Article:
Laguna, L, Sarkar, A orcid.org/0000-0003-1742-2122, Bryant, MG orcid.org/0000-0003-4442-5169 et al. (3 more authors) (2017) Exploring mouthfeel in model wines: Sensory-to-instrumental approaches. Food Research International, 102. pp. 478-486. ISSN 0963-9969

https://doi.org/10.1016/j.foodres.2017.09.009

Exploring mouthfeel in model wines: Sensory-to-instrumental approaches

Laura Laguna[1]*, Anwesha Sarkar[2], Michael Bryant[3], Rob Beadling[3], Begoña Bartolomé[1], M.Victoria Moreno-Arribas[1]

[1]Institute of Food Science Research (CIAL), CSIC-UAM

C/ Nicolás Cabrera 9, 28049, Madrid, Spain

[2]Food Colloids and Processing Group, School of Food Science and Nutrition, University of Leeds, LS2 9JT, United Kingdom

[3] Institute of functional surfaces (iFS), School of Mechanical Engineering, University of Leeds, LS2 9JT

[*]Corresponding author

Tel: (00)34 910017900- 822

Email: laura.laguna@csic.es

Abstract

Wine creates a group of oral-tactile stimulations not related to taste or aroma, such as astringency or fullness; better known as mouthfeel. During wine consumption, mouthfeel is affected by ethanol content, phenolic compounds and their interactions with the oral components. Mouthfeel arises through changes in the salivary film when wine is consumed. In order to understand the role of each wine component, eight different model wines with/without ethanol (8%), glycerol (10 g/L) and commercial tannins (1 g/L) were described using a trained panel. Descriptive analysis techniques were used to train the panel and measure the intensity of the mouthfeel attributes. Alongside, the suitability of different instrumental techniques (rheology, particle size, tribology and microstructure ,using Transmission Electron Microscopy (TEM)) to measure wine mouthfeel sensation was investigated. Panelists discriminated samples based on their tactile-related components (ethanol, glycerol and tannins) at the levels found naturally in wine. Higher scores were found for all sensory attributes in the samples containing ethanol. Sensory astringency was associated mainly with the addition of tannins to the wine model and glycerol did not seem to play a discriminating role at the levels found in red wines. Visual viscosity was correlated with instrumental viscosity ($R=0.815$, $p=0.014$). Hydrodynamic diameter of saliva showed an increase in presence of tannins (almost 2.5-3-folds). However, presence of ethanol or glycerol decreased hydrodynamic diameter. These results were related with the sensory astringency and earthiness as well as with the formation of nano-complexes as observed by TEM. Rheologically, the most viscous samples were those containing glycerol or tannins. Tribology results showed that at a boundary lubrication regime, differences in traction coefficient lubrication were due by the presence of glycerol. However, no differences in traction coefficients were observed in presence/absence of tannins. It is therefore necessary to use an integrative approach that combines complementary instrumental techniques for mouthfeel perception characterization.

Key words: wine mouthfeel, trained sensory panel, particle size, viscosity, astringency, tribology

1. Introduction

Wine is a unique and complex matrix that creates numerous sensations. These sensations appear even before the wine is consumed and persist even after the wine is swallowed (also called the finish of the wine). Aromas greatly influence the hedonic behaviour, starting with its initial smell in the glass, continuing with the wine being processed in the mouth, mixed with saliva and the after swallowing feelings, created by the breathing airflow (Munoz-Gonzalez, Martin-Alvarez, Moreno-Arribas, & Pozo-Bayon, 2014). Moreover, in wine, as consequence of oral-tactile stimulations, there is also another group of sensations not related with taste or aroma. These include astringency, body, burning, balance, pricking (Jackson, 2009), warmth and viscosity (Gawel, Oberholster, & Francis, 2000). These sensations are believed to be affected mainly by the ethanol content (King, Dunn, & Heymann, 2013), phenolic compounds (Ferrer-Gallego, Hernández-Hierro, Rivas-Gonzalo, & Escribano-Bailón, 2014; Quijada-Morin, Williams, Rivas-Gonzalo, Doco, & Escribano-Bailon, 2014) and their interaction with the oral components and/or oral physiological factors. Oral-tactile sensations arise mainly from the changes induced by the consumed food and/or beverage in the integrity of the salivary film perceived, which is perceived by the filiform papillae. As these papillae are highly innervated by free nerves endings (also called tactile sensors), they transfer any sensory input caused by the change in the salivary film by the trigeminal nerve through the trigeminal ganglion to the brainstem receptive areas (Jacobs et al., 2002). This is where the multimodal information is integrated (Verhagen & Engelen, 2006) and a perception of food ingestion is created.

Oral-tactile sensations are also known as mouthfeel sensations (DeMiglio, Pickering, & Reynolds, 2002) and usually are described by sensory analysis techniques, such as descriptive analysis, in which a trained panels define these sensations and score their intensities. In spite of the importance of wine tasting, the use of a sensory panel can be expensive and the training can be longer than instrumental characterization. Also, it is possible that the terms used by an expert with special sensory training may not be understood by others (Lehrer & Lehrer, 2016). Furthermore as panelists are trained or specialized in a determined product or set of products, what is a “heavy” wine for a California Pinot Noir trained panelist could be “light” for a French Burgundie panelist (Lehrer & Lehrer, 2016) and vice versa, making it difficult for cross- country comparisons.

Therefore, if wine mouthfeel could be quantitatively measured using an instrumental technique, that may allow wineries to have a faster, repeatable, harmonized and cheaper characterization complementary to the use of a panel of experts (Laguna, Bartolomé, & Moreno-Arribas, 2017; Laguna & Sarkar, 2017). This would be an innovative approach for enologists to modulate the astringency and quality characteristics of wines (Rinaldi, Gambuti, & Moio, 2012).

However, the key challenge lies in quantifying the sensory “mouth feel” feelings with instrumental technique taking into account the wine properties and its interactions with the human saliva. Our main hypothesis is that wine mouthfeel could be characterized by a combination of instrumental techniques based on the study of the interaction of saliva and wine components, fluid flow behaviour and frictional forces. Until now, changes in

rheological properties of wine upon consumption have not been well understood. Neto et al. 2015 measured the viscosity of wines at different temperatures with varying alcohol, dry extract and reducing sugar contents. Results showed that density and viscosity of wines decreased at higher temperatures. Regardless of temperature, wine viscosity was mainly affected by the dry extract, whereas wine density was mainly influenced by the alcohol content. It is worth noting that authors studied the wine in isolation and not in presence of saliva and did not perform any sensory analysis. Hence, it is unclear if such instrumental changes had any impact on the sensory perception. Prinz and Lucas (2000) studied the changes of viscosity of saliva by adding powdered tannic acid until saturation, and they observed a decrease in magnitude of the viscosity of saliva. However, such saturated tannic acid solution might not represent the wine matrix.

More importantly, wine mouthfeel does not only depend on flow properties (rheology). In mouth, saliva forms a pellicle that act as a lubricant. In presence of polyphenolic compounds, salivary proteins tend to form complexes (Hagerman & Butler, 1981) that causes rupturing of the salivary pellicle. As a consequence, there is an increased activation of mechanoreceptors, located within the mucosa (Horne, Hayes, & Lawless, 2002; Kallithraka, Bakker, & Clifford, 1997; Lesschaeve & Noble, 2005). Based on this, wine mouthfeel in presence of saliva can be characterised using mechano-surface techniques, such as tribology (Pradal & Stokes, 2016; Upadhyay, Brossard, & Chen, 2016). Using a Mini Traction Machine with polydimethyl siloxane material, "chemically pure" polyphenols (epigallocatechin gallate) were added to saliva (Rossetti, Bongaerts, Wantling, Stokes, & Williamson, 2009) and it was found that catechin-induced astringency was related to a loss of saliva lubrication. Later, Brossard, Cai, Osorio, Bordeu, and Chen (2016) studied the friction properties of saliva-wine system by using a purpose-built tribometer (device attached to a Texture Analyser) with a stainless steel-PDMS system. Authors compared the friction coefficient of saliva in presence of wines (real and model wine) indicating that the coefficient of friction of saliva increased in presence of wine. It is worth highlighting that in this study only four wines mixed with saliva were assessed, , and the surfaces of steel ball used might not be representative of the oral surfaces (Brossard et al., 2016). Therefore it is difficult to establish whether tribology is a predictive quantitative tool for astringency characterization in wines or not because of a low number of samples investigated with a large deviation (Pradal & Stokes, 2016). Furthermore, currently rare attention has been paid in literature to understand the change in salivary film in presence of other wine components, especially those known to alter the mouthfeel sensations, such as tannins or alcohol.

In order to gain deeper in the understanding of the influence of individual wine components on mouthfeel, this study has two main objectives: (i) to study the oral sensations perceived and described by a trained panel using model wine with special emphasis on mouthfeel characteristics, (ii) to use a combination of instrumental techniques (dynamic light scattering, rheology, tribology and electron microscopy) that can help to unravel those oral sensations in ex vivo or in vitro representative conditions.

2. Material and methods

2.1. Model wine

Model wine components were chosen based on real red wine components. Samples were created with either presence of absence of ethanol (E) (ethanol absolute food grade, AppliChem, Panreac, Barcelona, Spain), glycerol (G) (Mineral Waters, Purflee, United Kingdom) and tannins (T) (oak tannin, Agrovin, S.A., Ciudad Real, Spain) in a model wine matrix (W). W contained commercial inactive dry yeast (Superbouquet MN, Agrovin, Ciudad Real, Spain), seed extract (Vitaflavan, Les Dérivés Résiniques et Terpéniques, France), reduced L-glutathione (Solgar, Leonia, N.J. EEUU) and tartaric acid (Mineral Waters, Purflee, United Kingdom). All components used were food grade and were dissolved in still water (Agua Mineral Fuente Alta, Spain).

Initially the ethanol level chosen was 14%, however, that resulted in overpowering of the senses due to the flavor intensity of pure ethanol and thus it was not drinkable. For that reason, ethanol level was chosen on the basis of the minimum alcohol of wine (8%) from a legal perspective.

Samples were formulated one day before the analysis, filtered and adjusted to pH 3.8 using tartaric acid (1%) and kept in darkness at 17 ºC until analysis. The eight different formulations studied are shown in Table 1.

2.2. Descriptive sensory analysis

A panel of 13 assessors (10 women and 3 men, 21-50 years old) participated in the quantitative sensory analysis (QDA®) (Stone, Sidel, Oliver, Woolsey, & Singleton, 2008) of the wine model solutions.

The panel members had various experience participating in wine sensory sessions. To start with, they attended an informative session about sensory analysis and a detailed explanation about sensory threshold, mouthfeel perception, QDA technique and the time implication.

Their sensory thresholds were tested twice for tannins, glycerol and ethanol. Tartaric acid solutions (to achieve a pH=3.8) with dispersed tannins (at a concentration of: 0.01, 0.025, 0.05, 0.1.0.25.0.75 and 1.2 w/v%), ethanol (at concentration of: 0.1, 0.2, 0.4, 0.6, 0.8, 1, 2 and 4 v/v%) and glycerol (at a concentration of: 0.5, 1, 2, and 4 w/v%) were used. The purpose of these threshold tests were not only to assure that the assesors were able to perceive the comoponents at the levels presented in the model wine, but also to help them identify in the upcoming sensory sessions, the potential mouthfeel changes. During the test of sensory threshold, panelists wore blindfolds and nose clips. Recently, it has been published that most of the terms used in the mouthfeel wheel might not be adequate to characterized astringency (Vidal, Giménez, Medina, Boido, & Ares, 2015). Therefore, the descriptors for the model wines were developed by the panel members using the checklist method (Lawless & Heymann, 2010).

Panelists were instructed to focus on the mouthfeel characteristics, but if they believed that a particular taste or aroma was a key wine discriminating attribute, they were encouraged to write them down. After the first session, the panel leader collected and wrote all the

attributes on a board. The panel discussed the appropriateness of the selected attributes, their definitions, and procedures for assessing them. At the end of the session, a consensus on the list of attributes and procedures was reached. A second session to remind and check the agreement of all panelists was done. Following this, 8 sessions of training were attended by the panelists over a period of three weeks (2 sessions per week, until stdv<2.0 points was achieved in a 10 cm unstructured scale). In order to help them in this training, components at higher concentrations were given to compare against water at the beginning of the initial sessions. Therefore, solutions of ethanol (15%, maximum concentration of ethanol present in wines), glycerol (4%, double the concentration present in dessert wines) and tannins (1%) were presented. Tannin solution was labelled by the panelist as astringent, dry, wood taste and bitter; alcohol solution was labelled as hot and alcoholic and glycerol solution was labelled as viscous and sweet.

For the formal assessment (by triplicate), the panelists attended three sessions on different days. In each session, panelists received the samples in two blocks, with a delay of 30 minutes between blocks. They evaluated first the samples without ethanol, and later the ones containing ethanol. This was done because the residual ethanol flavours can linger after finishing the taste of a sample, and it could stun the sense for the non-ethanol containing samples. Panelists were advised to rest one minute in between samples and were offered water, crackers and carrots as palate cleansers.

Panelists rated visual attributes before consumption (sediments, colour, viscosity), in-mouth attributes (taste: sweetness, bitterness, acid taste and wood taste; mouthfeel: astringency, dryness, earthiness, hot feeling, alcoholic feeling and viscosity) and after feeling (overall persistency, alcohol persistency and wood after taste). However, three of those attributes: sediment, in mouth viscosity and sweetness were removed after the third session because no consensus was obtained among the panelists. In Table 2, the descriptors and the extremes of the scale are shown.

For all the training sessions and formal assessment, panelists used a 10 cm unstructured scales to score the selected attributes for the model wine. Twenty milliliters of model wine was presented in a wineglass labeled with 3-digit random codes. All tests were conducted with samples at 17°C that is the red wine serving temperature.

2.3. Particle size measurement

Dynamic light scattering was used to measure the size of aggregates (if any) formed due to the interaction between wine components and salivary proteins (human saliva, HS), using a Zetasizer Nano (Malvern instrument, Malvern, UK), equipped with a 4 mW He-Ne laser (output wavelength of 633 nm). The test was carried out with the addition of fresh HS from ten donors to the eight different model wine formulations in a ratio 1:1 (w/w). This part of the study has approved by Faculty Ethics committee at University of Leeds [ethics reference (MEEC 15-052)]. Hydrodynamic diameter (Z-average diameter) of human salivary proteins in absence or presence of different model wines without dilution was measured using back-scattering technology at a detection angle of 173ºC. The model wine and HS were mixed at 37 ºC for 10 min to ensure interaction (if any), which is higher than the general residence time of wine.

Each sample was run three times; each run consisted of three acquisitions that lasted for 60 s/acquisition. The result was reported as the mean and the standard deviation calculated from the nine readings from an individual sample.

2.4. Rheology

The rheological test was carried out for the wine samples in presence of fresh HS provided by one donor (model wine:saliva= 1:1 (w/w)). As the viscoelasticity of saliva decreases with storage (Stokes & Davies, 2007), the HS was used immediately after collection. The shear rate was measured in a rotational Kinexus rheometer (Malvern, UK). The rheometer was equipped with a 60 mm of cone (1˚) and plate geometry with a gap of 0.03 mm. One milliliter of a mixture of HS and model wine was placed with a pipette onto a pre-heated plate (37 °C). A temperature cover was used to maintain the samples at the specified temperature (37 °C) and avoid evaporation. In order to avoid protein-air adsorption a solution of 0.1% of SDS was applied on the edge of the cone-plate geometry (Stokes & Davies, 2007). Flow curves were obtained for samples at a shear rate ranging from 0.01-100 s^{-1}. Data from the flow curves were fitted to the Ostwald de Waele fit ($\sigma = K\dot{\gamma}^n$), where K (Pa s^n) is the consistency index and n is the flow index. At least three measurements were performed per sample.

2.5. Tribology experiments

It is recognized that no fluid is capable of mimicking all the properties of real HS (Rossetti et al., 2009; Stokes & Davies, 2007). However, due to the large quantity of saliva needed for each individual tribological experiment, a solution mimicking the ionic strength, pH and mucin concentration of saliva (SS) was used in this study. The SS contained 0.636g of K_2HPO_4, 1.594 g of NaCl, 0.202g of KCl, 0.021g of uric acid, 0.198g of urea and 3 g of mucin (porcine gastric mucin II, (Sigma Chemical Co., St. Louis, MO, USA) in 1 L of Milli Q water (purified by a Milli-Q system) (Sarkar, Goh, & Singh, 2009). Porcine gastric mucin was used as it simulated the rheological properties of human saliva at the afore-mentioned concentration. Milli-Q water (water purified by treatment with a Milli-Q apparatus; Millipore Corp., Bedford, MA, USA) was used as the solvent for simulated saliva preparation.

Friction measurements were performed at 37 ^{0} C using a Mini-Traction Machine (MTM, PCS Instruments Ltd., UK) operated under low-load conditions. The tribo-pairs consisted of Polydimethylsiloxane (PDMS, PCS instruments Ltd, UK) ball with a diameter of 19.5 mm and a flat disc (46 mm), latter with a thickness of 5 mm; both of which rotated about their axis producing a sliding-rolling contact. For all experiments, a normal load (L) of 1 N was applied. Stribeck type analysis was conducted in an attempt to identify the lubricating properties of each wine-SS mixture by varying the sliding speed of the plate from 1 to 1000 mm/s and then was decreased stepwise from 1000 mm/s to 1mm/s and the resultant traction coefficient was observed. Three replicates were done per sample.

Prior to each of the test, each ball and plate was submerged in SS for 15 minutes to facilitate the adsorption of the simulated saliva film. Surfaces were then placed within the tribometer and 30 mL of each model wine added. For the tribology analysis, it was decided to select samples with and without T and with and without EG were selected. Then, the following

samples W, WT, WEG and WEGT were analyzed. The same analysis was also completed for contacts immersed in distilled water and SS

2.6. Transmission electron microscopy

Negative-Stain Transmission Electron Microscopy (TEM) images were used to visualize the microstructure of the polyphenols and HS complexes. Immediately after mixing, the sample was fixed onto a copper mesh grid and stained with a phosphotungstic acid solution (2%) for 4 min and air dried at room temperature after excess liquid had been removed by a filter paper.

2.7. Statistical treatment

Analysis of variance (one way-ANOVA) was applied to study the differences between the wine formulations in descriptive sensory analysis, particle size and rheology. For each test, the dependent variable was the results obtained by the trained panel, the Zetasizer or by the rheometer, and the independent variables were the model wine formulations. Tukey test was used for post hoc mean comparisons. To investigate components' influence on descriptive sensory attributes, analysis of variance for one dependent variable with ethanol, tannis and glycerol as fixed factors was performed. For the descriptive sensory analysis, all the sensory attributes were used as dependent variables, whereas the independent variables were the wine components: ethanol, tannins and glycerol.

Pearson's correlation of the instrumental analysis and mean intensity scores in the sensory descriptive test were computed.

These test were done using IBM SPSS Statistics for Windows, Version 22.0. (Armonk, NY: IBM Corp).

3. Results

3.1. Sensory descriptive analysis by a trained panel

The mean scores of the sensory analysis results are shown in Figure 1. All sensory attributes were significantly affected by at least one of the wine components under study. For better visualization, samples with and without ethanol were plotted separately.

[FIGURE 1]

Visual attributes. Samples with tannins (WET, WEGT, WT and WGT) were perceived higher in amber colour intensity than samples without tannin (W, WG, WEG, WE). Visual viscosity was influenced by the presence of ethanol and tannin ($F_{ethanol/visual\ viscosity}$=21.49; $p<0.001$; $F_{tannin/visual\ viscosity}$=14.33; $p<0.001$), but not their interaction ($p>0.05$). Contrary to the widely accepted information that glycerol, provides viscosity, it did not influence the visual viscosity significantly ($p_{glycerol}$=0.142).

In-mouth attributes. Sample W was scored as the most acid one, although pH was adjusted for all the samples to 3.8 measured in all samples. Bitter taste was higher for samples containing ethanol and tannin ($F_{ethanol/bitter}$=21.49; $p<0.001$; $F_{tannin/bitter}$=14.33;$p<0.001$),. However, the interaction between ethanol and tannin was not statistically significant ($p_{ethanol*tannin/bitter}$ =0.387). Glycerol at the concentrations used did not influence bitterness ($p_{glycerol/bitter}$ =0.455).

Earthiness was scored significantly higher for samples containing tannins ($F_{tannins/earthiness}$=21. 37, p<0.001).

Regarding the attributes taste and aftertaste of wood two groups were clearly identified: with and without tannins (significantly p <0.05, see Figure 1). Model wines containing tannins had mean wood taste intensity of ~ 4 points, while samples without tannins were rated from 0.5 to 2. The aftertaste of wood, was rated slightly lower than wood taste (~ 3.5) for samples with tannins and almost zero for samples without tannins. Although wood taste was mainly caused by tannins ($F_{tannins/wood\ taste}$ = 38.13; p<0.001), the presence of ethanol also influenced such taste significantly ($F_{ethanol/wood\ taste}$ = 4.68; p=0.031), and had interactions with tannins (p=0.010).

Although samples 'perceived astringency was mainly governed by the tannin content ($F_{tannin/astringency}$=28.31; p<0.001), ethanol also had a significant influence on this attribute ($F_{ethanol/astringency}$=6.77; p<0.01).

Ethanol was the only component that caused hot sensation ($F_{ethanoll/hot\ sensation}$=161.86; p<0.001). Therefore, WEG, WE, WET and WEGT samples were scored with 6 points of intensity difference (Figure 1).

Similar to the astringency, dryness was affected by ethanol and tannins, but unlike the case with the astringency, the effect of ethanol was higher ($F_{ethanol/dryness}$= 35.43, p=0.01) than that of tannin ($F_{tannin/dryness}$ = 11.56, p=0.01). No effect by the interaction of components was found. Therefore, the WET, WE, WEG and WEGT samples were scored with greater intensity than the WT and WGT samples (Figure 1).

3.2.Particle size

Figure 2 shows the hydrodynamic diameter of the salivary proteins in absence or presence of wine components. As it can be observed, the hydrodynamic diameter of HS proteins was 96.61 nm.

[FIGURE 2]

An increase of diameter was observed for the sample WEG a. A, almost 2.5-3-folds increase in hydrodynamic diameter of salivary proteins was shown in presence of tannins (WT), which might suggest some degree of aggregation of the salivary proteins. Interestingly, the increase in the hydrodynamic diameter caused by tannins was lower in presence of ethanol and glycerol (decreased from 288. 86 nm to 184.2 nm).

3.3.Dynamic viscosity

For better visualization, samples with and without ethanol have been represented separately (Figure 3a and Figure 3b). Additionally, a table with the viscosity at a shear of 1 s^{-1} and the fitting the curve to Ostwald de Waele fit ($\sigma = K\dot{\gamma}^n$) is shown.

The HS was the most viscous sample and when water was added in the same ratio as compared to that of the wine models (1:1 w/w), a dilution effect was observed with HS becoming less viscous. Therefore, HS+water was used to compare the wines and not just HS. As it can be observed in Figure 3, the most viscous samples were those containing

glycerol or tannins (WG, WGT, WET). Sample W had similar viscosity as compared to that of HS+water, and WE was the comparatively less viscous.

In summary, the three components (E, G, T) added to W influenced the viscosity of the systems significantly ($p<0.05$). Ethanol significantly decreased the viscosity ($F_{ethanol}=19.93$, $p=0.001$), whilst glycerol and tannins provided a viscosity increment ($F_{glycerol}=12.31$, $p=0.002$; $F_{tannins}=43, 76$, $p=0.001$).

[FIGURE 3]

3.4.Tribology

Figure 4 shows the friction coefficient versus entrainment speed for each sample analyzed. For easiness of interpretation, l trend line was fitted.

At lower entrainment speed (< 100 mm/sec), typically defined as a boundary lubrication regime, differences in the traction coefficient were observed. Surfaces wetted by distilled water demonstrated the highest traction coefficients due to their hydrophilic nature when compared to other samples. Within the boundary lubricated regimes, 'W' and 'WG' demonstrated the highest and lowest traction coefficient respectively. As sliding speed increased (>100 mm/sec), the traction coefficient for each sample decreased, typically explained through the transition into a 'mixed' lubrication regime. As expected all samples appeared to converge towards a similar traction coefficient value with further increase in the entrainment velocity. For all samples, except distilled water, some hysteresis was observed within the traction coefficient. A higher traction coefficient was typically observed with decreasing entrainment speed with little differences was observed between the wine samples at both higher and lower sliding speeds. This indicates that some structural change within the lubricant might have occurred as a result of frictional dissipation at the contacting interfaces. Although traction coefficients were higher during the reverse traction phase when compared to the forward traction phase, model wine samples imparted some lubricity when compared to distilled water.

Figure 4 further demonstrates the lubricating capacity of SS, showing the ability of mucins to lubricate. An atypical traction plot was observed as a function of entrainment speed with no distinct transition from a boundary to mixed lubrication regime observed. This is presumably due to the electrostatic affinity ot the anionic mucins to the positively charges surfaces (i.e. PDMS), potential hydrophobic interactions between mucin and PDMS as well as the exhibition of highly non-Newtonian properties of the SS. Differences in the reverse traction phases were again observed, with a prolonged transition from mixed to boundary lubricated regimes seen and a lower traction coefficient observed. Whilst SS imparted superior lubrication properties when compared to model wines and distilled water, it is evident that some structural changes had occurred to the lubricant during the forward traction phase.

[FIGURE 4]

3.5. Complexes observed by TEM

Figure 5 shows the TEM images of HS, HS/water mixture and HS/model wines mixtures.

[FIGURE 5]

As it can be observed, aggregates were formed in HS/model wine mixtures (Figures 5c, 5d, 5e, 5f). Aggregates were absent in HS/water mixtures (Figure 5b).. These aggregates were observed more neatly in those samples with extra addition of tannins (5d: WT; 5f: WEGT), which is consistent with dynamic light scattering data (Figure 2). It cannot be ignored that the saliva pellicle structure had changed in samples even in presence of wine matrix components alone (Figure 5c).

4. Discussion

The present study constitutes one of the first approaches to integrate sensory evaluation and a range of complementary instrumental techniques (rheology, tribology, dynamic light scattering and electron microstructure) for evaluating the role of individual and/or group of major wine components on mouthfeel. As expected, components added (glycerol, tannins and ethanol) created different sensory properties, captured by the trained panel. Our study also showed how rheology, particle size and tribology results, in the boundary regimes, were able to quantitatively discriminate the samples and related them with the sensory assessment.

Rheological results showed that some of the wine components changed the behaviour of the saliva due to its own physical properties and not essentially due to a saliva-wine ingredient interaction. In other words, it was observed that samples with glycerol had higher viscosities than samples with ethanol (see Figure 3). This was expected as viscosity of ethanol (η_{20^oC}=0.0012 Pa.s) is three orders of magnitude lower than that of glycerol (η_{20^oC}=1.069 Pa.s). Yanniotis, Kotseridis, Orfanidou, and Petraki (2007) measured wine at 16 ^{0}C with different ethanol and glycerol content (0–15% v/v, 0–20 g/L, respectively). At these concentrations, the viscosity almost varied linearly with ethanol and glycerol concentration. For example, for every 1% (v/v) increase in ethanol concentration, viscosity increased by $0.047x10^{-3}$ Pa·s and for every g/L increase in glycerol concentration, viscosity increased by $0.005x10^{-3}$ Pa·s.

It was possible to measure instrumentally viscosity differences of model wine and HS mixtures; these were correlated with the visual viscosity reported by the trained panel s ($R_{intrumental/visual\ viscosity}$=0.815, p=0.014). However, visual and instrumental viscosity were influenced differently by the wine components. For example, visual viscosity was influenced by ethanol and tannins but not by glycerol..

In human saliva, Prinz and Lucas (2000) added powdered tannic acid until saturation to fresh saliva, showing a decrease in viscosity of saliva. In our case, 0.1% of tannic solution was added to saliva (HS+WT), and in comparison with HS +water, the viscosity increased. This difference can be explained based on the fact that Prinz and Lucas (2000) used powdered tannic acid with a supersaturation effect, whilst the quantity of tannic acid used in the model wines in this study, was much lower, producing higher viscosity but not precipitating the salivary proteins.

Saliva in presence of wine with tannins, showed an increase in the hydrodynamic diameter, which was in congruence with formation of nano-complexes as observed by TEM micrographs 5d). This suggests that these complexes were potentially responsible for the

viscosity increases which were eventually broken down in the direction of flow supporting the Non-Newtonian behavior.. Similar polyphenols-saliva aggregates were found by Brossard et al. (2016) in red wines-saliva mixtures. These complexes can be attributed to the polyphenolic compounds in wines forming complexes with salivary proline-rich proteins (PRP) (Hagerman & Butler, 1981). The consensus is that these complexes, saliva protein and wine polyphenols, are formed via hydrogen bonding between hydroxyl groups of phenolic compounds and carbonyl and amide groups of proteins, also by hydrophobic interaction between the benzoic ring of phenolic compounds and the apolar side chains of amino acids such leucine, lysine or proline in salivary proteins (Laguna & Sarkar, 2017; Santos-Buelga & De Freitas, 2009).

As shown in the sensory results, presence of saliva-tannin complexes provoked earthiness and astringency sensation, furthermore a significant relation was observed between earthiness and particle size ($R_{\text{hydrodinamic diameter/earthiness}}$=0.706, p=0.049). This means that particles were not only large but also ”gritty” and “particulate” to affect perception, which influenced the sensory perception (Engelen, Van Der Bilt, Schipper, & Bosman, 2005). Such gritty particle nature of the nano-complexes might have altered the lubricating properties of the salivary pellicles thus activating the mechanoreceptors, located within the mucosa (Horne et al., 2002; Kallithraka et al., 1997; Lesschaeve & Noble, 2005).

Hydrodynamic diameter of HS did not change significantly in presence of ethanol and glycerol (Figure 5e and Figure 2). This suggests that ethanol and glycerol, at levels present in wines, did not alter the salivary protein conformation and did not result in anynano-complex formation.

From the tribological results, it can be observed how the simulated saliva (SS) reduced the dynamic coefficient of friction between the PDMS surfaces as previously reported (Bongaerts, Rossetti, & Stokes, 2007; Laguna, Farrell, Bryant, Morina, & Sarkar, 2017). At the boundary lubrication regime of the wine model tested, it can be observed that WG was the sample with less traction coefficient, therefore, glycerol had a lubricant effect .. This is in agreement with previous literature, where glycerol was associated with various attributes, such as oiliness, persistence and mellowness (Lubbers, Verret, & Voilley, 2001).

To our knowledge there has been only one previous study that has analyzed wine samples using tribology techniques. The conclusion of the authors (Brossard et al., 2016) were in contrast with our present results. Brossard et al. (2016) stated that sensory perception via a physical stimulus can be quantified using tribology techniques. In our study, although panelists discriminated samples with and without tannins addition, tribology was not effective to discriminate the samples with/ without the presence of tannins. It needs to be remembered that Brossard et al. (2016), used four samples and a sliding speed of 0.075 mm/sec, whilst the tongue movement speed has been at speeds of up to 200 mm/s (Hiiemae & Palmer, 2003). Secondly, PDMS-steel tribopaires were used as opposed to PDMS-PDMS tribopaires alter used in this study. Furthermore, besides phenolic compounds, there are other components in wine, which can also contribute to astringency perception. For example the tartaric acid present, caused the change of pH (model wine pH=3.8 whilst

distilled water pH=7), altering the properties of the simulated saliva, i.e. mucins might be self-aggregating (Macakova, Yakubov, Plunkett, & Stokes, 2011) nearing the isoelectric point. Hence, the difference between the wine samples were not evident in these tribology results, as lubricating properties of SS containing mucin was diminishing owing to the low pH used in all wines in this study overshadowing the effects of tannin composition.

As a limitation of this study it should be mentioned that it was not feasible to use human saliva for the tribology test, and mucin 3 % wt% solution with salivary ionic composition and pH was used to simulate human saliva. However, the physical and chemical properties of this simulated saliva differ from those of HS. Even though, this study provides a first comprehensive understanding wine-saliva interaction.

Conclusions

In this paper, quantitative (rheology, particle size, tribology) and qualitative (microstructure) instrumental techniques were assessed to relate with wine mouthfeel properties, latter described by a trained panel. Overall, using a model wine matrix with the addition of tannins, glycerol and ethanol and evaluating the samples by sensory and instrumental techniques, it can be observed that particle size measurement correlates with sensory earthiness perception. However rheology and tribology techniques pose some challenges with respect to correlation with sensory perception. Although rheology was able to discriminate among samples, the changes captured were far too sensitive for the in-mouth perception. Tribology also complements the results obtained by the trained panel; it was able to display the difference in lubrication due to glycerol. However, due to the low pH of the samples, the differences found in astringency by the trained panel, were not found using a tribometer.

Therefore, these results suggest that instrumental methods cannot completely account for the complexity of the human perception, but can help to understand some of the in-mouth saliva-sine interaction quantitatively. Such tools offer a promising step towards standardizing testing protocols in wineries

With this consideration in mind, adaptation of the proposed techniques to represent oral conditions (e.g. use of bio-relevant surfaces and real human saliva in tribology measurements) need to be explored to study the surface and mechanical properties of the change in salivary film upon wine consumption.

Acknowledgements

The authors of this manuscript were funded by the Spanish MINECO project (AGL2015-64522-C2-R-01 and RTC-2016-4556-1). Laura Laguna would like to thank the Spanish "Juan de la Cierva" program for her contract (ref FJCI-2014-19907). Authors would like to thank the assistance of Lidia Catedra and Man Wai Lai. Also we would like to thank Maria Dolores Álvarez to kindly let us use the rheometer Kinexus and the appropriate geometries. The authors would like to gratefully acknowledge the contributions of Martin Fuller for his technical support in electron microscopy at the Bio-imaging Facility within

the Faculty of Biological Sciences of the University of Leeds. Finally, special thanks are addressed to Mr Phillip John Bentley (The University of Leeds) for his help in English revision and to the trained panel for their time and valuable answers.

References

Anger, H. (1977). F. J. Francis Und F. M. Clydesdale: Food Colorimetry: Theory And Applications. 477 Seiten, 195 Abb., 40 Tab. Westport, Connecticut, The Avi Publishing Company, Inc., 1975. Preis: 36.00 $. Food / Nahrung, 21(1), 90-91.

Bongaerts, J. H. H., Rossetti, D., & Stokes, J. R. (2007). The Lubricating Properties of Human Whole Saliva. Tribology Letters, 27(3), 277-287.

Brossard, N., Cai, H., Osorio, F., Bordeu, E., & Chen, J. (2016). "Oral" Tribological study on the astringency sensation of red wines. Journal of Texture Studies, n/a-n/a.

DeMiglio, P., Pickering, G. J., & Reynolds, A. G. (2002). Astringent sub-qualities elicited by red wine: the role of ethanol and pH. Paper presented at the Proceedings of the international Bacchus to the future conference, St Catharines, Ontario.

Engelen, L., Van Der Bilt, A., Schipper, M., & Bosman, F. (2005). Oral size perception of particles: Effect of size, type, viscosity and method. Journal of Texture Studies, 36(4), 373-386.

Ferrer-Gallego, R., Hernández-Hierro, J. M., Rivas-Gonzalo, J. C., & Escribano-Bailón, M. T. (2014). Sensory evaluation of bitterness and astringency sub-qualities of wine phenolic compounds: synergistic effect and modulation by aromas. Food Research International, 62, 1100-1107.

Gawel, R., Oberholster, A., & Francis, I. L. (2000). A 'Mouth- feel Wheel': terminology for communicating the mouth- feel characteristics of red wine. Australian Journal of Grape and Wine Research, 6(3), 203-207.

Gawel, R., & Waters, E. J. (2008). The Effect of Glycerol on the Perceived Viscosity of Dry White Table Wine. Journal of Wine Research, 19(2), 109-114. doi:10.1080/09571260802622191

Hagerman, A. E., & Butler, L. G. (1981). The specificity of proanthocyanidin-protein interactions. Journal of Biological Chemistry, 256(9), 4494-4497.

Hiiemae, K. M., & Palmer, J. B. (2003). Tongue movements in feeding and speech. Critical Reviews in Oral Biology & Medicine, 14(6), 413-429.

Horne, J., Hayes, J., & Lawless, H. T. (2002). Turbidity as a measure of salivary protein reactions with astringent substances. Chemical Senses, 27(7), 653-659.

Jackson, R. S. (2009). Chapter 4 - Taste and Mouth-Feel Sensations Wine Tasting (Second Edition) (pp. 129-175). San Diego: Academic Press.

Jacobs, R., Wu, C. H., Goossens, K., Van Loven, K., Van Hees, J., & Van Steenberghe, D. (2002). Oral mucosal versus cutaneous sensory testing: a review of the literature. Journal of oral rehabilitation, 29(10), 923-950.

Kallithraka, S., Bakker, J., & Clifford, M. N. (1997). Effect of pH on astringency in model solutions and wines. Journal of Agricultural and Food Chemistry, 45(6), 2211-2216.

King, E. S., Dunn, R. L., & Heymann, H. (2013). The influence of alcohol on the sensory perception of red wines. Food Quality and Preference, 28(1), 235-243.

Laguna, L., Bartolomé, B., & Moreno-Arribas, M. V. (2017). Mouthfeel perception of wine: Oral physiology, components and instrumental characterization. Trends in Food Science & Technology, 59, 49-59.
Laguna, L., Farrell, G., Bryant, M., Morina, A., & Sarkar, A. (2017). Relating rheology and tribology of commercial dairy colloids to sensory perception. Food & Function.
Laguna, L., & Sarkar, A. (2017). Oral tribology: update on the relevance to study astringency in wines. Tribology-Materials, Surfaces & Interfaces, 1-8.
Lawless, H. T., & Heymann, H. (2010). Consumer field tests and questionnaire design Sensory Evaluation of Food (pp. 349-378): Springer.
Lehrer, K., & Lehrer, A. (2016). The language of taste. Inquiry, 59(6), 752-765.
Lesschaeve, I., & Noble, A. C. (2005). Polyphenols: factors influencing their sensory properties and their effects on food and beverage preferences. American Journal of Clinical Nutrition, 81(1), 330S-335S.
Lubbers, S., Verret, C., & Voilley, A. (2001). The Effect of Glycerol on the Perceived Aroma of a Model Wine and a White Wine. LWT - Food Science and Technology, 34(4), 262-265.
Macakova, L., Yakubov, G. E., Plunkett, M. A., & Stokes, J. R. (2011). Influence of ionic strength on the tribological properties of pre-adsorbed salivary films. Tribology International, 44(9), 956-962. doi:10.1016/j.triboint.2010.12.006
Morrot, G., Brochet, F., & Dubourdieu, D. (2001). The color of odors. Brain and language, 79(2), 309-320.
Munoz-Gonzalez, C., Martin-Alvarez, P. J., Moreno-Arribas, M. V., & Pozo-Bayon, M. A. (2014). Impact of the nonvolatile wine matrix composition on the in vivo aroma release from wines. J Agric Food Chem, 62(1), 66-73. doi:10.1021/jf405550y
Nurgel, C., & Pickering, G. (2005). Contribution of glycerol, ethanol and sugar to the perception of viscosity and density elicited by model white wines. Journal of texture studies, 36(3), 303-323.
Pickering, G. J., Heatherbell, D., Vanhanen, L., & Barnes, M. (1998). The effect of ethanol concentration on the temporal perception of viscosity and density in white wine. American journal of enology and viticulture, 49(3), 306-318.
Pradal, C., & Stokes, J. R. (2016). Oral tribology: bridging the gap between physical measurements and sensory experience. Current Opinion in Food Science, 9, 34-41.
Prinz, J., & Lucas, P. (2000). Saliva tannin interactions. Journal of oral rehabilitation, 27(11), 991-994.
Quijada-Morin, N., Williams, P., Rivas-Gonzalo, J. C., Doco, T., & Escribano-Bailon, M. T. (2014). Polyphenolic, polysaccharide and oligosaccharide composition of Tempranillo red wines and their relationship with the perceived astringency. Food Chem, 154, 44-51.
Rinaldi, A., Gambuti, A., & Moio, L. (2012). Precipitation of salivary proteins after the interaction with wine: the effect of ethanol, pH, fructose, and mannoproteins. J Food Sci, 77(4), C485-490. doi:10.1111/j.1750-3841.2012.02639.x
Rossetti, D., Bongaerts, J. H. H., Wantling, E., Stokes, J. R., & Williamson, A. M. (2009). Astringency of tea catechins: More than an oral lubrication tactile percept. Food Hydrocolloids, 23(7), 1984-1992. doi:10.1016/j.foodhyd.2009.03.001
Santos-Buelga, C., & De Freitas, V. (2009). Influence of phenolics on wine organoleptic properties Wine chemistry and biochemistry (pp. 529-570): Springer.

Sarkar, A., Goh, K. K. T., & Singh, H. (2009). Colloidal stability and interactions of milk-protein-stabilized emulsions in an artificial saliva. Food Hydrocolloids, 23(5), 1270-1278.
Stokes, J. R., & Davies, G. A. (2007). Viscoelasticity of human whole saliva collected after acid and mechanical stimulation. Biorheology, 44(3), 141-160.
Stone, H., Sidel, J., Oliver, S., Woolsey, A., & Singleton, R. C. (2008). Sensory evaluation by quantitative descriptive analysis. Descriptive Sensory Analysis in Practice, 23-34.
Upadhyay, R., Brossard, N., & Chen, J. (2016). Mechanisms underlying astringency: introduction to an oral tribology approach. Journal of Physics D: Applied Physics, 49(10), 104003.
Verhagen, J. V., & Engelen, L. (2006). The neurocognitive bases of human multimodal food perception: sensory integration. Neuroscience & Biobehavioral Reviews, 30(5), 613-650.
Vidal, L., Giménez, A., Medina, K., Boido, E., & Ares, G. (2015). How do consumers describe wine astringency? Food Research International, 78, 321-326.
Yanniotis, S., Kotseridis, G., Orfanidou, A., & Petraki, A. (2007). Effect of ethanol, dry extract and glycerol on the viscosity of wine. Journal of Food Engineering, 81(2), 399-403.

Figure captions

Figure 1. Mean descriptive sensory scores of a) W (grey solid line), WT (grey dashed line), WG (black dashed line), WGT (black solid line) and b) WEGT (grey solid line), WE (grey dashed line), WEG (black dashed line), WET (black solid line).

Figure 2. Mean and standard deviation of the Z-average diameter of the mixture of human saliva (HS) and model wine. Bars with the same letter do not differ significantly ($p>0.05$) according to Tukey's test.

Figure 3. Dynamic viscosity of model wine with human saliva (1:1) of a) HS (●), HS+water (1:1) (○) and mixture of HS with model wine W (▲), WG (♦), WT (■), WGT (□) and b) HS (●), HS+water (1:1) (○) and mixture of HS with model wine WE (Δ), WEG (◊), WET (□) and WEGT (□). In the left corner of each graph is presented the average of three values and the deviation values ($\alpha = 0.05$) of viscosities at 1 Pa.s (η_1), the consistency index (K) and flow index (n). Means (in the same column) with the same letter do not differ significantly ($p < 0.05$) according to Tukey test.

Figure 4. Traction coefficient dependence at various entraintment speed of wine model with HS at a) forward traction and b) reverse traction for samples of W(■), WG (●), WT (▲), WEGT (▼), HS (♦) and distilled water (◀).

Figure 5. Negative-stain TEM micrographs of a) HS, b) HS+water, c) HS+W d) HS+WT, e) HS+WEG and f) HS+WEGT