900	4.18 ± 0.0	3.74 ± 0.1
23	110	7.03 ± 0.3	6.56 ± 0.2	66	2000	4.17 ± 0.0	3.72 ± 0.1
24	115	7.01 ± 0.3	6.53 ± 0.2	67	2100	4.14 ± 0.0	3.70 ± 0.1
25	120	7.01 ± 0.3	6.48 ± 0.2	68	2200	4.12 ± 0.0	3.69 ± 0.1
26	125	6.98 ± 0.3	6.42 ± 0.2	69	2300	4.10 ± 0.0	3.68 ± 0.1
27	130	6.97 ± 0.3	6.39 ± 0.3	70	2400	4.09 ± 0.0	3.66 ± 0.1
28	135	6.95 ± 0.3	6.33 ± 0.3	71	2500	4.08 ± 0.0	3.64 ± 0.1
29	140	6.92 ± 0.3	6.28 ± 0.3	72	2600	4.06 ± 0.0	3.64 ± 0.1
30	145	6.90 ± 0.3	6.23 ± 0.3	73	2700	4.05 ± 0.0	3.62 ± 0.1
31	150	6.87 ± 0.3	6.16 ± 0.3	74	2800	4.04 ± 0.0	3.62 ± 0.1
32	155	6.84 ± 0.3	6.11 ± 0.4	75	2900	4.04 ± 0.0	3.60 ± 0.1
33	160	6.82 ± 0.3	6.05 ± 0.4	76	3000	4.03 ± 0.0	3.58 ± 0.1
34	165	6.78 ± 0.3	6.00 ± 0.4	77	4000	3.99 ± 0.0	3.55 ± 0.0
35	170	6.76 ± 0.3	5.95 ± 0.4	78	5000	3.94 ± 0.0	3.50 ± 0.0
36	175	6.73 ± 0.3	5.88 ± 0.4	79	6000	3.91 ± 0.0	3.48 ± 0.0
37	180	6.70 ± 0.3	5.85 ± 0.4	80	7000	3.90 ± 0.0	3.45 ± 0.0
38	185	6.68 ± 0.3	5.83 ± 0.4	81	8000	3.88 ± 0.0	3.44 ± 0.0
39	190	6.66 ± 0.3	5.77 ± 0.4	82	9000	3.87 ± 0.0	3.42 ± 0.0
40	195	6.64 ± 0.4	5.73 ± 0.4	83	12000	3.86 ± 0.0	3.41 ± 0.0
41	200	6.58 ± 0.4	5.68 ± 0.4	84	15000	3.85 ± 0.0	3.40 ± 0.0
42	220	6.51 ± 0.3	5.57 ± 0.3	85	18000	3.84 ± 0.0	3.39 ± 0.0
43	240	6.39 ± 0.4	5.44 ± 0.3	86	wine	3.84 ± 0.0	3.38 ± 0.0

^apH values for each condition are presented as means ± standard deviation of triplicates from three independent experiments with saliva from three different subjects. T, treatment; RW, red wine; WW, white wine. ^bAt the start, 3 mL of plain saliva (no wine).

7.0.^{3,6} Under these conditions, very minor volumes of wine (a few microliters) were sufficient to significantly reduce the pH of saliva. This sole observation appears to conflict with the view that the buffer capacity of saliva serves to counteract changes in mouth pH associated with food consumption.⁹

Saliva, like many other bodily fluids, is a finely buffered system that takes up or releases H⁺ to minimize changes in its concentration.³ A significant decline in pH (whatever *significant* may mean!) can produce diverse biological effects in the organism. In effect, considering that pH is logarithmically related to H⁺ concentration, a pH decline from 7.0 to 6.9, which in the in vitro assay was produced by adding less than 70 μL of red wine or 35 μL of white wine to 3 mL of saliva,

represents roughly a 25% increase in the H⁺ concentration in the wine/saliva mixture compared to plain saliva. In fact, every drop of 0.1 pH unit represents a 25% increase in H⁺ concentration. By the same token, the H⁺ concentration was doubled or the pH dropped by 0.3 pH unit (in reference to plain saliva) after the addition of the first 135 μL of red wine or 70 μL of white wine to the 3 mL aliquot of saliva. Likewise, by mixing the red or white wine with saliva at a 1:1 volume ratio (i.e., 3 mL in the in vitro assay), pH values of the wine/saliva mixtures became close to that of the corresponding wine in the mixture. Under these latter conditions, compared to plain saliva, the H⁺ concentrations in the red wine/saliva and white wine/saliva mixtures were increased over 900- and 2500-fold,

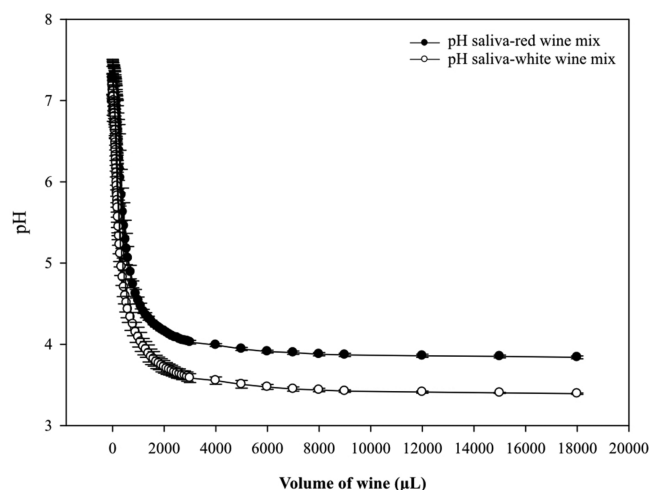


Figure 1. pH curve after in vitro addition of either red or white wine to saliva. The pH was continuously registered during successive additions of definite volumes of wine to a 3 mL aliquot of saliva. Curves represent means of triplicates from three independent experiments performed with saliva of three different subjects. Each pH value is depicted as the mean \pm standard deviation.

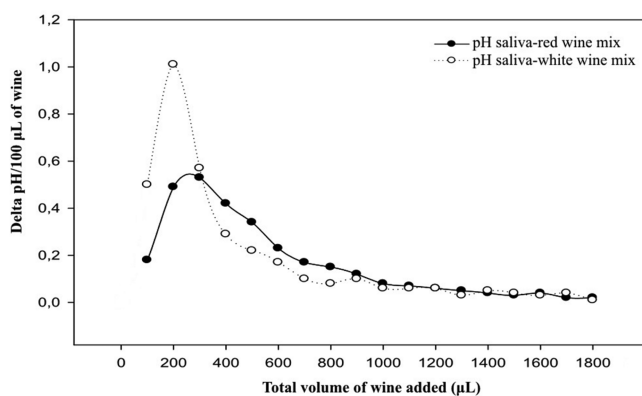


Figure 2. Changes in the pH of wine/saliva mixtures during initial additions of wine aliquots to a definite volume of saliva in vitro. On the basis of the continuous fall in pH in the experiment shown in Table 2, differences in pH observed at 100 μ L wine intervals in the range between the basal condition (no wine addition and 3 mL of saliva) and a total addition of 1.8 mL of wine. Note in either curve an initial peak representing the transition from the buffering influence of saliva to the buffering influence of wine.

respectively. Volumes of 4 mL or more of either red or white wine mixed with 3 mL of saliva lowered pH values to roughly those of the corresponding wines. Overall, highly reproducible pH curves were produced in the in vitro assay for different samples of saliva taken either from a single subject or from different subjects. Extrapolation of data from these in vitro measurements made in 3 mL of saliva to the in vivo condition, involving an estimated volume of saliva in the mouth of 1 mL,¹⁶ suggests that a third of the indicated volumes of wine would provoke the above-mentioned decreases in salivary pH or increases in salivary H⁺ concentration. Altogether, the in vitro assay clearly showed that resistance of saliva to pH change (buffering capacity) when mixed with either the red or white wine was not evident.

Certainly, saliva mixed with wine in a test tube should not be equated straightforwardly to saliva in the mouth during tasting. In effect, saliva is mostly organized as a complex film that covers

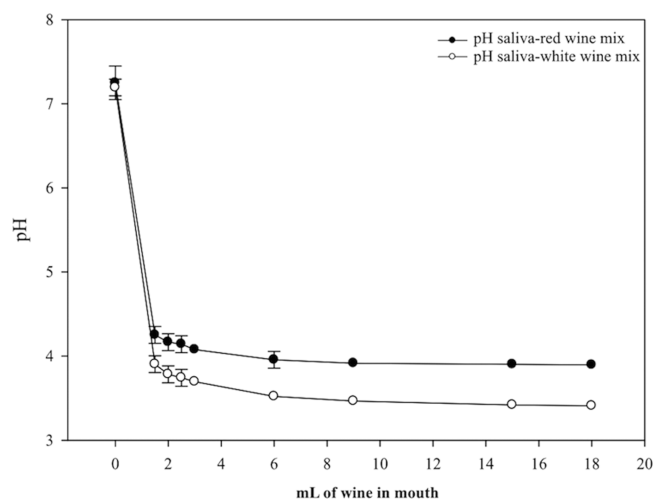


Figure 3. pH curve of wine/saliva mixtures after addition of wine to mouth saliva. Volumes of wine in the range of 1.5 and 18 mL (abscissa) were thoroughly mixed with saliva in the mouths of volunteers ($n = 3$) and returned to a glass vessel for pH determination. Curves represent means of triplicates from three independent experiments performed with saliva of different subjects. The pH is depicted as the mean \pm standard deviation.

Table 3. pH of Wine/Saliva Mixtures Produced by Placing Different Volumes of Wine into the Mouth^a

wine (mL)	pH RW–saliva mix	pH WW–saliva mix
0	7.2 \pm 0.2 e	7.2 \pm 0.1 e
1.5	4.3 \pm 0.1 d	3.9 \pm 0.1 d
2	4.2 \pm 0.1 cd	3.8 \pm 0.1 d
2.5	4.1 \pm 0.1 bcd	3.7 \pm 0.1 cd
3	4.1 \pm 0.0 abcd	3.7 \pm 0.0 bcd
6	4.0 \pm 0.1 abc	3.5 \pm 0.0 abc
9	3.9 \pm 0.0 ab	3.5 \pm 0.0 ab
15	3.9 \pm 0.0 ab	3.4 \pm 0.0 a
18	3.9 \pm 0.0 a	3.4 \pm 0.0 a
wine only	3.9 \pm 0.0 a	3.4 \pm 0.0 a

^apH values represent means \pm standard deviation of triplicates and three subjects. Values with different letters in a column are significantly different (Tukey's test, $p < 0.05$).

all hard and soft tissues in mouth and serves as the first interphase between the organism and the oral environment, including foods.^{16,17} In addition to its multiple functions in digestion, the salivary film maintains the oral surface, protects it against noxious influences, and repairs tissue damage.³ Oral sensoriality is also critically dependent on a normal salivary film. For instance, astringency, a tactile sensation in the mouth mostly associated with red wine tasting, has been mechanistically associated with molecular interactions between certain food components (tannins) and some protein components of the salivary film.^{18–20} In addition, the abundant aqueous component of the salivary film serves as a transport medium for chemical and biochemical signals, evoking a variety of oral sensations associated with taste, flavor, and mouthfeel.^{21,22} Thus, the salivary film is part of a responsive system that is linked by a neural network consisting of sensory receptors, afferent nerve fibers to the central nervous system, and efferent fibers to the salivary glands.^{23,24} Such suprastructure regulates salivary secretion activity in response to a variety of signals, from the inside and the outside of the organism.^{21,22,24} Citric

acid (1–8%) is the archetype of topical stimulants of salivary secretion in humans.²⁵ Such secretory response occurs within seconds after topical application of acid on the tongue and remains while the acid stimulus persists.²⁶ Once the acid stimulus disappears, the salivary flow will return to a normal rate within no more than 2–3 min.²⁶ Successive acid stimulations at time intervals longer than 3 min have proven to be independent of each other.²⁶ Due to its acidic character, among other features, it is quite likely that wine stimulates salivary secretion. Thus, the *in vivo* assay in this study was designed to measure pH in series of wine/saliva mixtures produced at 5 min intervals by swilling definite volumes (1.5–18 mL) of red wine or white wine in the mouth for a constant short time (15 s). As in the *in vitro* assay, the *in vivo* assay also showed a decrease in the pH of the wine/saliva mixtures in close connection with the volume of red or white wine placed in mouth.

The lowest volume of wine used in the *in vivo* assay (1.5 mL) was selected to ensure its complete mixing with the whole volume of saliva in the mouth.¹⁶ Such a small volume of wine was sufficient to lower the pH from about 7 in saliva to about 4 in the wine/saliva mixtures, that is, to pH levels near the ones of the corresponding red or white wine. Volumes of wine over 2 mL produced just marginal additional pH drops in the wine/saliva mixtures, so that the maximal volume of wine in the assay (18 mL) resulted in the pH of the corresponding red or white wine. Considering that wine tasting is frequently performed with volumes of wine around 10–25 mL,^{27–29} our observations clearly indicate that the pH of the wine/saliva mixture in the mouth during wine tasting is that of the wine. In that respect, however, some considerations are necessary. First, during tasting, the observed pH would be the one of the wine for up to at least 15 s after the wine is placed in the mouth. That means that over that period of time, which is derived from the experimental conditions in the *in vivo* assay, the buffering capacity of saliva is unable to prevent a pH decrease to its theoretically minimum value in the wine/saliva mixture. Second, after that time (15 s) and before 2–3 min, the corresponding increase in H⁺ concentration becomes neutralized jointly by the HCO₃⁻ ion provided by some domains of the secretory salivary epithelia (with formation of carbonic acid), by the carbonic anhydrase-catalyzed conversion of carbonic acid to carbonic anhydride and water (with diffusion of CO₂ out of saliva) and, concomitantly, by the acid-stimulated increase in salivary flow rate.³⁰ Such a tripartite system would play a major role in the response of the organism against the abrupt acid challenge from wine. According to data in this study, such a system seems to act since the wine comes into contact with the mouth. In effect, as shown in this study, similar pH drops were produced by mixing equivalent volumes of wine either with 3 mL of saliva in the *in vitro* assay or with the saliva present in the mouth (estimated to be 1 mL)¹⁶ in the *in vivo* assay. The red wine and the white wine behaved likewise. Quite likely, pH neutralization was already in progress during the 15 s mixing time of wine and saliva in the mouth.

As commented just above, during wine tasting, full recovery of the normal salivary pH (around 7) after exposure to the wine would take 2–3 min.²⁶ During all that period of time, a number of excitatory responses associated with wine sensoriality are dynamically triggered through a variety of oral sensory and tactile receptors to the nervous central system.^{23,24} At least some of those wine-evoked sensations and perceptions can be primarily influenced by pH-dependent interactions between a

diversity of wine components and specialized structures in the mouth, including highly selective sensory receptors and the salivary film.^{31–34} According to our findings, proper consideration of those various interactions should necessarily include pH values as low as that of the wine being tasted. Moreover, even the finest adjustments to pH that a winemaker may decide to do in a red or white wine will remain unaltered at least for a short while when the beverage is being tasted in the mouth.

AUTHOR INFORMATION

Corresponding Author

*(R.L.-S.) Phone: + (56 2) 29786477. Fax: + (56 2) 29785796. E-mail: rlopez@med.uchile.cl.

Funding

This study was supported by Grant Fondecyt-Chile 1150240 (E.O.-S.).

Notes

The authors declare no competing financial interest.

REFERENCES

- (1) Mato, I.; Suárez-Luque, S.; Huidobro, J. A review of the analytical methods to determine organic acids in grape juices and wines. *Food Res. Int.* **2005**, *38*, 1175–1188.
- (2) Flanzy, C. *Enología: Fundamentos Científicos y Tecnológicos*; Ediciones Mundi-Prensa: Madrid, Spain, 2000; 783 pp.
- (3) Humphrey, S. P.; Williamson, R. A review of saliva: normal composition, flow, and function. *J. Prosthet. Dent.* **2001**, *85*, 162–169.
- (4) Van Nieuw Amerongen, A.; Bolscher, J. G. M.; Veerman, E. C. I. Salivary proteins: prospective and diagnostic value in cariology? *Caries Res.* **2004**, *38*, 247–253.
- (5) De Smet, K.; Contreras, R. Human antimicrobial peptides: defensins, cathelicidins and histatins. *Biotechnol. Lett.* **2005**, *27*, 1337–1347.
- (6) Schipper, R.; Silletti, E.; Vingerhoeds, M. Saliva as research material: biochemical, physicochemical and practical aspects. *Arch. Oral Biol.* **2007**, *52*, 1114–1135.
- (7) Moss, M. E.; Zero, D. T. An overview of caries risk assessment, and its potential utility. *J. Dent. Educ.* **1995**, *59* (10), 932–940.
- (8) Mandel, I. D. *J. Dent. Res.* **1987**, *66*, 623–627.
- (9) Lenander-Lumikari, M.; Loimaranta, V. Saliva and dental caries. *Adv. Dent. Res.* **2000**, *14*, 40–47.
- (10) Morales-Bozo, I.; Urzúa-Orellana, B.; Domínguez, P.; Aguilera, S.; López-Solís, R. Patterns and variability in electrophoretic polypeptide profiles of human saliva in a healthy population. *J. Physiol. Biochem.* **2006**, *62*, 179–188.
- (11) Nordbö, H.; Darwish, S.; Bhatnagar, R. Rate of viscosity changes in five salivary protein fractions following pH alterations. *Scand. J. Dent. Res.* **1984**, *4*, 302–305.
- (12) García-Barceló, J. *Técnicas Analíticas para Vinos*; Ediciones FAB: Barcelona, Spain, 1990; 1713 pp.
- (13) Mercurio, M.; Damberg, R.; Herderich, M.; Smith, P. High throughput analysis of red wine and grape phenolics: adaptation and validation of methyl cellulose precipitable tannin assay and modified somers color assay to a rapid 96 well plate format. *J. Agric. Food Chem.* **2007**, *55*, 4651–4657.
- (14) Glories, Y. *Recherches sur la Matière Colorantes des Vins Rouges*. Thèse doctorat d'état, Université de Bordeaux II, France, 1978; 364 pp.
- (15) Hong, Y.; Wei, Z.; Xin-Qian, J.; Bing-Jun, Y.; Dao-Wei, Z. Analysis of organic acids accumulated in *Kochia scoparia* shoots and roots by reserve-phase high performance liquid chromatography under salt and alkali stress. *Chem. Res. Chin. Univ.* **2006**, *22*, 315–318.
- (16) Müller, K.; Figueroa, C.; Martínez, C.; Medel, M.; Obrequeslier, E.; Peña-Neira, A.; Morales-Bozo, I.; Toledo, H.; López-Solís, R. Measurement of saliva volume in the mouth of members of a trained

sensory panel using a beetroot (*Beta vulgaris*) extract. *Food Qual. Pref.* **2010**, *21*, 569–574.

(17) Watanabe, S.; Dawes, C. Salivary flow rates and salivary film thickness in five-year-old children. *J. Dent. Res.* **1990**, *69*, 1150–1153.

(18) Obrique-Slier, E.; Peña-Neira, A.; López-Solís, R.; Zamora-Marín, F. Tannin-protein interaction is more closely associated to astringency than tannin-protein complex precipitation: experience with two oenological tannins and gelatin. *Int. J. Food Sci. Technol.* **2010**, *45*, 2629–2636.

(19) Obrique-Slier, E.; Peña-Neira, A.; López-Solís, R. O. Interactions of enological tannins with the protein fraction of saliva and astringency perception are affected by pH. *LWT—Food Sci. Technol.* **2012**, *45*, 88–93.

(20) Lee, C. A.; Ismail, B.; Vickers, Z. M. The role of salivary proteins in the mechanism of astringency. *J. Food Sci.* **2012**, *77*, 381–387.

(21) Matsuo, R. Role of saliva in the maintenance of taste sensitivity. *Crit. Rev. Oral Biol. Med.* **2000**, *11*, 216–229.

(22) Spielman, A. I. Interaction of saliva and taste. *J. Dent. Res.* **1990**, *69*, 838–843.

(23) Bradley, R. M.; Kim, M. Reflex connections. In *The Role of the Nucleus of the Solitary Tract in Gustatory Processing*; Bradley, R. M., Ed.; CRC Press/Taylor & Francis: Boca Raton, FL, USA, 2007; 162 pp.

(24) Bradley, R. M.; Fukami, H.; Suwabe, T. Neurobiology of the gustatory–salivary reflex. *Chem. Senses* **2005**, *30*, 70–71.

(25) Femiano, F.; Rullo, R.; Di Spirito, F.; Lanza, A.; Festa, V. M.; Cirillo, N. A comparison of salivary substitutes versus a natural sialogogue (citric acid) in patients complaining of dry mouth as an adverse drug reaction: a clinical, randomized controlled study. *Oral Surg. Oral Med. Oral Pathol. Oral Radiol. Endod.* **2011**, *112*, 15–20.

(26) Durán, V.; Domínguez, P.; Morales, I.; López, R. O. Kinetic assessment of salivary secretory response to citric acid. Differences with pilocarpine. *Rev. Med. Chile* **1998**, *126*, 1330–1337.

(27) Schöbel, N.; Radtke, D.; Kyereme, J.; Wollmann, N.; Cichy, A.; Obst, K.; Kallweit, K.; Kletke, O.; Minovi, A.; Dazert, S.; Wetzel, C. H.; Vogt-Eisele, A.; Gisselmann, G.; Ley, J. P.; Bartoshuk, L. M.; Spehr, J.; Hofmann, T.; Hatt, H. Astringency is a trigeminal sensation that involves the activation of G protein-coupled signaling by phenolic compounds. *Chem. Senses* **2014**, *39*, 471–487.

(28) Nolden, A. A.; Hayes, J. E. Perceptual qualities of ethanol depend on concentration, and variation in these percepts associates with drinking frequency. *Chemosens. Percept.* **2015**, *8*, 149–157.

(29) Hopfer, H.; Nelson, J.; Ebeler, S. E.; Heymann, H. Correlating wine quality indicators to chemical and sensory measurements. *Molecules* **2015**, *20*, 8453–8483.

(30) Fejerskov, O.; Kidd, E. The role of saliva. In *Dental Caries. The Disease and Its Clinical Management*, 2nd ed.; Bardow, A., Lagerlof, F., Nauntofte, B., Tenovou, J., Eds.; Blackwell, Munksgaard Ltd.: Oxford, UK, 2008; 640 pp.

(31) Zhang, Y.; Hoon, M. A.; Chandrashekar, J.; Mueller, K. L.; Cook, B.; Wu, D.; Zuker, C. S.; Ryba, N. J. Coding of sweet, bitter, and umami tastes: different receptor cells sharing similar signaling pathways. *Cell* **2003**, *112*, 293–301.

(32) Hoon, M. A.; Adler, E.; Lindemeier, J.; Battey, J. F.; Ryba, N. J.; Zuker, C. S. Putative mammalian taste receptors: a class of taste-specific GPCRs with distinct topographic selectivity. *Cell* **1999**, *96*, 541–551.

(33) Sakurai, T.; Misaka, T.; Ueno, Y.; Ishiguro, M.; Matsuo, S.; Ishimaru, Y.; Asakura, T.; Abe, K. The human bitter taste receptor, hTAS2R16, discriminates slight differences in the configuration of disaccharides. *Biochem. Biophys. Res. Commun.* **2010**, *402*, 595–601.

(34) Ash, A.; Wilde, P. J.; Bradshaw, D. J.; King, S. P.; Pratten, J. R. Structural modifications of the salivary conditioning film upon exposure to sodium bicarbonate: implications for oral lubrication and mouthfeel. *Soft Matter* **2016**, *12*, 2794–2801.