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Original article

Astringency sub-qualities of red wines and the influence of wine–saliva aggregates

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Summary Astringency is a sensory attribute, related to the quality and mouthfeel of red wines. However, the origin of astringency sub-qualities, such as the typical drying astringency found in immature grapes, is still unknown. Astringency of red wines with similar tannin content but different astringency sub-qualities, from different harvest dates, is studied. Astringency was characterised in terms of friction coefficient, polyphenol content, sensory analysis and tannin/salivary–proteins aggregates characterisation. A different evolution during ripening was found for both Cabernet Sauvignon and Carménère, and tannin–protein aggregates showed differences in size, shape and surface. The velvety sub-quality appears to be related to aggregates with low precipitation, and with specific surface characteristics as roundness and Feret diameter. Results from this work propose an effect of aggregates on sensory perception and opens the possibility to explore their effect on oral lubrication.

Keywords Wine astringency, grape ripeness, tannin–protein aggregates, red wine, oral lubrication.

Introduction

Astringency may be described as the dry, rough and puckering sensation experienced in the mouth and is perceived as an increase of frictional forces between surfaces within the oral cavity (Upadhyay *et al.*, 2016). In enology, astringency is a crucial sensory attribute that contributes to mouthfeel of red wines, and it is a significant contributor to consumer acceptance (Gawel, 1998; Bajec & Pickering, 2008; Basalekou *et al.*, 2019). Astringent properties of grapes must be considered for planning harvest date and winemaking to fully exploit the potential of grapes. During ripening, the reduction of the drying astringency found in immature grapes is expected (Rousseau & Delteil, 2000). However, the real influence of ripeness on astringency is still not completely clear. The extractability of grape tannins is affected not only by agronomic factors but also by the evolution of other grape components such as carbohydrates and polysaccharides. Overall, the selection of the optimal grape astringency during ripening is a crucial decision in winemaking.

Astringency can be estimated by analytical methods based on tannin concentration. However, the conventional analytical methods are indirect and do not allow an accurate prediction of astringency (Ishikawa & Noble, 1995; Llaudy *et al.*, 2004; Kennedy *et al.*, 2006; Sáenz-Navajas *et al.*, 2010; Sáenz-Navajas *et al.*, 2019). These methods are incapable of describing astringency sub-qualities, and the mechanisms that explain the different descriptors of astringency are still controversial (Ma *et al.*, 2014). This is considered a significant limitation because consumers have been demanding a softer astringency along with a full body in red wines (Meléndez *et al.*, 2013).

Oral lubrication studies (friction coefficient) have emerged as a possible tool to estimate astringency (Prakash *et al.*, 2013; Brossard *et al.*, 2016; Upadhyay *et al.*, 2016). Results obtained have contributed to important advances in trying to mimic oral conditions

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and astringency determination by lubrication tests (Brossard *et al*., 2016). However, these results revealed complex and specific interactions between tannins and saliva proteins with or without the precipitation of the complex (Rossetti *et al*., 2009; Cala *et al*., 2012; Brossard *et al*., 2016). This was confirmed by photomicrographs that showed that wines with different astringency had different conformation of aggregates depending on the wine variety and astringency subquality (Brossard *et al*., 2016).

Astringency would depend upon the molecular assembly of tannins and proteins in aggregates (Scollary *et al*., 2012). The aggregation and precipitation of tannin–protein complexes increases with ionic strength, lower pH and decreases with higher ethanol content (Boulet *et al*., 2016). In addition, polysaccharides (de Freitas & Mateus, 2012; Quijada-Morín *et al*., 2014) and anthocyanins (Gonzalo-Diago *et al*., 2014; Paissoni *et al*., 2020) modulate astringency but their effect on aggregates and oral lubrication is still under research.

Based on this, this work explores the changes in astringency during grape ripening based on the evolution of both wine analytical parameters and changes in the aggregates produced by red wine tannins and saliva proteins. Red wines of Cabernet Sauvignon and Carménère cultivars, with similar tannin content but different astringency sub-quality (rough and soft/velvety, respectively) (Fernandez *et al*., 2007; Brossard *et al*., 2016), were evaluated. The main objective of this study was to contribute to the understanding of wine astringency through the study of astringency subqualities and tannin/protein aggregates.

Materials and methods

Harvest dates, winemaking process and wines characterisation

Experiments were conducted in two commercial irrigated vineyards (*Vitis vinifera L*.) located in Maipo valley, Chile. Two cultivars with different astringency sub-quality were evaluated: Cabernet Sauvignon (rough) and Carménère (soft/velvety). All grapes belonged to vintage 2018.

Harvest was carried out at four dates representing different phenolic ripeness stages: (i) grape grower traditional harvest (GTH) defined by the grape grower based on composition (13%–14% v/v of potential alcohol, titratable acidity 3.5 to 4.5 g L^{-1} H_2SO_4) and grape tasting, (ii) 1 month before GTH (GTH-1m), (iii) 15 days before GTH (GTH-15d) and (iv) 15 days after GTH (GTH+15d; only for Cabernet Sauvignon). For each date, wine samples were made in three replicates following traditional red wine winemaking practices at pilot scale. The latter consists of the following: (i) crushing, destemming and tank filling; (ii) primary alcoholic fermentation and maceration; (iii) separation of wine and pomace by free-run and pressing; (iv) and malolactic fermentation.

Chemical characterisation of wines considered alcohol content (v/v), pH, colour intensity, total phenolic index (OD 280 nm), anthocyanins (mg L^{-1}) and total tannins content (mg L^{-1}). Anthocyanins and proanthocyanidins wine composition and their mean degree of polymerisation (mDP: average number of subunits per molecule) were determinated by HPLC-DAD (Hitachi LaChrom Elite, Tokyo, Japan) as proposed by Kennedy & Jones (2001) (Table 1).

Saliva collection

Unstimulated whole saliva (UWS) was collected according to Brossard *et al*. (2016). Twenty volunteers aged between 20 and 30 years were recruited from the campus of Zhejiang Gongshang University. All subjects were healthy, non-smoking and with a normal Body Mass Index (BMI). Subjects were instructed to passively accumulate saliva in the mouth and then spit it into a clean glass container for a period of 15 min. Subjects were allowed to drink 20 mL of bottled water after each 5 min saliva collection. Immediately after collection, saliva samples from all subjects were mixed and centrifuged for 10 min at 500 ***g*** to remove suspended particles. Supernatants were placed in 10 mL aliquots and stored immediately in a freezer at −80°C. Before lubrication tests, an appropriate number of saliva containers were warmed up to 28°C in a water bath to mimic the temperature of the oral surface (Engelen & de Wijk, 2012).

Characterisation of wine astringency

Tribology analysis

Texture analysis was made with a purpose-built tribometer, consisting of a simple device which is attached to a commercial Texture Analyser TA.XTplus (Stable Micro Systems, Surrey, UK). Its feasibility had already been reported in a previous study (Brossard *et al*., 2016; Upadhyay & Chen, 2019). Lubrication tests were conducted at a sliding speed of 0.075 mm s^{-1} and at a normal force of 0.58 N, since these parameters have shown a high correlation with sensory astringency in our previous research (Brossard *et al*., 2016). Mixtures of unstimulated human saliva and wines were prepared at a ratio of 1:1 (saliva:wine). The effect of soluble aggregates on lubrication was evaluated by separating the insoluble aggregates from the wine–saliva mixture and measuring the friction coefficient of the supernatant after centrifugation at 500 ***g*** for 10 min.

The average force of the dynamic friction was calculated by the texture analyser software (Exponent,

TABLE 1 Chemical composition of Cabernet Sauvignon and Carménère wines from different harvest dates

Wines[a]	Alcohol (v/v)	pH	Colour intensity (CI)	Anthocyanins (mg L^{-1} ME)[b]	Total Phenols (OD 280 nm)	mDP	Galloylated tannins (mg/L CE)	Non-galloylated tannins (mg L^{-1} CE)	Total tannins (g L^{-1} CE)*,[c]
Cabernet Sauvignon									
GTH-1m	10.1 ± 0.4^{d}	3.6 ± 0.0^{c}	6.4 ± 0.8^{c}	71.6 ± 0.6^{cd}	34.8 ± 4.8^{d}	14.6 ± 0.1^{a}	8.9 ± 4.5	14.1 ± 0.8	1.0 ± 0.2^{d}
GTH-15d	11.7 ± 0.4^{c}	3.7 ± 0.0^{b}	7.6 ± 0.4^{c}	59.4 ± 8.7^{d}	40.9 ± 2.2^{cd}	12.5 ± 0.1^{ab}	9.7 ± 5.5	18.5 ± 6.5	1.3 ± 0.1^{bcd}
GTH	12.7 ± 0.3^{b}	3.9 ± 0.0^{b}	6.4 ± 1.1^{c}	109.6 ± 11.5^{c}	41.1 ± 1.0^{cd}	7.7 ± 0.1^{cd}	18.3 ± 6.2	18.1 ± 4.3	1.1 ± 0.1^{cd}
GTH+15d	12.5 ± 0.1^{b}	4.1 ± 0.1^{a}	7.5 ± 0.3^{c}	73.0 ± 5.4^{cd}	46.8 ± 2.7^{bc}	6.1 ± 0.2^{d}	23.0 ± 7.2	18.8 ± 2.3	1.4 ± 0.1^{bc}
Carménère									
GTH-1m	12.7 ± 0.2^{b}	3.8 ± 0.1^{b}	15.2 ± 2.0^{b}	158.0 ± 29.3^{b}	53.5 ± 0.7^{ab}	10.1 ± 0.2^{bc}	7.7 ± 12.1	17.9 ± 4.7	1.6 ± 0.0^{b}
GTH-15d	13.5 ± 0.3^{ab}	3.9 ± 0.0^{b}	15.4 ± 2.0^{b}	216.0 ± 2.3^{a}	60.3 ± 2.8^{a}	10.2 ± 0.1^{bc}	4.2 ± 20.2	19.1 ± 5.9	2.0 ± 0.0^{a}
GTH	14.1 ± 0.3^{a}	4.1 ± 0.0^{a}	20.5 ± 0.5^{a}	245.0 ± 19.4^{a}	60.9 ± 4.8^{a}	10.5 ± 0.0^{bc}	10.9 ± 20.6	17.7 ± 11.7	1.9 ± 0.1^{a}
P-value	<0.0001*	<0.0001*	<0.0001*	<0.0001*	<0.0001*	<0.0001*	0.998	0.152	<0.0001*

Different letters indicate a significant difference at $P < 0.05$ between wines and harvest dates treatments calculated by Tukey's Means Comparison Method.

[a]Wines produced at different harvest dates: (1) 1 month before of grape-grower traditional harvest (GTH-1m), (2) 15 days before (GTH-15d), (3) traditional harvest defined by the grape-grower (GTH), and (4) 15 days after (GTH+15d).

[b]Milligrams by litre expressed in malvidin equivalent (ME), determined by HPLC-DAD.

[c]Milligrams by litre expressed in catechin equivalent (CE), determined by HPLC-DAD.

*Total tannins also include Procyanidin B2 and Polymer-Adducts.

Stable Microsystems, version 3.2), and it was used for friction coefficient calculation.

Sensory analysis

Sensory evaluation of wines followed the methodologies described by Brossard *et al*. (2016). First, the astringency terms proposed by Gawel *et al*. (2000) and Vidal *et al*. (2003) were discussed and the following terms were chosen: astringency intensity (overall astringency): '*Overall level of astringent sensation, encompassing all the terms* proposed by Gawel *et al*. (2000)', the astringency sub-quality drying: *'feelings of lack of lubrication or desiccation in the mouth*' and volume (full): *'A feeling of a force pressing against the mouth surfaces and tongue*'.

A panel of 20 volunteers, 11 women and 9 men, aged between 20 and 30 years, all healthy non–smokers with a normal Body Mass Index (BMI) was used. A total of 7 training sessions (1.5 h) were conducted prior to the formal assessment of wines. Firstly, panellists got familiarised with samples and tasting procedures in one session where volunteers were asked to discuss their perception in an open session. Skin and seed tannin grape extracts in ascending concentrations were used to train the panel. In each training session, five unknown samples of skin and seed grape extracts (10 mL) were ranked in astringency intensity, drying and volume. Data were analysed with the Page test for ranked tendency ($\alpha = 0.05$), according to ISO 8586. Formal assessment of wines took place only after the panel was able to significantly rank different concentrations of tannins.

During the formal wine tastings, samples were presented in random order dark glasses; three unstructured 15 cm line graphic scales were used to score astringency intensity, drying and volume. Samples from each original replicate experiment were tasted in different sessions. Data were analysed with ANOVA and Tukey's comparison at a significance level of $\alpha = 0.05$, according to ISO 8586.

Characterisation of saliva/wine aggregates

Particle size distribution

The microaggregates size distribution (μm) was obtained using a Mastersizer 3000 using a HydroMV setup module (Malvern Instruments, MS 3000, MAL1114945). Aggregates size was measured in terms of the volume over surface average aggregate diameter ($D_{3,2} = 1 \pm 0.05$ μm). The aggregate detection considered the refractive indexes of red wines and saliva mixtures (1:1) (1.338) using 50 μL of sample in each evaluation, and water was used as dispersant (1.33).

Zeta-potential

Saliva/wine mixtures were injected directly into the measurement chamber of a Zetasizer (Malvern Instruments, Worcester, UK) for measuring the ζ-potential of aggregates. The ζ-potential was obtained as the average of 3 separate injections, with 3 readings per injection. All the samples were equilibrated at 28°C before measurement.

Morphology analysis

Microstructure features of saliva and wine mixtures were observed using light microscopy (LM) and scanning electron microscopy (SEM). LM was used to

provide a visual confirmation of microstructure of the precipitated aggregates. Each red wine was mixed with human saliva at a ratio 1:1. The sample was placed on a slide immediately after mixing and covered with a coverslip for microstructure observation. Photomicrographs were taken using an Olympus BX51 microscope plus Leica DM 3000 Led camera (Leica Microsystems Ltd., Heerbrugg, Switzerland) at 10× magnification.

A Scanning Electronic Microscope (SEM, Phenom, G2 Pro) was used to provide a surface characterisation of aggregates, using wine and saliva mixtures at a 1:1 ratio. Prior to SEM evaluation, the mixture samples were freeze dried for 48 h. Photomicrographs were taken at 690× magnification. The acquired images were analysed using the software Image Pro-plus 3.0 (Bethesda, Md., U.S.A.), and subsequently, Feret diameter (μm) and roundness (%) were evaluated according to Glicerina *et al*. (2013).

Statistical analysis

Statistical analysis was carried out using SAS (SAS Institute, Release 9.2.). Analysis of Variance (ANOVA) was performed to evaluate the effect of harvest date and Tukey's means comparison was used to determine significant differences between treatments. Principal Component Analysis (PCA) was used to describe the relation between instrumental results and sensory results. The significance level used was $P < 0.05$ throughout the study. All analyses were performed in triplicate.

Results and discussion

Evolution of friction coefficient in wines with different astringency sub-qualities

Two grape cultivars, Cabernet Sauvignon and Carménère (rough and soft/velvety astringency, respectively), were evaluated as a model of different sensory astringency sub-qualities. In addition, different grape harvest dates were chosen in this work as a model of evolution of astringency, and the wines were evaluated tribologically. The friction coefficients of human saliva and its mixtures with red wines are shown in Table 2. The non-centrifuged mixtures of both varieties showed an opposite evolution of the friction coefficient during ripening. Cabernet Sauvignon showed a significant decrease in the friction coefficient as ripeness increases, while Carménère had a significant increase ($P < 0.0001$). Notably, the first two harvest dates showed significant differences between both red wines, with Cabernet Sauvignon showing higher friction coefficients. However, for the third and fourth harvest dates (GTH and GTH+15d), Cabernet Sauvignon did not show significant differences when compared to Carménère.

Interestingly, a different behaviour was obtained for the centrifuged samples (Table 2). An increase of

TABLE 2 Comparision of friction coefficient of wine:saliva mixtures obtained at a sliding speed of 0.075 mm/s for Cabernet Sauvignon and Carménère wines from different harvest dates

	Friction Coefficient (μ)			
Wines[a]	**W:S**[b] **mixture**	**W:S Centrifuged**	***P-value***	**Precipitate (g)**
Cabernet Sauvignon				
GTH-1m	0.52 ± 0.03^{abA}	0.33 ± 0.03^{bcB}	*0.019**	0.14 ± 0.01^{ab}
GTH-15d	0.46 ± 0.03^{bcA}	0.39 ± 0.03^{bcA}	*0.279*	0.15 ± 0.01^{a}
GTH	0.39 ± 0.03^{bcdA}	0.41 ± 0.04^{bcA}	*0.831*	0.13 ± 0.02^{abc}
GTH+15d	0.31 ± 0.05^{cdA}	0.46 ± 0.04^{bB}	*0.005**	0.11 ± 0.01^{bcd}
Carménère				
GTH-1m	0.23 ± 0.02^{dA}	0.38 ± 0.02^{bcB}	*0.007**	0.10 ± 0.01^{cde}
GTH-15d	0.25 ± 0.03^{dA}	0.40 ± 0.04^{bcB}	*0.048**	0.08 ± 0.02^{e}
GTH	0.35 ± 0.03^{bcdA}	0.44 ± 0.03^{bcA}	*0.167*	0.09 ± 0.01^{de}
Control samples				
Water	0.68 ± 0.12^{a}	0.68 ± 0.12^{a}		
Saliva	0.26 ± 0.01^{d}	0.26 ± 0.01^{c}		
P-value	<0.0001*	<0.0001*		<0.0001*

Data expressed as mean ± standard error of triplicate fractionation procedures, compared by one-way ANOVA ($P < 0.05$); different letters indicate a significant difference at $P < 0.05$ between wines and harvest dates treatments calculated by Tukey's Means Comparison Method. Lowercase letters correspond to comparison between harvest dates, uppercase corresponds to comparison between centrifugated and non-centrifugated mixtures.

[a]Wines produced at different harvest dates: (1) 1 month before of grape-grower traditional harvest (GTH-1m), (2) 15 days before (GTH-15d), (3) traditional harvest defined by the grape-grower (GTH) and (4) 15 days after (GTH+15d).

[b]Human saliva and wine mixture (W:S) in a ratio 1:1.

*Significant value, with a significance level of $P < 0.05$

friction coefficient with ripening was obtained for both Cabernet Sauvignon and Carménère wines. Although the precipitates obtained (Table 2) also showed significant differences in weight ($P = 0.0001$), these results did not show a similar pattern as the friction results for non-centrifuged samples. These differences in friction coefficient for wine:saliva mixtures suggest a role of non-precipitated or soluble aggregates (de Freitas & Mateus, 2001; Kallithraka *et al.*, 2001; de Wijk & Prinz, 2005; Schwarz & Hofmann, 2008; Ma *et al.*, 2014; Brossard *et al.*, 2016).

When comparing the lubrication results of non-centrifugated mixtures to control samples (Table 2), GTH-1m (first harvest date Cabernet Sauvignon) – with lower tannin content (Table 1) – produces a high friction coefficient similar to water; while GTH+15d (last harvest date Cabernet Sauvignon) – with a higher tannin content (Table 1) – produces a lower friction coefficient more similar to saliva. On the other hand, for Carmenère, the first two dates showed friction coefficients similar to saliva even with high tannin contents (Table 1). These results suggest that the friction coefficient seems to be neither simply nor directly related to tannin content, and tannin–protein soluble and insoluble aggregates could be responsible for oral lubrication modulation.

Characterisation of aggregates

The aggregates were characterised during ripening to analyse their potential role in both oral lubrication and astringency perception. The aggregates size distribution and ζ-Potential are shown in Table 3. The harvest date has no significant effect on aggregates mean area (D3,2) ($P = 0.088$) of the mixtures of wines with saliva. The particle size distribution of the 90% of the total aggregates (D90, based on the median particle size by volume) showed significant differences between the first and last harvest dates of Cabernet Sauvignon and Carménère ($P = 0.024$), respectively, with the highest size of aggregates for Cabernet Sauvignon at GTH-1m and lowest size for Carménère at GTH. Therefore, a similar behaviour for both red wines along ripening was evidenced, with particle sizes ranging between 16.2 and 12.5 μm for Cabernet Sauvignon and between 12.7 and 10.7 μm for Carménère.

To evaluate aggregates stability, the ζ-potential was measured. ζ-potential can be defined as the electrokinetic potential of protein solutions that can be measured by electrophoretic mobility (O'Sullivan *et al.*, 2015). Solutions with low ζ-potential (negative or positive) tend to coagulate or flocculate, due to poor physical stability (Lu & Gao, 2010). ζ-potential of both red wines showed significant differences along ripening ($P = 0.011$) and was negative and distributed within the range of −7.6 to −8.7 mV (Table 3), being interestingly higher at harvest date (GTH) for both varieties. These data suggest that the aggregates are relatively more stable at this date, although both evidenced a rapid formation of aggregates when the mixtures were made.

The effect of different harvest dates was also reflected on the aggregate's microstructure under microscopic observation. Figure 1 shows the light

TABLE 3 Particle characterisation of wine:saliva aggregates from Cabernet Sauvignon and Carménère wines coming from different harvest dates

			Particle size (μm)[b]			Particle surface (SEM)	
Wines[a]	**ζ-Potential (mV)**	**D [3,2] (μm)**	**D10**	**D50**	**D90**	**Feret Diameter (μm)**	**Roundness (%)**
Cabernet Sauvignon							
GTH-1m	-7.9 ± 0.3^{ab}	3.3 ± 0.1	1.7 ± 0.4	2.8 ± 0.7	16.2 ± 2.1^{a}	9.4 ± 0.2^{d}	3.2 ± 0.2^{e}
GTH-15d	-7.8 ± 0.2^{b}	3.5 ± 0.1	1.9 ± 0.1	3.5 ± 1.0	15.7 ± 1.0^{ab}	15.4 ± 0.0^{c}	14.7 ± 0.8^{c}
GTH	-8.2 ± 0.1^{ab}	3.6 ± 0.1	2.3 ± 0.6	3.6 ± 1.1	14.1 ± 0.5^{ab}	21.9 ± 0.2^{b}	15.3 ± 0.8^{c}
GTH+15d	-7.8 ± 0.2^{b}	3.5 ± 0.1	2.3 ± 0.5	3.6 ± 1.0	12.5 ± 0.8^{ab}	31.1 ± 0.0^{a}	58.0 ± 0.0^{a}
Carménère							
GTH-1m	-7.7 ± 0.3^{b}	3.9 ± 0.3	2.5 ± 0.4	4.2 ± 0.2	12.7 ± 1.2^{ab}	32.2 ± 0.1^{a}	50.6 ± 0.3^{b}
GTH-15d	-7.8 ± 0.0^{b}	3.7 ± 0.2	2.2 ± 0.4	4.9 ± 1.6	11.4 ± 1.9^{ab}	30.4 ± 0.1^{a}	11.0 ± 0.2^{d}
GTH	-8.7 ± 0.2^{a}	3.5 ± 0.0	1.9 ± 0.1	4.1 ± 0.8	10.7 ± 1.6^{b}	19.2 ± 0.1^{bc}	12.3 ± 0.4^{d}
P-value	0.011*	0.088	0.594	0.591	0.024*	<0.0001*	<0.0001*

Different letters indicate a significant difference between wines and harvest dates treatments at $P < 0.05$ calculated by Tukey's Means Comparison Method.

[a]Wines produced at different harvest dates: (1) 1 month before of grape-grower traditional harvest (GTH-1m), (2) 15 days before (GTH-15d), (3) traditional harvest defined by the grape-grower (GTH) and (4) 15 days after (GTH+15d).

[b]Particle size shown as particle mean area (D 3,2) and as average values at the 10th (D10), 50th (D50) and 90th (D90) percentiles of the distribution (i.e. at D90, 90% of particles are below this size).

*Significant value, with a significance level of $P < 0.05$

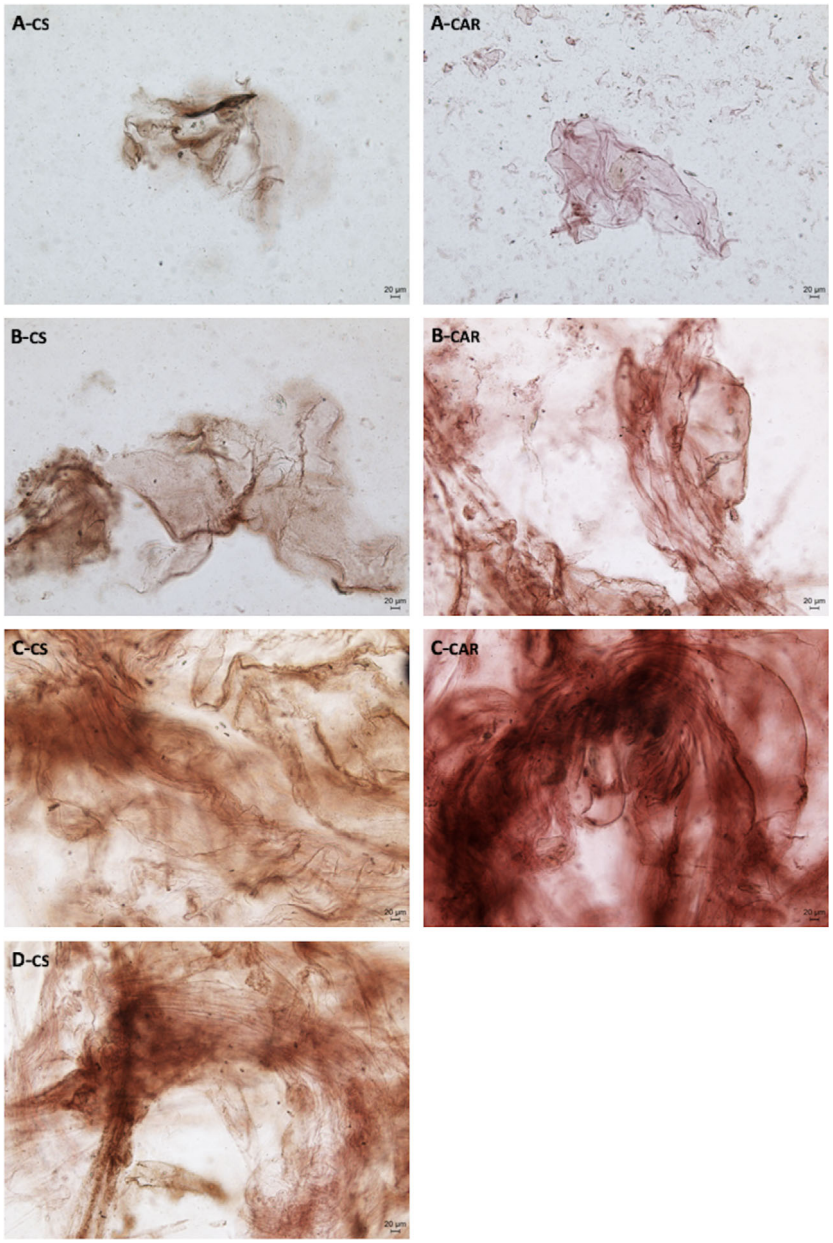

Figure 1 Light microscope micrographs of aggregates of wine:saliva mixtures in a ratio 1:1. The letters represent the mixture of saliva with Cabernet Sauvignon (CS) and Carménère (CAR), produced at different harvest dates: (a) GTH-1M, (b) GTH-15D, (c) GTH and (d) GTH+15D. The scale bar represents 20 μm.

microscope (LM) images of the wine:saliva aggregates. An increase in aggregates size was observed for both Cabernet Sauvignon and Carménère as maturation progresses, suggesting a sustained growth in the interactions with saliva proteins. Comparing both varieties, Carménère aggregates seem to show an increase of compact globular forms, while for Cabernet Sauvignon, an increase in open and flat structures is observed. The last could be related to different proanthocyanidin composition of skin and seed tannins of different grape varieties (Chira *et al.*, 2009; Obreque-Slier *et al.*, 2012).

Further examination of aggregates surface under SEM (Figure 2) reveals very different surface characteristics for both varieties. The aggregates of Cabernet Sauvignon show a smoother surface, with small granules (serrated and globular) on their surface, except for the fourth harvest date (GTH+15d) that presents aggregates more similar to Carménère. On the contrary, Carménère aggregates surface seems rougher and uneven in texture, with small spheres that protrude from their surface (flatted and globular). The micrograph analysis (Table 3) shows that Feret diameter (μm) and roundness (%) of the particles on the

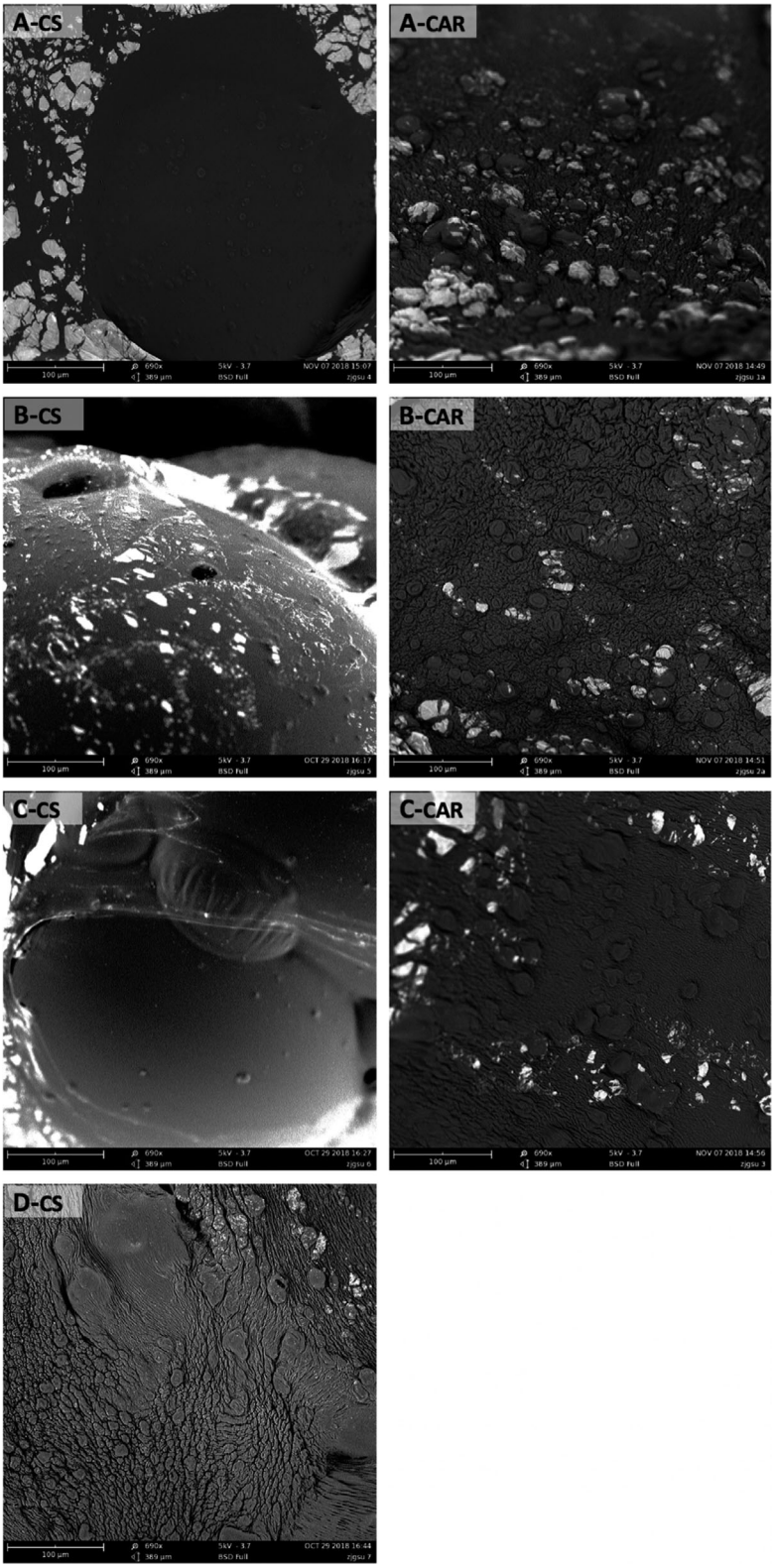

Figure 2 Scanning electronic microscope micrographs of aggregates of wine:saliva mixtures in a ratio 1:1. Letters represent the mixture of saliva with Cabernet Sauvignon (CS) and Carménère (CAR), produced at different harvest dates: (a) (GTH-1M), (b) GTH-15D, (c) GTH and (d) GTH+15D. The scale bar represents 100 μm.

aggregates surfaces have also significant differences, with an increase of both during ripening of Cabernet Sauvignon ($P < 0.0001$ and $P = 0.0002$, respectively) and a decrease of both parameters for Carménère along ripening ($P = 0.0005$ and $P = 0.0003$, respectively). Interestingly, this tendency of both varieties follows the same trend of the friction coefficients (Table 2) and the similarity of the surfaces of Cabernet Sauvignon (GTH+15d) and Carménère (GTH) aggregates is also echoed in similar results for friction coefficients (Table 2).

These results suggest that similarly to what it was proposed by de Wijk & Prinz (2006), the astringency mechanism can also be related to flocculation of particles and that their effect depends not only on their size and shape, but also on their texture.

Instrumental and sensory correlations

The results for astringency perception (intensity and dryness) and volume are shown in Table 4. Cabernet Sauvignon and Carménère showed significant differences in astringency intensity and dryness ($P = 0.029$ and $P = 0.017$) along ripening. Global astringency for Cabernet Sauvignon showed a tendency to decrease during advanced stages of ripeness, with the lowest intensity for the winemaker harvest date (GTH). Carménère showed a clear increase in the intensity of astringency with maturation. It should be noted that astringency intensity results of both varieties showed a similar trend to what it was observed for the friction coefficients of the first three harvest dates (Table 2). Dryness did not show clear trends for both varieties; however, in the case of Cabernet Sauvignon, it is interesting that the minimum value was obtained at GTH. On the other hand, although no significant differences ($P = 0.061$) were obtained, the volume descriptor showed an increase during ripening for both varieties.

TABLE 4 Sensorial characterisation of Cabernet Sauvignon and Carménère wines coming from different harvest dates

	Sensory Analysis[b]		
Wines[a]	**Astringency**	**Dryness**	**Volume**
Cabernet Sauvignon			
GTH-1m	6.1 ± 0.1^{ab}	4.4 ± 0.2^{abc}	4.2 ± 0.3^{a}
GTH-15d	6.8 ± 0.2^{a}	6.3 ± 0.1^{a}	4.5 ± 0.3^{a}
GTH	5.2 ± 0.0^{ab}	3.7 ± 0.1^{bc}	5.5 ± 0.1^{a}
GTH-15da	5.9 ± 0.1^{ab}	4.8 ± 0.2^{abc}	6.1 ± 0.1^{a}
Carménère			
GTH-1m	4.6 ± 0.2^{b}	3.2 ± 0.3^{c}	3.8 ± 0.3^{a}
GTH-15d	5.8 ± 0.1^{ab}	5.6 ± 0.1^{ab}	4.6 ± 0.2^{a}
GTH	6.2 ± 0.1^{ab}	5.2 ± 0.1^{abc}	4.8 ± 0.0^{a}
P-value	0.029*	0.005*	0.061

[a]Wines produced at different harvest dates: (1) 1 month before of grape-grower traditional harvest (GTH-1m), (2) 15 days before (GTH-15d), (3) traditional harvest defined by the grape-grower (GTH) and (4) 15 days after (GTH+15d).

[b]Sensory descriptors determined by a trained panel in triplicate.

*Significant value, with a significance level of $P < 0.05$

Principal component analysis (PCA) with significant parameters was carried out for both varieties (Figure 3; Figure 4). Significant parameters considered include chemical characterisation, characterisation of the aggregates and the sensorial data. The Cabernet Sauvignon PCA showed that the four harvest dates may easily be identified in each quadrant, and data points of each date are very close (Figure 3). The first harvest date (GTH-1m) is characterised by descriptors such as friction coefficient, mDP and precipitate weight; the second date (GTH-15d) is related to the sensory attributes of astringency intensity and drying. The third harvest date (GTH) is not associated with a particular vector while the final harvest date (GTH+15d) is characterised by volume, roundness and Feret diameter.

A closer look to this PCA showed that the friction coefficient was negatively correlated to the Feret diameter of the particles, and the diameter was positively correlated with roundness. Feret diameter is frequently used for particle shape characterisation (Pourghahramani & Forssberg, 2005), as it becomes more important in the last harvest date of Cabernet Sauvignon implies the particles increase their roundness. As it was shown previously, these particles morphologically changed from sharp and irregular (Figure 2 and Table 3, panels A-CS, B-CS and C-CS) to rounder (Figure 2 and Table 3, panel D-CS) along ripening, which means that for GTH+15, the particles are large and round. It has been reported that for rounded particles, the friction is lower than for sharp particles (Tyle, 1993; de Wijk & Prinz, 2005; Engelen *et al.*, 2005).

For the Carménère PCA, the separation of the different harvest dates studied is as obvious as in Cabernet Sauvignon (Figure 4). However, harvest dates behave differently. The first harvest date is characterised by roundness and even Feret diameter to some degree, while higher grape ripeness (GTH-15d and GTH) is characterised by descriptors such as drying, tannin content, volume, astringency intensity and friction coefficient. That is, grape ripening is related to an increase in both astringency and friction coefficient in Carménère.

This PCA also shows that roundness and astringency are negatively correlated, the same as friction coefficient with Feret diameter. This last correlation was also observed in the Cabernet Sauvignon PCA, but the descriptors were associated with opposite harvest dates. This suggests that in Carménère, the descriptors related to the shape of the particles are

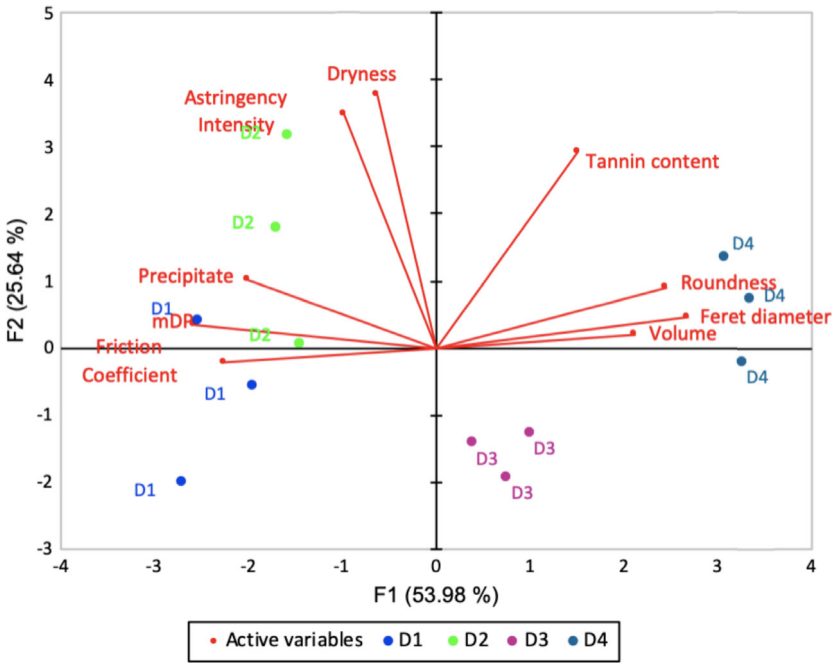

Figure 3 PCA analysis of cabernet sauvignon samples. this analysis includes sensory description of astringency and dryness by a trained panel, chemical characterisation and particle characterisation. Legend letters represent different harvest dates: (D1) (GTH1M), (D2) GTH-15D, (D3) GTH and (D4) GTH+15D.

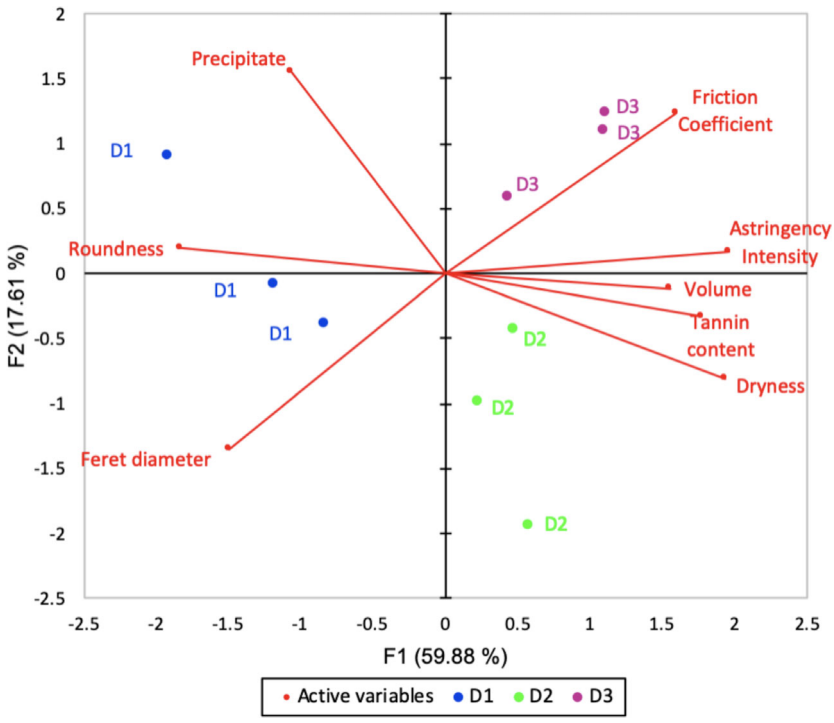

Figure 4 PCA analysis of carmenere samples. this analysis includes sensory description of astringency and dryness by a trained panel, chemical characterisation and particle characterisation. Legend letters represent different harvest dates: (D1) (GTH-1M), (D2) GTH-15D and (D3) GTH.

important during the first harvest date studied, while astringency and friction coefficient become important at the traditional harvest date (GTH) and later. This might be explained by a loss of roundness along its ripening, contrary to what it was observed for Cabernet Sauvignon. However, the role of the significant increase of tannin content along ripening for Carménère wines needs to be taken into consideration (Table 1) as tannin content also increases the astringency through the formation of more protein–tannin

aggregates (Green, 1993; Ma *et al*., 2014; Brossard *et al*., 2016; Upadhyay *et al*., 2016; García-Estévez *et al*., 2018; Soares *et al*., 2018).

Implications on astringency mechanisms and astringency sub-qualities

The correlations shown in the PCA (Figure 3; Figure 4) suggest an important role of both tannin content and aggregates on both astringency perception and oral lubrication. Also, it is possible to observe a common pattern that inversely relates both roundness and Feret diameter of aggregates to the coefficient of friction. When comparing Carménère, usually described with a velvety astringency but that showed a high astringency intensity (Table 4), the amount of precipitated aggregates is significantly lower than in Cabernet Sauvignon (Table 2). This suggests that the aggregates could have the capacity of modulating the perceived astringency (intensity and sub-quality) in function of their amount and surface characteristics. This might imply that astringency intensity could be related to the number of aggregates present in mouth (Figure 5a), and the astringency sub-quality could be related to the characteristics of aggregates themselves (Figure 5b), as proposed in Figure 5.

Pradal & Stokes (2016) propose that three situations can be envisaged when adding food systems to a pre-adsorbed salivary film: 1. food compounds and saliva do not interact, and lubrication properties of saliva remain intact; 2. food compounds and saliva interact, and lubrication properties of saliva are altered and the friction coefficient increases; and 3. food compounds and saliva interact synergistically, and lubrication properties of saliva increase or decrease the friction coefficient (this effect is produced by 'muco-adhesive' molecules). In the case of tannin–protein interactions, it has been proposed that precipitates have an effect on mouth friction, as well as a 'free' effect only dominated by the interaction itself (non-precipitation), both of them associated with an increase in friction and loss of mouth lubrication (Kallithraka *et al*., 2001; Vidal *et al*., 2003; Pascal *et al*., 2008). Although most existing evidence shows that tannins and saliva combined provide less lubrication than saliva alone, the possibility of finding a synergic relation between tannins and saliva proteins with an increase in mouth lubrication has never been explored.

Another parameter to be taken in consideration to explain the different astringency sub-qualities of Cabernet Sauvignon and Carménère could be related to the significant difference in anthocyanin and tannin content (Table 1). Anthocyanins, pigments responsible for the red/violet colour of red wines (Khoo *et al*., 2017; Soares *et al*., 2019), can interact with flavan-3-ols through copigmentation and or polymerisation (Yoshida *et al*., 2009; Soares *et al*., 2019). Copigmentation could have an influence in astringency, but its role remains controversial. It has been reported that anthocyanins can increase astringency sub-qualities such as 'fine grain' (Vidal *et al*., 2004; Sáenz-Navajas *et al*., 2017; Paissoni *et al*., 2018) and also that coumaroylated and acetylated anthocyanins contribute to both astringency and bitterness (Gonzalo-Diago *et al*., 2014). According to our results, the concentration of anthocyanins of our Carménère wines doubles the concentration measured in Cabernet Sauvignon for all the studied dates (Table 1). It has also been reported that anthocyanins are capable to form soluble complexes with salivary protein, and this interaction increases the astringency sensation (Ferrer-Gallego *et al*., 2015; Paissoni *et al*., 2018). Astringency intensity in wine also shows a strong

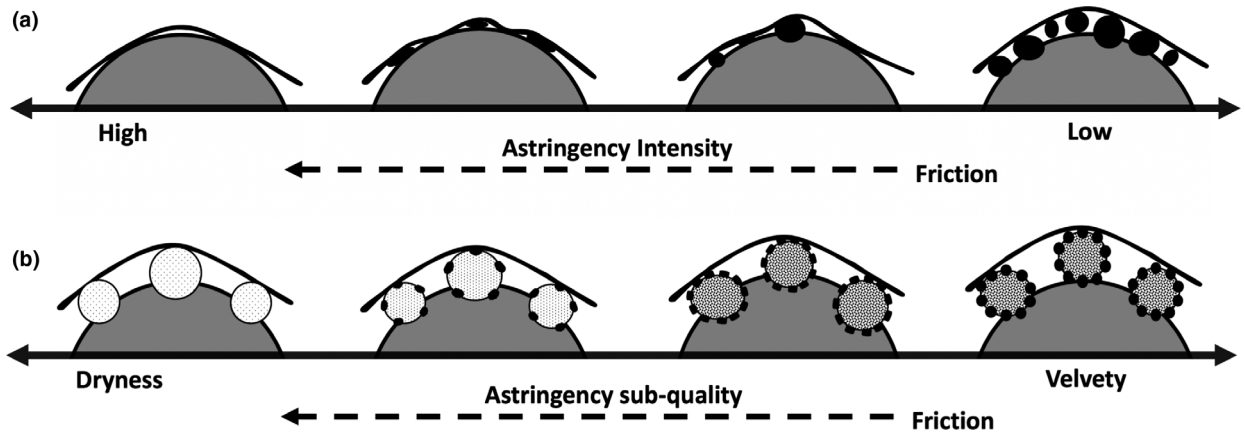

Figure 5 Schematic representation of the role of aggregates on friction. Letter (a) represents the proposed mechanism for astringency intensity, and specifically, the black spheres depict the number of aggregates between the tongue and palate; (b) represents the proposed mechanism for dryness, and specifically, the spheres with texture and elements on their surface represent the surface characteristics of the aggregates.

positive relationship with tannins concentration (Harbertson *et al*., 2014, Kallithraka *et al*., 2011) as it was shown for Carménère (Table 4). All these factors might explain the increase of astringency intensity obtained in Carménère along ripening (Table 4).

It is known that the size of tannin molecules or mean degree of polymerisation (mDP) is positively correlated to protein precipitation (Le Bourvellec & Renard, 2012; Watrelot & Norton, 2020). While mDP values remained mostly the same for Carménère during the three harvest dates, the values obtained for Cabernet Sauvignon decrease to one half when the first and the last harvest dates are compared (Table 1). These results support the tendency to a decrease in the astringency sensation with time shown by Cabernet Sauvignon in this study (Table 4). The results obtained for Carménère, however, need to be further studied because they do not explain the increase in astringency observed in this study (Table 4).

From the point of view of this study limitations, although previous studies support the use of tribology as an approximation to understand oral processing, it still needs improvement. In particular, the use of stainless steel sliding probes, differences in instrumental and *in vivo* speeds or the use of centrifuged saliva are not exactly the same conditions as in the human oral cavity. Future work is required to validate these findings and test hypothesis for different wines and conditions.

Conclusions

This preliminary study has examined the feasibility of tannin–protein aggregates as modulators of sensory astringency and oral lubrication. Results from this work on the friction coefficient suggest that both soluble and insoluble aggregates could be responsible for oral lubrication modulation. Here, we propose a mechanism for astringency intensity and its sub-qualities that illustrates the role of the aggregates. The model for astringency sub-qualities takes into consideration not only the presence of the particles, but also their shape, size and texture. These aggregates could be sensed as velvety or harsh depending on their effect on the friction coefficient. Findings of this work reveal a complex ripening evolution that can lead to opposite sub-qualities. However, other variables such as anthocyanins and tannin mDP also need to be taken into consideration in future studies to better explain the differences between Cabernet Sauvignon and Carménère wines. We hope this data contributes to the understanding of mouthfeel, wine astringency sub-qualities, but also to the knowledge of astringency evolution during grape ripening. This aspect could help to better plan harvest dates, to produce specific wine styles and to meet consumer expectancies.

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Conflict of interest

The authors declare that they do not have any conflict of interest.

Author contributions

Natalia Brossard: Conceptualization (lead); Data curation (lead); Formal analysis (lead); Funding acquisition (lead); Investigation (lead); Methodology (lead); Project administration (lead); Resources (lead); Software (lead); Supervision (lead); Validation (lead); Writing-original draft (lead); Writing-review & editing (lead). **Beatriz Gonzalez-Muñoz:** Formal analysis (supporting); Writing-original draft (equal); Writing-review & editing (equal). **Carolina Pavez:** Formal analysis (supporting); Software (supporting); Validation (supporting); Writing-review & editing (supporting). **Arianna Ricci:** Data curation (equal); Formal analysis (equal); Software (equal); Validation (equal); Writing-review & editing (equal). **Xinmiao Wang:** Data curation (supporting); Formal analysis (supporting); Writing-review & editing (supporting). **Fernando Osorio:** Conceptualization (lead); Formal analysis (equal); Methodology (lead); Validation (equal); Writing-review & editing (equal). **Edmundo Bordeu:** Conceptualization (lead); Formal analysis (lead); Methodology (lead); Supervision (lead); Writing-review & editing (lead). **Giuseppina Paola Parpinello:** Conceptualization (lead); Formal analysis (lead); Methodology (lead); Resources (lead); Supervision (lead); Writing-review & editing (lead). **Jianshe Chen:** Conceptualization (lead); Formal analysis (lead); Methodology (lead); Resources (lead); Supervision (lead); Validation (equal); Writing-review & editing (lead).

Ethical statements

Informed Consent: Written informed consent was obtained from all study participants.

Peer review

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Data availability statement

Data are available on request from the authors.

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