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Astringency sub-qualities *drying* and *pucker* are driven by tannin and pH – Insights from sensory and tribology of a model wine system

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ABSTRACT

Astringency mouthfeel is an important indicator of wine sensory quality that has been associated with colloidal interactions between tannins and salivary proteins, and a depletion in the lubricating salivary film. However, astringency is a complex sensation that has several contributing sub-qualities that may each have different physicochemical origins. We find that the model wine sample set varying in tannin, pH and polysaccharide content exhibit variations in the main sub-qualities: *drying*, *rough* and *pucker*. A range of soft-tribological methods involving saliva are used to gain mechanistic insight into these sub-qualities. Results suggest that *rough* is a secondary sub-quality that can be elicited by either *drying* or *pucker*, while these two sensations are driven by high tannin and low pH, respectively. Samples with 'chemically-equalised' astringency, which have similar colloidal stability upon interaction with saliva (saliva precipitation index), are also found to have varying sub-qualities and tribological responses. The boundary friction of saliva-wine mixtures in Stribeck curve aligned with *drying*, and the rate of increase in boundary friction of a salivary pellicle upon contacting wine aligned with *pucker*. *Rough* is not found to scale with any physical measure we explored. Quartz crystal microbalance indicated that the tannin, rather than pH, interacts with the salivary protein film to cause an increase in surfaces adsorbed mass in the absence of significant shear. The results indicate that while tannin and acid both contribute to the perception of astringency, the mechanisms by which they do this have different origins that lead to differences in sub-qualities.

1. Introduction

Astringency plays a significant role in the sensory experience of a wide range of foods and beverages, including wine, tea, soymilk, coffee, fruits, nuts and legumes (Bajec & Pickering, 2008). As an important indicator of wine sensory quality, a balanced level of astringency is the requisite of high-quality red wines; while in excess, it detracts from other sensory precepts; with too little, the wine can be described as flat, insipid and uninteresting (Bajec et al., 2008; Gawel, 1998). Astringency in food and beverages is typically associated with plant-based polyphenols, which undergo colloidal interactions with salivary proteins during oral consumption (Jöbstl, O'connell, Fairclough, & Williamson, 2004). While the formation of colloidal polyphenol-protein aggregates and loss of a lubricating salivary film have been considered a precursor to the sensation of astringency, it can still arise without such interactions occurring that suggests astringency is a much more complex sensation that is not dependent on a single physical or chemical mechanism (Rossetti, Bongaerts, Wantling, Stokes, & Williamson, 2009).

Sensorily, astringency is often described as 'harshness' and 'pungency', frequently accompanied by sensations of bitterness and/or sourness (Green, 1993). It is considered not as one simple sensory experience, but at least three distinct cues: drying of the oral cavity, sensations of increased roughness of the oral tissues, and a puckering or drawing sensation felt in the buccal musculature (Lawless & Corrigan, 1994). More recent studies on wine have expanded these sub-qualities into dozens of complex terms presented in mouthfeel wheels (Bajec et al., 2008; Gawel, Oberholster, & Francis, 2000). Over the past few decades, attention has been focussed on the mechanisms of astringency

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from the perspective of chemical assays (precipitation-based or chromatography-based), physical measures (i.e. surface techniques) and even cell/neural sciences (Ma et al., 2014; Soares, Brandão, Mateus, & De Freitas, 2017). A limitation of these studies was that they failed to pull apart the varying percepts of astringency and used the term generically. The loss of saliva lubricity as a model for astringency became popular after Bate-Smith (1954) first classified astringency as a chemically induced tactile sensation, and later reverified by Breslin, Gilmore, Beauchamp, and Green (1993). More recent studies have revealed that astringency could have multi-modal mechanisms (Carpenter, 2013; Gibbins & Carpenter, 2013; Payne, Bowyer, Herderich, & Bastian, 2009; Schöbel et al., 2014; Soares et al., 2016). These followed the finding that the loss of saliva lubricity is not necessary to obtain an astringent perception, and that astringency is unlikely to be a purely tactile percept (Rossetti et al., 2009).

There are numerous individual compounds known to elicit astringent sensations. However, these components are usually just one of many compounds in a complexly composed food system. Tannin is a focus of much research related to astringency and is important to wine among other food products. It interacts with salivary proteins through hydrophobic and hydrogen bonds, which subsequently results in protein aggregation and precipitation (Gawel, 1998; Jöbstl et al., 2004). For wine, the tannin quantity, quality and the wine matrix itself (i.e. ethanol, acidity and polysaccharide) exert significant impact on perceived astringency, especially the type of sub-quality which becomes dominant (Demiglio, Pickering, & Reynolds, 2002; Fontoin, Saucier, Teissedre, & Glories, 2008; Frost, Harbertson, & Heymann, 2017; Gawel, Smith, & Waters, 2016; Vidal et al., 2004). Currently, these astringency sub-qualities can only be assessed by sensory methods. The mechanism of their perceptive and the componential drivers remain unclear. Further, there is currently no instrumental approach available to directly measure these astringency sub-qualities.

Emerging tribology tools show promise in helping understand food oral processing (Stokes, Boehm, & Baier, 2013) and their impact on sensory perception (Krop, Hetherington, Holmes, Miquel, & Sarkar, 2019; Stokes, 2012a, 2012b). These methods can also mimic aspects of in-mouth lubrication by applying saliva in the system that is suggested to allow mouthfeel qualities, such as astringency, to be studied by simply monitoring the friction change. Studies on tea, milk and wine reveal some potential for scaling astringency by using these tribology measurements (Brossard, Cai, Osorio, Bordeu, & Chen, 2016; De Wijk & Prinz, 2005; De Wijk & Prinz, 2006; Joyner, Pernell, & Daubert, 2014; Laguna et al., 2017; Rossetti et al., 2009; Watrelot, Kuhl, & Waterhouse, 2018). However, as noted in a review by Pradal and Stokes (2016), "it is difficult to explain sensory effects with tribology alone" due to the complexity in the colloidal properties of foods and beverages as well as sensory perception itself. As mentioned above, most studies use a single definition of astringency and do not pull it apart into different sub-qualities. This is required because 'overall' sensation of astringency does not universally scale with any physical-measurement technique, including tribology. We hypothesise that by breaking it into its sub-qualities, measurable indicators for these sub-qualities may be found. Further, there are limited examples where tribology protocols have been implemented in wine-related studies involving systematic variations in individual components that drive the sub-qualities. Only a single tribological protocol was used in each study, including either the astringents' interaction with bulk level of saliva (Brossard et al., 2016; Laguna et al., 2017; Watrelot et al., 2018) or a thin layer of salivary pellicle (Rossetti et al., 2009). Whether using different protocols is able to help discriminate different astringency sub-qualities remain known.

The present work aims to develop appropriate tribology measuring techniques, which include those that consider saliva-wine interactions, that allows the drivers for different astringency sub-qualities to be determined. This will be achieved by controlling the levels of tannin, acidity and polysaccharide in a model wine (MW) system, to explore how different tribology measurements may relate to sensorily perceived differences. Outcomes from this research should provide new knowledge about the mechanisms of different astringency sub-quality perception and what role saliva may play. This research, and the methods developed, has the potential to assist winemakers in managing the wine matrix to attain preferable mouthfeel and astringency characteristics to meet market requirements.

2. Material and methods

2.1. Health and safety

Considering that the saliva is bodily fluid with the potential risk of infection, staff who were involved with collection were compulsorily vaccinated for Hepatitis B. Work surfaces and equipment involved in saliva collection were protected by disposable plastic films, lab tissue and subsequently disinfected with 75% ethanol. All associated consumables were disposed of appropriately. Saliva was collected at a standardised collection location and transferred into the lab in a sealed Ziplock bag.

This study was reviewed and granted prior approval by the Low Risk Ethics Sub-Committee at The University of Queensland (Ethics approval number: 2018000888).

2.2. Reagents

Food grade ethanol absolute (EMPROVE®, Merck, Bayswater, VIC, Australia), grape seed tannin extract (GSE; 65% w/w total polyphenols in gallic acid equivalent, 34% w/w tannin in epicatechin equivalent, Table S1; Tarac Technologies, Nuriootpa, SA, Australia), maltodextrin (dextrose equivalent = 19, Manildra Group, Bomaderry, NSW, Australia), and tartaric acid (McKenzie's, Altona, VIC, Australia) were used for the preparation of MW solutions.

All the reagents for sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) were purchased from Bio-Rad (Gladesville, NSW, Australia). Organic solvents for tribology/rheology/quartz crystal microbalance with dissipation monitoring (QCM-D) were purchased from Merck (Castle Hill, NSW, Australia). Sandpaper for sensory touching standards was purchased from Bunnings Warehouse (Flexovit P600, 3M P320, Flexovit P180, Harris Taskmasters 80 grit, Brisbane, QLD, Australia).

2.3. Saliva collection

Whole mouth saliva following mechanical or acid stimulation was collected according to (Bongaerts, Rossetti, & Stokes, 2007b); Knaś et al. (2016). Mechanically stimulated saliva was collected only for saliva precipitation index (SPI) determination (Rinaldi, Gambuti, & Moio, 2012a). SPI is based on measuring the binding of salivary proteins to polyphenols using an SDS-PAGE method, and it showed a strong correlation to astringency in red wines. Six non-smoker participants (3 males and 3 females) were instructed to refrain from eating or drinking for 2 h before saliva collection in the morning at 8–10 a.m. Prior to collection, participants were asked to rinse their mouth with potable water for 10 s and subsequently collect saliva by chewing a silicone tube (30 mm for length and 6.4 mm for diameter). The participant placed the tube in their mouth with 5 ml of water, chewed for 15 s and expectorated. This step was repeated to ensure that no residue water was retained in the saliva collected that could have a dilution effect. The tube was chewed for a further 30 s and saliva produced was expectorated as waste. This helped participants get familiar with the collecting procedure in order to avoid potential swallowing. Finally, participants were asked to continue chewing for another 2 min while collecting saliva in a 50 mL centrifuge tube served in ice. Tubes containing saliva were immediately centrifuged at 5000 rpm, 4 °C for 10 min. Then the supernatant was pooled across the donors and stored (−80 °C) before analysis.

For use in instrumental applications, acid-stimulated saliva was collected over 2 and a half minutes from a single donor to minimise the compositional variation among individuals (Bajec et al., 2008; Dinnella, Recchia, Fia, Bertuccioli, & Monteleone, 2009; Fleming, 2015). The acid-stimulated saliva was used in consideration of the acidic nature of wine matrix that boosts the production of acid-stimulated saliva, which better mimics the real in-mouth experience during wine tasting. The acid-stimulated saliva also shows enhanced elasticity and viscosity (Stokes et al., 2007), which is rich in surface-adsorbing proteins and can facilitate the formation of lubricious pellicle. Saliva was collected at a collecting station close to the tribology/rheology/QCM-D facilities to minimise the storage time (Stokes & Davies, 2007). After rinsing the mouth, an aqueous citric acid (2%) solution (100 μL) was dripped at the posterior part of a tongue to stimulate saliva. Saliva secreted in the first 30 s was discarded to get rid of the acid which could have an influence in the physicochemical property and lubrication behaviour of saliva collected. Then the collection continued for 2 min into a tube served on ice. Fresh acid-stimulated saliva was collected prior to each test, and the maximum storage time did not exceed 10 min.

2.4. Model wine samples preparation

A total of 6 MW samples were prepared as demonstrated in Fig. 1a. The matrix of the base MW was a hydroalcoholic solution (10% v/v ethanol) supplemented with tartaric acid (5 g/L). MW1, MW3 and MW5 contained 2 g/L tannin ('medium' tannin), while MW2 and MW6 contained 3 g/L tannin ('high' tannin). The final pH of MW1, MW4 and MW5 were adjusted to 3.0, and MW2, MW3 and MW6 to 3.6 with potassium hydroxide. The saliva precipitation index (SPI, to be detailed in the following section) was used to match MW4, MW5 and MW6 to the same SPI as MW3 by either lowering tannin level (MW4) or adding polysaccharide (MW5 and MW6). Maltodextrin was selected as a model polysaccharide for its good solubility in hydroalcoholic solution, commercial availability and simple chemical composition.

2.5. Chemical assays

Titratable acidity, SPI, and ethanol index of model wines were measured based on the methods proposed by the International Organisation of Vine and Wine OIV (2015), Rinaldi et al. (2012a) and Glories (1984), respectively.

The method of Rinaldi et al. (2012a) was used to determine SPI using the following stages: saliva-MW binding, SDS-PAGE electrophoresis, densitometric tracking and SPI calculation. For binding, pre-collected mechanically stimulated saliva (200 μL) and MW (100 μL) were mixed in a 1.5 mL centrifuge tube and warmed in a water-bath (37 °C for 5 min). The resulting mixture was centrifuged (10,000 g for 10 min) and the supernatant was used for analysis. The SDS-PAGE was performed on a Bio-Rad Criterion™ vertical electrophoresis cell coupled with a PowerPac power supply. A constant voltage (200 V/gel) was set for the Criterion™ Stain Free gel. Before starting the mixture was denatured by diluting in 2x Bio-Rad sample buffer and heating (95 °C for 5 min). A 4–20% 18 well precast stain-free gel (Bio-Rad) was used because of its good separation capacity and immediate imaging.

Band molecular weight was calculated from the linear regression equation of log molecular weight against mobility, compared with the migration rates of Precision Plus Protein™ standards (Bio-Rad, Hercules, California, United States). The mixtures and standards (10 μL) were loaded and each binding assay was performed in quadruplicate (at least). Band densitometric tracking was performed in a Criterion Stain Free™ Imaging System.

Bands of selected molecular weight (54–59 KDa and 15 KDa) were calculated for density reduction after binding. GSE aqueous solutions (0.1, 1.0, 2.5, 3.5, 5.0, 7.5 g/L) were prepared as standards to bind saliva (Fig. S1). The reduction percentage in salivary bands after binding with samples were expressed as g/L GSE.

The ethanol index estimates the percentage of polyphenols combined with polysaccharides. For ethanol index, wine (100 μL) was added to ethanol (900 μL) and allowed to stand for 24 h. After centrifugation, the supernatant was diluted to 1:5 ratio and absorbance was measured at 280 nm (A′280). Absorbance at 280 nm for the diluted original wine (1:50) was also measured (A280). The ethanol index was expressed as:

$$\text{Ethanol index} = \frac{A280 - A'280}{A280} \times 100\% \quad (1)$$

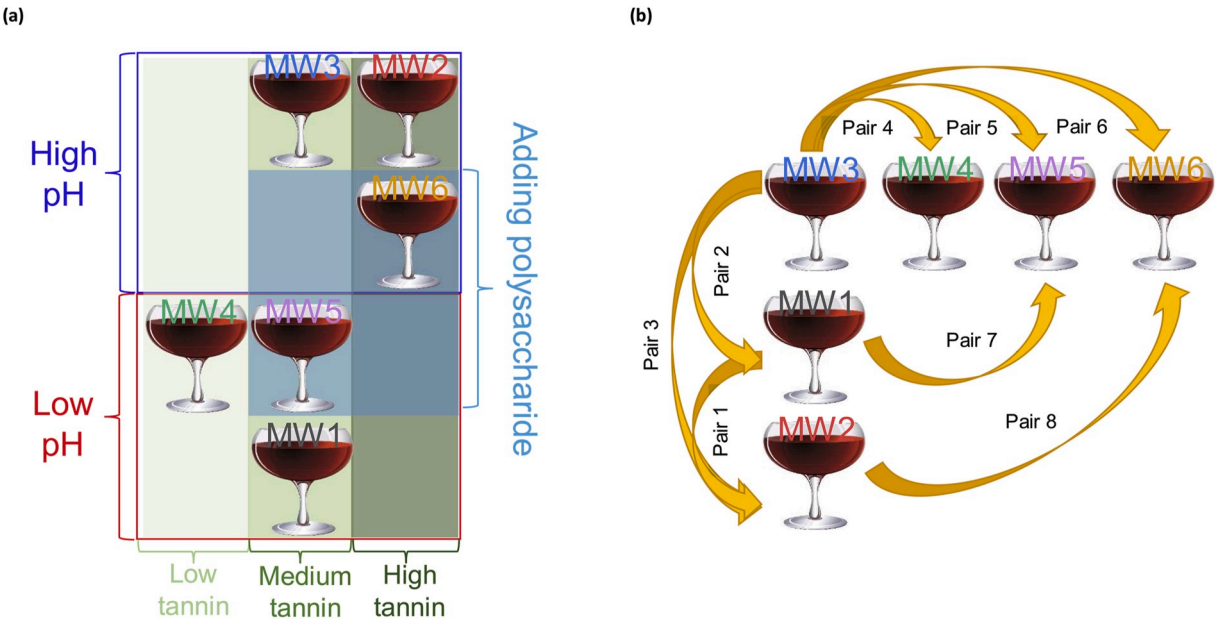


Fig. 1. Demonstrations for MW matrix design (a) and paired comparison (b). Pair 1 compared MWs with high-acidity-low-tannin and with low-acidity-high-tannin; pair 2 compared the acidity's effect at a medium tannin level; pair 3 compared tannin's effect at a low acidity level; pair 4, pair 5 and pair 6 correspond to when the MW's potential for precipitating salivary protein were the same (same SPI); pair 7 and pair 8 compare directly the effects of adding polysaccharide to a high-acidity matrix at the same tannin level, or to a high-tannin matrix at the same acidity level.

2.6. Sensory panel and testing location

The sensory panel comprised 15 assessors, 11 women and 4 men, aged between 19 and 56 with a mean age of 40. The panel were selected from a pool of Brisbane-based experienced sensory panellists, who had been pre-screened for sensory acuity (Meilgaard, Civille, & Carr, 2016). Panellists were paid AUD$60 per session (2 h) and gave informed consent for their participation in the study.

Assessments took place in the purpose-built sensory laboratory of the Health and Food Sciences Precinct, Coopers Plains, Brisbane, QLD, Australia. The laboratory is equipped with a separate sample preparation kitchen and 12 individual tasting booths which are temperature (21 °C) and light controlled (red fluorescent lamp lighting was used). An adjoining 'round table' discussion room suits panel training.

2.7. Sensory analysis

A total of 8 (2 h) sessions were conducted, including 2 training sessions prior to formal assessments. During training, panellists were provided with some information about astringency sensation and various sensory reference samples for demonstration and discussion. Reference samples included aqueous solutions of: alum (5 g/L) for 'overall astringency'; caffeine (0.8 g/L) for 'bitterness'; and, tartaric acid (1.5 g/L) for 'sourness'. Subsequently, the panel were presented the MW samples for vocabulary development. The red wine mouthfeel wheel, together with conceptual definitions of typical astringency terms were provided to aid lexicon development (Gawel et al., 2000; Lawless et al., 1994). Terms including *drying*, *rough* and *pucker, grippy, harsh, sappy, sour and viscous* were tentatively selected at the end of the first training session. Following a discussion, *sour* and *viscous* were not adopted because they did not directly scale with astringency. Terms *grippy* and *harsh* were excluded as these correlated with a 'very strong astringent sensation', which emphasised the intensity only, and the term *sappy* was further excluded as it emphasised a flavour quality related to acidity and bitterness rather than mouthfeel sensation. By consensus, only *drying*, *rough* and *pucker* were selected. Their definitions and sensory reference standards were developed for use in formal evaluation (shown in Table 1).

During formal assessments the standards and definitions were presented and reviewed by the panel prior to assessment of samples. Sessions were divided into three blocks corresponding to the three attributes (*drying, rough*, or *pucker*). Within a block, eight pairs of samples were successively presented, and panellists were asked to and identify which sample in the pair was higher for a given attribute. The sample pairs presented are shown in Fig. 1b. Only one attribute was assessed per block and between blocks, panellists were allowed a 15-min rest and served a small piece of cheese (15 g) and filtered water to assist palate cleansing. The order of blocks (attributes) were randomly allocated across the session, for each panellist. The order of samples within a pair and the order of pairs within a block were also balanced for each panellist.

The assessing protocol started with a mouth rinsing with spring water to remove food debris and maintain a 'neutral' mouth state. Then the entire sample was put in mouth and moved for 4–5 s, followed by expectorating and re-rinsing mouth for 10 s before proceeding to the next sample. A 1–2 min rest was taken for between each pair. Paper questionnaires were used to collect responses. Reference standards and MW samples (10 mL) were served in 50 mL plastic cups covered with a lid and coded according to the experimental design. In total, 4 replicates of each sample pair were assessed over the course of the experiment.

2.8. Tribology measurements

All the tribological measurements were performed at 37 °C (lube temperature that simulated oral temperature) on a Mini Traction Machine (MTM2, PCS Instruments Ltd., UK). The tribo-pair consisted of a PDMS ball (9.5 mm) and a PDMS disc (23 mm in radius, 4 mm in thickness). The root-mean-square (rms) roughness was measured as 26 nm and 9 nm respectively by an atom force microscope. More details of tribo-pair preparation can be found in a previous publication (Bongaerts, Fourtouni, & Stokes, 2007a). The friction coefficient μ was calculated as

$$\mu = \frac{F}{L} \tag{2}$$

where F is the lateral friction force exerting on the ball and L is normal load setting 1 N (low-load condition) for all the measurements. The slide-to-roll ratio (SRR) defined as

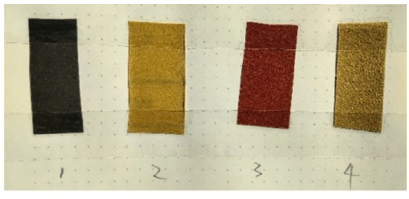
$$\mathrm{SRR} = \frac{V_{ball} - V_{disc}}{U} \tag{3}$$

was set to 50%. The entrainment speed was defined as

$$U = \frac{V_{ball} + V_{disc}}{2} \tag{4}$$

V_{ball} and V_{disc} represent the surface velocities of the ball and disc, respectively. A primary tribo-pair selection was performed and only those with a friction coefficient between 1.6 and 1.8 ($U = 5$ mm/s, $L = 1$ N) in dry condition were adopted. This helped ensure the good data reproducibility among replicates. For tribometer setups, the PDMS tribo-pair was first installed and the ball was lowered onto the disc. The pot was covered with a lid during each run to minimise alcohol evaporation. Three different protocols were developed to mimic the in-mouth lubrication model. They were hereby 1) dynamic tribology protocol with bulk saliva (DTP-BS), 2) Stribeck curve protocol and 3) dynamic

Table 1
Astringency sub-qualities: definitions and standards used for sensory assessments.

Term	Conceptual definition	Strength	Standard
drying	Lack of lubrication or moistness resulting in increased friction between oral surfaces (Lawless & Corrigan, 1994)	Low	1 g/L GSE aqueous solution
		Medium	2 g/L GSE aqueous solution
		High	3 g/L GSE aqueous solution
rough	The physical bumpiness of the tissues, not unlike coarse sandpaper (Fleming, 2015)	Sand papers of various coarse grades	 600 grit 320 grit 180 grit 80 grit
pucker			
	The tightening or drawing sensation that can be felt in the cheeks and muscles of the face (Lawless et al., 1994)	Low	0.5 g/L alum + 1 g/L tartaric acid
		Medium	1 g/L alum + 2 g/L tartaric acid
		High	2 g/L alum + 4 g/L tartaric acid

tribology protocol incorporating a salivary pellicle (DTP-SP). MWs without mixing saliva were also tested with DTP-BS and Stribeck curve as control.

2.8.1. Dynamic tribology protocol with bulk saliva

The DTP-BS mimicked the wine-tasting situating where the larger volume of free saliva is involved (Fig. 2a). The entrainment speed was set to $U = 5$ mm/s 2 mL of freshly collected acid-stimulated saliva was injected into a container followed by adding 20 mL MW sample. Then the mixture (MW + S) was homogenised in a syringe by thrusting for 3 times and all of mixture was poured into the cell. The reduction of friction coefficient was monitored continuously for 10 min. Data was recorded every second and fitted by an exponential-linear model:

$$\mu = e^{-t/p_1} + p_2 t + p_3 \tag{5}$$

to characterise the friction decrease as a function of time.

2.8.2. Stribeck curve protocol

The Stribeck curve protocol was initiated immediately after the end of DTP-BS (Fig. 3a). The friction coefficient was measured as a function of the sample entrainment speed (U) that was varied from 1 mm/s to 1000 mm/s, and then reversed from 1000 mm/s to 1 mm/s. Ten data points were recorded per decade on a logarithmic scale. 4 data points at the very beginning were discarded because of the tremendous deviation among replicates. A simplified 'master curve' model (only characterised the boundary and mixed regimes)

$$\mu = \frac{\mu_b}{1 + \left(\frac{U}{B}\right)^m} \tag{6}$$

from a previous publication was used to fit the Stribeck curves (Bongaerts et al., 2007a).

2.8.3. Dynamic tribology protocol incorporating a salivary pellicle

The salivary pellicle formation of DTP-SP (Fig. 4a) followed that of previous studies with minor modifications (Bongaerts et al., 2007b; Rossetti et al., 2009; Selway & Stokes, 2013; Yakubov, Macakova, Wilson, Windust, & Stokes, 2015). The entrainment speed was set to $U = 5$ mm/s. Initially, 300 μL of acid-stimulated saliva was evenly spilled on the surface of the ball-and-disc contact (edge area). This allowed the saliva to form an evenly adsorbed annulus. After 15 min, 20 mL of MW sample was injected into the pot without brutally pouring to the salivary annulus. The fluid completely submerged the contact between ball and disc for 20 min. Friction data were logged every 5 s. Only the data after MW flushing was plotted and fitted by a plateau model:

$$\mu = p \cdot \tanh\left(\frac{q \cdot t}{p}\right) + r \cdot t \tag{7}$$

to characterise the friction increase as a function of time.

For all measurements, each tribo-pair was used for one sample only. Each test was performed at least in quadruplicate. Friction coefficients and model-fitting parameters (Table S2) were compared among MWs and their mixtures with saliva.

2.9. Quartz crystal microbalance with dissipation monitoring

A QCM-D instrument (QSense Explorer model E4, Vastra Frolunda, Sweden) coupled with a peristaltic pump (Ismatec IPC-N 4, Cole-Parmer GmbH, Germany) was used to characterise the change in viscoelastic properties of the adsorbed salivary film after reacting with the model wines. Gold-quartz sensors (750-1072-G5 CrAu, nominal resonance frequency = 5 MHz, Inficon, Bad Ragaz, Switzerland) were spin-coated by a thin hydrophobic layer of PDMS, which were prepared following the protocols detailed elsewhere (Rodahl, Höök, Krozer, Brzezinski, & Kasemo, 1995).

The signal is recorded as the change in frequency (△F) and the change in dissipation of the oscillation energy (△D). The signal from the 3rd, 5th, 7th, 9th, 11th, 13th overtones was recorded following a protocol shown in Fig. 5a: 1) 2% SDS at a flowrate of 500 μL/min till the drift of △F was less than 2 Hz/h (or △D less than 0.2x10E-6/hour); 2) ultrapure water at a flowrate of 500 μL/min till reaching the same stability; if not attained in 1 h, step 1 repeated; 3) Initiate a new measurement by ultrapure water at a flowrate of 500 μL/min; 4) at 1 min, switch the fluid to freshly collected acid-stimulated saliva prefiltered via a cell strainer (40 μm, Biologix Group Limited, China); 5) at 5 min, switch the fluid to 70 μM NaCl; 6) at 8 min, slow the flowrate down to 50 μL/min; 7) at 10 min, switch the fluid to the MW sample; 8) at 19 min, switch the fluid back to 70 μM NaCl; 9) at 30 min, stop the measurement. The pump was stopped between switching fluids. Each MW sample was measured twice or three times.

The chamber temperature was set at 37 °C. A Voigt model was adopted to explain the viscoelastic properties of the adsorbed salivary film on the sensor. The adsorbed film is modelled as a Voigt viscoelastic element with a complex elasticity modulus G_f as:

$$G_f = \mu_f + i2\pi f \eta_f = G'_f + iG''_f \tag{8}$$

The μ_f is the film shear elasticity modulus; f is the sensing frequency, η_f is the film viscosity; G'_f is the apparent film storage modulus; G''_f is the apparent film loss modulus. The expressions for signal changes due to film adsorption in liquid media can be characterised as:

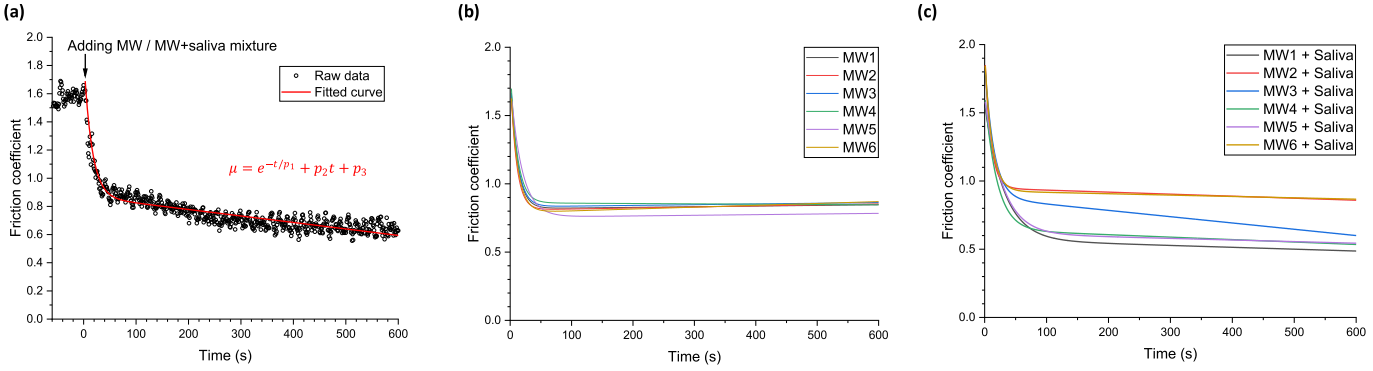


Fig. 2. Dynamic tribology protocol with bulk saliva. The raw friction data (○) was fitted by a curve (red line) for each measurement of model wine or model + saliva mixture (a). Sample was poured into the tribometer at 0s. Coloured curves were plotted the average of parameters of model wines (b) or model wine + saliva mixtures (c). Original tribology data refers to Fig. S3 and Fig. S4. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

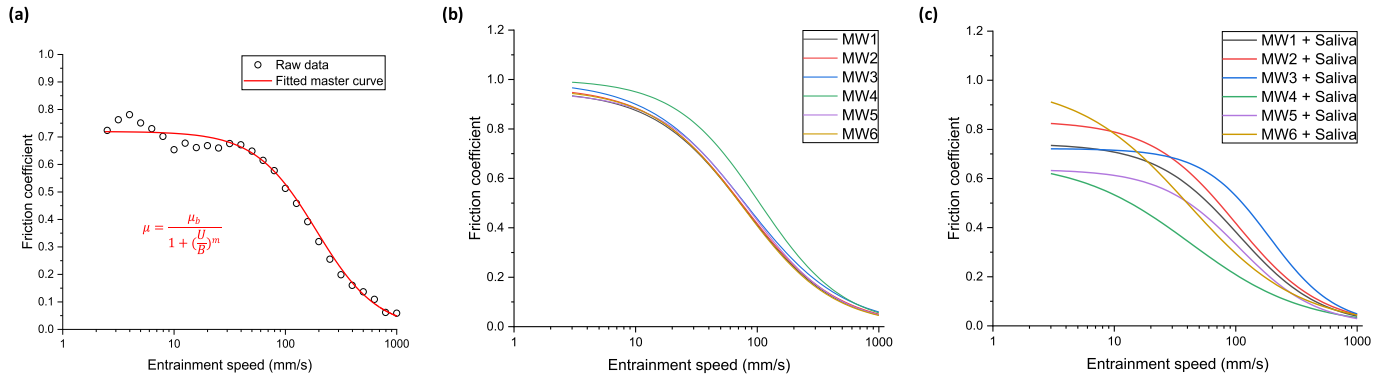


Fig. 3. Stribeck curves of model wine and model wine + saliva mixtures. The raw friction data (○) at different entrainment speeds was fitted by a curve (red line) for each measurement of model wine or model + saliva mixture (a). Coloured curves were plotted by using the average of parameters of model wines (b) or model wine + saliva mixtures (c). Original tribology data refers to Fig. S5 and Fig. S6. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

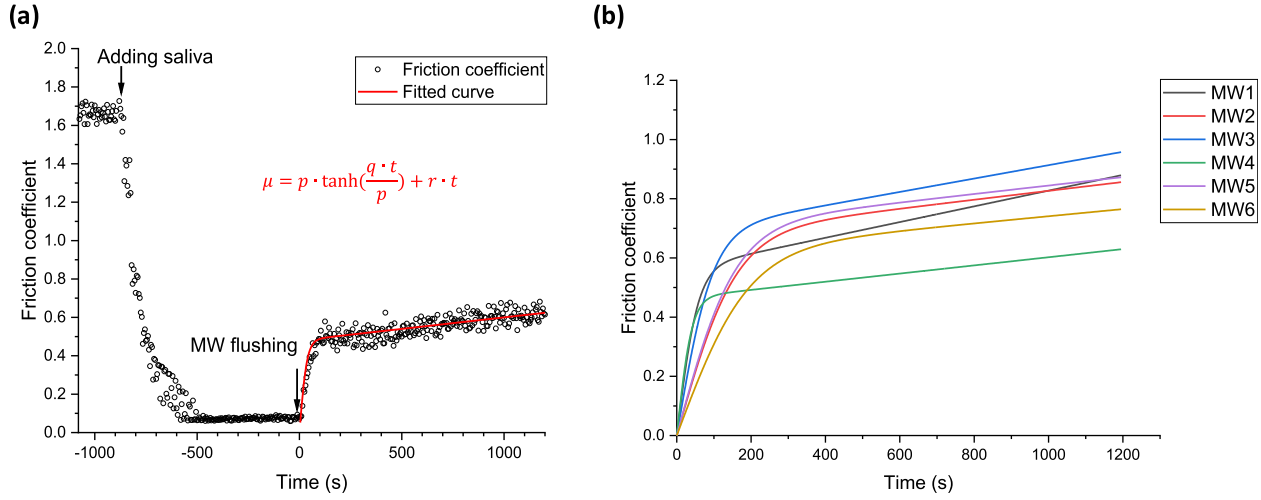


Fig. 4. Dynamic tribology protocol with salivary pellicle. The raw friction data (○) was fitted by a curve (red line) for each measurement (a). Sample was poured into the tribometer at 0s. Coloured curves were plotted by using the average of parameters (b). Original tribology data refers to Fig. S7. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

$$\frac{\Delta f}{f} = -\frac{h_f \rho_f}{h_0 \rho_0}\left\{1 - \eta_f \rho_b \times \frac{(\eta_f/\rho_f)\omega^2}{{\mu_f}^2 + \omega^2 {\eta_f}^2}\right\} \quad (9)$$

$$\Delta D = -\frac{h_f}{h_0 \rho_0}\left\{\eta_b \rho_b \times \frac{\mu_f \omega}{{\mu_f}^2 + \omega^2 {\eta_f}^2}\right\} \quad (10)$$

where f is the sensing frequency; $\omega = 2\pi f$, h_0 and ρ_0 are the thickness and density of quartz crystal; η_b and ρ_b are the bulk liquid viscosity and density and h_f, ρ_f, μ_f, η_f are the adsorbed film's thickness, density, shear elasticity modulus and viscosity.

The changes in △F and △D under different fluids were compared. The NaCl eluted salivary film was defined as stage 1 (S1); The MW eluted salivary film was defined as stage 2 (S2); The NaCl eluted salivary film after MW elution was defined as stage 3 (S3). The average values of △F and △D were calculated in 11.5–12.5 min for S1, 22.5–23.5 min for S2 and 29–30 min for S3. Then comparisons were made as S2–S1 and S3–S1 to reflect the film properties over different stages.

2.10. Statistics

Statistical significance for non-sensory data was examined in one-way analysis of variance (ANOVA) by SPSS 25 (IBM Corp, Armork, NY, USA). For sensory paired-comparison, significance level was determined by a statistic table based on the number of correct answers (Roessler, Pangborn, Sidel, & Stone, 1978).

3. Results and discussion

3.1. Achieving 'chemically-equalised' astringency across the sample set

The initial intention of the experiment was to sensorily match the overall astringency of wines with varying composition and pH. This proved to be challenging and was unsuccessful however, given that the variation in astringency sub-qualities made the sensory definition of 'equivalent astringency' problematic. As an alternative, the saliva precipitation index (SPI) method was selected as a control parameter across the MW samples as it has been shown to have a better correlation with an overall sensation of astringency compared to other chemical assays in a wine matrix (Rinaldi et al., 2012a). For convenience only, we refer to samples with the same SPI as having 'chemically-equalised' astringency. The pooled saliva from different donors used for the whole sample set ensured that protein variation from different donors was eliminated. The content of each MW in the sample set is detailed in Table 2. The levels of tannin and acidity used in this study were selected to be comparable to that found in commercial wine (Waterhouse, Sacks, & Jeffery, 2016). The adjustments of SPI were based on the concept that higher acidity alone can enhance the protein precipitation by 1) intensifying the

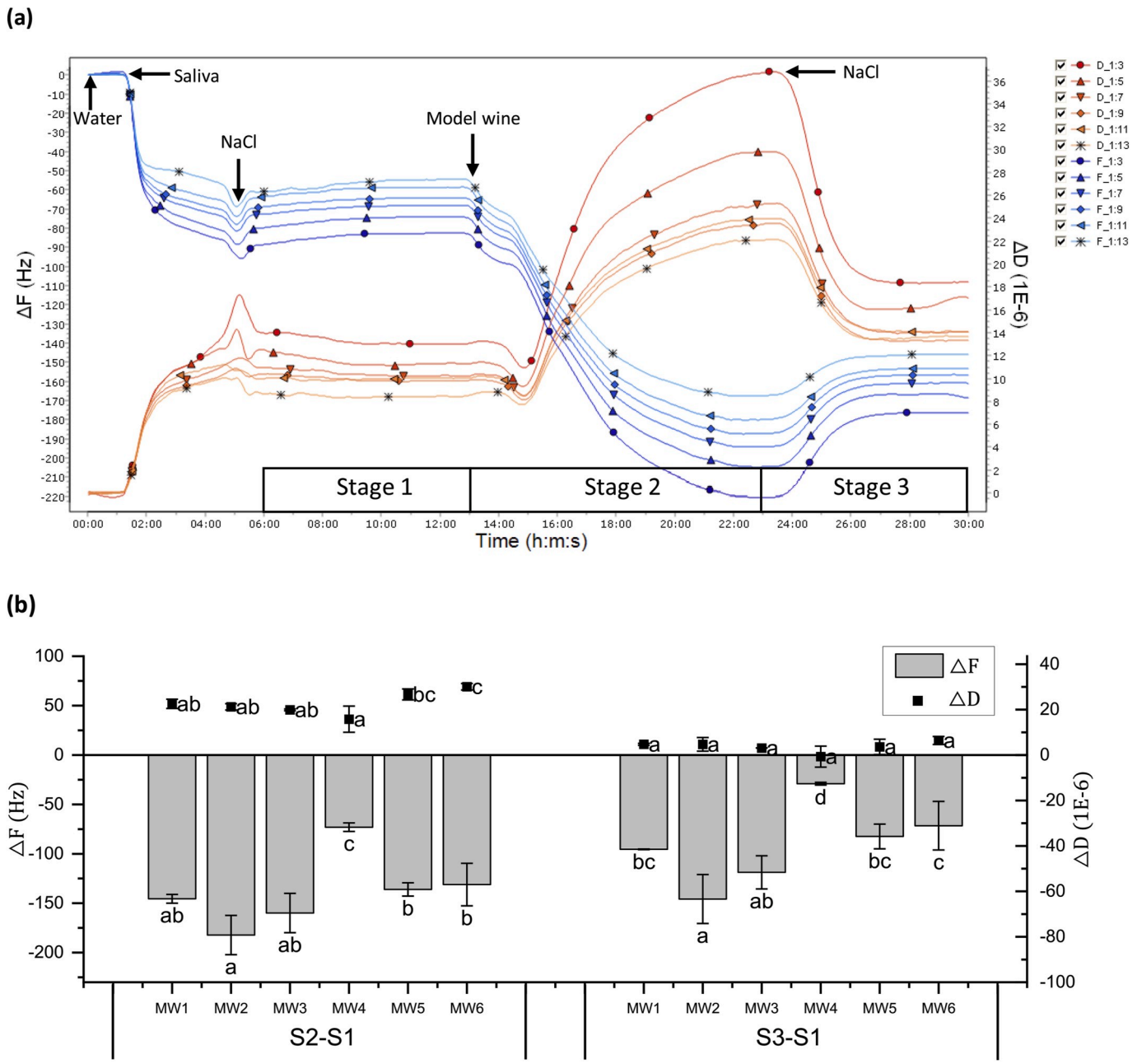


Fig. 5. Representative QCM-D curves (MW1) for salivary pellicle exposed to model wine samples (a). Changes in frequency (△F) and dissipation (△D) over different stages (salivary pellicle rinsed by NaCl, Stage 1; model wine elution, Stage 2; second rinse by NaCl, Stage 3) were compared (b). Different letters indicate significant difference over samples in Stage 2-Stage 1 (S2–S1), or Stage 3-Stage 1 (S3–S1) (Duncan one-way ANOVA at $p < 0.05$). Original QCM-D data refers to Fig. S9.

Table 2
Chemical indexes of model wines.

	Titratable acidity (g/L tartaric acid)		SPI (g/L GSE equivalent)		pH	GSE (g/L)	Maltodextrin (g/L)	Ethanol index (%)	
MW1	4.43 ± 0.05	b	6.54 ± 1.02	c	3.0	2.0	–	9.09 ± 0.88	a
MW2	3.06 ± 0.01	a	5.47 ± 0.60	b	3.6	3.0	–	16.81 ± 1.80	b
MW3	3.02 ± 0.03	a	4.01 ± 0.48	a	3.6	2.0	–	18.42 ± 1.58	b
MW4	4.36 ± 0.01	b	4.01 ± 0.87	a	3.0	0.9	–	10.23 ± 2.34	a
MW5	4.33 ± 0.00	b	4.60 ± 0.45	ab	3.0	2.0	20	18.20 ± 1.14	b
MW6	3.08 ± 0.09	a	3.94 ± 0.94	a	3.6	3.0	20	24.00 ± 1.16	c

Data is expressed by mean ± standard deviation. Different letters within one column indicate significant difference (Tukey HSD one-way ANOVA at $p < 0.05$). Total acidity is expressed as g/L tartaric acid equivalent. SPI is expressed as g/L GSE aqueous solution equivalent.

interaction between polyphenols and salivary proteins or 2) denaturing the salivary proteins (Neta, Johanningsmeier, & Mcfeeters, 2007). By reducing the tannin concentration of MW4 (from 2.0 g/L to 0.4 g/L, 0.2 g/L or 0.1 g/L using a stepwise approach), there was a point at which the SPI of MW4 (high acidity, lowered tannin) was just equal to that of MW3 (low acidity, medium tannin). Polysaccharide was used to lower the SPI because it 1) forms protein/polyphenol/polysaccharide soluble ternary complex, or 2) competes with polyphenol in aggregating salivary protein (Brandão et al., 2017; Watrelot, Schulz, & Kennedy, 2017). By a gradual polysaccharide supplementation (from 0 g/L to 30 g/L, 5 or 2 g/L per step), there was supposed to be a point where the SPIs of MW5 (high acidity, low tannin, polysaccharide supplemented) and MW6 (low acidity, high tannin, polysaccharide supplemented) were equal to that of MW3.

As seen from Fig. S2, the final GSE level in MW4 was determined to be 0.9 g/L. At this tannin concentration, the SPI of MW4 was equal to MW3, as the high acidity's precipitation effect in MW4 was compensated by its low tannin level. The final maltodextrin in MW5 and MW6 were determined to be 20 g/L for both. At this maltodextrin concentration, the SPI of MW5 and MW6 were equal to MW3, as high acidity's (MW5) or high tannin's (MW6) precipitation effects were compensated by the maltodextrin supplemented. The SPI of MW3-MW6 were all close to 4 g/L GSE equivalent (Table 2). Previous work also proved the feasibility of modulating wine SPI by either reducing tannin or adding polysaccharide (Rinaldi, Gambuti, & Moio, 2012b).

The titratable acidity of samples was reversely scaled with pH and the addition of maltodextrin did not alter the titratable acidity (comparing MW5 to MW1, MW6 to MW2). There was a certain proportion of endogenic polysaccharide combined with tannin in all the MWs. At the same tannin concentration (MW1 and MW3), a pH increase doubled the ethanol index, which indicated the higher proportion of polysaccharide-tannin aggregates were formed. Adding polysaccharide to MW5 and MW6 significantly increased their ethanol index (Table 2).

3.2. Sensory paired-comparison of astringency sub-qualities in model wines

A preliminary bench-top tasting confirmed that descriptive analysis might not be not be suitable as the differences between samples were subtle while sensory fatigue was great. Thus, the paired-comparison was selected on account of its power to discriminate and to reduce sensory fatigue and carryover effect of the MWs (Fleming, 2015). The design aims to reveal differences in the sub-qualities *drying*, *rough* and *pucker* among matrices varying in pH, tannin or polysaccharide addition (Fig. 1). As shown in Table 3, with the exception of Pair 8, at least one difference in sub-quality existed within any individual pair. *Drying* was the most distinguishable, of which the difference was discerned in 6 of 8 pairs (Pair 1–4, 6–7). Differences for *pucker* was discerned in four pairs (Pair 1, Pair 2, Pair 4 and Pair 5), and *rough* in just three pairs (Pair 3, 5 and 6).

Importantly, the data demonstrates that *pucker* and *drying* are independent sub-qualities of astringency. In Pair 1 (MW1 vs MW2) for example, MW2 was the more *drying* sample while MW1 was the higher for *pucker*. In contrast, Pair 2 results showed that MW1 was both more *drying* and more *pucker* than MW3. In other pairs (for example Pair 3, Pair 5), a significant difference was observed for one of the two attributes, but not for the other.

In contrast to *drying* and *pucker*, the *rough* sensation was not independent of the other two sub-qualities, as it was often detected accompanied with either more *drying* or *pucker*, and never on its own. This is supported by the following sets of observations. In Pair 1, the higher *pucker* in MW1 but higher *drying* in MW2 resulted in the counterbalanced *rough* within this pair (where neither sample was considered more *rough*). This was conversely observed in Pair 4. In Pairs 3, 5 and 6, the higher *drying* or higher *pucker* itself resulted in higher *rough*. In Pair 8, the similar *drying* and similar *pucker* resulted in the similar *rough*.

Table 3
Number of agreeing responses (n = 60) and sample rankings in the paired-comparison test.

Pair	Sample	Number of answers for which sample is more …		
		drying	*rough*	*pucker*
Pair 1	MW1	23	26	44*****
	MW2	37*	34	16
Pair 2	MW1	37*	25	42****
	MW3	23	35	18
Pair 3	MW2	41***	40**	36
	MW3	19	20	24
Pair 4	MW3	38*	33	16
	MW4	22	27	44*****
Pair 5	MW3	28	23	12
	MW5	32	37*	48*****
Pair 6	MW3	17	18	28
	MW6	43****	42****	32
Pair 7	MW1	39**	31	32
	MW5	21	29	28
Pair 8	MW2	30	31	34
	MW6	30	29	26
Rankings	MW1	c	ab	b
	MW2	d	b	a
	MW3	b	a	a
	MW4	a	ab	b
	MW5	ab	b	b
	MW6	d	b	a

Samples reaching significance levels (two-tailed T-test) within pairs were bolded. Significance was defined as 37 agreeing responses ($p < 0.1$, *), 39 agreeing responses ($p < 0.05$, **), 41 agreeing responses ($p < 0.01$, ***), 42 agreeing responses ($p < 0.005$, ****), and 44 agreeing responses ($p < 0.001$, *****) within 60 trials (Roessler et al., 1978). Each astringency sub-quality was ranked hierarchically following a logical order over the pairs. Different letters in ascending order indicate the ranking of the current sub-quality among samples.

Except Pair 2 and Pair 7, 6 out of 8 pairs support our hypothesis. This, again, emphasises that astringency is not one simple sensory experience but is made up of distinct cues, where linkages exist among sub-qualities (Lawless et al., 1994). It follows to say that these cues are supported by distinct mechanisms of perception because they are sensorily independent of each other.

While a sensory ranking test was not employed, the paired-comparisons allowed for sample rankings to be made in hierarchical order for each sub-quality and these are given in Table 3:

- The most *drying* samples were MW2 and MW6, followed by MW1, then MW3, then MW4; *drying* of MW3 and MW5 were equal.
- The ranking of *drying* was in line with the tannin levels of MWs.
- As for *rough*, MW2, MW5 and MW6 were ranked higher than MW3.
- MW1, MW4 and MW5 were more *pucker* than MW2, MW3 and MW6
- The ranking of *pucker* corresponded to the acidity of MWs.
- Polysaccharide modulated the *drying* in the high acidity sample (MW5) but not the high tannin sample (MW6)

Evidently, the samples with the same SPI ('chemically-equalised' astringency) are never sensorially the same (Pair 4, Pair 5 and Pair 6). As a counter example, samples varying in SPI (Pair 8, MW2 and MW6) are indistinguishable in terms of the three sub-qualities, which could be because the sensory differences existed in other astringency sub-qualities other than *drying*, *rough* or *pucker*. The saliva-precipitation-based method (SPI) was unable to pick up the drivers of different astringency sub-qualities. This highlights the inadequacy of single instrumental measures for predicting the complex sensation of astringency, and while SPI may provide a measure of salivary protein-polyphenol interactions, it does not capture all the physical processes occurring during oral processing that includes those associated with oral tribology.

3.3. Tribology results from different protocols for MW samples

Different tribology protocols were used because there are two main hypotheses addressing the development of astringency sensation when considering the role of salivary lubrication. One of them emphasise the precipitation of salivary proteins and lubricity loss in bulk saliva, while the other postulates that the reduced lubricating properties of the mucosal pellicle and friction increase are responsible for astringency (Canon, 2019). We emphasise that both these processes are likely to occur at the same time upon ingestion of astringent compounds that bind with salivary proteins, but also that other components in wines may also interact with saliva and/or affect lubricity in a variety of ways that should also influence mouthfeel perception. Accordingly, we used DTP-BS and Stribeck curve to reflect the properties of bulk saliva when reacting with wine astringents, and DTP-SP to reflect the properties of a thin layer of salivary proteins when exposed to wine astringents.

3.3.1. Dynamic tribology protocol with bulk saliva: monitoring lubrication in the boundary regime

The DTP-BS (Fig. 2) shows the rate-of-decrease in boundary friction and a stable friction coefficient after the PDMS substrate was fully lubricated by MW samples. A preliminary test on water showed an instant reduction in friction coefficient to around 1.3. For saliva + water mixture, it took around 150 s to get fully lubricated (lowest friction coefficient ≈ 0.06) (Fig. S8a). For MWs, they immediately lubricated the PDMS tribo-pair in about 30 s, and then maintained a very steady friction coefficient (≈0.85) (Fig. 2b). The MW + S (Fig. 2c) took a longer time to lubricate the tribo-pair and reached to lower frictions. The saliva-MW ratio of 1:10 (2 mL saliva +20 mL MW) was selected because the resting saliva presented in mouth was estimated to be 1 mL, while an estimated sip of wine was 10 mL (Obreque-Slier, Espínola-Espínola, & López-Solís, 2016).

The MW2+S and MW6+S (highest tannin samples) resulted in the highest friction coefficient after lubrication ($\mu_{DTP\text{-}BS(endpoint)}$) compared to the other samples (Table 4). Parameters from an exponential-linear model showed the high acidity samples (MW1, MW4 and MW5) possessed the highest p_1 which indicated a sluggish rate-of-decrease in friction. The p_3 (intercept) was basically in line with the $\mu_{DTP\text{-}BS(endpoint)}$.

Salivary macromolecules (mucins, statherin, Cystatin S and PRPs) assemble on oral surfaces through hydrophobic interactions and provide surface lubrication (Yakubov et al., 2015). The idea of DTP-BS is that the MW precipitates or denatures salivary proteins thus reducing the saliva's surface affinity (Ramos-Pineda, Carpenter, García-Estévez, & Escribano-Bailon, 2019). The less surface affinity the saliva possesses, the more difficult it is to lubricate the tribo-pair with saliva (higher friction or sluggish speed of lubrication). During the pre-mixing procedure, a certain amount of lubricious salivary protein was precipitated or denatured, depending on the reactivity of MW matrix. The MWs with highest tannin level (MW2 and MW6) precipitated the highest amount of lubricative salivary protein, resulting in the poorest lubricity (highest $\mu_{DTP\text{-}BS(endpoint)}$). Another observation was that the slower rate of friction decrease (higher p_1) seemed to respond to MWs with higher acidity. This may be because the higher acid matrix approached the isoelectric point of salivary proteins, thus causing conformational changes (Kallithraka, S., Bakker, J., & Clifford, M. N., 1997a, 1997b; Thomas & Lawless, 1995). Even the conformationally changed proteins were not precipitated or complexed with tannin, their hydrophobic binding to PDMS was reduced so that they took a longer time to form the lubricious layer.

3.3.2. Stribeck curve: comparing friction in multiple regimes

The Stribeck curve monitors friction at multiple entrainment speeds. Water and water + saliva mixtures were also tested on this tribo-pairs as references (Fig. S8b). Only boundary regime and mixed regime were shown owing to the slower range of entrainment speed selected. Fig. 3a shows that the Stribeck curves (i.e. friction coefficient versus entrainment speed) for the model wines lubricating between PDMS substrates are indistinguishable between the MWs without mixing saliva. In contrast, a decrease in the friction coefficient values were observed in these MW + S mixtures (Fig. 3b).

Table 4
Dynamic tribology protocol with bulk saliva: endpoint friction coefficients ($\mu_{DTP\text{-}BS(endpoint)}$) and model-fitting parameters (p_1, p_2, p_3).

	$\mu_{DTP\text{-}BS}$ (endpoint)		Model fitting					
			p_1		p_2		p_3	
MW1	0.86 ± 0.00	cd	11.23 ± 1.53	a	5.1E-05 ± 1.3E-05	b	0.82 ± 0.00	abcd
MW2	0.84 ± 0.01	cd	9.69 ± 1.57	a	7.4E-05 ± 7.1E-06	b	0.81 ± 0.02	abcd
MW3	0.87 ± 0.04	cd	11.15 ± 0.77	a	5.3E-05 ± 1.1E-04	b	0.83 ± 0.08	bcd
MW4	0.88 ± 0.07	d	11.24 ± 0.89	a	−2.3E-05 ± 1.2E-04	b	0.86 ± 0.12	cd
MW5	0.79 ± 0.01	bcd	18.64 ± 4.21	ab	5.1E-05 ± 9.0E-05	b	0.75 ± 0.05	abcd
MW6	0.88 ± 0.02	d	11.73 ± 5.23	a	1.4E-04 ± 1.2E-04	b	0.79 ± 0.12	abcd
MW1+S	0.54 ± 0.05	a	29.96 ± 7.14	b	−1.5E-04 ± 2.7E-04	ab	0.58 ± 0.13	a
MW2+S	0.87 ± 0.06	cd	9.66 ± 1.51	a	−1.5E-04 ± 8.3E-05	ab	0.95 ± 0.08	d
MW3+S	0.65 ± 0.12	abc	15.23 ± 7.80	ab	−4.6E-04 ± 1.2E-04	a	0.87 ± 0.04	cd
MW4+S	0.53 ± 0.08	a	20.06 ± 6.51	ab	−1.8E-04 ± 7.9E-05	ab	0.64 ± 0.09	abc
MW5+S	0.58 ± 0.08	ab	28.78 ± 10.11	b	−7.2E-05 ± 9.4E-05	b	0.59 ± 0.04	ab
MW6+S	0.82 ± 0.10	cd	11.54 ± 3.14	a	−1.0E-04 ± 7.4E-05	ab	0.93 ± 0.11	d

Data is expressed by mean ± standard deviation. Different letters within one column indicate significant difference (Tukey HSD one-way ANOVA at $p < 0.05$).

At the lowest entrainment speed, which is a marker for the boundary-lubrication regime, the friction coefficient ($\mu_{Stribeck(U=4\ mm/s)}$ and μ_b) varied from 0.6 (MW4+S, MW5+S) to 0.9 (MW6+S) (Table 5). Highest tannin mixtures MW2+S and MW6+S showed similar response as the MWs without saliva. The $\mu_{Stribeck(U=4\ mm/s)}$ for medium tannin mixtures (MW1+S and MW3+S) decreased to a significantly lower level (~0.75). The lowest tannin mixture (MW4+S) or medium tannin with polysaccharide mixture (MW5+S) decreased to a lowest level down to 0.6. The transition from boundary to mixed regime (B) of MW samples was ~80 mm/s. After mixing saliva, the MW3+S gained an increased B (~180 mm/s), while MW4+S and MW6+S gained a decreased B (~40 mm/s).

Overall, the Stribeck curves of the wine-saliva mixture more closely resemble those of wine-alone than the ultra-low friction found for bulk saliva (Bongaerts et al., 2007b). The boundary and mixed regimes of the Stribeck curves are distinguishable, which may relate to tannin content; samples with high tannin may bind more with salivary proteins and reduce their capacity for adsorbing and lowering friction, and thus these mixtures have higher boundary friction. Previous studies indicate that wines of different tannin levels could be distinguished with various friction coefficients in the boundary regime (Brossard et al., 2016; Laguna et al., 2017). However, in the current work, where astringency is decomposed into 3 distinctive sub-qualities, the boundary friction of the

Table 5
Stribeck curves of model wines and their mixtures with saliva: friction coefficient at 4 mm/s ($\mu_{Stribeck\ (U=4\ mm/s)}$) and model-fitting parameters (μ_b, B and m).

	$\mu_{Stribeck(U=4\ mm/s)}$		Model fitting					
			μ_b		B		m	
MW1	0.95 ± 0.12	de	0.95 ± 0.09	c	78.65 ± 11.54	ab	1.16 ± 0.06	abcd
MW2	0.95 ± 0.09	de	0.97 ± 0.05	c	77.72 ± 26.46	ab	1.13 ± 0.05	abcd
MW3	0.98 ± 0.06	de	0.99 ± 0.07	c	79.93 ± 18.80	ab	1.09 ± 0.09	abc
MW4	1.01 ± 0.11	e	1.00 ± 0.10	c	104.46 ± 18.08	b	1.25 ± 0.17	cd
MW5	0.98 ± 0.10	de	0.95 ± 0.09	c	85.26 ± 11.52	ab	1.20 ± 0.13	bcd
MW6	0.98 ± 0.07	de	0.96 ± 0.07	c	76.61 ± 10.91	ab	1.17 ± 0.09	abcd
MW1+S	0.75 ± 0.09	b	0.74 ± 0.10	ab	104.84 ± 26.80	b	1.28 ± 0.18	cd
MW2+S	0.87 ± 0.10	cd	0.83 ± 0.12	bc	100.23 ± 30.11	b	1.24 ± 0.12	cd
MW3+S	0.77 ± 0.14	bc	0.72 ± 0.11	ab	189.56 ± 56.95	c	1.58 ± 0.16	e
MW4+S	0.60 ± 0.09	a	0.67 ± 0.03	ab	42.34 ± 18.55	a	0.93 ± 0.15	a
MW5+S	0.66 ± 0.09	ab	0.64 ± 0.09	a	106.57 ± 17.00	b	1.35 ± 0.17	de
MW6+S	0.96 ± 0.18	de	0.99 ± 0.10	c	40.89 ± 7.73	a	0.95 ± 0.10	ab

Data is expressed by mean ± standard deviation. Different letters within one column indicate significant difference (Tukey HSD one-way ANOVA at $p < 0.05$).

saliva-MW mixture scales with the ranking of *drying* but not *rough* or *pucker* (Table 3). It is hypothesised that tannin is the driver of *drying* because higher tannin level depleted more salivary proteins in bulk saliva, which eventually resulted in a less lubricative absorbed layer of salivary protein on the surface of tribo-pair.

3.3.3. Dynamic tribology protocol incorporating a salivary pellicle: monitoring the friction increase on a thin film

The idea of DTP-SP is that the saliva pre-adsorbed PDMS disc can mimic the lubricative oral mucosal pellicle, which could respond differently to MW samples (Rossetti et al., 2009). This dynamic tribology protocol may reflect the kinetics of salivary pellicle breakdown after the MW flushing (Fig. 4). As seen in Fig. S8c, the salivary film retained very low friction (≈0.04) but broke down when the sample was loaded. Sample MW4 had the lowest $\mu_{DTP\text{-}SP\ (endpoint)}$, although no significant difference was observed among other samples. The high acidity samples, MW1 and MW4, showed the lowest p and highest q, which indicated that they caused a quicker surge in friction soon after the samples were loaded (Table 6).

Saliva adsorbs onto PDMS to form a heterogeneous 2-layer film; the outer layer is reported to be highly extended and hydrated, while the inner side is a dense anchoring layer (Macakova, Yakubov, Plunkett, & Stokes, 2010, 2011). As the MW contacts this adsorbed film, an increase in friction occurs due to mechanical desorption from the PDMS, as suggested for tea-polyphenols (Rossetti et al., 2009). This may be caused by the interaction (aggregation) of wine components (e.g. tannins) with salivary proteins on the adsorbed lubricious saliva layer, leading to a marked friction increase (Brossard et al., 2016; Laguna et al., 2017; Rossetti et al., 2009). The results of the present study suggest that the pre-adsorbed salivary film is more sensitive to acid. While saliva is known to buffer acidic solutions, here it is only present in small amounts (Bongaerts et al., 2007b; Rossetti et al., 2009; Yakubov et al., 2015), which has limited buffering capacity against the pH change (Obreque-Slier et al., 2016). In this case, the acidity may also cause precipitation of salivary proteins (acidic PRPs with isoelectric point 3–3.5) when the pH goes below the isoelectric point of the proteins, and this

Table 6
Dynamic tribology protocol incorporating a salivary pellicle: endpoint friction coefficients ($\mu_{DTP\text{-}SP(endpoint)}$) and model-fitting parameters (p, q, and r).

	$\mu_{DTP\text{-}SP\ (endpoint)}$		Model fitting					
			p		q		r	
MW1	0.85 ± 0.08	bc	0.56 ± 0.08	ab	1.1E-02 ± 4.8E-03	bc	2.6E-04 ± 9.4E-05	a
MW2	0.82 ± 0.04	bc	0.69 ± 0.08	ab	4.5E-03 ± 8.4E-04	a	1.4E-04 ± 5.2E-05	a
MW3	0.90 ± 0.07	c	0.69 ± 0.07	ab	7.6E-03 ± 2.8E-03	abc	2.2E-04 ± 8.4E-05	a
MW4	0.63 ± 0.09	a	0.48 ± 0.04	a	1.3E-02 ± 7.1E-03	c	1.2E-04 ± 6.7E-05	a
MW5	0.85 ± 0.09	bc	0.71 ± 0.07	b	4.5E-03 ± 1.1E-03	a	1.3E-04 ± 8.1E-05	a
MW6	0.74 ± 0.12	ab	0.63 ± 0.18	ab	3.2E-03 ± 1.0E-03	a	1.1E-04 ± 7.0E-05	a

Data is expressed by mean ± standard deviation. Different letters within one column indicate significant difference (Tukey one-way ANOVA at $p < 0.05$).

effect could even be more dominant than the effect from tannin's hydrophobic binding (Neta et al., 2007; Rinaldi et al., 2012b; Rudney, Staikov, & Johnson, 2009). This could explain why high acidity samples (MW1 and MW4) increased their friction faster than other samples.

The current findings support the hypothesis that the rate at which lubricity lost is an important parameter for temporal mouthfeel perception (Selway et al., 2013). *Pucker* could be such a transient sensation linking to the quicker disruption of salivary pellicle. Further, the less sample difference on $\mu_{DTP\text{-}SP(endpoint)}$ in DTP-SP, compared to the significantly different $\mu_{Stribeck(U=4\ mm/s)}$ in the Stribeck curve or DTP-BS, also supports that independent mechanisms exist between sensations of *drying* and *pucker*.

3.4. Monitoring the properties of salivary film by QCM-D

The QCM-D technique can simultaneously determine the △F and □D that enables not only the hydrated mass/thickness but also the viscoelastic properties of the adsorbed salivary layers to be determined (Wang, Ho, & Huang, 2007). Fig. 5a displays the typical curves of △F and △D (3rd, 5th, 7th, 9th, 11th, 13th overtones) over a measurement. After adding saliva, a gradual decrease of △F and increase of △D were observed, indicating adsorption of a soft salivary layer on the sensor. After rinsing with physiological levels of NaCl, the △F slightly increased and the △D slightly decreased (Stage 1) because of the removal of bulk phase. The dynamic response and values of △F and △D following saliva adsorption and rinsing in NaCl solution are remarkably similar to those obtained previously by Macakova et al. (2010) following the same sequence, thus providing confidence in our methodology as well as showing that viscoelastic film-forming properties of saliva are not significantly different from two entirely different donors and laboratories.

At the start of 'stage 2', the MW was introduced. In all the samples, a dramatic plunge in △F and surge in △D occurred, indicating the absorption or associations of MW components with the salivary film. As the film was rinsed using NaCl solution in stage 3, the △F and △D partially recovered but it did not go back to that found in Stage 1. This is likely to be indicating that there is still a mass of absorbed material on the surface. An increase in mass does not seem to positively correspond to the tribology measurements, which show an increase in friction associated with a *loss* of salivary surface film. However, the QCM-D results do not involve rubbing forces, and thus while they indicate an increase in adsorbed mass due to interactions with the MW, the film may be fragile to shear or rubbing forces. The results certainly compare with other QCM-D studies involving tea polyphenols and casein- or parotid-saliva coated substrates (Weerawatanakorn, Huang, & Ho, 2014; Yao, Lin, Chen, Lin, & Tao, 2010), as well as interfacial rheology measurements of Rossetti, Yakubov, Stokes, Williamson, and Fuller (2008) who observed

that the elasticity of salivary films at air-liquid interfaces is strengthened by interactions with tea polyphenols.

Fig. 5b summaries the values in stage 2 and stage 3 relative to stage 1. Low tannin MW4 showed the lowest changes in △F and △D after stage 2 (S2–S1, Fig. 5b). By contrast, the highest tannin sample MW2 displayed the greatest decrease in △F, indicating the highest amount of tannin was adsorbed on the salivary film. The samples with polysaccharide (MW5 and MW6) showed a higher △D and lower △F than those without polysaccharide (MW1 and MW2) in S2–S1, indicating that the less mass associated with the salivary film.

After rinsing with NaCl in Stage 3, the low tannin sample MW4 showed the smallest change in △F compared to those in Stage 1 (S3–S1), perhaps indicating that the salivary film has not been altered significantly compared to its structure back in Stage 1. The highest tannin sample MW2 had the highest reduction in △F, which was considered to display the strongest interaction between tannin and salivary protein.

As observed with tea-polyphenols, the significant decrease in △F relative for most wines indicates that components in the samples interact with salivary proteins and contribute to the viscoelastic network structure of the surface film (Yao et al., 2010). This process seemed to be more irreversible in the high tannin sample MW2 compared with low tannin sample MW4. The addition of polysaccharide, however, seemingly made this procedure less drastic because the changes in △D and of MW6 in S3–S1 were significantly lower than that of MW2. This may be because the polysaccharide competed with tannin to bind salivary proteins and reduced the surface aggregation caused by high tannin concentration (Brandão et al., 2017). Also, this surface became less rigid in existence of the polysaccharide.

The effect of acidity was not pronounced in the QCM-D responses, as no significant difference was found between MW1 (high acidity, medium tannin) and MW3 (low acidity, medium tannin) in any stage or with any parameter. This supports the previous finding that acidity may not be the main factor causing salivary protein aggregation in the presence of the strong hydrophobic interaction between protein surface and tannin (Muthuramalingam, 2017; Weerawatanakorn et al., 2014). A possible explanation could be that tannin has more pronounced effect on binding with the salivary film in a QCM-D system that can be reflected in □F or △D. The acidity may cause the conformational change of the salivary protein thus influencing their affinity to the surface of substrate. This was only reflected in a slower rate of change in lubrication (higher p_1) in DTP-BS and quicker collapse of salivary film (lower p and higher q) in DTP-SP. These tribology observations agree with the previous finding that lowering the pH causes the desorption of saliva protein on the hydrophilic silica surfaces (Sotres, Lindh, & Arnebrant, 2011). As a result, the strength and wear-resistance of the salivary film are reduced, but currently this can only be monitored by a tribology system where a loading force is applied on the film.

4. Conclusions

In this study, saliva-involved tribological measures were developed to gain insights into the mechanistic drivers of wine astringency in terms of its underlying sub-qualities. Compositional variations in model wines altered the sensorily perceived sub-qualities among the samples, as well as their tribological response. This occurred even in samples with 'chemically-equalised' astringency (by SPI). Specifically, the sub-quality *rough* seems to be a combination of *drying* and *pucker*. By using three different tribology protocols involving saliva, it was revealed the *drying* sub-quality is driven by tannin content and scales with the boundary friction coefficient for PDMS substrates lubricated with a mixture of wine and saliva in Stribeck curve. In contrast, perception of *pucker* is driven by acidity and scales with the rate of increase in friction on a saliva-coated substrate (DTP-SP method) due to disruption of the salivary film. The QCM-D shows that tannins bind to a saliva-coated substrate in the absence of significant shear forces, and thus rubbing action is perhaps a necessary condition for desorption of the salivary film.

Many previous studies suggest astringency to have a singular mechanism, which is usually based on colloidal interactions between salivary proteins and astringent components. We do not find this adequate to explain differences in the astringent sub-qualities. A unique insight here is that the colloidal interactions between wine components and salivary proteins affects lubrication of wine-saliva mixtures and the boundary-friction at salivary-coated substrates differently. The mechanisms by which they do this may have subtly different origins, which may contribute to differences it the sub-qualities and astringency as a whole. As this is the first study to deconvolute wine astringency into its sub-qualities and consider these in the context of oral processing using *in vitro* tribology measurements, future work is required to validate findings and test hypothesis on broader sample sets.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

Shaoyang Wang: Conceptualization, Methodology, Investigation, Formal analysis, Writing - original draft, Funding acquisition. **Sandra M. Olarte Mantilla:** Conceptualization, Investigation, Writing - review & editing, Supervision. **Paul A. Smith:** Conceptualization, Formal analysis, Writing - review & editing, Supervision. **Jason R. Stokes:** Conceptualization, Formal analysis, Resources, Writing - review & editing, Supervision. **Heather E. Smyth:** Conceptualization, Methodology, Formal analysis, Investigation, Resources, Writing - original draft, Writing - review & editing, Supervision, Project administration, Funding acquisition.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodhyd.2020.106109.

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